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The effect of development time on phytochemical characteristics of red mizuna (*Brassica rapa* L. var. *nipposinica*) sprouts

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Abstract. Germinating seeds constitute a natural source of substances that can be used to supplement food and increase its functionality. In this work, red mizuna (*Brassica rapa* L. var. *nipposinica*) in the form of sprouts was used as research materials. The main goal of this study was to investigate the effect of germination time on the phytochemical profile of health-promoting compounds in red mizuna sprouts. The metabolomic profile of water-methanol mizuna sprout extracts was analyzed by UPLC-ESI-MS/MS. In this work qualitative analysis of four mizuna sprout extracts collected after 4, 6, 8 and 11 days after sowing was carried out. Moreover, the phytochemical characterization of red mizuna seeds before the sprouting process was undertaken. Thirty-nine compounds were interpreted and the fact of the variability of the qualitative profile over time was confirmed. This is the first report to provide information on the differences in the phytochemical profile between sprouts of red mizuna subjected to the influence of germination time, in order to determine the harvesting and consumption maturity of the tested plant material.

Keywords: functional foods, cruciferous sprouts, red mizuna sprouts, germination time, phytochemical profile, UHPLC-MS/MS analysis.

INTRODUCTION

The highest consumption of cruciferous vegetables can be found among the inhabitants of China, about 100 g per day. In Europe, these values are much lower, varying between 15 and 30 g per day, depending on the country. The countries of Eastern Europe, including Poland, are in the lead, where cabbage has been eaten the most. However, recently, a decreasing tendency in its consumption has been observed. From 2002 to 2004, it was about 8.8 kg per person per year (about 24 g per day), while in 2011 it was only 6.6 kg (about 18 g per day). The share of other cruciferous vegetables, in the diet of Polish people, is much smaller and shows a downward trend. Based on this data, it can be noticed that the consumption of cruciferous plants, in general, is decreasing, which is not a good phenomenon, due to their beneficial health-promoting properties (Cieślik et al., 2017).

Mizuna cabbage belongs to cruciferous plants and is currently not very common in Poland. Its red variety is consumed mainly in the form of sprouts. It is also known by other names, such as kyona, Japanese mustard or spider mustard. This rare plant is cultivated mainly in Japan, China and Korea. Mizuna forms dense leaf rosettes and the leaves are placed on long, thin petioles. The leaves are finely dissected, similar to those of rockets (Kalisz et al., 2012). In places where it is known and grown, mizuna is very popular in salads, soups and hot pot dishes (Akter et al., 2018). These young plants are a source of amino acids, fiber, minerals, vitamins, phenolic compounds, glucosinolates and other bioactive substances (Park et al., 2020).

Many studies have shown that seeds during germination, significant nutritional, biological and medicinal values, evidencing an increase in antioxidant, antidiabetic, anti-inflammatory and hypolipidemic activity (Toro et al., 2021). In addition to the culinary use of these plants, traditional medicine has explored the potential of the *Brassicaceae* family to prevent and treat a diverse group of acute or chronic diseases, particularly cancer. According to literature data, it is the metabolic products of glucosinolate that are responsible for the anticancer effect (Al Mijan et al., 2021; Melim et al., 2022; Rouzaud et al., 2004).

In the area of the increasing popularity of functional food, the presence of unprocessed food products in the diet, such as sprouts, seems to be extremely important. Generally, various methods of obtaining sprouts, known for a long time, are characterized by simplicity and do not require large financial outlays. These nutrient-dense food sources can be produced without using pesticides; hence, they have low environmental impacts and a broad acceptance among

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health-conscious consumers. A very important aspect, when supplementing with sprouts, is the fact that this product is eaten raw, without thermal processing, so there is almost no loss or degradation of heat-sensitive micronutrients or vitamins during food processing (Ebert, 2022). An additional advantage of sprouts is their year-round availability on the market, as opposed to fruit and vegetables that occur seasonally. Due to the processes during germination, they contain more easily digestible proteins, lipids and sugars (Krzepiłko et al., 2017). Additionally, this process activates hydrolytic enzymes and releases nutrients from their phytate chelates, making them bioavailable (Ebert, 2022). Moreover, germinated seeds are characterized by intensive metabolism, which can be modified to produce specific ingredients or active compounds (Lewicki, 2010).

