Research Article

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Biochemical and mineral profile of various parts of fruits Styrian oil squash (*Cucurbita pepo* styriaca group) determined during ripening phases

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Abstract: The natural mutant *Cucurbita pepo* Styriaca Group originates from Styria (Austria), known for its hull-less seeds and dark green pumpkin oil with a roasted nut flavour. Seeds and fruit tissues, such as mesocarp, endocarp, exocarp are notable for their nutritional and bioactive compounds, with potential applications in nutraceuticals and pharmaceuticals. Our research focused on these components throughout five distinct ripening stages over two consecutive years 2020–2021, examining the dynamics of proteins, amino acids, lipids, minerals, and heavy metals. Protein content varied significantly, the highest in the mesocarp and the lowest in the seeds. Glutamic acid and aspartic acid dominated the amino acid profile, while essential amino acids were most abundant in seeds, with leucine, lysine, phenylalanine, valine, and isoleucine reaching notable concentrations. Mineral composition shifted during maturation: potassium and phosphorus declined, calcium increased, and elements such as magnesium, copper, and zinc rose in later stages. Heavy metals remained within safe limits across all phases. Biochemical and mineral profiles vary significantly between tissue types and between maturation stages. Our findings highlight young, unripe fruits as a valuable source of proteins and lipids, with the largely neglected endocarp emerging as a promising raw material for diverse applications. Harnessing these underutilized components could open new avenues in the agri-food sector, animal nutrition, and the pharmaceutical and cosmetic industries, aligning with global trends and local opportunities.

Keywords: endocarp; mesocarp; exocarp; amino acids; fatty acids; elements

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1 Introduction

Pumpkin cultivation in Europe dates back to ancient time and the Middle Ages, with its use expanding during World War II due to food insecurity [1]. The genus *Cucurbita*, belonging to the Cucurbitaceae family, comprises a highly diverse group of species. Five domesticated species – *Cucurbita pepo*, *Cucurbita moschata*, *Cucurbita maxima*, *C. argyrosperma*, and *C. ficifolia* – are cultivated worldwide and rank among the top vegetable crops globally [2–4]. The most significant form today is the oil pumpkin *C. pepo* L., particularly the Styrian type (*C. pepo* Styriaca Group), a phylogenetically young cultivar that originated in 19th-century Styria, Austria [5]. A spontaneous mutation that emerged in the late 19th century gave rise to hull less "naked seeds," governed by a single recessive gene identified by Brancucci and Bänziger, whose retention of only hypodermal lignified tissues makes them uniquely suitable for

producing the characteristic dark green, roasted flavor Styrian pumpkin oil [6, 7].

Pumpkin is increasingly recognized as a functional food due to its high content of nutrients and bioactive compounds [8–15]. Its anatomical parts – not only seeds, but also peel (exocarp), and flesh (mesocarp) – provide a balanced profile of molecules contribute to diverse health-promoting effects [12, 16–18]. *C. pepo* plants (fruits) have a numerous biological activities such as antibacterial [19, 20], antimicrobial [21], antioxidant [22–25], antitumor [26–28], antihelmintic and anti-inflammatory [29, 30], diuretic [31], hypoglycemic (antidiabetic) [32–34], hypolipidemic and anti-hypertensive activities [35–38], antiarthritic and antidepressant activities [39], allopathic therapeutics [40]. Pumpkin seed oil has also a high antioxidant capacity, higher than commonly used oils (sunflower seed oil, rape seed oil, etc.).

The seeds of pumpkin (*Cucurbita* spp.) have traditionally been regarded as agro-industrial by-products [41, 42]. They are consumed in roasted or raw form and incorporated into various culinary preparations, including cereals, breads, cakes, snacks, salads, soups, and other food products. In addition, pumpkin seed flour is frequently utilized as an ingredient in bakery formulations [43]. Food manufacturers are now testing their incorporation into various savoury products, and consumers are responding positively. Increasing awareness of their substantial content of proteins, dietary fibres, minerals, polyunsaturated fatty acids, and phytosterols has led to their recognition as valuable resources for the food industry [44–49]. Moreover, the seeds' potential health-promoting properties – including their effects on blood glucose regulation [50], immune function, cholesterol levels, hypotriglyceridemic and hepatoprotective effect [51], prostate and bladder function [52], mood disorders [53, 54], cognitive impairments, and parasitic infections [19, 55] – are receiving scientific validation.

The processes of maturation and development in fruits and vegetables have attracted considerable scientific interest, not only because of the complexity and dynamic nature of the biochemical, physiological, and structural changes involved, but also due to their relevance as essential components of human nutrition, functional foods, and nutraceutical formulations [56, 57]. Fruit maturity is an important quality factor since it influences both appearance and nutritional value. The ripening process involves alterations in the levels and composition of various bioactive compounds and secondary metabolites that affect the taste, fragrance, texture, and appearance [58]. The ripening stage can also influence antioxidant activity. As ripening occurs, these traits provide the first impression of a fruit's quality [59].

Although significant progress has been achieved in elucidating the molecular and genetic mechanisms underlying fruit and vegetable development, our current understanding of the associated metabolic changes remains largely focused on primary metabolic pathways. In contrast, many compounds recognized for their beneficial effects on human health belong to the group of secondary, or specialized, metabolites. The biosynthesis and accumulation of quality-related metabolites in fruits and vegetables typically follow well-defined and predictable patterns during the course of development.

A substantial portion of fruits – peels, seeds, pomace, and cores – becomes by-products during industrial processing. These components often contain higher concentrations of valuable nutrients including dietary fiber, polyphenols, essential oils, pigments, and minerals. In the frame of modern sustainability-oriented approaches converting this biomass into high-value ingredients. Therefore, this study aimed to determine the accumulation of protein content and its amino acids and lipids in different parts of pumpkin fruits (endocarp, mesocarp, exocarp and seeds) as potential sources for various practical uses, and their increasing and decreasing during ripening five phases. At the same time determination of mineral components and heavy metals and their acceptable range for uses.

2 Materials and methods

2.1 Plant material

Two genotypes of oil *C. pepo* Styriaca Group were used for the experiments. The nomenclature of plant names is according to Danihelka et al. [60]. The genotypes were planted in a 100 × 100 mm clip on 1 ha. Exocarp, mesocarp, endocarp segments, and seeds were separated from 30 fruits of each genotype. Biological material was analyzed for 2020 and 2021. At the same time, it was taken in 5 phases of different fruit development – 10-, 25-, 40-, 55-, and 70-day processes from growth, maturation, and ripening. Chemical analyses of all the samples were provided in the accredited laboratory of EL Company Ltd. in Spišská Nová Ves (Slovakia).

2.2 Lyophilisation

Freeze-drying removed water from pumpkin fruits at extremely low temperatures and pressures. This process took place in three stages: 1. Slow freezing–pumpkin fruits were first deep-frozen to a temperature between −50 and −100 °C. This process takes 12–35 h. 2. Primary drying–then there was a slow increase in temperature and a decrease in pressure, which caused the sublimation of water. The fruits lost 0–95 % of water. 3. Secondary drying–the remaining water molecules were removed, while the

temperature increased above 0 °C. After this drying, only 1–4% of water remained in the fruits. We used a pilot Lyophiliser Epsilon 2-10D LSCplus from CHRIST (Germany).

2.3 Determination of proteins

The crude protein content was determined by the Kjeldahl method, which consists of three basic parts such as mineralization, distillation of ammonia, and titration. The procedures described by Thiex et al. [61]. 1 g of dried plant sample mixed with 4 g of Na_2SO_4 and 0.3 g of $CuSO_4$. After this, 10 mL of H_2SO_4 was added to this mixture. All procedures are conducted on the Kjeldahl distillation system.

