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Proximate Analysis and Antioxidant Properties of Young Plants of *Sinapis alba* L. Depend on the Time of Harvest and Variety

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Abstract: White mustard plant (*Sinapis alba* L.) is an easy-to-grow species with low soil requirements and is often sown as a catch crop in Northern Europe to reduce nitrate leaching, especially during the winter. There are studies showing the high nutritional value of mustard seeds, which have a wide application, mainly in food production. Still little is known about the young shoots or plants of different cultivars of white mustard, although in Asian countries, eating them raw is quite common. The aim of the research was to determine the proximate composition, antioxidant activity and polyphenolic compound content in young green plants of the Polish cultivars of white mustard: Borowska (traditional, with a high content of erucic acid and glucosinolates), Bamberka (non-erucic with glucosinolates) and Warta (non-erucic with low glucosinolates content; double-improved). Young plants were harvested in three terms. The first harvest took place at the plover stadium and the next ones at 7-day intervals (31, 38 and 45 day after sowing). In freeze-dried plant material, proximate composition and antioxidant activity with the ABTS and FRAP methods, as well as phenolic compound content, were measured. The highest concentration of protein was measured in cultivars Warta and Borowska after 31 and 38 days of sowing. Harvest time and cultivar affected antioxidant activity and total polyphenol content in young mustard plants. Thirty-eight days after sowing, the examined cultivars of the young plants of mustard had the highest antioxidant activity and total polyphenolic compound content. Green young mustard plants have strong antioxidant properties at the basic level, they are classified as functional foods and are similar to other edible leafy plants such as celery, spinach and Brussels sprouts.

Keywords: white mustard; cultivar; antioxidant activity; polyphenolic compounds

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1. Introduction

The name of mustard plant "mustard" comes from the Latin *mustum ardens* due to the sharp, burning sensation attributed to their main metabolites, glucosinolates (GLS) [1]. For gastronomic purposes, three species of mustard are mainly cultivated worldwide: white mustard (*Sinapis alba*), red mustard (*Brassica juncea*) and black mustard (*Brassica nigra*) [2]. The most common species in Europe is white mustard [3]. White mustard (*Sinapis alba* L. syn. *Brassica hirta*), also called yellow or light mustard, is an annual oil plant belonging to the *Brassicaceae* family. It is the most fertile mustard crop cultivated in Poland and Europe and less demanding, and the cultivated variety is characterized by fidelity and stability of yield [4]. In Europe, white mustard is usually grown for seed or green manure for

plowing in stubble catch crops. Mustard seeds are usually used as a spice plant and for oil production in the food industry. Leaves and seeds of mustard can also be used for production of various dishes [3]. In Turkey, mustard is rarely used as a spice, while fresh leaves are commonly used as an ingredient of various dishes [5]. Leaves are often eaten with boiled eggs and used to prepare salads, cakes or vegetable soups [6–8]. Thus, mustard seeds and other parts of this plant are the part of the human diet in these regions [9,10].

Traditional varieties of white mustard seeds contain a large amount of glucosinolates (approx. 160 μM/g of seeds); mainly sinalbin, i.e., specific compounds from the group of sulphur-containing glucosides. On the other hand, oil obtained from seeds contains an excessive amount of erucic acid (approx. 40–45%). Due to the potential harmful effects of these compounds on the human body, in 2006 a variety of Bamberka was introduced into cultivation in Poland characterized by a reduced content of erucic acid. In 2012, the variety Warta was introduced to the farmers. This variety is characterized by a low content of erucic acid and a much lower content of glucosinolates in seeds, as compared to other traditional varieties [11,12].

It is well known that *Brassicae* vegetables (broccoli, cauliflower, cabbage) have strong antioxidant activity because of the content of glucosynolates and polyphenolic compounds. In the human organism they can reduce oxidative stress, and thus may reduce the risk of cancer, cardiovascular diseases and neurodegenerative diseases [13–17]. In the scientific literature are widely documented the antioxidative properties of various classes of polyphenolic compounds [18,19]. They have the ability to neutralize free radicals and chelate metal ions, as well as convert hydroperoxides to stable compounds [20,21]. The results of research published by various authors indicate a high content of phenolic compounds in the Brassicaceae family [15,16]. Cartea et al. [17] emphasize that cabbage leaves are particularly rich in polyphenolic compounds. Jo et al. [22] indicated a correlation between the content of polyphenols in *Brassica juncea* mustard leaves and their antioxidant activity. The variable content of polyphenols in plants depending on the time of harvest is pointed out by Yao et al. [23] and Ribeiro et al. [24]. In turn, Soengas et al. [25] emphasize that the antioxidant potential of *Brassica* vegetables can be differentiated by genotype. Similarly, in some studies it was reported that the chemical composition of mustard may depend on the genotype used, and may also change depending on the development stage of plants [14,26–28].

Due to the increasing number of noncommunicable diseases, the interest of both scientists and consumers in the bioactive properties of food products has increased in recent years. This leads to a constant search for new food products supporting the natural antioxidant defence. Green mustard plants harvested in the young stage of development may be one of them. In order to increase the predictability of the content of desirable compounds from the point of view of human health, it is essential to evaluate the phytochemical changes that occur in white mustard during growth and development, as these changes can affect their functional properties. So far, there is insufficient scientific literature on changes in the chemical composition and antioxidant properties of different varieties of white mustard, depending on the developmental stage of the plants.

The objective of this research was to determine the proximate composition, antioxidant activity and polyphenolic compound content in green white mustard plants of different varieties: Borowska (traditional cultivar; high in erucic acid and glucosynolates), Bamberka (improved non-erucic with glucosynolates) and Warta (non-erucic with low glucosinolates content double-improved), depending on the development of the plants in the green stage.

2. Materials and Methods

2.1. Plant Material

The research material consisted of plants of three cultivars of white mustard obtained from a one-factor field experiment. The factors of the experiment were white mustard cultivars Borowska, Bamberka, and Warta [29]. The Borowska cultivar, which was entered into the national register of cultivars in Poland in 1958, belongs to very old and fertile

cultivars with a large amount of erucic acid and glucosinolates in the seeds. The Bamberka cultivar (inscribed in the national register in 2006) is characterized by a reduced content of erucic acid. The Warta cultivar is one of the youngest cultivars (entered into the national register in 2012) and its seeds contain a low content of erucic acid and a significantly lower content of glucosinolates, compared to the other varieties of white mustard.

2.2. Field Experiment

The experiment was located on the experimental station of the University of Agriculture in Krakow (50°04′ N, 19°51′ E, 211 m MSL, slope 2°). The forecrop was peppermint. Before setting up the experiment, plowing was performed in autumn, and harrowing in spring. For all variants, the same fertilization was applied in the amount of N 60 kg/ha, P_2O_5 30 kg/ha, K_2O 80 kg/ha. Seeds were sown using a garden drill so as to obtain a density of 150 plants/m^2. Certified seed material was used for sowing. Sowing was done in the first week of May. The row spacing was 13 cm. No pesticides were used in the cultivation. The green plants were cut by hand about 1 cm above the soil surface with the use of pruning shears. The selection of plants was done randomly. Thus, different plants were used for subsequent studies. At the first harvest time, the plants had four pairs of primary leaves and the estimated length was 11 cm. Three harvests were made three times at weekly intervals e.g., 31, 38 and 45 days after sowing of seeds (first, second and third harvest time, respectively).

