



Uniwersytet Przyrodniczy w Poznaniu

Wydział Nauk o Żywności i Żywieniu

mgr inż. Monika Anna Marcinkowska

***Lotne produkty degradacji glukozynolanów jako związki
sensorycznie aktywne w warzywach kapustowatych***

*Volatile products of glucosinolate degradation as sensory-active
compounds in Brassica vegetables*

Rozprawa doktorska wykonana pod kierunkiem

prof. dr hab. Henryka H. Jelenia

Pracownia Badania Związków Lotnych i Aktywnych Sensorycznie

Katedra Technologii Żywności Pochodzenia Roślinnego

Wydział Nauk o Żywności i Żywieniu

Poznań, 2023

Rozprawa doktorska

Autor: mgr inż. Monika Anna Marcinkowska

Temat rozprawy doktorskiej: *Lotne produkty degradacji glukozynolanów jako związki sensorycznie aktywne w warzywach kapustowatych*

Title of the PhD dissertation: *Volatile products of glucosinolate degradation as sensory-active compounds in Brassica vegetables*

Realizacja pracy: Uniwersytet Przyrodniczy w Poznaniu, Wydział Nauk o Żywności i Żywieniu, Pracownia Badania Związków Lotnych i Aktywnych Sensorycznie oraz w okresie stażu naukowego: Leibniz Institute for Food Systems Biology at the Technical University of Munich

Źródła finansowania badań: Harmonia 7, *Kluczowe związki odpowiedzialne za zapach i smak wybranych warzyw z rodziny kapustowatych. Tworzenie i przemiany w procesach technologicznych*, Narodowe Centrum Nauki, nr grantu 548 2015/18/M/NZ9/00372.

podpis

Promotor: prof. dr hab. inż. Henryk Jeleń

Składam najserdeczniejsze podziękowania:

Panu prof. dr hab. inż. Henrykowi Jeleniowi

za życzliwość, liczne konsultacje i poświęcony czas

Pracownikom i Doktorantom

Pracowni Badania Związków Lotnych i Aktywnych Sensorycznie

za liczne i konstruktywne dyskusje naukowe i doskonałą atmosferę pracy

Rodzicom i Najbliższym

za nieskończone pokłady wiary w moje możliwości, wspieranie w trudnych chwilach
i dobro, którego nie sposób tutaj przekazać.

I would like to extend my sincere thanks to

**employees, friends, and PhD students from the Leibniz Institute
for Food Systems Biology at the Technical University of Munich,**

who generously provided knowledge, inspiration,
and a warm welcome during my stay in Munich.

Wykaz stosowanych skrótów

AEDA	analiza wielokrotnych rozcieńczeń (ang. <i>aroma extract dilution analysis</i>)
DB-5	faza stacjonarna: 5% difenylo / 95% dimetylopolisiloksan (ang. <i>5% diphenyl / 95% dimethylpolysiloxan</i>)
FD	rozcieńczenie zapachu (ang. <i>flavor dilution</i>)
FFAP	faza stacjonarna: 2-nitrotereftalan glikolu polietylenowego (ang. <i>free fatty acid phase</i>)
FPA	analiza profilu smakowo-zapachowego (ang. <i>flavor profile analysis</i>)
GC	chromatografia gazowa (ang. <i>gas chromatography</i>)
GC × GC	kompleksowa dwuwymiarowa chromatografia gazowa (ang. <i>comprehensive two-dimensional gas chromatography</i>)
GC-MS	chromatografia gazowa - spektrometria mas (ang. <i>gas chromatography - mass spectrometry</i>)
GC-O	chromatografia gazowa - olfaktometria (ang. <i>gas chromatography - olfactometry</i>)
OAV	wartość aktywności zapachowej (ang. <i>odor activity value</i>)
OTC	stężenie progowe zapachu (ang. <i>odor threshold concentration</i>)
RI	indeks retencji (ang. <i>retention index</i>)
SAFE	zagęszczanie aromatu w obecności rozpuszczalnika (ang. <i>solvent assisted flavor evaporation</i>)
SIDA	analiza rozcieńczeń stabilnych izotopów (ang. <i>stable isotope dilution analysis</i>)
SPME	mikroekstrakcja do fazy stałej (ang. <i>solid phase microextraction</i>)
TIC	całkowity prąd jonowy (ang. <i>total ion current</i>)

Spis treści

Wykaz stosowanych skrótów	4
Słowa kluczowe.....	6
Key words	6
Streszczenie	7
Summary	9
Wstęp.....	11
Hipoteza i cel pracy	21
Omówienie wyników pracy przeglądowej i badań	22
Wykaz artykułów wchodzących w skład rozprawy doktorskiej	22
Rola związków siarki w kreowaniu aromatu warzyw.....	23
Zbadanie cech sensorycznych wybranych izotiocyjanianów	29
Blokowanie natywnego enzymu poprzez zastosowanie soli metali ciężkich	35
Kluczowe związki aktywne zapachowo odpowiedzialne za aromat surowej i gotowanej kalarepy (<i>Brassica oleracea</i> var. <i>gongylodes</i> L.).....	39
Wnioski.....	45
Bibliografia.....	47
Aktywność naukowa	60
Dorobek publikacyjny	60
Zestawienie danych według listy Journal Citation Reports i Web of Science	60
Udział w konferencjach naukowych.....	61
Staże naukowe	62
Stypendia	62
Udział w projektach badawczych	62
Kopie publikacji naukowych wchodzących w zakres rozprawy doktorskiej.....	63
Pisemne oświadczenia współautorów publikacji naukowych stanowiących rozprawę doktorską	127

Słowa kluczowe

warzywa kapustowate, kalarepa, glukozynolany, mirozynaza, związki sensorycznie aktywne, izotiocyjaniiny, nityle, siarczki, progi wyczuwalności sensorycznej, SAFE, AEDA chromatografia gazowa z olfaktometrią, wartość aktywności zapachowej (OAV)

Key words

Brassica vegetables, kohlrabi, glucosinolates, myrosinase, sensory-active compounds, isothiocyanates, nitriles, sulfides, odor thresholds, SAFE, AEDA, gas chromatography – olfactometry, odor activity value (OAV)

Streszczenie

Aromat żywności stanowi jedną z najważniejszych cech sensorycznych żywności, która świadczy o wysokiej jakości produktu i determinuje wybór konsumentów. Według najnowszych wytycznych żywieniowych spożywanie warzyw i owoców stanowi zaraz po aktywności fizycznej trzon piramidy żywienia, przy czym ważne są również proporcje. Warzywa i owoce powinny stanowić aż połowę spożywanej żywności, z czego około 75% powinny stanowić warzywa. Poza substancjami odżywczymi zawierają one również liczne związki bioaktywne, które wykazują szerokie działanie prozdrowotne. Spożywanie warzyw kapustowatych niesie za sobą szereg korzystnych właściwości, m.in.: wykazują one działanie przeciwnowotworowe, przeciwzapalne, przeciwbakteryjne, przeciwutleniające, kardioprotekcyjne, przeciwdziałają otyłości czy schorzeniom żołądka. Wykazano, że pozytywny wpływ na zdrowie człowieka związany jest z działaniem produktów rozpadu glukozynolanów.

Lotne produkty rozpadu glukozynolanów pod kątem sensorycznym wciąż stanowią mało poznaną grupę związków chemicznych. Główne cele niniejszej rozprawy doktorskiej obejmowały: przegląd literaturowy pod kątem związków lotnych siarki, uzupełnienie danych sensorycznych dla izotiocyjanianów, znalezienie metody inaktywacji mirozynazy do celów analizy związków lotnych oraz identyfikacja i kwantyfikacja kluczowych związków lotnych i aktywnych zapachowo, które są odpowiedzialne za aromat kalarepy surowej i gotowanej.

Przegląd literatury dotyczący związków lotnych siarki, o aktywności zapachowej w warzywach kapustowatych został rozszerzony o warzywa cebulowe oraz trufle i grzyby shiitake ze uwagi na podobieństwa w drogach ich powstawania. Zaraz po estrach związki siarki stanowią drugą, najliczniejszą grupę lotnych związków organicznych występujących w żywności. Dodatkowo tworzą one dominującą grupę związków identyfikowanych jako kluczowe związki odpowiedzialne za aromat żywności w tym również warzyw kapustowatych, cebulowych, trufli i grzybów shiitake. Związki lotne, zawierające siarkę powstają głównie w procesach enzymatycznych ale również podczas ich degradacji termicznej z prekursorów takich jak aminokwasy siarkowe, a także z glukozynolanów. Ograniczona stabilność, podatność na utlenianie oraz niskie progi detekcji powodują, że analiza jakościowa i ilościowa lotnych związków siarki jest wyzwaniem.

Analiza sensoryczna (FPA) 19 izotiocyjanianów dostarczyła informacji na temat charakteru zapachów i stężeń progowych zapachu dla poszczególnych związków. Wyznaczono

także współczynniki podziału gaz/ciecz dla powietrza i czterech matryc ciekłych – wody, buforu ślinowego, sztucznej śliny oraz śliny ludzkiej. Zebrane dane pozwalają na ocenę migracji do fazy nadpowierzchniowej izotiocyjanianów w czasie spożywania/przeżuwania warzyw kapustowatych.

Aktywność mirozynazy – enzymu katalizującego reakcję hydrolizy glukozynolanów w procesie przygotowania próby do analizy związków lotnych i zapachowych powinna być zahamowana. Z uwagi na trudności w hamowaniu aktywności mirozynazy pod kątem analizy związków zapachowych za pomocą metod opartych na działaniu temperatury podjęto w pracy badanie przydatności do tego celu soli nieorganicznych. Oceniono działanie wybranych soli nieorganicznych (kationów Cu^{2+} , Fe^{2+} , Fe^{3+} , Ag^{+} , Mn^{2+} , Ca^{2+} , Na^{+}) na ilość powstawania lotnych produktów rozpadu glukozynolanów w układzie modelowym oraz w tkance kalarepy. Jako optymalny inhibitor mirozynazy spośród badanych czynników wybrano bezwodny siarczan(VI) sodu, który zastosowano przy realizacji kolejnego zadania badawczego.

Skład związków lotnych występujących w kalarepie był już wcześniej znany, jednakże nie podjęto próby wyznaczenia kluczowych związków aktywnych zapachowo, odpowiedzialnych za aromat tego warzywa. W celu wyizolowania związków przygotowano ekstrakt z surowej i gotowanej kalarepy, który następnie zagęszczono. Tak przygotowaną próbę poddano analizie GC-O, dzięki zastosowaniu analizy wielokrotnych rozcieńczeń wyznaczono 55 związków aktywnych zapachowo w kalarepie surowej i gotowanej. Wykorzystanie analizy GC-O umożliwiło zidentyfikowanie 6 związków lotnych i aktywnych zapachowo, których obecność w kalarepie nie była wcześniej znana. Analiza ekstraktu z dodatkiem standardów znakowanych izotopami umożliwiła wyznaczenie stężeń analitów w badanej próbce kalarepy i wyznaczenie OAV. Określenie OAV pozwoliło na wyznaczenie ośmiu kluczowych związków odpowiedzialnych za aromat surowej i gotowanej kalarepy, wśród których znajdowały się 3 izotiocyjaniany. W większości obliczone OAV były wyższe dla próby kalarepy surowej i niższe dla kalarepy gotowanej ze względu na utratę związków lotnych podczas gotowania.

Summary

The aroma of food is one of the most important sensory features of food, which proves the product's high quality and determines the consumers' choice. According to the latest nutritional guidelines, consuming vegetables and fruits is the backbone of the nutrition pyramid right after physical activity nevertheless, proportions are also substantial. Vegetables and fruits should constitute at least half of the food eaten of which about 75% should be vegetables. In addition to nutrients, they also contain numerous bioactive compounds that have a wide range of health benefits. Consumption of Brassica vegetables brings a number of health-promoting effects, including anti-cancer, anti-inflammatory, antibacterial, antioxidant, cardioprotective, anti-obesity, and stomach diseases properties. It has been shown that the positive impact on human health is associated with the activity of glucosinolate breakdown products.

Volatile products of the decomposition of glucosinolates in sensory terms are still a little-known class of chemical compounds. The main objectives of this doctoral dissertation included: a literature review for volatile sulfur compounds, enhancement of the sensory data for isothiocyanates, finding a method for quenching myrosinase activity for the analysis of volatile compounds and identification and quantification of key volatile and odor-active compounds that are responsible for the aroma of raw and cooked kohlrabi.

The review of the literature on volatile odor-active sulfur compounds in Brassica vegetables was extended to Allium vegetables, truffles, and shiitake mushrooms due to the similarities in the pathways of their formation. After esters, sulfur compounds are the second most numerous class of volatile organic compounds found in food. In addition, they form the dominant group of compounds identified as key odor-active compounds in food including Brassica vegetables, Allium vegetables, truffles, and shiitake mushrooms. Volatile sulfur-containing compounds are formed mainly in enzymatic processes, but also during their thermal degradation from precursors such as sulfur amino acids, as well as from glucosinolates. Limited stability, susceptibility to oxidation, as well as low detection thresholds makes the qualitative and quantitative analysis of volatile sulfur compounds a challenge.

Sensory analysis (FPA) of 19 isothiocyanates provided information on the nature of the odor-active compounds and the odor threshold concentrations for the individual compounds. Gas/liquid partition coefficients were also determined for air and four liquid matrices - water, saliva buffer, artificial saliva, and human saliva. The collected data allow evaluation of the

migration to the headspace of isothiocyanates during the consumption/mastication of Brassica vegetables.

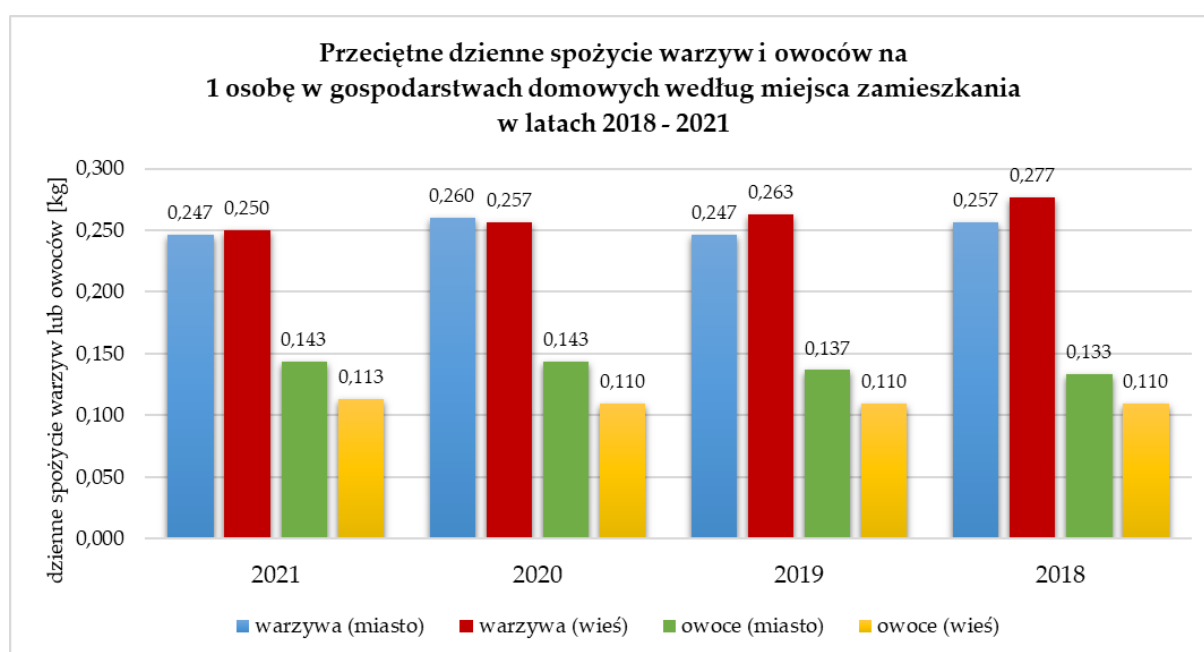
The activity of myrosinase - an enzyme catalyzing the reaction of glucosinolate hydrolysis in the process of sample preparation for the analysis of volatile and odor-active compounds should be inhibited. Due to the difficulties in inhibiting the activity of myrosinase in terms of the analysis of odor-active compounds using methods based on thermal treatment, the study of the suitability of inorganic salts for this purpose was undertaken. The effect of selected cations of inorganic salts (Cu^{2+} , Fe^{2+} , Fe^{3+} , Ag^{+} , Mn^{2+} , Ca^{2+} , Na^{+}) on the amount of formation of volatile glucosinolate breakdown products in the model system and in green kohlrabi tissue was evaluated. Anhydrous sodium sulfate was selected as the optimal myrosinase inhibitor from among the tested agents, which was used in the subsequent research task.

The composition of the volatile compounds found in kohlrabi was already tested, but no attempt was made to determine the key odor-active compounds responsible for the odor of this vegetable. In order to isolate the compounds, an extract of raw and cooked kohlrabi was prepared and concentrated. The sample prepared in this way was subjected to GC-O analysis and due to aroma extract dilution analysis, 55 aroma-active compounds were determined in raw and cooked kohlrabi. The application of GC-O analysis enabled the identification of 6 volatile and odor-active compounds whose presence in kohlrabi was previously unknown. The analysis of the extract with the addition of isotope-labeled standards allowed the determination of analyte concentrations in the kohlrabi sample and the calculation of the OAVs. Determination of the OAVs was crucial in the determination of eight key compounds responsible for the aroma of raw and cooked kohlrabi, including 3 isothiocyanates. For the most part, the calculated OAVs were higher for the raw kohlrabi sample and lower for the cooked kohlrabi due to the loss of volatile compounds during cooking.

Wstęp

Aktywność fizyczna oraz spożywanie warzyw i owoców stanowią trzon zasad prawidłowego żywienia i w konsekwencji prowadzą do zdrowego stylu życia [1, 2]. Biorąc pod uwagę aspekt żywieniowy, warzywa i owoce odgrywają kluczową rolę w diecie człowieka, ze względu na wysoką wartość odżywczą, natomiast zawarte w roślinach fitozwiązki, makro i mikroelementy są odpowiedzialne za utrzymywanie prawidłowej homeostazy organizmu człowieka [3, 4]. Produkty pochodzenia roślinnego stanowią również cenne źródło związków biologicznie aktywnych, które wykazują szereg prozdrowotnych funkcji, jednak do najważniejszych należą: obniżanie stanu zapalnego i potencjalne działanie zapobiegawcze w różnych przewlekłych stanach chorobowych takich jak otyłość, cukrzyca typu 2, choroby układu krążenia i niektóre rodzaje chorób nowotworowych [5].

Zgodnie z zaleceniami Światowej Organizacji Zdrowia oraz Międzynarodowej Agencji Badań nad Rakiem codzienne spożycie warzyw i owoców powinno być nie mniejsze niż 400 gramów, jednakże nawet w wielu europejskich państwach takich jak: Holandia, Niemcy, Szwecja, Dania, Norwegia codzienne spożycie warzyw i owoców jest niższe [6, 7]. Według danych Głównego Urzędu Statystycznego w Polsce na przestrzeni ostatnich czterech lat obserwuje się nieznaczne zmniejszenie spożycia warzyw. Natomiast codzienne spożycie warzyw i owoców jest bliskie 400 gramów na dzień, w szczególności dla osób mieszkających w mieście. Zestawienie danych zaprezentowano na poniższym wykresie:

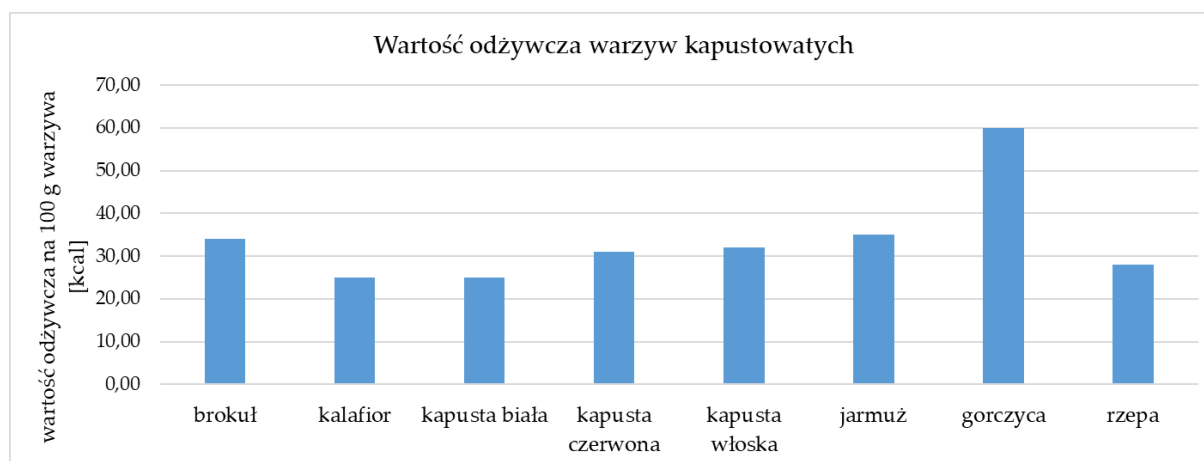


Rys. 1 Przeciętne dzienne spożycie warzyw i owoców w Polsce na 1 osobę w gospodarstwach domowych w latach 2018 – 2021 (na podstawie danych Głównego Urzędu Statystycznego 2018-2021)

Z roku na rok naukowcy z całego świata próbują odkryć nowe lub potwierdzić istniejące przesłanki dotyczące prozdrowotnych funkcji żywności pochodzenia roślinnego [8]. Dodatkowo wiele badań skupia się wokół sprawdzenia wpływu różnych czynników, np.: stadium wzrostu rośliny, jakości środowiska, w którym roślina wzrasta, warunków przechowywania, sposobu przetwarzania na finalną ilość związków bioaktywnych [9–11]. Z ekonomicznego punktu widzenia walory prozdrowotne nie są jednakże jedynymi, które przyciągają potencjalnych klientów, dla których żywność musi być przede wszystkim atrakcyjna pod względem wyglądu, tekstury, a ponadto smaczna i aromatyczna [12, 13]. W związku z tym producenci żywności, w interesie także konsumentów, zainteresowani są wykorzystaniem różnych wariantów analizy sensorycznej i technik instrumentalnych w celu rutynowej kontroli jakości surowców lub finalnego produktu [14–16].

Spośród warzyw dostępnych na rynku, istotną grupę stanowią warzywa kapustowate, które obejmują wiele ważnych gospodarczo gatunków uprawianych na całym świecie. Od czasów starożytności do współczesności warzywa te stanowiły ważny element diety człowieka i były spożywane głównie w formie surowej lub przetworzonej. Warzywa kapustowate reprezentują grupę monofiletyczną rozprzestrzenioną na całym świecie, z wyjątkiem Antarktydy i obejmują około 338 rodzajów i 3709 gatunków [17]. Do warzyw kapustowatych należy szeroka grupa surowców roślinnych takich jak: kapusta, brokuł, kalafior, kalarepa, jarmuż, gorczyca, a także rzepak i wiele innych [18]. Dodatkową zaletą jest fakt, że warzywa te są powszechnie dostępne przez cały rok [19].

Pod kątem żywieniowym warzywa kapustowate charakteryzują się niską zawartością tłuszczu, można je sklasyfikować jako żywność niskokaloryczną [20]. Poniżej przedstawiono wykres kaloryczności wybranych warzyw kapustowatych (rys 2.).



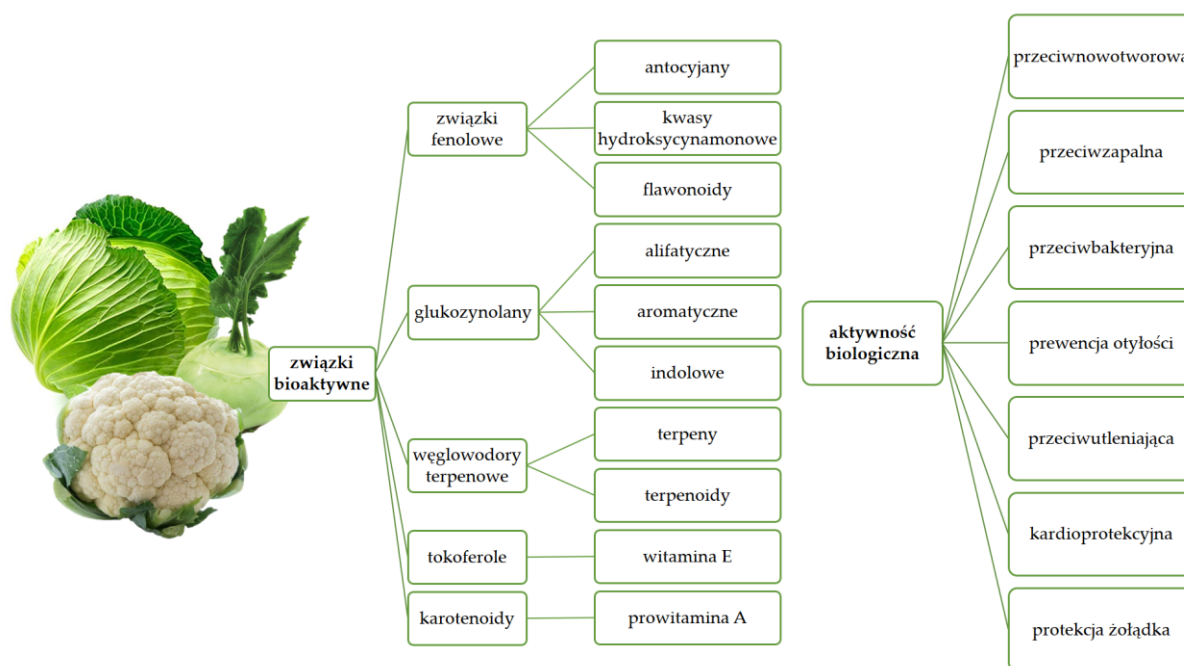
Rys. 2. Wartość odżywcza wybranych warzyw kapustowatych [kcal / 100 g warzywa] (na podstawie [21])

Spośród związków chemicznych wchodzących w skład warzyw, największy udział ma woda (której zawartość mieści się w zakresie 89 – 92%), natomiast pozostałe składniki odżywcze stanowią: węglowodany, błonnik, białka, tłuszcze, a także makroelementy takie jak wapń i magnez, mikroelementy, karotenoidy, tokoferole, witamina C i kwas foliowy [21]. Udział składników odżywczych w różnych gatunkach warzyw kapustowatych jest do siebie zbliżony. Poniżej zaprezentowano profile głównych składników wybranych warzyw kapustowatych takich jak: brokuł, kalafior, kapusta biała, kapusta włoska, rzepa i jarmuż (rys.3).



Rys. 3 Profil składników odżywczych wybranych warzywach kapustowatych (na podstawie [21])

Dodatkowo warzywa kapustowate są źródłem różnorodnych wyspecjalizowanych metabolitów, z czego największą rolę odgrywają glukozynolany i produkty ich rozpadu. Aktywność biologiczną wykazują również inne grupy związków chemicznych, które są obecne w tych warzywach między innymi: związki fenolowe (flawonoidy, kwasy fenolowe, proste fenole, kwasy hydroksycynamonowe), tokoferole, karotenoidy, terpeny [21]. W ostatnich latach skład chemiczny warzyw kapustowatych był przedmiotem intensywnych badań, ze względu na obecność cennych metabolitów, które spełniają szereg korzystnych funkcji biologicznych, co przedstawiono na rys. 4.



Rys. 4 Główne funkcje biologiczne warzyw kapustowatych oraz występujące w nich związki bioaktywne (na podstawie [21])

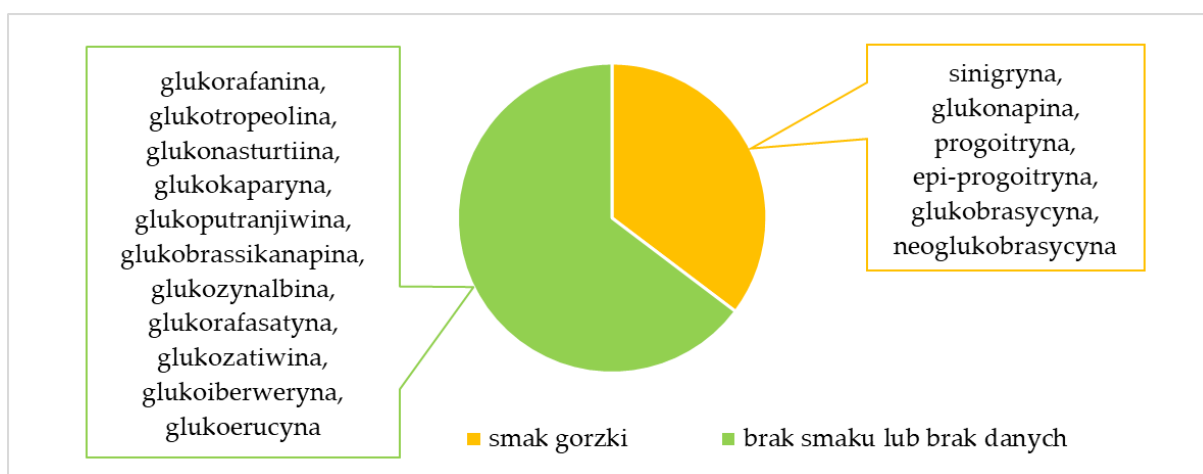
Spośród licznych funkcji biologicznych metabolitów występujących w warzywach kapustowatych największe zainteresowanie skupiają związki, które spełniają funkcje prozdrowotne takie jak: ograniczenie stresu oksydacyjnego, właściwości przeciwutleniające i przeciwzapalne, indukcja enzymów detoksykacyjnych, stymulacja układu odpornościowego, działanie przeciwcukrzycowe, obniżanie poziomu cholesterolu, redukcja proliferacji komórek nowotworowych, a także zahamowanie mutacji rakotwórczych [22, 23]. Glukozynolany i produkty ich rozpadu, głównie izotiocyjaniiny pełnią kluczową rolę w chemoprewencji wielu nowotworów, między innymi: płuc [24], żołądka [25], jelita grubego [26], piersi [27], endometrium [28], szyjki macicy [29], pęcherza moczowego [30], prostaty [31] oraz wątroby [32]. W rezultacie warzywa kapustowate mogą zostać wykorzystane jako potencjalne składniki w profilaktycznych dietach przeciwnowotworowych [33].

Pomimo licznych walorów prozdrowotnych, spożycie warzyw kapustowatych powinni ograniczyć i kontrolować pacjenci z niedoczynnością tarczycy. Duże stężenia glukozynolanów i produktów ich degradacji takich jak izotiocyjaniany, tiocyjaniany i oksazolidyno-2-tiony mogą wykazywać działanie antyodżywcze i goitrogenne (wołotwórcze). Związki powstające podczas hydrolizy tioglikozydów hamują szlak metaboliczny odpowiedzialny za transport jodu i wbudowywanie go w białka hormonalne – tyreoglobuliny. W konsekwencji prowadzi to do zwiększenia proliferacji komórek tarczycy i pobudzenia wydzielania hormonu tyreotropowego (TSH) [20, 22, 34]. Według Narodowego Centrum Edukacji Żywnościowej pacjenci cierpiący na niedoczynność tarczycy nie powinni całkowicie eliminować warzyw kapustowatych z codziennej diety, jednakże konieczne jest ograniczenie w ich spożywaniu oraz odpowiednie przetworzenie. Warto zwrócić uwagę, że zmniejszeniu zawartości związków antyodżywczych o 30 – 40% w tych warzywach sprzyja gotowanie w dużej ilości wody bez przykrycia [35]. Goitrogenne działanie związków obecnych w warzywach kapustowatych jest ściśle zależne od podaży jodu w diecie, dlatego może być łagodzone poprzez zwiększenie spożywania żywności bogatej w jod [36, 37].

Aspekt zdrowotny nie jest jedynym, który wpływa na preferencje żywieniowe konsumentów. Wybory żywieniowe zależą od wcześniejszych doświadczeń, środowiska żywieniowego, a także czynników biologicznych, które są częściowo uwarunkowane genetycznie. Warzywa kapustowate powodują awersję u niektórych konsumentów ze względu na ich charakterystyczny aromat, na który składa się smak i zapach. Celem wykształcenia się u człowieka zmysłu smaku było przede wszystkim odróżnienie pokarmu od potencjalnie trujących substancji. W konsekwencji przyjemne odczucia takie jak smak słodki i umami wskazywały na obecność w żywności cukrów i węglowodanów. Za zapewnienie homeostazy wodno-elektrolitowej organizmu odpowiadał smak słony, natomiast smak gorzki i kwaśny umożliwiały wykrycie potencjalnie szkodliwych związków chemicznych w żywności takich jak wyspecjalizowane metabolity czy toksyny mikrobiologiczne [38, 39].

Glukozynolany i produkty ich enzymatycznej hydrolizy – izotiocyjaniany nadają warzywom kapustowatym charakterystyczny ostry i gorzki smak, który przyczynia się do ograniczenia lub wyeliminowania tych produktów z codziennej diety [40, 41]. Dla przykładu dla synigryny, która jest glukozynolanem o gorzkim smaku, próg detekcji w wodzie wynosi 106 mg/kg [42]. Badania sensoryczne glukozynolanów i izotiocyjanianów stanowią wyzwanie, ze względu na szereg aspektów, które należy mieć na uwadze. Po pierwsze panel sensoryczny składa się z odpowiednio wytrenowanych osób w ocenie zapachu czy smaku, jednak każda

z nich posiada inne, indywidualne predyspozycje do oceny sensorycznej próbek [43], a percepcja smaku gorzkiego uwarunkowana jest genetycznie [44]. Jeden z 25 receptorów reagujących na smak gorzki – hTAS2R38 do aktywacji wymaga fragmentu cząsteczki $-N=C=S$ [45]. Co więcej, ze względu na występowanie tych związków w złożonej matrycy tkanki roślinnej, badania nad smakiem wymagają użycia wzorców o wysokiej czystości, które są trudno dostępne lub całkowicie niedostępne. Wysoki koszt standardów glukozynolanów, akceptowalny przy wykorzystaniu ich jako wzorców chromatograficznych, na potrzeby analizy sensorycznej, gdzie wymagane są znacznie większe ilości wzorca, sprawia, że dane literaturowe na temat ich właściwości sensorycznych są szczątkowe. Dostępność izotiocyjanianów jest większa, natomiast brak w literaturze danych na temat ich właściwości smakowych, zwłaszcza takich, które wykonane zostały w tych samych warunkach dla dużej grupy związków. Przyczyny te sprawiają, że wiedza na temat smaku większości izotiocyjanianów i glukozynolanów, które występują w warzywach kapustowatych jest niedostępna lub w znacznym stopniu ograniczona. Na wykresie 5 zestawiono, które z glukozynolanów zostały zaklasyfikowane jako gorzkie [46, 47].



Rys. 5 Smak glukozynolanów (na podstawie [46, 47])

Również pod względem aromatu warzywa kapustowate stanowią interesujący przedmiot badań, ze względu na charakterystyczny, siarkowy, ostry i drażniący zapach, który najczęściej przypisywany jest lotnym produktom hydrolizy enzymatycznej glukozynolanów, które zawierają atom siarki w swojej budowie (głównie izotiocyjaniany i siarczki) [47–49]. Odczucia sensoryczne, których dostarczają warzywa w postaci surowej lub po ugotowaniu dla niektórych konsumentów są nieakceptowalne w takim stopniu, że stanowią wystarczający argument do pomijania ich w codziennej diecie [40]. Przez ostatnie dekady dokonano wielu analiz jakościowych i ilościowych pod kątem związków lotnych występujących w warzywach

kapustowatych. Przegląd literaturowy pod kątem identyfikacji i kwantyfikacji związków lotnych dostarcza wielu informacji na temat zróżnicowania metabolitów i związków lotnych w tych warzywach, jednakże nie wyjaśnia, które z nich są bezpośrednio odpowiedzialne za ich charakterystyczny zapach. Zastosowanie wyłącznie metod instrumentalnych lub elektronicznych nosów pomimo ich wielu zalet, nadal nie jest metodą powszechnie wykorzystywaną do analiz zapachu, ze względu na istniejące ograniczenia [50].

Wyznaczenie kluczowych związków aktywnych zapachowo (ang. *key odorants*) w próbkach żywności jest procesem złożonym i pracochłonnym. Złotym standardem w analizie kluczowych związków aktywnych zapachowo jest połączenie analizy sensorycznej (olfaktometrii) z instrumentalną – tzw. sensomika (ang. *sensomics*), która obejmuje następujące etapy:

1. izolacja i zagęszczanie aromatu w obecności rozpuszczalnika (SAFE) (rys. 6) i analiza sensoryczna uzyskanego ekstraktu;
2. analiza ekstraktu przy użyciu chromatografii gazowej sprzężonej z olfaktometrią (GC-O) na kolumnach różniących się polarnością;
3. analiza wielokrotnych rozcieńczeń (AEDA) – wyznaczenie aktywnych fragmentów chromatogramu nawet przy wielokrotnych rozcieńczeniach wyjściowego ekstraktu;
4. identyfikacja związków odpowiedzialnych za aktywne zapachowo fragmenty chromatogramu przy użyciu baz danych, standardów, analiz GC-O oraz GC-MS lub GC × GC-MS;
5. wykonanie analizy GC-MS lub GC × GC-MS dla serii rozcieńczeń standardów oraz przygotowanie krzywych kalibracyjnych;
6. przygotowanie ekstraktu SAFE wraz z dodatkiem standardów znakowanych izotopami;
7. analiza danych chromatograficznych ekstraktu i wyznaczenie stężeń poszczególnych analitów odpowiedzialnych za zapach danej próby (SIDA);
8. przeprowadzenie analizy sensorycznej kluczowych związków aktywnych zapachowo w celu uzyskania stężeń progowych zapachu w wodzie lub znalezienie odpowiednich danych literaturowych;
9. wyznaczenie wartości aktywności zapachowej (OAV) dla każdego związku, któremu można przypisać charakterystyczny zapach [51].



Rys. 6 Przygotowanie ekstraktu do analizy chromatograficznej; a – homogenizacja i ekstrakcja rozpuszczalnikiem organicznym; b – SAFE; c – zagęszczanie ekstraktu do finalnej objętości 1 cm³

Mając na uwadze analitykę żywności, analiza związków lotnych żywności jest szeroko eksploatowana. Baza danych Web of Science (WoS) dla hasła FOOD – and – VOLATILES daje 8633 dokumenty, FOOD – and – AROMA ACTIVE COMPOUNDS daje 592 dokumenty, natomiast hasło FOOD – and – SENSOMICS daje 29 wyników. Sam termin sensomics w WoS ukazuje 130 publikacji (dane z 06.06.2023), aczkolwiek prac z użyciem tego podejścia jest więcej. Głównym powodem relatywnie małej popularności tego podejścia analitycznego jest stopień złożoności opisanych analiz i wymagania metodyczne dotyczące identyfikacji oraz przede wszystkim analizy ilościowej badanych związków. Sensomika była wykorzystana do analizy związków zapachowych występujących w szerokiej kategorii produktów, takich jak: napoje alkoholowe, produkty mięsne, ryby i owoce morza, produkty zbożowe i piekarnicze, produkty mleczne, tłuszcze i nasiona oleiste, owoce, warzywa, grzyby, przyprawy i zioła, kakao i czekolada, kawa, herbata, i kilka innych, w tym sos sojowy, ocet balsamiczny, miód, karmel i popcorn [52]. Najważniejsze zastosowania sensomiki to profilowanie kluczowych związków odpowiedzialnych za aromat danej żywności, weryfikowanie jej jakości podczas procesów przetwórczych i przechowywania [15, 53, 54], a także optymalizacja procesów biotechnologicznych, między innymi fermentacji [55, 56].

W przyszłości potencjał analizy olfaktometrycznej do identyfikacji kluczowych związków może przyczynić się do zwiększenia jej roli jako techniki analitycznej zwłaszcza w doskonaleniu upraw hodowlanych odmian interesujących pod kątem aromatu, kontroli autentyczności żywności, czy potencjału wykorzystania biotechnologii w tworzeniu związków zapachowych na drodze enzymatycznej [52].

Dalsze rozwijanie wiedzy oparte na chromatografii gazowej i olfaktometrii w przyszłości może umożliwić otrzymanie rekombinantów zapachowych, które potencjalnie mogą służyć np. do celów leczniczych takich jak: rehabilitacja pacjentów z hiperestezją węchową, terapia pacjentów z niedoborem odporności lub chorobą nowotworową, którzy często cierpią z powodu znacznego ograniczenia przyjmowania pokarmu w następstwie upośledzonych zdolności chemosensorycznych lub w celu sensorycznie indukowanej regulacji apetytu [52].

W literaturze naukowej dopiero na przestrzeni ostatnich kilku lat zaczęły powstawać prace, których celem było badanie związków odpowiedzialnych za kreowanie aromatu warzyw kapustowatych. Do chwili obecnej kluczowe związki lotne i aktywnie sensorycznie wyznaczono jedynie dla kilku warzyw kapustowatych takich jak: brokuł [57], kalafior [58], kapusta właściwa [59], rzepak [60, 61], gorczyca [61] czy kimchi, którego głównym składnikiem jest kapusta pekińska lub kapusta chińska [62]. Biblioteki danych sensorycznych, zawierających indeksy retencji na kolumnie polarnej i niepolarnej, progi wyczuwalności sensorycznej, deskryptor jakości zapachu a także występowanie w danej matrycy żywności tym samym wzbogaciły się o nieznane wcześniej związki takie jak: izotiocyjaniany, nityle, siarczki i inne związki lotne. Niektóre związki chemiczne były wcześniej znane jako związki aktywne zapachowo, ale zostały zidentyfikowane w roślinie innego gatunku lub całkowicie innym produkcie żywnościowym [63, 64].

Z punktu widzenia chemii analitycznej warzywa kapustowate stanowią wymagającą próbę ze względu na liczne metabolity, które nierzadko występują w śladowych ilościach [65]. Dla przykładu stężenia niektórych związków lotnych i aktywnych zapachowo nie osiągają wartości progu detekcji dla różnych metod analitycznych, jednocześnie są na tyle wysokie, że ich OAV umożliwia swobodną detekcję przez ludzki nos [66]. Problemem w analizie tkanek roślinnych są również aktywne enzymy, które przyczyniają się do uzyskiwania wysokich odchyleń standardowych nawet w przypadku prób pobranych z tego samego warzywa [67]. Konieczne jest zatem znalezienie optymalnej metody w celu inaktywacji działania enzymów endogennych. Co więcej, podczas analizy związków lotnych i aktywnych zapachowo należy mieć na uwadze związki termolabilne, które mogą tworzyć artefakty i utrudniać przeprowadzenie ostatecznej klasyfikacji związków lotnych i aktywnych zapachowo odpowiedzialnych za aromat konkretnej żywności [68].

Warzywa kapustowate stanowią niewątpliwie interesującą matrycę, która interesuje naukowców z różnorodnych dyscyplin naukowych. Z tej przyczyny istnieje obecnie wiele prac

naukowych, w których wykorzystuje się te warzywa, badając je pod różnym kątem. Mimo licznych zainteresowań, szczególnie w aspekcie aktywności biologicznej i prozdrowotnej, w literaturze naukowej wciąż brakuje podstawowych informacji sensorycznych zarówno na temat metabolitów aktywnych zapachowo oraz związków, które odpowiadają za charakterystyczny smak tych warzyw. Co więcej w zależności od metody przeprowadzania testów sensorycznych, uzyskiwane dane na temat jakości zapachu czy OTC mogą się znacznie różnić pomiędzy laboratoriami. Dla przykładu zakres dostępnych w literaturze danych OTC wyznaczonego w wodzie dla izotiocyjanianu allilu mieści się w zakresie od 0,046 do 3,8 mg/kg [69].

Przedstawione dane dowodzą, że badania nad związkami odpowiedzialnymi za aromat warzyw kapustowatych w porównaniu do bardzo dużej liczby publikacji dotyczących różnych aspektów związków bioaktywnych tych warzyw (głównie glukozynolanów) stanowią marginalny odsetek prac, biorąc pod uwagę popularność i rozpowszechnienie tych warzyw wśród konsumentów.

Hipoteza i cel pracy

W oparciu o dane literaturowe postawiono hipotezę badawczą, że na aromat warzyw kapustowatych w znacznym stopniu wpływają lotne produkty degradacji glukozynolanów, głównie izotiocyjany. Jako warzywo modelowe w badaniach wykorzystano kalarepę (*Brassica oleracea* var. *gongylodes* L.).

Cele niniejszej rozprawy doktorskiej były następujące:

- przegląd literaturowy dotyczący związków sensorycznie aktywnych w warzywach ze szczególnym uwzględnieniem prekursorów, biosyntezy oraz sposobu przetwarzania;
- zbadanie cech sensorycznych wybranych izotiocyjanianów (stężenia progowe zapachu, jakość zapachu) oraz współczynników podziału gaz/ciecz warunkujących ich progi wyczuwalności sensorycznej i uwalnianie w odniesieniu do różnych matryc (woda, bufor ślinowy, sztuczna ślina, ślina ludzka);
- optymalizacja procesu blokowania aktywności tioglukozydazy (mirozynazy) dla celów analitycznych poprzez zastosowywanie soli nieorganicznych;
- izolacja, identyfikacja, oznaczenie ilościowe i charakterystyka związków lotnych i aktywnych zapachowo powstających w kalarepie surowej oraz gotowanej w wodzie.

Omówienie wyników pracy przeglądowej i badań

Wykaz artykułów wchodzących w skład rozprawy doktorskiej

- **Marcinkowska, M.;** Jeleń, H.H. Role of Sulfur Compounds in Vegetable and Mushroom Aroma. *Molecules* **2022**, 27, doi:10.3390/molecules27186116. IF = 4.927
- **Marcinkowska, M.;** Jeleń, H.H. Determination of the odor threshold concentrations and partition coefficients of isothiocyanates from Brassica vegetables in aqueous solution. *LWT* **2020**, 131, doi:10.1016/j.lwt.2020.109793. IF = 6.056
- **Marcinkowska, M.;** Jeleń, H.H. Inactivation of thioglucosidase from *Sinapis alba* (white mustard) seed by metal salts. *Molecules* **2020**, 25, doi:10.3390/molecules25194363. IF = 4.927
- **Marcinkowska, M.;** Frank, S.; Steinhaus, M.; Jeleń, H.H. Key Odorants of Raw and Cooked Green Kohlrabi (*Brassica oleracea* var. *gongylodes* L.). *J. Agric. Food Chem.* **2021**, 69, 12270–12277, doi:10.1021/acs.jafc.1c04339. IF = 5.895

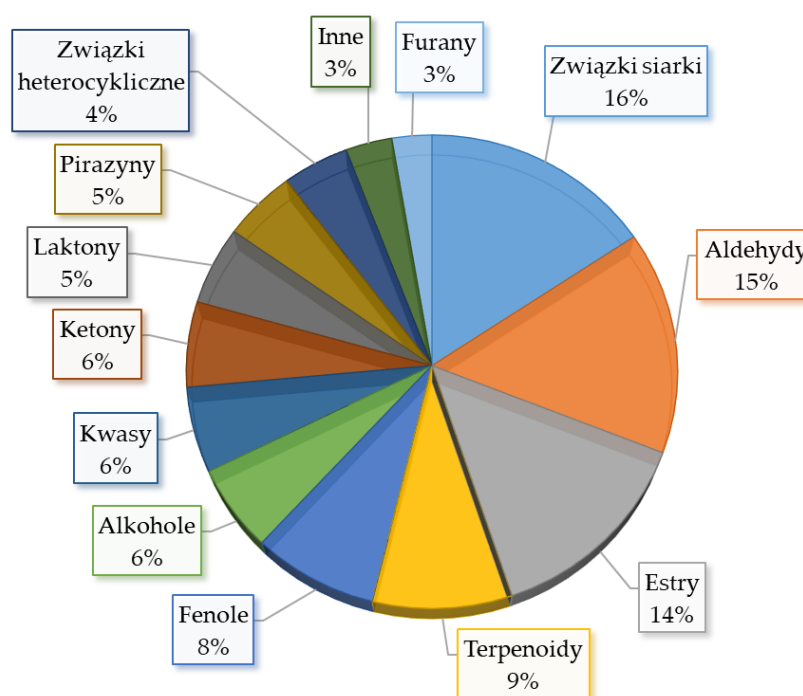
W ramach niniejszej pracy doktorskiej przygotowano trzy publikacje eksperymentalne oraz jedną pracę przeglądową. Omówienie najważniejszych aspektów poszczególnych prac przedstawiono w następujących podrozdziałach – każdy z nich odnosi się do odrębnie opublikowanych wyników badań:

- Rola związków siarki w kreowaniu aromatu warzyw;
- Zbadanie cech sensorycznych wybranych izotiocyjanianów;
- Blokowanie natywnego enzymu poprzez zastosowanie soli metali ciężkich;
- Kluczowe związki aktywne zapachowo odpowiedzialne za aromat surowej i gotowanej kalarepy (*Brassica oleracea* var. *gongylodes* L.).

Na podstawie wyszczególnionych prac sformułowano i zestawiono wnioski, które podsumowują najważniejsze wyniki przeprowadzonych badań. Wszystkie publikacje wchodzące w skład niniejszej rozprawy doktorskiej zostały załączone w całości z zachowanym formatowaniem czasopisma w części „Kopie publikacji naukowych wchodzących w zakres rozprawy doktorskiej”.

Rola związków siarki w kreowaniu aromatu warzyw [Publikacja 1]

Siarka jest ważnym pierwiastkiem, który tworzy wiele różnorodnych związków nieorganicznych i organicznych. Najważniejsze funkcje budulcowe odgrywają aminokwasy siarkowe - metionina i cysteina, które dodatkowo stanowią ważne źródło siarki w diecie człowieka [70]. Występujące w żywności związki siarki stanowią (zaraz po estrach) największą grupę lotnych związków organicznych. Nie wszystkie związki lotne siarki są aktywne zapachowo, jednakże w ujęciu kluczowych związków odpowiedzialnych za aromat żywności stanowią największą grupę (rys. 7).



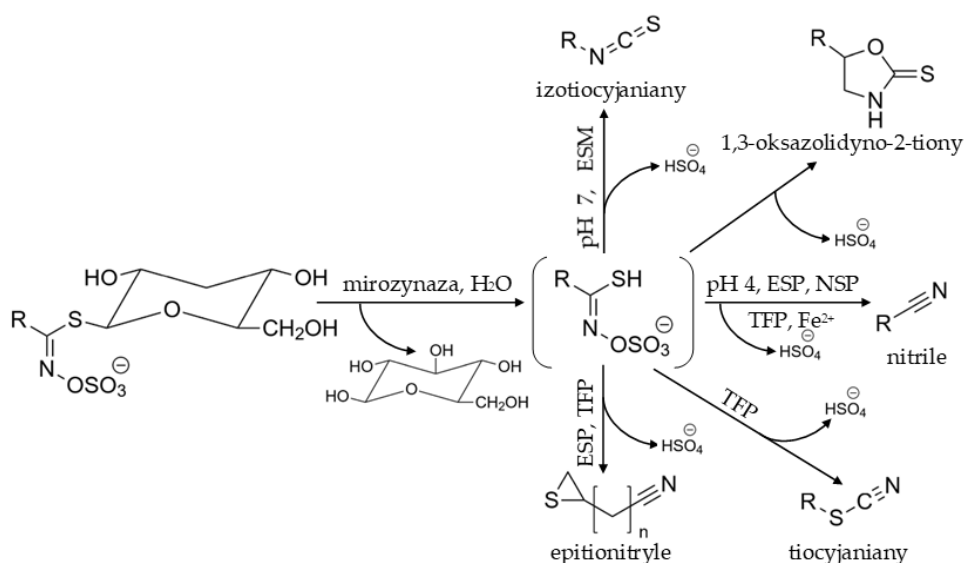
Rys. 7 Kluczowe związki lotne i aktywne zapachowo wykryte w żywności (na podstawie [52])

Związki zawierające siarkę są szeroko dostępne w warzywach kapustowych, cebulowych a także w grzybach shiitake czy truflach. Celem pracy przeglądowej było zebranie i podsumowanie informacji na temat podstawowych funkcji związków siarki oraz przedstawienia ich udziału w tworzeniu ogólnego aromatu żywności w odniesieniu do warzyw i grzybów. Pierwotnie cel pracy nie obejmował grzybów, jednakże ze względu na wiele podobieństw pod względem szlaków biosyntezy związków zawierających siarkę i ich właściwości sensorycznych do wymienionych wyżej warzyw, zostały one dodatkowo włączone do pracy przeglądowej. Dodatkowo omówiono również wpływ przetwarzania żywności na związki siarki oraz drogi powstawania związków aktywnych sensorycznie.

Prekursorami lotnych związków siarki są aminokwasy i glukozynolany. Spośród dwudziestu aminokwasów białkowych tylko cysteina i metionina zawierają w swojej budowie

atom siarki [71]. Główną rolą cysteiny w roślinach jest utrzymywanie poziomu siarki nieorganicznej pozyskiwanej z środowiska. Aminokwas ten jest jedynym donorem siarczzków potrzebnym do biosyntezy kluczowych biomolekuł, w szczególności przeciwutleniaczy (glutathionu), kofaktorów, witamin czy związków obronnych – glukozynolanów, tionin, fitoaleksyn. Metionina warunkuje rozwój i potencjalnie odpowiada za biosyntezę substancji regulujących wzrost (auksyny, brassinosteroidy, cytokininy). Jest również ważnym prekursorem biosyntezy karnityny, cysteiny, lecytyny, tauryny i fosfolipidów [72]. Glukozynolany to wyspecjalizowane metabolity roślinne, których cząsteczka składa się z β -D-glukozy, sulfonowanego oksymu oraz łańcucha bocznego, który determinuje klasę glukozynolanu (alifatyczny, aromatyczny, indolowy). Glukozynolany w warzywach odgrywają rolę naturalnych pestycydów, które zostają uaktywnione w momencie, gdy tkanka roślinna ulega zniszczeniu i w konsekwencji degradują enzymatycznie do związków lotnych [73].

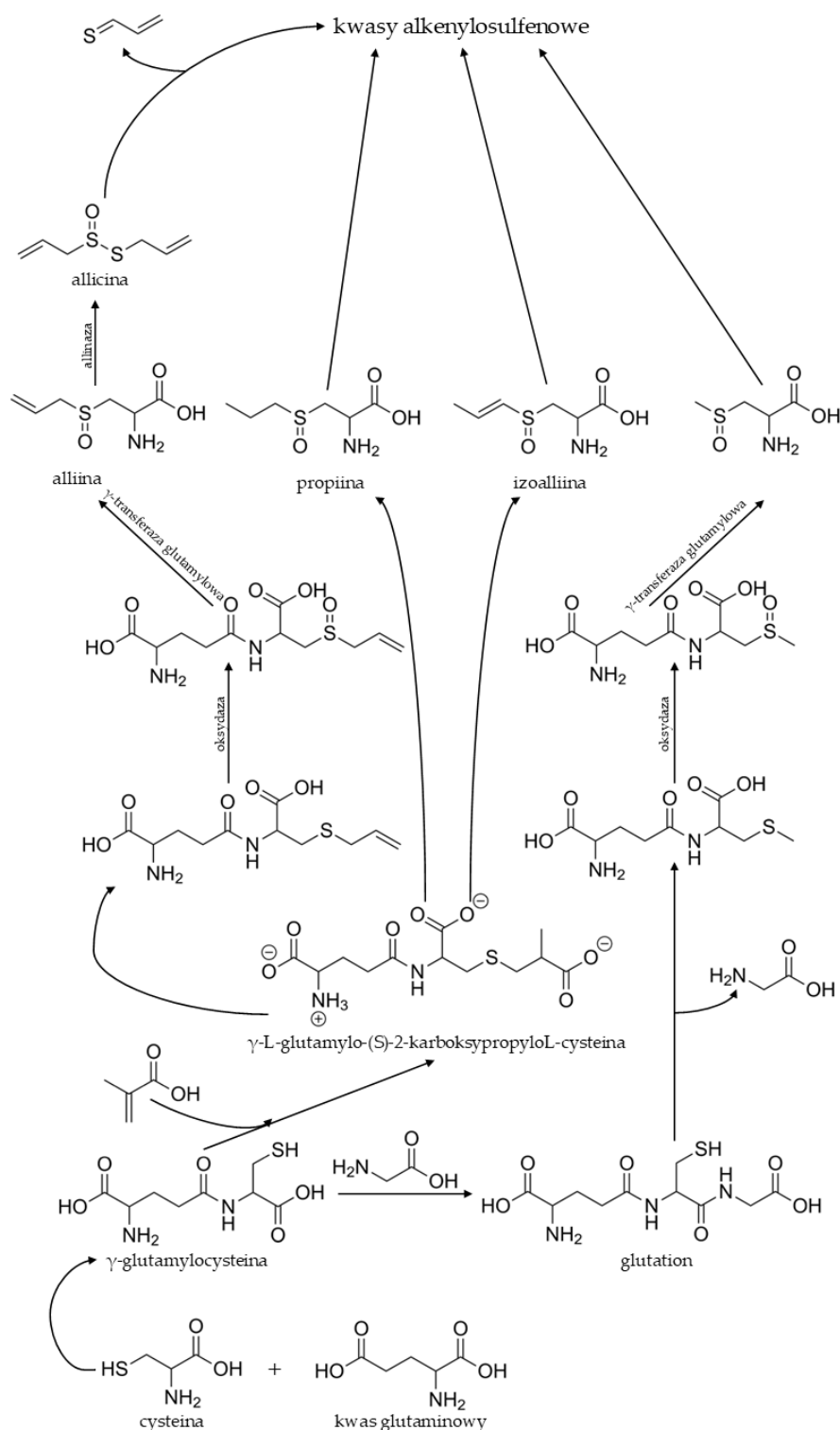
Biosynteza siarkowych związków lotnych zachodzi przy udziale enzymów w warzywach kapustowatych i cebulowych. Degradacja enzymatyczna glukozynolanów jest zależna od warunków, w jakich jest prowadzona i może prowadzić odpowiednio do różnych związków lotnych takich jak: izotiocyjaniany, nityle, tiocyjaniany, epitionitryle (rys. 8) [74, 75].



