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The relation between phytochemical composition and sensory traits of selected Brassica vegetables

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ABSTRACT

Main groups of sensory active compounds - glucosinolates, isothiocyanates, phenolics and sugars in 3 cultivars of broccoli, 5 of Brussels sprout, 3 of cauliflower, and 4 of kohlrabi, both raw and cooked, were analyzed and correlated with selected sensory traits. The differences in the concentration of these components were significant between different vegetables and also noticeable between cultivars of one vegetable. The bitterness of Brussels sprouts and broccoli was correlated with glucosinolates, though Brussel sprouts contained definitely higher concentration (>1 g/kg fw) of these substances than their concentration in broccoli (<0.3 g/kg fw), or other vegetables. A positive correlation between sugar concentration and general desirability was observed in Brussels sprouts (0.91 — raw), kohlrabi (0.53 — raw; 0.80 — cooked), and raw cauliflower (0.85). According to sensory analysis, the high correlation between sweetness level and general desirability was observed, however, sweetness intensity was not correlated with total sugars concentration. A lack of any correlation between phenolic content and taste was observed. The results presented in this work emphasized the diversity of investigated Brassica vegetables from both phytochemical and sensory point of view.

1. Introduction

Diets rich in vegetables and fruit are highly encouraged by nutritionists, due to their beneficial effects on human health. The reasons behind this include an abundance of secondary metabolites in vegetables and fruit of proven beneficial biological activities, mainly antioxidative, anti-inflammatory, and anticancer. Increment of Brassica vegetables consumption is particularly important in the light of research concerning their role in cancer prevention. Literature data point out the particular anti-tumor potential of Brassica vegetables are caused by the presence of thiocyanates - products of glucosinolate hydrolysis (Hecht, 2000; Wieczorek, Walczak, Skrzypczak-Zielińska, & Jeleń, 2018).

This study was focused on broccoli, cauliflower, Brussels sprouts, and kohlrabi because of their popularity in the daily diet and the specific sensory properties. The characteristic features of Brassica vegetables are their bitter taste and a sulfurous smell. These flavor attributes largely determine their acceptance by consumers.

It is believed that glucosinolates and their breakdown products - isothiocyanates - are responsible for the characteristic taste and smell of Brassica vegetables (Drewnowski & Gomez-Carneros, 2000; Fenwick, Heaney, & Mullin, 1983; Van Doorn et al., 1998), however, literature data in this matter are ambiguous and many sources negate this hypothesis (Bell & Wagstaff, 2014; Molina-Vargas, 2013; Schreiber, Jin, & Winkler, 2011; Zabaras, Roohani, Krishnamurthy, Cochet, & Delahunty, 2013). The problem seems to be particularly significant, mainly due to the bioactive properties of isothiocyanates which have undergone extensive research on their anticancer properties in recent years (Traka & Mithen, 2009). The progress in analytical techniques allowed to develop the concept of sensomics (Dresel, Dunkel, & Hofmann, 2015), which allowed to broaden knowledge about the relationships determining consumer acceptance of foods and the impact of technological or culinary treatments on their changes. Brassica vegetables are rich in flavor-active compounds, and the multitude and variety of those components make research on them demanding (Wieczorek et al., 2018). There are still only a few studies (Bell & Wagstaff, 2014; Zabaras et al., 2013) on sensory tests that are correlated with the content of main flavor groups such as phenols, glucosinolates, and sugars for Brassica vegetables. This stage is a necessary introduction to further research on specific

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compounds responsible for the characteristic bitterness of these vegetables.

The presented study focuses on profiles of glucosinolates, phenolics, sugars, and isothiocyanates and their relation to selected sensory (taste) traits and consumers' desirability of selected Brassica vegetables. All analyses were performed for both raw and cooked broccoli, cauliflower, Brussel sprouts and kohlrabi.

2. Materials and methods

Three cultivars of fresh broccoli (Covina, 2970, Malibu), three cultivars of cauliflower (Charlotte, Oviedo, Liria), five cultivars of Brussels sprout (Ajax, Maximus, Profitus, Neptuno, Marte) and four of kohlrabi (Konmar, Kolibri, Konan, Kordial) were used for the analysis. Varieties of broccoli, cauliflower and kohlrabi were harvested in September, Brussels sprouts cultivars were harvested in December. The vegetables were delivered to the laboratory within 24 h after harvest. Brussel sprouts were directly packed into plastic bags (0.5 kg each); broccoli and cauliflower florets were fragmented into smaller parts, packed into plastic bags (0.3 kg each); and kohlrabi were peeled and chopped into 2 cm × 8 cm x 1 cm cubes, then packed into plastic bags (0.5 kg). All vegetables after packing were directly transferred to −20 °C freezers and stored there before analysis (no more than 3 weeks).

2.1. Cooking process

All vegetables were analyzed raw and after cooking. Around 200 g of frozen plant material was placed in 1 L of boiling pure water with 7 g of salt. Cooking times differed among vegetables to reflect their preparation in a kitchen. It was 5 min for the broccoli, 7 min for the cauliflower, 10 min for the kohlrabi and 8 min for Brussels sprouts. The whole cooking procedure for each vegetable was performed in 3 replicates, which were used in further studies.

2.2. HPLC analysis of sugars

The raw or cooked vegetable samples were frozen in liquid nitrogen and comminuted in a laboratory mill, then 100 mL of pure water was added to 20 g of vegetable. Extraction was performed for 15 min in 60 °C. After this time extract was made up to 200 mL with water. The extract was filtered and analyzed using HPLC-RID.

Determination of carbohydrates was carried out on an Agilent Technologies 1200 series HPLC system consisting of an autosampler (G1329B), binary pump (G1312B) and refractive index detector (RID, G1362A) (Agilent Technologies, Waldbronn, Germany). The analysis was performed isocratically at a flow rate of 0.6 mL/min at 80 °C on the Rezex RPM-Monosaccharide Pb^{+2} 300 × 7.8 mm column (Phenomenex, Torrance, CA, USA). Water as mobile phase was used. Standards (sucrose, glucose, fructose, raffinose, stachyose, 1-kestose, nystose, arabinose, sorbitol – all from Sigma-Aldrich, Poznań, Poland) were used to identify peaks in chromatograms and perform quantitative analysis based on external calibration. Agilent ChemStation for LC 3D systems was used for data processing.

2.3. HPLC-MS/MS analysis of glucosinolates

Vegetable (20 g) ground in liquid nitrogen was extracted using water/methanol (8/2, v/v) solution for 30 min. The extract was centrifuged, then transferred to round bottom flask and placed in rotary evaporator (Heidolph, Poznan, Poland) to remove methanol (50 °C; 250 mbars). Then the sample was frozen and the extract was lyophilized and subjected to analysis.

