

Article

Nutritional Value and Antioxidant Activity of Fresh Pumpkin Flowers (*Cucurbita* sp.) Grown in Poland

Renata Biezanowska-Kopec^{1,*}, Anna Magdalena Ambroszczyk², Ewa Piatkowska¹ and Teresa Leszczyńska¹ 

¹ Department of Human Nutrition and Dietetics, Faculty of Food Technology, University of Agriculture in Krakow, Balicka Str. 122, 30-149 Kraków, Poland; ewa.piatkowska@urk.edu.pl (E.P.); teresa.leszczynska@urk.edu.pl (T.L.)

² Department of Vegetable and Medicinal Plants, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, 29 Listopada Ave. 54, 31-425 Kraków, Poland; aambroszczyk@interia.pl

* Correspondence: renata.biezanowska-kopec@urk.edu.pl; Tel.: +48-12-6624818

Abstract: Pumpkin flowers, in their composition, contain many bioactive ingredients that have a beneficial effect on the human body. The aim of the research was to evaluate the antioxidant activity and chemical composition of flowers of various species and varieties of pumpkins: Amazonka, Ambar, Atlantic Giant, Bambino (*Cucurbita maxima* L.), Butternut, Muscade de Provence, Rouge vif d'Etampes (*Cucurbita moschata* Duch.), and Miranda (*Cucurbita pepo* L.). The flowers came from flowering pumpkin shoots, grown in Poland (Krakow). The total polyphenols, carotenoids, total sugar contents, antioxidant activity, and fatty acid composition were determined. The content of dry matter, protein, ash, fat, and selected minerals were also determined. Pumpkin flowers of the Atlantic Giant variety were characterized by the highest content of total polyphenols and sugars and antioxidant activity. They also showed the highest percentage of n-myristic acid (C14:0) and docosanoic acid (C22:0). The energy value of fresh pumpkin flowers, of all varieties, was low and averaged 22 kcal/100 g. Fresh pumpkin flowers are a significant source of iron, covering 60–80% of the EAR standard for adults (Atlantic Giant and Bambino varieties).

Keywords: fresh pumpkin flowers; antioxidant activity; polyphenols; health properties



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1. Introduction

Pumpkin, next to zucchini, melon, and cucumber, belongs to the Cucurbitaceae family and is one of the oldest cultivated plants in the world. All the listed parts of the pumpkin (seeds, fruits, and flowers) are edible and contain nutrients that give them specific health-promoting properties [1–3]. Edible flowers are increasingly becoming an integral part of the human diet. They are a good source of antioxidant compounds, which are a very important component of one's daily diet [4]. The nutritional potential of edible flowers is very high. The health benefits associated with the consumption of edible flowers are believed to be due to the content of natural bioactive compounds with pro-health properties, including phenolic acids; flavonoids; anthocyanins; and other phenolic compounds with proven antioxidant, antimicrobial, and antibacterial properties [5–9]. The use of edible flowers in the kitchen can also be due to the decorative, taste, nutritional, and health-promoting values. They are used in many recipes, both in the decorative form and as an addition to various meals. In gastronomy, we can use the flowers of ornamental plants (e.g., violets, daylilies, magnolias, nasturtiums, and geraniums), flowers of trees and shrubs (e.g., acacia and cherry), flowers of herbs (e.g., lavender, coriander, and sage), and flowers of some vegetables (e.g., zucchini and pumpkin). Flowers are prepared in many ways. They can be both fresh and processed, such as jellies and jams. They can be boiled, fried, grilled, and candied; also, when frozen in ice cubes, they are added to drinks. Edible flowers are also used as an addition to tea, ice cream, sorbets, cocktails, salads, melted cheese, and honey [10,11]. An example of edible flowers that is now more and more often available

on the market (also in frozen form) and which has gained great popularity in the food industry is pumpkin flowers [8,11–14]. Since ancient times, pumpkin flowers have been eaten locally as a vegetable in Mexico and India [13]. There are 27 types of pumpkins, but the most commonly cultivated in Poland is giant pumpkin (*Cucurbita maxima* L.), pumpkin (*Cucurbita pepo* L.), and butternut squash (*Cucurbita moschata* Duch.). The giant pumpkin includes many varieties, for example, Amazonka, Ambar, Atlantic Gigant, and Bambino. Botanical varieties such as cucurbit, zucchini, patison, nakedseeded pumpkin (Miranda), and spaghetti pumpkin belong to the species of pumpkin while Butternut squash, Muscade de Provence, and Rouge vif d'Etampes are among cultivars of squash. Taking into account the plants' traits, pumpkins are divided into bushy (e.g., courgette, patison, giant Amazon squash) and creeping (most) pumpkin [15].

