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CHANGES IN AMINOACID AND PHENOLIC PROFILE OF AMARANTH SEEDS SUBJECTED TO SOUS VIDE TREATMENT

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Abstract: *The aim of this study is to evaluate the impact of sous-vide method on amaranth seeds amino acids and individual phenolics content. The sous-vide method was applied at different temperatures (75 °C, 80 °C and 85 °C) for different times (60 minutes, 120 minutes, 180 minutes and 240 minutes), at different solid/liquid ratios (1/4, 1/5, 1/6). Eight phenolic acids (rosmarinic acid, p-coumaric acid, chlorogenic acid, vanillic acid, caffeic acid, p-hydroxybenzoic acid, protocatechuic acid, and gallic acid) and four flavonoids (kaempferol, luteolin, myricetin, and quercetin) were studied. The extraction and identification of free amino acids were carried out using 15% trichloroacetic acid. Variance analysis of the amino acid content in the composition of sous-vide treated amaranth seeds highlighted five essential amino acids (valine, leucine, isoleucine, threonine, and phenylalanine), and the variance analysis of the composition of amaranth seeds treated with the sous-vide method revealed a high total content, particularly influenced by temperature. The Box-Behnken model was applied to study the combined effects of three parameters (temperature, time, and solid-to-liquid ratio) on the total amino acid and polyphenol content during sous-vide treatment. The models developed for total amino acids and polyphenols were statistically significant according to ANOVA, and the regression for both models was higher than 0.75.*

Keywords: *amaranth, sous-vide, amino acids, phenolics*

1. Introduction

The use of sous-vide thermal treatment is a cooking technique that involves preparing food in heat-resistant vacuum-sealed bags at controlled temperatures, which enhances texture and nutritional values while significantly extending the shelf life compared to other traditional food preparation methods. Among other constituents, vegetables are important sources of active substances. The sous-vide method is a professional food preparation technology that is applicable in both home environments and in catering, molecular gastronomy, or the food industry. It is also known as lapping, vacuum cooking, vacuum-sealed cooking, or cook-chill with vacuum package [1], [2].

Although there is little research on the impact of the sous-vide method on plant-based foods in terms of bioactive compounds or their antioxidant capacity, this study aims to determine the influence of sous-vide technological parameters on the amino acid content and phenolic compound content in amaranth seeds prepared with the sous-vide method. Some studies suggest specific technological parameters based on starch content. For instance, Baldwin [3] showed that vegetables without starch can be prepared using sous-vide at temperatures of 82-85 °C, while starchy vegetables can be cooked at 80 °C [3]. Amaranth seeds belong to a category of pseudo-cereals with high

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nutritional value, largely due to their amino acid, flavonoid, and phenolic compound content [4], [5].

Amaranth seeds are considered pseudo-cereals and are characterized by high nutritional and functional values, which are associated with their antioxidant capacity and the amount of protein and lipids they contain [4], [6], [7].

Amaranth is a valuable source of fatty acids, with 16.54% of the total determined fatty acids being saturated, while 83.45% were unsaturated. Amaranth seeds are also a valuable source of amino acids, with nine essential amino acids reported: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Amaranth seeds are a good source of polyphenols and are recommended in balanced diets because polyphenols are easily broken down in the intestines due to the abundant presence of the enzyme beta-glucosidase, which releases the aglycone fragment of molecules [8]. The extraction of compounds from the matrix is an important process for achieving the structural composition of amaranth seeds prepared using the sous-vide method. There are different extraction methods for compounds in plant-based products. One extraction method uses solvents such as ethanol, methanol, ethyl acetate, or hexane, among others. Conventional solvents like methanol and hexane are recognized for their high extraction yield, but for environmental safety, ethanol is a safer alternative. Extracting compounds from plant matrices using an ultra-turrax has many advantages, such as a narrow and uniform particle size distribution, being a fast and inexpensive extraction method, and being able to generate high extraction rates at normal temperatures by crushing matrices into smaller particles [9], [10].

The scope of this study is to evaluate the influence of sous-vide treatment on amino acids and individual phenolics from amaranth seeds.

2. Material and methods

2.1. Materials

Organic amaranth seeds (*Amaranthus cruentus*) were sourced from the Dried fruits warehouse in Timișoara, Romania. The seeds were verified based on their botanical traits and stored in paper bags until the experiment.