Rys. 8 Powstawanie głównych, lotnych produktów rozpadu glukozynolanów w zależności od warunków prowadzenia hydrolizy enzymatycznej; ESP – białko epitiospecyficzne; ESM – modyfikujące białko epitiospecyficzne związane z mirozynazą; NSP – nitylospecyficzne białko; TFP – białko tworzące tiocyjaniany; R – łańcuch boczny glukozynolanu pochodzący od aminokwasu (na podstawie [74, 75])

Natomiast w warzywach cebulowych bezzapachowe i nielotne prekursory stanowią pochodne cysteiny: sulfotlenki (+)-S-alk(en)ylo cysteiny i peptyd γ -glutamylowy [76]. Za wytwarzanie charakterystycznego zapachu cebuli podczas zniszczenia tkanki warzywa

odpowiadają głównie sulfotlenki, które tak jak np. alliina może przekształcać się pod wpływem allinazy w allicynę, która jest niestabilna i ulega dalszej degradacji do kwasów alkenylosulfenowych lub bezpośrednio do siarczków allilu. Szlak metaboliczny powstawania związków lotnych w warzywach cebulowych zaprezentowano na rys. 9 [77].



Rys. 9 Tworzenie związków lotnych i aktywnych zapachowo w reakcjach enzymatycznych warzyw cebulowych (na podstawie [78–81])

Podczas degradacji prekursorów powstają lotne związki zawierające w swojej budowie siarkę, które wykazują się obiecującymi właściwościami chemicznymi. Do tej grupy związków należą: izotiocyjaniany, nitryle, epitionitryle, siarczki, polisiarczki, tiole i inne np. metional czy związki heterocykliczne zawierające siarkę, które występują w truflach i grzybach shiitake. Izotiocyjaniany od lat zyskują zainteresowanie ze względu na ich liczne właściwości, które pozytywnie wpływają na zdrowie człowieka (działanie antyzapalne, prewencyjne i terapeutyczne) jak również pomagają ochronić plony czy zabezpieczyć żywność przed zepsuciem. Pod kątem sensorycznym izotiocyjaniany stanowią wciąż niedostatecznie poznaną grupę związków, jednakże dotychczasowe prace opisują, że ich główne noty zapachowe skupiają się wokół zapachu siarki, ostrego, drażniącego, charakterystycznego dla zapachu czosnku czy kapusty [64, 82].

W porównaniu do izotiocyjanianów o funkcjach bioaktywnych i cechach sensorycznych nitryli i epitionitryli wiadomo niewiele. Według źródeł literaturowych ekspozycja na nitryle jest niekorzystna dla człowieka i wiąże się z wieloma zaburzeniami zdrowotnymi, jednakże obecność nitryli w spożywanej żywności może wywoływać efekt prozdrowotny, ale mniejszy niż w przypadku izotiocyjanianów. Nitryle mają podobny zapach w odniesieniu do ich odpowiednich aldehydów. Występujące w warzywach nitryle i epitionitryle wykazywały różnorodny zapach od przyjemnych zapachów świeżo skoszonej trawy, ziół, bulionu warzywnego do nieprzyjemnych nut opisujących zapach jako: ostry, drażniący, przypominający siarkę lub zapach potu [83].

Ważną klasą lotnych związków siarki stanowią siarczki i polisiarczki, chociaż ich obecność jest związana zazwyczaj z nieprzyjemnym i nieakceptowanym zapachem. Siarczki odgrywają jednak kluczową rolę w tworzeniu aromatu żywności, co potwierdza fakt, że wiele z nich jest stosowanych jako dodatki do żywności wchodzących w skład kompozycji smakowo-zapachowych [84]. Czasami obecność siarczków w żywności jest niespodziewana i nieakceptowalna (np. w wodzie pitnej, napojach alkoholowych, sokach), związana z niepożądanymi cechami sensorycznymi w danym produkcie [85–87]. Warzywa zawierające wysokie stężenia siarczków organicznych charakteryzują się silnymi właściwościami przeciwutleniającymi oraz wykazują funkcje antybakteryjne. W ujęciu sensorycznym siarczki mają do siebie podobny, ostry drażniący zapach i ostry smak [64, 88].

Spośród lotnych produktów rozpadu glukozynolanów tiole stanowią ważną grupę w ujęciu sensorycznym, ponieważ stanowią kluczowe związki zapachowe odpowiedzialne np.

za aromat surowego brokuła. Dodatkowo tiole mogą być utlenione do odpowiednich siarczków [57]. Zarówno w wyniku enzymatycznego i nieenzymatycznego procesu powstawania związków lotnych zawierających w swojej strukturze atom siarki można również znaleźć w truflach i grzybach shiitake. Powstające heterocykliczne, lotne związki posiadają charakterystyczny zapach siarki [89]. Lotnym związkiem siarki, który również zasługuje na uwagę ze względu na powszechne występowanie w żywności jest metional, którego biosynteza rozpoczyna się od aminokwasu – metioniny. Metional charakteryzuje się niskim progiem wyczuwalności sensorycznej oraz przyjemnym zapachem, który przypomina gotowane ziemniaki [64].

Procesy przetwarzania żywności stanowią ważny etap w produkcji żywności, ponieważ bezpośrednio wpływają na zachowanie jakości, przedłużenie trwałości lub ograniczenie masy w celu redukcji kosztów transportu [90]. W pracy przeglądowej wyróżniono i opisano szereg procesów przetwarzania warzyw i grzybów (suszenie, blanszowanie, gotowanie, gotowanie na parze, smażenie, mrożenie, fermentacja) oraz ich wpływ na profil związków lotnych siarki. Procesy przetwórcze żywności (w szczególności wykorzystujących obróbkę termiczną lub fermentację) wiążą się ze zmianą parametrów sensorycznych żywności wraz z wytworzeniem wielu związków lotnych zawierających atom siarki w swojej budowie. Wysoka temperatura stanowi ważny czynnik, który nie tylko powoduje wytworzenie związków lotnych zawierających siarkę, ale również decyduje o stabilności tych związków. Labilność termiczna wielu związków siarki przyczynia się do dynamiki zmiany wzajemnych proporcji związków lotnych i w konsekwencji przyczynia się do zmiany finalnego aromatu końcowego produktu [35].

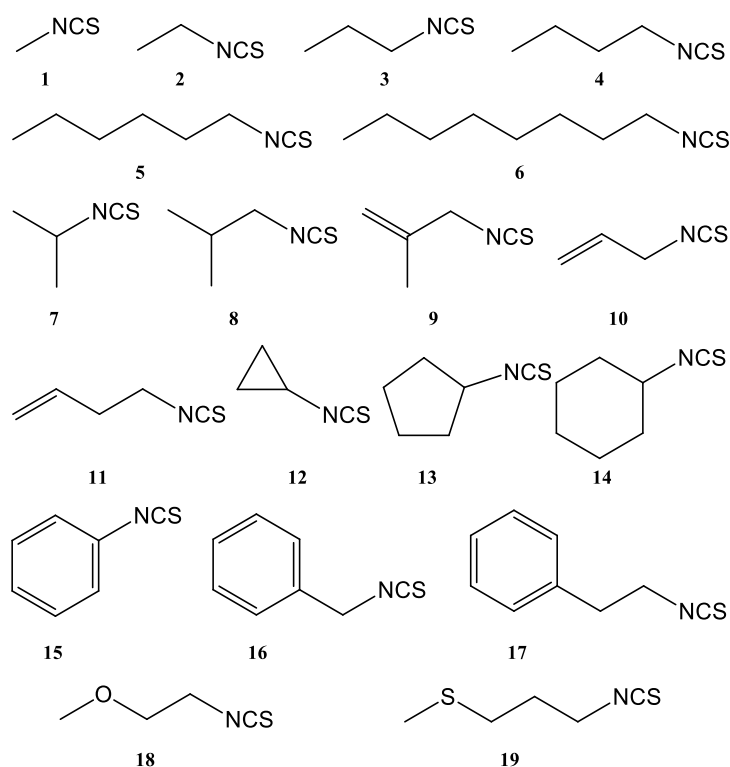
Ze względu na niskie progi wyczuwalności sensorycznej związki siarki stanowią często kluczowe związki odpowiedzialne za charakterystyczny zapach żywności. Analiza ilościowa związków siarki w warzywach lub grzybach stanowi duże wyzwanie analityczne z uwagi na dynamiczne zmiany podczas wytwarzania tych związków w żywych tkankach oraz degradację związków siarki w podwyższonej temperaturze. Dodatkowo związki siarki są termolabilne, dlatego istnieje możliwość tworzenia artefaktów w wysokiej temperaturze panującej w porcie nastrzykowym GC. Wiele związków lotnych siarki tworzonych jest podczas procesów przetwórczych żywności w których wykorzystuje się podwyższoną temperaturę [91]. Podsumowanie wpływu obróbki termicznej na degradację związków zawierających siarkę występujących w warzywach i grzybach zestawiono na rys. 10.



Zbadanie cech sensorycznych wybranych izotiocyjanianów [Publikacja 2]

Spożywanie warzyw kapustowatych niesie za sobą wiele ważnych korzyści prozdrowotnych, które w dużej mierze zawdzięczamy glukozynolanom i produktom ich przemian [99], jednakże produkty degradacji glukozynolanów stanowią wciąż niedostatecznie zbadaną grupę związków w ujęciu sensorycznym. Według literatury izotiocyjaniany charakteryzują się ostrym, drażniącym zapachem [100], który może odgrywać kluczową rolę w kreowaniu aromatu tych warzyw. W celu potwierdzenia tej hipotezy należy przeprowadzić szereg analiz sensorycznych, które pozwolą wyznaczyć stężenie progowe zapachu (OTC) oraz jakość zapachu.

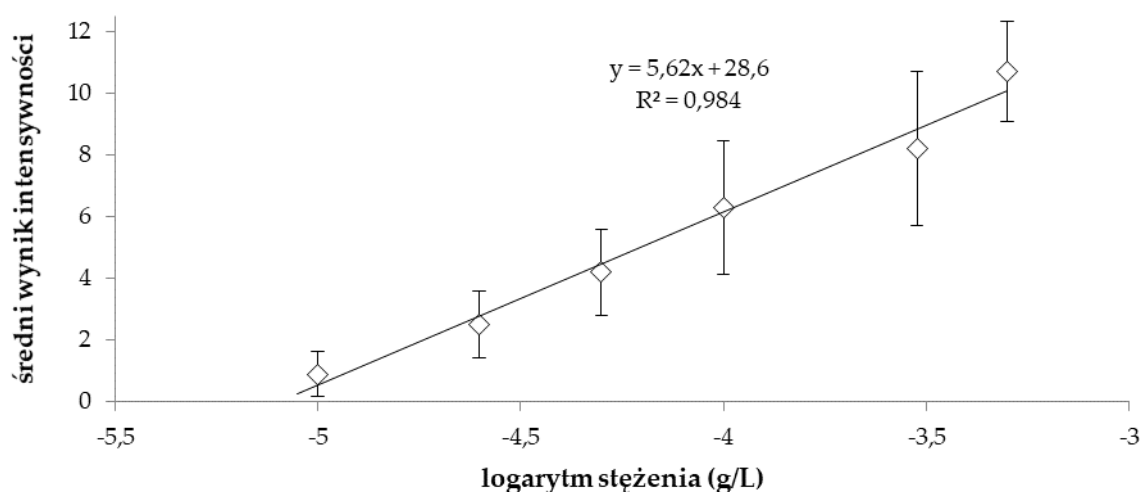
Przegląd literatury dotyczący progów wyczuwalności sensorycznej związków powstających podczas rozpadu glukozynolanów dowiódł, że dane pomiędzy poszczególnymi pracami różnią się od siebie, podobnie jak metody których użyto do ich wyznaczenia. Obecnie nie istnieją sposoby pomiaru progów wyczuwalności sensorycznej, które nie angażują udziału człowieka, ponieważ nadal ludzki nos stanowi najczulszy detektor wrażliwy na niezwykle małe stężenia (ng/L) [66]. Celem pracy było uzupełnienie biblioteki danych sensorycznych (deskryptora zapachu, progu wyczuwalności sensorycznej w wodzie) w odniesieniu do dziewiętnastu izotiocyjanianów metodą analizy profilu smakowo-zapachowego (FPA) [101]. Wzory chemiczne badanych izotiocyjanianów przedstawiono poniżej.



Rys. 11 Wzory chemiczne izotiocyjanianów wykorzystanych w badaniach

Zgoda Komisji Bioetycznej przy Uniwersytecie Medycznym im. Karola Marcinkowskiego w Poznaniu uzyskana 6 września 2018 roku zapoczątkowała tworzenie i trening panelu sensorycznego, w skład którego wchodziłi głównie pracownicy, doktoranci i studenci Wydziału Nauk o Żywności i Żywieniu Uniwersytetu Przyrodniczego w Poznaniu. Ich zadaniem była ocena jakości zapachu (lub jego braku) oraz jego intensywności (w skali od zera do dwunastu punktów) w sekwencji wodnych roztworów izotiocyjanianów ułożonych zgodnie ze wzrastającym stężeniem. W sekwencjach znajdowały się również próby bez dodatku izotiocyjanianów.

Następnie, osobno dla każdego z dziewiętnastu badanych izotiocyjanianów zarejestrowane wartości intensywności dla danej sekwencji próbek zostały uśrednione, natomiast odpowiednie deskryptory policzone. Z uzyskanych danych dla każdego izotiocyjanianu wyznaczono krzywą Webera-Fechnera (wykres zależności średniej intensywności zapachu od logarytmu stężenia). Jako przykład poniżej przedstawiono wykres dla izotiocyjanianu 3-butenylu (rys. 12).



Rys. 12 Wykres Webera-Fechnera do wyznaczenia progu wyczuwalności dla izotiocyjanianu 3-butenylu (n = 10)

Na podstawie liniowej zależności wyznaczano równanie Webera-Fechnera, na podstawie którego obliczono próg wyczuwalności sensorycznej w roztworze wodnym (temperatura pomiarów 45°C) dla każdego z badanych związków chemicznych. Izotiocyjaniany zostały podzielone na kategorie pod względem ich budowy chemicznej. Wyróżniono izotiocyjaniany n-alifatyczne, rozgałęzione, alifatyczne nienasycone, cykliczne, aromatyczne oraz inne (w której znalazły się izotiocyjaniany, które nie pasowały do grup wcześniej wymienionych). W tabeli zestawiono również deskryptory zapachu, które najczęściej wymieniały osoby

oceniające próby wodnych roztworów, które zostały uporządkowane zgodnie ze stężeniami izotiocyjanianów w analizowanych próbach (tabela 1.).

Tab. 1 Progi wyczuwalności sensorycznej izotiocyjanianów w wodzie w temperaturze 45°C

L.p. ^a	Klasa izotiocyjanianu ^b	Nazwa	Stężenie progowe zapachu [g/L]	Równanie Webera-Fechnera $I = a \cdot \log c + b$			Jakość zapachu, najczęstsze deskryptory ^c
				a	b	R ²	
1	A	izotiocyjanian metylu	$0,04 \times 10^{-3} \pm 0,21 \times 10^{-3}$	4,8	22	0,992	kapusta, gotowana brukselka, brokuł, siarkowódór
2	A	izotiocyjanian etylu	$0,2 \times 10^{-3} \pm 0,6 \times 10^{-3}$	7,0	27	0,973	czosnek, kapusta, brokuł, siarkowódór
3	A	izotiocyjanian propylu	$0,03 \times 10^{-3} \pm 0,40 \times 10^{-3}$	5,0	23	0,990	rzeżucha, siarka, czosnek, chrzan
4	A	izotiocyjanian butylu	$0,01 \times 10^{-3} \pm 0,05 \times 10^{-3}$	7,2	37	0,985	kapusta, suszony czosnek, kiszony ogórek
5	A	izotiocyjanian heksylu	$0,007 \times 10^{-3} \pm 0,052 \times 10^{-3}$	4,3	23	0,974	grzyby, kwiaty, rzepa, kalarepa
6	A	izotiocyjanian oktylu	$0,005 \times 10^{-3} \pm 0,030 \times 10^{-3}$	3,6	21	0,946	słodki, kwiatowy, owocowy, kolendra
7	B	izotiocyjanian izopropylu	$0,02 \times 10^{-3} \pm 0,08 \times 10^{-3}$	5,6	27	0,979	bulion, kapusta, czosnek, chemiczny
8	B	izotiocyjanian izobutylu	$0,009 \times 10^{-3} \pm 0,085 \times 10^{-3}$	5,1	27	0,984	musztarda, czosnek, siarka, chemiczny, farba olejna
9	B&AU	izotiocyjanian metallilu	$0,03 \times 10^{-3} \pm 0,16 \times 10^{-3}$	6,3	30	0,987	siarka, czosnek, rzeżucha, ostry i drażniący
10	AU	izotiocyjanian allilu	$0,09 \times 10^{-3} \pm 0,22 \times 10^{-3}$	9,1	38	0,980	siarka, czosnek, chrzan
11	AU	izotiocyjanian 3-butenylu	$0,01 \times 10^{-3} \pm 0,07 \times 10^{-3}$	5,6	29	0,984	siarka, czosnek, chrzan
12	C	izotiocyjanian cyklopropylu	$0,01 \times 10^{-3} \pm 0,08 \times 10^{-3}$	5,2	26	0,983	bulion, szczypiorek, por, czosnek
13	C	izotiocyjanian cyklopentylu	$0,02 \times 10^{-3} \pm 0,10 \times 10^{-3}$	5,1	25	0,980	czosnek, farba olejna, metaliczny, kalarepa, czarna rzepa
14	C	izotiocyjanian cykloheksylu	$0,03 \times 10^{-3} \pm 0,19 \times 10^{-3}$	6,0	29	0,980	słodki, tworzywo sztuczne, mięta, farba olejna, anyż
15	AR	izotiocyjanian fenylu	$0,05 \times 10^{-3} \pm 0,22 \times 10^{-3}$	7,4	33	0,982	siarka, tworzywo sztuczne, zioła, koper włoski
16	AR	izotiocyjanian benzylu	$0,06 \times 10^{-3} \pm 0,22 \times 10^{-3}$	7,8	34	0,965	czosnek, rzeżucha, por, kielki brokuła
17	AR	izotiocyjanian 2-fenylloetyl	$0,01 \times 10^{-3} \pm 0,05 \times 10^{-3}$	6,3	32	0,992	siarka, szczypiorek, rzeżucha
18	M	izotiocyjanian 2-metoksyetyl	$0,07 \times 10^{-3} \pm 0,32 \times 10^{-3}$	5,3	23	0,978	siarka, czosnek, siarkowódór, ostry i drażniący
19	M	izotiocyjanian 3-(metylio)propylu	$0,006 \times 10^{-3} \pm 0,058 \times 10^{-3}$	4,3	23	0,996	siarka, tworzywo sztuczne, ryba, bulion, chrzan

^a – numery odnoszą się do wzorów izotiocyjanianów przedstawionych na rys. 11. ^b – A – n-alifatyczny; B – rozgałęziony; AU – alifatyczny nienasycony; C – cykliczny; AR – aromatyczny; M – inny. ^c – odczuwana jakość zapachu – deskryptory zostały uporządkowane zgodnie ze wzrostem stężenia izotiocyjanianu.

Dane uzyskane podczas przeprowadzonych analiz sensorycznych zostały również opracowane pod kątem częstości występowania konkretnych deskryptorów zapachu w odniesieniu do wszystkich badanych izotiocyjanianów. Osoby oceniające jakość zapachu wodnych roztworów izotiocyjanianów najczęściej określały je jako przypominający: czosnek, siarkę, siarkowodór, kapustę lub rzeżuchę, co potwierdza że izotiocyjaniany mogą być odpowiedzialne za ostry zapach warzyw. Co ciekawe, wraz ze zmianą stężenia ten sam związek chemiczny może pachnieć nieco inaczej, co jest niezwykle istotne podczas innych analiz angażujących zmysł węchu takich jak np. GC-O. Poniżej przedstawiono wykres, który odzwierciedla częstość użycia danego deskryptora zapachu – pole powierzchni jest proporcjonalne do częstości użycia (rys. 13).



Rys. 13 Noty zapachowe związane z percepcją izotiocyjanianów, pole powierzchni odzwierciedla częstość odczuwanego zapachu

Dodatkowo dla dziewiętnastu izotiocyjanianów wykonano pomiary współczynników podziału, które będą mogły zostać wykorzystane np. w badaniach chemosensorycznych. W celu zbadania zachowania izotiocyjanianów w układzie faz gaz/ciecz wyznaczono współczynniki podziału dla różnych matryc (woda, bufor ślinowy, sztuczna ślina, ludzka ślina), przy czym fazę gazową stanowiło powietrze. Zebrane dane są przydatne do oceny, czy izotiocyjaniany jako produkty degradacji glukozynolanów mają większe powinowactwo do matrycy śliny, czy podczas przeżuwania w jamie ustnej – w większym stopniu będą dążyły do przedostawania się do fazy gazowej.

Tab. 2 Współczynniki podziału gaz/ciecz (K) określone dla izotiocyjanianów w różnych matrycach (woda, bufor ślinowy, sztuczna ślina ślina ludzka)

L.p. ^a	Nazwa	R _t [min.] ^b	K _{woda}	K _{bufor ślinowy}	K _{sztuczna ślina}	K _{ślina ludzka}	wartość p ^c
1	izotiocyjanian metylu	6,792	0,08 ± 0,03	0,108 ± 0,007	0,18 ± 0,09	0,17 ± 0,02	0,02
2	izotiocyjanian etylu	6,890	0,13 ± 0,04	0,17 ± 0,01	0,3 ± 0,1	0,38 ± 0,04	0,0005
3	izotiocyjanian propylu	7,448	0,18 ± 0,05	0,24 ± 0,01	0,3 ± 0,1	0,65 ± 0,09	0,00003
4	izotiocyjanian butylu	8,422	0,24 ± 0,07	0,32 ± 0,02	0,4 ± 0,2	0,9 ± 0,2	0,00001
5	izotiocyjanian heksylu	10,382	0,36 ± 0,09	0,46 ± 0,04	0,5 ± 0,2	3,0 ± 0,6	0,0000001
6	izotiocyjanian oktylu	12,018	0,45 ± 0,06	0,36 ± 0,03	0,2 ± 0,1	4,0 ± 0,7	0,000000007
7	izotiocyjanian izopropylu	6,419	0,20 ± 0,06	0,27 ± 0,01	0,4 ± 0,2	0,9 ± 0,1	0,000002
8	izotiocyjanian izobutylu	7,661	0,25 ± 0,07	0,33 ± 0,01	0,4 ± 0,2	1,1 ± 0,2	0,000002
9	izotiocyjanian metallilu	8,622	0,17 ± 0,06	0,23 ± 0,02	0,4 ± 0,2	0,12 ± 0,03	0,02
10	izotiocyjanian allilu	8,072	0,13 ± 0,04	0,17 ± 0,01	0,3 ± 0,1	0,06 ± 0,01	0,003
11	izotiocyjanian 3-butenylu	9,109	0,18 ± 0,05	0,25 ± 0,02	0,4 ± 0,2	0,41 ± 0,06	0,01
12	izotiocyjanian cyklopropylu	8,371	0,14 ± 0,04	0,19 ± 0,01	0,3 ± 0,1	0,02 ± 0,02	0,007
13	izotiocyjanian cyklopropylu	10,195	0,23 ± 0,06	0,31 ± 0,02	0,4 ± 0,2	1,2 ± 0,2	0,00004
14	izotiocyjanian cykloheksylu	11,239	0,28 ± 0,06	0,36 ± 0,03	0,5 ± 0,2	2,0 ± 0,4	0,0000003
15	izotiocyjanian fenylu	11,696	0,23 ± 0,05	0,30 ± 0,04	0,4 ± 0,2	0,1 ± 0,09	0,01
16	izotiocyjanian benzylu	14,021	0,19 ± 0,03	0,24 ± 0,03	0,4 ± 0,2	0,04 ± 0,04	0,001
17	izotiocyjanian 2-fenylloetylu	14,911	0,18 ± 0,03	0,21 ± 0,05	0,4 ± 0,1	0,43 ± 0,07	0,002
18	izotiocyjanian 2-metoksyetylu	10,302	0,08 ± 0,02	0,11 ± 0,01	0,2 ± 0,1	0,13 ± 0,06	0,02
19	izotiocyjanian 3-(metyltio)propylu	13,274	0,12 ± 0,02	0,15 ± 0,02	0,3 ± 0,1	0,20 ± 0,02	0,02

^a – numery odnoszą się do wzorów izotiocyjanianów przedstawionych na rys. 11 oraz tab. 1. ^b – R_t – czas retencji.

^c – wartość p – porównanie śliny ludzkiej z pozostałymi matrycami.

Niskie wartości progów detekcji rzędu $\mu\text{g/L}$ uzyskane w roztworze wodnym potwierdzają, że lotne i aktywne zapachowo produkty hydrolizy glukozynolanów mogą mieć znaczący wpływ na tworzenie aromatu warzyw kapustowatych. FPA oraz wykresy Webera-Fechnera wykazały tendencję, że wraz ze wzrostem łańcucha nienasyconego izotiocyjanianu n-alifatycznego jego OTC maleje (poza izotiocyjanianem etylu). Najwyższe wartości współczynników podziału wyznaczono dla izotiocyjanianu oktylu ($4,0 \pm 0,7$) i izotiocyjanianu heksylu ($3,0 \pm 0,6$) co sugeruje, że te związki najmniej oddziałują z fazą ciekłą. Najniższe wartości uzyskano natomiast dla izotiocyjanianu cyklopropylu ($0,02 \pm 0,02$) i izotiocyjanianu benzylu ($0,04 \pm 0,04$), co może wskazywać na występujące oddziaływania pomiędzy nimi a białkami obecnymi w ślinie ludzkiej.

Współczynniki podziału gaz/ślina ludzka różnią się od wyników uzyskiwanych w pozostałych badanych matrycach, co wykazano dla izotiocyjanianów po raz pierwszy. Ma to szczególne znaczenie dla percepcji tych związków zapachowych. Dostępne dane, na podstawie których wnioskujemy o uwalnianiu związku do fazy nadpowierzchniowej dotyczą prostych matryc, najczęściej wody. Percepcja aromatu ortonazalna wiąże się z wężaniem produktu i związki zapachowe docierają do nabłonka węchowego od strony nosa. Sytuacja zmienia się diametralnie w sytuacji spożywania produktu (percepcja retronazalna), gdy związki zapachowe danego produktu wchodzi w interakcję ze śliną, z niej są uwalniane do fazy nadpowierzchniowej i docierają od strony jamy gębowej do nabłonka węchowego. Pomimo tego, że ślina w 99% składa się ona z wody, to zachowanie związków lotnych w tej matrycy znacznie różni się od zachowania w wodzie, co związane jest najprawdopodobniej z interakcjami badanych związków z białkami występującymi w ślinie. Będzie to skutkowało także zróżnicowanymi progami wyczuwalności sensorycznej określonej w sposób orto i retronazalny. Z uwagi na konieczność rozprowadzenia roztworów izotiocyjanianów w jamie ustnej członków panelu sensorycznego (często o nieznanej toksyczności) nie określano retronazalnych progów wyczuwalności sensorycznej dla tych związków.

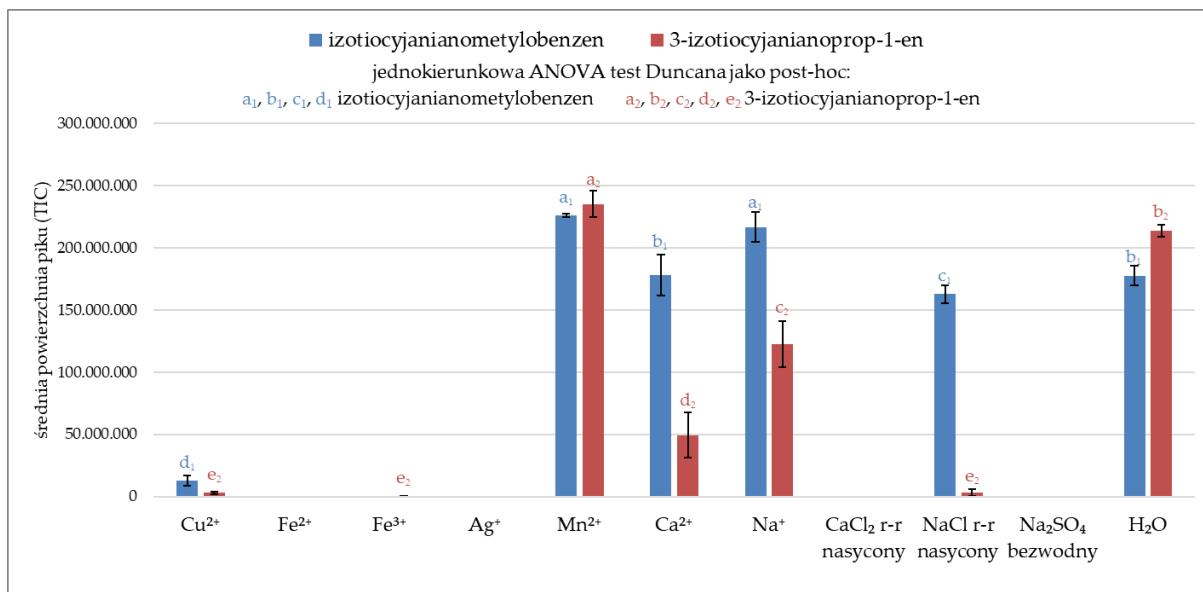
Blokowanie natywnego enzymu poprzez zastosowanie soli metali ciężkich [Publikacja 3]

W ujęciu chemii analitycznej warzywa stanowią złożoną matrycę głównie ze względu na zmienną zawartość związków chemicznych, które niekiedy są również specyficzne dla danego gatunku, odmiany, warunków środowiskowych, części rośliny, sposobu uprawy, regionu uprawy, stadium wzrostu, pory roku, przechowywania i sposobu przetwarzania [102]. Dodatkowo obecność natywnych enzymów w tkankach roślin przyczynia się do uzyskiwania wysokich odchyleń standardowych między poszczególnymi pomiarami zarejestrowanymi nawet dla tego samego warzywa, z uwagi na dynamiczne zmiany zachodzące w próbie wynikające z trudności hamowania procesów enzymatycznych na etapie przygotowania prób. W konsekwencji prowadzi to do trudności w analizie i opracowaniu uzyskanych wyników.

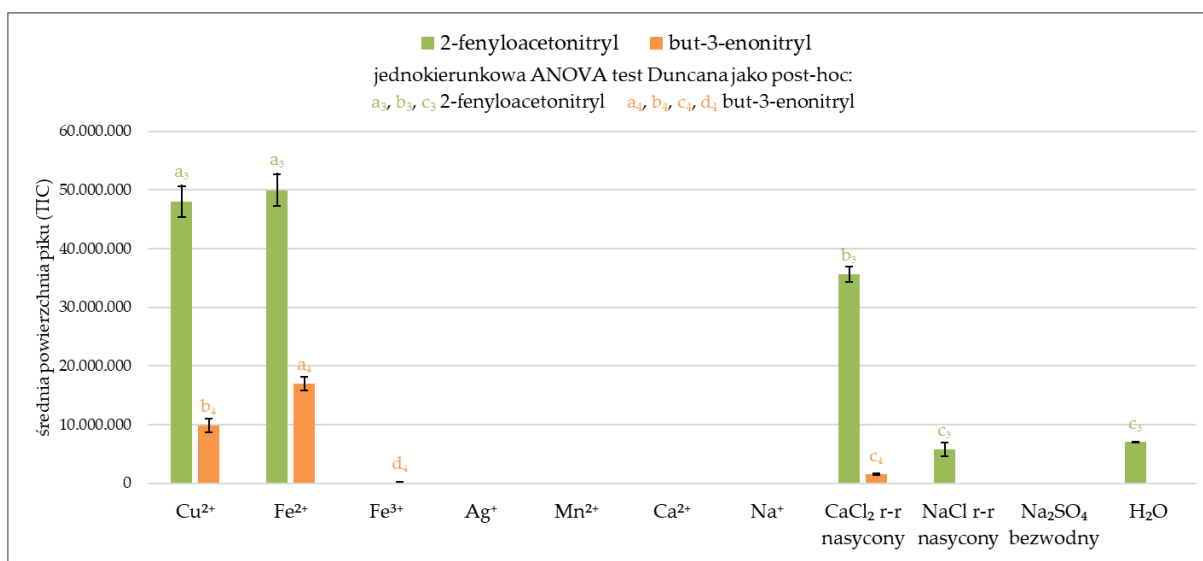
W okresie pracy nad publikacją istniało jedynie kilka prac, które skupiały swoją uwagę na metodach blokowania aktywności enzymatycznej mirozynazy (zmiana odczynu środowiska, metoda termiczna, zastosowanie ciśnienia lub połączenie tych technik). Zastosowanie procesów z wykorzystaniem podwyższonej temperatury (np. gotowanie w kuchence mikrofalowej, gotowanie na parze, gotowanie w wodzie) prowadziło do termicznej denaturacji enzymu [103], jednakże jak wspomniano wcześniej, nie było korzystne mając na uwadze kolejny etap badań, tj. analizę związków lotnych i aktywnych zapachowo (wykluczenie możliwości analizy związków termolabilnych). Zastosowanie ciśnienia wymagało użycia specjalnej aparatury [104], która nie była dostępna w laboratorium. Metody oparte na stosowaniu niskiej temperatury (ciekły azot) nie są wystarczające do hamowania aktywności mirozynazy [105]. Ze względu na białkową budowę enzymu, postanowiono sprawdzić inne czynniki powodujące jego denaturację – zastosowanie soli bezwodnych w celu usunięcia środowiska wodnego lub soli metali ciężkich w celu denaturacji enzymu. W konsekwencji celem omawianej pracy było zbadanie wpływu nieorganicznych soli metali na blokowanie aktywności mirozynazy (natywnej oraz dodawanej sztucznie do wody lub tkanki warzywa) poprzez badanie jakościowe i ilościowe związków lotnych powstałych podczas rozpadu glukozynolanów.

Wstępne badania polegały na zastosowaniu układu modelowego, w którym do jednakowej ilości i objętości wodnego roztworu enzymu dodawano badaną sól lub jej wodny roztwór i kolejno w ustalonym czasie dodawano określoną ilość wodnego roztworu glukozynolanu (synigryna i glukotropeolina). Reakcje prowadzono w ściśle określonym czasie, w szklanych, szczelnie zamkniętych fiolkach do analizy GC. Następnie frakcję gazową próby analizowano przy użyciu GC-MS. Wyniki zestawiono w postaci dwóch wykresów

reprezentujących główne lotne produkty rozpadu glukozynolanów takie jak izotiocyjaniany (rys. 14a) oraz nityle (rys. 14b).



(a)



(b)

Rys. 14. Lotne produkty rozpadu glukozynolanów w potencjalnych czynnikach blokujących aktywność enzymu; r-r – roztwór; a, b, c, d, e – wynik analizy statystycznej (jednokierunkowa ANOVA test Duncana jako post-hoc, wartości oznaczone tą samą literą w grupie Duncana, nie różnią się istotnie, numer indeksu dolnego i kolor liter odpowiadają legendzie wykresu ($\alpha = 0,05$); ($n = 3$); (a) Izotiocyjaniany (izotiocyjanianometylobenzen pochodzący z glukotropeoliny i 3-izotiocyjaniianoprop-1-en produkt rozpadu synigryny); (b) Nityle (2-feniloacetonitryl z glukotropeoliny i but-3-enonitryl z sinigryny)

Badania wstępne na układzie modelowym dostarczyły informacji, że zastosowanie soli z jonami żelaza(II), żelaza(III), srebra(I), nasyconego roztworu chlorku wapnia oraz bezwodnego siarczanu(VI) sodu powodują skuteczne zablokowanie reakcji enzymatycznej

w kierunku tworzenia izotiocyjanianów. Jednakże użycie soli z jonami żelaza(II) czy nasyconego roztworu chlorku wapnia (powszechnie stosowanego w analizie związków lotnych i zapachowych w zróżnicowanych matrycach) powodowały degradację glukozynolanów w kierunku nitryli. Zwiększoną ilość nitryli zaobserwowano również podczas użycia soli z jonami miedzi(II) i nasyconego roztworu chlorku wapnia, co nie było wcześniej zbadane i opisane w literaturze naukowej. Dodatkowo przeprowadzono eksperyment modelowy, w którym zaobserwowano, że w przypadku jonów żelaza(II), miedzi(II) oraz nasyconego roztworu chlorku wapnia mirozynaza nie musi być obecna w układzie, żeby zaobserwować powstawanie nitryli w środowisku wodnym.

Tkanka roślinna stanowi złożoną matrycę, której skład chemiczny determinuje degradację glukozynolanów do izotiocyjanianów [106]. Kolejny etap badań polegał na sprawdzeniu skuteczności w blokowaniu natywnego enzymu występującego w kalarepie poprzez detekcję i identyfikację związków lotnych za pomocą GC-MS. W tym celu do określonej ilości próby zblendowanej kalarepy dodano czynnik blokujący, a następnie określoną objętość roztworu glukozynolanu, który naturalnie nie występował w warzywie. Każde powtórzenie i etap eksperymentu wykonano w ściśle określonym czasie. Zielona kalarepa zawiera natywne glukozynolany: glukoerucynę, glukoiberwerynę oraz glukorafaninę [107]. Mogą się one przekształcić w odpowiednie lotne produkty degradacji – izotiocyjaniany oraz nitryle (kolejno 4-metylosulfanylobutanonitryl, 1-izotiocyjaniano-3-metylosulfanylopropan, 1-izotiocyjaniano-4-metylosulfanylbutan). Sztucznie dodane glukozynolany do próby kalarepy to synigryna i glukotropeolina, natomiast w zależności od potencjalnego czynnika blokującego reakcję enzymatyczną produktami mogły być izotiocyjaniany (odpowiednio 3-izotiocyjanianoprop-1-en i izotiocyjanianometylobenzen) i nitryle (but-3-enonitryl i 2-fenylacetynitryl). W badaniach uwzględniono również wpływ czynnika blokującego na lipooksygenazę, która jest enzymem katalizującym utlenianie wielonienasyconych kwasów tłuszczowych (wyniki zestawiono w publikacji).

Glukozynolany są podatne na hydrolizę enzymatyczną, która zależy od środowiska prowadzenia reakcji np.: odczynu pH, dostępności jonów żelaza(II), obecności specyficznych białek [74]. Warunki panujące wewnątrz tkanek warzyw kapustowatych faworyzują powstawanie izotiocyjanianów. Spośród szeregu eksperymentów modelowych prowadzonych początkowo w środowisku wodnym i kolejno w tkance kalarepy stwierdzono, że najsilniejszym czynnikiem ograniczającym tworzenie izotiocyjanianów były jony: żelaza(II), żelaza(III), srebra(I), roztwór nasyconego roztworu chlorku wapnia oraz zastosowanie bezwodnego

siarczanu(VI) sodu. Niestety użycie soli zawierających jony żelaza(II) lub nasyconego roztworu chlorku wapnia powodowały znaczącą zmianę ścieżki degradacji w kierunku tworzenia nitryli. Natomiast jony żelaza(III) obecne w tkance warzywa przyczyniały się do znaczącego wzrostu związków lotnych pochodzących z utlenienia tłuszczów. W konsekwencji sole żelaza(III) zostały wykluczone z dalszych rozważań. Z kolei jony srebra(I) są toksyczne dla środowiska, powodowały ciemnienie tkanki warzywa i stwarzały trudności związane z utylizacją próbek pozostałych po analizie. W związku z tym za optymalny czynnik blokujący uznano bezwodny siarczan(VI) sodu, który finalnie zastosowano w dalszych badaniach związków lotnych odpowiedzialnych za aromat kalarepy.

Kluczowe związki aktywne zapachowo odpowiedzialne za aromat surowej i gotowanej kalarepy (*Brassica oleracea* var. *gongylodes* L.) [Publikacja 4]

Zielona kalarepa to dwuletnie warzywo należące do rodziny warzyw *Brassicaceae* i początkowo uprawiana była w północno-zachodniej Europie, rozprzestrzeniając się dalej na całą Europę i świat. Bulwa kalarepy jest wykorzystywana w sałatkach i zupach, ale może być również spożywana w wersji surowej, pieczonej lub smażonej. Jej liście i łodygi są również jadalne i mogą zostać ugotowane, podobnie jak kapusta zielona. Kalarepa zawiera wyspecjalizowane metabolity takie jak: glukozynolany, antocyjany, karotenoidy i fenylopropanoidy. Dodatkowo zawiera także duże ilości węglowodanów, aminokwasów, kwasów organicznych, witamin i minerałów [108, 109].

Glukozynolany stanowią ważną grupę metabolitów, ponieważ pod wpływem natywnego enzymu mirozynazy mogą zostać przekształcone w związki charakteryzujące się aktywnością sensoryczną takie jak: izotiocyjaniiny, nityle, siarczki. Mając na uwadze zapach, izotiocyjaniiny najczęściej charakteryzowane są jako zapachy nieprzyjemne, ostre, drażniące. Jednakże niektóre z nich wykazują przyjemniejsze noty zapachowe takie jak zapach: grzybów, cebuli, zielonych warzyw lub rosołu [82, 110].

W literaturze naukowej istnieją prace gdzie zidentyfikowano i oznaczono ilościowo związki lotne występujące w kalarepie, jednakże brak w nich podejścia sensorycznego, które tłumaczy jakie związki są odpowiedzialne za aromat kalarepy. Ponadto należy pamiętać, że nie wszystkie związki lotne są aktywne zapachowo, natomiast niektóre z nich mają niskie progi wyczuwalności sensorycznej (np. związki zawierające siarkę czy pirazyny) ale występują w warzywie w ilościach śladowych, co utrudnia ich oznaczenie. Poniżej przedstawiono najważniejsze wyniki uzyskane podczas poszczególnych etapów mających na celu wyznaczenie kluczowych związków sensorycznie aktywnych odpowiedzialnych za aromat w dwóch wariantach kalarepy surowej i gotowanej.

Związki lotne z kalarepy wyizolowano w wyniku homogenizacji próby surowej i gotowanej z dodatkiem bezwodnej soli siarczanu(VI) sodu oraz rozpuszczalnika organicznego (dichlorometanu). Następnie wykorzystano metodę SAFE w celu oddzielenia związków lotnych od nielotnych oraz zagęszczenie ekstraktu do finalnej objętości 1 cm³. Podczas izolacji związków lotnych jakość zapachu prób była na bieżąco kontrolowana sensorycznie za pomocą pasków bibuły. Po odparowaniu rozpuszczalnika zarówno dla ekstraktów jak i destylatów zapach był charakterystyczny dla surowej i gotowanej kalarepy.

Tab. 3. Związki aktywne zapachowo zidentyfikowane spośród związków lotnych wyizolowanych z kalarepy surowej i gotowanej

lp.	nazwa	zapach ^a	RI ^b		współczynnik FD	
			FFAP	DB-5	surowa	gotowana
1	5-(metanosulfinylo)pentanonitryl	rosołu, cebuli, siarki	943	1563	256	256
2	pentan-1-tiol	palony, gumy	1022	850	1	< 1
3	disiarczek dimetylu	kapusty, siarki	1094	803	< 1	8
4	(3Z)-heks-3-enal	zielonej trawy	1141	802	16	< 1
5	but-3-enonitryl	ostry, musztardowy	1191	900	4	32
6	2-pentylofuran	warzywny	1243	1015	256	< 1
7	okt-1-en-3-on	grzybowy	1287	974	64	32
8	2-acetylo-1-pirolina	popcornu	1330	904	256	64
9	trisiarczek dimetylu	siarki, kapusty	1352	963	16	128
10	(3E)-heks-3-en-1-ol	zielonej trawy	1395	814	< 1	1
11	2-izopropyl-3-metoksypirazyna	zielonego groszku, ziemisty	1418	1096	512	128
12	kwasy octowe	octu	1450	631	16	16
13	izotiocyanian 3-butenylo	ostry, czosnku	1453	981	16	8
14	(2E,4E)-hepta-2,4-dienal	tlusty	1487	1158	< 1	1
15	4-(metylosulfanylo)butan-2-on	ziemisty	1514	989	8	< 1
16	oktan-1-ol	cytrusów	1545	981	32	4
17	(1S)-1-fenyletano-1-tiol	spaleniowy	1626	1122	2	< 1
18	2-acetylotiazol	pieczony	1635	1015	32	16
19	disiarczek 2-metylo-3-furylo metylo	mięśny, kapusty	1645	1187	64	4
20	γ-heksalakton	owocowy, słodki	1655	1052	1	64
21	tiofen-2-karbaldehyd	pieczony	1685	981	64	< 1
22	2-fenyletano-1-tiol	grupy	1690	1167	32	< 1
23	3-hydroksy-4-metylo-2H-furan-5-on	warzywny, cebulowy	1695	985	32	64
24	3-metylosulfanylopropan-1-ol	gotowanych ziemniaków	1715	963	4	32
25	4-(metylosulfanylo)butanonitryl	rosołu, czosnku, siarki	1721	1063	128	64
26	kwasy heksanowe	potu	1805	1015	64	< 1
27	5-(metylosulfanylo)pentanonitryl	gotowanego brokuła, kapusty	1845	1193	256	128
28	4-(metylosulfanylo)butan-1-ol	kapusty, czosnku	1858	965	64	< 1
29	12-metylotridekanal	wołowiny	1863	1015	16	< 1
30	p-cymen-8-ol	stęchły, benzyny	1863	1208	< 1	512
31	1,3-benzotiazol	gumy, kapusty	1900	1245	8	16

Tab. 3. Związki aktywne zapachowo zidentyfikowane spośród związków lotnych wyizolowanych z kalarepy surowej i gotowanej c.d.

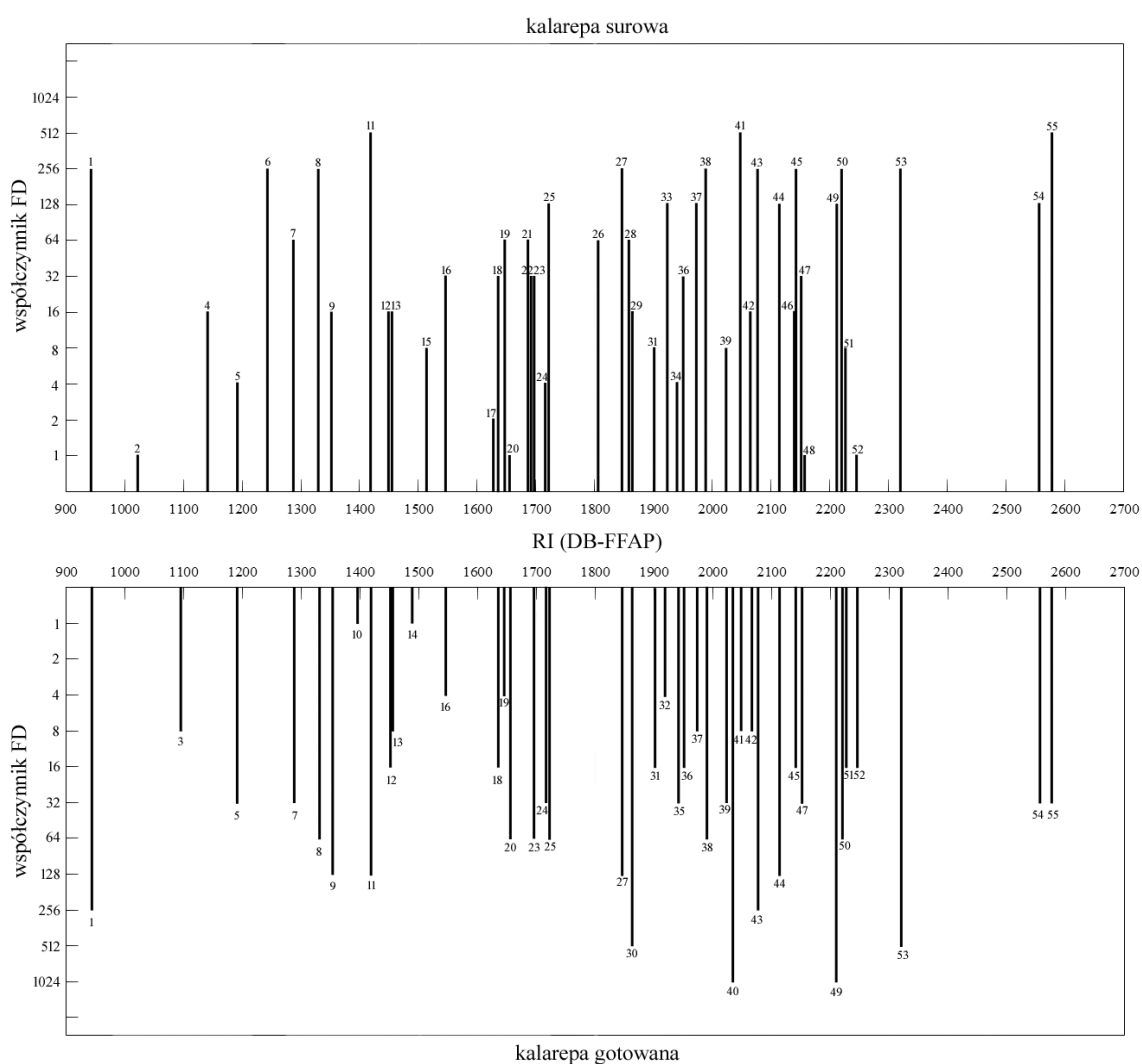
lp.	nazwa	zapach ^a	RI ^b		współczynnik FD	
			FFAP	DB-5	surowa	gotowana
32	sulfon dimetylowy	siarki, tłusty	1918	959	< 1	4
33	fenyloacetonitryl	grzybów	1922	1107	128	< 1
34	kwas heptanowy	potu, jelki	1939	1089	4	< 1
35	2-fenyloetan-1-ol	kwiatów, miodu	1940	1112	< 1	32
36	3-hydroksy-2-metylopiran-4-on (maltol)	karmelu	1950	1245	32	16
37	1-izotiocyaniano-3-(metylosulfanylo)propan	siarki, czosnku, grzybów	1972	1231	128	4
38	trans-4,5-epoksy-(E)-2-dekenal	metaliczny, słodki	1989	1379	256	64
39	γ -nonalakton	słodki, kokosowy	2022	1387	8	32
40	4-metoksybenzaldehyd (aldehydanyżowy)	anyżu	2033	1257	< 1	1024
41	3-fenylopropanonitryl	kwaśny, tłusty	2047	1241	512	8
42	pirolidyn-2-on	mysi, kwaśny	2065	1052	16	8
43	izotiocyanian benzylu	ziołowy, potu, ostry	2076	1363	256	256
44	tiocyanian benzylu	rosołu, maślane, rzeżuchy	2113	1352	128	< 1
45	1-izotiocyaniano-4-(metylosulfanylo)butan	grzybowy, rosołu	2141	1404	256	16
46	bis(2-metylo-3-furylo)disiarczek	mięsny	2138	1540	16	< 1
47	2-metoksy-4-winylofenol	goździków, dymu	2150	1300	32	32
48	2-fenoksyetanol	kwiatowy	2156	1240	1	< 1
49	4-hydroksy-2,3-dimetylo-2H-furan-5-on (sotolon)	lubczyku, przypraw	2210	1107	128	1024
50	2-izotiocyanianoetylobenzen	rosołu, warzywny	2220	1430	256	64
51	etano-1,2-ditiol	czosnku, kapusty	2225	817	8	16
52	nieznany	potu, gumy	2244	1021	1	16
53	4-metylo-5-tiazoleetanol	rosołu, siarki, orzechowy	2320	1269	256	512
54	kwas fenylooctowy	miodowy	2555	1252	128	32
55	4-hydroksy-3-metoksybenzaldehyd (wanilina)	słodki, waniliowy	2576	1395	512	32

^aPotwierdzenie struktury oparto o jakość zapachu, wartości RI (DB-FFAP, DB-5) i widma masowe; dane porównano z danymi uzyskanymi dla związków odniesienia analizowanych w tych samych warunkach. ^bWartości RI obliczono na podstawie czasu retencji związku aktywnego sensorycznie i czasu retencji sąsiednich n-alkanów metodą interpolacji liniowej.

Zastosowanie analizy GC-O oraz AEDA wykazało 55 związków aktywnych zapachowo w kalarepie surowej i gotowanej (tabela 3). Współczynniki FD dla kalarepy surowej mieściły

się w zakresie od 1 do 512, natomiast dla kalarepy gotowanej od 1 do 1024, jednakże dla większości związków aktywnych zapachowo większe wartości FD wyznaczono dla próby z kalarepy surowej. Biorąc pod uwagę wartości FD w próbce otrzymanej z kalarepy surowej dominowały noty takie jak: zapach zielonego groszku, ziemisty, kwaśny, oleisty, słodki, waniliowy, natomiast w kalarepie gotowanej dominującymi były zapachy: skoszonej trawy, anyżu, lubczyku, przypraw, stęchły, benzyny, rosołu, siarki czy orzechów.

Dla wszystkich aktywnych sensorycznie regionów chromatogramu (rys. 15) obliczono wartości RI na kolumnach różniących się polarnością: DB-FFAP (polarna) i DB-5 (niepolarna). W tym celu wykorzystano czasy retencji mieszaniny n-alkanów, uprzednio nastrzykniętej na ten sam system GC-O. Wstępna próba identyfikacji struktury związków aktywnych zapachowo opierała się o przegląd baz danych (the Leibniz-LSB@TUM Odorant Database [64]) i literatury pod kątem RI oraz jakości zapachu.