They contain many ingredients that have a beneficial effect on the human body. These compounds include polyphenols, phytosterols (α -spinasterol), carotenoids, mono- and polyunsaturated fatty acids, vitamin C and E, and selenium [16]. These compounds show many pharmacological activities, such as antitumor, antigenotoxic, antimutagenic, antibacterial, and anti-inflammatory, are also used in the treatment of diseases caused by oxidative stress, [17–19]. In gastronomic technology, they can be used for frying and stuffing, as well as an addition to, e.g., cakes, omelettes, or salads.

In the available literature, there is little information on the study of flowers of various types of pumpkin, and in particular on determining the profile of fatty acids and the content of selected minerals in them.

The aim of the study was analysis of the chemical composition and estimation the antioxidant activity and the polyphenol content of selected varieties of pumpkin flowers. The selected minerals content and the composition of fatty acids were determined. The energy value of these flowers was calculated. The novelty of the article is a comparison of the chemical composition, fatty acid content, and selected mineral components of flowers of eight pumpkin varieties as potential functional products. These flowers can be the basis for various types of vegetable salads.

2. Materials and Methods

2.1. Research Material

The subject of the research was the flowers of eight pumpkin varieties: Amazonka, Ambar, Atlantic Giant, Bambino, Miranda, Muscade de Provence, Butternut squash, and Rouge vif d'Etampes (Figure 1). The flowers came from flowering pumpkin shoots, sown on the plots of the Experimental Station of Vegetable and Medicinal Plants in Mydlniki, Faculty of Horticulture, University of Agriculture in Krakow, Poland. Two flowers were collected from each plot of 20 plants. The flowers were picked in the morning, before the heat of the day. They were harvested on the same day (pumpkin flowers bloom only 1–2 days). The plants were not subjected to any stress factors (neither by chemical preparations nor unfavorable weather conditions such as drought or torrential downpours). The flowers were randomly collected in July in perforated plastic bags and transported to the laboratory.



Figure 1. Cont.

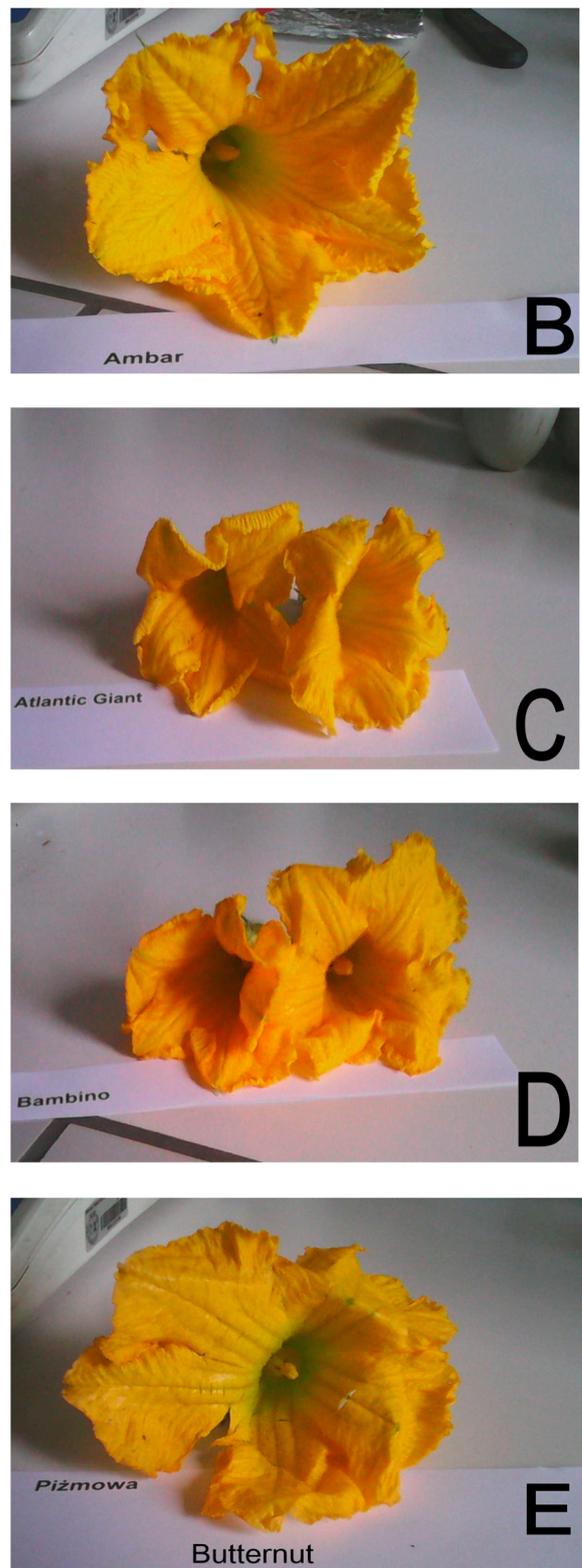


Figure 1. Cont.