It has been confirmed that the content of primary and secondary metabolites depends on different preharvest factors, including development stage, harvest time, postharvest handling and storage conditions. The manipulation of these parameters can be used to control the levels of desired compounds and improve the phytochemical quality of sprouts. It has been shown that it is possible to increase the concentration of active compounds in sprouts of this family, among others, in kale, radish and broccoli, by applying the aforementioned factors (Aloo et al., 2021; Ortega-Hernández et al., 2021). Such a stimulation process deserves special attention, because Cruciferae sprouts are a rich source of vitamins, mineral salts, antioxidants and other bioactive compounds. Despite so many reports about cruciferous plants and their metabolites (mainly broccoli and radish), a literature review revealed many thematic deficiencies in the studies of sprouts from this family. The main objective of this study was to interpret the secondary metabolites from red mizuna sprouts of different germination times by HR-UPLC-ESI-QTOF-MS. The analyses performed were used as a database for the structures elucidation of new molecules, based on the suggested molecular formula, structural information provided by MS/MS fragmentation, and the literature.

MATERIALS AND METHODS

Chemical reagents

Methanol and acetonitrile for LC-MS were purchased from Merck (Darmstadt, Germany). Methanol hyper grade was acquired from Fisher Chemical (Loughborough, UK). Formic acid, LC-MS grade, was purchased from Sigma-Aldrich, (St. Louis, MO, USA). Ultrapure water was obtained in-house with a purification system (Milli-Q-Simplicity-185, Millipore Corp.).

Cultivation of seeds for sprouts

The seeds for sprouts of red mizuna (*Brassica rapa* L. var. *nipposinica*) were purchased from a domestic seed company, Toraf, Maciejów 34, 46-211 Kujakowice Górne. Mizuna seeds germinated in the conditions of a potential consumer, i.e. in home cultivation. The seeds was soaked in MilliQ water for exactly 14 hours. After draining the water, the seeds were sown on sterile gauze (sterile gauze 1 m^2, Heltiso, 17-thread). Half a gram of seeds was sown on each of the sprout cultures. The plant material was harvested after 4 (96 h), 6 (144 h), 8 (192 h) and 11 (264 h) days after sowing. In addition, red mizuna seeds were also collected after 14 h of soaking in MilliQ water, before the visually visible germination process.

Preparation and extraction of plant material for HPLC-MS analysis

Fresh seeds and sprouts were frozen, lyophilized (Gamma 2-16 LSC lyophilizer, Christ), milled (analytical mill, IKA A100) and stored in a dry and dark place. Then prepared material was extracted using an automatic extractor Dionex ASE 200 Accelerated Solvent Extraction System. The extraction process conditions were as follows; extraction solvent 80% methanol, solvent pressure 1500 psi, extraction cell temperature 40 °C, extraction cycles 3. The seeds and sprouts extracts were evaporated to drops under reduced pressure at 40 °C (Heidolph Hei-Vap Advantage, rotary evaporator). The prepared extracts were kept at -20 °C. Immediately before the LC-MS/MS analysis, the samples were dissolved in 30% methanol. Then the extracts were sonicated for 10 min at 25 °C for better dissolution (sonicator SONOREX DIGITEC DT 510 H, Bandelin, Germany) and centrifuged for 10 min at 11,000 rpm (laboratory centrifuge Polygen Sigma 3-16 KL, Sigma, Germany). Finally, the supernatant from each sample was subjected to UHPLC-ESI-QTOF-MS for analysis.

Ultra-high-resolution mass spectrometry UHPLC-ESI-QTOF-MS conditions

The qualitative analysis of individual aqueous-methanol extracts was carried out by ultra-high resolution mass spectrometry (UHRMS) using a Dionex UltiMate 3000RS (Thermo Scientific, Darmstadt, Germany) system with a charged aerosol detector (CAD) interfaced with a high-resolution quadrupole time-of-flight mass spectrometer (HR/Q-TOF/MS, Impact II, Bruker Daltonik GmbH, Bremen, Germany). The chromatographic separation was performed on a CORTECS T3 column (150 × 2.1 mm, 2.7 µm, Waters, Manchester, UK). The column temperature was maintained at 40 °C. The mobile phases were acidified (0.1% formic acid) water (solvent A) and acidified (0.1% formic acid) acetonitrile (solvent B). The chromatographic method consisted of the following linear gradient: 5% B from 0 to 0.3 min, and the concentration of B was then increased to 90% from 0.3 to 26.5 min. The sample injection volume was 3.0 µL, and the flow rate was set at 500 µL/min. The mass scan range was set at 100–2000 m/z units. Ions source parameters were as follows; capillary voltage 4.0 kV, dry gas 6.0 L/min, dry temperature 200 °C. Elec-

trospray ionization (ESI) was performed in positive and negative ion modes. Data acquisition and processing were performed using Bruker Data Analysis version 4.4 SR1 (Bruker Daltonik GmbH, Bremen, Germany).

