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**Innowacyjne techniki impregnacji i optymalizacja procesów
w kształtowaniu właściwości fizykochemicznych,
funkcjonalnych, enzymatycznych i sensorycznych
liofilizowanych przekąsek jabłkowych**

Innovative impregnation techniques and process optimization
for shaping the physicochemical, functional, enzymatic, and sensory properties
of freeze-dried apple snacks

Doctoral dissertation in the field of agricultural science
in the discipline of food technology and nutrition

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Dedication

To my beloved wife, Yolanda Victoria Rajagukguk, for always being by my side not only as a life partner, but also as a co-worker since our undergraduate days. We have faced every challenge together, worked tirelessly side by side, and now we celebrate a dream we have built and achieved together. I could not have done this without her.

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List of publications and funding sources

A1. Arnold, M., & Gramza-Michałowska, A. (2024). Recent development on the chemical composition and phenolic extraction methods of apple (*Malus domestica*)—a review. *Food and Bioprocess Technology*, 17(9), 2519-2560. <https://doi.org/10.1007/s11947-023-03208-9>

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A3. Arnold, M., Białas, W., Kulczyński, B., Multisona, R. R., Suliburska, J., Świeca, M., Wojdyło, A., & Gramza-Michałowska, A. (2025). Product development study of freeze-dried apples enriched with sea buckthorn juice and calcium lactate. *Molecules*, 30(7), 1504. <https://doi.org/10.3390/molecules30071504>

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A4. Arnold, M., Tylewicz, U., Suliburska, J., Świeca, M., Wojdyło, A., & Gramza-Michałowska, A. (2025). Vacuum and ultrasound-assisted impregnation of Gala apple with sea buckthorn juice and calcium lactate: functional properties, antioxidant profile, and activity of polyphenol oxidase and peroxidase of freeze-dried products. *Polish Journal of Food and Nutrition Sciences*, 75(3), 283-302. <https://doi.org/10.31883/pjfn/209424>

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A5. Arnold, M., Suliburska, J., Świeca, M., Wojdyło, A., & Gramza-Michałowska, A. (2025). Storage stability of bioactive-enriched freeze-dried Gala apples: functional properties, sensory evaluation, and enzymatic browning. *Food and Bioprocess Technology*. <https://doi.org/10.1007/s11947-025-03975-7>

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List of abbreviations

AAS	: atomic absorption spectrometry
ABTS	: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
ANOVA	: analysis of variance
a-value	: greenness/redness
a_w	: water activity
BI	: browning index
BI ₄₂₀	: browning index (absorbance at 420 nm)
b-value	: blueness/yellowness
CaL	: calcium lactate
CON	: conventional impregnation (research stage 2)
DPPH	: 2,2-diphenyl-1-picrylhydrazyl
FA	: freeze-dried fresh apple
FA+AA	: freeze-dried fresh apple after dipping with 1% ascorbic acid
FAO	: Food and Agriculture Organization
FRAP	: ferric-reducing antioxidant power
FW	: fresh weight
GAE	: gallic acid equivalent
IDF	: insoluble dietary fiber
IMP	: freeze-dried apple after impregnation (research stage 3)
LDPE	: low-density polyethylene
L-value	: lightness
MLR	: multiple linear regression
OD	: osmotic dehydration
ORAC	: oxygen radical absorbance capacity
PCA	: principal component analysis
PCL	: photochemiluminescence
PCL-ACL	: photochemiluminescence – lipid soluble antioxidant capacity
PCL-ACW	: photochemiluminescence – water soluble antioxidant capacity
PCL-IAC	: photochemiluminescence – integral antioxidant capacity
PET	: polyethylene terephthalate
POD	: peroxidase
PPO	: polyphenol oxidase
RH	: relative humidity
SB	: sea buckthorn
SDF	: soluble dietary fiber
SDGs	: Sustainable Development Goals
TDF	: total dietary fiber
TE	: Trolox equivalent
TPC	: total phenolic content
UPLC-PDA	: ultra-performance liquid chromatography – photodiode array
US	: ultrasound-assisted impregnation
VI	: vacuum impregnation
WI	: whiteness index
YI	: yellowness index
ΔE	: total color difference

Streszczenie

Wysoka częstość występowania osteoporozy i niedoboru wapnia stała się impulsem do opracowania żywności funkcjonalnej z lokalnych zasobów, wspierającej cele zrównoważonego rozwoju wg. Organizacji Narodów Zjednoczonych. Celem niniejszego badania było opracowanie liofilizowanej przekąski jabłkowej przeciwutleniaczami z soku z rokitnika zwyczajnego (SB) oraz wapniem przy zastosowaniu procesu impregnacji, a także ocena aktywności enzymów brązowienia w celu oceny jakości produktu. Aby osiągnąć ten cel, przeprowadzono badania składające się z trzech etapów. W etapie 1 zoptymalizowano warunki impregnacji pod ciśnieniem atmosferycznym, stosując różne stężenia soku z rokitnika i mleczanu wapnia (CaL), różny stosunek inuliny do soku z rokitnika oraz temperaturę i czas procesu. Wykazano, że w porównaniu z liofilizowanym świeżym jabłkiem (FA) i świeżym jabłkiem po zanurzeniu w 1% kwasie askorbinowym (FA+AA), najniższe straty aktywności przeciwutleniaczy i zwiększoną zawartość wapnia uzyskano w produkcie przygotowanym przy zastosowaniu 93,8% soku z rokitnika (w/w), 4% CaL (w/w) w temperaturze 30°C przez 120 minut. W etapie 2 (dostosowując warunki z etapu 1 z 0 i 4% CaL), stwierdzono, że impregnacja próżniowa (VI) i impregnacja wspomagana ultradźwiękami (US) wpłynęły na poprawę aktywności przeciwutleniającej i zawartość wapnia w produkcie liofilizowanym w porównaniu z impregnacją konwencjonalną (atmosferyczną), FA i FA+AA. Najwyższą aktywność przeciwutleniającą zaobserwowano w VI dla 200 mbar i US dla 30 minut przy 0% CaL, natomiast najwyższą zawartość wapnia stwierdzono w VI dla 200 mbar i US dla 20 minut przy 4% CaL. W etapie 3 oceniono wpływ 3-miesięcznego przechowywania na właściwości funkcjonalne, enzymatyczne i sensoryczne liofilizowanych jabłek po impregnacji (IMP) z dostosowaniem wybranych warunków z etapu 1. IMP wpłynęło korzystnie na aktywność przeciwutleniającą i zawartość wapnia niż FA i FA+AA. We wszystkich etapach liofilizowane próbki impregnowane wykazały zmieniony skład związków fenolowych (zdominowany przez pochodne izoramnetyny) i karotenoidów w porównaniu z FA i FA+AA. Stwierdzono, że proces brązowienia enzymatycznego był zależny od zastosowanego roztworu impregnującego, VI i US oraz okresu przechowywania. Przeprowadzona ocena sensoryczna próbek liofilizowanych i impregnowanych, zwłaszcza z 4% CaL, wskazała na ich wyższą chrupkość w porównaniu z FA i FA+AA zarówno świeżo przygotowanymi, jak i po przechowywaniu. W toku przeprowadzonych badań potwierdzono, że opracowany produkt wykazuje potencjał jako alternatywna żywność funkcjonalna mająca na celu zmniejszenie ryzyka osteoporozy i niedoboru wapnia.

Słowa kluczowe: przeciwutleniacze, jabłko, wapń, impregnacja, rokitnik zwyczajny, zrównoważony rozwój.

Summary

The high prevalence of osteoporosis and calcium deficiency has driven the development of functional foods from local resources, supporting United Nations' Sustainable Development Goals. This study aimed to develop freeze-dried apples enriched with antioxidants from sea buckthorn (SB) juice and calcium via impregnation, while also assessing browning enzyme activities to evaluate product quality. To achieve the goal, a three-stage study was carried out. Stage 1 optimized impregnation conditions at atmospheric pressure using various concentrations of SB juice and calcium lactate (CaL), inulin:SB juice ratios, temperatures, and processing times. It was shown that, compared to freeze-dried fresh apple (FA) and fresh apple after dipping in 1% ascorbic acid (FA+AA), the minimal antioxidant activity loss and increased calcium content were obtained in the product prepared using 93.8% SB juice (w/w), 4% CaL addition (w/w) at 30°C for 120 min. In stage 2 (adapting the conditions from stage 1 with 0 and 4% CaL), it was found that vacuum (VI) and ultrasound-assisted impregnation (US) significantly improved the antioxidant activity and calcium content of the freeze-dried product as compared to conventional (atmospheric) impregnation, FA, and FA+AA. The highest antioxidant activity was observed in VI for 200 mbar and US for 30 min at 0% CaL, while the highest calcium content was found in VI for 200 mbar and US for 20 min at 4% CaL. Stage 3 evaluated the effects of 3-month storage on the functional, enzymatic, and sensory properties of freeze-dried apples after impregnation (IMP) adapting selected stage 1 conditions. IMP had a more beneficial effect on the antioxidant activities and calcium content than FA and FA+AA. In all stages, the freeze-dried impregnated samples showed an altered composition of phenolics (dominated by isorhamnetin derivatives) and carotenoids compared to FA and FA+AA. It was found that the enzymatic browning process was dependent on the impregnating solution used, VI and US, and the storage period. The freeze-dried impregnated samples, especially those with 4% CaL, showed higher crispiness than FA and FA+AA both freshly prepared and after storage in sensory evaluation. The research confirmed that the developed product has potential as an alternative functional food aimed at reducing the risk of osteoporosis and calcium deficiency.

Keywords: antioxidants, apple, calcium, impregnation, sea buckthorn, sustainability.

1. Introduction

1.1. Osteoporosis and calcium deficiency

Osteoporosis is a common skeletal disorder, characterized by reduced bone mineral density and microstructural quality, which leads to an increased risk of fragility fractures (Martiniakova et al., 2022). It has become one of the major causes of premature deaths and deaths in elderly (Bassatne et al., 2020). Osteoporosis is a global problem; based on 86 studies reported across five continents, a meta-analysis study highlighted 18.3% of osteoporosis prevalence in the world, with the age range 15-105 years (Salari et al., 2021). Women are more prone to develop osteoporosis and subsequent fractures than men; after the age of 50, osteoporosis affects 50% of women and 20% of men (Rizzoli & Chevalley, 2024). Additionally, the calcium intake of 72% of Polish population, aged 20-80 years, was lower than suggested 1000 mg/day (746 mg/day) (Skowrońska-Józwiak et al., 2016).

The risk factors of osteoporosis are divided into two categories: a) non-modifiable, such as gender, age, race and genetic characteristics, and b) modifiable, such as weight, smoking habit, alcohol consumption, physical inactivity, and calcium deficiency (Salari et al., 2021). It is recommended to control eating habits to achieve the highest bone mass density and manage the osteoporosis problem. Some studies found that macronutrients, micronutrients, polyphenols, and carotenoids play an important role in maintaining bone health and subsequently preventing osteoporosis (Arnold et al., 2021; Kulczyński et al., 2024; Martiniakova et al., 2022). Therefore, there is a need to develop functional food, enriched with bioactive compounds, in order to prevent osteoporosis and calcium deficiency.

1.2. Functional food based on local resources and impregnation process

Among 17 United Nations' Sustainable Development Goals (SDGs) to be achieved by 2030 (United Nations, 2015), this study supports goal number 2 and 3. Goal number 3 is to ensure healthy lives and promotes well-being for all at all ages. This goal has a target to reduce by one third premature mortality from non-communicable diseases (including osteoporosis) through prevention and treatment and to promote mental health and well-being. While goal number 2 focuses on ending hunger, achieving food security and improved nutrition, as well as promoting sustainable agriculture.

Therefore, a functional food rich in antioxidant and calcium based on local resources was developed in this study, intended for people with calcium deficiency and osteoporosis.

Among the available resources of antioxidants available in Poland and Europe, sea buckthorn (SB; *Hippophae rhamnoides*, synonym: *Elaeagnus rhamnoides*) and apple (*Malus domestica*) fruits are potential to be developed as functional food to achieve the goals. SB grows wild and survives on poor soil and extreme temperatures, ranging from -40 to 40°C. It is also well cultivated for industrial purposes in Asia, e.g. Central Asia, China, Tibet, India, Mongolia, the Caucasus, Turkey, Russia; in Europe, including Germany, Finland, Estonia, Poland, Sweden, Norway, and the Netherlands; and on desert area of North America (Ciesarová et al., 2020; Kania-Dobrowolska et al., 2023). The SB cultivation, supported by the Food and Agriculture Organization (FAO) and governments, contributes to food security and sustainability in line with the SDGs (FAO, 2020). The productivity of SB is about 4-5 ton/ha and in some cases could reach 20-25 ton/ha (Ciesarová et al., 2020). The chemical composition of SB have been reviewed previously (Ciesarová et al., 2020; Dong et al., 2021; Zhu et al., 2025); its pulp, seeds, fruit and juice contains more than 190 bioactive compounds, such as but is not limited to vitamins (A, B₁, B₂, B₉, C, E, K), fatty acids, lipids, phenolic compounds (flavonoids, phenolic acids, tannins), carotenoids (zeaxanthin, lycopene, β -cryptoxanthin, α -, β -, γ -carotene, lutein), minerals (K, Ca, P, Mg, Na).

Owing to abundant bioactive compounds, some studies on health effects of SB fruit were reported. SB fruit was studied to show anti-osteoporosis effects in ovariectomized mice study as it promotes osteogenic differentiation (Park et al., 2022), displayed by triterpenoids, with ursolic aldehyde exhibited the most significant effect on osteoblast differentiation (Lee et al., 2023). The flavonoids from SB beneficially impact the bone health in terms of bone remodeling, mineralization, and oxidative stress, according to the latest review (Martiniakova et al., 2024). Additionally, SB also contributes to reducing the risk of cardiovascular disease, diabetes, inflammatory diseases, thrombosis, and cancer, which were reviewed by Xu et al. through some *in vitro* and *in vivo* studies (Xu et al., 2011). Though edible fresh, SB is commonly found frozen, juiced, dried, or used in various products like baked goods, confectionery, dairy, jams, and beverages (Kania-Dobrowolska et al., 2023).

To provide the population with a significant intake of calcium and antioxidants, it is necessary to find the food that is widely consumed by the population of a region, for example, apples. Apple has become one of the most popular fruits consumed and cultivated worldwide. In 2021, Poland was reported to be the fourth largest apple producer, contributing 4.4% worldwide apple production, after China (49.4%), Turkey (4.8%), and USA (4.8%) (FAO, 2025). The major cultivars produced in European Union-20 and UK in the period of 2017-

2021 were Golden Delicious (19.1%), Gala (12.9%), Idared (6.7%), Red Delicious (6.7%), and Shampion (5.9%) (European Commission, 2022).

Due to its bioactive compounds, especially phenolics, apples provide health benefits, including antidiabetic, antioxidative, antihypertensive, cholesterol-lowering, cardiovascular protective, and anti-inflammatory effects (Geană et al., 2021; Patocka et al., 2020). The major phenolic groups found in apple (flesh and peel) are hydroxybenzoic acids, hydroxycinnamic acids, flavanols and their oligo- and polymeric structures, flavonols, dihydrochalcones, and anthocyanins. Furthermore, although calcium is still present in the apple (2.48-6.00 mg/100 g fresh weight (FW)), its contribution to daily recommended value is low (Feliciano et al., 2010; USDA, 2019). Different cultivars vary in chemical composition; consumers should choose affordable, locally available apples with good nutritional value to support overall health (dissertation paper A1).

Based on the knowledge above, freeze-dried apple snack enriched with antioxidants from SB juice and calcium was developed. Both conventional (at atmospheric pressure) and non-conventional impregnation (e.g. vacuum (VI) and ultrasound-assisted impregnation (US)), using different impregnating solutions (hypotonic to hypertonic solutions) have been reported previously to effectively enrich the bioactive compounds in various fruits (Kulczyński et al., 2021; Tappi et al., 2017; Vasile et al., 2022). VI could promote the impregnating solution to flow into the porous matrix due to pressure difference, while US relies on cavitation to enhance mass transfer. Additionally, organic calcium salt, calcium lactate (CaL), was also used in the impregnating solution in this study to increase the calcium content of resulting products. CaL has a relatively high bioavailability and good solubility; it is commonly used in supplements and functional foods to increase the calcium uptake (Wawrzyniak et al., 2022).

In addition, at some stages in this study, inulin was also included as one of the components in the impregnating solution. Previous studies found that impregnation process of fruits using hypertonic solution (sugars), also called osmotic dehydration (OD), could promote water loss, yet also significantly decreased antioxidant activities of product (Tylewicz et al., 2020). As an alternative to sugar (particularly sucrose), inulin could be included in the impregnating solution as an alternative sweetener with lower caloric value and might act as prebiotic improving calcium absorption (Jiménez-Hernández et al., 2017).

1.3. Preventing enzymatic browning in apple products

Enzymatic browning is one of the primary issues found in apple products, including fresh cut, juice, puree, and even in dried or freeze-dried apples. The main enzymes responsible for this browning are polyphenol oxidase (PPO) and peroxidase (POD). The enzymatic browning creates undesirable brown color and reduces the quality and sensory properties of fruits and vegetables that causes 50% of fresh fruit loss (Moon et al., 2020). The activity of PPO and POD varies between apple cultivars, depending on the self-defense mechanism, physicochemical characteristics, and chemical composition of each cultivar, such as vitamin C and natural antioxidants (dissertation paper A2). Therefore, selection of apple cultivars that have low PPO and POD activities becomes the initial step to prevent enzymatic browning. It is worth noticing that each region has different available apple cultivars.

Treating apples with anti-browning agents and natural products was reported to prevent enzymatic browning. As an antioxidant, vitamin C blocks enzymatic browning by the reduction of *o*-quinones back to the phenolic substrate (Lee et al., 2022). Phenolic compounds may act in enzymatic browning either as substrates (low molecular weight phenolic compounds) or inhibitors by interacting with active sites of browning enzymes, interacting with enzymes via hydrogen bonds or hydrophobic interaction, as well as electron donation to intermediate quinone to terminate further oxidation (Sae-leaw & Benjakul, 2019; Serra et al., 2021). Dipping or impregnating apples in the solution containing vitamin C and antioxidants, such as SB juice might have potential to prevent the PPO and POD activities.

1.4. Summary of introduction

This study focuses on the development of functional snack from locally available Gala apples in Poland and Europe, enriched with antioxidants from sea buckthorn juice and calcium via impregnation process, with potential benefits for people with osteoporosis and calcium deficiency. The end product not only contains enriched bioactive compounds but is also sensorially acceptable.

This chapter is written based on publications:

A1: Arnold, M., & Gramza-Michałowska, A. (2024). Recent development on the chemical composition and phenolic extraction methods of apple (*Malus domestica*)—a review. *Food and Bioprocess Technology*, 17(9), 2519-2560. <https://doi.org/10.1007/s11947-023-03208-9>

A2: Arnold, M., & Gramza-Michałowska, A. (2022). Enzymatic browning in apple products and its inhibition treatments: a comprehensive review. *Comprehensive Reviews in Food Science and Food Safety*, 21(6), 5038-5076. <https://doi.org/10.1111/1541-4337.13059>

2. Aims and hypotheses

2.1. Aims

This research aimed to evaluate the physicochemical, functional, enzymatic, and sensory properties of freeze-dried Gala apples enriched with antioxidants from SB juice and calcium through the impregnation process as a potential functional snack that may contribute to osteoporosis prevention and calcium intake enhancement. The main goal was achieved by accomplishing specific aims:

1. to optimize the impregnation process conditions of Gala apple using hypotonic to hypertonic solutions, containing different concentrations of SB juice, inulin, and CaL, at different times and temperatures, aimed to achieve freeze-dried products with improved antioxidant properties, calcium content, nutritional value, reduced enzymatic browning, and acceptable sensory properties (dissertation paper A3).
2. to evaluate the effect of vacuum and ultrasound-assisted impregnation of Gala apple using SB juice and CaL on the physicochemical properties, antioxidant properties, calcium content, and enzymatic browning of freeze-dried products (dissertation paper A4).
3. to evaluate the effect of 3-month storage on the functional properties, enzymatic browning, and sensory properties of freeze-dried products, after impregnation using a solution with selected composition of SB juice, CaL, and inulin (dissertation paper A5).

2.2. Hypotheses

In this research, the following hypotheses were verified:

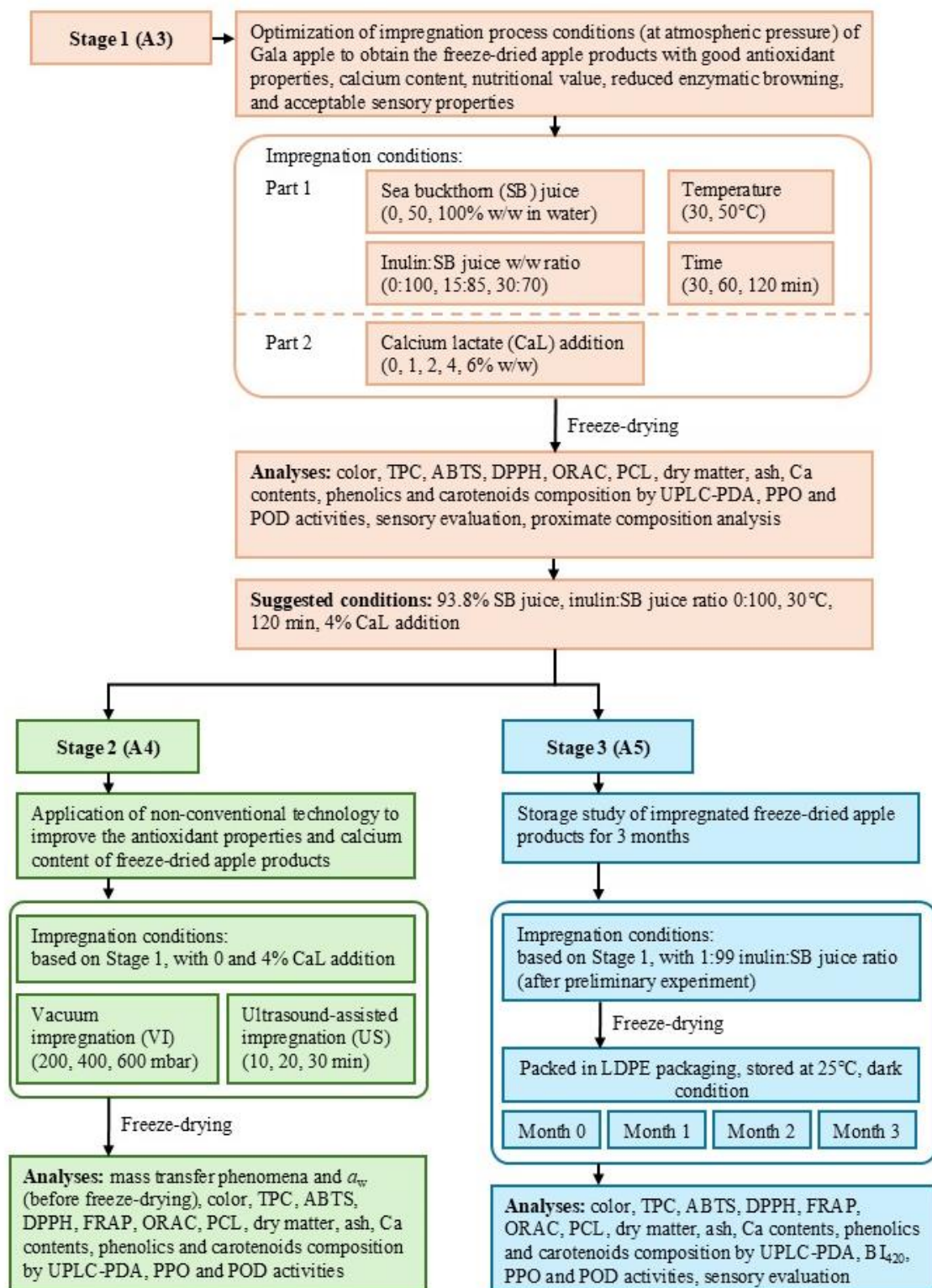
H1a&b: The antioxidant properties, calcium content, nutritional value, browning enzymes activities, and sensory properties of freeze-dried apple products are significantly influenced by temperature, time, and composition of impregnating solutions, containing various concentrations of SB juice, inulin (H1a), and CaL (H1b) (dissertation paper A3, A4).

H2 : Application of vacuum and ultrasound-assisted impregnation significantly affects the physicochemical, functional properties (antioxidant properties, calcium content) and browning enzymes activities of freeze-dried apple products (dissertation paper A4).

H3 : The functional properties, enzymatic browning, and sensory properties of freeze-dried products alter over the storage period, yet their antioxidant properties, calcium content, and sensory properties are still acceptable after 3 months of storage (dissertation paper A5).

2.3. Research scheme

This study consisted of three research stages (Figure 1).



All abbreviations are described on the “List of abbreviations” section.

Figure 1. Research scheme for dissertation

3. Materials and methods

3.1. Materials

Fresh apples (*Malus domestica* cv. Gala) were acquired from organic farm in Rabowice, Poland (dissertation paper A3, A5) and VOG s.a.c. in Terlan, Italy (dissertation paper A4). The C-shaped apple flesh (5 mm thick) was used in the impregnation process, obtained after cleaning, peeling (2 mm thick), coring using an apple peeler and slicer. The apple flesh was then dipped in 1% ascorbic acid (Stanlab sp. z o.o., Poland for dissertation paper A3 and A5; Sigma Aldrich, USA for dissertation paper A4) for 2 min to prevent early enzymatic browning. Freeze-dried apple without and with dipping with ascorbic acid were prepared as the controls. Pasteurized organic SB juice (*Hippophae rhamnoides*) was purchased from Oleofarm sp. z o.o., Poland (dissertation paper A3 and A5) and KoRo, Germany (dissertation paper A4). Inulin (Orafti HIS) was purchased from Hortimex Plus sp. z o.o., Poland (dissertation paper A3 and A5). Calcium lactate (CaL) was purchased from Agnex sp. z o.o., Poland (dissertation paper A3 and A5) and Sigma-Aldrich, USA (dissertation paper A4). The total soluble solid of raw materials and impregnating solutions were also measured using refractometer PAL-1 Atago, Japan.

3.2. Methods

The methods included in the research were as follows:

3.2.1. Impregnation process (atmospheric, vacuum, and ultrasound-assisted) and storage

The impregnation of Gala apple was conducted conventionally at atmospheric pressure (dissertation paper A3, A4, A5), as well as using non-conventional techniques (dissertation paper A4): vacuum (VI) and ultrasound-assisted (US) impregnation. In general, 50 g of frozen (dissertation paper A3, A5) or fresh apple flesh (dissertation paper A4), after dipping with 1% ascorbic acid, were placed in polyethylene terephthalate (PET) jars, containing 250 g of impregnating solution. The impregnating solution consisted of various or selected concentrations of sea buckthorn juice, water, CaL, and inulin. The impregnation process was carried out in triplicate, shaken continuously (Laboplay SWB 22N, Poland for dissertation paper A3 and A5; Shaker 709, ASAL, Italy for dissertation paper A4) at various or selected temperature and time, depending on the research stage. The suggested

impregnation conditions obtained in stage 1 (dissertation paper A3), became the basis for impregnation process in stage 2 (dissertation paper A4), and 3 (dissertation paper A5).

For stage 2 (dissertation paper A4), VI was performed at various pressures (200, 400, and 600 mbar) using a vacuum pump and automatic controller system (AVCS, S.I.A., Italy). The VI process consisted of 4 min to decrease the pressure, 10 min of holding time at those pressures, 4 min to achieve the atmospheric pressure, and 10 min relaxation at atmospheric pressure at 30°C. US was performed for 10, 20, and 30 min (25 kHz, 1000 W, 30°C) in an ultrasound bath (Xtra ST 600H, Elmasonic, Germany), continued with atmospheric impregnation for 110, 100, and 90 min, respectively, to achieve 120 min of total impregnation process. All impregnated samples in all stages were frozen at -40°C for 24 h, and freeze-dried for 48 h (Alpha 1-4 LCS, Christ, Germany for dissertation paper A3 and A5; Lio2000, CinquePascal S.r.l., Italy for dissertation paper A4).

For stage 3 (dissertation paper A5), the freeze-dried products were packed in low-density polyethylene (LDPE) bag, sealed, and stored for 3 months in a dark condition in an incubator (CLW 240 STD, Pol-Eko Aparatura, Poland) at 25±0.5°C and relative humidity (RH) 50±5%. The analyses of freeze-dried products were conducted in month 0, 1, 2, and 3.

3.2.2. Analyses of samples

The following analyses were carried out, with the detailed methods that could be found in the published dissertation articles (A3, A4, A5):

- Mass transfer phenomena and water activity

Weight gain/loss, water gain/loss, and solid gain/loss were calculated, and water activity (a_w) (WaterLab, Steroglass S.r.l., Italy) analysis was carried out in stage 2 (dissertation paper A4) after the impregnation process, prior to freeze-drying (Tylewicz et al., 2020).

- Color determination

The color of all freeze-dried apple products (before grinding) in all stages, as well as the SB juice, were analyzed using CIELAB scale (Chroma Meter CR-5, Konica Minolta, Japan for dissertation paper A3 and A5; Hunterlab, USA for dissertation paper A4). The L-value (lightness), a-value (greenness/redness), b-value (blueness/yellowness) of the sample were measured, and ΔE (total color difference), BI (browning index), WI (whiteness index), YI (yellowness index) were calculated (dissertation paper A2).

- Total phenolic content and antioxidant activities

Extraction of antioxidants: The antioxidant extraction of SB juice and powdered freeze-dried products in all stages was carried out using 80% methanol (v/v in water) at the sample:solvent ratio of 1:10 (w/v, for SB juice) and 1:20 (w/v, for freeze-dried products). The samples were shaken in a water bath (Laboplay SWB 22N, Poland) at 50°C for 2 h. Centrifugation (4°C, 12000 ×g, 10 min) was subsequently conducted to collect the supernatant, that was used for determination of total phenolic content (TPC) and antioxidant activities. The extraction process was carried out in duplicate.

Total phenolic content: TPC was determined by Folin-Ciocalteu assay (Singleton & Rossi, 1965), expressed as mg gallic acid equivalent (GAE) per 100 g of product.

Antioxidant activities: include ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radical cation scavenging activity (Re et al., 1999), DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity (Sánchez-Moreno et al., 1998), ferric-reducing antioxidant power (FRAP) (Benzie & Strain, 1999), oxygen radical absorbance capacity (ORAC) (Gramza-Michałowska & Korczak, 2013), and photochemiluminescence (PCL) – lipid-soluble (ACL), water-soluble (ACW), and integral (IAC) antioxidant capacities, using the designated reagents and Photochem® apparatus (Analytik Jena, Germany) (Balogh et al., 2010; Gramza-Michałowska et al., 2019), with all results presented as mg Trolox equivalent (TE) per 100 g of product, except FRAP (mmol Fe(II)/100 g of product). All reagents, except PCL reagent kits, were acquired from Sigma Aldrich, Germany.

- Calcium content

The atomic absorption spectrometry (AAS; Hitachi Z2000, Japan) was carried out to determine the calcium content of the samples (Suliburska & Krejpcio, 2014), using reference material (Soya Bean Flour, INCT-SBF-4) with 95.7% accuracy for calcium for validation. The result is presented as mg Ca per 100 g of product.

- Quantification of antioxidant composition by UPLC-PDA

The antioxidant composition, particularly phenolics and carotenoids, was quantified in duplicate by ultra-performance liquid chromatography – photodiode array (UPLC-PDA, ACQUITY Ultra Performance Liquid Chromatography Waters Corporation, USA). The extraction and quantification method were carried out according to previous studies (Tkacz et al., 2020, 2022; Wojdyło et al., 2018). Standard references (Extrasynthese, France) were used to compare the retention times and spectra.

- Polyphenol oxidase (PPO) and peroxidase (POD) activities, browning index (BI₄₂₀)

PPO and POD activities and BI₄₂₀ of freeze-dried samples were measured based on previous study (Sikora et al., 2020). The results of PPO and POD activities are expressed as unit (U) of enzyme activity per g of product, where one unit means the amount of enzyme that causes absorbance changes of 0.001 per minute.

- Sensory evaluation

The sensory evaluation of selected freeze-dried samples was carried out in stage 1 (dissertation paper A3) and 3 (dissertation paper A5). Trained panelists (20 panelists for dissertation paper A3; 7 panelists for dissertation paper A5) were asked to fill a questionnaire, in which, a 10 cm linear scale was used to assess the sensory attributes: appearance, aroma, taste, texture, and overall acceptance (Lawless & Heymann, 2010).

- Proximate composition analysis

Proximate composition analysis was performed on selected freeze-dried samples to determine protein (PN-75/A-04018:1975, 1975), lipid (PN-EN ISO 3947:2001, 2001), carbohydrate – determined through calculation: 100 - (protein + lipid + fiber + ash + moisture), fiber (Asp et al., 1983), ash, and moisture contents (AOAC, 2000). All contents are expressed as g per 100 g of product. The energy value, expressed in kcal/100 g product, was calculated using the conversion factors described in Regulation (EU) No. 1169/2011.

- Statistical analyses

Statistical analyses with 95% level of significance were performed employing the software Statistica 13.1 (StatSoft Inc., Poland), including one-way ANOVA followed by Tukey's post hoc test and T-test to analyze the mean differences between samples, Pearson's correlation to evaluate the correlation between variables, and multiple linear regression (MLR) analysis. Shapiro Wilk test to check the normal distribution, followed by Levene's test for homogeneous data of variance were performed before statistical difference analysis. The visualization of Pearson's correlation and principal component analysis (PCA) was conducted using OriginPro 10.1 (OriginLab Corporation, USA). Additionally, for stage 1 (dissertation paper A3), response surface methodology (Response Surface Cubic model) by Design Expert 13 (Stat-Ease, USA) was used to evaluate the effect of impregnating solution composition and process factors on the antioxidant properties of the freeze-dried apples.

4. Research findings

4.1. Stage 1: Optimization of conditions of impregnation process at atmospheric pressure

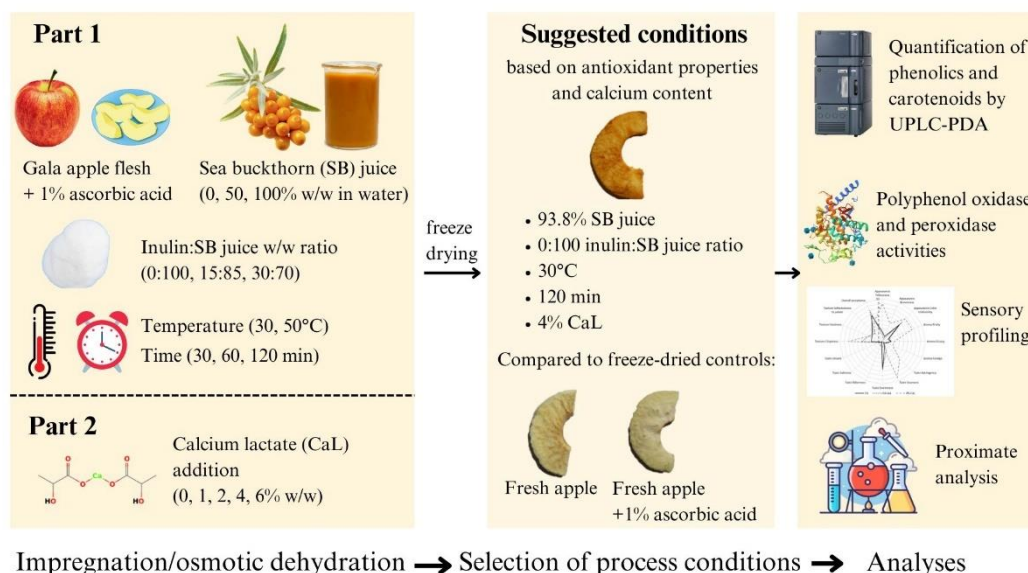


Figure 2. Graphical abstract of research stage 1 (dissertation paper A3) (Arnold et al., 2025)

This research focused on optimization of conditions to produce freeze-dried Polish Gala apple with improved antioxidant properties and calcium content through impregnation process. As described in graphical abstract (Figure 2), this research consisted of two parts. The first part investigated the effect of various impregnating solutions (hypotonic to hypertonic, 0.00 to 33.43°Brix), containing SB juice (0, 50, 100% w/w in water) and varied inulin:SB juice ratios (0:100, 15:85, 30:70), prepared at different times (30, 60, 120 min) and temperatures (30, 50°C). The antioxidant extract was prepared using 80% methanol. The optimized conditions to achieve the highest TPC and each antioxidant activity in freeze-dried impregnated samples were obtained by response surface methodology. Among the antioxidant activities, the conditions to obtain the optimum ORAC value (93.8% SB juice in water, 0:100 inulin:SB juice ratio, 30°C, and 120 min) were chosen for the second part of research, noting that peroxy radicals – the biological relevant radicals, were involved in ORAC assay (Zhong & Shahidi, 2015). The inulin was not suggested by the optimization as the inulin:SB juice ratio of 15:85 and 30:70 significantly decreased the antioxidant activity ($p < 0.05$), indicating that lower ratio could be considered in the future study. Furthermore, SB juice involvement, without or with inulin, could help increasing the TPC and antioxidant activity of freeze-dried products, compared to those with water and inulin only.

In the second part, using the impregnation conditions from first part, 1–6% CaL (w/w) were added to the solution. The higher CaL concentration added to the solution, the higher calcium content of freeze-dried products; however, the TPC and antioxidant activities decreased at the same time. The 4% CaL was then suggested, considering the minimal yet acceptable loss of antioxidant properties and increased calcium content (2209.13 mg Ca/100 g) in comparison to those at 0% CaL, freeze-dried fresh apple (FA) and after dipping with 1% ascorbic acid (FA+AA). Thus, the suggested impregnation conditions were 93.8% SB juice in water, 0:100 SB:juice ratio, 30°C, 120 min, and 4% CaL (also called as 4% CaL treatment).

The antioxidant compositional changes, especially phenolics and carotenoids, were observed in 4% CaL compared to FA and FA+AA using UPLC-PDA. For phenolics, isorhamnetin-3-*O*-glucoside and isorhamnetin-3-*O*-rutinoside were the two dominant compounds in 4% CaL, originated from SB juice (Tkacz et al., 2020), which were not detected in FA and FA+AA. While the total carotenoids of 4% CaL (38.63 mg/100 g product) were about 17 times higher than that of controls, with carotene as the dominated compound group (74% of total carotenoids). The carotenoids might also be responsible for the yellow-orange color of the freeze-dried apples after the impregnation process. Both PPO and POD activities of 4% CaL were also reduced respectively by 88.9 and 43.2% in comparison to FA, which might be due to the presence of lipophilic and hydrophilic antioxidants in 4% CaL.

From proximate analysis, the TDF, as well as SDF and IDF of 4% CaL were significantly higher than controls. The energy value of 4% CaL was 285.34 kcal/100 g, 15.5–17.9% lower than the energy values of FA+AA and FA. Through sensory evaluation, 4% CaL was characterized with darker yellow and brown color, grassy and SB aroma, more intensive astringency and sourness taste, and higher crispiness texture than FA and FA+AA. Although moderate overall acceptance of 4% CaL (5.02) was observed, it could be further improved, especially to balance the taste from SB in the end products.

Conclusions and suggestions for stage 1

1. Inulin at inulin:SB juice ratio of 15:85 and 30:70 significantly decreased the antioxidant activity of resulting freeze-dried samples if compared to those at 0:100 ratio. Ratios lower than 15:85 could be considered in further studies.
2. CaL addition at 1–6% significantly increased the calcium content yet decreased the TPC and antioxidant activities of freeze-dried products.
3. The suggested impregnation conditions considering minimal loss of antioxidant properties with increased calcium content of freeze-dried products were 93.8% SB juice

(w/w in water), 0:100 inulin:SB juice ratio, 30°C, 120 min, and 4% CaL (w/w). Samples prepared with these conditions could recover the loss of antioxidant activity during treatment, especially when compared to those with only water and inulin.

4. Impregnation using suggested conditions significantly affected the composition of phenolics and carotenoids, reduced PPO and POD activity, improved crispiness and nutritional composition of the freeze-dried products.
5. Further studies to balance the flavor should be considered.

Based on this research, the following hypothesis was confirmed:

H1a&b. The antioxidant properties, calcium content, nutritional value, browning enzymes activities, and sensory properties of freeze-dried apple products are significantly influenced by temperature, time, and composition of impregnating solutions, containing various concentrations of SB juice, inulin, and CaL.

This section is written based on publication:

A3: Arnold, M., Białas, W., Kulczyński, B., Multisona, R. R., Suliburska, J., Świeca, M., Wojdyło, A., & Gramza-Michałowska, A. (2025). Product development study of freeze-dried apples enriched with sea buckthorn juice and calcium lactate. *Molecules*, 30(7), 1504. <https://doi.org/10.3390/molecules30071504>

4.2. Stage 2: Application of vacuum and ultrasound-assisted impregnation

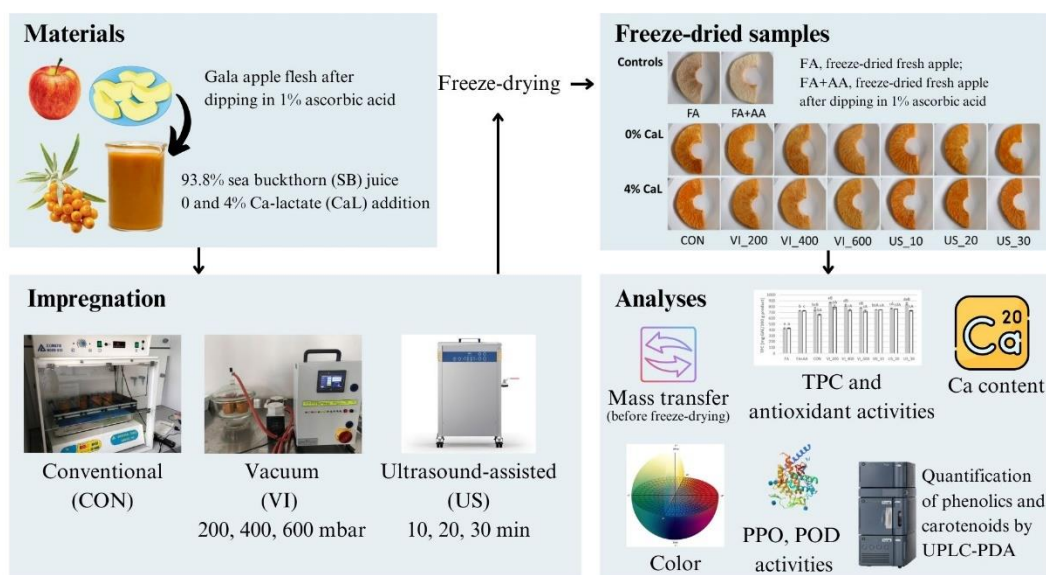


Figure 3. Graphical abstract of research stage 2 (dissertation paper A4)

The focus of this research stage was to improve the antioxidant properties and calcium content of freeze-dried Gala apple by applying vacuum (VI) and ultrasound-assisted impregnation (US) (Figure 3). The impregnation conditions of US and VI were adapted from

the suggested conditions of stage 1 (dissertation paper A3), with two concentrations of CaL (0 and 4%, w/w) added to the impregnating solutions. FA, FA+AA, and freeze-dried products prepared by conventional impregnation (CON) at atmospheric pressure were included as the controls.

Impregnated samples showed significant color difference (ΔE) as compared to FA+AA, however, the ΔE of CON was similar to VI and US, both at 0% and 4% CaL. Furthermore, VI and US affected the mass transfer and resulted in products with higher antioxidant activities and calcium content than CON, FA, and FA+AA. Similar trend as stage 1 (dissertation paper A3), the addition of 4% CaL in all impregnated samples generally decreased the TPC and antioxidant activities of resulting freeze-dried products, except the TPC and ORAC values in US_10 and US_20, and ABTS in CON, where the values were similar between CaL concentrations ($p>0.05$). Among VI treatments, VI_200 exhibited the highest TPC and antioxidant activities (at 0% CaL) and calcium content (at 4% CaL). While among US treatments, the highest TPC and antioxidant activities were found in US_30 at 0% CaL, and the highest calcium content was found in US_20 at 4% CaL. These results were confirmed by principal component analysis (PCA).

The selected samples mentioned above (VI_200 and US_30 at 0% CaL; VI_200 and US_20 at 4% CaL), together with CON (0 and 4% CaL), FA, and FA+AA proceeded to the quantification of phenolics and carotenoids by UPLC-PDA. Isorhamnetin-3-*O*-rutinoside (53.35–64.23 mg/100 g at 0% CaL; 36.70–67.65 mg/100 g at 4% CaL) and isorhamnetin-3-*O*-glucoside (30.98–38.28 mg/100 g at 0% CaL; 22.79–41.15 mg/100 g at 4% CaL) were the main flavonols in impregnated samples (CON, VI, and US). Among the samples, the highest total carotenoids content (34.88 mg/100 g) was found in VI_200 at 0% CaL, dominated by sum of carotene (21.62 mg/100 g).

The PPO and POD activities of selected samples were also analyzed. FA exhibited the highest PPO activity, accounted for 3794.97 U/g. Selected samples at 0% CaL generally limited the PPO and POD activities better than those at 4% CaL. There was no PPO activity observed in CON and US_30 at 0% CaL, while VI_200 still showed PPO activity which might be caused by cell deterioration by VI that promotes PPO exposure. POD activity was completely inhibited in CON, VI_200, and US_30 at 0% CaL, which could be associated with the high antioxidant activity and reducing agents that react with H_2O_2 – substrate of POD (Rojas-Graü et al., 2008).

Conclusions and suggestions for stage 2

1. VI and US improved the antioxidant properties and calcium content of freeze-dried apples in comparison to CON, FA, and FA+AA. Addition of 4% CaL improved the calcium content yet decreased the antioxidant activities of the freeze-dried products.
2. For VI, VI_200 was suggested to significantly improve the antioxidant activity (at 0% CaL) and calcium content (at 4% CaL). VI is also saving more time than CON, which might be beneficial for production efficiency and effectiveness.
3. For US, US_30 at 0% CaL showed the highest antioxidant activity, while US_20 at 4% CaL showed the highest calcium content. With the same processing time as CON (120 min), US could improve the antioxidant properties and calcium content of resulting freeze-dried products.
4. VI and US influenced antioxidant composition of the product with isorhamnetin-3-*O*-rutinoside and isorhamnetin-3-*O*-glucoside dominating flavonols, while the carotenes were the main compounds in carotenoids.
5. CON and US_30 at 0% CaL completely inhibited PPO and POD activities.
6. Although VI and US could improve the antioxidant properties and calcium content, further studies on *in vitro* digestion process and *in vivo* should be conducted to confirm the bioaccessibility and bioavailability of calcium and antioxidants.

Based on this research, the following hypotheses were confirmed:

H1b. The antioxidant properties, calcium content, and browning enzymes activities of freeze-dried apple products are significantly influenced by composition of impregnating solutions, containing various concentrations of CaL.

H2. Application of vacuum and ultrasound-assisted impregnation significantly affects the physicochemical, functional properties (antioxidant properties, calcium content) and browning enzymes activities of freeze-dried apple products.

This section is written based on publication:

A4: Arnold, M., Tylewicz, U., Suliburska, J., Świeca, M., Wojdyło, A., & Gramza-Michałowska, A. (2025). Vacuum and ultrasound-assisted impregnation of Gala apple with sea buckthorn juice and calcium lactate: functional properties, antioxidant profile, and activity of polyphenol oxidase and peroxidase of freeze-dried products. *Polish Journal of Food and Nutrition Sciences*, 75(3), 283-302. <https://doi.org/10.31883/pjfn/209424>

4.3. Stage 3: Storage study for three months

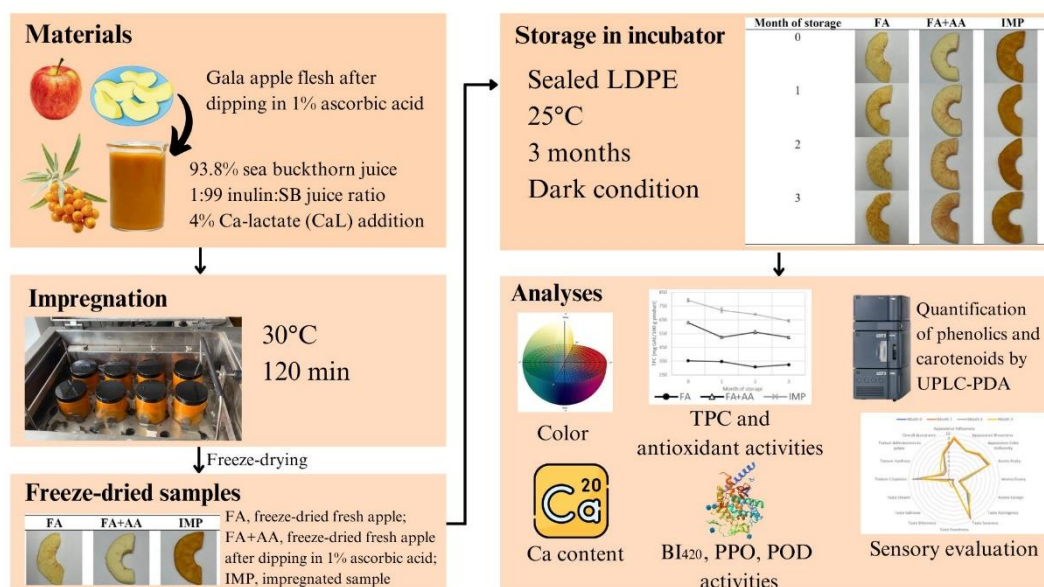


Figure 4. Graphical abstract of research stage 3 (dissertation paper A5)

During 3 months of storage at 25°C, this study monitored monthly changes in the color, TPC, antioxidant activities, calcium content, enzymatic browning, composition of phenolics and carotenoids, and sensory properties of developed freeze-dried apple products. The 3-month storage period was selected because it was long enough to observe significant changes in quality attributes mentioned above, which aligned with previous storage study of dried apples (Djekic et al., 2018). As explained in graphical abstract (Figure 4), there were 3 treatments of freeze-dried apples: FA, FA+AA, and that after impregnation using 93.8% SB juice, 1:99 inulin:SB juice ratio, 4% CaL addition, at 30°C for 120 min (also called IMP), considering the results from stage 1. In the preliminary experiment, the 1:99 inulin:SB juice ratio did not cause significant decrease of antioxidant activity (ORAC) as compared to 0:100, and it was selected among various inulin:SB juice ratios lower than 15:85, as suggested in stage 1. Methanol 80% was used for antioxidant extraction.

The freshly-prepared IMP exhibited significantly higher TPC (791.29 mg GAE/100 g product) and antioxidant activities (ABTS 864.01; DPPH 928.56; ORAC 4994.59; PCL-ACL 952.38; PCL-IAC 1568.35 mg TE/100 g product; and FRAP 6.35 mmol Fe(II)/100 g product) than FA and FA+AA as controls ($p < 0.05$), except the PCL-ACW (615.97 mg TE/100 g), which was 15.7% lower than that of FA+AA but 14.6% higher than that of FA+AA. The previous study (stage 1, dissertation paper A3) exhibited that the impregnated samples

showed either lower or similar TPC and antioxidant activities as compared to FA+AA. This might be explained by different harvesting time of apples and different batches of SB juice.

A general decreasing trend in TPC and antioxidant activities was observed across all samples during the 3-month storage period. Comparing the TPC and antioxidant activities of each treatment between months 0 and 3, the results showed that FA+AA better maintained its TPC and antioxidant activities (ABTS, DPPH, and FRAP) than IMP and FA. However, IMP still exhibited higher values of TPC and most antioxidant activities over 3 months than FA and FA+AA. Phenolics degradation from oxidation and enzymatic browning, as well as polymerization might be the causes of decreasing TPC and antioxidant activities over storage (Butkeviciute et al., 2022). Oxygen may still pass through the LDPE bag and further trigger the oxidation of products' antioxidants through enzymatic browning. Polymerization of monomeric phenolics may reduce the active group sites and further limit the antioxidant's ability to function effectively. The isomerization of carotenoids in IMP, might also occur during storage, which then affects product's antioxidant activity (Barba et al., 2018).

Initially, the calcium content of IMP (2793.86 mg Ca/100 g product) was over 81 times higher than that of FA and FA+AA. As comparison to month 3, the calcium content of IMP decreased 9.8% to 2520.13 mg Ca/100 g product. Nevertheless, there was an unclear pattern of calcium content during 3 months of storage in all freeze-dried samples. In terms of enzymatic browning, the BI₄₂₀, PPO and POD activities of IMP were still observed and significantly higher than controls during storage period, which were different compared to the results of stage 1 (dissertation paper A3), where antioxidants from SB juice could limit the PPO and POD activities of the products. This phenomenon could be explained by some factors. First, the impregnation may disrupt the structure integrity of the cells and expose more PPO and POD, while high TPC in IMP could also act as substrate of the browning enzymes, instead of inhibiting them. Second, increasing moisture content and oxygen may promote enzymatic browning, confirmed by significant negative correlation between dry matter (DM) and PPO, and POD. Third, different batch or harvesting time of apples and SB juice may result in different chemical compositions and responses to enzymatic browning.

The phenolics and carotenoids compositions of selected samples (month 0 and 3) were analyzed by UPLC-PDA. Similar to stage 1 and 2 (dissertation paper A3 and A5), isorhamnetin-3-*O*-rutinoside and isorhamnetin-3-*O*-glucoside are the two major flavonols detected in the impregnated samples, while zeaxanthin isomer and sum of carotene dominated the carotenoids in IMP. Although a 20.5% significant decrease of total phenolics

($p < 0.05$) and insignificant 4.2% decrease of total carotenoids ($p > 0.05$) were observed after storage, IMP still exhibited higher contents than FA and FA+AA, which agreed with the results of TPC and most antioxidant activities. The PCA biplot showed that IMP samples were characterized by sum of flavan-3-ols, flavonols, total phenolics and carotenoids.

For sensory, IMP was described by trained panelists with a dominant sourness taste, low sweetness, SB fruity aroma, high yellowness appearance, and high crispiness, which agreed with PCA, showing that IMP notably differed from FA and FA+AA. In contrast, FA and FA+AA exhibited significantly lower crispiness in all months of storage ($p < 0.05$). The maintained crispiness of IMP during storage might be caused by calcium involvement that maintains the membrane integrity and firmness of cell wall (Jain et al., 2019). Although the overall acceptance of IMP was lower than that of controls at the beginning of storage period because of imbalance taste and aroma, its high crispiness even until month 3 could maintain its moderate to rather high overall acceptance (5.87-6.36).

Conclusions and suggestions for stage 3

1. FA, FA+AA, and IMP showed decreasing TPC and antioxidant activities over 3 months of storage. However, the values of IMP were still higher than FA and FA+AA.
2. IMP showed significantly higher calcium content than FA and FA+AA over storage period. Consuming about 143 g of IMP (2793.86 mg Ca/100 g product) could fulfill the RDA for adults (1000 mg Ca/100 g, with 25% calcium absorption). CaL addition preserved the crispiness and sensory acceptability of IMP throughout storage.
3. PPO and POD activities of IMP were still observed during storage, which values were higher than controls. While total phenolics and carotenoids by UPLC-PDA decreased 20.5% ($p < 0.05$) and 4.2% ($p > 0.05$) respectively after 3 months.
4. Future studies should focus on a) bioaccessibility of antioxidants and calcium via *in vitro* digestion and *in vivo* studies, b) varied packaging materials and storage parameters, c) shelf-life prediction, and d) sensory improvement or way to consume IMP.

Based on this research, the following hypothesis was confirmed:

H3. The functional properties, enzymatic browning, and sensory properties of freeze-dried products alter over the storage period, yet their antioxidant properties, calcium content, and sensory properties are still acceptable after 3 months of storage.

This section is written based on publication:

A5: Arnold, M., Suliburska, J., Świeca, M., Wojdyło, A., & Gramza-Michałowska, A. (2025). Storage stability of bioactive-enriched freeze-dried Gala apples: functional properties, sensory evaluation, and enzymatic browning. *Food and Bioprocess Technology*. <https://doi.org/10.1007/s11947-025-03975-7>

5. Conclusions and practical implications

Freeze-dried Gala apple products enriched with sea buckthorn antioxidants and calcium lactate were developed via impregnation process, as a potential and an alternative functional food for people with osteoporosis and calcium deficiency. Local resources such as Gala apple (*Malus domestica* cv. Gala) and sea buckthorn (*Hippophae rhamnoides*) juice were utilized as the main components to create functional foods, intended to reduce the risk of non-communicable disease – osteoporosis and calcium deficiency. The main conclusions addressing the research hypotheses can be found as follows:

1. Impregnating Gala apples with 93.8% SB juice, 0:100 inulin:SB juice ratio, 4% CaL at 30°C for 120 min produced freeze-dried products with improved functionality – higher calcium and fiber contents, minimal antioxidant loss, reduced PPO/POD activity, high crispiness, and acceptable sensory properties.
2. Vacuum (VI) and ultrasound-assisted impregnation (US) significantly enhanced mass transfer and product functionality compared to conventional impregnation, FA, and FA+AA, and affected browning enzyme activities. VI at 200 mbar allowed to produce the product with the highest antioxidant activities (0% CaL) and calcium content (4% CaL). US for 30 min (0% CaL) and 20 min (4% CaL) resulted in the highest antioxidant properties and calcium content, respectively. PPO and POD were fully inhibited by conventional and US impregnation at 0% CaL.
3. Over 3 months of storage, antioxidant properties declined in all samples (FA, FA+AA, and IMP), though IMP (prepared with 93.8% SB juice, 1:99 inulin:SB juice, 4% CaL, 30°C, 120 min) maintained higher activities, phenolics and carotenoids contents. The PPO and POD activities of IMP persisted, consistent with BI₄₂₀ and color changes. The 4% CaL retained crispiness and moderate to rather high acceptability.

The present research focused only on *in vitro* analysis of the resulting freeze-dried apple products. Thus, further research is still needed to further confirm the product's potential for osteoporosis prevention and enhancement of calcium uptake, as well as to advance it to market readiness. The suggested research includes:

1. *In vitro* digestion, *in vivo* and human studies to assess the bioaccessibility and bioavailability of antioxidants and calcium.
2. Shelf-life prediction under varied storage conditions and packaging types.

3. Sensory improvement, focusing on flavor balance and exploring the alternative modes of consumption.

The above research demonstrated clear opportunities for translating scientific findings into marketable products and processes:

1. Functional snack development – the optimized impregnation and freeze-drying methods enable production of fortified apple snacks meeting consumer demand for clean-label, health-promoting foods and can target consumers at risk of osteoporosis.
2. Sustainability and local sourcing – the research findings support business models based on local raw materials (apples and sea buckthorn), aligning with corporate sustainability goals and growing consumer preference for regionally sourced products.

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Articles in the dissertation



Recent Development on the Chemical Composition and Phenolic Extraction Methods of Apple (*Malus domestica*)—A Review

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Abstract

Apple (*Malus domestica*) is a reliable source of nutrients to increase the bioactive compounds intake in the human body. In the market, the development of apple is still growing, not only in the hybridization of new cultivars, but also in the functional food products, based on apple's bioactive compounds. In this paper, we review the recent studies about the chemical composition of apples, including carbohydrates, proteins, lipids, dietary fibers, minerals, vitamins, phenolics, and other compounds, from various cultivars, grown in different countries or regions. Among the bioactive compounds in apple, the phenolic compounds, including hydroxybenzoic acids, hydroxycinnamic acids, flavanols and their oligo- and polymeric structure, flavonols, dihydrochalcones, and anthocyanins, majorly contribute to beneficial biological impacts. Therefore, the extraction process might be the most critical step to recover all the phenolics from apple and could be used in various food product developments. In this paper, the comparison of conventional and developed phenolic extraction methods is also reviewed in various apple products (flesh, peel, pomace, pulp, etc.). The selection of food grade and green solvents in the optimal phenolic extraction methods could reduce the environmental issues, thus supports sustainability and can be safer for consumers. To sum up, this paper may help the readers, both at general household and industrial levels, to understand the nutritional composition of various apple cultivars from different regions and to select the optimum conditions for apple's phenolic extraction, based on recent studies.

Keywords Antioxidant · *Malus domestica* · Bioactive compounds · Cultivar · Non-conventional extraction · Phenolics

Introduction

Due to its pleasant taste and high nutritional values, apple (*Malus domestica*) becomes one of the most popular fruits consumed worldwide. It is also one of the most cultivated crops in the world due to its ease to grow and harvest. The production of apple in the world increased around 21%, from 77 to 93 million tons from 2011 to 2021, respectively (FAO, 2023). In 2021, China was reported to be the largest producer of apple, contributed 49.37% of worldwide apple production, followed by Turkey (4.82%) USA (4.80%), and Poland (4.37%) (FAO, 2023).

There is a vast number of apple cultivars produced and available all the year-round. Gala (863,000 tons),

Red Delicious (625,000 tons) and Honey Crisps (542,000 tons) was reported by the World Apple and Pear Association (WAPA) as the dominant cultivars produced in USA (WAPA, 2021). While in European Union-20 (20 Member States, excluding 7 Member States that are very small producers and for which there is no data) and UK, especially in the period of 2017–2021, the major cultivars produced were Golden Delicious (19.1%), Gala (12.9%), Idared (6.7%), Red Delicious (6.7%), and Champion (5.9%) (European Commission, 2022). In southern hemisphere (Argentina, Australia, Brazil, Chile, New Zealand, and South Africa), Gala became the most produced cultivar in 2019 with 1,931,000 tons, followed by Fuji (774,000 tons) and Red Delicious (642,000 tons) (WAPA, 2020). In 2019, the worldwide availability of apple was 22 g/capita/day, ranged from 37 g/capita/day (Europe) to 5 g/capita/day (Africa) (FAO, 2022). The abundant apple production and its availability across the world could be an effective way to increase the intake of nutritional compounds in human diet through various apple products.

Regarding apple as one of sources in human diet, it is necessary to ensure the safety of the apples. According to

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European regulation (EC No. 85/2004), to be safely consumed, apple must be intact, clean, free from pests or pathogens, and free from foreign smell and/or taste (European Commission, 2004). The other safety concerns for apple fruit and products are pesticides residues and the secondary metabolite produced by *Penicillium expansum* (most common postharvest pathogen in apple), called patulin, which is a mycotoxin that causes chronic toxic problems, such as genotoxicity, immunotoxicity, and neurotoxicity (Lončarić et al., 2021). According to EC No. 1881/2006, the presence of patulin in apple juice and fermented drink derived from apples is limited to no more than 50 µg/kg, while in solid apple products to 25 µg/kg, and apple products for infant to 25 µg/kg (European Commission, 2006).

Around 70–75% of apples are consumed as fresh fruit (Zhang et al., 2021). It is recommended by the WHO to consume 400 g edible fruits or vegetables in order to improve overall health, and to prevent the nutrient deficiencies and non-communicable diseases (WHO, 2023). With the weight of apple whole fruit around 149–223 g (USDA, 2019), consuming 2–3 apples daily would be enough to fulfill the recommendation. Furthermore, about 25–30% of apples are processed into various products: (a) liquid form, such as apple juice or concentrate, apple cider, and apple vinegar; (b) solid form, such as freeze/dried apple, apple canning, apple sauce/jam, and apple slice. During the apple processing in industry, 25–30% of pomace are generated (Zhang et al., 2021). Each edible part of apple contributes to bioactive compounds, including carbohydrates, fibers, fatty acids, organic acids, polyphenols, carotenoids, and terpenes which provide health-promoting values in human diet (Acquavia et al., 2021). The major phenolics found in apple are hydroxybenzoic acids, hydroxycinnamic acids, flavanols and their oligo- (procyanidins) and polymeric structures (proanthocyanidins), flavonols, dihydrochalcones, and anthocyanins (Kschonsek et al., 2018). Apple shows functional and health promoting effects, such as antioxidant potential, anti-inflammatory activity, cholesterol-lowering effect, cardiovascular protective effect, antidiabetic activity, and anticancer activity due to its major phenolic compounds (Patocka et al., 2020).

In order to perceive the optimum health-promoting benefits from apple's phenolics, the phenolic extraction process for edible parts of apple, such as whole fruit, flesh, peel, and juice, and by-product, such as pomace should be carried out correctly and efficiently. The environmental problems due to high utilization of organic solvent should be reduced by the development of extraction techniques. It is also important to use the solvents which are food grade (Zhang et al., 2021). The obtained phenolic extract could be applied in various food product developments to improve their functional properties. Therefore, this present study focused on the review of apple's chemical compositions, total phenolics,

antioxidant potential of edible parts of apples from different cultivars and locations. The optimization of phenolic extraction using conventional and developed extraction methods was also reviewed in this study since different types of apple products from different cultivars might have different optimum extraction conditions. It is worth noticing that, to date, the development of apple fruit in the world is still growing, either in hybridization of new cultivars, or in food product development based on apple's bioactive compounds. Through this review, the readers, especially food industries, could select the extraction methods to produce apple products' phenolics that are suitable for their needs, based on recent studies.

Botanical Characteristics

Apple (*Malus domestica*) belongs to *Rosaceae* family. The botanical characteristics of apple have been described by (Jackson & Palmer, 2011). Depending on the rootstock, the apple tree has 1.5 to 7 m in height, with basal diameter ranges from 1 to 4.5 m. It is a hermaphrodite plant; some cultivars are partly self-fertile, but cross-pollination may also happen. It can also grow in wide range of soil types. The expected yield varied from 20 to 70 tons/ha, in 3–10 years of full production period, depending on the cultivar or rootstock and site. Based on various estimations, there are over 7500 apple cultivars discovered from various regions in the world, but only around 20 cultivars are widely cultivated in horticulture industry (Shlyavas et al., 2019). Several grown cultivars include, but are not limited to Royal Gala, Golden Delicious, Red Delicious, Jonagold, Braeburn, McIntosh, Fuji, Granny Smith, and Cripps Pink (Pink Lady®) (Jackson & Palmer, 2011; Simmonds & Howes, 2016). Apple grows and is adaptable to various climates, but grows better in moderate humidity and cool temperature, in the 35–50° latitude (Hammad & Malik, 2021). Higher humidity may increase the chance of diseases, such as scab and Nectria canker. The symptoms and favorable condition of apple diseases, such as apple scab, black rot, cedar apple rust, flyspeck, powdery mildew, apple mosaic, and core rot were described by Manavalan (2021). Temperature affects the quality of apples. Some cultivars (i.e., Granny Smith) grows better in warmer area, while Cox's Orange Pippin and Red Delicious have better flavor, color, and shape in cooler climates. Apple production can be found in various regions, for example, in Asia (mostly in China), Europe, North America, Africa, Middle East, and also southern hemisphere area (FAO, 2023). In warmer countries, apple is also grown for example in Ethiopia (Jemaneh & Chandravanshi, 2021), South Africa (Tharaga et al., 2021), Indonesia (Efendi, 2014; Utami et al., 2019), Costa Rica (Navarro et al., 2018), Brazil (Pio et al., 2019), and Argentina (Castro et al., 2016). The dormancy-management practices of apple tree should be applied in tropical countries to allow adequate flowering, growth, and productivity (Pio et al., 2019).

Chemical Composition

Apple contains an abundance of chemical compounds with proven health-promoting properties for humans, both by providing nutritional value and preventing various diseases. Bioactive compounds of apple fruit, such as organic acids, fibers, minerals, and vitamins, are shown in Table 1, while phenolic compounds are summarized in Table 2.