2.3. Proximate Analysis

Obtained plants of mustard were cleaned and dried. Dry mass (DM) in fresh samples of mustard was determined based on the AOAC methods [30]. Part of samples were frozen by freeze-drying in a lyophilizer (Christ Alpha 1-4, Gefriertrocknungsanlangen, Germany). The proximate analysis of freeze-dried samples was measured according to the AOAC official methods. The concentration of protein was determined with the Kjeldahl method (AOAC no. 978.04), crude fat content in accordance with the Soxhlet method (AOAC no. 935.38) and ash (AOAC no. 930.05). The total carbohydrate content of dry mass was calculated based on the formula: total carbohydrates = 100 − (protein + raw fat + ash) [31]. In freeze-dried samples the total phenolic content and antioxidant activity was measured.

2.4. Extract Preparation, Antioxidant Capacity and Total Polyphenol Content

About 0.5 g of lyophilized grounded mustard samples were used for the preparation of acidified methanolic extract (70% methanol acidified with 0.1% formic acid v/v). All samples were extracted by shaking in a laboratory shaker (Elpan, type 357, Lubawa, Poland) for two hours, without light. After 2 h of extraction, samples were centrifuged (Centrifuge type MPW-340, Warsaw, Poland). Thus, the obtained samples were kept at −22 °C for further analyses.

The total polyphenol concentration in the acidified methanolic extract of mustard plants was measured with Folin-Ciocalteu reagent [32]. The results are expressed as the chlorogenic acid equivalent (CGA) in mg per 100 g of dry sample.

The antioxidant activity of methanolic extracts of varieties of mustard was measured using the method with $ABTS^{\bullet+}$ radical (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)) [33] and the FRAP method (ferric-reducing antioxidant power) [34]. Results obtained for $ABTS^{\bullet+}$ and FRAP methods were compared to the concentration–response curve of the standard Trolox dilution and the results obtained are expressed in µM Trolox/1g DM of sample.

2.5. HPLC Analysis of Mustard Plant

The HPLC analysis of polyphenols was conducted using Prominence-i LC-2030C 3D Plus system (Shimadzu, Kyoto, Japan) equipped with a diode array detector (DAD). The separation was performed on the Luna Omega 5 µm Polar C18, 100 A, 250 × 10 mm column (Phenomenex, CA, USA) at 40 °C. The mobile phase was a mixture of two eluents: A—0.1%

formic acid in water (v/v) and B—0.1% formic acid in methanol (v/v). The flow rate of the mobile phase was 1.2 mL/min. The analysis was carried out with the following gradient conditions: from 20% to 40% B in 10 min, 40% B for 10 min, from 40% to 50% B in 10 min, from 50% to 60% B in 5 min, 60% B for 5 min, from 60 to 70% B in 5 min, from 70% to 90% B in 5 min, 90% B for 5 min, from 90% to 20% B (the initial condition) in 1 min and 20% B for 4 min, resulting in a total run time of 60 min. The injection volume was 20 µL.

The detection of 4-hydroxybenzoic acid, myricetin, quercetin, luteolin and isorhamnetin was done at 254 nm, rutin at 256 nm, vanillic acid at 260 nm, kaempferol at 264 nm, apigenin and acacetin at 267 nm, gallic acid at 271 nm, hispidulin at 273 nm, syringic acid at 274 nm, catechin and epicatechin at 278 nm, naringin and carnosol at 283 nm, hesperidin and carnosic acid at 284 nm, p-coumaric acid at 310 nm, caffeic acid, ferulic acid and sinapinic acid at 323 nm, chlorogenic acid at 326 nm, as well as rosmarinic acid at 329 nm. The data were integrated and analyzed using the LabSolution software (Shimadzu, Kyoto, Japan).

2.6. Identification of Some Phenolic Compounds

In order to identify the polyphenolic compounds, which were not determined based on available standards on HPLC, from which the signal originates in the peak, the retention time and signals from the spectrum, UV (λmax) and scientific articles were used [35–39]. Peak identification was assessed using the Reaxys platform [40] using the test spectrum included therein.

2.7. Statistical Analysis

Proximate composition analyses were carried out four times. HPLC analyses were carried out in duplicate The total polyphenolic compound content and antioxidant activity were performed in triplicate. Results are reported as the means ± SD. Two-way factorial analysis of variance (MANOVA) was used to test the differences. Significance of the obtained differences were verified with a Duncan test at the level of significance of $p \leq 0.05$. The results were subjected to analysis with the use of STATISTICA v.13.3 (StatSoft Inc., Tulsa, OK, USA).

3. Results

The content of dry mass and the proximate composition of plants of the white mustard varieties harvested in various stage of development are presented in Table 1.

Table 1. Dry mass and proximate composition of different varieties of white mustard [g/100 g DM].

Treatment	Cultivar	Harvest Date the Number of Days after Sowing			Means for Cultivar
		31	38	45	
Dry mass [g/100 g FM *]	Warta	9.99 ± 0.51 a	11.22 ± 0.62 a	17.40 ± 2.39 a	12.87 ± 3.60 A
	Bamberka	10.33 ± 1.05 a	11.13 ± 2.00 a	17.95 ± 0.85 a	13.14 ± 7.37 A
	Borowska	10.80 ± 1.43 a	10.36 ± 0.48 a	17.80 ± 0.71 a	12.99 ± 3.62 A
	Means for harvest time	10.38 ± 1.06 A	10.90 ± 1.23 A	17.72 ± 1.45 B	
Protein	Warta	36.28 ± 1.07 f	34.80 ± 1.13 ef	29.53 ± 0.76 c	33.53 ± 3.16 C
	Bamberka	22.96 ± 0.47 a	33.27 ± 1.00 e	24.39 ± 0.88 a	26.87 ± 4.82 A
	Borowska	33.82 ± 1.58 e	26.12 ± 1.53 b	31.40 ± 1.68 d	30.44 ± 3.65 B
	Means for harvest time	31.82 ± 6.13 B	31.40 ± 4.11 B	28.44 ± 3.27 A	