Intact glucosinolates were separated and quantified on a HPLC-MS/MS system (3200 MS/MS, AB Sciex, Framingham, MA, USA) equipped with an ESI source and operating in a negative ion mode. The system uses the Amide Column, 130 Å, (100 mm x 2.1 mm x 1.7 μm; Waters, Eschborn Germany). Glucosinolates were analyzed using a targeted approach: twelve most frequently occurring glucosinolates were determined. Those were: gluconasturin, glucoraphanin, glucoiberin, progoitrin, glucoerucin, sinalbin, glucosibarin, glucotreapolin, sinigrin, glucobrassicin, epigoitrin, and gluconapin. All standards of glucosinolates originated from Extrasynthese (Genay, France) company The mobile phase consisted of water containing methanol (A) and water (B). The flow rate was 0.4 mL/min. The syringe and needle were washed before and after each sample injection. Compounds present in each sample were identified based on standard retention time, molecular weight, and structural information obtained from MS/MS. The data analysis collection was carried out using the Analysts 1.6.2 software.

2.4. HPLC-MS analysis of phenolic compounds

To 0.6 g of vegetable lyophilisate, 5 mL of methanol/water (7/3, v/v) was added and sonicated for 20 min in 60 °C (Sulaiman et al., 2017). Then, the extract was centrifuged for 20 min (10 000 rpm, MPW 223e centrifuge, MPW Med Instruments, , Warsaw, Poland). The extraction was repeated and filtrates were collected. The extracts were concentrated to 5 mL, under a stream of nitrogen, then filtered through nylon filter membranes (0.45 μm, diam. 47 mm, Sigma Aldrich, Poznan, Poland) and analyzed using HPLC-MS/MS. To calculate the concentration of a phenolic compound in fresh weight vegetables were weighed before and after lyophylisation. Based on weight differences, the amount was calculated for dry mass.

Reversed-phase (C18) ultra-high-performance liquid chromatography-electrospray ionization mass spectrometry (RP-UHPLC-ESI-MS) analysis was performed using a DionexUltiMate 3000 UHPLC (Thermo Fisher Scientific, Sunnyvale, CA, USA) coupled to a Bruker maXis impact ultrahigh-resolution orthogonal quadrupole-time-of-flight accelerator (qTOF) system equipped with an ESI source and operated in the negative-ion mode (Bruker Daltonik, Bremen, Germany). The RP chromatographic separation was achieved with a Synergi 4 μm Fusion-RP 80 Å, LC column 150 × 3.0 mm (Phenomenex, Torrance, CA, USA). The mobile phase A was comprised of water, while mobile phase B was comprised of acetonitrile/water (95/5, v/v); both mobile phases contained 16 mmol/L acetic acid. The flow rate was 0.3 mL/min with a gradient elution of 1%–100% B over 20 min. The column temperature was set at 40 °C. The syringe and needle were washed before and after injection of each sample (water/methanol, 1/1). The carry-over between samples was not observed. The ESI-MS settings were according previously published paper (Mildner-Szkudlarz, Siger, Szwengiel, & Bajerska, 2015). Molecular ions: [M]- and [M+H]- were extracted from full scan chromatograms (±0.005 m/z) and peak areas were integrated with QuantAnalysis 2.1 (Bruker Daltonik, Bremen, Germany). The compounds presented in each sample were identified based on the retention time of standards (gallic acid, caffeic acid, syringic acid, kaempferol, luteolin, quetcetin, matairesinol, pinoresinol, all purchased from Sigma-Aldrich, Poznan, Poland) and/or molecular mass and structural information from the MS detector during MS/MS experiments (p-coumaric acid, sinapic acid, 4-hydroxybenzoate-O-glucoside, 4-coumarylquinate, chlorogenic acid, 1-O-sinapoyl-beta-glucose). Tandem mass spectrometric data was used for searching molecular structure using two computational methods. We used CSI:FingerID, which combines fragmentation tree computation and machine learning (Shen, Dührkop, Böcker, & Rousu, 2014; Dührkop, Shen, Meusel. Rousu & Böcker, 2015) and the in silicofragmenter MetFrag (Ruttkies, Schymanski, Wolf, Hollender, & Neumann, 2016). The possible molecular formulae for an MS precursor ion of not identified (NI) compound were calculated with the SmartFormula3D (Bruker, Germany) command by combining two spectra MS and MS/MS.

2.5. SPME-GC × GC-ToFMS analysis of isothiocyanates

Isothiocyanates from the analyzed vegetables were isolated using

HS-SPME and analyzed using SPME-GC × GC-Tof MS for the comparative analysis only. Allyl isothiocyanate, isopropyl isothiocyanate, methyl isothiocyanate, phenethyl isothiocyanates, benzyl isothiocyanate, hexyl isothiocyanate, isobutyl isothiocyanate were purchased from Sigma-Aldrich (Poznan, Poland). Isothiocyanates were identified based on comparison of their retention times, retention indices and mass spectra to standards or to NIST 2.0 mass spectra library. For the comparison purposes peak areas values were used, all samples from one vegetable were running over one-day in randomized order. Divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibers were used for extraction. Vegetables were cut to pieces of about 0.5 cm with a kitchen knife and 4 g was placed in an SPME vial. The extraction temperature was 60 °C and the fiber was exposed for 30 min. After this time, the compounds isolated using SPME were desorbed in the injector port of a GC × GC-ToFMS system (Pegasus4D, LECO, St. Joseph, MI, USA). The chromatograph was equipped with a DB-5 primary column (25 m × 0.2 mm × 0.33 μm Agilent Technologies, Santa Clara, CA, USA) and Supelcowax 10 (1.3 m × 0.1 mm × 0.1 μm, Supelco Bellefonte, USA) as the second column. The injector temperature was 250 °C, the gas flow was 0.8 mL/min. The primary oven temperature was programmed as follows: 40 °C (1 min) 6 °C/min to 200 °C (0 min) 25 °C/min to 235 °C (5 min). Secondary oven: 65 °C (1 min) 6 °C/min to 225 °C (0 min) 25 °C/min to 260 °C (5 min). Transfer line temperature was 260 °C. The modulation time was 4 s. The time-of-flight mass spectrometer was operating at a mass range of m/z 38–388 and detector voltage −1700 V at 150 spectra/s. Data were collected and processed using LECO ChromaTOF v.4.44 software. Total analysis time was 34.07 min.

2.6. Sensory analysis

The sensory analysis was performed by 10 panelists from the Department of Food Chemistry and Instrumental Analysis, Poznań University of Life Sciences. All members of the panel were trained according to ISO 8586:2012 standards, also all of them had a minimum of 3 years' experience in sensory analysis. Sensory analysis, as procedure involving human participants was performed in accordance with ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments of comparable ethical standards.

