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RESEARCH REGARDING CHOCOLATE BAR ENRICHED DEVELOPMENT WITH SOUS-VIDE TREATED AMARANTH SEEDS

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Abstract: *The idea of using amaranth seeds as part of a functional food is not new. Numerous studies on the quality of pseudocereals share a common element: the concept of a health-beneficial food. The aim of this study was to develop four types of chocolate bars that were enriched with amaranth seeds, which had been pre-treated with the sous-vide method. The percentage values of the amounts of amaranth seeds varied to be evaluated both sensorially and physico-chemically. Another focus of this study was to examine the effect of adding sous-vide treated amaranth seeds to dark chocolate in terms of nutritional and sensory properties. The results highlighted major differences between the samples, both in terms of texture and physico-chemical properties. The samples were made using 100% cocoa dark chocolate and amaranth seeds that were thermally treated at 85°C for 180 minutes, using a hydration ratio of 1/6, where 1 represents the amount of amaranth (10g) and 6 represents the amount of water in which the amaranth seeds were hydrated during the sous-vide treatment (60ml). All four samples with 5%, 10%, 20%, and 50% amaranth seeds were stored at 4°C during the determinations, along with the control sample, which was represented by the dark chocolate without the addition of sous-vide treated amaranth seeds.*

Keywords: *amaranth, texture, sous vide, chocolate bar*

1. Introduction

The concern for health and, by extension, for healthy foods is becoming a daily goal for an increasing number of consumers. Choices regarding food and the environmental consequences represent the guiding line for the development of new food products by companies in the food industry. Thus, the new products being developed are focused on improving nutritional content (by adding new functional ingredients) while simultaneously reducing environmental impact by using plant-based ingredients. The creation of "relatively sustainable brands" is a concept increasingly adopted by the food industry [1,2]. The idea of using pseudocereals as a sustainable ingredient in the production of functional foods is not new and is supported by studies that highlight their health benefits. Amaranth seeds, regardless of variety, are little known in Europe and even less so in Romania. The interest of European consumers in "healthy" ingredients is continuously growing, and thus, amaranth seeds are starting to be sought after by consumers of all age groups and social statuses, including those who are not vegan. However, a major disadvantage of amaranth seeds is their low organoleptic properties. Although their nutritional potential is high, studies that indicate concrete methods to improve their organoleptic properties are few, offering limited possibilities for obtaining food products with enhanced sensory characteristics. The combination of amaranth seeds with dark chocolate represents a new direction in the creation of functional foods with significantly improved sensory

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characteristics as well as nutritional benefits. Although amaranth seeds are less known to consumers at the European level, dark chocolate is a popular snack used in the composition of confectionery and gastronomy products worldwide. Dark chocolate is mainly composed of cocoa butter, sometimes with small additions of cocoa liquor or small amounts of sugar and lecithin. This unique profile of dark chocolate gives it a distinctive texture, with high density and melting point, as well as specific sensory properties. Most of the triacylglycerol structure is composed of monounsaturated and saturated fatty acids, such as palmitic acid, stearic acid, and oleic acid. This type of symmetrical structure of saturated fatty acids with unsaturated fatty acids indicates that it is an ingredient with desirable characteristics [3,4]. In the industry, the use of dark chocolate as an ingredient in confectionery products is done only under special conditions due to structural issues such as fatty acid oxidation, low resistance to mold attacks, or the difficulty of tempering for further processing [5]. The thermal treatment methods applied to the ingredients that make up the dark chocolate bar with amaranth seeds are crucial in obtaining a product that is sensory appealing to consumers. The tempering method of the chocolate was carried out considering the optimal temperature of 29°C. This value is an essential parameter in preserving the specific taste and maintaining antioxidant capacity, while the sous-vide method serves to protect the physico-chemical composition, considering the vitamin and amino acid content rather than the sensory characteristics. Dark chocolate is a familiar ingredient for consumers, so in combination with amaranth seeds, which are considered an exotic and little-known ingredient, it results in a food product that can satisfy different nutritional needs through consumption and offer an alternative for a new type of snack created using innovative methods.