Figure 1. Flowers of various species and varieties of pumpkins: (A)—Amazonka; (B)—Ambar; (C)—Atlantic Giant; (D)—Bambino; (E)—Butternut; (F)—Miranda; (G)—Muscade de Provence; (H)—Rouge vif d’Etampes.

2.2. Methods

One part of the sample of flowers was used for direct analysis of dry matter, ash, and preparation of 70% methanol extracts to determine the total polyphenol content and antioxidant activity. The rest of the material was frozen at $-20\text{ }^{\circ}\text{C}$ and then subjected to the lyophilization process in a Christ Alpha (A-4) 6581 07-053 freeze dryer.

The dried material was ground using a laboratory mill (Knifetec Sample Mill 1095) and used for further testing. The chemical composition of pumpkin flowers was determined according to AOAC [20], i.e., protein (method No. 950.36), crude fat (method No. 935.38), dietary fiber (method No. 991.43), dry matter (drying method at $105\text{ }^{\circ}\text{C}$), and ash (method No. 930.05). Carbohydrates were calculated from the following equation [21]:

$$\text{Carbohydrates} = 100 - (\text{water} + \text{protein} + \text{crude fat} + \text{ash}) \quad (1)$$

2.2.1. Determination of Fatty Acid Composition

The fatty acid content was determined by gas chromatography and expressed as a percentage of the total amount of fatty acids. Extraction of lipids from the samples was

performed using the method of Folch [22]. The tested oils were esterified with a mixture of chloroform:methanol (2:1 *v/v*) in the presence of BF_3 as the reaction catalyst. The obtained fatty acid methyl esters were separated on an HP-Agilent 6890N Gas Chromatograph (Shimadzu Corporation, Japan) with a flame ionization detector (FID) and an Rtx 2330 capillary column (100 m length, ID 0.25 mm). The retention times of various acid methyl esters were determined using standards: Supelco 37 No. 47885-U (Sigma Aldrich, St. Louis, MO, USA).

2.2.2. Determination of Minerals

The analysis of the content of selected minerals in pumpkin flowers products, i.e., calcium, iron, magnesium, and zinc, was determined by the method of flame technique of atomic absorption spectrophotometry with atomization (VARIAN AA240FS). The samples were dispensed using an automatic sample dispensing system (SIPS-20). Gas flow: 14 dm^3 (air), 3.5 dm^3/min (acetylene). Before the analysis, the wet digestion of samples with the addition of 4 cm^3 of concentrated HNO_3 in closed pressure vessels was carried out using a Mars Xpress microwave oven (170 °C, 1200 W, 15 min). The examined elements were determined in the Fast Sequential mode during a single sample aspiration. In order to prepare a standard solution containing appropriate ions, the MERCK standards, 1000 mg/dm^3 , were used and subjected to appropriate dilution. After mineralization, samples were diluted with deionized water and their absorbance was determined at the appropriate wavelengths: 422.7 nm (Ca^{2+}), 202.6 nm (Mg^{2+}), 248.0 nm (Fe^{2+}), and 213.9 nm (Zn^{2+}).

2.2.3. Determination of Total Sugars

For carbohydrate determination, proteins were removed from the ethanolic extract after treatment with basic lead acetate. The carbohydrate extracts were then determined by the anthrone method of Yemm and Wills [23]: 1 mL of the extract was incubated in 5 mL of anthrone solution (0.12 g anthrone in 100 mL 6.5 M H_2SO_4) at 90 °C for 10 min. The absorbance of the green product was measured at 630 nm. Results are expressed in μg glucose equivalent (GE) with reference to the standard.

2.2.4. Determination of Carotenoid Content

The amount of β -carotene was measured according to the PN-90/A-75101/12 standard [24] by extracting carotenoids from the test sample using a mixture of acetone:n-hexane (4:6 *v/v*), carotenoid separation on a glass chromatographic column (3 cm inner diameter \times 33 cm), and colorimetric determination of β -carotene on the Spectro 2000RS spectrophotometer by LaboMed at a wavelength of 450 nm. A standard curve of potassium dichromate solution was also determined. Carotenoid content is expressed as an equivalent of beta carotene ($\text{mg}/100$ g FW).