2.4 Determination of amino acids

The amino acid (AA) profile was determined by ion-exchange liquid chromatography (Model AAA-400 amino acid analyzer, Ingos, Czech Republic) using post-column derivatization with ninhydrin and a VIS detector [62]. Separation was provided on a glass column (inner diameter 3.7 mm, length 350 mm) that was filled manually with a strong cation exchanger in the LG ANB sodium cycle (Laboratory of Spolchemie) with an average particle size of 12 µM and 8 % porosity. The column was tempered within the range of 35–95 °C. The elution of the studied amino acids took place at a column temperature set to 74 °C. A double-channel VIS detector with an inner cell volume of 5 µL was set to two wavelengths: 440 and 570 nm. A solution of ninhydrin (Ingos, Czech Republic) was prepared in 75 % v/v methyl cellosolve (Ingos, Czech Republic) and 2 % v/v 4 M acetic buffer (pH 5.5). Tin chloride ($SnCl_2$) was used as a reducing agent. The prepared ninhydrin solution was stored in an inert atmosphere (N_2) in the dark at 4 °C. The flow rate was 0.25 (mL/min) and the reactor temperature was 120 °C. Amino acid values were expressed as g/kg of dry sample.

2.5 Determination of lipid content

The lipid samples were subjected to transesterification to obtain fatty acid methyl esters (FAMEs). The resulting FAMEs were quantified by gas chromatography on an Agilent 6890 N system (Agilent Technologies, Santa Clara, USA) equipped with a flame ionization detector (FID) operating at 250 °C, with hydrogen (40 mL/min) and air (450 mL/min) supplied at constant flow. Separation was achieved using a DB-23 capillary column (60 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent Technologies, Santa Clara, CA, USA). Identification of the FAME peaks was carried out by comparison with a C4–C24 FAME standard mixture (Supelco, Bellefonte, PA, USA). Fatty acid (FA) composition was expressed as the percentage of total lipids.

2.6 Determination of minerals/heavy metals content

For elemental analysis, samples were subjected to wet ashing in a microwave digestion system (Milestone 1200, Milestone, Italy). Approximately 0.25 g of the material was digested with 6 mL of nitric acid and 2 mL of hydrochloric acid (Analytika Praha Ltd., Czech Republic). Following mineralization, the solution was filtered through a 0.45 µm membrane filter and quantitatively transferred to a 25 mL volumetric flask, which was then made up to volume with ultrapure water. The determination of elements was carried out using ICP-OES (Ultima 2, Horiba Scientific, France) following the methodology described by Diviš et al. [63].

2.7 Statistical analyses

Data were analyzed with the ANOVA test, and differences between means were compared through the Tukey-Kramer test ($P < 0.05$). The variability of all these parameters was evaluated using descriptive statistics. The results were presented as means with standard error (SE). The PAST 2.17 software was used.

3 Results and discussion

Regarding fruit morphology, a pumpkin consists of several parts (exocarp, mesocarp, endocarp, seeds, remnants of reduced placenta). All of them are potentially interesting raw materials for several uses. However, not all of them are industrially processed and used. Part of the endocarp (where seeds are located) is practically unused, not industrially processed, and goes to waste. We set out to prove that it has high contents of biologically beneficial substances, primary and secondary metabolites, and minerals. The mesocarp forms the largest part of the pumpkin fruit. In our experiments, we determined the percentage of mesocarp from the total fruit in the range of 81–92 %, the same as by Brindza et al. [64]. The proportion of seed weight from the weight of fresh fruits was only 3.2–4.5 % for the species *C. pepo* Styriaca Group. In the same year, Brindza et al. [65] evaluated different landraces of pumpkins and determined the proportion of individual parts of the fruits. Of the total fruit weight, they determined the proportion of seed weight in the range of 1.65–6.58 %, the proportion of endocarp weight in the range of 6.33–12.95 %,

the proportion of exocarp weight in the range of 6.14–16.03 % and the proportion of mesocarp in the range of 69.99–88.50 %. The weight ratio of individual parts of the Styrian pumpkin was also evaluated during the years 2020 and 2021 by Horčinová Sedláčková and Avagyan [66] and Horčinová Sedláčková et al. [67]. From the obtained experimental data, the ratio of the basic anatomical parts of the fruit was determined, represented by 89–90 % mesocarp, 6–8% endocarp, and 3 %–4 % is the most economically used part of the fruit – seeds.

3.1 Protein complex

Some parts of the oil squash plant are a very valuable source of protein. A basic source of protein from all parts of the plant, oil squash seeds (33.77 % in 2021; 36.89 % in 2020). A relatively high content of all essential amino acids, especially valine, lysine, isoleucine, leucine, and phenylalanine, was determined in the seeds. The content of these essential amino acids is higher than 10 g/kg. From the results in Table 1, a higher content of proteins as well as essential amino acids is found at the beginning of fruit formation, and with their gradual formation, their content in the fruits decreases.

We determined 20.89 % of protein in the endocarp, which is almost 5 times more than in the mesocarp (4.93 %). In the exocarp, there is a relatively high protein content, in the range of 13.73–22.21 % (Table 1). These two parts of the-fruit are very valuable for potential use as sources of biologically valuable proteins in large-scale cultivation. Adewusi et al. [68] determined comparable protein content in exocarp (23.95 % dw) with our results for the 2020 year (22.21 %). On the other hand, *C. pepo* fruits had (15.50 % dw), meanwhile during ripening phases, decreased protein content following 23.06 % > 12.11 % < 12.34 % > 12.05 % > 9.92 %.

Pumpkin seeds are considered a very good source of protein. Adewusi et al. [68] in their experiments determined a lower content of crude protein in *C. pepo* seeds (27.48 % dw), Murkovic et al. [69] and Ardabili et al. [70] recorded 25–37 %, in comparison with both evaluated years, 2020 (36.89 %) and 2021 (33.77 %) for *C. pepo* Styriaca Groups. Pumpkin seeds from different regions of Cameron contained 28–40 % protein. Our results of protein content in the seeds are comparable to other studies.

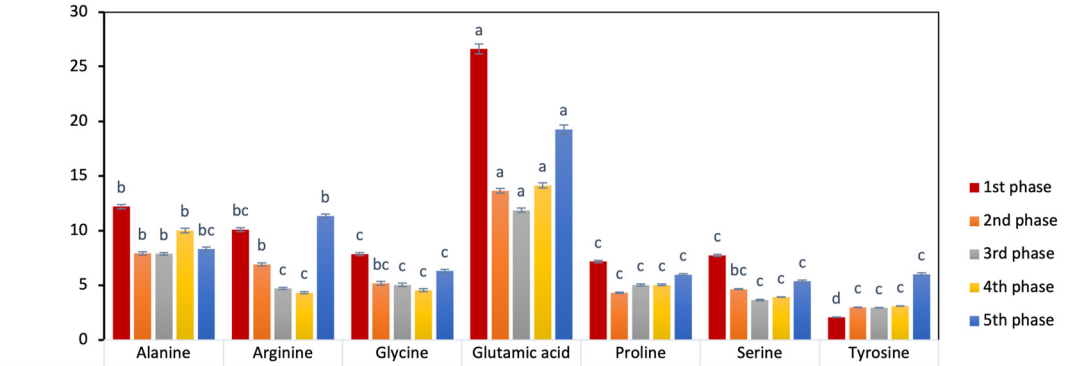
Amino acids play a key role in human nutrition as fundamental building blocks for numerous primary and secondary metabolites, serving either as sources of nutraceutical compounds or as essential dietary constituents [71–74].