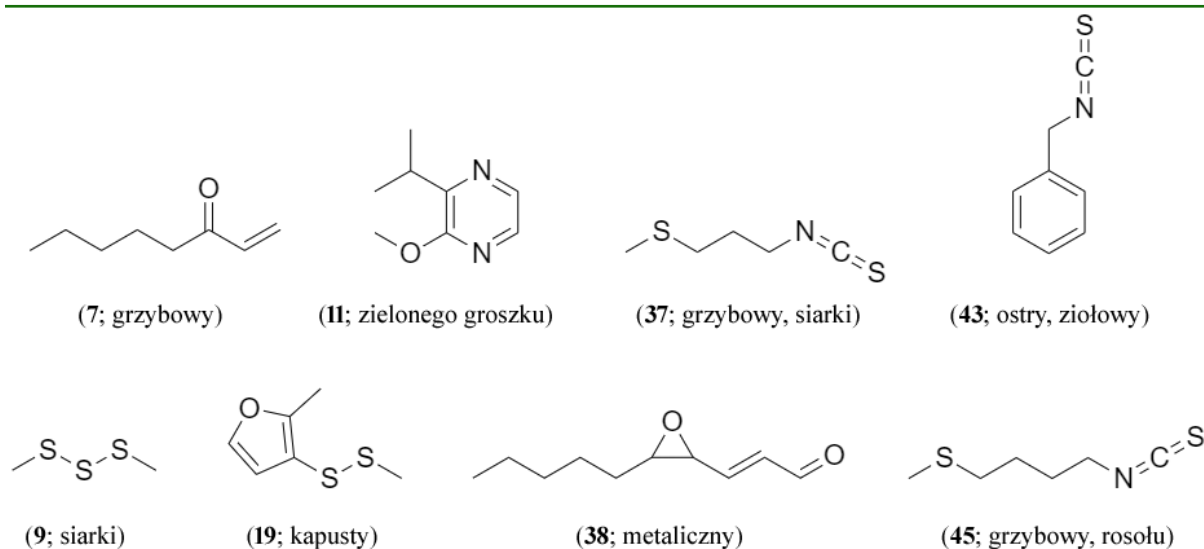
Rys. 15. Aromagramy FD związków aktywnych zapachowo izolowanych z surowej i gotowanej kalarepy z ich współczynnikami FD i wskaźnikami retencji (RI) na kolumnie DB-FFAP, numeracja zgodna z tabelą 3

Tożsame RI oraz jakość zapachu zostały potwierdzone przy użyciu GC-O (kolumny DB-FFAP i DB-5) oraz odpowiednich związków odniesienia, które zostały nastrzyknięte w stężeniu zbliżonym do badanego ekstraktu. Struktura związków aktywnych zapachowo została ostatecznie potwierdzona poprzez analizy chromatograficzne: GC-MS lub GC × GC-MS. Związki aktywne zapachowo występujące w surowej i gotowanej kalarepie wraz z deskryptorem zapachu, wartościami RI obliczonymi na kolumnach różniących się polarnością, a także współczynniki FD zostały przedstawione w tabeli 3.

Mając na uwadze prekursory oraz mechanizm powstawania związki aktywne zapachowo występujące w kalarepie można podzielić na kilka grup, które głównie są związane ze szlakiem metabolicznym lipidów i aminokwasów. Wcześniejsze badania dowiodły, że dominującymi związkami lotnymi w kalarepie są: izotiocyjaniiny, nityle, siarczki oraz nienasycone aldehydy [68]. W literaturze naukowej raportowano również obecność związków lotnych należących do: alkoholi, ketonów zawierających siarkę, tiazoli, pirazyn, i pochodnych furanu [111]. Zastosowanie analiz GC-O umożliwiło zidentyfikowanie związków lotnych aktywnych zapachowo, których obecność w kalarepie nie była wcześniej znana, np.: okt-1-en-3-on (7), 2-izopropyl-3-metoksypirazyne (11), disiarczek 2-metylo-3-furylu metylu (19), kwas heksanowy (26), *trans*-4,5-epoksy-(*E*)-2-dekenal (38) i 3-hydrokso-4,5-dimetylofuran-2(5*H*)-on (sotolon) (49).

AEDA została przeprowadzona w celu ustalenia, które ze związków aktywnych zapachowo mają największy udział w tworzeniu aromatu kalarepy. Wyznaczono 28 związków aktywnych zapachowo, które charakteryzowały się wysokimi współczynnikami FD (≥ 64) i oznaczono je ilościowo wykorzystując nowoczesne techniki analityczne (GC-MS, GC × GC-MS, 2D-GC-HRMS z jonizacją elektronową i chemiczną). Następnie dzieląc wartość stężenia otrzymanego w próbie przez OTC otrzymano OAV dla każdego z badanych związków aktywnych zapachowo, które odzwierciedlają udział poszczególnych analitów w ogólnym aromacie kalarepy.

Związki takie jak: okt-1-en-3-on (7), 2-izopropyl-3-metoksypirazyne (11), 1-izotiocyjaniano-3-(metylosulfanylo)propan (37), izotiocyjanian benzylu (43), trisiarczek dimetylu (9), disiarczek 2-metylo-3-furylu metylu (19), *trans*-4,5-epoksy-(*E*)-2-dekenal (38) i 1-izotiocyjaniano-4-(metylosulfanylo)butan (45) opisane wysokimi OAV mają największy udział w tworzeniu ogólnego aromatu kalarepy zarówno surowej jak i gotowanej, co przedstawiono na poniższym schemacie:



Rys. 16. Związki aktywne zapachowo scharakteryzowane przez najwyższe wartości OAV w kalarepie, w nawiasie podano oznaczenie zgodne z numeracją w tabeli 3 i zapach

Ze względu na niskie OAV nityryle mają niewielki wpływ na aromat kalarepy, pomimo wysokich wartości FD dla tych związków. AEDA została przeprowadzona jako metoda przesiewowa, która umożliwiła wyodrębnienie kluczowych związków odpowiedzialnych za aromat kalarepy. Natomiast izotiocyaniany stanowią główny produkt degradacji glukozynolanów, występują w surowym oraz gotowanym warzywie w wysokich stężeniach i osiągają wysokie OAV. Co więcej, wzrost stężenia izotiocyanianów po ugotowaniu warzywa był już wcześniej opisywany [112]. Trisiarczek dimetylu stanowi ważny produkt termicznej degradacji izotiocyanianów i stanowi jeden z kluczowych związków odpowiedzialnych za aromat kalarepy.

Aromat kalarepy zmienia się po ugotowaniu warzywa. Przede wszystkim wartości OAV dla związków kluczowych (7, 9, 11, 19, 38, 43) oraz dla innych związków aktywnych zapachowo, które mają mniejszy udział w tworzeniu ogólnego aromatu (8, 26, 44, 49) są wyraźnie wyższe dla próby otrzymanej z surowego warzywa. Jest to związane z procesem gotowania, który prowadzi do uwalniania związków chemicznych do wody i utraty związków lotnych. Dodatkowo, jedynie trzy związki aktywne zapachowo, których OAV > 1 (37, 45, 50) wykazywały zbliżony wkład w całościowy aromat kalarepy surowej i gotowanej. Związkiem aktywnym zapachowo, którego OAV wzrosła wskutek gotowania, jest 3-fenylpropanonitryl (41), co może być związane z inaktywacją tioglukozydazy podczas gotowania kalarepy.

Wnioski

Poniżej przedstawiono wnioski, które sformułowano na podstawie dokonanego przeglądu literatury, oraz przeprowadzonych badań, które szczegółowo opisano w publikacjach wchodzących w skład niniejszej rozprawy doktorskiej.

- 1) Przegląd literaturowy pod kątem lotnych i aktywnych zapachowo związków siarki dowiódł, że stanowią one kluczowe związki, odpowiedzialne za charakterystyczny aromat warzyw kapustowatych, cebulowych, a także niektórych grzybów (shiitake i trufli). Liczba badań nad profilem związków aktywnych zapachowo dla warzyw kapustowatych jest bardzo mała. Związki lotne zawierające siarkę powstają głównie podczas procesów enzymatycznych, a ich ograniczona stabilność termiczna, podatność na utlenianie, niskie progi detekcji powodują, że ich analiza jakościowa i ilościowa jest problematyczna.
- 2) Wyznaczone stężenia progowe zapachu dla 19 izotiocyjanianów zawierają się w przedziale od $0,005 \cdot 10^{-3}$ do $0,2 \cdot 10^{-3}$ g/L, co może predysponować je jako potencjalne związki wpływające na aromat warzyw kapustowatych. Wyznaczone współczynniki podziału dla różnych matryc, w tym wody i śliny wykazują bardzo zróżnicowane wartości dla danego związku i będą determinowały ich aktywność sensoryczną w procesie konsumpcji warzyw kapustowatych. Uzyskane wyniki pozwalają na ocenę roli tych związków w percepcji orto- i retronazalnej. Biorąc pod uwagę ślinę ludzką, największe powinowactwo do fazy ciekłej mają: izotiocyjanian cyklopropylu i izotiocyjanian benzylu, natomiast do fazy gazowej w pierwszej kolejności będą przechodziły: izotiocyjanian oktylu i izotiocyjanian heksylu.
- 3) Spośród wybranych nieorganicznych soli metali największą skuteczność inhibicji w matrycy tkanki roślinnej w stosunku do mirozynazy i lipooksygenazy wykazywały: jony srebra(I) i bezwodny siarczan(VI) sodu. Jako optymalny inhibitor wybrano bezwodny siarczan(VI) sodu, uwzględniając zasady zielonej chemii (związane głównie z trudnością utylizacji próbek zawierających jony srebra(I)) oraz zadowalającą skuteczność i łatwość wykorzystania w kolejnym etapie pracy badawczej.
- 4) Wyznaczono 55 związków aktywnych zapachowo w kalarepie w dwóch wariantach: surowej i gotowanej. Zastosowanie połączenia chromatografii gazowej i olfaktometrii (GC-O) umożliwiło zidentyfikowanie 6 związków

lotnych i aktywnych zapachowo, których obecność w kalarepie nie była wcześniej znana. Porównanie OAV umożliwiło wyznaczenie ośmiu kluczowych związków odpowiedzialnych za aromat surowego i gotowanego warzywa. Trzy z tych związków to izotiocyjaniany, a jeden to produkt ich degradacji. W większości obliczone OAV są wyższe dla próby kalarepy surowej i niższe dla kalarepy gotowanej ze względu na utratę związków lotnych podczas gotowania.

Bibliografia

1. López-González, L.; Becerra-Tomás, N.; Babio, N.; Martínez-González, M.Á.; Díaz-López, A.; Corella, D.; Goday, A.; Romaguera, D.; Vioque, J.; Alonso-Gómez, Á.M.; et al. Variety in Fruits and Vegetables, Diet Quality and Lifestyle in an Older Adult Mediterranean Population. *Clin. Nutr.* **2021**, *40*, 1510–1518, doi:<https://doi.org/10.1016/j.clnu.2021.02.024>.
2. Markovič, R.; Žiška, P.; Jaromír, Š. Physical Activity in the Time of Covid-19 Pandemic. *Stud. Univ. Babeş-Bolyai Educ. Artis Gymnast.* **2021**, *66*, 19–28, doi:10.24193/subbeag.66(2).13.
3. Mostafidi, M.; Sanjabi, M.R.; Shirkhan, F.; Zahedi, M.T. A Review of Recent Trends in the Development of the Microbial Safety of Fruits and Vegetables. *Trends Food Sci. Technol.* **2020**, *103*, 321–332, doi:<https://doi.org/10.1016/j.tifs.2020.07.009>.
4. Nahak, G.; Suar, M.; Sahu, R.K. Antioxidant Potential and Nutritional Values of Vegetables: A Review. *Res. J. Med. Plant* **2014**, *8*, 50–81, doi:10.3923/rjmp.2014.50.81.
5. Wallace, T.C.; Bailey, R.L.; Blumberg, J.B.; Burton-Freeman, B.; Chen, C. y. O.; Crowe-White, K.M.; Drewnowski, A.; Hooshmand, S.; Johnson, E.; Lewis, R.; et al. Fruits, Vegetables, and Health: A Comprehensive Narrative, Umbrella Review of the Science and Recommendations for Enhanced Public Policy to Improve Intake. *Crit. Rev. Food Sci. Nutr.* **2020**, *60*, 2174–2211, doi:10.1080/10408398.2019.1632258.
6. Frank, S.M.; Webster, J.; McKenzie, B.; Geldsetzer, P.; Manne-Goehler, J.; Andall-Brereton, G.; Houehanou, C.; Houinato, D.; Gurung, M.S.; Bicaba, B.W.; et al. Consumption of Fruits and Vegetables Among Individuals 15 Years and Older in 28 Low- and Middle-Income Countries. *J. Nutr.* **2019**, *149*, 1252–1259, doi:10.1093/jn/nxz040.
7. Zamora-Ros, R.; Béraud, V.; Franceschi, S.; Cayssials, V.; Tsilidis, K.K.; Boutron-Ruault, M.-C.; Weiderpass, E.; Overvad, K.; Tjønneland, A.; Eriksen, A.K.; et al. Consumption of Fruits, Vegetables and Fruit Juices and Differentiated Thyroid Carcinoma Risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study. *Int. J. Cancer* **2018**, *142*, 449–459, doi:<https://doi.org/10.1002/ijc.30880>.

8. Liu, R.H. Health-Promoting Components of Fruits and Vegetables in the Diet. *Adv. Nutr.* **2013**, *4*, 384S–392S, doi:10.3945/an.112.003517.
9. Septembre-Malaterre, A.; Remize, F.; Poucheret, P. Fruits and Vegetables, as a Source of Nutritional Compounds and Phytochemicals: Changes in Bioactive Compounds during Lactic Fermentation. *Food Res. Int.* **2018**, *104*, 86–99, doi:https://doi.org/10.1016/j.foodres.2017.09.031.
10. Lee, S.; Choi, Y.; Jeong, H.S.; Lee, J.; Sung, J. Effect of Different Cooking Methods on the Content of Vitamins and True Retention in Selected Vegetables. *Food Sci. Biotechnol.* **2018**, *27*, 333–342, doi:10.1007/s10068-017-0281-1.
11. Sarker, U.; Oba, S. Response of Nutrients, Minerals, Antioxidant Leaf Pigments, Vitamins, Polyphenol, Flavonoid and Antioxidant Activity in Selected Vegetable Amaranth under Four Soil Water Content. *Food Chem.* **2018**, *252*, 72–83, doi:https://doi.org/10.1016/j.foodchem.2018.01.097.
12. Politowicz, J.; Lech, K.; Lipan, L.; Figiel, A.; Carbonell-Barrachina, Á.A. Volatile Composition and Sensory Profile of Shiitake Mushrooms as Affected by Drying Method. *J. Sci. Food Agric.* **2018**, *98*, 1511–1521, doi:10.1002/jsfa.8622.
13. Hoppu, U.; Puputti, S.; Sandell, M. Factors Related to Sensory Properties and Consumer Acceptance of Vegetables. *Crit. Rev. Food Sci. Nutr.* **2021**, *61*, 1751–1761, doi:10.1080/10408398.2020.1767034.
14. Fu, M.; Shen, X.; Peng, H.; Zhou, Q.; Yun, J.; Sun, Y.; Ho, C.-T.; Cai, H.; Hou, R. Identification of Rancidity Markers in Roasted Sunflower Seeds Produced from Raw Materials Stored for Different Periods of Time. *LWT* **2020**, *118*, 108721, doi:https://doi.org/10.1016/j.lwt.2019.108721.
15. Plutowska, B.; Wardencki, W. Application of Gas Chromatography–Olfactometry (GC–O) in Analysis and Quality Assessment of Alcoholic Beverages – A Review. *Food Chem.* **2008**, *107*, 449–463, doi:https://doi.org/10.1016/j.foodchem.2007.08.058.
16. Yu, J.; Lu, K.; Zi, J.; Yang, X.; Xie, W. Characterization of Aroma Profiles and Aroma-Active Compounds in High-Salt and Low-Salt Shrimp Paste by Molecular Sensory Science. *Food Biosci.* **2022**, *45*, 101470, doi:https://doi.org/10.1016/j.fbio.2021.101470.

17. Šamec, D.; Salopek-Sondi, B. Chapter 3.11 - Cruciferous (Brassicaceae) Vegetables. In; Nabavi, S.M., Silva, A.S.B.T.-N. and N.N.S., Eds.; Academic Press, **2019**; pp. 195–202 ISBN 978-0-12-812491-8.
18. Salehi, B.; Quispe, C.; Butnariu, M.; Sarac, I.; Marmouzi, I.; Kamle, M.; Tripathi, V.; Kumar, P.; Bouyahya, A.; Capanoglu, E.; et al. Phytotherapy and Food Applications from Brassica Genus. *Phyther. Res.* **2021**, *35*, 3590–3609, doi:<https://doi.org/10.1002/ptr.7048>.
19. Xie, F.; Zha, J.; Tang, H.; Xu, Y.; Liu, X.; Wan, Z. Combining Ability and Heterosis Analysis for Mineral Elements by Using Cytoplasmic Male-Sterile Systems in Non-Heading Chinese Cabbage (*Brassica Rapa*). *Crop Pasture Sci.* **2018**, *69*, 296–302.
20. Górska-Warsewicz, H.; Rejman, K.; Kaczorowska, J.; Laskowski, W. Vegetables, Potatoes and Their Products as Sources of Energy and Nutrients to the Average Diet in Poland. *Int. J. Environ. Res. Public Health* **2021**, *18*, doi:10.3390/ijerph18063217.
21. Favela-González, K.M.; Hernández-Almanza, A.Y.; De la Fuente-Salcido, N.M. The Value of Bioactive Compounds of Cruciferous Vegetables (Brassica) as Antimicrobials and Antioxidants: A Review. *J. Food Biochem.* **2020**, *44*, 1–21, doi:10.1111/jfbc.13414.
22. Saban, G.M. The Benefits of Brassica Vegetables on Human Health. *J Hum. Heal. Res* **2018**, *1*, 104.
23. Wu X, Huang H, Childs H, Wu Y, Yu L, Pehrsson PR. Glucosinolates in Brassica Vegetables: Characterization and Factors That Influence Distribution, Content, and Intake. *Annu Rev Food Sci Technol.* **2021**, *12*, 485-511. doi: 10.1146/annurev-food-070620-025744.
24. Khan, N.; Mukhtar, H. Dietary Agents for Prevention and Treatment of Lung Cancer. *Cancer Lett.* **2015**, *359*, 155–164, doi:<https://doi.org/10.1016/j.canlet.2015.01.038>.
25. Wu, Q.-J.; Yang, Y.; Wang, J.; Han, L.-H.; Xiang, Y.-B. Cruciferous Vegetable Consumption and Gastric Cancer Risk: A Meta-Analysis of Epidemiological Studies. *Cancer Sci.* **2013**, *104*, 1067–1073, doi:<https://doi.org/10.1111/cas.12195>.
26. Macharia, J.M.; Mwangi, R.W.; Rozmann, N.; Zsolt, K.; Varjas, T.; Uchechukwu, P.O.; Wagara, I.N.; Raposa, B.L. Medicinal Plants with Anti-Colorectal Cancer

- Bioactive Compounds: Potential Game-Changers in Colorectal Cancer Management. *Biomed. Pharmacother.* **2022**, *153*, 113383, doi:<https://doi.org/10.1016/j.biopha.2022.113383>.
27. Liu, X.; Lv, K. Cruciferous Vegetables Intake Is Inversely Associated with Risk of Breast Cancer: A Meta-Analysis. *The Breast* **2013**, *22*, 309–313, doi:<https://doi.org/10.1016/j.breast.2012.07.013>.
 28. Lu, Y.-T.; Gunathilake, M.; Kim, J. The Influence of Dietary Vegetables and Fruits on Endometrial Cancer Risk: A Meta-Analysis of Observational Studies. *Eur. J. Clin. Nutr.* **2023**, *77*, 561–573, doi:10.1038/s41430-022-01213-3.
 29. Wang, L.; Zhang, J.; Yuan, Q.; Xie, H.; Shi, J.; Ju, X. Separation and Purification of an Anti-Tumor Peptide from Rapeseed (*Brassica Campestris* L.) and the Effect on Cell Apoptosis. *Food Funct.* **2016**, *7*, 2239–2248, doi:10.1039/C6FO00042H.
 30. Liu, B.; Mao, Q.; Lin, Y.; Zhou, F.; Xie, L. The Association of Cruciferous Vegetables Intake and Risk of Bladder Cancer: A Meta-Analysis. *World J. Urol.* **2013**, *31*, 127–133, doi:10.1007/s00345-012-0850-0.
 31. Rawla, P. Epidemiology of Prostate Cancer. *World J. Oncol.* **2019**, *10*, 63–89, doi:10.14740/wjon1191.
 32. Yang, M.-J.; Cha, S.-S.; Lee, J.-J. Effects of Purple Kohlrabi (*Brassica Oleracea* Var. Gongylodes) Flesh and Peel Ethanol Extracts on the Antioxidant Activity and Antiproliferation of Human Cancer Cells. *Korean J. Community Living Sci.* **2015**, *26*, 405–414.
 33. Paolini, M.; Nestle, M. Pitfalls of Enzyme-Based Molecular Anticancer Dietary Manipulations: Food for Thought. *Mutat. Res. Mutat. Res.* **2003**, *543*, 181–189, doi:[https://doi.org/10.1016/S1383-5742\(02\)00092-3](https://doi.org/10.1016/S1383-5742(02)00092-3).
 34. Babić Leko, M.; Gunjača, I.; Pleić, N.; Zemunik, T. Environmental Factors Affecting Thyroid-Stimulating Hormone and Thyroid Hormone Levels. *Int. J. Mol. Sci.* **2021**, *22*, doi:10.3390/ijms22126521.
 35. Al-juhaimi, F.; Ghafoor, K.; Özcan, M.M.; Jahurul, M.H.A.; Babiker, E.E.; Jinap, S.; Sahena, F.; Sharifudin, M.S.; Zaidul, I.S.M. Effect of Various Food Processing and Handling Methods on Preservation of Natural Antioxidants in Fruits and Vegetables. *J.*

36. Jarosz, M. Dietetyka. Żywność, Żywienie w Prewencji i Leczeniu. **2017**.
37. Jarosz, M.; Stolińska, H.; Wolańska, D. *Żywienie w Niedoczynności Tarczycy: Porady Lekarzy i Dietetyków*; Wydawnictwo Lekarskie PZWL, 2015; ISBN 8320048745.
38. Negri, R.; Di Feola, M.; Di Domenico, S.; Scala, M.G.; Artesi, G.; Valente, S.; Smarrazzo, A.; Turco, F.; Morini, G.; Greco, L. Taste Perception and Food Choices. *J. Pediatr. Gastroenterol. Nutr.* **2012**, 54, 624–629, doi:10.1097/MPG.0b013e3182473308.
39. Chmurzynska, A. *Nutrigenomika*. **2022**, ISBN 9788301223359.
40. Wieczorek, M.N.; Walczak, M.; Skrzypczak-Zielińska, M.; Jeleń, H.H. Bitter Taste of Brassica Vegetables: The Role of Genetic Factors, Receptors, Isothiocyanates, Glucosinolates, and Flavor Context. *Crit. Rev. Food Sci. Nutr.* **2018**, 58, 3130–3140, doi:10.1080/10408398.2017.1353478.
41. Westphal, A.; Riedl, K.M.; Cooperstone, J.L.; Kamat, S.; Balasubramaniam, V.M.; Schwartz, S.J.; Böhm, V. High-Pressure Processing of Broccoli Sprouts: Influence on Bioactivation of Glucosinolates to Isothiocyanates. *J. Agric. Food Chem.* **2017**, 65, 8578–8585, doi:10.1021/acs.jafc.7b01380.
42. Gemert, L.J. *Flavour Thresholds : Compilations of Flavour Threshold Values in Water and Other Media*; Oliemans Punter, 2011; ISBN 9789081089432.
43. Sipos, L.; Nyitrai, Á.; Hitka, G.; Friedrich, L.F.; Kókai, Z. Sensory Panel Performance Evaluation - Comprehensive Review of Practical Approaches. *Appl. Sci.* **2021**, 11, doi:10.3390/app112411977.
44. Dong, D.; Jones, G.; Zhang, S. Dynamic Evolution of Bitter Taste Receptor Genes in Vertebrates. **2009**, 9, 1–9, doi:10.1186/1471-2148-9-12.
45. Sandell, M.; Hoppu, U.; Mikkilä, V.; Mononen, N.; Kähönen, M.; Männistö, S.; Rönnemaa, T.; Viikari, J.; Lehtimäki, T.; Raitakari, O.T. Genetic Variation in the HTAS2R38 Taste Receptor and Food Consumption among Finnish Adults. *Genes Nutr.* **2014**, 9, 433, doi:10.1007/s12263-014-0433-3.
46. Bell, L.; Oloyede, O.O.; Lignou, S.; Wagstaff, C.; Methven, L. Taste and Flavor

- Perceptions of Glucosinolates, Isothiocyanates, and Related Compounds. *Mol. Nutr. Food Res.* **2018**, 62, 1–41, doi:10.1002/mnfr.201700990.
47. Drewnowski, A.; Gomez-Carneros, C. Bitter Taste, Phytonutrients, and the Consumer: A Review. *Am. J. Clin. Nutr.* **2000**, 72, 1424–1435, doi:10.1093/ajcn/72.6.1424.
48. Bertóti, R.; Böszörményi, A.; Alberti, Á.; Béni, S.; M-Hamvas, M.; Szőke, É.; Vasas, G.; Gonda, S. Variability of Bioactive Glucosinolates, Isothiocyanates and Enzyme Patterns in Horseradish Hairy Root Cultures Initiated from Different Organs. *Mol.* **2019**, 24.
49. Lin, C.-M.; Jf, K.; D, W.; Wei, C.-I. Bactericidal Activity of Isothiocyanate against Pathogens on Fresh Produce. *J. Food Prot.* **2000**, 63, 25–30, doi:10.4315/0362-028X-63.1.25.
50. Kiani, S.; Minaei, S.; Ghasemi-Varnamkhasti, M. Application of Electronic Nose Systems for Assessing Quality of Medicinal and Aromatic Plant Products: A Review. *J. Appl. Res. Med. Aromat. Plants* **2016**, 3, 1–9, doi:https://doi.org/10.1016/j.jarmap.2015.12.002.
51. Steinhaus, M. Gas Chromatography–Olfactometry: Principles, Practical Aspects and Applications in Food Analysis. In *Advanced Gas Chromatography in Food Analysis*; Tranchida, P.Q., Ed.; The Royal Society of Chemistry, **2019**; p. 0 ISBN 978-1-78801-127-3.
52. Dunkel, A.; Steinhaus, M.; Kotthoff, M.; Nowak, B.; Krautwurst, D.; Schieberle, P.; Hofmann, T. Nature’s Chemical Signatures in Human Olfaction: A Foodborne Perspective for Future Biotechnology. *Angew. Chem. Int. Ed. Engl.* **2014**, 53, 7124–7143, doi:10.1002/anie.201309508.
53. Song, H.; Liu, J. GC-O-MS Technique and Its Applications in Food Flavor Analysis. *Food Res. Int.* **2018**, 114, 187–198, doi:10.1016/j.foodres.2018.07.037.
54. Füllemann, D.; Steinhaus, M. Characterization of Odorants Causing Smoky Off-Flavors in Cocoa. *J. Agric. Food Chem.* **2020**, 68, 10833–10841, doi:10.1021/acs.jafc.0c04633.
55. Methner, Y.; Dancker, P.; Maier, R.; Latorre, M.; Hutzler, M.; Zarnkow, M.; Steinhaus, M.; Libkind, D.; Frank, S.; Jacob, F. Influence of Varying Fermentation

- Parameters of the Yeast Strain *Cyberlindnera Saturnus* on the Concentrations of Selected Flavor Components in Non-Alcoholic Beer Focusing on (E)- β -Damascenone. *Foods* **2022**, *11*, 1038, doi:10.3390/foods11071038.
56. Reglitz, K.; Féchir, M.; Mall, V.; Voigt, J.; Steinhaus, M. The Impact of Caramel and Roasted Wheat Malts on Aroma Compounds in Top-Fermented Wheat Beer. *J. Inst. Brew.* **2022**, *128*, doi:10.1002/jib.701.
 57. Wieczorek, M.N.; Majcher, M.A.; Jeleń, H.H. Identification of Aroma Compounds in Raw and Cooked Broccoli. *Flavour Fragr. J.* **2021**, *36*, 576–583, doi:10.1002/ffj.3669.
 58. Engel, E.; Baty, C.; le Corre, D.; Souchon, I.; Martin, N. Flavor-Active Compounds Potentially Implicated in Cooked Cauliflower Acceptance. *J. Agric. Food Chem.* **2002**, *50*, 6459–6467, doi:10.1021/jf025579u.
 59. Usami, A.; Motooka, R.; Takagi, A.; Nakahashi, H.; Okuno, Y.; Miyazawa, M. Chemical Composition, Aroma Evaluation, and Oxygen Radical Absorbance Capacity of Volatile Oil Extracted from Brassica Rapa Cv. “Yukina” Used in Japanese Traditional Food. *J. Oleo Sci.* **2014**, *63*, 723–730, doi:10.5650/jos.ess14033.
 60. Gracka, A.; Jeleń, H.H.; Majcher, M.; Siger, A.; Kaczmarek, A. Flavoromics Approach in Monitoring Changes in Volatile Compounds of Virgin Rapeseed Oil Caused by Seed Roasting. *J. Chromatogr. A* **2016**, *1428*, 292–304, doi:https://doi.org/10.1016/j.chroma.2015.10.088.
 61. Ortner, E.; Granvogl, M.; Schieberle, P. Elucidation of Thermally Induced Changes in Key Odorants of White Mustard Seeds (*Sinapis Alba* L.) and Rapeseeds (*Brassica Napus* L.) Using Molecular Sensory Science. *J. Agric. Food Chem.* **2016**, *64*, 8179–8190, doi:10.1021/acs.jafc.6b03625.
 62. Cha, Y.J.; Kim, H.; Cadwallader, K.R. Aroma-Active Compounds in Kimchi during Fermentation. *J. Agric. Food Chem.* **1998**, *46*, 1944–1953, doi:10.1021/jf9706991.
 63. Yang, P.; Song, H.; Wang, L.; Jing, H. Characterization of Key Aroma-Active Compounds in Black Garlic by Sensory-Directed Flavor Analysis. *J. Agric. Food Chem.* **2019**, *67*, 7926–7934, doi:10.1021/acs.jafc.9b03269.
 64. Kreissl, J.; Mall, V.; Steinhaus, P.; Steinhaus, M. Leibniz-LSB@TUM Odorant Database, Version 1.0. Leibniz-Institute for Food Systems Biology at the Technical

University of Munich: Freising, Germany Available online: <https://www.leibniz-lsb.de/en/databases/leibniz-lsb-tum-odorant-database> (dostęp 18.05.2021r.).

65. McGorin, R.J. The Significance of Volatile Sulfur Compounds in Food Flavors. In *Volatile Sulfur Compounds in Food*; ACS Symposium Series; American Chemical Society, **2011**; Vol. 1068, pp. 1–3 ISBN 9780841226166.
66. Pelosi, P.; Zhu, J.; Knoll, W. From Gas Sensors to Biomimetic Artificial Noses. *Chemosensors* **2018**, *6*.
67. Yábar, E.; Pedreschi, R.; Chirinos, R.; Campos, D. Glucosinolate Content and Myrosinase Activity Evolution in Three Maca (*Lepidium Meyenii* Walp.) Ecotypes during Preharvest, Harvest and Postharvest Drying. *Food Chem.* **2011**, *127*, 1576–1583, doi:<https://doi.org/10.1016/j.foodchem.2011.02.021>.
68. Marcinkowska, M.; Jeleń, H.H. Inactivation of Thioglucosidase from *Sinapis Alba* (White Mustard) Seed by Metal Salts. *Molecules* **2020**, *25*, doi:10.3390/molecules25194363.
69. Gemert, L.J. Van *Compilations of Odour Threshold Values in Air, Water and Other Media*; second enlarged and revised edition; Oliemans Punter & Partners BV: Utrecht, **2011**.
70. Doleman, J.F.; Grisar, K.; Van Liedekerke, L.; Saha, S.; Roe, M.; Tapp, H.S.; Mithen, R.F. The Contribution of Alliaceous and Cruciferous Vegetables to Dietary Sulphur Intake. *Food Chem.* **2017**, *234*, 38–45, doi:10.1016/j.foodchem.2017.04.098.
71. Bin, P.; Huang, R.; Zhou, X. Oxidation Resistance of the Sulfur Amino Acids: Methionine and Cysteine. *Biomed Res. Int.* **2017**, *2017*, 9584932, doi:10.1155/2017/9584932.
72. Shekari, G.; Javanmardi, J. Application of Cysteine, Methionine and Amino Acid Containing Fertilizers to Replace Urea: The Effects on Yield and Quality of Broccoli. *Adv. Crop Sci. Technol.* **2017**, *05*, doi:10.4172/2329-8863.1000283.
73. Ahuja, I.; Rohloff, J.; Bones, A.M. Defence Mechanisms of Brassicaceae: Implications for Plant-Insect Interactions and Potential for Integrated Pest Management BT - Sustainable Agriculture Volume 2. In; Lichtfouse, E., Hamelin, M., Navarrete, M., Debaeke, P., Eds.; Springer Netherlands: Dordrecht, **2011**; pp. 623–670.

74. Hanschen, F.S.; Lamy, E.; Schreiner, M.; Rohn, S. Reactivity and Stability of Glucosinolates and Their Breakdown Products in Foods. *Angew. Chem. Int. Ed. Engl.* **2014**, *53*, 11430–11450, doi:10.1002/anie.201402639.
75. Birchfield, A.; McIntosh, C. Metabolic Engineering and Synthetic Biology of Plant Natural Products – A Minireview. *Curr. Plant Biol.* **2020**, *24*, 100163, doi:10.1016/j.cpb.2020.100163.
76. Jones, M.G.; Hughes, J.; Tregova, A.; Milne, J.; Tomsett, A.B.; Collin, H.A. Biosynthesis of the Flavour Precursors of Onion and Garlic. *J. Exp. Bot.* **2004**, *55*, 1903–1918, doi:10.1093/jxb/erh138.
77. Block, E. The Organosulfur Chemistry of the Genus Allium – Implications for the Organic Chemistry of Sulfur. *Angew. Chemie Int. Ed. English* **1992**, *31*, 1135–1178, doi:https://doi.org/10.1002/anie.199211351.
78. Block, E. The Organosulfur Chemistry of the Genus Allium – Implications for the Organic Chemistry of Sulfur. *Angew. Chemie Int. Ed. English* **1992**, *31*, 1135–1178, doi:10.1002/anie.199211351.
79. Li, J.; Dadmohammadi, Y.; Abbaspourrad, A. Flavor Components, Precursors, Formation Mechanisms, Production and Characterization Methods: Garlic, Onion, and Chili Pepper Flavors. *Crit. Rev. Food Sci. Nutr.* **2021**, *0*, 1–23, doi:10.1080/10408398.2021.1926906.
80. Mazza, G.; Ciaravolo, S.; Chiricosta, G.; Celli, S. Volatile Flavour Components from Ripening and Mature Garlic Bulbs. *Flavour Fragr. J.* **1992**, *7*, 111–116, doi:https://doi.org/10.1002/ffj.2730070303.
81. Chin, H.-W.; Lindsay, R.C. Mechanisms of Formation of Volatile Sulfur Compounds Following the Action of Cysteine Sulfoxide Lyases. *J. Agric. Food Chem.* **1994**, *42*, 1529–1536, doi:10.1021/jf00043a026.
82. Marcinkowska, M.; Jeleń, H.H. Determination of the Odor Threshold Concentrations and Partition Coefficients of Isothiocyanates from Brassica Vegetables in Aqueous Solution. *LWT* **2020**, *131*, doi:10.1016/j.lwt.2020.109793.
83. Lan, L.; Huang, S.; Liu, Y.; Sun, B.; Tian, H. Preparation and Odor Characteristics of

-
- Nitriles Derived from Aldehydes. *Flavour Fragr. J.* **2020**, 35, 425–434, doi:10.1002/ffj.3581.
84. Munday, R. Harmful and Beneficial Effects of Organic Monosulfides, Disulfides, and Polysulfides in Animals and Humans. *Chem. Res. Toxicol.* **2012**, 25, 47–60, doi:10.1021/tx200373u.
 85. Antonopoulou, M.; Evgenidou, E.; Lambropoulou, D.; Konstantinou, I. A Review on Advanced Oxidation Processes for the Removal of Taste and Odor Compounds from Aqueous Media. *Water Res.* **2014**, 53, 215–234, doi:10.1016/j.watres.2014.01.028.
 86. Wu, J.; Liu, Y.; Zhao, H.; Huang, M.; Sun, Y.; Zhang, J.; Sun, B. Recent Advances in the Understanding of Off-Flavors in Alcoholic Beverages: Generation, Regulation, and Challenges. *J. Food Compos. Anal.* **2021**, 103, 104117, doi:10.1016/j.jfca.2021.104117.
 87. Cheng, Y.; Li, G.; Wu, H.; Huang, L.; Wang, H. Identification of Light-Induced Key Off-Flavors in Ponkan Mandarin Juice Using MDGC-MS/O and GC-MS/PFPD. *J. Agric. Food Chem.* **2021**, 69, 14259–14269, doi:10.1021/acs.jafc.1c05465.
 88. Chhikara, N.; Devi, H.R.; Jaglan, S.; Sharma, P.; Gupta, P.; Panghal, A. Bioactive Compounds, Food Applications and Health Benefits of *Parkia Speciosa* (Stinky Beans): A Review. *Agric. Food Secur.* **2018**, 7, 1–9, doi:10.1186/s40066-018-0197-x.
 89. Schmidberger, P.C.; Schieberle, P. Changes in the Key Aroma Compounds of Raw Shiitake Mushrooms (*Lentinula Edodes*) Induced by Pan-Frying As Well As by Rehydration of Dry Mushrooms. *J. Agric. Food Chem.* **2020**, 68, 4493–4506, doi:10.1021/acs.jafc.0c01101.
 90. Onwude, D.I.; Hashim, N.; Janius, R.; Abdan, K.; Chen, G.; Oladejo, A.O. Non-Thermal Hybrid Drying of Fruits and Vegetables: A Review of Current Technologies. *Innov. Food Sci. Emerg. Technol.* **2017**, 43, 223–238, doi:10.1016/j.ifset.2017.08.010.
 91. Marcinkowska, M.A.; Jeleń, H.H. Role of Sulfur Compounds in Vegetable and Mushroom Aroma. *Molecules* **2022**, 27, doi:10.3390/molecules27186116.
 92. Wu, C.M.; Wang, Z. Volatile Compounds in Fresh and Processed Shiitake Mushrooms (*Lentinus Edodes* Sing.). *Food Sci. Technol. Res.* **2000**, 6, 166–170, doi:10.3136/fstr.6.166.

93. Cedrowski, J.; Dąbrowa, K.; Przybylski, P.; Krogul-Sobczak, A.; Litwinienko, G. Antioxidant Activity of Two Edible Isothiocyanates: Sulforaphane and Erucin Is Due to Their Thermal Decomposition to Sulfenic Acids and Methylsulfinyl Radicals. *Food Chem.* **2021**, 353, 129213, doi:<https://doi.org/10.1016/j.foodchem.2021.129213>.
94. Schwab, W.; Davidovich-Rikanati, R.; Lewinsohn, E. Biosynthesis of Plant-Derived Flavor Compounds. *Plant J.* **2008**, 54, 712–732, doi:10.1111/j.1365-313X.2008.03446.x.
95. Chen, C.-W.; Ho, C.-T. Thermal Degradation of Allyl Isothiocyanate in Aqueous Solution. *J. Agric. Food Chem.* **1998**, 46, 220–223, doi:10.1021/jf970488w.
96. Williams, M.P.; Nelson, P.E. Kinetics of the thermal degradation of methylmethionine sulfonium ions in citrate buffers and in sweet corn and tomato serum. *J. Food Sci.* **1974**, 39, 457–460, doi:<https://doi.org/10.1111/j.1365-2621.1974.tb02924.x>.
97. Kubec, R.; Velíšek, J.; Doležal, M.; Kubelka, V. Sulfur-Containing Volatiles Arising by Thermal Degradation of Alliin and Deoxyalliin. *J. Agric. Food Chem.* **1997**, 45, 3580–3585, doi:10.1021/jf970071q.
98. Shu, C.K.; Hagedorn, M.L.; Mookherjee, B.D.; Ho, C.T. Volatile Components of the Thermal Degradation of Cystine in Water. *J. Agric. Food Chem.* **1985**, 33, 438–442, doi:10.1021/jf00063a028.
99. Sun, B.; Tian, Y.-X.; Jiang, M.; Yuan, Q.; Chen, Q.; Zhang, Y.; Luo, Y.; Zhang, F.; Tang, H.-R. Variation in the Main Health-Promoting Compounds and Antioxidant Activity of Whole and Individual Edible Parts of Baby Mustard (*Brassica Juncea* Var. *Gemmifera*). *RSC Adv.* **2018**, 8, 33845–33854, doi:10.1039/C8RA05504A.
100. Zhou, Q.; Tang, H.; Jia, X.; Zheng, C.; Huang, F.; Zhang, M. Distribution of Glucosinolate and Pungent Odors in Rapeseed Oils from Raw and Microwaved Seeds. *Int. J. Food Prop.* **2018**, 21, 2296–2308, doi:10.1080/10942912.2018.1514632.
101. 2170 Flavor Profile Analysis. In *Standard Methods For the Examination of Water and Wastewater.* **2018**.
102. Sikorska-Zimny, K.; Beneduce, L. The Glucosinolates and Their Bioactive Derivatives in Brassica: A Review on Classification, Biosynthesis and Content in Plant Tissues, Fate during and after Processing, Effect on the Human Organism and Interaction with

-
- the Gut Microbiota. *Crit. Rev. Food Sci. Nutr.* **2021**, *61*, 2544–2571, doi:10.1080/10408398.2020.1780193.
103. Oloyede, O.O.; Wagstaff, C.; Methven, L. Influence of Cabbage (*Brassica Oleracea*) Accession and Growing Conditions on Myrosinase Activity, Glucosinolates and Their Hydrolysis Products. *Foods* **2021**, *10*, doi:10.3390/foods10122903.
 104. Okunade, O.A.; Ghawi, S.K.; Methven, L.; Niranjana, K. Thermal and Pressure Stability of Myrosinase Enzymes from Black Mustard (*Brassica Nigra* L. W.D.J. Koch. Var. *Nigra*), Brown Mustard (*Brassica Juncea* L. Czern. Var. *Juncea*) and Yellow Mustard (*Sinapsis Alba* L. Subsp. *Maire*) Seeds. *Food Chem.* **2015**, *187*, 485–490, doi:10.1016/j.foodchem.2015.04.054.
 105. Wieczorek, M.N.; Pieczywek, P.M.; Cybulska, J.; Zdunek, A.; Jeleń, H.H. Chemical Changes in the Broccoli Volatilome Depending on the Tissue Treatment. *Molecules* **2022**, *27*, doi:10.3390/molecules27020500.
 106. Hanschen, F.S.; Klopsch, R.; Oliviero, T.; Schreiner, M.; Verkerk, R.; Dekker, M. Optimizing Isothiocyanate Formation during Enzymatic Glucosinolate Breakdown by Adjusting PH Value, Temperature & Dilution in Brassica Vegetables & *Arabidopsis Thaliana*. *Sci. Rep.* **2017**, *7*, 1–15, doi:10.1038/srep40807.
 107. Park, W.T.; Kim, J.K.; Park, S.; Lee, S.W.; Li, X.; Kim, Y.B.; Uddin, M.R.; Park, N. Il; Kim, S.J.; Park, S.U. Metabolic Profiling of Glucosinolates, Anthocyanins, Carotenoids, and Other Secondary Metabolites in Kohlrabi (*Brassica Oleracea* Var. *Gongylodes*). *J. Agric. Food Chem.* **2012**, *60*, 8111–8116, doi:10.1021/jf301667j.
 108. Sathasivam, R.; Kim, M.C.; Yeo, H.J.; Nguyen, B. Van; Sohn, S.I.; Park, S.U.; Kim, J. Accumulation of Phenolic Compounds and Glucosinolates in Sprouts of Pale Green and Purple Kohlrabi (*Brassica Oleracea* Var. *Gongylodes*) under Light and Dark Conditions. *Agronomy* **2021**, *11*, doi:10.3390/agronomy11101939.
 109. Ben Sassi, A.; Cheikh M'hamed, A.; Chahdoura, H.; Saidani Tounsi, M.; Mastouri, M.; Ben Salem, H. Variation in Biochemical Profile and Health Beneficial Compounds and Biological Activities of *Brassica Oleracea* Var *Gongylodes* L. Morphological Parts. *J. Food Meas. Charact.* **2020**, *14*, 1192–1200, doi:10.1007/s11694-019-00368-1.
 110. Kroener, E.M.; Buettner, A. Sensory-Analytical Comparison of the Aroma of Different Horseradish Varieties (*Armoracia Rusticana*). *Front. Chem.* **2018**, *6*, 1–11,

doi:10.3389/fchem.2018.00149.

111. Yi, G.E.; Robin, A.H.K.; Yang, K.; Park, J.I.; Kang, J.G.; Yang, T.J.; Nou, I.S. Identification and Expression Analysis of Glucosinolate Biosynthetic Genes and Estimation of Glucosinolate Contents in Edible Organs of Brassica Oleracea Subspecies. *Molecules* **2015**, *20*, 13089–13111, doi:10.3390/molecules200713089.
112. Wieczorek, M.N.; Jelen, H.H. Volatile Compounds of Selected Raw and Cooked Brassica Vegetables. *Molecules* **2019**, *24*, doi:10.3390/molecules24030391.

Aktywność naukowa

Dorobek publikacyjny:

- **Marcinkowska, M.**; Jeleń, H.H. Role of Sulfur Compounds in Vegetable and Mushroom Aroma. *Molecules* **2022**, 27, doi:10.3390/molecules27186116. IF = 4.927
- Jeleń, H.H.; **Marcinkowska, M.**; Marek, M. Determination of Volatile Terpenes in Coriander Cold Pressed. *Molecules* **2021**, 26. IF = 4.927
- **Marcinkowska, M.**; Jeleń, H.H. Determination of the odor threshold concentrations and partition coefficients of isothiocyanates from Brassica vegetables in aqueous solution. *LWT* **2020**, 131, doi:10.1016/j.lwt.2020.109793. IF = 6.056
- **Marcinkowska, M.**; Jeleń, H.H. Inactivation of thioglucosidase from *Sinapis alba* (white mustard) seed by metal salts. *Molecules* **2020**, 25, doi:10.3390/molecules25194363. IF = 4.927
- **Marcinkowska, M.**; Frank, S.; Steinhaus, M.; Jeleń, H.H. Key Odorants of Raw and Cooked Green Kohlrabi (*Brassica oleracea* var. *gongylodes* L.). *J. Agric. Food Chem.* **2021**, 69, 12270–12277, doi:10.1021/acs.jafc.1c04339. IF = 5.895
- **Marcinkowska, M.**; Wieczorek, M.; Jeleń, H.H. Dobre praktyki w analityce związków zapachowych żywności. *Analityka* **2020**, 3, 4–11.
- Jeleń, H.H.; Gaca, A.; **Marcinkowska, M.** Use of Sorbent-Based Vacuum Extraction for Determination of Volatile Phenols in Beer. *Food Anal. Methods* **2018**, 11, 3089–3094, doi:10.1007/s12161-018-1277-z. IF = 3.498

Zestawienie danych według listy Journal Citation Reports i Web of Science

W prezentowanym cyklu publikacji:

- Sumaryczny impact factor wg. listy Journal Citation Reports (rok 2022) wynosi: **21.81**
- Łączna liczba cytowania publikacji według bazy Web of Science (06.06.2023): **25**

Łącznie z publikacjami, które nie wchodzą w skład rozprawy doktorskiej:

- Sumaryczny impact factor wg. listy Journal Citation Reports (rok 2022) wynosi: **30.23**
- Liczba cytowania publikacji według danych bazy Web of Science (06.06.2023): **42**
- Indeks Hirsha według bazy Web of Science (06.06.2023): **5**

Udział w konferencjach naukowych

- **Marcinkowska, M.**; Jeleń, H.H. *Determination of the odor threshold concentrations of isothiocyanates*. Wystąpienie konferencyjne (poster) o zasięgu międzynarodowym zaprezentowane na: *12th Wartburg Symposium on Flavour Chemistry & Biology*. Niemcy, Eisenach **2019**, 21-24 maja.
- Jeleń, H.H.; Wieczorek, M.; Majcher, M.; **Marcinkowska, M.** *Brassica Vegetables aroma. From chemistry to perception*. Wystąpienie konferencyjne (ustne) o zasięgu międzynarodowym zaprezentowane na: *19th IUFoST World Congress of Food Science and Technology*. Indie, Mumbai **2018**, 23-27 października.
- Jeleń, H.H.; Mocydłarz, A.; **Marcinkowska, M.**; Wieczorek, M. *Vacuum assisted sorbent extraction for the analysis of isothiocyanates in Brassica vegetables*. Wystąpienie konferencyjne (ustne) o zasięgu międzynarodowym zaprezentowane na: *The 3rd International Flavor and Fragrance Conference*. Chile, Viña del Mar **2019**, 1-4 października.
- Jeleń, H.H.; Wieczorek, M.; Majcher, M.; **Marcinkowska, M.** *Cooking with GCxGC a tool for monitoring changes in flavor, volatile and nonvolatile metabolites in Brussel sprouts subjected to thermal processing*. Wystąpienie konferencyjne (ustne) o zasięgu międzynarodowym zaprezentowane na: *International Symposium on Capillary Chromatography and GCxGC Symposium*. Włochy, Riva del Garda **2020**, 24-29 maja.
- **Marcinkowska, M.**; Jeleń, H.H. *Mirozyna jako problem analityczny w analizie związków lotnych kalarepy*. Wystąpienie konferencyjne (ustne) zaprezentowane na: *V Konferencji Naukowej ENZYMOS Enzymy w nauce i przemyśle*. **2021**, 5 lutego.
- **Marcinkowska, M.**; Frank, S.; Steinhaus, M.; Jeleń, H.H. *Characterization of aroma-active compounds in raw and cooked green kohlrabi*. Wystąpienie konferencyjne (ustne) o zasięgu międzynarodowym zaprezentowane na: *QUO VADIS Life Sciences*. Polska, Opole **2021**, 23-27 czerwca.
- Jeleń, H.H.; **Marcinkowska, M.** *Vacuum assisted sorbent extraction (vase) – a new approach in the analysis of flavor compounds*. Wystąpienie konferencyjne (ustne) o zasięgu międzynarodowym zaprezentowane na: *QUO VADIS Life Sciences*. Polska, Opole **2021**, 23-27 czerwca.

Staże naukowe

- Leibniz Institute for Food Systems Biology at the Technical University of Munich (LSB) – 6 miesięczny staż naukowy realizowany w ramach grantu nr 548 2015/18/M/NZ9/00372 w następujących terminach:
 - wrzesień 2019 – grudzień 2019 (3 miesiące)
 - luty 2020 – kwiecień 2020 (2 miesiące)
 - wrzesień 2020 – październik 2020 (miesiąc)

Stypendia

- Stypendium naukowe dla doktorantów 2018/2019
- Stypendium Rektora dla doktorantów 2018/2019
- Stypendium naukowe dla doktorantów 2020/2021
- Stypendium Rektora dla doktorantów 2020/2021

Udział w projektach badawczych

- Harmonia 7, *Kluczowe związki odpowiedzialne za zapach i smak wybranych warzyw z rodziny kapustowatych. Tworzenie i przemiany w procesach technologicznych*, Narodowe Centrum Nauki, nr grantu 548 2015/18/M/NZ9/00372 – wykonawca.
- OPUS 18, *Redefiniowana analiza związków zapachowych i lotnych żywności. Co kryje się za volatilomiką żywności?*, Narodowe Centrum Nauki, nr grantu 2019/35/B/NZ9/01582 – wykonawca.

Kopie publikacji naukowych wchodzących w zakres rozprawy doktorskiej

Review

Role of Sulfur Compounds in Vegetable and Mushroom Aroma

Monika A. Marcinkowska  and Henryk H. Jeleń 

Faculty of Food Science and Nutrition, Poznań University of Life Sciences, Wojska Polskiego 31,
60-624 Poznań, Poland

* Correspondence: henrykj@up.poznan.pl; Tel.: +48-618-487-273

Abstract: At the base of the food pyramid is vegetables, which should be consumed most often of all food products, especially in raw and unprocessed form. Vegetables and mushrooms are rich sources of bioactive compounds that can fulfill various functions in plants, starting from protection against herbivores and being natural insecticides to pro-health functions in human nutrition. Many of these compounds contain sulfur in their structure. From the point of view of food producers, it is extremely important to know that some of them have flavor properties. Volatile sulfur compounds are often potent odorants, and in many vegetables, belonging mainly to *Brassicaceae* and *Allium* (*Amaryllidaceae*), sulfur compounds determine their specific flavor. Interestingly, some of the pathways that form volatile sulfur compounds in vegetables are also found in selected edible mushrooms. The most important odor-active organosulfur compounds can be divided into isothiocyanates, nitriles, epithionitriles, thiols, sulfides, and polysulfides, as well as others, such as sulfur containing carbonyl compounds and esters, R-L-cysteine sulfoxides, and finally heterocyclic sulfur compounds found in shiitake mushrooms or truffles. This review paper summarizes their precursors and biosynthesis, as well as their sensory properties and changes in selected technological processes.

Keywords: sulfur compounds; vegetable aroma; glucosinolates; isothiocyanates; nitriles; sulfides; polysulfides; thiols; *Brassica* vegetables



Citation: Marcinkowska, M.A.; Jeleń, H.H. Role of Sulfur Compounds in Vegetable and Mushroom Aroma. *Molecules* **2022**, *27*, 6116. <https://doi.org/10.3390/molecules27186116>

Academic Editor: María Angeles Pozo-Bayón

Received: 19 July 2022
Accepted: 14 September 2022
Published: 19 September 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

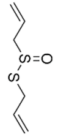
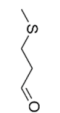
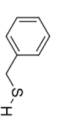
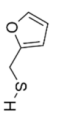
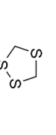
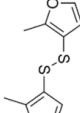
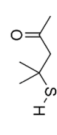
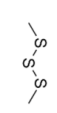
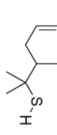
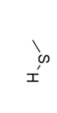
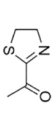
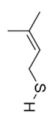
The intake of vegetables plays a crucial role in maintaining the homeostasis of the human body due to bioactive compounds that are present in plant tissues. Sulfur is a fundamental element, ensuring the sustainable development of plants and people. Amino acids, such as methionine and cysteine, derived originally from plants, represent the main source of sulfur for the human diet [1]. Vegetables contain vitamins, polyphenols, fiber, and micronutrients, which are necessary for daily diets, and they can contribute to the prevention of chronic diseases [2,3]. Sulfur-containing compounds are commonly present in Brassica vegetables, namely broccoli, cabbage, and cauliflower, or Allium vegetables, for example, garlic, leeks, and onions. The structure of organosulfur compounds is based on sulfur atoms that are bound with a cyanate group or a carbon atom in a chain or cyclic arrangement. The bioactive compounds present in Allium species are alk(en)yl cysteine sulfoxides; S-allyl cysteine; thiosulfinates; diallyl; mono-, di-, and tri-sulfides; and vinylidithiins, while for cruciferous vegetables the largest group are glucosinolates (GLSs) and their breakdown produces isothiocyanates (ITCs) [4]. An equally important role of GLSs in plants is their self-protection strategies. The first mechanism of food protection involves repelling pests by releasing ITCs, whereas the attraction of the natural enemies of insects is linked with the effects of nitriles [5]. Depending on the condition of GLSs degradation, such as the company of additional cofactors and proteins, presence of metal ions, or pH value, it is possible to obtain other rearranged products, namely epithionitriles, nitriles, oxazolidine-2-thiones, and thiocyanates [6]. Volatile sulfur compounds contribute significantly to the characteristic aroma of vegetables belonging to discussed above Brassica and Allium, being usually among their key odorants. It makes them a very unique group

of plant metabolites, which are interesting from a flavor chemistry and also analytical point of view due to their diversity, low odor thresholds, instability, and reactivity.

Although mushroom-like flavor is usually associated with eight carbon unsaturated alcohols and ketones (mainly 1-octene-3-ol, 3-octanone, 1-octene-3-one), sulfur compounds are also important components of some mushrooms, particularly shiitake and truffles, which are discussed in this review. Due to their high price, truffles are used as food seasoning rather than vegetables, unlike shiitake. Heterocyclic organosulfur compounds provide burned, sulfuric, onion-like, rubber-like, and truffle-like odor notes [7,8]. Although mushrooms are consumed mainly for their sensory features, especially aroma, they also are valuable sources of valuable bioactive compounds. Shiitake mushrooms are a low energy-dense food and supply nutritionally relevant amounts of B group vitamins and minerals; they may be a proper source of vitamin D₂, and their consumption has some medicinally beneficial properties, such as improved immunity [9]. Truffles are valuable sources of dietary fiber, essential amino acids, metals, and ergosteroids and have many health-promoting properties, particularly antioxidant, anti-inflammatory, antimicrobial property, immunomodulatory, antitumor properties, and anti-depressant properties [10].