2.2. Souse vide technique

The samples were treated at 75 °C, 80 °C, and 85 °C for durations of 60, 120, 180, and 240 minutes. For each sample cooked at varying temperatures and times, different amaranth seed-to-water hydration ratios (1/4, 1/5, 1/6) were applied. A Sirman sous-vide device was used to prepare the samples.

2.3. Amino acids determination

For the extraction and identification of free amino acids, 1.75 ± 0.1 g of amaranth seeds were mixed with 15 mL of 15% trichloroacetic acid (TCA). The pH of the mixture was adjusted to 2.2 using 15% TCA, which corresponds to the isoelectric precipitation point of proteins. The extract was then diluted to 25 mL with 15% TCA [11]. The supernatant was collected and filtered through 0.45 µm microfilters. A 100 µL aliquot of the filtered supernatant was analyzed for organic components using the EZfaast GC-MS kit, following the manufacturer's instructions. The amino acid chromatogram for the 100 nmol/L standard is presented in Figure 1.

2.4. Determination of individual phenolic compounds

Phenolic acids (rosmarinic acid, *p*-coumaric acid, chlorogenic acid, vanillic acid, caffeic acid, *p*-hydroxybenzoic acid, protocatechuic acid, and gallic acid) and flavonoids (kaempferol, quercetin, luteolin, and myricetin) were identified from the methanolic extract (1 g of sample extracted with 25 mL of methanol) using a Shimadzu high-performance liquid chromatograph (Kyoto, Japan) equipped with a diode array detector. The separation was performed on a Zorbax SP-C18 column, measuring 150 mm in length and 4.6 mm in internal diameter, using particles with a 5 µm diameter. Elution was achieved with a solvent system composed of 0.1% acetic acid in water (solvent A) and acetonitrile (solvent B), at a flow rate of 1 ml•min^{-1}. Phenolic compounds such as gallic acid, vanillic acid, protocatechuic acid, and *p*-hydroxybenzoic acid were detected at 280 nm, while chlorogenic acid, *p*-coumaric acid, caffeic acid, rosmarinic acid, myricetin, quercetin, luteolin, and kaempferol were measured at 320 nm [12]. Figure 2 shows the HPLC-DAD chromatogram at 280 nm and 320 nm for the 100 mg/L standards.

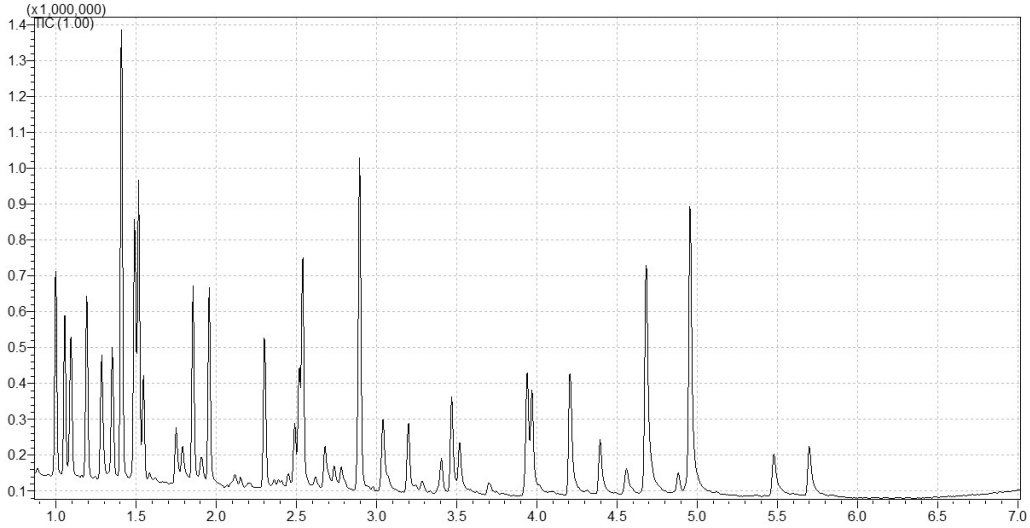

Fig. 1. Amino Acid Chromatogram (100 nmol/mL concentration standard):
Alanine – 0.998 min, Sarcosine – 1.054 min, Glycine – 1.091 min, Valine – 1.282 min, Leucine – 1.490 min, Isoleucine – 1.544 min, Threonine – 1.749 min, Serine – 1.790 min, Proline – 1.855 min, Asparagine – 1.956 min, Aspartic acid – 2.491 min, Methionine – 2.540 min, Phenylalanine – 2.896 min, Glutamic acid – 3.040 min, Glutamine – 3.405 min, Histidine – 4.395 min, Lysine – 4.563 min, Tyrosine – 4.684 min, Tryptophan – 4.958 min, Cystine – 5.703 min.