2. Materials and methods

2.1. Materials

100% cacao dark chocolate, BIO certified, country of origin: Dominican Republic. Organic amaranth seeds (*Amaranthus cruentus*) purchased from the company Driedfruits, based in Timișoara.

2.2. Sample preparation

The recipe for obtaining the four samples in the form of chocolate bars with amaranth seeds was applied in the laboratory, considering the specific parameters for each ingredient. The control sample consisted only of dark chocolate, without the addition of amaranth seeds. For tempering the chocolate, a Thermomix TM5 cooking device produced by Vorwerk, Germany, with a chocolate tempering function, was used. The dark chocolate was placed in the cooking bowl, and the temperature was set to 30°C, the blade speed for homogenization was set to 1500 rotations per minute, and a time of 8 minutes was set for tempering. The temperature set at 30°C is ideal for the complete melting of unstable polymorphic crystals. After reaching the temperature parameter and the expiration of the time, the Thermomix cooking device stops, and the chocolate is allowed to cool by 1°C. Thus, at 29°C, the chocolate is poured into a heat-resistant silicone mold with dimensions of 7.5 x 3 x 3 cm per cavity. The amount of dark chocolate poured into the mold to obtain the control sample is 100g. The control sample was stored in the refrigerator at 4°C until the analyses were performed.

Sample 1 was obtained by tempering dark chocolate at 29°C using 95g of dark chocolate to which 5g of amaranth seeds, cooked using the sous-vide method with a 1/6 hydration ratio, 180 minutes exposure time, and 85°C exposure temperature, were added. The homogenization of the two ingredients was performed using the Thermomix TM5 cooking device, with the homogenization blade set at a speed of 1000 rotations per minute for 3 minutes. After

homogenization, the resulting mixture was poured into a heat-resistant silicone mold with dimensions of 7.5 x 3 x 3 cm per cavity. Sample 1 was stored in the refrigerator at 4°C until the analyses were conducted. Sample 2 was obtained under similar conditions to those used for Sample 1, except that 90g of dark chocolate and 10g of amaranth seeds cooked using the sous-vide method with a 1/6 hydration ratio, 180 minutes exposure time, and 85°C exposure temperature was used. Sample 3 was obtained under similar conditions to those used for Samples 1 and 2, except that 80g of dark chocolate and 20g of amaranth seeds cooked using the sous-vide method with a 1/6 hydration ratio, 180 minutes exposure time, and 85°C exposure temperature was used. Sample 4 was obtained under similar conditions to those used for Samples 1, 2, and 3, except that 50g of dark chocolate and 50g of amaranth seeds cooked using the sous-vide method with a 1/6 hydration ratio, 180 minutes exposure time, and 85°C exposure temperature was used.

2.3. Texture Analysis

The test for determining hardness was conducted using a TVT 6700 texture analyzer (Perten Instruments, Stockholm, Sweden). A compression cylinder with a height of 45mm and a diameter of 3mm was attached to this device. This cylinder was used to press with a specific predetermined force. To analyze the samples, they were sized to a height of 30mm and a diameter of 30mm. The principle of the method is defined by applying force to the dark chocolate samples with amaranth seeds. The compression of the 5 samples was performed on the surface of a pressing table. Using the software, the device was programmed to apply a compressive force to the sample twice. The first compression was performed with a displacement of 18 mm, applying a speed of 2 mm/s. The second compression was performed with a displacement of 18 mm, maintaining the same compression speed. Using this method, the device records in real-time the resistance force against the analyzed samples and simultaneously records the calculated parameters. The result consists of recording the force values, as well as calculating texture parameters derived from the integration of the two recorded values.