Sulfur compounds as metabolites show various chemical structures due to their differences in molecular weight, stability, polarity, and volatility. They are present in food and may be formed or altered as a result of enzymatic and chemical reactions in technological processes and during storage. As a result of heating, smoking, cooking, frying, or roasting, many complex reactions, namely oxidation, degradation, hydrolysis, dehydration, condensation, and decarboxylation, may take place. Volatile sulfur compounds (VSCs) in food can be divided into several classes according to their functional groups (Table 1) [11]. Thiols (mercaptans) constitute a wide group with antioxidant properties that are labeled as part of the sulfhydryl functional group [12]. Methanethiol represents the parent compounds, as a result of easy oxidation into sulfides (dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide) [13]. Further oxidation of sulfides can lead to sulfoxides or sulfones. The variety of sulfur compounds is connected to the ability of sulfur atoms for taking different oxidation states, ranging from -2 in sulfides, 0 in sulfoxides, and $+2$ in sulfones [14]. In addition to the sulfur compounds resulting from the breakdown of GLSs mentioned above, an important group is S-heterocyclic compounds. Thiophenes, with a smoky, sulfurous, and roasted aroma, are created during thermal processes, especially when bioavailability of sulfur is sufficient by a high concentration of cysteine [15]. Thiazolines and thiazoles are formed under thermal processes in the Maillard reaction. Thiazoles are associated with a roasted aroma in food [16]. The 2-acetyl-2-thiazoline is a crucial flavor compound with a lower detection threshold at 1 ppm level, with a pleasant popcorn-like, roasted, nutty aroma [17].

Table 1. Selected sulfur key odorants in food formed in enzymatic and chemical (mainly thermal) reactions.

Name	Structure	Odor Threshold	Odor Quality	Occurrence	Name	Structure	Odor Threshold	Odor Quality	Occurrence
diallyl thiosulfinate (allicin)		NA	Freshly crushed garlic	Garlic, onion	3- (methylthio)propanal (methional)		1.3 µg/L in water	Cooked potato-like	Beef, truffle, coffee, cheese
benzenemethanethiol (phenylmethanethiol)		0.01 µg/L in 67% ethanol-water solution	Roasted, garden cress seed	Beef, wine, Baijiu	2-furfurylthio(2- furanmethanethiol)		0.004 µg/kg in water-alcohol solution	Sulfuric, burnt	Popcorn, coffee, meat, sesame seeds
1,2,4-trithiolane		1.27 µg/L in water	Shiitake-like, sulfury, onion-like	Shiitake mushrooms, truffle, stinky beans	bis-(2-methyl-3- furyl)disulfide(1,2- bis(2-methyl-3- furyl)disulfane)		0.00002 µg/L in water	Meat-like	Beef, coffee, brown rice, tea, kohlrabi
4-mercapto-4- methyl-2- pentanone		0.0001 µg/L in water	Blackcurrant-like, sulfury	Green tea, edible flowers	dimethyl trisulfide (DMS)		0.009 µg/L in water	Sulfuric, cabbage-like	Brassica vegetables, wine, cheese, coffee
1-p-menthene-8- thiol		0.0002 µg/L in water	Grapefruit-like, sulfuric	Grapefruit, orange, honey pomelo	methanethiol		0.59 µg/L in water	Sulfuric, cabbage-like	Coffee, cheese, potato, spinach, durian
2-acetyl-2- thiazoline		1 µg/L in water	Roasted, popcorn-like	Meat, rice, shiitake mushroom	3-methyl-2- butene-1-thiol		0.00076 µg/L in water	Beer-like, animal-like	Beer, coffee, durian

NA—not available. Based on references [18–26].

Sulfur compounds present in such complex matrices as food are an analytical challenge due to their often sub-parts-per-billion concentrations. As a result of low sensory detection thresholds, VSCs are emphatically responsible for the aroma creation of food. Diallyl disulfide (DADS) is an aroma impact compound for garlic and allyl isothiocyanate causes a typical mustard flavor. However, not all sulfur compounds are a backbone of vegetable aroma; some of them are responsible for sensory nuances in total flavor. Moreover, flavor sensory impact relies on specific odor threshold and level of content in particular food [27]. Nevertheless, sulfur compounds have a significant impact in creating food aroma also because they are the second largest category in volatile constituents in food after esters (Figure 1). Sulfur compounds can be grouped into many distinct categories, which include ITCs, nitriles, epithethriles, sulfides, polysulfides, and thiols, as well as a wide group of miscellaneous compounds.

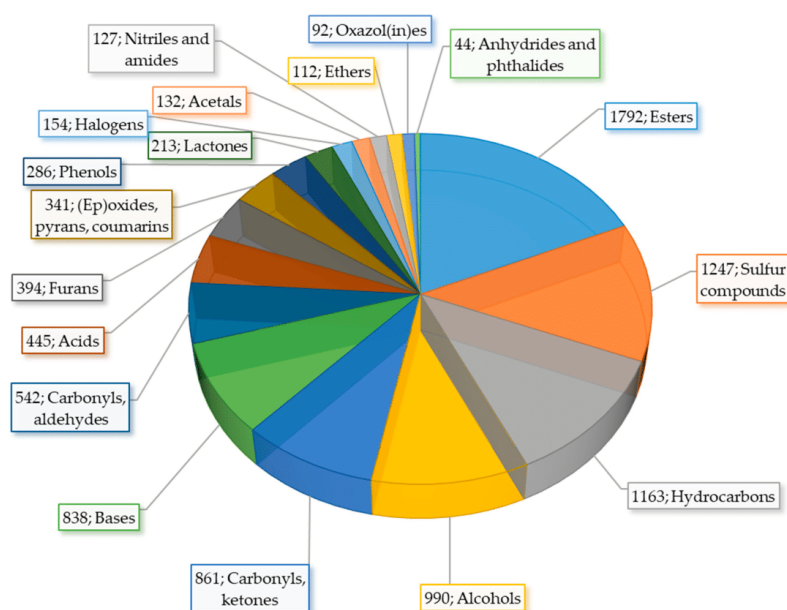


Figure 1. Volatile compounds in food according to volatile compounds in food database based on [28].

The main aim of this study is to summarize information and highlight the basic function of sulfur compounds in the creation of the aroma of vegetables and mushrooms. Although the main focus is on vegetables as a source of sulfur aroma compounds (mainly *Allium* and Brassica), mushrooms were included in the review, as some of their volatiles characteristics are shared with *Allium* vegetables, partially the pathways in the formation of aroma compounds. The formation of sulfur aroma compounds and their precursors are summarized, as well as their role as key odorants. The influence of food processing, namely cooking, baking, freezing, and frying, on sulfur compounds will be discussed. Apart from their aroma, sulfur compounds are also crucial in the creation of the taste of vegetables, especially Brassica; however, the present review is focused solely on aroma compounds.

2. Precursors of Volatile Sulfur Compounds

2.1. Amino Acids

The proteins that build every living organism consist of 20 standard or canonical amino acids—aliphatic, which are the most prevalent, aromatic, and heterocyclic. Nine

of them must be supplied through the diet, as they cannot be produced in the human organism [29]. Amino acids control the nutrostat system, take part in regulating blood pressure, can be used as an alternative source of fuels and biosynthetic materials, and show crucial importance as antioxidants and protective agents, especially when they contain sulfur in their structure [30–32].

Amino acids that have a sulfhydryl group in their structure—sulfur amino acids (SAAs)—are represented by cysteine and methionine [33]. The main function of cysteine in plants is maintaining an inorganic sulfur level obtained from the environment. This amino acid is the sole sulfide donor needed for the biosynthesis of crucial biomolecules, in particular, antioxidants (glutathione), cofactors, vitamins, or defense compounds—glucosinolates, thionins, phytoalexins. Methionine belongs to the group of essential amino acids, and it determines the development and is potentially responsible for the biosynthesis of growth-regulating substances (auxins, brassinosteroids, and cytokinins) [34]. It is also an important precursor to the biosynthesis of carnitine, cysteine, lecithin, taurine, and phospholipids. SAAs, such as methionine and cysteine, are the principal source of sulfur in the human diet supplied with food of plant origin. It was estimated that approximately 70% of sulfur obtained from plant products is accumulated in amino acids [1]. However, alliaceous and cruciferous vegetables have lower sulfur levels accumulated in amino acids in favor of specialized metabolites (Figure 2).

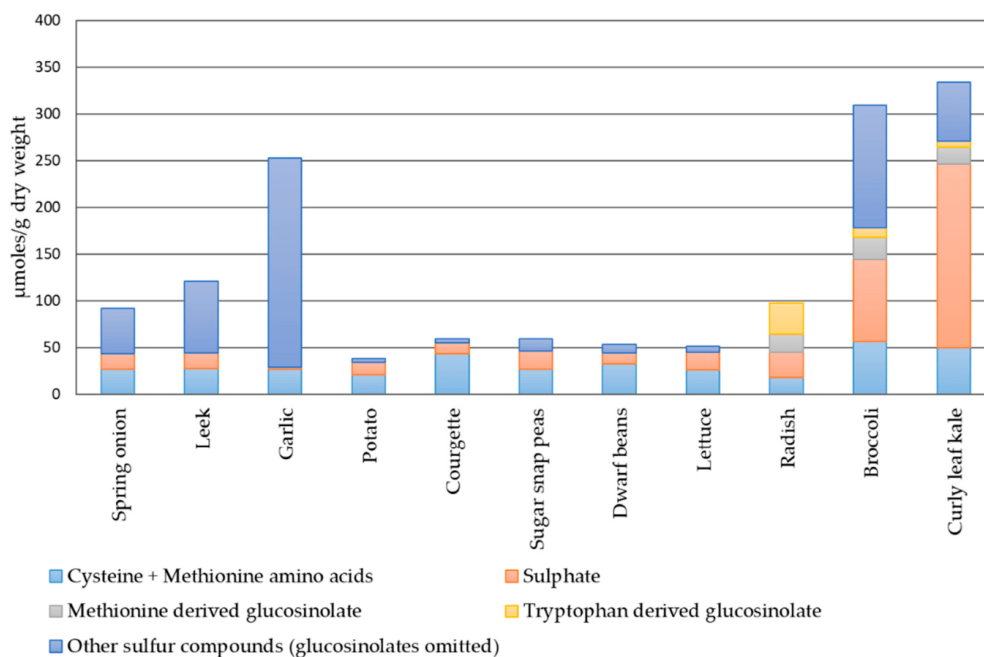


Figure 2. Sulfur distribution in selected vegetables based on [35].

2.2. Glucosinolates

Glucosinolates are amino acid-derived secondary plant metabolites containing a sulphate and a thioglucose moiety (Figure 3). Their origin from particular amino acid structures determines their classification into aliphatic (usually methionine derived), aromatic (phenylalanine and tyrosine derived), or indolyl (originating from tryptophan). The GLSs biosynthesis comprises three stages: (i)—synthesis of chain elongated aminoacids; (ii)—glucose common to all glucosinolates addition and; (iii)—side chain modification [36]. In the

process of side chain elongation, methionine can be elongated to homo, then di homo, and finally tri homo-methionine. The formation of glucone moiety starts from aminoacids conversion into oximes. Starting points for oximes formation are tyrosine, phenylalanine, methionine (with potential chain extension), and tryptophan. This yields the basic type sidechains, as listed in Table 2. The oximes are oxidized via aci-nitro compound, which serves as an acceptor for a thiol donor (preferably cysteine). The activated form of glucose (UDPG, uridine diphosphate glucose) is incorporated forming desulphoglucosinolate in S-glucosylation, transferred into glucosinolate in the presence of 3'-phosphoadenosine-5'-phosphosulfate [36,37]. For a detailed description on GLS biosynthesis, one can refer to the thorough review in [38].

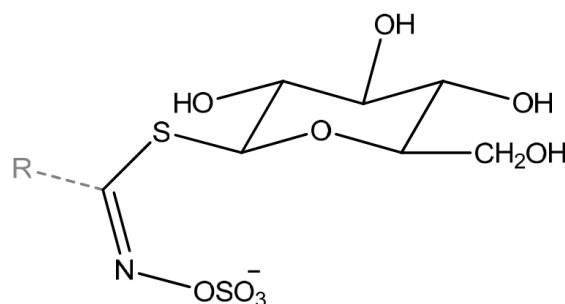
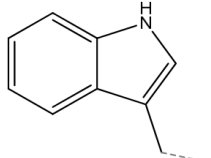
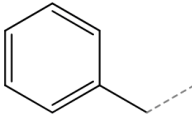
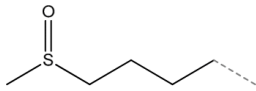
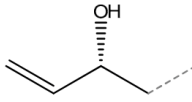


Figure 3. Structure of GLSs; R expresses side chain.

GLSs are naturally occurring compounds found in 16 dicotyledonous families. As mentioned above, significant amounts of GLSs occur in plants of the *Brassicaceae* family, though they are also present at a high level in the *Capparaceae* and *Caricaceae* families. By 2012, around 132 natural GLSs were documented [39]. The content of GLSs in the plant depends on plant organs, species, and vegetable development stage, for example, broccoli sprouts have 10–100 times higher concentrations of glucoraphanin, in contrast to mature broccoli.

GLSs are non-volatile compounds and their taste is associated with cruciferous vegetables. Though GLSs are non-odoriferous, their taste influences the characteristic flavor of Brassica vegetables. It is generally approved that bitterness is related to the presence of these sulfur-containing compounds in the plant [40]. Nevertheless, there is currently limited research focusing on the sensory evaluation of pure compounds and estimation of their impact on overall flavor assessment. The sensory evaluation of GLSs is problematic due to the personal abilities of panelists (non-tasters, bitter blind), limited quantities, or the lack of suitable and pure standards that could be used in taste sensory tests. These reasons contribute to a lack of taste knowledge for most of the studied GLSs [41]. The existing studies showed that there are differences in the perception of GLSs bitterness. Taste evaluation of four GLSs indicated that most taste panelists (71% for sinigrin and 79% for gluconapin) found a bitter taste. However, in the case of glucobrassicin and progoitrin, only 21% and 9% of panelists, respectively, stated a bitter taste. A bitter mouthfeel of GLSs may be reduced by the application of debittering processes, such as selective breeding, non-bitter cultivars, or customized growth conditions [42].

Table 2. Chemical structure of glucosinolate side chain [43,44].

Name	Structure of Side Chain	Systematic Name	Type of Glucosinolate
Glucobrassicin		3-indolylmethyl glucosinolate	Indole
Glucotropeolin		benzyl glucosinolate	Aromatic
Glucoraphanin		4-methylsulfinylbutyl glucosinolate	Aliphatic
Progoitrin		2(R)-2-hydroxy-3-butenyl glucosinolate	Aliphatic
Sinigrin		allyl glucosinolate	Aliphatic

According to the scientific literature, the great importance on bitter taste sensation has the concentration of plant constituents with a sweet mouthfeel, e.g., sugars and amino acids. In reference to the first, a sensation of bitter taste in cruciferous vegetables can be reduced by sweetness caused by the presence of free sugars, such as fructose, galactose, glucose, or sucrose. Vegetables differ in the ratio of free sugars to GLSs; higher ratio value results in desirable consumer preferences. Amino acids, such as alanine, proline, serine, and threonine, induce a sweet taste against leucine and valine, which are responsible for bitterness mouthfeel [42]. Some research showed an association between the content of sweet-tasting amino acids and reducing the effect in a bitter taste. However, the correlation between amino acid levels and GLSs content in vegetables requires more attention and additional research [41].

The moiety of GLSs consist of an unchanging part β -D-thioglucoside-N-hydroxysulfates and side chain, which is different for each GLS because it is a derivative of one of eight amino acids (Figure 3). GLSs are divided, according to the amino acids from which the side chain is formed, into aliphatic (Ala, Leu, Ile, Met, Val), aromatic (Phe, Tyr), and indole (Trp) GLSs; nevertheless, side chain is generally subjected to many modifications, such as acylation, desaturation, elongation, glycosylation, hydroxylation, and O-methylation [43]. The side chain variety of selected GLSs is shown in Table 2. The side chain of GLSs determines the character and structure of volatile compounds released after GLSs enzymatic or thermal degradation.

The enzyme myrosinase catalyzes the breakdown of GLSs. To initiate the hydrolysis reaction, it is necessary to disrupt the plant tissue (chewing, chopping, crushing, cutting) because GLSs are found in the cytoplasm, whereas the enzyme is stored in vacuoles. The first stage of hydrolysis degradation is the cleavage of the β -D-glucose molecule and obtaining an unstable intermediate product called an aglycone. Aglycone is subject to a spontaneous Lossen rearrangement, causing detachment of sulfate ion and depending on many factors (e.g., pH, presence of Fe^{2+} ions, or specialized proteins) described below,

obtaining various aroma sulfur volatile compounds, such as epithionitrile, ITC, nitrile, oxazolidine-2-thione, and thiocyanate [6,38,45].

3. Biosynthesis of Sulfur Volatiles in Vegetables

The formation of VSCs in vegetables has been intensively explored in *Allium* vegetables. The second group of vegetables with important and abundant VSCs described is *Brassicaceae*. These two groups of vegetables dominate, though S-methylmethionine derived compounds are formed in asparagus and other vegetables. The *Allium* genus comprises about 700 species, of which garlic (*Allium sativum*) and onion (*Allium cepa*) are the most important vegetables appreciated mainly for their unique flavor.

The formation of *Allium* flavor include: (i) enzyme-mediated degradation of non-volatile precursors; (ii) secondary reactions of primary flavor compounds, and; (iii) thermal degradation of precursors. Cysteine as a sulfur containing amino acid plays a crucial role in formation of flavor compounds of *Allium* vegetables along with glutamine, as (+)-S-alk(en)yl cysteine sulfoxides (CSOs) and their γ -glutamyl peptide (γ GPs) are the main non-volatile, odorless precursors [46]. Detailed review papers on biosynthesis of flavor compounds and their precursors have been published [46–48].

Garlic, onion, and other members of the *Allium* contain 1–5% dry weight of nonprotein SAA secondary metabolites. The disruption of the cell results in the release of allinase (C-S lyase) are present in vacuole and the reaction with S-alk(en)yl-L-cysteine S-oxides (volatile compounds precursors) located in the cytoplasm [49]. Four sulfoxides occur in *Allium* species: S-2-propenyl-L-cysteine sulfoxide (alliin, ACSO), S-(E)-1-propenyl-L-cysteine sulfoxide (isoalliin, 1-PeCSO), S-methyl-L-cysteine sulfoxide (methiin, MCSO, also present in Brassica vegetables), and S-propyl-L-cysteine sulfoxide (propiin, PCSO). Differences in flavor within *Allium* is caused in various amounts of these precursors [47]. As summarized by Block, based on experiments with labelled $^{35}\text{SO}_4^{2-}$ fed to onion plants, the sulfate is reduced and assimilated into cysteine in the chloroplasts. Then incorporation of glutamic acid yields γ -glutamylcysteine, which reacts with methacrylic acid (from valine) to yield γ -glutamyl-S-2-carboxypropylcysteine, which can undergo sequential decarboxylation to γ -glutamyl-S-1-propenylcysteine. Subsequently oxidation to γ -glutamyl-S-1-propenylcysteine-S-oxide takes place and it is cleaved by γ -glutamyl transpeptidase [EC 2.3.2.1] to S-(E)-1-propenylcysteine S-oxide [47].

The main enzymes, involved in the formation of *Allium* volatiles—allinases [EC 4.4.1.4]—are α,β -eliminating lyases that catalyze the decomposition of the above-mentioned S-oxides to ammonium pyruvate and allicin and its homologues. Sulfenic acids, which are highly unstable, are key enzymatically formed intermediates in *Allium* chemistry. The initial stages of sulfenic acids enzymatical formation has been summarized on Figure 4.

It is assumed that the flavor components of garlic and onion mainly include S-allyl cysteine sulfoxide (allicin); S-allyl-cysteine; and the sulfides of diallyl-, methyl allyl-, and dipropyl mono-, di-, tri-, and tetrasulfides, all being produced by sulfur-containing precursors—mainly S-alkenyl cysteine sulfoxides (ACSO) through an enzyme-mediated degradation process [48]. Allicin ($\text{C}_6\text{H}_{10}\text{OS}_2$), a key molecule in garlic flavor, is responsible for the typical smell and taste of freshly cut or crushed garlic. It accounts for 70% (w/w) of the total thiosulfinate compounds present/formed during the crushing of garlic cloves [50]. Allicin is very unstable and can easily be converted to diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), and diallyl tetrasulfide (DATTS).

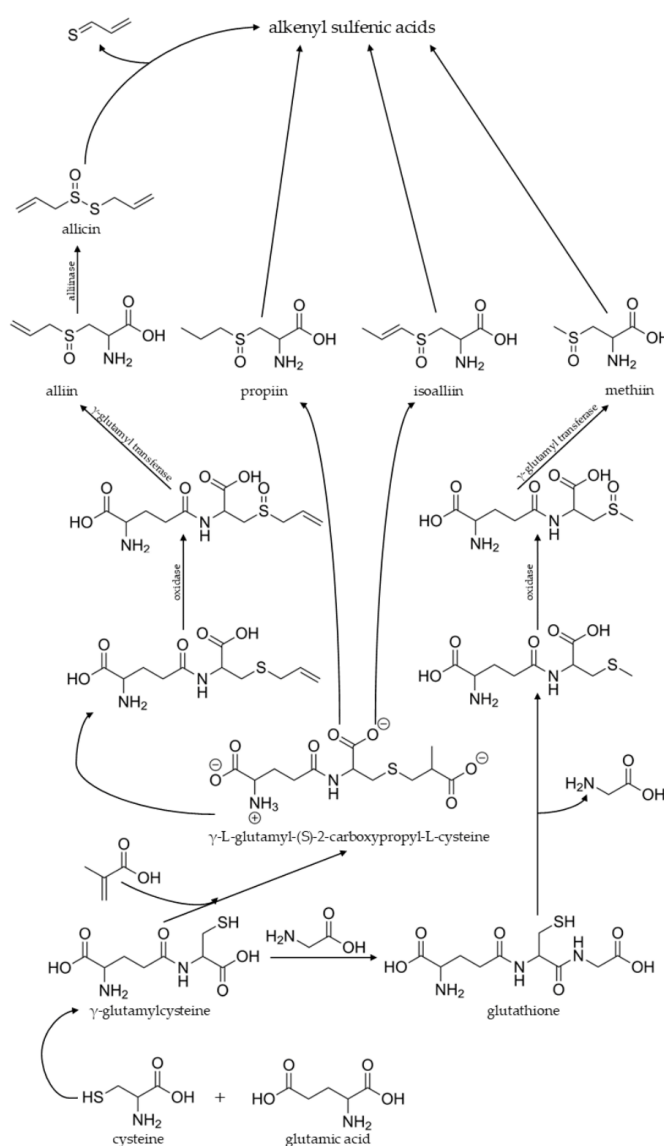


Figure 4. Initial stages of Allium flavor compounds formation by enzymatic reactions. Based on [47–49,51].

Due to the instability of primary flavor compounds of Allium, they can decompose or undergo condensation to secondary ones—these derived from alk(en)yl thiosulfinates. Allicin is the best example, as it undergoes degradation to a variety of sulfides (mainly DADS). It is sensitive to pH (>11), solvents, and temperature. At temperatures exceeding 50 °C, it decomposes rapidly [52]. It was investigated that heating S-methyl-L-cysteine and S-methyl-L-cysteine sulfoxide in a model system with varying water content and temperatures resulted in formation of volatile breakdown products of these precursors [53] that can

S-methylmethionine was identified in asparagus by Challenger and Hayward (1954), and it has been shown that it undergoes decomposition during food storage/thermal treatment into dimethyl sulfide being a part of boiled asparagus, processed tomatoes, corn, and beer flavor (Figure 6). The S-methylmethionine has been found in a range of 2.8–176 mg/kg in tomato, cabbage, turnip cabbage, beetroot, celery, and leek [55].

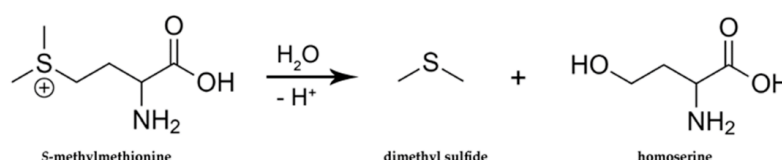


Figure 6. Thermal degradation of S-methylmethionine.

Sulfur containing acids were identified in asparagus and characterized in the 1970s, as precursors of aroma compounds [56,57]. They were 1,2-dithiolane-4-carboxylic acid (asparagusic acid), 3,3-dimercaptoisobutyric acid (dihydroasparagusic acid), and S-acetyldihydro asparagusic acid. It was found that sulfur compounds are formed in asparagus in the intact plant cells, which is an exceptional case in the formation of sulfur compounds, which are usually formed by enzymatic reactions. In asparagus, 13 sulfur containing acids were detected (of which asparagusic acid is predominant) and three esters (methyl 1,2-dithiolane-4-carboxylate being the dominant one). After performing [U-¹⁴C], labelled experiments valine was postulated as a starting molecule that is transformed via corresponding oxo acid, isobutyric acid, and methacrylic acid into 3-mercaptoisobutyric acid, 3-methylthioisobutyric acid, and, to a lesser extent, asparagusic acid [56]. In the cooking process, asparagus yields compounds belonging to different chemical classes: sulfur compounds (17), pyrroles, pyridines, pyrazines, furanes (25), aldehydes (12), ketones (15), alcohols (26), phenols (11), lactones (5), acids (10), and dimethyl sulfide and methyl 1,2-dithiolane-4-carboxylate (3000 ppb and 7000 ppb, respectively) [57].

Sulfides are formed in vegetables from the methionine or cysteine pathway or transforming other sulfur substrates, e.g., ITC rearrangement reactions [58]. For example, vegetables belonging to the *Amaryllidaceae* family include S-alk(en)yl-L-cysteine sulfoxides in the cytosol of storage mesophyll cells, whereas alliinase is sequestered in the vacuole of vascular bundle sheath cells vacuoles of plant cells [59]. The mechanism of sulfide formation is similar to that of the GLS-myrosinase system. Tissue disruption is required to allow the enzyme to contact the substrate and a series of unstable products is then created. Sulfenic acids are converted into thiosulfinates and, finally, into a complex mixture of compounds, in which mono-, di-, and polysulfides predominate [60]. Some studies show a close relationship between sulfide formation and the raised temperature necessary for industrial processes, for example, in preparing tomato paste or cooking cabbage [27]. The extension of the influence of industrial processes will be discussed in the following paragraphs.

After tissue disruption alliinase reacts with ASCOs and cleaves their C–S bond, obtaining sulfenic acid, pyruvic acid, and ammonia. Notoriously unstable sulfenic acids undergo additional chemical reactions according to the non-enzymatic pathway (self-condensation resulting in thiosulfinates) and enzymatic mechanisms (for example 1-propenyl sulfenic acid lachrymatory factor synthase (LFS) leads to propanethial S-oxide and, finally, in the presence of water forms propanal). Concisely, a series of sulfur organic compounds contributing to aroma creation (odor sensation, mouthfeel) and bioactive properties of *Allium* plants are spontaneously obtained from sulfenic acids in the absence of the enzyme that catalyzes the conversion of sulfenic acids [59,61]. Figure 7 shows the formation of sulfur compounds from CSOs, indicating the pathway to lachrymatory factor (in onion, propanethial-S-oxide), as well as a simplified route, leading to polysulfides and thiosulfinates.

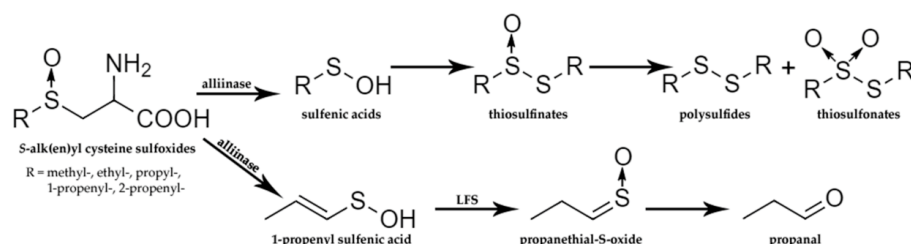


Figure 7. Transformations of cysteine sulfoxides (CSOs) after tissue disruption. LFS—lachrymatory factor synthase based on [61].

4. Main Groups of Volatile Sulfur Compounds

4.1. Isothiocyanates

In recent years, knowledge of ITCs has increased significantly. As a result of the growing interest in human health and research, it was possible to learn about many of the desired properties of these compounds. Most studies of ITCs properties are a concern to their chemopreventive and chemotherapeutic action [62]. Moreover, antimicrobial properties have been demonstrated, which can be used in agriculture for plant protection and food preservation as well [63]. Anti-inflammatory effects have been examined, respectively, for example, allyl ITC [64] and benzyl ITC [65].

Although GLSs are non-volatile and odorless precursors of their hydrolysis products ITCs [66], the pungent flavor of Brassica vegetables is associated with the presence of ITCs [67]. ITCs have specific aroma of a wide variety. There is a relationship between chemical structure and the odor threshold value. According to the increasing number of carbon atoms in aliphatic molecules, odor threshold value decreases, with the exception of ethyl ITC, which has a higher odor threshold value than methyl ITC. The chemical structure of ITCs (e.g., aliphatic saturated and unsaturated, aromatic, branched, cyclic, and miscellaneous) determines not only odor thresholds but odor quality too. Quite the non-obvious quality of the smell that ITCs described were sweet, fruity, floral, herbs, mushroom, or even fish descriptors. Of all recorded odor descriptors, the most frequent for ITCs are sulfur, garlic, and pungent smell [68] (Figure 8). Nevertheless, the sensory evaluation of ITCs odor is still limited, and there are not many studies that would allow us to compare odor quality and thresholds.

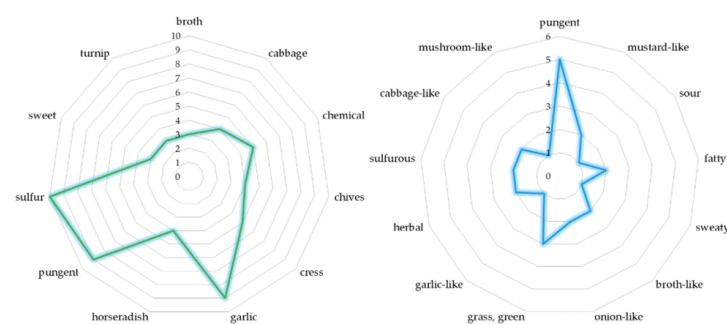


Figure 8. The most common odor descriptors for isothiocyanates (green) [68], nitriles, and epithionitriles (blue) [26,61–64] found in vegetables.

There are generally very limited, if any, data on the taste of ITC. They occur naturally in a complex food matrix, often in a large number. Insufficient information on bi-modal sensory activity of ITC, combining both their aroma and taste leaves many questions on their role in flavor formation unanswered. The quantitative determination of GLSs and

ITCs separately and statistical analysis showed correlations only between some compounds and bitter taste [41].

4.2. Nitriles and Epithionitriles

Nitriles and epithionitriles are produced by the rearrangement of unstable aglycone in the presence of specified cofactors, such as nitrile specifier proteins (NSP), epithiospecifier proteins (ESP), or specific environmental conditions [69,70]. The presence of nitriles and epithionitriles are significantly connected with edible parts and the stage of growth for crops from the *Brassicaceae* family. ITCs are the most abundant GLSs; hydrolysis produces edible parts, such as leaves in white cabbage, Savoy cabbage, or heads of Brussels sprouts, whereas nitriles and epithionitriles are mainly found in considerable concentrations in sprouts and seeds [71].

Although in scientific reports, occupational exposure to nitriles is commonly associated with numerous health disorders (e.g., cardiovascular, gastrointestinal, hepatic, neurologic, renal) [72], food borne nitriles also show cytotoxic and genotoxic potential [73]. The presence of nitriles in Brassica can be associated with health-promoting effects [74]. Compared to ITC and their bioactivity, relatively little is known about nitriles. Due to reduced beneficial health-promoting activities of nitriles, in comparison with ITCs, some strategies are used to select particular varieties of vegetables. They are characterized by either reducing the activity of ESP or increasing the activity of the native enzyme, myrosinase [70].

Nitriles as volatile compounds, in comparison to corresponding aldehydes, have comparable odors to corresponding aldehydes [75]. Nitriles and epithionitriles found in vegetables show diverse aromas from pleasant grassy, herbal, broth-like notes to unacceptable ones, such as pungent, sulfurous, or sweaty. Figure 8 summarizes the most common descriptors of nitriles and epithionitriles found in vegetables.

Taking into consideration that the taste of nitrile compounds is unknown, it is not possible to identify how nitrile derivatives influence taste in major vegetables, if at all. In the present day, we do not have data that indicate the bitter taste of nitriles and epithionitriles [41]. Therefore, further research is needed, in particular sensory analysis and taste evaluation of these GLS hydrolysis products.

4.3. Sulfides and Polysulfides

The presence of sulfide volatiles in food samples is commonly associated with an unpleasant and unacceptable odor. Indeed the cause of rejection of cauliflower is the presence of dimethyl trisulfide and dimethyl sulfide, among other odorants [76]. High concentrations of sulfides are present in vegetables from the *Brassicaceae* and *Amaryllidaceae* families [77,78]. Vegetables containing abundant organic sulfides in different species have strong antioxidant activities and these important bioactive compounds exhibit antibacterial and immune activities [79].

Sulfides play a crucial role in the creation of organoleptic properties of food, which is why many synthetic sulfides are commonly used as food flavors (more than 80 mono-, di-, and polysulfides) and additives in processed foodstuff [60]. Sulfides are also associated with the off-flavors of many other products, such as drinking water, alcoholic beverages, and juices [80–83]. The sensory properties of sulfides are similar to each other because these compounds are most often characterized by a pungent smell and a spicy taste [26,84,85].

4.4. Thiols and Miscellaneous Sulfur Compounds

Volatile thiols are mainly associated with off-flavors in various types of food and beverages [86,87]. Among the breakdown products of GLSs, thiols are an important group that determines the smell of vegetables, especially *Brassica* vegetables. Research shows that in raw broccoli, methanethiol and 1-pentanethiol are two aromatic active compounds with the highest flavor dilution factor. What is more, thiols can be oxidized to the corresponding sulfides [88]. Thiols were also found in green kohlrabi but were not the key flavor active

compounds [89]. The regulation of thiol biosynthesis in plants means their redox status is related to plant stress tolerance; nevertheless, the biochemical pathways have not been completely explained so far [90].

Interesting aroma-active compounds that contain a sulfur atom in their structure are odorants found in truffles and shiitake mushrooms, in which volatile compounds are formed in the enzymatic and non-enzymatic way. The compounds responsible for the aroma of shiitake mushrooms come from the fatty acid pathway and the amino acid pathway. Linolenic acid and linoleic acid are the precursors of the fatty acid pathway. They are converted by enzymes—lipoxygenase, hydroperoxide lyase, and alcohol dehydrogenase—to generate aldehydes and alcohols (mostly C6, C8, and C9 volatiles). Multi-step oxidation and polymerization of sulfur-containing amino acids provide precursors, namely lentinic acid, which is a precursor substance to form heterocyclic sulfur volatiles in the presence of enzymes, in particular, γ -glutamyl transpeptidase and CSO lyase [91]. Sulfur volatiles obtained from shiitake mushrooms responsible for distinctive, sulfurous aroma is related to the presence of 1,2,4-trithiolane, 1,2,4,6-tetrathiepane, and lenthionine [92]. However, in truffles, the origin of sulfur-containing volatiles is complex and unclear at the same time. Aroma-active compounds might be derived from the truffle itself but also the microbial population residing in truffle-fruited bodies. Organic volatile compounds are biosynthesized, mainly by sulfate reduction, amino acid, and fatty acid catabolism pathways [93,94].

An interesting flavor compound that has been found in many vegetables, as well as shiitake mushrooms and truffles, is methional. Biosynthesis begins with the amino acid methionine, which is converted to methional through Strecker degradation, in which intermediates in the Maillard reaction interact with the methionine. Methional as a volatile compound containing a sulfur atom in its structure is characterized by a low detection threshold, and the quality of its smell is defined as pleasant, reminiscent of a boiled potato. What is more, methional can be further converted into sulfide and disulfide compounds [95,96]. Table S1 (Supplementary Files) lists the main aroma sulfur compounds with their odor qualities, representing different classes found in vegetables and mushrooms.

5. Sulfur Compounds as Key Odorants in Vegetables

In the fundamental review on food key odorants, presence in various food products sulfur compounds are 16% of all (226) of them [97]. Along with aldehydes and esters, sulfur compounds form 45% of all key odorants (Figure 9). However, one has to remember that key odorants form roughly only <3% of all volatiles in foods.

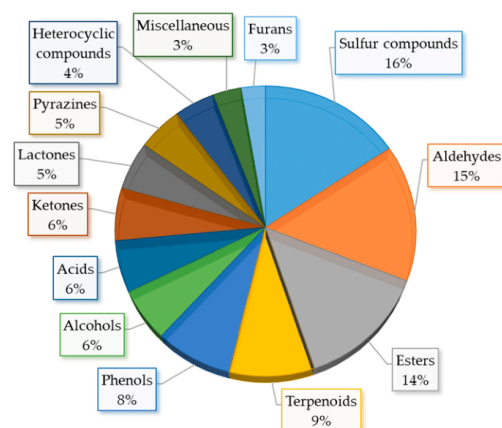


Figure 9. Key food odorants detected in food samples based on [97].

VSCs in food form a significant group of compounds, regarding their number and, what is probably more important, their odor thresholds and odor notes. As estimated for a piece of research conducted prior to 1991, a total of 633 VSC were detected in food [98], and one can presume that the number increased within last 30 years.

Although in many publications VSCs are regarded as compounds of potential influence on the aroma of vegetables, there are not many papers devoted to elucidation of their key odorants. The use of gas chromatography—olfactometry (GC-O)—to detect aroma compounds of foods and methods to quantify the sensory sensation, started the sensomics approach to characterize foods [99]. After vacuum distillation - the most frequent technique being SAFE (solvent-assisted flavor evaporation), it involves the application of GC-O to detect odor active regions in chromatogram and then identify compounds with the highest sensory role in the creation of the overall aroma of a product. Usually to present the aroma importance of particular compounds, FD (flavor dilution) is determined by subsequent GC-O analyses of a serially diluted extract of aroma compounds (AEDA, aroma extract dilution analysis). Moreover, the concept of OAV value (defined as ratio of compound concentration to its odor threshold) is used in the characterization of odorants [99].

Literature data on key odorants in vegetables can be divided into early works on crucial sensory active compounds, especially in *Allium* vegetables; some works are on other vegetables, mainly Brassica, and there are works, especially in recent years, on compounds migrating in the cold pressing of rapeseed oils, especially after roasting seeds [69,100–102].

The early works on VSC presence and also their sensory properties were summarized by Boelens (1993), who provided data for onion, garlic, and leek, but also truffle, tomato, and potato. In raw onion, thiopropanal-S-oxide, as well as propyl methanethiosulfonate and propyl propanethiosulfonate with OT 1700 and 1500 ng/L for the latter two compounds, respectively, were detected. Dipropyl disulfide and *cis*- and *trans*-1-propenyl propyl sulfide contributed to the aroma of cooked onion. After heating, they tend to form dimethylthiophenes with a distinct fried onion aroma. The most important odorants in garlic were di(2-propenyl)disulfide and di(2-propenyl)trisulfide. In leek, compounds possessing leek aroma were propanethiol, methylpropylsulfide, methylpropyldisulfide, and also 3,4-dimethyl-2,5-dioxo-2,5-dihydrothiophene, which can possibly hydrolyze in water to yield hydrogen sulfide [98].

Several Brassica vegetables were investigated using chromatography and olfactometry. In cauliflower, the main compounds responsible for sulfur odor notes detected by GC-O were methanethiol, perceived by sniffers as “sulfur, cooked cabbage”, dimethyl sulfide (DMS) described as “cauliflower”, allyl isothiocyanate described as “black mustard-like and pungent”, and dimethyl trisulfide (DMTS) with the odor note as “sulfur, cauliflower, cabbage”. Of 63 odor active compounds, 13 were sulfur compounds [103]. Key odorants of raw and cooked kohlrabi (*Brassica oleracea* var. *gongylodes* L.) were analyzed, and a total of 55 odor active compounds were detected and identified using GC-O. Twenty-eight compounds with the highest FD were quantified and OAV were determined. Of eight compounds with the highest OAV, five were sulfur compounds (dimethyl trisulfide, methyl 2-methyl-3-furyl disulfide, and three ITCs—1-isothiocyanato-3-(methylsulfanyl)propane, benzyl isothiocyanate, and 1-isothiocyanato-4-(methylsulfanyl)butane). The same compounds formed the backbone of both raw and cooked kohlrabi; differences were noted in OAV values and minor compounds [89]. Raw and cooked broccoli was subjected to GC-O and AEDA to assess the main odorants. Among 30 odor active compounds in raw broccoli, 19 were sulfur-containing ones. They were also characterized with the highest FD values. Two compounds with the highest FD were methanethiol and 1-pentanethiol (FD 1024). They were followed with dimethyl sulfide, dimethyl trisulfide, 2-methyl methanethio sulphonate, 4-methylpentyl isothiocyanate, hexyl isothiocyanate, dimethyl tetrasulfide, and 3-methylthiopropyl isothiocyanate (all FD 256). In contrast, cooked broccoli florets had only nine odor active compounds, among which dimethyl sulfide and dimethyl trisulfide were detected [88]. For *Brassica rapa* cv. Yukina, 12 odor active compounds were detected among 50 volatiles using GC-O. Among them, eight compounds contained sulfur.

The highest FD factors were obtained for dimethyl tetrasulfide, 3-phenylpropanenitrile (FD = 64), methional (FD = 32), and dimethyl trisulfide (FD = 16) [101].

For fermented Brassica vegetables, GC-O was used to assess the odor active compounds present in Kimchi. It is a traditional Korean fermented vegetable product in which Chinese cabbage is a main ingredient. Minor components can be red pepper, garlic, ginger, and fish sauce. For a Kimchi, in which Chinese cabbage was 86.1%, which also included garlic (1.4%) and <1% of leek, green onion, ginger, and carrot, 23 sulfur compounds were detected among 160 volatiles. The highest abundances were noted for DADS, methylallyl disulfide, dimethyl trisulfide, and dimethyl disulfide, whereas the highest odor intensities were noted for dimethyl trisulfide, DADS, and DATS [104]. In another fermented vegetable called Yongchuan Douchi (fermented soybean)—a traditional Chinese product—49 aroma active compounds were detected by GC-O. Twenty-two compounds were characterized by determining FD and OAV. Among these 20 key odorants, two sulfur compounds were playing an important role, where dimethyl trisulfide was characterized by an OAV of 8818—the highest OAV. For 3-(methylthio)propionaldehyde OAV was 229 [105]. Tempeh prepared from soy, fermented with *R. oligosporus* for 1 and 5 days, showed 21 odor active compounds. Among the three were sulfur compounds—dimethyl sulfide, dimethyl trisulfide, and 3-(methylthio)propanal. After 5 day fermentation, 3-(methylthio)propanal was characterized by the highest OAV (680) of all 21 compounds. Dimethyl trisulfide was also among the seven most potent odorants with OAV 120 [106]. In fermented fresh garlic, known as black garlic in Asia, 52 aroma compounds were detected after SAFE and SPME extraction and when assessed using FD, 24 compounds with the log2FD >2 were selected. Nine of them were sulfur compounds with the highest log2FD values noted for allyl methyl trisulfide (8), 2-vinyl-4H-1,3-dithiine (6), DATS (5), 3-vinyl-1,2-dithiacyclohex-4-ene (5), and 3-(methylthio)propionaldehyde. The highest OAV value calculated for black garlic were noted as 5-heptyldihydro-2(3H)-furanone (536) followed by DADS (188) and (E,Z)-2,6-nonadien-1-ol (134) [107].

Aged garlic extract (ethanolic, AGE) aged for more than 10 months was compared to fresh garlic. When AEDA was performed, fresh garlic compounds with the highest FD factor were 2-vinyl-4H-1,3-dithiine (FD 65,536), followed by S-methylmethanethiosulfinate (FD 256), 3-vinyl-4H-1,2-dithiine, methional (both FD 128), diallyldisulfide, allyl methyl trisulfide (FD 64), and allyl mercaptan (FD 32). All identified odorants of fresh garlic were sulfur compounds. Moreover, 2-vinyl-4H-1,3-dithiine was the main odorant in AGE, however, it was followed by phenolic compounds absent in fresh garlic [24].

Dimethyl trisulfide was detected by GC-O as an odor active compound in dried bell peppers [108]. Methional was characterized with an FD of 512 and was detected in Hungarian sweet bell pepper powder, however, the most intense odorants in it were β -ionone (FD 32768), furaneol (FD 16384), 2 and 3-methylbutanoic acid, and sotolon (FD 8192). The role of methional as an odor active compounds in Moroccan sweet bell pepper powder was more significant [109]. In red bell pepper, a secondary alkanethiol—2-heptanethiol with low OT (10 $\mu\text{g/L}$)—was detected [110]. Several sulfur compounds were detected in packed rocket leaves close to expiration date by GC-O: methanethiol, dimethyl sulfide, dimethyl disulfide, and 2,4-dithiapentane [111]. Interesting sulfur-containing acids were characterized in asparagus, and in cooked asparagus, dimethyl sulfide and methyl 1,2-dithiolane-4-carboxylate were predominant [56], but their role in the formation of asparagus flavor has not been elucidated by sensomics. The odorants of cooked asparagus explored by GC-O and Charm analysis revealed 3-(methylthio)propanal and S-methyl thioacetate as important odorants, though the highest Charm values were noted for 2-methoxy-3-isopropyl pyrazine [112]. Sulfur compounds also play an important role in the aroma of truffles. When aroma active compounds of white alba truffles (WAT) were compared to burgundy truffles (BT), among the 56 odorants obtained from SAFE extracts, 5 were sulfur compounds: bis(methylthio)methane, dimethyl trisulfide, 3-(methylthio)propanal, 3-(methylthio)propanol and 1,2,4-trithiolane. Their sensory importance was high: for WAT, the compound with the highest FD factor was 3-(methylthio)propanal (4096), followed

by bis(methylthio)methane (FD 1024). Interestingly, the last compound played a minor role in the aroma of BT when considering FDs. In the headspace, dimethyl sulfide and methanethiol were also detected. When OAV was calculated for key aroma compounds the highest value was observed for bis(methylthio)methane (817,000) in WAT, though not quantifiable for BT. Moreover, 3-(methylthio)propanal was characterized with very high OAV values (807 and 500), respectively [113].

The roasting process of rape seeds results in a grand increase in sulfur compounds responsible for aroma. When expressed as OAV, values for 2-furanmethanethiol in roasted rapeseeds was 14,200, followed by dimethyl trisulfide (13300) increased from unquantifiable values in raw rapeseed. Moreover, very high OAV were noted for methanethiol (1160), dimethyl sulfide (962), and 3-(methylthio)propanal (54). Among the 10 compounds with the highest OAV values, 5 were sulfur compounds. Similar results in profile of sulfur key odorants were noted for roasted mustard seeds, in which 2-furanmethanethiol was also the most pronounced odorant with an OAV of 36,300 [102]. When native, cold-pressed rapeseed oil was investigated using the sensomics approach, 54 aroma active compounds were noted, among them 47 were identified. Dimethyl trisulfide (FD 128) and 2-propionylthiazole (FD 512) were two sulfur compounds with high importance of flavor creation, though the highest FD values were noted for 2-isopropyl-3-methoxy pyrazine (IPMP) and 2-isobutyl-3-methoxypyrazine (IBMP) (both FD of 20148). Using HS-AEDA, dimethyl sulfide was also detected (FD 4). When OAV was calculated for odorants characterized by FD, the highest value was for IPMP (330), followed by dimethyl trisulfide (37) and dimethyl sulfide (37) [114]. Dimethyl trisulfide was one of the compounds responsible for fusty/musty defects in cold-pressed rapeseed. The FD values for this compound in fusty/musty rape seeds (2048) and oil obtained from these seeds (512) exceeded this in the control oil (128). When expressed in OAV, dimethyl trisulfide in fusty/musty oil (1900) and seeds (2900) exceeded the OAV for control oil (37) even more [115]. Pollner and Schieberle analyzed key odorants in cold pressed rapeseed oils from unpeeled and peeled seeds and among 60 compounds with FD > 2 dimethyl sulfide and dimethyl trisulfide were detected (FD 2 and 32, respectively, in oil from unpeeled seeds and FD 128 and 16 in oil from peeled seeds). The OAV values for DMS in commercial rapeseed oils (from peeled and unpeeled seeds) were as high as 480 and in 6 out of 10 samples were >200 [116]. Dimethyl sulfide was the key odorant in rapeseed oil obtained from traditional and high oleic rapeseed varieties, with dimethyl trisulfide also detectable [117].

In rapeseed oil obtained from microwaved seeds, four thiols with low odor thresholds were identified: 2-methyl-3-furanthiol (OT 0.481 µg/L), 2-furfurylmethanethiol (OT = 0.061 µg/L), phenylmethanethiol (OT 0.029 µg/L), and 3-sulfanyl-1-hexanol (OT 0.006 µg/L). The OAV in various rapeseed oils ranged from 2–28, 56–626, 104–3589, and 213–7565, respectively [118].

6. Influence of Vegetable Processing on Sulfur Compounds

Industrial processes are an important element in food production because they directly influence the preservation of its quality, extend the shelf life, or reduce in transport mass [119]. Dehydration and fermentation as preservation methods were utilized for centuries not only to preserve and extend the shelf life of food products but also to impart new, attractive sensory properties. Thermal processes are utilized due to the reduction in microbial population, destroying native enzymes and finally rendering food more palatable. Conversely, the main function of freezing technologies is the preservation of food as a result of lowered water activity and reduced rates of chemical reactions [120]. Nevertheless, there is no doubt that industrial processes have a considerable impact on the aroma of processed products, in particular, vegetables and mushrooms. The prudent choice of appropriate food processing methods from farm to consumer can guarantee that the health-promoting properties of specific bioactive compounds are preserved [121]. The research on quantitative aspects of flavor changes, involving especially sulfur compounds is limited. The majority of papers published focus on GLSs. A few selected industrial processes are presented in the following subsections.

Many VSCs are formed during a variety of industrial processes, in particular, thermal processes. Figure 10 shows the thermal degradation and the production of the most important groups of VSCs, such as GLSs, ITCs, alliin, thiols, sulfides, polysulfides, lenthionine, and sulfur-containing flavor compounds, derived from SAA as cysteine and methionine. This makes the influence of high temperatures a substantial factor in creation, as well as stability of VSCs. Thermal lability of many sulfur compounds contributes to dynamic changes in mutual proportions of volatile compounds in products and flavor changes in it.

6.1. Drying

Drying is probably the oldest method of food preservation. Today, there are many drying options: combined convective hot-air and non-thermal drying involve other technologies, such as reduced pressure, ultrasound, pulsed electric field, and ultraviolet technologies [119]. Vegetables and other food extracts can be used as additives to commercial food products [129].

Among various drying techniques (convective drying, freeze-drying, vacuum microwave drying, combined drying) of shiitake mushrooms, freeze-drying showed the best results in terms of volatile compounds content and sensory parameters [130]. The spray freeze-drying (SFD) method, first introduced as a new method for biopharmaceutical powder preparation, was used to produce the aromatic powder obtained from shiitake mushrooms. It turned out that, as a result of SFD, the content of volatile and aroma-active compounds in food was reduced. Concerning sulfur compounds, the percentage of recovery ranged from 30.9 to 68.3%. Other important key odorants showed similar or higher recoveries. The greatest losses after the SFD of volatile compounds containing sulfur concerned 2,3,5,6-tetrathiaheptane, dimethyl, trisulfide and disulfide, and methyl [129].

Raw shiitake mushrooms are almost odorless, therefore, mushroom aroma is created in enzymatic and non-enzymatic reactions. Sulfur volatiles involving straight-chain dimethyl disulfide, dimethyl trisulfide, 1-(methylthio)dimethyl disulfide, and cyclic sulfur compounds, including 1,2,4-trithiolane and lenthionine, are formed by enzymatic processes. The formation of cyclic sulfur compounds via non-enzymatic reactions, such as polymerization and degradation, is also possible for example formation 1,2,4-trithiolane from dimethyl disulfide [122].

6.2. Blanching

The main aim of blanching is the deactivation of degradation enzymes by short-term heat treatment. Blanching also eliminates air and inhibits oxidative processes, and it can remove some food-spoilage microorganisms. Generally, the temperature is set at a range of 85–100 °C (in water, steam, and less often by microwaving or application of infrared or radio waves). The thermal process takes up 60 to 150 s, and it is followed by rapid cooling, for example, by immersing the product in cold water. This short-term heating affects the composition and sensory properties, for example, blanching Brussels sprouts caused a significant decrease in the dry matter, antioxidant activity, total polyphenol, and ascorbic acid content [131,132]. Among the heat treatment processes, blanching is one of the mildest and recommended to minimize the loss of GLSs and their derivatives [133].

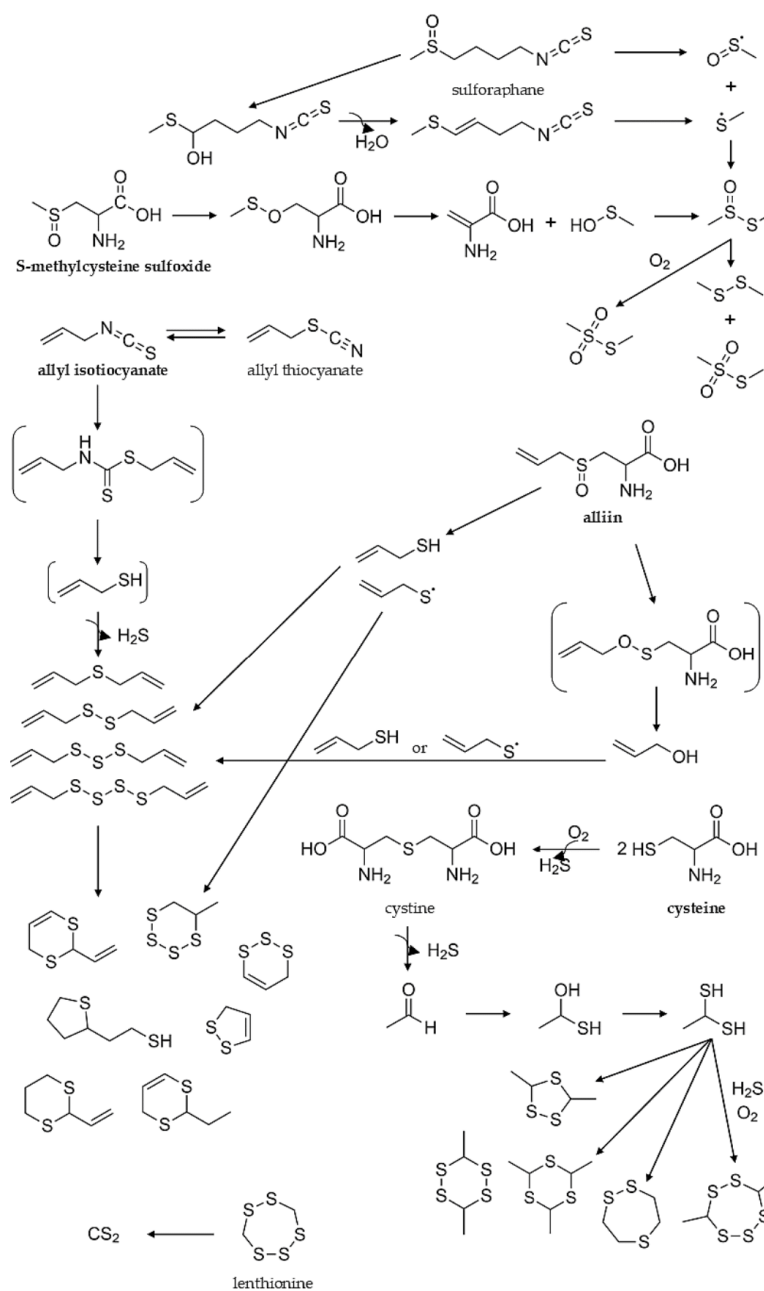


Figure 10. Thermal degradation of sulfur compounds in vegetables and mushrooms [122–128].