The composition of amino acids varied depending on the stage of ripeness. We recorded the highest content of amino acids in the first stage of fruit ripening (Figures 1 and 2). In the second, third, and fourth phases, we noticed a decreasing trend in the content of all amino acids. In the fifth stage of fruit ripening, we noticed a slight increase in the content of all amino acids, which was also related to an increase in the protein content (Table 2). By analyses seven essential amino acids were determined in the pumpkin fruits during 5 ripening phases in the following order: leucin > lysin > valine > phenylalanine > isoleucine > threonine > histidine for 1st, 3rd, 4th phases the same sequence and for 2nd stage histidine had

Table 1: Composition of the protein content of selected parts of fruits *Cucurbita pepo* Styriaca Group.

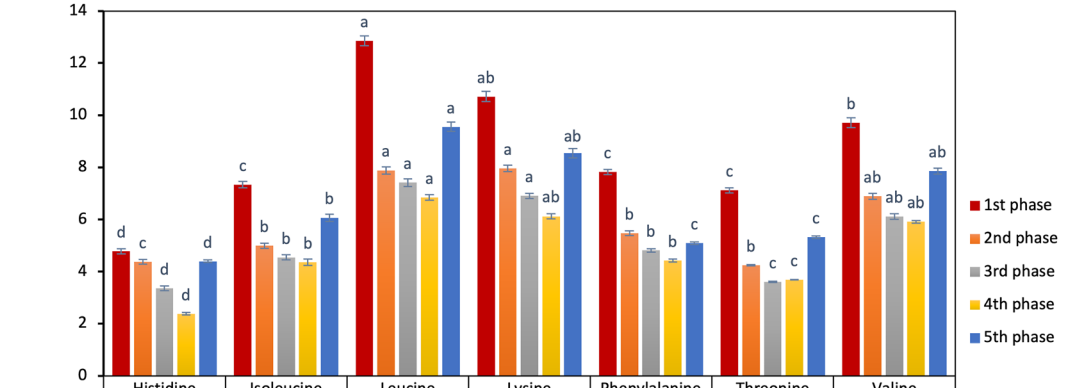
Components (g/kg)	Endocarp	Mesocarp	Exocarp		Seeds	
			Years			
	2021	2020	2020	2021	2020	2021
Proteins (%)	20.89 ± 0.45	4.93 ± 0.18	22.21 ± 0.48	13.73 ± 0.38	36.9 ± 0.60	33.77 ± 0.45
ARG	6.27 ± 0.21	1.60 ± 0.07	8.30 ± 0.25	7.15 ± 0.14	47.70 ± 0.29	47.21 ± 0.51
GLY	6.16 ± 0.18	1.50 ± 0.05	7.30 ± 0.22	6.60 ± 0.13	15.80 ± 0.36	16.13 ± 0.35
HIS	2.91 ± 0.10	1.40 ± 0.05	4.60 ± 0.15	3.29 ± 0.12	8.70 ± 0.15	9.01 ± 0.17
ISO	6.40 ± 0.20	1.30 ± 0.02	6.70 ± 0.15	5.61 ± 0.13	13.10 ± 0.29	13.34 ± 0.32
ASP	20.46 ± 0.45	4.80 ± 0.15	16.10 ± 0.28	n/d	23.00 ± 0.51	27.53 ± 0.48
GLU	29.94 ± 0.51	6.10 ± 0.16	15.50 ± 0.26	10.89 ± 0.25	47.80 ± 0.55	48.22 ± 0.54
LEU	8.34 ± 0.22	2.20 ± 0.08	10.20 ± 0.25	8.61 ± 0.22	22.00 ± 0.45	22.11 ± 0.45
LYS	7.33 ± 0.25	2.40 ± 0.08	7.40 ± 0.21	6.86 ± 0.21	10.40 ± 0.19	11.40 ± 0.21
PHE	6.53 ± 0.15	2.70 ± 0.06	3.70 ± 0.09	5.53 ± 0.18	14.40 ± 0.35	16.81 ± 0.38
PRO	6.07 ± 0.15	2.10 ± 0.06	7.20 ± 0.60	6.25 ± 0.19	14.70 ± 0.28	12.87 ± 0.22
SER	5.13 ± 0.13	1.10 ± 0.02	3.90 ± 0.09	4.59 ± 0.23	9.40 ± 0.25	12.33 ± 0.23
THR	4.36 ± 0.16	1.50 ± 0.05	4.30 ± 0.15	4.49 ± 0.20	7.90 ± 0.25	10.77 ± 0.19
TYR	3.93 ± 0.10	1.10 ± 0.02	4.40 ± 0.18	4.03 ± 0.18	8.10 ± 0.15	7.68 ± 0.14
ALA	9.77 ± 0.21	1.70 ± 0.07	7.20 ± 0.16	6.56 ± 0.19	13.50 ± 0.24	15.58 ± 0.22
VAL	8.1 ± 0.24	0.90 ± 0.02	7.90 ± 0.16	7.31 ± 0.16	19.00 ± 0.38	14.02 ± 0.22

n/d, Not detected; ALA, alanine; ARG, arginine; ASP, aspartic acid; GLY, glycine; GLU, glutamine; HIS, histidine; ISO, isoleucine; LEU, leucine; LYS, lysine; PHE, phenylalanine; PRO, proline; SER, serine; THR, threonine; TYR, tyrosine; VAL, valine.



	Alanine	Arginine	Glycine	Glutamic acid	Proline	Serine	Tyrosine
1st phase	12.19	10.09	7.85	26.6	7.17	7.73	2.05
2nd phase	7.91	6.91	5.16	13.62	4.32	4.63	2.98
3rd phase	7.87	4.69	5.03	11.87	5.02	3.64	2.92
4th phase	10.02	4.31	4.54	14.12	5.03	3.89	3.08
5th phase	8.31	11.34	6.31	19.26	5.96	5.36	6.00

Figure 1: Composition of essential amino acids content (g/kg) of fruits *Cucurbita pepo* styriaca group during 5 ripening phases.



	Histidine	Isoleucine	Leucine	Lysine	Phenylalanine	Threonine	Valine
1st phase	4.78	7.33	12.85	10.72	7.82	7.11	9.71
2nd phase	4.37	4.99	7.88	7.95	5.47	4.24	6.88
3rd phase	3.35	4.54	7.41	6.9	4.81	3.6	6.11
4th phase	2.37	4.36	6.84	6.12	4.42	3.68	5.91
5th phase	4.39	6.06	9.55	8.54	5.09	5.32	7.85

Figure 2: Composition of non-essential amino acids content (g/kg) of fruits *Cucurbita pepo* styriaca group during 5 ripening phases.

the highest content as threonine and for the 5th stage phenylalanine had the lower content as threonine. Sequences of seven non-essential amino acids during fruit ripening phases followed. Glutamic acid > alanine > arginine > glycine > serine > proline > tyrosine for the 1st and 2nd phases, the 3rd and 4th had proline and glycine higher contents than arginine, and the 5th stage had proline and tyrosine comparable contents. Staveckienė et al. [75] investigated the amino acid composition in the fruits of four *Solanum* species throughout the ripening process. Their findings indicated that the highest amino acid concentration was observed at the final ripening stage (Stage III) in *S. melanocerasum* Willd. (2.62 g/100 g). In contrast, for *S. villosum* Mill., *S. nigrum* L., and *S. retroflexum* Dunal, the maximum levels were recorded at the initial stage of ripening (Stage I), amounting to 1.82, 2.45, and 2.65 g/100 g, respectively. Among the essential amino acids, proline ranked as the third most abundant, appearing predominantly in *S. melanocerasum* and *S. villosum* at Stage I, and in *S. nigrum* and *S. retroflexum* at Stage II. In our study, however, proline was detected in smaller quantities compared with glutamic acid, alanine, arginine, and glycine.

