ACCEPTED ARTICLE

The Relations between Some Phytochemical Properties and Fatty Acid Content of Pumpkin (*Cucurbita pepo* L.) Seeds

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Running title: Some Phytochemical Properties in different pumpkin seeds

ABSTRACT

Pumpkin (*Cucurbita pepo* L.) seeds are popular for their dietary and health benefits. However, there were limited data on the pathway between phytochemical and nutritional values of pumpkin seeds. For this purpose, the seeds of some Turkish pumpkin genotypes (NVS-1, NVS-2, KNY, KYS-1, KYS-2, BRS, EDR and KRK) were analysed for their amino acids, organic acids, fatty acids, and mineral content. The wide variation between seeds in organic acids (KYS-2, 8.105 ng μl^{-1}; KRK, 1.939 ng μl^{-1}) and amino acids (KYS-2, 32.99 nmol μl^{-1}; KNY, 15.65 nmol μl^{-1}) content was observed. C18:2n6 and C18:1n9 were the most predominant fatty acids in the seeds, whereas C16:1n7 was the least abundant. Considering the mineral contents, seeds were relatively rich in potassium (2560.3-6697.5 mg kg^{-1}), phosphorus (529.8-1120,9 mg kg^{-1}), and magnesium (426- 1124,5 mg kg^{-1}). Moreover, the path diagram of phytochemical properties, nutritional value, and fatty acids of pumpkin seeds was determined. Consequently, the seeds of pumpkin cultivars were examined to find the best potential for a high nutritional value and contribution to the food industry.

Keywords: Pumpkin seeds, organic acid, amino acid, fatty acid, minerals.

INTRODUCTION

Pumpkin (*Cucurbita pepo* L.) is commercially grown in many regions of the world. The seeds of pumpkin have economic significance and are consumed as snack food in the Mediterranean region, particularly in Turkey and other Middle Eastern countries (Al-Khalifa, 1996). Recently, pumpkin silage is also used in cow feed owing to its valuable source of bioactive compounds (Halik *et al.*, 2018). Moreover, many growers and breeders have focused on pumpkins and their seeds, mainly aiming for drought resistance and high nutritional values, including proteins as well as high oil content (Idouraine *et al.*, 1996). For example, pumpkin seed is an abundant source of fatty acids and 98% of them contain oleic, linoleic, stearic and palmitic acids (Younis *et al.*, 2000; Murkovic *et al.*, 2004). One great source of polyunsaturated fatty acids is pumpkin seed oil,

representing about 84% of the total fatty acids of seeds (Procida *et al.*, 2013). Owing to these core phytochemical components, pumpkin seeds were valued as an oil-rich source for nutritional purposes (Stevenson *et al.*, 2007; Gohari *et al.*, 2011). The oil of pumpkin seed is currently used for its medicinal properties, i.e., it is involved in the regulation of hypertension as well as the mitigation of hypercholesterolemia and prevents benign prostatic hyperplasia proliferation (Zuhair *et al.*, 2000; Fu *et al.*, 2006; Gossell-Williams *et al.*, 2006).

More than 2 billion people worldwide suffer from mineral deficiencies in many developing countries. Pumpkin seeds have been indicated as considerable potential sources of potassium and, to a lesser extent, sources of sodium, magnesium, calcium, phosphorus, zinc, iron, manganese, and copper (Rezig *et al.*, 2012). Pumpkin seeds have also biochemical importance as a source of phenylalanine, tyrosine and tryptophan (Tinoco *et al.*, 2012). As for amino acid content, it has been reported that arginine and aspartic, glutamic acids were higher than threonine, methionine, tyrosine, and histidine in seeds of previously studied pumpkin lines (Amin *et al.*, 2019). Antioxidant activity of organic acids provide protection against a range of ailments in humans (Nawirska-Olszanska *et al.*, 2014). To the best of our knowledge, data on organic acids in pumpkin seeds have not been reported up-to-date. To qualify pumpkin seeds as a functional food, it is important to ascertain the levels of organic acids in them. On the other hand, there is limited knowledge regarding the interrelationships among the phytochemicals present in pumpkin seeds, despite their rich nutritional content. Thus, understanding the relationship between the fatty acids and phytochemical components in pumpkin seeds is of utmost importance. In addition to being an excellent source of fatty acids, pumpkin seeds also serve as a significant phytochemical reserve. However, a more detailed understanding of how phytochemical compounds are related to the fatty acid composition of pumpkin seeds is necessary. Furthermore, uncovering these relationships will aid in emphasizing the potential health benefits and nutritional value of pumpkin seeds, serving as a valuable food source for the food industry, nutritionists, and consumers. Therefore, the primary goal of the research was to determine the pathway between phytochemical and nutritional values of pumpkin seeds. Another major objective was to contribute to their potential industrial applications and to make recommendations as a prospective material for plant breeders to develop enhanced functional crops.

MATERIAL AND METHODS

Material

The seeds of pumpkin (*Cucurbita pepo* L.) genotypes were collected from the following prominent provinces of commercial pumpkin seed production in Turkey in 2020; Nevşehir (NVS-1 and NVS-2), Konya (KNY), Kayseri (KYS-1 and KYS-2), Bursa (BRS), Edirne (EDR), and

Kırklareli (KRK). In these provinces, pumpkin seeds are produced from commercial populations of national importance, but they have not been registered as cultivars. Moreover, these provinces account for 86% of Turkey's overall pumpkin seed production. Seeds are sown in late April to early May in these production areas, depending on weather and soil conditions, with harvesting occurring after about four months. The minimum, maximum, and average temperatures of these provinces ranged between 11ºC and 17ºC, 27ºC and 30ºC, and 20ºC and 24ºC, respectively.