Three varieties of broccoli, 3 varieties of cauliflower, 5 varieties of Brussels sprouts and 4 varieties of kohlrabi were assessed by the sensory panel to point out the general desirability, evaluate the bitter and sweet taste and quantify the specific odour notes in selected varieties. The aroma analyses were performed using 10 aroma descriptors which were: "raw" broccoli, cauliflower, kohlrabi, Brussels sprouts respectively, "green", "sulfur", "seaweed", "plant aroma", "putrid", "mushroom-like", "earthy". Attributes were selected by a panel during preliminary sessions with raw vegetables. About 50 g of raw or cooked vegetable was placed in polystyrene cup closed with plastic cover. Every panelist collected all 3, 4 or 5 varieties of one vegetable and started the assessment procedure using ANALSENS 5000E software (CARET Sp. z o.o., Poznan, Poland). Sensory analysis for each vegetable was performed in separate days, such as one vegetable per day, and session of each vegetable was repeated 3 times. The panelists quantified the intensity of each descriptor using a scale anchored from zero (no perception) to 10 (very strong intensity). For examination of investigated metabolites influence on selected sensory traits of examined vegetables these related to taste and desirability were selected.

2.7. Statistical analysis

All analytical measurements were performed in triplicate for all individual cultivars of each vegetable. In each experiment, the replicates of each group were summarized as the mean value of three replicates and standard deviation was calculated. All statistical analyses were performed in the R environment, and using the SIMCA 14.0 software (Umetrics, Umea, Sweden).

3. Results

3.1. Profiles of major metabolites in Brassica vegetables

Chemical compounds from the following groups were analyzed: glucosinolates, phenolic compounds, sugars and isothiocyanates, as these, according to literature, have the most pronounced influence on taste. The composition of isothiocyanates in the analyzed vegetables was earlier described (Wieczorek & Jeleń, 2019) and those results were used for statistical analysis.

A typical chromatogram of glucosinolates determined without desulphatation step is shown on Fig. 1. The high quality of the chromatogram, even though it was achieved without time consuming desulphatation step demonstrates usefulness of the proposed method. Omitting the desulphatation step not only simplifies the analytical procedure, saves time required for desulphathase and sample preparation but allows reliable quantitation in short chromatographic runs. Detailed information of the concentration of individual glucosinolates and their concentrations in 3 varieties of broccoli, 5 varieties of Brussels sprouts, 3 varieties of cauliflower and 3 varieties of kohlrabi in their raw and cooked form has been provided in Supplementary files: Fig. S1 and Table S1. The profile and concentration of glucosinolates varied depending on the type of vegetables and also cultivar type. Brussel sprouts was characterized with the highest concentration of glucosinolates among examined vegetables. In Brussel sprouts the most abundant glucosinolate was sinigrin. Depending on variety its concentration in fresh vegetables ranged from 0.3 to 1.7 g/kg fw. Brussel sprouts varieties were also characterized by high concentrations of glucobrassicin 0.6–1.1 g/kg fw depending on a variety). Obtained results were in accordance with the data reported by Heaney et al. (1980), authors investigated 22 varieties of Brussel sprouts and similarly to obtained herein results the content between cultivars differed significantly. Nevertheless, the amount of sinigrin and glucobrassicin was significantly higher that other GLS (Heaney & Fenwick, 1980). In all broccoli accessions, the major glucosinolate was glucoraphanin, present in a concentration range of 0.1–0.18 g/kg fw in raw cultivars. This vegetable was the richest source of the mentioned glucosinolate. Glucoraphanin and glucobrassicin accounted for >90% of all glucosinolates in broccoli. Results presented by Li et al. (2021), who investigated 80 different genotypes of broccoli in order to determine the glucosinolates concentration showed similar results in the majority of broccoli cultivars glucoraphanin was most abundant one, followed by glucobrassicin. Authors highlighted, that the amount of analyzed glucosinolates depends mostly on the genotype, as well as climate or cultivation conditions. In this work, the varieties analyzed were harvested in the same field and during the same season, so the variation between them is only an effect of genotype. Profile of glucosinolates in cauliflower was dominated by glucobrassicin, followed by sinigrin. Again results obtained were in accordance with previously reported data (Engel, Baty, le Corre, Souchon, & Martin, 2002), the major glucosinolates were also sinigrin and glucobrassicin, however significant variation was observed between 11 cultivars investigated in the study. In analyzed kohlrabi varieties glucoerucin was the most abundant glucosinolate, followed by glucobrassicin. Previously published data about kohlrabi which aimed to performed the profiling of secondary metabolites in this vegetable both pale green and purple demonstrated slightly different findings. Glucotropeaolin was predominant glucosinolate detected in pale green kohlrabi flesh, while in purple kohlrabi flesh the main GLS was glucoerucin (Park et al., 2017). The profile and differences in glucosinolates composition between the analyzed vegetables has been presented in Fig. 2.

Sugar content in examined cultivars has been presented in Fig. S2 and Table S2. Sugars are important metabolites involved not only in developments of sweetness of fruit and vegetables, but also involved in

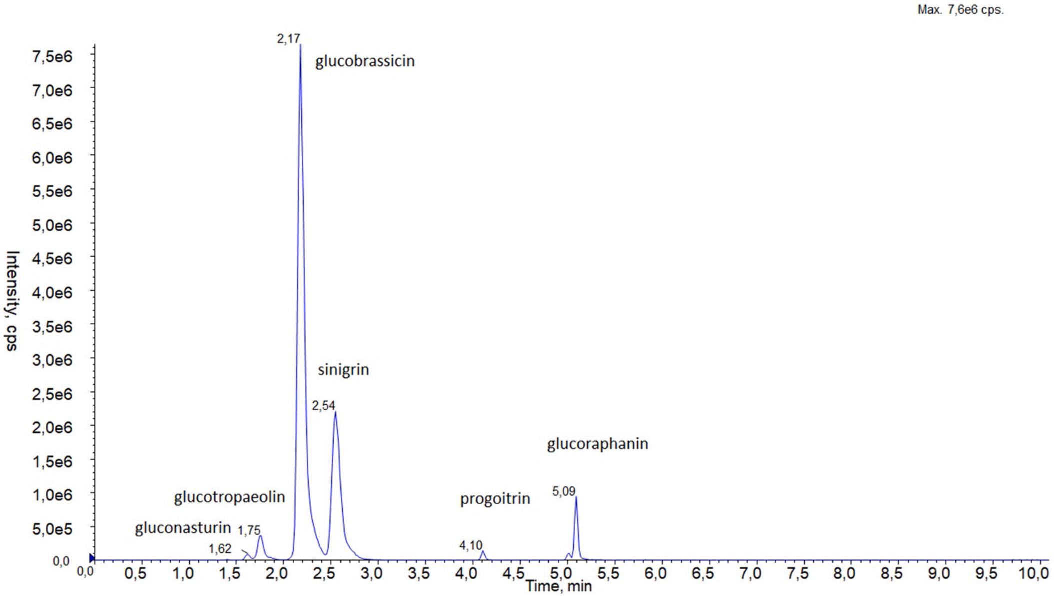


Fig. 1. LC-MS/MS total ion current (TIC) chromatogram from the analysis of glucosinolates in Brussels sprouts var. Maximus.