Table 1. *Cont.*

Treatment	Cultivar	Harvest Date the Number of Days after Sowing 31	38	45	Means for Cultivar
Crude fat	Warta	0.83 ± 0.41 a	0.82 ± 0.44 a	1.37 ± 0.56 b	1.01 ± 0.50 A
	Bamberka	1.35 ± 0.09 b	1.19 ± 0.24 ab	2.77 ± 0.44 c	1.77 ± 0.79 B
	Borowska	1.56 ± 0.09 b	1.37 ± 0.05 b	2.76 ± 0.17 c	1.90 ± 0.65 B
	Means for harvest time	1.25 ± 0.39 A	1.13 ± 0.35 A	2.30 ± 0.79 B	
Total carbohydrates	Warta	46.69 ± 1.37 a	49.50 ± 1.24 b	57.34 ± 0.79 ef	51.18 ± 4.82 A
	Bamberka	59.06 ± 0.54 fg	51.87 ± 1.43 c	60.12 ± 0.89 g	57.02 ± 3.94 C
	Borowska	46.66 ± 1.57 a	56.82 ± 1.68 e	53.90 ± 1.94 d	52.46 ± 4.73 B
	Means for harvest time	50.81 ± 6.20 A	52.73 ± 3.45 B	57.12 ± 2.91 C	
Ash	Warta	16.20 ± 0.24 ef	14.87 ± 0.32 d	11.76 ± 0.51 a	14.28 ± 1.97 A
	Bamberka	16.63 ± 0.06 f	13.66 ± 0.68 c	12.72 ± 0.40 b	14.34 ± 1.79 A
	Borowska	17.96 ± 0.53 g	15.69 ± 0.18 e	11.95 ± 0.41 a	15.20 ± 2.62 B
	Means for harvest time	16.93 ± 0.84 C	14.74 ± 0.96 B	12.14 ± 0.59 A	

* FM—fresh mass; results are expressed as mean ± SD; n = 4; mean values with different letters (a–g) within the individual rows and columns (without last column) are statistically different $p \leq 0.05$; mean values with capital letter (A–C) within last column (for cultivar) or rows (for harvest time) are statistically different at $p \leq 0.05$.

Dry mass content was affected by time of harvest. Forty five days after the sowing, the plants obtained a higher content of dry mass as compared to plants harvested in thirty-one and thirty-eight day after sowing. There were no differences between the mustard varieties in terms of dry mass content. The protein content in white mustard plants depended on both the date of harvest and the cultivar (Table 1). In the 45 days after sowing, the concentration of proteins was significantly lower as compared to the 31 and 38 days after sowing. The double-improved Warta cultivar with an average content of 33.53% DM was the richest in protein. It was also found that individual cultivars reacted slightly differently in terms of the content of this component to the term of harvest (Table 1). Statistically significant differences in crude fat content were found in the tested cultivars of white mustard. The green plants of the Warta cultivar were characterized by a lower crude fat content compared to the Bamberka and Borowska cultivars.

The content of the total carbohydrates significantly increased in mustard plants 38 and 45 days after sowing. However, the reaction to the date of harvest of individual varieties was slightly different, because the Borowska variety reached the highest total carbohydrate content during the second harvest. Bamberka turned out to be the cultivar richest in total carbohydrates with an average content of 57.02% DM. The content of ash significantly decreased with the growth of the plant and, thus, the delay of the harvest date (Table 1). However, a slightly different effect of each cultivar to the harvest time was observed. The highest ash content was found in the Borowska cultivar from the first harvest, and the lowest in the Warta harvested after 45 days of sowing.

Table 2 presents the results of antioxidant activity determined by the ABTS and FRAP methods and the content of total polyphenols.

Table 2. Antioxidant activity [µmolTrolox/1 g DM] and total polyphenol [mg/100 g] content in the tested cultivars of white mustard in particular harvest dates.

Compound	Cultivar	Number of Days after Sowing			Means for Cultivar
		31	38	45	
ABTS	Warta	217 ± 4 e	1755 ± 3 b	163 ± 1 a	1855 ± 25 A
	Bamberka	225 ± 3 f	255 ± 1 g	193 ± 3 c	225 ± 27 C
	Borowska	173 ± 7 b	273 ± 2 h	200 ± 1 d	216 ± 45 B
	Means for harvest time	205 ± 24 B	234 ± 45 C	185 ± 17 A	
FRAP	Warta	1511 ± 98 c	1549.39 ± 5 c	1399.37 ± 6 b	1486.54 ± 84 B
	Bamberka	1368 ± 12 b	1890.06 ± 8 d	922.57 ± 7 a	1393.63 ± 419 A
	Borowska	1539 ± 10 c	1963.47 ± 15 e	1523.29 ± 10 c	1675.25 ± 217 C
	Means for harvest time	1477 ± 94 B	180 ± 192 C	1282 ± 275 A	
Total polyphenols	Warta	827 ± 24 f	7582 ± 14 c	6048 ± 14 a	7301 ± 986 A
	Bamberka	796 ± 14 d	8194 ± 21 e	8249 ± 21 f	8135 ± 133 B
	Borowska	689 ± 15 b	8964 ± 29 g	6036 ± 8 a	7297 ± 1304 A
	Means for harvest time	7709 ± 628 B	824 ± 600 C	6778 ± 1104 A	

Results are expressed as mean ± SD; n = 3; mean values with different letters (a–h) within the individual rows and columns (without last column) are statistically different $p \leq 0.05$; mean values with capital letter (A–C) within last column (for cultivar) or rows (for harvest time) are statistically different at $p \leq 0.05$.

Based on the results of antioxidant activity measured with the ABTS and FRAP methods, it can be confirmed that white mustard plants obtained the highest results after the 38 days of sowing, with the exception of the Warta cultivar (Table 2). After the 45 day of sowing, the antioxidant activity measured with the ABTS method decreased by about 21%, compared to the 38 days after sowing, while with FRAP it was by about 29%. On the other hand, white mustard plants harvested after 31 days of sowing were characterized by lower antioxidant activity, measured with the methods mentioned above, as compared to the plants harvested after 38 days of sowing (ABTS 13% and FRAP 18% lower). Differences between the tested mustard cultivars in terms of antioxidant activity were also found. In the ABTS method, the maximum values were obtained for the Bamberka cultivar, and in the FRAP method, the Borowska cultivar (Table 2). Similar relationships were also observed for the content of polyphenols. The maximum content of polyphenols was measured after 38 days of sowing and it was, on average, 8247 mg/100 g. In the third harvest (45 days after sowing), the polyphenols content decreased by about 18% and in the first by about 7%, compared to the second harvest. In terms of polyphenol content, the Bamberka cultivar (8135 mg/100 g) came first.

In HPLC analysis, there were 20 polyphenolic compounds identified, which are presented in Table 3. The exceptions were cultivar Bamberka (not detected: caffeic acid) and cultivar Warta (not detected: gallic acid). Comparing the content of total polyphenols in samples collected at specified dates, the cultivar Warta was characterized by the significantly lowest, however the cultivar Borowska by the significantly highest content of total polyphenols, at all three harvest times. Based on the results in Table 3 it can be suggested that in all samples, the dominant compound was rutin. However in Figures 1–3 the highest peaks are shown. These peaks are probably for luteolin-7-O-glucoside, apigenin-7-O-glucoside apigenin-7-O-glucuronide. The others with the high level were epicatechin and sinapinic acid (the exceptions were cultivars Borowska and Bamberka, collected in the second and third time of harvest).