Methods

Proximate analysis

The pumpkin seeds were dried to a constant weight prior to subsequent analyses. Moisture, ash contents and crude protein (protein factor 6.25) were determined following the AOAC procedure (1995). Total carbohydrate content and energy levels were calculated according to Idouraine *et al.* (1996).

Amino acid analysis

Following the procedures described by Aristoy and Toldra (1991). The amino acid derivatives were analyzed by HPLC (Agilent 1200, USA) on a Zorbax Eclipse-AAA 4.6 x 150 mm, 3.5 µm columns at a temperature 40 °C with detection at 254 nm. The amino acids were expressed in nmol μl^{-1}.

Organic acid analysis

A method of Gunes *et al.* (2014) was used to identify organic acids.. In summary, ultra turraks were used to homogenize 1.0 g of the seed sample in 10 mL of deionized water. The mixture was centrifuged at 1200 rpm for 50 min. Subsequently, supernatants were filtered through 0.22 µm filters (Millex Millipore). The supernatants were transferred to glass vials and injected into HPLC (Agilent 1200, USA) for separation using columns (Zorbax Eclipse-AAA 4.6 x 250 mm, 5 µm) with UV detector at 220 nm absorbance. The column temperature was at 25 ˚C and the flow rate was 1 mL min^{-1}. Organic acids were determined with 25 mM potassium phosphate (pH 2.5) as the mobile phase.

Fatty acid analysis

Five sets of samples of each genotype were analysed described by Folch *et al.* (1957). The samples (c. 1 g) were homogenized in a solution of chloroform/methanol (2:1, v/v) containing 0.01% (w/v) of butylated hydroxytoluene (Sigma, Gas Chromatography (GC), B1378) as antioxidant 20 vol. (w/v) for 1 min. The homogenized was carried out at 20-22°C on ice, filtration and incubation etc. After the organic solvent was evaporated under a nitrogen stream, the amount of lipid was

measured gravimetrically. Fatty acid methyl esters (FAMEs) were prepared as described by Metcalfe and Schmitz (1961). The crude lipid extract was saponified with NaOH in methanol and FAMEs were prepared by transmethylation with boron trifluoride (BF_3) in methanol (Kaymak, 2014; Kaymak *et al.*, 2022). The samples and reference solution were analysed by a GC (Hewlett–Packard 6890, USA) equipped with a flame ionization detector and a 7673A injector tower. Methyl esters were separated on a DB 23 capillary column (Agilent, 60 m, 0.25 mm i.d. and 0.25 µm). The temperature program was set for 35 min at 190°C, after which it increased by 30°C per min until 220°C, where it remained for 5 min. Hydrogen gas (2mL min^{-1} and split ratio was 30:1) was used as a carrier. By comparing their retention times and peak with a standard mix of fatty acids (FAs) ("Supelco 37" component FAME mix, Cat No. 47885-U) the characteristic FAs were identified and quantified (David *et al.*, 2003).

Mineral content

The mineral contents were determined according to AOAC official procedures 922.02 and 975.03 (AOAC, 1995). Moreover, tissue P, K, Ca, Mg, S, Na B, Fe, Mn, Zn, Cu, Cd, Mo, Ni and Pb were determined by using an Inductively Couple Plasma Spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT, USA). Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) was used for determined the total N by Kjeldahl method (Bremner, 1996).

Statistical analyses

All analyses performed in this study were replicated five times. Statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA) software. The data were presented as mean and assessed by variance analysis procedures (PROC GLM). The significant differences between the mean values were compared by Duncan's multiple range tests at $P \leq 0.05$ level. Principal component analysis (PCA) was carried out to evaluate the relationships among phytochemical content of seeds. In addition, AMOS 20 software (IBM Corporation, Chicago, IL) was used to develop a structural equation model to show complex relationships among studied variables.

RESULT AND DISCUSSION

Table 1 shows the chemical analysis results of the seeds in eight pumpkin genotypes. The moisture content significantly ($P \leq 0.05$) differed among genotypes and varied from 4.45% (KRK) to 4.93% (NVS-1 and 2). The protein contents revealed that genotype KRK (36.12%) indicated the highest protein content while genotype KYS-2 (28.63%) had the lowest. The observed differences are