masking bitterness. The intensity of sweetness sensation differs between various sugars. For example, fructose and sucrose exhibit compression of sweetness with growing concentration, while glucose, lactose, maltose and trehalose exhibit expansion of sweetness with increasing concentration. To calculate the correlation between sugar concentration and general desirability, the quantities of particular sugars were converted according to relative sweetness values (Clemens et al., 2016). The results in the tables presented in the supplementary material are original values. The comparison between the four analyzed vegetables revealed that the level of total sugars in Brussels sprouts is almost two times higher than in broccoli and cauliflower, and slightly higher in comparison to kohlrabi. Broccoli, cauliflower, and kohlrabi had a similar composition profiles of sugars. Glucose and fructose were dominant. Small amount of sucrose and trace amounts of other sugars were present. Kohlrabi contained a noticeably higher concentration of sugars than broccoli or cauliflower. The profile of sugars in broccoli and kohlrabi were similar to outcomes obtained by other researchers (Park et al., 2017; Rosa, David, & Gomes, 2001). Brussels sprouts differed significantly from other analyzed plants, in all varieties, the main sugar was sucrose, and smaller amounts of fructose, glucose, and raffinose occurred. This is the only vegetable in the current research, in which raffinose was detected.

Phenolic compounds revealed the presence of mainly phenolic acids and their glycosides. The analysis in the presented study was performed by non-targeted LC-HRMS approach in aim to compare investigated cultivars, followed by quantitation of main phenolic compounds detected. Detailed information on the phenolics in particular varieties have been shown in Table S3. In broccoli the number of phenolic compounds was the highest. Differentiation between analyzed varieties was significant, however, the most abundant phenolic in all varieties was 1-O-sinapoyl-beta-D-glucose which derives from beta-D-glucose and *trans*-sinapic acid. Its content was between 1.39 and 4.42 mg/100 g dw in the analyzed cultivars (Table S3, Fig. S3, supplementary material). 1-O-sinapoyl-beta-D-glucose was also the most abundant phenolic compound in Brussel sprouts. In all raw cauliflower varieties, most the frequently occurring phenolic was 4-hydroxybenzoate-O-glucoside which derives from benzoic acid. Charlotte cultivar contained the highest concentration, and it was 67 mg/kg dw, while the precursor 4-hydroxybenzoic acid was present in the range 0.12 mg/kg dw in Oviedo var. to 0.12 mg/kg dw in Liria var. As in broccoli, a large amount of 1-*O*-sinapoyl-beta-D-glucoside, as well as derivatives of cinnamic acid (p-coumaric acid, ferulic acid, and sinapic acid) were present (Table S2., Fig. S2). Kohlrabi, in comparison to other analyzed vegetables, was a relatively poor source of phenolics. The most abundant in all the 4 varieties was 4-hydroxybenzoate-O-glucoside, while its precursor, 4-hydroxybenzoic acid was not detectable.

To draw some conclusions on the similarity of examined vegetables based on investigated and discussed metabolites a PCA analysis was performed. Based on the PCA diagram (Fig. 3), the profile of analyzed chemicals was similar in broccoli and cauliflower, and these two vegetables formed an undistinguishable cluster in PCA indicating similarities in discussed compounds profiles. Moreover, when raw and cooked broccoli and cauliflower are compared their profiles based on PCA are also very similar. On the contrary profiles of kohlrabi and Brussel sprouts were different from cauliflower and broccoli forming separate clusters. One can also observe differences in phytochemicals monitored caused by cultivars, especially in case of Brussel sprouts. For this vegetable the profiles of raw and cooked vegetables were also quite similar with the exception of one cultivar (raw) located in a distance from remaining ones. For kohlrabi PCA clearly indicates the different profile from remaining vegetables and also differences between raw and cooked vegetables.

3.2. Impact of thermal processing on the phytochemical composition

Thermal processing of vegetables generally enhances some nutritional values, texture and acceptability of food product, as well as decreases concentration of some anti-nutritional components (Cartea, Francisco, Soengas, & Velasco, 2011). Changes induced by heat treatment whether beneficial or not, depend on the type of vegetable, as well as preparation factors, such as cooking time or cooking method. Changes in selected metabolites (as an average of investigated cultivars) in a result of cooking has been shown on Fig. 4.

Thermal inactivation of myrosinase, the enzyme responsible for the hydrolysis of glucosinolates does not stop their breakdown. Glucosinolates might be degraded by different factors, such as strong acids, bases or different types of salts (Hanschen, Lamy, Schreiner, & Rohn, 2014). Heat treatment affects the concentration of glucosinolates because of their leaching into the heating medium, a decrease of myrosinase activity and thermal breakdown. In the presented research, as in many published before, a decrease in the content of glucosinolates after heat treatment was observed, however with few exceptions for selected