Basic Composition

Apple has been known as a beneficial fruit thanks to its balanced nutritional composition. The nutritional value of edible parts of apple is shown in Table 1. The content of each nutrient varied depending on the cultivars. Water dominated the composition of apple, ranged from 81.06 to 85.60 g/100 g. Apple provided moderate calories of

Table 1 Proximate analysis and content of dietary fiber, organic acids, minerals, and vitamins of apple fruit's edible parts per 100 g fresh weight (FW)

Nutrients	National Food Institute of Denmark (2022) ^a	USDA (2019) ^b	Almeida and Gomes (2017) ^c	Feliciano et al. (2010)		Unit
				Traditional apples ^d	Exotic apples ^e	
Energy	55	52	57	N.A	N.A	kcal/100 g
Water	84.90	85.60	84.50	81.06	81.73	g/100 g
Protein	0.30	0.26	0.35	0.08	0.07	
Total lipid	0.20	0.17	0.10	N.A	N.A	
Ash	0.30	0.19	0.23	N.A	N.A	
Carbohydrate	12.10	13.80	14.80	N.A	N.A	
Sugars	10.90	10.40	N.A	11.86	11.60	
Dietary fiber	2.20	2.40	2.00	2.97	2.29	
Soluble fiber	N.A	N.A	N.A	0.37	0.41	
L-Malic acid	N.A	N.A	N.A	181.80	324.68	mg/100 g
Citric acid	N.A	N.A	N.A	3.25	5.60	
Calcium (Ca)	4.13	6.00	N.A	2.48	3.22	
Iron (Fe)	0.12	0.12	N.A	0.15	0.15	
Magnesium (Mg)	4.49	5.00	N.A	3.90	3.46	
Phosphorus (P)	9.52	11.00	N.A	8.41	10.19	
Potassium (K)	118.00	107.00	N.A	100.90	99.46	
Sodium (Na)	0.60	1.00	N.A	0.82	0.94	
Zinc (Zn)	0.02	0.04	N.A	0.03	0.03	
Copper (Cu)	0.03	0.03	N.A	0.07	0.04	
Manganese (Mn)	0.06	0.04	N.A	0.04	0.04	
Vitamin C	8.26	4.60	N.A	8.88	8.28	
Vitamin E (α -tocopherol)	0.25	0.18	N.A	0.18	0.14	
Thiamin (Vit. B ₁)	0.01	0.02	N.A	0.01	0.01	
Riboflavin (Vit. B ₂)	0.01	0.03	N.A	0.00	0.00	
Niacin (Vit. B ₃)	0.12	0.09	N.A	0.12	0.13	
Pantothenic acid (Vit. B ₅)	0.07	0.06	N.A	<0.10	<0.10	
Pyridoxine (Vit. B ₆)	0.05	0.04	N.A	0.03	0.04	
Folate (Vit. B ₉)	9.00	3.00	N.A	<5.00	<5.00	μg/100 g
Vitamin A (retinol)	0.00	3.00	N.A	<4.50	<4.50	
β -carotene	25.00	27.00	N.A	11.92	10.24	

N.A. data not available

^aBased on analytical data for all apple cultivars (the cultivars are not described)

^bBased on analytical data for Red Delicious, Golden Delicious, Gala, Granny Smith, and Fuji apples

^cBased on analytical data for apples from the Protected Geographical Indication “Maçã de Alcobaça,” Portugal: Casa Nova, Fuji, Galaxy, Golden Delicious, Granny Smith, Jonagored, Reinette, and Starking apples

^dBased on analytical data for traditional apples from Portugal: Bravo de Esmolfe, Malápio Fino, Malápio da Serra, and Pêro Pipo apples

^eBased on analytical data for exotic apples from Portugal: Fuji, Starking, Reineta Parda, Gala Galaxy, and Golden apples

Table 2 Phenolic composition of flesh and peel of various apple cultivars

Phenolic group	Name	Part of fruit	Cultivars	Notes (D or N.D.)	References
Hydroxybenzoic acids	Gallic acid	Flesh	Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
			Golden Delicious, Red Delicious, Royal Gala	D in all cultivars	Chen et al. (2012)
		Peel	Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
			Golden Delicious, Red Delicious, Royal Gala	D in all cultivars	Chen et al. (2012)
	Protocatechuic acid	Flesh	Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
		Peel	Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
Hydroxycinnamic acids	Chlorogenic acid	Flesh	Antonówka, Delikates, Early Geneva, Gloster, Jonagored, Ligol, Paulared, Papierówka, Quinte, Rubinola, Sunrise	D in all cultivars	Zielińska and Turemko (2020)
			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
			King Luscious, Amasya, Ervin Spur, Sky Spur, Arap Kizi, Lutz Golden, Granny Smith	D in all cultivars	Karaman et al. (2013)
			Golden Delicious, Red Delicious, Royal Gala	D in all cultivars	Chen et al. (2012)

Table 2 (continued)

Phenolic group	Name	Part of fruit	Cultivars	Notes (D or N.D.)	References
Caffeic acid	Peel		Chouka 4, Fuji, JPP35, Nakano no Kirameki, Nakano Shinku, No. 37	D in all cultivars	Sato et al. (2017)
			Big Time, Cripps Pink, Cripps Red, Firm Gold, Fuji, Gala, Galaxy, Golden Delicious, Granny Smith, Hi-Early, Lady Williams, Naga Fu no. 2, Purple Wave, Red Braeburn, Sansa, Splendour, Wandadale, Western Dawn, Western Tang	D in all cultivars	Bondonno et al. (2020)
			Aldas, Auksis, Connel Red, Ligol, Lodel, Rajka	D in all cultivars	Raudone et al. (2017)
			Antonówka, Delikates, Early Geneva, Gloster, Jonagored, Ligol, Paulared, Papierówka, Quinte, Rubinola, Sunrise	D in all cultivars	Zielińska and Turemko (2020)
			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
			King Luscious, Amasya, Ervin Spur, Sky Spur, Arap Kizi, Lutz Golden, Granny Smith	N.D. in Ervin Spur and Granny Smith	Karaman et al. (2013)
			Golden Delicious, Red Delicious, Royal Gala	D in all cultivars	Chen et al. (2012)
			Aldas, Auksis, Connel Red, Ligol, Lodel, Rajka	D in all cultivars	Raudone et al. (2017)
		Flesh	King Luscious, Amasya, Ervin Spur, Sky Spur, Arap Kizi, Lutz Golden, Granny Smith	D in all cultivars	Karaman et al. (2013)
			Golden Delicious, Red Delicious, Royal Gala	D in all cultivars	Chen et al. (2012)
			Chouka 4, Fuji, JPP35, Nakano no Kirameki, Nakano Shinku, No. 37	D in all cultivars	Sato et al. (2017)
			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
	Peel		King Luscious, Amasya, Ervin Spur, Sky Spur, Arap Kizi, Lutz Golden, Granny Smith	N.D. in all cultivars	Karaman et al. (2013)

Table 2 (continued)

Phenolic group	Name	Part of fruit	Cultivars	Notes (D or N.D.)	References
	<i>p</i> -Coumaric acid	Flesh	Golden Delicious, Red Delicious, Royal Gala	D in all cultivars	Chen et al. (2012)
			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
		Peel	Golden Delicious, Red Delicious, Royal Gala	D in all cultivars	Chen et al. (2012)
			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
	Ferulic acid	Flesh	Golden Delicious, Red Delicious, Royal Gala	D in all cultivars	Chen et al. (2012)
			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
		Peel	Golden Delicious, Red Delicious, Royal Gala	D in all cultivars	Chen et al. (2012)
			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)

Table 2 (continued)

Phenolic group	Name	Part of fruit	Cultivars	Notes (D or N.D.)	References
Flavanols and procyanidins	Catechin	Flesh	Antonówka, Delikates, Early Geneva, Gloster, Jonagored, Ligol, Paulared, Papierówka, Quinte, Rubinola, Sunrise	D in all cultivars	Zielińska and Turemko (2020)
			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
			King Luscious, Amasya, Ervin Spur, Sky Spur, Arap Kizi, Lutz Golden, Granny Smith	D in all cultivars	Karaman et al. (2013)
			Golden Delicious, Red Delicious, Royal Gala	D in all cultivars	Chen et al. (2012)
			Chouka 4, Fuji, JPP35, Nakano no Kirameki, Nakano Shinku, No. 37	D in all cultivars	Sato et al. (2017)
			Aldas, Auksis, Connel Red, Ligol, Lodel, Rajka	D in all cultivars	Raudone et al. (2017)
		Peel	Antonówka, Delikates, Early Geneva, Gloster, Jonagored, Ligol, Paulared, Papierówka, Quinte, Rubinola, Sunrise	D in all cultivars	Zielińska and Turemko (2020)
			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
			King Luscious, Amasya, Ervin Spur, Sky Spur, Arap Kizi, Lutz Golden, Granny Smith	N.D. in all cultivars	Karaman et al. (2013)
			Golden Delicious, Red Delicious, Royal Gala	D in all cultivars	Chen et al. (2012)
			Aldas, Auksis, Connel Red, Ligol, Lodel, Rajka	D in all cultivars	Raudone et al. (2017)
			Antonówka, Delikates, Early Geneva, Gloster, Jonagored, Ligol, Paulared, Papierówka, Quinte, Rubinola, Sunrise	D in all cultivars	Zielińska and Turemko, (2020)
	Epicatechin	Flesh	Antonówka, Delikates, Early Geneva, Gloster, Jonagored, Ligol, Paulared, Papierówka, Quinte, Rubinola, Sunrise	D in all cultivars	Zielińska and Turemko, (2020)

Table 2 (continued)

Phenolic group	Name	Part of fruit	Cultivars	Notes (D or N.D.)	References
Peel			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
			King Luscious, Amasya, Ervin Spur, Sky Spur, Arap Kizi, Lutz Golden, Granny Smith	D in all cultivars	Karaman et al. (2013)
			Golden Delicious, Red Delicious, Royal Gala	D in all cultivars	Chen et al. (2012)
			Chouka 4, Fuji, JPP35, Nakano no Kirameki, Nakano Shinku, No. 37	D in all cultivars	Sato et al. (2017)
			Aldas, Auksis, Connel Red, Ligol, Lodel, Rajka	D in all cultivars	Raudone et al. (2017)
			Antonówka, Delikates, Early Geneva, Gloster, Jonagored, Ligol, Paulared, Papierówka, Quinte, Rubinola, Sunrise	D in all cultivars	Zielińska and Turemko (2020)
			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
			King Luscious, Amasya, Ervin Spur, Sky Spur, Arap Kizi, Lutz Golden, Granny Smith	D in all cultivars	Karaman et al. (2013)
			Golden Delicious, Red Delicious, Royal Gala	D in all cultivars	Chen et al. (2012)
			Big Time, Cripps Pink, Cripps Red, Firm Gold, Fuji, Gala, Galaxy, Golden Delicious, Granny Smith, Hi-Early, Lady Williams, Naga Fu no. 2, Purple Wave, Red Braeburn, Sansa, Splendour, Wandadale, Western Dawn, Western Tang	D in all cultivars	Bondonno et al. (2020)
			Aldas, Auksis, Connel Red, Ligol, Lodel, Rajka	D in all cultivars	Raudone et al. (2017)

Table 2 (continued)

Phenolic group	Name	Part of fruit	Cultivars	Notes (D or N.D.)	References
Procyanidins		Flesh	Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
			Big Time, Cripps Pink, Cripps Red, Firm Gold, Fuji, Gala, Galaxy, Golden Delicious, Granny Smith, Hi-Early, Lady Williams, Naga Fu no. 2, Purple Wave, Red Braeburn, Sansa, Splendour, Wandadale, Western Dawn, Western Tang	D in all cultivars	Bondonno et al. (2020)
			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
			Golden Delicious, Red Delicious, Royal Gala	D in all cultivars	Chen et al. (2012)
			Chouka 4, Fuji, JPP35, Nakano no Kirameki, Nakano Shinku, No. 37	D in all cultivars	Sato et al. (2017)
		Peel	Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
			Golden Delicious, Red Delicious, Royal Gala	D in all cultivars	Chen et al. (2012)

Table 2 (continued)

Phenolic group	Name	Part of fruit	Cultivars	Notes (D or N.D.)	References
Flavonols	Quercetin	Flesh	Big Time, Cripps Pink, Cripps Red, Firm Gold, Fuji, Gala, Galaxy, Golden Delicious, Granny Smith, Hi-Early, Lady Williams, Naga Fu no. 2, Purple Wave, Red Braeburn, Sansa, Splendour, Wandadale, Western Dawn, Western Tang	D in all cultivars	Bondonno et al. (2020)
			Antonówka, Delikates, Early Geneva, Gloster, Jonagored, Ligol, Paulared, Papierówka, Quinte, Rubinola, Sunrise	N.D. in all cultivars	Zielińska and Turemko (2020)
			King Luscious, Amasya, Ervin Spur, Sky Spur, Arap Kizi, Lutz Golden, Granny Smith	N.D. in all cultivars	Karaman et al. (2013)
		Peel	Ambrosia, Fuji, Golden (Spain), Golden (France), Granny, Pink Lady®, Red, Royal	D in all cultivars	Alarcón-Flores et al. (2015)
			Antonówka, Delikates, Early Geneva, Gloster, Jonagored, Ligol, Paulared, Papierówka, Quinte, Rubinola, Sunrise	D in all cultivars	Zielińska and Turemko (2020)
			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
	Isoquercetin	Flesh	King Luscious, Amasya, Ervin Spur, Sky Spur, Arap Kizi, Lutz Golden, Granny Smith	D in all cultivars	Karaman et al. (2013)
			Ambrosia, Fuji, Golden (Spain), Golden (France), Granny, Pink Lady®, Red, Royal	D in all cultivars	Alarcón-Flores et al. (2015)
			Antonówka, Delikates, Early Geneva, Gloster, Jonagored, Ligol, Paulared, Papierówka, Quinte, Rubinola, Sunrise	D in all cultivars	Zielińska and Turemko (2020)
			Golden Delicious, Red Delicious, Royal Gala	D in all cultivars	Chen et al. (2012)
		Peel	Aldas, Auksis, Connel Red, Ligol, Lodel, Rajka	D in all cultivars	Raudone et al. (2017)
			Antonówka, Delikates, Early Geneva, Gloster, Jonagored, Ligol, Paulared, Papierówka, Quinte, Rubinola, Sunrise	D in all cultivars	Zielińska and Turemko (2020)

Table 2 (continued)

Phenolic group	Name	Part of fruit	Cultivars	Notes (D or N.D.)	References
	Rutin	Flesh	Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
			Golden Delicious, Red Delicious, Royal Gala	D in all cultivars	Chen et al. (2012)
			Aldas, Auksis, Connel Red, Ligol, Lodel, Rajka	D in all cultivars	Raudone et al. (2017)
			Antonówka, Delikates, Early Geneva, Gloster, Jonagored, Ligol, Paulared, Papierówka, Quinte, Rubinola, Sunrise	D in all cultivars	Zielińska and Turemko (2020)
		Peel	Aldas, Auksis, Connel Red, Ligol, Lodel, Rajka	D in all cultivars	Raudone et al. (2017)
			Antonówka, Delikates, Early Geneva, Gloster, Jonagored, Ligol, Paulared, Papierówka, Quinte, Rubinola, Sunrise	D in all cultivars	Zielińska and Turemko (2020)
			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
			Aldas, Auksis, Connel Red, Ligol, Lodel, Rajka	D in all cultivars	Raudone et al. (2017)
		Flesh	Antonówka, Delikates, Early Geneva, Gloster, Jonagored, Ligol, Paulared, Papierówka, Quinte, Rubinola, Sunrise	D in all cultivars	Zielińska and Turemko (2020)
			Golden Delicious, Red Delicious, Royal Gala	D in all cultivars	Chen et al. (2012)
Dihydrochalcones	Phloridzin	Flesh	Chouka 4, Fuji, JPP35, Nakano no Kiremeki, Nakano Shinku, No. 37	D in all cultivars	Sato et al. (2017)
			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)

Table 2 (continued)

Phenolic group	Name	Part of fruit	Cultivars	Notes (D or N.D.)	References
Anthocyanins	Cyanidin-3-galactoside	Peel	Antonówka, Delikates, Early Geneva, Gloster, Jonagored, Ligol, Paulared, Papierówka, Quinte, Rubinola, Sunrise	D in all cultivars	Zielińska and Turemko (2020)
			Golden Delicious, Red Delicious, Royal Gala	D in all cultivars	Chen et al. (2012)
			Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	D in all cultivars	Kschonsek et al. (2018)
			Big Time, Cripps Pink, Cripps Red, Firm Gold, Fuji, Gala, Galaxy, Golden Delicious, Granny Smith, Hi-Early, Lady Williams, Naga Fu no. 2, Purple Wave, Red Braeburn, Sansa, Splendour, Wandadale, Western Dawn, Western Tang	D in all cultivars	Bondonno et al. (2020)
		Flesh	Antonówka, Delikates, Early Geneva, Gloster, Jonagored, Ligol, Paulared, Papierówka, Quinte, Rubinola, Sunrise	D in all cultivars	Zielińska and Turemko (2020)
			Chouka 4, Fuji, JPP35, Nakano no Kirameki, Nakano Shinku, No. 37	N.D. in Fuji	Sato et al. (2017)
			Antonówka, Delikates, Early Geneva, Gloster, Jonagored, Ligol, Paulared, Papierówka, Quinte, Rubinola, Sunrise	D in all cultivars	Zielińska and Turemko (2020)
			Lještarka, Božićnica, Kolerova, Srčika, Ivanlija, Duga, Zimnjara, Citronka, Zlatica, Paradija, Adamova Zvijezda, Slavonska Srčika, Cortland, Russet, Wild	D only in Lještarka, Božićnica, and Cortland	Jakobek and Barron (2016)

D detected, N.D. not detected

52 kcal/100 g (USDA, 2019). Protein and lipid did not contribute much to the energy value of apple due to the relatively small content. However, the compositions of fatty acids and amino acids found in various apple cultivars were reported by previous studies. Salas et al. (2016) reported that fatty acids are the main precursors of volatile compounds in apples which concentration mostly increased during 210 days of cold storage, especially Golden Delicious

from Chihuahua, Mexico, which were dominated by linoleic acid (85–125 mg/kg), followed by palmitic acid (27–45 mg/kg), linolenic acid (5–17 mg/kg), oleic acid (3–12 mg/kg), and stearic acid (5–8 mg/kg). Similar results were found as among 16 analyzed fatty acids, palmitic, stearic, and linoleic acids were dominant in Bravo de Esmolfe apple from Portugal (Pires et al., 2018). Additionally, fatty acids (linoleic, oleic, and palmitic acid were abundantly found), carotenoids,

and α -tocopherol were also found in the seeds of apples grown in Norway (Akšić et al., 2021). For amino acids, Feng et al. (2014) found that most amino acids, except histidine, lysine, threonine, and glycine, were found in higher level in the apples from inner-tree canopy. Total protein content of Eastern Himalayan apples, especially Dorsett Golden and Anna cultivars, was higher than that of Western Himalayan ones (Raj et al., 2021). This phenomenon can be explained as the higher annual temperature of Eastern Himalayan region positively affect the nutritive composition of the apples, while higher precipitation in West Himalayan region affected it negatively. Additionally, the composition and concentration of amino acids varied among cultivars depending on location, season, and crop load (Peck et al., 2016).

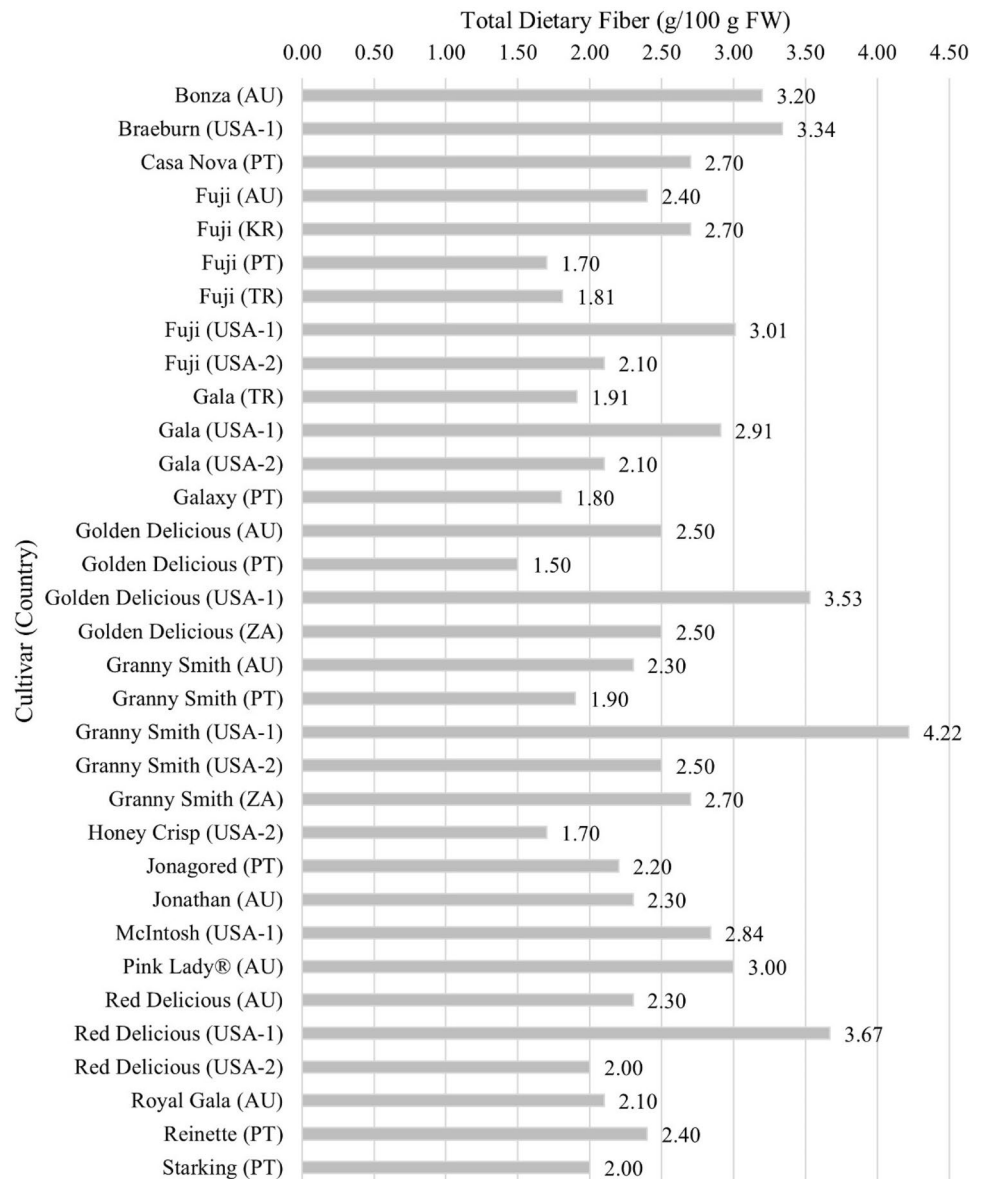
The sugars (10.40 to 11.86 g/100 g) were also found in apple and dominantly contributed to the energy value and the taste of apple (Table 1). Most fruits contain higher content of glucose and fructose than sucrose (Li et al., 2020a). The content of fructose and glucose of dried Bravo de Esmolfe apple flesh was around 14 times and 6 times higher than sucrose content, respectively (Pires et al., 2018). In Norway, a study also compared 74 apple cultivars grown in Ullensvang area to 29 cultivars in Njøs; it was found that the apples in Ullensvang showed higher average content of sugars (744.54 g/kg dry weight (DW)), sugar alcohol (60.33 g/kg dw), and organic acid (90.81 g/kg DW) than those in Njøs (Akšić et al., 2022). The sugar and major organic acid (malic acid) contents were higher in the sun-exposed apples, compared to the shaded ones, due to increased total photosynthesis in the leaf (Jing et al., 2020). It is worth noticing that organic acids were also found in all apple cultivars from Turkey (Aslik, Cebegirmez, Bey Elmasi, Arapkizi) with following order: malic > fumaric > succinic > tartaric acids (Celik et al., 2018). A study also reported that malic acid, which is more responsible for the sourness of apple (Petkova et al., 2019), predominated the organic acid composition of Bravo de Esmolfe apple, followed by quinic, oxalic, and shikimic acids (Pires et al., 2018). In the analysis of 10 apple cultivars grown in Austria, the average showed that malic acid predominated the organic acid composition, followed by citric (4.6%), pyruvic (0.4%), oxalic (0.3%), maleic (0.3%), and shikimic acid (0.1%) (Oszmiański et al., 2020). While in China, 85.8% of analyzed 106 sour apple cultivars were reported to have organic acids with following order: malic > oxalic > citric > lactic > succinic > fumaric acid (Yan et al., 2018). A comparative analysis of chemical composition of apples cultivated in Western and Eastern Himalaya reported that among the analyzed cultivars, Dorsett Golden apple in Western Himalaya possessed the lowest content of fructose (355.50 mg/g fresh weight (FW)) and glucose (76.85 mg/g FW), which could be then recommended for the diabetic patients (Raj et al., 2021). The sugar content in apple fruits

varied due to some factors such as climate conditions, fruit positioning (Feng et al., 2014), genotype (Akagić et al., 2019), growing region, cultivar, maturity, seasons, blooming period, altitude, temperature, storage condition (Raj et al., 2021; Yoon et al., 2020), quality, and intensity of light (Jing et al., 2020).

The dietary fiber content in edible parts of apples from different cultivars and countries is shown in Fig. 1. Dietary fibers were also found in apples in both soluble and insoluble forms. The total dietary fibers of traditional and exotic apples grown in Portugal were 2.97 and 2.29 g/100 g, respectively, while the soluble fiber contents were 0.37 and 0.41 g/100 g, respectively (Feliciano et al., 2010). Another study reported that total dietary fiber of various apple cultivars was about 1.79 g/100 g, with 0.40 g/100 g of soluble, and 1.39 g/100 g of insoluble fiber (Patocka et al., 2020). The higher ratio of insoluble to soluble fiber in Red Delicious apple (4.37), compared to Granny Smith (3.21), might be the reason why more energy is required to remove the water during the drying process of Red Delicious apple, in comparison to that of Granny Smith (Joardder et al., 2015). It is also noteworthy that consuming apple with the peels is better as the dietary fiber content of apple peel is twice to three times higher than the apple flesh (Patocka et al., 2020).

In comparison to other fruits, apple contains higher fiber contents (pectin, cellulose, hemicellulose, and lignin) (Feliciano et al., 2010). Apple's pectin helps in cholesterol and blood glucose reduction (Asale et al., 2021). A study on pectin content in pulp of 35 apple cultivars grown in Czech Republic found that Strymka cultivar possessed the highest content (3.26 g/100 g FW), while the lowest (1.11 g/100 g FW) was found in Lebelovo cultivar that was harvested in Tišnov (Balík et al., 2012). In the analysis of 17 apple cultivars grown in Siena, Italy, the pectin content varied from 4.05 (Rossa Casetta) to 19.72 mg/g FW (Solaio) (Berni et al., 2019). Recent study found that among the new Hungarian apple cultivars, Damra exhibited the highest pectin content (1.15 g/100 g), followed by Feronia (0.82 g/100 g) and Bellona (0.74 g/100 g) (Ebadi et al., 2022). The pectin content of those three cultivars was significantly higher than that of Jonagored (0.59 g/100 g), Idared (0.57 g/100 g), and Fuji (0.56 g/100 g) as controls or commercial cultivars. The authors also added that the picking or harvesting time significantly influenced the pectin content of abovementioned cultivars, as for examples, Damra showed the highest pectin content in third picking time because it is a late ripening cultivar, while the pectin content of Feronia was the highest in the first picking time because it is an early ripening cultivar. With high pectin content as one of the quality parameters, the authors concluded that those new cultivars are suitable for fresh consumption (Ebadi et al., 2022).

Fig. 1 Total dietary fiber content in edible parts of apples from different cultivars and countries. Abbreviations: FW, fresh weight; AU, Australia (Food Standards Australia & New Zealand, 2021); KR, South Korea (National Institute of Agricultural Sciences of South Korea, 2022); PT, Portugal (Almeida & Gomes, 2017); TR, Turkey (Ministry of Agriculture & Forestry of Turkey, 2022); USA-1, Washington, United States of America (Condezo-Hoyos et al., 2014); USA-2, United States of America (USDA, 2019); ZA, South Africa (South African Medical Research Council, 2018)



Minerals

Minerals such as potassium, magnesium, calcium, sodium, and phosphorus, and trace elements such as zinc, manganese, copper, and iron were found in apple from various regions, reported by some studies (Jemaneh & Chandravanshi, 2021; Petkova et al., 2019). In Table 1, potassium dominates the mineral composition of apple, ranged from 99.46 to 119.00 mg/100 g. This was in agreement with Akšić et al. (2022), who found that potassium and zinc are two dominant minerals in apples grown in Norway. Sachini et al. (2020) reported that potassium from apple cultivars from southern Brazil contributed high to the percentage of daily recommendation value, followed by phosphorus and magnesium, while calcium contributed lower. However, the mineral content varied depending on the cultivars,

production cycle, and growing region (Sachini et al., 2020). In apples grown in Ethiopia, and imported apples to Ethiopia from Israel and South Africa, the varied mineral contents between red and green apples were observed (Jemaneh & Chandravanshi, 2021). It was highlighted that green apple fruits had higher calcium and aluminum content (1065–36,275 mg Ca/kg, 77.8–129 mg Al/kg) than red apple fruits (1013–36,143 mg Ca/kg, 52.5–89.6 mg Al/kg). In contrast, nickel content in green apple fruits were lower than in red apple fruits. The authors also reported that the analyzed red and green apples did not contain non-essential toxic metals cadmium and lead. It is worth noticing that according to European regulation (1881/2006/EC), the concentration of lead and cadmium in fruit is limited at 0.10 and 0.05 mg/kg wet weight, respectively (European Commission, 2006).

In apple products, minerals, especially potassium and calcium, together with polysaccharides and proteins contributed to the haze formation of apple cider, juice, and pommeau (alcoholic drink) by taking a role as ligands for polyphenols (Millet et al., 2017).

Vitamins

Various vitamins were present in the apple, including vitamins A, B₁, B₂, B₃, B₅, B₆, B₉, C, and E (Table 1). Those vitamins take a role in human's body metabolism and immune support to protect against infection problems, inflammation, and possibly some cancers (Alpert, 2017). Vitamin A has a role in immune system, particularly in innate and cell-mediated immunity and humoral antibody responses (Alpert, 2017). The carotenoids were found in higher content in the apple peel as it contributes to the fruit coloration together with chlorophyll (Delgado-Pelayo et al., 2014), while in flesh, the concentration of carotenoid is low (Acquavia et al., 2021). Feliciano et al. (2010) found that the β -carotene (provitamin A) content of unpeeled Bravo de Esmolfe apple was almost twice higher than that of the peeled one. B complex vitamins are important to ensure that the body's cells working appropriately; among them, vitamin B₃ is the most abundant vitamin found in apple fruit (Table 1). Vitamin B₃ plays some roles in lipid metabolism, synthesis of HDL (high-density lipoprotein) cholesterol, DNA synthesis, blood glucose regulation, and reducing the risk of cardiovascular disease (Andrade et al., 2018). Vitamin B₉ (folate) was also found in apple (3–9 μ g/100 g) (Table 1). Folate deficiency has been associated to some health problems, such as Alzheimer's and cardiovascular disease, osteoporosis, increased breast and colorectal cancer, poor cognitive ability, abnormalities in white and red blood cells, and hearing loss (Alpert, 2017). Vitamin C contained in apples can improve the human immunity, prevent anemia, and act as antioxidant and anti-aging agents (Patocka et al., 2020). Vitamin E (e.g., α -tocopherol) has also been known as a lipid-soluble antioxidant which has the ability to scavenge reactive oxygen species (ROS) (Mutalip et al., 2018). However, in comparison to polyphenols, vitamin C and E contributed less to the biological impact of apple (Feliciano et al., 2010). The vitamin C content of various apple cultivars has been reported by several studies. All analyzed 15 apple cultivars expressed higher vitamin C content in peel than flesh (Kschonsek et al., 2018). Between Granny Smith, Fuji Rose, and Golden Delicious cultivars, the highest content vitamin C content was found in Golden Delicious (31.48 mg/100 g), while the lowest one was found in Granny Smith (14.97 mg/100 g) (Asale et al., 2021). Furthermore, in the study about the effect of location within tree canopy on apples chemical compositions, it was found that the ascorbic acid contents of various apple peels from outer-canopy were

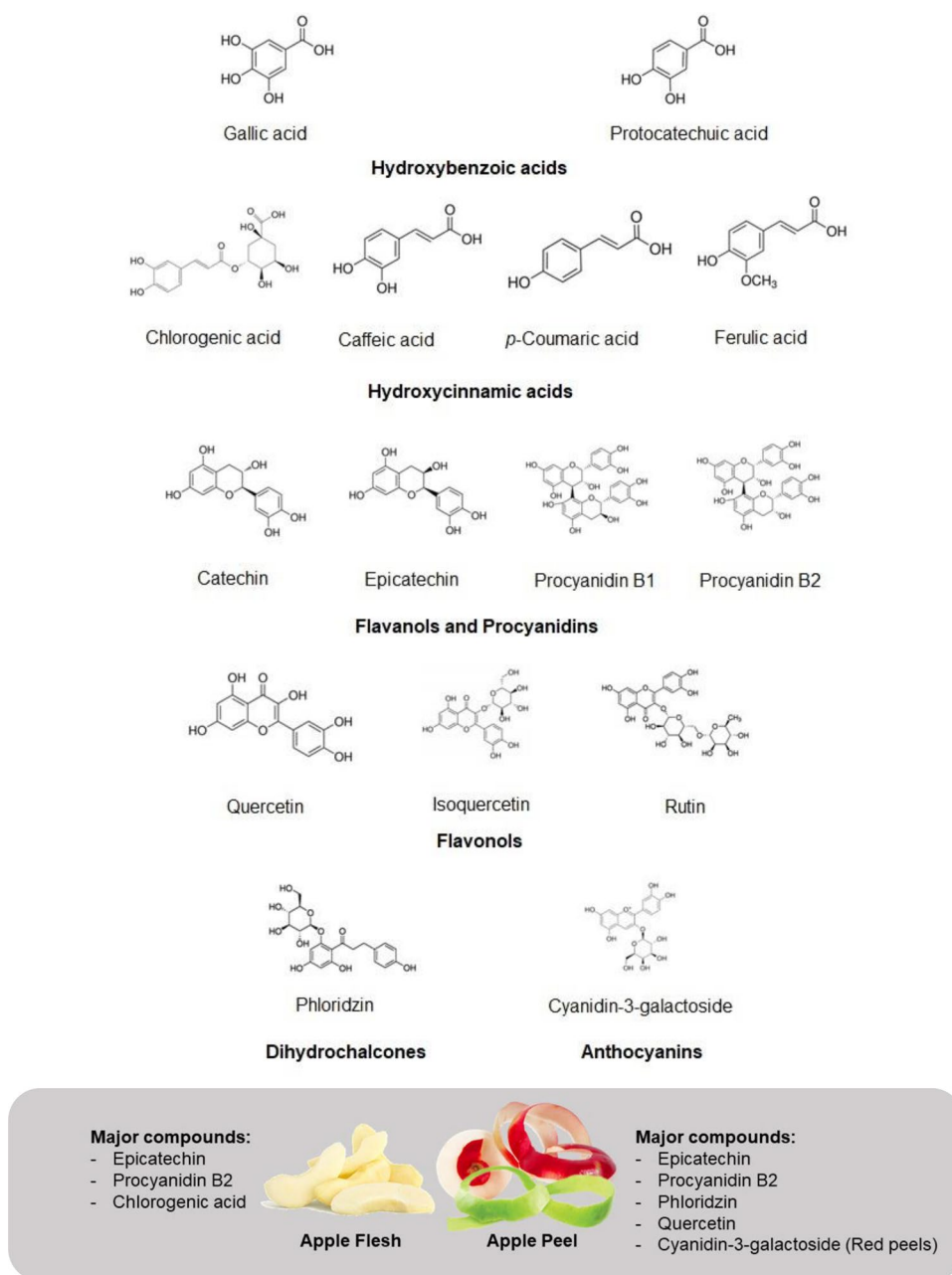
1.6–1.9 times higher than from the inner ones (Feng et al., 2014). It is also worth noticing that apple peels contained higher content of vitamin C than the flesh (Hamadziripi et al., 2014). For vitamin E, the content α -tocopherol, which is the fat-soluble vitamin most abundantly found in apple, was analyzed in the 24 apple cultivars harvested in South Tyrol, Italy (Bianchi et al., 2020). Among them, the authors found that the lowest α -tocopherol content was found in Freiherr von Berlepsch cultivar (0.13 mg/100 g FW), while the highest one was detected in Brixner Plattling cultivar (0.33 mg/100 g FW).

Phenolics

The phenolic composition (Fig. 2) of apple flesh and peel from various cultivars have been reported by several studies, summarized in Table 2. The major phenolic groups found in apple are hydroxybenzoic acids (gallic acid, protocatechuic acid), hydroxycinnamic acids (chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid), flavanols (catechin and epicatechin) and their oligo- (procyanidins) and polymeric structures (proanthocyanidins), flavonols (quercetin, isoquercetin, rutin, kaempferol, astragalin), dihydrochalcones (phloridzin, phloretin-xyloglucoside), and anthocyanins (cyanidin-3-galactoside). However, a study also reported the other phenolic groups, such as flavones and isoflavones contents in apple (Alarcón-Flores et al., 2015). Recently, many studies reported the phenolic composition of various apple cultivars grown in different countries or regions such as Himalaya (Raj et al., 2021), Australia (Bondonno et al., 2020), Austria (Oszmiański et al., 2020), Bosnia and Herzegovina (Akagić et al., 2019), China (Wang et al., 2023), Costa Rica (Navarro et al., 2018; Navarro-Hoyos et al., 2021), Croatia (Lončarić et al., 2021), Italy (Tarola et al., 2019; Wandjou et al., 2020a, b), Japan (Sato et al., 2017), Lithuania (Raudone et al., 2017), Moldova and Romania (Geană et al., 2021), Nepal (Pandey et al., 2020), Norway (Akšić et al., 2022), Poland (Oszmiański et al., 2018), and Portugal (Pires et al., 2018). The phenolic composition of apple may differ from one to another depending on growing location, genetic and cultivars, and environmental factors (Oszmiański et al., 2018). Additionally, the difference in natural antioxidant composition between cultivars, including phenolics, as well as physicochemical properties also affect the effectiveness of enzymatic browning inhibition in apple products (Arnold & Gramza-Michałowska, 2022).

A study also reported that mechanical vibration during transport (28 Hz, 6 h) and storage (14 days, 6 °C) of apple might affect the quality and physicochemical parameters of selected apple cultivars (Gala, Idared, Topaz, and Red Prince), including total phenolic content (TPC), antioxidant capacity, pH value, color, firmness, total soluble solids and dry matter (Walkowiak-Tomczak et al., 2021). Increased

Fig. 2 Chemical structures of major phenolic compounds found in apple fruits



TPC and antioxidant potential of all cultivars were observed after the mechanical vibration and storage, indicating that after fruit transport and storage, the apples still remain a good source of bioactive compounds.

Among all phenolic compounds, in Australian apples, the total procyanidins (including flavanols and procyanidins) predominated the phenolic composition in the flesh (51.5%), followed by hydroxycinnamic acids (39.6%), while in peel, flavonols predominated (66.7%), followed by total procyanidins (24.7%) (Fig. 3A) (Bondonno et al., 2020). For apple cultivars from Southeast Europe, flavanols and procyanidins contributed more than 70% of total polyphenols in flesh and

peel; phenolic acids were mainly present in flesh (6–25%); flavonols and dihydrochalcones accounted 1–13% and 1–10%, respectively in the peel; and 1–7% of anthocyanins were found in the red apples peels (Jakobek et al., 2013). Furthermore, in apples grown in Germany, Kschonsek et al. (2018) found that in apple peel, flavonols predominated the composition by more than 70%, followed by flavanols, phenolic acids, and phloridzin, while phenolic acids (more than 40%) predominated in the flesh, followed by flavanols and phloridzin, with missing flavonols (Fig. 3B). It is also worth noticing that apple peels generally had 5 times higher content of total individual phenolics than apple flesh (Kalinowska et al., 2020).

Hydroxybenzoic Acids

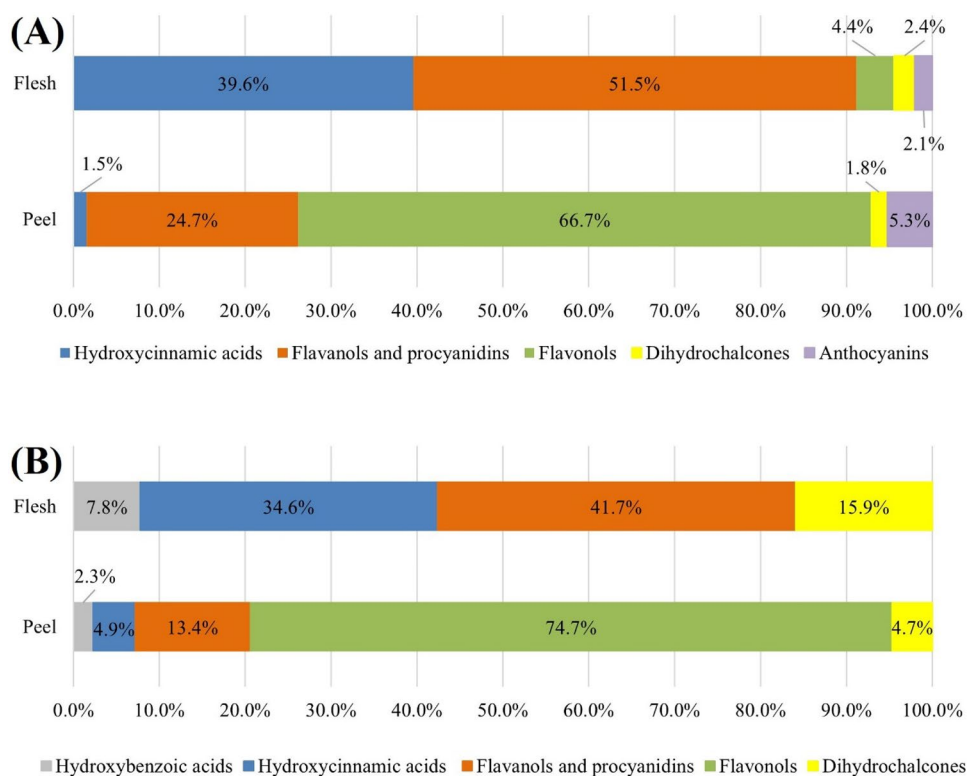
In hydroxybenzoic acids group, the gallic acid content in the apples was reported by several studies. Kschonsek et al. reported that there was no difference in gallic acid content between the flesh of old and new cultivars in Germany, while in the peel, old cultivars possessed higher content than new ones (Kschonsek et al., 2018). Furthermore, based on the finding from Kschonsek et al. (2018) (see Fig. 3B), it was found that the contribution of hydroxybenzoic acids to all analyzed phenolics was 2.3% in the flesh and 7.8% in the peel, but the hydroxybenzoic acids content of the peel was about 3 times higher than the content in the flesh. A study about fruit bagging reported that fruit bagging did not significantly decrease gallic acid content in Red Delicious apple peel ($p > 0.05$), but decreased gallic acid content in Golden Delicious and Red Gala apple peels significantly ($p < 0.05$) (Chen et al., 2012). Besides gallic and protocatechuic acid, other hydroxybenzoic acids such as syringic (0.9 to 2.8 mg/100 ml) and vanillic acid (1.1 to 31.4 mg/100 ml) were reported in apple cultivars grown in Van region, Turkey (Celik et al., 2018).

Hydroxycinnamic Acids

For hydroxycinnamic acids group, as seen in Fig. 3A, it contributed more to the flesh (39.6%) than the peel (1.5%) of Australian apples. Many studies reported the

hydroxycinnamic acids composition in apples (Table 2). Generally, chlorogenic acid has been reported by previous studies to predominate the hydroxycinnamic acids composition in apple. The hydroxycinnamic acid composition in the peel of Spanish cultivar Verde Doncella was dominated by chlorogenic acid (around 55%), followed by *p*-coumaric acid (34%) and caffeic acid (11%) (Krawitzky et al., 2014). The total hydroxycinnamic acids of this cultivar was lower than Red Delicious cultivar (Krawitzky et al., 2014). Wang et al. also found chlorogenic acid, together with catechin, phloretin, and quercetin, as the dominant phenolics in core, flesh, and peel of 20 apple cultivars in China (Wang et al., 2023). It is worth noticing that chlorogenic and caffeic acid were found in higher levels in the peels of red to dark-red-peeled apples, while procyanidins and flavanols were found in lower levels compared to the peels of green-peeled apples (De Paepe et al., 2015). Unfortunately, chlorogenic acid, together with flavanols and procyanidins were reported to be a good substrate for polyphenol oxidase (PPO), the enzymatic browning in apple (Kschonsek et al., 2019; Tinello et al., 2018), which can reduce the quality of the apple. The authors indicated that Szampion apple is good to produce light-colored juices and products due to its lower catechin, chlorogenic acid contents, and PPO activity in comparison to other cultivars. An article, however, did not detect the chlorogenic acid in the peels of Ervin Spur and Granny Smith, and caffeic acid in the peels of all analyzed cultivars (King Luscious, Amasya, Ervin Spur, Sky Spur, Arap Kizi, Lutz Golden, Granny Smith) (Karaman

Fig. 3 The contribution of phenolics in flesh and peel of various apple cultivars grown in **A** Australia—average of 91 cultivars, adapted from (Bondonno et al., 2020) (percentage based on fresh weight of apples); **B** Germany—average of 15 cultivars, adapted from (Kschonsek et al., 2018) (percentage based on weight of freeze-dried apples). Notes: Hydroxybenzoic acids content was not evaluated in (A). Anthocyanins content was not evaluated in (B); Flavonols were not detected in the flesh in (B)



et al., 2013). Furthermore, Chen et al. (2012) found that bagging treatment lowered the concentration of hydroxycinnamic acids (chlorogenic, caffeic, *p*-coumaric, gallic, and ferulic acid) in the peels of three apple cultivars (Golden Delicious, Red Delicious, Red Gala), except the chlorogenic acid in Red Delicious apple peel ($p > 0.05$). While in the flesh, the result varied in all cultivars.

Flavanols and Procyanidins

The composition of flavanols such as catechin, epicatechin, and procyanidins in apple flesh and peel have been reported by many studies (Table 2). In Fig. 3A, flavanols and procyanidins contributed 51.5% in the flesh, more than in the peel (24.7%) of Australian apples. However, about the content, Feng et al. (2014) found that higher levels of flavanols and procyanidins were found in the peels compared to the flesh in all analyzed cultivars (McIntosh, Gala, and Mutsu). The authors also reported that McIntosh apple (flesh and peel) from outer-canopy showed higher catechin, epicatechin, and procyanidin B2 contents compared to that from inner-canopy; while procyanidin B1 content was similar, regardless the position (Feng et al., 2014). The average catechin and epicatechin contents were respectively 3.4 and 5.2 times more in the peel than the flesh in old cultivars in Germany. While in new cultivars, the bigger difference was found as the catechin and epicatechin contents were 4.9 and 10.1 times more in the peel than the flesh (Kschonsek et al., 2018). Furthermore, among 11 first-quality grade apple cultivars, a study reported that epicatechin predominated the flavanols and phloridzin composition in both flesh and peel (Zielińska & Turemko, 2020). The authors also reported that the epicatechin content in the peel ranged from 94.79 µg/g FW (Delikates) to 297.77 µg/g FW (Quinte), while in the flesh ranged from 4.99 µg/g FW (Gloster) to 325.04 µg/g FW (Quinte). On the other hand, Panzella et al. (2013) found that procyanidin B2 predominated the flavanols composition of all apple cultivars (whole fruit) from Southern Italy, ranged from 11.87 mg/100 g FW (Gold Chief® Gold Pink) to 83.12 mg/100 g (Cape 'e Ciuccio). Similar result was also found in all 10 apple cultivars grown in Austria (Oszmiański et al., 2020). In comparison to Red Delicious cultivar, Verde Doncella cultivar from Spain showed lower concentrations of flavanols and quercetin derivatives in peel, pomace, and juice (Krawitzky et al., 2014).

The flavanols content of four new red-fleshed cultivars (RS-1, Redlove Era 107/06, 117/06, and 119/06) and white-fleshed cultivars (Brookfield Gala, Zhen Aztec Fuji, Story, Golden Smoothee, and Granny Smith) was compared by a study (Bars-Cortina et al., 2017). The authors observed that the flavanols content of flesh and peel of red-fleshed cultivars were lower than those of white-fleshed cultivars, which

might be due to the competitive synthesis between anthocyanins and proanthocyanidins via flavonoid pathway. With a competitive interaction from substrate between anthocyanidin reductase and anthocyanidin synthase enzymes, it could result in the lower flavanols and proanthocyanidins contents in the red-fleshed cultivars, which accordingly contain higher anthocyanins content than white-fleshed cultivars.

A study in Japan found that pale-red-fleshed cultivars, such as Nakano Shinku and Nakano no Kirameki, possessed high content of catechin and epicatechin; deep-red-fleshed cultivars, such as Chouka 4 and JPP35, were characterized by high accumulation of cyanidin-3-*O*-galactoside; and white-fleshed cultivars (Fuji and no. 37) showed the lowest phenolic compounds compared to others (Sato et al., 2017). The authors suggested to also consider the development of pale-red-fleshed cultivars in functional food although the deep-red-flesh cultivars are the major developed cultivars in Japan.

Regarding food safety, unfortunately, a study found that the apples with higher contents of catechin, epicatechin, and gallic acid were positively correlated to higher content of patulin (Lončarić et al., 2021). The authors added that these results can be explained due to pro-oxidative impact of flavanol, which leads to the formation of ROS in *Penicillium expansum* cells and activated the cellular antioxidant defense system and stimulated the production of patulin to reduce the ROS level inside the cells. However, the presence of phloridzin may reduce the fungal infection in apple (see the “Dihydrochalcones” section), that may also reduce the patulin formation.

Flavonols

Flavonols contributed more to total phenolics in Australian apples peels (66.7% of phenolic composition), while in the flesh only 4.4% (Fig. 3A). The other authors even highlighted that the flavonols were missing in the flesh of various apple cultivars in Germany (Kschonsek et al., 2018). It was in agreement with several studies that did not detected the quercetin in the flesh of any analyzed apple cultivars (Karaman et al., 2013; Zielińska & Turemko, 2020). However, Alarcón-Flores et al. (2015) detected quercetin in the flesh of all apple cultivars, ranged from 2.5 mg/kg DW (Golden (France)) to 4.2 mg/kg DW (Royal). Raudone et al. still detected quercitrin (quercetin-3-rhamnoside) and rutin (quercetin-3-*O*-rhamnoside) in the flesh of six Lithuanian cultivars by using high-performance liquid chromatography (HPLC) method; however, both quantities were very low and did not significantly contribute to the reducing activity (Raudone et al., 2017). De Paepe et al. (2015) studied that overall classic/new cultivars of apple, grown in Belgium, were typified by higher flavonols content, and lower contents of dihydrochalcones, proanthocyanidins, and chlorogenic acid.

Besides quercetin, isoquercetin, and rutin (Table 2), some articles reported the presence of kaempferol derivatives (including kaempferol glucoside or astragalin, kaempferol pentoside, kaempferol rhamnoside, etc.) (Alarcón-Flores et al., 2015; Jakobek et al., 2013), isorhamnetin derivatives (isorhamnetin galactoside, isorhamnetin glucoside) (Oszmianański et al., 2020), and myricetin (Alarcón-Flores et al., 2015) in some apple cultivars. Not only in fruits, quercetin and rutin were also found in peel, bark, and stem bark of apple cultivars originated from Jumla and Mustang, Nepal (Pandey et al., 2020). Among the analyzed samples, Richard apple peel from Mustang expressed the lowest quercetin (2.66 mg/100 g DW) and rutin (3.59 mg/100 g DW), while the highest quercetin and rutin contents were found in Chocolate stem bark from Jumla (171.05 mg/100 g DW) and Jonathan stem bark from Jumla (374.50 mg/100 g), respectively. The peel of Lještarka apple from Croatia were reported to have the highest total flavonols (1222.7 mg/kg FW), in comparison to other analyzed cultivars from Croatia and USA (Jakobek & Barron, 2016). The authors also found that the peel of red-peeled apples generally had higher flavonols content than the peel of yellow-green-peeled apples.

Dihydrochalcones

Dihydrochalcones, e.g., phloridzin, contributed to the total phenolics slightly higher in the flesh than peel of Australian apples (Fig. 3A), but phloridzin was found in higher content in apple peel (8.4 mg/100 g FW; ranged from 1.7 to 31.2 mg/100 g FW) than apple flesh (0.7 mg/100 g FW; ranged from 0.0 to 2.9 mg/100 g FW) (Bondonno et al., 2020). On the other hand, a study found that the phloridzin content was higher in the flesh than the peel, particularly in the Cox Orange, Ontario, and Roter Boskoop cultivars grown in Germany (Kschonsek et al., 2018). Besides phloridzin, which was reported by many studies (Table 2), other dihydrochalcones are also found in apple, for example, phloretin-2'-*O*-xyloglucoside and both of them are the major dihydrochalcones found in Finnish apple juice (He et al., 2022).

Phloridzin is one of the responsible bioactive compounds in apple that exhibits antidiabetic activity as it reduces intestinal sugar uptake by inhibition of Na/glucose cotransporter 1, which then is beneficial for people with type 2 diabetes mellitus (Niederberger et al., 2020). Apples, in the form of juice and fruit, have been the main contributors of phloridzin intake in European consumers (0.7–7.5 mg phloridzin/day) (Niederberger et al., 2020). Other than that, phloridzin can also be the chemotaxonomy marker for apple cultivar identification as well as its products, i.e., to identify the fraudulent admixtures of apple juice (Liaudanskas et al., 2015). In terms of food safety, phloridzin is the major polyphenol responsible for resistance to fungal infection, which

is explained by the formation of the hydrolyzed product, phloretin, that oxidizes and forms fungitoxic *o*-quinone (Lončarić et al., 2021).

Anthocyanins

Anthocyanins contributed to total phenolics more in the peels (5.3%) than in the flesh (2.1%) of apples grown in Australia (Fig. 3A). The main anthocyanin found in apple is cyanidin-3-galactoside (Meng et al., 2015). However, cyanidin-hexoside, cyanidin-pentoside, and other cyanidin derivatives were also reported (Jakobek et al., 2013). Anthocyanins content was correlated to the *a**-value (redness) of apple (Sethi et al., 2020). Although most articles reported that anthocyanins were found only in the red peel, several articles reported that anthocyanins were found in the flesh of several cultivars in Australia (Bondonno et al., 2020) and red-fleshed apples, such as Ljubeničarka which is grown in Southeast Europe (318.9 mg/kg FW) (Jakobek et al., 2013), and other apple species from China—*Malus pumila* Niedzwetzkyana (Dieck) (84.28 mg/kg FW) (Katiyo et al., 2018) and. Zielińska and Turemko (2020) also detected cyanidin-3-galactoside in the flesh of all analyzed apple cultivars (low level) and the peel of all analyzed apple cultivars (including the yellow-green-peeled apples—Papierówka and Antonówka, low level). Karaman et al. (2013) did not detect the cyanidin in the flesh of all analyzed cultivars (King Luscious, Amasya, Ervin Spur, Sky Spur, Arap Kizi, Lutz Golden, Granny Smith), but it was detected in the peel of all cultivars, except Lutz Golden and Granny Smith. Furthermore, Jakobek and Barron (2016) only detected the cyanidin-3-galactoside in several red-peeled apple peels (Lještarka, Božićnica, and Cortland), but not in neither the other analyzed red-peeled nor yellow-green-peeled apples peels.

Other Compounds

Other compounds, such as pigments (chlorophyll and carotenoids) in apple were studied (Delgado-Pelayo et al., 2014). The authors found that the most abundant compounds in the flesh and peel of 13 apple cultivars (yellow/red/green-peeled apples) were diesterified xanthophylls and chlorophyll *a*. In the flesh, diesterified xanthophylls ranged from 3.98 µg/g DW (green-peeled apple—Granny Smith) to 25.15 µg/g DW (yellow-peeled apple—Golden Montaña); while in the peel ranged from 5.04 µg/g DW (green-peeled apple—Granny Smith) to 38.39 µg/g DW (red-peeled apple—Ariane). For chlorophyll *a*, in the flesh, it ranged from 0.81 µg/g DW (red-peeled apple—Fuji from France) to 47.00 µg/g DW (green-peeled apple—Granny Smith); while in the peel ranged from 18.39 µg/g DW (yellow-peeled apple—Golden Rosett) to 1049.26 µg/g DW (green-peeled apple—Granny Smith). Furthermore, the authors also explained that green-peeled apple cultivars possessed high chlorophyll content

in the peel; however, it was also found in high level in some red-peeled apple cultivars, such as Fuji from Italy, Pink Lady®, and Starking Red Chief. While yellow-peeled apple cultivars (Golden Delicious, Golden Montaña, and Golden Rosett) lack of chlorophyll (Delgado-Pelayo et al., 2014).

To sum up, with the updated knowledge of qualitative and quantitative content of chemical composition in various apple cultivars, the consumers and apple products industries can select the promising apple cultivars that are rich in nutritional values to be consumed as direct consumption or production of apple products. In selecting the promising cultivar, it is also important to consider its availability in consumers' region as it is related to the price of the apples. The apple cultivar with higher availability and affordable price yet is still rich in nutritional values can contribute to wider scale of consumption to promote general health of consumers. For apple products industries, e.g., apple juice industries, the by-products from production process such as apple pomace or peels that still contains valuable bioactive compounds can open a wider range of production instead of just being a waste.

Total Phenolics and Antioxidant Activity

Apples have been studied to possess various bioactive compounds which showed antioxidant activity. Many articles reported the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of the extracts of apple cultivars from different countries (Table 3). TPC is commonly determined using Folin-Ciocalteu assay that is well-known, simple, and reproducible, yet there are some drawbacks that need to be concerned, such as pH sensitivity, temperature, reaction time, and selectivity (Zhong & Shahidi, 2015). Regarding its selectivity, the overestimation of total phenolics may happen as nonphenolic reducing agents, such as reducing sugars and amino acids that present in the extract could affect the result of TPC. Therefore, HPLC could also be conducted to confirm the TPC result.

Generally, studies reported higher TPC and TFC were found in the peel than the flesh. The barrier function of the peels against the external biotic and abiotic stress might be the reason of this phenomenon (Kschonsek et al., 2018). In the study on 13 apple cultivars grown in India, Sethi et al. (2020) reported that the average TPC and TFC in the peel were respectively 2.8 and 2.68 times higher than those in the flesh. The similar result was found by Zhang et al. (2012), who found the order of part of fruits according to their TPC and TFC: peel > core > flesh, in apple cultivars grown in China.

A study on the whole fruit of 104 apple cultivars from Europe (Switzerland, Italy, Netherlands) reported the average TPC of 150 mg catechin equivalents (CAE)/100 g FW, ranged from 52 to 378 mg CAE/100 g FW (Ceymann et al., 2012).

The content was comparable and could be more specifically described by Zielińska and Turemko (2020) as they found TPC of the peels of Polish apple cultivars, ranged from 182 mg CAE/100 g FW (Ligol) to 328 mg CAE/100 g FW (Quinte); while the TPC of flesh ranged from 54 mg CAE/100 g FW (Ligol) to 174 mg CAE/100 g FW (Quinte). In terms of TFC, in the peel, it ranged from 55 mg CAE/100 g FW (Ligol) to 130 mg CAE/100 g FW (Paulared); while in the flesh, it ranged from 18 mg CAE/100 g FW (Ligol) to 83 mg CAE/100 g FW (Papierówka). It is also noteworthy that the TPC and TFC are not always higher in peel than the flesh. Several cultivars, such as Topaz (Italy) had higher TPC (Tarola et al., 2019) and Royal Delicious (India) had higher TFC (Sethi et al., 2020) in the flesh than peel. Furthermore, a study reported that the mild heat treatments (40–50 °C) increased 70% TPC of Eva apple fruit, and at the same time reduced the browning activity (Rodríguez-Arzuaga et al., 2019). It is noteworthy that higher TPC might be related to higher browning activity because phenolics are the substrates of PPO. However, in Eva apple, the higher TPC did not increase the browning development as the reduction PPO activity of Eva apple was observed after the mild heat treatment.

Various assays were conducted by recent studies on various parts of fruit and cultivars (Table 3). In brief, the antioxidant activity can be analyzed using (a) reactive oxygen species (ROS) scavenging-based (e.g., 2,2-diphenyl-1-picrylhydrazyl—DPPH, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid—ABTS, oxygen radical absorbance capacity—ORAC, and chemiluminescence), (b) redox potential-based assays (e.g., ferric reducing antioxidant power—FRAP, cupric ion reducing antioxidant capacity—CUPRAC, and cyclic voltammetry method—CV), and (c) metal chelation capacity (e.g., chelating of ferrous ion—CA) (Zhong & Shahidi, 2015). Compared to the other assays or methods, ORAC assay is considered more relevant as a reference for antioxidant effectiveness as it uses biological relevant oxidant—peroxyl radicals, that is generated by azo compounds, commonly the hydrophilic 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) (Zhong & Shahidi, 2015). Those different methods or assays were conducted by previous studies in apples as each assay has different hydrophilicity or lipophilicity on natural antioxidants in apples as described in the “Phenolics” and “Other Compounds” sections.

Generally, the antioxidant activity of apple's extract is in accordance with its TPC. The correlation between antioxidant activity and sum of polyphenols or phenolic acids in apple can be analyzed. For examples, using Pearson correlations, the sum of polyphenols of Austrian apple cultivars had high correlation to ABTS, DPPH, and FRAP with *r*-value of 0.89, 0.95, and 0.93 (*p* < 0.05), respectively; while the total phenolic acids also had high correlation to those respective assays, with *r*-value of 0.69, 0.70, and 0.68 (*p* < 0.05) (Oszmiański et al., 2020).

Table 3 Content of total phenolics, total flavonoids, total anthocyanins and antioxidant potential of various apple cultivars

Analyzed cultivars	Country	Part of fruit	Assay	Findings	References
Fuji Rose, Golden Delicious, Granny Smith	Ethiopia	Whole fruit	TPC TFC DPPH FRAP	H: Granny Smith ↔ Fuji Rose; L: Golden Delicious H: Fuji Rose ↔ Granny Smith; L: Golden Delicious H: Fuji Rose ↔ Granny Smith; L: Golden Delicious H: Fuji Rose; L: Golden Delicious	Asale et al. (2021)
Gala, Golden Delicious, Granny Smith, Red Delicious, Starking	Mexico	Whole fruit	TPC TFC TAC	H: Granny Smith; L: Golden Delicious H: Starking; L: Gala H: Red Delicious; L: Golden Delicious ↔ Granny Smith (not detected)	Corona-Leo et al. (2021)
Beliy Naliv, Crestesc, Florina, Gloster, Golden Delicious (Moldova), Golden Delicious (Romania), Idared, Jonathan (Moldova), Jonathan (Romania), Montuan, Renet Simirenco, Richard, Spartian, Starkrimson	Moldova and Romania	Whole fruit	ABTS DPPH TPC TFC DPPH	H: Red Delicious; L: Granny Smith H: Red Delicious; L: Starking H: Starkrimson; L: Gloster ↔ Montuan H: Beliy Naliv ↔ Montuan ↔ Jonathan (Moldova); L: Idared ↔ Gloster ↔ Golden Delicious (Romania) H: Starkrimson ↔ Jonathan (Romania) ↔ Beliy Naliv; L: Montuan ↔ Gloster	Geană et al. (2021)
Anna, Jonagold	Costa Rica	Flesh Peel	TPC DPPH ORAC TPC DPPH ORAC	H: Jonagold; L: Anna H: Jonagold; L: Anna H: Jonagold; L: Anna H: Anna; L: Jonagold; Peel > Flesh for Anna; Peel < Flesh for Jonagold H: Anna; L: Jonagold; Peel > Flesh for Anna; Peel < Flesh for Jonagold H: Anna; L: Jonagold; Peel > Flesh for Anna; Peel < Flesh for Jonagold	Navarro-Hoyos et al. (2021)
Berner Rose, Brünnerling Type 1, Brünnerling Type 2, Brünnerling Type 4, Croncels Type 2, Galloway Pepping, Goldgelbe Sommerrenette, Salzburger Type 1, Schmidberger Renette, Transparente de Croncels	Austria	Whole fruit	ABTS DPPH FRAP	H: Brünnerling Type 1; L: Brünnerling Type 2 H: Brünnerling Type 1; L: Schmidberger Renette H: Brünnerling Type 1; L: Schmidberger Renette	Oszmiałski et al. (2020)

Table 3 (continued)

Analyzed cultivars	Country	Part of fruit	Assay	Findings	References
Gale Gala, Oregon Spur II, Red Chief, Royal Delicious, Royal Gala, Scarlet Gala, Scarlet Spur I, Silver Spur, Starkrimson, Super Chief, Top Red, Vance Delicious, Well Spur	India	Flesh	TPC	H: Scarlet Spur I; L: Royal Delicious	Sethi et al. (2020)
			TFC	H: Royal Delicious; L: Royal Gala ↔ Gale Gala ↔ Red Chief ↔ Vance Delicious	
			CUPRAC	H: Silver Spur ↔ Oregon Spur II ↔ Well Spur ↔ Starkrimson ↔ Scarlet Spur I ↔ Scarlet gala; L: Red Chief ↔ Royal Delicious ↔ Royal Gala ↔ Super Chief ↔ Vance Delicious ↔ Top Red	
			DPPH	H: Oregon Spur II; L: Royal Gala ↔ Top Red ↔ Scarlet Gala ↔ Vance Delicious ↔ Super Chief ↔ Well Spur ↔ Starkrimson	
		Peel	FRAP	H: Silver Spur ↔ Oregon Spur II; L: Scarlet Gala ↔ Royal Gala ↔ Vance Delicious ↔ Well Spur ↔ Super Chief	
			TPC	H: Well Spur ↔ Oregon Spur II; L: Top Red ↔ Red Chief ↔ Scarlet Spur I; Peel > Flesh	
			TFC	H: Well Spur; L: Royal Delicious; Peel > Flesh (except Royal Delicious; Flesh > Peel)	
			CUPRAC	H: Top Red; L: Royal Delicious ↔ Scarlet Gala; Peel > Flesh	
			DPPH	H: Well Spur ↔ Royal Gala; L: Silver Spur ↔ Royal Delicious ↔ Vance Delicious; Peel > Flesh	
			FRAP	H: Oregon Spur II; L: Silver Spur ↔ Vance Delicious ↔ Scarlet Gala ↔ Top Red ↔ Starkrimson ↔ Super Chief ↔ Gale Gala; Varied results of Peel vs. Flesh	

Table 3 (continued)

Analyzed cultivars	Country	Part of fruit	Assay	Findings	References
Annurca, Mela Rosa dei Monti Sibillini (MR1—good shape, color, size; MR2—with malformations)	Italy	Whole fruit	TPC ABTS	H: MR1; L: Annurca H: MR1; L: Annurca	Wandjou et al. (2020b)
Antonówka, Delikates, Early Geneva, Gloster, Jonagored, Ligol, Paulared, Papierówka, Quinte, Rubinola, Sunrise	Poland	Flesh	DPPH TPC TFC CA CV DPPH FRAP TPC TFC CA CV	H: MR1; L: Annurca H: Quinte; L: Ligol ↔ Gloster H: Papierówka; L: Ligol H: Antonówka; L: Ligol H: Quinte; L: Ligol ↔ Gloster H: Quinte; L: Gloster H: Quinte; L: Gloster H: Quinte; L: Ligol; Peel > Flesh H: Paulared; L: Ligol; Peel > Flesh H: Antonówka; L: Ligol; Peel > Flesh (except Antonówka and Rubinola: Flesh > Peel) H: Paulared ↔ Jonagored; L: Antonówka ↔ Ligol; Peel > Flesh	Zielińska and Turemko (2020)
Anciano, Campo Pianacce, Casolana, Ficareto, Filare Delle Pianacce, Gialla Pianacce, Golden Delicious, Piatta Cantine, Podere Pianacce, Red Delicious, Rossa Casetta, Rugginosa Delle Pianacce, Solaio, Sotto Muro Casetta, Strada Pianacce, Tocchi, Tre Colli, Vecchio Pollaio, Vialle Casetta	Italy	Whole fruit	TPC FRAP	H: Solaio; L: Casolana H: Solaio; L: Red Delicious ↔ Golden Delicious ↔ Casolana ↔ Sotto Muro Casetta ↔ Strada Pianacce ↔ Rossa Casetta	Berni et al. (2019)
Braeburn, Cerina, Florina, Gelato Cola, Gold Rush, Jonagored, Limoncella, Renetta Ostris, Rosada, Topaz	Italy	Flesh Peel	TPC DPPH TPC	H: Topaz; L: Rosada H: Jonagored; L: Rosada H: Florina; L: Topaz; Peel > Flesh (except Topaz: Flesh > Peel)	Tarola et al. (2019)
Braeburn, Berlepsch, Cox Orange, Dülmener Rosenapfel, Elstar, Golden Delicious, Goldparmäne, Granny Smith, Gravensteiner, James Grieve, Jonagold, Jonathan, Oldenburger, Ontario, Roter Boskoop	Germany	Flesh Peel	DPPH TPC ABTS ORAC TPC ABTS ORAC	H: Topaz; L: Gold Rush; Peel > Flesh H: Jonathan; L: Golden Delicious H: Jonathan; L: Golden Delicious ↔ Braeburn H: Jonathan; L: Golden Delicious H: Oldenburger; L: Golden Delicious; Peel > Flesh H: Oldenburger; L: Golden Delicious; Peel > Flesh H: Oldenburger; L: Golden Delicious; Peel > Flesh	Kschonsek et al. (2018)

Table 3 (continued)

Analyzed cultivars	Country	Part of fruit	Assay	Findings	References
Altländer Pfannkuchenapfel, Boikenapfel, Booskop, Charlamowsky, Dulmener Rosenapfel, Geflammtter Kardinal, Gelber Richard, Horneburger Pfannkuchenapfel, Kaiser Alexander, Kaiser Wilhelm, Landsbergen, Lausitzer Nelkenapfel, Renette, Parkers Pepping, Riesenboiken, Rote Sternrenette, Roter Delicious Type Starkinson, Roter Eiserapfel, Roter Herbstkalvill, Roter Trier Weinapfel, Weisser Winterkalvill, Wintergoldparman, Wintergoldparmane Aldas, Auksis, Connel Red, Ligol, Lodel, Rajka	Poland	Whole fruit	ABTS FRAP	H: Wintergoldparmane; L: Landsbergen Renette H: Wintergoldparmane; L: Landsbergen Renette	Oszmiański et al. (2018)
	Lithuania	Flesh	TPC	H: Lodel ↔ Auksis ↔ Aldas; L: Rajka	Raudone et al. (2017)
		Peel	FRAP	H: Aldas ↔ Auksis; L: Ligol	
			TPC	H: Lodel ↔ Auksis ↔ Aldas; L: Rajka; Peel > Whole fruit > Flesh	
		Whole fruit	FRAP	H: Aldas ↔ Auksis; L: Ligol; Peel > Whole fruit > Flesh	
			TPC	H: Lodel ↔ Auksis ↔ Aldas; L: Rajka	Balík et al. (2012)
			FRAP	H: Aldas ↔ Auksis; L: Ligol	
		Flesh	PCL-TAC	All cultivars ↔	
		Peel	PCL-TAC	H: Bernské Růžové; L: Jadernička Moravská; Peel > Flesh	
Albrechtovo, Bernské Růžové, Hvězdnatá Reneta, Jadernička Moravská, Lebelovo, Matčino, Starkrimson, Vilémovo	Czech Republic				
Fuji, Golden Delicious, Guoguang, Wanglin	China	Core	TPC	H: Guoguang; L: Golden Delicious ↔ Fuji	Zhang et al. (2012)
			TFC	H: Guoguang; L: Golden Delicious ↔ Fuji	
			DPPH	H: Guoguang ↔ Wanglin; L: Golden Delicious	
			FRAP	H: Guoguang; L: Golden Delicious ↔ Fuji	
		Flesh	TPC	H: Guoguang; L: Golden Delicious	
			TFC	H: Guoguang; L: Golden Delicious	
			DPPH	H: Wanglin ↔ Guoguang ↔ Fuji; L: Golden Delicious	
			FRAP	H: Guoguang; L: Golden Delicious	
		Peel	TPC	H: Fuji; L: Wanglin ↔ Golden Delicious; Peel > Core > Flesh	
			TFC	H: Guoguang ↔ Fuji; L: Golden Delicious; Peel > Core > Flesh	
			DPPH	H: Fuji ↔ Guoguang ↔ Wanglin; L: Golden Delicious; Peel > Core > Flesh (Fuji and Golden Delicious); Core > Peel > Flesh (Guoguang and Wanglin)	
			FRAP	H: Fuji ↔ Guoguang; L: Golden Delicious; Peel > Core > Flesh (except Fuji; Peel > Flesh > Core)	

ABTS 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), CA chelating of ferrous ion activity, CUPRAC cupric ion reducing antioxidant capacity, CV cyclic voltammetry, DPPH 2,2-diphenyl-1-picrylhydrazyl, FRAP ferric reducing antioxidant power, H the highest content (TPC and TFC) or the highest activity (antioxidant activity), L the lowest content (TPC and TFC) or the lowest activity (antioxidant activity), ORAC oxygen radical absorbance capacity, PCL-TAC photochemiluminescence-total antioxidant capacity, TAC total anthocyanins content, TFC total flavonoid content, TPC total phenolic content, ↔ not significantly different

CV method and different assays, such as CA, FRAP, and DPPH assays were conducted on apples from Poland (Zielińska & Turemko, 2020). It was observed that the peels of all cultivars had higher antioxidant potential than the flesh in CV, DPPH, and FRAP assays. By DPPH assay, the intermediate radical-scavenging activity of flesh was observed, ranged from 2.23 to 4.65 μmol Trolox equivalent (TE)/g FW for Gloster and Quinte, respectively. While in the peels, it ranged from 5.27 μmol TE/g FW (Antonówka) to 8.65 μmol TE/g FW (Jonagored). In FRAP assay, the peels of all cultivars expressed at least twice higher value than the flesh. Similar result was also found in CV assay, which is the rapid, simple, low-cost, and sustainable method to analyze the reducing capacity of antioxidants, in comparison to FRAP and DPPH assays. The CA of peels was also higher than that of flesh in all cultivars, except Antonówka and Rubinola. The authors also discussed the correlation of phenolic compounds and antioxidant activity. It was reported that in the peels, TPC and TFC are positively correlated to the results of FRAP and CV, while cyanidin-3-galactoside was highly correlated to the results of DPPH. While in the flesh, TPC and TFC had strong correlation with FRAP, DPPH, CV, and CA. Additionally, epicatechin, chlorogenic acid, and cyanidin-3-galactoside had high and positive correlations with results of FRAP, DPPH, and CV; while with CA, the correlations were weaker.