Experimental Design and Statistical Analysis

In this study, a three-level Box–Behnken design was employed to investigate and optimize the effects of the independent variables-temperature (X1), time (X2), and the solid-to-liquid ratio (RSL) (X3) – on amino acid and phenolic content. The coded variable levels of -1, 0, and 1 corresponded to 75 °C/120 min/1:6, 80 °C/180 min/1:5, and 85 °C/180 min/1:4, respectively. All calculations and graphical representations were performed using Design Expert 11 statistical

software (trial version, Minneapolis, MN, USA). To validate the optimal extraction conditions, experiments were conducted in triplicate. The Box–Behnken design was based on a second-order (quadratic) polynomial response surface model, using the following equation: $y = b_0 + \sum_{i=1}^{n}(b_i x_i) + \sum_{i=1}^{n}(b_{ii} x_{ii}^2) + \sum_{ij=1}^{n}(b_{ij} x_i x_j)$ (1)

where y is the predicted response (aminoacid and phenolics), x_i stands for the coded levels of the design variable (temperature (X_1), time (X_2), and liquid solid ratio (RSL) (X_3)), b_0 is a constant, b_i is the linear effects, b_{ii} is the quadratic effects and b_{ij} is the interaction effects.

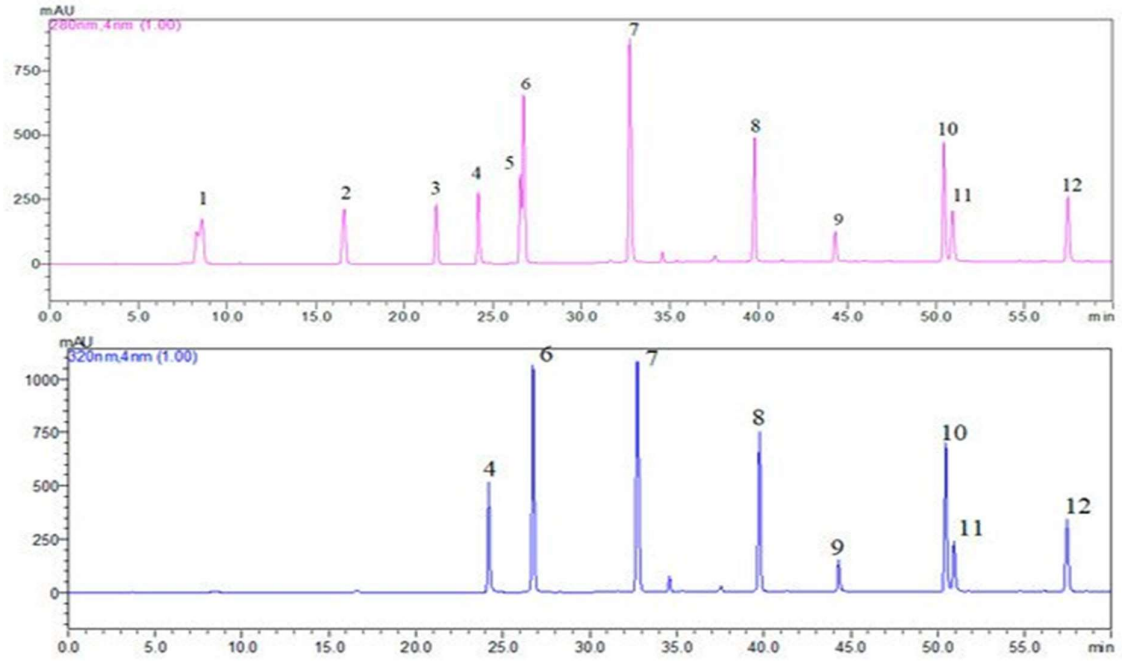

Fig. 2. HPLC-DAD Chromatogram at 280 nm and 320 nm for the Standard (100 mg/L) for gallic acid (peak 1 (8.578 min)), protocatechuic acid (peak 2 (16.6 min)), p-hydroxybenzoic acid (peak 3 (21.8 min)), caffeic acid (peak 4 (24.2 min)), vanillic acid (peak 5 (26.6 min)), chlorogenic acid (peak 6 (26.7 min)), p-coumaric acid (peak 7 (32.7 min)), rosmarinic acid (peak 8 (39.8 min)), myricetin (peak 9 (44.4 min)), luteolin (peak 10 (50.5 min)), quercetin (peak 11 (50.9 min)), and kaempferol (peak 12 (57.4 min)).

3. Results and discussion

3.1. Amino acids

Amaranth seeds are an important source of plant-based proteins rich in essential amino acids. This study examined the amino acid content in amaranth seeds cooked using the sous-vide technique. It was established that the total amino acid content in amaranth seeds reaches a maximum value of 5546.3 mg/100 g of product, depending on the sous-vide thermal treatment parameters applied to the seeds. The essential amino acid content is characterized by a high level of valine and leucine. Figure 3 shows the amino acid profile for the amaranth seed sample subjected to sous-vide treatment at a temperature of 75 °C for 60 minutes, with a solid/liquid ratio of 1/4. Valine is one of the essential branched-chain amino acids with a structural role in protein synthesis. Valine