2.4. Color Parameters Evaluation

The color parameters of the analyzed samples were evaluated using a portable colorimeter (Chroma Meter, model CR-410, Konica Minolta, Japan). The results were expressed based on the following parameters: L* which indicates lightness (a lower value indicates a darker color, with black: L* = 0 and white: L* = 100), a* (indicating the balance between red > 0 and green < 0), and b* (balance between yellow > 0 and blue < 0). The CIELAB color parameters were obtained in three replicates after calibrating the equipment on a white reference plate [6].

2.5. Determination of Water Content

This analysis aimed to accurately determine the moisture content in the prepared samples and was conducted using the standard method with the following laboratory tools and devices: Kern analytical balance, Biobase Bov-V45F drying oven, and porcelain desiccator containing an efficient dehydrating agent. The sample analysis involved precisely weighing each sample and placing it in a porcelain crucible with a known weight. The crucible with the sample was placed in the oven at a temperature of 102-104°C. The drying time from when the oven reached the working temperature was 2 hours. After the time expired, the crucibles with samples were removed from the oven and allowed to cool in the desiccator.

2.6. Determination of Total Polyphenol Content

The Folin-Ciocalteu method was used to determine the total polyphenol content. This

method is based on the chemical reduction of a mixture of tungsten and molybdenum oxides called the Folin-Ciocalteu reagent. The result is compounds with a maximum absorbance of 765nm and a blue color. The intensity of the wavelength is directly proportional to the concentration of phenols in the solution. The method described by Horincar et al. [7] was used, where 0.1mL of extract was used, to which 1.9mL of distilled water and 0.1 mL of Folin-Ciocalteu reagent were added. After 10 minutes of reaction, 0.8 mL of 5% sodium carbonate was added. A wavelength of 750 nm was used to read the absorbance after the mixture had rested at room temperature for 60 minutes. The content of polyphenolic compounds was expressed as mg gallic acid equivalents (GAE)/g dry weight, using an equation from the gallic acid standard curve.

2.7. Determination of Antioxidant Activity (DPPH)

The determination of antioxidant activity was conducted using the method described by Horincar et al. [7], which utilizes DPPH (2,2-diphenyl-1-picrylhydrazyl). This method was used to determine the antioxidant activity of the samples. 3.9 mL of DPPH solution was measured, reacting with 100µL of diluted sample for 30 minutes at room temperature, without light. The control sample was prepared by adding 3.9 mL of DPPH to 100 µL of methanol. The absorbance of the solution was measured at a wavelength of 515 nm.

2.8. Determination of Total Flavonoid Content

For the analysis of total flavonoid content, a colorimetric technique was used based on the property of aluminum chloride to form stable acid complexes with the C-3, C-4, and C-5 hydroxyl groups of flavonoids. The method used was described by Albishi et al. [8] and determined the total flavonoid content in the supernatant of the samples. A volume of 0.2 mL of diluted extract (1:10) was measured and homogenized with 2mL of pure methanol. The mixture was allowed to react for 5 minutes, after which 0.1mL of 5% aluminum chloride was added. The absorbance was read at a wavelength of 510nm. The total flavonoid content was expressed in mg quercetin equivalents (QE)/g.

2.9. Sensory Analysis

To conduct the sensory analysis of the samples, a structured hedonic scale with 9 levels of perception (1 = dislike very much to 9 = like very much) was used. Participants evaluated the sensory characteristics of the 4 samples and the control sample. The external appearance, cross-sectional appearance, color, aroma, and taste were evaluated. This study was conducted at the beginning of 2024 in Suceava, Romania. Potential consumers participating in the study were randomly selected at "Ștefan cel Mare" University of Suceava. The eligibility of the participant group was established based on the criteria set in the study by Meilgaard et al. [9]. These criteria require that study participants have no aversion to chocolate products, have no food allergies, and be willing to participate in the study.

Before starting the tasting session, participants were thoroughly informed about the study conditions and specifically that an ingredient approved for human consumption and sold in specialty stores in Romania had been added to the chocolate. The ingredient introduced into the chocolate mass was only revealed at the end of the tasting to avoid influencing the final result. Thus, only after the tasting did the participants learn that the ingredient combined with dark chocolate was a different mass of amaranth seeds cooked using the sous-vide method. Participants agreed to the conditions and chose to participate voluntarily. A total of 36 volunteers participated, of whom 20 were female, with an age range between 18 and 34 years.