The increase in protein levels during ripening may result from the activation of enzymes such as cellulase and polygalacturonase, which facilitate fruit softening. Conversely, protein content can decline by up to 30 % due to biochemical degradation and the formation of secondary metabolites during maturation. While this reduction does not affect nutritional value, it may alter the fruit's flavor profile [76].

Table 2: Correlation dependence between the accumulated content of some amino acids during the development of fruits *Cucurbita pepo* Styriaca group, expressed by the method of Pearson's correlation coefficients.

AA	ALA	ARG	GLY	GLU	HIS	ISO	LEU	LYS	PHE	PRO	SER	THR	TYR
ARG	0.265												
GLY	0.658*	0.825**											
GLU	0.798*	0.783*	0.962**										
HIS	0.148	0.830**	0.785*	0.647*									
ISO	0.650*	0.864**	0.994**	0.971**	0.802*								
LEU	0.712*	0.786*	0.996**	0.972**	0.764*	0.990**							
LYS	0.582	0.820**	0.973**	0.913**	0.884*	0.975**	0.974**						
PHE	0.739*	0.581*	0.907**	0.875*	0.733*	0.893*	0.936**	0.939**					
PRO	0.777*	0.693*	0.920**	0.944**	0.491	0.901**	0.916**	0.807*	0.773*				
SER	0.754*	0.773*	0.971**	0.977**	0.753*	0.979**	0.986**	0.968**	0.946**	0.876*			
THR	0.719*	0.823**	0.987**	0.986**	0.762*	0.995**	0.992**	0.968**	0.915**	0.907**	0.994**		
TYR	−0.485	0.478	−0.050	−0.063	0.109	0.004	−0.126	−0.105	−0.434	−0.027	−0.160	−0.067	
VAL	0.668*	0.836*	0.990**	0.967**	0.815*	0.996**	0.992**	0.987**	0.925**	0.878*	0.990**	0.996**	−0.067

AA, amino acids; ALA, alanine; ARG, arginine; ASP, aspartic acid; GLY, glycine; GLU, glutamic acid; HIS, histidine; ISO, isoleucine; LEU, leucine; LYS, lysine; PHE, phenylalanine; PRO, proline; SER, serine; THR, threonine; TYR, tyrosine; VAL, valine; ** Correlation is significant at $p \leq 0.01$; * correlation is significant at $p \leq 0.05$.

These changes observed along the ripening gradient described Tlili et al. [77] who monitored that protein content in *Rhus tripartitum* increased in two varieties from 4.81 % to 9.37 % and from 6.16 % to 10.5 % during early ripening, followed by a decline to 6.75 % and 7.88 %, respectively–representing a reduction of over 25 %.

Badr et al. [78] investigated the amino acid composition of *C. pepo* exocarp powder, and the most abundant amino acid was aspartic acid (2.64 %), followed by glutamic acid (2.53 %) and leucine (1.21 %). We determined comparable amounts of amino acids mentioned above. On the other hand, we did not evaluate tryptophan content, meanwhile, Badr et al. [78] recorded the highest percentage in the exocarp against mesocarp and seeds. Glycine, glutamic acid, isoleucine, leucine, lysine, phenylalanine, proline, serine, and threonine are highly correlated with other amino acids instead except histidine, which is only positively correlated (Table 2). Tyrosine is non-significantly negatively correlated ($r = -0.485$) with alanine and phenylalanine ($r = -0.434$) and correlated with arginine ($r = 0.478$).

Proteins are present in considerable amounts in pumpkin seeds, with concentrations reported between 31 % and 51 % [79]. Mansour et al. [80] demonstrated that dehulled and defatted seeds of *C. pepo* Kakai 35 exhibit a highly favorable amino acid composition, providing substantial quantities of essential amino acids, including isoleucine (2.66), leucine (6.13), lysine (5.20), cysteine (1.52), methionine (1.25), tyrosine (2.94), phenylalanine (4.00), threonine (2.75), tryptophan (1.56), valine (3.40), and histidine (3.62). In addition, non-essential amino acids are also abundant, such as arginine (16.70), aspartic acid (10.19), glutamic acid (18.13), serine (5.46), proline (4.34), glycine (5.86), and alanine (4.29) (g/16 gN). Generally, oilseeds from the Cucurbitaceae family are recognized as valuable sources of essential amino acids and demonstrate good protein digestibility [81]. Proteins serve as the fundamental structural component of the human body and represent an indispensable nutrient in human diets. They provide nitrogen and amino acids required for the synthesis and maintenance of approximately 25,000 proteins encoded by the human genome, as well as for the production of metabolically active nitrogen-containing compounds such as hormones, neurotransmitters, nucleic acids, glutathione, and keratin. Moreover, the carbon skeleton of amino acids contributes to diverse metabolic processes and may serve as an energy source. Proteins are macromolecular organic substances composed of amino acids, which are needed by the body for the growth and constant renewal of the body's proteins and other metabolically active substances. The recommended physiological ratio of the three essential nutrients–macronutrients in the diet of an adult is: protein 10–15 %, fat 25–35 %, carbohydrates 45–60 % of the daily energy intake [82].

3.2 Lipid complex

The application of gas chromatography detected 18 FAs in lipid fractions extracted from *C. pepo* Styriaca Group samples: lyophilized endocarp, lyophilized mesocarp, lyophilized exocarp, seeds, and fruits during ripening phases and formation. The results of FA composition, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and

polyunsaturated fatty acids (PUFAs) of the studied samples are shown in Table 3. It should be noted that the contents differed significantly ($p < 0.05$) depending on the morphological part of the plant.

The findings demonstrate that the lipid composition of various parts of the oil pumpkin plant exhibits considerable diversity. Among the fatty acids, essential ones are predominant, particularly linoleic (C18:2), linolenic (C18:3), palmitic (C16:0), and oleic (C18:1), which highlight the nutritional relevance of its intake. SFAs were represented mainly by palmitic acid (C16:0), 11.70 % (seeds) – 28.85 % (exocarp), and stearic acid (C18:0), 3.06 % (mesocarp) – 6.73 % (exocarp) of oil. There is significant content of myristic (C14:0) and behenic (C22:0) acids in the exocarp (4.88 and 3.16 %, respectively). The content of other SFAs can be regarded as low. *C. pepo* petals and endocarp were distinguished by the highest amounts of palmitic acid (C16:0). Among MUFAs, oleic acid (C18:1 9c), 6.64 % (exocarp) – 30.30 % (seeds) of oil undoubtedly dominated in pumpkin parts, with the highest content during the 5th stage of fruit development. The difference between the contents of MUFAs in the endocarp and mesocarp was irrelevant. Generally, other MUFAs were identified in rather small quantities in all studied samples. It is noteworthy that the lipid fraction of the *C. pepo* Styriaca group exhibited a particularly high proportion of polyunsaturated fatty acids (PUFAs), despite the identification of only two representatives of this class. Linoleic acid (C18:2 9c, 12c), a member of the *n*-6 family, was the predominant PUFA, with levels ranging from 15.74 % in the exocarp to 50.90 % in the seeds, where it constituted the principal component of the lipid fraction. The second most abundant PUFA was α-linolenic acid (C18:3 9c,12c, 15c), which was especially prevalent in the endocarp (19.20 %) and mesocarp (26.80 %). Consequently, the *C. pepo* Styriaca group can be considered a valuable dietary source of α-linolenic acid, an *n*-3 fatty acid.