most likely caused by the results of genetic background. However, environmental factors particularly location may have played a role in the differences (Idouraine *et al.*, 1996; *Charaya et al.*, 2023). Accordingly, the differences in the results of the protein content among genotypes could also arise from climatic conditions ranged between 11ºC and 30ºC during growing period. In the study, the protein content of *Cucurbita pepo* seeds results is in line with Idouraine *et al.* (1996), Younis *et al.* (2000), Rezig *et al.* (2012), and Nawirska-Olszanska *et al.* (2014). Crude oil content was over 40% in genotypes BRS, NVS-1 and NVS-2 and no lower than 37% in the remaining genotypes. Idouraine *et al.* (1996) reported that the observed variations might be related to growing conditions, crop practices and harvest time. Additionally, the oil contents of the genotypes are in the high range (13-33%) cited by Stevenson *et al.* (2007) and Rezig *et al.* (2012). These outcomes are also equivalent to those reported by Idouraine *et al.* (1996) and Kaymak (2012) but lower than those reported by Nawirska-Olszanska *et al.* (2014). As elucidated in Table 1, the seeds from genotype KYS-2 showed the highest level of total carbohydrates (21.54%) whereas genotype KRK represented the lowest level (14.76%). No significant variation in the carbohydrates was observed between seed samples, but these values were higher than that recorded by Idouraine *et al.* (1996) and lower than those recorded by Younis *et al.* (2000). This may vary concerning some environmental factors as indicated by Idouraine *et al.* (1996). Ash content ranged from 9.40% (NVS-2) to 11.60% (NVS-1) and varied significantly among genotypes. Calculated energy was high and varied significantly among genotypes. The energy levels of present genotypes are similar to those reported by Idouraine *et al.* (1996). Moreover, genotype KYS-1 (545.30 kcal 100^{-1}) showed the lowest energy, while genotype NVS-2 (578.99 kcal 100^{-1}) had the highest energy. It can be clearly said that the chemical analysis of seed from the eight pumpkin genotypes exhibited higher oil and protein content compared to previous studies.

The total yield of amino acids in the seeds of pumpkin genotypes ranged from 15.65 nmol μl^{-1} (KNY) to 32.99 nmol μl^{-1} (KYS-2). The genotype KYS-2 had the highest of all the amino acids (Table 2). The lowest amino acid values changed according to the genotypes. Namely, glutamate, glycine, tyrosine, tryptophan, leucine, hydroxyproline and proline were recorded at lower contents in the KYS-1 genotype; isoleucine in EDR; phenylalanine and methionine in KRK. Thus, these variations may result from the effect of different factors, including ripe stage, plant age, and crop conditions (Song *et al.*, 2013). It is found that 22 amino acids in the seeds of pumpkin genotypes. All of the essential amino acids (EAA) including threonine, valine, methionine, isoleucine, leucine, phenylalanine, tryptophan, lysine, histidine, and arginine were detected in tested pumpkin genotypes. Firstly, arginine and threonine turned out to be the predominant EAA, accounting for

1.00–2.13 nmol μl^{-1} and 1.05–2.12 nmol μl^{-1}, respectively. Secondly, histidine, methionine, tryptophan and leucine were in a small amount at 0.52–1.10 nmol μl^{-1}, 0.40–1.02 nmol μl^{-1}, 0.45–1.14 nmol μl^{-1} and 0.49–1.20 nmol μl^{-1}, respectively. Finally, valine, phenylalanine, isoleucine and lysine were in a trace amount at 0.16–0.32 nmol μl^{-1}, 0.38–0.94 nmol μl^{-1}, 0.35–0.85 nmol μl^{-1} and 0.32–0.72 nmol μl^{-1}, respectively. The composition of EAA in the eight pumpkin genotypes is quite similar to that reported by previous studies (Mansour et al. 1993; Idouraine *et al.*, 1996; Glew *et al.*, 2006), but the concentrations differed.

As explained in Table 3, 11 organic acids were determined. The results showed, the highest organic acid content, except for oxalic, malonic, malic and citric acids were determined in the KYS-2 genotype. Lactic and maleic acids were the predominant organic acids in KYS-2, accounting for 3106.1 ng μl^{-1} and 2644.8 ng μl^{-1}, respectively. The lowest amino acid values were changed based on the pumpkin genotypes. Low amounts of oxalic acid in tested pumpkin genotypes were observed. Lactic, propionic, and tartaric acids differed in the tested pumpkin seeds. In addition, organic acids, especially lactic and propionic acid, are known to exhibit good antibacterial activity. Due to these properties, E.C., FAO/WHO and US-FDA has been approved as a food additive (Surekha and Reddy, 2000). Tartaric acid is also one of the most important organic acids, which has been widely applied in fields such as; food industry, pharmaceutical industry, chemical industry (Zhang *et al.*, 2011).

Malic and citric acid, the main organic acids of horticultural crops, are responsible for the flavour, taste and microbial stability of the products (Saavedra and Barbas, 2003). Moreover, citric acid retard enzymatic activity by the way playing an important role in conjunction with antioxidants to chelate trace metals (McCluskey *et al.*, 2004; Bellion *et al.*, 2006). Additionally, malic acid is used as a common parameter to food control points in the food process and evaluate the quality of agricultural products (Kim, 2006). The tested pumpkin genotype seeds are relatively rich in organic acids, and the richness of organic acids in pumpkin seeds enables us to classify them as a functional food.

In seeds of pumpkin genotypes, palmitic (C16:0), oleic (C18:1n-9) and linoleic (C18:2n-6) acids were the highest in concentration, followed by stearic acid (C18:0) at less than 10%, and the other fatty acids at an even lower content (<1%) (Table 4). Similar results have been documented by Rezig *et al.* (2012), which found the major fatty acids of pumpkin seeds were oleic (44.11%), linoleic (34.77%) and palmitic (15.97%) acids. Some researchers made the same observations as four dominant fatty acids linoleic, oleic, palmitic, and stearic acids were determined in the seed oil of pumpkin cultivars (Younis *et al.*, 2000; Stevenson *et al.*, 2007). The total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), n-6 and n-3 polyunsaturated fatty acids

(PUFA) were also different. Similarly, Procida *et al.* (2013) reported that the content of these four predominant fatty acids ranged from 97.5 to 98.7% of the total fatty acid content of tested pumpkin seed oils of various origins. The results of our study are in agreement with different crop studies made by Safdari-Monfared *et al.* (2019) and Akçalı (2022), who recorded that total fatty acid content decreased due to the increasing temperature and sowing dates depending on different ecologies.