Despite the short treatment period with temperature, the content of GLSs in cabbage after blanching in relation to fresh vegetables decreased [134]. The loss of GLSs may be

explained by leaching into the heating water, enzymatic hydrolysis, or thermal degradation of GLSs [135]. The use of blanching for radish also caused a reduction in the number of volatile compounds, in comparison with raw radish. Concerning organosulfur compounds, the amount of thiophene decreased three times; 1-menthen-8-thiol was observed at a similar level using E-nose analysis. Gas chromatography with mass spectrometry showed that there are no consistent trends in major sulfur volatile compounds between raw and blanched vegetables, with respect to specific classes of compounds. For example, the contents of methyl-, 3-butenyl-, hexyl-, 3-methylthiopropyl- isothiocyanate increased after blanching, whereas pentyl-, 4-methylpentyl- isothiocyanate and erucin, and berteroin had lower values after blanching. However, the content of sulfides during blanching decreases, which is best illustrated by dimethyl disulfide, dimethyl trisulfide, methyl (methylthio)methyl disulfide, and dimethyl tetrasulfide [136].

6.3. Cooking

Some *Brassica* vegetables are not commonly eaten raw but, after processing, are most often by cooking. Unfortunately, food processing, especially thermal processes, significantly changes the composition of bioactive and odor-active compounds, as well as the taste of the vegetable itself. Increased temperature also affects the possibility of denaturing the myrosinase enzyme and ESP. The inactivation of myrosinase and ESP mainly leads to an increasing trend in the production of nitriles relative to ITCs; however, in general, few hydrolysis products are formed from GLSs. Cooking promotes the formation of sulfur volatiles with the typical sulfurous odor of boiled *Brassica* vegetables, which is mainly related to the release of sulfides, such as dimethyl sulfide. Unfortunately, the effect of cooking on changing the taste is unknown [41].

Boiling in water is the most common method of cooking shiitake mushrooms. Changes in volatile compounds during boiling in water at 70 °C depend on the cooking time and on the form of the mushrooms used (raw or dried). In general, the short cooking time of raw mushrooms (up to 1 h) favors an increase in the concentration of volatile compounds; only the amount of 1-octen-3-one and dimethyl trisulfide decreased. A similar tendency was noticed during the cooking of dried mushrooms, however, apart from dimethyl trisulfide, the concentration of lenthionine decreased in the first hour of cooking. In the case of using raw mushrooms, long-term cooking (for 3 h) resulted in a decrease in the concentration of aroma-active compounds, such as 1-octen-3-one, 3-octanone, dimethyltrisulfide, 1-(methylthio)dimethyl disulfide, and 1,2,4,5-tetrathiane, while the concentration of dimethyl disulfide, 1-octen-3-ol, and 1,2,4-trithiolane, increased. In contrast, most long-term cooking of dried mushrooms increased in the number of volatile compounds, however, a decrease in the amount of three of them (dimethyl trisulfide, 1,2,4,5-tetrathiane, and lenthionine) was also observed. What is more, lenthionine is unstable during water boiling [122].

6.4. Steaming

Steaming involves exposing a vegetable to the steam generated from boiled water, whereby the vegetable is separated from the boiling water with which it is not in direct contact. Steaming is milder than cooking in boiling water; additionally, it resulted in lower rates of cell lysis and myrosinase inactivation. Steaming broccoli, green cabbage, cauliflower, and Brussels sprouts for 20 min has no significant effect on the total GLSs content, unlike cooking. During the first two minutes of the steaming process, myrosinase activity remains unchanged, but as steaming progresses, after 7 min, myrosinase activity is lost by 90.4% [137].

Studies have shown that ESP usually denatures at a lower temperature than myrosinase, which results in the advantage of ITCs over nitriles in volatile compounds obtained as a result of the breakdown of GLSs [41]. The steaming of broccoli and cauliflower decreased the overall odor profile in both vegetables with a reduction in the intensity of most volatiles but sulfur volatiles after steaming were more perceptible. However, the process favors the production of dimethyl trisulfide, which becomes the dominant odorant in steamed

vegetables. The odor intensity of ITCs created from corresponding GLSs increased three times, in comparison with raw vegetables, which confirms that the activity of myrosinase during steaming was retained [138].

While the use of steaming is commonly used in the preparation of vegetables, no use of steaming has been found for shiitake mushrooms or truffles.

6.5. Frying

Mushrooms need to be processed (e.g., cooked or fried) as food because they are not usually eaten raw. The heat treatment process favors the conversion of non-volatile precursors into volatile compounds. Nine odor active compounds were lost or degraded during frying and one of these compounds was methional. The greatest losses were related to the compounds responsible for the fungal odor of 1-octen-3-one and phenolic from 2-methoxyphenol. On the other hand, the concentration of several sulfur compounds were characteristic of the smell of shiitake mushrooms, e.g., lenthionine, dimethyl trisulfide, and 1,2,4,5-tetrathiane. Moreover, after frying, an increase in the compounds responsible for the desired odor notes was observed, such as caramel-like from 4-hydroxy-2,5-dimethyl-3(2H)-furanone and fatty fried from (*E,E*)-2,4-decadienal [139].

Vegetables are often prepared by frying by preheating oil in a pan and adding the selected vegetable. Stir-frying *Brassica* vegetables, such as broccolini or kale, reduces the total amount of GLSs. The loss of GLSs in frying is not as great as in cooking, where GLSs leaks directly into the water, which is the heating medium. Interestingly, frying these vegetables does not cause a considerable decrease in the content of ITCs, such as sulforaphane in broccolini or iberin content in kale, which means that the active enzyme myrosinase remained in the vegetable tissues even after the heating treatment was completed. A slight decrease in the number of ITCs compared, for example, to another thermal process, which is cooking, means that frying and steaming are classified as mild heating methods [140].

6.6. Freezing

Freezing is one of the most popular food processing methods, mainly used to extend its shelf-life. The quality of the frozen product depends on many factors, including the quality of the product itself, and the speed of the freezing process. Quick freezing leads to large amounts of small ice crystals and less tissue disruption, as opposed to slow freezing, which causes the formation of large intracellular crystals and the destruction of vegetable tissue) [132]. Freezing Brussels sprouts have a major impact on the biochemical pathways and, as a consequence, the volatile profile and aroma properties of raw vegetable samples. After freezing, reduced concentrations were observed for alcohols and bioactive ITC compounds; nevertheless, the number of nitriles, and aldehydes increased considerably. Changes in the ratio of alcohols to aldehydes are related to lipoxygenase activity, however, the increase in the number of nitriles to ITCs after freezing remains unclear at the moment. To maintain the health-promoting properties of *Brassica* vegetables (e.g., high content of ITCs), it is recommended to consume them when they have not been previously frozen [141].

Freezing has a significant effect on the truffle flavor, as opposed to the negligible effect of storing the mushrooms at the same time, but at a temperature of 4 °C [142]. Descriptive sensory analysis has shown that the aroma of black truffles can be defined by eight major descriptors, such as characteristic fresh truffle aroma, sulfurous, mushroom-like, moldy, animal-like, boiled potatoes-like, buttery, and cheesy. It has been observed that even the mildest freezing conditions at −20 °C for 24 h caused significant changes in the aroma of black truffles. More precisely, the characteristic aroma of truffles was reduced, but the intensity of the aroma remained at the same level due to the increase in odor notes, such as sulfurous, mushroom-like, boiled potatoes-like. The change in the odor of the frozen samples compared to the fresh truffle samples was explained on the basis of quantitative analysis. Frozen samples contained higher amounts of such compounds as diacetyl, 1-octen-3-one, 1-octen-3-ol, 2-methylisoborneol, and dimethyltrisulfide; however, isoamyl

alcohol, ethyl 3-methylbutyrate, and methionol decreased [143]. Summing up, freezing has a significant impact on the qualitative (descriptive quantitative analysis) and quantitative impact on the aroma of truffles [7].

6.7. Fermentation

Fermentation is both a food preservation method and a food processing method (improving not only the structure but also the flavor) that has been used since ancient times. The microorganisms are capable of processing some of the metabolites present in food by fermentation, resulting in the formation of novel flavor compounds, which impart a unique texture, flavor, and aroma to the processed food. It is mainly bacteria, which produce lactic acid or acetic acid, fungi (mostly yeasts), or molds (species *Mucor*, *Aspergillus*), that are used in food fermentation processes [144].

Brassica fermentation is a very popular food processing method to improve the flavor of raw vegetables. During fermentation, biochemical reactions and transformations occur with the participation of vegetable enzymes (e.g., myrosinase), native bacteria, and added fermentation microorganisms [145]. GLSs concentration decreases during the fermentation process, which is most likely the result of GLSs biotransformation into other metabolites. No GLSs was found in sauerkraut and fermented white cabbage, as GLSs degrades quickly, from 2 to 5 days of fermentation. Fermentation carried out by lactic acid bacteria not only produces lactic acid but also enriches the fermentation product with bioactive compounds. What is more, fermentation supports the biotransformation of glucoraphanin to sulforaphane [146]. A synergistic effect of microorganisms (*Lactobacillus plantarum* and *Leuconostoc mesenteroides*) was also observed, which resulted in a 16-fold increase in sulforaphane concentration in fermented white cabbage, compared to samples where the microorganisms were used separately [147].

In order to refine the characteristic mushroom aroma of shiitake mushrooms, the food industry uses various food processing methods, including fermentation. The results of fermented shiitake mushroom samples showed that the acidity, total free amino acid content, and total flavor nucleotides increased throughout the fermentation process, indicating that *Saccharomyces cerevisiae*, *Aspergillus oryzae*, *Aspergillus niger*, and *Lactobacillus plantarum* species had the ability to improve the flavor substances of the shiitake mushroom. *Lactobacillus plantarum* species showed the greatest influence on the improvement of taste and aroma in the fermentation process. The taste activity value of free amino acids and flavor nucleotides were both higher than one, suggesting that those flavor substances have a significant influence on the fermented food taste [144].

7. Conclusions and Perspective

Over the years, there have been many attempts to determine what molecular determinants affect the smell of chemical compounds—molecular mass, polarity, presence of hydrogen bonding, stability, symmetry of the molecule, and volatility. Sulfur as a heteroatom in molecules, compared to an oxygen atom from the same group of the periodic table, significantly increases sensory properties. It is believed that this effect is associated with the electronic capabilities of these two atoms. Unlike oxygen, sulfur has the option of expanding d-orbital to 10 electrons on the valence shell [11].

Flavor research indicates that VSCs are responsible for the remarkable flavor of many food products. For vegetables of Allium and Brassica, but also for some mushroom they are crucial for their characteristic aroma. However, considering the dynamic formation of sulfur-containing volatiles in enzymatic reactions, their instability, and rapid decomposition of some intermediates, analysis of them is extremely challenging. It starts from matrix issues, easily undergoing oxidation processes, low detection limits required to quantify aroma relevant concentrations, and also the possibilities of the creation of artefacts in gas chromatography injection port. VSCs are listed among the most potent odorants found in raw materials and foods. With their extremely low odor thresholds, they contribute to the characteristic flavor of many foods. However, their very often low odor thresholds and

low concentrations make the analytical process difficult. For vegetables, especially Brassica, further research using the sensomics approach is necessary to fully explore their flavor potential. There is still insufficient data on odor/taste thresholds of many VSC and even GLSs to fully explore the flavor of Brassica.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/molecules27186116/s1>. Table S1. Variety of Sulfur Compounds in Vegetables and Mushrooms.

Author Contributions: Conceptualization, literature reviewing, writing, and editing of the manuscript M.A.M. Conceptualization, writing, and revision of the manuscript H.H.J. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the National Science Centre (Poland) under Grant No. 548 2015/18/M/NZ9/00372.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Prasad, R. Major Sulphur Compounds in Plants and their Role in Human Nutrition and Health—An Overview. *Proc. Indian Natl. Sci. Acad.* **2014**, *80*, 1045–1054. [\[CrossRef\]](#)
2. Albuquerque, T.G.; Nunes, M.A.; Bessada, S.M.F.; Costa, H.S.; Oliveira, M.B.P.P. 14—Biologically active and health promoting food components of nuts, oilseeds, fruits, vegetables, cereals, and legumes. In *Chemical Analysis of Food*, 2nd ed.; Pico, Y., Ed.; Academic Press: Cambridge, MA, USA, 2020; pp. 609–656, ISBN 978-0-12-813266-1.
3. Shashirekha, M.N.; Se, M.; Rajarathnam, S. Status of Bioactive Compounds in Foods, with Focus on Fruits and Vegetables. *Crit. Rev. Food Sci. Nutr.* **2013**, *55*, 1324–1339. [\[CrossRef\]](#)
4. Barba, F.J.; Orlén, V. Processing, bioaccessibility and bioavailability of bioactive sulfur compounds: Facts and gaps. *J. Food Compos. Anal.* **2017**, *61*, 1–3. [\[CrossRef\]](#)
5. Bohinc, T.; Goreta Ban, S.; Ban, D.; Trdan, S. Glucosinolates in plant protection strategies: A review. *Arch. Biol. Sci.* **2012**, *64*, 821–828. [\[CrossRef\]](#)
6. Hanschen, F.S.; Lamy, E.; Schreiner, M.; Rohn, S. Reactivity and stability of glucosinolates and their breakdown products in foods. *Angew. Chem. Int. Ed. Engl.* **2014**, *53*, 11430–11450. [\[CrossRef\]](#)
7. Splivallo, R.; Ebeler, S.E. Sulfur volatiles of microbial origin are key contributors to human-sensed truffle aroma. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 2583–2592. [\[CrossRef\]](#)
8. Luo, D.; Wu, J.; Ma, Z.; Tang, P.; Liao, X.; Lao, F. Production of high sensory quality Shiitake mushroom (*Lentinus edodes*) by pulsed air-impingement jet drying (AID) technique. *Food Chem.* **2021**, *341*, 128290. [\[CrossRef\]](#)
9. Dai, X.; Stanilka, J.M.; Rowe, C.A.; Esteves, E.A.; Nieves, C.; Spaiser, S.J.; Christman, M.C.; Langkamp-Henken, B.; Percival, S.S. Consuming Lentinula edodes (Shiitake) Mushrooms Daily Improves Human Immunity: A Randomized Dietary Intervention in Healthy Young Adults. *J. Am. Coll. Nutr.* **2015**, *34*, 478–487. [\[CrossRef\]](#)
10. Patel, S.; Rauf, A.; Khan, H.; Khalid, S.; Mubarak, M.S. Potential health benefits of natural products derived from truffles: A review. *Trends Food Sci. Technol.* **2017**, *70*, 1–8. [\[CrossRef\]](#)
11. Shankaranarayana, M.L.; Raghavan, B.; Abraham, K.O.; Natarajan, C.P.; Brodnitz, H.H. Volatile sulfur compounds in food flavors. *C R C Crit. Rev. Food Technol.* **1974**, *4*, 395–435. [\[CrossRef\]](#)
12. Demirkol, O.; Adams, C.; Ercal, N. Biologically important thiols in various vegetables and fruits. *J. Agric. Food Chem.* **2004**, *52*, 8151–8154. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Ganesan, B.; Weimer, B.C.; Qian, M.C.; Burbank, H.M. Compounds associated with cheese flavor. In *Improving the Flavour of Cheese*; Woodhead Publishing Ltd.: Sawston, UK, 2007; pp. 26–51. [\[CrossRef\]](#)
14. Surur, A.S.; Schulig, L.; Link, A. Interconnection of sulfides and sulfoxides in medicinal chemistry. *Arch. Pharm.* **2019**, *352*, 1800248. [\[CrossRef\]](#)
15. Parker, J.K. 1—Introduction to aroma compounds in foods. In *Flavour Development, Analysis and Perception in Food and Beverages*; Woodhead Publishing Series in Food Science, Technology and Nutrition; Parker, J.K., Elmore, J.S., Methven, L., Eds.; Woodhead Publishing: Sawston, UK, 2015; pp. 3–30, ISBN 978-1-78242-103-0.
16. Jayasena, D.D.; Ahn, D.U.; Nam, K.C.; Jo, C. Flavour chemistry of chicken meat: A review. *Asian-Australas. J. Anim. Sci.* **2013**, *26*, 732–742. [\[CrossRef\]](#)

17. Nursten, H.E. *The Maillard reaction: Chemistry, Biochemistry, and Implications* 2005; Royal Society of Chemistry (Great Britain), Ed.; Royal Society of Chemistry: London, UK.
18. Borlinghaus, J.; Foerster, J.; Kappler, U.; Antelmann, H.; Noll, U.; Gruhlke, M.C.H.; Slusarenko, A.J. Allicin, the odor of freshly crushed garlic: A review of recent progress in understanding allicin's effects on cells. *Molecules* **2021**, *26*, 1505. [\[CrossRef\]](#)
19. Iranshahi, M. A review of volatile sulfur-containing compounds from terrestrial plants: Biosynthesis, distribution and analytical methods. *J. Essent. Oil Res.* **2012**, *24*, 393–434. [\[CrossRef\]](#)
20. Adams, A.; Kimpe, N. De Maillard Flavor Compounds. *Chem. Rev.* **2006**, *106*, 2299–2319. [\[CrossRef\]](#)
21. Meng, Q.; Hatakeyama, M.; Sugawara, E. Formation by yeast of 2-furanmethanethiol and ethyl 2-mercaptopropionate aroma compounds in Japanese soy sauce. *Biosci. Biotechnol. Biochem.* **2014**, *78*, 109–114. [\[CrossRef\]](#)
22. Flaig, M.; Qi, S.; Wei, G.; Yang, X.; Schieberle, P. Characterization of the Key Odorants in a High-Grade Chinese Green Tea Beverage (*Camellia sinensis*; *Jingshan cha*) by Means of the Sensomics Approach and Elucidation of Odorant Changes in Tea Leaves Caused by the Tea Manufacturing Process. *J. Agric. Food Chem.* **2020**, *68*, 5168–5179. [\[CrossRef\]](#)
23. Czerny, M.; Christlbauer, M.; Christlbauer, M.; Fischer, A.; Granvogl, M.; Hammer, M.; Hartl, C.; Hernandez, N.M.; Schieberle, P. Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. *Eur. Food Res. Technol.* **2008**, *228*, 265–273. [\[CrossRef\]](#)
24. Abe, K.; Hori, Y.; Myoda, T. Characterization of key aroma compounds in aged garlic extract. *Food Chem.* **2020**, *312*, 126081. [\[CrossRef\]](#)
25. Li, J.X.; Schieberle, P.; Steinhaus, M. Insights into the key compounds of durian (*Durio zibethinus* L. 'Monthong') pulp odor by odorant quantitation and aroma simulation experiments. *J. Agric. Food Chem.* **2017**, *65*, 639–647. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Kreissl, J.; Mall, V.; Steinhaus, P.; Steinhaus, M. *Leibniz-LSB@TUM Odorant Database, Version 1.0*; Leibniz-Institute for Food Systems Biology at the Technical University of Munich: Freising, Germany; Available online: <https://www.leibniz-lsb.de/en/databases/leibniz-lsb-tum-odorant-database> (accessed on 18 May 2021).
27. McGorin, R.J. The Significance of Volatile Sulfur Compounds in Food Flavors. In *Volatile Sulfur Compounds in Food*; ACS Symposium Series; American Chemical Society: Washington, DC, USA, 2011; Volume 1068, pp. 1–3, ISBN 9780841226166.
28. Available online: www.leffingwell.com/bacis1.htm (accessed on 13 September 2022).
29. Martinez, Y.; Li, X.; Liu, G.; Bin, P.; Yan, W.; Más, D.; Valdiviño, M.; Hu, C.-A.A.; Ren, W.; Yin, Y. The role of methionine on metabolism, oxidative stress, and diseases. *Amino Acids* **2017**, *49*, 2091–2098. [\[CrossRef\]](#)
30. Górka-Warzewicz, H.; Laskowski, W.; Kulykovets, O.; Kudlińska-Chylak, A.; Czacotko, M.; Rejman, K. Food Products as Sources of Protein and Amino Acids—The Case of Poland. *Nutrients* **2018**, *10*, 1977. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Lieu, E.L.; Nguyen, T.; Rhyne, S.; Kim, J. Amino acids in cancer. *Exp. Mol. Med.* **2020**, *52*, 15–30. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Luisi, G.; Stefanucci, A.; Zengin, G.; Dimmito, M.P.; Mollica, A. Anti-Oxidant and Tyrosinase Inhibitory In Vitro Activity of Amino Acids and Small Peptides: New Hints for the Multifaceted Treatment of Neurologic and Metabolic Disfunctions. *Antioxidants* **2018**, *8*, 7. [\[CrossRef\]](#)
33. Bin, P.; Huang, R.; Zhou, X. Oxidation Resistance of the Sulfur Amino Acids: Methionine and Cysteine. *Biomed Res. Int.* **2017**, *2017*, 9584932. [\[CrossRef\]](#)
34. Shekari, G.; Javanmardi, J. Application of Cysteine, Methionine and Amino Acid Containing Fertilizers to Replace Urea: The Effects on Yield and Quality of Broccoli. *Adv. Crop Sci. Technol.* **2017**, *5*, 283. [\[CrossRef\]](#)
35. Doleman, J.F.; Grisar, K.; Van Liedekerke, L.; Saha, S.; Roe, M.; Tapp, H.S.; Mithen, R.F. The contribution of alliaceous and cruciferous vegetables to dietary sulphur intake. *Food Chem.* **2017**, *234*, 38–45. [\[CrossRef\]](#)
36. Halkier, B.A.; Du, L. The biosynthesis of glucosinolates. *Trends Plant Sci.* **1997**, *2*, 425–431. [\[CrossRef\]](#)
37. Augustine, R.; Bisht, N.C. *Regulation of Glucosinolate Metabolism: From Model Plant Arabidopsis thaliana to Brassica Crops BT—Glucosinolates*; Mérillon, J.-M., Ramawat, K.G., Eds.; Springer International Publishing: Cham, Switzerland, 2017; pp. 163–199, ISBN 978-3-319-25462-3.
38. Chhajer, S.; Mostafa, I.; He, Y.; Abou-Hashem, M.; El-Domiaty, M.; Chen, S. Glucosinolate Biosynthesis and the Glucosinolate-Myrosinase System in Plant Defense. *Agronomy* **2020**, *10*, 1786. [\[CrossRef\]](#)
39. Al-Gendy, A.; Nemataallah, K.; Zaghloul, S.; Ayoub, N. Glucosinolates profile, volatile constituents, antimicrobial, and cytotoxic activities of *Lobularia libyca*. *Pharm. Biol.* **2016**, *54*, 3257–3263. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Westphal, A.; Riedl, K.M.; Cooperstone, J.L.; Kamat, S.; Balasubramaniam, V.M.; Schwartz, S.J.; Böhm, V. High-Pressure Processing of Broccoli Sprouts: Influence on Bioactivation of Glucosinolates to Isothiocyanates. *J. Agric. Food Chem.* **2017**, *65*, 8578–8585. [\[CrossRef\]](#) [\[PubMed\]](#)
41. Bell, L.; Oloyede, O.O.; Lignou, S.; Wagstaff, C.; Methven, L. Taste and Flavor Perceptions of Glucosinolates, Isothiocyanates, and Related Compounds. *Mol. Nutr. Food Res.* **2018**, *62*, 1700990. [\[CrossRef\]](#)
42. Drewnowski, A.; Gomez-Carneros, C. Bitter taste, phytonutrients, and the consumer: A review. *Am. J. Clin. Nutr.* **2000**, *72*, 1424–1435. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Halkier, B.A.; Gershenzon, J. Biology and biochemistry of glucosinolates. *Annu. Rev. Plant Biol.* **2006**, *57*, 303–333. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Bischoff, K.L. Chapter 40—Glucosinolates. In *Nutraceuticals*; Gupta, R.C.B.T.-N., Ed.; Academic Press: Boston, MA, USA, 2016; pp. 551–554, ISBN 978-0-12-802147-7.

45. Barba, F.J.; Nikmaram, N.; Roohinejad, S.; Khelifa, A.; Zhu, Z.; Koubaa, M. Bioavailability of Glucosinolates and Their Breakdown Products: Impact of Processing. *Front. Nutr.* **2016**, *3*, 24. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Jones, M.G.; Hughes, J.; Tregova, A.; Milne, J.; Tomsett, A.B.; Collin, H.A. Biosynthesis of the flavour precursors of onion and garlic. *J. Exp. Bot.* **2004**, *55*, 1903–1918. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Block, E. The Organosulfur Chemistry of the Genus *Allium*—Implications for the Organic Chemistry of Sulfur. *Angew. Chemie Int. Ed. Engl.* **1992**, *31*, 1135–1178. [\[CrossRef\]](#)
48. Li, J.; Dadmohammadi, Y.; Abbaspourrad, A. Flavor components, precursors, formation mechanisms, production and characterization methods: Garlic, onion, and chili pepper flavors. *Crit. Rev. Food Sci. Nutr.* **2021**, *1*–23. [\[CrossRef\]](#)
49. Mazza, G.; Ciaravolo, S.; Chiricosta, G.; Celli, S. Volatile flavour components from ripening and mature garlic bulbs. *Flavour Fragr. J.* **1992**, *7*, 111–116. [\[CrossRef\]](#)
50. Miron, T.; Rabinkov, A.; Mirelman, D.; Weiner, L.; Wilchek, M. A spectrophotometric assay for alliin and alliinase (Alliin lyase) activity: Reaction of 2-nitro-5-thiobenzoate with thiosulfates. *Anal. Biochem.* **1998**, *265*, 317–325. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Chin, H.-W.; Lindsay, R.C. Mechanisms of formation of volatile sulfur compounds following the action of cysteine sulfoxide lyases. *J. Agric. Food Chem.* **1994**, *42*, 1529–1536. [\[CrossRef\]](#)
52. Chong, K.; Zamora, M.; Tilakawardane, D.; Buckley, N.; Rego, J.; Liu, Y. Investigation of Alliin Stability in Aqueous Garlic Extract by High Performance Liquid Chromatography Method. *J. Sci. Res. Rep.* **2015**, *4*, 590–598. [\[CrossRef\]](#)
53. Kubec, R.; Dřhová, V.; Velíšek, J. Thermal Degradation of S-Methylcysteine and Its Sulfoxide Important Flavor Precursors of *Brassica* and *Allium* Vegetables. *J. Agric. Food Chem.* **1998**, *46*, 4334–4340. [\[CrossRef\]](#)
54. Birchfield, A.; McIntosh, C. Metabolic Engineering and Synthetic Biology of Plant Natural Products—A Minireview. *Curr. Plant Biol.* **2020**, *24*, 100163. [\[CrossRef\]](#)
55. Scherb, J.; Kreissl, J.; Haupt, S.; Schieberle, P. Quantitation of S-Methylmethionine in Raw Vegetables and Green Malt by a Stable Isotope Dilution Assay Using LC-MS/MS: Comparison with Dimethyl Sulfide Formation after Heat Treatment. *J. Agric. Food Chem.* **2009**, *57*, 9091–9096. [\[CrossRef\]](#)
56. Tressl, R.; Holzer, M.; Apetz, M. Formation of flavor components in asparagus. 1. Biosynthesis of sulfur-containing acids in asparagus. *J. Agric. Food Chem.* **1977**, *25*, 455–459. [\[CrossRef\]](#)
57. Tressl, R.; Bahri, D.; Holzer, M.; Kossa, T. Formation of flavor components in asparagus. 2. Formation of flavor components in cooked asparagus. *J. Agric. Food Chem.* **1977**, *25*, 459–463. [\[CrossRef\]](#)
58. Zhou, Q.; Tang, H.; Jia, X.; Zheng, C.; Huang, F.; Zhang, M. Distribution of glucosinolate and pungent odors in rapeseed oils from raw and microwaved seeds. *Int. J. Food Prop.* **2018**, *21*, 2296–2308. [\[CrossRef\]](#)
59. Yoshimoto, N.; Saito, K. S-Alk(en)ylcysteine sulfoxides in the genus *Allium*: Proposed biosynthesis, chemical conversion, and bioactivities. *J. Exp. Bot.* **2019**, *70*, 4123–4137. [\[CrossRef\]](#)
60. Munday, R. Harmful and beneficial effects of organic monosulfides, disulfides, and polysulfides in animals and humans. *Chem. Res. Toxicol.* **2012**, *25*, 47–60. [\[CrossRef\]](#)
61. Lokke, M.M.; Edelenbos, M.; Larsen, E.; Feilberg, A. Investigation of volatiles emitted from freshly cut onions (*Allium cepa* L.) by real time proton-transfer reaction-mass spectrometry (PTR-MS). *Sensors* **2012**, *12*, 16060–16076. [\[CrossRef\]](#) [\[PubMed\]](#)
62. Martin, S.L.; Royston, K.J.; Tollefsbol, T.O. The Role of Non-Coding RNAs and Isothiocyanates in Cancer. *Mol. Nutr. Food Res.* **2018**, *62*, e1700913. [\[CrossRef\]](#) [\[PubMed\]](#)
63. Dufour, V.; Stahl, M.; Bayse, C. The antibacterial properties of isothiocyanates. *Microbiology* **2015**, *161*, 229–243. [\[CrossRef\]](#) [\[PubMed\]](#)
64. Wagner, A.E.; Boesch-Saadatmandi, C.; Dose, J.; Schultheiss, G.; Rimbach, G. Anti-inflammatory potential of allyl-isothiocyanate—Role of Nrf2, NF-(κ) B and microRNA-155. *J. Cell. Mol. Med.* **2012**, *16*, 836–843. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Lee, Y.M.; Seon, M.R.; Cho, H.J.; Kim, J.-S.; Park, J.H.Y. Benzyl isothiocyanate exhibits anti-inflammatory effects in murine macrophages and in mouse skin. *J. Mol. Med.* **2009**, *87*, 1251–1261. [\[CrossRef\]](#)
66. Bertóti, R.; Böszörményi, A.; Alberti, Á.; Béni, S.; M-Hamvas, M.; Szőke, É.; Vasas, G.; Gonda, S. Variability of Bioactive Glucosinolates, Isothiocyanates and Enzyme Patterns in Horseradish Hairy Root Cultures Initiated from Different Organs. *Molecules* **2019**, *24*, 2828. [\[CrossRef\]](#)
67. Lin, C.M.; Kim, J.; Du, W.X.; Wei, C.I. Bactericidal Activity of Isothiocyanate against Pathogens on Fresh Produce. *J. Food Prot.* **2000**, *63*, 25–30. [\[CrossRef\]](#)
68. Marcinkowska, M.; Jeleń, H.H. Determination of the odor threshold concentrations and partition coefficients of isothiocyanates from *Brassica* vegetables in aqueous solution. *LWT* **2020**, *131*, 109793. [\[CrossRef\]](#)
69. Marcinkowska, M.; Jeleń, H.H. Inactivation of thioglucosidase from *sinapis alba* (white mustard) seed by metal salts. *Molecules* **2020**, *25*, 4363. [\[CrossRef\]](#)
70. Hanschen, F.S.; Schreiner, M. Isothiocyanates, nitriles, and epithionitriles from glucosinolates are affected by genotype and developmental stage in *Brassica oleracea* varieties. *Front. Plant Sci.* **2017**, *8*, 1095. [\[CrossRef\]](#) [\[PubMed\]](#)
71. Kołodziejcki, D.; Piekarska, A.; Hanschen, F.S.; Pilipczuk, T.; Tietz, F.; Kusznierevicz, B.; Bartoszek, A. Relationship between conversion rate of glucosinolates to isothiocyanates/indoles and genotoxicity of individual parts of *Brassica* vegetables. *Eur. Food Res. Technol.* **2019**, *245*, 383–400. [\[CrossRef\]](#)
72. Tani, H. Allyl nitrile: Toxicity and health effects. *J. Occup. Health* **2017**, *59*, 104–111. [\[CrossRef\]](#)

73. Kupke, F.; Herz, C.; Hanschen, F.S.; Platz, S.; Odongo, G.A.; Helmig, S.; Bartolomé Rodríguez, M.M.; Schreiner, M.; Rohn, S.; Lamy, E. Cytotoxic and genotoxic potential of food-borne nitriles in a liver in vitro model. *Sci. Rep.* **2016**, *6*, 37631. [\[CrossRef\]](#) [\[PubMed\]](#)
74. Hanschen, F.S.; Klopsch, R.; Oliviero, T.; Schreiner, M.; Verkerk, R.; Dekker, M. Optimizing isothiocyanate formation during enzymatic glucosinolate breakdown by adjusting pH value, temperature & dilution in *Brassica* vegetables & *Arabidopsis thaliana*. *Sci. Rep.* **2017**, *7*, 40807. [\[CrossRef\]](#) [\[PubMed\]](#)
75. Lan, L.; Huang, S.; Liu, Y.; Sun, B.; Tian, H. Preparation and odor characteristics of nitriles derived from aldehydes. *Flavour Fragr. J.* **2020**, *35*, 425–434. [\[CrossRef\]](#)
76. Feng, Y.; Albiol Tapia, M.; Okada, K.; Castaneda Lazo, N.B.; Chapman-Novakofski, K.; Phillips, C.; Lee, S.Y. Consumer Acceptance Comparison Between Seasoned and Unseasoned Vegetables. *J. Food Sci.* **2018**, *83*, 446–453. [\[CrossRef\]](#) [\[PubMed\]](#)
77. Oh, J.; Cho, I.H. The aroma profile and aroma-active compounds of *Brassica oleracea* (kale) tea. *Food Sci. Biotechnol.* **2021**, *30*, 1205–1211. [\[CrossRef\]](#)
78. Bastaki, S.M.A.; Ojha, S.; Kalasz, H.; Adeghate, E. Chemical constituents and medicinal properties of *Allium* species. *Mol. Cell. Biochem.* **2021**, *476*, 4301–4321. [\[CrossRef\]](#)
79. Wu, Z.Q.; Li, K.; Ma, J.K.; Huang, Q.; Tian, X.; Li, Z.J. Antioxidant activity of organic sulfides from fresh *Allium macrostemon* Bunge and their protective effects against oxidative stress in *Caenorhabditis elegans*. *J. Food Biochem.* **2020**, *44*, e13447. [\[CrossRef\]](#)
80. Antonopoulou, M.; Evgenidou, E.; Lambropoulou, D.; Konstantinou, I. A review on advanced oxidation processes for the removal of taste and odor compounds from aqueous media. *Water Res.* **2014**, *53*, 215–234. [\[CrossRef\]](#) [\[PubMed\]](#)
81. Wu, J.; Liu, Y.; Zhao, H.; Huang, M.; Sun, Y.; Zhang, J.; Sun, B. Recent advances in the understanding of off-flavors in alcoholic beverages: Generation, regulation, and challenges. *J. Food Compos. Anal.* **2021**, *103*, 104117. [\[CrossRef\]](#)
82. Cheng, Y.; Li, G.; Wu, H.; Huang, L.; Wang, H. Identification of Light-Induced Key Off-Flavors in Ponkan Mandarin Juice Using MDGC-MS/O and GC-MS/PFPD. *J. Agric. Food Chem.* **2021**, *69*, 14259–14269. [\[CrossRef\]](#)
83. Aboshi, T.; Musya, S.; Sato, H.; Ohta, T.; Murayama, T. Changes of volatile flavor compounds of watermelon juice by heat treatment. *Biosci. Biotechnol. Biochem.* **2020**, *84*, 2157–2159. [\[CrossRef\]](#) [\[PubMed\]](#)
84. Chhikara, N.; Devi, H.R.; Jaglan, S.; Sharma, P.; Gupta, P.; Panghal, A. Bioactive compounds, food applications and health benefits of *Parkia speciosa* (stinky beans): A review. *Agric. Food Secur.* **2018**, *7*, 46. [\[CrossRef\]](#)
85. Lv, C.; Wang, C.; Li, P.; Huang, Y.; Lu, X.; Shi, M.; Zeng, C.; Qin, S. Effect of Garlic Organic Sulfides on Gene Expression Profiling in HepG2 Cells and Its Biological Function Analysis by Ingenuity Pathway Analysis System and Bio-Plex-Based Assays. *Mediat. Inflamm.* **2021**, *2021*, 7681252. [\[CrossRef\]](#)
86. Lund, M.N.; Ray, C.A. Control of Maillard Reactions in Foods: Strategies and Chemical Mechanisms. *J. Agric. Food Chem.* **2017**, *65*, 4537–4552. [\[CrossRef\]](#)
87. Perestrelo, R.; Silva, C.; Câmara, J.S. Off-Flavors in Alcoholic Beverages: An Overview. In *Food Aroma Evolution*; CRC Press: Boca Raton, FL, USA, 2019; pp. 595–622.
88. Wiecezorek, M.N.; Majcher, M.A.; Jeleń, H.H. Identification of aroma compounds in raw and cooked broccoli. *Flavour Fragr. J.* **2021**, *36*, 576–583. [\[CrossRef\]](#)
89. Marcinkowska, M.; Frank, S.; Steinhaus, M.; Jeleń, H.H. Key Odorants of Raw and Cooked Green Kohlrabi (*Brassica oleracea* var. *gongylodes* L.). *J. Agric. Food Chem.* **2021**, *69*, 12270–12277. [\[CrossRef\]](#)
90. Zagorchev, L.; Seal, C.E.; Kranner, I.; Odjakova, M. A central role for thiols in plant tolerance to abiotic stress. *Int. J. Mol. Sci.* **2013**, *14*, 7405–7432. [\[CrossRef\]](#)
91. Li, W.; Chen, W.C.; Wang, J.B.; Feng, J.; Wu, D.; Zhang, Z.; Zhang, J.S.; Yang, Y. Screening candidate genes related to volatile synthesis in shiitake mushrooms and construction of regulatory networks to effectively improve mushroom aroma. *J. Sci. Food Agric.* **2021**, *101*, 5618–5626. [\[CrossRef\]](#) [\[PubMed\]](#)
92. Wang, S.L.; Lin, S.Y.; Du, H.T.; Qin, L.; Lei, L.M.; Chen, D. An insight by molecular sensory science approaches to contributions and variations of the key odorants in shiitake mushrooms. *Foods* **2021**, *10*, 622. [\[CrossRef\]](#) [\[PubMed\]](#)
93. Murat, C.; Payen, T.; Noel, B.; Kuo, A.; Morin, E.; Chen, J.; Kohler, A.; Krizsán, K.; Balestrini, R.; Da Silva, C.; et al. Pezizomycetes genomes reveal the molecular basis of ectomycorrhizal truffle lifestyle. *Nat. Ecol. Evol.* **2018**, *2*, 1956–1965. [\[CrossRef\]](#)
94. Splivallo, R.; Deveau, A.; Valdez, N.; Kirchhoff, N.; Frey-Klett, P.; Karlovsky, P. Bacteria associated with truffle-fruited bodies contribute to truffle aroma. *Environ. Microbiol.* **2015**, *17*, 2647–2660. [\[CrossRef\]](#)
95. Singh, K.; Tripathi, S.; Chandra, R. Maillard reaction product and its complexation with environmental pollutants: A comprehensive review of their synthesis and impact. *Bioresour. Technol. Reports* **2021**, *15*, 100779. [\[CrossRef\]](#)
96. Jansky, S.H. Potato Flavor. *Am. J. Potato Res.* **2010**, *87*, 209–217. [\[CrossRef\]](#)
97. Dunkel, A.; Steinhaus, M.; Kotthoff, M.; Nowak, B.; Krautwurst, D.; Schieberle, P.; Hofmann, T. Nature's chemical signatures in human olfaction: A foodborne perspective for future biotechnology. *Angew. Chem. Int. Ed. Engl.* **2014**, *53*, 7124–7143. [\[CrossRef\]](#) [\[PubMed\]](#)
98. Boelens, M.H.; van Gemert, L.J. Volatile character-impact sulfur compounds and their sensory properties. *Perfum. Flavorist* **1993**, *18*, 29–39.
99. Grosch, W. Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. *Chem. Senses* **2001**, *26*, 533–545. [\[CrossRef\]](#)

100. Abe, K.; Myoda, T.; Nojima, S. Identification and Characterization of Sulfur Heterocyclic Compounds That Contribute to the Acidic Odor of Aged Garlic Extract. *J. Agric. Food Chem.* **2021**, *69*, 1020–1026. [\[CrossRef\]](#)
101. Usami, A.; Motooka, R.; Takagi, A.; Nakahashi, H.; Okuno, Y.; Miyazawa, M. Chemical composition, aroma evaluation, and oxygen radical absorbance capacity of volatile oil extracted from *Brassica rapa* cv. “yukina” used in Japanese traditional food. *J. Oleo Sci.* **2014**, *63*, 723–730. [\[CrossRef\]](#)
102. Ortner, E.; Granvogl, M.; Schieberle, P. Elucidation of Thermally Induced Changes in Key Odorants of White Mustard Seeds (*Sinapis alba* L.) and Rapeseeds (*Brassica napus* L.) Using Molecular Sensory Science. *J. Agric. Food Chem.* **2016**, *64*, 8179–8190. [\[CrossRef\]](#) [\[PubMed\]](#)
103. Engel, E.; Baty, C.; le Corre, D.; Souchon, I.; Martin, N. Flavor-Active Compounds Potentially Implicated in Cooked Cauliflower Acceptance. *J. Agric. Food Chem.* **2002**, *50*, 6459–6467. [\[CrossRef\]](#) [\[PubMed\]](#)
104. Cha, Y.J.; Kim, H.; Cadwallader, K.R. Aroma-Active Compounds in Kimchi during Fermentation. *J. Agric. Food Chem.* **1998**, *46*, 1944–1953. [\[CrossRef\]](#)
105. Wang, S.; Chang, Y.; Liu, B.; Chen, H.; Sun, B.; Zhang, N. Characterization of the key aroma-active compounds in yongchuan douchi (*Fermented soybean*) by application of the sensomics approach. *Molecules* **2021**, *26*, 3048. [\[CrossRef\]](#) [\[PubMed\]](#)
106. Jeleń, H.; Majcher, M.; Ginja, A.; Kuligowski, M. Determination of compounds responsible for tempeh aroma. *Food Chem.* **2013**, *141*, 459–465. [\[CrossRef\]](#) [\[PubMed\]](#)
107. Yang, P.; Song, H.; Wang, L.; Jing, H. Characterization of Key Aroma-Active Compounds in Black Garlic by Sensory-Directed Flavor Analysis. *J. Agric. Food Chem.* **2019**, *67*, 7926–7934. [\[CrossRef\]](#)
108. van Ruth, S.; Boscaini, E.; Mayr, D.; Pugh, J.; Posthumus, M. Evaluation of three gas chromatography and two direct mass spectrometry techniques for aroma analysis of dried red bell peppers. *Int. J. Mass Spectrom.* **2003**, *223–224*, 55–65. [\[CrossRef\]](#)
109. Zimmermann, M.; Schieberle, P. Important odorants of sweet bell pepper powder (*Capsicum annuum* cv. *annuum*): Differences between samples of Hungarian and Moroccan origin. *Eur. Food Res. Technol.* **2000**, *211*, 175–180. [\[CrossRef\]](#)
110. Simian, H.; Robert, F.; Blank, I. Identification and Synthesis of 2-Heptanethiol, a New Flavor Compound Found in Bell Peppers. *J. Agric. Food Chem.* **2004**, *52*, 306–310. [\[CrossRef\]](#)
111. Raffo, A.; Moneta, E.; Ferrari Nicoli, S.; Paoletti, F. GC-olfactometric characterisation of off-odours in commercially packaged rocket leaves. *Food Packag. Shelf Life* **2020**, *25*, 100540. [\[CrossRef\]](#)
112. Ulrich, D.; Hoberg, E.; Bittner, T.; Engewald, W.; Meilchen, K. Contribution of volatile compounds to the flavor of cooked asparagus. *Eur. Food Res. Technol.* **2001**, *213*, 200–204. [\[CrossRef\]](#)
113. Schmidberger, P.C.; Schieberle, P. Characterization of the Key Aroma Compounds in White Alba Truffle (*Tuber magnatum* pico) and Burgundy Truffle (*Tuber uncinatum*) by Means of the Sensomics Approach. *J. Agric. Food Chem.* **2017**, *65*, 9287–9296. [\[CrossRef\]](#)
114. Matheis, K.; Granvogl, M. Characterisation of the key aroma compounds in commercial native cold-pressed rapeseed oil by means of the Sensomics approach. *Eur. Food Res. Technol.* **2016**, *242*, 1565–1575. [\[CrossRef\]](#)
115. Matheis, K.; Granvogl, M. Characterization of Key Odorants Causing a Fusty/Musty Off-Flavor in Native Cold-Pressed Rapeseed Oil by Means of the Sensomics Approach. *J. Agric. Food Chem.* **2016**, *64*, 8168–8178. [\[CrossRef\]](#)
116. Pollner, G.; Schieberle, P. Characterization of the Key Odorants in Commercial Cold-Pressed Oils from Unpeeled and Peeled Rapeseeds by the Sensomics Approach. *J. Agric. Food Chem.* **2016**, *64*, 627–636. [\[CrossRef\]](#) [\[PubMed\]](#)
117. Gracka, A.; Jeleń, H.H.; Majcher, M.; Siger, A.; Kaczmarek, A. Flavoromics approach in monitoring changes in volatile compounds of virgin rapeseed oil caused by seed roasting. *J. Chromatogr. A* **2016**, *1428*, 292–304. [\[CrossRef\]](#) [\[PubMed\]](#)
118. Yang, Y.; Yu, P.; Sun, J.; Jia, Y.; Wan, C.; Zhou, Q.; Huang, F. Investigation of volatile thiol contributions to rapeseed oil by odor active value measurement and perceptual interactions. *Food Chem.* **2022**, *373*, 131607. [\[CrossRef\]](#)
119. Onwude, D.I.; Hashim, N.; Janius, R.; Abdan, K.; Chen, G.; Oladejo, A.O. Non-thermal hybrid drying of fruits and vegetables: A review of current technologies. *Innov. Food Sci. Emerg. Technol.* **2017**, *43*, 223–238. [\[CrossRef\]](#)
120. Barrett, D.M.; Lloyd, B. Advanced preservation methods and nutrient retention in fruits and vegetables. *J. Sci. Food Agric.* **2012**, *92*, 7–22. [\[CrossRef\]](#)
121. Al-Juhaimi, F.; Ghafoor, K.; Özcan, M.M.; Jahurul, M.H.A.; Babiker, E.E.; Jinap, S.; Sahena, F.; Sharifudin, M.S.; Zaidul, I.S.M. Effect of various food processing and handling methods on preservation of natural antioxidants in fruits and vegetables. *J. Food Sci. Technol.* **2018**, *55*, 3872–3880. [\[CrossRef\]](#) [\[PubMed\]](#)
122. Wu, C.M.; Wang, Z. Volatile Compounds in Fresh and Processed Shiitake Mushrooms (*Lentinus edodes* Sing.). *Food Sci. Technol. Res.* **2000**, *6*, 166–170. [\[CrossRef\]](#)
123. Cedrowski, J.; Dąbrowa, K.; Przybylski, P.; Krogul-Sobczak, A.; Litwinienko, G. Antioxidant activity of two edible isothiocyanates: Sulforaphane and erucin is due to their thermal decomposition to sulfenic acids and methylsulfinyl radicals. *Food Chem.* **2021**, *353*, 129213. [\[CrossRef\]](#) [\[PubMed\]](#)
124. Schwab, W.; Davidovich-Rikanati, R.; Lewinsohn, E. Biosynthesis of plant-derived flavor compounds. *Plant J.* **2008**, *54*, 712–732. [\[CrossRef\]](#)
125. Chen, C.-W.; Ho, C.-T. Thermal Degradation of Allyl Isothiocyanate in Aqueous Solution. *J. Agric. Food Chem.* **1998**, *46*, 220–223. [\[CrossRef\]](#)
126. Williams, M.P.; Nelson, P.E. Kinetics of the thermal degradation of methylmethionine sulfonium ions in citrate buffers and in sweet corn and tomato serum. *J. Food Sci.* **1974**, *39*, 457–460. [\[CrossRef\]](#)

127. Kubec, R.; Velišek, J.; Doležal, M.; Kubelka, V. Sulfur-Containing Volatiles Arising by Thermal Degradation of Alliin and Deoxyalliin. *J. Agric. Food Chem.* **1997**, *45*, 3580–3585. [\[CrossRef\]](#)
128. Shu, C.K.; Hagedorn, M.L.; Mookherjee, B.D.; Ho, C.T. Volatile components of the thermal degradation of cystine in water. *J. Agric. Food Chem.* **1985**, *33*, 438–442. [\[CrossRef\]](#)
129. Her, J.Y.; Kim, M.S.; Kim, M.K.; Lee, K.G. Development of a spray freeze-drying method for preparation of volatile shiitake mushroom (*Lentinus edodes*) powder. *Int. J. Food Sci. Technol.* **2015**, *50*, 2222–2228. [\[CrossRef\]](#)
130. Politowicz, J.; Lech, K.; Lipan, L.; Figiel, A.; Carbonell-Barrachina, Á.A. Volatile composition and sensory profile of shiitake mushrooms as affected by drying method. *J. Sci. Food Agric.* **2018**, *98*, 1511–1521. [\[CrossRef\]](#)
131. Soares, A.; Carrascosa, C.; Raposo, A. Influence of Different Cooking Methods on the Concentration of Glucosinolates and Vitamin C in Broccoli. *Food Bioprocess Technol.* **2017**, *10*, 1387–1411. [\[CrossRef\]](#)
132. Martínez, S.; Armesto, J.; Gómez-Limia, L.; Carballo, J. Impact of processing and storage on the nutritional and sensory properties and bioactive components of *Brassica* spp. A review. *Food Chem.* **2020**, *313*, 126065. [\[CrossRef\]](#) [\[PubMed\]](#)
133. Biondi, F.; Balducci, F.; Capocasa, F.; Visciglio, M.; Mei, E.; Vagnoni, M.; Mezzetti, B.; Mazzoni, L. Environmental conditions and agronomical factors influencing the levels of phytochemicals in *Brassica* vegetables responsible for nutritional and sensorial properties. *Appl. Sci.* **2021**, *11*, 1927. [\[CrossRef\]](#)
134. Tao, Y.; Han, M.; Gao, X.; Han, Y.; Show, P.L.; Liu, C.; Ye, X.; Xie, G. Applications of water blanching, surface contacting ultrasound-assisted air drying, and their combination for dehydration of white cabbage: Drying mechanism, bioactive profile, color and rehydration property. *Ultrason. Sonochem.* **2019**, *53*, 192–201. [\[CrossRef\]](#) [\[PubMed\]](#)
135. Hanschen, F.S.; Platz, S.; Mewis, I.; Schreiner, M.; Rohn, S.; Kroh, L.W. Thermally induced degradation of sulfur-containing aliphatic glucosinolates in broccoli sprouts (*Brassica oleracea* var. *Italica*) and model systems. *J. Agric. Food Chem.* **2012**, *60*, 2231–2241. [\[CrossRef\]](#)
136. Hong, S.J.; Boo, C.G.; Lee, J.; Hur, S.W.; Jo, S.M.; Jeong, H.; Yoon, S.; Lee, Y.; Park, S.S.; Shin, E.C. Chemosensory approach supported-analysis of wintering radishes produced in Jeju island by different processing methods. *Food Sci. Biotechnol.* **2021**, *30*, 1033–1049. [\[CrossRef\]](#)
137. Nugraheni, P.Y.; Verkerk, R.; Widanarko, B.; Dekker, M. A Mechanistic Perspective on Process-Induced Changes in Glucosinolate Content in *Brassica* Vegetables: A Review. *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 823–838. [\[CrossRef\]](#)
138. Frank, D.; Piyasiri, U.; Archer, N.; Heffernan, J.; Poelman, A.A.M. In-Mouth Volatile Production from *Brassica* Vegetables (*Cauliflower*) and Associations with Liking in an Adult/Child Cohort. *J. Agric. Food Chem.* **2021**, *69*, 11646–11655. [\[CrossRef\]](#)
139. Schmidberger, P.C.; Schieberle, P. Changes in the Key Aroma Compounds of Raw Shiitake Mushrooms (*Lentinula edodes*) Induced by Pan-Frying as Well as by Rehydration of Dry Mushrooms. *J. Agric. Food Chem.* **2020**, *68*, 4493–4506. [\[CrossRef\]](#)
140. Baenas, N.; Marhuenda, J.; García-Viguera, C.; Zafrilla, P.; Moreno, D.A. Influence of cooking methods on glucosinolates and isothiocyanates content in novel cruciferous foods. *Foods* **2019**, *8*, 257. [\[CrossRef\]](#)
141. Wiczeorek, M.N.; Jelen, H.H. Volatile compounds of selected raw and cooked *Brassica* vegetables. *Molecules* **2019**, *24*, 391. [\[CrossRef\]](#) [\[PubMed\]](#)
142. Mustafa, A.M.; Angeloni, S.; Nzekoue, F.K.; Abouelenein, D.; Sagratini, G.; Caprioli, G.; Torregiani, E. An overview on truffle aroma and main volatile compounds. *Molecules* **2020**, *25*, 5948. [\[CrossRef\]](#) [\[PubMed\]](#)
143. Culleré, L.; Ferreira, V.; Venturini, M.E.; Marco, P.; Blanco, D. Chemical and sensory effects of the freezing process on the aroma profile of black truffles (*Tuber melanosporum*). *Food Chem.* **2013**, *136*, 518–525. [\[CrossRef\]](#) [\[PubMed\]](#)
144. Chen, Z.; Gao, H.; Wu, W.; Chen, H.; Fang, X.; Han, Y.; Mu, H. Effects of fermentation with different microbial species on the umami taste of Shiitake mushroom (*Lentinus edodes*). *LWT* **2021**, *141*, 110889. [\[CrossRef\]](#)
145. Luang-In, V.; Deesenthum, S.; Udomwong, P.; Saengha, W.; Gregori, M. Formation of Sulforaphane and Iberin Products from Thai Cabbage Fermented by Myrosinase-Positive Bacteria. *Molecules* **2018**, *23*, 955. [\[CrossRef\]](#)
146. Yu, Y.; Xu, Y.; Li, L.; Yu, Y.; Xu, Z. Dynamic evolution of flavor substances and bacterial communities during fermentation of leaf mustard (*Brassica juncea* var. *multiceps*) and their correlation. *LWT* **2022**, *167*, 113796. [\[CrossRef\]](#)
147. Sun, J.; Wang, Y.; Pang, X.; Tian, S.; Hu, Q.; Li, X.; Liu, J.; Wang, J.; Lu, Y. The effect of processing and cooking on glucoraphanin and sulforaphane in brassica vegetables. *Food Chem.* **2021**, *360*, 130007. [\[CrossRef\]](#)



Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt



Determination of the odor threshold concentrations and partition coefficients of isothiocyanates from Brassica vegetables in aqueous solution

Monika Marcinkowska, Henryk H. Jeleń^{*}

Faculty of Food Science and Nutrition, Poznań University of Life Sciences, Wojska Polskiego 31, 60-624, Poznań, Poland

ARTICLE INFO

Keywords:

Odor quality
Artificial saliva
Sensory evaluation
Flavor profile analysis
Glucosinolate derivatives

ABSTRACT

Isothiocyanates (ITCs) are products of the enzymatic breakdown of glucosinolates and may cause pungent flavor notes in vegetables. Limited data on odor threshold concentrations (OTCs) and odor quality descriptors of ITCs exist in literature. These parameters were determined in aqueous samples for 19 isothiocyanates found mainly in Brassica vegetables. Thresholds and odor quality descriptors were ascertained by panelists using flavor profile analysis (FPA). The odor threshold concentrations of ITCs range from 0.005×10^{-3} (octyl ITC) to 0.2×10^{-3} (ethyl ITC) g/L and ITCs were mainly characterized by sulfur, garlic, and Brassica vegetable-like notes. Correlation coefficients were between 0.946 (octyl ITC) and 0.996 (3-(methylthio)propyl ITC) and proved convincing results of OTCs using FPA.

Partition coefficients were determined in water, artificial saliva and human saliva to provide an insight into behavior of ITCs in two-phase liquid/air systems. Partition coefficients (K) for human saliva are significantly different from other matrices nevertheless the profile of ITCs K values in water and saliva buffer solution were similar. In human saliva the highest K values were observed for octyl, hexyl and cyclohexyl ITCs, and the lowest for cyclopropyl, benzyl, allyl ITCs. Obtained data provide essential and previously unknown sensory properties for future flavor studies.