molecules are converted into branched and methylated derivatives of alcohols and esters. The average value was obtained using sous-vide treatment parameters of 85 °C, 120 minutes, and a solid/liquid ratio of 1/6, with an average value of 631.77 mg/100 g of product. Leucine is an essential amino acid vital in various metabolic processes that support protein synthesis, prevent muscle loss and catabolism, preserve glycogen in muscle tissue, and maintain nitrogen balance. The average value was obtained using sous-vide treatment parameters of 80 °C, 60 minutes, and a solid/liquid ratio of 1/6, with an average value of 247.22 mg/100 g of product. Isoleucine is an isomer of leucine and belongs to the group of essential branched-chain amino acids. This amino acid is recognized as very important in physical recovery by participating in hemoglobin synthesis and regulating blood glucose levels. The average value was obtained using sous-vide treatment parameters of 80 °C, 180 minutes, and a solid/liquid ratio of 1/4, with an average value of 28.71 mg/100 g of product. Threonine is the last essential amino acid discovered in 1935, playing an important role in supporting the immune system by participating in antibody production. It directly participates in the synthesis of glycine and serine. The average value was obtained using sous-vide treatment parameters of 80 °C, 120 minutes, and a solid/liquid ratio of 1/5, with an average value of 3.06 mg/100 g of product. Phenylalanine is an essential amino acid that the human body converts into tyrosine, playing an important role in the production of dopamine and adrenaline. Monitoring the ratio between phenylalanine and tyrosine is an effective method for determining dietary intake. The average value was obtained using sous-vide treatment parameters of 75 °C, 120 minutes, and a solid/liquid ratio of 1/4, with an average value of 58.95 mg/100 g of product.

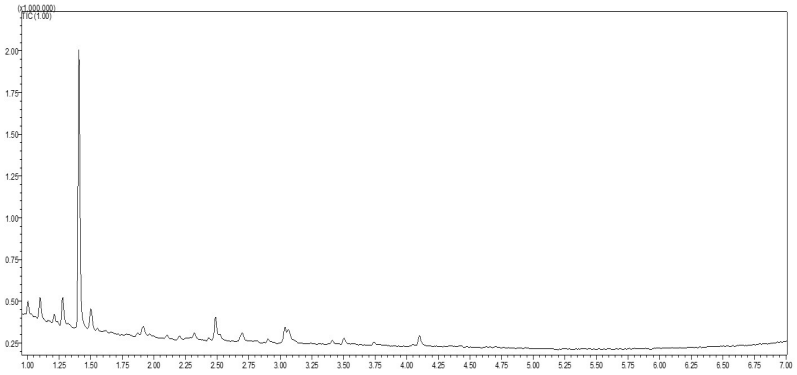

Fig. 3. Amino acid profile for the amaranth seed sample subjected to sous-vide treatment at a temperature of 75 °C for 60 minutes, with a solid/liquid ratio of 1/4: alanine – 0.998 min, glycine – 1.091 min, valine – 1.282 min, internal standard – 1.407 min, leucine – 1.490 min, serine – 1.790 min, asparagine – 1.956 min, aspartic acid – 2.491 min, phenylalanine – 2.896 min, glutamic acid – 3.040 min, glutamine – 3.405 min.