The results propound that pumpkin seeds are a good source of the essential fatty acid, linoleic acid. Concerning genotypes, wide variations were found related to the major fatty acids of tested seeds (Table 4). SFA, MUFA, and n-6 and n-3 PUFA also differed among genotypes. Both n-6 and n-3 PUFA ranged from 26.29% (KYS-2) to 46.83% (KNY), and 0.16% (BRS) to 0.23% (KNY), respectively; MUFA ranged from 35.60% (KNY) to 43.92% (KYS-2). In addition, all seed oils contain low amounts (16-19%) of SFA. Such variations may be the result of variations in cultivar, soil, and seasonal variation, stage of maturity, harvest time, drying conditions, and storage (Al-Khalifa 1996; De Mello *et al.*, 2000). Furthermore, it is known that variations in oil and fatty acid content are governed more by the genotypes than the growing location (Bhardwaj and Hamama, 2009). Nawirska-Olszanska *et al.* (2014) also declared that the composition of fatty acids differed depending on the variety and the species of the pumpkin seeds.

From Table 5, it is clear that the tested pumpkin seeds are excellent sources of potassium magnesium (Mg) and phosphor (P), respectively. However, sodium (Na), sulfur (S), calcium (Ca), iron (Fe), copper (Cu), boron (B), manganese (Mn) and zinc (Zn) levels were observed to be low. In addition, cadmium (Cd) contents were the lowest, and lead (Pb) was lower in the genotypes but did not prove to be statistically significant. The nitrogen (N) content of seeds ranged from 2.28% (KYS-2) to 2.88% (KRK). However, environmental factors might be responsible for a small portion of the differences observed (Idouraine *et al.*, 1996). The human body needs a variety of minerals for almost all aspects of body function, such as potassium known for decreasing blood pressure, magnesium known for its role in the structure, iron known for its essential component of many of proteins and enzymes, copper known its crucial role in redox reactions and the scavenging of free radicals, zinc known for its role in the structure of proteins and cell membranes of the human body. Overall, the seeds of the Turkish pumpkin genotypes appeared to be a good source of minerals. Although reporting different levels, Idouraine *et al.* (1996), Juranovic *et al.* (2003), Glew *et al.* (2006) and Rezig *et al.* (2012) indicated similar trends for K, Mg, P, Na, and the remaining minerals.

To assess the presence of any relationships among the 64 variables identified in the study, a correlation analysis was carried out, which indicated the presence of multicollinearity among these

variables. To mitigate this multicollinearity issue, Principal Component Analysis (PCA) was conducted. The PCA analysis revealed that the 9 parameters with the highest factor loadings explained 94.93% of the total variance (Fig.1). A path analysis was also conducted to assess the direct and indirect effects of the obtained 9 parameters on the dependent variables (C18:1n9 and C18:2n6) (Fig.2). The path analysis showed that the Root Mean Square Approximation (RMSA) value was significant at the 1% level.

The goodness-of-fit index (GFI), which measures the extent to which the covariance matrix is captured by the model, was calculated as 0.865 (Excellent fit). Additionally, the comparative fit index (CFI), which assesses whether there is any relationship between the variables and aims to demonstrate the difference between the constructed model and the zero model, was computed as 0.915 (Excellent fit). Considering the path coefficients, it is seen in Fig.2 that the indirect effect of lactic acid on the dependent variables (C:18 1n9 and C:18 2n6) has the highest coefficient (801695.05) in terms of contribution to the model. Serine (52011.58), Alanine (277365.62), Leucine (52106.96), Tryptophan (46267.06), Glycine (42350.87), Glutamate (31918.84), Isoleucine (21414.17) and Malic acid (34.38) followed lactic acid, respectively.

CONCLUSIONS

The seeds of the Turkish pumpkin genotypes examined differed in most of the chemical parameters. Some genotypes were distinguished from others by having higher protein, total carbohydrates, and crude oil content. The seeds of the eight Turkish pumpkin genotypes are also discovered to be a good source of K, Mg, P, and Na minerals. It is found that the studied genotypes-dependent fatty acid content. Based on the results, the genotypes NVS-1, NVS-2, KYS-1, and KYS-2 appeared to have the best potential in biochemical content for human health. Through significant variances in the biochemical composition of Turkish pumpkin genotypes, these genotypes can be selected, developed commercially, and registered as nutrient-rich cultivars.

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Table 1. Proximate composition of seeds of Turkish pumpkin genotypes [a].