1. Introduction

There is no doubt that eating vegetables has a positive influence on human health consequently they are affirmed as the desired food among consumers. Vegetables of the family Brassicaceae include a high level of beneficial health compounds for instance vitamins, minerals, polyphenols, carotenoids, and glucosinolates (GLS). They have been cultivated worldwide due to their easy adaptation to the harsh environmental conditions. Moreover, Brassica vegetables are widely consumed after being treated by a different food processing methods (cooking, baking, freezing, drying, steaming, microwaving, canning). Advantageous health properties, in particular prevention or reversal of cancer, hepatic steatosis, cardiovascular disease, antioxidant, anti-inflammatory, gastroprotective and anti-obesity activity activities are connected with the content of phytochemicals – mainly glucosinolates and their hydrolysis products (Šamec, Pavlović, Radojčić Redovniković, & Salopek-Sondi, 2018). Even if the scientific literature focuses its attention and reports on many beneficial properties of GLS breakdown products, attention should be paid to the anti-nutritional effect of these

compounds (Cartea & Velasco, 2008). Glucosinolate derivatives can adversely affect the thyroid gland. Especially organisms with hypothyroidism are prone to negative effects of glucosinolate rich products (Pasko et al., 2018). Brassica vegetables such as broccoli, cabbage, cauliflower, etc. are a rich source of GLS which are precursors of bioactive compounds like isothiocyanates (ITCs) characterized by above mentioned positive health effects (Wu et al., 2018).

Glucosinolates are nitrogen and sulfur-containing plant secondary metabolites that are present in a significant concentration in Brassica vegetables. The variation in the structure of GLS gives an opportunity to divide this type of compounds into three groups of aliphatic, aromatic and indolic glucosinolates, corresponding to the structure of the side chain (Hansch, Kühn, Nickel, Rohn, & Dekker, 2018). In plant cells GLS are stored in vacuole in contrast to β -D-thioglucosidase, an endogenous hydrolyzing enzyme called myrosinase, which is located in a distinct compartment of a cell (Hansch, Lamy, Schreiner, & Rohn, 2014). When a vegetable cell is damaged enzyme and GLS can interact and the hydrolysis is started, resulting in a diversity of biodegradation products, namely nitriles, epithionitriles, thiocyanates, indoles,

^{*} Corresponding author.

E-mail address: henrykj@up.poznan.pl (H.H. Jeleń).

<https://doi.org/10.1016/j.lwt.2020.109793>

Received 14 January 2020; Received in revised form 18 June 2020; Accepted 19 June 2020

Available online 24 June 2020

0023-6438/© 2020 Elsevier Ltd. All rights reserved.

oxazolidines, and isothiocyanates. A product formed in an enzymatic reaction between glucosinolate and myrosinase is related to the structure of glucosinolates and the biodegradation conditions (e.g. pH, the presence of proteins and metal ions). When hydrolysis happens at neutral pH the default products are volatile odor - active compounds – isothiocyanates (Oliviero, Verkerk, & Dekker, 2018).

Odor represents the main factor of the food quality and customer satisfaction as well. That is why flavor evaluation is a matter of concern, especially for the food industry. ITCs, which are products of enzymatic breakdown of glucosinolates may cause a pungent flavor note, therefore it is an important group of volatile compounds, which play a leading role in generation of aroma of Brassica vegetables (Wieczorek, Walczak, Skrzypczak-Zielińska, & Jeleń, 2019). Nevertheless nowadays or in the immediate future, there are no appropriate apparatuses available for detecting and measuring odor threshold (OT). The best way to measure OT is by directly utilizing sensory analysis and more specifically using the human nose. The human nose provides a superior quality detector with enormous sensitivity to certain chemicals at extremely low concentrations in range from some mg/L to few ng/L, which is lower than any analytical instrument is able to detect (Pelosi, Zhu, & Knoll, 2018).

Numerous research of odor thresholds of various groups of compounds has been published thus far however, literature data have shown that the thresholds of food odorants are inconsistent and differ as a result of different experimental methodologies, impurities of the standards and the choice of panelists in sensory studies. Odor threshold data in water previously available for allyl isothiocyanate have covered a very high variation from study to study in a wide range of concentrations from 0.046 to 3.8 mg/kg (Gemert, 2011).

The flavor profile analysis (FPA) is a standard sensory evaluation method applied to describe odor, taste, and odor threshold concentrations (OTCs) in water (APHA, 2170 Flavor Profile Analysis, 2017). Principally FPA was developed for analyzing food, beverages, and pharmaceuticals and was adapted for assessing samples of drinking water (Krasner, 1988). This method of sensory evaluation based on a group of trained sensory panelists who analyze the samples by flavor descriptor and intensity level value chosen from point scale with defined standards (Schweitzer, Tomboulian, Atasi, Chen, & Khiari, 2004). The average intensity for each sample to the logarithm of the corresponding compound concentration is represented as a Weber-Fechner curve, which allows determination of the OTC (Diaz, Fabrellas, Ventura, & Galceran, 2005).

No data about most of the odor parameters of glucosinolates breakdown compounds has been published previously in the literature. The aim of the present study, therefore, was to investigate the OTCs of 19 ITCs included their descriptors using FPA as the main technique based on defined aqueous solutions of standard reference compounds. This will expand the library of odor threshold concentrations in water and partition coefficients (K) that can be used by the flavor study e.g. chemosensory perception. The results obtained can be used to establish the influence of variation and concentration ITCs compounds in the generation of the aroma of Brassica vegetables. It will be also an important data for GC-O (gas chromatography – olfactometry) studies on the determination of key odorants in Brassica vegetables and quantifying them using odor aroma value (OAV) concept, where ratio of particular compound concentration to its OTC is calculated.

2. Materials and methods

This study was approved on 6 Sep 2018 by the Bioethical Commission at the Medical University of Poznań (No. 869/18) and conducted according to the Declaration of Helsinki. All assessors were recruited from The University of Life Sciences in Poznań and provided informed consent for this study.

2.1. Preparation of glassware

Stoppered conical glass flasks with a capacity of 0.25 L were carefully cleaned to remove odors. Glassware was washed with soapy water, rinsed with tap water, ultrapure Milli-Q water with a conductivity of 18.2 MΩ/cm and with reagent-grade acetone. Conical glass flasks were air-dried and baked at 200 °C overnight. Then they were finally covered with aluminum foil to avoid odors adsorption, dust, and other contaminants.

2.2. Reference odorants and chemicals

The reference compounds (allyl isothiocyanate, 2-phenethyl isothiocyanate, benzyl isothiocyanate, butyl isothiocyanate, 3-butenyl isothiocyanate, cyclopentyl isothiocyanate, isobutyl isothiocyanate, isopropyl isothiocyanate, ethyl isocyanacetate, hexyl isothiocyanate, octyl isothiocyanate, methyl isothiocyanate, cyclohexyl isothiocyanate, 3-(methylthio)propyl isothiocyanate, phenyl isothiocyanate, propyl isothiocyanate, cyclopropyl isothiocyanate, methallyl isothiocyanate, 2-methoxyethyl isothiocyanate) were obtained from Sigma-Aldrich (Poznań, Poland). Components to prepare saliva buffer and artificial saliva: calcium chloride dihydrate, sodium chloride, sodium bicarbonate, potassium phosphate dibasic trihydrate, potassium chloride, pig gastric mucin were from Sigma-Aldrich (Poznań, Poland). Other reagent grade chemicals such as hydrochloric acid, acetone, and methanol were also purchased from Sigma-Aldrich (Poznań, Poland). Milli-Q water was from The Simplicity UV Water Purification System (Merck, Darmstadt, Germany).

2.3. Standard solutions

The reference odorants were dissolved in methanol by weighing approximately 0.02 g of reference odorant separately into a 0.01 L volumetric flask and diluting to volume. Several dilutions were prepared in odor-free Milli-Q water immediately before sensory evaluation by using the flavor profile analysis (FPA) method. The initial concentrations of samples were selected according to previously published odor threshold values for allyl isothiocyanate fixed in water. Suitable aliquots of stock solutions were added to glass conical flasks with a capacity of 0.25 L with ultrapure water.

2.4. Sensory methodology

The main technique which was used to determine the odor threshold concentrations of isothiocyanates was flavor profile analysis (FPA). Appropriate aliquots of methanol stock solutions were mixed into conical flasks (0.25 L) with ultrapure odor-free water and immediately closed to prevent loss of volatile reference odorants. Concentrations in a range between 1×10^{-3} and 5×10^{-3} g/L with a total volume of 0.1 L per sample were obtained. Erlenmeyer flasks were covered with aluminum foil to avoid contamination caused by the adsorption of odors from panelist's hands through the glass and maintain the temperature of the samples during the sensory session. Prior to the sensory session, all samples were heated to 45 ± 1 °C for half an hour on the heating plate in order to achieve an equilibrium state.

Volunteers were recruited mainly from the Food Chemistry and Instrumental Analysis Department of The University of Life Sciences in Poznań. The qualified panel consists of carefully selected seven to ten assessors (from a group of 15, age 27 - 50) per sensory session. The panelists had no specific health problems that could affect their sense of the odors and they were not permitted to eat or drink before 1 h of the sensory experiment. No extraneous interference cosmetics were allowed, perfume, hand cream, or chewing gum. The panels took place in a specific room retained free from distracting odors. Sensory evaluation experiments were performed individually from 12:00 noon to 15:00 p.m.

Prepared and preheated set of aqueous samples was presented to the trained panel. Panelists were informed to swirl the contents, smell each sample, in the specific order and they were allowed to use free-odor water without reference odorant sample to sniff between samples to avoid overloading of the senses. The sequence of aqueous solutions was set from the most diluted to the most concentrated with random blank samples. Judges were not informed about increasing concentration in the sample sequences (Cancho, Fabrellas, Diaz, Ventura, & Galceran, 2001). Attention was paid to record the first sensation of the smells. Evaluators were instructed to determine odors in samples and assign an intensity for each odor based on a point scale with defined scale presented below in the table (Table 1).

Assessors were asked to smell aqueous samples in established order, describe intensity value (I), and assess the quality of odor value to the corresponding concentration. The intensity of odor values given by the sensory panelist was recorded and averaged excluding $I = 0$ values. A Weber-Fechner curve was plotted which shows a correlation between the average intensity value and the concentration logarithm. Odor threshold concentrations (OTCs) were calculated for individual ITCs from the equations of Weber-Fechner curve at an intensity of $I = 1$. The lowest concentration detected by one or more assessors was defined as the experimental threshold concentration (OTC_{exp}) (Diaz et al., 2005).

2.5. Matrices preparation

Different matrices like water, human saliva (HSaliva), artificial saliva (ASaliva), and saliva buffer (SBuffer) was prepared. Water was purified by Simplicity UV Water Purification System (Millipore) to conductivity of 18.2 MΩ/cm.

SBuffer solution was obtained by dissolving in ultrapure water the subsequent constituents: calcium chloride dihydrate $CaCl_2 \cdot 2H_2O$ (0.441 g/L), sodium chloride NaCl (0.877 g/L), sodium bicarbonate $NaHCO_3$ (5.208 g/L), potassium phosphate dibasic trihydrate $K_2HPO_4 \cdot 3H_2O$ (1.369 g/L), potassium chloride KCl (0.477 g/L).

ASaliva was made up using a saliva buffer described above by addition of pig gastric mucin (2.16 g/L). The pH of saliva buffer and artificial human saliva was adjusted by hydrochloric acid HCl to 7.3 ± 0.1 , which corresponds to the pH of human saliva sample described below (Pagès-Hélary, Andriot, Guichard, & Canon, 2014).

HSaliva was collected from 7 volunteers and they were not allowed to eat or drink for at least 1 h before saliva collections at habitual periods. Received saliva samples were combined to obtain a total volume of 70 mL and inhibitor of bacterial and fungal contamination NaN_3 was added immediately at a concentration of 0.02 g/L. Mixed saliva was centrifuged (MPW-223e, MPW Med. instruments, Warszawa, Poland) at 1000 g for 15 min and the precipitate was separated from the clear liquid. Before further usage, the pH was measured and it was 7.3 ± 0.1 (Muñoz-González et al., 2014).

2.6. Static equilibrium headspace analysis

Equal quantity of methanol stock solutions were added into matrices (Water, HSaliva, ASaliva, and SBuffer solution) in two volume variation

Table 1
Flavor profile analysis (FPA) scale for assessment of isothiocyanate aqueous samples; * - odor perception without its quality.

Intensity of odor	Corresponding level value
No odor perception, odorless	0
Odor threshold*	1
Very slight	2
Weak	4
Weak/Moderate	6
Moderate	8
Strong	10
Very strong	12

1 and 10 mL in four repetitions for each volume diverse. The headspace vials of total volume 22 mL were immediately closed with silicone septa caps. The headspace tray with the samples was loaded at automatic headspace sampler (Agilent Technologies 7697A Headspace Sampler, Agilent Technologies, Wilmington, DE) with defined equilibration temperature of 40 °C and time 20 min to reach a static equilibrium state. Samples were introduced to gas chromatography system (Agilent Technologies 7890A GC System, Agilent Technologies, Wilmington, DE) by transfer line set at 140 °C and analyzed by GC. The injector temperature was established at 220 °C in split mode (split ratio 1:5). A ZB-Wax column (60 m, 530 μm × 1 μm; Phenomenex, Torrance, CA) was used and a constant flow of He as a carrier was kept at 10 mL/min. Oven temperature was programmed from 40 °C (1min.) ramped at 10 °C/min to 140 °C (2 min), then 20 °C/min to 240 °C (7 min). Total runtime was 20 min. FID detector was kept at 240 °C with H_2 flow of 30 mL/min, air – 300 mL/min and makeup gas (N_2) – 5 mL/min. FID acquisition rate of 20 Hz was used. Chemstation B.04.03 by Agilent Technologies (Agilent Technologies, Santa Clara, CA), was used to process chromatographic data. Partition coefficients (K_{Water} , $K_{SBuffer}$, $K_{ASaliva}$, $K_{HSaliva}$) for tested ITCs were determined based on the equilibrium partitioning in closed systems (EPICS) method applied originally by Gossett and Lincoff (Valsaraj, Gupta, Thibodeaux, & Harrison, 1988). This approach is focused on partition of volatile organic compounds within liquid and air phase, where the total number of moles (M) equals the sum of the total mass in the gas ($C_g V_g$) and liquid ($C_l V_l$) phase expressed in moles as shown by formula (1):

$$M = C_l V_l + C_g V_g \quad (1)$$

where M – total mass in the vial (mol); C_l – liquid phase concentration ($mol \cdot L^{-1}$); V_l – total liquid volume (L); C_g – gas phase concentration ($mol \cdot L^{-1}$); V_g – total gas volume (L). In regard to that method, the same amount of the moles of the compound added into two sealed vials that differ in volume of water is suitable to determine the K values according to equation (2).

$$K = \frac{(C_{g1})V_{l1} - V_{l2}}{V_{g2} - (C_{g1})V_{g1}} \quad (2)$$

Absolute concentrations are not required and the ratio of gas phase concentrations are reflected by GC peak areas (Hansen, Zhou, Yaws, & Aminabhavi, 1995).

3. Results

3.1. Flavor profile analysis - odor threshold concentrations

The Weber-Fechner curves were plotted separately for each isothiocyanate standard in aqueous solutions. Fig. 1 presents the average intensity score as a function logarithm of the concentration of hexyl isothiocyanate as an example. The flavor profile analysis (FPA) plots contain between five and seven points depending on the ITC investigated and its OTC. The Weber-Fechner plots were linear with the correlation coefficients (R^2) between 0.946 (octyl ITC) and 0.996 (3-(methylthio)propyl ITC) (Table 2).

The OTCs were determined for the nineteen ITCs, collected in Fig. 2, the values were obtained from the equation of every tested compound and ranged from 0.005×10^{-3} g/L (octyl ITC) to 0.2×10^{-3} g/L (ethyl ITC). The lowest concentration detected by minimum one panelist is defined as experimental threshold concentration (OTC_{exp}) and the values coincide with the previous OTC results. The OTC_{exp} was between 0.001×10^{-3} g/L (octyl ITC) to 0.1×10^{-3} g/L (ethyl ITC, allyl ITC).

3.2. Flavor profile analysis - flavor descriptors

According to the sensory panel assessment, the flavor descriptors were associated with the chemical structure of the ITC molecule –

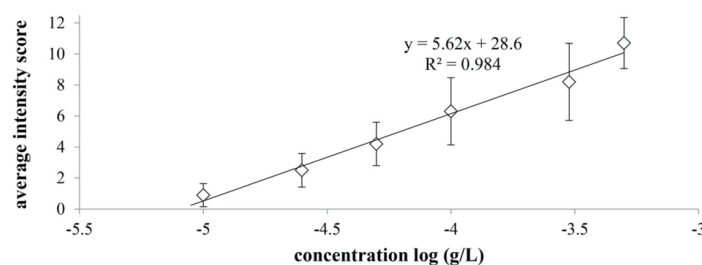


Fig. 1. Weber-Fechner curve for determination of odor threshold of 3-butenyl isothiocyanate (n = 10).

Table 2

Odor thresholds in water (OTs) at 45 °C, Weber–Fechner equations where I – intensity score; a – slope; b – intercept; log c – concentration logarithm (g/L); R² – coefficient of determination, odor qualities, and data on isothiocyanates (ITCs) presence in Brassica plants.

No ^a	Class ^b	Compound	OT [g/L]	Weber-Fechner equation I = a · log c + b			Odor quality ^c	Natural occurrence
				a	b	R ²		
1	A	Methyl ITC	$0.04 \times 10^{-3} \pm 0.21 \times 10^{-3}$	4.8	22	0.992	cabbage, cooked Brussels sprouts, broccoli, hydrogen sulfide	Brassicaceae, Capparaceae (Iranshahi, 2012)
2	A	Ethyl ITC	$0.2 \times 10^{-3} \pm 0.6 \times 10^{-3}$	7.0	27	0.973	garlic, cabbage, broccoli, hydrogen sulfide	Brassicaceae, Tropaeolaceae (Iranshahi, 2012)
3	A	Propyl ITC	$0.03 \times 10^{-3} \pm 0.40 \times 10^{-3}$	5.0	23	0.990	crisp, sulfur, garlic, horseradish	Capparaceae (Iranshahi, 2012)
4	A	Butyl ITC	$0.01 \times 10^{-3} \pm 0.05 \times 10^{-3}$	7.2	37	0.985	cabbage, green/dried garlic, pickled cucumber	Brassicaceae, Capparaceae (Iranshahi, 2012)
5	A	Hexyl ITC	$0.007 \times 10^{-3} \pm 0.052 \times 10^{-3}$	4.3	23	0.974	mushroom, floral, turnip, kohlrabi	Brassicaceae (Iranshahi, 2012)
6	A	Octyl ITC	$0.005 \times 10^{-3} \pm 0.030 \times 10^{-3}$	3.6	21	0.946	sweet, floral, fruity, coriander	Brassicaceae (Iranshahi, 2012)
7	B	Isopropyl ITC	$0.02 \times 10^{-3} \pm 0.08 \times 10^{-3}$	5.6	27	0.979	broth, cabbage, garlic, chemical	Brassicaceae, Capparaceae, Gyrostemonaceae, Euphorbiaceae, Moringaceae, Salvadoraceae, Tovariaceae, Tropaeolaceae (Iranshahi, 2012)
8	B	Isobutyl ITC	$0.009 \times 10^{-3} \pm 0.085 \times 10^{-3}$	5.1	27	0.984	mustard, garlic, sulfur, chemical, oil paint	Brassicaceae, Moringaceae, Gyrostemonaceae, Tropaeolaceae (Iranshahi, 2012)
9	B&AU	Methylallyl ITC	$0.03 \times 10^{-3} \pm 0.16 \times 10^{-3}$	6.3	30	0.987	sulfur, garlic, cress, pungent	-
10	AU	Allyl ITC	$0.09 \times 10^{-3} \pm 0.22 \times 10^{-3}$	9.1	38	0.980	sulfur, garlic, horseradish	Brassicaceae, Capparaceae (Iranshahi, 2012)
11	AU	3-Butenyl ITC	$0.01 \times 10^{-3} \pm 0.07 \times 10^{-3}$	5.6	29	0.984	sulfur, garlic, horseradish	Brassicaceae (Iranshahi, 2012)
12	C	Cyclopropyl ITC	$0.01 \times 10^{-3} \pm 0.08 \times 10^{-3}$	5.2	26	0.983	broth, chives, leek, garlic	Brassicaceae (Peng et al., 2014)
13	C	Cyclopentyl ITC	$0.02 \times 10^{-3} \pm 0.10 \times 10^{-3}$	5.1	25	0.980	garlic, oil paint, metallic, kohlrabi, black turnip	Brassicaceae (Tomson, Kruma, Galoburda, & Talou, 2013)
14	C	Cyclohexyl ITC	$0.03 \times 10^{-3} \pm 0.19 \times 10^{-3}$	6.0	29	0.980	sweet, plastic, mint, oil paint, anise	Brassicaceae (Iranshahi, 2012)
15	AR	Phenyl ITC	$0.05 \times 10^{-3} \pm 0.22 \times 10^{-3}$	7.4	33	0.982	sulfur, plastic, herbs, fennel	Brassicaceae (Iranshahi, 2012)
16	AR	Benzyl ITC	$0.06 \times 10^{-3} \pm 0.22 \times 10^{-3}$	7.8	34	0.965	garlic, cress, leek sprouts, broccoli sprouts	Brassicaceae, Capparaceae, Caricaceae, Moringaceae, Phytolaccaceae, Resedaceae, Salvadoraceae, Sterculiaceae, Tovariaceae, Tropaeolaceae (Iranshahi, 2012)
17	AR	2-Phenethyl ITC	$0.01 \times 10^{-3} \pm 0.05 \times 10^{-3}$	6.3	32	0.992	sulfur, chives, cress	Brassicaceae, Resedaceae, Pedalicaceae, Valerianaceae (Iranshahi, 2012)
18	M	2-Methoxyethyl ITC	$0.07 \times 10^{-3} \pm 0.32 \times 10^{-3}$	5.3	23	0.978	sulfur, garlic, hydrogen sulfide, pungent	-
19	M	3-(Methylthio) propyl ITC	$0.006 \times 10^{-3} \pm 0.058 \times 10^{-3}$	4.3	23	0.996	sulfur, plastic, fish, vegetable broth, horseradish	Brassicaceae (Kala, Ali, Ahmad, Gilani, & Khan, 2018)

^a Numbers correspond to those in Fig. 2.

^b A – n-aliphatic; B – branched; AU – aliphatic unsaturated; C – cyclic; AR – aromatic; M – miscellaneous.

^c Odor qualities (sensation) perceived, associated with changes in concentration (from low to high) of analyzed ITCs (n = 7–10).

mainly presence of sulfur atom in their structure. Garlic, sulfur, hydrogen sulfide, cabbage, cress are the main descriptors characterizing tested ITCs. The frequency of occurrence of a given descriptor during sensory evaluation is expressed in the area of the diagram and expressed in the area of particular boxes representing main odor notes (Fig. 3). The

odor descriptors presented in Table 2 are listed from the most concentrated to the least standard solutions to those being the most concentrated for particular compound.

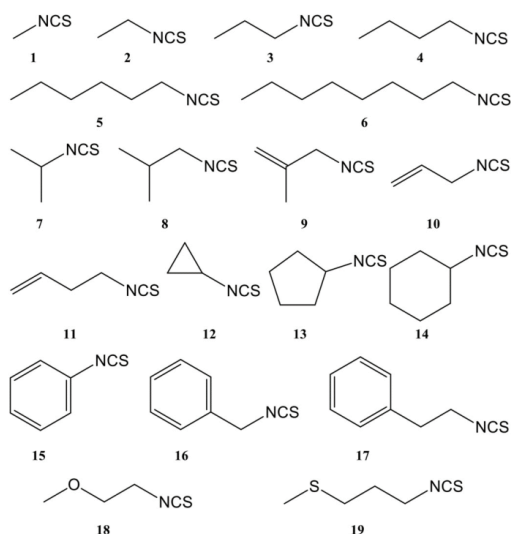


Fig. 2. Formulae of isothiocyanates (ITCs) used in experiments (1 – Methyl ITC; 2 – Ethyl ITC; 3 – Propyl ITC; 4 – Butyl ITC; 5 – Hexyl ITC; 6 – Octyl ITC; 7 – Isopropyl ITC; 8 – Isobutyl ITC; 9 – Methylallyl ITC; 10 – Allyl ITC; 11 – 3-Butenyl ITC; 12 – Cyclopropyl ITC; 13 – Cyclopentyl ITC; 14 – Cyclohexyl ITC; 15 – Phenyl ITC; 16 – Benzyl ITC; 17 – 2-Phenethyl ITC; 18 – 2-Methoxyethyl ITC; 19 – 3-(Methylthio)propyl ITC).

3.3. Static equilibrium headspace analysis – partition coefficients

The gas/liquid partition coefficients calculated for nineteen ITCs in different matrices (water, saliva buffer, artificial saliva, human saliva) are provided in Table 3. Partition coefficients obtained for the human saliva matrix are significantly different ($p < 0.003$) and were

characterized by higher values compared to other matrices. In human saliva the highest K were observed to octyl ITC (4.0 ± 0.7), hexyl ITC (3.0 ± 0.6) and cyclohexyl ITC (2.0 ± 0.4) respectively while in artificial saliva, it were cyclohexyl ITC (0.5 ± 0.2) and hexyl ITC (0.5 ± 0.2). The lowest partition coefficient were cyclopropyl ITC (0.02 ± 0.02), benzyl ITC (0.04 ± 0.04), allyl ITC (0.06 ± 0.01) and in artificial saliva it were methyl ITC (0.18 ± 0.09), octyl ITC (0.2 ± 0.1), 2-methoxyethyl ITC (0.2 ± 0.01). In comparison to the profile ITCs K in water and saliva buffer solution, similar values were observed.

4. Discussion

4.1. Correlation of OTC and chemical structure

To our knowledge, this is the first study done on the sensory properties of the majority of ITCs present mainly in Brassica vegetables. ITC compounds were divided into groups according to the chemical structure to aliphatic (saturated/unsaturated), branched, cyclic, aromatic, and miscellaneous. The Weber-Fechner plots for n-aliphatic saturated ITCs showed a relationship between OTCs and the number of carbon atoms. The OTCs decrease with the increase of the saturated chain length. According to the EPA DSSTox database, the octanol/water partition coefficient (LogP) values are 0.94 (methyl ITC), 1.47 (ethyl ITC), 2.94 (butyl ITC), 3.98 (hexyl ITC) respectively (“EPA DSSTox,” 2020). This tendency was related to the increased hydrophobicity of isothiocyanates as the carbon chain is lengthened. What is more, the partition coefficients determined for the gas-liquid system showed an increased affinity for the air phase as the number of carbon atoms in the isothiocyanate molecule increases. The above data showed that if the partition coefficient is high, the less amount of compound is needed to be perceptible by the human nose, which explains the lower detection threshold. The exception to this rule was ethyl isothiocyanate, which was the least noticeable in this group of ITCs. The discrepancy of the ethyl ITC OT detection relative to other n-aliphatic unsaturated ITCs may be due to a different mechanism of perception of this compound or different receptors responsible for detection. In relation to ITCs which consist of isothiocyanate group and three carbon atoms (C-3 ITCs) OTC increased for ITC cyclic, branched saturated, aliphatic saturated,

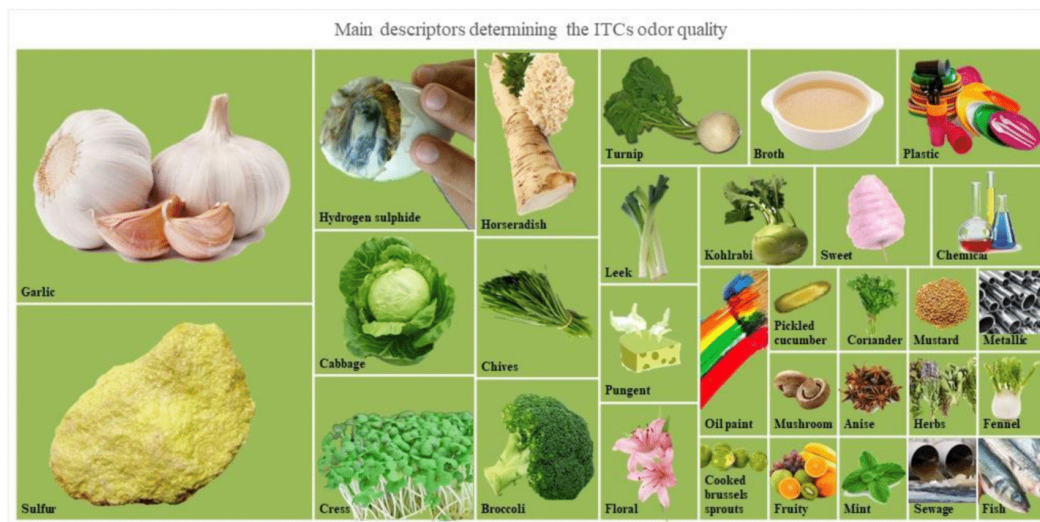


Fig. 3. Odor notes associated with orthonasal perception of investigate isothiocyanates. The area of squares reflects the frequency of perceived odor.

Table 3

Partition coefficients (K) determined for isothiocyanates (ITCs) in different matrices (n = 4); Rt – retention time, No – numbers correspond to those in Fig. 2 & Table 2.

No	Compound	Rt [min.]	K _{Water}	K _{Buffer}	K _{ASaliva}	K _{HSaliva}	p-value
1	Methyl ITC	6.792	0.08 ± 0.03	0.108 ± 0.007	0.18 ± 0.09	0.17 ± 0.02	0.02
2	Ethyl ITC	6.890	0.13 ± 0.04	0.17 ± 0.01	0.3 ± 0.1	0.38 ± 0.04	0.0005
3	Propyl ITC	7.448	0.18 ± 0.05	0.24 ± 0.01	0.3 ± 0.1	0.65 ± 0.09	0.00003
4	Butyl ITC	8.422	0.24 ± 0.07	0.32 ± 0.02	0.4 ± 0.2	0.9 ± 0.2	0.00001
5	Hexyl ITC	10.382	0.36 ± 0.09	0.46 ± 0.04	0.5 ± 0.2	3.0 ± 0.6	0.0000001
6	Octyl ITC	12.018	0.45 ± 0.06	0.36 ± 0.03	0.2 ± 0.1	4.0 ± 0.7	0.000000007
7	Isopropyl ITC	6.419	0.20 ± 0.06	0.27 ± 0.01	0.4 ± 0.2	0.9 ± 0.1	0.000002
8	Isobutyl ITC	7.661	0.25 ± 0.07	0.33 ± 0.01	0.4 ± 0.2	1.1 ± 0.2	0.000002
9	Methylallyl ITC	8.622	0.17 ± 0.06	0.23 ± 0.02	0.4 ± 0.2	0.12 ± 0.03	0.02
10	Allyl ITC	8.072	0.13 ± 0.04	0.17 ± 0.01	0.3 ± 0.1	0.06 ± 0.01	0.003
11	3-Butenyl ITC	9.109	0.18 ± 0.05	0.25 ± 0.02	0.4 ± 0.2	0.41 ± 0.06	0.01
12	Cyclopropyl ITC	8.371	0.14 ± 0.04	0.19 ± 0.01	0.3 ± 0.1	0.02 ± 0.02	0.007
13	Cyclopentyl ITC	10.195	0.23 ± 0.06	0.31 ± 0.02	0.4 ± 0.2	1.2 ± 0.2	0.00004
14	Cyclohexyl ITC	11.239	0.28 ± 0.06	0.36 ± 0.03	0.5 ± 0.2	2.0 ± 0.4	0.0000003
15	Phenyl ITC	11.696	0.23 ± 0.05	0.30 ± 0.04	0.4 ± 0.2	0.1 ± 0.09	0.01
16	Benzyl ITC	14.021	0.19 ± 0.03	0.24 ± 0.03	0.4 ± 0.2	0.04 ± 0.04	0.001
17	2-Phenethyl ITC	14.911	0.18 ± 0.03	0.21 ± 0.05	0.4 ± 0.1	0.43 ± 0.07	0.002
18	2-Methoxyethyl ITC	10.302	0.08 ± 0.02	0.11 ± 0.01	0.2 ± 0.1	0.13 ± 0.06	0.02
19	3-(Methylthio)propyl ITC	13.274	0.12 ± 0.02	0.15 ± 0.02	0.3 ± 0.1	0.20 ± 0.02	0.02

alkoxy-containing, and aliphatic unsaturated. For compounds that contain four carbon atoms and a -N=C=S group (C-4 ITCs) differing in chemical structure it was observed that the OTC decrease sequentially for ITCs branched unsaturated, thioether-containing, branched saturated, saturated aliphatic, aliphatic unsaturated groups. Nevertheless, when uncertainties for each threshold were calculated using 'linest' function the obtained values determined based on human response were high compared to measurements performed for instrumental methods (detectors) with highly reproducible curves (in terms of slope and intercept) between replicates. Such high variability in perception of ITCs from person to person may be similar to bitter taste sensitivity divergence among people resulting in very different acceptability of Brassica vegetables among consumers (Wieczorek et al., 2019).

4.2. Relation of odor quality and chemical structure

The odor of unsaturated aliphatic ITCs is described as characteristic for Brassica vegetables. In case of saturated ITCs, the aroma corresponding to Brassica vegetables referred to compounds with the number of carbon atoms $C \leq 3$ in the whole range of concentrations, whereas when the number of carbon atoms increases $C > 6$ the dominant descriptor were sweet and herbal. The examined aromatic ITCs (phenyl ITC, benzyl ITC, 2-phenethyl ITC) smelled like vegetables and herbs. For the branched saturated ITCs, the aroma of Brassica vegetables was present only at low concentrations, high concentrations were characterized by a chemical odor. Additionally for methylallyl ITC and 2-methoxyethyl ITC, odor quality was described mainly as sulfur, garlic, hydrogen sulfide, pungent and gained descriptors responsible for the smell of Brassica vegetables, even though their natural occurrence had not been reported.

4.3. Interaction effects in air-liquid phase systems

Partition coefficients were studied to provide information on the behavior of ITCs in liquid - air two-phase systems, which would be useful in prediction of their release behavior from foods and in-mouth release, which would affect retronasal perception of these compounds. Indeed human saliva as a matrix resulted in significantly different ($p < 0.003$) K compared to other matrices, even artificial saliva. Although human saliva consists of more than 99% water, it is still difficult to reproduce it in laboratory conditions. This oral fluid consists of numerous electrolytes (bicarbonate, calcium, magnesium, phosphates, potassium, and sodium), immunoglobulins, proteins, enzymes, mucins, and nitrogenous products (ammonia and urea) (Humphrey & Williamson, 2001). The differences are probably related to the composition of artificial saliva in

which only mucin was present, no amylases, or other proteins, whereas the pH was the same as of human saliva. Increasing the value of gas-liquid partition coefficients depended on the complexity of the matrix ranging from water, buffer, artificial saliva, and human saliva. Looking at the partition coefficients for human saliva, it can be noticed that their values were higher in relation to water, buffer and artificial saliva. This may be due to a decrease in polarity in subsequent, more complex matrices. The differences in composition regarding macromolecules could result in viscosity differences high enough to influence partition coefficients. Regarding human saliva, the highest partition coefficients were obtained for ITC octyl (4.0 ± 0.7) and hexyl ITC (3.0 ± 0.6), respectively, which can be explained as a result of the lack of interaction with the liquid phase, which is also suggested by low OT values. The lowest K values were obtained for cyclopropyl ITC (0.02 ± 0.02) and benzyl ITC (0.04 ± 0.04) which may indicate the interaction of these compounds with salivary proteins.

5. Conclusions

FPA method described first by Cairncross and Sjostrom (1950) for the food and beverages, a standard method used for evaluation of odors in drinking water (APHA, 2170 Flavor Profile Analysis, 2017) allowed the determination of OTCs for investigated ITCs. The method in its concept is similar to method detection levels in chemical analyses, however it has to be remembered that sensory thresholds vary across individuals, in many cases substantially, and the measurements are prone to differences related to contamination, background interferences, experimental conditions and practice. To minimize probability of bias and errors strict conformation to method instructions and control requirements should be followed (Meng & Suffet, 1992; Suffet et al., 1988).

The low values for orthonasal OTs for 19 ITCs ($\mu\text{g/L}$) obtained in water matrix confirms that volatile hydrolysis products of glucosinolates can have considerable influence on aroma generation in Brassica vegetables. However, not every ITC for which the OTC has been determined has a natural occurrence in Brassica vegetables and their role as key odorants in these vegetables still requires a comprehensive study. It should be emphasized that the OTCs obtained refer to aqueous solutions and while similar values can be expected for vegetables, they will be different from the threshold values determined by gas chromatography with olfactometry. FPA and plotted Weber-Fechner curves show that an increase in carbon atoms of n-aliphatic unsaturated ITC molecule causes a decrease in the OTC. This correlation does not apply to the ethyl isothiocyanate, which requires more chemosensory and chemoreceptor research in the future. This work provides valuable data for fragrance

research, e.g. profiling key odor compounds in various matrices using e.g. GC-O.

No clear relationship was observed between the OTC and the water/air coefficient. Salting-out effect occurring in the artificial saliva buffer sample have no influence on profile of K. This study emphasizes the effect of human saliva on retention or release of aroma compounds present in Brassica vegetables. By comparison, human saliva and artificial saliva, the compounds which are more likely to transit to air phase are octyl ITC, hexyl ITC, cyclohexyl ITC, cyclopentyl ITC, and isobutyl ITC. The results suggest that these ITCs can be sensed mainly by orthonasal sensing. The strongest effect of retention by proteins present in saliva concerns cyclopropyl ITC, benzyl ITC, and allyl ITC. That results indicate a possibility that retained ITCs may have a major influence on mouthfeel flavor sensation however, both cases require detailed on receptor research.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

CRediT authorship contribution statement

Monika Marcinkowska: Conceptualization, Investigation, Methodology, Writing - original draft. **Henryk H. Jeleń:** Conceptualization, Methodology, Writing - review & editing, Formal analysis, Funding acquisition.

Acknowledgements

This work was supported by the National Science Centre (Poland) [grant number 548 2015/18/M/NZ9/00372]. We would like to thank volunteers from the Food Chemistry and Instrumental Analysis Department of The University of Life Sciences in Poznań.

References

- APHA 2170 Flavor profile Analysis. (2017). In R. B. Baird, A. D. Eaton, & E. W. Rice (Eds.), *Standard methods for the examination of water and wastewater* (23rd ed.). <https://doi.org/10.2105/SMWW.2882.216>.
- Caincross, S. E., & Sjostrom, L. B. (1950). Flavor profiles: A new approach to flavor problems. *Food Technology*, 4(8), 308–311.
- Cancho, B., Fabrellas, C., Diaz, A., Ventura, F., & Galceran, M. T. (2001). Determination of the odor threshold concentrations of iodinated trihalomethanes in drinking water. *Journal of Agricultural and Food Chemistry*, 49(4), 1881–1884. <https://doi.org/10.1021/jf001252m>.
- Cartea, M. E., & Velasco, P. (2008). Glucosinolates in Brassica foods: Bioavailability in food and significance for human health. *Phytochemistry Reviews*, 7(2), 213–229. <https://doi.org/10.1007/s11101-007-9072-2>.
- Diaz, A., Fabrellas, C., Ventura, F., & Galceran, M. T. (2005). Determination of the odor threshold concentrations of chlorobrominated anisoles in water. *Journal of Agricultural and Food Chemistry*, 53(2), 383–387. <https://doi.org/10.1021/jf049582k>.
- EPA DSSTox. (2020, April 23). Retrieved from <https://www.epa.gov/chemical-research/distributed-structure-searchable-toxicity-dssto-database>.
- Gemert, L. J. V. (2011). *Compilations of odour threshold values in air, water and other media*. Utrecht: Olie-mans Punter & Partners BV.
- Hanschen, F. S., Kühn, C., Nickel, M., Rohn, S., & Dekker, M. (2018). Leaching and degradation kinetics of glucosinolates during boiling of Brassica oleracea vegetables

- and the formation of their breakdown products. *Food Chemistry*, 263, 240–250. <https://doi.org/10.1016/j.foodchem.2018.04.069>.
- Hanschen, F. S., Lamy, E., Schreiner, M., & Rohn, S. (2014). Reactivity and stability of glucosinolates and their breakdown products in foods. *Angewandte Chemie International Edition*, 53(43), 11430–11450. <https://doi.org/10.1002/anie.201402639>.
- Hansen, K. C., Zhou, Z., Yaws, C. L., & Aminabhavi, T. M. (1995). A laboratory method for the determination of Henry's law constants of volatile organic chemicals. *Journal of Chemical Education*, 72(1), 93. <https://doi.org/10.1021/ed072p93>.
- Humphrey, S. P., & Williamson, R. T. (2001). A review of saliva: Normal composition, flow, and function. *The Journal of Prosthetic Dentistry*, 85(2), 8.
- Iranshahi, M. (2012). A review of volatile sulfur-containing compounds from terrestrial plants: Biosynthesis, distribution and analytical methods. *Journal of Essential Oil Research*, 24(4), 393–434. <https://doi.org/10.1080/10412905.2012.692918>.
- Kala, C., Ali, S. S., Ahmad, N., Gilani, S. J., & Khan, N. A. (2018). Isothiocyanates: A review. *Research Journal of Pharmacognosy*, (2) <https://doi.org/10.22127/rjp.2018.58511>.
- Kranner, S. W. (1988). Flavor-profile Analysis: An objective sensory technique for the identification and treatment of off-flavors in drinking water. *Water Science and Technology*, 20(8–9), 31–36. <https://doi.org/10.2166/wst.1988.0221>.
- Meng, A.-K., & Suffet, I. H. (1992). Assessing the quality of flavor profile analysis data. *Journal of American Water Works Association*, 84(6), 89–96.
- Muñoz-González, C., Feron, G., Guichard, E., Rodríguez-Bencomo, J. J., Martín-Alvarez, P. J., Moreno-Arribas, M. V., et al. (2014). Understanding the role of saliva in aroma release from wine by using static and dynamic headspace conditions. *Journal of Agricultural and Food Chemistry*, 62(33), 8274–8288. <https://doi.org/10.1021/jf503503b>.
- Oliviero, T., Verkerk, R., & Dekker, M. (2018). Isothiocyanates from Brassica vegetables: effects of processing, cooking, mastication, and digestion. *Molecular Nutrition & Food Research*, 62(18), 1701069. <https://doi.org/10.1002/mnfr.201701069>.
- Pagès-Hélary, S., Andriot, I., Guichard, E., & Canon, F. (2014). Retention effect of human saliva on aroma release and respective contribution of salivary mucin and α -amylase. *Food Research International*, 64, 424–431. <https://doi.org/10.1016/j.foodres.2014.07.013>.
- Pasko, P., Okoń, K., Krośniak, M., Prochowik, E., Żmudzki, P., Kryczyk-Kozioł, J., et al. (2018). Interaction between iodine and glucosinolates in rutabaga sprouts and selected biomarkers of thyroid function in male rats. *Journal of Trace Elements in Medicine & Biology*, 46, 110–116. <https://doi.org/10.1016/j.jtemb.2017.12.002>.
- Pelosi, P., Zhu, J., & Knoll, W. (2018). From gas sensors to biomimetic artificial noses. *Chemosensors*, 6(3), 32. <https://doi.org/10.3390/chemosensors603032>.
- Peng, C., Zhao, S.-Q., Zhang, J., Huang, G.-Y., Chen, L.-Y., & Zhao, F.-Y. (2014). Chemical composition, antimicrobial property and microencapsulation of Mustard (Sinapis alba) seed essential oil by complex coacervation. *Food Chemistry*, 165, 560–568. <https://doi.org/10.1016/j.foodchem.2014.05.126>.
- Samec, D., Pavlović, I., Radojčić Redovniković, I., & Salopek-Sondi, B. (2018). Comparative analysis of phytochemicals and activity of endogenous enzymes associated with their stability, bioavailability and food quality in five Brassicaceae sprouts. *Food Chemistry*, 269, 96–102. <https://doi.org/10.1016/j.foodchem.2018.06.133>.
- Schweitzer, L., Tomboulis, P., Atasi, K., Chen, T., & Khari, D. (2004). Utility quick test for analyzing materials for drinking water distribution systems for effect on taste-and-odor. *Water Science and Technology*, 49(9), 75–80. <https://doi.org/10.2166/wst.2004.0538>.
- Suffet, I. H., Brady, B. M., Bartels, J. H., Burlingame, G., Mallevialle, J., & Yohe, T. (1988). Development of the profile analysis method into a standard method for sensory analysis of water. *Water Science and Technology*, 20(9/9), 1–9.
- Tomsone, L., Kruma, Z., Galoburda, R., & Talou, T. (2013). Composition of volatile compounds of horseradish roots (Armoracia rusticana L.) depending on the genotype. *Proceedings of the Latvia University of Agriculture*, 29(1), 1–10. <https://doi.org/10.2478/plua-2013-0001>.
- Valsaraj, K. T., Gupta, A., Thibodeaux, L. J., & Harrison, D. P. (1988). Partitioning of chloromethanes between aqueous and surfactant micellar phases. *Water Research*, 22(9), 1173–1183. [https://doi.org/10.1016/0043-1354\(88\)90013-9](https://doi.org/10.1016/0043-1354(88)90013-9).
- Wieczorek, M. N., Walczak, M., Skrzypczak-Zielińska, M., & Jeleń, H. H. (2019). Bitter taste of Brassica vegetables: The role of genetic factors, receptors, isothiocyanates, glucosinolates, and flavor context. *Critical Reviews in Food Science and Nutrition*, 58(18), 3130–3140. <https://doi.org/10.1080/10408398.2017.1353478>.
- Wu, Y., Shen, Y., Wu, X., Zhu, Y., Mupunga, J., Bao, W., et al. (2018). Hydrolysis before stir-frying increases the isothiocyanate content of broccoli. *Journal of Agricultural and Food Chemistry*, 66(6), 1509–1515. <https://doi.org/10.1021/acs.jafc.7b05913>.

Inactivation of Thioglucosidase from *Sinapis alba* (White Mustard) Seed by Metal Salts

Monika Marcinkowska  and Henryk H. Jeleń * 

Faculty of Food Science and Nutrition, Poznań University of Life Sciences, Wojska Polskiego 31, 60-624 Poznań, Poland; monika.marcinkowska@up.poznan.pl

* Correspondence: henrykj@up.poznan.pl; Tel.: +48-618-487-273

Received: 27 August 2020; Accepted: 21 September 2020; Published: 23 September 2020



Abstract: The glucosinolates which are specialized plant metabolites of *Brassica* vegetables are prone to hydrolysis catalyzed by an endogenous enzyme myrosinase (thioglycoside hydrolase, thioglucosidase) that exists in *Brassica* plant tissue causing volatile isothiocyanates release. Currently existing literature data on the inactivation of myrosinase is insufficient in particular for use in the analysis of volatile and odor compounds in vegetables rich in glucosinolates. In this study, the impact of different metal salts in effective inactivation of enzyme activity was investigated by solid-phase microextraction (SPME) and GC/MS system in aqueous samples and kohlrabi matrix. A saturated solution of calcium chloride which is commonly used to stop enzyme activity in plant tissue inactivates the myrosinase–glucosinolate system. However, even without the participation of myrosinase, it changes the reaction pathway towards nitrile formation. The model experiment shows that optimum efficiency in inhibition of the enzyme system shows iron(III) ions, silver ions, and anhydride sodium sulfate resulting in no volatile products derived from glucosinolates. However, in the kohlrabi matrix, the strongest enzyme inhibition effect was observed for silver salt resulting in no volatile products, also both anhydrous Na_2SO_4 and saturated CaCl_2 solution seem to be useful inhibitors in flavor studies.

Keywords: myrosinase; thioglucosidase; enzyme inactivation; glucosinolates hydrolysis; kohlrabi; isothiocyanates; nitriles; SPME

1. Introduction

Enzymes (mainly myrosinase and lipoxygenase) play a crucial role in the creation of *Brassica* vegetable flavor. Myrosinase can convert glucosinolates into volatile products that not only contribute to the aroma of vegetables, but also have documented health-promoting properties (anticarcinogenic activity, chemoprotective, prevention of cardiovascular and neurodegenerative disorders) [1]. The lipoxygenase enzyme family provide catalytic oxidation of polyunsaturated fatty acids with a *cis,cis*-1,4-pentadiene moiety resulting in a number of important metabolites and odor active compounds [2,3]. Meat-like flavors associated mainly with Maillard reaction can be created by a double enzyme (Flavourzyme) in the two-step hydrolysis of *Brassica* proteins [4]. Knowledge of enzymes allows for controlling the enzymatic processes in plant tissue and thus maintain the quality by using a wide range of anti-browning agents against enzymatic browning of fruit and vegetable products [5].

On the other hand, enzymes pose an analytical challenge, especially in plant-derived food. The study which is based on analysis of vegetable tissues is enormously complex due to variable content of compounds which are specific for plant species, varieties, environmental conditions, part of a plant, cultivation, the region of origin, the status of growth, the season of cultivation, postharvest, and food processing [6], and varying activity of endogenous enzymes such as myrosinase [7]. Myrosinase is

endogenous thioglucoside glucohydrolase that is present in glucosinolate-rich *Brassicaceae* vegetables such as broccoli, brussels sprout, cabbage, cauliflower, kohlrabi, mustard, and radish; however it was also found in bacteria, fungi and even in the human gut microflora [8]. Glucosinolates in presence of myrosinase are prone to hydrolysis after tissue disruption (blending, cutting) into β -D-glucose and an intermediate product (aglycon). This latter compound is spontaneously converted into isothiocyanates, nitriles, thiocyanates, epithionitriles, indoles, or 1,3-oxazolidine-2-thiones. The type of glucosinolate product obtained during hydrolysis strictly depends on the reaction conditions and the original glucosinolate side-chain structure (aliphatic, aromatic, and indolic) [9,10].

Myrosinase is an enzyme that converts glucosinolates into glucosinolate degradation compounds, most of which are volatile and odor active. An active enzymatic reaction in the vegetable tissue is an analytical problem as it leads to inaccurate results and large variations between samples. Screening of volatiles is not the only branch of analytical chemistry where blocking of thioglucosidase enzyme is important. Quantitative analysis of metabolites (glucosinolates) in plant tissues requires the enzyme to be blocked, which prevents their further degradation during the analysis and leads to deviations in the obtained results. The inactivation of the enzyme at an exact time is also important to increase the reproducibility of results obtained between laboratories.

Currently, there are only few studies about the inactivation methods of myrosinase. The only well-known approach of deactivating this enzyme activity is pH changing [11], thermal treatment [12], pressure enzyme inactivation, or fusion of these latter two methods [13]. However, both methods have disadvantages; the first approach cannot be used simultaneously with the analysis of thermolabile compounds and pressure inactivation needs specific equipment that is unique and not available for all laboratories.

The aim of this study was to investigate the impact of different metal salts in inactivation of myrosinase activity. The utilization of metal salts is simple to use, widely available, and relatively cheap. This knowledge will be useful for future research in flavor studies especially for identification and quantification of volatile compounds extracted from plant tissue and to improve analytical extraction protocols for glucosinolate-containing plants by increasing reproducibility when preparing samples for analysis.

2. Results

2.1. Preliminary Experiments with Commercial Thioglucosidase

A preliminary experiment relied on analyzing volatile glucosinolate breakdown products in aqueous system. To select the most effective agent for the inactivation of thioglucosidase, the volatile products were determined by GC/MS system. The peak area represents the total ion current (TIC). As shown in Figure 1a not all of the investigated metal salts were effective at blocking myrosinase, resulting in a similar amount of obtained isothiocyanates as in the control test (H_2O). Manganese ions proved to be completely ineffective at blocking the enzymatic reaction for both glucosinolates, glucotropaeolin, and synigrin. The adequate molar concentration of calcium and sodium ions and saturated solution of sodium chloride exhibited inhibition effect for synigrin, nevertheless they were ineffective in the case of blocking glucotropaeolin hydrolysis.

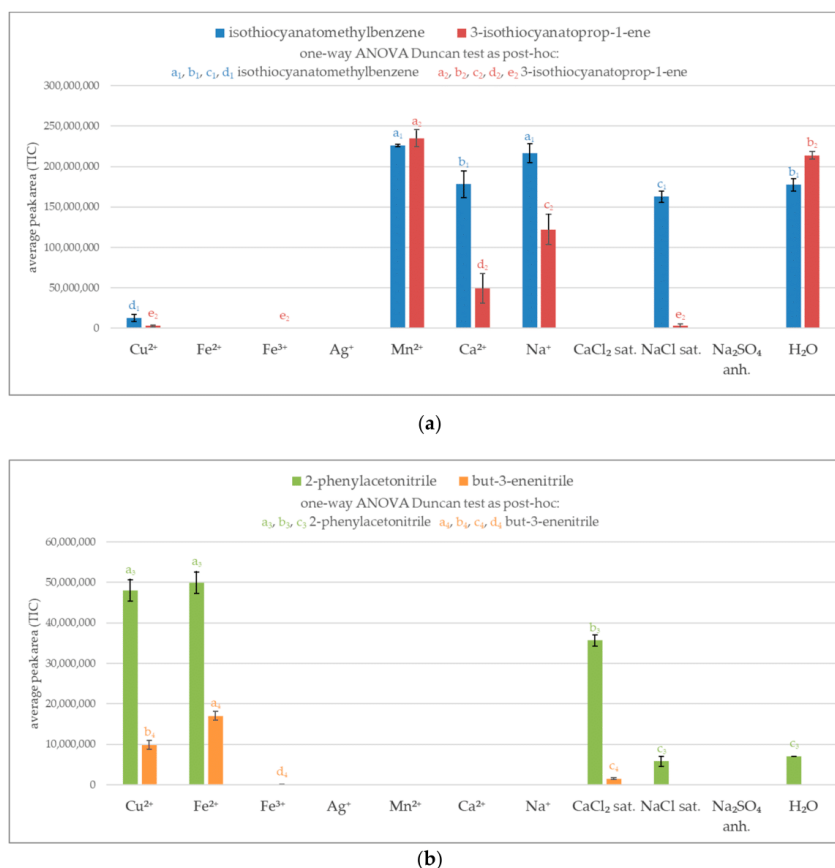


Figure 1. Volatile products of glucosinolates breakdown in miscellaneous enzyme inactivation media; sat.—saturated solution; anh.—anhydrous; a, b, c, d, e—the result of statistical analysis (one-way ANOVA Duncan test as post-hoc), values followed by the same letter in a Duncan grouping are not significantly different, the subscript number and letter color are corresponding to the chart legend ($\alpha = 0.05$); (n = 3); (a) Isothiocyanates (isothiocyanatomethylbenzene derived from glucotropaeolin and 3-isothiocyanatoprop-1-ene is a product from sinigrin); (b) Nitriles (2-phenylacetonitrile from glucotropaeolin and but-3-enitrile from sinigrin).

The maximum efficiency in inhibition of enzyme system was noticed for iron ions (Fe^{2+} and Fe^{3+}), silver ions, saturated solution of calcium chloride, and anhydrous sodium sulfate. The use of these salts did not produce volatile isothiocyanates for both glucotropaeolin and sinigrin. However, some of the potential enzyme-blocking agents produced volatile nitriles, for example iron(II) and saturated calcium chloride. Except for these salts, copper(II) ions show the ability to produce a considerable amount of nitriles (Figure 1b).

Previous preliminary experiments show that silver ions were one of the most effective methods to inactivate myrosinase enzymatic activity resulting in no volatile products derived from glucosinolate. Figure 2a shows silver ions dependent inhibition of sinigrin hydrolysis curve where concentration is presented as a logarithm function. The same experiment was performed on glucotropaeolin glucosinolate (Figure 2b). The average peak areas were measured in extracted-ion chromatogram

mode (EIC) for products of enzymatic hydrolysis: 3-isothiocyanatoprop-1-ene ($m/z = 99.2$) and for isothiocyanato methylbenzene ($m/z = 91.2$).