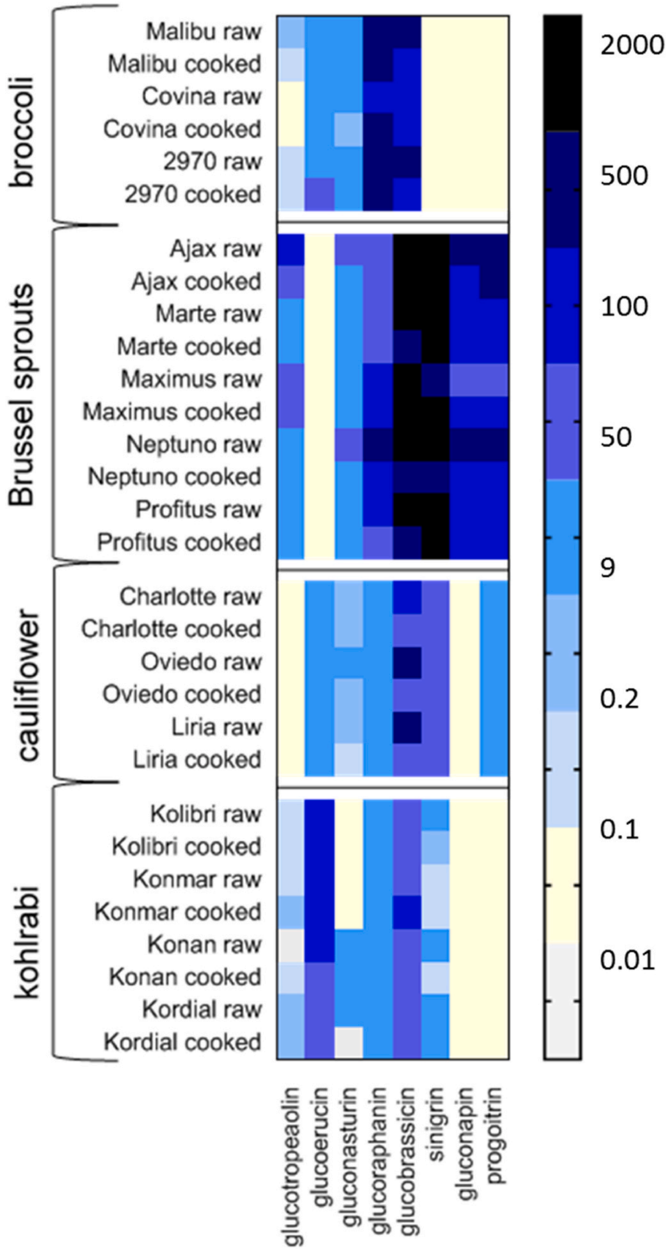


Fig. 2. Heatmap of the concentration of glucosinolates [mg/kg fresh weight] in broccoli, Brussels sprouts, cauliflower and kohlrabi varieties. The color key represents differences in the concentration of those components. Based on Tab S1 (A,B,C,D) – supplementary materials.

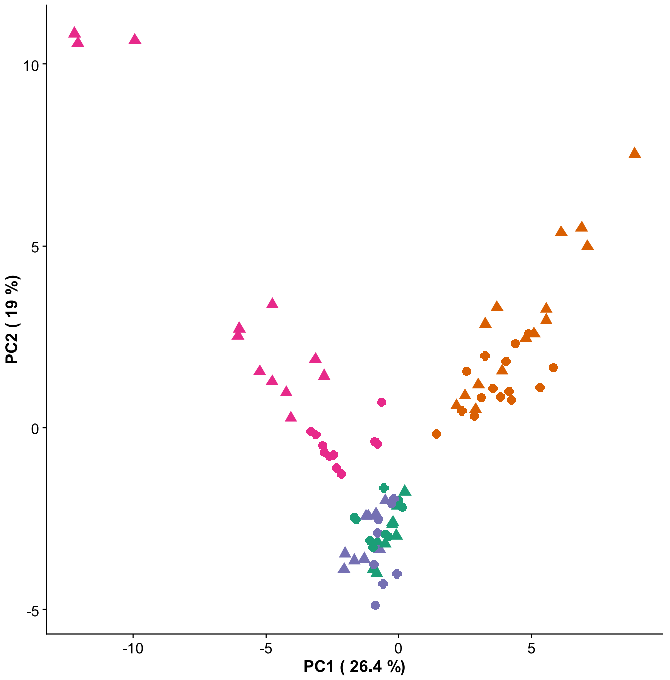


Fig. 3. Principal component analysis (PCA) graphs of phytochemicals extracted from raw and cooked varieties of broccoli, Brussels sprouts, cauliflower and kohlrabi: ▲ - broccoli raw; - ●broccoli cooked; ▲ - raw Brussel sprouts; ● - cooked Brussel sprouts; ▲ - raw cauliflower; ● - cooked cauliflower; ▲ - raw kohlrabi; ●- cooked kohlrabi.

cultivars (Fig. S1, Table S1). The greatest differences between raw and cooked vegetables were noticed in cauliflower varieties, where several-fold decrement in glucobrassicin concentration was detected. Interestingly, the level of this glucosinolate in broccoli and kohlrabi did not change after cooking. As presented in Fig. 4, the decrement of sinigrin concentration in the investigated vegetables was not significant. Ali (2015) also reported that heat treatment had a negative effect on nutrient composition in cauliflower and caused strong losses of protein, minerals, and phytochemical content. According to Kapusta-Duch et al (2016) boiling broccoli and cauliflower resulted in a significant reduction in the concentration of glucosinolates. Also, parameters like cooking time or size of cut vegetables influence the content of glucosinolates in boiled vegetables (Martínez, Armesto, Gómez-Limia, & Carballo, 2020). Results concerning isothiocyanates and the influence of thermal treatment for their concentration in subjected cultivars of broccoli, Brussels sprouts, cauliflower and kohlrabi were earlier described by our group (Wieczorek & Jeleń, 2019).

The stability of phenolic compounds depends on plant matrix, heating conditions and time of thermal processing (Martínez et al., 2020). The boiling process also significantly influences phenolics by weakening the cell wall matrix and leads to the release of bound phenolics (Ilyasoğlu & Burnaz, 2015; Mazzeo et al., 2011). It also causes a variety of chemical reactions resulting in the changes in content and structure of phenolic compounds. On the other hand, the increase in phenolic concentration might be due to better availability for extraction which is caused by the softening of vegetable matrix and release of phenolic compounds due to thermal inactivation of the polyphenol oxidase enzyme (Martínez et al., 2020). The influence of cooking on phenolic content in the analyzed plants was ambiguous – for cauliflower and kohlrabi in all varieties cooking resulted in decrease of phenolics, whereas for broccoli and Brussel sprouts there was a decrease or increase depending on a variety. A few tendencies were noticed: the amount of 3-hydroxybenzoate-*o*-glucoside decreased in all vegetables after boiling, while hydroxybenzoic acid increased in most cultivars. The concentration of chlorogenic acid, sinapic acid, *p*-coumaric acid in most of the samples was higher in boiled vegetables. Previous studies, in majority, reported a significant decrease in the total phenolic content after boiling (Florkiewicz, Socha, Filipiak-Florkiewicz, & Topolska, 2019; Murador, Mercadante, & De Rosso, 2016; Şengül, Yildiz, & Kavaz, 2014). However, there were also reports on an increase in total phenolic content after heat treatment (Martínez et al., 2020). Tables and graph with the concentration of phenolics in raw and cooked vegetables has been presented in Fig. S3 and Table S3.