Table 3. Profile of polyphenolic compounds in white mustard young plants [mg/100 g DM].

Compound	Borowska			Bamberka			Warta		
	Number of Days after Sowing								
	31	**38**	**45**	**31**	**38**	**45**	**31**	**38**	**45**
Acacetin	6.22 ± 0.29 e	3.59 ± 0.09 b	3.23 ± 0.02 a	4.93 ± 0.19 d	4.03 ± 0.16 c	4.95 ± 0.03 d	4.25 ± 0.09 c	4.98 ± 0.02 d	4.73 ± 0.20 d
Catechin	40.06 ± 2.39 b	33.42 ± 1.13 a	44.08 ± 0.92 bc	69.05 ± 0.40 e	76.01 ± 4.92 f	58.00 ± 2.60 d	58.89 ± 0.09 d	48.01 ± 0.25 c	40.07 ± 0.37 b
Epicatechin	95.79 ± 0.36 bc	58.43 ± 1.75 a	98.83 ± 7.47 bc	92.56 ± 0.02 b	98.61 ± 5.71 bc	139.17 ± 2.94 d	100.88 ± 0.56 c	177.75 ± 0.94 e	175.51 ± 0.30 e
Hispidulin	2.02 ± 0.05 a	2.25 ± 0.00 b	2.01 ± 0.06 a	8.78 ± 0.00 g	2.08 ± 0.05 a	2.37 ± 0.00 c	2.53 ± 0.01 d	3.41 ± 0.00 f	3.04 ± 0.00 e
Kaempferol	5.63 ± 0.07 a	6.58 ± 0.02 d	6.61 ± 0.00 d	5.81 ± 0.05 ab	6.10 ± 0.03 bc	8.83 ± 0.53 f	6.37 ± 0.07 cd	10.10 ± 0.05 g	8.04 ± 0.03 e
Luteolin	3.88 ± 0.02 a	7.07 ± 0.05 c	4.38 ± 0.02 ab	4.53 ± 2.97 ab	6.59 ± 0.03 bc	4.47 ± 0.35 ab	3.00 ± 0.04 a	5.08 ± 0.02 abc	3.44 ± 0.02 a
Myricetin	10.52 ± 0.03 e	7.85 ± 0.05 c	12.40 ± 0.15 f	10.12 ± 0.03 d	6.78 ± 0.08 a	13.26 ± 0.05 g	7.25 ± 0.03 b	10.05 ± 0.21 d	6.97 ± 0.07 a
Naringin	2.62 ± 0.07 b	2.88 ± 0.02 bc	6.39 ± 0.27 e	2.88 ± 0.02 bc	1.89 ± 0.03 a	4.57 ± 0.31 d	3.11 ± 0.03 c	17.92 ± 0.28 g	11.15 ± 0.1 f
Quercetin	5.65 ± 0.42 a	8.17 ± 0.02 d	7.14 ± 0.02 b	7.90 ± 0.03 cd	7.53 ± 0.02 bc	5.51 ± 0.20 a	8.16 ± 0.04 d	10.52 ± 0.31 e	8.24 ± 0.02 d
Rutin	1365.07 ± 13.8 d	2013.88 ± 1.61 f	2286.00 ± 1.24 h	2103.88 ± 4.86 g	1760.55 ± 13.3 e	1084.55 ± 50.9 c	837.22 ± 0.53 b	1115.81 ± 0.78 c	583.12 ± 0.13 a
Caffeic acid	7.24 ± 0.07 d	6.25 ± 0.03 c	9.30 ± 0.03 f	nd	nd	nd	5.52 ± 0.19 b	7.79 ± 0.02 e	5.27 ± 0.02 a
Chlorogenic acid	1.90 ± 0.02 b	6.10 ± 0.00 g	1.97 ± 0.06 b	7.90 ± 0.03 h	5.38 ± 0.10 f	3.31 ± 0.02 d	1.76 ± 0.01 a	2.53 ± 0.08 c	3.48 ± 0.02 e
Ferulic acid	12.72 ± 0.66 b	9.59 ± 0.05 a	19.60 ± 0.02 e	12.61 ± 0.19 b	9.90 ± 0.77 a	22.45 ± 0.56 f	13.67 ± 0.19 c	29.04 ± 0.03 g	14.99 ± 0.08 d
Gallic acid	8.23 ± 0.19 b	9.41 ± 0.21 c	11.22 ± 0.71 d	13.64 ± 0.32 f	12.31 ± 0.26 e	7.18 ± 0.43 a	Nd	nd	nd
p-Coumaric acid	4.98 ± 0.02 b	4.40 ± 0.09 ab	8.08 ± 0.18 c	4.62 ± 0.05 ab	4.06 ± 0.08 a	10.44 ± 0.84 d	8.32 ± 0.10 c	16.45 ± 0.02 e	7.88 ± 0.12 c
Sinapinic acid	23.15 ± 0.67 b	10.48 ± 0.31 a	132.58 ± 0.02 c	27.29 ± 1.99 b	20.58 ± 1.32 b	126.66 ± 7.59 c	183.87 ± 7.63 e	356.53 ± 0.17 f	159.41 ± 0.57 d
Syringic acid	2.32 ± 0.07 b	2.33 ± 0.07 b	5.76 ± 0.23 d	1.85 ± 0.05 a	8.17 ± 0.16 f	24.86 ± 0.30 h	7.71 ± 0.16 e	4.24 ± 0.02 c	20.69 ± 0.17 g
Vanillic acid	3.03 ± 0.21 a	3.27 ± 0.05 a	7.66 ± 0.03 d	5.96 ± 0.13 c	4.82 ± 0.07 b	22.48 ± 0.18 f	9.83 ± 0.92 e	8.37 ± 0.21 d	3.39 ± 0.08 a
Carnosol	47.84 ± 0.55 e	17.18 ± 0.26 d	16.01 ± 0.05 bc	17.05 ± 0.05 d	16.62 ± 0.10 cd	16.14 ± 0.51 bc	13.32 ± 0.07 a	15.52 ± 0.30 b	15.59 ± 0.15 b
Carnosolic acid	44.13 ± 4.13 f	23.42 ± 0.14 e	19.89 ± 0.94 d	5.70 ± 0.10 b	9.18 ± 0.28 c	48.71 ± 1.36 g	1.50 ± 0.11 a	5.38 ± 0.05 b	3.75 ± 0.22 ab
TOTAL	1692.99 ± 10.13 c	2236.53 ± 0.88 f	2703.14 ± 10.36 h	2407.05 ± 0.05 g	2061.18 ± 26.48 e	1607.90 ± 51.06 c	1277.15 ± 6.51 b	1849.47 ± 1.70 d	1078.75 ± 1.26 a

Results are expressed as mean ± SD; n = 2; mean values with different letters (a–h) within the individual rows are statistically different $p \leq 0.05$.