Another study reported the antioxidant potential of apples cultivar in Germany using ABTS and ORAC assays (Kschonsek et al., 2018). Among 15 analyzed cultivars (5 new cultivars and 10 old cultivars), Oldenburger and Jonathan possessed the highest TPC and antioxidant potential by both ABTS and ORAC assays in the peel and flesh, respectively. Golden Delicious in contrast showed the lowest TPC and antioxidant potential by both mentioned assays. The other findings reported by the authors were about the comparison between old and new cultivars. The flesh of old cultivars had higher TPC and vitamin C content than of the new cultivars which led to the higher antioxidant capacity determined by ABTS and ORAC. While in the peel, higher content of vitamin C was found in the old cultivars, but there was no significant difference of TPC and antioxidant potential found between the old and new cultivars. This phenomenon could be explained as new cultivars possessed lower content of flavanols (in flesh), total phenolic acids (in flesh and peel), and phloridzin (in flesh and peel) than the old cultivars, whereas monomeric and oligomeric flavanols and phloridzin are strong antioxidants (Kschonsek et al., 2018). It is also worth noticing that vitamin C explains only around 0.4% of the total antioxidant capacity of apples (Feliciano et al., 2010). Similar result was also found by Asale et al. (2021), as the whole fruit of Golden Delicious apple possessed the lowest antioxidant potential by DPPH and FRAP assays. Although it contained the highest vitamin C content,

but it contained the lowest TPC and TFC among analyzed cultivars grown in Southern Ethiopia.

Wandjou et al. explored the antioxidant composition and activity of ancient apple cultivar Mela Rosa dei Monti Sibillini (MR) from Sibillini Mountains, central Italy (Wandjou et al., 2020b). The authors compared two choices of MR: first choice which has good shape, color, and size, while second choice is normally discarded due to unpleasant shape and color. Interestingly, the second choice showed majorly higher polyphenols and triterpenes when HPLC–DAD analysis was performed, yet possessed a bit lower TPC (Folin-Ciocalteu method) and antioxidant activity (ABTS and DPPH assays) than the first choice, indicating that the MR apples with malformation are also potential to be reused and recovered from nutraceutical and cosmeceutical properties. In another publication, the same authors also compared the antioxidant composition and activity of MR (pulp and peel) in freeze-dried and dried (45 °C) forms (Wandjou et al., 2020a). Generally, the lyophilized apple showed higher antioxidant potential than the dried materials. Also higher levels of phenolics, especially catechin, epicatechin, procyanidin B2, phloridzin, and triterpenes are found the peel extract than pulp extract.

In Czech Republic, the antioxidant capacity mean of apple peels was approximately 7 times higher than that of the flesh, using photochemiluminescence (PCL) method (Balík et al., 2012). The value ranged from 19.11 mmol TE/kg FW (Jadernička Moravská) to 56.65 mmol TE/kg FW (Bernské Růžové) in the peel. While in the flesh, it ranged from 4.02 mmol TE/kg FW (Vilémovo) to 6.59 mmol TE/kg FW (Bernské Růžové), but there was no significant difference between them. While in Podlasie province of Poland, Kalinowska et al. (2020) observed the TPC and antioxidant activity (DPPH, ABTS, FRAP, CUPRAC) of the peel's extract of new, scab-resistant cultivar—Gold Milenium, in comparison to that of old, scab-sensitive cultivar—Papierówka. The results showed that the peel's extract of Papierówka possessed higher TPC, antioxidant activity than that of Gold Milenium. This result maybe correlated to the findings of Shah and Gupta, who found that secondary metabolites (including antioxidants) were synthesized in scab-infected apple peel as a defensive response, which resulted in higher TPC and TFC in comparison to non-infected apple peel (Shah & Gupta, 2023).

Corona-Leo et al. (2021) approached the antioxidant potential (ABTS and DPPH assays), TPC, TFC, and total anthocyanins content of five apple cultivars (Gala, Golden Delicious, Granny Smith, Red Delicious, and Starking) in Mexico from their bioaccessibility index after in vitro digestion. The results showed that the antioxidant activity, TPC, TFC, total anthocyanins content after in vitro digestion were significantly lower than those before digestion, indicating that those values before digestion may overestimate the

bioavailability of the antioxidants. The other publications about antioxidant bioaccessibility of apples after *in vitro* digestion are summarized in Table 4.

Considering the higher TPC, TFC, and antioxidant potential in the peels of most apple cultivars, it is suggested to consume the fruit with the peels. It does not mean that apple peel contributed to phenolic intake more than the flesh as the peel represents only around 10% of the whole fruit and it is not always consumed. The apple flesh then contributes more to the phenolic intake of consumer. Therefore, apple cultivar which has higher phenolic content and antioxidant potential in the flesh is suggested to improve the phenolic contents in consumer's body. Additionally, it is also important to consider the bioavailability of the antioxidants in apples.

Apple Phenolics Extraction

Phenolics are the important metabolites of apple as they could show health-promoting effects when ingested. The phenolic extraction procedure should be accurate and precise to maximize the yield of targeted compounds and their antioxidant activity. Therefore, extraction might be the most critical step in the analysis of apple's metabolites, as the ideal extraction should recover all the targeted metabolites without any chemical addition (Acquavia et al., 2021).

Various apple products (juice, mash, pomace, whole fruit) were extracted using various extraction methods, such as conventional liquid–liquid extraction (LLE) and solid–liquid extraction (SLE), as well as development of SLE, such as microwave-assisted extraction (MAE), radio frequency-assisted extraction (RFAE), ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), pulsed electric field (PEF)-assisted extraction, and pressurized liquid extraction (PLE) (Table 5). The independent factors that affect the phenolic extraction, such as type and concentration of solvent, temperature of extraction, sample to solvent ratio, particle size, and preparation or pretreatment of apple samples (grinding, milling, blending) were varied in the research design (Perussello et al., 2017). As the dependent factors, TPC, TFC, and antioxidant activities were then analyzed to find the optimum conditions or to be compared to conventional methods, such as Soxhlet extraction (Ferrentino et al., 2018), or simply compared to control (without developed treatment) (Lohani & Muthukumarappan, 2016; Quang et al., 2014). Some researchers statistically checked the optimization of extraction condition by employing response surface methodology (RSM) (Alberti et al., 2014; Franquin-Trinquier et al., 2014). The result could be confirmed by HPLC–DAD and LC–MS/MS (the sensitive and selective analysis) to check the phenolic compositions (Acquavia et al., 2021).

Conventional Extractions

Various types of products can be produced from apple fruit. The protocol could differ according to the product type. For liquid samples, such as apple juice, it could be analyzed directly after the filtration and/or centrifugation process (Acquavia et al., 2021), or could be extracted by LLE using conventional solvents, such as acetone, ethanol, and methanol (Sharma et al., 2015). From apple juice of Red Delicious and McIntosh apples, Sharma et al. (2015) carried out the phenolic extraction using surfactant mediated LLE. The extraction using surfactant, especially Brij-58 surfactant resulted in higher TPC and antioxidant activity (DPPH assay) of extracted juice than using organic solvents and water. However, further studies on interactional behavior of surfactant mediated assemblies and scale-up procedure at industrial level is still needed.

Solid–liquid extraction (SLE) was conducted by most of the previous studies to extract the phenolics from the solid parts of apple (peel, pomace, and whole fruit) using acetone, methanol, ethanol, or water (Bars-Cortina et al., 2017; Çam & Aaby, 2010; Casazza et al., 2015). Although most studies reported that using methanol resulted in higher phenolic yield, the usage of acetone, ethanol, and water were reported to be safer and more environmentally friendly than methanol (Casazza et al., 2020; Quang et al., 2014). However, the preliminary study on phenolic extraction of whole apple fruit (Ligol cultivar) for 4 h reported that employing ethanol as solvent yielded more flavonoids than employing methanol and acetone (Liaudanskas et al., 2018). Rana et al. (2015) carried out the extraction using 50% acetone (30 min at 60 °C), which was more efficient than 50% methanol and 50% ethanol to extract the apple pomace. By using water as solvent to extract phenolics from pomace, a study found that extraction conducted at 100 °C for 37 min with 100 mL/g of solvent to solid ratio was optimized in terms of TPC yield and limitation of 5-hydroxymethylfurfural (Çam & Aaby, 2010). This compound is an intermediate Maillard reaction product and was limited in some foods, including apple juice (max. 20 mg/kg) according to European Fruit Juice Association (Li et al., 2019). Furthermore, a study investigated that SLE of apple pomace (Champion) using Tween 80 as surfactant was optimum to obtain the highest TPC at concentration of 1.14% in water, 104 mL/g of solvent to material ratio, pH of 3.8, for 65 min of extraction (Skrypnik & Novikova, 2020). Using these optimum conditions, the predicted TPC was 7.75 mg gallic acid equivalent (GAE)/g dry weight (DW), while the experimental value was 7.68 mg GAE/g DW.

The SLE should be done efficiently and selectively. For example, the extraction using only one type of solvent is not very efficient to extract all the polyphenols as other hydrophilic compounds could be also extracted, and less polar phenolics could remain inside the matrix (Acquavia

Table 4 Bioaccessibility of natural antioxidants in apples during in vitro digestion model

Apple products	Cultivar (Country)	Type of analysis	Bioaccessibility (%) during in vitro digestion			Notes	References
			Oral digestion	Gastric digestion	Intestinal digestion		
Whole fruit	Gala (Mexico)	TPC	-	24.98	57.45	-	Corona-Leo et al. (2021)
		TFC	-	41.63	73.15		
		TAC	-	204.23	83.09		
		ABTS	-	14.59	18.22		
		DPPH	-	16.10	33.07		
	Starking (Mexico)	TPC	-	7.23	35.02		
		TFC	-	23.14	50.08		
		TAC	-	152.81	46.07		
		ABTS	-	14.93	22.44		
		DPPH	-	15.57	28.45		
	Red Delicious (Mexico)	TPC	-	26.61	81.43		
		TFC	-	44.28	83.41		
		TAC	-	126.49	82.70		
		ABTS	-	15.53	23.38		
		DPPH	-	10.80	20.76		
	Golden Delicious (Mexico)	TPC	-	25.52	29.63		
		TFC	-	16.62	48.30		
		TAC	-	-	-		
		ABTS	-	8.08	18.91		
		DPPH	-	4.84	12.89		
	Granny Smith (Mexico)	TPC	-	18.78	20.79		
		TFC	-	37.62	43.08		
		TAC	-	-	-		
		ABTS	-	20.20	21.98		
		DPPH	-	15.32	24.05		
Oven-dried apple	Fuji (Chile)	TPC	28.09	31.65	27.11	a, b	Pavez-Guajardo et al. (2020)
Freeze-dried pomace	Pink Lady (Australia)	Total flavanols	-	161.50	24.70	b	Liu et al. (2019)
		Total flavonols	-	352.20	156.60		
		Total phenolic acids	-	227.20	407.60		
		Total dihydrochalcones	-	182.80	6.30		
Freeze-dried extruded pomace	Pink Lady (Australia)	Total flavanols	-	80.20	48.60	b, c	Liu et al. (2019)
		Total flavonols	-	258.60	136.00		
		Total phenolic acids	-	245.60	190.30		
		Total dihydrochalcones	-	311.80	255.50		
Whole fruit	Jonaprinz (Luxembourg)	Total chlorogenic acid	-	109.47	31.61	-	Bouayed et al. (2012)
	Jonagold (Luxembourg)	Total chlorogenic acid	-	103.67	34.19		
	Mutzu (Luxembourg)	Total chlorogenic acid	-	92.07	56.52		
	Golden Delicious (Luxembourg)	Total chlorogenic acid	-	83.93	33.31		

a, The bioaccessibility was calculated based on data obtained in referred source. Bioaccessibility (%)=(value after digestion/value before digestion)*100. b, Final intestinal digestion (ileum) is considered. c, Extruded apple pomace at 15% barrel moisture is considered (Liu et al., 2019)

ABTS 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), DPPH 2,2-diphenyl-1-picrylhydrazyl, TAC total anthocyanins content, TFC total flavonoid content, TPC total phenolic content

Table 5 Methods used for phenolics extraction from various apple products

Apple products	Sample form	Reported optimum conditions				Analyses conducted		Notes	References
		Extraction method	Solvent	Concentration	Temperature (°C)	Time	Sample to solvent ratio	Other parameters	
Juice (Red Delicious; McIntosh; India)	Fresh	Surfactant-mediated LLE	Brij-58	7 mM (aqueous solution)	RT	10 min	n.i	2 mL of BHT (0.8% in methanol) was added to prevent oxidative stress	Sharma et al. (2015)
Mash/juice (Envy; New Zealand)	Mashed (juice for analysis)	UAE	Water	-	30	7.3 min	1:1 (w/w)	20 W/g	Quang et al. (2014)
Peel (Ruby S; South Korea)	Freeze dried powder	UAE	Ethanol	50% in water	20	25.3 min	0.05 g/mL	40 kHz	Park et al. (2022)
Peel (Jonagold; Italy)	Dried powder	MAE	Ethanol	68% (v/v) in water	150	90 min	0.1 g/mL	Inert atmosphere	Casazza et al. (2020)
Peel (McIntosh; Canada)	Microwave-hot air dried powder	RFAE	Ethanol	42.67% in water	50	10 min	0.0114 g/mL	400 W, 320 mL N ₂ /min of mixing speed, inert atmosphere	Jusoh et al. (2017)
				37.33% in water	50	10 min	0.02 g/mL	400 W, 165 mL N ₂ /min of mixing speed, inert atmosphere	
Peel (Golden Delicious; Jonagold; Renetta Canada; Raventze; Italy)	Oven dried powder	HPTE	Methanol	Pure	150	150 min	0.2 g/mL	Under N ₂ atmosphere	Casazza et al. (2015)
Pomace (Champion, Gloster, Imant, Jonagored, Ligoli; Russia)	Dried powder	Surfactant-mediated SLE	Tween 80	1.14% in water	RT	65 min	0.0096 g/mL	pH 3.8	Skrypnik and Novikova (2020)

Table 5 (continued)

Apple products	Sample form	Reported optimum conditions					Analyses conducted		Notes	References
		Extraction method	Solvent	Concentration	Temperature (°C)	Time	Sample to solvent ratio	Other parameters		
Pomace (Fruitus Meran S.p.A.; Italy)	Fresh / freeze dried powder / oven dried powder	SFE	Ethanol (as co-solvent)	5% (w/w)	45	120 min	-	30 MPa, freeze dried powder is better than fresh or oven dried powder	TPC, DPPH	It is better than Soxhlet with ethanol and boiling water maceration methods Ferrentino et al. (2018)
Pomace (Tree Top, Inc.; USA)	Naturally fermented powder	PEF-assisted extraction	Water	-	25	500 μ s	0.125 g/mL	2 kV/cm	TPC, DPPH	Followed by extraction: methanol (80), sample to solvent ratio (2:10 g/mL), 25 °C, 1 h TPC and antioxidant activity (DPPH) increased 37.4% and 86% than control Lohani and Muthukumarappan (2016)
Pomace (Appledale Processors Co-op. Ltd.; Australia)	Homogenized	SLE	Water	-	85	30 min	0.05 g/mL	-	TPC, ABTS, DPPH, FRAP	Candrawinata et al. (2015)
Pomace (Conde-sur-Vire; France)	Dried	UAE	Malate buffer	50 mM	40	40 min	0.15 g/mL	0.764 W/cm ² , pH 3.8	TPC	It is better than conventional SLE Pingret et al. (2012)
Whole fruit (without seed, Ligoli; Lithuania)	Freeze dried powder	UAE	Ethanol	70% (v/v) in water	44.61	26.90 min	0.083 g/mL (+ 20 mL for rinsing)	480 W	TFC	- Liaudanskas et al. (2018)
Whole fruit (without seed, Gala; Brazil)	Freeze dried powder	SLE	Acetone	65% in water	10	20 min	0.017 g/mL	-	TPC, TFC, DPPH, FRAP	Alberti et al. (2014)
			Methanol	84.5% in water	28	15 min	0.017 g/mL	-	TPC, TFC, DPPH, FRAP	-

Table 5 (continued)

Apple products	Sample form	Reported optimum conditions				Analyses conducted		Notes	References
		Extraction method	Solvent	Concentration	Temperature (°C)	Time	Sample to solvent ratio	Other parameters	
Whole fruit (unpeeled, Braeburn; France)	Freeze dried powder	PLE	Methanol	Pure	RT	15 min	0.007 g/mL	3 extraction cycles, using pressurized N ₂ controlled at 1 MPa for 30 s	It is better than using acetone 70% In comparison to SLE, PLE is better
		SLE	Methanol:BHT	99:1 (v/v)	RT	20 min	0.01 g/mL	-	It is better than using acetone:water:BHT 79:30:1

ABTS 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), *BHT* butylated hydroxytoluene, *DPPH* 2,2-diphenyl-1-picrylhydrazyl, *FRAP* ferric reducing antioxidant power, *HPTE* high pressure and temperature extraction, *MAE* microwave-assisted extraction, *n.i.* not informed, *PEF* pulsed electric field, *PLE* pressurized liquid extraction, *RFAE* radio frequency-assisted extraction, *RT* room temperature, *SFE* supercritical fluid extraction, *SLE* solid-liquid extraction, *TAA* total ascorbic acid content, *TFC* total flavonoid content, *TPC* total phenolic content, *UAE* ultrasound-assisted extraction

et al., 2021). To minimize this issue, a multi-step extraction process could be conducted. Firstly, hexane could be used to discard the lipids, carotenoids, and chlorophyll from the apple. Secondly, extraction using methanol could dissolve the sugars, organic acids, and low molecular weight phenolics. Lastly, aqueous acetone (4:6) could be used to extract the polymerized polyphenols from the residue of previous step (Acquavia et al., 2021).

A study compared different modified SLE methods to extract the pomace from Australian Pink Lady cultivars (Li et al., 2020a, b). The authors used aqueous methanol to extract the phenolics of the pomace, and after which, the residue was subjected to either sequential base and acid hydrolysis or sequential acid and base hydrolysis and extraction with 1:1 (w/w) diethyl ether/ethyl acetate (DE/EA) solution in order to liberate the bound phenolics from the pomace. As comparison, direct base or acid hydrolysis followed by DE/EA extraction was evaluated. Among the methods conducted, the authors suggested the direct base hydrolysis using NaOH, followed by DE/EA extraction to extract high content of polyphenols (10.62 mg GAE/g DW), considering the simplicity and efficiency.

Despite the modifications of SLE have been studied as above, generally, conventional extraction has some drawbacks to be concerned, such as long extraction time (19 h at 25 °C) (Casazza et al., 2015), degradation of phenolics due to excessive extraction time, high solvent requirement (leading to corrosion of equipment and extra waste treatment), flammability problem, poor selectivity (Perussello et al., 2017), and food safety issues (Zhang et al., 2021). Therefore, the development of extraction, including MAE, RFAE, UAE, SFE, PEF-assisted extraction, and PLE have been studied, especially to reduce the extraction time, organic solvent consumption, as well as to increase the phenolic yield.

Non-conventional Extractions

Microwave-Assisted Extraction

In comparison to conventional extraction method, microwave-assisted extraction (MAE) has been known as an eco-friendly and rapid-heating method with lower solvent utilization. It also produced more yield with higher purity, has higher level of automation, reduced wastes, and has suitable reproducibility (Rezaei et al., 2013). The principle of this method is the fast microwave energy transfer to the materials via molecular interaction with the electromagnetic field, which then weakens the apple tissue and subsequently increasing the phenolic yield during solvent extraction (Perussello et al., 2017).

The MAE was applied for extracting the phenolics from apple pomace (Chandrasekar et al., 2015; Rezaei et al., 2013) and peel (Casazza et al., 2015, 2020). Casazza et al.

(2015) have reported that MAE (using methanol, solid to liquid ratio 0.2 g_{DM}/mL_{solvent}, at 110 °C for 60 min) yielded higher TPC in Raventze peel than conventional SLE (using methanol, solid to liquid ratio 0.2 g_{DM}/mL_{solvent}, at 25 °C, for 19 h, dark condition). The faster extraction time could be also highlighted as an advantage as MAE was 19 times faster than conventional SLE. In another study, Casazza et al. (2020) used ethanol to extract the phenolic from Jonagold peel, and found that the optimum conditions based on TPC, TFC, and antioxidant activity (ABTS assay) were using ethanol (32% water concentration), 90 min extraction time, and found that the degradation due to high temperature (up to 150 °C) could be limited by inert atmosphere. The authors also paid attention on the solid residue after the extraction, and concluded that that extraction process allowed to obtain solid residue less pollutant and higher caloric value than the unprocessed apple skin, manifesting that it could simplify the waste handling, increasing the efficiency of its potential use in waste-to-energy plants (Casazza et al., 2020).

By employing RSM with central composite design (CCD), Chandrasekar et al. (2015) analyzed the optimum conditions to extract the phenolics from Red Delicious and Jonathan apple pomace using MAE with acetone 70% and ethanol 60%. The authors varied the microwave power (100–900 W), solvent to sample ratio (4–12 mL/g dry pomace), and extraction time (30–180 s). They found that using acetone 70%, the increasing microwave power (265 to 735 W) significantly increased the TPC of both cultivars, additionally longer extraction time (61 to 149 s) increased the phenolics yield of Jonathan pomace. On the other hand, the solvent volume did not significantly affect the extraction process. When ethanol 60% was employed, the solvent to sample ratio, microwave power, and extraction time significantly affected the TPC of both Red Delicious and Jonathan pomace. In conclusion, the highest TPC (15.8 mg GAE/g) was found in Red Delicious pomace, extracted under optimum conditions (735 W of power, 149 s of extraction time, and 10.3 mL of ethanol 60% per gram dry sample (Chandrasekar et al., 2015).

It is noteworthy that the microwave power and extraction time are the two most fluctuating parameters in MAE, which keep changing depending on plants, bioactive compounds, and different plant part of the same plant (Kala et al., 2016). Furthermore, excessive microwave power and exposition time might result in higher solution temperature, which then degrade the phenolics (Perussello et al., 2017). It was confirmed by Rezaei et al. (2013) that the TPC of Red Delicious pomace decreased with excessive power (more than 90 W) and extraction time (more than 15 min).

Radio Frequency-Assisted Extraction

The radio frequency-assisted extraction (RFAE) mechanism is similar to MAE. In comparison to MAE, RFAE is more

advantageous due to longer wavelength and lower frequency that result in greater penetration depth and suitable for bulk material with better uniform heating (Jiao et al., 2018). RFAE for 10 min at 50 °C was used to recover the phenolics from McIntosh apple peel (Jusoh et al., 2017). The authors studied the effects of ethanol concentration (10–70%), mixing speed (10–320 mL N₂/min), solid to liquid ratio (0.002–0.02 g/mL), and radio frequency power (200–400 W) on TPC and antioxidant activity (DPPH assay) (Table 5). By performing RSM, the highest TPC value (121.87 mg GAE/g DW) was achieved after the extraction using 42.67% ethanol, 230 mL N₂/min of mixing speed, 0.0114 g/mL solid to liquid ratio, and 400 W of power. While to achieve the highest antioxidant activity by DPPH assay, 37.33% ethanol, 165 mL N₂/min of mixing speed, 0.02 g/mL solid to liquid ratio, and 400 W of power were used. Besides phenolic, RFAE was reported to increase the yield of pectin from apple pomace, better than MAE and conventional extraction (Zheng et al., 2021). Further investigation on apple's phenolic extraction using RFAE method is still needed, especially from various apple cultivars.

It is also important to know that when the plant matrix is exposed to radio frequency for a longer time (i.e., more than 10 min), the phenolics might degrade, resulted in lower TPC, and some impurities might be released which affected the yield (Kochadai et al., 2022).

Ultrasound-Assisted Extraction

The mechanism of ultrasound-assisted extraction (UAE) is based on the application of acoustic waves to the apple products, which can rupture the plant cells and then releasing more phenolics content to the medium (Perussello et al., 2017). UAE has been studied to have lower extraction time, higher process efficiency, and more sustainable, which has potential for industrial application for phenolic extraction (Acquavia et al., 2021; Perussello et al., 2017).

UAE was carried out in phenolic extraction from apple pomace (Pingret et al., 2012; Pollini et al., 2021; Wang et al., 2018a), mash (Quang et al., 2014), whole fruit (Liudanskas et al., 2018), flesh (Wiktor et al., 2016), peel (Park et al., 2022; Wang et al., 2022), leaves (Stefova et al., 2019), and wood or bark (Withouck et al., 2019), using various solvents. The ultrasound could be applied during extraction, which was reported by most of the studies, but sometimes applied to pretreat the samples before the extraction (Wang et al., 2018a; Wiktor et al., 2016; Withouck et al., 2019). Wiktor et al. (2016) pretreated the flesh of Ligol apple (without peel) using contact (direct soundwave irradiation) and immersion (indirect soundwave irradiation) ultrasound treatment. It was found that the highest TPC and antioxidant activity (DPPH assay) was found in extract using immersion method (40 kHz, 5 min). In contrast, the contact ultrasound

treatment, particularly for 30 min (which was the longest treatment time analyzed), decreased around 30% TPC and 92% of antioxidant activity in comparison to control. This might be explained as the contact ultrasound may magnify the enzymes activity that degrades the phenolics. Furthermore, the immersion method affected the color of apple in less extent in comparison to contact one. Another study pretreated apple pomace using ultrasound (24 kHz, 400 W, varied pretreatment time of 0–30 min, in 0–50% ethanol solution) under controlled moderate temperature of pretreatment (initial temperature 20 °C, never exceeded 37 °C) to prevent thermal degradation, and continuously was extracted conventionally using the same medium for another 30 min (total 60 min including the 30 min of UAE pretreatment) at 20 °C (Wang et al., 2018a). The highest TPC was obtained in the pomace pretreated with ultrasound for 30 min, and subsequently extracted using 50% of ethanol. The same authors in other publication recovered the catechin from flesh and peel of Granny Smith and Red Delicious with UAE (Wang et al., 2018b). Besides the flesh and skin, apple cultivars could also affect the relative content of catechin in TPC that indicates the selectivity of catechin extraction compared to other phenolics. However, the mechanism of the observed extraction selectivity remained unclear.

The selectivity to extract anthocyanins in Idared and Lještarka peels could also be improved by acidifying the methanol using 0.1% HCl as the solvent, with optimized UAE parameters (15 min, 0.1 g/mL of sample to solvent ratio), noting that anthocyanins are more stable in acidic environment (Jakobek et al., 2015). While to extract phenolics in general, such as flavonols, anthocyanins, dihydrochalcones, flavanols from peels, or flavanols, procyanidins, dihydrochalcones, and hydrocinnamic acids from flesh of those apples, UAE with 80% methanol was recommended.

An aqueous buffer—50 mM malate buffer (pH 3.8) was employed by Pingret et al. (2012) as the solvent in phenolic UAE of pomace. The authors varied the temperature (10–40 °C), sonication time (5–55 min), and ultrasonic intensity (0.431–0.764 W/cm²) as the independent factors, and as dependent one, TPC was analyzed. The UAE conducted at 40 °C for 40 min, with 0.764 W/cm² resulted in 30% higher TPC, in comparison to conventional SLE. The authors also explained that temperature and sonication time are the most influential variables, as TPC linearly increased as temperature and sonication time increase. Ultrasonic intensity also showed the same trend, but it was less predominant. However, it is worth noticing that excessive sonication time might degrade the phenolics, and hence might decrease the yield (Perussello et al., 2017).

In whole fruit of Ligol apple, the optimization of UAE using RSM was carried out to extract the phenolics (Liaudanskas et al., 2018). The ethanol 70% was used as the solvent. The effect of optimization of temperature, extraction

time, and ultrasonic power was analyzed to find the highest TFC content of the extract. The optimum UAE was fulfilled at 44.61 °C for 26.90 min, with 480 W of ultrasonic power. With this optimum condition, the experimental TFC (6.58 mg rutin equivalent (RE)/g) was near to the predicted one (6.69 mg RE/g). This optimum condition was then applied to 6 apple cultivars grown in Lithuania (Aldas, Aukasis, Connel Red, Ligol, Lodel, Rajka), and HPLC analysis showed that Aldas apple possessed the highest total phenolics (Liaudanskas et al., 2018).

In comparison to other non-conventional phenolic extraction methods (ultraturax extraction, accelerated solvent extraction or PLE, and pulsed electric field), UAE method applied during extraction for 60 min at 60 °C was observed as the most effective method to extract the TPC from Red Delicious pomace, especially when 50% ethanol was used as the solvent (Pollini et al., 2021). However, a study also reported that PLE with solid-phase extraction absorbent (da Silva et al., 2020) extracted more apple's TPC than UAE.

Recently, instead of using ethanol or other organic solvents, a study optimized the UAE (0.242 kW.h/kg of energy input) of Gala peel's phenolics using CO₂ water (0–7.05 mmol/L) (Wang et al., 2022). The idea of using CO₂ water is because the dissolved CO₂ could act as nucleus for new cavitation bubbles generated during UAE, then the resulting cavitation effect could eventually improve the phenolic extraction. Among the analyzed concentration of CO₂ in water, UAE using 5.28 mmol CO₂/L extracted the highest TPC, total proanthocyanidins, and antioxidant activity (DPPH assay), and additionally high TFC. This technique can be considered by the industry as CO₂ water is more economically affordable than organic solvents.

According to our knowledge, the comparison between ultrasound treatment applied before and during extraction of phenolics, especially in apples, has not been conducted, and could be explored in future studies. Besides their effects on the phenolics yield and composition, the time and energy consumed by both applications should be studied and considered to be economically beneficial. However, either before and during extraction, it is advisable to maintain the temperature low or moderate (50 °C or lower) to prevent the degradation of phenolics during UAE. The short time (50–100 s) but several pulses of ultrasound can also be applied to maintain the temperature (Wang et al., 2018b).

Supercritical Fluid Extraction

In supercritical fluid extraction (SFE), the nontoxic and safe gas CO₂ (with low critical temperature, around 31 °C) was used as the main solvent to extract the phenolics of apple products (Fernandes et al., 2022). The organic co-solvents (acetone, ethanol, methanol) could be applied to increase the solvating power of CO₂, noting that the CO₂ alone is

selective to non-polar compounds, which might be the limitation to extract the phenolics with higher polarity. In comparison to conventional extractions, the degradation of phenolics could be decreased and the quality of phenolics could be maintained as SFE was conducted in lower temperature, without light and air exposure (Perussello et al., 2017). Additionally, apart from phenolics, the SFE method was also selective to extract and isolate triterpene acids, which also possess antioxidant, anti-inflammatory, and anticancer activities (Zhang et al., 2021).

The SFE method was used to recover the phenolic of apple pomace (Ferrentino et al., 2018) and peel (Massias et al., 2015). Furthermore, a study also analyzed the total phenolics and antioxidant activity of phenolics in the oil extracted from apple seeds by SFE (Ferrentino et al., 2020). Ferrentino et al. (2018) optimized the SFE of apple pomace (fresh, freeze dried, and oven dried) with controlled time (2 h) and various pressure (20 and 30 MPa), temperature (45 and 55 °C), with and without addition of ethanol 5% as co-solvent. The authors found that freeze dried pomace extracted at optimum conditions (45 °C, with 30 MPa of pressure, and applying ethanol 5% as co-solvent) resulted in higher TPC and antioxidant activity (DPPH assay). The TPC, antioxidant activity by DPPH assay, and yield of extract obtained by performing SFE at this optimum condition was compared with conventional Soxhlet extraction and boiling water maceration. The yield obtained by SFE was lower than both conventional extractions, but interestingly, the TPC and antioxidant activity of SFE extract were higher than by both conventional methods. This result indicated that SFE selectively extracted less but more active polyphenols, in comparison to analyzed conventional methods. The result was then confirmed as HPLC analysis (between SFE and Soxhlet) showed that higher signals intensity and relative areas of SFE (in the retention time between 20 and 25 min, in which phloridzin, epicatechin, quercetin, and phloretin are detected) were obtained than of Soxhlet extraction (Ferrentino et al., 2018). Additionally, Ferrentino et al. (2020) extracted the oils from apple seeds using SFE (24 MPa, 40 °C, 1 L/h of flow rate, 140 min), which resulted in higher TPC and antioxidant activities (DPPH and FRAP assays) of oil than that using Soxhlet extraction (using n-hexane, 10/150 g/mL of sample to solvent ratio, 6 h, at boiling temperature).

Massias et al. (2015) investigated the SFE (25 MPa, 50 °C, 75:22:3 mol ratio of CO₂: ethanol: water) of phenolics in dried (lyophilized) and ground Golden Delicious peels. The use of ethanol as cosolvent was adapted due to the fact that phloridzin and quercetin glycoside that are abundant in apple peels, contain a sugar component that is too polar to be soluble in only CO₂. As comparison, maceration using ethanol and conventional solvent extraction using methanol + acetone 70% (v/v) were conducted. The result was higher total phenolics extracted by SFE ranged from 550 to 800 mg/100 g dry peel (depending on solvent

to peel ratio—37 to 73 wt basis, mass of dried peel—15 or 30 g, and SFE procedure—static or dynamic) than maceration (177 mg/100 g dry peel). While total phenolics of 792 mg/100 g dry peel were observed after extraction by conventional solvent extraction.

Pulsed Electric Field-Assisted Extraction

PEF (pulsed electric field-assisted extraction) application to plant materials, such as apple, causes the cell disruption due to the short and high voltage pulses that can increase the yield and phenolic extraction efficiency. It is an eco-friendly method as the solvent consumption is reduced. As PEF is non-thermal method, it also minimizes the damage of the nutrients in the foods. The problem of this method is the difficulty to scale-up (Perussello et al., 2017; Zhang et al., 2021).

The study on the effect of PEF treatment on the phenolic extraction from apple was reported in pomace (Lohani & Muthukumarappan, 2016), flesh (Wiktor et al., 2015), mash (Turk et al., 2010), and whole fruit (Ribas-Agustí et al., 2019). Lohani and Muthukumarappan (2016) applied the mild PEF to release the bound phenolics of apple pomace powder. The varied flour (pomace powder) to water ratios (FWR 5–12.5%, w/v), treatment time (500–1250 µs), and electric field intensity (1–3 kV/cm) were optimized based on the results of TPC and antioxidant activity (DPPH assay). The factors affecting the release of phenolics were in following order: FWR > electric field intensity > treatment time. The authors found that the optimized conditions (12.5% FWR, 500 µs, 2 kV/cm) resulted in the increased TPC and antioxidant activity values of 37.4% and 86%, respectively, compared to the control. Those phenolics were dominantly consisted of protocatechuic acid, followed by chlorogenic and salicylic acid, analyzed by UHPLC-DAD.

Wiktor et al. (2015) analyzed the impact of PEF treatment on TPC, antioxidant activity (DPPH assay) and color of Ligol apple flesh, grown in Warsaw, Poland. The authors varied the electric field intensities (0–5 kV/cm) and pulse numbers (0–100 pulses), which corresponded to the energy input of 0–80 kJ/kg (based on calculation). After the PEF treatment, the polyphenols were extracted using 80% ethanol for further analyses. It was reported that the highest TPC and antioxidant activity were observed in the samples treated by 1.13 kJ/kg (1.85 kV/cm and 10 pulses). Excessive energy above 40 kJ/kg (5 kV/cm, 50–100 pulses) negatively impacted the TPC and antioxidant activity of samples up to 35.93 and 32.95%, respectively. The authors explained that this phenomenon might happen as the electric energy was not sufficient for PPO deactivation, which resulted in TPC degradation. The color of samples was analyzed 0 and 60 min after the PEF treatment and was compared to the control (untreated sample). The total color change at 0 min

(ΔE_0) ranged from 0.48 to 8.58, while at 60 min (ΔE_{60}) ranged from 1.25 to 21.87) which could be related to the different chemical compositions of apples. Higher electric field intensity might increase the chance of enzymatic browning. The samples treated at 3 and 5 kV/cm showed decreasing L^* -value (brightness), indicating more intensive browning, which might be caused by the higher release of PPO and its substrates.

The negative impact of PEF on phenolic composition was reported in different study. From the mash of Golden Delicious apple from France, phenolic extraction was conducted using PEF treatment (Turk et al., 2010). The mash size (small and large: 120 and 630 mm³, respectively) and electric field intensity (0 and 450 V/cm) were varied. The juice was collected from the PEF-treated mash. The juice yield was checked, and the polyphenolic compounds of obtained juice were then analyzed using HPLC. It was found that PEF-treatment increased the juice yield in comparison to control, regardless the mash size. Unfortunately, in comparison to untreated sample, the PEF treatment decreased 53.4 and 17.1% of total polyphenol concentration for small and large mash size, respectively. In comparison to the loss from small size mash, the lower loss from the large size mash could be explained by the decreasing oxidative area, so the oxidation of hydroxycinnamic acids could be lower.

In an in vitro simulated digestion study, the bioaccessible and non-bioaccessible fractions of phenolic compounds of Golden Delicious whole fruit were analyzed after the PEF treatment (0 and 24 h after 0.01, 1.8, and 7.3 kJ/kg of energy input), and was compared to the untreated apple (Ribas-Agustí et al., 2019). By employing UHPLC-DAD-MS, the TPC and individual phenolic content were determined. The phenolic extraction using methanol was conducted only to undigested apples, but not conducted to bioaccessible and non-bioaccessible fractions (after digestion) as there was no significant difference result from direct analysis in the preliminary study. The result showed that 0.01 kJ/kg treatment after 24 h resulted in increased bioaccessible (61%) and non-bioaccessible (35%) 5-caffeoylquinic acid, as well as TPC of bioaccessible (26%) and non-bioaccessible phenolic compounds (19%) in comparison to untreated apple. The increase of bioaccessible phenolic contents could be explained by the increased contents in the undigested apples. In comparison to untreated apple, higher energy inputs (1.8 and 7.3 kJ/kg) were observed to decrease the overall TPC of bioaccessible and non-bioaccessible fractions up to 37% and 22%, respectively after 0 h of treatment, and 44% and 22%, respectively after 24 h of treatment. Furthermore, the bioaccessibility of phenolics was also determined by calculating the ratio of bioaccessible compounds to the compounds of undigested one. The bioaccessibility of overall phenolics increased from 14% (untreated) to 27% (24 h after 7.3%). Therefore, the functional properties of apple could be

improved by PEF treatment by either increasing TPC of bio-accessible and non-bioaccessible fractions or the phenolic bioaccessibility (Ribas-Agustí et al., 2019).

To conclude, some factors such as processing parameters, solvent types, and apple's composition affect the efficacy of apple's phenolic extraction. Although some studies reported negative impact of PEF on extracted phenolic composition due to some possible mechanisms, PEF is still a good alternative for phenolic extraction as it reduces the extraction time and solvent usage as reported by other studies. Further studies are still needed to optimize the conditions, especially in various apple cultivars.

Pressurized Liquid Extraction

PLE (pressurized liquid extraction) is another green extraction technique with shorter extraction time, lower solvent requirement, and higher extraction rate and yield in comparison to conventional extractions. The method was based on the high pressure and temperature applied to the solvents (above boiling point of solvent, but it keeps the solvent still in the liquid state), which leads to penetration within the matrix tissue, hence increases the phenolic extraction efficiency. The expensive equipment cost to withstand the high pressure utilized limits the usage of PLE (Deen et al., 2019). In PLE, the solid sample, such as apple, is extracted using solvent under following conditions: 40–200 °C of temperature, 500–3000 psi, and 5–15 min of extraction time (Deen et al., 2019).

In previous studies, the PLE was performed to extract the phenolics from apple fruit (Franquin-Trinquier et al., 2014) and pomace (da Silva et al., 2020, 2023). A study performing RSM to optimize the PLE of phenolics from Braeburn apple fruit (Franquin-Trinquier et al., 2014). At room temperature and 1 MPa, the authors varied the solvent (pure methanol, acetone 70%), sample mass (50–550 mg), extraction time (1–15 min), and number of extraction cycles (1–3 cycles), and compared the resulting TPC with manual solvent extraction. The extraction using PLE was optimum when 50 mg sample was extracted using pure methanol for 15 min and three cycles. While for the manual extraction, the usage of methanol-butylated hydroxytoluene (BHT), 100 mg sample, 20 min, resulted the highest TPC. The TPC resulted from PLE was 19.5 times higher than manual extraction.

High pressure and high temperature extraction (HPTE) was analyzed by Casazza et al. (2015) to extract the phenolics of peels of 4 different apple cultivars (Golden Delicious, Jonagold, Renetta Canada, Raventze), in comparison to MAE and conventional SLE. It was found that the antioxidant activity (DPPH assay) of extract obtained by HPTE was higher than MAE and SLE, in all cultivars.

The development of PLE coupled on-line with solid-phase extraction (SPE) was reported to extract the

phenolics of apple pomace (da Silva et al., 2020). By using solid adsorbents in SPE, the specific phenolic classes and compounds could be separated with the mechanism similar to a chromatographic separation. The PLE-SPE consisted of several steps: (1) activation (using methanol or ethanol) and conditioning (using water) of adsorbents, (2) first extraction stage using water, (3) second extraction stage using a lower polarity solvent (methanol or ethanol) than that in the first extraction stage. During the extraction, the authors used various solid adsorbents (Septra, Isolute, Strata X, and Oasis), amount of water in the first extraction stage (0–120 mL), temperature (60–80 °C), and activation or elution solvent (methanol or ethanol). HPLC analysis was used to identify the compounds (two phenolic acids, 10 flavonoids) in the sample. Generally, the best adsorbent to recover the phenolics was Septra. There was a small effect of temperature on the extraction as higher temperature only significantly affected the content of chlorogenic acid (decreased), total phenolic acids (decreased), quercetin derivative (decreased), and phloridzin (increased). Furthermore, the authors included that ethanol could be used as the substitute (or partially substitute) for methanol, as no significant difference was found in total phenolic acids and total flavonoids. Additionally, in comparison to other methods (PLE, UAE, shaker, magnetic stirring), regardless the solvent used for extraction (water, ethanol, methanol), PLE-SPE exhibited higher or similar recovery of total phenolic acids and total flavonoids (da Silva et al., 2020).

Recently, in another article, the same authors also used PLE-SPE method, online with HPLC-photodiode array, so called 2D-PLE-SPE-HPLC-PDA, to extract the apple pomace (da Silva et al., 2023). The authors used Septra as the adsorbent in SPE column, five different extraction solvent gradients (different concentrations of ethanol and water, and time of each solvent), and also analyzed the effect of temperature (40–80 °C) and static time (0–30 min) in the later steps. The solvent gradient (25 min: 100% water; 100–160 min: 100% ethanol), static time (20 min), and extraction temperature (80 °C) were suggested to obtain higher mass transfer of target analytes, such as furfurals, chlorogenic acids, flavonoids, and phloridzin. This developed method was compared to other methods (PLE only, UAE, and stirring). The developed method extracted similar (furfural, chlorogenic acid) or higher (phloridzin, flavonoids) extraction yield than PLE alone. UAE still showed significantly higher extraction yield of all target analytes than this developed method, except phloridzin (not significantly different). The developed method provides advantages, such as high efficiency (especially for phloridzin), elimination of sample preparation procedure, high automation, low human intervention, real-time process monitoring, and compounds fractionation of samples.

Other Factors Affecting Extractions

The pretreatment using enzyme could accelerate the phenolic extraction by degrading the cell walls that retain the phenolic in the polysaccharide-lignin network via hydrogen or hydrophobic bonding. The enzymatic maceration of Szampion apple pomace using commercial pectinase (Pectinex Yield Mash, Pectinex Smash XXL, Pectinex XXL, Pectinex Ultra-SPL, Pectinex AFP L-4) was carried out (Oszmiański et al., 2011). The obtained purees were mixed with the apple juices and increased the TPC of juices in comparison to the raw juice.

Fermentation pretreatment have been reported for improving the phenolic extraction efficiency. The microbial enzymes, such as pectinase, cellulase, β -galactosidase, naringinase, α -rhamnosidase and hesperidinase could be beneficial in phenolic extraction by rupturing the cell walls to release the phenolics, or deglycosylation of phenolics into their corresponding aglycones (Huynh et al., 2018). In comparison to control, the higher TPC and antioxidant activities were observed after the solid-state fermentation pretreatment of apple peel using four isolated *Aspergillus* spp. (black rot fungi) (Gulsunoglu et al., 2020) have been reported. The natural fermentation (without inoculum) was also reported to pretreat the apple prior to phenolic extraction. Natural fermentation pretreatment resulted in a slight rupture of apple pomace tissue, observed by optical microscopy (Lohani & Muthukumarappan, 2016). On the other hand, Gulsunoglu et al. (2020) did not find any significant differences on antioxidant activities (CUPRAC and DPPH assays) during 7 days of incubation of naturally fermented apple peel, while significant increases were found in the peel fermented by *Aspergillus* spp., except *A. aculeatus* ZGM6 (not significant).

Ferrentino et al. (2018) compared freeze dried, fresh, and oven dried apple pomace and their effects on TPC and antioxidant activity (DPPH assay) of extracted phenolics by SFE. Generally, it was found that freeze drying pretreatment significantly enhanced the TPC and antioxidant activity, followed by oven drying and without drying (fresh). This can be explained as freeze drying pretreatment minimize the thermal degradation of phenolics in comparison to oven drying, leading to higher content of recoverable phenolics. Rana et al. (2015) investigated that the TPC of freeze dried pomace (5.78 mg GAE/g DW) was higher than that oven dried and sun dried pomace, after the extraction using 70% ethanol (60 °C, 30 min). The TFC correspondingly showed similar result with TPC.

It is important to notice that high temperature not only can effect positively by increasing the yield of phenolics, but also negatively due to higher chance of phenolic degradation (Perussello et al., 2017). A negative impact of drying process on phenolics content of apple was reported by

previous study. Prior to phenolic extraction using acetone 80%, Red Delicious apple pomace was blanched and subsequently dried in a cabinet drier at various temperatures, from 50 to 80 °C (Heras-Ramírez et al., 2012). In comparison to undried pomace, the TPC, TFC, and antioxidant activity (ABTS) significantly decreased ($p < 0.05$) after the drying process of blanched and unblanched apple pomace due to thermal degradation. The degradation of phenolics could be reduced by increasing the drying temperature, which also means reducing the drying time at the same time. However, blanching process could help maintaining the stability of remaining phenolics during the drying process. This phenomenon might be explained by the PPO deactivation through blanching (Heras-Ramírez et al., 2012).

It is noteworthy that smaller particle size could enhance the extraction process by promoting better mass transfer, which results in higher bioactive compounds release in the solvent, in a shorter time of extraction. Prior to extraction, most studies prepared the apple samples in powder form by grinding the samples after drying (Table 5). Grinding process not only increases the apple product's surface contact to the solvent, but also to homogenize the particle size from the pomace as different sizes and shapes of stem, seeds, peels were found in it (Perussello et al., 2017).

Conclusions and Future Works

Apple has been known as one of the most consumed fruits in the world and has been investigated by previous studies to be the source of functional ingredients, such as carbohydrates, fibers, minerals, vitamin C, vitamin B complex, and phenolics. The growing location, cultivars, and environmental factors affect the chemical composition of the fruit. Major phenolics found in apple are hydroxybenzoic acids, hydroxycinnamic acids, flavanols and their oligo- and polymeric structures (procyanidins and proanthocyanidins), flavonols, dihydrochalcones, and anthocyanins. More phenolics are generally found in higher concentration in the peel. Therefore, consuming unpeeled apple is recommended to increase daily intake of phenolics. In choosing the apple, it is also necessary to consider the bioaccessibility of antioxidants after the digestion. As different cultivars showed different phenolic compositions, through this review, the consumers may select the suitable apple cultivars that are available in their regions, and affordable, yet still have high nutritional value, in order to achieve wider scale of consumption to promote general health of consumers. In the future, the bioactive compounds composition and their bioaccessibility are still widely open to be explored as the hybridization of new apple cultivars is still developing in different regions.

The antioxidant activity of apple's phenolics majorly contributes to the health promoting impacts. Thus, the phenolic extraction should be conducted correctly to maximize its benefits. This paper reviewed the optimization of phenolic extraction conducted with different methods. The developed methods, including MAE, RFAE, UAE, SFE, PEF-assisted extraction, and PLE have been studied and optimized to shorten the extraction time, decrease the solvent consumption and waste, and increase the phenolic yield and antioxidant activity of apple extracts, in comparison to the conventional extraction. Concerning the food grade solvents, acetone and ethanol were used by several studies for phenolic extraction as opposed to methanol. Besides optimization of non-conventional extraction methods using other new apple cultivars, further studies can be conducted to characterize those extraction processes with kinetic modeling.

The extracted apple phenolics could be beneficial for various food product developments to improve their functional properties. However, some challenges may be found during the adaptation from scientific literatures to industrial scale, either in non-conventional extraction method of apple polyphenols or the development of functional food products based on apple phenolics. Both researchers and professionals in industries need to concern not only about the scale-up cost, but also the safety, effectiveness, and quality of the apple phenolics to be applied as food ingredients. The approval of Generally Recognized as Safe (GRAS) in the food regulations could be crucial to widely commercialize the apple phenolics, both as food ingredients or apple phenolic-based functional foods, which subsequently contributes to better general health in the population. To support the safety concern of apple phenolics, the strategies to limit the presence of harmful substances from apples, such as patulin and pesticide residues can be further studied. Furthermore, it is suggested by researchers and food industries in different countries to study, develop, and formulate the apple phenolics-based functional foods following the common healthy food types, or even healthy traditional foods, consumed in that regions, so that the consumer acceptability on those functional foods would be higher.

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Data Availability Not applicable.

Declarations

Competing Interests The authors declare no competing interests.

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Enzymatic browning in apple products and its inhibition treatments: A comprehensive review

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Abstract

Apple (*Malus domestica*) is widely consumed by consumers from various regions. It contains a high number of phenolic compounds (majorly hydroxybenzoic acids, hydroxycinnamic acids, flavanols, flavonols, dihydrochalcones, and anthocyanins) and antioxidant activity, which are beneficial for human health. The trends on healthy and fresh food have driven the food industry to produce minimally processed apple, such as fresh-cut, puree, juice, and so on without degrading the quality of products. Enzymatic browning is one of the problems found in minimally processed apple as it causes the undesirable dark color as well as the degradation of phenolics and antioxidant activity, which then reduces the health benefits of apple. Proper inhibition is needed to maintain the quality of minimally processed apple with minimal changes in sensory properties. This review summarizes the inhibition of enzymatic browning of apple products based on recent studies using the conventional and nonconventional processing, as well as using synthetic and natural antibrowning agents. Nonconventional processing and the use of natural antibrowning agents can be used as promising treatments to prevent enzymatic browning in minimally processed apple products. Combination of 2–3 treatments can improve the effective inhibition of enzymatic browning. Further studies, such on as other potential natural antibrowning agents and their mechanisms of action, should be conducted.

KEYWORDS

antibrowning, apple, enzymatic browning, nonconventional processing, peroxidase, polyphenol oxidase

1 | INTRODUCTION

Due to its delicious taste and excellent nutritional value, the apple (*Malus domestica*) has become one of the most widely consumed and cultivated fruits in the world.

Apple contains bioactive ingredients, especially phenolic compounds that include hydroxybenzoic acids, hydroxycinnamic acids, flavanols, flavonols, dihydrochalcones, and anthocyanins, which contribute to health benefits, such as antioxidative activity, anti-inflammatory

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activity, hypocholesterolemic effect, cardiovascular protective effect, antidiabetic activity, and anticancer activity (Patocka et al., 2020).

Recently, the growing interest in healthy food has driven consumers to consume fresh foods with high nutritional value, minimally processed, and free from synthetic additives (Wibowo et al., 2019). Enzymatic browning is one of the main problems in minimally processed foods, such as fresh-cut fruits (apple, peach, pear, banana, avocado, etc.) and vegetables (lettuce, potato, eggplant, etc.), fruit juices, and so on (Moon et al., 2020). It is caused by two vital enzymes, polyphenol oxidase (PPO) and peroxidase (POD) (Zhu et al., 2022). Around 50% of fresh fruit loss is caused by enzymatic browning; it not only creates undesirable brown color, but also reduces the nutritional quality and sensory properties of fruits (Moon et al., 2020). Therefore, researchers and food industries have recently been investigating the most suitable procedure to minimize the enzymatic browning in minimally processed fruits.

Previous studies reported on the methods to inhibit enzymatic browning, but they did not focus on apple, instead on various other fruits and vegetables (Moon et al., 2020; Tinello & Lante, 2018; Xu et al., 2020). A study also reviewed the enzymatic and nonenzymatic browning in various fruit juices (Zhu et al., 2022). Therefore, the present study aims to review recent studies using the conventional and nonconventional processing as well as using synthetic and natural antibrowning agents to prevent the enzymatic browning of apple products.

2 | ENZYMATIC BROWNING AND ITS ANALYSES

Enzymatic browning (Figure 1) is mainly caused by PPO (EC1.10.3.1 – diphenol oxidase or catechol oxidase; EC1.10.3.2 – laccase; EC1.14.18.1 – cresolase or tyrosinase) and POD (EC1.11.1.7) (Muñoz-Pina et al., 2022; Singh et al., 2018). PPO is an oxidoreductase enzyme containing copper (the active site) in its structure as the prosthetic group. In brief, the enzymatic browning of fruits, such as apple, is caused by the oxidation of apple's phenolics as the substrates (Sommano et al., 2020). The substrates, mostly phenolic compounds, are bound by the tonoplast (a lipoprotein membrane) in vacuole, while the enzymes (PPO and POD) are in the chloroplasts and cell walls. Once physical stress (cutting, peeling, etc.) is introduced to the apple, as well as aging stress, the cells create reactive oxygen species (ROS), which then ruptures the lipoprotein membrane. PPO and POD then interact with the released phenolic substrates. In the presence of oxygen, PPO catalyzes the hydroxylation of monophenols, such as phenol,

tyrosine, and *p*-cresol to *o*-diphenols (monophenol oxidase activity), and furthermore, *o*-diphenols, such as catechol, dopamine, and adrenaline, to *o*-quinones (diphenol oxidase activity) (Singh et al., 2018; Sommano et al., 2020). Phenolic compounds in apple, including chlorogenic acid, can be a good substrate of enzymatic browning (Tang et al., 2020). The *o*-quinones, which are already dark in color, subsequently undergo nonenzymatic secondary reactions by condensation to synthesize the brown color pigment, melanin (Singh et al., 2018).

POD is a heat-resistant oxidoreductase that catalyzes the oxidation of phenolic compounds in the presence of hydrogen peroxide (H_2O_2) to the final dark-pigment product, melanin (Sikora et al., 2020). It was reported that the oxidation of phenolic compounds in PPO-catalyzed reactions may synthesize the H_2O_2 , which then can increase the POD activity in fruits (Singh et al., 2018). POD causes not only undesirable changes in color and nutritional quality, but also texture and flavor, which is unacceptable to consumers. However, the contribution of POD to enzymatic browning is less than that of PPO since the presence of H_2O_2 in apple is low (Rodríguez-Arzuaga et al., 2019). The PPO and POD contents may vary in different fruits and cultivars depending on the ratio of bound and soluble enzymes (Singh et al., 2018).

The analyses used to measure the enzymatic browning activity of apple, including spectrophotometry, kinetic analysis, colorimetry, and sensory analysis, are shown in Table 1. Most of studies measured the PPO and POD activities in various apple products from various cultivars, such as fresh-cut (Kumar et al., 2018; Remorini et al., 2015), juice (Mannozi et al., 2019; Marszałek et al., 2018; Wibowo et al., 2019), puree (Sukhonthara et al., 2016; Sulaiman, Soo, Yoon, et al., 2015), and dried apples (Bußler et al., 2017). To analyze the PPO activity, the extracted PPO from apple was introduced to the substrate, commonly catechol (Tinello & Lante, 2018). However, pyrogallol could also be a substrate in PPO activity analysis of apple juice (Murtaza et al., 2018). Besides catechol and pyrogallol, a study used the other substrates to analyze the PPO activity of Mexican Golden Delicious juice, for example, 4-methylcatechol, caffeic acid, ferulic acid, chlorogenic acid, and L-3,4-dihydroxyphenylalanine (L-Dopa) (Marrufo-Hernández et al., 2017). It was found that Golden Delicious' PPO could only oxidize six out of seven analyzed substrates, with 4-methylcatechol as the substrate with the best specificity (measured as the activity at the highest absorbance/wavelength of each compound) and the second-best affinity (after pyrogallol, based on Michaelis–Menten kinetic parameter K_m). Ferulic acid could not be oxidized by Golden Delicious' PPO, which might be due to the lack of *o*-hydroxyl groups in the aromatic ring. Also, based on affinity, the authors found that

Selection of apple cultivars with lower content of browning enzymes

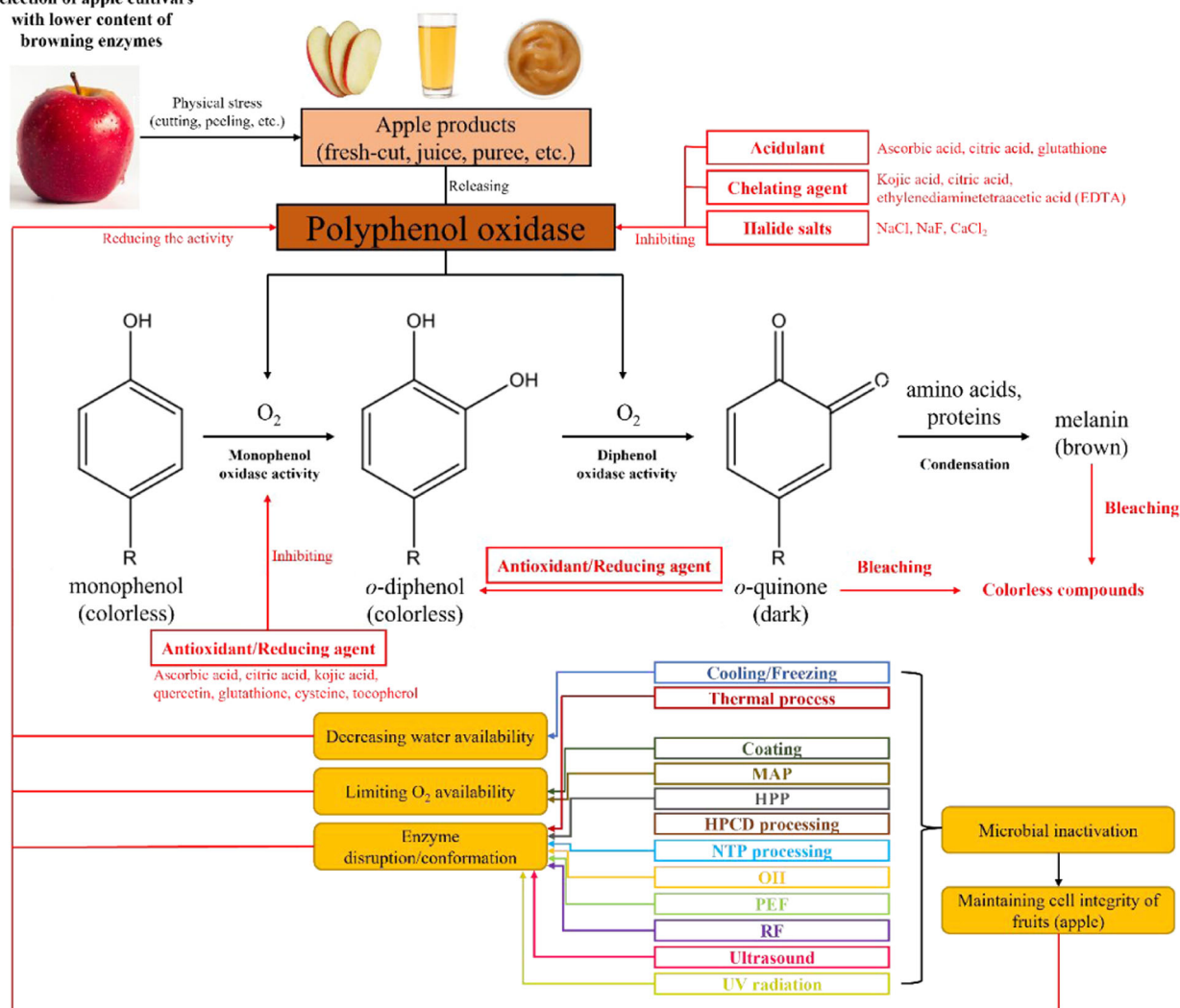


FIGURE 1 Enzymatic browning by polyphenol oxidase and its inhibition process in apple products. HPCD: high pressure carbon dioxide; HPP: high pressure processing; MAP: modified atmosphere packaging; NTP: Non-thermal plasma; OH: ohmic heating; PEF: pulsed-electric field; RF: radio frequency; UV: ultraviolet

PPO from different cultivars had different best substrates, which are chlorogenic acid for Red Delicious and Fuji, and 4-methylcatechol for Monroe, Bramley's seedling, and Red Fuji. This might indicate that each PPO type has the optimum substrate. It is worth noticing that, as mentioned before, PPO can be classified into three types based on their substrate and mechanism of oxidation: (a) tyrosinase that has the cresolase and catecholase activities; (b) catechol oxidase that catalyzes the oxidation from *o*-diphenols to *o*-quinones; and (c) laccase that can oxidize various aromatic compounds through a radical-catalyzed reactions mechanism (Taranto et al., 2017). Laccase has the ability to oxidize *o*- and *p*-diphenols to produce *o*- and *p*-quinones (Sae-leaw & Benjakul, 2019). Therefore, observing the optimum substrates of PPO from apples is suggested due to varied PPO types or phenolics composition in various apple cultivars.

For POD activity, the extracted POD is mixed with substrate, such as guaiacol (Rodríguez-Arzuaga et al., 2019), pyrogallol (Serra et al., 2021), *o*-phenylenediamine and *p*-phenylenediamine (Wibowo et al., 2019; Junjie Yi et al., 2017), and H₂O₂. Both PPO and POD activities are measured spectrophotometrically at 25°C between 398 (C. Chen et al., 2016) and 420 nm (Junjie Yi et al., 2017) for PPO activity, and 430 (Serra et al., 2021) to 485 nm (Wibowo et al., 2019) for POD activity. Other than PPO and POD activities, phenylalanine ammonia lyase (PAL; EC4.3.1.5) activity and copper chelating activity can also be measured. PAL is the key enzyme that enhances the biosynthesis of phenolics via the phenylpropanoid pathway when the stress (i.e. cutting) is introduced to fruits, like apple. The synthesized phenolics work as substrates of PPO and POD, and then enhance browning (Remorini

TABLE 1 Analyses used to measure the enzymatic browning activity of apple

Analyses	Equations	Expressed as	References
Spectrophotometry			
PPO activity	$U = \frac{\Delta A}{t}$	U/kg or U/g	(C. Chen et al., 2021)
	$\text{PPOresidualactivity} = \frac{\Delta A_{\text{treatment}}}{\Delta A_{\text{control}}} \times 100\%$	% PPO residual activity	(Junjie Yi et al., 2017)
	$\text{PPOinhibition} = \frac{\Delta A_{\text{control}} - \Delta A_{\text{treatment}}}{\Delta A_{\text{control}}} \times 100\%$	% PPO inhibition	(Tinello et al., 2018)
POD activity	$U = \frac{\Delta A}{t}$	U/kg or U/g	(Serra et al., 2021)
	$\text{PODresidualactivity} = \frac{\Delta A_{\text{treatment}}}{\Delta A_{\text{control}}} \times 100\%$	% POD residual activity	(Junjie Yi et al., 2017)
	$\text{PODinhibition} = \frac{\Delta A_{\text{control}} - \Delta A_{\text{treatment}}}{\Delta A} \times 100\%$	% POD inhibition	(Bußler et al., 2017)
PAL activity	$U = \frac{\Delta A}{t}$	U/kg or U/g	(C. Chen et al., 2021; Gao et al., 2018)
Copper chelating activity	$\text{Copperchelatingactivity} = \frac{\Delta A_{\text{control}} - \Delta A_{\text{treatment}}}{\Delta A_{\text{control}}} \times 100\%$	% copper chelating activity	(B. Lee et al., 2016)
BI	$\text{BI} = A_{\text{at}420\text{nm}}$	A at 420 nm (no unit)	(Remorini et al., 2015)
Kinetic analysis			
<i>k</i> -value (PPO and POD)		<i>k</i> -value	(Marszałek et al., 2017)
<i>D</i> -value (PPO and POD)	$D = \frac{2.303}{k}$	<i>D</i> -value	(Marszałek et al., 2017)
Colorimetry			
ΔE	$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$	No unit	(Szczepańska et al., 2021)
BI	$\text{BI} = 100 \times \frac{x-0.31}{0.172}$, where $x = \frac{a+1.75L}{5.645L+a-3.012b}$	No unit	(Szczepańska et al., 2021)
<i>C</i>	$C = \sqrt{a^2 + b^2}$	No unit	(Szczepańska et al., 2021)
<i>h</i>	$h = \arctan \frac{b}{a}$	°	(Szczepańska et al., 2021)
WI	$\text{WI} = L - 3b$ or $\text{WI} = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}$	No unit	(C. Chen et al., 2016; Kumar et al., 2018)
YI	$\text{YI} = 142.86 \times \frac{b}{L}$	No unit	(Klimczak & Gliszczyńska-Świątło, 2017)
Sensory analysis			
Appearance score	Average of score; score 1–9 (bad-very good); trained panelists	No unit	(Rodríguez-Arzuaga et al., 2021)
Browning score	Average of score; score 1–9 (slight-severe); trained panelists	No unit	(Rodríguez-Arzuaga et al., 2021)

Note: A: absorbance at certain wavelength (398–420 nm for PPO activity; 430–485 nm for POD activity; 290 nm for PAL activity; 632 nm for copper chelating activity); *a*-value: redness/greenness; *b*-value: yellowness/blueness; BI: browning index; *C*: chroma value; *D*-value: treatment time needed to inactivate 90% of enzyme at a given pressure and temperature; *h*: hue angle; *k*-value: inactivation rate constant; *L*-value: lightness/darkness; PPO: polyphenol oxidase; POD: peroxidase; *t*: time (minute); U: unit of enzyme (absorbance difference per minute); WI: whiteness index; YI: yellowness index, ΔE : total color difference.

et al., 2015). PAL activity is measured at 290 nm (Gao et al., 2018). Copper chelating activity can be determined using pyrocatechol violet as metal-chelating indicator, and measured spectrophotometrically at 632 nm (B. Lee et al., 2016). Sometimes, total phenolic content (TPC) and antioxidant activity (e.g., 2,2-diphenyl-1-picrylhydrazyl [DPPH] assay) could be also conducted to support the data (Rasouli & Saba, 2018), but it is worth noticing that phenolics could serve either as PPO substrates or inhibitors (Serra et al., 2021). Furthermore, the inactivation rate (*k*-value) of PPO and POD following conventional first-order equation and

the time needed to inactivate 90% PPO and POD at certain temperature and pressure (*D*-value) were also analyzed by several studies (Manzocco et al., 2017; Marszałek et al., 2017).

Browning index (BI) can be measured by spectrophotometry or colorimetry (Table 1). It is worth noticing that BI is more specific to brown color than total color difference (ΔE), as ΔE does not indicate the direction of color change (Klimczak & Gliszczyńska-Świątło, 2017). The ΔE can also be affected by other color changes, for example, the color from antibrowning agent after

treating the apples. Generally, the higher enzymatic browning activity is confirmed by higher ΔE . However, the ΔE is not always in accordance with enzymatic browning activity. For example, fresh-cut apple dipped in unripe grape juice had lower PPO inhibition (higher PPO activity) than that in sodium metabisulfite, but the apple dipped in unripe grape juice showed lower ΔE due to the whitening effect of the juice (Tinello et al., 2018). Furthermore, Tao et al. (2019) observed an increased b -value (yellowness) after treating the fresh-cut apple with 10 $\mu\text{mol/L}$ curcumin solution, that might increase the ΔE or BI of apple, while actually curcumin reduced the PPO and POD activities even at lower concentration (2 $\mu\text{mol/L}$).

Colorimetry, especially ΔE and BI (by spectrometry and colorimetry), may not be fully intended to measure the enzymatic browning in apple, because nonenzymatic browning that is influenced by heat treatment, such as Maillard reaction or caramelization, may also enhance the brown color of treated apples (Bochnak & Świeca, 2020; Tian, Wang, et al., 2018). However, colorimetry can be considered as a valid, indirect method to estimate inhibition of PPO activity of fruits (Lante et al., 2016). Furthermore, the sensory analysis with appearance and browning scores (Rodríguez-Arzuaga et al., 2021) or without score (only fresh-like appearance by visual observation) (Jin et al., 2020) conducted by trained panelists were also performed to check the enzymatic browning of apple. However, there are some limitations if the sensory evaluation is used to analyze the enzymatic browning of apple: (i) it does not specifically measure the browning changes caused by enzymes and (ii) casual panelist can notice the color difference when ΔE is higher than 2 to 3.5, although trained panelist can differentiate color if ΔE is lower than 2 (Illera et al., 2018). Therefore, it is suggested to measure enzymatic activities (e.g., PPO and POD activities) as primary analyses, confirmed by colorimetry, sensory analysis, TPC, and antioxidant activity as secondary analyses.

3 | INHIBITION OF ENZYMATIC BROWNING

The methods to inhibit the enzymatic browning in apple products (Figure 1) are divided into selection of apple cultivars, conventional processing, treatment with antibrowning agents and natural products, nonconventional processing, and combination of treatments.