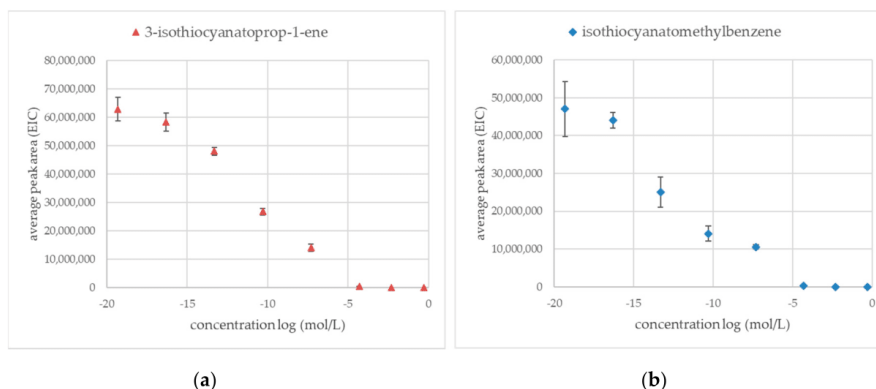


Figure 2. Area of the peaks (EIC) to the logarithm of the concentration of silver ions in the aqueous solution (mol/L); Statistical analysis: One-way ANOVA Duncan test as post-hoc ($\alpha = 0.05$); ($n = 3$) (a) 3-isothiocyanatoprop-1-ene derived from sinigrin ($m/z = 99.2$); values obtained for concentration log in the range of 0 to -5 are not significantly different (b) Isothiocyanato methylbenzene derived from glucotropaeolin ($m/z = 91.2$); values obtained for concentration log in the range of 0 to -5 , -7 , to -11 , and -15 to -20 are not significantly different.

The silver ions were an effective inhibition factor for the inactivation of thioglucosidase for both glucosinolate sinigrin and glucotropaeolin. The lowest concentration of silver salt, which is effective in blocking thioglucosidase enzyme activity was 5×10^{-5} mol/L. Results obtained for the lowest investigated concentration of silver salt (5×10^{-20} mol/L) equals average peak areas measured for the control sample (water, enzyme, sinigrin, or glucotropaeolin).

Results obtained from the first experiment indicated that the favored volatile products for some enzyme-blocking agents (metal salts) are nitriles. An investigation of the interaction between glucosinolate and inactivation agent to investigate non enzymatic reaction pathway was performed. To this stage, metal salts were selected with the best ability in blocking enzymatic hydrolysis of glucosinolates. In that part of the study, volatile compounds after the addition of glucosinolate to an aqueous solution of metal salts without enzyme (thioglucosidase from *Sinapis alba*) addition were measured. The control sample was water and glucosinolate (sinigrin potassium salt or glucotropaeolin potassium salt) in which no volatiles were produced.

It was observed that aqueous silver and iron(III) solutions did not result in any volatile products derived by chemical degradation of glucosinolates. On the other hand, iron(II) and copper(II) ions or saturated solution of calcium chloride produced nitriles (2-phenylacetone nitrile and but-3-enenitrile). When both sinigrin and glucotropaeolin were used, the highest amount of nitrile was obtained in the iron(II) solution. In a blocking agent environment, more volatile products were formed from sinigrin than glucotropaeolin from the same amount of glucosinolate, nevertheless, results obtained in saturated sodium chloride solution were similar. The results are presented in Figure 3.

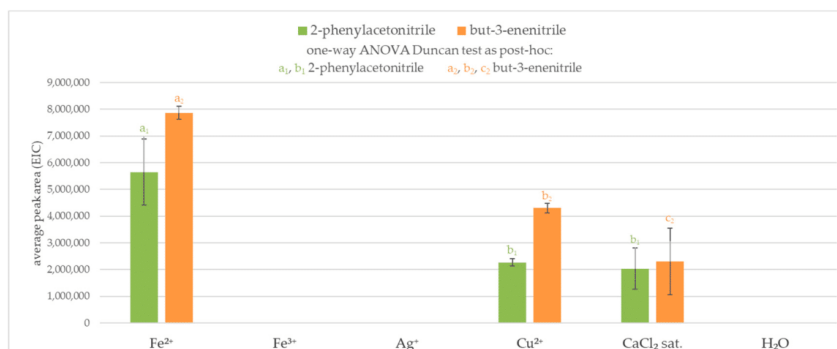


Figure 3. Volatile products from the interaction between glucosinolate (sinigrin or glucotropaeolin) and an enzymatic reaction blocking agent (without enzyme presence); 2-phenylacetoneitrile ($m/z = 117.2$); but-3-enenitrile ($m/z = 41.3$); a, b, c—the result of statistical analysis (one-way ANOVA Duncan test as post-hoc), values followed by the same letter in a Duncan grouping are not significantly different, the subscript number and letter color are corresponding to the chart legend ($\alpha = 0.05$); ($n = 3$).

2.2. Study of the Effect of Metal Salts on the Native Enzyme in Kohlrabi Tissue

There is no doubt that plant tissue is a different environment than water, that is why the behavior of glucosinolates (absent in kohlrabi and used previously in the model experiment) was investigated in a model matrix in which this class of compounds occur naturally. The same batches of kohlrabi were used for established volume (3 mL) of aqueous metal salts solutions or sufficient amount of anhydride sodium sulfate salt. The control sample was water and glucosinolate (sinigrin potassium salt or glucotropaeolin potassium salt) without any metal salt addition. Compared to the model experiment, a larger volume of salt solution was used—allowing the complete immersion of plant tissue in the aqueous solution and an adequately increased amount of glucosinolate. It was observed that kohlrabi samples after extraction and analysis differed depending on the metal salt used for the analysis. Results of GC/MS analyses are shown in Figure 4.

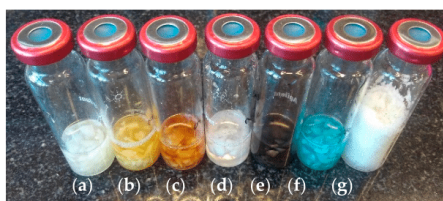


Figure 4. Kohlrabi matrix samples with addition of sinigrin potassium salt after GC/MS analysis (a) control (H₂O); (b) iron(II) sulfate (FeSO₄); (c) iron(III) sulfate (Fe₂(SO₄)₃); (d) saturated solution of calcium chloride (CaCl₂); (e) silver nitrate (AgNO₃); (f) copper sulfate (CuSO₄); (g) anhydrous sodium sulfate salt (Na₂SO₄).

The main volatiles derived from glucosinolates in various environmental conditions in the kohlrabi matrix are presented in Table 1. But-3-enenitrile was formed only in samples with addition of synigrin with the presence of iron(II) and copper(II) ions. The most abundant peak for 3-isothiocyanatoprop-1-ene was recorded in control sample (H₂O), low amounts of this isothiocyanate were also noted for a saturated solution of calcium chloride and anhydrous sodium sulfate. Iron(III) and silver ions caused the complete blockage of the enzyme that hydrolyzes the added synigrin.

The presence of 2-phenylacetonitrile was not recorded in the sequence with the addition of synigrin, while it was detected in the samples with the addition of glucotropaeolin. The highest amount of 2-phenylacetonitrile was found for copper(II) ions, half less when iron(II) ions were used, and negligible amounts were found in the calcium chloride and iron(III) solutions. Isothiocyanatomethylbenzene was also found only in the samples to which glucotropaeolin was added. However, the presence of this isothiocyanate was not confirmed in the samples with the addition of synigrin. The only peak for isothiocyanatomethylbenzene was in the control sample (H₂O).

The profile of 4-methylsulfanylbutanenitrile and 5-methylsulfanylpentanenitrile average peak area depending on various environmental conditions for samples with the addition of synigrin and glucotropaeolin was similar. The higher content of 4-methylsulfanylbutanenitrile than the control was observed in both samples with the addition of synigrin and glucotropaeolin in aqueous solutions of copper(II) sulfate, and iron(II) salts. In aqueous solution of silver salt 4-methylsulfanylbutanenitrile was not found. Concerning the control sample, an increased amount of this compound is observed in the presence of copper ions, while in the medium of silver and iron(III) ions, the presence of this nitrile was not observed.

For the 1-isothiocyanato-3-methylsulfanylpropane and 1-isothiocyanato-4-methylsulfanylbutane, the average peak area values follow a comparable trend. Isothiocyanates were observed only in the control sample (H₂O), in a saturated solution of sodium chloride and anhydrous sodium sulfate. Average peak areas for the 1-isothiocyanato-4-methylsulfanylbutane are analogous, and for 1-isothiocyanato-3-methylsulfanylpropane peak area obtained for samples with saturated sodium chloride solution is slightly reduced compared to the control sample.

Miscellaneous volatiles not related to the breakdown of glucosinolates were shown in Table 2. Addition of silver ions resulted in no volatiles produced. Sulfides ((methyldisulfanyl)methane and (methyltrisulfanyl)methane) were obtained mainly in the control sample and samples with anhydrous sodium sulfate and iron(II) sulfate. The presence of benzaldehyde was found in almost all tests except those with an aqueous solution of silver nitrate and anhydrous sodium sulfate. Compared to the control sample, an increased concentration of volatile compounds (hexanal, 2-pentylfuran, heptan-2-one, (E)-non-2-enal) was observed with the use of iron(III) ions. The highest mean peak value for hexanal was observed for iron(III) ions, copper ions, and iron(II) ions, respectively. Pent-1-en-3-ol was detected in tests with the addition of iron(II) ions, saturated sodium chloride solution and anhydrous sodium sulfate.

Table 1. Volatile compounds derived from glucosinolates in variable conditions within the kohlrabi tissue.

Compound Name	RR _{Exp}	RR _{lit}	H ₂ O	Fe ²⁺	Ag ⁺	Fe ³⁺	CaCl ₂ sat. ¹	Cu ²⁺	Na ₂ SO ₄ anh. ²
sinigrin potassium salt									
but-3-enitrile	1169	1166 [14]	84767 @b	1081935 *a	16794 #b	-	52008 #b	1231764 \$a	23012 \$b
3-isothiocyanatoprop-1-ene	1356	1383 [15]	21509589 #a	-	-	68156 \$b	390208 \$b	59443 @b	257598 \$b
4-methylsulfanybutanenitrile	1785	1733 [16]	482607 #b	658725 *b	-	390468 #b	540572 \$b	979739 #a	459707 #b
5-methylsulfanypentanenitrile	1948	1891 [15]	664729 *c	-	-	814106 \$b	1155252 \$b	2520488 #a	893718 #bc
1-isothiocyanato-3-methylsulfanypropane	1976	1983 [17]	6175537 #a	-	-	24958 @b	5770687 *a	-	6790716 #a
1-isothiocyanato-4-methylsulfanybutane	2167	2135 [16]	4351726 *a	-	-	-	4149057 #a	-	4135976 #a
glucotropaeolin potassium salt									
4-methylsulfanybutanenitrile	1785	1733 [16]	414374 #d	588352 *bc	-	376014 #d	561198 \$b	936512 *a	470188 #cd
2-phenylacetone	1931	1912 [18]	-	169090 *b	-	114952 #c	124882 *c	3746581 #a	-
5-methylsulfanypentanenitrile	1948	1891 [15]	687165 #b	-	-	814106 \$b	888626 *b	2335283 *a	679047 \$b
1-isothiocyanato-3-methylsulfanypropane	1976	1983 [17]	6697967 #ab	-	-	-	5169479 *b	26058 *c	7497730 \$a
1-isothiocyanato-4-methylsulfanybutane	2167	2135 [16]	4277184 #a	-	-	-	3682303 *a	-	3509385 \$a

¹: saturated solution of calcium chloride; ²: anhydrous sodium sulfate; RR_{Exp}: relative retention index calculated by n-alkane series using the experimental value (Supelcowax-10 column); RR_{lit}: relative retention index calculated by n-alkane series using the literature value; relative standard deviation: *, RSD ≤ 25%; \$, 25% < RSD ≤ 50%; @, RSD > 50%; values express the mean peak area; a, ab, b, bc, c, cd, d: the result of statistical analysis (one-way ANOVA Duncan test as post-hoc), values followed by the same letter in a Duncan grouping are not significantly different ($\alpha = 0.05$); (n = 3).

Table 2. Miscellaneous volatile compounds from kohlrabi tissue.

Compound Name	RRI _{exp}	RRI _{lit}	H ₂ O	Fe ²⁺	Ag ⁺	Fe ³⁺	CaCl ₂ sat. 1	Cu ²⁺	Na ₂ SO ₄ anh. 2
sinigrin potassium salt									
(methylsulfonyl)methane	1068	1064 [15]	317715 #b	69214 #c	-	5106 %c	-	2482 %c	419816 %a
hexanal	1075	1082 [18]	47284 \$b	169473 #b	-	3789656 #a	42992 *b	233815 @b	61231 #b
pent-1-en-3-ol	1141	1158 [18]	5905 %c	119966 %c	-	-	70899 \$b	-	187903 #a
(E)-hex-2-enal	1218	1216 [18]	-	-	-	-	13660 @b	-	36592 %a
2-pentylfuran	1222	1232 [18]	-	26154 *b	-	-	-	2988 #c	2493 %c
heptan-2-one	1325	1182 [18]	-	-	-	242172 *a	-	-	-
(Z)-hex-3-en-1-ol	1364	1380 [18]	-	10077 @b	-	-	102469 *c	-	58909 #a
(methylsulfonyl)methane	1391	1376 [18]	2974175 #a	-	-	-	-	-	83767 \$b
benzaldehyde	1530	1518 [18]	46994 %a	1311 #c	-	25629 *b	16560 #b	2234 #c	-
(E)-non-2-enal	1539	1535 [18]	20479 *b	-	-	147120 #a	-	-	-
glucotropaeolin potassium salt									
(methylsulfonyl)methane	1068	1064 [15]	615854 *a	93952 #c	-	-	3438 *c	3848 %c	342112 *b
hexanal	1075	1082 [18]	71835 #b	140440 \$b	-	3789656 #a	27612 #b	202812 *b	57077 #b
pent-1-en-3-ol	1141	1158 [18]	9007 *b	100647 *b	-	-	190000 %a	2830 #b	159107 %a
(E)-hex-2-enal	1218	1216 [18]	-	-	-	-	69644 %a	-	39481 %a
2-pentylfuran	1222	1232 [18]	-	10856 *b	-	138788 #a	-	-	-
heptan-2-one	1325	1182 [18]	-	-	-	242172 *a	-	-	-
(Z)-hex-3-en-1-ol	1364	1380 [18]	-	3675 \$b	-	-	142612 #a	-	45657 \$b
(methylsulfonyl)methane	1391	1376 [18]	3243687 #a	-	-	-	-	-	88756 \$b
benzaldehyde	1530	1518 [18]	43684 #a	3781 *c	-	25629 *b	23531 #b	18571 *bc	-
(E)-non-2-enal	1539	1535 [18]	29224 *b	-	-	147120 #a	-	-	-

1. saturated solution of calcium chloride; 2. anhydrous sodium sulfate; RRI_{exp}: relative retention index calculated by n-alkane series using the experimental value (Supelcowax-10 column); RRI_{lit}: relative retention index calculated by n-alkane series using the literature value; relative standard deviation: *; RSD ≤ 10%; #: 10% < RSD ≤ 25%; \$: 25% < RSD ≤ 50%; @: RSD > 50%; values express the mean peak area; a, b, bc, c: the result of statistical analysis (one-way ANOVA Duncan test as post-hoc); values followed by the same letter in a Duncan grouping are not significantly different (α = 0.05); (n = 3).

3. Discussion

The composition of Brassica vegetable tissue usually promotes the formation of isothiocyanates [19]. Glucosinolates are prone to hydrolysis by myrosinase and as a result of degradation various volatile compounds can be obtained, which are strictly dependent on the conditions (e.g., pH value, availability of iron(II) (Fe^{2+}) and the presence of specific proteins that interact with myrosinase) [20,21]. Several heavy metals were reported to exhibit inactivation of enzymatic reaction on different types of enzymes that are used especially in biosensors studies [22]. Saturated sodium chloride and calcium chloride solutions are known to inactivate enzyme system and they are commonly used in flavor study [23,24]. An alternative method to stop the enzyme reaction is to remove water by using anhydrous sodium sulfate [25]. However, myrosinase has so far been poorly studied, particularly concerning the volatile analysis and flavor study.

Results of the preliminary experiments showed that the addition of metal salts may have different consequences such as decreasing or stopping of enzyme activity, change of breakdown pathways, or salting-out effect. Silver and iron(III) ions are enzyme reaction inhibitors as heavy metals. Silver ions completely block the action of myrosinase, even at a concentration of 5×10^{-5} mol/L, regardless of the glucosinolate used. Their action can be explained by the conformational change of myrosinase protein. Anhydrous sodium sulfate is also effective because it removes water without which the enzyme is inactive. A saturated solution of sodium chloride is a valuable inhibitor concerning aliphatic glucosinolates however, in the case of aromatic glucosinolates it is ineffective. A slight inhibitory effect with a similar tendency was observed in equimolar solutions of sodium and calcium ions. This effect can be explained by changing the enzyme's active center, which blocks the enzyme's activity only in relation to a specific substrate—the group of aliphatic glucosinolates.

The increased average peak area of isothiocyanates in samples with additional manganese salt is related to the salting-out effect. It is well known that iron(II) ions as well as the acidic environment promote the formation of nitriles, which is explained by the formation of a complex with glucosinolate. Copper also forms considerable amounts of nitriles because, like iron(II), it is a transition metal that willingly forms supramolecular structures. What is more, both the aqueous solution of iron(II) and copper(II) are acidic ($\text{pH} < 7$), because their salts are derived from strong acids and transition metals. Nevertheless, the previously tested aqueous solution of manganese chloride does not change the reaction pathway to nitriles, which suggests that when using transition metal salt ions, the formation of the complex is a more important factor than the pH value of the reaction medium. Interestingly, a saturated sodium chloride solution indeed blocked the possibility of obtaining isothiocyanates, but nitriles were recorded instead. The use of saturated sodium chloride solution to inactivate the enzyme may falsely increase the proportion of nitriles in the analysis of volatile compounds directly from vegetables in the sample. Metal salts which do not form any volatile compounds such as isothiocyanates and nitriles from the glucosinolate can be considered as a suitable blocking agent.

An experiment with glucosinolate and selected metal salts was executed to show that nitriles can be formed without the enzyme presence. The highest amount of nitriles was obtained when iron(II) ions were used, which is consistent with current scientific reports [26,27]. The presence of nitriles was also noted in samples with copper ions and a saturated sodium chloride solution. The absence of the enzyme in the samples tested suggests that the degradation of glucosinolates can be non-enzymatic. Thus, the degradation of glucosinolates can take place catalytically through the formation of a complex structure [26]; however, the full confirmation of this hypothesis still requires further research.

Measurements of myrosinase activity are usually done by utilizing isolated enzyme and water environment however to establish the best blocking agent for myrosinase it is needed to study this phenomenon in cellular conditions [28]. But-3-enenitrile and 3-isothiocyanatoprop-1-ene are formed from synigrin. 2-phenylacetone nitrile and isothiocyanatomethylbenzene are obtained from glucotropaeolin. These two glucosinolates were artificially added to the vegetable. Additionally, kohlrabi contains native glucosinolates (glucoerucin, glucoiberberin, glucoraphanin [29]) which form the corresponding volatile products (4-methylsulfanylbutanenitrile, 1-isothiocyanato-3-

methylsulfanylpropane, 1-isothiocyanato-4-methylsulfanylbutane). 5-methylsulfanyl pentanenitrile derived from glucoberveroin.

In kohlrabi bulb tissue several volatiles were observed that are related to the action of lipoxygenase (e.g., hexanal, (E)-hex-2-enal, pent-1-en-3-ol, (E)-hex-2-enal, 2-pentylfuran, heptan-2-one, (Z)-hex-3-en-1-ol, (E)-non-2-enal) [30], therefore, we can additionally evaluate the effectiveness of the metal salts used to block this enzyme. According to the preliminary experiment, the strongest inhibition effect of thioglucosidase inactivation in the kohlrabi matrix has a silver and iron(III). However, the iron(III) salt causes an important increase in some volatile compounds derived from lipid oxidation, primarily hexanal, 2-pentylfuran and heptan-2-one, which may distort the results of the analyzes (profile of volatiles). Enzyme inactivation can be caused by changing the conformation of the enzyme protein and destroying the key and lock enzyme system. Likewise, in the kohlrabi matrix environment of copper and iron(II) salts, analogous products are formed as in the model system. The change of the pathway of glucosinolates degradation by these salts is most likely caused by the catalytic decomposition of an intermediate complex compound.

4. Materials and Methods

4.1. Materials and Reagents

The reference compounds (3-isothiocyanatoprop-1-ene (CAS 57-06-7), but-3-enenitrile (CAS 109-75-1), isothiocyanatomethylbenzene (CAS 622-78-6), 2-phenylacetone nitrile (CAS 140-29-4)) of the highest available GC purity (>98%) were obtained from Sigma-Aldrich (Poznań, Poland). Thioglucosidase from *Sinapis alba*, sinigrin potassium salt, anhydrous sodium sulfate, iron(II) sulfate heptahydrate, SPME fiber divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) were purchased from Sigma-Aldrich (Poznań, Poland), glucotropaeolin potassium salt was purchased from Extrasynthese (Genay Cedex, France), copper(II) sulfate pentahydrate was purchased from Chempur (Piekary Śląskie, Poland) and silver nitrate was purchased from POCh S.A. (Gliwice, Poland). Fresh kohlrabi was purchased at a local grocery store (Poznań, Poland).

4.2. Preliminary Experiments with Commercial Thioglucosidase from *Sinapis alba*: Screening of Potential Metal Inhibitors

One milliliter of liquid was added (0.5 mol/L of $\text{Cu}^{2+}_{(\text{aq})}$, $\text{Fe}^{2+}_{(\text{aq})}$, $\text{Fe}^{3+}_{(\text{aq})}$, $\text{Ag}^{+}_{(\text{aq})}$, $\text{Mn}^{2+}_{(\text{aq})}$, $\text{Na}^{2+}_{(\text{aq})}$, and $\text{Ca}^{2+}_{(\text{aq})}$, saturated solutions of NaCl, CaCl_2 or water) was placed in a 20 mL vial. 50 μL of aqueous thioglucosidase from *Sinapis alba* (2.9 g/L) was added to the vial. Afterward 50 μL of an aqueous solution of glucosinolate (2 mmol/L of sinigrin potassium salt or glucotropaeolin potassium salt) was added and the vial was immediately closed with a cap. Fifty microliters of aqueous thioglucosidase from *Sinapis alba* (2.9 g/L) was added to the vial. A sufficient amount of anhydride Na_2SO_4 was placed in a 20 mL vial. Afterward 50 μL of an aqueous solution of glucosinolate (2 mmol/L of sinigrin potassium salt or glucotropaeolin potassium salt) was added and the vial was immediately closed with a cap.

The stock solution of silver nitrate (0.5 mol/L AgNO_3) was diluted step by step to obtain the following concentrations: 5×10^{-2} , 5×10^{-3} , 5×10^{-4} , 5×10^{-5} , 5×10^{-6} , 5×10^{-8} , 5×10^{-11} , 5×10^{-14} , 5×10^{-17} , 5×10^{-20} mol/L. One milliliter of silver solution was placed in a 20 mL vial and 50 μL of an aqueous thioglucosidase from *Sinapis alba* (2.9 g/L) was added to the vial. Subsequently, 50 μL of aqueous solution of glucosinolate (2 mmol/L of sinigrin potassium salt or glucotropaeolin potassium salt) was added and the vial was immediately closed with cap.

1 mL of liquid was added (0.5 mol/L of $\text{Cu}^{2+}_{(\text{aq})}$, $\text{Fe}^{2+}_{(\text{aq})}$, $\text{Fe}^{3+}_{(\text{aq})}$, $\text{Ag}^{+}_{(\text{aq})}$, and saturated solution of CaCl_2) was placed in a 20 mL vial. 50 μL of an aqueous solution of glucosinolate (2 mmol/L of sinigrin potassium salt or glucotropaeolin potassium salt) was added and the vial was immediately closed with a cap. These tests were negative control, while the positive control sample was prepared with 1 mL of water and adequate glucosinolate addition.

4.3. Endogeneous Thioglucosidase Activity of Kohlrabi Extract and Application of Metal Inhibitors

Kohlrabi bulb with no water added was homogenized using a table-top stainless steel blender. 2 g of kohlrabi was placed in a 20 mL vial. The enzymatic reaction blocking agent ($\text{Cu}^{2+}_{(\text{aq})}$, $\text{Fe}^{2+}_{(\text{aq})}$, $\text{Fe}^{3+}_{(\text{aq})}$, $\text{Ag}^{+}_{(\text{aq})}$, anhydrous Na_2SO_4 , and saturated solution of CaCl_2) was added whenever in the exact time (1 min and 30 sec) after blending and glucosinolates addition (200 μL of 2 mmol/L of sinigrin potassium salt or glucotropaeolin potassium salt). The vial was immediately closed with a cap. The control sample was prepared with 2 g of kohlrabi, 3 mL of water, and adequate glucosinolate addition.

4.4. Solid-Phase Microextraction and GC-MS Analysis

Extraction of volatiles by SPME and their subsequent analysis were performed on gas chromatograph coupled with triple quadrupole mass spectrometer (7890A/7000B, Agilent Technologies, Santa Clara, CA, USA) working in a single quadrupole mode. The GC/MS system was equipped with an automatic MultiPurpose MPS-2 sampler (Gerstel, Germany). The headspace solid-phase microextraction (SPME) was performed at 40 °C and the sample was incubated for 5 min prior to extraction, and then the fiber was exposed in the vial for 30 min. After extraction SPME fiber was removed from the vial and subsequently was placed in a desorption port (230 °C) of GC-MS system. Samples were injected in a splitless mode.

A Supelcowax-10 column (60 m $\times$ 0.25 mm $\times$ 0.25 μm , Supelco, Bellefonte, PA) was used and a constant flow of He as a carrier gas was kept at 1 mL/min. The oven temperature was 40 °C for 1 min, ramped at 8 °C/min to 200 °C and 20 °C/min to 280 °C, and held at 280 °C for 5 min. The transfer line temperature was 260 °C. The mass spectrometer was operating in an electron ionization (EI) mode at 70 eV, in a scan range of m/z 33–333. The temperature of MS ion source was 230 °C. Instrument control, data acquisition, and peak area count calculation were performed by Agilent Technologies MassHunter Workstation (B 07.00, Agilent Technologies, Santa Clara, CA, USA) software with a library of the National Institute of Standards and Technology (version 2.0) (NIST, Gaithersburg, MD, USA). Then the spectra of volatile compounds derived from synigrin and glucotropaeolin were compared with those obtained after the reference compounds analysis. All samples were run in triplicate ($n = 3$).

5. Conclusions

A saturated solution of calcium chloride and anhydrous sodium sulfate salts were effective in inhibiting synigrin and glucotropaeolin hydrolysis in the vegetable matrix. Compared to the preliminary experiment in aqueous system, no major discrepancies were observed for a saturated sodium chloride solution and the formation of an increased amount of nitriles compared to the control. The discrepancies in results obtained from the preliminary experiment and during the use of the vegetable matrix were mainly due to the differences between the chemical composition of these two environments.

Summing up, the most effective inhibitors are silver ions concerning both myrosinase and lipoxygenase deactivation. Milder blocking conditions, resulting in a similar profile of volatile compounds in the control provides the use of anhydrous sodium sulfate.

Author Contributions: Conceptualization, M.M. and H.H.J.; methodology, M.M. and H.H.J.; formal analysis, H.H.J.; investigation, M.M.; writing—original draft preparation, M.M.; writing—review and editing, H.H.J.; funding acquisition, H.H.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Science Centre (Poland), grant number 548 2015/18/M/NZ9/00372.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Bell, L.; Oloyede, O.O.; Lignou, S.; Wagstaff, C.; Methven, L. Taste and Flavor Perceptions of Glucosinolates, Isothiocyanates, and Related Compounds. *Mol. Nutr. Food Res.* **2018**, *62*, 1700990. [\[CrossRef\]](#) [\[PubMed\]](#)
- Terp, N.; Göbel, C.; Brandt, A.; Feussner, I. Lipoygenases during *Brassica napus* seed germination. *Phytochemistry* **2006**, *67*, 2030–2040. [\[CrossRef\]](#) [\[PubMed\]](#)
- Baysal, T.; Demirdöven, A. Lipoygenase in fruits and vegetables: A review. *Enzym. Microb. Technol.* **2007**, *40*, 491–496. [\[CrossRef\]](#)
- Guo, X.; Tian, S.; Small, D.M. Generation of meat-like flavourings from enzymatic hydrolysates of proteins from *Brassica* sp. *Food Chem.* **2010**, *119*, 167–172. [\[CrossRef\]](#)
- Moon, K.M.; Kwon, E.-B.; Lee, B.; Kim, C.Y. Recent Trends in Controlling the Enzymatic Browning of Fruit and Vegetable Products. *Molecules* **2020**, *25*, 2754. [\[CrossRef\]](#)
- Sikorska-Zimny, K.; Beneduce, L. The glucosinolates and their bioactive derivatives in *Brassica*: A review on classification, biosynthesis and content in plant tissues, fate during and after processing, effect on the human organism and interaction with the gut microbiota. *Crit. Rev. Food Sci. Nutr.* **2020**, *1*–28. [\[CrossRef\]](#)
- Yábar, E.; Pedreschi, R.; Chirinos, R.; Campos, D. Glucosinolate content and myrosinase activity evolution in three maca (*Lepidium meyenii* Walp.) ecotypes during preharvest, harvest and postharvest drying. *Food Chem.* **2011**, *127*, 1576–1583. [\[CrossRef\]](#)
- del Carmen Martínez-Ballesta, M.; Carvajal, M. Myrosinase in Brassicaceae: The most important issue for glucosinolate turnover and food quality. *Phytochem. Rev.* **2015**, *14*, 1045–1051. [\[CrossRef\]](#)
- Kołodziejwski, D.; Piekarska, A.; Hanschen, F.S.; Pilipczuk, T.; Tietz, F.; Kusznierevich, B.; Bartoszek, A. Relationship between conversion rate of glucosinolates to isothiocyanates/indoles and genotoxicity of individual parts of *Brassica* vegetables. *Eur. Food Res. Technol.* **2019**, *245*, 383–400. [\[CrossRef\]](#)
- Abellán, Á.; Domínguez-Perles, R.; Moreno, D.; García-Viguera, C. Sorting out the Value of Cruciferous Sprouts as Sources of Bioactive Compounds for Nutrition and Health. *Nutrients* **2019**, *11*, 429. [\[CrossRef\]](#)
- Yen, G.-C.; Wei, Q.-K. Myrosinase activity and total glucosinolate content of cruciferous vegetables, and some properties of cabbage myrosinase in Taiwan. *J. Sci. Food Agric.* **1993**, *61*, 471–475. [\[CrossRef\]](#)
- Dal Prá, V.; Jardim, N.S.; Dolwitsch, C.B.; Mazutti, M.A.; Viana, C.; Bohrer, D.; Nascimento, P.C.; Carvalho, L.D.; Silva, M.D.; Carvalho, C.D.; et al. A review of influence of environment and process parameters on glucosinolate-myrosinase system from *Brassica*. *J. Appl. Pharm. Sci.* **2013**, *3*, 121–128. [\[CrossRef\]](#)
- Okunade, O.A.; Ghawi, S.K.; Methven, L.; Niranjani, K. Thermal and pressure stability of myrosinase enzymes from black mustard (*Brassica nigra* L. W.D.J. Koch. var. *nigra*), brown mustard (*Brassica juncea* L. Czern. var. *juncea*) and yellow mustard (*Sinapsis alba* L. subsp. *maire*) seeds. *Food Chem.* **2015**, *187*, 485–490. [\[CrossRef\]](#)
- Zhou, Q.; Jia, X.; Yao, Y.-Z.; Wang, B.; Wei, C.-Q.; Zhang, M.; Huang, F. Characterization of the Aroma-Active Compounds in Commercial Fragrant Rapeseed Oils via Monolithic Material Sorptive Extraction. *J. Agric. Food Chem.* **2019**, *67*, 11454–11463. [\[CrossRef\]](#) [\[PubMed\]](#)
- Chen, W.; Karangwa, E.; Yu, J.; Xia, S.; Feng, B.; Zhang, X.; Jia, C. Characterizing Red Radish Pigment Off-Odor and Aroma-Active Compounds by Sensory Evaluation, Gas Chromatography-Mass Spectrometry/Olfactometry and Partial Least Square Regression. *Food Bioprocess Technol.* **2017**, *10*, 1337–1353. [\[CrossRef\]](#)
- Miyazawa, M.; Maehara, T.; Kurose, K. Composition of the essential oil from the leaves of *Eruca sativa*. *Flavour Fragr. J.* **2002**, *17*, 187–190. [\[CrossRef\]](#)
- Kroener, E.-M.; Buettner, A. Sensory-Analytical Comparison of the Aroma of Different Horseradish Varieties (*Armoracia rusticana*). *Front. Chem.* **2018**, *6*, 149. [\[CrossRef\]](#)
- Babushok, V.I.; Linstrom, P.J.; Zenkevich, I.G. Retention Indices for Frequently Reported Compounds of Plant Essential Oils. *J. Phys. Chem. Ref. Data* **2011**, *40*, 043101. [\[CrossRef\]](#)
- Hanschen, F.S.; Klopsch, R.; Oliviero, T.; Schreiner, M.; Verkerk, R.; Dekker, M. Optimizing isothiocyanate formation during enzymatic glucosinolate breakdown by adjusting pH value, temperature and dilution in *Brassica* vegetables and *Arabidopsis thaliana*. *Sci. Rep.* **2017**, *7*, 40807. [\[CrossRef\]](#)
- Román, J.; Castillo, A.; Mahn, A. Molecular Docking of Potential Inhibitors of Broccoli Myrosinase. *Molecules* **2018**, *23*, 1313. [\[CrossRef\]](#)
- Marcinkowska, M.; Jelen, H.H. Determination of the odor threshold concentrations and partition coefficients of isothiocyanates from *Brassica* vegetables in aqueous solution. *LWT* **2020**, *131*, 109793. [\[CrossRef\]](#)

22. Bachan Upadhyay, L.S.; Verma, N. Enzyme Inhibition Based Biosensors: A Review. *Anal. Lett.* **2013**, *46*, 225–241. [[CrossRef](#)]
23. Buttery, R.G.; Teranishi, R.; Ling, L.C. Fresh tomato aroma volatiles: A quantitative study. *J. Agric. Food Chem.* **1987**, *35*, 540–544. [[CrossRef](#)]
24. Steinhaus, M. Chapter 9 Gas Chromatography–Olfactometry: Principles, Practical Aspects and Applications in Food Analysis. In *Advanced Gas Chromatography in Food Analysis*; The Royal Society of Chemistry: London, UK, 2020; pp. 337–399, ISBN 978-1-78801-127-3.
25. Steinhaus, M. Characterization of the Major Odor-Active Compounds in the Leaves of the Curry Tree *Berbera koenigii* L. by Aroma Extract Dilution Analysis. *J. Agric. Food Chem.* **2015**, *63*, 4060–4067. [[CrossRef](#)] [[PubMed](#)]
26. Hanschen, F.S.; Lamy, E.; Schreiner, M.; Rohn, S. Reactivity and Stability of Glucosinolates and Their Breakdown Products in Foods. *Angew. Chem. Int. Ed.* **2014**, *53*, 11430–11450. [[CrossRef](#)]
27. Hanschen, F.S.; Kühn, C.; Nickel, M.; Rohn, S.; Dekker, M. Leaching and degradation kinetics of glucosinolates during boiling of Brassica oleracea vegetables and the formation of their breakdown products. *Food Chem.* **2018**, *263*, 240–250. [[CrossRef](#)]
28. Verkerk, R.; Dekker, M. Glucosinolates and Myrosinase Activity in Red Cabbage (*Brassica oleracea* L. Var. *Capitata* f. *rubra* DC.) after Various Microwave Treatments. *J. Agric. Food Chem.* **2004**, *52*, 7318–7323. [[CrossRef](#)]
29. Carlson, D.G.; Daxenbichler, M.E.; Vanetten, C.H.; Kwolek, W.F.; Williams, P.H. Glucosinolates in crucifer vegetables: Broccoli, brussels sprouts, cauliflower, collards, kale, mustard greens, and kohlrabi. *J. Am. Soc. Hortic. Sci.* **1987**, *112*, 173.
30. Molteberg, E.; Magnus, E.; Bj, J.; Nilssoni, A. Sensory and Chemical Studies of Lipid Oxidation in Raw and Heat-Treated Oat Flours. *Cereal Chem.* **1996**, *73*, 579–587.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

Key Odorants of Raw and Cooked Green Kohlrabi (*Brassica oleracea* var. *gongylodes* L.)

Monika Marcinkowska, Stephanie Frank, Martin Steinhaus, and Henryk H. Jelen*



Cite This: <https://doi.org/10.1021/acs.jafc.1c04339>



Read Online

ACCESS |

Metrics & More

Article Recommendations

Supporting Information

ABSTRACT: Volatile compounds of raw and cooked green kohlrabi were investigated using a sensomics approach. A total of 55 odor-active compounds were detected and identified in raw and cooked green kohlrabi using GC-O. Twenty-eight odor-active compounds with high flavor dilution (FD) factors ranging from 64 to 1024 were quantitated, and odor activity values (OAVs) were determined. Eight compounds showed high OAVs in raw and cooked kohlrabi: five sulfur compounds (dimethyl trisulfide, methyl 2-methyl-3-furyl disulfide, and three isothiocyanates (1-isothiocyanato-3-(methylsulfanyl)propane, benzyl isothiocyanate, and 1-isothiocyanato-4-(methylsulfanyl)butane)), two lipid oxidation products (1-octen-3-one and *trans*-4,5-epoxy-(2*E*)-dec-2-enal), and 2-isopropyl-3-methoxypyrazine. Among these, the sulfur compounds contributed most to the overall smell of the raw and cooked vegetables. The quantitation analysis indicates that the eight odorants are the backbone compounds for raw and cooked kohlrabi. The OAVs for the backbone compounds and also for minor odorants are clearly higher in raw kohlrabi than in the cooked one. Differences can be explained by the influence of the cooking process.

KEYWORDS: kohlrabi (*Brassica oleracea* var. *gongylodes* L.), aroma extract dilution analysis (AEDA), stable isotope dilution assay (SIDA), odor activity value (OAV), GC-O, isothiocyanate (ITC), nitrile

■ INTRODUCTION

Kohlrabi (*Brassica oleracea* var. *gongylodes* L.) belongs to the vast family of Brassicaceae vegetables. Kohlrabi was initially cultivated in northwestern Europe and later spread throughout Europe and around the world to the USA, Canada, Asia, and Africa.¹ Kohlrabi is consumed year-round. The main edible part of the plant is its bulbous stem that is delicate and slightly sweet in taste.² Nowadays several kohlrabi species are identified that differ in their bulb and stalk color, which can invariably be green, purple, or white. The flesh, however, is always pale. Usually kohlrabi bulbs are peeled and eaten raw. Kohlrabi may also be cooked, used for sauces, soups, or even as a pickled vegetable. Kohlrabi leaves are also edible. In Poland, they are used to prepare salads, soups, green smoothies, or pesto.

Due to its nutritional benefits and low calorific value, kohlrabi flesh and leaves are highly recommended for consumption. The health benefits of kohlrabi are diverse and include anti-inflammatory, antimicrobial, and antioxidant effects; reduction of oxidative stress; cancer inhibition; prevention of asthma, cancer, and Alzheimer's disease; management of type-II diabetes, and improve heart health.² These positive effects are attributed to the vegetable's contents of antioxidants; vitamin C; folic acid; minerals such as iron, potassium, and selenium; carotenoids, particularly zeaxanthin and lutein; and glucosinolates.³

Glucosinolates are special organosulfur plant metabolites that occur in significant amounts in *Brassica* vegetables. They consist of an oxime sulfate group, the carbon atom of which is bound via a sulfur atom to a β -D-glucopyranosyl group and on the other side carries an aliphatic or aromatic side chain

derived from an amino acid.^{4,5} Tissue disruption brings glucosinolates in direct contact with the endogenous enzyme myrosinase, which cleaves the glycosidic bond. The aglycone spontaneously reacts to yield compounds such as isothiocyanates, nitriles, thiocyanates, epithionitriles, indoles, and 1,3-oxazolidine-2-thiones.^{5,6}

Glucosinolate breakdown products can greatly affect the flavor of *Brassica* vegetables. Favored by hydrolysis at neutral pH, isothiocyanates⁵ include volatile compounds with a strong garlic and sulfur odor, frequently found at a low odor threshold concentration (OTC) in the range of $\mu\text{g/L}$ in water.⁷ An aroma extract dilution analysis (AEDA) recently applied to horseradish of different varieties resulted in a considerable number of isothiocyanates with high flavor dilution (FD) factors. While most of the identified isothiocyanates were pungent, some additionally showed specific odor qualities such as mushroom-like (1-isothiocyanato-3-methylsulfanylpropane), onion-like (3-isothiocyanatopro-1-ene (allyl isothiocyanate)), or green-like (2-isothiocyanatobutane).⁸ Nitriles are formed from glucosinolates in the presence of nitrile-specific proteins or iron(II) ions under acidic conditions.⁵ The presence of nitrile compounds in Nakajimana (*Brassica rapa* cv. *nakajimana*) has been associated with pungency and bitterness,

Received: July 18, 2021

Revised: September 12, 2021

Accepted: September 16, 2021



ACS Publications

© XXXX American Chemical Society

A

<https://doi.org/10.1021/acs.jafc.1c04339>
J. Agric. Food Chem. XXXX, XXX, XXX–XXX

Table 1. Odor-Active Compounds Identified among Volatiles Isolated from Raw and Cooked Green Kohlrabi

no.	odorant	odor quality ^a	RI ^b		FD factor ^c	
			FFAP	DB-5	raw	cooked
1	5-(methanesulfinyl)pentanenitrile	broth-like, onion-like, sulfurous	943	1563	256	256
2	pentane-1-thiol	burned, rubber-like	1022	850	1	<1
3	dimethyl disulfide	cabbage-like, sulfurous	1094	803	<1	8
4	(3Z)-hex-3-enal	green, grassy	1141	802	16	<1
5	but-3-enenitrile	pungent, mustard-like	1191	900	4	32
6	2-pentylfuran	vegetable-like	1243	1015	256	<1
7	oct-1-en-3-one	mushroom-like	1287	974	64	32
8	2-acetyl-1-pyrroline	popcorn-like	1330	904	256	64
9	dimethyl trisulfide	sulfurous, cabbage-like	1352	963	16	128
10	(3E)-hex-3-en-1-ol	green, grassy	1395	814	<1	1
11	2-isopropyl-3-methoxypyrazine	pea-like, earthy	1418	1096	512	128
12	acetic acid	vinegar-like	1450	631	16	16
13	3-butenyl isothiocyanate	pungent, garlic-like	1453	981	16	8
14	(2E,4E)-hepta-2,4-dienal	fatty	1487	1158	<1	1
15	4-(methylsulfonyl)butan-2-one	potato-like, earthy	1514	989	8	<1
16	octan-1-ol	green, citrus-like	1545	981	32	4
17	(1S)-1-phenylethane-1-thiol	burned	1626	1122	2	<1
18	2-acetylthiazole	roasty	1635	1015	32	16
19	methyl 2-methyl-3-furyl disulfide	meat-like, cabbage-like	1645	1187	64	4
20	γ -hexalactone	fruity, sweet	1655	1052	1	64
21	thiophene-2-carbaldehyde	roasty	1685	981	64	<1
22	2-phenylethane-1-thiol	rubber-like	1690	1167	32	<1
23	3-methylfuran-2(5H)-one	vegetable-like, onion-like	1695	985	32	64
24	methionol	cooked potato-like	1715	963	4	32
25	4-(methylsulfonyl)butanenitrile	broth-like, garlic-like, sulfurous	1721	1063	128	64
26	hexanoic acid	sweaty	1805	1015	64	<1
27	5-(methylsulfonyl)pentanenitrile	broccoli-like, cabbage-like	1845	1193	256	128
28	4-(methylsulfonyl)butan-1-ol	cabbage-like, garlic-like	1858	965	64	<1
29	12-methyltridecanal	beef-like, tallow-like	1863	1015	16	<1
30	<i>p</i> -cymen-8-ol	musty, petrol-like	1863	1208	<1	512
31	1,3-benzothiazole	rubber-like, cabbage-like	1900	1245	8	16
32	dimethyl sulfone	sulfurous, fatty	1918	959	<1	4
33	phenylacetone nitrile	mushroom-like	1922	1107	128	<1
34	heptanoic acid	sweaty, rancid	1939	1089	4	<1
35	2-phenylethan-1-ol	floral, honey-like	1940	1112	<1	32
36	maltol	caramel-like	1950	1245	32	16
37	1-isothiocyanato-3-(methylsulfonyl)propane	sulfurous, garlic-like, mushroom-like	1972	1231	128	4
38	<i>trans</i> -4,5-epoxy-(<i>E</i>)-2-decenal	metallic, sweet	1989	1379	256	64
39	γ -nonalactone	sweet, coconut-like	2022	1387	8	32
40	<i>p</i> -anisaldehyde	woodruff-like, anise-like	2033	1257	<1	1024
41	3-phenylpropane nitrile	sour, fatty	2047	1241	512	8
42	pyrrolidin-2-one	mouse-like, sour	2065	1052	16	8
43	benzyl isothiocyanate	herb-like, sweaty, pungent	2076	1363	256	256
44	benzyl thiocyanate	broth-like, buttery, cress-like	2113	1352	128	<1
45	1-isothiocyanato-4-(methylsulfonyl)butane	mushroom-like, broth-like	2141	1404	256	16
46	bis(2-methyl-3-furyl)disulfide	meat-like	2138	1540	16	<1
47	2-methoxy-4-vinylphenol	clove-like, smoky	2150	1300	32	32
48	2-phenoxyethan-1-ol	floral	2156	1240	1	<1
49	sotolon	lovage-like, seasoning-like	2210	1107	128	1024
50	(2-isothiocyanatoethyl)benzene	broth-like, vegetable-like	2220	1430	256	64
51	ethane-1,2-dithiol	garlic-like, cabbage-like	2225	817	8	16
52	unknown	sweaty, rubber-like	2244	1021	1	16
53	4-methyl-5-thiazoleethanol	broth-like, sulfurous, nutty	2320	1269	256	512
54	phenylacetic acid	honey-like, beeswax-like	2555	1252	128	32
55	vanillin	sweet, vanilla-like	2576	1395	512	32

^aStructure assignment was based on odor quality, RIs (DB-FFAP, DB-5), and mass spectrum; data was compared with the data obtained from reference compounds analyzed under the same conditions. ^bRetention index; RIs were calculated from the retention time of the odorant and the retention time of adjacent *n*-alkanes by linear interpolation. ^cFlavor dilution factor.

B

<https://doi.org/10.1021/acs.jafc.1c04339>
J. Agric. Food Chem. XXXX, XXX, XXX–XXX

whereas sulfur-containing volatiles were perceived as pungent and burnt rubber-like.⁹

Food aroma and appearance are among the most important factors driving consumers' preferences and food choices. These product features can be directly assessed at the moment of decision-making, as opposed to texture and taste. Many publications have focused on the identification and quantitation of primary and specialized metabolites^{10,11} present in kohlrabi, including volatiles.¹² However, to date no study has been conducted on its odor-active compounds.

The main objectives of the current study include (i) screening for odor-active compounds in raw and cooked green kohlrabi, (ii) quantitation of major odorants to assess their relevance on the basis of odor activity values (OAVs), and (iii) finally a comparison of the odorant profiles of raw and cooked green kohlrabi to gain insight into changes in odor induced by the cooking process.

MATERIALS AND METHODS

Green Kohlrabi (*Brassica oleracea* var. *gongylodes* L.). Raw kohlrabi samples were obtained from a local grocery store (Freising, Germany). All vegetables were stored at 5 °C until use.

Reference Odorants. Compounds 1–5, 9–15, 17–24, 26–28, 30–33, 35, 37, 39, 41–43, 45–46, and 49–55 were obtained from Merck (Darmstadt, Germany). Compounds 6–7 and 47 were purchased from Alfa Aesar (Karlsruhe, Germany). Compound 16 was obtained from Carl Roth (Karlsruhe, Germany), 25, and 44 from Santa Cruz Biotechnology (Heidelberg, Germany), and 34, 36, 40, and 48 were from Fluka (Munich, Germany). Compounds 8,¹³ 29,¹⁴ and 38¹⁵ were synthesized according to methods described in the literature.

Stable Isotopically Substituted Odorants. The following isotopically substituted standards were synthesized according to the literature cited: (²H₄)-7,¹⁶ (¹³C₂)-8,¹⁷ (²H₃)-11,¹⁸ (¹³C₂)-49,¹⁹ (²H₃)-3-(methylthio)propan-1-ol,¹⁶ (²H₃)-5-methylthiophene-2-carbaldehyde,²⁰ (¹³C₂)-40,²¹ (²H₆)-9,²² (²H₃)-55,¹⁸ and (²H₄)-38.²³ (²H₃)-6 was purchased from aromaLAB (Martinsried, Germany), (²H₈)-naphthalene and (¹³C₂)-54 were obtained from Merck, and (²H₃)-26 was purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada).

Gas Chromatography. A GC equipped with a cold on-column injector, a flame ionization detector, a sniffing port, and DB-FFAP (30 m × 0.32 mm, film thickness 0.25 μm; Agilent, Waldbronn, Germany) or DB-5 (30 m × 0.32 mm, film thickness 0.25 μm; Agilent) columns was used for GC-O analyses. Identification and quantitation were carried out using a GC-O/FID system, a GC × GC-MS system, a GC-(CI)MS, and a heart-cut GC × GC-HRMS system. Detailed specifications of the GC systems are provided in the [Supporting Information](#).

Isolation of Green Kohlrabi Volatiles. Raw kohlrabi bulbs were peeled, cut into strips with a knife, and blended to a pulp with a Moulinex Moulinette table blender (Krups GmbH, Frankfurt am Main, Germany). A preliminary experiment revealed that the panelists rated dichloromethane extract higher than the diethyl ether one according to the similarity of aroma to the vegetable sample. The kohlrabi pulp (100 g) was immediately subjected to solvent extraction with dichloromethane (500 mL) in an ice bath. Anhydrous sodium sulfate (400 g) was added promptly to stop the enzymatic reactions. The mixture was homogenized with a hand blender and then kept under vigorous stirring (1 h). The extracts were filtered and the filtrate was subjected to solvent-assisted flavor evaporation (SAFE)²⁴ at 40 °C. The vacuum degree of the SAFE apparatus was on the order of magnitude of about 10⁻³ to 10⁻⁴ Pa and was achieved using a vacuum pump set (Oerlikon Leybold Vacuum, Cologne). The distillate was concentrated using a Vigreux column to reach a final volume of 1 mL. The cooked kohlrabi volatile isolate was prepared in an analogous manner from kohlrabi (100 g) cooked (20 min) in tap water (2 L) with the addition of sucrose (10 g) and sodium chloride

(10 g). The kohlrabi volatile isolates were stored at –20 °C until analysis.

AEDA. Raw and cooked kohlrabi volatile isolates were prepared as described above and then stepwise diluted with dichloromethane to obtain 1:2, 1:4, 1:8, 1:16, ..., and 1:1024 dilutions. The diluted samples were analyzed by GC-O. Finally, each odorant was assigned an FD factor representing the dilution factor of the highest diluted sample, in which the odorant was detected during GC-O.

Odorant Quantitation. For the quantitation of compounds 1, 6–9, 11, 19–21, 23, 25–28, 30, 33, 37, 38, 40, 41, 43–45, 49, 50, and 53–55, dichloromethane (500 mL) was added to raw and cooked kohlrabi blends (100 g) under ice-cooling. Stable isotopically substituted odorants of the target analytes (0.05–10.65 μg) in a dichloromethane solution (0.1–1 mL) were added as internal standards. Anhydrous sodium sulfate (400 g) was added, and the mixture was homogenized with a hand blender made of stainless steel. After magnetic stirring in an ice bath (1 h), the mixture was filtered through sea sand and cotton wool. The residue was washed with dichloromethane. Volatile compounds were isolated from the combined organic phases by SAFE at 40 °C. The distillates were concentrated to final volumes of between 0.1 and 1 mL. The concentrates were analyzed using the GC-MS system (6, 9, 11, 25, 27–28, 30, 33, 37, 41, 43–45, 50, 53–55), the GC-(CI)MS system (1, 7, 19–21, 23, 26, 40, 49), or the heart-cut GC-GC-HRMS system (8, 38). Peak areas of the target compounds and the internal standards were acquired from the extracted ion chromatograms using unique quantifier ions. The concentrations of each target compound in the kohlrabi samples were calculated from the area counts of the target compound peak, the area counts of the standard peak, the amount of kohlrabi blend used, and the amount of the standard added by employing a calibration line equation (see Supporting Information, [Table S1](#)) earlier obtained from the analysis of mixtures of the analyte and the standard in five concentration ratios (4:1, 2:1, 1:1, 1:2, and 1:4).

Odor Threshold Concentrations. Odor threshold concentrations (OTCs) not available in the literature were determined orthonasally in pure water according to the American Society for Testing and Materials (ASTM) standard practice for determination of odor and taste thresholds by a forced-choice ascending concentration series method of limits.²⁵ The panel consisted of 15–20 assessors, male and female, aged 24–65 years. OTC determinations were performed in a sensory room at 22 ± 1 °C.

RESULTS AND DISCUSSION

Screening for Odor-Active Compounds and Structural Identification. During isolation of the volatiles from raw and cooked green kohlrabi by solvent extraction, SAFE, and concentration, the odor was continuously monitored. The extracts, the distillates, and the concentrated kohlrabi volatile isolates were orthonasally assessed on a strip of filter paper after evaporation of the solvent. The characteristic odors of raw and cooked kohlrabi were still clearly perceivable at all stages tested.

The application of GC-O analyses and AEDA revealed a total of 55 odor-active compounds in the raw and cooked kohlrabi samples. Data is summarized in [Table 1](#). The results obtained from the coupling of the GC-O with the AEDA are presented as an FD chromatogram for raw and cooked kohlrabi samples ([Figure S1](#)).

The FD factors ranged between 1 and 512 in the sample obtained from raw kohlrabi and between 1 and 1024 in the sample obtained from cooked kohlrabi. Moreover, for all of these compounds, FD factors for raw kohlrabi were much higher than those for the cooked one. In the raw kohlrabi sample, the highest FD factors were obtained for three compounds with odor descriptors such as pea-like, earthy (11), sour, fatty (41), and sweet, vanilla-like (55), while in the

C

<https://doi.org/10.1021/acs.jafc.1c04339>
J. Agric. Food Chem. XXXX, XXX, XXX–XXX

cooked kohlrabi, the dominant odor notes were woodruff-like, anise-like (40), lovage-like, seasoning-like (49), musty, petrol-like (30), and broth-like, sulfurous, nutty (53). Somewhat lower FD factors in raw kohlrabi were determined for broth-like, onion-like, sulfurous (1), vegetable-like (6), popcorn-like (8), broccoli-like, cabbage-like (27), metallic, sweet (38), herb-like, sweaty, pungent (43), mushroom-like, broth-like (45), broth-like, vegetable-like (50), and broth-like, sulfurous, nutty (53). In cooked kohlrabi, it was broth-like, onion-like, sulfurous (1), sulfurous, cabbage-like (9), pea-like, earthy (11), broccoli-like, cabbage-like (27), and herb-like, sweaty, pungent (43). Preliminary structure proposals were developed by comparing the RIs and odor descriptors of the odorants detected during AEDA to the data of reference compounds collected from the Leibniz-LSB@TUM Odorant Database²⁶ and from a literature review. Outcomes were confirmed by analyzing the corresponding reference compounds in a proper dilution in parallel to the isolates of kohlrabi volatiles by GC-O with capillary columns of different polarities (DB-FFAP, DB-5). Final identification was accomplished by GC-MS or GC × GC-MS analysis in the EI and CI modes.

The odor-active compounds in kohlrabi can be divided into several groups according to their precursors and formation mechanisms. The main pathways are related to lipid and protein metabolism. Prior studies revealed that the major volatile constituents of kohlrabi are isothiocyanates such as (3-isothiocyanatopropyl)(methyl)sulfane and 1-isothiocyanato-4-(methylsulfanyl)butane; nitriles such as 4-(methylsulfanyl)butanenitrile and 5-(methylsulfanyl)pentanenitrile; sulfides such as dimethyl disulfide, dimethyl trisulfide, and 1,4-dimethyltetrasulfane; and unsaturated aldehydes such as (2E)-hex-2-enal, (2E)-non-2-enal, and (2E,4E)-hepta-2,4-dienal.²⁷ Sulfur-containing ketones such as 4-(methylsulfanyl)butan-2-one and 1-(methylthio)pentan-3-one, thiazoles, alcohol, pyrazines, and furan derivatives have also been reported.²⁸ Previous research, however, was targeted at the identification and quantitation of major volatiles in kohlrabi and not focused on odor-active compounds. Nevertheless, most odorants identified in our study had been reported as kohlrabi volatiles before. In contrast, compounds 7, 11, 19, 26, 38, and 49 were previously unknown in kohlrabi.