2.10. Statistical Analysis

All analyses were performed in triplicate and reported as mean values with standard deviation. The results were compared using analysis of variance (ANOVA) with a 5% deviation rate. The Principal Component Analysis (PCA) was performed using Unscrambler X 10.1 (CAMO Process AS, Oslo, Norway), all the parameters were weighed and normalized to perform the analysis.

3. Results and discussion

3.1. Determination of Water Content

Water is an important component in chocolate-based products. The water content can vary depending on the complementary ingredient mixed with the chocolate, the type of thermal processing, or the degree of freshness. In this study, the amaranth seeds were thermally treated using the sous-vide method and were hydrated with 60 ml of water per 10 g of amaranth seeds [10]. Due to the specific thermal treatment, there is no loss of water. Thus, the water used for hydrating the amaranth seeds during the sous-vide thermal treatment is fully absorbed by the seeds and cannot evaporate [10]. When mixing dark chocolate with amaranth seeds, a certain amount of water in samples 1, 2, 3, and 4 may significantly influence both the physical-chemical and microbiological quality of the samples. Some sample parameters (degree of crystallization, color, aroma, and taste) can be influenced by water content [11]. According to current standards, the water content in chocolate specialties ranges from 3% to 37%, depending on the type of filling, with the note that the water content on the product's surface can be up to 3%. In this study, the control sample, consisting of plain chocolate without added amaranth seeds, had a moisture content of 0.59%. In Table 1 are presented the value of moisture content. Sample 1, with 5% amaranth seeds, had 3.85%, and sample 2, with 10% amaranth seeds, had 7.21%, which were the most favorable in terms of moisture content.

Table 1.

Physicochemical parameters of chocolate bars with amaranth seed

S	L*	a*	b*	DPPH	Hardness	Moisture (%)	TFC mg QE/g dry weight	TPC mg GAE/100g dry weight
S0	25.89	5.77	4.30	14	7255	0.60	296.37	194.66
S1	26.53	5.84	4.17	18	6907	3.86	289.63	191.66
S2	25.46	6.10	4.25	18	7942	7.21	292.84	192.10
S3	25.53	5.52	4.41	18	5220	14.32	294.84	192.92
S4	31.96	8.32	11.45	19	1904	43.74	300.28	201.32

Samples 3 and 4 had high moisture values of 14.32% and 43.74%, respectively. These values were directly influenced by the percentage of amaranth seeds cooked using the sous-vide method.

Since the sous-vide thermal treatment involves cooking the amaranth seeds at 85°C, which also serves as pasteurization, the amaranth seeds can be safely used in food products, including dark chocolate.

3.2. Color Parameter Evaluation

After the samples were stored for 5 days at 4°C, they were analyzed colorimetrically. The temperature of 4°C represents an optimal parameter for the crystallization of samples for color parameter determination. The vegetable fat, which is the main component in the chocolate samples, tends to recrystallize on the surface [12] at temperatures above 20°C, which can lead to inconclusive colorimetric results.

Recrystallization of fats can alter light scattering and visually affect color characteristics [13]. The control sample showed the darkest color along with sample 1, which contains 5% amaranth seeds. For samples 2 and 3, the amaranth seed content had a negligible effect with an L* value ≤ 0.5 (Table 1). Only in sample 4, where the amaranth seed content is 50%, was the color lighter with an L* value ≤ 2.1.