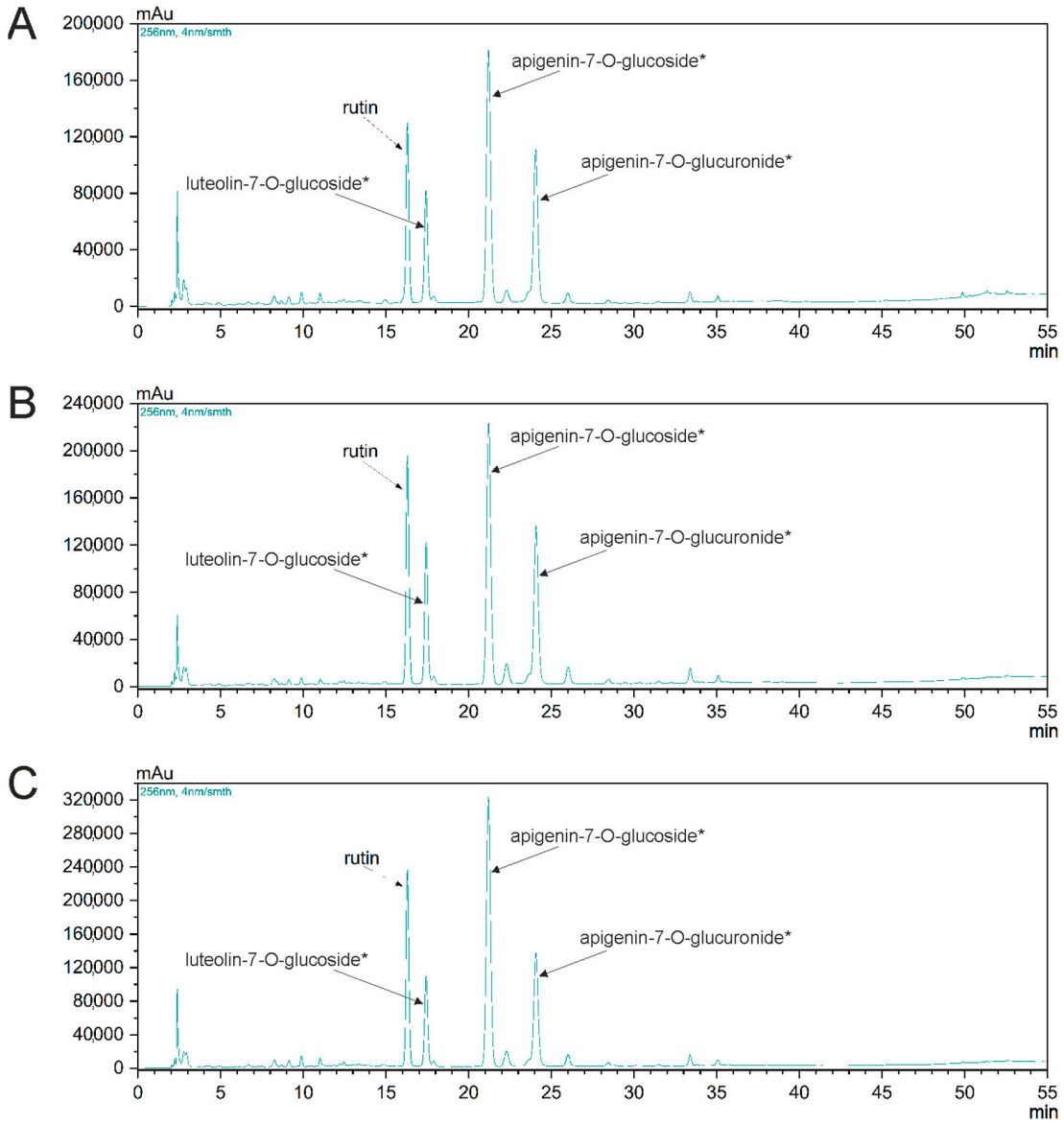

Figure 1. Example of chromatogram obtained for mustard cultivar Warta. (**A**) Thirty-one days after sowing. (**B**) Thirty-eight days after sowing. (**C**) Forty-five days after sowing. * The identification was made based on the Reaxys platform and references [36–40].

Among the samples of cultivar Borowska, after 45 days of sowing, the significantly higher content of the total polyphenols was determined. This tendency was characterized by the following compounds: rutin, gallic acid, kaempferol, vanillic acid, naringin and syringic acid. The opposite trend concerned carnosolic acid, carnosol and acacetin. The samples of cultivar Bamberka collected after 31 days of sowing had the largest level of total polyphenols, therefore those collected after 45 days of sowing had the lowest. This tendency was observed in rutin, gallic acid, chlorogenic acid, quercetin and carnosol; however, the opposite was in carnosolic acid, syringic acid and kaempferol. In cultivar Warta, the highest concentration of total polyphenolic compounds was found in samples collected at the second harvest time, in comparison to other dates. What is more, the content of almost all individual polyphenolics was the largest in samples collected after 38 days of sowing. The exceptions were catechin, chlorogenic acid, syringic acid, vanillic acid and carnosol.