Genotypes	Moisture (%)	Protein (%)	Crude oil (%)	CHO [b] (%)	Ash (%)	Energy [c] (100 kcal^{-1})
KYS-1	4.49 ± 0.001 f	35.35 ± 1.20 ab	37.30 ± 1.34 b	17.05 ± 2.26 [NS]	10.30 ± 0.28 ab	545.30 ± 7.81 b
KYS-2	4.53 ± 0.001 d	28.63 ± 0.43 c	39.64 ± 0.24 ab	21.54 ± 1.38	10.20 ± 0.71 ab	557.39 ± 1.65 ab
NVS-1	4.93 ± 0.001 a	31.29 ± 0.27 abc	41.96 ± 1.03 ab	15.15 ± 1.19	11.60 ± 0.42 a	563.39 ± 3.47 ab
NVS-2	4.93 ± 0.001 a	30.43 ± 1.14 bc	43.32 ± 3.12 a	16.86 ± 1.84	9.40 ± 0.14 b	578.99 ± 16.15 a
KRK	4.45 ± 0.003 g	36.12 ± 1.63 a	39.37 ± 3.34 ab	14.76 ± 1.22	9.75 ± 0.50 b	557.85 ± 18.70 ab
EDR	4.51 ± 0.001 e	33.50 ± 3.80 abc	39.28 ± 0.03 ab	16.82 ± 4.25	10.40 ± 0.42 ab	554.81 ± 1.56 ab
BRS	4.65 ± 0.001 c	30.31 ± 1.92 bc	40.38 ± 0.29 ab	18.86 ± 2.83	10.45 ± 1.20 ab	560.10 ± 6.27 ab
KNY	4.84 ± 0.001 b	30.13 ± 3.83 bc	39.95 ± 4.42 ab	20.12 ± 7.68	9.80 ± 0.57 b	560.57 ± 24.38 ab

[a] Expressed on dry weight basis (mean ± SD, n = 5). Mean values with the different letter within columns are significantly different ($P \leq 0.05$). [b] CHO = carbohydrates, calculated by difference: 100 - (protein + crude fat + ash). [c] Energy determined by multiplying fat by 9 and CHO and protein by 4.

Table 2. Amino acid composition of seeds of Turkish pumpkin genotypes (nmol µl^{-1}).

Amino acids	Genotypes (n=5)							
	KYS-1	KYS-2	NVS-1	NVS-2	KRK	EDR	BRS	KNY
Aspartate	0.95 c	1.71 a	0.90 c	1.20 ab	0.89 c	0.92 c	1.27 ab	0.84 c
Glutamate	0.48 d	1.06 a	0.59 cd	0.71 b	0.52 d	0.53 d	0.69 bc	0.56 d
Asparagine	2.54 ab	2.77 a	1.41 d	2.08 bc	2.18 abc	1.91 cd	2.07 bc	1.44 d
Serine	1.97 c	4.17 a	1.92 c	2.73 b	2.07 c	2.33 bc	2.82 b	2.07 c
Glutamine	0.90 bcd	1.44 a	0.70 d	0.92 bcd	0.77 cd	0.93 bc	1.07 b	0.70 d
Histidine	0.67 b	1.10 a	0.55 c	0.70 b	0.64 b	0.72 b	0.71 b	0.52 c
Glycine	0.49 d	1.17 a	0.69 bc	0.71 bc	0.58 cd	0.61 cd	0.76 b	0.52 d
Threonine	1.37 b	2.12 a	1.32 bc	1.47 b	1.38 b	1.22 bc	1.39 b	1.05 c
Arginine	1.21 bc	2.13 a	1.00 d	1.50 b	1.22 bc	1.18 bc	1.32 ab	1.04 d
Alanine	1.60 c	3.09 a	1.38 d	1.90 b	1.61 c	1.58 c	1.90 b	1.33 d
Tyrosine	0.50 e	1.47 a	0.69 d	0.90 bc	0.77 cd	0.71 d	0.97 b	0.67 d
Cystine	0.61 bc	1.02 a	0.68 b	0.62 bc	0.70 b	0.64 bc	0.69 b	0.50 c
Valine	0.20 bc	0.32 a	0.16 c	0.20 bc	0.22 bc	0.22 bc	0.22 b	0.22 bc
Methionine	0.41 c	1.02 a	0.58 bc	0.53 bc	0.40 c	0.53 bc	0.61 b	0.46 bc
Tryptophan	0.45 d	1.14 a	0.58 cd	0.70 bc	0.49 d	0.54 d	0.79 b	0.52 d
Phenylalanine	0.46 c	0.94 a	0.47 c	0.67 b	0.38 c	0.44 c	0.68 b	0.47 c
Isoleucine	0.56 b	0.85 a	0.51 b	0.50 b	0.50 b	0.35 c	0.51 b	0.37 c
Leucine	0.49 d	1.20 a	0.54 cd	0.75 b	0.51 cd	0.62 c	0.80 b	0.52 cd
Lysine	0.45 bc	0.72 a	0.39 cd	0.48 bc	0.40 bcd	0.40 bcd	0.50 b	0.32 d
Hydroxyproline	0.39 c	0.87 a	0.41 c	0.56 b	0.40 c	0.43 c	0.59 b	0.43 c
Sarcosine	1.44 c	2.59 a	1.21 d	1.71 b	1.49 c	1.48 c	1.69 b	1.06 d
Proline	0.04 d	0.09 a	0.07 b	0.05 c	0.05 c	0.05 d	0.06 bc	0.05 c
Total	18.18	32.99	16.74	21.57	18.17	18.34	22.13	15.65

The number in parenthesis indicates the number of specimens of each genotype sampled. The different lowercase letters within rows indicate a significant difference ($P \leq 0.05$) between the genotypes.