Boiling caused the general decrease of concentration of sugars; however, the qualitative profile remained unchanged (Fig. S2 – supplementary material). The most significant differences were observed in broccoli and cauliflower followed by kohlrabi, whereas in some Brussels sprouts varieties sugar content after cooking remained virtually unchanged. This might result from the structure of Brussels sprouts and difficulties in the passage of sugars from the inner part of the vegetable

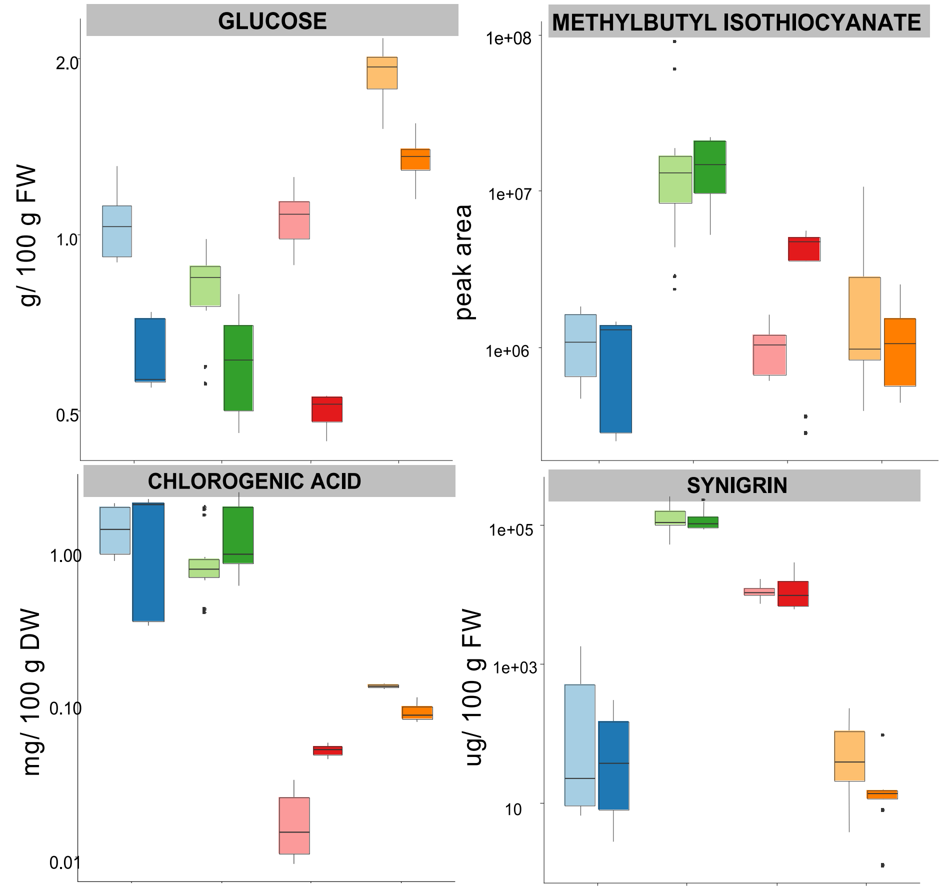


Fig. 4. The boxplots of 4 representative components from the analyzed phytochemical groups: sugars (glucose); isothiocyanates (2-methylbutyl isothiocyanates); phenolics (chlorogenic acid); glucosinolates (sinigrin) and changes in their concentration after boiling presented in the supplementary material. 🟦 – broccoli raw; 🟦- broccoli cooked; 🟩 - Brussel sprouts raw; 🟩- Brussel sprouts cooked; 🟥- cauliflower raw; 🟥- cauliflower cooked; 🟧- kohlrabi raw; 🟧- kohlrabi cooked.

into the water. Cooking was done on frozen vegetables and one must remember that this process can influence the release of water soluble compounds from the matrix (Bureau et al., 2015; Delchier, Reich, & Renard, 2012). Nevertheless in this study all vegetables were treated in the same way, since the major point of the study was the comparison of their composition, freezing factor was not further investigated.

3.3. *The influence of phytochemical composition and sensory attributes on consumers' preferences*

Sensory profile evaluation of particular vegetables revealed differences between raw ones, exhibited in number of flavor attributes (ranging from 6 to 12 depending on a vegetable), as well as their intensities. Differences were also noted between raw and cooked vegetables in number of flavor attributes; for cooked ones the flavor had generally more notes compared to raw ones. Significant differences between cultivars were noticed - the highest observed for raw broccoli (Fig. S4). In the case of Brussels sprouts, Maximus variety was best rated among the analyzed cultivars in a general desirability test. Raw Maximus Brussels sprouts were characterized by the typical aroma of raw Brussels sprouts, while the cooked vegetable was the sweetest one in comparison with the other 4 cultivars – Ajax, Marte, Neptuno and Profitus. The least desirable variety Ajax, described also as the most bitter one (Fig. S4) was characterized by the highest progoitrin content (see Table S1), which is bitter itself and additionally its hydrolysis product – goitrin was described in literature as "extremally bitter". This variety contained also the highest concentration of sinigrin and glucobrassicin, both described as "bitter" (Bell, Oloyede, Lignou, Wagstaff, & Methven, 2018). The intensity of sweetness in this cultivar was also evaluated as the lowest, comparing with others, what was associated with the lowest sugar content (Fig. S2).

Three varieties of broccoli (both raw and cooked) were assessed by our sensory panel. Covina var. achieved the highest score in general desirability evaluation of raw and boiled vegetables, it was also described as the sweetest variety. The amount of sugars in this cultivar was not significantly higher, than in other varieties. Additionally, the glucosinolates level was only slightly lower than in other broccoli cultivars. The dominant glucosinolates in broccoli's florets was glucoraphanin The bias was observed in cauliflower evaluation, where in the raw plant the highest value was noted for Charlotte var., however, a minimally smaller rate was observed for Oviedo var., which was described as the sweetest one. In the evaluation of boiled cauliflower, the Oviedo got the highest rate. It was the variety with the highest content of sugars and lowest content of glucosinolates. Also, the sulfur odour was not significant in this variety (Fig. S4) The highest-rated kohlrabi variety was Konmar, which was also the sweetest one, however the sugar content in this cultivar was not noticeable higher than in other varieties, and it makes it difficult to point out components responsible for its flavor.