3.1 | Selection of apple cultivars

Each cultivar of apple was studied to have different enzymatic browning activities; each also responded differ-

ently after the enzymatic browning inhibition treatment (Table 2). Without inhibition treatment, the plant itself has self-defense against enzymatic browning by producing natural antioxidants (Sommano et al., 2020). Vitamin C is one of the antioxidants that removes free radicals, including ROS, thus decreasing oxidative stress (Arnold et al., 2021) and inhibits browning reaction (Sommano et al., 2020). The other possible mechanism of vitamin C to block enzymatic browning is by the reduction of o -quinones (the product from PPO) back to the phenolic substrates (M. Lee et al., 2022). Besides vitamin C, phenolic compounds from apple could modify the enzymatic activity, whether as inhibitors or substrates; lower molecular weight phenolics and smaller monomeric compounds (i.e., catechin, p -coumaric acid, chlorogenic acid) serve as the more efficient substrate of PPO (Serra et al., 2021). This was in accordance with the findings of the authors, as Granny Smith and Cripps Pink apples that contain the lowest level of catechin, p -coumaric acid, and chlorogenic acid, browned the least. Polyphenols, such as catechin, epicatechin, and chlorogenic acid, were also studied as good substrates for PPO (Tang et al., 2020). As inhibitors, polyphenols could (a) interact with active sites of PPO due to similar structure to substrates, (b) interact with enzymes via hydrogen bonds or hydrophobic interaction, and (c) donate the electron to intermediate quinone, thus terminating further oxidation (Sae-leaw & Benjakul, 2019). As each apple cultivar has different chemical (including phenolic) compositions, this might be the possible reason for different enzymatic browning activities, both before and after inhibition treatments. Furthermore, genetic relation between apple cultivars corresponding to the enzymatic genotype-specific activity of PPO might also affect the difference or similarity of PPO activity between cultivars or even within *Rosaceae* family (e.g., apple and pear) (Putnik et al., 2017).

Besides the difference of phenolics composition of different cultivars, the difference in physicochemical characteristics may affect the effectiveness of browning inhibition treatment. A study found that porosity of various apple cultivars might influence the cold plasma penetration within the empty spaces in the tissue to inhibit browning (Tappi et al., 2019). In another study (Remorini et al., 2015), Granny Smith slices, with lower PPO activity than Red Delicious, need the combination of ClO_2 + ascorbic acid treatment to prevent the browning during 96 h of storage, because when it was only treated with ClO_2 , the PPO activity increased significantly. While in Red Delicious, there was no difference between PPO activity of ClO_2 + ascorbic acid treatment and ClO_2 treatment, as both treatments could successfully inhibit the enzymatic browning during 96 h. Therefore, the cultivar selection is really important to be considered by the industries that apply specific

TABLE 2 The effect of apple cultivars on enzymatic browning of apple products

Products	Cultivars	Main findings	References
Fresh-cut	Gala, Golden Delicious	BI: Golden Delicious < Gala	(Jancikova et al., 2021)
Fresh-cut	Braeburn, Cripps Pink, Delorina, Durello, Fiesta, Florina, Fuji, Gloster, Golden Delicious, Granny Smith, Jonathan, Mondial, Gala, Red Rome, Topaz	Granny Smith and Cripps Pink browned minimally; Fiesta and Mondial Gala browned severely based on PPO and POD activities, ΔE , and ΔC	(Serra et al., 2021)
Fresh-cut	Golden Delicious, Jonagold	Biofortification using lower amount of selenium (0.075 kg/ha.m canopy height) reduced the PPO activity of both cultivars. PPO activity of Jonagold was lower than that of Golden Delicious.	(Groth et al., 2020)
Fresh-cut	Elstar, Golden Delicious	Different cultivars showed different ΔE , PPO, and POD activities (after thermal and dipping treatment)	(Shrestha et al., 2020)
Fresh-cut	Fuji (Type P and Type T)	Browning was not related to PPO activity	(Tang et al., 2020)
		Hyperoside, POD, SOD, H_2O_2 , ascorbic acid, and expression of 9 genes were higher in type P	
Fresh-cut	Fuji, Modi, Pink Lady, Red Delicious	PPO activity: Modi < Pink Lady = Red Delicious < Fuji	(Tappi et al., 2019)
Fresh-cut	Caricia, Eva, Princesa	Each cultivar responded differently toward mild heat treatment (based on PPO and POD activities)	(Rodríguez-Arzuaga et al., 2019)
Fresh-cut	Golden Delicious (organic and conventional)	PPO activity, <i>L</i> -value, and hue angle were similar	(Neri et al., 2019)
Fresh-cut	Bay 4152, Gala Mitslugla, Gravin Goldach, Sonnen Glanz	PPO activity was not affected by color of flesh	(Hutabarat & Halbwirth, 2019)
		Each cultivar had different PPO and POD activities	
Flesh	Delbard Estival, Kohanz	PPO activity of Kohanz was still similar to control 3 weeks after spraying with chelated and nano-chelated Zn	(Rasouli & Saba, 2018)
Fresh-cut	Cripps Pink, Golden Delicious	ΔE : Golden Delicious < Cripps Pink	(Putnik et al., 2017)
Fresh-cut	Granny Smith, Jonagold, Pink Lady	PPO activity: Granny Smith < Jonagold < Pink Lady (after HPP); no difference (after thermal)	(Junjie Yi et al., 2017)
		POD activity: Jonagold < Granny Smith < Pink Lady (after HPP); no difference (after thermal)	
		ΔE : Granny Smith < Pink Lady < Jonagold (after HPP); Pink Lady < Jonagold < Granny Smith (after thermal)	
Fresh-cut	Fuji, Golden Delicious, Granny Smith	Golden Delicious and Fuji were more responsive to UV-A-LED treatment (stronger antibrowning effect) than Granny Smith	(Lante et al., 2016)
Fresh-cut	Granny Smith, Red Delicious	Granny Smith was less sensitive to browning (had lower BI, PPO, and POD activities) than Red Delicious (after dipping with ClO_2 + ascorbic acid)	(Remorini et al., 2015)
Puree	Royal Gala, Scirose	Different cultivars showed different PPO activity at different temperatures and time of HPP treatments	(Sulaiman, Soo, Yoon, et al., 2015)
Pulp	Aori27, Elstar, Fuji, Mellow	Aori21 showed the lowest ΔE and PPO activity	(Holderbaum et al., 2010)

Abbreviations: BI: browning index; C: chroma value; HPP: high pressure processing; LED: light-emitting diodes; PPO: polyphenol oxidase; POD: peroxidase; SOD: superoxide dismutase; UV-A: ultraviolet A; ΔE : total color difference.

technology or treatment not only to focus on prevention of the enzymatic browning of apple products, but also on economic benefits.

A study measured the PPO, POD activities, ΔE , and ΔC of fresh-cuts of 14 Italian apple cultivars (Serra et al., 2021). Among all cultivars, Granny Smith and Cripps Pink, which had the lowest content of catechin, *p*-coumaric, and chlorogenic acids (PPO substrates), showed lower PPO and POD activities during 24 h after cutting, supported by low ΔE and ΔC . Fiesta and Mondial Gala were two cultivars which were more prone to enzymatic browning according to those analyses. The authors also analyzed the phenolic compositions of each cultivar. Topaz and Red Rome cultivars were concerned in this study. Topaz possessed the highest content of proanthocyanidins (0.45 mg/g FW) and high quantified phenolic concentration (0.63 mg/g FW), which confirmed that Topaz showed lower PPO and POD activities than other cultivars. However, higher ΔE and ΔC than other cultivars were observed in Topaz, which might be due to alternative oxidation pathways. While for Red Rome, although it exhibited the highest chlorogenic acid content and high quantified phenolic concentration, it browned minimally (low ΔE and ΔC , moderate PPO and POD activity), which might be due to its less maturity than others (Serra et al., 2021). Maturity, selection of cultivars, and preharvest condition (climate, irrigation, rain conditions) may affect the quality and PPO activity of fresh-cut fruits and vegetables (Nogales-Delgado, 2021). However, a study found that the maturity did not significantly affect the PPO activity of Elstar, Fuji, and Mellow pulps, but PPO activity decreased significantly along with the maturity (ripeness) of Aori27 pulp, supported by decreased ΔE (after 24 h of storage) and TPC, compared to the immature and half-mature Aori27 pulp (Holderbaum et al., 2010). Another research studied the effect of preharvest spraying (three to four weeks before harvesting) of chelated and nano-chelated Zn to the trees of two apple cultivars, Delbard Estival and Kohanz, on enzymatic browning of the flesh of fruits (Rasouli & Saba, 2018). The assessment was conducted at spray time, three or four weeks after spraying, and three weeks of storage (4°C, RH 95%). Both treatments successfully decreased PPO activity, BI, and ΔE of both cultivars with increased ascorbic acid, total antioxidant activity (DPPH), and superoxide dismutase (SOD). The difference between both cultivars was found in the three weeks after spraying, as the PPO activity of Zn-coated Kohanz was still similar to control.

As shown in Table 2, different apple cultivars resulted in different responses after the enzymatic browning inhibition treatments, such as UV-A-treatment (Lante et al., 2016), dipping treatment with ascorbic acid and ClO_2 solution (Remorini et al., 2015), high pressure processing (HPP) (Sulaiman, Soo, Yoon, et al., 2015; Junjie Yi et al., 2017),

and modified atmosphere (Putnik et al., 2017) and thermal treatment (Junjie Yi et al., 2017). Compared to other cultivars, Granny Smith was more stable against browning after the dipping treatment (100 mg/L ClO_2 + 3% ascorbic acid, 4°C, 96 h of storage) and HPP (600 MPa, 3 min) (Junjie Yi et al., 2017). In comparison to Pink Lady and Jonagold, Granny Smith showed lower ΔE after HPP and thermally treated (85°C, 5 min), but no difference was found in PPO and POD activities between those three cultivars after thermal treatment, manifesting that increased ΔE in Granny Smith was caused by nonenzymatic browning like Maillard reaction or alternative oxidative reaction (e.g., degradation of carotenoid and unsaturated fatty acids) (Junjie Yi et al., 2017). However, another study reported that Granny Smith is less responsive to UV-A-LED treatment (weaker antibrowning effect) than Golden Delicious and Fuji (Lante et al., 2016). Golden Delicious appeared as the preferable cultivar against browning, both without treatment (Jancikova et al., 2021; Putnik et al., 2017) and after enzymatic browning inhibition treatment (Lante et al., 2016). Additionally, a study compared the PPO activity and color of organic and conventional Golden Delicious fresh-cut from Italy (Neri et al., 2019). It was found that although the TPC was 30% higher in organic apples, there was no difference in PPO activity and color of fresh-cut between both apples.

Tang et al. (2020) compared two Fuji apple types, type P (flushed-skin) and type T (striped-skin). The authors concluded that type P had slower browning rate than type T. Interestingly, they found that PPO activity, TPC, and expression of 5 genes did not change during 2 h after cutting in both types. Type P apple has higher levels of hyperoside (quercetin-3-galactoside), POD, SOD, and H_2O_2 than type T. This indicated that browning was not related to PPO activity and TPC, but higher hyperoside level in type P might delay the enzymatic browning.

It is also worth noticing that the cultivar selection in inhibiting the enzymatic browning is not only important in apple products, but also in other fruits and vegetables. For examples, eggplant that is affected by the difference of phenolic content, initial PPO specific activity and isoforms, the so-called PPO1 that is more responsible for cresolase and catecholase activities and PPO2 for catecholase activity but lack cresolase activity (Mishra et al., 2013); litchi's pericarp, due to variations in bioactivity (Mishra et al., 2012); pear, due to the genetic variations that affect the fruit composition that is then related to variance in enzymes activity and bioactive constituents (Saba & Moradi, 2016); rambutan's spinterns and peel (Yingsanga et al., 2008); potato tubers, due to difference in lipid metabolism, amino acid metabolism, signal transduction-related metabolites, for example, jasmonic acid, glutamate, and gibberellins, as well as anti-browning compounds, such as L-aspartic

acid, L-glutamate, and kynurenic acid (Qiao et al., 2022).

3.2 | Conventional processing

Heating and cooling are important to control the enzymatic browning of apple. Thermal processing, such as blanching and pasteurization, could be beneficial to inhibit enzymatic browning of fruits via denaturation of protein, which is the base component of enzymes (Moon et al., 2020). However, high temperature and long heating treatment may destroy the nutritional compounds, such as vitamins, and affect the texture or aroma of fruits, which may not be acceptable to consumers (Singh et al., 2018). Table 3 presents the thermal processing and its effect on enzymatic browning of apple fresh-cut, flesh, juice, and puree. Due to the advantages and drawbacks of thermal processing mentioned above, thermal processing was conducted mostly as the comparison method of non-conventional processing and in combination with other treatments (see section 3.4 and 3.5, respectively).

PPO activity in fresh-cut products could be effectively inhibited at temperatures from 50–80°C (Nogales-Delgado, 2021). Mild heat treatment at 40–50°C for 20–90 min was carried out to inhibit the enzymatic browning of Caricia, Eva, and Princesa fresh-cut apples (Rodríguez-Arzuaga et al., 2019). It was highlighted that mild heat treatment increased the TPC of Eva apple by 70% after 7 days of storage at 2°C, and accordingly inhibited 96% PPO activity (POD activity decreased only on day 0, while on day 7, >100% POD residual activity was observed), but decreased the firmness. However, different results were found in Caricia and Princesa, as mild heat treatment did not reduce the PPO activity (PPO activity >100%) of both cultivars, and POD activity of Princesa. The authors explained that the difference in the phenolic composition of those three cultivars might result in the different responses to heat treatment. However, the phenolic composition was not conducted in this study to confirm the possible reasons. As mentioned in section 3.1, phenolics can act as substrates or inhibitors of browning enzymes. Besides the phenolic composition, the effect of mild heat treatment on the browning enzymes (e.g., PPO, POD, PAL) of each cultivar might differ (Rodríguez-Arzuaga et al., 2019). The response of PAL to the mild heat treatment, for example, which is the enzyme related to phenolic metabolism, may be induced differently in each cultivar. A study obtained different responses to thermal treatment in phenolic compounds of New Zealand-grown Mahana Red apples, the genetic mutation of Braeburn (Aguayo et al., 2015). While the quercetin and phloridzin contents increased, the *p*-coumaric and procyanidins con-

tents decreased. This phenomenon was affected by the differences in phenolic metabolism.

Another study applied heat treatment from 30–80°C for 10–60 min to apple juice (Murtaza et al., 2018). The PPO activity was analyzed using two different substrates: catechol and pyrogallol. It was found that PPO activity was inhibited at 80°C. The reduced PPO activity of juice (42–0.95% in catechol; 72–15% in pyrogallol) was observed when the juice was heated at 80°C for 10–60 min. Interestingly, PPO activity was increased to the highest level when the juice was heated at 50°C and 60°C along with longer time when using catechol and pyrogallol, respectively. This indicated that mild heat treatment in apple might also enhance the enzymatic browning. However, if too high a temperature was applied to apple, this might affect the browning through nonenzymatic browning. A study conventionally heated apple puree at 107°C, which resulted in lower L-value (higher darkness) (Rinaldi et al., 2021), although enzymatic browning might be inactivated.

Illera et al. (2018) measured the PPO activity and its kinetic model of Golden Delicious cloudy juice after mild heating treatment (35–45°C) at atmospheric pressure for up to 120 min. The maximum inhibition of PPO activity (40% inhibition) was found after 2 h of heating at 45°C. It was also found that with increasing temperature, the inactivation rate constant (*k*-value) increased from 0.00102 to 0.00194 per min, and the treatment time needed to inactivate 90% of enzyme (D-value) decreased from 980 to 515 min.

Storing apple at low temperature could also help inhibiting enzymatic browning. Some studies combined some treatments with cold storage at 10–2°C, and prevented the enzymatic browning of apple products up to 42 days, depending on the treatment (Kumar & Sethi, 2021; Rodríguez-Arzuaga et al., 2021). Moreover, freezing could also prevent the enzymatic browning as it decreases the water available for taking part in enzymatic activity, and thus reduces the browning (Singh et al., 2018). A study stored the Golden Delicious fresh-cuts at –40°C for 300 days after dipping or vacuum impregnation treatment using lemon juice (Neri et al., 2019). The frozen storage reduced the hue angle of frozen apple fresh-cuts by up to 8%. However, freezing could decrease the firmness of apple up to 58%. It is worth noticing that the thawing process could also activate the PPO in plant tissue of apples, which further leads to browning (Neri et al., 2019).

3.3 | Treatment with antibrowning agents and natural products

Various antibrowning agents have been studied for enzymatic browning inhibition in apple products. In liquid

TABLE 3 The inhibitory effect of thermal processing and synthetic antibrowning agents on enzymatic browning of apple products

Products	Cultivars	Treatment	Main findings	References
Thermal processing				
Puree	-	Thermal (1700 L/h, 107°C, 40 s)	↓L-value	(Rinaldi et al., 2021)
Fresh-cut	Fuji	Thermal (hot water blanching, 85°C, 30–120 s)	↓PPO activity	(X. Zhang et al., 2020)
Fresh-cut	Caricia, Eva, Princesa	Thermal (40–50°C, 20–90 min)	↓PPO activity (Eva)	(Rodríguez-Arzuaga et al., 2019)
			↑PPO activity (Caricia, Princesa)	
			↓POD activity (Caricia, Eva)	
			↑POD activity (Princesa)	
Cloudy juice	Mixture of Belgian apple cultivars	Thermal (pasteurization, 72°C, 15 s or 85°C, 30 s)	↓PPO activity	(Wibowo et al., 2019)
			↓POD activity	
			↑L-value	
Juice	Elstar	Thermal (90°C, 60 s)	↓PPO activity	(Abdelmaksoud et al., 2018)
Cloudy juice	Golden Delicious	Thermal (35–45°C, 120 min)	↓PPO activity	(Illera et al., 2018)
			↑k-value (PPO)	
			↓D-value (PPO)	
Juice	-	Thermal (30–80°C, 10–60 min)	↓PPO activity (80°C, 10–60 min)	(Murtaza et al., 2018)
			↑PPO activity (50–60°C, 10–60 min)	
Flesh and juice	Red Fuji	Thermal (60–70°C, 5–20 min)	↓PPO activity	(Tian, Wang, et al., 2018)
Flesh and juice	Red Fuji	Thermal (65–75°C, 5–20 min)	↓BI	(Tian, Yan, et al., 2018)
Clarified juice	Starking Delicious	Thermal (70–80°C, 15–120 s)	↓PPO activity	(Akgün & Ünlütürk, 2017)
Juice	Granny Smith, Jonagold, Pink Lady	Thermal (85°C, 5 min)	↓PPO activity	(Junjie Yi et al., 2017)
			↓POD activity	
Puree	Royal Gala	Thermal (65–75°C, up to 25 min)	↓PPO activity	(Sulaiman, Soo, Farid, et al., 2015)
Synthetic antibrowning agents				
Fresh-cut	Granny Smith	Spread by oil in water nanoemulsion incorporated with quercetin + quercetin-3-rhamnoside + ascorbic acid + α -tocopherol (each 100 μ M)	↓PPO activity	(Mendoza-Wilson et al., 2022)
			↓ Δ L-value	

(Continues)

TABLE 3 (Continued)

Products	Cultivars	Treatment	Main findings	References
Cloudy juice	Golden Delicious	Addition of 1.12–2.25 mM compound 11 (tetraazahydroxypyridinone derivative)	↓ΔE	(Muñoz-Pina et al., 2022)
Fresh-cut	-	Dipped in 0.5 mM compound 5d and 5f (gallic acid-benzylidenehydrazine hybrids) (5 min)	↓ΔE	(Peng et al., 2022)
			↑L-value	
Fresh-cut	Red Fuji	Exposed to H ₂ S gas from 0.7 mM NaHS solution (24 h)	↓PPO activity	(C. Chen et al., 2021)
			↓POD activity	
			↓PAL activity	
Fresh-cut	Fuji	Dipped in 15 g/L citric acid or 20 g/L GABA (10 min)	↓BI (GABA)	(Zhao et al., 2021)
			↔BI (citric acid)	
Flesh	Red Delicious	Extracted PPO was added with 0.40 g/L ascorbic acid	↓PPO activity	(Villamil-Galindo et al., 2020)
Fresh-cut	-	Dipped in 1% gallotannin or 1% ascorbic acid (2 min)	↓PPO activity	(Adeyemi et al., 2019)
			↔POD activity	
			↓BI	
Whole fruit	Granny Smith	Extracted and purified mPPO and sPPO were mixed with 0.1–10 mM ascorbic acid, glutathione, L-cysteine, EDTA, citric acid, NaCl, KI	↓mPPO, sPPO activities (ascorbic acid, glutathione, L-cysteine, EDTA, citric acid)	(Han et al., 2019)
			No inhibition detected on mPPO (KI)	
			No inhibition detected on sPPO (NaCl, KI)	
Fresh-cut	Braeburn	Dipped in 10 g/L ascorbic acid (8 min)	↓BI	(Martínez-Hernández, Castillejo, et al., 2019)
Fresh-cut	Fuji	Dipped in 0.5 mg/mL compound 11 solution (hydroxypyridinone derivative from kojic acid) (room temperature, 60 s)	↓BI	(Shao et al., 2018)
Fresh-cut	Golden Delicious	Dipped in a solution of: a) 1% (w/v) ascorbic acid and 1% (w/v) citric acid, b) 6% (w/v) NatureSeal®, c) 0.1% (w/v) sodium metabisulfite (25°C, 15 min)	↓PPO activity	(Tinello et al., 2018)
			↓ΔE	
Cloudy juice	Golden Delicious	Addition of 0.3 g/L ascorbic acid	↓PPO activity	(Klimczak & Gliszczynska-Świgło, 2017)

(Continues)

TABLE 3 (Continued)

Products	Cultivars	Treatment	Main findings	References
Fresh-cut	Granny Smith	Dipped in 0.01–0.1 mol/L halide salts (4°C, 5 min)	↓PPO activity	(Li et al., 2015)
Fresh-cut	Granny Smith, Red Delicious	Dipped in 100 mg/L of ClO ₂ + 3% ascorbic acid (2 min)	↓PPO activity	(Remorini et al., 2015)
			↓POD activity	
			↓BI	
Fresh-cut	Fuji	Dipped in 0.5% ascorbic acid (5 min)	↓ΔE	(Liang et al., 2014)
Juice	Fuji	Addition of 0.005–10 g/L whey protein isolate, or ascorbic acid, L-cysteine	↓PPO activity	(Jianhua Yi & Ding, 2014)
			↑L-value	

Abbreviations: BI: browning index; D-value: treatment time needed to inactivate 90% of enzyme at a given pressure and temperature; EDTA: ethylenediaminetetraacetic acid; GABA: γ -aminobutyric acid; k-value: inactivation rate constant; L-value: lightness/darkness; mPPO: membrane-bound polyphenol oxidase; PAL: phenylalanine ammonia lyase; PPO: polyphenol oxidase; POD: peroxidase; sPPO: soluble polyphenol oxidase; ΔE: total color difference; ↑: higher than control (increased); ↓: lower than control (decreased); ↔: not significantly different from control.

form (e.g., juice), the antibrowning agents could be added directly, while in solid form (e.g., fresh-cut), dipping and coating the apple with antibrowning solution, or by exposing the apple with the gas from antibrowning solution. As the demand for using natural products for obtaining safer, healthier foods is growing, some of the antibrowning agents from natural sources were also studied.

3.3.1 | Synthetic antibrowning agents

Besides thermal processing, Table 3 also presents various publications that applied exogenous synthetic antibrowning agents in apple products, which could act as antioxidants or reducing agents (e.g., ascorbic acid, citric acid, kojic acid, quercetin, glutathione, cysteine, tocopherol), acidulant (e.g., ascorbic acid, citric acid, glutathione), chelating agents (kojic acid, citric acid, ethylenediaminetetraacetic acid (EDTA)), and halide salts (e.g., NaCl, NaF, CaCl₂). The safety concerns of those synthetic antibrowning agents are summarized in Table 4.

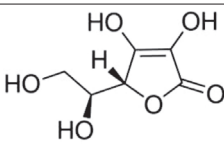
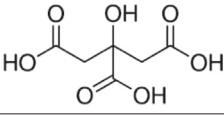
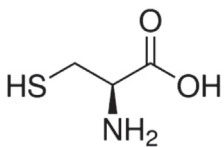
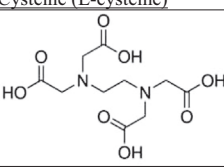
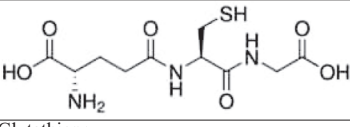
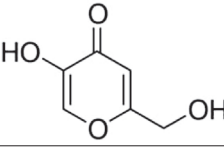
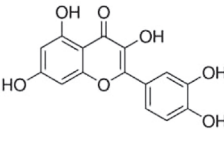
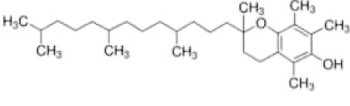
The antioxidants could help inhibiting browning by reacting with oxygen, as well as with intermediate products, thereby breaking the chain reaction and suppressing melanin formation (Moon et al., 2020). A study applied the oil-in-water nanoemulsion incorporated with four antioxidants together—quercetin, quercetin-3-rhamnoside, ascorbic acid, and α -tocopherol (each 100 μ M)—to Granny Smith fresh-cut (Mendoza-Wilson et al., 2022). This composition inhibited 41% PPO activity, and inhibited browning (based on ΔL -value) between 74% to 81% during 6 h, better than while using only two or three antioxidants. Quercetin inhibited the PPO activity through a competitive or noncompetitive mechanism, which showed synergistic effect with other antioxidants,

including vitamins C and E. Quercetin-3-rhamnoside, as a selective and competitive inhibitor, interacted with amino acids that are part of the PPO active site and could form the complex with PPO (including with His108 that coordinates one of copper atoms of PPO), which were predominated by hydrogen bonds. It was also found that all antioxidants reduced the *o*-quinones generated from various substrates. Besides vitamins C and E, recently, researchers also studied the antibrowning effect of riboflavin (vitamin B₂), which works as antioxidant (Hyun et al., 2022; Zha et al., 2022).

Han et al. (2019) analyzed the inhibition of membrane and soluble PPOs (mPPO and sPPO) using reducing agents (ascorbic acid, glutathione, and L-cysteine), chelators (EDTA and citric acid), and halides (NaCl and KI). Among all the inhibitors, all the reducing agents showed better mPPO and sPPO inhibition than chelators and halides. Glutathione appeared to be the best inhibitor of sPPO with the lowest IC₅₀ (0.48 mM); while for mPPO, a similar IC₅₀ was found between reducing agents. Glutathione and L-cysteine can reduce the *o*-quinones to colorless diphenols or by irreversible reaction with catechol to prevent browning complexes from forming. EDTA and citric acid were effective in inhibiting mPPO and sPPO at higher concentrations (more than 44 mM) by chelating and removing copper atoms from enzyme molecules. Ascorbic acid and citric acid are also acidulants (Moon et al., 2020), which reduce the pH; (to be noted is that most PPOs were optimum in pH range of 6.0–7.0 (Zhou et al., 2020)).

Han et al. (2019) also found that NaCl and KI did not show any inhibitory effect against sPPO even at a concentration of 100 mM. The inhibitory effects of other halide salts against enzymatic browning were carried out by dipping Granny Smith fresh-cuts into the various halide salt

TABLE 4 The synthetic antibrowning agents used to inhibit enzymatic browning in apple products and their safety regulations

Anti-browning agents	Type based on mechanism	Safety regulation	References
 Ascorbic acid	Reducing agent, acidulant	GRAS substance under 21 CFR 182.3013. Based on EC 1333/2008, it is registered as E300, with maximum limit in food is quantum satis ^a .	(Code of Federal Regulations, 2022; European Commission, 2008; M. Lee et al., 2022; Moon et al., 2020)
$\text{Cl}-\text{Ca}-\text{Cl}$ Calcium chloride (CaCl_2)	Halide salt	GRAS substance under 21 CFR 184.1193. Based on EC 1333/2008, it is registered as E509, with maximum limit in food is quantum satis.	(Code of Federal Regulations, 2022; European Commission, 2008; Li et al., 2015)
 Citric acid	Reducing agent, acidulant, chelating agent, bleaching agent	GRAS substance under 21 CFR 184.1033. Based on EC 1333/2008, it is registered as E330, with maximum limit in food is quantum satis.	(Azevedo et al., 2018; Code of Federal Regulations, 2022; European Commission, 2008; Han et al., 2019)
 Cysteine (L-cysteine)	Reducing agent	GRAS substance under 21 CFR 184.1271. Based on EC 1333/2008, it is registered as E920, with maximum limit in food is quantum satis.	(Code of Federal Regulations, 2022; European Commission, 2008)
 Ethylenediaminetetraacetic acid (EDTA)	Chelating agent	May be safely used in designated foods following regulation 21 CFR 172.120. Based on EC 1333/2008, it is registered as E385 ^b , with maximum limit in food is quantum satis.	(Code of Federal Regulations, 2022; European Commission, 2008)
 Glutathione	Reducing agent	GRAS substance based on section 201(s) of the Federal Food, Drug, and Cosmetic Act of the United States Food and Drug Administration (US-FDA).	(Han et al., 2019; Sonthalia et al., 2018)
$\text{H}-\text{S}-\text{H}$ Hydrogen sulfide (H_2S)	Regulator of antioxidant system, energy and lipid metabolisms.	Toxic gas, but could be safe as the H_2S trace remained low in storage and preservation of fruits and vegetables.	(C. Chen et al., 2021)
 Kojic acid	Reducing agent, chelating agent, bleaching agent	Kojic acid does not cause safety issues if consumed in an amount and in a manner consistent with historical use.	(Burdock et al., 2001; Moon et al., 2020; Singh et al., 2018)
 Resorcinol	Reducing agent	GRAS substance under 21 CFR 330.12.	(Code of Federal Regulations, 2022; Mendoza-Wilson et al., 2022)
$\text{Na}-\text{Cl}$	Halide salt	GRAS substance under 21 CFR 182.1. Sodium level in the diet should be concerned.	(Code of Federal Regulations, 2022; Li et al., 2015)
$\text{Na}-\text{F}$	Halide salt	GRAS substance under 21 CFR 355.10.	(Code of Federal Regulations, 2022; Li et al., 2015)
 Hexamethylenetetramine (Hexamine)	Reducing agent	GRAS substance under 21 CFR 182.3890. Based on EC 1333/2008, it is registered as E307, with maximum limit in food is quantum satis.	(Code of Federal Regulations, 2022; European Commission, 2008; Mendoza-Wilson et al., 2022)

Abbreviations: CFR: Code of Federal Regulations; EC: European Commission; GRAS: generally recognized as safe.

^aQuantum satis: no maximum numerical level is specified and substances shall be used in accordance with good manufacturing practice, at a level not higher than is necessary to achieve the intended purpose and provided the consumer is not misled.^bRegistered in the form of calcium disodium EDTA.

solutions (0.01–0.1 mol/L, 4°C, 5 min) (Li et al., 2015). Halide salts, especially NaF and NaCl, decreased PPO activity with the associated maintenance of polyphenols arising from their lower oxidation of polyphenols. Halides may bind to the secondary substrate-binding site of PPO, called site B or inhibitory site, then subsequently affects the structure of active site (site A) of PPO, modulates the affinity of catechol or substrate to site A, and thus exerts an inhibitory effect (Li et al., 2015; Lim et al., 2012). The halide ions from halide salts can also make a complex with the copper atom of the active site, thus inhibiting the browning (Robert et al., 1998). The authors (Li et al., 2015) concluded that browning could be delayed by salts following this order: chloride = phosphate > sulfate > nitrate, with no difference between the ions of sodium, potassium, and calcium. They also observed that browning rapidly appeared by dipping apple in KNO₂ and NaNO₃ solutions, indicating that those salts were not the effective antibrowning agents. CaCl₂ could effectively inhibit PPO and prolonged the postharvest life of apple, but imparted a bitter taste. In addition, as NaCl treatment was not effective enough to inhibit the enzymatic browning of fresh-cut potato, a recent study soaked the potato in NaCl solution before the cutting process (Ma et al., 2021). The pre-cut NaCl solution treatment conducted in this research effectively inhibited the browning of potato fresh-cut by influencing PPO activity and changing the contents of amino acids, highlighting that amino acids may react with quinones and affect the PPO activity, or may act as chelating agents forming a colorless adduct (Ali et al., 2016; Ma et al., 2021). This new way to improve the effectiveness of antibrowning agents, which may be not only limited to NaCl, in inhibiting the enzymatic browning in potato may also be studied further in apples.

Dipping Fuji fresh-cut in 15 g/L citric acid did not decrease significantly the BI after 4 days of storage (4°C) in comparison to dipping in water and the untreated one (Zhao et al., 2021). However, the authors highlight that dipping the apple in 20 g/L γ -aminobutyric acid (GABA) successfully inhibits the enzymatic browning as the BI on day 4 was still similar to the untreated sample on day 0 (Zhao et al., 2021), and a similar result was also found in *Malus pumila* apple fresh-cut using the same concentration and treatment time (Gao et al., 2018). Besides exhibiting hypotensive, calming, and relaxing effects that are beneficial for humans, GABA could also benefit as antibrowning agent in apple by regulating the expression level of browning-related enzymes and maintained the TPC at a relatively low level by regulating the related polyphenol genes, and thus could suppress the severe browning of apple fresh-cuts (Zhao et al., 2021). Dipping Fuji fresh-cut in 0.5% citric acid solution also enhanced browning (lower WI and higher BI than control) in treated apple during 15 days of storage, which might

be due to water loss (C. Chen et al., 2016). Another study using fresh-cut apple (*Malus pumila*) also found that low concentration of ascorbic acid (<7.5 μ M) could activate tyrosinase (PPO), while in higher concentrations, it acted as inhibitor (Wen et al., 2021). It is also worth noticing that if ascorbic acid is fully oxidized to dehydroascorbic acid, o-quinones cannot be reduced to diphenols, which then continues to melanin formation (Moon et al., 2020).

The addition of 1.12–2.25 mM compound II as tetraaza-hydroxypyridinone derivative in apple juice successfully decreased more than 50% of ΔE in comparison to control, with 2.25 mM as the best concentration for browning inhibition (Muñoz-Pina et al., 2022). The author also concluded that compound II as tetraaza-hydroxypyridinone derivative is exactly named as: 6-[2'-(3'-hydroxy-2''-methyl-4'-pyr-idinone)ethyl]-3,6,9-triaza-1(2,6)-pyridinacyclodecaphane also completely stopped the TPC decrease and delayed loss of antioxidant capacity during first 30 min. Other studies also found a successful browning inhibition of apple fresh-cut after the treatment using compound 5d and 5f of gallic acid-benzylidenehydrazine hybrids by chelating copper (Cu²⁺) of tyrosinase (PPO) and reducing Cu²⁺ to Cu⁺ (Peng et al., 2022), as well as compound II of hydroxypyridinone derivative from kojic acid by binding with copper of tyrosinase (Shao et al., 2018). Kojic acid may also interfere with the uptake of oxygen required for enzymatic browning and bleach melanin because it reduces the colored pigment to the colorless one (Singh et al., 2018).

A study carried out the enzymatic browning inhibition of Red Fuji fresh-cut by H₂S gas treatment (from 0.7 mM NaHS solution) for 24 h of exposure (C. Chen et al., 2021). In comparison to control, the treated apples had lower PPO, POD, and PAL activities during 12 days of storage at 4°C. It was also interesting to note that as the five analyzed enzymes, including PPO and PAL activities, of both control and treated apples increased during the first 6 days, they then decreased afterward. To explain the mechanism, transcriptome analysis was conducted, revealing that H₂S inhibited the enzymatic browning by regulating the antioxidant system, energy metabolism, and lipid metabolism (C. Chen et al., 2021).

3.3.2 | Natural products

New enzymatic browning inhibitors are now being researched to replace synthetic additives with natural compounds that are eco-friendly, affordable, and safe for human health while also improving the sensory, nutritional, and health aspects of products (Tinello & Lante, 2018). Antibrowning agents of apple products could also

be found in natural products from plants and agricultural byproducts (Table 5).

After optimization, it was found that 1.2% yerba mate leaves extract in combination with 1% ascorbic acid and 0.9% citric acid optimally inhibited enzymatic browning in Granny Smith fresh-cut, with decreased BI, and 78% of panelists liked the color, flavor, texture of treated apple (Rodríguez-Arzuaga & Piagentini, 2018). Due to yerba mate's high polyphenols content (Rzasa-Duran et al., 2022), combined with ascorbic and citric acid, the antioxidant capacity (DPPH assay and ferric reducing antioxidant power [FRAP]) of treated apple increased, therefore reducing the enzymatic browning activity (Rodríguez-Arzuaga & Piagentini, 2018). Besides acting as antioxidants, polyphenols could also decrease PPO activity by serving as competitive or noncompetitive inhibitors (Tinello & Lante, 2018). By using the same concentrations of above-mentioned solution, the treated apple showed higher general appearance score and lower browning score by sensory analysis until day 7 at 2°C and day 5 at 10°C (Rodríguez-Arzuaga et al., 2021).

Polyphenols, especially procyanidins, were studied as the bioactive compounds of unripe grape juice (GJ) that are responsible in inhibiting PPO activity of Golden Delicious fresh-cut, together with citric acid and L-malic acid (Tinello et al., 2018). The anti-browning effect of GJ was compared to that of 1% ascorbic acid + 1% of citric acid (AACA), 6% NatureSeal® (NS), and 0.1% sodium metabisulfite (SM). It was found that the order of PPO inhibition from the strongest to the weakest was NS>AACA>SN>GJ>control, indicating that dipping apples in GJ still could replace the inhibition of enzymatic browning by conventional processing (blanching) and synthetic additives. Another study added 0.25–1.25% *Rosa roxburghii* juice, rich in ascorbic acid (1.67%), flavonoids (quercetin and morin), and phenolic acids (*p*-coumaric acid) to inhibit PPO activity of cRed Fuji cloudy juice (K. Yu et al., 2021). Quercetin and *p*-coumaric acid worked as mixed inhibitors that could bind the enzymes and enzyme–substrate complex. Lower BI in comparison to equivalent exogenous ascorbic acid was also observed after 48 h, confirming better antibrowning effect than exogenous ascorbic acid. Synergistic effect of 0.05% ascorbic acid (as reducing agent) and (0.1–0.4%) cinnamon essential oil nanoemulsion was found as this combination almost completely inactivated PPO (<20% PPO activity) during 48 h of storage at 4°C (Xu et al., 2020). Lemon juice, which is rich in ascorbic and citric acid, was applied to organic and conventional Golden Delicious fresh-cut to inhibit PPO activity by dipping (19% and 14% PPO inhibition, respectively for apple) and by vacuum impregnation (32% and 16% PPO inhibition) (Neri et al., 2019). However, decreased *L*-value (higher darkness) of apple was found

after treated by vacuum impregnation, but lemon juice could help reducing the darkness effect in comparison to vacuum impregnation with water. Dipping treatment caused an increase in *L*-value of apple and hue angle to green color.

Although catechin was studied to be the substrate of PPO, which could induce browning (Remorini et al., 2015), another study reported that 1–3 g/L commercialized green tea extract that is rich in catechin could decrease PPO activity, Δ BI, and Δ YI of Golden Delicious juice, during 48 h at 4–6°C (Klimczak & Gliszczynska-Świgło, 2017). Although higher concentration resulted in better PPO inhibition (completely inhibited PPO activity at 3 g/L after 24 h), increased ΔE was observed. Furthermore, recently, a study found that addition of 250–1000 μ g/ml of *Galla rhois* water extract to apple (*Malus pumila*) juice successfully inactivated PPO by increasing antioxidant capacity (DPPH assay and FRAP, better than ascorbic acid), due to its phenolic compounds, especially galloylglucose- and gallate-derivates (M. Lee et al., 2022).

Besides natural extracts and juices, the valorization of agricultural byproducts, such as strawberry byproducts, tomato skins, mangosteen rinds, and rice bran (Table 5), which are still rich in bioactive compounds were also observed to inhibit enzymatic browning in apple products. Although 0.40 g/L ascorbic acid (45% PPO inhibition) inhibited PPO in Red Delicious flesh better than strawberry byproducts extract (0.24 g gallic acid equivalent (GAE)/L, 30% PPO inhibition), strawberry byproducts could be a low-cost antibrowning agent (Villamil-Galindo et al., 2020). Strawberry byproducts that contained agrimoniin inhibited PPO via reversible uncompetitive inhibition, while ascorbic acid via competitive inhibition.

Mangosteen rinds and tomato skins successfully decreased the BI and PPO activity of apple products, due to their cyanidin-3-sophoride (noncompetitive inhibitor-binding with free PPO and PPO–substrate complex) and lycopene (antioxidant, sequester of free radicals, protector of DNA and cellular membranes from oxidative damage), respectively (Hemachandran et al., 2017; Martínez-Hernández, Castillejo, et al., 2019). Sukhonthara et al. (2016) found that phenolic acids in defatted and full-fatted rice bran successfully inhibited PPO activity of Fuji puree, with full-fatted rice bran (47% PPO inhibition) inhibited better than the defatted one (19% PPO inhibition). Supplementation of 0.5–2% shallot (*Allium ascalonicum* L.) extract in apple (*Malus pumila*) juice decreased BI and increased the total soluble solid, TPC, total flavonoid content (TFC), and antioxidant activities (DPPH assays and FRAP) of resulting juice (Phaiphan et al., 2019). Similar results were also found by another study (B. Lee et al., 2016).

TABLE 5 The inhibitory effect of natural products on enzymatic browning of apple products

Products	Cultivars	Natural sources	Bioactive compounds	Treatment	Main findings	References
Fresh-cut	Granny Smith	Yerba mate (<i>Ilex paraguariensis</i>) leaves	Polyphenols	Dipped in 1.2% yerba mate water extract + 0.9% citric acid + 1.0% ascorbic acid (3 min, 1:3 m/v ratio)	↓BI	(Rodríguez-Arzuaga et al., 2021; Rodríguez-Arzuaga & Piagentini, 2018)
Cloudy juice	Red Fuji	<i>Rosa roxburghii</i> juice	Ascorbic acid, flavonoids (quercetin, morin), phenolic acids (<i>p</i> -coumaric)	Addition of juice (0.25–1.25%, w/w)	↓PPO activity	(K. Yu et al., 2021)
Flesh	Red Delicious	Strawberry (<i>Fragaria</i> × <i>ananassa</i> Duch. cv. Festival) by-products	Polyphenols (agrimoniin)	Extracted PPO was added with 0.24 g GAE/L extract	↓BI ↓PPO activity	(Villamil-Galindo et al., 2020)
Fresh-cut	-	Soursop (<i>Annona muricata</i>) leaves	Polyphenols	Dipped in 1% extract (2 min)	↓PPO activity ↔ POD activity	(Adeyemi et al., 2019)
Cloudy juice	Fuji	Cinnamon (<i>Cinnamomum</i> sp.) essential oil nanoemulsion	Cinnamaldehyde	Addition of essential oil (0.1–0.4%) with or without ascorbic acid (0.05%)	↓BI ↓PPO activity	(Xu et al., 2020)
Fresh-cut	Braeburn	Tomato (<i>Solanum lycopersicum</i> L. var. <i>lycopersicum</i> cv. Caniles™) skin	Lycopene	Dipped in 0.5–2 g/L lycopene microspheres (8 min)	↓BI	(Martínez-Hernández, Castillejo, et al., 2019)

(Continues)

TABLE 5 (Continued)

Products	Cultivars	Natural sources	Bioactive compounds	Treatment	Main findings	References
Fresh-cut	Golden Delicious (organic and conventional)	Lemon (<i>Citrus limon</i> L.) juice	Ascorbic acid, citric acid	Dipped in juice (0.5 w/v citric acid, 1 min, 1:4 w/w fruit-solution ratio); vacuum impregnation in juice (0.5 w/v citric acid, 738 mbar, 10 s holding time, 1 min contact time, 1:4 w/w fruit-solution ratio)	↓PPO activity	(Neri et al., 2019)
					↑L-value (dipping) ↓L-value (vacuum impregnation)	
Fresh-cut	Golden Delicious	Unripe grape (<i>Vitis vinifera</i> cv. Merlot) juice	Citric acid, L-malic acid, polyphenols (procyanidins)	Dipped in juice (25°C, 15 min)	↑hue angle (dipping) ↓PPO activity	(Tinello et al., 2018)
Fresh-cut	Red Delicious	Mangosteen (<i>Garcinia mangostana</i>) rind	Cyanidin-3-sophoroside	Dipped in 0.1-2% cyanidin-3-sophoroside (5 min)	↓ΔE ↓PPO activity	(Hemachandran et al., 2017)
Cloudy juice	Golden Delicious	Green tea (<i>Camellia sinensis</i>) leaves	Catechin (ECG, EGCG, GCG)	Addition of extracts (1-3 g/L)	↓BI ↓PPO activity	(Klimczak & Gliszczynska-Swiglo, 2017)
					↓ΔBI ↓ΔYI ↑ΔE with ↑concentration	
Puree	Fuji	Rice (<i>Oryza sativa</i> , defatted and full-fatted) bran	Phenolic acids (<i>p</i> -coumaric showed the highest inhibitory effect)	Blended with each extract (1:1 w/w)	↓PPO activity (full-fatted bran decreased PPO activity better than defatted)	(Sukhonthara et al., 2016)
Fresh-cut	Fuji	Pine (<i>Cedrus deodara</i>) needle	2R,3R-dihydromyricetin	Dipped in 0.5% 2R,3R-dihydromyricetin (5 min)	↓ΔE	(Liang et al., 2014)
Fresh-cut	Fuji	Pine (<i>Cedrus deodara</i>) needle	Polyphenols (methylconiferin, ferulic acid-β-d-glucoside)	Dipped in 0.05% extract (25°C, 5 min)	↑L-value	(Z. L. Yu et al., 2014)

Abbreviations: BI: browning index; ECG: (–)-epicatechin gallate; EGCG: (–)-epigallocatechin gallate; GAE: gallic acid equivalent; GCG: (–)-gallic acid equivalent; L-value: lightness/darkness; PPO: polyphenol oxidase; POD: peroxidase; YI: yellowness index; ΔE: total color difference; †: higher than control (increased); ‡: lower than control (decreased); ↔: not significantly different from control.

3.3.3 | Coating

Coating of apple fresh-cut was carried out by recent studies using κ -carrageenan, chitosan, sodium alginate, carboxymethyl cellulose (CMC), rice starch/fish protein, aloe vera gel, zein, soy protein isolate (SPI), whey protein isolate (WPI), and emulsion, without and with chemical or natural additives to suppress the enzymatic browning activity (Table 6). Polysaccharide-based coatings have been studied by prior research to be effective in fresh-cut preservation; not only exhibiting desirable mechanical properties, and film-forming ability, but also having selective gas permeability (Pilon et al., 2015). Polysaccharide-based coatings also showed antimicrobial action (Pilon et al., 2015) that might be beneficial to inhibit enzymatic browning, noting that microbial growth could increase enzymatic browning activity by worsening the cell integrity of apple tissue, provoking cell lysis, and leading to contact between browning enzymes and substrates (Nogales-Delgado, 2021).

To improve the antibrowning effect, some studies added natural or synthetic antibrowning agents to the polysaccharide-based coating solution. There are some studies published using synthetic antibrowning agents. Chitosan nanoparticles (110 and 300 nm) were sprayed to coat Gala fresh-cut, and as comparison, dipping treatment using 2 g/L chitosan in 2% citric acid and control (untreated) were used to analyze the PPO and POD activities (Pilon et al., 2015). The authors found that generally, apple treated with both sizes of chitosan nanoparticles exhibited slightly lower PPO and POD activities than the dipping treatment and control during 10 days of storage (5°C). This phenomenon might be explained as chitosan acts as oxygen barriers, supported by the presence of citric acid and sodium tripolyphosphate in the composition of chitosan nanoparticles. Furthermore, coating apple fresh-cut with 0.5% chitosan with 1% of three organic acids (acetic, lactic, and levulinic acid) and 2% N-acetyl-L-cysteine or L-cysteine was studied as the best composition to inhibit the enzymatic browning and microbial activity (*Listeria monocytogenes*, *Escherichia coli*, *Salmonella* spp.), better than with only one or two organic acids and without cysteine (Jin et al., 2020). This coating composition could maintain the color and fresh-like appearance and texture of apple fresh-cut up to 35 days of storage (4°C).

Aloe vera gel-based coating incorporated with synthetic and natural antibrowning agents was studied by some studies (Table 6). Coating based on aloe vera gel (40%) with 1% ascorbic and 1% citric acid (AVG), without or with hydroxypropyl methylcellulose (AVG/HPMC) and lemon essential oil (AVG/LEO) successfully maintained the color (higher *L*-value, lower *a*-value) of Fuji fresh-cut in comparison to control (dipped in 0.5% chlorinated water) during 9 days of storage at 4°C (Farina et al., 2020). Fresh-

cut apple coated with both AVG/HPMC and AVG/LEO showed better values of appreciation by sensory analysis than with AVG or control. Kumar et al. (2018) compared the PPO and POD inhibition effect between coating based on aloe vera gel and CMC, incorporated with antibrowning agents. Although all treated samples exhibited enzymatic browning inhibition, the maximum inhibition of PPO and POD activities were found in Royal Delicious fresh-cut coated with CMC + 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.5% CaCl₂ during 7 days of storage (5°C). It also generally showed higher WI and lower BI than control and other treatments during storage. The result was also confirmed by sensory analysis, where it showed the lowest browning score (score 1 out of 5).

Some studies incorporated the antibrowning agents from natural sources into the polysaccharide-based coating. The effect of κ -carrageenan or chitosan-based coating, without or with 20% extracts of *Clitoria ternatea*, *Brassica oleracea*, and *Ipomea batatas* (rich in anthocyanins) on enzymatic browning inhibition of Golden Delicious fresh-cut was conducted (Jancikova et al., 2021). All the treatments generally had lower BI (better browning inhibition) in comparison to control (untreated) after 7 days of storage (4°C). The κ -carrageenan-based coating, without or with extract, showed lower BI, higher antioxidant properties (DPPH assay and FRAP), and higher TPC than chitosan-based coating. Another study incorporated 0.5% shallot onion peel or stalk waste extract in 1% sodium alginate + 1% CMC coating film of fresh-cut apple (Thivya et al., 2021). Coating with film with shallot stalk extract appeared to be an effective way to inhibit browning of apple (low BI, 3.45–17.19) during 12 days of storage (4°C), in comparison to control (without extract, BI 3.45–24.08) and shallot peel extract (BI 3.45–23.04). The BI of apple coated with film with shallot peel extract was either higher than or similar to BI of apple with control coating film during storage. Furthermore, another research incorporated 6% of pink pepper phenolic extract to rice starch/fish muscle protein (15/85) blend film to inhibit enzymatic browning in Gala fresh-cut (Romani et al., 2018). Due to pink pepper phenolics, dominated by protocathechuic acid and *p*-hydroxybenzoic acid, the antioxidant activity (DPPH assay) increased, and thus could suppress more the enzymatic browning (lower BI) of coated apple during storage (4°C, 12 days).

Protein-based coatings, without or with incorporation of antibrowning agents, were also reported by recent studies to control the enzymatic browning of apples. Without addition of antibrowning agent, zein (15% w/v), a protein found in maize, was used as the cling-wrap material plasticized with 35% (w/w of zein) polyethylene glycol 400 and was compared to chitosan-based cling-wrap (1.5% w/v) with 40% (w/w of chitosan) glycerol (Bharathi et al., 2020).

TABLE 6 The inhibitory effect of coating on enzymatic browning of fresh-cut apples

Type of incorporated anti-browning agent	Cultivars	Coating materials	Main findings	References
Natural	Golden Delicious	Coated in κ -carrageenan or chitosan-based film-forming solutions, without and with incorporation of 20% <i>Clitoria ternatea</i> , <i>Brassica oleracea</i> , and <i>Ipomea batatas</i> extracts	↓BI	(Jancikova et al., 2021)
Natural	-	Wrapped by films from 1 % (w/v) sodium alginate + 1 % (w/v) CMC, incorporated with 0.5% shallot onion (<i>Allium cepa</i> var. <i>aggregatum</i>) peel or stalk extract	↓BI (stalk)	(Thivya et al., 2021)
Synthetic and natural	Fuji	Coated with 40% (v/w) aloe vera leaves (<i>Aloe barbadensis</i> Miller) gel + 1% (v/w) ascorbic acid + 1% (v/w) citric acid, without and with 0.1% (v/w) HPMC or 1% (v/w) lemon essential oil	↑L-value	(Farina et al., 2020)
			↓a-value	
Synthetic	Granny Smith	Coated with the solution containing 0.5% chitosan + 1% of three organic acids (acetic, lactic, and levulinic acids) + 2% N-acetyl-L-cysteine or L-cysteine	↑L-value	(Jin et al., 2020)
			↓a-, b-values	
			Good appearance (visual observation)	
-	Red Delicious	Wrapped by 15% (w/v) zein-based or 1.5% (w/v) chitosan-based cling wraps	↓ΔL-value	(Bharathi et al., 2020)
Synthetic	Gala	Coated with the solution containing 6% (w/v) WPI + 40% (w/w of WPI) glycerol + 3% (w/w of WPI) MMT + 5–10% (w/w of WPI) citric acid	↓PPO activity	(Azevedo et al., 2018)
			↓POD activity	
			↓BI	
			↑L-value	
			↓a-value	
-	Fu Shi	Coated with the solution containing 5% (w/v) WPNFs + 4% (w/v) glycerol + 3% (w/v) trehalose	↓BI	(Feng et al., 2018)
Synthetic	Royal Delicious	Coated with 15% (v/v) aloe vera leaves (<i>Aloe barbadensis</i> Miller) gel or 1% (w/v) CMC + antibrowning agents (ascorbic acid, 4-hexylresorcinol, CaCl ₂ , cysteine)	↓PPO activity	(Kumar et al., 2018)
			↓POD activity	
			↓BI	

(Continues)

TABLE 6 (Continued)

Type of incorporated anti-browning agent	Cultivars	Coating materials	Main findings	References
			↑WI	
			↓browning score (sensory analysis)	
Natural	Gala	Coated with 15/85 (w/w) rice starch/fish protein blends incorporated with 6% (v/w) pink pepper (<i>Schinus terebinthifolius</i> Raddi) phenolic extract	↓BI	(Romani et al., 2018)
Synthetic	Golden Delicious	Coated with the solution containing 30 g/L SPI + 9.0 g/L glycerol + 4 g/L ferulic acid, prepared at pH 7	↓BI	(Alves et al., 2017)
Synthetic	Gala	Sprayed with 110 and 300 nm chitosan nanoparticles; dipped in a solution of 2 g/L chitosan dissolved in 2% citric acid (2 min)	↓PPO activity (110 and 300 nm are better than dipping)	(Pilon et al., 2015)
			↓POD activity	
Synthetic and natural	Golden Delicious	Electrosprayed with water-in-oil emulsion (10% water or aqueous phase, 85% olive oil, 5% lecithin) or chocolate (70% chocolate, 15% milk butter, 15% PGPR); in the aqueous phase of water-in-oil emulsion, 10% molasses or ascorbic acid were also added	↑WI	(Khan et al., 2014)
			↓C	

Abbreviations: a-value: redness/greenness; b-value: yellowness/blueness; BI: browning index; C: chroma value; CMC: carboxymethyl cellulose; HPMC: hydroxypropyl methylcellulose; L-value: lightness/darkness; MMT: montmorillonite; PGPR: polyglycerol polyricinoleate; PPO: polyphenol oxidase; POD: peroxidase; SPI: soy protein isolate; WI: whiteness index; WPI: whey protein isolate; WPNFs: whey protein nanofibrils ↑: higher than control (increased); ↓: lower than control (decreased).

The Red Delicious fresh-cut showed lower ΔL -value during 24 h of storage, with higher antioxidant characteristic and better freshness when it was wrapped by zein-based cling-wrap than with chitosan-based cling-wrap.

To improve the effectiveness of browning inhibition, some studies added synthetic antibrowning agents. A study used 30 g/L soy protein isolate (SPI) with 9.0 g/L glycerol as plasticizer in the formulation of film forming solution to inhibit the browning of Golden Delicious fresh-cut (Alves et al., 2017). Although the apple coated with this formulation already showed lower BI than the uncoated one, which may be explained by the reduction of oxygen permeability, the incorporation of ferulic acid as antioxidant and antimicrobial agent at 4.0 g/L improved the antibrowning activity. Interestingly, pH 7.0 affected significantly the properties of formed film, which also then positively affected the antibrowning activity. This is because at pH 5.1, which is near to the isoelec-

tric point of SPI (pH 4.6), the formed SPI-based film is less-homogenous and may result in coating breakage, causing oxygen exposure to the apple surface. Additionally, ferulic acid was reported to improve the antibrowning activity if it was incorporated in SPI-based film than in aqueous solution through dipping technique. Furthermore, coating using whey protein isolate (WPI), which has antioxidant and oxygen barrier properties, was studied to inhibit enzymatic browning of Gala fresh-cut (Azevedo et al., 2018). As improvement of WPI-based film properties and browning inhibition, the authors incorporated 3% (w/w of WPI) montmorillonite, which is a natural clay with low cost and toxin-free mineral used in foods, as well as the citric acid (5-10% w/w of WPI). With this formulation, the PPO, POD activities, and BI of the coated apple decreased significantly, while L-value increased, which might also be related to bleaching effect of citric acid.

Instead of WPI, a group of researchers assembled the WPI into whey protein nanofibrils (WPNFs) at 80°C, pH 2.0 for 10 h under 220 rpm constant magnetic stirring (Feng et al., 2018). In the form of WPNFs, the digestibility, hydrophobic and antioxidant properties increased in comparison to WPI. As a result, the film formulation of WPNFs (5% w/v), especially with addition of glycerol (4% w/v) as plasticizer and trehalose (3% w/v) as a food-grade compound to support protein dispersion as well as to improve the color and aroma of fruits, exhibited the best browning inhibition (the lowest BI) of Fu Shi fresh-cut. During 10 days of storage at 4°C, this film formulation also maintained the TPC and weight of apple better than the WPI-coated and uncoated apple. Moreover, the authors also mentioned that the WPNFs-based film coating did not influence the consumer acceptance in sensory analysis. However, the addition of antibrowning agent, neither natural nor synthetic one into the coating, was not studied in the authors' research.

Water-in-oil emulsion was applied as coating of Golden Delicious fresh-cut using electrospraying method, which generates charged microdroplets as a result of electrostatic attraction, leading to higher transfer efficiency than the conventional spraying, and reduces the processing cost (Khan et al., 2014). Olive oil was preferred in the emulsion than sunflower oil due to its antioxidant activity. Molasses or ascorbic acid was added to the aqueous phase of emulsion as the antibrowning agents (Table 6). This water-in-oil emulsion generally showed higher WI and lower chroma value than control, even without antibrowning agents. However, the water-in-oil emulsion did not reduce the moisture losses as it penetrated inside the pores of apple fresh-cut, leading to a very thin film. Therefore, chocolate-based coating was used as alternative to reduce water vapor flux more effectively than water-in-oil emulsion. The other applications of emulsified coating to prevent enzymatic browning of apple were reviewed by a previous study (Galus & Kadzińska, 2015).

To sum up, generally, the application of coating in apple fresh-cut, regardless of the coating materials used, was scientifically proved to inhibit the enzymatic browning activity by limiting the contact between oxygen with the apples. The inhibition activity could be improved by incorporating antibrowning agents, either natural or synthetic one. In this review, various natural antibrowning agents, including from the food waste or byproduct, such as shallot peel or stalk, showed good antibrowning activity. Further studies are open to utilize and valorize the unused part of plants to act as potential antibrowning agents instead of being a waste, especially in coating of apples. The valorization of food waste as antibrowning agents may also be economically beneficial for industries.

3.4 | Nonconventional processing

Nonconventional processing was conducted by recent studies to inhibit the enzymatic browning of apple products (Table 7) without significantly changing the nutritional value and sensory properties resulting due to shorter treatment time and lower treatment temperature than conventional thermal processing. Nonconventional processing also decontaminates food from microbes, noting that higher microbial activity might increase enzymatic browning activity due to reduction of cell integrity (Nogales-Delgado, 2021). The possible mechanisms of nonconventional processing to inhibit enzymatic browning are summarized in Table 8.

3.4.1 | High pressure processing (HPP)

The enzyme or protein structure could be affected (denaturation, aggregation, or gelation) by HPP (Sulaiman, Soo, Yoon, et al., 2015), which then could inhibit browning enzymes in apple products. The application of HPP (300–600 MPa, 1–3 pulses, 22°C) in Golden Delicious juice successfully maintained the BI up to 4 weeks of storage (4°C), depending on parameters (Szczepańska et al., 2021). After 4 weeks, the juice treated with HPP with 1 pulse at 300 MPa still exhibited lower BI (70.83) than fresh juice (73.14), which was also the lowest in comparison to other treatments. However, Maillard reaction might also take place during 12 weeks of storage that resulted in increased BI. Another study applied HPP (600 MPa, 34°C) for 60 min to fresh-cut Royal Gala, which could decrease only 11% of PPO activity, whereas treatment time of 5–30 min resulted in increased PPO activity (Sulaiman, Soo, Yoon, et al., 2015). The same authors also found the positive impact when HPP (600 MPa) was conducted at higher temperature (62°C) for 15 min. By kinetic analysis, the same pressure but with increasing temperature (57–71°C) resulted in increased *k*-value and decreased *D*-value of apple's PPO. Another study also applied 600 MPa for 3 min to the juices of 3 apple cultivars (Junjie Yi et al., 2017). The juices of Granny Smith and Jonagold showed significant lower PPO and POD residual activity after the treatment, which were 55% and 75% (PPO), and 66% and 59% (POD), respectively. However, HPP did not always successfully reduce the enzymatic browning in apple. The authors found negligible decrease of PPO activity and negligible increase of POD activity in Pink Lady juice ($p > 0.05$) after the HPP (Junjie Yi et al., 2017). While as comparison, thermal treatment (85°C, 5 min) completely deactivated PPO and POD activities of all juices of those 3 cultivars. Another study observed similar results, as HPP at 400–600 MPa

TABLE 7 The inhibitory effect of nonconventional processing on enzymatic browning of apple products

Products	Cultivars	Processes and parameters	Main findings	References
Juice	Red Delicious	PEF (85–110 V/cm, 60 Hz, 50°C for periodic on–off PEF treatment or 50–65°C for constant exposure PEF treatment)	↓PPO activity	(Samaranayake et al., 2022)
Fresh-cut	Fuji	UV-C treatment (254 nm, 5 min, 35 cm distance, treated pre- or post-processing (cutting, peeling, coring))	↓PPO activity (after 8 d of storage; post-processing is better)	(Yuan et al., 2022)
			↓PAL activity (post-processing is better)	
			↓BI (post-processing is better)	
Puree	-	HPP (600 MPa, 3 min)	↔L-value	(Rinaldi et al., 2021)
		OH (2000 L/h, 107°C, preheating 25–60°C)	↓L-value	
Juice	Golden Delicious	Multi HPP (300 MPa: 1 pulse, 5 min; 300 MPa: 3 pulses, 5 min each; 450 MPa: 1 pulse, 5 min; 600 MPa: 1 pulse, 5 min, 22°C)	↓BI	(Szczepańska et al., 2021)
			↓ΔE	
Fresh-cut	Fuji	Plasma-activated water (6–10 kV, 7.0 kHz, 5 min)	↓BI	(Liu et al., 2020)
Fresh-cut	Fuji	RF (27.12 MHz, 6 kW, up to 85°C, 30–120 s, 120 mm electrode gap, 50×8×50 mm sample geometry)	↓PPO activity	(X. Zhang et al., 2020)
			ΔE: RF = thermal (85°C, 120 s)	
Cloudy juice	Golden Delicious	Cold plasma (spark discharge plasma, 7.9375–10.875 kV, 1–5 min)	↓PPO activity	(Illera et al., 2019)
Juice	-	HPCD (31.1–55°C, 7.38–25 MPa, 20 min)	↓PPO activity	(Murtaza et al., 2019)
			↓BI	
Fresh-cut	Fuji, Modi, Pink Lady, Red Delicious	Cold plasma (gas plasma, 22°C, RH 60%, 30–60 min)	↓PPO activity (Fuji, Modi, Pink Lady)	(Tappi et al., 2019)
			↑PPO activity (Red Delicious)	
Cloudy juice	Mixture of Belgian apple cultivars	PEF (12.5 kV/cm, 76.4 kJ/L or 12.3 kV/cm, 132.5 kJ/L)	↓PPO activity	(Wibowo et al., 2019)
			↓POD activity	
			↑L-value	
		HPP (400–600 MPa, 3 min)	↔PPO activity	

(Continues)

TABLE 7 (Continued)

Products	Cultivars	Processes and parameters	Main findings	References
			↔POD activity	
			↑L-value	
Juice	Elstar	Ohmic heating (40 V/cm, 80°C)	↓PPO activity	(Abdelmaksoud et al., 2018)
			ΔE: Ohmic > conventional thermal (90°C, 60 s)	
Cloudy juice	Golden Delicious	HPCD (35–45°C, 10–20 MPa, up to 120 min)	↓PPO activity	(Illera et al., 2018)
			↑k-value (PPO)	
			↓D-value (PPO)	
Cloudy juice	Golden Delicious	SCCD (45°C, 10–60 MPa, 30 min)	↓PPO activity	(Marszałek et al., 2018)
			↓POD activity	
			↓ΔE	
Flesh and juice	Red Fuji	RF (27.12 MHz, 3.5 kW, 35 mm pole spacing, 5–20 min)	↓BI (10–20 min)	(Tian, Yan, et al., 2018)
Cloudy juice	Starking Delicious	UV-LED treatment (254 and 280 nm, and coupled emissions 254/356, 254/405, 280/365, 280/405, 254/289/365/405 nm, 40 min)	↓PPO activity	(Akçün & Ünlütürk, 2017)
			↓ΔE	
Fresh-cut	Granny Smith	Plasma processed air (1.2 kW, 20 L/min, 2.5–10 min)	↓PPO activity	(Bußler et al., 2017)
			↓POD activity	
			↑BI	
Freeze dried	Granny Smith		↓PPO activity	
			↓POD activity	
			↑BI	
Warm air dried	Granny Smith		↓PPO activity	
			↓POD activity	
			↑BI	
Clarified juice	Golden Delicious	HPCD (20–45°C, 6–18 MPa, up to 30 min)	↓PPO activity	(Manzocco et al., 2017)
			↓BI	
			↑k-value (PPO)	
			↓D-value (PPO)	
Cloudy juice	Golden Delicious	SCCD (35–65°C, 10–60 MPa, 10–30 min)	↑k-value (PPO and POD)	(Marszałek et al., 2017)
			↓D-value (PPO and POD)	
Juice	Granny Smith, Jonagold, Pink Lady	HPP (600 MPa, 3 min)	↓PPO activity (Granny Smith, Jonagold)	(Junjie Yi et al., 2017)

(Continues)

TABLE 7 (Continued)

Products	Cultivars	Processes and parameters	Main findings	References
			↔PPO activity (Pink Lady)	
			↓POD activity (Granny Smith, Jonagold)	
			↔POD activity (Pink Lady)	
			ΔE: HPP < thermal	
Fresh-cut	Fuji, Golden Delicious, Granny Smith	UV-LED treatment (2.5×10^{-3} W/m ²) using UV-A light (390 nm, 25°C, up to 60 min)	↑%RΔE (stronger anti-browning effect)	(Lante et al., 2016)
Clarified juice	Royal Gala	PEF (24.8 kV/cm, 60 pulses, 169 μs, 53.8°C)	↓PPO activity	(Sulaiman et al., 2016)
			↓ΔE	
Fresh-cut	Royal Gala, Scirose	HPP (34–71°C, 600 MPa, 5–60 min)	↓PPO activity (Royal Gala, 34°C, 600 MPa, 60 min)	(Sulaiman, Soo, Yoon, et al., 2015)
			↑k-value (PPO Royal Gala, 57–71°C, 600 MPa)	
			↓D-value (PPO Royal Gala, 57–71°C, 600 MPa)	
Fresh-cut	Pink Lady	Gas plasma treatment (atmospheric gas, 22°C, RH 50%, 10–30 min)	↓PPO activity	(Tappi et al., 2014)
Juice	Fuji	PEF (25–35 kV/cm, 75 μs, 2 or 0.2 μs of pulse rise time)	↓PPO activity	(Bi et al., 2013)
			↓POD activity	
			↑L-value	
Cloudy juice	Golden Delicious	PEF (30–40 kV/cm, 50–200 pulses, 40°C)	↓PPO activity	(Ertugay et al., 2013)
			↑L-value (40 kV/cm)	
			↓a-, b-values (40 kV/cm)	
Cloudy juice	Gala	UV-C treatment (254 nm, 0–53 kJ/L)	↔PPO activity	(Müller et al., 2013)

Abbreviations: a-value: redness/greenness; b-value: yellowness/blueness; BI: browning index; D-value: treatment time needed to inactivate 90% of enzyme at a given pressure and temperature; HPCD: high pressure carbon dioxide; HPP: high pressure processing; k-value: inactivation rate constant; L-value: lightness/darkness; LED: light-emitting diodes; MAP: modified atmosphere packaging; OH: ohmic heating; PAL: phenylalanine ammonia lyase; PEF: pulsed-electric field; PPO: polyphenol oxidase; POD: peroxidase; RF: radio frequency; RH: relative humidity; SCCD: supercritical carbon dioxide; UV: ultraviolet; ΔE: total color difference; %RΔE: percent reduction in color difference; ↑: higher than control (increased); ↓: lower than control (decreased); ↔: not significantly different from control.

TABLE 8 Possible mechanisms of nonconventional processing in inhibiting enzymatic browning in apple products

Non-conventional processing	Possible mechanisms to inhibit enzymatic browning	References
High pressure processing (HPP)	- High pressure causes protein denaturation or conformation, thus changes the structure of browning enzymes and reduces browning. - Microbial inactivation.	(Song et al., 2022; Sulaiman, Soo, Yoon, et al., 2015)
High pressure carbon dioxide (HPCD) processing	- The increased pressure (above critical pressure of CO₂; lower than HPP) and temperature in HPCD resulted in the loss of α-helix conformation of browning enzymes. - Microbial inactivation.	(Murtaza et al., 2019; Silva et al., 2020)
Modified atmosphere packaging (MAP)	- Limiting the availability of O₂ by introducing CO₂ or N₂ or other alternative gases (e.g., Ar) to the packaging of fresh-cuts. - Microbial inactivation. - Combination with other technique (e.g., dipping with antibrowning agents) is needed to effectively prevent browning.	(Cortellino, Gobbi, et al., 2015; Cortellino, Rizzolo, et al., 2015)
Non-thermal plasma (NTP) processing	- Enzyme's secondary structure is lost due to breaking of particular bonds or chemical changes of the side chains caused by interactions with multiple active species in the plasma. - Microbial inactivation.	(Tinello & Lante, 2018)
Ohmic heating (OH)	- Electric current is passed through food materials and promotes thermal effect. - Removing metallic prosthetic groups of enzymes. - Influencing the interactions between protein chains. - Modifying enzyme surface charge or surrounding environment. - Microbial inactivation.	(Shao et al., 2021)
Pulsed-electric field (PEF)	- High-voltage electric field alters the α-helix conformation, leading to the changes of enzyme's active site, thus prevents enzymatic browning. - Microbial inactivation.	(Roobab et al., 2022)
Radio frequency (RF)	- Electromagnetic energy rapidly generates heat within food matrix by molecular friction caused by charged ion shift and dipole rotation, which then inactivates endogenous enzymes. - Microbial inactivation.	(Soto-Reyes et al., 2022)
Ultrasound	- Vibrational energy from ultrasound causes the formation and collapse of bubbles which then leads to cavitation. - Inducing the enzymes' structural change in the secondary and tertiary structures by interrupting of hydrogen bond, van der Waals, hydrophobic interaction, electrostatic force in protein/enzyme. - Microbial inactivation.	(Jiang et al., 2020; Nicolau-Lapeña et al., 2019)
Ultraviolet (UV) radiation	- Related to the direct photo-oxidation caused by absorption of light by amino acid residues, the resulting protein denaturation, and the creation of high molecular weight aggregates. - Melanin degradation. - Microbial inactivation.	(Tinello & Lante, 2018)

Note: The microbial inactivation is indirectly related to enzymatic browning inhibition as the reduction of cell integrity in fruits and vegetables caused by microbial activity may increase the enzymatic browning activity (Nogales-Delgado, 2021).

for 3 min did not significantly reduce the PPO and POD activities of mixed cultivars apple juice, and negatively affected vitamin C content (Wibowo et al., 2019). However, during 3 weeks of storage, the PPO and POD activities decreased, which might be due to the complex formation between polyphenols of juice and active site of browning enzymes, which could prevent the reaction between enzymes and substrate. The authors also compared HPP with pasteurization and pulse electric field (PEF), which in contrast, successfully decreased the PPO and POD activities. The lightness (*L*-value) of juice increased after the application of all 3 treatments, despite no significant effect observed in PPO and POD activities after HPP.