2.2.5. Total Polyphenols and Antioxidant Activity

Total polyphenols was determined by the Folin-Ciocalteu method in 70% methanol extract according to Swain and Hillis [25]. The colored compounds, formed as a result of the reaction between phenolic compounds and the product components present in the extract, were determined spectrophotometrically (using a Rayleigh UV-1800 spectrophotometer) at a wavelength of 760 nm. The standard calibration curve was obtained using several concentrations of chlorogenic acid. Total polyphenol content is expressed as mg of chlorogenic acid equivalent (CAE) per 100 g of fresh mass (FM).

Antioxidant activity was investigated using the stable free radical $\text{ABTS}^{\bullet+}$ according to Re et al. [26]. The amount of the colored radical $\text{ABTS}^{\bullet+}$ (2,2'-azino-bis (3-ethylbenzthialoline-6-sulphonic acid), which remained after the reaction with the antioxidant compounds of the extract (in 70% methanol), was determined spectrophotometrically (Rayleigh UV-1800 spectrophotometer) at a wavelength of 734 nm. Results are expressed in μmol Trolox equivalents (TE) per 1 g of fresh mass (FM). A calibration curve was constructed using Trolox over a concentration range of 10–800 μM .

2.2.6. Statistical Analysis

The obtained results were statistically analyzed using a Microsoft Excel spreadsheet and the Statistica 10 package. The statistical analysis was performed using one-way analysis of variance (ANOVA) for the significance level $p \leq 0.05$. Data are presented per 100 g of fresh mass of flowers.

3. Results

Results of chemical composition of flowers of various species and varieties of pumpkins flowers are presented in Table 1. On the basis of the obtained dry matter results, the average DM (dry mass) content in the pumpkin flowers was 6.42 g/100 g. The average protein content in the fresh mass of pumpkin flowers was 1.31 g/100 g. The highest content of this component was found in the Rouge vif d'Etampes variety (1.50 g/100 g), while the lowest was found in the variety of butternut squash flowers (1.14 g/100 g). The fat content in the analyzed pumpkin flowers ranged from 0.33 g/100 g (Miranda variety) to 0.21 g/100 g (Ambar variety). Among the analyzed fatty acids in pumpkin flowers, no heptadecanoic acid (C17:0) or, in most cases, erucic acid (C22:1) were found. C22:1 acid was only found in flowers of Muscade de Provence and Butternut squash. Additionally, docosanoic acid (C22:0) was not found in the flowers of Ambar and Atlantic Giant varieties. The individual fatty acids in the pumpkin flowers varied within fairly wide ranges (Table 1). The presence of n-myristic acid (C14:0) was found at an amount from 2.48% (Bambino variety) to 0.01% (Rouge vif d'Etampes variety), n-palmitic acid (C16:0) from 33.43% (Ambar variety) up to 13.56% (Muscade de Provence variety), and palmitoleic acid (C16:1) from 2.58% (Ambar variety) to 0.06% (Amazon variety). No amounts of octadecanoic acid (C18:0) were found in the flowers of Ambar pumpkin, while these values were the highest in the Muscade de Provence variety (5.12%). Similarly, the above variety was characterized by the highest oleic acid content (C18:1). The range of linoleic acid (C18:2) ranged from 23.69% (Butternut variety) to 10.63% (Miranda variety), and α -linolenic acid (C18:3) ranged from 62.59% (Miranda variety) to 38.28% (Ambar variety).

The average total carbohydrate content in the fresh mass of pumpkin flowers was 3.84 g/100 g. Miranda (3.90 g/100 g) had the highest content of this nutrient, whilst the Atlantic Giant variety (3.02 g/100 g) had the lowest. Among the total carbohydrates, sugars ranged from 2.43% to 1.36%. The energy value in 100 g of fresh pumpkin flowers ranged from 18.67 kcal to 24.25 kcal (Table 1).

The ash content in fresh pumpkin flowers was on average 1.31 g/100 g. The highest mineral content was found in the Atlantic Giant variety (1.92 g/100 g), and the lowest was found in Miranda (0.85 g/100 g) (Table 1). Among the macronutrients, calcium ranged from 37.77 mg (Miranda) to 14.01 mg (Rouge vif d'Etampes), magnesium ranged from 35.83 mg (Miranda) to 17.72 mg (Ambar), and potassium ranged from 349.66 mg (Muscade de Provence) to 199.73 mg (Amazon). Among the micronutrients, Fe ranged from 5.29 mg to 1.26 mg (for the Butternut and Ambar varieties, respectively), Zn ranged from 0.78 mg to 0.48 mg (for Miranda and the Amazonka and Ambar varieties, respectively), and Mn ranged from 0.38 mg to 0.18 mg (Butternut and Ambar, respectively). The highest energy value of fresh flowers was only 24.25 kcal (Miranda cultivars), and the Atlantic Giant flowers (18.67 kcal) had the lowest energy (Table 1).