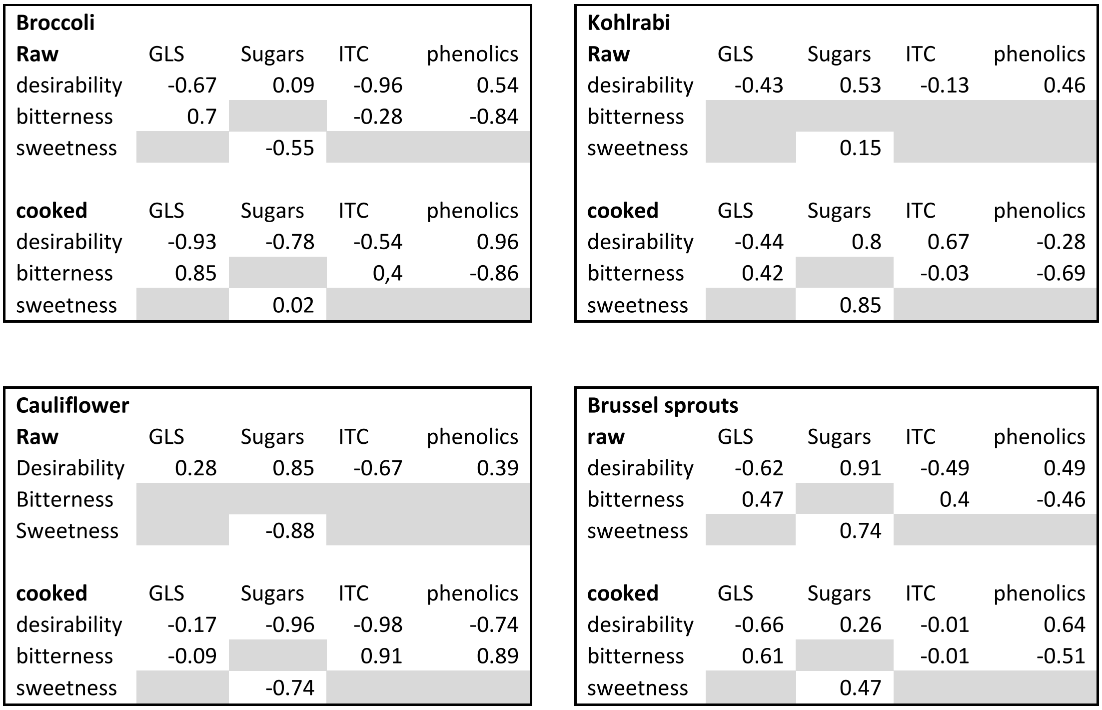
The results from the sensory profile analysis (values of particular traits) were correlated with metabolites (amounts) and presented in Fig. 5. The first group of interest were glucosinolates and their effect on taste. The taste threshold for sinigrin has been reported as 106 mg/L and for goitrin 12 mg/L. Values for other glucosinolates and their degradation products have not been discussed in the literature. It is probably due to the complicated cleanup procedure which is necessary to obtain appropriate amounts and purity for a sensory evaluation. Some glucosinolates, like glucoraphanin - the most abundant glucosinolate in broccoli - have shown a lack of any association with the bitter taste in Brassica vegetables (Traka & Mithen, 2009). The same situation occurred with glucoraphanin degradation product – sulforaphane – which despite the high concentration of its precursor was not visible on the chromatogram from sensitive GC × GC analysis (Wieczorek & Jeleń, 2019). It should be highlighted that glucoraphanin is a compound of special interest because of its widely proven health benefits and its lack of dependence between its concentration and bitterness level, as it was confirmed by earlier research (Baik et al., 2003; Bell, Methven, Signore, Oruna-Concha, & Wagstaff, 2017).

Significant correlation between the concentration of glucosinolates and consumer desirability was observed for broccoli and Brussels sprouts (Fig. 5). Since Brussels sprouts is a vegetable with very high GLS concentration, their influence on bitterness seems to be obvious. It is noticeable that the variety which was least desirable by the sensory panel – Ajax – contained the highest amount of glucosinolates and the most desirable one – Maximus – contained the smallest concentration of glucosinolates, comparing with other cultivars. For broccoli the overall positive correlation was noted between amounts of glucosinolates and bitterness sensation and negative for glucosinolates and desirability for both raw and cooked, however for cooked broccoli the relations were stronger. Interestingly, the amount of glucosinolates in broccoli is significantly lower than for Brussel sprouts (Table S1). In vegetables with the lowest amounts of glucosinolates, which was cauliflower and kohlrabi, no correlation with bitterness was observed. The concentration of sinigrin in cauliflower was lower than 0.05 g/kg fresh weight, as mentioned before, the taste threshold for sinigrin is ~0.1 g/L, there is a small probability that it influenced the taste of cauliflower accessions. In both vegetables - raw cauliflower and raw kohlrabi, no bitterness was detected by the sensory panel. In their cooked forms, minimal level of bitterness was detected.

The correlation between the concentration of isothiocyanates and sensory parameters was also investigated. Isothiocyanates were mostly correlated with pungent taste/aroma (Bell et al., 2018), however, during a preliminary session with our panelists, no pungent taste was observed in the analyzed vegetables. Isothiocyanates were not determined quantitatively, their effect on taste was determined based on their peak areas. As presented in Fig. 5, a high negative correlation was observed in broccoli and cauliflower between the content of isothiocyanates and general desirability. The role of isothiocyanates in flavor creation is dual, they are volatile substances influencing aroma and at the same time, the thiocyanate group present in isothiocyanates interacts with TRPV1 receptor, what indicates their taste-active character (Wieczorek et al., 2018). It could also suggest that they might contribute to the taste more than glucosinolates whose taste threshold is relatively high.

Phenolic compounds are responsible for the bitterness of many foods and beverages. Some studies linked several phenolic components to bitterness (Drewnowski & Gomez-Carneros, 2000). Unlike in the research conducted by Zabaras et al. (Zabaras et al., 2013), according to our results, no positive link between phenolic abundance and bitterness was found, except for cooked cauliflower.

Sugars present in the analyzed Brassica vegetables accession might show a dual impact on taste, on one hand, they enhance the sweet sensation and on the other hand, they can mask the bitterness of other components. Several studies indicated that an increase in the amount of sugars causes the reduction in the intensity of the bitter taste (Beck, Jensen, Bjoern, & Kidmose, 2014; Ley, 2008). According to PCA data projection, sweetness rate shown on Fig. 6 played an important role in general desirability. However, the correlation between sweetness and concentration of sugars varied among examined vegetables: high correlation was observed for cooked kohlrabi (which was also associated with high desirability) and raw Brussel sprouts and cauliflower, whereas negative correlations were noted for cooked cauliflower. Some interesting observation about sugar concentration and sweetness perception was made: Brussels sprouts was the richest source of sugars among the analyzed vegetables; however, the perception rate of sweetness was



Broccoli

Raw	GLS	Sugars	ITC	phenolics
desirability	-0.67	0.09	-0.96	0.54
bitterness	0.7		-0.28	-0.84
sweetness		-0.55		

cooked	GLS	Sugars	ITC	phenolics
desirability	-0.93	-0.78	-0.54	0.96
bitterness	0.85		0,4	-0.86
sweetness		0.02		

Kohlrabi

Raw	GLS	Sugars	ITC	phenolics
desirability	-0.43	0.53	-0.13	0.46
bitterness				
sweetness		0.15		

cooked	GLS	Sugars	ITC	phenolics
desirability	-0.44	0.8	0.67	-0.28
bitterness	0.42		-0.03	-0.69
sweetness		0.85		

Cauliflower

Raw	GLS	Sugars	ITC	phenolics
Desirability	0.28	0.85	-0.67	0.39
Bitterness				
Sweetness		-0.88		

cooked	GLS	Sugars	ITC	phenolics
desirability	-0.17	-0.96	-0.98	-0.74
bitterness	-0.09		0.91	0.89
sweetness		-0.74		

Brussel sprouts

raw	GLS	Sugars	ITC	phenolics
desirability	-0.62	0.91	-0.49	0.49
bitterness	0.47		0.4	-0.46
sweetness		0.74		

cooked	GLS	Sugars	ITC	phenolics
desirability	-0.66	0.26	-0.01	0.64
bitterness	0.61		-0.01	-0.51
sweetness		0.47		

Fig. 5. Correlation values between main phytochemical content and sensory attributes.