RESULTS

The information of all characterized metabolites in aqueous-methanolic extracts of seed and sprouts, by HPLC-ESI-Q-TOF-MS, is given in Table 1 and Figure 1. In this work, a total of 39 phytoconstituents were identified, which were interpreted based on several parameters, such as retention time, MS spectra, MS/MS fragmentation and literature data. The first significant group of compounds that were characterized among the analytes is glucosinolates (GLS). These secondary metabolites are a specific class of compounds with sulfur, occurring mainly in the plants of the Brassicaceae family (Pecio et al., 2023). Glucosinolates are interpreted in the negative ion mode. Data analysis showed the presence of 11 GLSs belonging to different groups, such as 5 aliphatic GLSs, 4 indolic GLSs, and 2 aromatic GLSs. All identified glucosinolates showed the characteristic fragment ions at m/z 274,9899 and m/z 259,0128 (Zhou et al., 2017). The two dominant compounds gave signals as a peak of **7** (m/z 358) and its isomer, a peak of **9**. They have been characterized as sinigrin or allyl glucosinolate at m/z 358 (Baenas et al., 2019) and they are the dominant compounds in all tested preparations. Importantly, the older the culture, the greater the signal of this analyte. In addition, three more compounds have been identified from the first-mentioned class of glucosinolates, such as gluconapin (peak **13**, m/z 372), glucoiberin (peak **14**, m/z 422) and glucoiberverin (peak **15**, m/z 406). Peaks **17**, **21**, **27**, and **33** are compounds from the indole group of GLSs, which have been marked as 4-hydroxyglucobrassicin (m/z 463), glucobrassicin (m/z 447), 4-metoxyglucobrassicin (m/z 477) and its isomeric form, respectively. Further, two aromatic GLSs were observed at peaks **22** and **25**. The first of these signals was interpreted as a glucolimnathin at m/z 438, while the second was determined as a gluconasturtiin at m/z 422 (Kusznierewicz et al., 2013; Baenas et al., 2012). These biologically active compounds are present in most of the analyzed samples. The exception is the seed extract, where peaks **21**, **22**, **25** and **33** were not detected.

Peaks **24**, **26**, **28**, **34**, **35**, **36**, and **37** give typical ions fragmentation in the negative ionization mode, m/z 223 and m/z 205. These analytes were identified as sinapic acid derivatives. The phytochemicals in this group were classified as mono- (peaks **24**, **26**, **28**), di- (peaks **34**, **35**, **36**), and trisinapoyl (peak **37**) conjugates. Depending on the analyte, they lose a fragment of 224 u (sinapic acid moiety), 162 u (hexose moiety), 132 u (glucose moiety), or 324 u (dihexose moiety) (Olszewska et al., 2020). In the first-mentioned class, three isomers of sinapoyl hexose at m/z 385 (peaks **24**, **26**, **28**) are distinguished. Among the disinapoyl derivatives, compounds with the sugar moiety of glucose (peaks **35**, **36**) and gentiobiose (peak **34**) were found. As for the presence of some mono- and di- conjugates in individual preparations, peaks **26**, **28** and **36** were not located in the seed extract. Compound **37**, belonging to the last group of sinapic acid derivatives, was interpreted as trisinapoyl gentiobiose at m/z 959, and its presence was noted in extracts from 6-, 8- and 11-day-old sprouts. Furthermore, peak **31** was identified in the positive mode by the pseudomolecular ion [M+H]+ at m/z 310 and its fragment at m/z 251 as an amine derivative of sinapic acid, sinapine (Mayengbam et al., 2014). Figure 1 shows sinapine as the main compound in the red chromatograms. It has been observed that the signal intensity of this analyte decreases significantly with the germination time. In addition to sinapic acid derivatives, other phenols have been identified, peaks of **11**, **18**, **19** and **23**. Analyte **11** was classified as free phenolic acid and was interpreted as p-coumaric acid. Three consecutive signals were assigned to phenolic acids glycosides and defined as dihydroxybenzoic acid hexose (peak **18**), vanilloyl hexose (peak **19**) and salicylic acid hexose (peak **23**) (Li et al., 2018). These compounds were identified in extracts from 4-, 6-, 8- and 11-day-old sprouts.