Table 3. Organic acid composition of seeds of Turkish pumpkin genotypes (ng µl^{-1})

	Genotypes (n=5)							
Organic acids	KYS-1	KYS-2	NVS-1	NVS-2	KRK	EDR	BRS	KNY
Oxalic acid	27.4 d	15.6 e	21.2 de	39.3 c	39.4 c	52.6 b	45.2 c	59.8 a
Propionic acid	342.9 b	461.7 a	350.6 b	237.1 d	290.9 c	356.5 b	251.1 cd	464.7 a
Tartaric acid	17.3 bc	14.3 c	24.7 a	24.9 a	18.7 bc	13.9 c	20.2 ab	17.3 bc
Butyric acid	168.9 e	489.4 a	263.0 c	196.1 de	178.5 de	216.9 d	214.6 de	321.5 b
Malonic acid	57.9 d	71.9 ab	61.8 bcd	80.3 a	58.9 cd	70.9 abc	61.8 bcd	56.8 d
Malic acid	14.6 c	23.3 abc	27.1 ab	24.0 abc	24.9 ab	30.3 ab	20.9 bc	31.8 a
Lactic acid	277.8 c	3106.1 a	384.8 e	878.7 c	310.3 e	321.9 e	1276.7 b	671.7 d
Citric acid	471.2 bc	100.4 g	406.4 de	518.0 ab	428.5 cd	547.4 a	280.7 f	351.5 e
Maleic acid	47.4 e	2644.8 a	648.7 c	250.7 d	49.1 e	53.7 e	1063.4 b	78.8 e
Fumaric acid	74.3 c	146.4 a	117.7 ab	73.5 c	67.9 c	99.4 bc	111.7 b	85.1 bc
Succinic acid	498.0 e	1033.1 a	835.1 b	716.1 c	472.4 e	623.6 d	716.8 c	537.8 e

The number in parenthesis indicates the number of specimens of each genotype sampled. The different lowercase letters within rows indicate a significant difference ($P \leq 0.05$) between the genotypes.

Table 4. Fatty acid composition of seeds of Turkish pumpkin genotypes (%).

	Genotypes (n=5)							
Fatty acids	KYS-1	KYS-2	NVS-1	NVS-2	KRK	EDR	BRS	KNY
C16:0	10.45 ±0.10 bc	11.38 ±0.10 abc	10.73 ± 0.33 abc	10.91 ± 0.83 abc	12.31 ± 0.11 a	11.18 ± 0.51 abc	11.95 ± 0.58 ab	10.29 ± 1.34 b
C16:1n7	0.19 ± 0.01 ns	0.12 ± 0.02	0.12 ± 0.00	0.14 ± 0.02	0.27 ± 0.17	0.20 ± 0.04	0.14 ± 0.00	0.12 ± 0.03
C18:0	6.95 ± 0.33 ab	7.70 ± 0.14 a	6.51 ± 0.20 abc	5.99 ± 1.41 a-d	5.45 ± 0.14 bcd	4.72 ± 0.64 d	4.90 ± 0.25 cd	6.58 ± 1.21 abc
C18:1n9	37.71 ± 0.30 ab	43.81 ± 3.55 a	37.25 ± 1.03 ab	38.19 ± 5.30 ab	37.69 ± 1.33 ab	42.23 ± 4.73 ab	42.37 ± 2.08 ab	35.49 ± 2.41 b
C18:2n6	44.02 ± 0.55 ab	36.29 ± 3.53 b	44.78 ± 1.61 ab	44.13 ± 7.65 ab	43.65 ± 1.15 ab	41.13 ± 5.92 ab	40.08 ± 1.24 ab	46.83 ± 0.29 a
C18:3n3	0.21 ± 0.00 ab	0.17 ± 0.01 ab	0.17 ± 0.01 ab	0.20 ± 0.01 ab	0.20 ± 0.04 ab	0.19 ± 0.02 ab	0.16 ± 0.00 b	0.23 ± 0.04 a
C20:0	0.47 ± 0.03 ab	0.54 ± 0.01 a	0.44 ± 0.06 ab	0.45 ± 0.14 ab	0.43 ± 0.00 ab	0.36 ± 0.10 b	0.39 ± 0.01 ab	0.46 ± 0.07 ab
SFA	17.87 ± 0.26 ns	19.61 ± 0.03	17.68 ± 0.58	17.35 ± 2.38	18.19 ± 0.03	16.25 ± 1.25	17.24 ± 0.84	17.34 ± 2.62
MUFA	37.91 ± 0.29 ab	43.92 ± 3.57 a	37.37 ± 1.03 ab	38.32 ± 5.28 ab	37.96 ± 1.16 ab	42.44 ± 4.69 ab	42.52 ± 2.08 ab	35.60 ± 2.38 b
n-6 PUFA	44.02 ± 0.55 ab	36.29 ± 3.53 b	44.78 ± 1.61 ab	44.13 ± 7.65 ab	43.65 ± 1.15 ab	41.13 ± 5.92 ab	40.08 ± 1.24 ab	46.83 ± 0.29 a
n-3 PUFA	0.21 ± 0.00 ab	0.17 ± 0.01 ab	0.17 ± 0.01 ab	0.20 ± 0.01 ab	0.20 ± 0.04 ab	0.19 ± 0.02 ab	0.16 ± 0.00 b	0.23 ± 0.04 a

C16:0 – palmitic acid, C16:1n-7, palmitoleic acid, C18:0 – stearic acid, C18:1n-9 – oleic acid C18:2n-6 – linoleic acid, C18:3n-3 – linolenic acid, C20:0 – arachidic acid, SFA – saturated fatty acids, PUFA – polyunsaturated fatty acids, MUFA – monounsaturated fatty acids; means followed by different small letters in rows are significantly different at $P \leq 0.05$., ns – not significant. The number in parenthesis indicates the number of specimens of each genotype sampled.