Based on the recently reviewed above-mentioned articles, the results might differ from one research to another. This indicates that the specific processing parameters and complex system in the apple products (juice or fresh-cut) may affect the effectiveness of HPP to prevent the browning process and maintain the quality of the apple products. However, in general, HPP can be the alternative of thermal processing of apple products. A similar finding was also found by a recent review about the effect of HPP on the sensory properties and consumer acceptability of juices and smoothies, in which browning of fruit juices was included (Song et al., 2022). Therefore, besides obtaining valid sensory information suggested by Song et al. (2022), the enzyme activities (e.g., PPO, POD, PAL activities) as well as the remaining nutritional value and antioxidant activity of apple products should be validated to ensure that the HPP-treated apple products are of good quality, still acceptable by consumers and beneficial for them.

3.4.2 | High pressure carbon dioxide (HPCD) processing

HPCD, also known as supercritical carbon dioxide (SCCD), combines pressure and CO₂, that could effectively rupture the microorganism and enzyme, without significantly changing the sensory properties (Tinello & Lante, 2018). The increased pressure and temperature in HPCD resulted in the loss of the α -helix conformation of PPO (Murtaza et al., 2019). The pressure used in this process is higher than the critical pressure for CO₂ (7.36 MPa) (Marszałek et al., 2017). This process was applied to inhibit the enzymatic browning in liquid foods, such as clarified (Manzocco et al., 2017) and cloudy apple juice (Illera et al., 2018; Marszałek et al., 2017, 2018; Murtaza et al., 2019).

By applying HPCD (10–20 MPa) at mild temperature (35–45°C) to Golden Delicious juice, Illera et al. (2018) found increasing *k*-value and decreasing *D*-value, along with increasing pressure and temperature. The authors compared the HPCD with thermal process, and it appeared

that HPCD (10 MPa, 35–45°C, 120 min) could inhibit PPO activity about 70–90%, better than thermal process (atmospheric pressure, 35–45°C, 120 min), which was about 20–40% PPO inhibition. Murtaza et al. (2019) also successfully reduced the PPO activity of apple juice about 80% or more at 25 MPa and 40–55°C. A similar kinetic model result was found when lower range of pressure (6–18 MPa) and temperature (20–45°C) was applied to Golden Delicious clarified juice (Manzocco et al., 2017). The authors also observed that HPCD decreased the initial count of total viable bacteria and yeast, as well as inhibited their growth during refrigerated storage up to 15 days, in comparison to untreated juice.

SCCD at increasing pressure from 10 to 60 MPa and at increasing mild temperature (35–65°C) successfully increased *k*-value and decreased *D*-value of PPO and POD (native enzymes and model system using commercial enzymes) in Golden Delicious cloudy juice (Marszałek et al., 2017). Those *k*- and *D*-values were significantly dependent on the source of enzyme as well, as native enzymes were more affected by SCCD (higher *k*-value, lower *D*-value) than commercial enzymes. They also noted that in comparison to PPO, POD was more thermally stable. In further research, the authors used the same range of pressure but fixed temperature (45°C) for 45 min to analyze the enzymatic browning inhibition of Golden Delicious cloudy juice during 10 weeks of storage (4°C) (Marszałek et al., 2018). Besides suppressing the PPO and POD activities during storage, SCCD also decreased ΔE , vitamin C content (decreased about 28%), and individual polyphenols (about 18%) in comparison to untreated juice.

HPCD, based on the reviewed articles, exhibited an effective microbial and enzymatic browning inactivation, especially when it is conducted at around 55°C (minimum thermal process). This technology successfully showed positive results in various fruit and vegetable juices (Silva et al., 2020). However, its application, process optimization in different apple cultivars, as well as its economic feasibility are still needed.

3.4.3 | Modified atmosphere packaging (MAP)

As O₂ is needed in enzymatic browning, the MAP could help limiting the availability of O₂ by introducing CO₂ or N₂ to the packaging of fresh-cut apples, and thus could inhibit enzymatic browning (Cortellino, Rizzolo, et al., 2015). The authors investigated the use of conventional MAP (N₂: 99%, O₂: 1% or N₂: 90%, CO₂: 5%, O₂: 5%) and alternative MAP (N₂O: 65%, Ar: 25%, O₂: 5%, CO₂: 5% or Ar: 80%, CO₂: 20%) to inhibit enzymatic browning in Golden Delicious fresh-cut, in comparison to air as

control (Cortellino, Rizzolo, et al., 2015). However, the result appeared that MAP alone using all gas compositions did not give significant effect on BI during 11 days of storage. Another study also found a similar result, as during the preliminary experiment, the MAP alone (O_2 : 1.5–5%, CO_2 : 2.5–7.5%) was not sufficient to prevent enzymatic browning of Red Delicious fresh-cuts (Kumar & Sethi, 2021). Therefore, both studies combined the MAP with dipping treatment to significantly reduce enzymatic browning activity of apple fresh-cut (see section 3.5).

3.4.4 | Non-thermal plasma (NTP) processing

NTP or cold plasma is an ionized gas that contains electrons, positive and negative ions, gas atoms, ground and excited state molecules, free radicals, and electromagnetic radiation quanta (UV photons and visible light) (Liu et al., 2020). Besides food decontamination from microbes, NTP can also involve in inactivation of food enzymes whose secondary structure is lost due to the breaking of particular bonds or chemical changes of the side chains caused by interactions with multiple active species in the plasma (Tinello & Lante, 2018). Recently, NTP was applied for enzymatic browning inactivation in apple fresh-cut (Bußler et al., 2017; Liu et al., 2020; Tappi et al., 2014, 2019), juice (Illera et al., 2019), and dried apple (Bußler et al., 2017).

Plasma-activated water treatment (6–10 kV, 5 min) was applied to Fuji fresh-cut (Liu et al., 2020). Although BI increased during 12 days of storage (4°C), the treated fresh-cut had lower BI than control (with no initial BI difference). It was noted that the treatment with 10 kV maintained the color best. Furthermore, the treatment did not affect the firmness, titratable acidity, TPC, and antioxidant activity, but successfully reduced total bacteria, coliforms, molds, and yeasts counts during storage. Another study used gas plasma to inhibit PPO activity in Fuji, Modi, and Pink Lady fresh-cuts, but did not significantly inhibit (30 min treatment time) or even increase PPO activity (>100%, 60 min) in Red Delicious fresh-cut (Tappi et al., 2019). The best PPO inhibition was found in Fuji fresh-cut, which were 50% and 90% PPO inhibition, after 30 and 60 min treatment time, respectively.

Another study used plasma processed air (1.2 kW, 20 L/min) for 2.5–10 min of treatment time to inhibit fresh-cut, freeze dried, and warm-air dried Granny Smith apple (Bußler et al., 2017). In treated fresh-cut, the PPO activity decreased to 48% after 2.5 min of treatment, followed by slight decrease to 42% after 10 min of treatment. POD inactivation result was similar to PPO inactivation. However, the reduction of PPO and POD did not accordingly reduce BI of fresh-cut. Instead, the BI of fresh-cut increased from

30 to 78 (2.5 min) and 75 (5–10 min), which might be caused by nonenzymatic reactions of secondary plant metabolites triggered by plasma-immanent species (Bußler et al., 2017). The authors also applied plasma processed air treatment to freeze-dried and warm air-dried apples that reduced the PPO and POD activities (<10% PPO and POD activities) during 19 days of storage (20°C). However, higher BI was found in treated samples, than untreated ones, which was similar to the phenomenon in fresh-cut. It is also worth noticing that although plasma-treated dried apples did not change the PPO and POD activities during storage, both freeze-drying and warm air-drying treatment caused nonenzymatic browning.

To Golden Delicious cloudy juice, a study applied spark discharge plasma to inhibit the enzymatic browning (Illera et al., 2019). The PPO activity decreased to 16 and 27.6% after 5 and 4 min of treatment, respectively, at 10.5 kV.

In summary, NTP treatment is a new developing trend, which based on results is effective to prevent the enzymatic browning in minimum processed apple products. It can also improve the safety (i.e., degrading allergen, agrochemicals, antinutrients, and toxin) and general quality of various postharvest fruits and vegetables at nearly ambient temperature (Y. Q. Chen et al., 2020). As apple can be found in different forms (i.e., juice, fresh-cut, dried apple) with different physiological and chemical changes, the interaction between NTP and apple products is complex. Further investigation of this process especially in exploring the specific mechanism of shelf-life extension, including browning prevention in various apple products and cultivars, as well as its scaling-up to industrial scale is still needed. Its combination with other treatments, such as various antioxidants, refrigeration, and so on, is also open for further studies. Last but not least, as NTP processing is a novel technology that involves specific physical and chemical processes in food products, new and updated regulations would need to be drafted (Niemira, 2020).

3.4.5 | Ohmic heating (OH)

OH, also known as Joule heating or electro-heating, is a developed thermal treatment of food by passing alternating electric current through food components. In conventional thermal treatment, the heating process occurs via convection and conduction from a heated surface to inner part, while in OH, the heating occurs volumetrically in nature (inside and outside heat transfer pattern), which potentially reduces the over-processing (Abdelmaksoud et al., 2018). The possible mechanisms of OH on enzyme are divided into two: (a) thermal effect (protein denaturation of conformation), (b) nonthermal effect (removing metallic prosthetic groups in enzymes, modifying tertiary

or quaternary structures), which then result in browning inhibition (Shao et al., 2021). The OH treatment was conducted to inhibit browning in apple juice (Abdelmaksoud et al., 2018), mash and juice (Mannozi et al., 2019), and puree (Rinaldi et al., 2021).

Abdelmaksoud et al. (2018) investigated that OH at optimum condition of 40 V/cm and 80°C inhibited PPO activity of apple juice better than conventional heating (90°C), which were 97.13% and 91.35% inhibition, respectively. However, OH caused higher ΔE than conventional heating, which might be due to migration of moisture or other chemical changes. Another study found that PPO and POD activities were not inhibited significantly if OH was applied at mild temperature (20–40°C to 20–60°C), but significantly at higher temperature (20–80°C) (Mannozi et al., 2019). But, it is worth noticing that too a high temperature (e.g., at 107°C) might darken (lower *L*-value) the apple product due to nonenzymatic browning, which was observed in apple puree (Rinaldi et al., 2021). The same author also found that the *L*- and *a*-values of OH-treated puree were similar to those of conventional heating-treated puree.

To summarize, in general, OH is a promising technique to inhibit the enzymatic browning activity and maintain the color of apple, in comparison to conventional heating as it promotes fast heating rate and uniform temperature distribution. However, the reported results were based on lab-scale application and it still can be optimized due to the complex. Further studies would be needed for industrial application as well as to minimize the possible negative impacts of electrochemical degradation on apple or fruit quality during OH treatment, such as (a) the released metallic ions due to electrode corrosion that could promote color deterioration, oxidation, and degradation of bioactive compounds, and (b) formation of air bubbles in juice at higher temperature of OH, as these bubbles could cause loss of juice and color deterioration (Shao et al., 2021).

3.4.6 | Pulsed-electric field (PEF)

PEF was recently applied to inhibit enzymatic browning, as well as microbial growth in apple semi-liquid or liquid products. It entails the use of a high-voltage electric field for a brief period of time (Ertugay et al., 2013). With this technique, the α -helix conformation occurs, leading to the changes of enzyme's active site, thus prevents enzymatic browning (Roobab et al., 2022). Generally, PEF generates heat (around 40°C), but is not too high in comparison to thermal processing, which then reduces the loss of nutrients (Zhu et al., 2022).

Although a study mentioned that pulsation may activate the PPO and POD activities and generates mild tempera-

ture (Zhu et al., 2022), another study found that, in Belgian apple juice, the PEF treatment with higher intensity (132.5 kJ/L, 12.3 kV/cm) caused inactivation of PPO and POD activities to large extent (below 10% of activities during 3 weeks of refrigerated storage), similar to thermal treatment (72–85°C, 15–30 s) (Wibowo et al., 2019). Lower intensity of PEF (76.4 kJ/L, 12.5 kV/cm) partially inhibited PPO and POD activities during storage. This might be due to the temperature generated by PEF, which were around 60 and 73°C for lower and higher intensity, respectively, similar to that of thermal treatment. This high temperature might affect the browning enzymes' structures; thus the activities were reduced. It was also found that the PEF-treated apple juice exhibited the highest remained vitamin C content.

PPO and POD activities were also almost completely inhibited at 35 kV/cm and 2 μ s of pulse retention time in Fuji juice (Bi et al., 2013). However, PEF caused a significant decrease of vitamin C content (up to 36.6%), but still could maintain the antioxidative activities (DPPH, FRAP, and oxygen radical absorbance capacity (ORAC)) of juice if conducted at 2 μ s of pulse retention time. Sulaiman et al. (2016) also found that PEF (24.8 kV/cm, 53.8°C, 169 μ s) also found similar antioxidant activity (DPPH) of Royal Gala clarified juice after the treatment. But unfortunately, the antioxidant activity decreased significantly during the shelf storage for 30 days, while in contrast, the juice treated by thermosonication and conventional thermal process showed insignificant decrease of antioxidant activity after storage. Furthermore, Ertugay et al. (2013) observed a complete PPO inhibition with higher *L*-value of Golden Delicious cloudy juice when PEF was conducted at 40 kV/cm at 100 or more pulses. PEF at 30–40 kV/cm also inhibited the growth of mold, yeast, and mesophilic bacteria (≤ 1 log CFU/mL) during 13 weeks of storage.

Furthermore, moderate electric field at field strength of 85–110 V/cm was recently reported to inhibit PPO activity of Red Delicious juice (Samaranayake et al., 2022). This study applied this treatment using periodic on-off treatment or constant exposure treatment. Both kinds of treatments effectively inhibited PPO activity by changing the protein structure of enzymes, due to the electrical and temperature (50–65°C) effects. Another study applied PEF at lower temperature (20°C, 0.5 kJ/kg) to the apple mash (Mannozi et al., 2019). After the treatment, the juice was obtained and it was found that PPO activity was inhibited significantly; however, POD activity and BI were similar to control.

To conclude, the enzymatic browning of apple products, especially in liquid or semiliquid forms, can be effectively inhibited by PEF technology at lower temperature than conventional heating, which is then beneficial to maintain the color, flavor, and nutritional value of the

products. Although its implementation in small to industrial scale have been reported, the impact of PEF-treated apple products on enzymatic browning and the remaining bioactive compounds during the storage could be studied and improved further in the future.

3.4.7 | Radio frequency (RF)

RF heating applies electromagnetic energy (1–300 MHz of frequency, with 13.56–40.68 MHz of frequencies are assigned by US Federal Communications Commission; lower than that of microwave), which rapidly generates heat within food matrix by molecular friction caused by charged ion shift and dipole rotation (Guo et al., 2019; Tian, Yan, et al., 2018; X. Zhang et al., 2020). To inactivate endogenous enzymes, RF can swiftly transmit energy to every portion of a bulk food product, such as a full apple (Tian, Yan, et al., 2018). Additionally, RF heating also promotes microbial inactivation via thermal effect, including irreversible denaturing enzymes, proteins, and nucleic acids, which then ensures the safety of the apple or the other food products (Soto-Reyes et al., 2022).

To inhibit PPO activity of Fuji fresh-cut, a study treated the apple with RF (27.12 MHz) (X. Zhang et al., 2020). After optimization, it was found that 120 mm electrode gap and 50×8×50 mm sample geometry (without superimposed arrangement) resulted in optimum heating rate and uniformity. The optimum RF condition was applied to the apple (30–120 s), and PPO inhibition was higher when longer time was applied to the apple (6–80% PPO inhibition, respectively). In comparison to hot water blanching (85°C, 120 s), RF showed higher PPO inhibition, but similar ΔE was observed.

The same frequency (27.12 MHz) was also applied to inhibit enzymatic browning in Red Fuji flesh for 5–10 or 5–20 min, conducted by the same author (Tian, Wang, et al., 2018; Tian, Yan, et al., 2018). The flesh was then squeezed to obtain the juice. Juice obtained from RF-treated mash showed lower BI, PPO activity and higher L-value than untreated one. The treatment time of 10 min was recommended as the BI was still similar up to 20 min. In comparison to thermal treatment (65–75°C for 5–20 min), RF showed lower BI (Tian, Yan, et al., 2018).

In summary, based on recent studies, RF heating offers faster heating rate and higher penetration depth, which then more efficiently inhibits the enzymatic browning of apple than conventional thermal treatment under identical operating conditions. It is also cost effective, although the high equipment cost might be the challenge (Guo et al., 2019). Its implementation in continuous industrial-scale production might need to be studied further (Y. Zhang et al., 2022).

3.4.8 | Ultrasound

Ultrasound is a short length acoustic wave at frequencies ranging from 18 kHz to 100 MHz (Masztalerz et al., 2020). It provides vibrational energy which causes formation and collapse of bubbles and produces temporary spots during bubble implosion process, which then leads to cavitation. These conditions induce the enzymes' structural change in the secondary and tertiary structures due to the interruption of hydrogen bond, van der Waals, hydrophobic interaction, electrostatic force in protein/enzyme, thus inhibiting the browning of fruits (Jiang et al., 2020; J. Zhang et al., 2022). Additionally, ultrasound can also inactivate the microbial activity in the fresh-cut fruits and vegetables by two mechanisms: (a) the cavitation effect that breaks the cell walls, membrane structures, DNA of microbes, and (b) the free radical formation, such as hydroxyl radicals, from which hydrogen peroxide (antibacterial agent) can be formed through some reactions, and hydroxyl radicals can also react with the DNA of microbes (Nicolau-Lapeña et al., 2019).

Recently, a study investigated the effect of ultrasound treatment (100–400 W, 5–30 min) on enzymatic browning of apple juice (J. Zhang et al., 2022). It was found that ultrasound at 100 W was too weak to change or destroy the enzymes, and in contrast, it only promoted the interaction between enzymes and phenolics, resulting in higher BI. However, at 300 W, the BI decreased. At this recommended intensity (300 W), the longer treatment time, the lower the BI. The authors also mentioned that ultrasound could inhibit the transformation of phenolics and thus inhibit the occurrence of browning. However, this study combined the ultrasound with β -cyclodextrin treatment and found synergistic effect in browning inhibition (see section 3.5).

A study applied ultrasound (1.3 W/g, 32°C) to Royal Gala puree (Sulaiman, Soo, Farid, et al., 2015). Under this condition, the PPO inhibition of puree was about 40%. However, if ultrasound was combined with heat treatment, the higher PPO activity inhibition and PPO inhibition rate were observed. Furthermore, the apple juice (*Malus pumila*) was subjected to ultrasound (2 W/cm², 15°C, pulsed mode of 2 s on and 2 s off) (Sun et al., 2015). By appearance and BI, it was observed that ultrasound could inhibit the enzymatic browning of juice. However, the result of PPO and POD activities were not in agreement with the appearance, as in treated juice, the PPO activity increased, and POD activity did not change significantly in comparison to untreated juice during 30 min of treatment. After considering other results in treated juice, such as decreased TPC, TFC, chlorogenic acid, and antioxidant activity (ORAC), ultrasound treatment was then not recommended by the authors to inhibit the browning of apple (Sun et al., 2015).

Although ultrasound technology may attract the industries to implement it as it is versatile and reasonably easy to use, the optimization of ultrasound treatment still needs to be studied, for example, by combining with other treatments (i.e., dipping with antibrowning agents, other nonconventional treatments), noting that the impact of ultrasound treatment alone is not effective enough to inhibit the enzymatic browning activity in apple, or instead promotes the activity. It is also worth considering that although longer ultrasound treatment time and higher frequency/energy may inhibit the browning, some bioactive compounds such as phenolics in apple may be impaired and the functionality of apple may then decrease.

3.4.9 | Ultraviolet (UV) radiation

The enzymatic browning inhibition using low-cost UV radiation treatment is mainly related to the direct photo-oxidation caused by absorption of light by amino acid residues (e.g., Trp, Tyr, His, Phe, Met, Cys), the resulting protein denaturation, and the creation of high molecular weight aggregates (Tinello & Lante, 2018). The melanin can be degraded due to UV treatment. There are three types of UV lights: UV-A at 315–400 nm, UV-B at 280–315 nm, and UV-C at 100–280 nm (Tinello & Lante, 2018). Between those three, the UV-C treatment is widely known to effectively kill the microbes in fresh-cut fruits.

Recently, a study investigated the effect of UV-C treatment at 254 nm for 5 min on browning inhibition of Fuji fresh-cut (Yuan et al., 2022). The treatment was conducted before or after processing (cutting, coring, peeling). It was found that UV-C treatment conducted both before and after processing effectively delayed the browning of fresh-cut, based on BI, PPO, and PAL activities, but better if it was treated after processing. The PPO activity of apple treated either before or after processing was lower than control after 8 days of storage at 4°C, while in contrast, it was higher than control before 8 days of storage. UV-C treatment reduced H₂O₂ accumulation and TPC, but the antioxidant activity (2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) or ABTS) of treated apple was higher than control, especially up to 6 days of storage (Yuan et al., 2022). Another study used the same wavelength of UV-C treatment, but could not find a significant PPO inhibition in Gala juice, even at 53 kJ/L (Müller et al., 2013). UV-C also reduced the bacterial activity and BI, as well as increased WI of Fuji fresh-cut during 15 days of cold storage, but in contradiction with PPO activity, which was higher in the treated apples than control (C. Chen et al., 2016).

UV light-emitting diodes (UV-LED) treatment using single or various coupled emissions (at 254–405 nm) for 40 min successfully inhibited the PPO activity of Starking

Delicious cloudy juice (Akgün & Ünlütürk, 2017). Among all treatments, juice treated with UV-LED using coupled emission of UV-C and UV-A (280/365 nm) exhibited the lowest PPO residual activity of 32.58%, confirmed by the lowest ΔE . The authors also compared it with pasteurization (70–80°C, 15–120 s). Although the PPO activity was reduced more by pasteurization (22–27% of PPO residual activity), treating the juice at 280/365 nm emission resulted in lower ΔE (Akgün & Ünlütürk, 2017). UV-LED treatment (2.5×10^{-3} W/m²) of various apple cultivars using UV-A (390 nm) also successfully generally showed stronger antibrowning effect (higher percent reduction in color difference (% ΔE)) with longer exposure time (about 60% ΔE after 60 min of exposure) (Lante et al., 2016).

UV radiation treatment is a well-known technology in postharvest storage of fruits and vegetables. Based on recent studies, it exhibited an impact on inhibition of enzymatic browning not only in apple products, but also in different fruits and vegetables, such as banana, strawberry, grape, mushrooms, and so on (W. Zhang & Jiang, 2019). However, some studies also found that this technology did not significantly delay the browning of analyzed apple products. Prior to its application in apple products, validation is needed to optimize the browning inhibition. Further research is widely open to combine the UV radiation treatment with other technologies and/or antibrowning agents. Furthermore, noting that UV radiation treatment may influence the bioactive compounds (especially phenolics) in apples or other fruits and vegetables, either increasing or degrading their contents (W. Zhang & Jiang, 2019), it is suggested to investigate the more specific mechanism and impact of UV radiation on enzymatic browning activities, noting that the phenolics can be a substrate or inhibitor of PPO enzyme.

3.5 | Other treatments and combination of treatments

In section 3.4, some nonconventional processing methods at certain parameters were not effective to inhibit the enzymatic browning of apple products if it was conducted alone, such as MAP, ultrasound, and HPP. Therefore, it should be combined with other treatments. The combination of 2–3 treatments could give the synergistic or improved effect on apple's enzymatic browning inhibition (Table 9).

In combination with dipping treatment with ascorbic and citric acid, MAP using conventional (N₂, O₂, CO₂) or alternative (Ar, N₂O) gas compositions resulting in lower BI (BI around 26–28) of Golden Delicious fresh-cut than MAP alone (BI around 40–45) during storage (4°C, 11 days) (Cortellino, Rizzolo, et al., 2015). In this case, the

TABLE 9 The inhibitory effect of combined treatments on enzymatic browning of apple products

Products	Cultivars	Treatment 1	Treatment 2	Treatment 3	Effect of combination	References
Fresh-cut	-	Dipping (0.025% carvacrol; 0.125% citric acid; 0.0078% curcumin; 0.0078 riboflavin; 1% ascorbic acid, 5 min)	Photodynamic treatment (blue LED illumination, 460–470 nm, 3 V, 1 W, 181 J/cm ²)	-	↓BI (carvacrol, citric acid, ascorbic acid)	(Hyun et al., 2022)
					↑BI (curcumin, riboflavin)	
Fresh-cut	Red Fuji	Dipped in 20–80 μM riboflavin solution	Photodynamic treatment (blue LED illumination, 460 nm, 30 W, 300 J/cm ²)	-	↓PPO activity	(Zha et al., 2022)
					↓POD activity	
					↓BI	
					↑WI	
Juice	-	Ultrasound (300 W, 5–30 min)	β-cyclodextrin (0.006 g/mL)	-	↓PPO activity	(J. Zhang et al., 2022)
					↓BI	
Fresh-cut	Granny Smith	Dipping (5% citric acid)	Cold plasma (200 MPa, 30 kV, 1–3 min)	Low-pressure cooling (1°C, 7 mbar)	↓PPO activity	(Adam et al., 2021)
					↑L-value	
Fresh-cut	Royal Delicious	Dipping (0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.2% CaCl ₂ , 5 min)	MAP (O ₂ : 1.5–5%, CO ₂ : 2.5–7.5%)	-	↓PPO activity	(Kumar & Sethi, 2021)
					↓POD activity	
					↓BI	
					↑WI	
					↓ΔE	
Fresh-cut	Elstar, Golden Delicious	Thermal by blanching with hot water (50–70°C, 3 min) or steam (65–85°C, 3 min)	Dipping in 1% ascorbic acid + 1% citric acid (3 min)	-	↓PPO activity	(Shrestha et al., 2020)
					↓POD activity	
Fresh-cut	Amasya	Coating (0.75% chitosan + 2% ascorbic acid + 2.5% (w/v) <i>Stevia rebaudiana</i> leaves water extract)	Passive MAP (O ₂ : 21%, CO ₂ and other gasses: 0.03%)	-	↓PPO activity	(Karagöz & Demirdöven, 2019)

(Continues)

TABLE 9 (Continued)

Products	Cultivars	Treatment 1	Treatment 2	Treatment 3	Effect of combination	References
Mash and juice	-	Microwave preheating (850 W, 40–80°C)	PEF (0.8 kV/cm, 500 μ s)	-	↓PPO activity	(Mannozi et al., 2019)
					↓POD activity	
					↓BI	
					↓ΔE	
		Microwave preheating (850 W, 40–80°C)	OH (114 V/cm, 20–80°C)	-	↓PPO activity	
					↓POD activity	
					↓BI	
					↓ΔE	
Cloudy juice	Braeburn	HPP (22°C, 300–500 MPa, 5 min)	Addition of α -cyclodextrin (10–40 mM) or β -cyclodextrin (5–15 mM)	-	↑ΔBI (HPP)	(Martínez-Hernández, Álvarez-Hernández, et al., 2019)
					↓ΔBI (with combination of α - and β -cyclodextrins)	
					↓POD activity	
Fresh-cut	Fuji	Dipping (2 μ mol/L curcumin, room temperature, 5 min)	Photodynamic treatment (420 nm, 15 W, 298 mW/cm ² , 420 s)	-	↓PPO activity	(Tao et al., 2019)
					↓POD activity	
					↑L-value	
Fresh-cut	-	Dipping (1% ascorbic acid + 1% citric acid + 1% CaCl ₂ , 2 min)	Coating (2:1 sodium caseinate:chitosan)	-	↓ΔE	(Volpe et al., 2019)
Flesh and juice	Red Fuji	RF (27.12 MHz, 3.5 kW, 35 mm pole spacing, 5–10 min)	PEF (15–35 kV/cm, 400 μ s)	-	↓PPO activity	(Tian, Wang, et al., 2018)
					↑L-value	
Fresh-cut	Cripps Pink, Golden Delicious	Dipping (1% ascorbic acid + 0.2% citric acid; 1% Ca-ascorbate, 3 min)	Ultrasound (40 kHz)	MAP (N ₂ : 90.5%, CO ₂ : 2.5%, O ₂ : 7%)	↓ΔE	(Putnik et al., 2017)
Fresh-cut	Golden Delicious	Dipping (0.5% ascorbic acid + 1% citric acid, 2 min)	MAP with conventional gas compositions (N ₂ : 99%, O ₂ : 1% or N ₂ : 90%, CO ₂ : 5%, O ₂ : 5%)	-	↓BI	(Cortellino, Rizzolo, et al., 2015)

(Continues)

TABLE 9 (Continued)

Products	Cultivars	Treatment 1	Treatment 2	Treatment 3	Effect of combination	References
			MAP with alternative gas compositions (N ₂ O: 65%, Ar: 25%, O ₂ : 5%, CO ₂ : 5% or Ar: 80%, CO ₂ : 20%)	-	↓BI (Ar: 80%, CO ₂ : 20%)	
					↔BI (N ₂ O: 65%, Ar: 25%, O ₂ : 5%, CO ₂ : 5%)	
Fresh-cut	Fuji	Dipping (0.5% citric acid, 5 min)	UV-C (254 nm, 5 min)	-	↑PPO activity	(C. Chen et al., 2016)
					↓BI	
					↑WI	
Puree	Royal Gala	Thermal (32–72°C)	Ultrasound (24 kHz, 1.3 W/g)	-	↓PPO activity	(Sulaiman, Soo, Farid, et al., 2015)

Abbreviations: BI: browning index; HPP: high pressure processing; *L*-value: lightness/darkness; LED: light-emitting diodes; MAP: modified atmosphere packaging; OH: ohmic heating; PEF: pulsed-electric field; PPO: polyphenol oxidase; POD: peroxidase; RF: radio frequency; UV: ultraviolet; WI: whiteness index; ΔE : total color difference; ↑: higher than control (increased); ↓: lower than control (decreased); ↔: not significantly different from control.

dipping treatment was reported to give the whitening effect on fresh-cut. Among all combinations, only dipping treatment with MAP using 80% Ar and 20% CO₂ showed similar BI to the dipping treatment without MAP (using air only). Another research dipped the Royal Delicious fresh-cut in a solution containing 4-hexylresorcinol, ascorbic acid, and CaCl₂ and combined it with MAP with various compositions (1.5–5% O₂, 2.5–7.5% CO₂, the rest was N₂) (Kumar & Sethi, 2021). In the preliminary study, the authors reported that MAP alone could not significantly prevent browning in apple. While in a previous publication (Kumar et al., 2018), the same authors already found the most effective concentration and combination of antibrowning agents mentioned above, which is 0.01% 4-hexylresorcinol + 0.5% ascorbic acid + 0.5% CaCl₂. Therefore, dipping treatment with this solution, combined with MAP of 5% O₂, 5% CO₂, and 90% N₂ was observed to be the recommendation to inhibit browning (high WI, low ΔE , BI, PPO and POD activities) during 42 days of storage (5°C) (Kumar & Sethi, 2021). Furthermore, the browning inhibition could be improved when apple juice was treated with ultrasound (300 W) in combination with 0.006 g/mL β -cyclodextrin treatment (J. Zhang et al., 2022). This synergistic impact resulted in up to 39% reduction in BI and 18% reduction in PPO activity, which might be due to increased solubility of β -cyclodextrin by ultrasound. Another study combined thermal process (up to 72°C) with ultrasound (24 kHz, 1.3 W/g) to inhibit browning of Royal Gala juice (Sulaiman, Soo, Farid, et al., 2015). Ultrasound conducted at 72°C inhibited PPO better (almost complete inhibition) than at 32–52°C (about 40–50% PPO inhibition). A study even combined dipping treatment (ascorbic and cit-

ric acid or Ca-ascorbate) with ultrasound (40 kHz), then packed in MAP (90.5% N₂, 2.5% CO₂, 7% O₂) in order to inhibit the browning optimally (Putnik et al., 2017). The lowest ΔE (0.29) of apple fresh-cut was found when Ca-ascorbate was used as dipping solution in this combination. Furthermore, as HPP at 300–500 MPa increased the ΔBI of Braeburn juice, its combination with α - or β -cyclodextrin was needed (Martínez-Hernández, Álvarez-Hernández, et al., 2019). The authors recommended to combine HPP (300 MPa) with 30 mM α -cyclodextrin to inhibit browning optimally. While lower concentration of β -cyclodextrin (due to limited solubility) and 500 MPa of HPP might be conducted to achieve the lowest apple juice browning.

Other researchers combined dipping treatment (with various antibrowning agents) with photodynamic treatment at 460–470 nm (Hyun et al., 2022; Zha et al., 2022) or 420 nm (Tao et al., 2019) and generally found improved browning inhibition effect on apple fresh-cut, in comparison to dipping treatment alone. Although treating apple mash using PEF alone (20°C, 0.5 kJ/kg) and OH alone (20–80°C) could partially inhibit enzymatic browning in obtained juice, the PPO, POD activities, BI and ΔE could be more reduced (either significantly or not significantly) when microwave preheating (850 W, 40–80°C) was combined (Mannozi et al., 2019). From ΔE , it also showed that the combination of microwave preheating with PEF or OH resulted in lower ΔE than microwave pre-heating (80°C) alone. Apple (*Malus pumila*) was cut and quickly ground (to produce puree) under vacuum (2.67 kPa) to limit the contact with O₂ and heated under vacuum at 90°C for 30 min (Kim et al., 2021). The authors found

that although thermal treatment with or without presence of oxygen inhibited completely PPO and POD activities, the thermal treatment without oxygen resulted in lower BI (BI 0.57) and higher antioxidant activities (DPPH, ABTS, FRAP) compared to thermal treatment with oxygen (BI 0.84).

Combination of treatments sometimes did not always show good results for enzymatic browning inhibition. A study found that although the BI of Fuji fresh-cut treated with combined 0.5% citric acid and UV-C (254 nm) treatment showed lower BI than the single treatment (citric acid or UV-C) alone and control especially during first 3 days of storage, the PPO activity was increased in comparison to single treatment and control (C. Chen et al., 2016).

4 | CONCLUSIONS

Minimally processed apple (*Malus domestica*) products are prone to enzymatic browning, which not only causes undesirable and unattractive color, but also loss of nutritional quality of apple, especially the phenolics that are the substrates of browning enzymes. There are some analyses that could be done to measure the enzymatic browning in apple. It is suggested to measure enzymatic activities (e.g.m PPO and POD activities) as primary analyses, confirmed by colorimetry, sensory analysis, TPC, and antioxidant activity as secondary ones. To inhibit the enzymatic browning, it can be initiated by selecting the cultivars that have low enzymatic browning activities, as different cultivars grown in different countries have different activities. Based on previous studies, thermal treatment could effectively inhibit enzymatic browning in apple, but reduced the nutritional value and sensory properties such as texture (firmness) and aroma. Dipping treatment using synthetic antibrowning agents was also effective, but currently, consumers seek natural and eco-friendly products. Therefore, dipping or coating treatment using natural or agricultural byproducts as antibrowning agents could be conducted to replace the use of chemicals. Non-conventional methods were also developed to inhibit the enzymatic browning in apple without significantly changing the nutritional values of resulting apple products as lower temperature and shorter treatment time was conducted. Synergistic effects of 2–3 treatments could even reduce the enzymatic browning activity further.

Nevertheless, noting that different apple cultivars have different characteristics and a complex system, it is not that simple to easily decide which method is the best because the enzymatic browning inhibition depends not only on the nutritional composition and native enzymes of apple, but also the parameters of conventional, noncon-

ventional technologies, as well as combined treatments. Through this review, the readers, including the consumers and apple product industries, may understand the enzymatic browning inhibition process, as well as may consider its application, from home-scale to industrial-scale applications. According to the needs and desired results, the readers may approach and further choose and decide on the most applicative technology based on the most similar conditions reviewed in this review article, including apple cultivars grown in their region, type of apple products, availability of antibrowning agents and technology, and so on, with further validation prior to its implementation on a bigger scale to ensure its effectiveness in inhibiting enzymatic browning in minimally processed apple products with guaranteed safety and quality. Furthermore, the other potential natural antibrowning agents, especially from agricultural byproducts to reduce the food waste, and their mechanisms of action should be investigated in further studies. As most studies focused on PPO and POD activities, the activity of other enzymes, such as PAL, lipoxygenase, phospholipase, and so on that are also related to enzymatic browning should be explored more. The development of apple cultivars in the future may also be opportunities for researchers to optimize the enzymatic browning inhibition. Last but not least, the regulations of recent nonconventional treated fruits and vegetables ensure that the consumers would feel safe to consume the treated products.

AUTHOR CONTRIBUTIONS

Marcellus Arnold: Conceptualization; Data curation; Investigation; Methodology; Visualization; Writing - original draft; Writing - review & editing. **Anna Gramza-Michałowska:** Conceptualization; Supervision; Writing - review & editing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Article

Product Development Study of Freeze-Dried Apples Enriched with Sea Buckthorn Juice and Calcium Lactate

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Abstract: Enriched or fortified foods are typically linked to ultra-processed foods, limiting the choice of functional food in the market. Addressing the market potential, particularly the elder population with osteoporosis, the functional food industry should consider developing a healthy snack enriched with bioactive substances. This study aimed to produce freeze-dried Polish Gala apple with improved antioxidant properties and calcium content via impregnation or osmotic dehydration process. The solutions containing various concentrations of sea buckthorn (SB) juice and inulin were prepared at different temperatures and times, then analyzed by response surface regression modelling. Subsequently, the effect of the addition of 0–6% calcium lactate (CaL) on antioxidant properties and calcium content was also studied. Freeze-dried apple, after impregnation with 93.8% SB juice, 0:100 inulin–SB juice ratio, at 30 °C for 120 min, with the addition of 4% CaL (hereafter called “4% CaL” treatment), possessed a minimum yet acceptable loss of antioxidant properties and increased calcium content (2209.13 mg Ca/100 g). UPLC-PDA revealed the altered compositions of phenolics (flavonols were dominated by isorhamnetin-3-O-glucoside and isorhamnetin-3-O-rutinoside) and carotenoids in 4% CaL. The 4% CaL also exhibited lower polyphenol oxidase and peroxidase activities, moderate sensory acceptability with soft texture, and better nutritional values with lower calories when compared to the controls. This work is a scalable study, covering aspects of process design, physicochemical, nutritional, and enzymatic properties, as well as sensory profiling, which has potential for industrial implementation.

Keywords: apple; antioxidant activity; calcium; impregnation; osteoporosis; sea buckthorn



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1. Introduction

In 2015, the United Nations declared 17 Sustainable Development Goals (SDGs), for which a target to reduce premature mortality from non-communicable diseases by one-third by 2030 through prevention and treatment and to promote mental health and well-being

was mentioned in goal number three of the SDGs [1]. Functional food to prevent non-communicable diseases, particularly osteoporosis, has been developed recently. Among bioactive compounds, increasing intake of calcium and natural antioxidants, such as vitamin C, phenolics, and carotenoids, through functional food products is suggested to minimize the risk of osteoporosis, especially in the elderly [2,3].

Impregnation and osmotic dehydration (OD) processes have been studied over the past decade to improve the nutritional value (e.g., calcium and antioxidants) of various plant-based products, including, but not limited to, pumpkins, pineapples, apples, etc. [4–6]. During OD, mass transfer allows for the incorporation of solutes from a hypertonic solution to the food matrix, as well as for water removal from the food matrix as a drying pre-treatment. Although mass transfer in OD could help enzymatic browning inhibition and improve the flavor and taste of a product, the loss of the food matrix's own solutes (i.e., vitamins, minerals, organic acids, etc.) may also happen during the process, which could be affected by the composition and concentration of osmotic agents, the physicochemical characteristics of the food matrix, and process parameters such as time, temperature, and agitation [7]. Various osmotic agents (i.e., salt, sugar, fruit juice concentrate) and process conditions of OD in fruits and vegetables have been reviewed previously [7].

Although the conventional OD process using sugars (sucrose and trehalose) promotes water loss, it significantly decreases the oxygen radical absorbance capacity (ORAC) value of dried strawberry and kiwi in comparison to untreated ones [8]. As opposed to sugar, inulin can be used as it has a lower caloric value than sugar and can act as prebiotic and dietary fiber [9]. The dried matter of strawberries after OD treatment using inulin only and inulin/chokeberry juice concentrate as osmotic solutions showed significantly higher polyphenol content and antioxidant activity via DPPH assay [10]. In OD-treated, oven-dried mango, using an emulsion prepared with inulin/piquin pepper oleoresin as an osmotic agent could recover the loss of free phenols, free flavonoid content, and antioxidant activity at certain levels, depending on temperature; as a comparison, using sucrose decreased their content and antioxidant activity significantly [9].

Recently, the utilization of various concentrated fruit juices, such as chokeberry, grape, apple, cranberry, etc., in OD treatment showed significant influence on the functional properties of various plant matrices [6,7,11]. Investigations of other fruit juices that are available in many regions and that are rich in bioactive compounds as potential osmotic agents are still needed. Sea buckthorn (SB) is a pro-health plant that is native to northwestern Europe through Central Asia to the Altai Mountains, western and northern China, and the northern Himalayas [12]. All parts of SB, especially its berries and juice, exhibit abundant bioactive compounds, including lipophilic (carotenoids and tocopherols) and hydrophilic antioxidants (flavonoids, tannins, ascorbic acid, phenolic acids), fiber, amino acids, and minerals (including calcium), offering a perspective on SB for upcoming research in the functional food field [13,14]. Thanks mainly to SB's antioxidants, various pro-health benefits, such as anti-inflammatory activity, anti-hypertension properties, wound-healing activity, and protection against radiation, were observed [14]. SB fruit also possessed anti-osteoporosis effects as its active fractions promoted osteoblast differentiation in mesenchymal stem cells and increased osteogenic gene expression, which resulted in improved bone mineral density in an osteoporosis mouse model [15].

To ensure the effective intake of antioxidants and calcium by the population, potential food sources that are highly available in many regions should be taken into consideration; e.g., apples. Apples are one of the most popular fruits consumed worldwide thanks to their pleasant taste and high nutritional value; they are also one of the most cultivated crops in the world [16]. However, apples still lack calcium. Therefore, using SB juice and calcium as

an osmotic or impregnation agent might be adapted to the apple to improve its calcium and antioxidant content in order to prevent osteoporosis. This study aimed to develop an alternative, locally sourced functional food product through the OD or impregnation process of apple flesh using SB juice, inulin, and calcium lactate. The suggested conditions to prepare the product were based on the antioxidant properties and calcium content of the final product. Some analyses, such as phenolic and carotenoid composition, sensory profiling, activity of polyphenol oxidase (PPO) and peroxidase (POD), and proximate analysis, were also conducted.

It is also worth noticing that the freeze-drying process was involved in this study. Freeze drying is an important and effective drying technique in the food industry, which has rapidly developed in the 21st century [17]. In comparison to other drying techniques, freeze drying provides significant advantages, such as preserving the quality of the food with minimum degradation of bioactive compounds or loss of nutritional value; easy reconstitution with a quick rehydration process; and lowering the moisture content, extending the shelf life of products. However, it also has some limitations, such as being time-consuming and costly. While Liu et al. [17] already reviewed some technical strategies (osmotic treatment, microwaving, etc.) to reduce the energy cost of the freeze-drying process with respect to various fruits and vegetables, the Food and Agriculture Organization suggested local sourcing as one of strategies to reduce costs in the food sector while creating a collaboration with local suppliers [18]. Our study is in agreement with this strategy, as utilizing local Polish apples and SB juice could be one of the ways to compromise the high production costs of freeze drying.

The outcome of this study could be beneficial for food producers, noting that consumer interest in functional food consumption is increasing. In contrast, enriched and/or fortified foods on the market are mostly associated with ultra-processed foods, such as fortified cereals, snack bars, etc., that contain a lot of additives and preservatives. This study may give new insight into the optimum conditions for producing freeze-dried apples enriched with bioactive compounds. This work is a scalable study, covering aspects of process design, physicochemical, nutritional, and enzymatic properties, as well as sensory profiling, which have potential for industrial implementation. Additionally, this research is also in line with the SDGs [1], especially goal number three, mentioned above, and number two supporting food security, as the product developed in this study was based on local and available resources—apple and SB juice. By applying responsible and sustainable production, it will be beneficial for the environment, such as minimizing transportation emissions, and additionally, it may strengthen the local economy by supporting regional farmers and producers.

2. Results and Discussion

2.1. First Part of Research

After the OD process using different process parameters and osmotic solution compositions (Table 1), total phenolic content (TPC) and antioxidant activities were analyzed by ABTS, DPPH, ORAC, and photochemiluminescence (PCL) of FA, FA + AA, and treated freeze-dried samples, and the results are displayed in Table S1. Generally, a higher concentration of SB juice resulted in higher TPC and antioxidant activities in treated freeze-dried samples, while increasing the inulin–SB juice ratio, regardless of the SB juice concentration and process parameters, resulted in lower TPC and antioxidant activities in freeze-dried samples. This might be because, during OD, the inulin was absorbed into the apples, which resulted in heavier freeze-dried products. Subsequently, lower concentrations of TPC and antioxidant activities per 100 g of freeze-dried products were obtained. Another possible mechanism is that hypertonic solutions, especially those with inulin, may force mass transfer, in which native phenolics leaked from the apples, while the SB juice from the solutions only partially recovered the loss of those apples' native phenolics. This loss of antioxidant

activity (ORAC) was also observed in OD of kiwi and strawberry using hypertonic sucrose or trehalose solutions [8]. In contrary, a study reported that the antioxidant activity by DPPH of frozen strawberry, osmotically dehydrated at 50 °C for 60 min, using 50 °Brix solutions of sucrose–inulin (1:1), inulin only, or inulin–chokeberry juice concentrate (1:4), increased by 26, 24, and 35%, respectively, in comparison to untreated strawberry, which was supported by increased TPC, expressed in gallic and chlorogenic acid equivalents in 100 g of dry matter [10]. This might be explained by the fact that apples and strawberries have different food matrices and the initial TPC of fruits may affect the final TPC after the OD process, noting that apples from different cultivars and regions exhibited different chemical compositions, including TPC, and antioxidant activities [16].

2.1.1. Optimization of Conditions to Obtain Optimum Total Phenolic Content and Antioxidant Activities

Based on the results from Table S1, optimization of conditions to obtain the optimal TPC and antioxidant activities of treated freeze-dried apples was analyzed and is shown in Table 2. Inulin involvement was not suggested in terms of the optimized conditions, indicating that the inulin–SB juice ratio of 15:85 and 30:70 decreased the values of TPC and antioxidant activities (ABTS, DPPH, ORAC, PCL-ACL (lipid-soluble compounds) and PCL-ACW (water-soluble compounds)) in freeze-dried products, which is in accordance with the previous explanation (Section 2.1). This means that an inulin–SB juice ratio of 0:100 was the optimized one. Published studies on the effect of inulin as an osmotic agent in osmotically dehydrated apples have been limited to the mass transfer phenomenon [19] and reduction in sugar uptake [20], rather than antioxidant activity or retention of the contents of bioactive compounds. However, in contrast, for other matrices, like freeze-dried strawberries after OD with a chokeberry juice concentrate–inulin (1:4 *w/w*) solution, the results exhibited about three times higher TPC and about two times higher DPPH antiradical activity than in freeze-dried control samples [21]. Additionally, the authors also observed similar TPC and DPPH antiradical activity in freeze-dried strawberries treated with 50% sucrose, 50% inulin, and a 50% sucrose–inulin mixture (1:1, *w/w*) solution. OD of mango using inulin and piquin pepper oleoresin also resulted in higher free phenol, flavonoid, and DPPH radical scavenging activity than when using a sucrose solution, especially at 40 °C; however, the free phenols and flavonoids were lower in comparison to the untreated control [9].

Furthermore, water, ranging from 1.2 to 31.9% (3.08 to 79.65 g in 250 g), was, interestingly, suggested in the composition of osmotic solutions in all TPC and antioxidant activities, except PCL-ACW (0% or 0 g in 250 g) (Table 2). In other words, SB juice at a concentration of 68.1–100% in water was suggested, depending on the type of assay. The addition of water may affect the total soluble solid (°Brix, Table 1) of the solution, which then affects mass transfer during the OD or impregnation process. The higher Brix value in osmotic solutions than in the food matrix not only improves the uptake of solutes from osmotic solutions, but also lets the water and solutes from the food matrix pass into the osmotic solution [22]. This may indicate that a higher total soluble solid or concentration of SB juice in the osmotic solution does not always improve the TPC and antioxidant activities of treated freeze-dried apples, as the loss of apples' solute or phenolics could also occur (Section 2.3.1). Similar results were reported by Kowalska et al. [23], as convective-dried Idared apples, osmotically dehydrated by 25 °Brix concentrated apple juice (CAJ), showed higher TPC than those treated with 50 °Brix CAJ, irrespective of the temperatures applied (40 and 60 °C); and those treated with 25 °Brix CAJ resulted in a 9–24% decrease in comparison to untreated dried apples. However, when concentrated cherry juice (CCJ) only, or in combination with fructooligosaccharide (CCJ-F) at 25 and 50 °Brix, was used, the TPC of treated dried apples increased with an increasing of the Brix value of CCJ or CCJ-F [23]. Therefore, the fruit juice type could affect TPC and, thus, the antioxidant activities of treated dried apples.

Table 1. Sample preparation for the first part of the research.

Code of Sample	Name of Sample	SB Juice Concentration (w/w in Water)	Inulin–SB Juice Ratio in Solution (w/w)	Osmotic Solution Components in Grams During OD (Total Mass = 250 g)			Total Soluble Solid of Osmotic Solution [°Brix]	Time of OD [min]	Temperature of OD [°C]
				SB Juice	Water	Inulin			
FA	Freeze-dried fresh apple			-	-	-	-	-	-
FA + AA	Freeze-dried fresh apple + 1% ascorbic acid			-	-	-	-	-	-
SB0_I0	OD of fresh apple + 1% ascorbic acid (50 g) with freeze drying	0%	0:100	0.00	250.00	0.00	0.00 ± 0.00	30, 60, 120	30, 50
SB0_I15			15:85	0.00	212.50	37.50	13.90 ± 0.00		
SB0_I30		50%	30:70	0.00	175.00	75.00	28.43 ± 0.06		
SB50_I0			0:100	125.00	125.00	0.00	3.83 ± 0.06		
SB50_I15			15:85	106.25	106.25	37.50	17.37 ± 0.06		
SB50_I30			30:70	87.50	87.50	75.00	31.53 ± 0.06		
SB100_I0		100%	0:100	250.00	0.00	0.00	7.37 ± 0.06		
SB100_I15			15:85	212.50	0.00	37.50	20.40 ± 0.10		
SB100_I30			30:70	175.00	0.00	75.00	33.43 ± 0.15		

SB, sea buckthorn; OD, osmotic dehydration.

Table 2. Optimized conditions to obtain optimized TPC and antioxidant activities in freeze-dried samples and their validations.

Optimized Conditions *		TPC	ABTS	DPPH	ORAC	PCL-ACL	PCL-ACW
SB juice [g]		170.35	225.31	207.35	234.51	246.92	250.00
Water [g]		79.65	24.69	42.65	15.49	3.08	0.00
Inulin [g]		0.00	0.00	0.00	0.00	0.00	0.00
Time [min]		73.54	119.37	117.72	120.00	120.00	117.82
Temperature [°C]		38.82	30.11	31.33	30.00	30.00	30.40
Freeze-dried samples		TPC [mg GAE/ 100 g product]	ABTS [mg TE/ 100 g product]	DPPH [mg TE/ 100 g product]	ORAC [mg TE/ 100 g product]	PCL-ACL [mg TE/ 100 g product]	PCL-ACW [mg TE/ 100 g product]
FA		301.08 ± 13.79 a	449.33 ± 17.37 a	425.99 ± 18.35 b	3423.52 ± 52.93 a	950.62 ± 28.04 b	613.59 ± 5.76 b
FA + AA		623.81 ± 21.06 c	784.18 ± 16.11 b	877.35 ± 30.93 c	3579.68 ± 49.61 b	1138.25 ± 26.18 c	762.84 ± 24.61 c
Optimized conditions **	Validation mean value	427.35 ± 1.59 b	464.54 ± 21.35 a	325.03 ± 0.80 a	3532.58 ± 17.30 b	677.05 ± 6.25 a	471.29 ± 5.11 a
	Predicted mean value	498.39	447.08	324.43	3627.31	668.41	465.28
	95% confidence interval	480.75–516.66	427.23–467.36	311.65–337.45	3356.32–3908.84	644.44–692.38	425.72–506.62
	95% prediction interval	449.35–551.47	413.71–480.35	289.08–361.33	2920.17–4392.88	611.31–725.51	377.01–560.78
Code of samples		Opt_TPC	Opt_ABTS	Opt_DPPH	Opt_ORAC	Opt_PCL-ACL	Opt_PCL-ACW

SB, sea buckthorn. * The total mass of SB juice, water, and inulin in optimized conditions is 250 g. ** Optimized conditions mean that the TPC and antioxidant activities were obtained from freeze-dried samples prepared according to their respective optimized conditions. Different lowercase letters indicate significant differences ($p < 0.05$) between controls (FA and FA + AA) and validation mean values within the same column.

Regarding treatment time (Table 2), the optimized time was about 120 min for ABTS, DPPH, ORAC, PCL-ACW, and PCL-ACL, while for TPC, 73.54 min was the optimized time. The trend in TPC is consistent with previous work, as the dried Champion apple showed increased TPC during 90 min of OD using concentrated chokeberry juice, and then decreased about 8% at 120 min [24]. Regarding temperature (Table 2), the optimized temperature was about 30 °C for ABTS, DPPH, ORAC, PCL-ACW, and PCL-ACL, while 38.82 °C was the optimized temperature for TPC. A higher temperature might either positively or negatively affect mass transfer during the OD or impregnation process [25]. Higher temperatures may accelerate water loss and solid gain during OD, which then may increase the nutritional value of the end products; however, a higher temperature may also decrease the antioxidant activity as degradation of phenolics may occur, as well as leaching of products, in this case the apple's phenolics. A higher temperature can also reduce the viscosity of the osmotic solution and thus affect mass transfer [25]. Similar results were also found with respect to oven-dried mango slices, osmotically dehydrated using an emulsion of inulin and piquin pepper oleoresin, where treatment at 40 °C showed higher TPC and antioxidant activity than treatment at 30 and 50 °C [9].

The predicted and validation mean values of the optimized conditions in each assay were evaluated (Table 2). The validation mean values of all antioxidant activities (ABTS, DPPH, ORAC, PCL-ACL, and PCL-ACW) fall within the range of 95% prediction and confidence intervals, indicating that the predicted optimized conditions are consistent with the validation of observed data. In contrast, TPC's validation mean value falls outside both intervals, which might be caused by the limitations of the Folin-Ciocalteu assay. It is a simple, reproducible, robust, and comparable assay, yet it is sensitive to pH, temperature, and reaction time. This assay also includes non-phenolic reducing agents, such as reducing sugars, some amino acids, organic acids, ascorbic acid, etc., that could interfere with the TPC results [26,27]. Nevertheless, TPC still showed high and significant positive correlations ($p < 0.05$) with all antioxidant activities, for which Pearson's coefficients ranged from 0.91 to 0.98 (Table S2).

Among the optimized conditions, the conditions to obtain the optimum ORAC value (Opt_ORAC; 93.8% SB juice in water, 0:100 inulin–SB juice ratio, 30 °C, 120 min, based on Table 2) were chosen for the second part of the research, as the ORAC assay uses peroxy radicals, which are biologically relevant free radicals [26]. Freeze-dried apples prepared in Opt_ORAC conditions showed significantly higher ORAC values (3532.58 mg TE/100 g product) than FA (3423.52 mg TE/100 g product, $p < 0.05$), yet still similar to FA + AA (3579.68 mg TE/100 g product, $p > 0.05$) (Table 2). This means that freeze-dried apples prepared in Opt_ORAC conditions involving SB juice could recover the loss of antioxidant activity during the treatment, especially when compared to treatments with 0–50% SB juice and inulin–SB juice ratios of 15:85 and 30:70 at different temperatures and times (Tables 1 and S1).

2.1.2. Color of Freeze-Dried Apples Prepared in Optimized Conditions

Visual perception, such as perception of color, plays an important role in the selection of healthy food products by consumers. The appearance, L, a, and b values, and ΔE , BI, WI, and YI of freeze-dried apples prepared in optimized conditions (Opt_TPC, Opt_ABTS, Opt_DPPH, Opt_ORAC, Opt_PCL-ACL, and Opt_PCL-ACW) and of the controls (FA and FA + AA) were measured and described in Table 3. FA + AA possessed the highest L value (81.06), higher than FA (70.96) and optimized samples (45.14–62.19). Dipping apples in an ascorbic acid solution prevents the melanin formation of apples by binding to intermediate products, e.g., o-quinone, which results in a higher lightness [28]. Furthermore, compared to the controls (FA and FA + AA), all optimized freeze-dried samples showed lower WI

(ranged from 24.61–33.58) and higher *a* values (redness; 20.18–23.79), *b* values (yellowness; 45.71–50.74), ΔE (35.20–45.43), BI (165.13–248.64), and YI (116.57–145.74). However, the higher ΔE , BI, and YI are not always related to higher enzymatic browning activity as it can be caused by the color of dipping solutions [29]. In this case, the phenomenon was caused by the orange color of SB juice in this study (*L* value: 41.77 ± 0.19 ; *a* value: 23.34 ± 0.03 ; *b* value: 47.92 ± 0.43), noting that SB juice contains carotenoids [14]. Polyphenol oxidase and peroxidase activities were observed to confirm the effect of SB juice impregnation on the enzymatic browning activity of treated freeze-dried apples (Section 2.3.3.). The orange color of the treated product could also be an alternative in terms of producing a functional food with appealing color, particularly for kids.

Different types of fruit juice (apple, grape, and cranberry) used in the OD process also affected the color of hot-air-dried apple chips [6]. However, the authors performed the hot-air drying process at 70 °C, which may have also influenced the final color of the apple chips through enzymatic browning, Maillard reaction, and ascorbic acid oxidation. In the present study, a non-thermal freeze-drying process was performed to prepare the end products, thus those thermal-related factors may not have influenced the color of treated freeze-dried apples.

2.2. Second Part of Research

In the second part, the conditions of Opt_ORAC from the first part were applied, with the addition of 0–6% CaL. The effect of CaL addition on the TPC, antioxidant activities (Table 4), and calcium content (Figure 1) of treated freeze-dried apples was observed. In Table 4, increasing the CaL concentration resulted in significant decreasing values of TPC and all antioxidant activities in treated freeze-dried apples. The highest decrease was found in freeze-dried apples prepared with 6% CaL, which possessed 38, 37, 24, 45, 35, 56, and 45% lower values of TPC, ABTS, DPPH, ORAC, PCL-ACL, PCL-ACW, and PCL-IAC, respectively, than those prepared with 0% CaL. This phenomenon may be caused by interaction between bioactive compounds, such as phenolics and non-reactive redox metal–calcium. A previous study showed that in food and beverages, calcium may the lower antioxidant activity of plant phenolics through complexation or by precipitation [30]. Additionally, the decrease may also be explained in terms similar to the case of inulin in Section 2.1, in which CaL was adsorbed in the apple flesh during the impregnation process, and subsequently, heavier freeze-dried products were obtained and lower TPC and antioxidant activities were observed per 100 g of end products. The increased weights of freeze-dried samples: 10.9, 19.9, 31.4, and 37.0% were observed in samples at 1, 2, 4, and 6% CaL, respectively, in comparison to the weight of 0% CaL sample.

Those results were also supported by the phenomenon in which the addition of 1–6% CaL significantly increased the calcium content of the freeze-dried apples, accounting for 828.11–3408.04 mg Ca/100 g of product (Figure 1A). The impregnation process promoted the adsorption of CaL, and subsequently, the calcium content increased in the freeze-dried products. In other studies, an increase in calcium content was also found in OD-treated plant matrices using a solution with CaL. Tappi et al. [31] observed increased calcium content in OD-treated apples in solutions containing 40% sucrose and 4% CaL, with and without 2% ascorbic acid. For OD of more than 2 h, samples treated in a solution with ascorbic acid showed a significantly higher increase in calcium content than those treated in a solution without ascorbic acid. In a study of pineapples, 6 h of OD using solutions of (a) 40% sucrose with 4% CaL and (b) 50% sucrose with 4% CaL resulted in pineapples with approximately 90 mg Ca/100 g of wet basis [4].

Table 3. The appearance and color analysis of freeze-dried apples: FA, FA + AA, and apples treated using optimized TPC and antioxidant activities' conditions.

Treatment	Appearance	L Value	A Value	b Value	ΔE	BI	WI	YI
FA		70.96 ± 0.52 d	5.99 ± 0.45 b	29.26 ± 0.63 a	11.17 ± 0.54 a	58.13 ± 1.90 b	58.33 ± 0.57 e	58.92 ± 1.25 b
FA + AA		81.06 ± 0.37 e	1.58 ± 0.10 a	27.57 ± 0.27 a	-	41.97 ± 0.53 a	66.51 ± 0.23 f	48.58 ± 0.42 a
Opt_TPC		62.19 ± 0.49 c	20.18 ± 0.27 c	50.74 ± 0.13 d	35.20 ± 0.44 b	165.13 ± 2.70 c	33.58 ± 0.41 d	116.57 ± 1.08 c
Opt_ABTS		52.27 ± 1.90 b	22.31 ± 0.47 de	48.56 ± 1.44 cd	41.28 ± 0.53 d	208.94 ± 4.42 e	28.31 ± 0.41 b	132.76 ± 1.55 e
Opt_DPPH		53.74 ± 2.36 b	20.21 ± 0.37 c	47.43 ± 0.57 bc	38.61 ± 1.57 c	190.16 ± 11.25 d	30.71 ± 1.32 c	126.25 ± 4.19 d
Opt_ORAC		47.74 ± 2.25 a	23.79 ± 1.42 f	48.72 ± 3.09 cd	45.43 ± 0.78 e	248.64 ± 12.57 f	24.61 ± 1.16 a	145.74 ± 4.36 f
Opt_PCL-ACL		47.64 ± 1.36 a	23.11 ± 0.73 ef	47.38 ± 2.16 bc	44.48 ± 0.43 e	237.00 ± 7.23 f	25.67 ± 0.70 a	142.02 ± 2.50 f
Opt_PCL-ACW		45.14 ± 0.13 a	21.9 ± 0.11 d	45.71 ± 0.11 b	45.08 ± 0.18 e	244.59 ± 2.17 f	25.31 ± 0.17 a	144.69 ± 0.68 f

ΔE is the total color difference between FA + AA and related samples. Different lowercase letters indicate significant differences ($p < 0.05$) between samples within the same column.

Table 4. The effect of calcium lactate addition on the TPC and antioxidant activities of freeze-dried apples treated using Opt_ORAC conditions (93.8% SB juice in water, 0:100 inulin–SB juice ratio, 30 °C, 120 min).

Treatment	TPC [mg GAE/100 g Product]	ABTS [mg TE/100 g Product]	DPPH [mg TE/100 g Product]	ORAC [mg TE/100 g Product]	PCL-ACL [mg TE/100 g Product]	PCL-ACW [mg TE/100 g Product]	PCL-IAC [mg TE/100 g Product]
0% CaL	453.33 ± 8.45 d	525.43 ± 24.91 d	346.51 ± 7.94 d	3532.58 ± 17.30 d	710.32 ± 11.84 c	601.40 ± 15.42 d	1311.72 ± 18.98 d
1% CaL	369.68 ± 8.98 c	425.26 ± 3.97 c	307.24 ± 1.90 c	2745.31 ± 177.85 c	583.02 ± 29.29 b	447.85 ± 3.37 c	1030.87 ± 26.24 c
2% CaL	347.41 ± 11.25 bc	394.12 ± 14.29 bc	285.48 ± 1.09 b	2474.84 ± 131.01 bc	503.19 ± 20.62 a	314.31 ± 6.94 b	817.50 ± 21.11 b
4% CaL	331.02 ± 13.99 b	385.69 ± 3.27 b	281.76 ± 7.25 b	2275.52 ± 37.04 b	475.81 ± 8.63 a	282.12 ± 7.46 a	757.93 ± 15.92 ab
6% CaL	280.46 ± 3.79 a	333.18 ± 6.67 a	262.82 ± 2.19 a	1942.63 ± 86.43 a	461.80 ± 25.31 a	265.86 ± 3.01 a	727.66 ± 28.25 a

CaL, calcium lactate. PCL-IAC is the sum of PCL-ACL and PCL-ACW. Different lowercase letters indicate significant differences ($p < 0.05$) between samples within the same column.

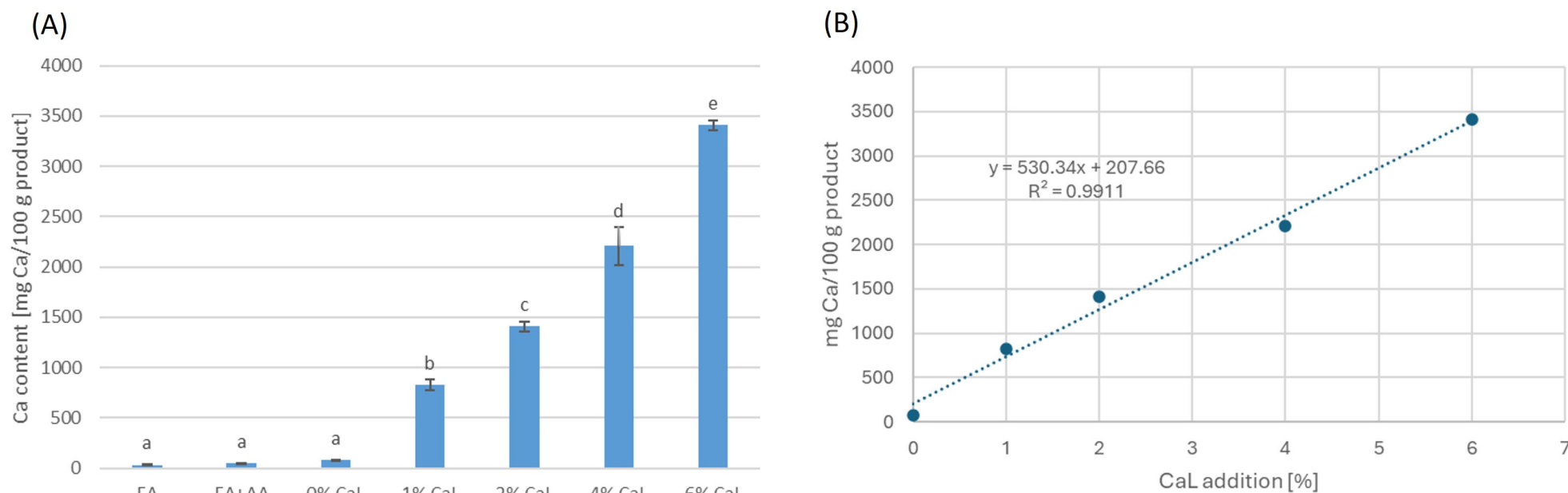


Figure 1. The effect of calcium lactate addition on the calcium content of freeze-dried controls (FA and FA + AA) and treated freeze-dried apples: (A) calcium content; (B) linear regression analysis (0–6% CaL addition). Different lowercase letters in (A) indicate significant differences ($p < 0.05$) between samples.

Furthermore, it was also noticed that at 0% CaL (Figure 1A), the calcium content was 79.44 mg Ca/100 g of product, which is about 2.4 and 1.8 times higher than that of FA and FA + AA, respectively, yet they were similar ($p > 0.05$). Despite the insignificance, this increase might be explained in terms of the SB juice used in the osmotic solution of this study, which also contains 12.7 mg Ca/100 g. The calcium content of the SB juice was slightly higher but comparable to the total in a review by Ciesarová et al. [13], who observed that the calcium content of SB juice ranged from 2.1 to 10.9 mg Ca/100 g, depending on the country of origin. Additionally, from the calcium content of samples prepared at 0–6% CaL, a linear regression model (Figure 1B) was obtained with the equation of [Ca content in mg Ca/100 g] = 530.34 [%CaL addition] + 207.66 ($R^2 = 0.9911$).

Besides antioxidant properties, the calcium content of end products was also considered. It was observed that the TPC and antioxidant activities, except PCL-ACW, of the samples prepared with 2 and 4% CaL were similar ($p > 0.05$) (Table 4). Noting that the freeze-dried apples prepared at 4% CaL contain 2209.13 mg Ca/100 g, which was 1.57 times higher than those prepared at 2% CaL, the suggested CaL concentration is 4% CaL. The current daily recommended dietary allowance (RDA) of calcium is 1000 mg Ca for adults 19–50 years of age; absorption of dietary calcium in adults is 25%, which decreases with age [32]. By assuming an absorption of 25%, consuming 100 g of freeze-dried apples prepared after impregnation using 93.8% SB juice in water, at a 0:100 inulin–SB juice ratio, with the addition of 4% CaL at 30 °C for 120 min (hereafter called 4% CaL treatment), could fulfill 55.2% of the RDA, with a minimum loss of antioxidant activity and TPC in the end products. The total soluble solid of this suggested solution was 8.63 ± 0.06 °Brix, which was slightly hypotonic when compared to FA + AA (10.13 °Brix) during the treatment process. Therefore, the term impregnation is used hereafter instead of OD, as with OD, mass transfer takes place when water from the food matrix passes into the hypertonic solution through apoplastic, symplastic, and transmembrane transports [7].

2.3. Analyses of Selected Samples

Based on the results of the first and second parts of the research, the freeze-dried products following 4% CaL treatment and the controls (FA and FA + AA) were selected for further analyses.