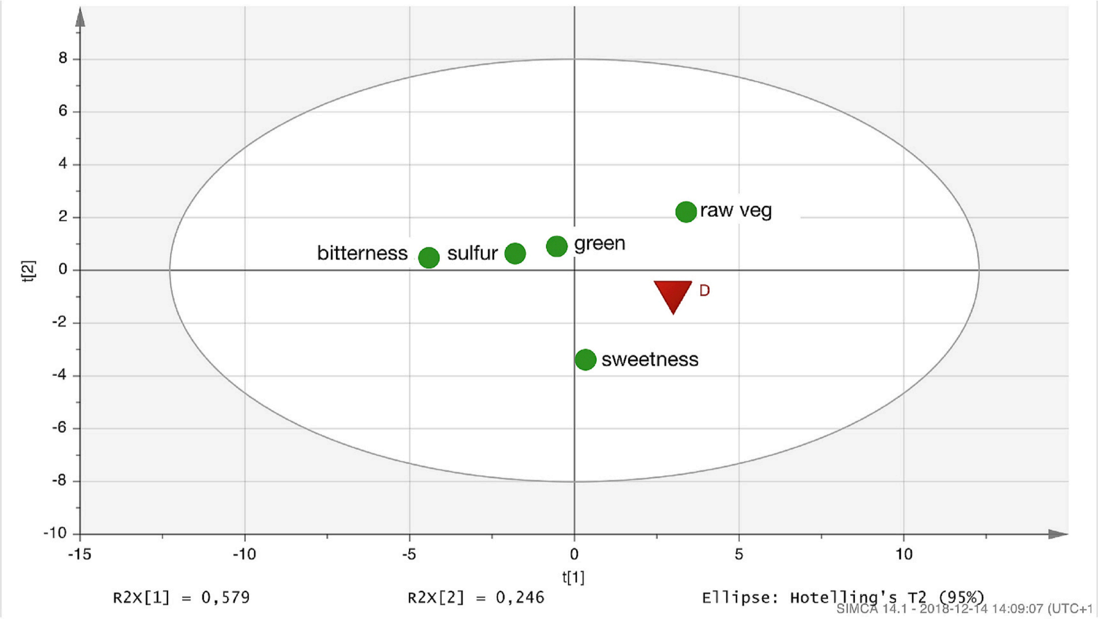


Fig. 6. Principal component analysis (PCA) of sensory descriptive of broccoli, Brussels sprouts, cauliflower and kohlrabi varieties (both raw and cooked). Created based on sensory analysis results. D – general desirability.

definitely lower than in kohlrabi, which contained a lower amount of sugars. Sucrose present in majority in Brussels sprouts was showing lower sweetness intensity than fructose which occurred in other vegetables (Fig. S3 – supplementary material). Glucose was present at a concentration similar to fructose in broccoli, cauliflower and kohlrabi which exhibit definitely lower sweetness intensity than sucrose or fructose (Clemens et al., 2016). However, sugars in Brussel sprouts and cauliflower were highly correlated with desirability for raw vegetables, but not for cooked ones. The sugar-to-glucosinolates ratio was also calculated and used for the correlation matrix, however, no significant correlation was observed and results were not included in the present study.

Few studies focusing on the association of glucosinolates with bitterness were published before. In Brussels sprouts, high sinigrin and progoitrin concentrations have been associated with consumer rejection and poor taste (Van Doorn et al., 1998). In turnip positive correlation between glucosinolates concentration and bitterness was observed (Nor et al., 2020). The presented results confirm thesis about the participation of glucosinolates in flavor formation in vegetables containing their high concentration. Brussels sprouts are a good example compared to other analyzed vegetables.

4. Conclusion

In this study a detailed analysis of phytochemical composition in conjunction with sensory evaluation of selected Brassica vegetables was presented. The relation between glucosinolates concentration and negative perception was proven in Brussel sprouts, in other investigated vegetables (broccoli, cauliflower, kohlrabi) no strong links were found. Sweetness was determined as another crucial factor responsible for the perception of vegetables, this sensory trait was correlated with sugars concentration in the majority of cultivars under study. Additionally, no relation between phenolic compounds and taste was observed. Sweetness and bitterness seemed to be the most important taste traits, which determined the desirability of the analyzed Brassica representative. Meaningful variation in metabolomics profiles was observed between different cultivars of one vegetable, which indicated that genetic manipulation might help to deliver products desired by consumers. However, similarities between broccoli and cauliflower in metabolomics profiles were shown, in contrast to different profiles characteristic for Brussel sprouts and kohlrabi.

Studies concerning the determination of different phytochemicals and their correlation with the results of a sensory analysis are difficult because of a poor ability of human to assess mixtures of flavor compounds, the nonlinearity of human senses response to stimuli and also multimodal action of some of the flavor compounds that can trigger both taste and aroma sensation, as in isothiocyanates. As presented, the taste of analyzed vegetables is an extremely complex issue hard to explain based on classic “correlation” approach. Moreover, correlations between different traits are not synonymous with causation. As presented, the amounts of taste-active substances differ significantly between vegetables. The concentration of taste-active substances is a crucial factor in the evoked sensory impressions. Furthermore, any generalization about glucosinolates responsible for bitterness is not correct because according to available literature, taste thresholds vary markedly among different substances, even belonging to the same chemical group. The currently poor state of knowledge concerning taste-threshold of the analyzed components is crucial for further considerations regarding taste-active components in Brassica vegetables. Interactions between phytochemicals and potential synergistic or masking effects are another obstacles in this type of research. To fully understand the role of glucosinolates and isothiocyanates in flavor creation, further research on their odour/taste thresholds is necessary along with a sensomic approach to decipher a role of particular compounds in overall sensory impression and desirability.

CRediT authorship contribution statement

Martyna N. Wieczorek: Conceptualization, Methodology, &, Investigation, glucosinolates, Sensory analysis, Isothiocyanates, Writing – original draft, Writing – review & editing. **Andreas Dunkel:** Methodology, Investigation. **Artur Szwengiel:** Methodology, Investigation, phenolics. **Katarzyna Czaczyk:** Methodology, Investigation, sugars. **Agnieszka Drożdżyńska:** Methodology, &, Investigation, glucosinolates, sugars. **Renata Zawirska - Wojtasiak:** Methodology, Investigation, Sensory analysis. **Henryk H. Jeleń:** Conceptualization, Methodology, Investigation, Isothiocyanates, Writing – review & editing, Formal analysis, Funding acquisition.

Declaration of competing

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.113028.

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