According to a previous study, green kohlrabi contains seven main glucosinolates. In the order of increasing concentrations, these are glucotropaeolin, neoglucobrassicin, 4-hydroxyglucobrassicin, glucoraphanin, glucobrassicin, glucoalyssin, and 4-methoxyglucobrassicin.¹¹ Other studies reported that kohlrabi additionally contains progoitrin, glucoiberin, glucoiberin, sinigrin, glucobrassicinapin, and gluconasturtiin.²⁸ In agreement with earlier assumptions,⁷ our data suggests that degradation products of these special metabolites, particularly isothiocyanates, might have a considerable impact on kohlrabi odor. Isothiocyanates and nitriles characterized by high FD factors include 1, 5, 13, 25, 27, 33, 37, 41, 43, and 45. These compounds are well-known degradation products of glucoraphanin (1), sinigrin (5), glucoiberin (13), glucoiberin (25, 37), glucoerucin (27, 45), glucotropaeolin (33, 43), and gluconasturtiin (41). Among these glucosinolates, glucoerucin has not been detected in green kohlrabi yet but has been reported in purple kohlrabi.¹¹

Further sulfur-containing odorants include the sulfides 3, 9, 19, and 46. While sulfides have been reported previously in *Brassica* vegetables (kohlrabi, cauliflower, Brussel sprouts, broccoli,¹² and white mustard seeds),²⁹ 19 and 46 are reported

for the first time in green kohlrabi. Interestingly, these compounds are usually associated with meat-like aromas. Miscellaneous sulfur-containing odorants are 2, 15, 17, 18, 21, 22, 24, 28, 32, 51, and 53. In summary, the vast majority of the odor-active compounds in kohlrabi include sulfur in their structure.

Green-grassy, metallic, and mushroom-like odors are provided by 4, 7, 10, 14, and 38. These compounds are products of the breakdown of unsaturated fatty acids such as linoleic and linolenic acids.^{30,31} *trans*-4,5-Epoxy-(2E)-dec-2-enal (38) is reported for the first time in kohlrabi. The aldehyde 29 (12-methyltridecanal) detected as an odorant in kohlrabi was previously found in Gouda-type cheese,³² and stewed beef, and it can be formed by microorganisms.³³

A green pea-like odor quality is associated with odorant 11, 2-isopropyl-3-methoxypyrazine. In fresh vegetables, the biosynthesis of this compound occurs through the use of S-containing amino acids; the cooking process resulted in a decrease in this compound. 2-Isopropyl-3-methoxypyrazine is not only an important odorant in grapes, wine,³⁴ and hops³⁵ but was also detected as a crucial odorant in cold-pressed rapeseed oil.³⁶ As summarized in a recent review,³⁷ methoxypyrazines as a group are important odorants in cold-pressed rapeseed oil.

Different pathways for the biosynthesis of sotolon (3-hydroxy-4,5-dimethyl-2(SH)-furanone, 49) have been proposed in the literature involving threonine as a starting molecule in *Vin jaune* or 4-hydroxyisoleucine in fenugreek.³⁸ Sotolon is one of the key odorants in foods such as raw sugarcane, fenugreek, flor-sherry, botrytized wines, lovage, and sweet, fortified wines exhibiting caramel, nutty, or curry notes.³⁹

A compound with a low OTC is 2-acetyl-1-pyrroline (8). Its odor is described as cooked rice-like or popcorn-like. 2-Acetyl-1-pyrroline is naturally present in both plant and animal products. In plants it is formed by biosynthesis, but in foods it is mainly generated from proline during thermal food processing in the Maillard reaction.⁴⁰

Odorant Quantitation and Calculation of OAVs. To further estimate the contribution of odor-active compounds to the overall odor of raw and cooked kohlrabi, a total of 28 odorants with high FD factors of ≥ 64 were quantitated, and their OAVs were calculated as a ratio of their concentration to the OTC. Quantitation was accomplished using different approaches (cf. Supporting Information, Table S1). The highest concentrations in both raw and cooked kohlrabi were determined for some isothiocyanates and nitriles such as odorant 37 (1.4 mg/kg in raw kohlrabi and 1.4 mg/kg in cooked kohlrabi), 45 (1.0 mg/kg in raw kohlrabi and 1.1 mg/kg in cooked kohlrabi), 25 (371 μ g/kg in raw kohlrabi and 379 μ g/kg in cooked kohlrabi), and 27 (272 μ g/kg in raw kohlrabi and 282 μ g/kg in cooked kohlrabi).

Thirteen odorants in the raw kohlrabi sample showed an OAV >1, while in cooked kohlrabi samples, 11 compounds exceeded their respective OTCs. OAV calculations confirmed isothiocyanates as dominant odorants in kohlrabi: high OAVs were determined for 1-isothiocyanato-4-(methylsulfanyl)butane (45), dimethyl trisulfide (9), 1-isothiocyanato-3-(methylsulfanyl)propane (37), and benzyl isothiocyanate (43). These compounds are associated with odors described as mushroom-like, broth-like, sulfurous, cabbage-like, garlic-like, and herb-like, sweaty, as well as with pungency. Based on OAVs, further important odorants were 1-octen-3-one (7), 2-

D

<https://doi.org/10.1021/acs.jafc.1c04339>
J. Agric. Food Chem. XXXX, XXX, XXX–XXX

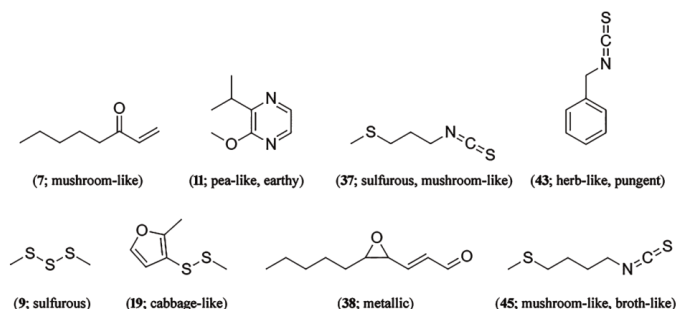


Figure 1. Odorants characterized by the highest OAV in green kohlrabi (for numbers refer to Table 2).

Table 2. Concentration, OTC, and OAV for Odorants in Raw and Cooked Green Kohlrabi

no.	odorant	concentration ($\mu\text{g/kg}$) ^a		OTC ($\mu\text{g/kg}$) ^b	OAV ^c	
		raw	cooked		raw	cooked
1	5-(methanesulfinyl)pentanenitrile	31.1 [■]	29.1 [■]	780	<1	<1
6	2-pentylfuran	2.43 [°]	≤OTC	4.8 ⁴⁷	1.0	≤1
7	oct-1-en-3-one	0.851 [°]	0.503 [°]	0.016	53	31
8	2-acetyl-1-pyrroline	≤OTC	≤OTC	0.053	<1	<1
9	dimethyl trisulfide	3.61 [■]	2.13 [▲]	0.0099	370	220
11	2-isopropyl-3-methoxyisobenzofuran	0.110 [°]	0.0931 [°]	0.0039	28	24
19	methyl 2-methyl-3-furyl disulfide	0.00690 [°]	0.00150 [°]	0.00026	27	6.0
20	γ -hexalactone	1.62 [°]	2.11 [°]	13 000 ⁴⁷	<1	<1
21	thiophene-2-carbaldehyde	0.343 [°]	0.306 [°]	200 ⁴⁷	<1	<1
23	3-methylfuran-2(5H)-one	0.0479 [°]	0.0245 [■]	10 000	<1	<1
25	4-(methylsulfonyl)butanenitrile	371 [°]	379 [°]	8700	<1	<1
26	hexanoic acid	59.7 [°]	39.2 [°]	4800	6.0	4.0
27	5-(methylsulfonyl)pentanenitrile	272 [°]	282 [°]	31 000	<1	<1
28	4-(methylsulfonyl)butan-1-ol	16.9 [°]	12.6 [◆]	100 ⁴⁸	<1	<1
30	<i>p</i> -cymen-8-ol	3.47 [◆]	2.75 [■]	52	<1	<1
33	phenylacetone	6.74 [°]	≤OTC	1000 ⁴⁸	<1	≤1
37	1-isothiocyanato-3-(methylsulfonyl)propane	1350 [°]	1360 [°]	5.0 ⁴⁸	270	270
38	<i>trans</i> -4,5-epoxy-(<i>E</i>)-2-decenal	6.98 [◆]	5.95 [°]	0.22	32	27
40	<i>p</i> -anisaldehyde	0.172 [°]	0.0913 [◆]	2.6	<1	<1
41	3-phenylpropanenitrile	99.1 [°]	139 [°]	15 ⁴⁸	7.0	9.0
43	benzyl isothiocyanate	154 [°]	130 [◆]	0.70 ⁴⁸	220	190
44	benzyl thiocyanate	22.4 [°]	2.02 [▲]	5.0 ⁴⁷	4.0	<1
45	1-isothiocyanato-4-(methylsulfonyl)butane	1050 [°]	1060 [°]	3.0 ⁴⁸	350	350
49	sotolon	4.13 [▲]	1.66 [■]	1.7	2.0	1.0
50	(2-isothiocyanatoethyl)benzene	39.9 [°]	43.4 [°]	6.0 ⁴⁸	7.0	7.0
53	2-(4-methyl-thiazol-5-yl)ethanol	2.55 [°]	6.81 [°]	100 ⁴⁸	<1	<1
54	phenylacetic acid	0.832 [▲]	1.82 [■]	68	<1	<1
55	vanillin	13.2 [■]	24.1 [◆]	53	<1	<1

^aConcentration—mean of triplicates; standard deviation [°]—SD ≤ 10%; [◆]—10% < SD ≤ 20%; [■]—20% < SD ≤ 30%; [▲]—SD > 30%; quantitation details are given in the Supporting Information. ^bOTC—reference number represents OTCs in water from the literature data used in the calculations. ^cOAVs were calculated as the ratio of the concentration in green kohlrabi (raw or cooked) to OTC.

isopropyl-3-methoxyisobenzofuran (11), and *trans*-4,5-epoxy-(2*E*)-dec-2-enal (38).

Surprisingly, a number of odor-active compounds identified in raw and cooked kohlrabi with high FD factors during the screening experiments showed OAVs <1; thus, their role in the overall odor of kohlrabi is disputable. These included 1, 8, 20, 21, 27, 28, 33, 40, 53, 54, and 55. The AEDA is a routine procedure in food flavor science and in this experiment, it is just used as a screening method. It is the first step in understanding the relevant volatile compounds in a food

matrix. The differences in high FD factors and low OAVs may be explained in several approaches. The threshold values for the aroma-active compounds were determined in water. Despite the high water content of kohlrabi, the matrix also contains sugars, fiber, protein (as well as vitamins and minerals), which may be the cause of the mechanisms of various sensory interactions, e.g., masking certain sensations. The OAVs were calculated using odor thresholds measured in water or oil, without considering the specificity of a particular product, whereas during a GC-O analysis the assessor

E

<https://doi.org/10.1021/acs.jafc.1c04339>
J. Agric. Food Chem. XXXX, XXX, XXX–XXX

evaluated the odorants directly from the olfactometry port. In addition, the tissue of fresh and cooked vegetables definitely differs in structure, which also undoubtedly affects the release or retention of volatile compounds. However, compounds with the highest OAV were also characterized with usually high or moderately high FD values (Table 1). This observation clearly exemplifies the importance of quantitation and calculation of OAVs for the substantiation of GC-O data.

When comparing the kohlrabi odorants with the odorants reported in other vegetables of the *Brassica* genus, one can see some similarities particularly associated with the presence of glucosinolate degradation products. Considering that compounds with OAVs >1 constitute the backbone of kohlrabi odor, we note that 9, 11, 26, 37, and 45 are also odor-active in *Brassica napus*;^{36,41,42} 7, 9, 26, and 45 also occur in *Brassica rapa*;^{43,44} 9, 37, 41, 45, and 50 were previously detected in odor-active amounts in *Brassica oleracea*;⁴⁵ and in cooked cauliflower compounds, 7, 9, and 45 were reported in odor-active amounts.⁴⁶

Comparison of Olfactory Profiles of Raw and Cooked Kohlrabi. Odor-active compounds estimated based on the FD factors reflecting the intensity of the sensation perceived on GC-O indicated both similarities and differences in the profile of odorants (Figure S1, Table 1). Two compounds of the highest FD factor (1024) detected in cooked kohlrabi, *p*-anisaldehyde (40) and sotolon (49), were either nonexistent or much weaker in raw kohlrabi. Also *p*-cymen-8-ol (30) with a celery-like smell was not detected in raw kohlrabi, but in the cooked samples, it had an FD factor of 512. Therefore, their potential formation/release during cooking is an open question. An opposite situation (in terms of FD) could be observed for some nitriles (33, 41), benzyl thiocyanate (44), and 2-pentylfuran (6).

However, we treat AEDA analysis as a screening method, and that is why when these samples were compared based on quantitation analysis, the OAVs of eight compounds dominated the flavor and formed the backbone odorants of both raw and cooked kohlrabi (Figure 1). However, kohlrabi after the cooking process varies from the raw one. First of all, the OAVs for backbone compounds (7, 9, 11, 19, 38, 43) and also for minor odorants (19, 26, 44, 49) are clearly higher in raw kohlrabi than in the cooked one. It is associated with the cooking process, which leads to the release of compounds directly into water and the loss of volatile odorants into the air due to thermal factors. Moreover, only three odorants with OAV >1 (37, 45, 50) express the same level in the fresh and cooked vegetables. Only 3-phenylpropanenitrile (41) from a minor odorant increases OAV after cooking, which could be associated with the inactivation of thioglucosidase during cooking. These observations clearly exemplify the importance of quantitation and calculation of OAVs for the substantiation of GC-O data.

As summarized in a thorough review,⁵ thermal degradation of glucosinolates is, among other factors, temperature dependent and results in the formation of high amounts of nitriles at high temperatures (>150 °C) and also isothiocyanates at lower temperatures. In the cooking process, myrosinase is inactivated, so the possible influence of temperature on the formation of nitriles and isothiocyanates could be observed. However, when nitrile concentrations were compared (Table 2) for 3 out of 5 compounds (1, 25, 27), they remained on the same level for fresh and cooked kohlrabi. The temperature used in cooking may be not sufficient to induce their

degradation. Only in the case of 3-phenylpropane nitrile (41), its concentration in cooked kohlrabi increased significantly.

To sum up, the role of nitriles in the creation of flavor of raw and cooked kohlrabi was negligible based on OAV evaluation. On the contrary, isothiocyanates (37, 43, 45), being other major degradation compounds of glucosinolates, play a crucial role in both raw and cooked kohlrabi aroma. They were present in high concentrations, which also resulted in high OAVs. As observed for nitriles, the cooking process did not induce thermal degradation of glucosinolates resulting in higher isothiocyanate concentration. The increase in isothiocyanate concentration in cooked vegetables has been previously reported;¹² and in these studies,⁵ glucobrassicin and glucoraphenin were identified as the most thermolabile glucosinolates.

Apart from the isothiocyanates mentioned above, dimethyl trisulfide (9) was characterized with very high OAVs (highest OAV of all compounds in raw kohlrabi). It is known as the thermal degradation product of isothiocyanates, and also plays an important role in the formation of aroma of white mustard seeds⁴⁹ and rapeseed oils.^{36,37}

Cooking of kohlrabi favors the release of some volatile compounds (1, 7, 9, 11, 19, 21, 23, 26, 28, 30, 38, 40, 43, 44, 49) from the vegetable tissue, which results in their decrease on cooking. On the other hand, there are volatile compounds (20, 41, 50, 53, 54, 55) whose concentrations slightly increase after cooking.

The results of our experiments allowed the determination of 55 compounds that are characteristic to both raw and cooked kohlrabi. Using the concept of molecular sensor science enabled the selection of compounds that play a crucial role in the formation of kohlrabi aroma. The comparison of OAVs allowed for the selection of the crucial odorants, dominated by sulfur compounds, but it turns out that eight backbone odorants are present in raw and cooked kohlrabi. Differences in the overall aroma are strongly associated with the OAVs, which are generally higher for raw kohlrabi and smaller for cooked kohlrabi due to the loss of volatiles during the cooking process and are attributed to compounds of lower OAVs.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jafc.1c04339>.

Miscellaneous chemicals and materials; GC systems; FD chromatograms of odorants isolated from raw and cooked green kohlrabi; and experimental data of quantitation parameters (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Henryk H. Jeleń — Faculty of Food Science and Nutrition, Poznań University of Life Sciences, 60-624 Poznań, Poland; orcid.org/0000-0002-6568-8155; Email: henryk.jelen@up.poznan.pl

Authors

Monika Marcinkowska — Faculty of Food Science and Nutrition, Poznań University of Life Sciences, 60-624 Poznań, Poland

F

<https://doi.org/10.1021/acs.jafc.1c04339>
J. Agric. Food Chem. XXXX, XXX, XXX–XXX

Stephanie Frank — Leibniz Institute for Food Systems Biology at the Technical University of Munich, 85354 Freising, Germany

Martin Steinhaus — Leibniz Institute for Food Systems Biology at the Technical University of Munich, 85354 Freising, Germany; orcid.org/0000-0002-9879-1474

Complete contact information is available at:
<https://pubs.acs.org/10.1021/acs.jafc.1c04339>

Funding

This study was supported by the National Science Centre (Poland) under Grant No. 548 2015/18/M/NZ9/00372.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank Julia Bock and Jörg Stein for their technical assistance.

ABBREVIATIONS

AEDA, aroma extract dilution analysis; CI, chemical ionization; EI, electron ionization; FD factor, flavor dilution factor; FFAP, free fatty acid phase; FID, flame ionization detector; GC, gas chromatography; GC × GC, comprehensive two-dimensional gas chromatography; GC-O, gas chromatography–olfactometry; ITC, isothiocyanate; MS, mass spectrometry; OAV, odor activity value; OTC, odor threshold concentration; RI, retention index; SAFE, solvent-assisted flavor evaporation; SIDA, stable isotope dilution assay

NOMENCLATURE

methyl disulfide, (methylthio)ethane; 2-acetyl-1-pyrroline, 1-(3,4-dihydro-2H-pyrrrol-5-yl)ethan-1-one; dimethyl trisulfide, dimethyltrisulfane; 2-isopropyl-3-methoxypyrazine, 2-methoxy-3-(propan-2-yl)pyrazine; 3-butenyl isothiocyanate, 4-isothiocyanatobut-1-ene; 2-acetylthiazole, 1-(1,3-thiazol-2-yl)ethan-1-one; methyl 2-methyl-3-furyl disulfide, 2-methyl-3-(methylthio)furan; γ -caprolactone, 5-ethylloxolan-2-one; methionol, 3-(methylthio)propan-1-ol; *p*-cymen-8-ol, 2-(4-methylphenyl)propan-2-ol; dimethyl sulfone, (methanesulfonyl)methane; maltol, 3-hydroxy-2-methyl-4H-pyran-4-one; *trans*-4,5-epoxy-(*E*)-2-decenal, (2*E*)-3-(3-pentylloxiran-2-yl)prop-2-enal; γ -nonalactone, 5-pentylloxolan-2-one; *p*-anisaldehyde, 4-methoxybenzaldehyde; benzyl isothiocyanate, (2-isothiocyanatoethyl)benzene; 4-methylthiobutyl isothiocyanate, (4-isothiocyanatobutyl)(methyl)sulfane; bis(2-methyl-3-furyl)disulfide, 3,3'-disulfanediyldis(2-methylfuran); 2-methoxy-4-vinylphenol, 4-ethenyl-2-methoxyphenol; sotoion, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one; phenethyl isothiocyanate, (2-isothiocyanatoethyl)benzene; 4-methyl-5-thiazoleethanol, 2-(4-methyl-1,3-thiazol-5-yl)ethan-1-ol; vanillin, 4-hydroxy-3-methoxybenzaldehyde; allyl isothiocyanate, 3-isothiocyanatoprop-1-ene

REFERENCES

(1) Ben Sassi, A.; Cheikh M'hamed, A.; Chahdoura, H.; Saidani Tounsi, M.; Mastouri, M.; Ben Salem, H. Variation in Biochemical Profile and Health Beneficial Compounds and Biological Activities of *Brassica oleracea* Var. Gongylodes L. Morphological Parts. *J. Food Meas. Charact.* **2020**, *14*, 1192–1200.

(2) Sorescu, A.-A.; Nuta, A.; Ion, R.-M. Pale-Green Kohlrabi, a Versatile Brassica Vegetable. In *Brassica Germplasm - Characterization, Breeding and Utilization*; BoD—Books on Demand, 2018.

(3) Manchali, S.; Chidambaram Murthy, K. N.; Patil, B. S. Crucial Facts about Health Benefits of Popular Cruciferous Vegetables. *J. Funct. Foods* **2012**, *4*, 94–106.

(4) Mao, X.; Zhao, X.; Huyen, Z.; Liu, T.; Yu, X. Relationship of Glucosinolate Thermal Degradation and Roasted Rapeseed Oil Volatile Odor. *J. Agric. Food Chem.* **2019**, *67*, 11187–11197.

(5) Hanschen, F. S.; Lamy, E.; Schreiner, M.; Rohn, S. Reactivity and Stability of Glucosinolates and Their Breakdown Products in Foods. *Angew. Chem., Int. Ed.* **2014**, *53*, 11430–11450.

(6) Marcinkowska, M.; Jelen, H. H. Inactivation of Thioglucosidase from *Sinapis alba* (White Mustard) Seed by Metal Salts. *Molecules* **2020**, *25*, No. 4363.

(7) Marcinkowska, M.; Jelen, H. H. Determination of the Odor Threshold Concentrations and Partition Coefficients of Isothiocyanates from Brassica Vegetables in Aqueous Solution. *LWT* **2020**, *131*, No. 109793.

(8) Kroener, E. M.; Buettner, A. Sensory-Analytical Comparison of the Aroma of Different Horseradish Varieties (*Armoracia rusticana*). *Front. Chem.* **2018**, *6*, No. 149.

(9) Bell, L.; Oloyede, O. O.; Lignou, S.; Wagstaff, C.; Methven, L. Taste and Flavor Perceptions of Glucosinolates, Isothiocyanates, and Related Compounds. *Mol. Nutr. Food Res.* **2018**, *62*, No. 1700990.

(10) Park, C. H.; Yeo, H. J.; Kim, N. S.; Eun, P. Y.; Kim, S. J.; Arasu, M. V.; Al-Dhabi, N. A.; Park, S. Y.; Kim, J. K.; Park, S. U. Metabolic Profiling of Pale Green and Purple Kohlrabi (*Brassica oleracea* Var. Gongylodes). *Appl. Biol. Chem.* **2017**, *60*, 249–257.

(11) Park, W. T.; Kim, J. K.; Park, S.; Lee, S. W.; Li, X.; Kim, Y. B.; Uddin, M. R.; Park, N. I.; Kim, S. J.; Park, S. U. Metabolic Profiling of Glucosinolates, Anthocyanins, Carotenoids, and Other Secondary Metabolites in Kohlrabi (*Brassica oleracea* Var. Gongylodes). *J. Agric. Food Chem.* **2012**, *60*, 8111–8116.

(12) Wiczorek, M. N.; Jelen, H. H. Volatile Compounds of Selected Raw and Cooked Brassica Vegetables. *Molecules* **2019**, *24*, No. 391.

(13) Buttery, R. G.; Ling, L. C.; Turnbaugh, J. G.; Juliano, B. O. Cooked Rice Aroma and 2-Acetyl-1-Pyrroline. *J. Agric. Food Chem.* **1983**, *31*, 823–826.

(14) Guth, H.; Grosch, W. 12-Methyltridecanal, a Species-Specific Odorant of Stewed Beef. *LWT - Food Sci. Technol.* **1993**, *26*, 171–177.

(15) Schieberle, P.; Grosch, W. Potent Odorants of the Wheat Bread Crumb Differences to the Crust and Effect of a Longer Dough Fermentation. *Z. Lebensm.-Unters. Forsch.* **1991**, *192*, 130–135.

(16) Lin, J.; Welti, D. H.; Arce Vera, F.; Fay, L. B.; Blank, I. Synthesis of Deuterated Volatile Lipid Degradation Products to Be Used as Internal Standards in Isotope Dilution Assays. 2. Vinyl Ketones. *J. Agric. Food Chem.* **1999**, *47*, 2822–2829.

(17) Kirchhoff, E.; Schieberle, P. Determination of Key Aroma Compounds in the Crumb of a Three-Stage Sourdough Rye Bread by Stable Isotope Dilution Assays and Sensory Studies. *J. Agric. Food Chem.* **2001**, *49*, 4304–4311.

(18) Semmelroch, P.; Laskawy, G.; Blank, I.; Grosch, W. Determination of Potent Odourants in Roasted Coffee by Stable Isotope Dilution Assays. *Flavour Fragrance J.* **1995**, *10*, 1–7.

(19) Blank, I.; Schieberle, P.; Grosch, W. Quantification of the Flavour Compounds 3-Hydroxy-4,5-Dimethyl-2(5*H*)-Furanone and 5-Ethyl-3-Hydroxy-4-Methyl-2(5*H*)-Furanone by Stable Isotope Dilution Assay. In *Progress in Flavour Precursor Studies: Analysis, Generation, Biotechnology: Proceedings of the International Conference, Würzburg, Germany, September 30–October 2, 1992*; Schreier, P., Winterhalter, P., Eds.; Allured Publishing Corporation, 1993; pp. 103–109.

(20) Holzmayer, S. Characterization of Key Aroma Compounds of Celeriac, Carrot, Navet, Onion and Leek and Their Contribution to the Aroma of the Meat Bouillon {Pot}-Au-Feu; Technische Universität München: München, 2017.

G

<https://doi.org/10.1021/acs.jafc.1c04339>
J. Agric. Food Chem. XXXX, XXX, XXX–XXX

- (21) Kiefl, J.; Pollner, G.; Schieberle, P. Sensomics Analysis of Key Hazelnut Odorants (*Corylus avellana* L. "Tonda Gentile") Using Comprehensive Two-Dimensional Gas Chromatography in Combination with Time-of-Flight Mass Spectrometry (GC×GC-TOF-MS). *J. Agric. Food Chem.* **2013**, *61*, 5226–5235.
- (22) Milligan, B.; Saville, B.; Swan, J. M. Trisulfides and tetrasulfides from Bunte Salts. *J. Chem. Soc.* **1963**, *680*, 3608–3614.
- (23) Guth, H.; Grosch, W. Deterioration of Soya-Bean Oil: Quantification of Primary Flavour Compounds Using a Stable Isotope Dilution Assay. *Lebensm. Wiss. Technol.* **1990**, *23*, S13–S22.
- (24) Engel, W.; Bahr, W.; Schieberle, P. Solvent Assisted Flavour Evaporation – a New and Versatile Technique for the Careful and Direct Isolation of Aroma Compounds from Complex Food Matrices. *Eur. Food Res. Technol.* **1999**, *209*, 237–241.
- (25) ASTM. Standard Practice for Determining Odor and Taste Thresholds by a Forced-Choice Ascending Concentration Series Method of Limits, E-679-04. *Annu. B. Stand.* **2008**, *11*, Vol 15.08, 36–42. DOI: 10.1520/E0679-19.2.
- (26) Kreissl, J.; Mall, V.; Steinhaus, P.; Steinhaus, M. *Leibniz-LSB@TUM Odorant Database*, Version 1.0; Leibniz-Institute for Food Systems Biology at the Technical University of Munich: Freising, Germany. <https://www.leibniz-lsb.de/en/databases/leibniz-lsb-tum-odorant-database> (accessed May 18, 2021).
- (27) Fischer, J. Sulphur- and Nitrogen-Containing Volatile Components of Kohlrabi (*Brassica oleracea* Var. Gongylodes L.). *Z. Lebensm.-Unters. Forsch.* **1992**, *194*, 259–262.
- (28) Yi, G. E.; Robin, A. H. K.; Yang, K.; Park, J. I.; Kang, J. G.; Yang, T. J.; Nou, I. S. Identification and Expression Analysis of Glucosinolate Biosynthetic Genes and Estimation of Glucosinolate Contents in Edible Organs of *Brassica oleracea* Subspecies. *Molecules* **2015**, *20*, 13089–13111.
- (29) Ortner, E.; Granvogl, M.; Schieberle, P. Elucidation of thermally induced changes in key odorants of white mustard seeds (*Sinapis alba* L.) and Rapeseeds (*Brassica napus* L.) using molecular sensory science. *J. Agric. Food Chem.* **2016**, *64*, 8179–8190.
- (30) Chetschik, I.; Granvogl, M.; Schieberle, P. Comparison of the Key Aroma Compounds in Organically Grown, Raw West-African Peanuts (*Arachis hypogaea*) and in Ground, Pan-Roasted Meal Produced Thereof. *J. Agric. Food Chem.* **2008**, *56*, 10237–10243.
- (31) Fischer, J. The Influence of Different Nitrogen and Potassium Fertilisation on the Chemical Flavour Composition of Kohlrabi (*Brassica oleracea* Var. Gongylodes L.). *J. Sci. Food Agric.* **1992**, *60*, 465–470.
- (32) Inagaki, S.; Fujikawa, S.; Wada, Y.; Kumazawa, K. Identification of the Possible New Odor-Active Compounds "12-Methyltridecanal and Its Analogs" Responsible for the Characteristic Aroma of Ripe Gouda-Type Cheese. *Biosci., Biotechnol., Biochem.* **2015**, *79*, 2050–2056.
- (33) Kersch, R.; Nürnberg, K.; Voigt, J.; Schieberle, P.; Grosch, W. Occurrence of 12-Methyltridecanal in Microorganisms and Physiological Samples Isolated from Beef. *J. Agric. Food Chem.* **2000**, *48*, 2387–2390.
- (34) Pickering, G. J.; Karthik, A.; Inglis, D.; Sears, M.; Ker, K. Determination of Ortho- and Retronasal Detection Thresholds for 2-Isopropyl-3-Methoxypyrazine in Wine. *J. Food Sci.* **2007**, *72*, S468–S472.
- (35) Steinhaus, M.; Wilhelm, W.; Schieberle, P. Comparison of the Most Odor-Active Volatiles in Different Hop Varieties by Application of a Comparative Aroma Extract Dilution Analysis. *Eur. Food Res. Technol.* **2007**, *226*, 45–55.
- (36) Matheis, K.; Granvogl, M. Characterisation of the key aroma compounds in commercial native cold-pressed rapeseed oil by means of the sensomics approach. *Eur. Food Res. Technol.* **2016**, *242*, 1565–1575.
- (37) Zhang, Y.; Wu, Y.; Chen, S.; Yang, B.; Zhang, H.; Wang, X.; Granvogl, M.; Jin, Q. Flavor of rapeseed oil: an overview of odorants, analytical techniques, and impact of treatment. *Compr. Rev. Food Sci. Food Saf.* **2021**, *20*, 3983–4018.
- (38) Peterson, D.; Reineccius, G. Biological Pathways for the Formation of Oxygen-Containing Aroma Compounds. In *Heteroatomic Aroma Compounds*; Reineccius, G. A., Reineccius, T. A., Eds.; ACS Symposium Series; American Chemical Society, 2002; Vol. 826, pp 227–242.
- (39) Maimone, M.; Manukyan, A.; Tranchida, P. Q.; Steinhaus, M. Odour-Active Compounds in the Traditional Armenian Soup Seasoning Herb *Heracleum Transcaucasicum*. *Eur. Food Res. Technol.* **2017**, *243*, 969–977.
- (40) Routray, W.; Rayaguru, K. 2-Acetyl-1-Pyrroline: A Key Aroma Component of Aromatic Rice and Other Food Products. *Food Rev. Int.* **2018**, *34*, 539–565.
- (41) Jia, X.; Wang, L.; Zheng, C.; Yang, Y.; Wang, X.; Hui, J.; Zhou, Q. Key Odorant Differences in Fragrant *Brassica napus* and *Brassica juncea* Oils Revealed by Gas Chromatography–Olfactometry, Odor Activity Values, and Aroma Recombination. *J. Agric. Food Chem.* **2020**, *68*, 14950–14960.
- (42) Pollner, G.; Schieberle, P. Characterization of the Key Odorants in Commercial Cold-Pressed Oils from Unpeeled and Peeled Rapeseeds by the Sensomics Approach. *J. Agric. Food Chem.* **2016**, *64*, 627–636.
- (43) Pierre, P. S.; Jansen, J. J.; Hordijk, C. A.; van Dam, N. M.; Cortesero, A.-M.; Dugravot, S. Differences in Volatile Profiles of Turnip Plants Subjected to Single and Dual Herbivory Above- and Belowground. *J. Chem. Ecol.* **2011**, *37*, No. 368.
- (44) Zhang, Y.; Lu, R.; Song, H.; Zheng, S.; Chen, Y.; Zhou, N. Effect of Stir Frying on the Antioxidant Capacity and Aroma Components of Bok Choy (Chinese Cabbage). *Int. J. Food Prop.* **2016**, *19*, 2536–2549.
- (45) Wei, S.; Xiao, X.; Wei, L.; Li, L.; Li, G.; Liu, F.; Xie, J.; Yu, J.; Zhong, Y. Development and Comprehensive HS-SPME/GC–MS Analysis Optimization, Comparison, and Evaluation of Different Cabbage Cultivars (*Brassica oleracea* L. Var. Capitata L.) Volatile Components. *Food Chem.* **2021**, *340*, No. 128166.
- (46) Engel, E.; Baty, C.; le Corre, D.; Souchon, I.; Martin, N. Flavor-Active Compounds Potentially Implicated in Cooked Cauliflower Acceptance. *J. Agric. Food Chem.* **2002**, *50*, 6459–6467.
- (47) Van Gemert, L. J. *Flavour Thresholds: Compilations of Flavour Threshold Values in Water and Other Media*; Oliemans Punter, 2011.
- (48) Van Gemert, L. J. *Compilations of Odour Threshold Values in Air, Water and Other Media*; second enlarged and revised edition; Oliemans, Punter & Partners BV: Utrecht, 2011.

Supporting Information

Miscellaneous Chemicals and Materials. Dichloromethane was purchased from CLN (Freising, Germany). Before use, it was freshly distilled through a column (120 cm × 5 cm) packed with Raschig rings. Anhydrous sodium sulfate and sodium chloride were purchased from Merck (Darmstadt, Germany). Sucrose was obtained from a local shop (Freising, Germany).

GC-O/FID System. A GC 8000 series gas chromatograph (Fisons Instruments, Mainz, Germany) was equipped with a cold on-column injector, flame ionization detector, and a sniffing port. A DB-FFAP column (30 m × 0.32 mm, film thickness 0.25 μm; Agilent, Waldbronn, Germany) or a DB-5 (30 m × 0.32 mm, film thickness 0.25 μm; Agilent) was used for separation. The carrier gas was helium at 45 kPa (DB-FFAP) and 55 kPa (DB-5). The oven temperature was 40 °C for 2 min, then ramped at 6 °C/min to 230 °C (DB-FFAP) or 240 °C (DB-5), and held for 5 min. The end of the capillary column was connected to a glass Y-splitter which divided the column effluent at a 1:1 volume ratio. One part of the effluent was transferred to the FID, while the other portion was routed to the sniffing port. During a GC-O analysis, the assessor placed the nose closely above the sniffing port and evaluated the odor of the effluent. Odor-active regions were marked and described by the assessor in the chromatogram printed by the FID recorder. Linear retention indices (RIs) of the odor-active compounds were calculated after co-injection with a mixture of *n*-alkanes from the retention times of the odorants and the adjacent two *n*-alkanes by linear interpolation.

GC-MS. A 6890 Plus gas chromatograph (Agilent) was equipped with a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland), a CIS4 injector (Gerstel, Mülheim/Ruhr, Germany), and a GC capillary column DB-FFAP (30 m × 0.25 mm, film thickness 0.25 μm; Agilent). The end of the column was connected to a second column, which was a DB-5 (2.5 m × 0.15 mm, film thickness 0.30 μm; Agilent). The carrier gas was helium at 2.0 mL/min

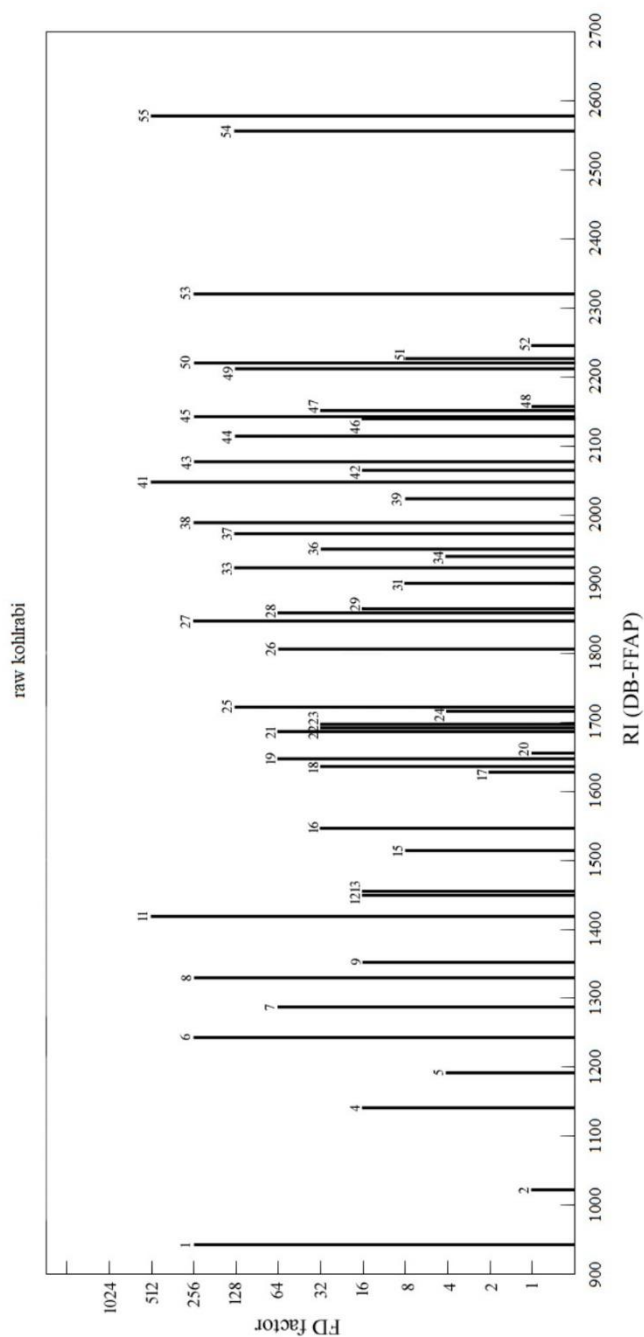
constant flow. At the beginning of the second column, a liquid nitrogen-cooled dual-stage quadrupole thermal modulator was used to collect the volatiles eluted from the first column in discrete portions (4 s) which were subsequently separated on the second column. The end of the second column was connected to the MS interface (250 °C) of a Pegasus III time-of-flight mass spectrometer (ToF-MS) (Leco, Mönchengladbach, Germany). Mass spectra were generated in the EI mode at 70 eV, in a range of 35–350 *m/z*, at a rate of 100 spectra/s. The oven temperature program was 40 °C (2 min), then 6 °C/min to 230 °C (5 min) for the first oven. The second oven was heated with an offset of +20 °C to the first oven. GC Image software (GC Image, Lincoln, NE, USA) was used for data analysis.

GC-(CI)MS. Mass spectra in the chemical ionization (CI) mode were acquired with a 7890B GC System (Agilent) connected to a 240 Ion Trap GC-MS mass spectrometer (Agilent) using methanol as a reagent gas. The system was equipped with a GC sampler 80 (Agilent). The column was a DB-FFAP (30 m × 0.32 mm, film thickness 0.25 µm; Agilent). The injection volume was 1 µL. The carrier gas was helium at 1 mL/min. The oven temperature program was 40 °C (2 min), then 8 °C/min to 230 °C (5 min).

Heart-Cut GC-GC-HRMS System. A Trace 1300 gas chromatograph (Thermo Fisher Scientific, Dreieich, Germany) was equipped with a TriPlus RSH autosampler (Thermo) and a programmed temperature vaporizing (PTV) injector. The injection volume was 1 µL. The carrier gas was helium at a constant flow of 1 mL/min. The column in the first oven was DB-FFAP (30 m × 0.25 mm, film thickness 0.25 µm; Agilent), and a DB-1701 (30 m × 0.25 mm, film thickness 0.25 µm; Agilent) in the second oven. For the quantitation of odorant **8**, the temperature program of the first oven was 40 °C (2 min), then 6 °C/min to 105 °C, then 40 °C/min to 230 °C (5 min). The temperature program of the second oven was 40 °C (2 min), then 6 °C/min to 150 °C, then 40 °C/min to 230 °C (5 min). For the quantitation of odorant **38**, the temperature program of the first oven was 40 °C (2 min), then 6 °C/min to 170 °C, and then

15 °C/min to 230 °C (5 min). The temperature program of the second oven was 40 °C (2 min), then 6 °C/min to 150 °C, and then 15 °C/min to 230 °C (5 min). The end of the second GC column was connected to a Q Exactive GC Orbitrap high resolution (HR) mass spectrometer (Thermo) operated in CI HR mode with isobutane as a reagent gas. For the quantitation of odorant **8**, positive CI was used with a scan range of 85 to 130 m/z , and the temperature of the ion source was 220 °C. For the quantitation of odorant **38**, negative CI was used with a scan range of 90 to 180 m/z , and the temperature of the ion source was 250 °C. Data were evaluated using the Xcalibur software (Thermo).

Figure S1 FD chromatograms of Odorants Isolated from Raw and Cooked Green Kohlrabi with their FD Factors and Retention Indices (RI) on a DB-FFAP Column, Numbers Refer to Table 1



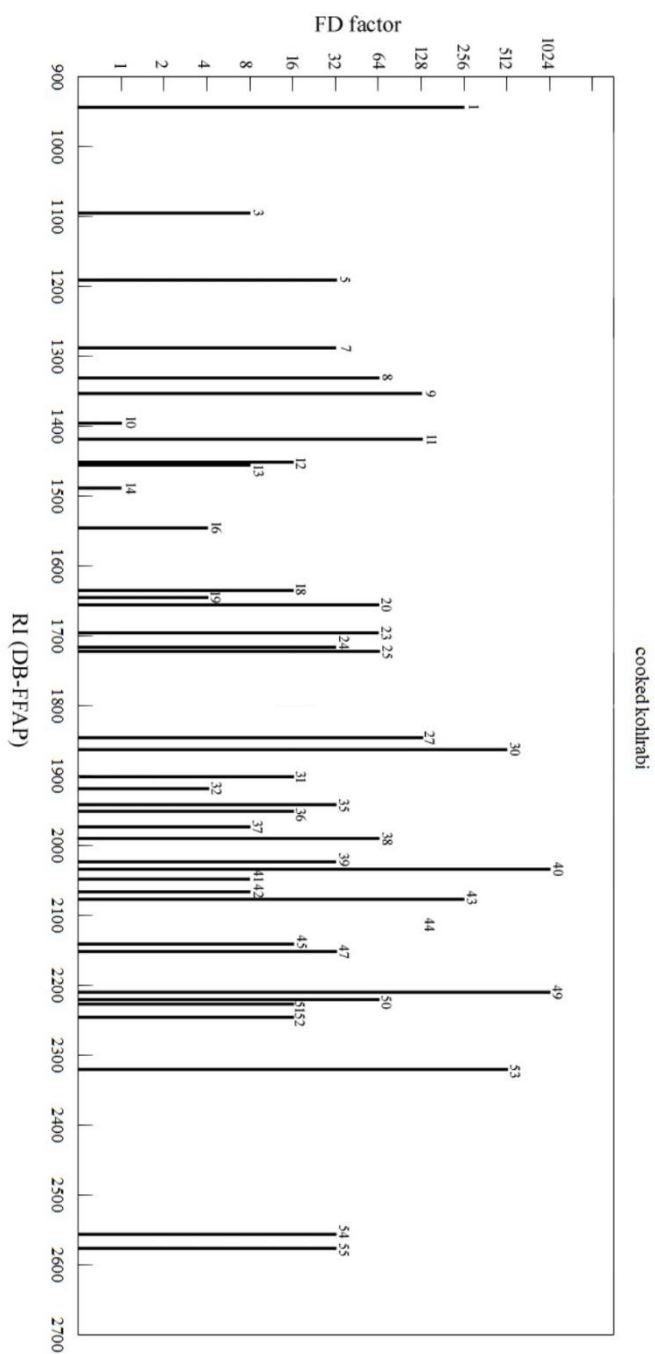


Table S1 Experimental Data of Quantitation Parameters (Internal Standards, Quantifier Ions, Calibration Lines).

odorant	isotopically substituted standard	quantifier ions (m/z)		calibration line equation ^a	R ²
		analyte	standard		
1	(² H ₈)naphthalene	82	136	y = 1.096x - 0.097	0.998
6	(² H ₃)- 6	81	84	y = 0.613x + 0.079	0.999
7	(² H ₄)- 7	127	131	y = 0.932x + 0.019	1.000
8	(¹³ C ₃)- 8	111	116	y = 1.390x - 0.065	1.000
9	(² H ₆)- 9	126	132	y = 0.819x + 0.092	0.993
11	(² H ₃)- 11	153	156	y = 0.654x + 0.131	0.998
19	(² H ₈)naphthalene	161	136	y = 1.133x - 0.129	0.999
20	(² H ₈)naphthalene	115	136	y = 1.118x - 0.146	0.998
21	(² H ₃)-5-methylthiophene-2-carbaldehyde	113	130	y = 1.082x - 0.003	1.000
23	(² H ₈)naphthalene	99	136	y = 0.640x + 0.053	0.994
25	(² H ₈)naphthalene	61	136	y = 1.275x - 0.060	0.999
26	(² H ₃)- 26	131	134	y = 0.938x + 0.055	0.996
27	(² H ₈)naphthalene	61	136	y = 1.056x - 0.035	1.000
28	(² H ₃)-3-(methylthio)propan-1-ol	120	109	y = 0.72x + 0.282	0.999
30	(² H ₈)naphthalene	150	136	y = 0.831x + 0.220	1.000
33	(² H ₈)naphthalene	91	136	y = 1.089x - 0.034	1.000
37	(² H ₈)naphthalene	101	136	y = 1.144x - 0.053	1.000
38	(² H ₄)- 38	171	167	y = 1.246x - 0.289	0.994
40	(¹³ C ₆)- 40	137	143	y = 1.104x - 0.009	1.000

Table S1 continued

odorant	isotopically substituted standard	quantifier ions (m/z)		calibration line equation ^a	R ²
		analyte	standard		
41	(² H ₈)naphthalene	91	136	y = 1.098x - 0.038	1.000
43	(² H ₈)naphthalene	91	136	y = 1.095 - 0.115	0.999
44	(² H ₈)naphthalene	91	136	y = 1.089x - 0.034	1.000
45	(² H ₈)naphthalene	61	136	y = 1.205x - 0.089	0.999
49	(¹³ C ₂)- 49	129	131	y = 0.996x + 0.092	0.999
50	(² H ₈)naphthalene	91	136	y = 0.972x + 0.013	0.999
53	(² H ₈)naphthalene	112	136	y = 1.104x - 0.045	1.000
54	(¹³ C ₂)- 54	136	138	y = 0.706x + 0.151	0.994
55	(² H ₈)- 55	151	154	y = 1.002x + 0.037	1.000

^ay = peak area standard / peak area analyte; x = concentration standard (µg/mL) / concentration analyte (µg/mL).

**Pisemne oświadczenia współautorów publikacji naukowych stanowiących
rozprawę doktorską**



mgr inż. Monika Anna Marcinkowska
Wydział Nauk o Żywności i Żywieniu
Uniwersytet Przyrodniczy w Poznaniu
Wojska Polskiego 31, 60-624 Poznań

Oświadczenie współautora

Oświadczam, że jestem współautorem następujących publikacji:

1. **M. Marcinkowska**, H.H. Jeleń. Role of sulfur compounds in vegetable and mushroom aroma. *Molecules*, 2022, 27, 6116
2. **M. Marcinkowska**, H.H. Jeleń. Determination of the odor threshold concentrations and partition coefficients of isothiocyanates from Brassica vegetables in aqueous solutions. *LWT – Food Science and Technology*, 2020, 131, 109793
3. **M. Marcinkowska**, H.H. Jeleń. Inactivation of thioglucosidase from *Sinapis alba* (White Mustard) seeds by metal salts. *Molecules*, 2020, 25, 4363
4. **M. Marcinkowska**, S. Frank, M. Steinhaus, H. Jeleń. Key odorants of raw and cooked green kohlrabi (*Brassica oleracea* var. *gongylodes* L.). *Journal of Agricultural and Food Chemistry*, 2021, 69, 12270-12277

Publikacje te wchodzą w skład mojej rozprawy doktorskiej.

Moja rola w realizacji wyżej wymienionych prac polegała na:

- współtworzeniu koncepcji pracy i metod analizy,
- przeprowadzeniu wszystkich eksperymentów i analiz
- opracowaniu i interpretacji wyników
- napisaniu manuskryptów (100% prac 2-4, 80% pracy 1)
- współredagowaniu korekt manuskryptów i odpowiedzi na recenzje.

Monika Marcinkowska

Poznań, 10.06.2023

Prof. dr hab. Henryk Jeleń

Wydział Nauk o Żywności i Żywieniu
Uniwersytet Przyrodniczy w Poznaniu
Wojska Polskiego 31, 60-624 Poznań

Oświadczenie współautora

Oświadczam, że jestem współautorem publikacji:

- M. Marcinkowska, H.H. Jeleń*. Role of sulfur compounds in vegetable and mushroom aroma. Molecules, 2022, 27, 6116

Publikacja ta wchodzi w skład rozprawy doktorskiej Pani mgr Moniki Marcinkowskiej

Moja rola w realizacji pracy polegała na współtworzeniu koncepcji pracy, ok. 20% jej treści, edycji wersji manuskryptu przygotowanej przez Panią Monikę Marcinkowską oraz współredagowaniu odpowiedzi na recenzje.

Henryk
Hubert Jeleń

Elektronicznie podpisany przez
Henryk Hubert Jeleń
DN: c=PL,
serialNumber=PNOP-62100103230,
cn=Henryk Hubert Jeleń,
givenName=Henryk Hubert, sn=Jeleń
Data: 2023.06.10 15:18:15 +02'00'

Poznań, 10.06.2023

Prof. dr hab. Henryk Jeleń

Wydział Nauk o Żywności i Żywieniu
Uniwersytet Przyrodniczy w Poznaniu
Wojska Polskiego 31, 60-624 Poznań

Oświadczenie współautora

Oświadczam, że jestem współautorem publikacji:

- M. Marcinkowska, H.H. Jeleń*. Determination of the odor threshold concentrations and partition coefficients of isothiocyanates from Brassica vegetables in aqueous solutions. LWT – Food Science and Technology, 2020, 131, 109793

Publikacja ta wchodzi w skład rozprawy doktorskiej Pani mgr Moniki Marcinkowskiej

Moja rola w realizacji pracy polegała na współtworzeniu koncepcji pracy i metod analizy, zapewnieniu środków związanych z jej realizacją, edycji wersji manuskryptu przygotowanej przez Panią Monikę Marcinkowską oraz współredagowaniu odpowiedzi na recenzje.

Henryk
Hubert Jeleń

Elektronicznie podpisany przez Henryk
Hubert Jeleń
DN: c=PL,
serialNumber=PNOP-62100103230,
cn=Henryk Hubert Jeleń,
givenName=Henryk Hubert, sn=Jeleń
Data: 2023.06.10 15:20:08 +02'00'

Poznań, 10.06.2023

Prof. dr hab. Henryk Jeleń

Wydział Nauk o Żywności i Żywieniu

Uniwersytet Przyrodniczy w Poznaniu

Wojska Polskiego 31, 60-624 Poznań

Oświadczenie współautora

Oświadczam, że jestem współautorem publikacji:

- M. Marcinkowska, H.H. Jeleń*. Inactivation of thioglucosidase from *Sinapis alba* (White Mustard) seeds by metal salts. *Molecules*, 2020, 25, 4363

Publikacja ta wchodzi w skład rozprawy doktorskiej Pani mgr Moniki Marcinkowskiej

Moja rola w realizacji pracy polegała na współtworzeniu koncepcji pracy i metod analizy, zapewnieniu środków związanych z jej realizacją, edycji wersji manuskryptu przygotowanej przez Panią Monikę Marcinkowską oraz współredagowaniu odpowiedzi na recenzje.



Poznań, 10.06.2023

Prof. dr hab. Henryk Jeleń

Wydział Nauk o Żywności i Żywieniu
Uniwersytet Przyrodniczy w Poznaniu
Wojska Polskiego 31, 60-624 Poznań

Oświadczenie współautora

Oświadczam, że jestem współautorem publikacji:

- M. Marcinkowska, S. Frank, M. Steinhaus, H. Jeleń*. Key odorants of raw and cooked green kohlrabi (*Brassica oleracea* var. *gongylodes* L.). Journal of Agricultural and Food Chemistry, 2021, 69, 12270-12277

Publikacja ta wchodzi w skład rozprawy doktorskiej Pani mgr Moniki Marcinkowskiej

Moja rola w realizacji pracy polegała na współtworzeniu koncepcji pracy, zapewnieniu części środków związanych z jej realizacją, edycji wersji manuskryptu przygotowanej przez Panią Monikę Marcinkowską oraz współredagowaniu odpowiedzi na recenzje.

Henryk
Hubert Jeleń

Elektronicznie podpisany przez
Henryk Hubert Jeleń
DN: c=PL,
serialNumber=PNCPL-62100103230,
cn=Henryk Hubert Jeleń,
givenName=Henryk Hubert, sn=Jeleń
Data: 2023.06.10 15:20:59 +02'00'

Poznań, 10.06.2023

Dr. Stephanie Frank

Leibniz Institute for Food Systems Biology
at the Technical University of Munich,
Lise-Meitner-Straße 34
85354 Freising, Germany

Statement of contribution

I declare that I am the co-author of the following manuscript:

- M. Marcinkowska, S. Frank, M. Steinhaus, H. Jeleń. Key odorants of raw and cooked green kohlrabi (*Brassica oleracea* var. *gongylodes* L.). *Journal of Agricultural and Food Chemistry*, 2021, 69, 12270-12277

This publication constitutes a part of the PhD thesis of Miss Monika Marcinkowska. I have contributed to the publication in the following way:

- **Supervision** of Miss Monika Marcinkowska during her research stays at the Leibniz Institute for Food Systems Biology at the Technical University of Munich between September and December 2019 and in February, March, September, and October 2020

- **Writing – review and editing** of the above mentioned manuscript based on the original draft prepared by Miss Monika Marcinkowska

June 5, 2023 Stephanie Frank

Date and signature

PD Dr. Martin Steinhaus

Leibniz Institute for Food Systems Biology
at the Technical University of Munich,
Lise-Meitner-Straße 34
85354 Freising, Germany

Statement of contribution

I declare that I am the co-author of the following manuscript:

- M. Marcinkowska, S. Frank, M. Steinhaus, H. Jeleń. Key odorants of raw and cooked green kohlrabi (*Brassica oleracea* var. *gongylodes* L.). *Journal of Agricultural and Food Chemistry*, 2021, 69, 12270-12277

This publication constitutes a part of the PhD thesis of Miss Monika Marcinkowska. I have contributed to the publication in the following way:

- **Supervision** of Miss Monika Marcinkowska during her research stays at the Leibniz Institute for Food Systems Biology at the Technical University of Munich between September and December 2019 and in February, March, September, and October 2020
- Provision of **Resources** used by Miss Monika Marcinkowska during her research stays at the Leibniz Institute for Food Systems Biology at the Technical University of Munich including instrumentation and chemicals
- **Writing – review and editing** of the above mentioned manuscript based on the original draft prepared by Miss Monika Marcinkowska

June 5, 2023 *Martin Steinhaus*

Date and signature