According to Brindza et al. [83], linoleic acid represented the predominant fatty acid in both the seeds and bark of *Cornus mas*. A comparable profile was observed in the seeds of *Asimina triloba* (L.) Dunal, which also exhibited a notably high proportion of linoleic acid. Nevertheless, this species was further characterized by a considerable contribution of oleic acid [84]. In the advancing maturity phase, there is a significant reduction in the content of some essential fatty acids such as palmitic (33.1 > 26.4 > 23.4 < 24.7 > 16.2 %) and linolenic acid (39 > 35 > 28.8 > 13.5 > 3.69 %). On the other hand, during fruit formation, the content of oleic acid (2.02 < 3.05 < 8.08 < 21.3 < 33.1 %) and linoleic acid (16.1 < 26.1 < 30.2 > 27.9 < 40.2 %) gradually increases (Table 3). A study by Staveckienė et al. [85] evaluated the content of fatty acids in fruits of four *Solanum* species across different maturity phases. Their results showed that the highest PUFA contents were observed in *S. nigrum* L. fruits in the ripening stage I, and the highest MUFA and SFA contents were observed in *S. melanocerasum* Willd. Fruits during ripening phases I and II, respectively. Myristic acid is highly negatively correlated ($r = -0.913$) with stearic acid. Palmitic acid positively correlated with pentadecanoic ($r = 0.936$), linolenic ($r = 0.858$), behenic ($r = 0.902$), and arachidic ($r = 0.828$) acids. On the other side, negatively correlated with linoleic ($r = -1.000$), oleic ($r = -0.843$), and stearic ($r = -0.545$) acids. Oleic acid is highly negatively correlated with linolenic ($r = -0.993$), arachidic ($r = -0.957$), and behenic ($r = -0.952$) acids and significantly correlated ($r = 0.828$) with linoleic acid. Linolenic acid is highly positively correlated with arachidic ($r = 0.959$) and behenic ($r = 0.9256$) acids (Table 4).

The composition of fatty acids depends on several factors: variety of areas in which the plants are grown, climate [86–88], and state of ripeness [85]. The variability in the oil content is very high, resulting from a broad genetic diversity [89]. Badr et al. [78] determined a higher content of crude lipid in *C. pepo* seeds (38.00 % dw), in comparison to fruits (0.18 % dw) and exocarp (6.57 % dw), meanwhile, pumpkin mesocarp is not a rich source of oil [90]. Pumpkin seeds have significantly higher fat values as demonstrated in the following studies: 37–45 % [69, 89], 41.59 % [70], and 44–53 % [81] in *C. pepo* species. The glyceride fraction contains over 80 % unsaturated fatty acids, mainly linoleic (C18:2; 42 %) and oleic (C18:1; 38 %) acids, and approximately 19 % saturated fatty acids, mainly palmitic (C16:0; 12.7 %) and stearic acids (C18:0; 6 %) [91]. The dominant fatty acids found in the seeds of the 100 breeding lines of *C. pepo* L. convar. *citrullina* – var. *styriaca* with dark green seeds without seed coat and long shoots are palmitic (C16:0; 9.5–14.5 %), stearic (C18:0; 3.1–7.4 %), oleic (C18:1; 21.0–46.9 %) and linoleic (C18:2; 35.6–60.8 %) acids. The average values in our experiments are comparable to the results by the authors Murkovic et al. [89]. The content of these four fatty acids ranges from 98.1 to 98.7 % of the total amount of fatty acids, and the other is found at levels below 0.5 %. As a measure of nutritional value, the ratio of PUFA to SFA in *C. pepo* Styriaca group seeds varied from 2.75 to 2.82. Srbinoska et al. [88] investigated two major pumpkin species, *C. maxima* and *C. pepo*, cultivated in the Republic of Macedonia. For *C. maxima* seed extracts, the PUFA/SFA ratio ranged between 2.51 and 2.78, depending on the solvent applied for extraction. In the case of *C. pepo* whole seed extracts, higher PUFA/SFA ratios were observed when diethyl ether and benzene served as solvents. The SFA and PUFA levels obtained in our extracts are consistent with the ranges previously documented in the literature [88, 89, 92]. Essential fatty acids that must be consumed through food include *n*-3 fatty acids (FAs)

Table 3: Composition of the lipid content of selected parts of fruits *Cucurbita pepo* Styriaca group.

Components (%)	Endocarp	Mesocarp	Exocarp	Seeds	Fruit ripening phases				
					1st stage	2nd stage	3rd stage	4th stage	5th stage
SFAs									
C8:0	<0.075	<0.075	<0.075	<0.075	<0.075	<0.075	<0.075	<0.075	<0.075
C10:0	<0.12	<0.12	0.17 ± 0.01	<0.12	<0.12	< 0.12	<0.12	<0.12	<0.12
C12:0	0.24 ± 0.016	<0.20	1.98 ± 0.07	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
C13:0	<0.13	<0.13	<0.13	<0.13	<0.13	<0.13	<0.13	<0.13	<0.13
C14:0	0.66 ± 0.037	1.84 ± 0.09	4.88 ± 0.13	<0.12	0.19 ± 0.013	0.28 ± 0.018	0.27 ± 0.018	0.35 ± 0.022	0.14 ± 0.01
C15:0	<0.07	<0.07	0.30 ± 0.03	<0.07	0.50 ± 0.03	0.30 ± 0.019	0.28 ± 0.018	0.19 ± 0.013	0.11 ± 0.0082
C16:0	19.10 ± 0.65	23.40 ± 0.78	28.85 ± 1.24	11.70 ± 0.43	33.10 ± 1.00	26.40 ± 0.86	23.40 ± 0.78	24.70 ± 0.81	16.20 ± 0.57
C17:0	<0.12	<0.12	0.53 ± 0.05	<0.12	0.25 ± 0.016	0.34 ± 0.021	0.57 ± 0.033	0.25 ± 0.016	<0.12
C18:0	3.23 ± 0.14	3.06 ± 0.14	6.73 ± 0.17	5.92 ± 0.24	4.16 ± 0.18	3.59 ± 0.16	3.98 ± 0.17	3.57 ± 0.16	4.96 ± 0.21
C20:0	<0.068	<0.068	1.26 ± 0.02	0.36 ± 0.022	1.80 ± 0.088	1.96 ± 0.094	1.30 ± 0.067	1.00 ± 0.053	0.59 ± 0.034
C22:0	1.01 ± 0.054	2.94 ± 0.13	3.16 ± 0.14	0.27 ± 0.018	2.42 ± 0.11	2.18 ± 0.10	2.07 ± 0.099	1.74 ± 0.085	0.53 ± 0.031
MUFAs									
C14: 1	<0.074	<0.074	<0.074	<0.074	<0.074	<0.074	<0.074	<0.074	<0.074
C16: 1	0.49 ± 0.029	1.45 ± 0.073	0.79 ± 0.05	<0.24	0.37 ± 0.023	0.47 ± 0.028	0.58 ± 0.034	5.11 ± 0.21	0.23 ± 0.015
C18: 1	10.90 ± 0.41	9.82 ± 0.37	6.64 ± 0.10	30.30 ± 0.97	2.02 ± 0.097	3.05 ± 0.14	8.08 ± 0.31	21.30 ± 0.72	33.1 ± 1.00
C20: 1	<0.068	<0.068	<0.068	<0.068	<0.068	<0.068	<0.068	<0.068	<0.068
C22: 1	<0.064	<0.064	0.77 ± 0.06	<0.064	0.21 ± 0.014	0.27 ± 0.018	0.24 ± 0.016	0.23 ± 0.015	< 0.064
PUFAs									
C18:2	45.20 ± 1.40	30.70 ± 0.98	15.74 ± 0.63	50.90 ± 1.50	16.10 ± 0.57	26.10 ± 0.85	30.20 ± 0.96	27.90 ± 0.90	40.20 ± 1.20
C18:3	19.20 ± 0.66	26.80 ± 0.87	19.80 ± 0.47	<0.24	39.00 ±1.2	35.00 ± 1,10	28.80 ± 0.93	13.50 ± 0.49	3.69 ± 0.16

SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids. SFAs, Octanoic acid C8:0; Decanoic acid C10:0; Lauric acid C12:0; Tridecanoic acid C13:0; Myristic acid C14:0; Pentadecanoic acid C15:0; Palmitic acid C16:0; Heptadecanoic acid C17:0; Stearic acid C18:0; Arachidic acid C20:0; Behenic C22:0; MUFAs: Myristoleic acid C14:1; Palmitoleic acid C16:1; Oleic acid C18:1; Eicosenoic acid C20:1; Erucic acid C22:1; PUFAs: Linoleic acid C18:2; Linolenic acid C18:3.