Table 1 shows the influence of sous-vide process parameters on the amino acid content of amaranth seeds. Regarding the influence of temperature on the amino acid profile, there is a significant effect ($p<0.05$) in the case of proline. As the applied temperature increases, the proline content rises, most likely due to protein breakdown. Concerning time, a significant decrease ($p<0.05$) can be observed in alanine and isoleucine levels. The solid/liquid ratio is a parameter for which the variance in amino acid composition did not show major changes. All *F* values are insignificant and are similar in value. The only notable difference is that, in the case of the 1/5 solid/liquid ratio, the highest concentrations for more than half of the analyzed amino acids were recorded.

3.2. Individual polyphenols

The composition of phenolic compounds in amaranth seeds treated with the sous-vide method depends on the parameters, as shown in Table 2. The temperature did not significantly alter the composition of phenolic compounds, except for myricetin and kaempferol, where differences were small. The solid/liquid ratio between the amaranth seeds and the hydration water did not significantly affect the phenolic content, recording insignificant *F* values. A possible explanation is that phenols belong to the class of organic compounds that are only slightly soluble in water. The exposure time of the amaranth seeds to the sous-vide treatment is the parameter that most influenced the variance in phenolic compound composition. The *F* value indicates that a longer exposure time directly influences the variance in the composition of certain phenols, such as protocatechuic acid or gallic acid, with a significant decrease ($p<0.05$). The content of the determined phenolic compounds is as follows: Gallic acid is an organic acid with antioxidant and anti-inflammatory properties, used in the food industry, particularly for its preservative and antioxidant properties. The average value was obtained using sous-vide thermal treatment parameters of 85 °C, 120 minutes, and a solid/liquid ratio of 1/5, with an average value of 72.13 mg/100 g of product. Protocatechuic acid is the major metabolite of polyphenols with a strong antioxidant effect. It is the phenolic compound that recorded the highest content in the analyzed samples. The average value was obtained using sous-vide treatment parameters of 80 °C, 120 minutes, and a solid/liquid ratio of 1/5, with an average value of 72.13 mg/100 g of product. Rosmarinic acid, myricetin, luteolin, and kaempferol are flavonoids that showed low content in all the extracts from the samples, regardless of the parameters used in the sous-vide technique.

Table 1.