Two acylated flavonoids were also identified in the metabolic profile of red mizuna sprouts. Peak **29** and **30** were interpreted as kaempferol-3-O-sinapoyl-triglucoside-7-O-diglucoside at m/z 1301 and kaempferol-3-O-sinapoyl-triglucoside-7-O-glucoside at m/z 1139, respectively (Ferreres et al., 2009). Their synthesis begins during the growth of the plant, therefore these compounds are not present in seed extract. Additionally, one compound (peak **32**) from the group of anthocyanins, also acylated with sinapic acid was determined, cyanidin 3-O-(sinapoyl) diglucoside-5-O-glucoside at m/z 979 (Moreno et al., 2010), its presence was found in all tested samples.

Amino acids constituted the dominant part of the compounds identified in the extracts. These primary metabolites were interpreted with electrospray ionization in the positive ion mode. In total, 8 compounds have been identified, 7 of them are essential amino acids. Analytes with deprotonated [M+H]+ molecules at m/z values of 156.076, 175.119, 147.076, 118.032, 132.102, 166.086 and 205.096 were tentatively identified as histidine (peak **1**), arginine (peak **2**), lysine (peak **3**), valine hexoside (peak **8**), leucine or isoleucine (peak **12**), phenylalanine (peak **16**) and tryptophane (peak **20**), respectively. The last compound identified in this group was the non-essential amino acid, tyrosine (peak **10**) (Aloo et al., 2021).

Moreover, among the less significant compounds, two organic acids and one derivative of glucose were identified. The first analytes were interpreted as malic acid and gluconic acid, they were responsible for peaks **5** and **4**, respectively (Placines et al., 2020). Both phytoconstituents were presented in 8- and 11-day-old sprout cultures. Peak **6** was described as sucrose, this compound was presented