2.3.1. Quantification of Phenolic and Carotenoid Composition of Freeze-Dried Apples by UPLC-PDA

The quantification of phenolic and carotenoid content in selected freeze-dried samples (FA, FA + AA, and 4% CaL) by the UPLC-PDA method is shown in Table 5. Phenolic substances aid in the prevention and treatment of osteoporosis not only by inhibiting oxidative stress, but also by affecting bone metabolism, reducing bone resorption, maintaining bone density, and lowering osteoclast differentiation [33]. In terms of phenolics, flavan-3-ols dominated the phenolic composition of FA and FA + AA at 51.7 and 54.8%, respectively, followed by phenolic acids, which accounted for about 38% in each sample, while flavonols (46.36 mg/100 g product) dominated the phenolic composition of the 4% CaL sample at 43.5%, followed by flavan-3-ols at 41.7%. The 4% CaL sample had a significantly higher content and more types of flavonols than FA (1.07 mg/100 g product) and FA + AA (0.96 mg/100 g product). Among flavonols, only quercetin-3-O-rhamnoside (compound 21) was found in all samples, while the rest were found only in 4% CaL. Impregnation using SB juice in the 4% CaL sample enriched the flavonols of the final product. The flavonol composition of 4% CaL was in agreement with that of the berries of six Polish SB cultivars [34], where compounds 10–24 were also found, with isorhamnetin-3-O-glucoside and isorhamnetin-3-O-rutinoside as the two dominant compounds. Those two compounds were also considered as major

flavonol glycosides in SB berries from Canada, Finland, and China [35]. Flavonol glycosides in SB have been studied with respect to prevention of chronic diseases, including diabetes, cancer, and cardiovascular disease [36]. Despite the increase in flavonol content, 4% CaL had 13.96 mg of phenolic acids, which was about 60–64% lower than FA and FA + AA ($p < 0.05$). The sum of dihydrochalcones in 4% CaL was also significantly lower than the sum of dihydrochalcones in the control groups, while for flavan-3-ols, 4% CaL possessed a higher catechin and procyanidin B1 content yet lower epicatechin and procyanidin C1 compared to the controls. The highest total phenolic content was found in 4% CaL (106.49 mg/100 g product), about 3.3% and 19.2% higher than FA + AA and FA, respectively. The total phenolic trend via the UPLC-PDA method was generally different for TPC and antioxidant activities via the Folin–Ciocalteu method, where the TPC and antioxidant activities of 4% CaL were lower than the controls, yet it is worth noticing that the UPLC-PDA and Folin–Ciocalteu methods are two different methods with their own advantages and limitations. Interference from non-phenolic compounds may overestimate TPC in the Folin–Ciocalteu method [26]; in this case, most likely from ascorbic acid. Liquid chromatography focuses only on specific or determined compounds of interest, and there might be other compounds that are not covered [37] in this study; for example, non-glycoside flavonoids (aglycones). It was also found that among all apple cultivars, the flesh of Oldenburger had the highest TPC and antioxidant activities (ABTS and ORAC), yet total polyphenols via high-performance liquid chromatography with diode array detection were not the highest [38]. Another limitation of the Folin–Ciocalteu method is also gallic acid usage as a standard, which reacts more strongly than other phenolics and thus may overestimate TPC [37]. The principle means of quantifying TPC in the Folin–Ciocalteu and liquid chromatography methods are also different; the first one focuses on reduction reactions using tungsten and molybdenum-containing reagents and is spectrophotometrically evaluated, while the second one focuses on separation and quantification of analytes present in the samples [39]. Additionally, another study observed weak correlations between the sum of flavonols and antioxidant activities by ABTS, DPPH, and FRAP methods in various apple cultivars in Poland, whereas the sum of flavan-3-ols and the sum of hydroxycinnamic acids exhibited higher positive correlations with antioxidant activities [40].

Carotenoids have been shown to have antiosteoporotic effects and a bone-building support effect [3]. The 4% CaL sample possessed a total of 38.63 mg of carotenoids per 100 g of product, about 17 times higher than that of the controls, which could be caused by the involvement of SB juice during the impregnation process. Carotene dominated the carotenoids in 4% CaL at 74%. It is worth noting that the composition of carotenoids varied with respect to commercialized SB juice in Poland [41]. Furthermore, the totals and compositions of carotenoids in FA and FA + AA in this study were comparable to the results observed previously, particularly for Royal Gala flesh [42,43].

In this study, phenolic and carotenoid compositional changes in freeze-dried apples were affected by both the impregnation process using SB juice and CaL and also by dipping in an ascorbic acid solution, which then may influence the properties of end products.

Table 5. UPLC-PDA quantification data for phenolic and carotenoid composition in freeze-dried apples: FA, FA + AA, and apples treated using Opt_ORAC conditions (93.8% SB juice in water, 0:100 inulin–SB juice ratio, 30 °C, 120 min) with the addition of 4% CaL.

Compounds	No. Compound	Total Content [mg/100 g Product]		
		FA	FA + AA	4% CaL
PHENOLICS				
Phenolic acids				
p-Coumaric acid- <i>O</i> -hexoside	1	30.71 ± 0.09 c	35.3 ± 0.03 b	13.04 ± 0.17 a
Ferulic acid- <i>O</i> -hexoside	2	3.88 ± 0.08 b	3.91 ± 0.05 b	0.92 ± 0.10 a
Sum of phenolic acids		34.59 ± 0.17 b	39.21 ± 0.02 c	13.96 ± 0.08 a
Flavan-3-ols				
(+)-Catechin	3	3.60 ± 0.18 a	6.25 ± 0.13 b	17.97 ± 0.19 c
Procyanidin B1	4	nd	nd	17.88 ± 0.04
(-)-Epicatechin	5	10.59 ± 0.63 b	13.06 ± 0.01 c	3.03 ± 0.10 a
Procyanidin C1	6	32.04 ± 1.32 b	37.21 ± 0.57 c	5.48 ± 0.33 a
Sum of flavan-3-ols		46.24 ± 0.51 a	56.53 ± 0.43 b	44.36 ± 0.58 a
Dihydrochalcones				
Phloretin-2- <i>O</i> -xyloglucoside	7	2.15 ± 0.00 c	1.61 ± 0.02 a	1.81 ± 0.04 b
Derivative of dihydrochalcones	8	3.83 ± 0.08 b	3.48 ± 0.11 a	nd
Phloretin-2- <i>O</i> -glucoside	9	1.47 ± 0.31 a	1.36 ± 0.07 a	nd
Sum of dihydrochalcones		7.46 ± 0.4 b	6.44 ± 0.2 b	1.81 ± 0.04 a
Flavonols				
Isorhamnetin-3,7- <i>O</i> -dihexoside	10	nd	nd	4.28 ± 0.08
Isorhamnetin-3- <i>O</i> -sophoroside-7- <i>O</i> -rhamnoside	11	nd	nd	1.93 ± 0.05
Quercetin-3- <i>O</i> -(dirhamnosyl)hexoside	12	nd	nd	3.68 ± 0.20
Quercetin-3-galactoside-7- <i>O</i> -rhamnoside	13	nd	nd	1.02 ± 0.20
Quercetin-3- <i>O</i> -rutinoside	14	nd	nd	1.24 ± 0.06
Isorhamnetin-3- <i>O</i> -glucoside-7- <i>O</i> -rhamnoside	15	nd	nd	2.65 ± 0.17
Quercetin-3- <i>O</i> -glucoside	16	nd	nd	4.56 ± 0.09
Isorhamnetin-3- <i>O</i> -(2-rhamnosyl)hexoside	17	nd	nd	2.50 ± 0.00
Kaempferol-3- <i>O</i> -hexoside-7- <i>O</i> -rhamnoside	18	nd	nd	4.06 ± 0.23
Isorhamnetin-3- <i>O</i> -(rhamnosyl)hexoside	19	nd	nd	0.72 ± 0.00
Isorhamnetin-3- <i>O</i> -rutinoside	20	nd	nd	8.23 ± 0.00
Quercetin-3- <i>O</i> -rhamnoside	21	1.07 ± 0.08 a	0.96 ± 0.04 a	1.81 ± 0.03 b
Isorhamnetin-3- <i>O</i> -glucoside	22	nd	nd	8.72 ± 0.02
Kaempferol-3- <i>O</i> -rutinoside	23	nd	nd	0.63 ± 0.02
Isorhamnetin-3- <i>O</i> -rhamnoside	24	nd	nd	0.32 ± 0.05
Sum of flavonols		1.07 ± 0.08a	0.96 ± 0.04a	46.36 ± 0.39 b
Total phenolics		89.36 ± 0.36 a	103.14 ± 0.26 b	106.49 ± 0.86 c
CAROTENOIDS				
Lutein isomer	25	0.28 ± 0.01 a	0.25 ± 0.04 a	nd
All-trans-lutein	26	0.19 ± 0.12 a	0.17 ± 0.02 a	nd
Zeaxanthin isomer	27	nd	nd	9.75 ± 1.71
Sum of carotene	28	1.75 ± 0.10 a	1.76 ± 0.05 a	28.57 ± 0.73 b
Phytofluene	29	0.07 ± 0.00 a	0.05 ± 0.01 a	0.31 ± 0.00 b
Total carotenoids		2.29 ± 0.24 a	2.23 ± 0.02 a	38.63 ± 2.45 b

nd, not detected. Different lowercase letters indicate significant differences ($p < 0.05$) between samples within the same row.

2.3.2. Sensory Profiling of Freeze-Dried Apples

In the development of functional food products, it is necessary to ensure that the products are not only nutritionally beneficial and promote health but also sensorially acceptable to consumers. Therefore, three selected freeze-dried samples (FA, FA + AA, and 4% CaL) were evaluated via sensory profiling and overall acceptance (Figure 2). For the attribute of appearance, 4% CaL was profiled as having darker yellowness, brownness, and higher color uniformity in comparison to FA and FA + AA as controls, noting that 4% CaL used SB juice containing carotenoids during its preparation. It is also worth noticing that the stability of carotenoids is influenced by environmental factors, including pH. A study reviewed that depending on the food matrix, acidic pH may either improve (due to the higher solubility of carotenoids) or decrease (due to isomerization) the intensity of the yellow-orange color of a food product [44]. Furthermore, the lightest brownness and yellowness were found in FA + AA, which was influenced by ascorbic acid and enzymatic browning inhibition. The utilization of SB juice might also be responsible for the more intense grassy and sea buckthorn aroma in 4% CaL compared to the controls. Despite the foreign aroma being more noticeable at 4% CaL, the score was rather low (2.55). In terms of taste, the 4% CaL samples possessed a high sourness intensity, a rather high astringency intensity, and a low bitterness and sweetness intensity. This was in accordance with a previous study, as sensations of astringency and sourness were more noticeable than sensations of sweetness and bitterness in SB puree from various cultivars [45]. Malic acid contributes to the sourness, while quinic acid is responsible for the astringency of SB berries [14]. Additionally, tastes of umami and saltiness were negligible, as the scores were below 1 in all samples. For texture, the addition of CaL in the 4% CaL treatment might play a significant role in terms of improving its crispiness (8.00), which was higher in comparison to the controls. CaL could form complexes with carboxyl groups of pectin, which is available in apples, and thus affect the firmness and texture of the product [25]. Furthermore, 4% CaL was also as soft as the controls and showed low adhesiveness to palate, which is then suitable for the elderly, who are prone to osteoporosis, noting that foods with soft texture and low adhesiveness could be easily swallowed by them [46]. For overall acceptance, 4% CaL was moderately acceptable (5.02) to the panelists, but still showed a lower score than FA (6.60) and FA + AA (7.82). The lower overall acceptability of 4% CaL might be caused by the imbalanced taste of SB juice as one of the ingredients, especially the high sourness intensity. This was supported by a previous study in which most of the panelists described the apple chips as crispy and sweet with a sourish apple taste [47]. Therefore, improvements in sensory properties should be investigated in further studies involving inulin, for example, but with an inulin–SB juice ratio lower than 15:85, as inulin could also increase the sweetness but with lower calories than sugar. Furthermore, the means of consuming this 4% CaL sample could also be modified—for example, as a topping for yogurt, ice cream, or fruit salad—in order to perceive a more balanced flavor, instead of it being consumed directly as chips. If necessary, involving safe and natural fruit flavors in the formulation may also compromise the sourness and astringency of the product, achieving more balanced sensory properties. Further studies on sensorial improvement without significantly deteriorating the health benefits of the product are encouraged.

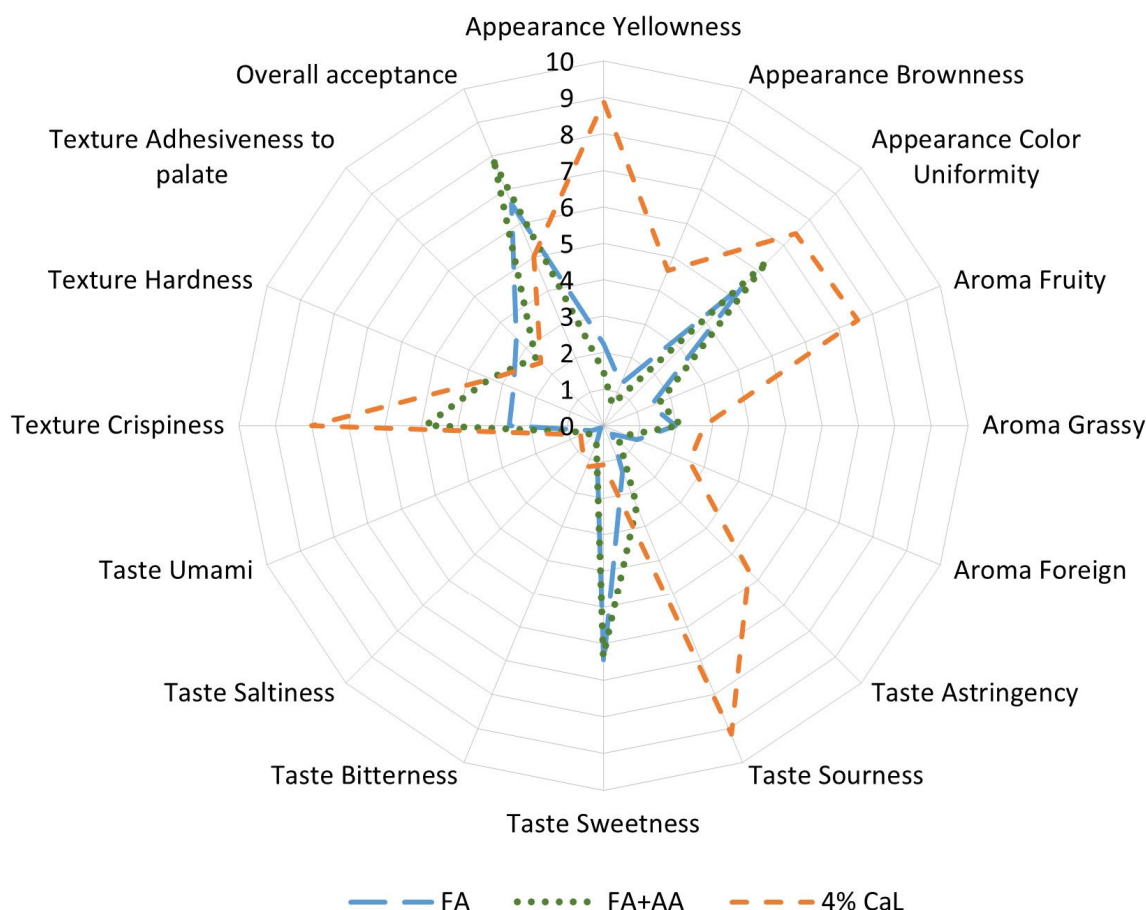


Figure 2. Sensory profiling of freeze-dried apples: FA, FA + AA, and apples treated using Opt_ORAC conditions (93.8% SB juice in water, 0:100 inulin–SB juice ratio, 30 °C, 120 min) with the addition of 4% CaL. Scores 0–10 for descriptors of each attribute are described in Table S3.

2.3.3. Activity of Enzymes

The PPO and POD activities of FA, FA + AA, and 4% CaL were also measured (Table 6). The FA + AA and 4% CaL significantly reduced 13.7 and 88.9% of PPO activity in comparison to FA. For POD activity, the 4% CaL treatment inhibited 43.2% of POD activity in comparison to FA. In the preliminary study, it is worth noting that the SB juice used in this research did not show either PPO or POD activity, which is as expected as the SB juice was pasteurized and might denature the enzymes. Therefore, the inhibition of PPO and POD activities in 4% CaL might be caused by the presence of SB juice, which, based on previous studies, contains lipophilic (carotenoids and tocopherols) and hydrophilic antioxidants (flavonoids, tannins, ascorbic acid, phenolics) [13,14] and may inhibit PPO and POD activity in apple products [29]. It is also worth noting that in Table 5, the sum of phenolic acids in FA and FA + AA were higher than in 4% CaL, which then may act as substrates of PPO and POD and could stimulate enzymatic browning. However, the relationship between phenolic acids and prevention of enzymatic browning is also cultivar-specific; some cultivars may have high phenolic acid content but low PPO and POD activities, while others may show the opposite effect [29].

The POD activity of FA + AA was more than twice as high as that of FA. A similar result was found in a previous study [48], where dipping Fuji apple slices in 1% ascorbic acid for 1 min increased the POD activity of the slices, while for solutions that combined ascorbic acid with other anti-browning agents, inhibition of POD activity was observed. The authors explained that ascorbic acid may also act as a pro-oxidant and subsequently exhibit the opposite effect with respect to hydrogen peroxide and peroxidation.

Table 6. Activity of enzymes and proximate analysis of freeze-dried apples: FA, FA + AA, and apples treated using Opt_ORAC conditions (93.8% SB juice in water, 0:100 inulin–SB juice ratio, 30 °C, 120 min) with the addition of 4% CaL.

Analyses	Unit	FA	FA + AA	4% CaL
Activity of enzymes				
PPO activity	U/g product	3894.81 ± 247.07 c	3358.52 ± 175.90 b	432.59 ± 28.57 a
POD activity	U/g product	717.78 ± 26.94 b	1642.22 ± 36.72 c	407.41 ± 12.83 a
Proximate analysis				
Protein	g/100 g product	4.38 ± 0.08 b	3.52 ± 0.19 a	5.14 ± 0.38 c
Lipid	g/100 g product	0.53 ± 0.03 a	0.68 ± 0.03 a	3.64 ± 0.18 b
Carbohydrate	g/100 g product	75.20 ± 0.27 b	73.29 ± 0.36 b	48.96 ± 1.47 a
Ash	g/100 g product	1.27 ± 0.11 a	1.12 ± 0.05 a	8.81 ± 0.29 b
Moisture	g/100 g product	6.39 ± 0.29 a	8.95 ± 0.34 b	15.36 ± 0.77 c
TDF	g/100 g product	12.22 ± 0.29 a	12.44 ± 0.62 a	18.09 ± 0.31 b
SDF	g/100 g product	2.57 ± 0.62 a	3.05 ± 0.47 a	4.18 ± 0.06 b
IDF	g/100 g product	9.65 ± 0.48 a	9.39 ± 0.36 a	13.90 ± 0.37 b
Energy value	kcal/100 g product	347.53	338.24	285.34

Carbohydrate was calculated using the formula of 100 g—(mass in g [protein + lipid + ash + moisture + TDF]) in 100 g of product. Energy value was calculated using the conversion based on Regulation (EU) No 1169/2011: carbohydrates—4 kcal/g, protein—4 kcal/g, lipid—9 kcal/g, and fiber (TDF)—2 kcal/g. Different lowercase letters indicate significant differences ($p < 0.05$) between samples within the same row.

The results observed in this study provided information with respect to the impregnation of apple flesh using SB juice and calcium lactate, which inhibited the PPO and POD activity of the resulting freeze-dried apples. However, the mechanism of inhibition and application in terms of other SB cultivar juices should be further studied. To date, according to our knowledge, the utilization of SB juice to prevent PPO and POD activity in plant matrices has not been carried out. Tkacz et al. [49] investigated other enzymatic activity of Polish cultivars with respect to SB fruit extracts, such as anti- α -amylase, anti-glucosidase, anti-lipase, and anti-lipoxygenase activity, which indicated that SB fruit may potentially be of benefit to general health. Instead of SB juice, in the preliminary study of Zhang et al. [50], the authors found that SB fruit extract showed an anti-browning effect in fresh-cut potato sticks based on ΔE and sensory analysis in comparison to the control group (water-treated). However, the effect was still weaker than for SB leaf extract [50]. The SB leaf extract was also incorporated into a carboxymethyl cellulose-based edible coating, which successfully inhibited the browning index, PPO, and POD activities in apples during storage [51]. Therefore, the potency of SB juice as a natural anti-browning agent, especially in terms of its mechanism of inhibition, could be further investigated in the future.

2.3.4. Proximate Analysis of Freeze-Dried Apples

The proximate analysis of FA, FA + AA, and 4% CaL is shown in Table 6. In comparison to the controls, 4% CaL freeze-dried apples exhibited significantly higher protein and lipid content, with lower carbohydrate content ($p < 0.05$). This could be due to the involvement of SB juice in the 4% CaL samples during the treatment. Dong et al. [14] reported that SB berry contains 3% lipids and major fatty acids, such as palmitoleic (32–52%), palmitic (26–36%), oleic (10–26%), linoleic (5–16%), and linolenic acids (6%), while the protein content of SB juice varied from 52–766 mg/100 g, with 42–46% of the total essential amino acids. SB juice also contains 2.7–5.3 g of sugar per 100 mL, dominated by glucose, fructose, and xylose [14]. The lowest moisture content of freeze-dried apples was found in FA (6.39 g/100 g), which was comparable to 6.8 g/100 g of freeze-dried Braeburn apple slices [52]. A similar result was also found by Wang et al. [6], who observed a slightly higher moisture content in dried apples treated by OD with different fruit juices, in comparison to that of dried untreated

apples. The ash content of 4% CaL was about 7–8 times higher than the controls, which was in accordance with the calcium content of the product (Figure 1A). Although inulin was not involved in the suggested conditions, the 4% CaL sample still possessed significantly higher soluble dietary fiber (SDF), as well as insoluble (IDF) and total dietary fiber (TDF), than the controls ($p < 0.05$), which could be due to the contribution of SB juice. The higher dietary fiber of 4% CaL, especially SDF, might be due to the pectin content in SB, which ranged from 0.21 to 0.68 g/100 g of fresh weight [49]. The 4% CaL treatment also possessed 285.34 kcal/100 g of product, which was about 15.5–17.9% lower than the energy values of FA + AA and FA, respectively. This, then, has the potential to be an alternative functional food or healthy snack with lower calories, reducing the risk of osteoporosis.

3. Materials and Methods

3.1. Raw Materials and Chemicals

Apples (*Malus domestica* cv. Gala) were acquired in September 2022 from organic farm ‘Dębski Sad’, Rabowice, Poland, and were then transported to the laboratory and processed immediately. The apples were washed, cleaned, cored, peeled (2 mm thick peel), and sliced using an apple peeler and slicer to obtain the C-shaped flesh slices (5 mm thick). The sliced apple flesh was dipped in a solution of 1% ascorbic acid (Stanlab sp. z o.o., Lublin, Poland) for 2 min at a sample–solution ratio of 1:4 to prevent early enzymatic browning, then blotted with absorbing paper and frozen ($-40\text{ }^{\circ}\text{C}$) until further analysis. The prepared flesh had total soluble solids of $10.13 \pm 0.06\text{ }^{\circ}\text{Brix}$ (PAL-1 Atago, Fukaya-shi, Japan). The fresh apple flesh ($13.27 \pm 0.06\text{ }^{\circ}\text{Brix}$) without dipping in ascorbic acid was also prepared and frozen ($-40\text{ }^{\circ}\text{C}$).

Pasteurized, 100% sea buckthorn (*Hippophae rhamnoides*) juice not from concentrate ($7.37 \pm 0.06\text{ }^{\circ}\text{Brix}$) was purchased from Oleofarm sp. z o.o., Wrocław, Poland. Inulin (Orafti HSI) was purchased from Hortimex Plus sp. z o.o., Konin, Poland. Food-grade calcium lactate was purchased from Agnex sp. z o.o., Białystok, Poland.

Chemicals, including 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azobis(2-methylpropionamidine) dihydrochloride (AAPH), fluorescein sodium salt, ($\pm$)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), Folin–Ciocalteu's phenol reagent, gallic acid, 65% nitric acid, methanol, potassium persulfate, sodium carbonate, lanthanum (III) chloride, monopotassium phosphate, disodium phosphate, polyvinylpyrrolidone (PVPP), catechol, and guaiacol, were purchased from Sigma-Aldrich, Hamburg, Germany. An ACL reagent kit (lipid-soluble substances) and ACW reagent kit (water-soluble substances) for the photochemiluminescence (PCL) assay were purchased from Analytik Jena, Jena, Germany. The solvents for the ultra-performance liquid chromatography (UPLC) method were bought from Sigma Aldrich, Hamburg, Germany. The standards for the phenolic and carotenoid compounds were obtained from Extrasynthese (Genay, France).

3.2. Osmotic Dehydration or Impregnation

The research plan was divided into two parts. In the first part, the effect of various osmotic solution compositions, such as sea buckthorn (SB) juice concentrations (0, 50, and 100% w/w in water) and inulin–SB juice ratios (0:100, 15:85, 30:70), and process parameters, such as temperature and time (Table 1), on the TPC and antioxidant activities of treated apple flesh was observed. The concentration of calcium lactate (CaL) was controlled at 0% in this first part. For this part, 50 g of frozen apple slices, after dipping in 1% ascorbic acid, were placed in a tightly closed polyethylene terephthalate (PET) jar. The freezing process may rupture the cellular structure of the flesh, which can improve the incorporation of substances. The osmotic solution with the abovementioned composition was added to the

PET jar at sample–solution ratio of 1:5. This ratio was chosen to maintain the concentration of solutions during the treatments. The samples were then placed in a water bath (Laboplay SWB 22N, Bytom, Poland) with continuous shaking (150 rpm and a vibration amplitude of 14 mm) at 30 and 50 °C for 30, 60, and 120 min. The treated samples were drained and blotted using absorbing paper and frozen (−40 °C) for 24 h. Afterwards, the samples were freeze dried for 48 h. Freeze-dried fresh apple (FA), freeze-dried fresh apple after dipping in 1% ascorbic acid (FA + AA), as well as freeze-dried treated apples (Table 1) were ground to powder and were ready for analysis. The OD or impregnation process was conducted in triplicate. The optimization of process conditions to obtain the highest TPC and antioxidant activities (ABTS, DPPH, ORAC, PCL) was conducted using Design Expert 13 (Stat-Ease, Minneapolis, MN, USA) (Section 3.4).

In the second part, the treatment process was similar to the first part, only that in this part, the optimized conditions to obtain the highest ORAC value were used (93.8% of SB juice, 0:100 inulin–SB juice ratio, 30 °C, 120 min, Section 2.1.1.), noting that ORAC is more relevant to *in vivo* conditions because it utilizes peroxy radical as the biologically relevant free radical source, reflecting the major mechanisms of antioxidant action regarding cell protection [53]. CaL at a concentration of 0, 1, 2, 4, 6% (*w/w*) was then added to the osmotic solution. This range of CaL concentration was chosen based on previous studies that showed that it significantly increased the calcium content of treated fruits and affected the mass transfer of water and solutes [4,31]. In this second part, the effect of the addition of CaL on the TPC, antioxidant activities, and calcium content of freeze-dried products was studied.

3.3. Analytical Methods

3.3.1. Extraction of Antioxidants

For extraction of antioxidants, the freeze-dried samples in powder form were weighed and extracted in 80% methanol (*w/w* in water), at a sample–solvent ratio of 1:20. The samples were shaken in a water bath at 50 °C for 2 h. Afterwards, the samples were cooled down, centrifuged (4 °C, 12,000× *g*, 10 min), and filtered. The extraction was conducted in duplicate. The extracts were used for determination of total phenolic content and antioxidant activities.

3.3.2. Determination of Total Phenolic Content and Antioxidant Activity

Total phenolic content (TPC) was determined following a previous study [54] using a Folin–Ciocalteu reagent. The absorbance of the samples was measured at $\lambda = 725$ nm. The results were expressed as mg of gallic acid equivalent (GAE) per 100 g of product.

The antioxidant activity of freeze-dried apple was determined in triplicate using the ABTS, DPPH, ORAC, and PCL methods. The ABTS radical cation scavenging activity was conducted according to a previous study [55]. The degree of reduction in terms of the ABTS radical cation was measured spectrophotometrically at $\lambda = 734$ nm after a 6 min incubation of complexes of sample extracts with ABTS and potassium persulfate, expressed as mg of Trolox equivalent (TE) per 100 g of product.

Determination of antioxidant activity using a DPPH assay was determined as described previously [56]. This assay was based on a DPPH solution absorbance decrease at $\lambda = 515$ nm in the presence of an antioxidant. The results were expressed as mg of TE/100 g of product.

The oxygen radical absorbance capacity (ORAC) assay was performed according to a previous study [57], which is based on the antioxidant ability to scavenge peroxy radicals, generated by AAPH using fluorescein solution. The readings were taken at a given excitation ($\lambda = 493$ nm) and emission wavelength ($\lambda = 515$ nm) on a F-2700 fluorescence

spectrophotometer (Hitachi, Tokyo, Japan). The results were presented as mg of TE/100 g of product.

The photochemiluminescence (PCL) assay was conducted using a Photochem[®] apparatus (Analytik Jena, Jena, Germany), as described previously [58]. This assay is based on the detection of superoxide anion radicals ($O_2^{\bullet-}$) generated upon exposure to light and the presence of a photosensitizer, chemiluminogenous compound–luminol (5-amino-2,3-dihydro-1,4-phthalazinedione). The antioxidant activities in lipid-soluble (ACL) and water-soluble (ACW) compounds and integral antioxidant capacity (IAC) were presented following the protocol, using the kits provided by the manufacturer. The results were expressed as mg of TE/100 g of product.

3.3.3. Color Measurement

The color of SB juice and the surface of freeze-dried apple samples (from part 1 of the research) before grinding were examined using the CIE $L^*a^*b^*$ scale using a colorimeter (Chroma Meter CR-5, Konica Minolta, Tokyo, Japan). The zero and white calibration was conducted prior to measurement. Results were expressed as L value (lightness), a value (greenness/redness), b value (blueness/yellowness), ΔE (total color difference), BI (browning index), WI (whiteness index), and YI (yellowness index) with the following equations [29]:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (1)$$

$$BI = 100 \times \frac{x - 0.31}{0.172}, \text{ where } x = \frac{a + 1.75 L}{5.645L + a - 3.012b} \quad (2)$$

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \quad (3)$$

$$YI = 142.86 \times \frac{b}{L} \quad (4)$$

where for ΔE of freeze-dried samples, the ΔL , Δa , and Δb are the mean difference of L, a, and b values, respectively, between FA + AA and other analyzed samples. The measurements were carried out over six repetitions from each optimized sample in the first part of the research.

3.3.4. Determination of Calcium Content

About 1 g of freeze-dried samples in powder form and SB juice underwent mineralization in a muffle furnace at 450 °C, which was then dissolved in 1 mol/L nitric acid. Each sample was analyzed in triplicate. The samples were diluted in 0.5% $LaCl_3$ solution. The calcium content of freeze-dried apples was determined by atomic absorption spectrometry (Hitachi Z2000, Tokyo, Japan) using the air–acetylene flame [59]. The methods were validated by a simultaneous analysis of the reference material (Soya Bean Flour, INCT-SBF-4) with 95.7% accuracy for Ca. The results were expressed as mg of Ca/100 g of product. Deionized water and acid-washed glassware were used in this study.

3.3.5. Analyses of Selected Samples

The selected samples based on the results of the first and second part were further analyzed by ultra-performance liquid chromatography–photodiode array (UPLC-PDA, Waters Corporation, Milford, MA, USA), sensory profiling, activity of enzymes, and proximate analysis.

Quantification of Phenolic and Carotenoid Composition of Freeze-Dried Apples by UPLC-PDA

The UPLC-PDA method using ACQUITY Ultra Performance Liquid Chromatography (Waters Corporation, Milford, MA, USA) was performed to quantify the phenolic and carotenoid composition of the samples, with extraction and quantification protocols carried out according to the procedure described previously [34,41]. For the extraction of phenolics, about 0.5 g of selected powdered samples were mixed with 5 mL of 70% methanol (*v/v* in water) with 2% ascorbic acid and 1% acetic acid, then sonicated for 15 min (35 kHz; RK514H Bandelin Sonorex, Darmstadt, Germany). The sonication was repeated after storage at 4 °C for 24 h. The samples were centrifuged (14,000× *g*, 4 °C, 10 min; MPW-352R, Warszawa, Poland), and the supernatants were filtered through a hydrophilic polytetrafluoroethylene (PTFE) 0.20 µm membrane (Millex Simplicity Filter, Merck, Darmstadt, Germany) and used for analysis. The separations of individual phenolics were performed at 30 °C using an ACQUITY UPLC BEH C18 column (1.7 µm, 2.1 mm × 100 mm, Waters Corporation, Milford, MA, USA). The sample extracts (5 µL) were injected and the elution was completed in 15 min with a flow rate of 0.420 mL/min. The mobile phase was solvent A (2% formic acid, *v/v*) and solvent B (100% acetonitrile). The program started with gradient elution with 98–65% of solvent A (to 12 min) and then 0% (to 14 min); the gradient returned to the initial composition (98% of A) until the 15 min mark, when it was held constant to re-equilibrate the column. The PDA spectra were measured at 280 (flavan-3-ols and dihydrochalcones), 320 (phenolic acids), and 360 nm (flavonols).

The whole extraction procedure for carotenoids was conducted in dark conditions. About 0.4 g of selected powdered samples with the addition of 10% MgCO₃ was shaken continuously with 4 mL hexane–acetone–methanol (2:1:1, *v/v/v*) with 1% butylated hydroxytoluene (300 rpm, 15 min; DOS-10L Digital Orbital Shaker, ELMI, Riga, Latvia). The samples were centrifuged (same as with the phenolics extraction), and supernatants were collected. The extraction was repeated two to three times. The combined supernatants were evaporated (XcelVap, Biotage, Uppsala, Sweden) and the residues were diluted in methanol–tetrahydrofuran (4:1, *v/v*). Before injection, the samples were filtered through a hydrophilic membrane (as previously described) and used for analysis. The ACQUITY UPLC BEH RP C18 column (1.7 µm, 2.1 mm × 100 mm, Waters Corporation, Milford, MA, USA), protected by a C18 guard column, was operated at 32 °C. The injection volume of sample extracts was 10 µL, and the flow rate was 0.500 mL/min. The mobile phase was solvent A (0.1% formic acid) and solvent B (7:3 acetonitrile–methanol, *v/v*). The gradient elution started with 25% A (to 0.60 min); 4.9% A (to 6.50 min); 0% A (to 13.60 min); and 25% A (to 16.60 min). The analysis was monitored at a wavelength of 450 nm.

The retention times and spectra were compared to those of standards. The results of the UPLC-PDA analysis were determined as the average of two replicates and expressed as mg per 100 g of freeze-dried product.

Sensory Profiling of Freeze-Dried Apples

The sensory profiling and overall acceptance evaluation were conducted by 20 trained panelists, using a 10 cm linear scale [60]. In the sensory evaluation room, the panelists were asked to assess the selected samples encoded with three-digit numbers. The attributes for sensory profiling were appearance, aroma, taste, and texture, which descriptors were listed in Table S3. The overall acceptance of samples was also analyzed. The study did not require ethical approval. Participants were informed of the study's purpose and that their contribution was entirely voluntary; therefore, they could stop the analysis at any time and their responses were anonymous. The authors did not ask respondents for sensitive data or personal information. No formal relationship was used to recruit participants to the study.

Mean, variance, and standard deviation were calculated for each attribute of all samples and sessions individually.

Activity of Enzymes

The activity of enzymes included assay of polyphenol oxidase (PPO) and peroxidase (POD) activities, which are the enzymes responsible for enzymatic browning. For the extraction, 0.3 g of freeze-dried powdered samples was mixed vigorously with 2 mL of 0.067 mol/L of Sorensen's phosphate buffer solution (SPBS; pH 7.0, determined previously as the optimal pH in freeze-dried apple for both PPO and POD activities) containing 0.02 g of PVPP. The extracts were then shaken for 30 min at 1400 rpm and centrifuged at $12,000\times g$. The supernatants were collected and used for further analysis. The whole extraction process for enzymes was carried out at 4 °C [61].

For the PPO activity, the final reaction mixture contained 50 µL of extract, 900 µL of 0.067 mol/L SPBS (pH 7.0), and 50 µL of 0.5 mol/L catechol as the substrate. For the POD activity, the final reaction mixture contained 100 µL of extract, 800 µL of 0.067 mol/L SPBS (pH 7.0), 50 µL of 0.04 mol/L H₂O₂, and 50 µL of 0.04 mol/L guaiacol as the substrate. The changes in the absorbance at 420 and 470 nm (DA) were monitored at 25 °C using a UV-Vis spectrophotometer (UV-1280, Shimadzu, Kyoto, Japan) for PPO and POD activity, respectively. One unit (U) of enzyme activity is defined as the amount of enzyme that changes an absorbance 0.001 per minute under the specified conditions. The PPO and POD activities were expressed in unit per g of product (U/g product).

Proximate Analysis of Freeze-Dried Apples

Proximate analysis of selected samples included protein, lipid, carbohydrate, ash, moisture, and fiber. The Kjeldahl method (Kjeltec-2200 System, Foss Tecator, Hoeganaes, Sweden) was applied for protein content determination (PN-75/A-04018:1975) [62]. The Soxhlet method was applied for lipid content determination (PN-EN ISO 3947:2001) [63]. Moisture and ash contents were determined following methods of the Association of Official Agricultural Chemists [64]. The soluble (SDF), insoluble (IDF), and total dietary fiber (TDF) contents were determined according to the Asp method [65]. The carbohydrate content was calculated by subtracting 100 from the sum of protein, lipid, TDF, moisture, and ash content per 100 g of product. The energy value, expressed in kcal/100 g product, was calculated using the conversion factors described in Regulation (EU) No. 1169/2011.

3.4. Response Surface Methodology and Statistical Analysis

A combined experimental design (mixture–process) was used to evaluate the effect of three mixture components (sea buckthorn juice, water, and inulin) and two process factors (time and temperature) on the antioxidant properties of the product by employing Design Expert 13 (Stat-Ease, USA). A randomized design was implemented using a Response Surface Cubic model with two-factor interactions (RSpCub $\times$ 2FI), totaling 162 experimental runs without blocking. The data for treated samples from Table S1, particularly SB0_I0, SB0_I15, SB0_I30, SB50_I0, SB50_I15, SB50_I30, SB100_I0, SB100_I15, and SB100_I30, treated at different times (30, 60, 120 min) and temperatures (30 and 50 °C) were analyzed (Tables S1 and 1). Each experimental run was replicated in triplicate to ensure data reproducibility and robustness.

Independent variables:

- Mixture components:
 - SB juice: 0–250 g;
 - Water: 0–250 g;
 - Inulin: 0–75 g;

- Total mixture mass fixed at 250 g.
- Process factors:
 - Time: 30–120 min;
 - Temperature: 30–50 °C.
- Dependent variables (responses):
- Total phenolic content (TPC, mg GAE/100 g product).
- Antioxidant activity assays:
 - ABTS (mg TE/100 g product);
 - DPPH (mg TE/100 g product);
 - ORAC (mg TE/100 g product);
 - PCL-ACL, PCL-ACW (mg TE/100 g product).

Multiple regression analysis based on response surface methodology (RSM) was applied to develop predictive polynomial regression models describing the relationships between independent variables (mixture components and process factors) and dependent variables (antioxidant responses). Model fitting and validation included calculation of regression coefficients, analysis of variance (ANOVA), and determination of prediction and confidence intervals (95% confidence level) to assess the reliability and accuracy of the developed models. The final models were used to predict optimal combinations of mixture components and processing parameters for maximizing antioxidant activities. *t*-test and one-way ANOVA followed by Tukey's post hoc test were performed using Statistica 13.1 (StatSoft Inc., Kraków, Poland), noting that a significant difference was considered when $p < 0.05$. The Pearson correlation coefficient was determined between antioxidant activities and TPC of the samples.

4. Conclusions

The development of freeze-dried apples impregnated with SB juice, inulin, and CaL at different temperatures and times was studied. Freeze-dried apples prepared by impregnation using 93.8% SB juice, a 0:100 inulin–SB juice ratio, 30 °C, 120 min, with the addition of 4% CaL (also called 4% CaL treatment), possessed a minimum yet acceptable loss of antioxidant properties and increased calcium content (2209.13 mg Ca/100 g). With the assumption of 25% calcium absorption and RDA of 1000 mg of Ca/day for adults, consuming 100 g of freeze-dried apples prepared with 4% CaL could fulfill 55.2% of the RDA. The results from UPLC-PDA indicated that the impregnation process using SB juice and CaL, as well as dipping in ascorbic acid, affected the compositional changes in phenolics and carotenoids of the final product. The 4% CaL treatment also produced a product with lower PPO and POD activities, moderate sensory acceptability with soft texture, and better nutritional values with lower calories when compared to FA and FA + AA. This product has the potential to be an alternative functional food product with respect to reducing the risk of osteoporosis and improving the intake of calcium and antioxidants. Thanks to the beneficial bioactive compounds in the freeze-dried product (4% CaL treatment), it can be beneficial not only for osteoporosis but for general health. In practice, the functional food industry may apply or further improve the suggested conditions to produce the product in a sustainable way, while consumers may include this product in their diet, either as a daily healthy snack or as an alternative food, noting that food diversification is always encouraged to support sustainability.

There are some improvements that could be further studied. Firstly, a lower ratio of inulin–SB juice than 15:85 could be considered not only to potentially minimize the loss of antioxidant activities of the freeze-dried apple, but also to improve the prebiotic effect, calcium absorption, and sensory properties of the end product. Secondly, impregnation using

non-conventional technologies, absorption or bioaccessibility of calcium and antioxidants via an in vitro digestion model or in vivo studies, as well as a storage study to observe the stability of bioactive compounds should be conducted. Thirdly, the utilization of potential fruit juices other than SB juice could also be investigated via impregnation or OD processes, noting that every region has its own potential natural resources and thus could also support food diversification in the future.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/molecules30071504/s1>: Table S1: TPC and antioxidant activities of freeze-dried apples, osmotically dehydrated with different concentrations of SB juice (0, 50, 100% in water), inulin–SB juice ratios (0:100, 15:85, 30:70), times (30, 60, 120 min), and temperatures (30, 50 °C); Table S2: Pearson’s correlation coefficients for TPC and antioxidant activities of freeze-dried apples; Table S3: Sensory profiling attributes and descriptors.

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Vacuum and Ultrasound-Assisted Impregnation of Gala Apples with Sea Buckthorn Juice and Calcium Lactate: Functional Properties, Antioxidant Profile, and Activity of Polyphenol Oxidase and Peroxidase of Freeze-Dried Products

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Vacuum impregnation (VI) and ultrasound-assisted impregnation (US) were applied to Gala apples with the aim of improving the antioxidant capacity and calcium content of freeze-dried apples. An impregnation solution was composed of 93.8% sea buckthorn (SB) juice with 0 and 4% (w/w) calcium lactate (CaL). Gala apples conventionally impregnated (CON) at 30°C for 120 min were also prepared for comparison. The VI was performed at 200, 400, and 600 mbar with 10 min of each holding and relaxation time. US treatment was performed for 10, 20, and 30 min and was continued with CON for 110, 100, and 90 min, respectively. The VI at 200 mbar and US for 30 min at 0% CaL produced freeze-dried samples with the highest antioxidant capacity, higher than that of the CON and the non-impregnated samples: fresh apple (FA) and those after dipping in 1% ascorbic acid (FA+AA). The addition of 4% CaL optimally improved the calcium content when VI at 200 mbar and US for 20 min were applied. Generally, the freeze-dried apples impregnated under these conditions had lower antioxidant capacity compared to those treated without CaL. The impregnation process (CON, VI, and US) affected the mass transfer and composition of phenolics and carotenoids in the freeze-dried apples. Additionally, polyphenol oxidase (PPO) and peroxidase (POD) activities were completely inhibited upon CON and US treatment for 30 min at 0% CaL. While at 4% CaL, US for 20 min reduced PPO activity by 19.4% as compared to FA+AA. The end products, particularly those obtained at 4% CaL, could serve as potential functional snacks for consumers with osteoporosis and calcium issues, considering their calcium content and antioxidant properties.

Keywords: bioactive compounds, calcium enrichment, enzymatic browning, functional snack, non-conventional technology

INTRODUCTION

The development of functional food products has been a recent trend aimed at minimizing the risk of non-communicable

diseases (NCD), including osteoporosis and calcium deficiency. It was previously observed that consumers aged 36–50 years consider functional foods as an essential means to prevent

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osteoporosis and other NCD [Plasek *et al.*, 2020]. Among many bioactive compounds, calcium and antioxidants (*e.g.*, carotenoids and phenolics) are the key ones in preventing those issues [Kulczyński *et al.*, 2024; Martiniakova *et al.*, 2022]. In addition, the manufacture of functional food products utilizing locally sourced raw materials is encouraged to support sustainability, particularly by minimizing the carbon footprint.

Apple is known as a fruit with high productivity in Europe and across the world. According to the Food and Agriculture Organization (FAO), 18% of worldwide apple production was accounted for by Europe in 2023, amounting to more than 17.5 million tons [FAO, 2025]. Apple is widely consumed, either fresh (60–75%) or processed (20–40%) into various products including juice, cider, jam, and dried products, *etc.* [De la Peña-Armas & Mateos-Aparicio, 2022]. Furthermore, consuming dried fruit instead of unhealthy snacks may be a viable way to meet the World Health Organization's (WHO) daily fruit intake recommendations and promote the adoption of a sustainable diet [Testa *et al.*, 2023]. In our preliminary study, we developed a locally sourced apple-based healthy snack conventionally impregnated (at an atmospheric pressure) using local sea buckthorn (SB) juice. The optimized impregnation conditions were as follows: 93.8% (*w/w*) SB juice in water, 4% (*w/w*) calcium lactate (CaL) addition, 120 min, and 30°C [Arnold *et al.*, 2025]. The application of these conditions resulted in freeze-dried products with improved calcium content and minimal loss of antioxidant activities, compared to those impregnated with inulin only and the untreated samples.

Non-conventional technologies, particularly vacuum impregnation (VI) and ultrasound-assisted impregnation (US), were studied to promote mass transfer during the impregnation process to a greater extent than the conventional (CON) or atmospheric impregnation, thus improving the functional properties of plant-based products, including apples [Panayampadan *et al.*, 2022; Santarelli *et al.*, 2020; Vasile *et al.*, 2022]. The VI can promote the mass transfer process between the impregnating solution and solid porous food by intensifying the capillary flow in the cells due to the pressure gradients during the process. When vacuum pressure is applied, the internal cell pore volume becomes larger than under atmospheric pressure and thereby increases internal volume. The hydrodynamic mechanism (HDM) is initiated simultaneously, and the impregnating solution starts flowing into the capillaries until mass transfer equilibrium is reached. Subsequent restoration to atmospheric pressure is then applied, and the food is allowed to remain immersed during the relaxation time. During this phase, HDM and compression-induced deformation of the solid matrix take place, with external pressure acting as the driving forces. The cell pores of food eventually become filled with the impregnating solution, altering the physicochemical and functional properties of the product [Panayampadan *et al.*, 2022]. Following the application of high-power US (high intensity, >1 W/cm²; low frequency, 18–100 kHz), the phenomena, such as sponge effect and cavitation, occur in liquids or solids containing moisture [Nowacka *et al.*, 2021]. These phenomena cause the formation

of microbubbles in the material and improve mass transfer during the impregnation process.

The application of VI to improve the functional properties of apple-based products has been recently reported using blueberry juice and CaL at 200 mbar [Castagnini *et al.*, 2021], apple-peer juice at 40–80 mbar [Paśławska *et al.*, 2019], grape juice concentrate at 133–667 mbar [González-Pérez *et al.*, 2022], and lemon juice at 738 mbar [Santarelli *et al.*, 2020]. In turn, applications in apple-based products using vitamin B₁₂ (24 kHz, 400 W) [Vasile *et al.*, 2022] and chokeberry juice concentrate (40 kHz, 0.1 W/g) [Masztalerz *et al.*, 2021] have been reported for the US treatment. Some studies also demonstrated synergistic effects of US and VI using a hibiscus extract solution [Dinçer, 2022], a mixture of black carrot phenolics and CaL [Yılmaz & Ersus Bilek, 2018], aloe vera juice [Trusinska *et al.*, 2024], and chokeberry juice concentrate [Masztalerz *et al.*, 2021].

To the best of our knowledge, to date, the application of VI and US to improve the functional properties of plant matrices has not been carried out using SB juice, without or with CaL. It is worth noting that SB is abundant in Europe and Asia, and is rich in lipo- and hydrophilic antioxidants, as well as other health-promoting compounds, such as vitamins and minerals, that can promote the general health of consumers [Ciesarová *et al.*, 2020]. Therefore, this study aimed to investigate the antioxidant activities and calcium content of freeze-dried Gala apples after the application of VI and US using SB juice without and with CaL, in comparison to CON treatment, highlighting the novelty of VI and US with SB juice to enhance the functional properties of apples based on our previous study [Arnold *et al.*, 2025]. The mass-transfer phenomena after impregnation (before freeze-drying), color, enzyme activities (including polyphenol oxidase (PPO) and peroxidase (POD)), and composition of antioxidants of freeze-dried products were analyzed as well.

MATERIALS AND METHODS

■ Raw materials and chemicals

Apples (*Malus domestica* Borkh. cv. Gala) were purchased from a local market in Cesena, originating from VOG s.a.c. in Terlan, South Tyrol, Italy. A homogeneous batch of apples (20 kg) was transported to the laboratory of the Department of Agricultural and Food Sciences (DISTAL) of the University of Bologna in Cesena, and randomly divided into 16 treatment groups: 2 controls – FA and FA+AA; and 14 impregnated samples – CON, VI (200, 400, 600 mbar), US (10, 20, 30 min), each prepared without and with CaL. An equal weight of apples was allocated to each group. All the samples were stored at 4°C and processed within a week. The apples were washed, peeled, cored, and sliced to a thickness of 5 mm to obtain the C-shaped slices. The slices were then dipped for 2 min in a 1% (*w/v*) ascorbic acid (Sigma Aldrich, St. Louis, MO, USA) solution, at a sample to solution ratio of 1:4 (*w/v*) to delay enzymatic browning. The raw fresh apple flesh (FA) and those dipped in a 1% (*w/v*) ascorbic acid solution (FA+AA) were used as the controls, each in triplicate. The organic sea buckthorn (SB) (*Hippophae rhamnoides* L.) juice was purchased from KoRo (Berlin, Germany), with SB fruits

grown in an organic farm in Brandenburg, Germany. The calcium L-lactate hydrate was purchased from Sigma Aldrich. Prior to impregnation treatment, the total soluble solid content of raw FA, raw FA+AA, SB juice, and the final impregnating solution was measured using a DBR 95 digital refractometer (XS Instruments, Carpi, Italy).

All chemicals were purchased from Sigma Aldrich, including 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azobis(2-methylpropionamide) dihydrochloride (AAPH), fluorescein sodium salt, sodium acetate trihydrate, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), iron(III) chloride hexahydrate, iron(II) sulfate heptahydrate, ($\pm$)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), Folin-Ciocalteu's phenol reagent, acetic acid, gallic acid, hydrochloric acid, 65% nitric acid, methanol, potassium persulfate, sodium carbonate, lanthanum(III) chloride, monopotassium phosphate, disodium phosphate, polyvinylpyrrolidone (PVPP), catechol, and guaiacol. ACL (lipid-soluble substances) and ACW (water-soluble substances) reagent kits were purchased from Analytik Jena (Jena, Germany). The solvents for ultra-performance liquid chromatography (UPLC) method were bought from Sigma Aldrich. The standards of phenolic compounds included phenolic acids (chlorogenic acid), flavan-3-ols (($\pm$)-(epi)catechin, procyanidin B₁), dihydrochalcones (phloretin 2-O-xyloglucoside), flavonols (quercetin 3-O-glucoside and isorhamnetin 3-O-glucoside) and carotenoids (lutein, zeaxanthin, β -carotene) were obtained from Extrasynthese (Genay, France).

■ Impregnation of apples using sea buckthorn juice and calcium lactate

The impregnation process was divided into three types: conventional (CON) and non-conventional which included vacuum (VI) and ultrasound-assisted impregnation (US). The impregnating solution and process were designed based on the optimization from a preliminary study [Arnold *et al.*, 2025]. The impregnating solution consisted of 93.8% (w/w) SB juice in water, with 0 and 4% (w/w) calcium lactate (CaL); these conditions were chosen as they ensured the optimum antioxidant capacity and calcium content of freeze-dried Gala slices when impregnated under CON conditions at 30°C for 120 min. The sample to solution ratio of 1:5 (w/w) was chosen to prevent concentration changes during the treatment. Each CON, VI, and US treatment was carried out in triplicate using independent samples of Gala apples (raw FA+AA) before freeze-drying, and the samples were analyzed accordingly.

In the CON treatment, the raw FA+AA was impregnated in the solution in a parafilm-wrapped beaker at an atmospheric pressure. The samples were continuously shaken at 100 rpm, 30°C, for 120 min (Shaker 709, ASAL, Milan, Italy). For the VI treatment, the solution was conditioned at 30°C for 5 min prior to the treatment. The raw FA+AA was then immersed in the beaker containing the solution and the VI was carried out in a closed chamber connected to a vacuum pump and an automatic controller system (AVCS, S.I.A.,

Bologna, Italy). The VI treatments were performed at three different absolute pressures 200, 400, and 600 mbar. The total duration of each VI treatment was 28 min, consisting of 4 min to decrease the pressure to 200, 400, or 600 mbar; 10 min of holding time at those pressures; 4 min to recover the pressure to the atmospheric pressure; and 10 min of relaxation at the atmospheric pressure at 30°C [Kidoń *et al.*, 2023]. For the US treatment, the raw FA+AA was immersed in the beaker containing the solution, and the US treatment was conducted for 10, 20, and 30 min (25 kHz, 1,000 W, 30°C) in an ultrasonic bath (Xtra ST 600H, Elmasonic, Singen, Germany). During the US treatment, the temperature was kept constant at 30°C by a recirculating cooler (FL601, Julabo, Seelbach, Germany) with a coil exchanger inserted in the treatment tank. The water temperature was controlled by a probe thermometer throughout the treatment. A negligible increase in temperature (up to 1°C depending on the treatment time) was observed during the treatment. Afterwards, the conventional impregnation process was conducted for additional 110, 100, and 90 min for US treatments of 10, 20, and 30 min, respectively to reach the total impregnation time of 120 min.

After the CON, VI, and US treatments, the samples were removed from the solutions, drained, and blotted using absorbing paper, and their weight was recorded for mass transfer analysis. Moreover, water activity was also measured on the whole samples, while color and chemical analyses were performed on freeze-dried samples. For the freeze-dried samples, the control and treated samples were frozen at -40°C for 24 h, and freeze-dried for 48 h (Lio2000, CinquePascal S.r.l., Milan, Italy). All samples with related abbreviations are described in **Table 1**.

Table 1. Codes of apple flesh control samples without (FA) and with dipping in an ascorbic acid solution (FA+AA), and apple flesh samples subjected to conventional (CON), and non-conventional impregnation (vacuum impregnation, VI, and ultrasound-assisted impregnation, US).

Sample codes	Dipping time (min)	VI pressure (mbar)	US time (min)	CON time (min)
FA	–	–	–	–
FA+AA	2	–	–	–
CON	2	–	–	120
VI_200	2	200	–	10*
VI_400	2	400	–	10*
VI_600	2	600	–	10*
US_10	2	–	10	110**
US_20	2	–	20	100**
US_30	2	–	30	90**

*For vacuum impregnation, the relaxation time was conducted adapting the conventional impregnation conditions (30°C, atmospheric pressure). **After ultrasound-assisted impregnation, the conventional impregnation was conducted subsequently to reach 120 min of total impregnation process.

■ Analysis of mass transfer phenomena and water activity

After the impregnation treatment, the weight gain/loss, water gain/loss, and solid gain/loss of the samples were calculated according to Equations (1), (2) and (3), respectively, with the note that positive and negative calculated values refer to the gain and loss, respectively:

$$\text{Weight gain/loss (\%)} = \frac{m_t - m_0}{m_0} \times 100 \quad (1)$$

$$\text{Water gain/loss (\%)} = \frac{m_t x_{wt} - m_0 x_{w0}}{m_0} \times 100 \quad (2)$$

$$\text{Solid gain/loss (\%)} = \frac{m_t x_{STt} - m_0 x_{ST0}}{m_0} \times 100 \quad (3)$$

where: m_0 is initial weight before the impregnation process (kg), m_t is weight after impregnation process at time t (kg), x_{w0} is initial water mass fraction (kg/kg), x_{wt} is water mass fraction after the impregnation process at time t (kg/kg), x_{ST0} is initial total solids (dry matter) mass fraction (kg/kg), and x_{STt} is total solids (dry matter) mass fraction after the impregnation process at time t (kg/kg).

The moisture and dry matter contents were determined by drying the samples at 70°C until the weight was constant [AOAC, 2002]. The water activity (a_w) was measured by an aWLife meter (WaterLab, Steroglass S.r.l., Perugia, Italy) at 25°C. All analyses were conducted in triplicate.

■ Color analysis

The color measurement of SB juice and the surface of freeze-dried samples before grinding was performed in ten repetitions using a ColorFlex EZ colorimeter (HunterLab, Reston, VA, USA) in the CIE $L^*a^*b^*$ scale. Prior to measurement, the colorimeter was calibrated using a black and white tile ($L^* 93.47$, $a^* 0.83$, $b^* 1.33$). The L^* (lightness), a^* (greenness/redness), and b^* (blueness/yellowness) of the sample were measured, and total color difference (ΔE), browning index (BI) [Buera *et al.*, 1985], whiteness index (WI) [Judd & Wyszecki, 1963], and yellowness index (YI) [Francis & Clydesdale, 1975] were calculated using Equations (4), (5), (6), and (7), respectively:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

where: ΔL^* , Δa^* , and Δb^* are the differences of L^* , a^* , and b^* , respectively, between FA+AA and other analyzed samples.

$$\text{BI} = 100 \times \frac{x - 0.31}{0.172} \quad (5)$$

$$\text{where: } x = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*}$$

$$\text{WI} = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (6)$$

$$\text{YI} = 142.86 \times \frac{b^*}{L^*} \quad (7)$$

■ Determination of total phenolic content and antioxidant capacity

The freeze-dried powder was extracted using 80% methanol (v/v) at a sample-to-solvent ratio of 1:20 (w/v). The SB juice (filtered through a 0.45 μm membrane, Membrane Solutions, Auburn, WA, USA) was diluted in 80% methanol (v/v) at a sample-to-solvent ratio of 1:10 (w/v). The samples were shaken in a water bath at 150 rpm, and 50°C for 120 min. After cooling to room temperature, the samples were centrifuged (4°C, 12,000 $\times g$, 10 min) and filtered to obtain the supernatant. The extraction was conducted in duplicate, and the obtained extracts were used to determine the total phenolic content and antioxidant capacity.

The total phenolic content (TPC) was determined in triplicate by the Folin-Ciocalteu assay following a previous report [Singleton & Rossi, 1965], in which the absorbance of the extracts was measured at $\lambda = 725$ nm using an SP-880 spectrophotometer (Metertech, Taipei, Taiwan). The TPC was expressed as mg gallic acid equivalent (GAE) *per* 100 g of product.

The ABTS, DPPH, ferric-reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC), and photochemiluminescence (PCL) assays were carried out in triplicate to determine the antioxidant capacity of the products. The ability of the samples to reduce the ABTS cation radical was measured spectrophotometrically using an SP-880 spectrophotometer (Metertech) set at $\lambda = 734$ nm after 6 min of incubation of the reaction mixture containing the extract and ABTS cation radicals generated with potassium persulfate, according to a previous report [Re *et al.*, 1999]. The results were expressed as mg Trolox equivalent (TE) *per* 100 g of product.

The DPPH radical scavenging activity was determined as described previously [Sánchez-Moreno *et al.*, 1998]. The absorbance of the reaction mixture containing the extract and DPPH radicals was measured at $\lambda = 515$ nm after incubation in the dark for 20 min. The results were expressed as mg TE *per* 100 g of product.

FRAP of the samples was determined according to the Benzie & Strain [1999] method. The FRAP reagent was prepared by mixing the acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM of $\text{FeCl}_3 \cdot \text{H}_2\text{O}$ solution at the ratio of 10:1:1 (v/v/v). The extract was added to the prepared reagent and incubated at 37°C for 4 min. The reduction of TPTZ-Fe^{3+} to TPTZ-Fe^{2+} with the presence of antioxidants was assessed at $\lambda = 593$ nm. The results were presented as mmol Fe(II) *per* 100 g of product.

The ORAC assay measured the AAPH-generated peroxy radicals scavenging ability of the antioxidants in the sample extracts, using a fluorescein solution [Ou *et al.*, 2001]. The readings were taken at a given excitation ($\lambda = 493$ nm) and emission wavelength ($\lambda = 515$ nm) on an F-2700 fluorescence spectrophotometer (Hitachi, Kyoto, Japan). The results were presented as mg TE/100 g of product.

The PCL assay was performed using a Photochem apparatus (Analytik Jena, Germany). In this assay, the superoxide anion radicals ($\text{O}_2^{\bullet -}$) were produced by optical excitation

of a photosensitizer. The antioxidant capacity of the samples against the radicals was detected with the presence of chemiluminogenous compound – luminol. The antioxidant capacity of lipid-soluble (ACL) and water-soluble (ACW) substances of the extract was measured according to the protocol and using the reagent kits from the manufacturer. Additionally, the integral antioxidant capacity (IAC) was calculated as the sum of ACL and ACW [Besco *et al.*, 2007]. The results were presented as mg TE per 100 g of product.

■ Determination of calcium content

The calcium content of SB juice, raw FA, raw FA+AA, and freeze-dried samples was determined in triplicate by atomic absorption spectrometry (Hitachi Z2000, Tokyo, Japan) using the air-acetylene flame, following a previous study [Suliburska & Krejpcio, 2014]. The complete mineralization of 1 g of the sample was performed in a muffle furnace at 450°C for 72 h. The obtained ash was then dissolved in 1 M nitric acid and diluted in 0.5% LaCl₃ solution. The methods were validated by simultaneous analysis of the reference material (Soya Bean Flour, INCT-SBF-4) with 95.7% accuracy for calcium. Deionized water and acid-washed glassware were used in this study. The results were presented as mg Ca per 100 g of product.

■ Quantification of phenolics and carotenoids

The extraction of phenolics and carotenoids of the selected freeze-dried samples, followed by their quantification using ultra-performance liquid chromatography with photodiode array detection (UPLC-PDA; ACQUITY UPLC system, Waters Corporation, Milford, MA, USA) was performed following a previous study [Tkacz *et al.*, 2020]. To extract phenolics, 0.5 g of powdered samples was mixed with 5 mL of 70% methanol (v/v, in water) with 2% (w/v) ascorbic acid and 1% (v/v) acetic acid, followed by 15 min of sonication (35 kHz; Sonorex RK514H, Bandelin, Berlin, Germany). The mixtures were stored at 4° for 24 h C, and sonication was repeated once afterwards. The supernatants from centrifugation at 14,000×g, 4°C for 10 min performed using an MPW-352R centrifuge (MPW MED. Instruments, Warsaw, Poland) were collected and filtered through a syringe filter with a polytetrafluoroethylene (PTFE) membrane, 0.20 µm (Millex Simplicity Filter, Merck, Darmstadt, Germany). The filtrate phenolic compound separation was carried out using an ACQUITY UPLC BEH C18 column (1.7 µm, 2.1×100 mm, Waters Corporation), which temperature was maintained at 30°C. The injection volume was 5 µL and the elution, using a mobile phase consisting of 2% (v/v) formic acid (solvent A) and 100% acetonitrile (solvent B) applied in a gradient system, was completed in 15 min with a flow rate of 0.42 mL/min. The gradient program started with 98–65% solvent A (to 12 min), and then to 0% (to 14 min), the gradient returned to the initial composition (98% A) until 15 min for held constant to re-equilibrate the column. Identification of compounds was conducted based on retention times and PDA spectra of standards and using literature data [Tkacz *et al.*, 2020]. For quantification, the absorbance was measured at 280 nm for

flavan-3-ols expressed as (±)-(epi)catechin equivalents (calibration curve in a concentration range of 5–10 mg/L; $R^2=0.9978$), procyanidin B₁ (5–10 mg/L; $R^2=0.9995$) and dihydrochalcones expressed as phloretin 2-O-xyloglucoside equivalents (10–20 mg/L; $R^2=0.9992$); at 320 nm for phenolic acids expressed as chlorogenic acid equivalents (10–25 mg/L; $R^2=0.9989$); and at 360 nm for flavonols expressed as quercetin 3-O-glucoside and isorhamnetin 3-O-glucoside equivalents (10–25 mg/L; $R^2=0.9995$ and 0.9957, respectively).

For carotenoid extraction, in dark conditions, 0.4 g of the powdered samples and 10% MgCO₃ solution were shaken with 4 mL of a mixture of hexane, acetone and methanol (2:1:1, v/v/v) with 1% butylated hydroxytoluene (w/v) (300 rpm, 15 min; DOS-10L Digital Orbital Shaker, ELMI, Riga, Latvia). Supernatants were collected after centrifugation (same as phenolics extraction). The extraction was repeated two (for FA and FA+AA) to three times (for CON, VI, and US). The combined supernatants underwent evaporation (XcelVap, Biotage, Uppsala, Sweden), and the residues were diluted in a mixture of methanol and tetrahydrofuran (4:1, v/v). Solutions were filtered through PTFE syringe filters before injection. UPLC-PDA separation of carotenoids was performed at 32°C using the ACQUITY UPLC BEH Shield RP C18 column (2.1×100 mm, 1.7 µm; Waters Corporation) with a C18 guard column. A volume of 10 µL was injected, and the flow rate of the mobile phase was 0.5 mL/min. The mobile phase was used in a gradient system of 0.1% (v/v) formic acid, (solvent A) and a mixture of acetonitrile and methanol (7:3, v/v) (solvent B). The elution started with 25% A (to 0.60 min); 4.9% A (to 6.50 min); 0% A (to 13.60 min); 25% A (to 16.60 min). The analysis was monitored at wavelength of 450 nm, and the spectra were measured. Carotenoids were identified by comparing their retention times and spectra with those of standards and using literature data [Tkacz *et al.*, 2020]. Quantification was performed based on standard curves for lutein (5–10 mg/L; $R^2=0.9994$), zeaxanthin (5–15 mg/L; $R^2=0.9979$), and β-carotene (10–25 mg/L; $R^2=0.9999$).

The results of UPLC-PDA analyses were calculated as the mean of two replicates and expressed as mg per 100 g of freeze-dried product.

■ Determination of polyphenol oxidase and peroxidase activity

Polyphenol oxidase (PPO) and peroxidase (POD) activities in selected freeze-dried samples were measured according to the method described previously [Sikora *et al.*, 2020]. The product extraction for enzyme activity measurements was performed at 4°C. About 0.3 g of the powdered freeze-dried sample was mixed vigorously with 2 mL of 0.067 M Sorensen's phosphate buffer solution (SPBS, pH 7.0, determined previously as the optimal pH in freeze-dried apple for both PPO and POD activities) and 0.02 g of PVPP. The mixtures were shaken at 1,400 rpm for 30 min (thermo-shaker TS-100C, Biosan, Riga, Latvia). The supernatant was collected for further analysis, after centrifugation at 12,000×g.

The final reaction mixture for PPO activity measurement consisted of 50 µL of extract, 900 µL of 0.067 M SPBS (pH 7.0), and 50 µL of 0.5 M catechol as the substrate. For the POD activity measurement, the final reaction mixture contained 100 µL of extract, 800 µL of 0.067 M SPBS (pH 7.0), 50 µL of 0.04 M H₂O₂, and 50 µL of 0.04 M guaiacol as the substrate. The absorbance changes (ΔA) with the time at 420 and 470 nm for PPO and POD activity, respectively, were monitored at 25°C using a UV-Vis spectrophotometer (UV-1280, Shimadzu, Kyoto, Japan). The absorbance was linear with the time at the first 3 min. The amount of enzyme that changes an absorbance 0.001 *per* min under the specified conditions was defined as one unit (U) of enzyme activity. The PPO and POD activities were determined in triplicate for each product, and results were expressed in unit of enzyme activity *per* g of product (U/g product).

Statistical analysis

The differences between treatments with 0 and 4% CaL were analyzed by a T-test. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare FA, FA+AA, CON, US, and VI at the same CaL concentration (before and after freeze-drying) and also to compare SB juice, raw FA, and raw FA+AA. Analyses were performed using the Statistica 13.1 software (StatSoft Inc., Krakow, Poland). Principal component analysis (PCA) was carried out to explore the distribution of antioxidant capacity, calcium, dry matter, and ash content, as impacted by impregnation processes. Visualization of PCA was conducted using OriginPro 10.1 (OriginLab Corporation, Northampton, MA, USA). The significance level of 95% was considered in this study.

RESULTS AND DISCUSSION

Physicochemical and functional properties of raw materials

This research used SB juice as the main ingredient of the impregnating solution of Gala apple flesh. The physicochemical parameters and antioxidant capacity of the SB juice, as well as raw apple flesh (FA), and FA after dipping in 1% (w/v) ascorbic acid solution (raw FA+AA) are shown in Table 2. The content of total soluble solids of SB juice reached 5.03°Brix, and was lower than those of the raw FA and FA+AA. This SB juice from fruits grown in an organic farm in Germany had a slightly lower content of total soluble solids than Polish (5.7–7.2°Brix) [Tkacz *et al.*, 2019] or Chinese (6.97–13.50°Brix) SB juice [Zhang *et al.*, 2024]. The impregnating solution consisting of 93.8% (w/w) SB juice in water with 0% CaL had 4.53±0.15°Brix, and that with 4% CaL had 7.47±0.06°Brix of total soluble solids, which was slightly hypotonic compared to raw FA+AA (8.80°Brix).

SB juice contained 11.83 mg Ca/100 g, which was more than twice the amount found in apples (Table 2). This value was comparable to the calcium content of SB juice from Poland (12.7 mg Ca/100 g) or other countries (2.1–10.9 mg Ca/100 g) [Ciesarová *et al.*, 2020]. In terms of TPC and antioxidant capacity, SB juice generally showed significantly higher values than

Table 2. Physicochemical properties and antioxidant capacity of sea buckthorn (SB) juice, raw fresh apple flesh, without (FA) and with dipping process in an ascorbic acid solution (FA+AA).