Variance analysis of aminoacids in amaranth seeds during the sous-vide treatment

Aminoacid (mg/100 g product)	Temperature (°C)			*F*	Time (min)			*F*	Solid/liquid ratio			*F*
	75	80	85		60	120	180		1/4	1/5	1/6	
Alanine	201.5^{a}	170.7^{a}	170.1^{a}	*0.5ns*	255.7^{a}	132.3^{b}	154.4^{b}	*14.69***	158.8^{a}	202.8^{a}	180.6^{a}	*0.79^{ns}*
Glycine	846.3^{a}	603.9^{a}	647.9^{a}	*3.2ns*	748.4^{a}	708.0^{a}	641.0^{a}	*0.45^{ns}*	722.9^{a}	728.1^{a}	645.5^{a}	*0.32^{ns}*
Valine*	723.6^{a}	416.3^{a}	640.3^{a}	*2.7ns*	766.2^{a}	621.2^{ab}	392.8^{b}	*4.37**	493.3^{a}	653.2^{a}	633.7^{a}	*0.73^{ns}*
Leucine*	250.7^{a}	234.0^{a}	245.0^{a}	*0.1ns*	258.2^{a}	262.9^{a}	208.6^{a}	*0.66ns*	225.8^{a}	254.4^{a}	249.4^{a}	*0.16^{ns}*
Isoleucine*	28.2^{a}	33.0^{a}	35.1^{a}	*0.1ns*	57.4^{a}	18.8^{b}	20.0^{b}	*6.90***	33.0^{a}	40.3^{a}	23.0^{a}	*0.72^{ns}*
Threonine*	3.00^{a}	3.18^{a}	3.8^{a}	*1.4ns*	1.59^{b}	2.4^{ab}	11.0^{a}	*4.16**	3.3^{a}	3.6^{a}	8.1^{a}	*0.88^{ns}*
Serine	65.6^{a}	44.8^{a}	110.6^{a}	*1.9ns*	78.9^{a}	62.8^{a}	79.3^{a}	*0.13ns*	61.1^{a}	73.9^{a}	86.0^{a}	*0.24^{ns}*
Proline	18.9^{a}	34.7^{ab}	39.7^{a}	*3.8**	31.1^{a}	33.0^{a}	29.3^{a}	*0.08ns*	35.6^{a}	30.5^{a}	27.3^{a}	*0.44^{ns}*
Asparagine	37.0^{a}	59.8^{a}	41.5^{a}	*0.8ns*	51.2^{a}	41.7^{a}	45.3^{a}	*0.11ns*	54.3^{a}	59.7^{a}	24.1^{a}	*2.25^{ns}*
Aspartic acid	1891.7^{a}	2062.6^{a}	1891.7^{a}	*1.0ns*	2007.8^{a}	1826.2^{a}	1859.7^{a}	*0.40ns*	1721.8^{a}	1858.5^{a}	2113.3^{a}	*1.62^{ns}*
Phenylalanine*	52.6^{a}	92.3^{a}	46.66^{a}	*1.2ns*	55.0^{a}	55.0^{a}	81.54^{a}	*0.44ns*	69.2^{a}	76.5^{a}	45.8^{a}	*0.48^{ns}*
Glutamic acid	1020.4^{a}	1080.4^{a}	1224.0^{a}	*0.3ns*	1234.2^{a}	994.9^{a}	1095.8^{a}	*041ns*	1005.6^{a}	1078.4^{a}	1240.8^{a}	*0.41^{ns}*
AA total	5140.8^{a}	4512.8^{a}	5272.0^{a}	*1.2ns*	5546.3^{a}	4531.3^{a}	5546.3^{a}	*2.28ns*	4652.9^{a}	4992.8^{a}	5279.2^{a}	*0.74^{ns}*

*essential aminoacids

Table 2.

Variance analysis of fatty acids in amaranth seeds during the sous-vide treatment

Compound (mg/100 g product)	Temperature (°C)			*F*	Time (min)			*F*	Solid/liquid ratio			*F*
	75	80	85		60	120	180		¼	1/5	1/6	
Galic acid	78.94^{a}	66.8^{a}	70.3^{a}	*2.10^{ns}*	81.1^{a}	73.3^{ab}	61.7^{b}	*6.9***	78.68^{a}	72.76^{a}	64.72^{a}	*2.80^{ns}*
Protocatechuic acid	476.1^{a}	234.8^{a}	170.3^{a}	*2.09^{ns}*	664.7^{a}	178.6^{b}	37.9^{b}	*19.4****	360.03^{a}	286.32^{a}	234.96^{a}	*0.27^{ns}*
Rosmarinic acid	3.01^{a}	2.84^{a}	5.2^{a}	*0.35^{ns}*	8.4^{a}	1.56^{ab}	1.1^{b}	*4.24**	5.79^{a}	2.90^{a}	2.41^{a}	*0.66^{ns}*
Myricetin	0.98^{a}	0.0^{b}	0.0^{b}	*4.56**	0.99^{a}	0.0^{b}	0.0^{b}	*1.5^{ns}*	0.78^{a}	0.19^{a}	0.0	*0.76^{ns}*
Luteolin	25.69^{a}	2.82^{a}	4.09^{a}	*2.11^{ns}*	28.0^{a}	3.18^{a}	1.39^{a}	*3.02^{ns}*	18.98^{a}	7.94^{a}	5.68^{a}	*0.57^{ns}*
Kaempferol	4.28^{a}	0.0^{b}	0.0^{b}	*3.82**	4.28^{a}	0	0	*1.8^{ns}*	3.17^{a}	0.90^{a}	0.19^{a}	*0.66^{ns}*
Total	589.0^{a}	307.3^{a}	250.0^{a}	*2.11^{ns}*	787.5^{a}	256.7^{b}	102.1^{b}	*17.0****	464.5^{a}	373.9^{a}	307.9^{a}	*0.34^{ns}*