Table 5. Mineral contents of seeds of Turkish pumpkin genotypes (mg kg^{-1}).

	Genotypes (n=5)							
Minerals	KYS-1	KYS-2	NVS-1	NVS-2	KRK	EDR	BRS	KNY
B	5.84 a	4.68 a	1.14 b	5.07 a	5.36 a	5.70 a	6.20 a	2.21 b
Ca	68.66 b	63.53 b	13.49 c	32.67 c	78.87 b	107.01 a	63.96 b	34.11 c
Cd	0.03 b	0.03 b	0.09 a	0.03 b	0.03 b	0.02 b	0.03 b	0.08 a
Cu	6.77 a	4.07 c	0.56 e	3.98 c	5.28 b	5.66 b	5.16 b	2.59 d
Fe	7.76 a	4.41 c	1.51 e	3.23 d	6.69 b	7.22 ab	6.26 b	2.54 d
K	5064.50 abc	4353.50 bc	6697.50 a	5277.00 abc	4535.40 bc	5789.50 ab	2560.35 d	3542.90 cd
Mg	1124.55 a	775.65 c	426.00 d	735.30 c	924.45 b	1109.10 a	919.65 b	479.93 d
Mn	4.82 a	2.70 b	0.77 c	2.63 b	5.12 a	5.40 a	3.06 b	2.85 b
Mo	0.51 bcd	0.66 abc	0.71 ab	0.27 d	0.34 cd	0.62 a-d	0.87a	0.50 bcd
Na	19.59 c	81.21 b	157.50 a	132.80 a	13.34 c	17.66 c	22.35 c	22.82 c
Ni	0.50 bc	1.29 a	0.63 b	0.41 bc	0.27 c	0.69 b	0.69 b	0.41 bc
P	1014.60 ab	651.00 cd	859.46 bc	529.80 d	921.00 ab	1120.95 a	890.55 ab	889.55 ab
Pb	0.11 ^{ns}	0.04	0.08	0.05	0.11	0.05	0.09	0.11
S	128.70 a	46.67 c	85.45 b	58.51 c	63.66 bc	59.28 c	72.63 bc	58.28 c
Zn	4.71 a	2.51 bc	1.66 dc	1.88 cd	4.32 a	5.21 a	3.09 b	0.88 d
N (%)	2.82 ab	2.28 c	2.42 bc	2.49 abc	2.88 a	2.67 abc	2.41 bc	2.40 bc

The number in parenthesis indicates the number of specimens of each genotype sampled. The different lowercase letters within rows indicate a significant difference ($P \leq 0.05$) between the genotypes, ns – not significant.