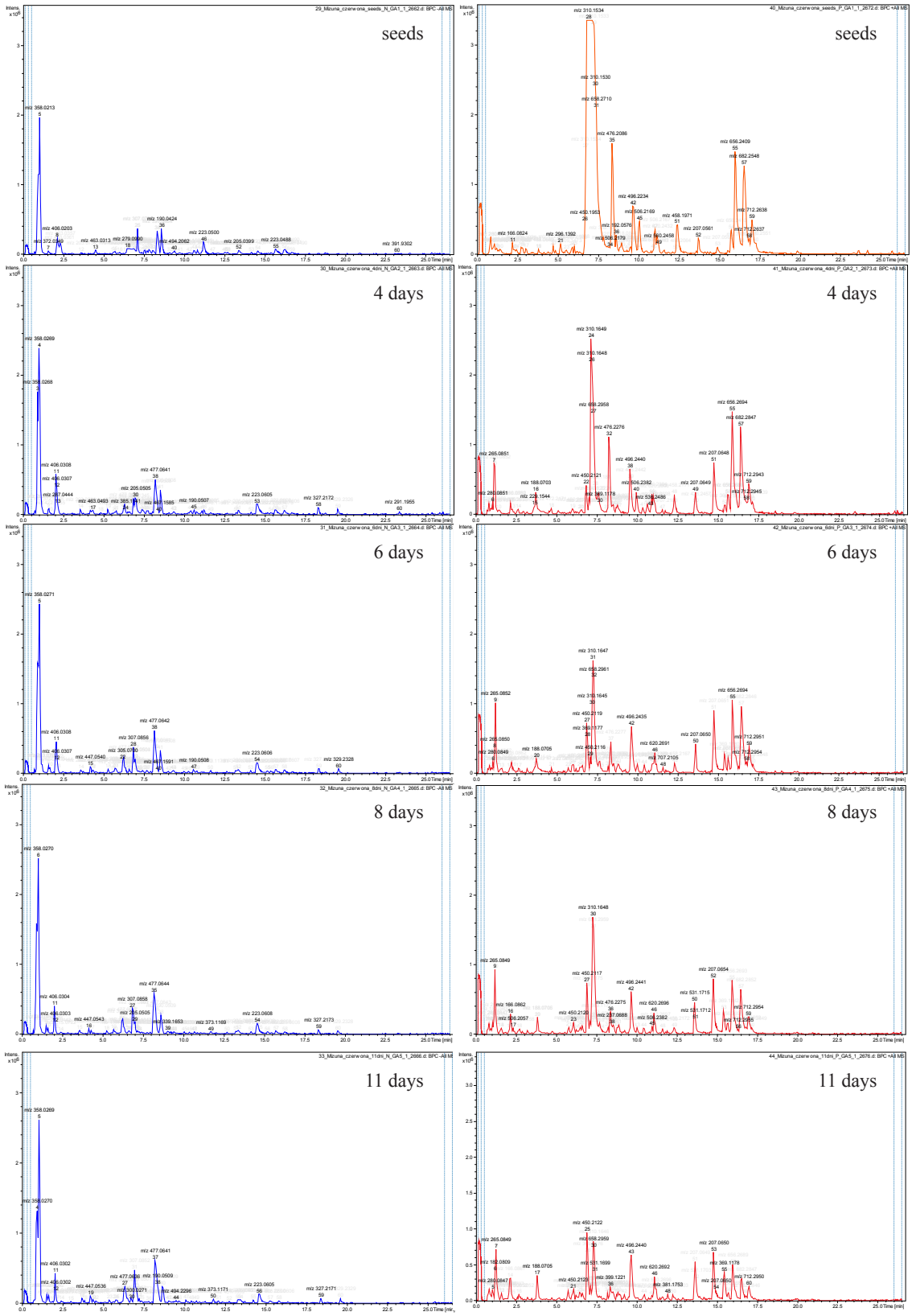

Figure 1. The base-peak chromatograms (BPC) of aqueous-methanolic extracts from red mizuna seeds and sprouts harvested 4, 6, 8 and 11 days after sowing, obtained using HR UHPLC-ESI-QTOF-MS in negative (blue chromatograms from top to bottom; seeds, 4, 6, 8 and 11 days after sowing) and positive (red chromatograms from top to bottom; seeds, 4, 6, 8 and 11 days after sowing) ionization modes.

Table 1. Phytochemical characteristics of the aqueous-methanolic extracts from red mizuna seeds and sprouts harvested after 4, 6, 8 and 11 days after sowing identified by UPLC-QTOF MS/MS.

No.	RT [min]	[M+H]+ m/z	[M-H]- m/z	Frag_source[ESIpos]	Frag_source[ESIneg]	Tentative identification	Ion Formula	Seeds	Days after sowing			
									4	6	8	11
1	0.76	156		156, 110		histidine	$C_6H_9N_3O_2$	-	+	+	-	-
2	0.79	175		175, 158, 130, 116		arginine	$C_6H_{14}N_4O_2$	+	+	+	+	+
3	0.82	147		147, 130, 120		lysine	$C_6H_{14}N_2O_2$	-	+	+	+	+
4	0.81		195		195, 179, 165	gluconic acid (maltonic acid)	$C_6H_{12}O_7$	-	-	-	+	+
5	0.85		133		133, 115	malic acid	$C_4H_6O_5$	-	-	-	+	+
6	1.02		341		387, 341, 195, 179	sucrose	$C_{12}H_{22}O_{11}$	+	-	-	-	-
7	1.03		358		358, 274, 259, 195	sinigrin (isomer I)	$C_{10}H_{17}NO_9S_2$	+	+	+	+	+
8	1.03	280		280, 118		val - hexose (valina)	$C_{11}H_{20}NO_7$	+	+	+	+	-
9	1.05		358		358, 274, 259, 195	sinigrin (isomer II)	$C_{10}H_{17}NO_9S_2$	-	-	-	+	+
10	1.2	182		182, 165, 147, 136		tyrosine	$C_9H_{11}NO_3$	-	+	+	+	-
11	1.36		163		163, 119	p-coumaric acid	$C_9H_8O_3$	-	+	+	+	+
12	1.37	132		132, 119		leucine/isoleucine	$C_6H_{13}NO_2$	+	+	+	-	-
13	1.59		372		372, 274, 259, 195	gluconapin	$C_{11}H_{19}NO_9S_2$	+	+	+	+	+
14	1.68		422		422, 342, 274, 259, 195	glucoiberin	$C_{11}H_{21}NO_{10}S_3$	+	+	+	+	+
15	2.06		406		406, 274, 259, 195	glucoiberverin	$C_{11}H_{20}NO_9S_3^-$	+	+	+	+	+
16	2.1	166		166, 146, 120		phenylalanine	$C_9H_{11}NO_2$	+	+	+	+	+
17	2.1		463		463, 285, 274, 267, 259	4-hydroxyglucobrassicin	$C_{16}H_{20}N_2O_{10}S_2$	+	+	+	+	+
18	2.44		315		315, 153	dihydroxybenzoic acid hexose	$C_{13}H_{16}O_9$	-	+	+	+	+
19	3.01		329		329, 167	vanilloyl hexose	$C_{14}H_{18}O_9$	-	-	+	+	+
20	3.84	205		205, 188		tryptophane	$C_{11}H_{12}N_2O_2$	+	+	+	+	+
21	4.15		447		447, 274, 259	glucobrassicin	$C_{16}H_{20}N_2O_9S_2$	-	+	+	+	+
22	4.31		438		438, 359, 274, 259, 195	glucolimnathin	$C_{15}H_{21}NO_{10}S_2$	-	+	+	+	+
23	4.54		299		299, 137	salicylic acid hexose	$C_{13}H_{16}O_8$	-	+	+	+	+
24	5.01		385		385, 247, 223, 205	sinapoyl hexose (isomer I)	$C_{17}H_{22}O_{10}$	+	+	+	+	+
25	5.27		422		422, 385, 274, 259, 195	gluconasturtiin	$C_{15}H_{21}NO_9S_2$	-	-	+	+	+
26	5.72		385		385, 247, 223, 205	sinapoyl hexose (isomer II)	$C_{17}H_{22}O_{10}$	-	+	+	+	+
27	6.27		477		477, 274, 259, 195	4-metoxyglucobrassicin (isomer I)	$C_{17}H_{22}N_2O_{10}S_2$	-	+	+	+	+
28	6.36		385		385, 247, 223, 205	sinapoyl hexose (isomer III)	$C_{17}H_{22}O_{10}$	-	+	+	+	+
29	7.17		1301		1301, 1139, 977, 933, 771, 650, 284, 205	kaempferol-3-O-sinapoyl-triglucoside-7-O-digluco-side	$C_{56}H_{70}O_{35}$	-	-	+	+	+
30	7.23		1139		1139, 977, 771, 446, 284	kaempferol-3-O-sinapoyl-triglucoside-7-O-glucoside	$C_{50}H_{60}O_{30}$	-	+	+	+	+