3.3. Design and Adjustment of the Box-Behnken Model for Total Amino Acid, and Polyphenol Content

The Box-Behnken model was applied to study the combined effects of three parameters (temperature, time, and solid-to-liquid ratio) on the total amino acid and polyphenol content during sous-vide treatment. The models were significant (p<0.05), with F-values of 2.34 for total amino acid content and 2.92 for total polyphenol content, respectively. The models developed for total amino acids and polyphenols were statistically significant according to ANOVA, and the regression for both models was higher than 0.75. Regarding total amino acid content, there were statistically significant effects between time and the solid-to-liquid ratio, while for total polyphenol content, a linear effect of time was observed

3.3.1. Adjustment of Second-Order Polynomial Equations

The relationship between texture parameters and the sous-vide technique was evaluated using second-order polynomial equations with the Box-Behnken model. The equations describing the correlation between inputs and outputs for total amino acid content, and total polyphenol content are presented below:

$$Total\_aminoacids = 4996{,}36 + 354{,}49 \cdot X_1 - 166{,}57 \cdot X_2 - 121{,}62 \cdot X_3 + 577{,}19 \cdot X_1 \cdot X_2 - 386{,}83 \cdot X_1 \cdot X_3 - 744{,}86 \cdot X_2 \cdot X_3 + 440{,}41 \cdot X_1^2 + 166{,}05 \cdot X_2^2 - 537{,}60 \cdot X_3^2 \quad (2)$$

$$Total\_individual\_phenolics = 70{,}55 - 1{,}51 \cdot X_1 - 8{,}81 \cdot X_2 + 6{,}51 \cdot X_3 - 4{,}80 \cdot X_1 \cdot X_2 - 0{,}83 \cdot X_1 \cdot X_3 + 1{,}05 \cdot X_2 \cdot X_3 + 5{,}61 \cdot X_1^2 - 3{,}83 \cdot X_2^2 + 1{,}29 \cdot X_3^2 \quad (3)$$

3.4. Principal component analysis

The principal component analysis (PCA) was conducted to evaluate the global effect of aminoacids and phenolics from a descriptive point of view. Figures 6 and 7 present the scores and compound loadings of PCA analysis performed.

It was found that the two principal components (PCs) explained 92% of the variations in the dataset. It can be observed that the sample treated at 75 °C are placed in the right part, while the samples treated at 80 °C and 85 °C are placed in the left part, the PC2 is dividing these two groups. The samples treated at 75 °C are influenced by hardness, while the samples treated at 80 °C and 85 °C are influenced the total content of aminoacids. The parameters placed in the outer ellipse of the correlation loadings have a higher influence on the projection than those placed in the inner ellipse.