Table 4: Correlation dependence between the accumulated content of some saturated and unsaturated fatty acids during the development of fruits *Cucurbita pepo* Styriaca group expressed by the method of Pearson's correlation coefficients.

Component	C14:0	C15:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0
C15:0	−0.062								
C16:0	0.230*	0.936**							
C16:1	0.747**	−0.309	0.016						
C18:0	−0.913**	−0.290	−0.545	−0.518					
C18:1	−0.253	−0.864**	−0.843	0.284*	0.594*				
C18:2	−0.229	−0.932	−1.000	−0.033	0.538*	0.828**			
C18:3	0.145*	0.906**	0.858**	−0.356	−0.507	−0.993	−0.844		
C20:0	0.208*	0.821**	0.828**	−0.295	−0.586	−0.957	−0.812	0.959**	
C22:0	0.463*	0.837**	0.902**	0.008	−0.741	−0.952	−0.893	0.926**	0.881**

** Correlation is significant at $p \leq 0.01$; * correlation is significant at $p \leq 0.05$. SFAs: Pentadecanoic acid C15:0; Palmitic acid C16:0; Stearic acid C18:0; Arachidic acid C20:0; Behenic C22:0; MUFAs: Palmitoleic acid C16:1; Oleic acid C18:1; PUFAs: Linoleic acid C18:2; Linolenic acid C18:3.

(alpha-linolenic acid) and *n*-6 fatty acids (linoleic acid). They are essential and must be taken in through food. These two FAs are the most abundant among other PUFAs in the human diet. Essential FAs have the following functions in the body: formation of eicosanoids, formation of cell membranes (as part of phospholipids), construction of nervous tissues, and importance in reproduction. Linoleic acid is key to maintaining the skin barrier in humans (it is part of the skin ceramides). Arachidonic acid is a precursor of *n*-6 eicosanoids, namely prostanoids series 2 and leukotrienes series 4, with potent pro-inflammatory effects. Other *n*-3 fatty acids are formed from alpha-linolenic acid in the metabolism by desaturation and elongation reactions, it is a precursor of *n*-3 long-chain PUFAs. The conversion of α-linolenic acid to EPA is about 8–12 %, while the conversion to DHA may be less than 1 %. DHA is a component of membrane structural lipids, especially phospholipids, in nervous tissue and the retina of the eye. Many studies have demonstrated beneficial effects of *n*-3 long-chain PUFAs on cardiovascular disease risk factors [93–95].

3.3 Content of minerals

The results document that oil squash is a source of mineral elements in addition to nutrients. From the results, it can be assumed that during fruit formation, the content of some macro-elements in fruits decreases (potassium, phosphorus) or increases (calcium). During the 1st stage of fruit formation, the amounts of some minerals were higher in comparison to the following phases (2nd, 3rd, 4th), and the last ripening 5th stage moderately increased (K, Mg, Cu, Zn). Microelements also have the same course of storage and processing of substances during fruit formation (Table 5).

Potassium, magnesium, and calcium are essential elements that influence fruit quality traits such as color, uniformity of ripening, hollow fruit, fruit shape, firmness, and acidity [96]. Calcium is involved in the normal functioning of cell membranes. It activates certain enzymes and plays an important role in regulating metabolic activities [97]. The highest content of Ca was contained in the exocarp (4,455 mg/kg) in comparison to seeds (795 mg/kg). Across the ripening

Table 5: The content of minerals in selected parts of *Cucurbita pepo* Styriaca group.

Component (mg/kg)	Endocarp	Exocarp	Seeds	Fruit ripening phases				
				1st stage	2nd stage	3rd stage	4th stage	5th stage
Ca	3,660	4,455	795	3,930	8,050	7,580	7,690	8,720
K	71,400	30,300	9,490	61,000	33,300	32,400	34,300	41,800
P	4,495	4,660	12,220	10,030	7,366	7,132	7,919	6,613
Mg	2,140	1,715	5,070	4,150	3,020	3,220	3,250	3,700
Mn	3.8	8.7	41.8	14.3	7.0	6.3	4.6	20
Na	80	29.8	16.3	62.8	19.8	19.2	20.7	19.4
Fe	60.8	48.25	94.7	n/d	n/d	n/d	n/d	n/d
Cu	20.5	5.5	14.4	12.6	6.4	7	8	9.6
Co	<0.1	<0.1	<0.1	n/d	n/d	n/d	n/d	n/d
Zn	45.7	14.75	91	71	31	28.4	31.3	39.3

n/d, not detected.

phases of fruits, its content changed following: 1st 3,930 < 2nd 8,050 > 3rd 7,580 < 4th 7,690 < 5th 8,720 mg/kg. Calcium is one of the most abundant minerals in the human body, accounting for 1–2% of total body weight in an adult. About 99 % of calcium is in teeth and bones, the remaining 1 % in serum. It forms the structure of bones and teeth, and is a key mediator for nerve transmission, myocardial function, muscle excitation and contraction, coagulation, cell division, intercellular signaling, and enzyme regulation.

Potassium (K) is the most abundant cation to balance negative ions and plays important roles in plant growth and development, where it contributes to charge balance, osmotic adjustment, and enzyme catalysis [98]. The highest content of K was contained in the endocarp (71,400 mg/kg) compared to the least content in the seeds (9,490 mg/kg). Across the ripening phases of fruits, its content changed following: 1st 61,000 > 2nd 33,300 > 3rd 32,400 < 4th 34,300 < 5th 41,800 mg/kg. Potassium is an essential intracellular fluid cation in the human body. It plays an important role in the distribution of water inside and outside cells, participates in the regulation of acid-base balance, and contributes to the formation of membrane potential and thus activity in nerve and muscle cells. In cells, it is involved in energy conversion, hormone secretion, and the regulation of protein and glycogen synthesis. The ratio of the mineral components sodium: potassium (Na:K) is an important indicator in diets because low Na and high K act synergistically to reduce blood pressure and the prevalence of arterial hypertension [99]. Just the low level of Na:K ratio makes pumpkins an interesting commodity. Analysis of microelements showed results of ratios Na:K in the various parts of the pumpkin fruits as follows: for endocarp 80:71,400 = 0.00112, exocarp 29.8:4,455 = 0.00668, seeds 16.3:9,490 = 0.00171, and fruits have values according to ripening phases 0.0197 (1st) > 0.00245 (2nd) < 0.00253 (3rd) < 0.00269 (4th) > 0.00222 (5th).