31	7.32	310	310, 251	sinapine	$C_{16}H_{24}NO_5^+$	+	+	+	+	+
32	7.82	979	979, 449, 287, 207	cyanidin 3-O-(sinapoyl) diglucoside-5-O-glucoside	$C_{51}H_{46}O_{20}$	+	+	+	+	+
33	8.19	477	477, 274, 259, 195	4-metoxyglucobrassicin (isomer II)	$C_{17}H_{22}N_2O_{10}S_2$	-	+	+	+	+
34	13.41	753	753, 529, 223, 205	disinapoyl-gentiobiose	$C_{34}H_{42}O_{19}$	+	+	+	+	+
35	14.59	591	591, 367, 223, 205	disinapoyl-glucose (isomer I)	$C_{28}H_{32}O_{14}$	+	+	+	+	+
36	14.73	591	591, 367, 223, 205	disinapoyl-glucose (isomer II)	$C_{28}H_{32}O_{14}$	-	+	+	+	+
37	15.22	959	959, 735, 529, 511, 223, 205	trisinapoyl-gentiobiose	$C_{45}H_{52}O_{23}$	-	-	+	+	+
38	18.38	327	327, 291, 229, 211	trihydroxy octadecadienoic acid	$C_{18}H_{32}O_5$	+	+	+	+	+
39	19.59	329	329, 229, 211	pinellic acid	$C_{18}H_{34}O_5$	+	+	+	+	+

+ present; - absent

RT – retention time; **m/z** – mass to charge ratio; **ESI pos** – electroionization in positive mode; **ESI neg** – electroionization in negative mode

only in the extract from mizuna seeds. In addition, a group of non-polar compounds, such as fatty acids, was identified in the phytochemical profile of mizuna seeds and sprouts. Among these constituents, two analytes were characterized; trihydroxy octadecadienoic acid (peak **38**) and pinellic acid (peak **39**).

DISCUSSION

The major, non-phenolic compounds reported in almost all the Brassicaceae family are glucosinolates (Aloo et al., 2021). These metabolites are synthesized from a small number of primary amino acids, including alanine, leucine, valine, methionine, tyrosine, phenylalanine and tryptophan (Sønderby et al., 2010). These compounds differ from one another in the structure of their aglycon moieties, generally classified as alkyl, aliphatic, alkenyl, hydroxyalkyl, aromatic or indole. It has been proven that myrosinase is present in all the cells of cruciferous plants. After cell damage (slicing, chewing), myrosinase generates a hydrolysis process that converts glucosinolates into compounds from the following groups; thiocyanates, isothiocyanates, nitriles, epithionitriles and oxazolidines, among others (Maina et al., 2020; Xie et al., 2022). Depending on environmental conditions (pH, presence and concentration of given ions, e.g. Fe^{2+}) and the type of glucosinolate, various active substances are formed. For example, in the case of sinigrin, a low pH generates the production of allyl cyanide, while at neutral and alkaline pH conditions, allyl isothiocyanate is the dominant breakdown product (Uda et al., 1986). Glucosinolate hydrolysis products significantly contribute to the characteristic smell and flavor of cruciferous plants, which is caused by the presence of sulfur in the molecule (Bennett et al., 2004).