Parameter	SB juice	Raw FA	Raw FA+AA
Total soluble solids (°Brix)	5.03±0.06 ^c	11.90±1.22 ^a	8.80±0.10 ^b
Dry matter (g/100 g)	7.84±0.02 ^c	15.11±0.19 ^a	12.80±0.29 ^b
Calcium content (mg/100 g)	11.83±0.44 ^a	3.51±0.16 ^c	5.58±0.38 ^b
TPC (mg GAE/100 g)	280.14±3.38 ^a	58.22±1.30 ^c	92.96±0.84 ^b
ABTS (mg TE/100 g)	103.52±4.93 ^a	69.71±2.20 ^b	66.95±0.39 ^b
DPPH (mg TE/100 g)	186.62±2.28 ^a	61.01±0.75 ^c	68.12±0.94 ^b
FRAP (mmol Fe(II)/100 g)	1.76±0.05 ^a	0.39±0.01 ^c	0.51±0.01 ^b
ORAC (mg TE/100 g)	915.7±72.7 ^a	416.7±5.3 ^b	408.7±23.3 ^b
PCL-ACL (mg TE/100 g)	150.77±0.70 ^a	90.13±3.49 ^b	90.28±0.47 ^b
PCL-ACW (mg TE/100 g)	68.33±0.33 ^a	55.41±0.41 ^b	67.33±1.44 ^a
PCL-IAC (mg TE/100 g)	219.10±0.38 ^a	145.54±3.90 ^c	157.61±1.56 ^b

The results are presented as mean ± standard deviation. Different lowercases mean significant differences ($p < 0.05$) between samples within the same row. TPC, total phenolic content; ABTS, ABTS radical cation scavenging activity; DPPH, DPPH radical scavenging activity; FRAP, ferric-reducing antioxidant power; ORAC, oxygen radical absorbance capacity; PCL-ACL and PCL-ACW, photochemiluminescence assay – antioxidant capacity of lipid- and water-soluble substances, respectively; PCL-IAC, integral antioxidant capacity in PCL assay; GAE, gallic acid equivalent; TE, Trolox equivalent.

raw FA and FA+AA. We hypothesize that, with better chemical composition than apple, SB juice, with or without 4% CaL, could be beneficial in improving the functional properties of apple in the form of freeze-dried chips through conventional and non-conventional impregnation processes.

Mass transfer phenomena and water activity

The mass transfer results of the impregnated FA+AA prior to freeze-drying are presented in Table 3. Regardless of the pressure and the CaL concentration used, the VI caused a significant weight and water gain of ascorbic acid-treated apple flesh. The lower the VI absolute pressure, the higher the weight and water gain. The highest ($p < 0.05$) weight gain and water gain were determined in VI₂₀₀ with 0% CaL and reached 18.72% and 17.49%, respectively. The weight and water gain in fresh-cut apples after VI were reported in a previous study. Various Polish cultivars of apple cubes showed weight gain of 15–32% after the VI treatment (150 mbar) using a solution containing 0.5% ascorbic acid, 0.5% citric acid, and 10% sucrose (w/w) [Kidoń *et al.*, 2023]. The authors also found that the weight gain was negatively correlated ($r = -0.85$) with firmness of apples before VI. In another study, VI performed at 738 mbar on Golden Delicious cubes (13.25–13.50°Brix) using hypotonic lemon juice (6.25°Brix) showed significantly higher weight

and water gain compared to those prepared by the dipping process [Neri *et al.*, 2019].

The water gain/loss of CON and US-treated FA+AA under the same CaL concentration was similar ($p \geq 0.05$) (Table 3). The values ranged from -3.46% (US_30, 4% CaL) to 1.41% (US_20, 0% CaL). About 1% weight loss was observed in FA+AA impregnated in 0% CaL, while 4% CaL resulted in about 2–4% weight loss, especially in the US-treated samples. There was no significant ($p \geq 0.05$) weight loss difference between FA+AA prepared with 0 and 4% CaL in CON and all US treatments, except for US_20, where 4% CaL caused significantly ($p < 0.05$) higher weight loss than 0% CaL. According to literature, the US treatment (probe, 24 kHz, 400 W) of Granny Smith apple, impregnated with hypotonic vitamin B₁₂ solution, showed minor water gain/loss, depending on the US power and treatment time (5–15 min) applied [Vasile *et al.*, 2022]. The same authors also observed that the treatment at 400 W with two different amplitudes of 20% and 100% resulted in up to 2% water loss and 5.1% water gain, respectively.

The total solid gain/loss in all treatments was close to zero, however slight but significant ($p < 0.05$) differences were found between the samples (Table 3). The highest solid loss was observed in US_10 with 0% CaL, and the highest solid gain in VI_200 and VI_400 with 4% CaL. In comparison to 0% CaL, the 4% CaL treatment caused significantly ($p < 0.05$) higher solid gain in VI and CON, and solid loss in US_10 and US_20. For US_30, there was no significant difference ($p \geq 0.05$) between the samples treated with and without CaL. A solid loss of apple was also highlighted in US-assisted impregnation using a hypotonic vitamin B₁₂ solution [Vasile *et al.*, 2022] and VI in a hypotonic lemon juice solution [Neri *et al.*, 2019].

In terms of a_w , the value determined for the treated FA+AA (CON, VI, and US) ranged from 0.989 to 0.998 for 0% CaL, and from 0.989 to 0.996 for 4% CaL (Table 3), whereas the a_w of raw FA and FA+AA were 0.994 and 0.998, respectively.

The results of this research indicated that the mass transfer phenomena and the a_w of the impregnated apples were affected by the composition of the solution, impregnation types, and process parameters. In the CON treatment, as the used solutions were rather hypotonic, the mass transfer, especially water transfer, may have occurred toward apple tissue by simple diffusion [Vasile *et al.*, 2022]. US and VI could cause cellular damage, allowing fluid to flow within the plant matrix [Yılmaz & Ersus Bilek, 2018]. The VI removes intercellular air and native liquid in the apple, and subsequently the emptied intercellular spaces and pores can be easily filled with the impregnating solution [Paśławska *et al.*, 2019], in this case, SB juice and CaL solution. Vacuum also intensifies capillary flows and favors mass transfer during impregnation, which occurs due to surface tension at the solid-solution interface [Vinod *et al.*, 2024]. The US treatment could also promote mass transfer during the impregnation process due to the cavitation phenomenon and continuous sponge effect of the apple tissue [Nowacka *et al.*, 2021]. Other factors, such as cultivars [Kidoń *et al.*, 2023], maturity of apples [Casim *et al.*, 2023], or even their cultivation method (organic and conventional apples) [Neri *et al.*, 2019], could also affect the mass transfer phenomena during impregnation, as different apples showed different physicochemical characteristics.

■ Color of freeze-dried apples

Next to functional properties, color also is one of the important factors that can attract consumer to buy and consume

Table 3. Mass transfer results and water activity of raw fresh apple flesh (FA), FA dipped in ascorbic acid solution (FA+AA), and FA+AA samples subjected to conventional (CON), and non-conventional impregnation (vacuum impregnation, VI, and ultrasound-assisted impregnation, US) in sea buckthorn juice without and with calcium lactate (CaL).

Treatment	Weight gain/loss (%)		Water gain/loss (%)		Solid gain/loss (%)		Water activity	
	0% CaL	4% CaL	0% CaL	4% CaL	0% CaL	4% CaL	0% CaL	4% CaL
Raw FA	–	–	–	–	–	–	0.994±0.001 ^{abc}	0.994±0.001 ^{abc}
Raw FA+AA	–	–	–	–	–	–	0.998±0.001 ^a	0.998±0.001 ^a
CON	−1.02±0.04 ^{dA}	−0.72±0.13 ^{cA}	0.36±0.04 ^{deA}	−1.08±0.11 ^{cB}	−1.38±0.01 ^{eB}	0.35±0.02 ^{cA}	0.995±0.003 ^{abcA}	0.996±0.002 ^{abA}
VI_200	18.72±1.67 ^{aA}	16.74±1.44 ^{aA}	17.49±1.47 ^{aA}	14.16±1.25 ^{aA}	1.23±0.20 ^{aB}	2.58±0.19 ^{aA}	0.989±0.001 ^{cA}	0.989±0.003 ^{cA}
VI_400	14.85±0.34 ^{bA}	13.89±0.81 ^{aA}	14.26±0.30 ^{bA}	11.47±0.70 ^{ab}	0.59±0.04 ^{bB}	2.42±0.11 ^{abA}	0.991±0.005 ^{bcA}	0.989±0.002 ^{cA}
VI_600	9.07±0.91 ^{cA}	8.96±0.86 ^{bA}	9.94±0.81 ^{cA}	6.93±0.74 ^{bA}	−0.87±0.10 ^{dB}	2.03±0.12 ^{bA}	0.996±0.002 ^{abA}	0.991±0.005 ^{bcA}
US_10	−1.05±0.17 ^{dA}	−2.39±0.58 ^{cA}	1.41±0.15 ^{dA}	−0.81±0.52 ^{cB}	−2.46±0.02 ^{dB}	−1.58±0.07 ^{eA}	0.998±0.001 ^{aA}	0.988±0.001 ^{cB}
US_20	−1.03±0.12 ^{dA}	−3.13±0.59 ^{cB}	0.39±0.10 ^{deA}	−1.96±0.52 ^{cB}	−1.41±0.01 ^{eB}	−1.16±0.07 ^{eA}	0.996±0.001 ^{abA}	0.988±0.001 ^{cB}
US_30	−1.91±1.15 ^{dA}	−4.07±1.26 ^{cA}	−1.71±1.00 ^{eA}	−3.46±1.10 ^{cA}	−0.21±0.15 ^{cA}	−0.62±0.16 ^{dA}	0.991±0.002 ^{bcA}	0.989±0.003 ^{cA}

The results are presented as mean ± standard deviation. Different lowercases mean significant differences ($p < 0.05$) between samples within the same column. Different uppercases mean significant differences ($p < 0.05$) between 0 and 4% CaL within the same impregnation treatment and parameter. Sample codes refer to Table 1.

Table 4. Color parameters of freeze-dried products: fresh apple flesh (FA), FA dipped in ascorbic acid solution (FA+AA), and FA+AA samples subjected to conventional (CON), and non-conventional impregnation (vacuum impregnation, VI, and ultrasound-assisted impregnation, US) in sea buckthorn juice without and with calcium lactate (CaL).

Color parameter	CaL concentration	FA	FA+AA	CON	VI_200	VI_400	VI_600	US_10	US_20	US_30
<i>L</i> *	0%	79.3±1.7 ^b	82.9±1.0 ^a	72.0±2.8 ^a	68.4±1.4 ^{ab}	69.8±1.2 ^{cdB}	71.9±1.3 ^{cb}	71.7±2.0 ^{cA}	70.8±2.2 ^{cdA}	70.4±2.1 ^{cdA}
	4%	79.3±1.7 ^b	82.9±1.0 ^a	68.6±1.9 ^{deB}	69.7±0.9 ^{dA}	72.9±1.1 ^{cA}	74.7±1.2 ^{cA}	68.0±2.6 ^{deB}	67.1±1.0 ^{cb}	66.9±1.4 ^{deB}
<i>a</i> *	0%	3.4±1.3 ^c	−0.2±0.4 ^d	12.3±2.7 ^{abA}	13.8±0.6 ^{abA}	12.4±1.4 ^{abA}	11.2±1.2 ^{bA}	12.5±2.8 ^{bcA}	13.4±2.3 ^{abA}	14.9±2.6 ^{aA}
	4%	3.4±1.3 ^e	−0.2±0.4 ^f	12.8±1.8 ^{abA}	11.4±0.7 ^{bcb}	9.9±1.1 ^{cdB}	8.1±1.0 ^{db}	13.2±1.9 ^{aA}	13.6±1.0 ^{aA}	13.7±1.4 ^{aA}
<i>b</i> *	0%	25.3±1.6 ^e	18.0±2.6 ^f	57.5±4.3 ^{abA}	53.1±1.4 ^{bcdA}	52.1±4.0 ^{cdA}	51.4±2.8 ^{dA}	57.0±4.2 ^{bcdA}	57.8±3.7 ^{abA}	60.9±5.6 ^{aA}
	4%	25.3±1.6 ^f	18.0±2.6 ^d	48.4±5.7 ^{bcb}	47.3±3.2 ^{bb}	47.2±2.3 ^{bb}	47.0±2.9 ^{bb}	48.6±3.5 ^{abb}	52.3±3.7 ^{ab}	52.8±3.4 ^{ab}
ΔE	0%	9.0±2.2 ^d	–	42.9±5.1 ^{abA}	40.5±1.4 ^{bca}	38.7±3.6 ^{bca}	37.0±2.8 ^{cA}	42.5±5.1 ^{abcA}	43.8±4.2 ^{abA}	47.2±6.2 ^{aA}
	4%	9.0±2.2 ^d	–	36.1±5.6 ^{abcb}	34.2±2.8 ^{bcb}	32.4±2.5 ^{bcb}	31.3±2.8 ^{cb}	36.6±4.6 ^{bbB}	40.2±3.7 ^{aA}	40.8±3.1 ^{ab}
BI	0%	41±4 ^d	24±4 ^d	151±27 ^{abca}	145±7 ^{bca}	135±14 ^{bca}	125±12 ^{ca}	150±26 ^{abca}	157±22 ^{abA}	175±33 ^{aA}
	4%	41±4 ^f	24±4 ^f	127±25 ^{bcdA}	117±11 ^{cdB}	107±10 ^{deB}	101±10 ^{eb}	130±22 ^{abca}	147±19 ^{abA}	149±15 ^{ab}
WI	0%	67.0±2.1 ^b	75.1±2.2 ^a	34.9±5.1 ^{deB}	36.6±1.3 ^{cdB}	38.5±3.2 ^{cdB}	40.4±2.7 ^{cb}	35.1±4.9 ^{deB}	33.9±4.2 ^{deA}	30.6±5.9 ^{ab}
	4%	67.0±2.1 ^b	75.1±2.2 ^a	40.8±5.3 ^{deA}	42.7±2.6 ^{cdA}	44.7±2.4 ^{cdA}	46.0±2.7 ^{ca}	40.3±4.5 ^{deA}	36.7±3.6 ^{aA}	36.2±2.9 ^{aA}
YI	0%	45.7±3.4 ^d	31.0±4.6 ^e	114.4±11.6 ^{abA}	111.0±3.0 ^{bca}	106.5±6.4 ^{bca}	102.1±5.9 ^{cA}	113.8±11.1 ^{bca}	116.7±9.7 ^{abA}	123.7±13.3 ^{aA}
	4%	45.7±3.4 ^e	31.0±4.6 ^f	101.0±12.6 ^{bcb}	96.9±6.2 ^{cdB}	92.5±5.3 ^{cdB}	89.8±5.8 ^{db}	102.5±10.9 ^{abcb}	111.5±9.3 ^{abA}	112.7±7.1 ^{ab}

The results are presented as mean ± standard deviation. Different lowercases mean significant differences (*p*<0.05) between samples within the same row. Different uppercases mean significant differences (*p*<0.05) between 0 and 4% CaL within the same impregnation treatment and color parameter. ΔE , total color difference compared to FA+AA sample; BI, browning index; WI, whiteness index; YI, yellowness index. Sample codes refer to Table 1.

the functional food products. The color of the freeze-dried control and impregnated apple flesh was described by several parameters (Table 4). Regarding controls, FA+AA exhibited higher L^* , lower a^* and b^* , as well as higher WI and lower YI compared to FA ($p < 0.05$). This might be explained by the role of ascorbic acid that could prevent melanin formation through intermediate products binding, e.g., *o*-quinone, thus increasing the lightness of the apple [Moon *et al.*, 2020].

The color parameters of freeze-dried impregnated apples (CON, VI, and US) were influenced by the SB juice in the impregnation solution, and more specifically by the carotenoids present in it. For SB juice, L^* , a^* , and b^* were 52.9 ± 0.1 , 22.0 ± 0.1 , and 63.5 ± 0.3 , respectively. The treated samples had lower L^* and WI, and higher a^* , b^* , BI, and YI than the controls ($p < 0.05$) (Table 4). The ΔE of the impregnated samples ranged from 37.0 to 47.2 in 0% CaL, and 31.3 to 40.8 in 4% CaL, indicating that the color difference between FA+AA and all impregnated apple products could be clearly noticed by observers. The color changes of apple chips or fresh apple pieces caused by the use of a colored impregnation solution or an osmotic dehydration solution were observed in other studies, e.g., apples with cranberry and grape juices [Wang *et al.*, 2022], beetroot juice [Aguirre-García *et al.*, 2020], and chokeberry juice [Lech *et al.*, 2018].

The enzymatic browning, which determines color, may occur in apple products, due to the presence of polyphenol oxidase (POD) and peroxidase (POD). Although the color parameters of apple products, especially L^* , ΔE , BI, WI, and YI, were used in some studies to observe the enzymatic browning, those parameters are not always in accordance with PPO and POD activities as the color of the impregnating or dipping solution may also affect those parameters [Arnold & Gramza-Michałowska, 2022]. The activity of PPO and POD was determined in our study and discussed below to confirm whether the enzymatic browning occurred in the freeze-dried products.

Regarding the application of non-conventional impregnations (VI and US), only freeze-dried samples prepared under certain treatment conditions showed significant differences in BI, WI, and YI compared to CON (Table 4). However, ΔE did not show any significant differences between CON and non-conventional treatments in either 0% or 4% CaL solution. This might be due to the intense color from SB juice in the samples, noting that for products with intense color, even the ΔE values of 6–7 may still be subtle to the eye [Tylewicz *et al.*, 2020]. In another report, the VI with isotonic CaL solutions caused a significant darkening of melon, which might be due to changes in the structural properties of the tissue as a consequence of vacuum application and/or gas-liquid exchange that can alter the refraction index [Tappi *et al.*, 2016]. The application of ultrasound in combination with VI using a solution containing 9% sucrose, 0.5% citric acid, and 0.5% ascorbic acid (*w/w*) also affected the ΔE of cranberry, showing that cranberries impregnated at 50 mbar had lower ΔE than those treated at 300 mbar [Mierzwa *et al.*, 2022].

Generally, the addition of 4% CaL tended to cause significant changes in the color parameters of the products, regardless

of the impregnation method (CON, VI, or US). The VI of fresh-cut melon using up to 5% of CaL also showed significant changes in L^* and hue angle, depending on the pressure levels [Tappi *et al.*, 2016]. In another study, similar chroma values were found for pineapples osmotically dehydrated in sugar solution with 2 and 4% CaL [Silva *et al.*, 2014].

■ Total phenolic content and antioxidant capacity of freeze-dried apples

The TPC and antioxidant capacity (determined by ABTS, DPPH, FRAP, ORAC, PCL-ACL, PCL-ACW, and PCL-IAC assays) of freeze-dried controls and impregnated samples is shown in Figure 1. In controls, FA+AA in general showed higher TPC and antioxidant capacity than FA, due to the presence of ascorbic acid. However, an insignificant increase was found in ABTS and ORAC assays ($p \geq 0.05$).

Among the impregnated samples (CON, VI, and US), the addition of 4% CaL in the solution significantly ($p < 0.05$) decreased the TPC and antioxidant capacity of the samples, except for the TPC and ORAC values in US_10 and US_20, and ABTS in CON, where the values were similar ($p \geq 0.05$) between CaL concentrations. This phenomenon could be explained by the presence of calcium in the samples, which increased the weight of the freeze-dried samples and subsequently reduced the TPC and antioxidant capacity *per* 100 g of the sample with 4% CaL [Arnold *et al.*, 2025]. Another possible mechanism could also be the total soluble solid change of the impregnating solution from 4.53°Brix in 0% CaL to 7.47°Brix in 4% CaL solution, which subsequently affected the mass transfer phenomenon and influenced the TPC and antioxidant capacity of freeze-dried products. Although both solutions were still mildly hypotonic than raw FA+AA (8.80°Brix), the higher total soluble solid difference between 0% CaL solution and raw FA+AA could have caused a higher increase in the TPC and antioxidant capacity of the samples than 4% CaL solution. In mango, adding sucrose to the grape-residue solution (raising total soluble solids) during osmosonication-assisted vacuum impregnation (US and VI combined) did not significantly increase the TPC, while US and VI alone without sucrose increased TPC [Batista de Medeiros *et al.*, 2019].

In Figure 1, it was observed that mainly the CON samples showed higher TPC and antioxidant capacity than FA+AA and FA. However, the significance of these differences varied depending on the type of antioxidant assay and CaL concentration. Although other studies reported increased antioxidant activity of apple products impregnated in hypertonic solutions, e.g., concentrated chokeberry juice without [Masztalerz *et al.*, 2021] or with sucrose [Kowalska *et al.*, 2020] and beetroot juice with sucrose [Aguirre-García *et al.*, 2020], the impregnation using hypotonic SB juice solution in this study could also increase the TPC and antioxidant activity of the end product, especially in the treatment with 0% CaL. Another study also observed faster migration of phenolic compounds into cucumber slices after impregnation in a hypotonic gallic acid solution than in a hypertonic gallic acid-glycerol solution, and further used various



Figure 1. Total phenolic content, TPC (A), ABTS radical cation scavenging activity (B), DPPH radical scavenging activity (C), ferric-reducing antioxidant power, FRAP (D), oxygen radical absorbance capacity, ORAC (E), and results of photochemiluminescence assay including antioxidant capacity of lipid-soluble substances, PCL-ACL (F) water-soluble substances, PCL-ACW (G), and integral antioxidant capacity, PCL-IAC (H) of freeze-dried products: fresh apple flesh (FA), FA dipped in ascorbic acid solution (FA+AA), and FA+AA samples subjected to conventional (CON) and non-conventional impregnation (vacuum impregnation, VI, and ultrasound-assisted impregnation, US) in sea buckthorn juice without and with calcium lactate (CaL). Different lowercase letters indicate significant differences ($p < 0.05$) between samples within the same CaL concentration. Different uppercase letters indicate significant differences ($p < 0.05$) between 0 and 4% CaL within the same impregnation treatment. GAE, gallic acid equivalent; TE, Trolox equivalent. Sample codes refer to Table 1.

hypotonic herbal solutions to improve the TPC and antioxidant activities (ABTS and FRAP assays) of cucumber slices [Giannakourou *et al.*, 2019].

In contrast to this study, our previous research found lower or similar TPC and antioxidant capacity of freeze-dried Polish Gala apples conventionally impregnated with hypotonic Polish SB juice, even without CaL addition, as compared to FA+AA and FA [Arnold *et al.*, 2025]. The variation in raw material origin may explain the differences in results, as apples and SB juice from different regions can exhibit distinct physicochemical compositions [Ciesarová *et al.*, 2020; Raj *et al.*, 2021]. The differences in physicochemical compositions of apples and SB juice may further influence the mass transfer during the impregnation process and subsequently the chemical composition and antioxidant properties of the end products.

In this study, the application of non-conventional techniques (VI and US) under certain treatment conditions improved the TPC and antioxidant capacity of freeze-dried samples in comparison to CON treatments under the same CaL concentration (**Figure 1**). For the VI treatment, samples prepared with VI_200 exhibited the highest TPC and antioxidant capacity in both 0% and 4% CaL. This indicated that the lower the pressure applied, the more empty intercellular spaces in apple tissue, and the better the mass transfer as more antioxidants from SB juice filled those spaces and eventually increased the antioxidant capacity of the freeze-dried product. This was in accordance with the weight gain (**Table 3**), where VI_200 exhibited the highest weight gain in 0% CaL (18.72%) and 4% CaL (16.74%).

In another study using hypotonic lemon juice (6.25°Brix), it was observed that the VI of organic and conventional Golden Delicious (13.25–13.50°Brix), even at 738 mbar and 10 s of holding time, could increase TPC by 8–11% and antioxidant activity by 10–13% (ABTS assay) [Santarelli *et al.*, 2020]. In the present study, in comparison to CON at the same CaL concentration, the TPC of samples after VI_200 increased 14.8% and 19.5% for 0 and 4% CaL, respectively. Among antioxidant assays, the highest increases after VI_200 were observed in PCL-ACW, which were 26.8% and 29.9% for 0 and 4% CaL, respectively. However, it is noteworthy that the type of impregnating solution, treatment time, and vacuum level in the VI treatment may influence the antioxidant capacity of end products. Pasławska *et al.* [2019] observed both negative and positive impacts of VI (40–80 mbar, 10–80 s) of Ligol apple in apple-pear juice or citric acid solution on the TPC and antioxidant activities (ABTS and FRAP assays) [Pasławska *et al.*, 2019]. In contrast, Trusinska *et al.* [2024], who used an isotonic aloe vera-trehalose solution (13 °Brix) during VI of Italian Golden Delicious slices, observed that VI (200 mbar, 10 min) significantly decreased vitamin C content by 34% and TPC by 32%, while similar antioxidant activity was observed in the ABTS assay. Nevertheless, based on the present study, VI especially at 200 mbar, could be an effective way not only to increase the TPC and antioxidant capacity of freeze-dried apples, but also to save more time as the treatment lasted shorter (28 min) than US or CON (120 min).

In the US treatment, the longer the treatment time, the higher the TPC and antioxidant capacity of the samples, especially in the 0% CaL solution. The US treatment (45 kHz, 900 W, 10–30 min) prior to VI was also reported to minimize the loss of TPC and vitamin C caused by VI alone [Trusinska *et al.*, 2024]. The US treatment may loosen the apple matrix, facilitating the impregnating solution to penetrate into the matrix [Indriani *et al.*, 2023], thus improving the antioxidant properties of the end freeze-dried products.

As the highest TPC and antioxidant capacity among the samples treated with 0% CaL were observed for US_30, the optimum US treatment with 4% CaL was US_20. In comparison to US_20 (in 4% CaL), the US_30 sample had significantly ($p < 0.05$) lower antioxidant capacity in all assays, while TPC did not differ significantly ($p \geq 0.05$). After the application of those optimum US conditions (US_30 for 0% CaL and US_20 for 4% CaL), the TPC of samples increased 11.7% and 13.8% for 0 and 4% CaL, respectively, compared to CON samples. Among antioxidant capacity assays, the highest increase was observed in PCL-ACW (29.7%) for 0% CaL and ORAC (35.5%) for 4% CaL.

During the US-assisted impregnation, the mass transfer in the samples was facilitated by cavitation, which is the formation, growth, and collapse of bubbles in the impregnating solution or solids (which contain moisture), produced by the pressure fluctuations generated by the ultrasound waves [Nowacka *et al.*, 2021]. The implosion of these microbubbles could promote mass transfer rates and further influence various mechanical properties [Batista de Medeiros *et al.*, 2019]. The pores of the apple flesh in this study could expand more with the action of collapsed bubble, resulting in more intercellular spaces inside the flesh, and further allowing more antioxidants from the impregnating solution (in this case, SB juice) would infuse through the pores; this was similarly explained in the influence of US on vacuum impregnated apple flesh using black carrot concentrate [Yilmaz & Ersus Bilek, 2018]. However, in the 4% CaL solution, the decline in antioxidant capacity after 30 min of the US treatment might be due to US-induced aggregation of the compounds of impregnating solution within the apple matrix, leading to an increased total soluble solid content with prolonged US treatment. This might cause irreversible excessive cell ruptures, so that during relaxation time, the excessively ruptured cells could not optimally retain the infused antioxidants from SB juice solution. Also, in some cases, viscous solutions make penetration of solutes more difficult compared to less viscous solutions [Tappi *et al.*, 2016]. Furthermore, a study observed that the application of US (33 kHz, 60 W) to cherry in water for up to 40 min increased the TPC and antioxidant activities (ABTS and DPPH assays) of cherry, due to cell disruption and more effective extraction of phenolics. However, after 60 min, the cell disruption might be excessive, resulting in detrimental effects, such as phenolics degradation [Muzaffar *et al.*, 2016]. Apples impregnated with black carrot concentrate also showed higher ion leakage when US treatment (33 kHz) was applied at 198 W, compared to those at 0–158 W, indicating a higher rate of cellular damage in plant tissue [Yilmaz & Ersus Bilek, 2018].

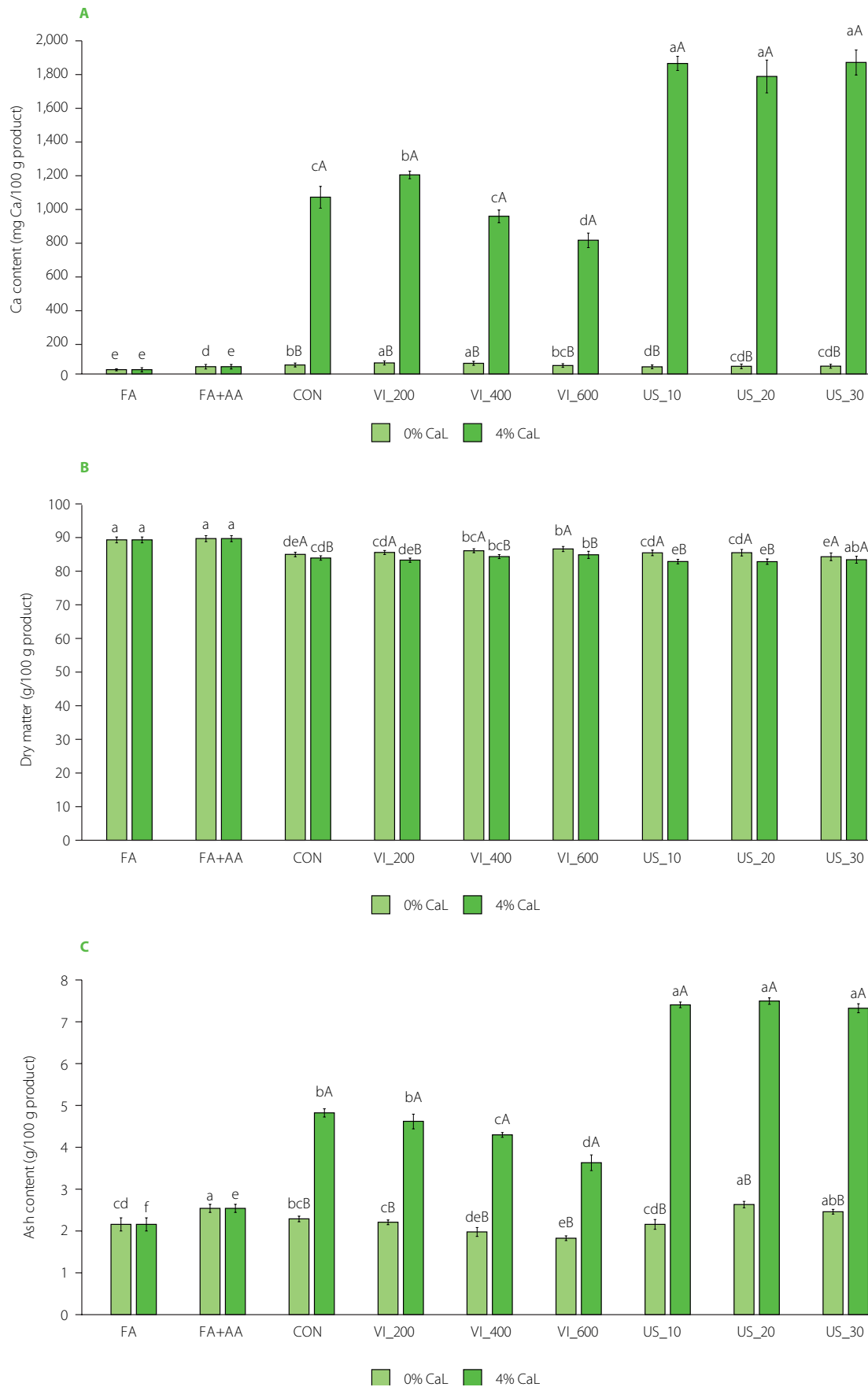


Figure 2. Content of calcium (A), dry matter (B), and ash (C) of freeze-dried products: fresh apple flesh (FA), FA dipped in ascorbic acid solution (FA+AA), and FA+AA samples subjected to conventional (CON) and non-conventional impregnation (vacuum impregnation, VI, and ultrasound-assisted impregnation, US) in sea buckthorn juice without and with calcium lactate (CaL). Different lowercase letters indicate significant differences ($p < 0.05$) between samples within the same CaL concentration. Different uppercase letters indicate significant differences ($p < 0.05$) between 0 and 4% CaL within the same impregnation treatment. Sample codes refer to Table 1.

■ Calcium content of freeze-dried apples

The calcium content of freeze-dried apples before and after the impregnation treatment is shown in **Figure 2**. The FA had a calcium content of 25.78 mg Ca/100 g, while FA+AA showed a higher calcium content of 43.65 mg Ca/100 g. The dipping process with ascorbic acid may have changed the calcium content of FA through mass transfer. In 0% CaL solution, all impregnated samples (CON, VI, and US) showed a higher calcium content than FA. However, when compared to FA+AA, only CON (54.22 mg Ca/100 g) and VI (51.09–66.65 mg Ca/100 g) freeze-dried apples had significantly ($p < 0.05$) higher values, while US samples (43.45–46.71 mg Ca/100 g) had similar ($p \geq 0.05$) values. In our previous study, using SB juice and Gala apples from other sources (fruits originating from Poland), a calcium content of 79.44 mg Ca/100 g was determined in a conventionally-impregnated sample without CaL addition [Arnold *et al.*, 2025]. The higher calcium content of SB juice than raw FA and FA+AA (**Table 2**) might explain the increasing calcium content in the impregnated samples without CaL.

The samples prepared at 4% CaL in the solution, as expected, showed a significantly ($p < 0.05$) higher calcium content than those treated at 0% CaL (**Figure 2**). Interestingly, among the VI treatments, only VI_200 effectively ($p < 0.05$) increased the calcium content of the freeze-dried apples compared to CON (from 1,194 to 1,060 mg Ca/100 g), while the VI_400 sample showed similar ($p \geq 0.05$) values and VI_600 showed 24.3% lower calcium content than CON. When comparing CON with VI_600 in the 4% CaL solution, the pressure used in VI_600 was not sufficient to enhance impregnation. Instead, it may have caused the impregnating solution, along with the calcium, to be drawn out of the apple tissue. This could explain the reduced calcium content observed in the sample, a phenomenon also reported in a previous study using Fuji apples [Assis *et al.*, 2019]. Casim *et al.* [2023] determined calcium contents of 3,600 and 5,200 mg Ca/kg in Granny Smith dried apples treated by atmospheric (4.5 h, 20°C) and vacuum impregnation (50 mm Hg, 10 min) using an isotonic solution containing 3% calcium gluconate and 2.24% CaL, which were higher than those of the untreated apples (39 mg Ca/kg).

US treatments (4% CaL) resulted in a higher calcium content of the freeze-dried apples, *i.e.*, 49.3–56.4% higher compared to VI_200 and 68.3–76.1% higher compared to CON (**Figure 2**). However, the duration of the US treatment did not significantly ($p \geq 0.05$) affect the calcium content. Therefore, US_20 (4% CaL), with 1,783 mg Ca/100 g, was suggested as optimal, considering it increased both calcium content and antioxidant capacity. The simultaneous application of US (130 W, 35 kHz) during VI (211 mmHg), using a solution containing 3% CaL and 0.8% black carrot concentrate, led to a 13.8% calcium content increase in Starking Delicious apple discs, compared to VI without US [Yilmaz & Ersus Bilek, 2018]. The authors also stated that the addition of Ca could inhibit microbial activity in the product by improving the resistance of cellular structures to microbial infection, and also due to the presence of lactate ions derived from Ca-lactate.

As additional information, considering 25% calcium absorption in adults, consuming 100 g of freeze-dried apples prepared at VI_200 and US_20 in 4% CaL solution may fulfill 29.8% and 44.6% of the recommended daily allowance for adults aged 19–50 [National Institutes of Health, 2022].

■ Relationship between total phenolic content, antioxidant capacity, and physicochemical parameters of freeze-dried apples

PCA was applied to better understand the relationship between TPC, antioxidant capacity, calcium, dry matter, and ash content of freeze-dried apples. The first two principal components accounted for 92.45% of the total variance (PC1 – 64.79%; PC2 – 27.66%). In the PCA biplot, three groups were noticeable, including a cluster with control samples (FA and FA+AA), a cluster with apples impregnated with 4% CaL, and a group with samples impregnated with 0% CaL (**Figure 3**). Distinctive subgroups of CON, VI, and US were also observed in the cluster with the apples impregnated with 4% CaL. Variables that distinguished freeze-dried apples treated at 0% CaL were TPC and antioxidant capacity determined by different antioxidant assays, while calcium and ash content characterized samples at 4% CaL. The calcium content of the products was affected not only by the concentration of CaL in the solution, but also by the technology applied during the impregnation process. The calcium content of the product was correlated with ash content, while TPC was associated with antioxidant capacity, especially when it was determined by ORAC, FRAP, ABTS, and DPPH assays.

■ Content of individual phenolics and carotenoids of freeze-dried apples

The composition of phenolics and carotenoids of the freeze-dried apple controls and samples with selected treatments from 0% CaL (CON, VI_200, and US_30) and 4% CaL (CON, VI_200, and US_20) is shown in **Table 5**. The total phenolics of the impregnated samples (226.18–328.02 mg/100 g product) determined by UPLC-PDA were more than twice as high as those of FA and FA+AA (95.03–99.53 mg/100 g product). The application of VI and US generally improved the total phenolics compared to CON, regardless of the CaL concentration, although increases for US_30 without CaL and VI_200 with CaL were insignificant ($p \geq 0.05$). Furthermore, CON and VI at 0% CaL showed significantly higher total phenolics than those treated at 4% CaL ($p < 0.05$), while US showed similar values. This trend of results was in accordance with the antioxidant capacity and TPC (**Figure 1**).

However, the increase was not found in all phenolic subgroups. The impregnated samples regardless of the CaL addition had lower sums of phenolic acids compared to controls, except VI_200 without CaL which showed a similar value to controls (**Table 5**). Controls also had more dihydrochalcones (6.56–6.88 mg/100 g product) than the treated apples. Although the application of VI and US could enhance the impregnation of a bioactive-containing solution into the apple, it could also lead to microstructural changes in the tissue, possibly degrading

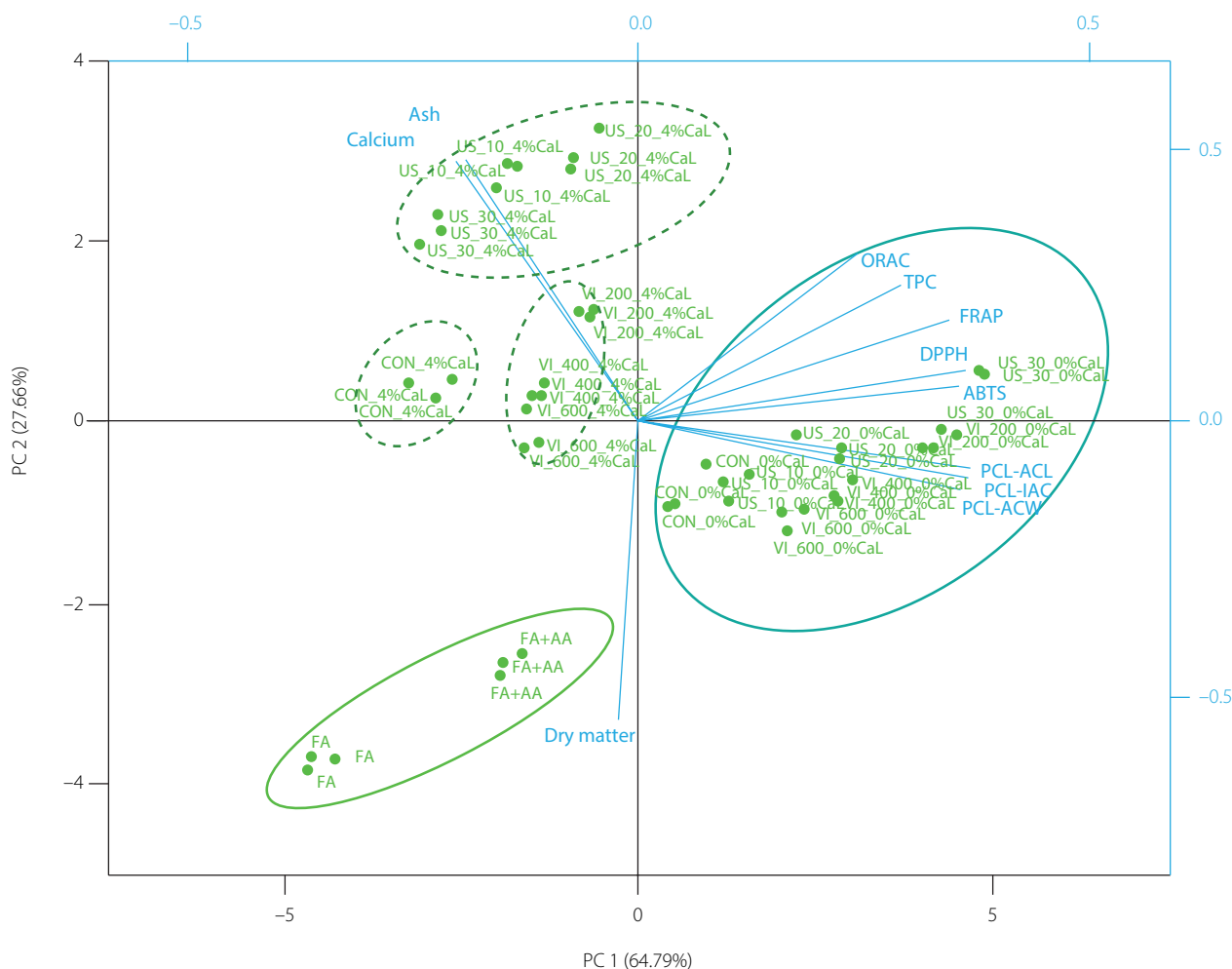


Figure 3. Biplot of principal component analysis of total phenolic content, antioxidant capacity, and physicochemical parameters of freeze-dried products: fresh apple flesh (FA), FA dipped in ascorbic acid solution (FA+AA), and FA+AA samples subjected to conventional (CON) and non-conventional impregnation (vacuum impregnation, VI, and ultrasound-assisted impregnation, US) in sea buckthorn juice without and with calcium lactate (CaL). Sample codes refer to Table 1.

or enhancing the migration of some native antioxidants, in this case lower molecular weight phenolics like phenolic acids and dihydrochalcones, out of the apple matrix [Vinod *et al.*, 2024; Yılmaz & Ersus Bilek, 2018]. Therefore, it is necessary for industries to control the processing parameters of VI and US to improve the functional properties of food products, noting that the sample matrix and process parameters will profoundly affect specific compound behavior.

Furthermore, the impregnated samples with 0% CaL showed the highest range of flavan-3-ols content, followed by 4% CaL and controls (Table 5). Catechin was generally the predominant compound among flavan-3-ols in the impregnated samples, due to the involvement of SB juice; sea buckthorn fruits are rich in this compound [Guo *et al.*, 2017]. Among phenolics, flavonols were the most abundant subgroup in the impregnated samples also due to SB juice, while only quercetin 3-O-rhamnoside was found in FA and FA+AA. The profile of SB fruit comprises mainly diverse simple glycosides of isorhamnetin and quercetin with less frequent occurrence of kaempferol [Ciesarová *et al.*, 2020]. Isorhamnetin 3-O-rutinoside (about 36%) and isorhamnetin 3-O-glucoside

(about 22%) were the major flavonols, which were also dominant in the end product of our previous study, using Polish Gala and SB juice from the Polish retail market [Arnold *et al.*, 2025], as well as agreed with the flavonol composition determined in various SB juices [Tkacz *et al.*, 2020]. The flavonol content of SB differs due to maturity, climate, cultivars, and geographical sites. It is worth noting that flavonoids play important roles in osteogenesis [Yuca *et al.*, 2024] and the prevention of chronic diseases, such as diabetes, cardiovascular diseases, and cancer, due to their antioxidant activity [Ciesarová *et al.*, 2020].

For carotenoids, just like with the phenolic compounds, the impregnated freeze-dried apples with SB juice showed higher total carotenoids (16.47–38.88 mg/100 g) than the controls (2.70–3.02 mg/100 g) (Table 5). Among carotenoids, carotenes dominated with the highest content in VI_200 at 0% CaL accounting for 21.62 mg/100 g product. When comparing between 0 and 4% CaL in each treatment (CON, VI, US), the CON and VI samples prepared at 0% CaL showed higher total carotenoids, while US had similar values. Carotenoids have been studied not only for their contribution to antioxidant activity, but also for their role in bone metabolism, such as bone mineral density and bone

Table 5. Phenolic and carotenoid compositions of freeze-dried products (mg/100 g): fresh apple flesh (FA), FA dipped in ascorbic acid solution (FA+AA) and FA+AA samples subjected to conventional (CON), and non-conventional impregnation (vacuum impregnation, VI), and ultrasound-assisted impregnation, US) in sea buckthorn juice without and with calcium lactate (CaL).

Compound	Control			0% CaL			4% CaL		
	FA	FA+AA	CON	CON	VI_200	US_30	CON	VI_200	US_20
Phenolics									
<i>p</i> -Coumaric acid <i>O</i> -hexoside	30.44±1.85 ^{ab}	32.24±0.42 ^a	25.34±0.04 ^{cd}	33.07±0.33 ^a	21.78±1.51 ^e		26.91±0.35 ^{bc}	27.45±0.24 ^{bc}	22.72±0.49 ^{de}
Ferulic acid <i>O</i> -hexoside	3.52±0.27 ^a	2.97±0.04 ^a	2.77±0.07 ^a	3.4±0.22 ^a	4.06±1.76 ^a		2.51±0.20 ^a	2.04±0.04 ^a	2.24±0.07 ^a
Sum of phenolic acids	33.96±2.13^{ab}	35.22±0.46^a	28.10±0.03^c	36.48±0.55^a	25.84±3.26^c		29.42±0.55^{bc}	29.50±0.20^{bc}	24.96±0.42^c
(+)-Catechin	2.26±0.18 ^d	3.02±0.09 ^d	44.90±1.85 ^a	35.41±0.49 ^b	36.93±3.50 ^b		34.24±2.02 ^b	25.00±0.18 ^c	37.58±0.06 ^b
Procyanidin B ₁	nd	nd	34.58±0.96 ^{ab}	42.72±0.53 ^a	32.86±8.01 ^{ab}		23.47±0.46 ^b	27.14±0.25 ^b	31.98±0.41 ^{ab}
(-)-Epicatechin	13.37±0.90 ^a	12.21±0.37 ^{ab}	8.38±0.99 ^c	11.88±0.36 ^{ab}	9.20±0.75 ^c		12.41±0.10 ^{ab}	10.23±0.52 ^{bc}	9.07±0.33 ^c
Procyanidin C ₁	42.20±0.70 ^a	36.94±0.49 ^b	20.35±0.15 ^{cd}	23.90±2.49 ^c	18.32±0.53 ^d		22.07±0.04 ^{cd}	22.01±0.33 ^{cd}	18.22±0.52 ^d
Sum of flavan-3-ols	57.84±1.43^d	52.16±0.21^d	108.2±0.25^{ab}	113.9±1.82^a	97.32±1.29^{abc}		92.18±2.34^{bc}	84.39±1.29^c	96.86±1.20^{bc}
Phloretin 2- <i>O</i> -xyloglucoside	2.51±0.09 ^{bc}	2.28±0.08 ^c	0.50±0.09 ^d	0.74±0.12 ^d	0.98±0.06 ^d		2.40±0.21 ^{bc}	3.03±0.05 ^b	4.27±0.39 ^a
Derivative of dihydrochalcones	2.87±0.52 ^a	2.80±0.11 ^a	nd	nd	nd		nd	nd	nd
Phloretin 2- <i>O</i> -glucoside	1.50±0.41 ^a	1.48±0.06 ^a	nd	nd	nd		nd	nd	nd
Sum of dihydrochalcones	6.88±1.02^a	6.56±0.03^a	0.50±0.09^e	0.74±0.12^e	0.98±0.06^{de}		2.40±0.21^{cd}	3.03±0.05^{bc}	4.27±0.39^b
Isorhamnetin 3,7- <i>O</i> -dihexoside	nd	nd	6.94±0.24 ^b	8.56±0.36 ^a	8.16±0.06 ^a		4.61±0.12 ^c	6.96±0.07 ^b	8.77±0.02 ^a
Isorhamnetin 3- <i>O</i> -sophoroside-7- <i>O</i> -rhamnoside	nd	nd	5.29±0.11 ^{bc}	6.36±0.33 ^a	6.03±0.28 ^{ab}		3.62±0.29 ^d	4.95±0.04 ^c	6.45±0.12 ^a
Quercetin 3- <i>O</i> -(di(rhamnosyl))hexoside	nd	nd	1.81±0.28 ^{bc}	2.25±0.36 ^{ab}	2.26±0.07 ^{ab}		1.32±0.02 ^c	1.87±0.12 ^{bc}	2.56±0.03 ^a
Quercetin 3-galactoside-7- <i>O</i> -rhamnoside	nd	nd	3.21±0.04 ^{ab}	3.84 ± 0.19 ^a	3.48±0.58 ^{ab}		1.70±0.13 ^c	2.80±0.11 ^b	3.79±0.06 ^a
Quercetin 3- <i>O</i> -rutinoside	nd	nd	3.50±0.00 ^b	4.51 ± 0.18 ^a	4.13±0.43 ^{ab}		2.35±0.26 ^c	3.33±0.31 ^b	4.39±0.03 ^a
Isorhamnetin 3- <i>O</i> -glucoside-7- <i>O</i> -rhamnoside	nd	nd	28.46±0.88 ^b	34.11±0.53 ^a	33.77±0.22 ^a		19.33±0.98 ^c	28.45±0.59 ^b	35.15±0.24 ^a
Quercetin 3- <i>O</i> -glucoside	nd	nd	4.71±0.83 ^{ab}	5.30±0.72 ^a	6.16±0.07 ^a		3.57±0.24 ^b	4.99±0.31 ^{ab}	6.26±0.22 ^a



Table 5 continued. Phenolic and carotenoid compositions of freeze-dried products (mg/100 g): fresh apple flesh (FA), FA dipped in ascorbic acid solution (FA+AA) and FA+AA samples subjected to conventional (CON), and non-conventional impregnation (vacuum impregnation, VI, and ultrasound-assisted impregnation, US) in sea buckthorn juice without and with calcium lactate (CaL).

Compound	Control			0% CaL			4% CaL		
	FA	FA+AA	CON	CON	VI_200	US_30	CON	VI_200	US_20
Kaempferol 3-O-hexoside-7-O-rhamnoside	nd	nd	3.77±0.02 ^{ab}	4.28±0.13 ^a	4.28±0.13 ^a	4.04±1.02 ^{ab}	2.65±0.07 ^b	3.92±0.30 ^{ab}	4.80±0.02 ^a
Isorhamnetin 3-O-(rhamnosyl)hexoside	nd	nd	1.28±0.02 ^b	1.52±0.01 ^a	1.52±0.01 ^a	1.51±0.06 ^a	0.99±0.06 ^c	1.28±0.06 ^b	1.58±0.00 ^a
Isorhamnetin 3-O-rutinoside	nd	nd	53.35±0.37 ^b	64.23±0.37 ^a	63.57±0.78 ^a	63.57±0.78 ^a	36.70±1.42 ^c	52.77±0.67 ^b	67.65±0.51 ^a
Quercetin 3-O-rhamnoside	0.85±0.04 ^e	1.09±0.11 ^{de}	1.69±0.12 ^{bc}	1.70±0.18 ^{ab}	1.88±0.09 ^{ab}	1.88±0.09 ^{ab}	1.16±0.00 ^{cde}	1.42±0.20 ^{bcd}	1.96±0.21 ^a
Isorhamnetin 3-O-glucoside	nd	nd	30.98±0.47 ^c	37.80±0.99 ^b	38.28±0.38 ^{ab}	38.28±0.38 ^{ab}	22.79±1.22 ^d	31.18±1.43 ^c	41.15±0.86 ^a
Kaempferol 3-O-rutinoside	nd	nd	1.12±0.06 ^a	1.31±0.17 ^a	1.13±0.02 ^a	1.13±0.02 ^a	0.74±0.09 ^b	1.05±0.07 ^{ab}	1.22±0.09 ^a
Isorhamnetin 3-O-rhamnoside	nd	nd	1.02±0.17 ^a	1.13±0.13 ^a	1.19±0.09 ^a	1.19±0.09 ^a	0.66±0.04 ^b	1.07±0.03 ^a	1.28±0.04 ^a
Sum of flavonols	0.85±0.04^e	1.09±0.11^e	147.13±1.00^c	176.91±0.41^b	175.59±0.61^b	175.59±0.61^b	102.18±4.56^d	146.02±2.36^c	187.00±1.27^a
Total phenolics	99.53±2.50^f	95.03±0.12^f	283.93±1.13^{cd}	328.02±0.98^a	299.72±15.23^{bc}	299.72±15.23^{bc}	226.18±7.25^e	262.93±3.79^d	313.09±3.28^{ab}
Carotenoids									
Lutein isomer	0.49±0.03 ^a	0.36±0.04 ^b	nd	nd	nd	nd	nd	nd	nd
All- <i>trans</i> -lutein	0.21±0.03 ^a	0.17±0.03 ^a	nd	nd	nd	nd	nd	nd	nd
Zeaxanthin isomer	nd	nd	5.51±1.14 ^b	12.93±0.49 ^a	10.79±0.00 ^a	10.79±0.00 ^a	3.98±0.81 ^b	4.43±1.37 ^b	11.49±0.04 ^a
Sum of carotenes	1.86±0.10 ^d	2.36±0.61 ^d	18.34±2.23 ^{ab}	21.62±0.00 ^a	12.54±0.00 ^c	12.54±0.00 ^c	12.26±0.16 ^c	16.48±0.93 ^{abc}	14.45±3.03 ^{bc}
Phytofluene	0.14±0.01 ^c	0.13±0.01 ^c	0.30±0.05 ^{ab}	0.34±0.02 ^{ab}	0.31±0.03 ^{ab}	0.31±0.03 ^{ab}	0.23±0.02 ^{bc}	0.36±0.02 ^a	0.28±0.06 ^{ab}
Total carotenoids	2.70±0.08^d	3.02±0.55^d	24.15±3.42^b	34.88±0.47^a	23.64±0.03^{bc}	23.64±0.03^{bc}	16.47±0.99^c	21.27±2.33^{bc}	26.22±3.13^b

The results are presented as mean ± standard deviation. Different lowercases mean significant differences ($p<0.05$) between samples within the same row. nd, Not detected. Sample codes refer to Table 1.

mineral content in various *in vitro* and *in vivo* studies, which may be beneficial for people with osteoporosis [Kulczyński *et al.*, 2024].

In this study, the impregnation process of Gala flesh using SB juice, without or with 4% CaL, either through conventional or non-conventional impregnation (VI and US) affected the composition of phenolics and carotenoids, which may in turn impact the functional properties of the end products.

■ Polyphenol oxidase and peroxidase activity of freeze-dried apples

PPO and POD are the enzymes responsible for browning in apple products, which causes discoloration and degradation of phenolics [Moon *et al.*, 2020]. Their inhibition is important for maintaining the quality of apple products. The PPO and POD activity of freeze-dried apple controls and samples with selected treatments from 0% CaL (CON, VI_200, and US_30) and 4% CaL (CON, VI_200, and US_20) are shown in **Figure 4**. For PPO activity, the highest value was found in FA (3,795 U/g). In comparison to FA, the FA+AA reduced the PPO activity by 11.2%, which was comparable to our previous results (13.7%) [Arnold *et al.*, 2025]. Another study observed higher PPO and POD activity in freeze-dried apple (Jonagold, without blanching) than in hot air-dried apple, where the enzymes were almost inactivated due to high temperature [Ahmad-Qasem *et al.*, 2017]. The authors explained that the enzymes may be preserved in a latent state at low temperature during freeze-drying, yet their activities can be recovered when they come into contact with an aqueous medium.

The PPO activity was not observed in CON and US_30 at 0% CaL (**Figure 4**), which might be caused by complete inhibition from the antioxidants in the samples treated at 0% CaL, confirmed by their high antioxidant capacity. Ultrasound treatment may also inactivate browning enzymes (*i.e.*, PPO) by changing

their secondary and tertiary structures [Iqbal *et al.*, 2020]. However, VI_200 at 0% CaL still showed PPO activity (3,486 U/g), a value similar to FA and FA+AA. In this case, despite the high antioxidant capacity found in VI_200, the applied high vacuum (pressure of 200 mbar) might deteriorate the texture of apple flesh [Kidoń *et al.*, 2023], and thus release more PPO during impregnation. Additionally, the sum of phenolic acids of VI_200 at 0% CaL was high, yet still similar to controls (**Table 5**), which may have acted as substrates of PPO.

In 4% CaL, US_20 reduced PPO activity by 19.4% compared with FA+AA, while CON and VI_200 showed similar values to FA+AA (**Figure 4**). Neri *et al.* [2019] applied lower vacuum pressure (738 mbar) during VI of organic and conventional Golden Delicious cubes using a lemon juice solution; this treatment successfully inhibited PPO activity by 32 and 16% in respective treated apple cubes. Additionally, the composition of organic acid contained in the impregnating solution may also influence the inhibition of PPO activity in treated apples [Santarelli *et al.*, 2020]. Another non-conventional technology, high-pressure processing of peaches in a CaL solution (0–2%), was applied and also increased the PPO activity of Clingstone peach, while caused no effect on Freestone peach [Techakanon & Barrett, 2017].

The POD activity of FA+AA was more than three times higher than of FA (**Figure 4**). The POD activity was completely inhibited in all selected treatments at 0% CaL, which might be linked to the high antioxidant activity and reducing agents that masked the POD activity by reacting with H₂O₂ as one of the POD substrates [Rojas-Graü *et al.*, 2008]. In contrast, at 4% CaL, POD activity was completely inhibited in CON but increased to 3,444 and 4,002 U/g for VI_200 and US_20, respectively. This might be caused by the combination of non-conventional technologies and the higher total soluble solid content in the solution



Figure 4. Polyphenol oxidase (PPO) and peroxidase (POD) activities of freeze-dried products: fresh apple flesh (FA), FA dipped in ascorbic acid solution (FA+AA), and FA+AA samples subjected to conventional (CON) and non-conventional impregnation (vacuum impregnation, VI, and ultrasound-assisted impregnation, US) in sea buckthorn juice without and with calcium lactate (CaL). Different lowercase letters indicate significant differences ($p < 0.05$) between samples within the same enzyme. Sample codes refer to Table 1.

containing 4% CaL (7.47 °Brix) than in 0% CaL (4.53 °Brix) that could affect the cell structure and thus promote the release of POD. Nevertheless, the antioxidants could not effectively inhibit the POD activity in those treatments. Additionally, a study explained that ultrasound could activate POD by partially unfolding the protein structure, exposing more of the catalytic center and increasing the affinity for the substrate without fully denaturing the protein [Li & Tang, 2021].

Furthermore, although ascorbic acid was reported by previous studies to prevent PPO and POD activities [Arnold & Gramza-Michałowska, 2022; Moon *et al.*, 2020], in some cases it could act as a pro-oxidant that increased PPO and POD activity in apples [Rojas-Graü *et al.*, 2008]. Nevertheless, despite the presence of POD activity observed in some treatments in this study, the POD contribution to enzymatic browning is lower than that of PPO because of the low concentration of H₂O₂ in apples [Rodríguez-Arzuaga *et al.*, 2019].

CONCLUSIONS

The impregnation of apple flesh using 93.8% SB juice in water (w/w), without or with 4% CaL was carried out conventionally and non-conventionally. Compared with CON, the application of VI and US affected the mass transfer phenomenon and thus resulted in freeze-dried apple products with higher antioxidant capacity and calcium content. The application of VI_200 enabled the development of freeze-dried apple samples with the highest antioxidant capacity (at 0% CaL) and calcium content (at 4% CaL), while the application of US_30 at 0% CaL and US_20 at 4% CaL also effectively improved the antioxidant capacity and calcium content, respectively. In comparison to CON, VI was also beneficial in terms of time-saving and improvement of functional properties, while US could improve the functional properties despite requiring the same treatment time as CON. The output of this study provides a suggestion to consumers and the food industry to consume or produce new alternative locally sourced healthy snacks that may potentially reduce the risk of osteoporosis and calcium deficiency, especially by applying non-conventional technologies. The demonstrated antioxidant properties, calcium content, and enzyme activities of the products suggest that VI and US have potential for application in the development of dried fruit snacks aimed at people with osteoporosis and calcium deficiency. However, it is worth noting that the industrial application of VI and US involves challenges that need to be considered, such as process scale-up, cost, uniformity, regulatory requirements, and the product's sensory properties and quality. Furthermore, as this study focused on *in vitro* analysis, further research is needed to evaluate the bioaccessibility and bioavailability of antioxidants and calcium, particularly through *in vitro* digestion, *in vivo* experiments, and human studies.

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CONFLICT OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Storage Stability of Bioactive-Enriched Freeze-Dried Gala Apples: Functional Properties, Sensory Evaluation, and Enzymatic Browning

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Abstract

A storage study of healthy snack potential for osteoporosis prevention was carried out. Freeze-dried apple flesh after impregnation (IMP) using 93.8% sea buckthorn (SB) juice, 1:99 of inulin:SB juice ratio, 4% calcium lactate addition, at 30°C for 120 min was prepared. The study aimed to monthly investigate the characteristic changes of IMP compared to freeze-dried fresh apple (FA) and that after dipping with 1% ascorbic acid (FA+AA) as controls, during 3 months of storage at 25°C in an incubator. Throughout the 3-month storage period, IMP consistently exhibited higher total phenolic content and stronger antioxidant activity compared to the control samples. After 3 months, significant degradation of these values was observed ($p < 0.05$), confirmed by results from composition of phenolics and carotenoids, as well as the presence of polyphenol oxidase and peroxidase activities, and the development of browning color or appearance. The freshly prepared IMP also contained more than 81 times calcium content than controls ($p < 0.05$). Sensory evaluation highlighted moderate to rather high overall acceptability of IMP, most-likely due to calcium involvement in maintaining its crispiness during storage compared to controls. Due to its significantly high antioxidant content, calcium enrichment, and stable sensory properties, IMP has the potential to serve as a functional snack for osteoporosis prevention and increased calcium intake. These findings support the potential application of SB-enriched apples as calcium-rich, antioxidant-preserving functional snacks.

Keywords Apple · Calcium · Enzymatic browning · Impregnation · Osteoporosis · Sea buckthorn · Sensory

Introduction

The demand for functional foods has increased, particularly for their role in preventing chronic diseases. Many researchers were interested in developing dried fruits and vegetables due to their significant contribution in providing essential

vitamins, minerals, antioxidants, fibers, and carbohydrates in consumers' diet, and also their longer shelf-life than the fresh ones (Vidinamo et al., 2021). Among the fruits, apple should be considered as the matrix to deliver the bioactive compounds because of its abundant availability in many regions, health-promoting effects, and its familiarity and acceptability by consumers (Arnold & Gramza-Michałowska, 2024).

Analyzing the quality changes in dried fruits and vegetables during storage is essential, as phytochemicals (e.g., carotenoids, phenolic compounds, phytosterols) may degrade due to various factors, impacting their health benefits and marketability (Vidinamo et al., 2021). Some studies have studied the quality changes of dried apples during storage, including probiotics survivability and sensory properties of conventionally-dried apples enriched with probiotics (25 days in cold storage) (Afzaal et al., 2020), mass changes in freeze- and air-dried Braeburn apples after osmotic dehydration using sucrose and chokeberry juice concentrate (7 and 12 months, stored at 25–45°C) (Cichowska & Kowalska, 2018), quercetin derivatives content in freeze-dried apple

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chips enriched with quercetin derivatives (12 months, stored at 20–40°C) (Schulze et al., 2014), physicochemical changes in oven-dried and dehydrated three apple cultivars (21 days at ambient temperature) (Ghinea et al., 2022), and antioxidant changes in dehydrated apple pomace (9 months, stored at 30°C) (Lavelli & Corti, 2011). Additionally, a study also reported that freeze-drying method was better than vacuum-microwave and convective drying in maintaining the antioxidant activities and content of bioactive compounds in red-fleshed apples (Wojdyło et al., 2020).

In our previous study (Arnold et al., 2025), freeze-dried Gala apple after impregnation using different concentration of sea buckthorn (SB) juice, inulin, and calcium lactate (CaL), prepared at different times and temperatures have been developed in order to find the potential locally sourced functional food alternatives for people with osteoporosis and calcium deficiency. It was highlighted that the optimized impregnation conditions: 93.8% SB juice (w/w in water), 0:100 of inulin:SB juice ratio, with addition of 4% CaL, prepared at 30°C for 120 min resulted in freeze-dried apple with minimum losses of antioxidant activities and TPC, higher calcium content than controls, and acceptable sensory profiles. Our previous study also noted a significant decrease of antioxidant activities and TPC of freeze-dried apples when higher temperature (50°C) and shorter period (30–60 min) of impregnation process was applied. SB, especially that is available in Poland, was studied to have abundant nutritional values, such as antioxidants (carotenoids and phenolics), fatty acids, fibers, and minerals (Rybicka et al., 2021; Tkacz et al., 2020). Despite its strong sourness and unpleasant odor, SB has been used to make jams, drinks, teas, baked products, yogurts, confectionery, and other food products in industries, which could be the functional foods due to its functional properties (Kania-Dobrowolska et al., 2023). It is worth noticing that osteoporosis could be caused by oxidative stress caused by free radicals, such as reactive oxygen species that induces the apoptosis of osteoblasts, osteocytes and ontogenesis, leading to increased bone remodeling and bone loss (Mottaghi & Nasri, 2021). The oxidative stress could be neutralized by endogenous and exogenous antioxidant; therefore, administering foods with high antioxidants is recommended, especially elderly or postmenopausal women who are prone to osteoporosis.

Till date, the storage study of functional freeze-dried fruits, especially after impregnation process, is still limited. Based on optimized freeze-dried apples after impregnation in our previous study (IMP), the influence of 3 months of storage on its quality changes compared to freeze-dried fresh apple (FA) and that dipped in ascorbic acid (FA+AA) as controls was investigated in this study. This study aims to monthly monitor the changes on color, total phenolic content (TPC), antioxidant activities, calcium content, enzymatic browning, composition of phenolics and carotenoids, as well

as sensory properties of those freeze-dried samples during 3 months of storage time at 25°C.

Materials and Methods

Raw Materials and Chemicals

Gala apples (*Malus domestica* cv. Gala) were sourced from the Dębski Sad organic farm in Rabowice, Poland. The apples were washed, cored, and peeled to a thickness of 2 mm, then sliced into C-shaped pieces (5 mm thick) using an apple slicer. To prevent early enzymatic browning, the sliced fleshed were dipped for 2 min into 1% ascorbic acid solution (Stanlab sp. z o.o., Poland) at 1:4 sample:solution ratio, then blotted with kitchen towel and frozen at −40°C until further analysis. The untreated raw fresh apple fleshed before freeze-drying is noted as RFA, while those dipped in 1% ascorbic acid is RFA+AA. The organic sea buckthorn (SB) 100% juice was purchased from Oleofarm sp. z o.o., Poland. Inulin (Orafti HSI) and food grade calcium lactate (CaL) were purchased respectively from Hortimex Plus sp. z o.o. and Agnex sp. z o.o., Poland.

Chemicals and reagents for determination of TPC, antioxidant activities (ABTS, DPPH, FRAP, and ORAC), calcium content, browning index (BI₄₂₀), and activity of enzymes were purchased from Sigma-Aldrich, Germany. ACL reagent kit (lipid-soluble substances) and ACW reagent kit (water-soluble substances) for photochemiluminescence (PCL) assay were purchased from Analytik Jena, Germany. For ultra-performance liquid chromatography (UPLC) method, the solvents were acquired from Sigma Aldrich, Germany, while the standards of phenolic and carotenoid compounds were bought from Extrasynthese (Genay, France).

Impregnation of Apples in SB Juice, Calcium Lactate, and Inulin

In our preliminary study (Arnold et al., 2025), the application of inulin:SB juice ratio lower than 15:85 was suggested to minimize the loss of antioxidant activity of impregnated apple chips, yet inulin may still act as prebiotic and improve the calcium absorption. A preliminary experiment tested different inulin:SB juice ratios (0:100, 0.5:99.5, 1:99, and 2.5:97.5) under controlled conditions (93.8% SB juice w/w in water, 4% CaL addition, 120 min at 30°C). These conditions were chosen for their high calcium content retention and minimal antioxidant loss in the freeze-dried apples. The ORAC values of 0:100, 0.5:99.5, and 1:99 was respectively 4775.57±58.72, 4730.00±84.83, and 4724.98±95.78 mg TE/100 g product which were statistically similar ($p>0.05$), while 2.5:97.5 showed significantly lower ORAC value of 4188.26±58.02 mg TE/100 g product ($p<0.05$). The

resulting freeze-dried sample with inulin:SB juice ratio 1:99 was chosen as it exhibited similar ORAC value ($p>0.05$) compared to that at the 0:100 ratio.

Therefore, in current study, 50 g of RFA+AA was placed in a tightly closed polyethylene terephthalate (PET) jars, impregnated with 250 g of solution containing 93.8% SB juice (w/w in water), 1:99 of inulin:SB juice ratio, addition of 4% CaL, shaken continuously (150 rpm, vibration amplitude 14 mm) for 120 min at 30 °C in a water bath (Laboplay SWB 22 N, Poland). The impregnation was conducted in triplicate. After impregnation process, the samples were drained and blotted with kitchen towel, and frozen at −40 °C for 24 h. The samples were then freeze-dried for 48 h. The freeze-dried fresh apple (FA), freeze-dried fresh apple dipped in 1% ascorbic acid (FA+AA), and the freeze-dried impregnated samples (IMP) were separately packed and sealed in low-density polyethylene (LDPE) bag, and stored in a dark condition inside an incubator (CLW 240 STD, Pol-Eko Aparatura, Poland) at 25±0.5 °C and relative humidity (RH) 50±5%. The analyses of the samples were carried out after 0, 1, 2, and 3 months of storage. The research scheme is depicted in Fig. 1.