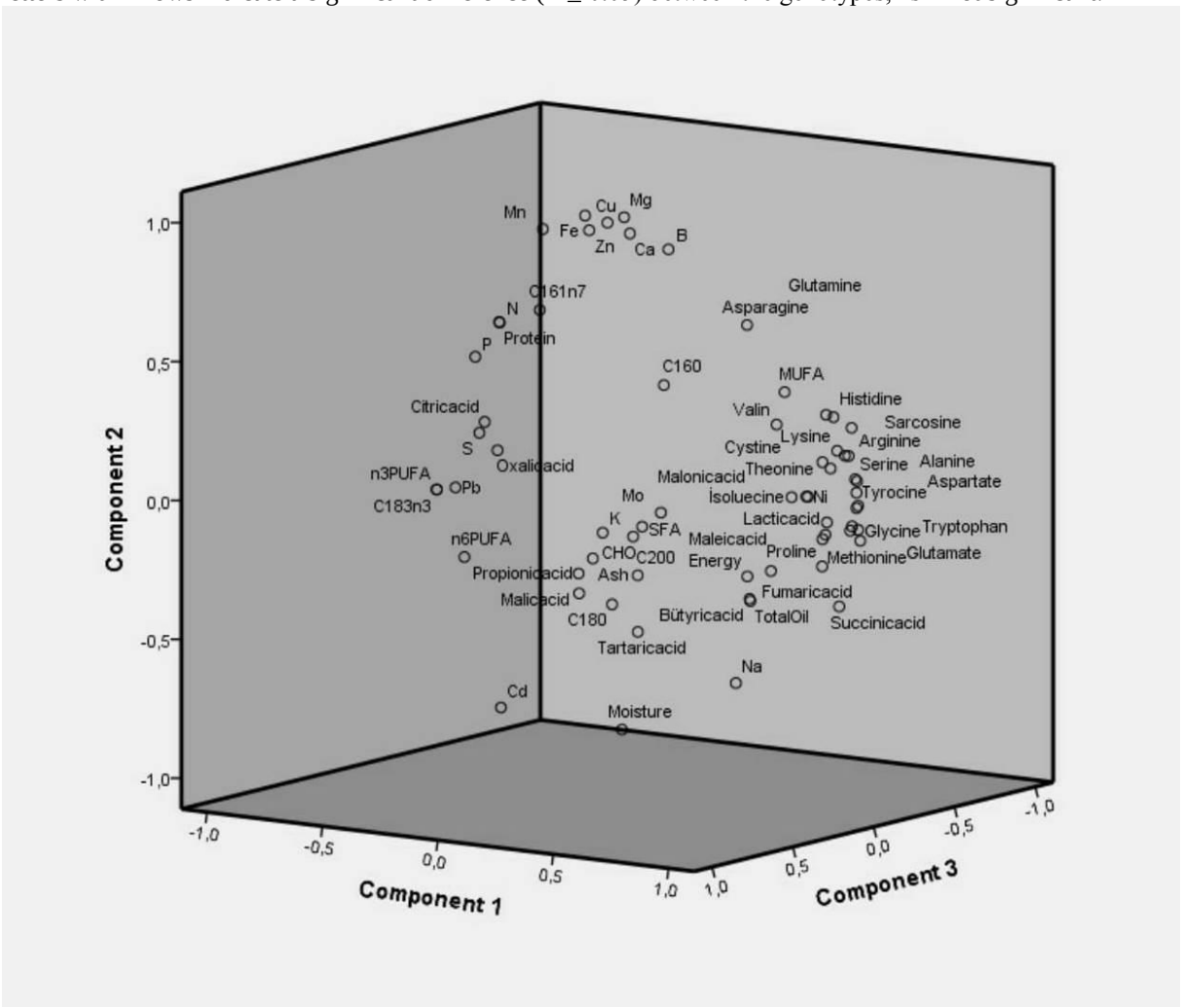

Figure 1. Principle component analyses of phytochemical content of pumpkin seeds.

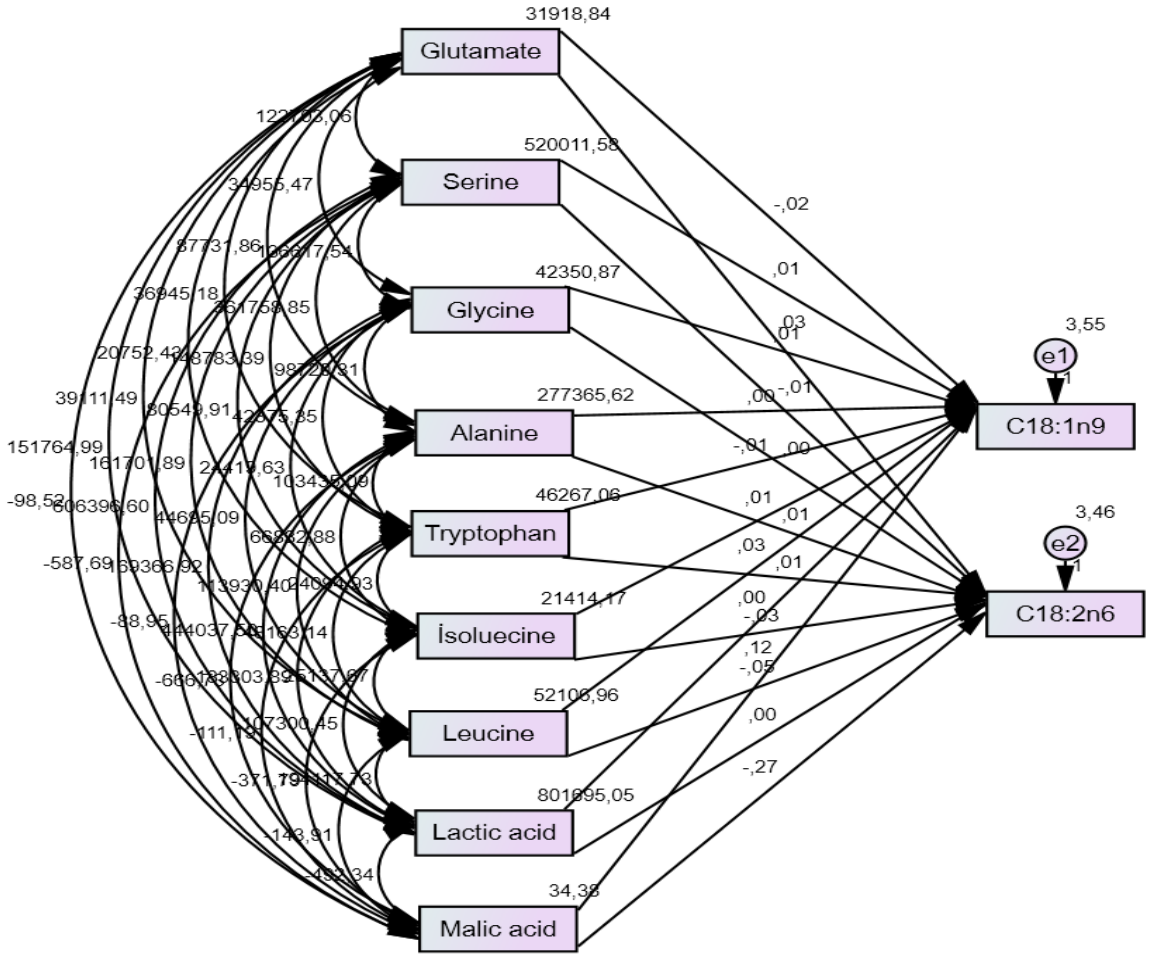

Figure 2. Pathway among C18:1n9, C18:2n6 and phytochemical properties of pumpkin seeds.