The carotenoid and total polyphenol content and antioxidant activity of flowers of various species and varieties of pumpkins are shown in the Table 2. Pumpkin flowers are distinguished by the beautiful yellow-orange color of the petals. This is due to the carotenoid content in them. The strongest color, and thus the highest carotenoid content, corresponded to the Miranda variety (45.82 mg), while the lowest amount was found in butternut squash flakes (15.27 mg). The average carotenoid content of the fresh mass of pumpkin flowers was 29.8 mg/100 g.

Table 1. Chemical composition of flowers of various species and varieties of pumpkins flowers (per 100 g of fresh weight).

| | Amazonka | Ambar | Atlantic Giant | Bambino | Miranda | Muscade de Provence | Butternut | Rouge vif d'Etampes |
|------------------------|----------------------------|-----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| Dry mass [%] | 6.12 ^a ± 0.28 | 6.12 ^a ± 0.63 | 6.32 ^a ± 0.81 | 6.62 ^a ± 1.96 | 6.51 ^a ± 0.5 | 6.58 ^a ± 1.6 | 6.29 ^a ± 0.03 | 6.81 ^a ± 0.46 |
| Protein [g] | 1.19 ^c ± 0.05 | 1.15 ^{cd} ± 0.00 | 1.16 ^{cd} ± 0.00 | 1.44 ^b ± 0.01 | 1.43 ^b ± 0.01 | 1.43 ^b ± 0.00 | 1.14 ^d ± 0.01 | 1.50 ^a ± 0.01 |
| Fat [g] | 0.29 ^{ab} ± 0.05 | 0.21 ^c ± 0.01 | 0.22 ^c ± 0.01 | 0.28 ^{ab} ± 0.02 | 0.33 ^a ± 0.01 | 0.25 ^{bc} ± 0.00 | 0.23 ^{bc} ± 0.03 | 0.31 ^a ± 0.02 |
| Fatty acids [%]: C14:0 | 0.31 ^c | 0.22 ^d | 1.25 ^b | 2.48 ^a | 1.54 ^b | 0.24 ^d | 0.27 ^d | 0.01 |
| C16:0 | 32.18 ^a | 33.43 ^a | 19.28 ^c | 17.91 ^d | 20.43 ^c | 13.56 ^e | 16.42 ^d | 24.23 ^b |
| C16:1 n-7 | 0.06 ^e | 2.58 ^a | 1.09 ^c | 1.59 ^b | 1.26 ^b | 0.95 | 1.06 ^c | 0.63 ^d |
| C17:0 | nd | nd | nd | nd | nd | nd | nd | nd |
| C18:0 | 2.98 ^b | nd | 2.19 ^c | 1.54 ^d | 2.03 ^c | 5.12 ^a | 3.47 ^b | 1.01 ^d |
| C18:1 n-9 | 3.67 ^b | 3.36 ^b | 0.72 ^d | 1.24 ^c | 0.78 ^d | 12.66 ^a | 2.96 ^b | 1.41 ^c |
| C18:2 n-6 | 19.32 ^b | 22.13 ^a | 14.2 ^d | 13.74 ^d | 10.63 ^e | 20.6 ^b | 23.69 ^a | 17.29 ^c |
| C18:3 n-3 | 39.94 ^d | 38.28 ^d | 61.27 ^a | 61.21 ^a | 62.59 ^a | 46.01 ^c | 50.32 ^b | 54.58 ^b |
| C22:0 | 1.54 ^a | nd | nd | 0.29 ^c | 0.74 ^b | 0.3 | 0.84 ^b | 0.84 ^b |
| C22:1 n-9 | nd | nd | nd | nd | nd | 0.56 ^b | 0.97 ^a | 0 |
| Carbohydrates [g] | 3.68 ^{ab} ± 0.2 | 3.68 ^{ab} ± 0.01 | 3.02 ^c ± 0.14 | 3.43 ^b ± 0.17 | 3.90 ^a ± 0.09 | 3.74 ^a ± 0.1 | 3.05 ^c ± 0.08 | 3.81 ^a ± 0.06 |
| Total sugars [g] | 2.30 ± 0.33 | 1.93 ± 0.01 | 2.43 ± 0.18 | 1.97 ± 0.02 | 1.36 ± 0.04 | 1.41 ± 0.08 | 1.64 ± 0.03 | 1.66 ± 0.03 |
| Ash [g] | 0.96 ^{cd} ± 0.2 | 1.07 ^{cd} ± 0.00 | 1.92 ^a ± 0.15 | 1.47 ^b ± 0.15 | 0.85 ^d ± 0.09 | 1.16 ^c ± 0.09 | 1.86 ^a ± 0.04 | 1.19 ^c ± 0.05 |
| Calcium [mg] | 26.16 ^b ± 3.91 | 19.86 ^c ± 1.01 | 13.78 ^d ± 1.54 | 17.77 ^{cd} ± 0.62 | 37.77 ^a ± 1.28 | 28.09 ^b ± 0.96 | 28.89 ^b ± 1.67 | 14.01 ^d ± 0.42 |
| Magnesium [mg] | 23.89 ^b ± 1.3 | 17.72 ^c ± 0.76 | 19.98 ^c ± 0.47 | 24.04 ^b ± 1.64 | 35.83 ^a ± 0.69 | 25.04 ^b ± 0.51 | 18.81 ^c ± 0.18 | 20.10 ^c ± 0.33 |
| Potassium [mg] | 199.73 ^d ± 7.25 | 251.63 ^c ± 19.35 | 236.96 ^c ± 9.02 | 310.75 ^b ± 4.34 | 312.75 ^b ± 12.6 | 349.66 ^a ± 9.74 | 306.15 ^b ± 15.1 | 308.30 ^b ± 0.67 |
| Iron [mg] | 2.02 ^e ± 0.13 | 1.26 ^f ± 0.01 | 5.07 ^{ab} ± 0.16 | 4.85 ^b ± 0.06 | 3.40 ^c ± 0.02 | 2.62 ^d ± 0.08 | 5.29 ^a ± 0.17 | 3.32 ^c ± 0.03 |
| Zinc [mg] | 0.48 ^a ± 0.01 | 0.48 ^a ± 0.04 | 0.55 ^a ± 0.09 | 0.64 ^a ± 0.06 | 0.78 ^a ± 0.07 | 0.50 ^a ± 0.01 | 0.73 ^a ± 0.35 | 0.50 ^a ± 0.02 |
| Manganese [mg] | 0.26 ^{bc} ± 0.02 | 0.18 ^c ± 0.01 | 0.36 ^a ± 0.02 | 0.36 ^a ± 0.02 | 0.31 ^{ab} ± 0.01 | 0.26 ^{bc} ± 0.03 | 0.38 ^a ± 0.06 | 0.21 ^c ± 0.03 |
| Energy value [kcal] | 22.06 ^{bc} ± 0.58 | 21.23 ^c ± 0.06 | 18.67 ^d ± 0.68 | 22.02 ^{bc} ± 0.51 | 24.25 ^a ± 0.3 | 22.92 ^b ± 0.35 | 18.88 ^d ± 0.03 | 23.99 ^a ± 0.12 |