Literature data show that biological activities, especially of glucosinolates in cruciferous sprouts, e.g. broccoli, have been very well described and studied. One of the important glucosinolates identified in broccoli is sulforaphane and it is present in its chemically stable form, called glucoraphanin. This phytocompound was first discovered in the 1950s, in red cabbage. The next step was to isolate sulforaphane from broccoli; done by Talalay and Zhang, when it was identified as a cancer-preventing agent (Al Mijan et al., 2021). Żuryń et al. (2016) studied the effect of this active compound on the cell cycle, apoptosis and expression of cyclin D1 and p21 in the A549 lung cancer cells line. The results showed that the leading cause of cancer cell death, induced by sulforaphane, was necrosis, which was most probably related to oxidative stress induction. Differential regulation of cell cycle proteins cyclin D1 and p21 was observed as a result of the therapy, which may be dependent on the applied concentration of sulforaphane. According to literature data, this is the first report documenting such an important issue in the participation of cyclin D1 in cell death, especially necrosis induction in A549 cells treated with sulforaphane. Broccoli sprouts extract indicated considerable antiproliferative activities towards line A549 (lung carcinoma cells), line HepG2 (hepatocellular carcinoma cells), and line Caco-2 (colorectal adenocarcinoma cells) using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, with IC50 values of 0.117, 0.168 and 0.189 mg/mL for 48 h, respectively (Le et al., 2019). Research from The Knight Cancer Institute at Oregon Health and Science University in Portland conducted clinical trials of broccoli sprout extract with sulforaphane, in a male prostate cancer case. Interestingly, extracts inhibited androgen receptor signaling in prostate cancer cells (Alumkal et al., 2015). In conclusion, the results of epidemiological studies conducted so far indicate that a diet rich in cruciferous plants may reduce the risk of the most commonly diagnosed cancers, such as breast, prostate, lung, stomach, or pancreatic cancer. Apart from extensive research on anti-cancer activity and chemopreventive activity, the sprouts of these plants also show other biological activities. Gawlik-Dziki et al. (2014) investigated the antioxidant activities of bread, with the addition of powdered broccoli sprouts. The results showed that bread enriched with powdered broccoli sprouts increased antioxidant activity. The high antibacterial activity of brassica sprout extracts was reported by Vale et al. (2015). Among the extracts from Galega kale, Pencea cabbage, Red cabbage and Broccoli, the latter two were the most effective against *Staphylococcus aureus*. A pilot study, led by Shiina et al. (2015), suggested that supplementation therapy of sulforaphane-rich broccoli sprout extract may have the potential to improve cognitive deficits in patients with schizophrenia. High levels of amyloid beta-protein fragments in plasma are associated with faster memory loss, which in many cases leads to Alzheimer's disease. Okada and Okada (2016) evaluated the neuroprotective effects of aqueous extracts from plant sprouts (including, broccoli sprouts) on the level of β-amyloid. Treatment with the extract decreased β-amyloid levels by 44.0%. According to literature data, research has also been conducted on the effect of supplementation with broccoli sprouts in neurodegenerative diseases, such as Parkinson's disease, multiple sclerosis, and ischemia (Schepici et al., 2020; Subedi et al., 2019).

A review of literature showed that among cruciferous plants, broccoli, radish, Brussels, kale, red cabbage, rutabaga, turnips, garden cress and white mustard were the most frequently studied for the presence of polyphenolic compounds (Baenas et al., 2012; Doniec et al., 2022; Ferreres et al., 2009; Pająk et al., 2014). Particularly noteworthy is the fact that sinapic acid and its derivatives are especially frequent in various brassica vegetables (Nićiforović et al., 2014). Vallejo et al. (2004) detected and quantified sinapic acid and its conjugates in fresh broccoli, among others; 2-disinapoylgentiobiose (25.5 μg/g), 1-sinapoyl-2-feruloylgentiobiose (32.7 μg/g), trisinapoylgentiobiose (40.8 μg/g), disinapoyl-2-feruloylgentiobiose (9.5 μg/g) and diferuloylgentiobiose (2.4 μg/g). In addition, sinapoyl-

acylated derivatives of qercetin-3-O-sophorotrioside-7-O-glucoside and kaempferol-3-O-sophorotrioside-7-O-glucoside were identified. Takaya et al. (2003) reported, that sinapic acid esters are the main components of the methanolic extract of the radish sprout (*Raphanus sativus* L.) and three of them were identified: methyl sinapate, 1,2-disinapoyl-β-D-glucopyranoside, and β-D-(3,4 disinapoyl) fructofuranosyl-α-D-(6-sinapoyl)-glucopyranoside. In the same year, other authors published that they had identified 8 sinapoyl-acylated flavonoid glycosides and 4 sinapoyl glycosides from common cauliflower by-products, consisting mainly of leaves (Llorach et al., 2003).