Analytical Methods

Color Analysis of Freeze-Dried Apples

The color of SB juice and the surface of FA, FA+AA, and IMP during storage were analyzed in ten repetitions using CIELAB scale (Chroma Meter CR-5, Konica Minolta, Japan). The color measurement of each sample was performed in a laboratory of food analysis, Department of

Gastronomy Science and Functional Foods, Poznań University of Life Sciences, under standardized conditions (standard daylight CIE illuminant D65, 20 °C, 50% RH). The zero and white calibration was carried out before measurement. The color parameters, such as L-value (lightness), a-value (greenness/redness), b-value (blueness/yellowness) of the sample was measured, and ΔE (total color difference), BI (browning index), WI (whiteness index), YI (yellowness index) was calculated following these equations (Arnold & Gramza-Michałowska, 2022):

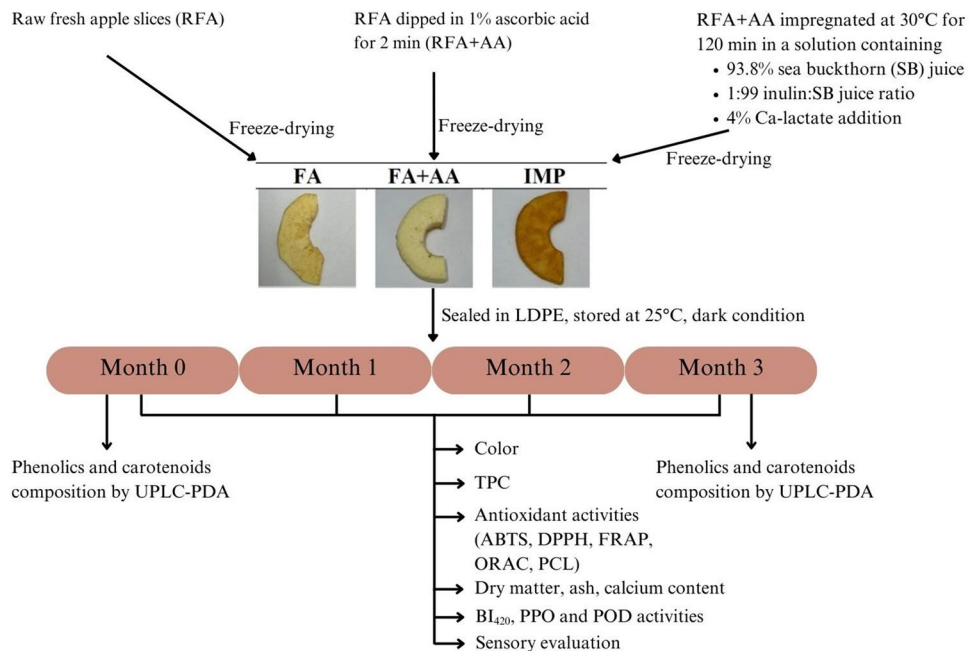
$$\begin{aligned} \Delta E &= \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \\ BI &= 100 \times \frac{x-0.31}{0.172} \text{ where } x = \frac{a+1.75L}{5.645L+a-3.012b} \\ WI &= 100 - \sqrt{(100-L)^2 + a^2 + b^2} \\ YI &= 142.86 \times \frac{b}{L} \end{aligned}$$

where for ΔE of freeze-dried samples, the ΔL , Δa , and Δb are the mean difference of L-, a-, and b-values, respectively, between before (month 0) and after storage (month 1-3) in each treatment.

Extraction of Antioxidants

The freeze-dried samples were ground into powder prior to extraction. The powdered samples were weighed and extracted at the 1:20 sample:solvent ratio (w/v) using 80% methanol (w/w in water). The samples were shaken in a water bath (50°C, 2 h), which conditions were selected following our previous study (Arnold et al., 2025). The extraction conditions above have been reported by previous studies to optimally extract the antioxidants of apple products,

Fig. 1 Research scheme of storage study of freeze-dried apples (FA, FA+AA, and IMP)



which were reviewed previously (Arnold & Gramza-Michałowska, 2024). Afterwards, the samples were cooled down, subjected to centrifugation (4°C, 12000 ×g, 10 min), and filtered to collect the supernatant that was used for determination of total phenolic content and antioxidant activities. The extraction was carried out in duplicate.

Determination of Total Phenolic Content and Antioxidant Activities of Freeze-Dried Apples

Total phenolic content (TPC), expressed as mg of gallic acid equivalent (GAE) per 100 g of product (freeze-dried product), was determined in triplicate by Folin-Ciocalteu assay following previous study (Kulczyński et al., 2020). The absorbance was measured at $\lambda=725$ nm and evaluated based on standard curve ($R^2=0.9985$).

The antioxidant activities of freeze-dried products, including ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), DPPH (2,2-Diphenyl-1-picrylhydrazyl), Ferric-reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC), and photochemiluminescence (PCL) methods were conducted in triplicate. The ability to reduce the ABTS cation radical in the sample was measured spectrophotometrically at $\lambda=734$ nm after incubation for 6 min of complexes containing extracts with ABTS and potassium sulfate (Re et al., 1999). The result was compared to the standard curve ($R^2=1$) and was expressed as mg Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) equivalent (TE) per 100 g of product.

The determination of DPPH radical scavenging activity, expressed as mg TE/100 g of product, was carried out following Sánchez-Moreno et al. (1998). The absorbance was measured spectrophotometrically ($\lambda=515$ nm) after 20 min of incubation at dark condition, then evaluated based on standard curve ($R^2=0.9997$).

FRAP assay of the samples was carried out according to previous study (Benzie & Strain, 1999). Briefly, the FRAP reagent was prepared by mixing the acetate buffer (pH 3.6), 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM of $\text{FeCl}_3 \times \text{H}_2\text{O}$ solution at the ratio of 10:1:1. The extracts of sample were added to the reagent solution before incubation (37°C, 4 min). The reduction of TPTZ-Fe^{3+} to TPTZ-Fe^{2+} with the presence of antioxidants was assessed spectrophotometrically ($\lambda=593$ nm) and evaluated following the standard curve ($R^2=0.9997$). The results were expressed as mmol Fe(II)/100 g of product.

The oxygen radical absorbance capacity (ORAC) assay is based on the ability of samples' antioxidants to scavenge the peroxy radicals, generated by 2,2'-Azobis(2-methylpropionamide) dihydrochloride (AAPH), using fluorescein solution (Gramza-Michałowska & Korczak,

2013). The readings were taken at a given excitation ($\lambda=493$ nm) and emission wavelength ($\lambda=515$ nm) on F-2700 fluorescence spectrophotometer (Hitachi, Japan), and the ORAC value was calculated following the standard curve ($R^2=0.9956$). The results were presented as mg TE/100 g of product.

The Photochem® apparatus (Analytik Jena, Germany) was used to perform photochemiluminescence (PCL) assay, according to Gramza-Michałowska et al. (Gramza-Michałowska et al., 2019). The antioxidant capacity of the samples against the radicals, which are superoxide anion radicals ($\text{O}_2^{\bullet-}$), produced by photosensitizer substance, was detected with the presence of chemiluminogenous compound—luminol (5-amino-2,3-dihydro-1,4-phthalazinedione). The lipid-soluble (ACL), water-soluble (ACW), and integral (IAC) antioxidant capacities of the samples were measured according to the protocol and using the reagent kits from the manufacturer. The results were presented as mg TE/100 g of product.

Determination of Dry Matter, Ash, and Calcium Content of Freeze-Dried Apples

Dry matter (DM) and ash contents of samples were determined by Association of Official Analytical Chemists (AOAC) methods (AOAC, 2000). The determination of calcium content of SB juice and freeze-dried samples by atomic absorption spectrometry (Hitachi Z2000, Japan) using the air-acetylene flame, was carried out in triplicate according to previous report (Suliburska & Krejpcio, 2014). About 1 g of freeze-dried sample underwent complete mineralization in a muffle furnace at 450°C. The obtained ash was then diluted in 1 mol/L nitric acid and was diluted in 0.5% LaCl_3 solution. The methods were validated by a simultaneous analysis of the reference material (Soya Bean Flour, INCT-SBF-4) with 95.7% accuracy for Ca. The results were expressed as mg Ca/100 g of product. Deionized water and acid-washed glassware were used in this study.

Browning Index, Polyphenol Oxidase and Peroxidase Activity of Freeze-Dried Apples

Besides the BI of the surface of freeze-dried apples by CIELAB scale (section "Color analysis of freeze-dried apples"), the browning index (BI_{420}) of powdered freeze-dried samples was also measured spectrophotometrically, expressed as the absorbance at $\lambda=420$ nm (Sikora et al., 2020). In brief, 0.5 g of powdered sample was mixed vigorously in 10 mL of 10% trichloroacetic acid. The samples were incubated and continuously shaken for 2 h at 35°C, and centrifuged at 12000 ×g for 15 min at 25°C. The supernatant was collected and its absorbance was measured at 420 nm.

Polyphenol oxidase (PPO) and peroxidase (POD) are the responsible enzymes in enzymatic browning of food products. Their activities in freeze-dried samples during storage were measured based on previous study (Sikora et al., 2020). In the beginning, the enzyme extraction from freeze-dried samples was performed at 4°C. About 0.3 g of powdered freeze-dried sample was mixed vigorously with 2 mL of 0.067 mol/L Sorensen's phosphate buffer solution (SPBS, pH 7.0, determined previously as the optimal pH in freeze-dried apple for both PPO and POD activities) and 0.02 g of polyvinylpyrrolidone. The extracts were shaken at 1400 rpm for 30 min. The supernatant of the sample was collected as the extract for further analysis, after centrifugation at 12000 ×g.

For the PPO activity, the final reaction mixture consisted of 50 µL of extract, 900 µL of 0.067 mol/L SPBS (pH 7.0), and 50 µL of 0.5 mol/L catechol as the substrate. For the POD activity, the final reaction mixture contained 100 µL of extract, 800 µL of 0.067 mol/L SPBS (pH 7.0), 50 µL of 0.04 mol/L H₂O₂, and 50 µL of 0.04 mol/L guaiacol as the substrate. The absorbance changes at 420 and 470 nm for PPO and POD activity, respectively, were monitored at 25 °C using a UV-Vis spectrophotometer (UV-1280, Shimadzu, Japan). The amount of the enzyme that changes an absorbance 0.001 per minute under the specified conditions is defined as one unit (U) of enzyme activity. The PPO and POD activities were expressed in unit per g of product (U/g product).

Composition of Antioxidants by UPLC-PDA

The composition of antioxidants, particularly phenolics and carotenoids were quantified using an ultra-performance liquid chromatography – photodiode array (UPLC-PDA, ACQUITY Ultra Performance Liquid Chromatography Waters Corporation, USA). The quantification method as well as the extraction of phenolics and carotenoids were carried out as per previous studies (Tkacz et al., 2020, 2022; Wojdyło et al., 2018). Phenolics extraction was carried out by mixing 0.5 g of powdered samples with 5 mL of 70% methanol (v/v in water), 2% ascorbic acid and 1% acetic acid. Sonication of mixtures for 15 min (35 kHz; RK514H Bandelin Sonorex, Germany) was conducted, then the mixtures were stored for a day at 4°C. Sonication was repeated afterwards. The mixtures were subjected to centrifugation (14000 ×g, 4 °C, 10 min; MPW-352R, Poland). The collected supernatants were filtered through a hydrophilic polytetrafluoroethylene (PTFE) membrane (0.20 µm; Millex Simplicity Filter, Merck, Germany), and used for analysis. The individual phenolic separation was carried out at 30 °C by employing an ACQUITY UPLC BEH C18 column (1.7 µm, 2.1 mm×100 mm, Waters Corporation, USA). A total sample volume of 5 µL were

injected and the elution was completed in 15 min at a flow rate of 0.420 mL/min. The mobile phase was solvent A (2% formic acid, v/v) and solvent B (100% acetonitrile). The program started with gradient elution with 98–65% solvent A (to 12 min), and then to 0% (to 14 min), the gradient returned to the initial composition (98% A) until 15 min for held constant to re-equilibrate the column. The PDA spectra were measured at 280 (flavan-3-ols and dihydrochalcones), 320 (phenolic acids) and 360 nm (flavonols).

The extraction of carotenoids was carried out in dark conditions. Powdered samples (0.4 g) with addition of 10% MgCO₃ were shaken with 4 mL of 2:1:1 (v/v/v) hexane:acetone:methanol with 1% butylated hydroxytoluene (300 rpm, 15 min; DOS-10L Digital Orbital Shaker, ELMI, Latvia). Supernatants were collected after centrifugation (same as phenolics extraction). The extraction was repeated two to three times. The combined supernatants were evaporated (XcelVap, Biotage, Sweden) and the residues were diluted in 4:1 (v/v) methanol:tetrahydrofuran. Supernatants were filtered through a hydrophilic membrane (as previously described) before injection. For carotenoids separation, ACQUITY UPLC BEH RP C18 column (1.7 µm, 2.1 mm×100 mm, Waters Corporation, USA), protected by C18 guard column, was operated at 32°C. A volume of 10 µL was injected with the flow rate of 0.500 mL/min. The mobile phase was solvent A (0.1% formic acid) and solvent B (7:3 acetonitrile:methanol, v/v). The gradient elution started with 25% A (to 0.60 min); 4.9% A (to 6.50 min); 0% A (to 13.60 min); 25% A (to 16.60 min). The analysis was monitored at 450 nm of wavelength.

The retention times and spectra were compared to those of standard references. The results of UPLC-PDA analysis were determined as the average of two replicates with the unit of mg per 100 g of freeze-dried product.

Sensory Profiling of Freeze-Dried Apples

The FA, FA+AA, and IMP fresh and stored samples were sensorially profiled in the sensory evaluation room (Department of Gastronomy Science and Functional Foods, Poznań University of Life Sciences) by 7 trained panelists, adapting a 10 cm linear scale (Lawless & Heymann, 2010). About 10 g of freeze-dried samples of each treatment were packed separately and sealed in LDPE plastic, encoded with three-digit numbers. The serving order of samples was random and neutral mineral water was provided as the taste neutralizer between samples. The panelists were asked to assess the samples using a questionnaire. The assessed attributes for sensory profiling included appearance, aroma, taste, texture, and overall acceptance with the descriptors described in Supplementary 1.

Statistical Analysis

The software Statistica 13.1 (StatSoft Inc., Poland) was used for statistical analysis. One-way ANOVA, followed by Tukey's post hoc test were performed to analyze the mean differences between samples with the significance level of 95%. Prior to statistical difference analysis, Levene's test for homogenous data of variance was conducted. Pearson's correlation (95% confidence level) was also performed to analyze the correlation between parameters throughout storage: antioxidant properties, content of calcium, dry matter, ash, PPO and POD activities, and compositions of phenolics and carotenoids by UPLC-PDA. Principal component analysis (PCA) was carried out to explore the distribution of a) physicochemical, b) sensory properties, as impacted by treatments throughout storage. Visualization of Pearson's correlation and PCA was conducted using OriginPro 10.1 (OriginLab Corporation, Northampton, MA, USA). Multiple linear regression analysis (MLR) was employed to identify composition of phenolics and carotenoids by UPLC-PDA and to predict the TPC and antioxidant activities (ABTS, DPPH, FRAP, ORAC, and PCL) of freeze-dried samples. TPC and antioxidant activity were the dependent variables in this predictive model, which were constructed using the sum of each group of phenolic compounds and total carotenoids by UPLC-PDA as independent variables (predictors). A hierarchical regression approach with forward stepwise regression was employed due to the large set of variables. In this approach, algorithms determined which predictors to use until a statistical stopping criterion is reached. The coefficient of determination (R^2) was checked to estimate the goodness of fit of the regression model.

Results and Discussion

The physicochemical and antioxidant activities of the SB juice, RFA, and RFA+AA were described in Table 1. SB juice as the main ingredient in the impregnation solution had 7.40°Brix of total soluble solid, which is slightly above the range of 5.7–7.2°Brix found in Polish SB juice (Tkacz et al., 2019) and slightly lower than that of Indian SB juice, which was 8.25°Brix (El-Sohaimy et al., 2022). Different harvesting region and time of SB berries may affect the physicochemical properties of the obtained juice (Georgescu et al., 2022; Jaśniewska & Diowski, 2021), while various processing technologies may also influence the properties of the juice (Salehi, 2020a, 2020b). The total soluble solid of SB juice or other fruit juices is essential to be measured as it may affect the mass transfer during impregnation process, thus may also influence the antioxidant properties of the final product. The final impregnation solution for IMP samples which consisted of 93.8% SB juice (w/w in water), 1:99 inulin:SB juice ratio, with addition of 4% CaL, possessed 9.53°Brix, slightly lower than the total soluble solid of RFA+AA (11.37°Brix) and RFA (14.20°Brix). The total soluble solid of RFA and RFA+AA were also comparable to that of other cultivars – fresh Golden Delicious, Starkrimson, and Florina (15.00–16.46°Brix) (Ghinea et al., 2022).

SB juice had over 2.5 times more calcium than RFA and RFA+AA, aligning with Ciesarová et al. (2020), who reported 2.1–10.9 mg Ca/100 g of juice. Variations in SB juice origin, cultivation area, soil composition, fertilizers application, and maturity influence its mineral profile. SB juice also showed significantly higher TPC and antioxidant activities than RFA or RFA+AA ($p < 0.05$) (Table 1). El-Sohaimy et al. (2022) found both fermented and non-fermented SB juice had greater antioxidant activities by

Table 1 Physicochemical properties and antioxidant activities of sea buckthorn (SB) juice, raw fresh apple flesh (RFA), and raw fresh apples flesh after dipping in 1% ascorbic acid (RFA+AA)

Analysis	Unit	SB juice	RFA	RFA+AA
Total soluble solid	°Brix	7.40 ± 0.46a	14.20 ± 0.17c	11.37 ± 0.18b
Dry matter	g/100 g	9.19 ± 0.27a	12.77 ± 1.44b	11.87 ± 0.02b
Calcium	mg/100 g	12.74 ± 0.40b	4.89 ± 0.60a	4.69 ± 0.29a
TPC	mg GAE/100 g	283.57 ± 4.41c	51.62 ± 0.36a	86.61 ± 1.10b
ABTS	mg TE/100 g	209.20 ± 2.09c	79.01 ± 0.98a	106.23 ± 1.59b
DPPH	mg TE/100 g	305.83 ± 1.23b	95.12 ± 1.10a	92.45 ± 0.90a
FRAP	mmol Fe(II)/100 g	2.00 ± 0.05c	0.40 ± 0.00a	0.53 ± 0.01b
ORAC	mg TE/100 g	970.10 ± 62.89b	516.13 ± 14.50a	543.00 ± 23.69a
PCL-ACL	mg TE/100 g	433.51 ± 16.14b	98.87 ± 0.98a	107.24 ± 1.67a
PCL-ACW	mg TE/100 g	160.96 ± 0.87c	78.71 ± 0.79a	100.52 ± 2.01b
PCL-IAC	mg TE/100 g	594.47 ± 15.95c	177.58 ± 0.29a	207.76 ± 2.62b

Different lowercases indicate a significant difference between samples at the same row ($p < 0.05$)

ABTS and DPPH methods than ascorbic acid as the standard, attributed to its rich phenolics, flavonoids, carotenoids, and vitamin C content. Fermentation process of SB juice with *Lactobacillus plantarum* further boosted most phenolic compounds (except ellagic and quercetin) and enhanced their potentials. Another study also observed that various SB juices from Polish retail markets exhibited different antioxidant activities by ABTS, FRAP, and ORAC (Tkacz et al., 2022). Given these properties, SB juice was selected as the main ingredient in the impregnation solution to enhance the health benefits of freeze-dried apple snacks.

Appearance and Color Analysis of Freeze-Dried Apples

The appearance and color of dried fruit are critical quality indicators, as they significantly influence consumer perception and acceptability (Arendse & Jideani, 2022). Figure 2 illustrates the appearance of freeze-dried samples at month 0, 1, 2, and 3. IMP samples could be described with the dark orange color. While FA+AA and FA samples had from white to yellowish color. All samples were getting darker during 3 months of storage which might be mainly due to the enzymatic browning by PPO and POD. The observed color was in accordance with the color analysis using CIELAB scale (Table 2). At the same month of storage, IMP samples showed the lowest L-value, WI, and the highest b-value, BI, and YI. While in contrary, FA+AA was generally described with the highest L-value, WI, and the lowest b-value, BI, and YI. This phenomenon might be explained due to the contribution of sea buckthorn's carotenoids found in IMP samples, while ascorbic acid in FA+AA was studied to give anti-browning and whitening effect on the apples. Ascorbic acid may reduce the dark *o*-quinones as the product from

PPO, back to the phenolic substrate (Lee et al., 2022). In terms of the effect of storage time on color of samples, it can be observed that FA+AA stored during 3 months showed the highest ΔE , followed by FA, and IMP; this indicated that there was significant changes of L-, a-, and b-values during 3 months of storage. The highest ΔE was observed in FA+AA at the third month of storage (23.33). This might be caused by the oxidation of ascorbic acid due to storage that produces dehydroascorbic acid, thus the *o*-quinones cannot be reduced to diphenols, and instead continues to melanin formation (Arnold & Gramza-Michałowska, 2022). In another study on freeze-dried apples, Ghinea et al. (2022) investigated that the trends on color change of apple chips, especially the ΔE , BI, and WI, during 3 weeks of storage were affected by cultivars and drying methods.

Total Phenolic Content and Antioxidant Activities of Freeze-Dried Apples

Functional foods rich in antioxidants help counteract oxidative stress, supporting bone remodeling and reducing bone loss, particularly in the elderly (Mottaghi & Nasri, 2021). In this study, the TPC and antioxidant activities of freeze-dried apples over 3 months are depicted in Fig. 3, which values were shown in Supplementary 2. At month 0, IMP samples had significantly higher TPC (791.29 mg GAE/100 g product) and antioxidant activities (ABTS 864.01; DPPH 928.56; ORAC 4994.59; PCL-ACL 952.38; PCL-IAC 1568.35 mg TE/100 g product; and FRAP 6.35 mmol Fe(II)/100 g product) than FA and FA+AA, except PCL-ACW where the PCL-ACW of IMP was 615.97 mg TE/100 g product; about 15.7% lower than FA+AA but 14.6% higher than FA ($p < 0.05$). Unlike our previous study (Arnold et al., 2025), the impregnated freeze-dried samples showed either lower or similar TPC and antioxidant activities compared to FA+AA, possibly due to the involvement of inulin (1:99 with SB juice) which may advantage the mass transfer, and also different apple harvesting times and SB juice batches (Łysiak et al., 2020; Tkacz et al., 2020).

Furthermore, this present study applied freeze-drying as the drying method of apple slice. Freeze-drying method of Gala apple was studied to better preserve the TPC and antioxidant activity (by DPPH) than other drying methods, particularly non-thermal ultrasound contact drying (US-CD) and hot-air drying (HAD) (Kahraman et al., 2021). Compared to freeze-drying, US-CD and HAD respectively decreased about 32 and 76% of apple's TPC, while in DPPH assay, the samples respectively showed 12 and 27% lower radical scavenging rate. Good antioxidant preservation in freeze-dried apple may benefit in better antioxidant retention during the storage period. Additionally, the TPC of FA at month 0 in present study was 352.44 mg/100 g product (Fig. 3, Supplementary 2), about five times higher than that

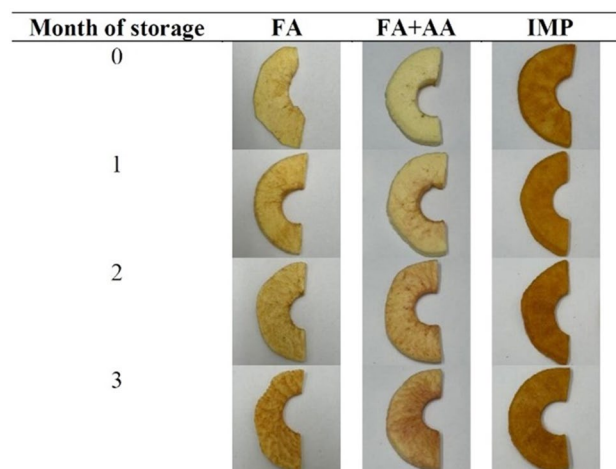


Fig. 2 Appearance of freeze-dried apples (FA, FA+AA, and IMP) stored for 0 to 3 months at 25°C and RH 50%

Table 2 Color analysis of freeze-dried apples (FA, FA+AA, and IMP) stored for 0 to 3 months at 25 °C and RH 50%

Month of storage	L-value			a-value			b-value			ΔE		
	FA	FA+AA	IMP	FA	FA+AA	IMP	FA	FA+AA	IMP	FA	FA+AA	IMP
0	69.98 ± 1.73cB	81.79 ± 1.88dC	56.14 ± 1.57bA	7.02 ± 0.82aB	1.52 ± 0.38aA	19.09 ± 0.79aC	32.07 ± 0.96aB	29.47 ± 1.20bA	49.00 ± 1.48aC	-	-	-
1	63.92 ± 1.50bB	76.18 ± 2.09cC	57.63 ± 0.71bA	8.98 ± 0.50bB	6.80 ± 1.33bA	20.67 ± 0.86bC	34.86 ± 0.52cB	26.58 ± 1.79aA	51.93 ± 1.27bC	7.02 ± 1.33aB	8.68 ± 1.03aC	3.75 ± 1.43aA
2	63.36 ± 1.63bB	68.47 ± 1.19bC	50.35 ± 1.17aA	11.74 ± 1.44dA	15.55 ± 0.92cB	21.01 ± 0.43bC	33.69 ± 0.97bB	27.71 ± 0.74aA	48.37 ± 0.97aC	8.51 ± 1.26bB	19.47 ± 1.09bC	6.23 ± 1.11bA
3	61.00 ± 1.65aB	63.27 ± 1.67aC	50.59 ± 1.56aA	10.53 ± 0.55cA	15.60 ± 1.51cB	20.23 ± 0.73bC	35.18 ± 1.24cB	30.37 ± 1.41bA	48.12 ± 2.00aC	10.29 ± 0.96cB	23.33 ± 2.19cC	6.03 ± 1.76bA
BI												
WI												
FA												
0	67.05 ± 2.38aB	44.93 ± 2.85aA	184.03 ± 4.26aC	55.48 ± 1.06cB	65.29 ± 1.44cC	31.49 ± 0.59cA	65.49 ± 1.81aB	51.50 ± 2.41aA	124.70 ± 1.72aC	FA	FA+AA	IMP
1	86.14 ± 3.00bB	48.84 ± 4.92bA	194.84 ± 7.85aC	49.02 ± 1.03bB	63.63 ± 2.52cC	29.85 ± 1.10bA	77.94 ± 1.64bB	49.95 ± 4.55aA	128.74 ± 2.89aC	FA	FA+AA	IMP
2	86.94 ± 4.71bB	67.61 ± 2.20cA	220.22 ± 7.42bC	48.83 ± 1.32bB	55.21 ± 0.88bC	27.55 ± 0.68aA	76.00 ± 2.86bB	57.82 ± 1.32bA	137.29 ± 2.61bC	FA	FA+AA	IMP
3	94.81 ± 2.09cB	81.86 ± 3.09dA	216.10 ± 17.45bC	46.40 ± 0.56aB	49.81 ± 1.22aC	28.10 ± 1.58aA	82.39 ± 1.22cB	68.56 ± 2.49cA	135.95 ± 6.04bC	FA	FA+AA	IMP

Different lowercases indicate a significant difference between month of storage within the same treatment and color parameter ($p < 0.05$)

Different uppercases indicate a significant difference between treatments within the same month of storage and color parameter ($p < 0.05$)

For calculation of ΔE , the ΔL , Δa , and Δb are the mean difference of L-, a-, and b-values, respectively, between before (month 0) and after storage (month 1-3) in each treatment

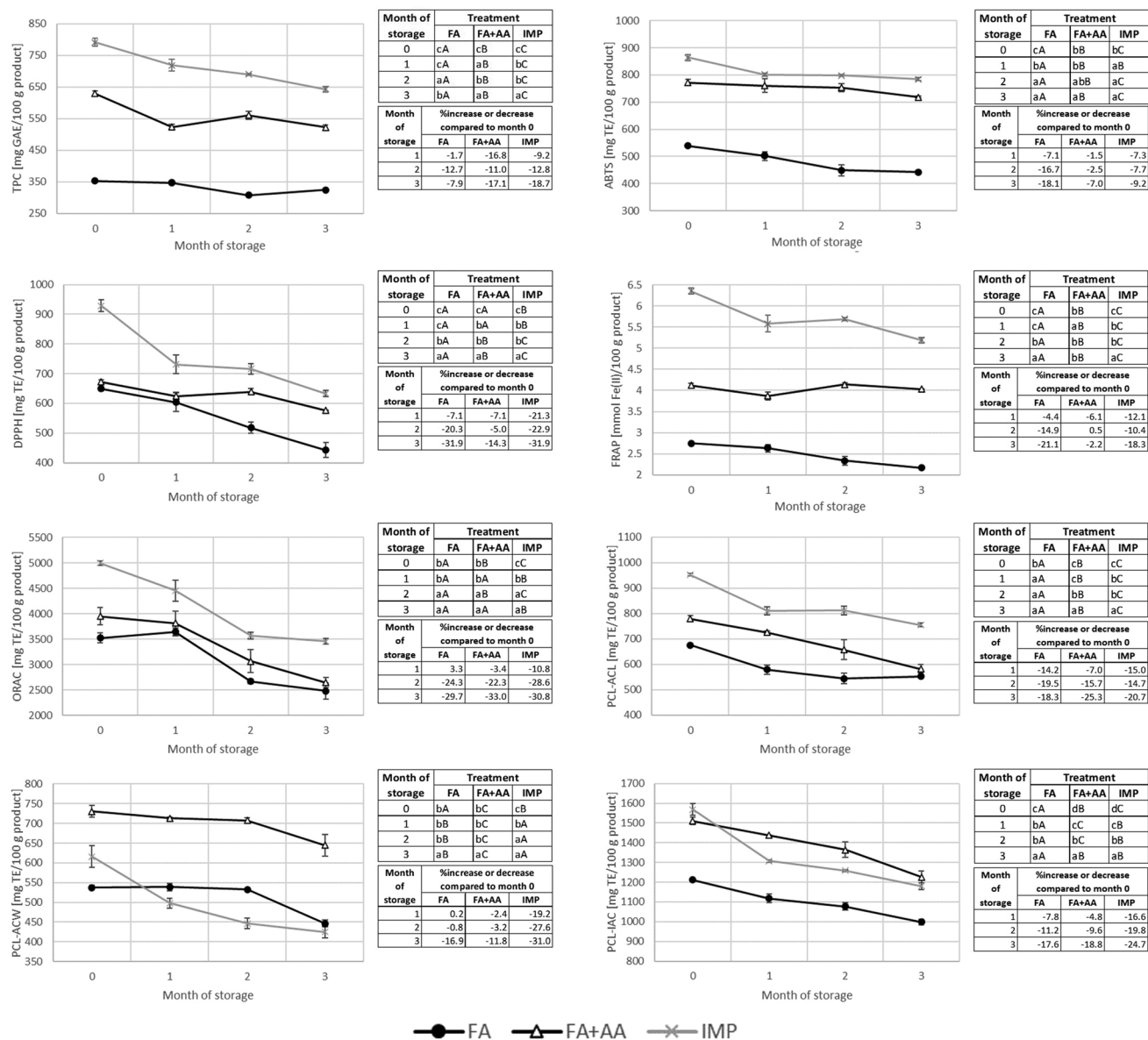


Fig. 3 Total phenolic content and antioxidant activities of freeze-dried apples (FA, FA+AA, and IMP) stored for 0 to 3 months at 25°C and RH 50%. Different lowercases indicate a significant difference between months of storage within the same treatment ($p < 0.05$).

of freeze-dried Gala as reported by Kahraman et al. (2021), which might be caused by different region and time of apple harvesting and slightly different extraction method. Although the authors used the same extraction parameters, particularly 1:20 of sample:solvent ratio, 80% methanol, and 2 hours of extraction time, they only used room temperature as extraction temperature which may limit cell breakdown and antioxidant release to the solvent.

Generally, the TPC and antioxidant activities exhibited a decreasing trend during 3 months of storage in all samples (Fig. 3, Supplementary 2). The discussion of the results in

Different uppercases indicate a significant difference between treatments within the same month of storage ($p < 0.05$). The %increase and decrease value in comparison to month 0 is respectively marked with positive and negative value

this part focuses on comparison to fresh sample (0 month) of each treatment or control. It was observed that a significant decrease of TPC was found in IMP (9.2%) and FA+AA (16.8%) after 1 month of storage. Among all samples, IMP showed the highest TPC decrease of 18.7% after 3 months of storage that accounted for 643.32 mg GAE/100 g product. After a month of storage, a significant decrease of 7.1 and 7.3% antioxidant activity by ABTS was also observed in FA and IMP, respectively. The subsequent decrease was insignificant for the second and third month of storage for IMP. For DPPH, 3 months of storage decreased about 31.9%

of FA and IMP, which respectively accounted for 442.56 and 632.75 mg/100 g product. Furthermore, FA and IMP again exhibited a higher decrease of 21.1 and 18.3% of antioxidant activity by FRAP, respectively. In other words, FA+AA could better maintain the TPC and antioxidant activity by ABTS, DPPH and FRAP during 3 months of storage than FA and IMP. Nevertheless, during the storage period, the TPC and antioxidant activities of IMP were still higher than FA and FA+AA ($p < 0.05$). About 30% decrease of ORAC value was observed after 3 months in all samples. Furthermore, after 3 months of storage, IMP exhibited the highest decrease of PCL-ACW (31.0%) and -IAC (24.7%). The highest decrease of PCL-ACL was found in FA+AA (25.34%).

The decreasing TPC and antioxidant activities during the storage period was confirmed by Pearson's correlation (Fig. 4a). TPC had the highest positive correlation with FRAP and ABTS ($r = 0.97$, $p < 0.05$). However, it is worth noting that in another study by Tappi et al. (2017), although there is a positive correlation between TPC and antioxidant activity (DPPH), the decrease in TPC did not always reflect the decrease of antioxidant activity. The other phenolics fraction produced as the consequences of phenolics degradation during storage may not have significant antioxidant activities. In this study, it was also seen that for example, when TPC of IMP significantly decreased from month 2 to month 3 ($p < 0.05$), but the result from ABTS showed similar values between these months ($p > 0.05$) (Fig. 3).

In comparison to another study with longer storage period, significant decreases of TPC, anthocyanins, and antioxidant activity by DPPH were also observed in freeze-dried Braeburn apples control and osmotically dehydrated (osmo-freeze-dried) with chokeberry juice concentrate after 12 months of storage at 25–45°C (Cichowska et al., 2018). The authors reported that a decrease of 40% of TPC, 86% of anthocyanins content, and an increase of EC₅₀ DPPH from 0.67 to 2.64 mg d.m./ml was found on osmo-freeze-dried samples stored at 25°C. Furthermore, storage study of freeze-dried Braeburn apple enriched with quercetin derivative from apple peel extract via vacuum impregnation

was carried out previously for 12 months at 20 and 40 °C (Schulze et al., 2014). The strongest decrease was found for quercetin-3-*O*-glucoside (28%) and for quercetin-3-*O*-arabinoside (91%) respectively at 20 and 40 °C, which may also impact the antioxidant activity of end product.

The decline in TPC and antioxidant activities of freeze-dried samples over 3 months of storage was mainly due to phenolics degradation from oxidation and enzymatic browning, and polymerization. The LDPE bag, while commonly used for food packaging due to its low cost and moisture resistance, has higher oxygen permeability than other plastics (Safandowska et al., 2024). The oxygen may then trigger the oxidation of antioxidants in the products, especially through enzymatic browning. PPO and POD activities (section "Polyphenol oxidase and peroxidase activity of freeze-dried apples".) contribute to phenolics degradation as their activities were observed during the storage period, especially PPO, as shown by browning appearance and indicators (Fig. 2, Table 2). Similar trends were observed previously in minimally processed Braeburn apples, impregnated with various isotonic solutions containing ascorbic acid, green tea extract, and sucrose, which showed decreased TPC and increased EC₅₀ by DPPH after 7 days of storage at 10 °C in some treatments (Tappi et al., 2017). The fluctuation was also observed during 7 days by the authors, which might be due to data variability in solid heterogeneous products. Despite the decreases due to enzymatic browning oxidation, samples containing green tea extract still showed higher TPC and antioxidants after storage; IMP samples with SB juice in this study also exhibited the same phenomenon. Additionally, polymerization of monomeric phenolic compounds may result in decrease number of active groups and further hinder the antioxidants' ability to function effectively (Butkeviciute et al., 2022). For carotenoids, their isomerization during storage may also occur and further affect the antioxidant activity (Barba et al., 2018). Although conditions were controlled in present study, degradation rates can still be influenced by factors like temperature, moisture, oxygen, sulfur dioxide, and fruit history (Vidinamo et al., 2021).

Fig. 4 a) Pearson's correlation and b) biplot – loading and score plot from principal component analysis of selected physicochemical properties of freeze-dried apples throughout storage. Statistically significant Pearson's correlation values were indicated by (*) ($p < 0.05$)

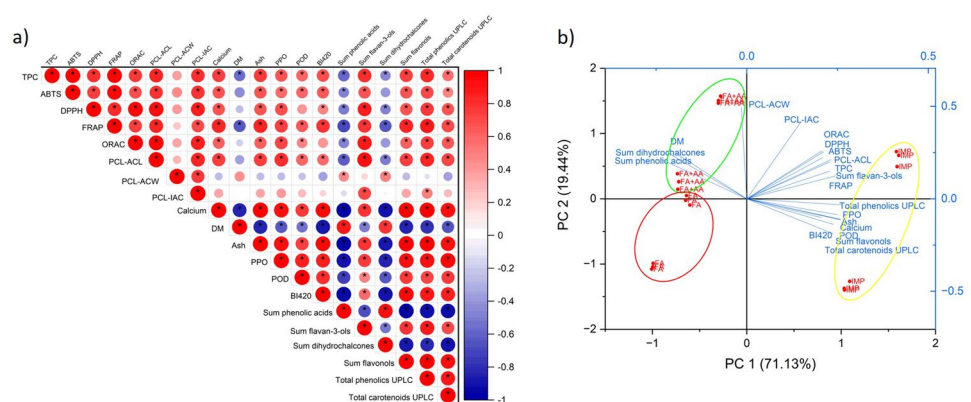


Table 3 Calcium content, dry matter, and ash content of freeze-dried apples (FA, FA+AA, and IMP) stored for 0 to 3 months at 25 °C and RH 50%

Month of storage	FA	FA+AA	IMP
Calcium content (mg Ca/100 g product)			
0	33.42 ± 4.10aA	34.12 ± 2.09bA	2793.86 ± 117.32bB
1	24.58 ± 1.39aA	34.54 ± 3.31bA	2362.19 ± 69.35aB
2	30.57 ± 3.31aA	44.91 ± 2.53cA	2331.33 ± 21.67aB
3	28.02 ± 4.89aA	26.00 ± 2.98aA	2520.13 ± 62.76aB
Dry matter (g/100 g)			
0	87.39 ± 0.27cC	86.27 ± 0.12cB	80.89 ± 0.07cA
1	84.54 ± 0.03bB	85.40 ± 0.07bC	80.31 ± 0.16bA
2	84.86 ± 0.11bC	84.47 ± 0.18aB	80.02 ± 0.06aA
3	83.34 ± 0.07aB	84.15 ± 0.13aC	80.01 ± 0.02aA
Ash content (g/100 g)			
0	1.12 ± 0.12aA	1.28 ± 0.21aA	9.90 ± 0.18cB
1	1.38 ± 0.14bA	1.26 ± 0.15aA	8.50 ± 0.11aB
2	1.09 ± 0.01aA	1.03 ± 0.05aA	8.44 ± 0.05aB
3	1.27 ± 0.03abA	1.20 ± 0.19aA	8.95 ± 0.06bB

Different lowercases indicate a significant difference between month of storage within the same treatment and color parameter, in the same analysis ($p < 0.05$)

Different uppercases indicate a significant difference between treatments within the same month of storage and color parameter, in the same analysis ($p < 0.05$)

Additionally, if compared to the dry matter of the products in Table 3, IMP samples had significantly lower dry matter (80.01–80.89 g/100 g product) that indicated higher moisture content than that of FA and FA+AA during storage. The longer storage time also led to lower dry matter. Noting that thermal process was not applied in sample preparation, other than microbial stability issue, the presence of moisture in products can lead to phenolics oxidation and deterioration in food products (Tran et al., 2020). This might be the reason why after 3 months, IMP generally showed the highest decrease of TPC and antioxidant activities if compared to controls.

Calcium Content of Freeze-Dried Apples

Other than antioxidants, calcium is also the bioactive compound that contributes to bone mineralization and osteoporosis prevention. The calcium content of fresh freeze-dried IMP (2793.86 mg Ca/100 g) was over 81 times higher than that of the control samples (Table 3). As expected, a remarkably high and significant correlation ($r = 0.999$, $p < 0.01$) between calcium ash content was observed. With assumption of 25% calcium absorption and recommended dietary allowances (RDAs) of 1000 mg Ca daily for adults (National Institutes of Health, 2022), consuming about 143 g of the

freeze-dried IMP could fulfill the RDAs of calcium. Calcium enrichment in apple or fruit products was studied not only to increase the calcium content but also prolong the shelf life of the products. Based on sensory evaluation, a study combined hot water treatment (48 and 55 °C, 2 min) and 6% calcium ascorbate dipping (2 min, 0 °C) to prolong the shelf life Braeburn apple fresh-cut from <7 days in those without treatment, to 14 days in a 4 °C of storage (Aguayo et al., 2015). It was explained that as a consequence of improved calcium content, the texture and taste of the apple fresh-cut of longer stored fresh-cut was still acceptable. In this study, 4% CaL involvement in IMP also had an effect in sensory properties of products during storage (section "Sensory evaluation").

If compared between month 0 and 3 of each treatment, the calcium content decreased significantly in IMP (9.8%) and FA+AA (23.8%), but insignificantly in FA. However, no clear pattern was noticed during the storage. Unclear pattern was also noticed in persimmon after pretreatment using 0–1% CaL during 40 days of storage (Naser et al., 2018). In another matrix, which is peach treated with slightly acidic electrolyzed water and $\text{Ca}(\text{NO}_3)_2$, it was studied that calcium content generally increased during 8 days of storage as the consequences of structural changes in cell wall, vacuole, and lamella of the peach that affect the exogenous calcium transit in the cell (Zhi et al., 2017).

Food storage may impact both content and chemical form of nutrients and bioactive compounds, thus affecting the bioavailability, either positively or negatively according to storage conditions (time and temperature) (Barba et al., 2018). Despite the retention of calcium content, calcium bioavailability could be affected by storage and its reaction with other compounds in food matrix. Although apples are considered as a low-oxalate fruit, the antinutrient such as oxalates and phytates in plant-based food, may adversely affect the calcium absorption in human's body as their complexes with calcium are not solubilized and ionized in gastrointestinal tract (Shkembi & Huppertz, 2022). Selecting food matrix in designing functional food with calcium enrichment should be then considered carefully. Moreover, further study on calcium bioaccessibility and bioavailability of stored product is still needed.

Polyphenol Oxidase and Peroxidase Activity of Freeze-Dried Apples

In Fig. 5a, b, and c, the results of BI_{420} , PPO and POD activity of all samples during 3 months of storage were depicted respectively to evaluate the enzymatic browning. The enzymatic browning could affect the browning color (Table 2, Fig. 2) and also the decrease of antioxidant activities and TPC during storage (Fig. 3). IMP had BI_{420} ranged from 0.452 to 0.593 during 3 months of storage, which were

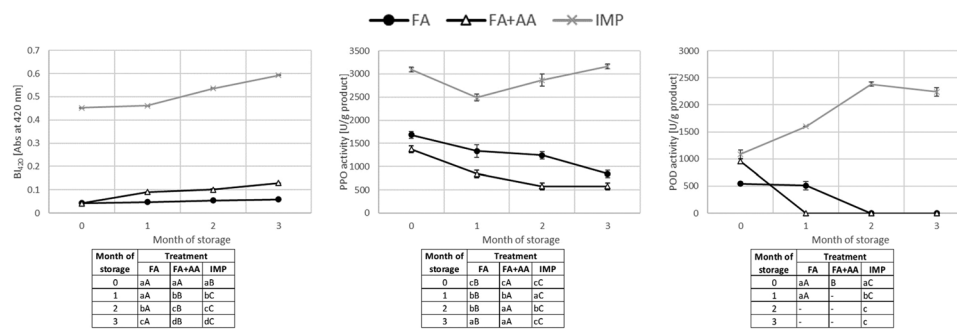


Fig. 5 Browning index at 420 nm, PPO and POD activities of freeze-dried apples (FA, FA+AA, and IMP) stored for 0 to 3 months at 25°C and RH 50%. Different lowercases indicate a significant difference

between months of storage within the same treatment ($p < 0.05$). Different uppercases indicate a significant difference between treatments within the same month of storage ($p < 0.05$)

more than five times higher than FA and FA+AA. The PPO activity of IMP was also the highest (2491.00–3163.80 U/g), followed by FA and then FA+AA during the storage. The PPO activity of FA and FA+AA decreased along with the increasing storage time, while for IMP, after it decreased in the first month, it increased again up to the third month. While for POD activity, the value was increased during storage for IMP, whereas it was not detected after one and two months for FA+AA and FA, respectively. Similar result was found for controls as the PPO and POD activities of freeze-dried control apple were decreasing during 19 days of storage at 20°C (Bußler et al., 2017).

The explanation of this phenomenon is that although the antioxidant activities of IMP were generally higher than FA+AA and FA (Fig. 3), the impregnation itself might disrupt the structure integrity of the product in IMP, thus resulted in more PPO and POD exposure. The same explanation was also highlighted in another study, where the application of high-pressure processing of peach, particularly to Clingstone cultivar, after CaL and CaCl₂ pretreatment resulted in higher PPO activity compared to control (Tehkanon & Barrett, 2017). However, similar or lower PPO activity, depending on calcium types and concentrations, was noticed in Freestone cultivar as the authors added. This indicated that cultivar of fruits may also affect the reaction of enzyme activity after a treatment. Another possible reason is also the higher moisture content or lower DM content observed in IMP (Table 3), confirmed by significant negative correlation between DM and PPO ($r = -0.64$), and POD ($r = -0.55$) ($p < 0.05$, Fig. 4a). Increasing moisture content, together with oxygen exposure, temperature, and time, may promotes the enzymatic browning in fruits (Al-Amrani et al., 2020). Additionally, the higher TPC (Fig. 3) and some phenolics types, particularly catechin (Table 4) in IMP may have tendency to act as the substrate of PPO and POD instead of inhibiting them, noting that browning enzymes can interact with wide range of phenolics as substrates (Arnold & Gramza-Michałowska, 2022; Zhang, 2023). Pearson's

correlation results support this phenomenon, where PPO and POD activities are positively correlated with TPC, all antioxidant activities (except PCL-ACW and PCL-IAC), and results from UPLC-PDA, particularly sum of flavan-3-ols, flavonols, total phenolics, and total carotenoids ($p < 0.05$, Fig. 4a). In general, the correlation between PPO activity, phenolic composition, and enzymatic browning exists but its strength may vary depending on cultivar (Groth et al., 2021).

Composition of Antioxidants by UPLC-PDA

The compositional changes of phenolics and carotenoids between fresh freeze-dried samples (month 0) and those after 3 months of storage were described in Table 4. For phenolics at month 0, the highest total phenolics was observed in IMP. IMP was dominated by flavonols (182.87 mg/100 g) which accounted for 61.3% of total phenols. The involvement of SB juice that is rich in flavonols as studied previously (Tkacz et al., 2020) could explain the high concentration of flavonols in IMP if compared to FA and FA+AA. The flavonols in SB are mainly present in the glycosylated forms of quercetin, isorhamnetin, and kaempferol (Wang et al., 2022). Among flavonols in IMP, compound 19 which is isorhamnetin-3-*O*-rutinoside that accounted for 80.50 and 69.33 mg/100 g products for month 0 and 3, respectively. Our preliminary study also found isorhamnetin-3-*O*-rutinoside and isorhamnetin-3-*O*-glucoside as two major compounds found in freeze-dried apples impregnated with 93.8% SB juice and 4% CaL (Arnold et al., 2025), which is in accordance to other research in SB juice (Tkacz et al., 2020). There was only quercetin-3-*O*-rhamnoside that was detected in FA and FA+AA for flavonols; the controls were more dominated by flavan-3-ols and phenolic acids both at month 0 and 3. A significant increase of flavan-3-ols, especially catechin, was also noticed in fresh IMP if compared to fresh controls, noting that SB juice and berries are abundant in catechin (Dong et al., 2021). A study found that dried SB, particularly from Russia, showed the highest catechin

Table 4 Phenolics and carotenoids compositions of freeze-dried apples stored after 0 and 3 months at 25°C and RH 50% by UPLC-PDA

Compounds	No. compound	Total content [mg/100 g product]					
		Month 0			Month 3		
		FA	FA+AA	IMP	FA	FA+AA	IMP
PHENOLICS							
Phenolic acids							
p-Coumaric acid- <i>O</i> -hexoside	1	34.57 ± 0.28d	35.72 ± 0.07d	13.14 ± 0.24b	29.43 ± 0.50c	35.09 ± 0.37d	10.60 ± 1.33a
Ferulic acid- <i>O</i> -hexoside	2	3.00 ± 0.15b	4.95 ± 0.17d	1.17 ± 0.12a	3.92 ± 0.02c	5.88 ± 0.16e	1.23 ± 0.17a
Sum of phenolic acids		37.56 ± 0.44c	40.67 ± 0.24d	14.31 ± 0.35a	33.34 ± 0.52b	40.97 ± 0.21d	11.83 ± 1.50a
Flavan-3-ols							
(+)-Catechin	3	2.66 ± 0.09a	4.38 ± 0.25a	43.74 ± 0.54d	8.63 ± 0.07b	7.53 ± 0.21b	18.27 ± 1.18c
Procyanidin B1	4	nd	nd	38.00 ± 0.29a	nd	nd	33.16 ± 3.54a
(-)-Epicatechin	5	10.99 ± 0.56b	15.81 ± 0.11c	7.95 ± 0.38ab	10.01 ± 0.30b	8.15 ± 0.03ab	5.02 ± 2.19a
Procyanidin C1	6	28.74 ± 0.21d	40.91 ± 1.64e	8.74 ± 0.18b	25.47 ± 2.32d	17.75 ± 0.49c	3.02 ± 0.87a
Sum of flavan-3-ols		42.38 ± 0.68a	61.10 ± 1.78b	98.43 ± 0.44c	44.12 ± 2.09a	33.43 ± 0.73a	59.47 ± 7.79b
Dihydrochalcones							
Phloretin-2- <i>O</i> -xyloglucoside	7	1.35 ± 0.26b	1.68 ± 0.13b	2.79 ± 0.14c	1.32 ± 0.10b	0.34 ± 0.02a	2.37 ± 0.24c
Derivative of dihydrochalcones	8	2.36 ± 0.17a	3.35 ± 0.08a	nd	3.42 ± 0.19a	3.54 ± 0.86a	nd
Phloretin-2- <i>O</i> -glucoside	9	1.41 ± 0.26a	2.36 ± 0.75a	nd	1.16 ± 0.14a	1.87 ± 0.32a	nd
Sum of dihydrochalcones		5.13 ± 0.70b	7.39 ± 0.69c	2.79 ± 0.14a	5.90 ± 0.43bc	5.76 ± 0.52bc	2.37 ± 0.24a
Flavonols							
Isorhamnetin-3,7- <i>O</i> -dihexoside	10	nd	nd	8.8 ± 0.15a	nd	nd	8.18 ± 1.06a
Isorhamnetin-3- <i>O</i> -sophoroside-7- <i>O</i> -rhamnoside	11	nd	nd	9.19 ± 0.79a	nd	nd	8.38 ± 1.01a
Quercetin-3- <i>O</i> -(dirhamnosyl)hexoside	12	nd	nd	1.34 ± 0.22a	nd	nd	1.56 ± 0.01a
Quercetin-3-galactoside-7- <i>O</i> -rhamnoside	13	nd	nd	3.29 ± 0.16a	nd	nd	3.2 ± 0.48a
Quercetin-3- <i>O</i> -rutinoside	14	nd	nd	9.39 ± 0.45a	nd	nd	8.68 ± 1.39a
Isorhamnetin-3- <i>O</i> -glucoside-7- <i>O</i> -rhamnoside	15	nd	nd	24.10 ± 0.43a	nd	nd	21.97 ± 2.31a
Quercetin-3- <i>O</i> -glucoside	16	nd	nd	6.69 ± 0.8a	nd	nd	6.08 ± 0.89a
Kaempferol-3- <i>O</i> -hexoside-7- <i>O</i> -rhamnoside	17	nd	nd	2.92 ± 0.13a	nd	nd	2.80 ± 0.58a
Isorhamnetin-3- <i>O</i> -(rhamnosyl)hexoside	18	nd	nd	3.91 ± 0.19b	nd	nd	3.44 ± 0.16a
Isorhamnetin-3- <i>O</i> -rutinoside	19	nd	nd	80.50 ± 1.14b	nd	nd	69.33 ± 2.84a
Quercetin-3- <i>O</i> -rhamnoside	20	0.84 ± 0.04a	1.12 ± 0.12a	3.31 ± 0.07b	0.87 ± 0.04a	1.07 ± 0.02a	3.29 ± 0.14b
Isorhamnetin-3- <i>O</i> -glucoside	21	nd	nd	28.42 ± 1.30a	nd	nd	25.8 ± 3.49a
Kaempferol-3- <i>O</i> -rutinoside	22	nd	nd	0.41 ± 0.15a	nd	nd	0.36 ± 0.08a
Isorhamnetin-3- <i>O</i> -rhamnoside	23	nd	nd	0.62 ± 0.07a	nd	nd	0.51 ± 0.12a
Sum of flavonols		0.84 ± 0.04a	1.12 ± 0.12a	182.87 ± 5.28b	0.87 ± 0.04a	1.07 ± 0.02a	163.57 ± 14.55b
Total phenolics		85.91 ± 1.77a	110.28 ± 2.10a	298.41 ± 5.33c	84.22 ± 2.14a	81.23 ± 1.02a	237.24 ± 24.08b
CAROTENOIDS							
Lutein isomer	24	0.05 ± 0.02a	0.16 ± 0.05a	nd	0.07 ± 0.03a	0.11 ± 0.02a	nd

Table 4 (continued)

Compounds	No. compound	Total content [mg/100 g product]					
		Month 0			Month 3		
		FA	FA+AA	IMP	FA	FA+AA	IMP
All-trans-lutein	25	0.04 ± 0.00a	0.11 ± 0.05a	nd	0.06 ± 0.04a	0.08 ± 0.02a	nd
Zeaxanthin isomer	26	nd	nd	9.48 ± 0.28a	nd	nd	9.20 ± 0.75a
Sum of carotene	27	8.48 ± 0.90b	2.88 ± 1.42a	6.85 ± 1.02ab	3.43 ± 2.37ab	1.85 ± 0.36a	6.42 ± 0.34ab
Phytofluene	28	0.33 ± 0.01d	0.13 ± 0.00abc	0.19 ± 0.00bc	0.09 ± 0.02ab	0.06 ± 0.01a	0.20 ± 0.05c
Total carotenoids		8.90 ± 0.94b	3.28 ± 1.42a	16.52 ± 0.73c	3.65 ± 2.38a	2.10 ± 0.35a	15.82 ± 0.46c

nd, not detected. Different lowercases mean significant differences ($p < 0.05$) between samples within the same row

content of 309.62 µg/g dw in comparison to other dried berries such as blackcurrant, gooseberry, bilberry, strawberry, and redcurrant; while that of dried SB from Romania was the second highest among the dried berries (Georgescu et al., 2022). The higher flavonoids content found in IMP may also improve the functionality of the product on osteogenesis, especially as structural support and therapeutic agent in promoting bone health (Yuca et al., 2024). Flavonoids also act as reducing agents, hydrogen donors, and singlet oxygen quencher, thus could reduce oxidative stress that is linked to various diseases, including osteoporosis (Vidinamo et al., 2021). Besides its role as a natural antioxidant, it was also studied that isorhamnetin inhibited osteoclastogenesis and bone resorption in rat femoral shaft and tendon tissue and mouse bone marrow cells to prevent osteoporosis; however, further studies on its mechanism of action still need to be done (Gong et al., 2020).

For the total carotenoids, IMP also possessed the highest value both at month 0 and month 3 compared to controls. Zeaxanthin isomer (57.4%) and sum of carotenes (41.5%) dominated the carotenoids composition of IMP at month 0. The composition and content of carotenoids in SB juice might vary depending on the harvesting time, cultivar, maturity, as studied by Tkacz et al. (2022). Recent reviews by Kulczyński et al. (2024) indicated that the effect of zeaxanthin on bone mass density, bone mineral content, and risk of fractures should be further studied as the results from recent human studies regarding this issue are still inconclusive, while carotenes, especially β-carotene is suggested to be included in the diet due to the positive impact with bone mass density and risk of fracture.

After 3 months of storage, despite a significant decrease in total phenolics (20.5% decrease) and insignificant one in total carotenoids (4.2% decrease), IMP still possessed higher contents than FA and FA+AA, which agrees with TPC and antioxidant activities (Fig. 3).

PCA was applied to better understand the relationships between selected physicochemical properties, including TPC, antioxidant activity, enzymatic browning, and

composition of phenolics and carotenoids by UPLC-PDA (Fig. 4b). The first two principal components accounted for 90.57% of total variances (PC1= 71.13%; PC2= 19.44%). The PCA results showed noticeable differences between FA, FA+AA, and IMP. On the right side of PCA biplot, IMP samples (yellow oval) were characterized by sum of flavan-3-ols, flavonols, total phenolics and carotenoids by UPLC-PDA, enzymatic browning parameters, and other functional properties such as antioxidant activities (except PCL-ACW) and calcium content. FA samples (red oval) that majorly showed lower antioxidant activity, sum of flavan-3-ols, flavonols, and total carotenoids by UPLC-PDA during storage were located on the lower left side of the PCA biplot. While FA+AA samples (green oval) that possessed high content of dihydrochalcones and phenolic acids were plotted on the upper left side of the PCA biplot.

As Pearson's correlations (Fig. 4a) could only display the bivariate linear relationship between two variables, multiple linear regression (MLR) was analyzed to study the relationship between each dependent variable – TPC and each antioxidant activities with multiple independent variables – composition of phenolics and total carotenoids by UPLC-PDA (Table 5). As forward stepwise regression was performed ($p < 0.05$), only the independent variables that best explain the dependent variables are included, while the independent variables that do not meet the criteria are eliminated. The regression models to determine TPC and all antioxidant activities were statistically significant ($p < 0.05$). The TPC regression consisted of significant variables, i.e. sum of phenolic acids, dihydrochalcones, and flavonols. Among the regressions of antioxidant activities, ABTS regression contained the largest number of significant variables, such as sum of phenolic acids, flavan-3-ols, dihydrochalcones, and flavonols. This indicates the suitability of ABTS to thoroughly explain the antioxidant activity of freeze-dried apple, based on MLR analysis. In contrast, the DPPH regression only consisted of sum of flavan-3-ols. Furthermore, total carotenoids was one of the significant variables to determine ORAC and PCL-ACL.

Table 5 Multiple linear regression (MLR) for evaluation of total phenolic content and antioxidant activities changes in freeze-dried apples

Dependent variables	R ²	Intercept		Independent variables				
				Sum phe-nolic acids	Sum flavan-3-ols	Sum dihydro-chalcones	Sum flavonols	Total carotenoids UPLC
TPC	0.928	−778.42 [#]	b	21.36 [#]	−1.22	78.00 [#]	6.40 [#]	-
			b*	1.56 [#]	−0.15	0.84 [#]	3.15 [#]	-
ABTS	0.899	−732.93 [#]	b	28.55 [#]	−3.35 [#]	67.52 [#]	7.33 [#]	-
			b*	2.33 [#]	−0.48 [#]	0.81 [#]	4.04 [#]	-
DPPH	0.740	315.54 [#]	b	-	5.92 [#]	-	-	-
			b*	-	0.86 [#]	-	-	-
FRAP	0.954	−3.99 [#]	b	0.19 [#]	-	-	0.04 [#]	−0.06
			b*	1.69 [#]	-	-	2.74 [#]	−0.26
ORAC	0.948	−2134.02 [#]	b	83.66 [#]	34.45 [#]	-	3.50	119.28 [#]
			b*	1.20 [#]	0.86 [#]	-	0.33	0.84 [#]
PCL-ACL	0.948	−135.61	b	11.02 [#]	3.96 [#]	19.64	1.44 [#]	14.18 [#]
			b*	0.99 [#]	0.62 [#]	0.26	0.87 [#]	0.62 [#]
PCL-ACW	0.927	−614.00 [#]	b	25.33 [#]	1.29	29.17	3.60 [#]	-
			b*	2.79 [#]	0.25	0.47	2.67 [#]	-
PCL-IAC	0.903	−432.91	b	35.18 [#]	7.19 [#]	-	4.47 [#]	-
			b*	2.15 [#]	0.77 [#]	-	1.84 [#]	-

Independent variables are the composition of phenolics and carotenoids by UPLC-PDA. Statistically significant values are marked by [#] (p<0.05)

Table 5 includes the standardized regression coefficient (b*), which shows the importance of each independent variable, in addition to the raw regression coefficient (b) of the MLR equation to predict TPC and antioxidant activities. Sum of phenolic acids possessed the highest b* value in ORAC (1.20) and PCL (0.99–2.79). Sum of flavonols was the most important variable for ABTS (4.04), TPC (3.15), and FRAP (2.74). While sum of flavan-3-ols was the most important variable for DPPH (0.86). In brief, TPC and antioxidant activities were best explained by different composition of phenolics and carotenoids in the freeze-dried apple.

Sensory Evaluation

Figure 6a, b, and c depicted the sensory evaluation of freeze-dried FA, FA+AA, and IMP respectively during 3 months of storage. Generally, IMP was described with a dominant sourness taste (8.21–9.36), low sweetness (1.43–1.64), sea buckthorn fruity aroma (8.00–8.50), high yellowness appearance (8.79–9.29), and high crispiness (7.07–9.00) during storage period. As comparison, significantly lower crispiness was found in both FA and FA+AA in all months of storage (p<0.05). The calcium treatment could maintain the membrane integrity and increase firmness of cell wall (Jain et al., 2019), thus the crispiness during storage could be maintained although the moisture content of IMP was higher (lower dry matter content) than controls (Table 3).

At the beginning of storage (especially from month 0–1), the overall acceptability of IMP was significantly lower than FA and FA+AA (p<0.05), this might be caused by the very high sourness (9.36 in both months), low sweetness (1.43), low to rather low notes of astringency taste (3.93–4.21), and low foreign aroma score (1.14) in IMP (Fig. 6c) due to SB juice involvement. The proportion of sour and sweet taste had a significant role in sensory properties of food products; a study found that Gala fresh-cut osmotically dehydrated with hypertonic solution containing sucrose and 15% chokeberry juice concentrate exhibited the best color and taste based on sensory evaluation, yet the antioxidant activities were not carried out (Samborska et al., 2019). The small concentration of inulin (1:99 of inulin:SB juice ratio) and the natural sweetness from the apple itself were inadequate to improve the sweetness and cover the dominant flavor from SB juice in IMP. According to the information of the manufacturer, the inulin used in this study has 30% sweetness of sucrose. Despite its valuable nutrients that are health-promoting, SB that was involved in IMP is not particularly tasty as it has sour taste and unpleasant odor (Kania-Dobrowolska et al., 2023). However, at the last month of storage, a rather high overall acceptability score was found in IMP (6.36), slightly higher yet statistically similar as in FA (5.50) and FA+AA (5.29) (p>0.05), which might be due to the maintained crispiness in IMP. The dry matter content of FA and FA+AA decreased 4.6% and 2.5% respectively after 3 months,

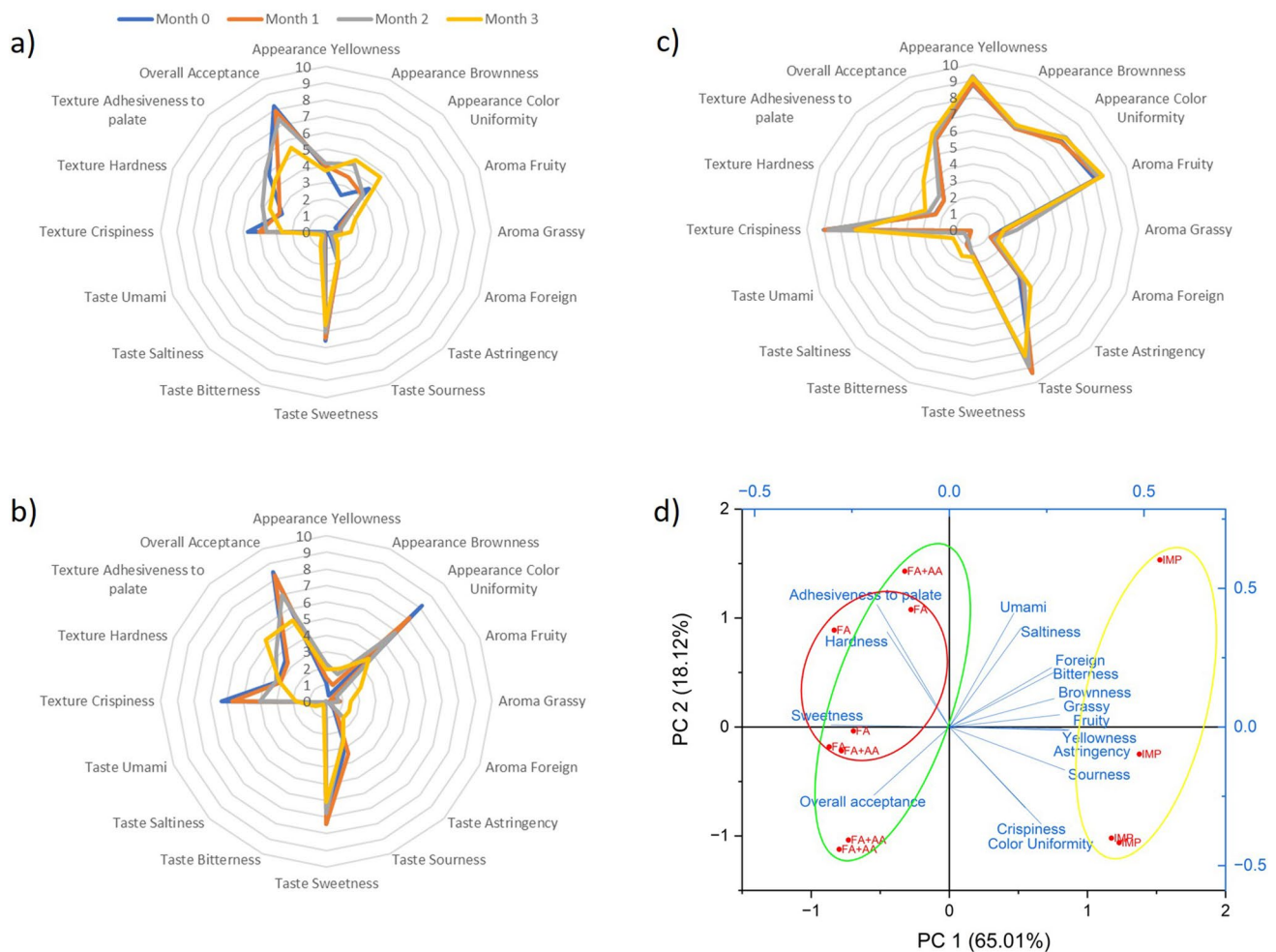


Fig. 6 Sensory profiles of freeze-dried apples: a) FA, b) FA+AA, and c) IMP, stored for 0 to 3 months at 25°C and RH 50%; and d) biplot – loading and score plot from principal component analysis of sensory

which may affect their crispiness due to increased moisture content. A study using South African Granny Smith apple, dried at 70 °C observed 0.5% of moisture increase after 3 month of storage at ambient temperature, using sealed polyester foil and LDPE (Arendse & Jideani, 2022). As consequences, the authors found an increase in extensibility or reduction of crispiness of the dried apple over the storage period.

Increasing brownness appearance was observed by the panelists in FA (2.43–4.71) and FA+AA (0.36–2.14) over the storage period. While only a negligible brownness increase was found in IMP (6.64–6.86), which might be caused by the dominant orange color from the carotenoids of SB juice. Enzymatic browning (Fig. 5) might be responsible for this phenomenon. PPO and POD activities were still observed in freeze-dried and warm-air dried apple during storage at room temperature (Bußler et al., 2017). A sensorial study found that increasing browning was

properties of freeze-dried apples throughout storage. Supplementary 1 described the scores 0–10 of descriptors of each attribute in Fig. 6a, b, and c

highly correlated with the decreasing general appearance of Granny Smith treated with yerba mate and stored at 2 and 10 °C for 18 and 15 days respectively (Rodríguez-Arzuaga et al., 2021). Additionally, reactive carbonyl species from Maillard reaction, especially methylglyoxal and glyoxal, were also studied to promote the non-enzymatic browning and off-flavors development in apple juice during 10-week storage at 4 and 35 °C, in which browning was more accelerated at 35 °C (Paravisini & Peterson, 2018).

PCA was performed using the sensory evaluation data from each treatment during 3 months of storage to determine the relationships between sensory descriptors and treatments (Fig. 6d). These data were reduced into two dimensions: PC1 (65.01%) and PC2 (18.12%), that cover total of 83.13% of total variances. The PCA results showed that IMP notably differed from FA and FA+AA. As previously described, the most important sensory descriptors of IMP (yellow oval) were taste of sourness, astringency,

umami, saltiness, and bitterness; aroma of SB (fruity), grassy, and foreign; crispiness texture; and brown-yellow uniform color in appearance. While adhesiveness to palate, hardness, sweetness, and overall acceptance characterized the FA (red oval) and FA+AA (green oval).

From sensory evaluation, IMP still have moderate to rather high overall acceptability during 3 months of storage. The sensory improvement or way to consume the freeze-dried IMP could be modified, for example as an additive in yogurt, salad, or ice cream, instead of as chips for direct consumption to obtain improved balanced flavor or perception.

Conclusions

The properties of IMP, which was the freeze-dried product after impregnating Gala flesh in 93.8% SB juice and 4% CaL, in comparison to FA and FA+AA as controls were studied monthly during 3 months of storage at 25°C. Based on TPC and most antioxidant activities, IMP could maintain the highest values if compared to controls over the storage time although the values decreased significantly during storage period. The results from UPLC-PDA exhibited that flavonols were the dominant phenolics, while zeaxanthin isomers and carotenes were the dominant carotenoids in IMP. The calcium content of IMP was also more than 81 times compared to controls. Based on appearance and color, browning color was developed during the storage, which was also confirmed by sensory evaluation and results of PPO and POD activity found in all products. Sensory evaluation revealed that CaL addition helped maintain the crispiness of IMP over 3 months, contributing to its overall acceptability. With these results, the IMP could be the potential alternative functional food product based on local resources – apples and SB juice with acceptable sensory properties better functional properties to prevent osteoporosis and improve calcium intake of consumers. Nevertheless, further studies are needed to improve and complement current study, for examples a) *in vitro* and *in vivo* studies to confirm the bioavailability of calcium and antioxidants and their role in osteoporosis prevention; b) studies on the effect of various packaging materials, modified atmosphere packaging, and other storage parameters (e.g. various temperatures, humidity) on physicochemical properties and microbial activity of freeze-dried apples; c) more advanced modeling and predictive analysis to predict the shelf-life at different storage conditions, as well as storage optimization to retain the quality freeze-dried apples; d) improvement of sensory or way to consume the IMP to compensate its sourness.

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Data Availability Data is provided within the manuscript or supplementary information files.

Declarations

Competing interests The authors declare no competing interests.

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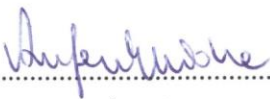

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