Nd—not detected; ^{a, b, c, ...}—values in the lines marked with different letters differ significantly when $p < 0.05$.

Table 2. Carotenoids, total polyphenols, and antioxidant activity of flowers of various species and varieties of pumpkins (per 100 g of fresh weight).

| | Amazonka | Ambar | Atlantic Giant | Bambino | Miranda | Muscade de Provence | Butternut | Rouge vif d'Etampes |
|-------------------------------------|-----------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|----------------------------|-----------------------------|
| Carotenoids [mg] | 40.17 ^b ± 2.87 | 27.38 ^c ± 4.67 | 30.95 ^c ± 3.93 | 39.01 ^b ± 3.69 | 45.82 ^a ± 3.38 | 21.36 ^d ± 4.31 | 15.27 ^e ± 1.33 | 18.44 ^d ± 2.8 |
| Total polyphenols [mg] ^a | 116.60 ^{cd} ± 1.79 | 156.18 ^b ± 3.88 | 182.07 ^a ± 16.57 | 104.36 ^d ± 34.11 | 127.78 ^{cd} ± 9.15 | 125.10 ^{cd} ± 5.68 | 107.79 ^d ± 1.61 | 146.30 ^{bc} ± 7.31 |
| TEAC [μmol Troloxu/1 g] | 11.55 ^c ± 0.18 | 11.99 ^b ± 0.05 | 12.66 ^a ± 0.09 | 10.53 ^d ± 0.15 | 11.67 ^{bc} ± 0.25 | 10.75 ^d ± 0.32 | 11.46 ^c ± 0.27 | 11.94 ^b ± 0.16 |

^{a, b, c, ...}—values in the lines marked with different letters differ significantly when $p < 0.05$.