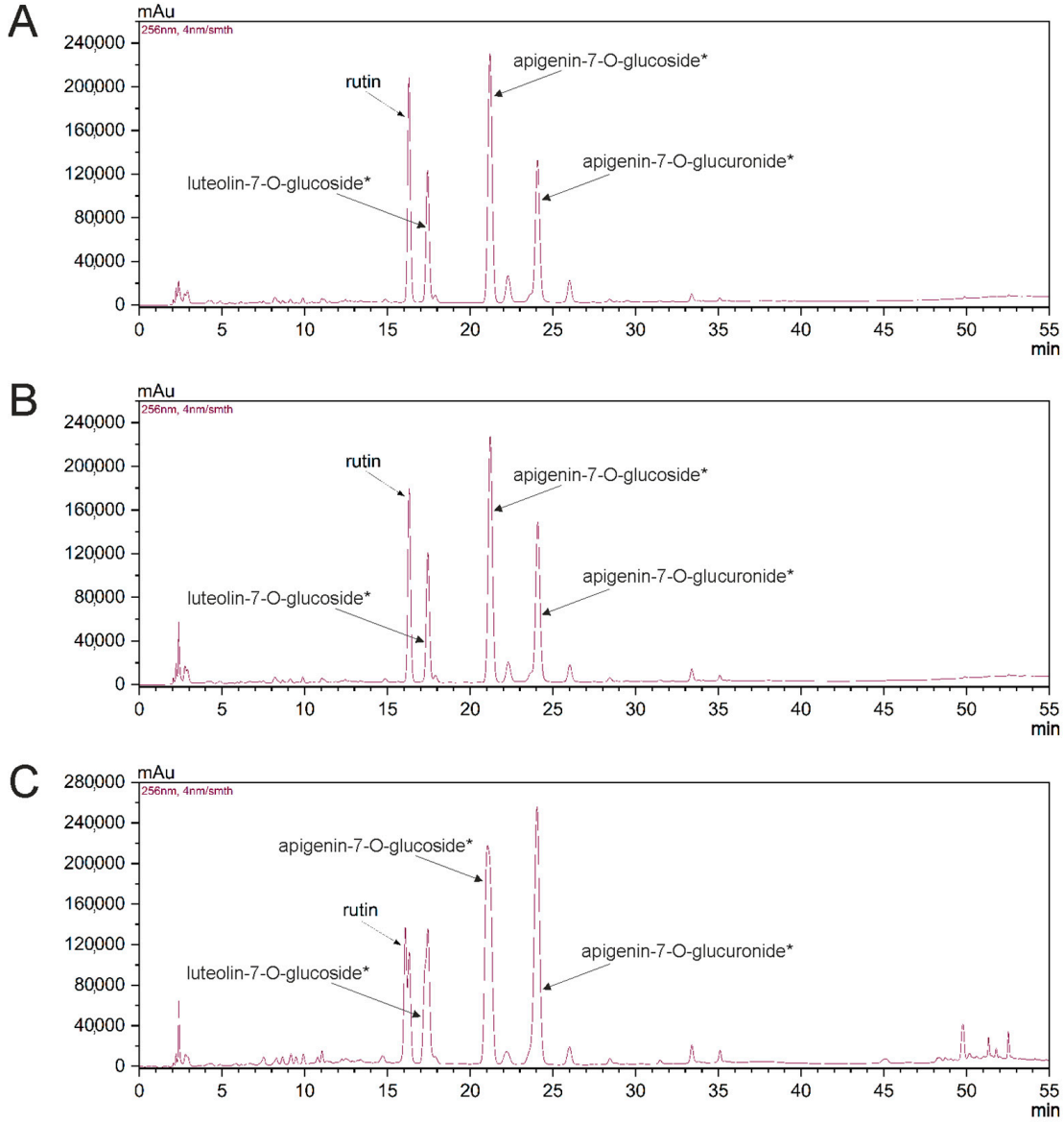

Figure 2. Example of chromatogram obtained for mustard cultivar Bamberka. (**A**) Thirty-one days after sowing. (**B**) Thirty-eight days after sowing. (**C**) Forty-five days after sowing. * The identification was made based on the Reaxys platform and references [36–40].

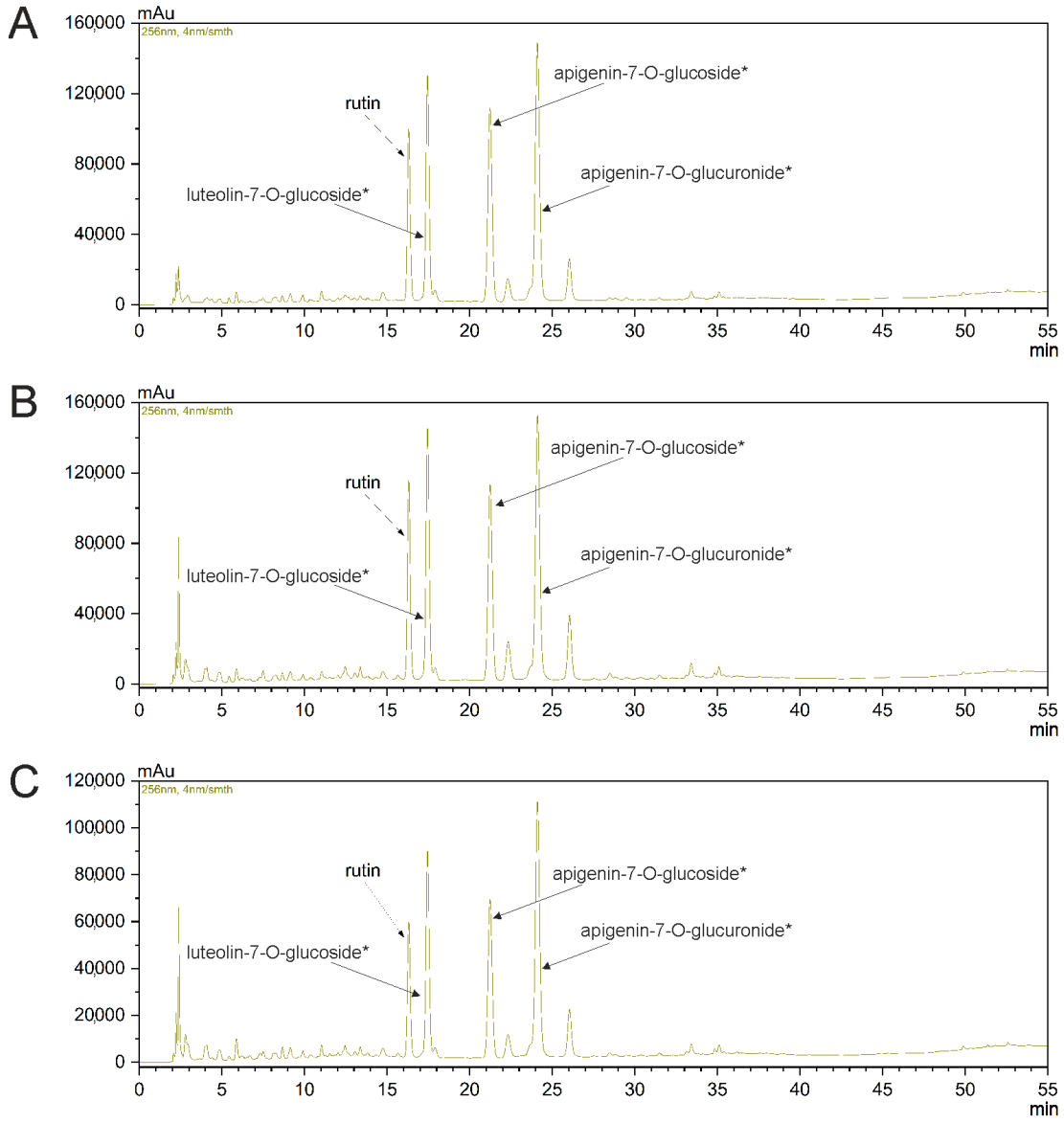

Figure 3. Example of chromatogram obtained for mustard cultivar Borowska. (**A**) Thirty-one days after sowing. (**B**) Thirty-eight days after sowing. (**C**) Forty-five days after sowing. * The identification was made based on the Reaxys platform and references [36–40].