Another quite important group of phenolic compounds, present in Brassicaceae plants, is anthocyanins. These compounds are frequently present in highly glycosylated and acylated forms in Brassicaceae sprouts (Garcia-Ibañez et al., 2023). Among the 6 major anthocyanidins such as; cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin, present in Cruciferous vegetables, cyanidin is the most widespread (Park et al., 2020). In 2010, seventeen acylated anthocyanins were identified in sprouts of commercial broccoli ('Marathon' and 'Nubia'), a variety produced for edible sprouts ('Intersemillas' sprouts) and purple broccoli sprouts ('Viola') (Moreno et al., 2010). Recently, it has also been published that the two major anthocyanins identified in broccoli sprouts and red cabbage were cyanidin 3-(sinapoyl)(sinapoyl) diglucoside-5-glucoside and cyanidin 3-(sinapyl) diglucoside-5-glucoside, respectively (Zhang, Jing, 2022).

Other significant bioactive compounds present in germinating seeds are amino acids. These primary metabolites have been identified in many cruciferous sprouts. Aloo et al. performed UHPLC-Q-TOF-MS2 analyses of the 70% ethanol extracts of broccoli sprouts and determined 20 compounds, including 11 amino acids. Five of them were essential amino acids: lysine, histidine, leucine, phenylalanine and tryptophan (Aloo et al., 2021). These active compounds are very important for human health because they are not synthesized by the organism and must be supplied externally in the diet (Hill et al., 2022).

The conducted research confirms the validity of the thesis assuming the possibility of modeling the presence and content of a given metabolite in sprouts of red mizuna using the time of the growth. A good example is sinapine (peak 31), the older the sprout breeding, the lower the signal of this analyte, which may indicate a decrease in the content of this metabolite in the material. Literature sources confirm the fact, that during the development of the plant, most of the sinapine accumulated is present in the seeds. When it comes to glucosinolates, according to general public knowledge, their content in the seeds themselves is lower than in the later stages of germination. This is shown by the increased signal of glucosinolates, peak 15 (m/z 406) and 33 (m/z 477) on the chromatograms in negative ionization mode. However, this amount, at some point in the vegetative development of the plant, begins to decrease. According to the research conducted by Giorgetti, in kale, total polyphenols, flavonoids, glucosinolates, and total antioxidant activity were highest in seedlings at 10 days of cultivation (Giorgetti et al., 2018). This is due to the reduction in the content of amino acids (the richest source of these compounds are seeds), which are specific substrates and a source of nitrogen for the synthesis of glucosinolates in the cell. Based on current knowledge, it can be assumed that with further growth of mizuna sprouts, the content of these compounds would decrease. Literature data indicate that the most optimal range for harvesting high-glucosinolate sprouts is the period between 4 and 10 days of germination (Lee et al., 2023). In turn, Baenas et al. (2012) observed a decrease in the content of phenolic compounds with an increase in total content in seeds from the 8th to 12th day of germination, by approximately 50% and 65%, respectively, in broccoli; by 30% in kohlrabi; by 35 and 55%, respectively, in red cabbage and turnips; and 70, 75 and 75% in rutabaga, turnip greens and radish, respectively.

To summarize, the identification of plant tissues and development phases with the greatest variety of phytochemical compounds could provide information support for the food industry to maximize the health-promoting properties of sprouts or for the pharmaceutical industry in the production of dietary supplements.

CONCLUSIONS

The present studies showed that the change trends for metabolites in seeds caused by the germination process occur and the obtained extracts are phytochemically differentiated. Analyses of the metabolic profile of selected sprouts may contribute to the development of research, whose goal should be to use red mizuna sprouts as completely unprocessed food, with a high nutritional index. Perhaps, these activities will cause the dissemination of red mizuna sprouts, currently unavailable on the domestic market. Therefore, further research is needed to understand the bioavailability and metabolism of compounds present in mizuna sprouts, which will allow science-based claims and recommendations for intake, effective dosages, and dietary guidelines for nutrition and health to be made.

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