The total polyphenol content in fresh pumpkin flowers was on average 133.26 mg CAE/100 g. The highest content was that of the Atlantic Giant variety (182.07 mg), while the lowest content was that of the Bambino variety (104.36 mg CAE/100 g) (Table 2). The antioxidant activity of fresh pumpkin flowers was on average 11.57 $\mu\text{mol TE/g}$. The highest antioxidant activity of fresh weight of the tested flowers was found in the Atlantic Giant variety (12.66 $\mu\text{mol TE/g}$), while the lowest, similar to polyphenols, was found in Bambino (10.53 $\mu\text{mol TE/g}$). The antioxidant potential of the studied pumpkin flower varieties depended on the polyphenol content ($R = 0.84$) (Table 2).

4. Discussion

On average, the fresh pumpkin flowers contained (per 100 g): water, 93.6 g; protein, 1.3 g; fat, 0.3 g; ash, 1.3 g; and carbohydrates, 3.8 g. Other authors presented a comparable (93.72–94.25%) [12] or a lower water content (85.03% by weight) in pumpkin flowers [27]. Such low humidity was demonstrated in the flowers of pumpkins harvested in India. The higher water content in fresh pumpkin flowers was reported by the US Department of Agriculture (95.15 g/100 g). In male pumpkin flowers harvested in the lowlands of Puerto Rico's Caribbean basin, the percentages of protein (1.91–1.37%) and carbohydrate (3.5–3.6%) were comparable to this study, while lower fat levels were shown (0.15–0.09%) [12]. Slightly lower protein content in fresh pumpkin flowers was reported by the US Department of Agriculture [28], according to which the protein content was 1.03 g/100 g. A lower fat content, compared to the results obtained in this paper, was published in the USDA report [28]. According to this report, the amount of fat in the fresh weight of these flowers was 0.07 g/100 g. The fatty acid composition is of particular importance for the nutritional value of the fat. Saturated fatty acids (SFA) and monoenoic fatty acids (MUFA) can be synthesized in the human body. Polyenoic acids (PUFAs) are not synthesized due to the lack of enzyme systems capable of introducing double bonds at the n-6 and n-3 positions of the carbon chain and must be supplied daily with food. Long-chain polyunsaturated fatty acids from the n-3 family (docosahexaenoic acid (DHA, 22:6 n-3) and eicosapentaenoic acid (EPA, 20:5 n-3)) are particularly important for the proper functioning of the nervous and circulatory systems [29,30]. The fatty acid profile presented by some other authors differs from that presented in this paper. Ghosh and Rana [27] showed the highest amounts of oleic acid (21%), myristic acid (15.99%), and stearic acid (15.19%) in pumpkin flowers from India. The ash content of minerals in pumpkin flowers varied, ranging from 0.85% to 1.92%. A lower ash content in 100 g of fresh pumpkin flowers, compared to the results obtained in the study, was reported in the USDA report (0.48 g) as well as by Toro-Velez et al. [12] (0.70–0.78). Fresh pumpkin flowers are a significant source of iron. According to this study, 100 g of fresh pumpkin flowers of the Atlantic Giant or Bambino variety cover 60–80% of the EAR (Estimated Average Requirement) iron level for adults [31]. Such an iron content is twice as high as that in 100 g of spinach (2.8 mg) and is comparable with the content in 100 g of parsley leaves (5.3 mg) [32]. In the pumpkin flowers collected in India, the authors showed a comparable content of Ca (17.6 mg/100 g), but much lower amounts of potassium (only 18.2 mg/100 g) [27]. In the studies of Toro-Velez et al. [12], comparable levels of potassium, magnesium, and iron were obtained, but the maximum amount of Fe was 2.07 mg/100 g. The content of calcium shown by the authors was higher (58.4–52.22 mg), while Mn and Zn were lower (0.21–0.13 mg and 0.17–0.13 mg, respectively). Total carbohydrates in fresh pumpkin flowers in this study were in the range of 3.02–3.90%. A similar carbohydrate content in fresh pumpkin flowers was published in a report by the US Department of Agriculture (3.28 g/100 g) [28], as well as by Toro-Velez et al. [12]. Total sugar (glucose, fructose, and sucrose) as determined by other authors was similar or higher than the results obtained in this study, which ranged from 2.18 to 7.76 g/100 g FW [16]. The energy value of fresh pumpkin flowers was low and amounted to an average of 21.75 kcal/100 g. Pumpkin flowers of Rouge vif d'Etampes were characterized by the highest energy value, with high protein, fat, and carbohydrate content at the same time. The highest energy value was found in the cultivar Miranda and Rouge vif d'Etampes,