4. Discussion

4.1. Proximate Composition

Most of the literature data concern the composition of mustard seeds of individual species, and not plants in vegetative stages, such as young shoots. The presented research results are often a novelty in the literature on the subject. An increase in the share of dry mass in white mustard plants was found depending on the time of harvest. After about 1.5 months of vegetation, e.g., 45 days after sowing, the plants obtained an average of about 17.72% DM. Similarly, Kisielewska and Harasimowicz-Herman [41] showed that the content of dry mass in the white mustard increases with the extension of the vegetation period of the plant. Mustard of the Nakielska cultivar, harvested after 45 days of vegetation, contained on average 10% of dry mass, and after 108 days of vegetation, on average 32.4% of dry mass. Nowakowski and Szymczak-Nowak [42] assessed the DM content in white mustard plants sown in stubble catch crops in the first decade of August. After about 2 months, they obtained a dry mass content of 15.21–16.79%. A higher content of dry matter

in our research may result from the use of different genotypes and the time of observation. Since white mustard is a long-day plant with a strong photoperiodic reaction [43] in spring sowing, it hurries to produce inflorescences faster, increasing the share of dry matter. In turn, research conducted by Kwon et al. [27] using monthly plants of two varieties of red mustard indicates a slightly lower dry matter content compared to those presented in this work (on average, after about 1 month, 10.38% DM), within the range 8.4–6.51%. This may be due to differences in genotypes, soil moisture, temperature during plant vegetation and applied fertilization. The obtained dry mass values of green mustard plants in the analyzed harvest dates are similar to data from the literature for other commonly used green vegetables. For comparison, Kapusta-Duch et al. [44], in frozen Brussels sprouts (*Brassica oleracea*), determined about 14 g/100 g of dry mass content, and according to a study by Rożek et al. [45], celery leaves contained on average 21.99% of dry mass in leaf blades and 12.83% of dry mass in petioles. These authors also observed an increase in the share of dry mass in celery in the second harvest.

The protein content in green white mustard plants in the presented research results ranged from 22–96–36.28% DM. Edelman and Colt [46] emphasize the high value of green leafy plant proteins due to the presence of a polypeptide complex: RUBISCO. RUBISCO is rich in essential amino acids, with typically eight of the designated nine percent meeting the FAO nutritional criteria. No data on the basic composition of white mustard plants were found in the literature, but similar studies were conducted by Kwon et al. [27] using monthly plants of two varieties of red mustard. In the cited studies, the crude protein content ranged from 1.66–1.81% for one cultivar and from 1.64–1.93% for the other. Sawicka et al. [43] report that 140 g of chopped mustard leaves contained 2.56 g of protein (without specifying the species used for the study). According to Cho et al. [47], shoots and leaves of common mustard, depending on the cultivar, contained from 1.1 g to 4.4 g of protein per 100 g of fresh weight. After converting the averaged results into protein content in dry matter, a result of 22.6% protein was obtained, similar to that for the white mustard cultivar Bamberka (31 day after sowing—22.96). The green parts of white mustard, therefore, contain more protein (22–96–36.28%) than common mustard, which is common in Asian countries. In the tested cultivars of white mustard, the protein content depended on both the date of harvest and the cultivar used. In the third term, there was a significant decrease in the protein content, on average by about 3%, compared to the earlier term. Singh and Sinhal [28] observed a similar downward trend in protein content as plants matured in the leaves of the red mustard (Brassica juncea). On the 70th day of vegetation, they contained 24.68% of protein, while on the 85th day it was 23.68%, and on the 130th day only 17.64%. In our research, the double-improved Warta cultivar, with an average content of 36.28% DM, was the richest in protein. It was also found that individual cultivars reacted slightly differently in terms of the content of this component to the date of harvest (Table 1). Similarly, Piętka et al. [12] noted the higher protein content in the meal of seeds of the Warta cultivar (43.1%) compared to the Bamberka cultivar (42%), presenting two-year results from 12 field experiments. Usually, for mustard seeds, the protein content, according to different authors, is 30–36.8% [48,49]. Paszkiewicz-Jasinska [26] points out that, similarly to the presented results, differences in protein content in total white mustard seeds were conditioned by the cultivar factor.

It is well known that white mustard seeds are a rich source of fat and, according to various authors, its content usually ranges from 29% [48] to 31.78% [49]. The literature indicates slight differences in the fat content between the seeds of the varieties used in the experiment: Borowska 25.60–27.56%, Bamberka 28.9–31.4% [12,50–52]. Paszkiewicz-Jasinska [26] emphasizes that differences in protein content in white mustard seeds depend on the genotype used.

However, there is insufficient scientific literature on the fat content of the green parts of white mustard. In the presented results, the fat content in mustard plants ranged from 0.82–2.76% DM. Kwon et al. [27], using monthly plants of two cultivars of red mustard, indicating the content of crude lipids in one of the tested plants in the range of 0.42–0.59%,

while in the other 0.40–0.61%. In turn, Sawicka et al. [43] in mustard leaves obtained 0.47 g of fat from 140 g of fresh mass of leaves used for research. On the other hand, research by Cho et al. [47] showed the content of fat in fresh *Brassica juncea* plants in the range of 0.2–0.7%, which gives more than 3% of fat in dry mass—more than the tested varieties of white mustard, where the highest determined fat content was 2.76% DM. In the presented results of our own research, statistically significant differences in fat content between varieties of white mustard were found. The Warta cultivar was characterized by a lower fat content in the green parts of the plant: on average, 1.01% DM compared to the Bamberka and Borowska varieties, 1.77 and 1.90 DM, respectively. On the contrary, in the seeds, Piętka et al. [12] indicate a higher oil content in the seeds of the Warta cultivar (32.5%) compared to the Bamberka cultivar (31.4%), presenting two-year results from 12 field experiments: The third harvest, on average, 1.17 DM compared to the second harvest. This may explain the fact that Karydogianni et al. [53], in experiments conducted with the use of black mustard biomass, obtained a higher crude fat content of 2.66 and 2.62% DM. The aim of these studies was to use the crop for fodder, so older plants were probably used in this experiment. It should also be emphasized that the total lipid content decreases in stressed plants with increasing triacylglycerol levels [54].

The content of total carbohydrates in the dry matter of plants significantly increased with the development of plants. During the first harvest, the mustard plants contained an average of 50.81 DM, total carbohydrates and a week later by 1.92% more, and after two weeks by 6.31% DM, compared to the first harvest. However, the reaction to the date of harvesting of individual varieties was slightly different, because the Borowska cultivar reached the highest carbohydrate content during the second harvest: on average 56.82% DM. Bamberka turned out to be the cultivar richest in total carbohydrates with an average content of 57.02% DM. In our study, the content of total carbohydrates in green plants of white mustard was in the range of 46.66–60.12% DM. Research by Karydogianni et al. [53] carried out on black mustard biomass (*Brassica nigra* L.) indicate the highest total carbohydrate content in unfertilized crops in the range of 68.25 and 67.25% DM in individual growing seasons. On the other hand, for red mustard, Cho et al. [47] indicate the carbohydrate content in the fresh plant at the level of 7–8%, which is an average of 68 g/100 g of dry mass. In our own research, an increase in the total sugar content was observed as the harvest was delayed. Similar observations were made by Hagen et al. [55] in curly kale leaves. In the harvest delayed by 6 weeks, the sugar content increased by 20% compared to the first date. Therefore, the lack of precise information in the literature on the date of harvesting green mustard plants may be the reason for the differences in the results. On the other hand, mustard seeds, due to their higher fat and protein content, contain much less carbohydrates. Sharma et al. [48] report the share of carbohydrates in *Sinapis alba* seeds at about 16%.