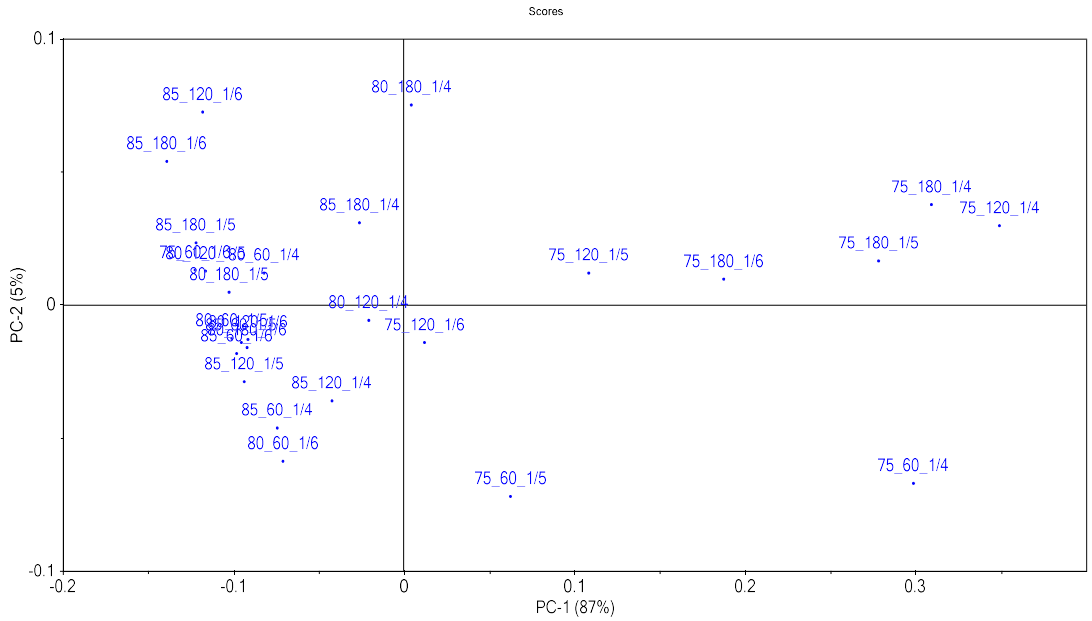

Fig. 4. Principal Component Analysis – Scores: the first number is temperature, the second is time, and the third is the solid-to-liquid ratio.

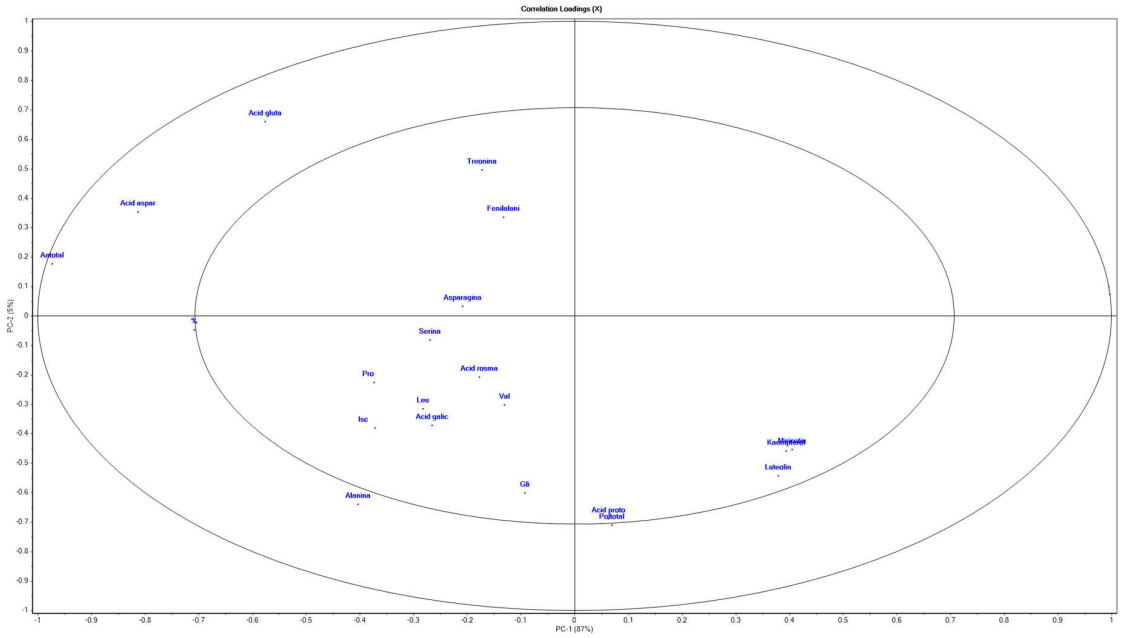

Fig. 5. Principal Component Analysis – The influence of the studied parameters during the sous-vide treatment

4. Conclusions

Based on the results obtained in this study, it can be said that amaranth seeds treated with the sous-vide method are an important source of phenolic compounds, and the use of the sous-vide method leads to a final product with good phytochemical content. Protocatechuic acid had the highest value among the individual polyphenols identified, followed by gallic acid. The F-value from the analysis of variance for phenolic compounds indicates that time is the most important parameter. As the duration increases, the phenolic compound content decreases. The lowest values were recorded for rosmarinic acid, myricetin, luteolin, and kaempferol due to the use of the sous-vide technique.

Five essential amino acids were reported in the variance analysis of amino acids from amaranth seeds treated with the sous-vide method: valine, leucine, isoleucine, threonine, and phenylalanine. Valine had the highest value, followed by leucine. The main parameters used in the sous-vide technique that influenced the amino acid composition were temperature and the solid-to-liquid ratio (1/5).

Acknowledgments

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