and the lowest was found in the flowers of the Atlantic Giant (18.67 kcal/100 g FM) and Butternut (18.88 kcal/100 FM). The lower energy value in fresh pumpkin flowers with respect to the values obtained in this paper was published in a report by the US Department of Agriculture [28]. According to this report, the energy value of fresh pumpkin flowers was 15 kcal/100 g. The consumption of foods rich in antioxidant compounds has a positive effect on the health of the body by inhibiting certain diseases. Carotenoids belong to the group of plant antioxidants which act as plant pigments and also antioxidants. To date, more than 600 carotenoid pigments have been classified. These compounds are divided into carotenes, including α -carotene; β -carotene; γ -carotene; lycopene; and xanthophylls, in which we distinguish lutein, zeaxanthin, violaxanthin, and capsanthin. They are mainly found in fruits, seeds, and flowers. The most common carotenoids in the daily diet include α -carotene, β -carotene, β -cryptoxanthin, lutein, lycopene, and zeaxanthin. As this research shows, the color of pumpkin flowers varies considerably and largely depends on the variety of pumpkin. The β -carotene content of fresh as well as stored pumpkin flowers was also determined by Toro-Velez et al. [12]. The researchers showed that during storage, the content of this component increased in comparison with fresh flowers, containing an average of 10–14 mg of β -carotene/100 g fresh weight of flowers. These values were similar to the least colored butternut squash flowers in our research. Zhou et al. [16] obtained the highest β -carotene content for *C. maxima*, and the lowest for *C. pepo*. The antioxidant activity of plants depends largely on the polyphenol content. This relationship in the studied pumpkin flowers is positive ($R = 0.84$), which means that the activity increases with the increase in the levels of these components. Levels of polyphenols in fresh pumpkin flowers, calculated as gallic acid equivalent (approx. 320–340 mg GAE/100 g FM), as well as antioxidant capacity (39–93 μ M TE/g) were presented by Toro-Velez et al. [12]. Zhou et al. [16] obtained a large variation in the total phenol content of fresh pumpkin flowers, which was related to the pumpkin species. These values were only 50.17 mg GAE/100 g FW for *C. pepo*, but 436.16 and 453.72 mg GAE/100 g FM for *C. maxima* and *C. moschata*, respectively. The most common compounds among the polyphenols are the flavonoids that act as pigments. They give the yellow color to plants found in nature [33]. In addition to giving the characteristic color of plant-based food, they have many other very beneficial properties, e.g., they protect plants against stress, fungi, mold, and ultraviolet radiation. They work as detoxifying agents, antibacterials, and defense compounds, and also play a role in thermal acclimatization of plants, anti-frost resistance, and resistance to drought [33,34]. The dietary role of flavonoids is, e.g., prevention against neurodegenerative diseases, coronary heart disease, and cancer [35,36]. The mechanism of antioxidant activity can manifest itself in two ways. One mechanism is responsible for transforming the radical into a more stable compound. This is done by providing the radicals with a hydrogen atom or electrons, resulting in radical reactions being cut-off. This group includes phenols of the gallates, hydroquinones, trihydroxy-butyrophenones, and tocopherols. The action of compounds such as ascorbic acid, flavonoids, vitamin A, β -carotene, and selenium is based on the capture of oxygen atoms and the chelation of ions that make up the radical molecule [7–9].

5. Conclusions

The fresh pumpkin flowers in this study showed some variability in their nutritional composition. They contain many natural bioactive compounds, such as phenolic acids, flavonoids, anthocyanins, and other phenolic compounds, with already proven antioxidant and antimicrobial properties. Among the determined fatty acids, α -linolenic acid (C18:3), linoleic acid (C18:2), and n-palmitic acid (C16:0) predominated. The lowest energy value, along with low levels of protein, fat, and carbohydrates, was shown by the pumpkin flowers of the Atlantic Giant and Butternut squash cultivars.

Due to their natural antioxidants (especially carotenoids and polyphenols) and high iron content, edible pumpkin flowers can be considered a new functional food, especially for vegetarian and vegan daily diets. The determination of the phenolic acid and heavy

metal profiles, as well as determination of antioxidant activity with other methods, will be taken into account in further research on the flowers of various pumpkin varieties.

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