Phosphorus plays a key role in regulating plants' physiological responses to abiotic stresses [100]. The highest content of phosphorus (P) was contained in the seeds (12,220 mg/kg) compared to the lower comparable contents in the endocarp (4,495 mg/kg) and exocarp (4,660 mg/kg). Across the ripening phases of fruits, its content changed following: 1st 10,030 > 2nd 7,366 < 3rd 7,132 > 4th 7,919 250 > 5th 6,613 mg/kg. Phosphorus is the major intracellular anion in the human body. Most of it is in bones and teeth (about 85 %), 14 % in soft tissues such as muscles, liver, heart, and kidneys, and 1 % is found in the extracellular fluid. It is involved in many physiological processes such as energy conversion, regulation of acid-base balance, cellular regulation and signaling, mineralization of bones and teeth, and is a component of cellular structures.

Magnesium (Mg) activates the enzymes of respiration and photosynthesis and is involved in the synthesis of DNA and RNA [101]. The highest content of Mg was contained in the seeds (5,070 mg/kg) compared to the least content in the exocarp (1,715 mg/kg). Across the ripening phases of fruits, its content changed following: 1st 4,150 > 2nd 3,020 < 3rd 3,220 < 4th 3,250 < 5th 3,700 mg/kg. Magnesium is mainly found in the intracellular fluid of the human body, its content in the body is about 25 g. About 50–60 % is in the bones, the remaining 40–50 % is found in the heart muscle, skeletal muscle, liver, and extracellular fluid (about 2 %). It is a factor in about 300 enzymes important in the metabolism of carbohydrates, fats, proteins, and nucleic acids, and is also active in the organs of the neuromuscular and cardiovascular systems. Physiological functions include glucose metabolism, fatty acid synthesis and breakdown, DNA and protein metabolism, control of inflammation, central nervous system function, energy production, and bone metabolism.

It is reversibly oxidized from Fe^{2+} to Fe^{3+} during electron transfer. It activates the catalase enzyme and is essential for the formation of chlorophyll [102]. The highest content of Fe was contained in the seeds (94.7 mg/kg) in comparison to the exocarp (48.25 mg/kg). Iron is essential in the human body for oxygen transport, glucose metabolism (as a component of cytochromes), enzyme activity, immune system functioning, and free radical reduction. In the human body, it occurs in four forms: incorporated in hemoglobin (in red blood cells and erythroblasts), bound to transferrin in serum, bound to proteins (myoglobin, catalase and peroxidase and cytochrome complex), storage forms of iron (ferritin and hemosiderin).

Manganese activates many enzymes involved in photosynthesis, respiration, and nitrogen metabolism. The best-defined function of manganese is in the splitting of water to liberate oxygen during photosynthesis [103]. The highest content of Mn was contained in the seeds (41.8 mg/kg) compared to the lowest content in the endocarp (3.8 mg/kg). Across the ripening phases of fruits, its content changed following: 1st 14.3 > 2nd 7.0 > 3rd 6.3 > 4th 4.6 < 5th 20 mg/kg. In the human body, manganese is a cofactor of many enzymes with different functions, such as superoxide dismutase, hydrolase, and kinase. It is involved in the metabolism of amino acids, lipids, and carbohydrates.

The correlation coefficients between the variables and their corresponding P-values were obtained, showing significant correlations ($p < 0.05$) for most of the parameters studied. Calcium negatively correlated with all minerals, but especially highly negatively correlated with sodium ($r = -0.973$) and phosphorus ($r = -0.970$). On the other side, potassium high positively correlated with zinc ($r = 0.996$), copper ($r = 0.969$), magnesium ($r = 0.954$), and sodium ($r = 0.948$). Phosphorus is highly positively correlated with sodium ($r = 0.944$), magnesium with copper (0.989) and zinc

Table 6: Correlation dependence between the accumulated content of some macro- and microelements during the development of fruits *Cucurbita pepo* Styriaca group expressed by the method of Pearson's correlation coefficients.

Component	Ca	K	P	Mg	Mn	Na	Cu
K	−0.856*						
P	−0.970**	0.815*					
Mg	−0.719	0.954**	0.654				
Mn	−0.111	0.595	0.024	0.734			
Na	−0.973**	0.948**	0.944**	0.833*	0.318		
Cu	−0.768	0.969**	0.735	0.989**	0.660	0.872*	
Zn	−0.893*	0.996**	0.859*	0.925**	0.527	0.972**	0.948**

** Correlation is significant at $p \leq 0.01$; * correlation is significant at $p \leq 0.05$. **

(r = 0.925), sodium (r = 0.972) and copper (0.948) each other with zinc (Table 6).

Experiments have shown that lyophilized exocarp and seeds are rich in potassium and phosphorus, as well as magnesium and zinc. Mala and Kurian [104] determined the nutritional composition of pumpkin exocarp, and the content of phosphorus was 319.13 mg/100 g which is lower than our value (4,660 mg/kg) and iron was 42.99 mg/100 g which is 10 times higher than our value 48.22 mg/kg.

Martínez-Valdivieso et al. [105] studied the mineral content of *C. pepo* seeds, fruits, and exocarp and found that Ca is higher in seeds as compared to fruit and exocarp. We reported another trend, where fruits and exocarp have a higher content of potassium, sodium, and calcium than seeds. We must report another trend, where fruits in the 1st stage of development have the highest content of minerals in comparison to other phases. Dalda-Şekerci et al. [106] studied 36 ornamental pumpkin genotypes collected from different regions of Turkey and determined in the seeds the most abundant macro element phosphorus P that was in the range of 13.902–28.686 mg/kg, the same in our experiments but with lower value 12,220 mg/kg, followed by potassium K (8,490–21.798 mg/kg) in comparison of K (9,490 mg/kg) for *C. pepo* Styriaca Group, and magnesium Mg (3,626–7,692 mg/kg) is comparable with our value 5,070 mg/kg. Dalda-Şekerci et al. [106] determined the average concentration of microelements ordered as Fe, Zn, Mn, Na, Cu, and B with 116.10, 64.21, 42.12, 35.74, 25.10, and 9.61 mg/kg, respectively. The maximum and minimum concentrations of microelements were iron (65.34–157.40 mg/kg), zinc (31.86–96.12 mg/kg), manganese (23.04–53.28 mg/kg), sodium (6.54–85.38 mg/kg^{-}, copper (17.04–42.30 mg/kg) and boron (6.48–13.26 mg/kg). Our results are comparable to the average values by Dalda-Şekerci et al. [94].

According to Mansour et al. [80] particularly phosphorus, potassium, magnesium, calcium, iron, and zinc are the main minerals (P 17.831; K 13.736; Mg 5.688; Ca 1.643; Fe 0.211; Na 0.332; Cu 0.016; Zn 0.190; Mn 0.080 in g/kg) in pumpkin seed of *C. pepo* Kakai 35. Values of mineral content were comparable with our results, whereas phosphorus (12,200 mg/kg), potassium (9,490 mg/kg), calcium (795 mg/kg), and sodium (16.3 mg/kg) were lower in *C. pepo* Styriaca Group. Selenium is of particular importance as its content ranges between 0.08 and 0.4 µg/g, one of the highest values found in plants [107, 108]. Other sources are reporting an even higher amount of selenium: 1.29 g/g [79]. Badr et al. [78] determined the content of mineral substances in the powder of exocarp *C. pepo,* and the values of calcium, iron, zinc, and copper were 5,571, 247.33, 42.92, and 12.91 mg/100 g of dry matter, respectively. Application of *C. moschata* seed meal as a good source of zinc to malnourished Wistar rats showed significant body weight gain and increased serum zinc levels [109]. Steiner-Asiedu et al. [110] researched the nutrient composition and protein quality of four different types of pumpkin fruits. Upon mineral analysis, found higher values of Zn 5.1–7.1 mg/100 g, Cu 1.4–7.9 mg/100 g and Fe 5.6–8.5 mg/100 g, which is comparable with our results. Nield et al. [111] analyzed the pericarp, embryo, and testae of mature squash *C. maxima* fruits to find that the orientation of the fruit did not influence the concentration of the minerals in the samples. Results also showed that the stigma and stalk regions differed in their Ca and K concentrations in the middle and innermost samples. The mineral concentrations in the endocarp did not differ from the other regions. K and Ca were more concentrated in pericarp samples than in embryo samples, whereas P and Mg had the opposite relationship.