The ash content significantly decreased with the growth of the plant and, thus, the delay of the harvest time. However, a slightly different response of individual cultivars to the harvest time was observed. The highest ash content was found in the Borowska cultivar from the first harvest (17.96% DM), and the lowest was in Warta harvested after 45 days of sowing (11.76% DM). Cho et al. [47] give an average ash content in fresh *Brassica juncea* plants of 1.4% (11.3% of dry weight). This is a lower content of minerals than in the tested white mustard (after conversion: 1.48–2.25% DM). In turn, in the research conducted by Kwon et al. [27] on green mustard plants after about 1 month, the crude ash content was significantly dependent on the genotype used in the research and ranged from 1.47% to 1.55%, while in the second genotype from 1.11% to 1. 20%. Karydogianni et al. [53] found that the crude ash content in the aboveground biomass of black mustard was not dependent on plant density; however, plots with low density reached slightly higher values of this feature and it was 14.05% and 13.95% DM in the first and second experimental periods, respectively. Plots with a high density of plants were 13.89% and 13.63% DM for the same experimental periods. In these studies, no significant effect of fertilization on the ash content was found. In our own research, similar values of ash were obtained in relation

to those quoted in the second and third collections, respectively 14.74% and 12.14 DM, which indicates that the literature needs to determine the length of vegetation of the tested plants with potential food use. On the other hand, the content of mineral compounds in seeds is higher and amounts to over 4% [48]. Sawicka et al. [50] report the ash content in the seeds of the Borowska cultivar—4.42% and Bamberka—4.33%.

4.2. Antioxidant Activity and Total Polyphenolic Compound Content

Compounds contained in plant foods, accumulating in plants before harvest, affect the quality of products obtained from them and have a beneficial effect on reducing oxidative stress [56]. For the best knowledge of authors in the published literature there are not many data concerning the antioxidant activity and polyphenolic compound content, including polyphenolic profile in young green parts of white mustard.

In the presented results, it was noticed that the highest amount of polyphenols was observed after 38 days of sowing (8246 mg/100 g), in which also, the maximum antioxidant capacity was obtained by ABTS and FRAP tests. This is an important finding of our study. The maximum antioxidant values using the ABTS method were obtained for the Bamberka cultivar (224.53 µmol Trolox/1 g DM). Soengas et al. [25] suggested that the antioxidant activity of *Brassica* vegetables may be differentiated by genotype. In a similar study, in which the research material was young shoots of buckwheat, the ability to scavenge free radicals was determined at the level of 277.62 µmol Trolox/1 g [57]. This value was similar to the result of the Borowska mustard from the harvest in the second harvest time and allows us to conclude that the green parts of the plants have a high antioxidant capacity. Kapusta-Duch et al. [44], in their research on Brussels sprouts, determined that fresh leaves of this plant have the ability to neutralize free radicals ABTS at the level of 61.6 µmol Trolox/g. On the other hand, in a study carried out on cabbage of the Dolsan cultivar using the ABTS method, 50.07% of RSA was determined [58]. Results of both analyses confirmed that the plants reached their maximum antioxidant capacity after the 38 days of sowing, obtaining 234.37 µmol Trolox/1 g DM in the ABTS method, and 1801 µmol Trolox/1 g DM in the FRAP method. Plants harvested a week later obtained values lower in the ABTS method by about 21%, and FRAP by 29%, in relation to the most favorable result. Similarly, the results of research by Drozdowska et al. [59] showed that the antioxidant activity of red cabbage decreases with growing time. In these studies, young red cabbage leaves had an antioxidant activity determined by the ABTS method by about 100% higher, and by about 35% by the FRAP method, compared to mature ones. It is believed that sprouts of brassica vegetables are characterized by high biological activity [60]. The results of research by various authors indicate a high content of phenolic compounds in the *Brassicaceae* family [15,16] emphasizing that cabbage leaves are particularly rich in polyphenolic compounds. The variable content of polyphenols depending on the time of harvest is pointed out by Yao et al. [23] and Ribeiro et al. [24]. According to the study by Jo et al. [22], the average content of polyphenols in green *Brassica juncea* mustard leaves was 1228.48 mg/100 g of raw material and the total amount of phenols correlated with antioxidant activity. There is a lot of scientific evidence indicating the strong antioxidant effect of polyphenolic compounds [18,19]. For comparison, according to Piątkowska et al. [57], young buckwheat leaves contained 497 mg/100 g of polyphenols. Blackcurrant fruit is considered to be one of the richest sources of polyphenols [61]. It contained an average of 1300 mg/100 g of total polyphenols. Comparing the content of polyphenols in mustard and blackcurrant, the high potential of mustard leaves as a source of polyphenols in a balanced diet was noticed.

Based on the results of the HPLC analysis (available standards for phenolic compounds) it can be suggested that rutin was the compound with a higher amount in all cultivars and in terms of harvest of plant of mustard. Albeit based on the Figures 1–3, it can be concluded that it is not the dominant polyphenol in green white mustard plants. The following compounds were probably identified: luteolin-7-O-glucoside, apigenin-7-O-glucoside apigenin-7-O-glucuronide. Since the identification was based on retention

times, spectra and publications, it should be confirmed in future. Additionally, it should be emphasized that the differences in the retention time between the data from the literature and those determined in these studies may result from the type and concentration of the mobile phase used (water/acetic acid, water acetic acid/acetontryle) [36–39]. This finding requires further research.

To the best knowledge of authors there are not many published article concerning the polyphenolic compounds profile in mustard in various stage of growing. Singh et al. [62] reported that the profile of polyphenolic compounds depends on the part of various factors including cultivar and part of the plant. Rochfort et al. [63] reported that immature leaves of pak choi (*Brassica rapa* L.) are rich sources of kaempferol (36.0–102.6 mg/100 g DM), but their content depends on the variety. Also Romani et al. [64] reported that in turni tops (cultivated in Italy) the following phenolic compounds were identified in higher amounts, as compared to other brassica vegetables: kaempferol, quercitin, ferulic acid and caffeic acid.. It was also reported that, in baby mustard, a higher content of the proanthocyanins and flavonoids was found in leaves [65].

5. Conclusions

It can be concluded that white mustard young plants are a relatively good source of protein and have strong antioxidants properties. They are also a rich source of polyphenolic compounds but more studies are required, especially to assess the content of individual polyphenolic compounds. With the delay in the harvest of green shoots of white mustard from 31 to 45 days after sowing, the content of ash and protein decreased, and the content of crude fat and carbohydrates increased. The highest level of antioxidant activity was found in mustard plants harvested 38 days after sowing. It can be suggested that white mustard can be used in the green stage as a source of nutrients. It is worth considering the harvest time and the appropriate cultivar to obtain the most valuable ingredients of plants.

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