3.4 Heavy metal content

In 2020 and 2021, individual parts of the plant were chemically analyzed for the content of heavy metals such as Pb (lead), Cd (cadmium), Hg (mercury), and As (arsenic). The determined values of the characters mentioned are shown in Figure 3. The results of the chemical analysis show that the content of all evaluated heavy metals was demonstrated in the individual

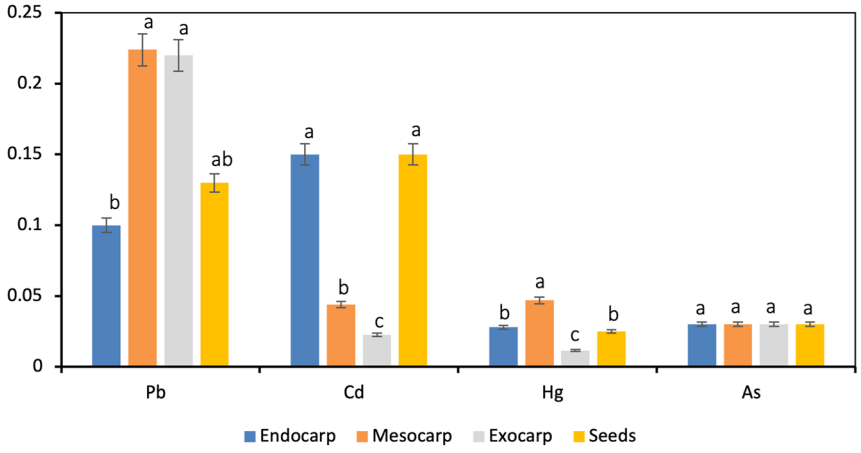


Figure 3: Heavy metal content (mg/kg) of selected parts of squash oil fruits *Cucurbita pepo* styriaca group.

parts of the oil squash fruits and during the ripening phases of the fruits (Figure 4). However, their representation does not exceed the hygienically permitted limits.

Many authors studied pumpkin seeds due to their content of minerals and heavy metals. Glew et al. [79] contained relatively large amounts of potassium (5,790 µg/g dw) and chromium (approx. 3 µg/g dw). However, the sodium content of pumpkin seeds was low (6.9 µg/g dw). Pumpkin seeds contained relatively large amounts of magnesium Mg (5,690); zinc Zn [113]; copper Cu (15.4); molybdenum Mo (0.805) and other minerals: phosphorus P (15,700); calcium Ca (346); iron Fe [106]; manganese Mn (49.3); aluminum Al (9.21); barium Ba (1.16); cobalt Co (0.29); strontium Sr (1.83); nickel Ni (0.53); arsenic As (0.45) (in µg/g dw). Noteworthy was the low amounts of calcium in the seeds (346 mg/kg), meanwhile experiments of Styrian pumpkin revealed high contents of calcium in the endocarp (3,660 mg/kg), exocarp (4,455 mg/kg) and fruits during ripening phases (3,930 < 8,050 > 7,580 < 7,690 < 8,720 mg/kg).

Carvalho et al. [112] reported in their study the mineral analysis of pumpkin mesocarp, which indicated that contained high levels of Mn (0.5 mg/kg), Fe (1.37 mg/kg), Cu (3.9 mg/kg), Pb (0.29 mg/kg), P (11.38 mg/kg), Ni (0.5 mg/kg), Ca (179 mg/kg), Mg (190 mg/kg), Na (159 mg/kg) and K (160 mg/kg). The levels of Pb (0.21–0.25 mg/kg) and Cu (2–5 mg/kg) are within the acceptable range for FAO [113]. Compared these results in the contents of mineral elements of endocarp in our study, we determined several times higher values, such as P (4,495 mg/kg), Ca (3,660 mg/kg), Mg (2,140 mg/kg), K (71,400 mg/kg). FAO/WHO Expert Committee on Food Additives [113] allocated a Provisional Tolerable Weekly Intake (PTWI) of 400–500 µg of cadmium (Cd) per person or expressed it in terms of intake per kg of body weight (7 µg per kg of body weight) and 3 mg of lead (Pb) per person, equivalent to 50 µg per kg of body weight. The levels of Pb and Cd are within the acceptable range of FAO (1993).

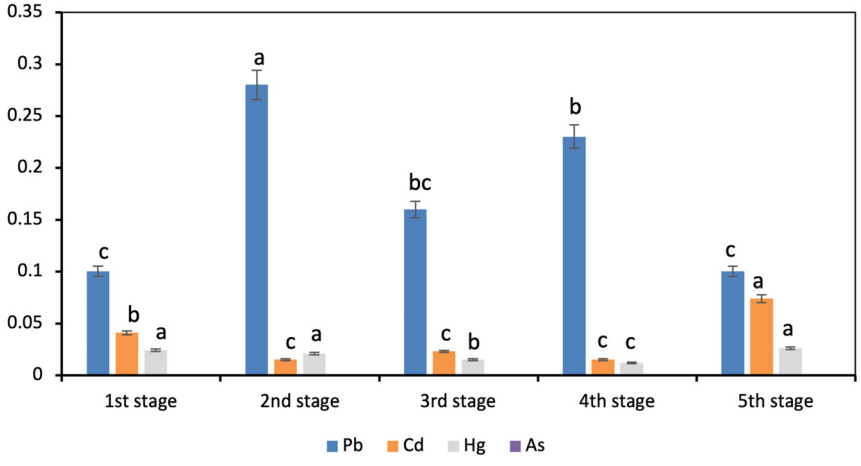


Figure 4: Heavy metal content in the fruits *Cucurbita pepo* styriaca group during ripening phases.

4 Conclusions

Results of this study provide additional and valuable insights into the accumulation of protein content, amino acids, fatty acids, minerals, and heavy metals content in the fruits with a comparison of various fruit parts, such as endocarp, mesocarp, exocarp, and seeds. Ripening fruit formation and development of fruits were associated with increasing contents in all evaluated parameters during the 1st ripening stage and subsequently decreasing up to the 4th stage, then in the 5th ripening phase, moderately increasing. Of the essential amino acids in all parts of the fruit, this study revealed the lowest content in the mesocarp, which is the most processed industry part, and the highest content in the seeds. Styrian pumpkin is a rich source of arginine, which is an amino acid that contributes to several aspects of human biochemistry. Biochemical and mineral profiles vary significantly between tissue types and between maturation stages. All of them parts are potentially interesting raw materials for several uses. However, not all of them are industrially processed and used. Part of the endocarp is practically unused, not industrially processed, and goes to waste. We set out to prove that endocarp has high contents of biologically beneficial substances, primary and secondary metabolites, and minerals. Results showed higher content compared to other parts. Moreover, it can be an important raw material for various uses. The content of all evaluated heavy metals was demonstrated in the individual parts of the oil squash fruits and did not exceed the hygienically permitted limits. Individual parts of oil pumpkin fruits can be more practically used in the agri-food industry, and livestock feeding, pharmaceutical, and cosmetic industry, just like in the world and our conditions.

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