3.3. Determination of Total Polyphenol Content

The results for total polyphenol content were obtained using conventional solvent extraction methods and expressed as mg gallic acid equivalent (GAE)/g dry weight. An important factor in extracting polyphenols from the analyzed samples was the concentration of the solvent (pure ethanol). Pure ethanol proved effective for extracting total polyphenols due to its ability to break bonds between dissolved substances in the plant matrix, allowing for good mass transfer of all compounds. The concentration was higher for sample 4 due to its high content of bioactive compounds. The highest total polyphenol value was 201.23 mg GAE/100 g dry weight for sample 4 with 50% amaranth seeds. The lowest total polyphenol content was recorded for the control sample with a value of 194.66 mg GAE/100 g dry weight. Optimal recovery of phenolic compounds varies from sample to sample and depends on the plant matrix variety and bioactive compounds, as well as the combinations of solvents, extraction times, and temperatures used [14].

3.4. Determination of Antioxidant Activity (DPPH)

The results for antioxidant activity of the samples were obtained using conventional solvent extraction methods as percentage of inhibition (%). The absorbance increased with the percentage of amaranth seeds in the sample composition.

3.5. Determination of Total Flavonoid Content

In the plant kingdom, flavonoids represent the most widespread class of polyphenols. The results obtained in this study highlight that the highest total flavonoid content was found in sample 4, which contains 50% dark chocolate and 50% amaranth seeds, with 300.28 mg quercetin equivalent (QE)/g dry weight, compared to the control sample, which had 296.36 mg QE/g dry weight. This result was obtained using pure ethanol for extraction. The difference in results between sample 4, with the highest concentration, and sample 1, with the lowest value of 289.63 mg QE/g dry weight, is considered acceptable. This study highlighted that quercetin concentration is similarly high in all samples, unaffected by the amount of amaranth seeds in the sample composition.

3.6. Morphological Analysis of Crystals

A major concern in the chocolate and chocolate product industry is the changes in the crystal structure that forms the chocolate mass. The phenomenon known as "fat bloom" is characterized by the loss of gloss and unattractive color, but at the same time, it results in a transition to a more stable crystalline structure [5,12]. Factors that directly influence improper crystallization and the occurrence of "fat bloom" can include tempering at inappropriate temperatures or storage at improper temperatures. Consequently, surface crystals form due to temperature disturbances and variations [15]. Another important parameter for uneven crystallization is the percentage of additives in the chocolate, as well as their nature. In this case, the chocolate sample containing 50% amaranth seeds exhibited an uneven crystallization structure (Figs. 1-2). This phenomenon was likely because the amaranth seeds, thermally treated using the sous-vide method, contain a certain amount of water. The water content in the amaranth seeds directly influences the morphological profile of the crystals.

Fig. 1. Morphological Analysis of Crystals – sample S_0 represents the control sample

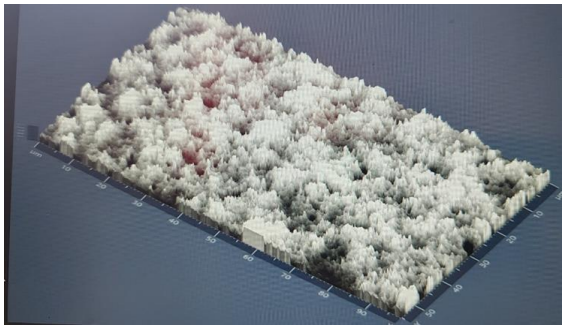

Fig. 2. Morphological Analysis of Crystals – sample S_4 the sample with 50% dark chocolate and 50% amaranth seeds

3.7. Melting Profile Analysis

Dark chocolate is a non-Newtonian solid suspension composed of cocoa powder and sugar [16]. The quality and stability of chocolate are strongly influenced by rheological parameters. The melting profile of chocolate is associated with certain production defects, such as flow losses or unwanted deformations [17]. Generally, two major factors influence the melting profile or flow behavior of chocolate: the chocolate formula and the homogenization process with other ingredients incorporated into the chocolate mass during production [18]. The thermogram representing the melting point is shown in Figure 3. For the control sample, the maximum melting temperature was 17.34°C, while the onset temperature was 13.22°C, and the end temperature was 14.52°C. The samples containing different percentages of amaranth seeds had a higher melting point, ranging between 18.22°C and 20.18°C, as shown in Table 2.

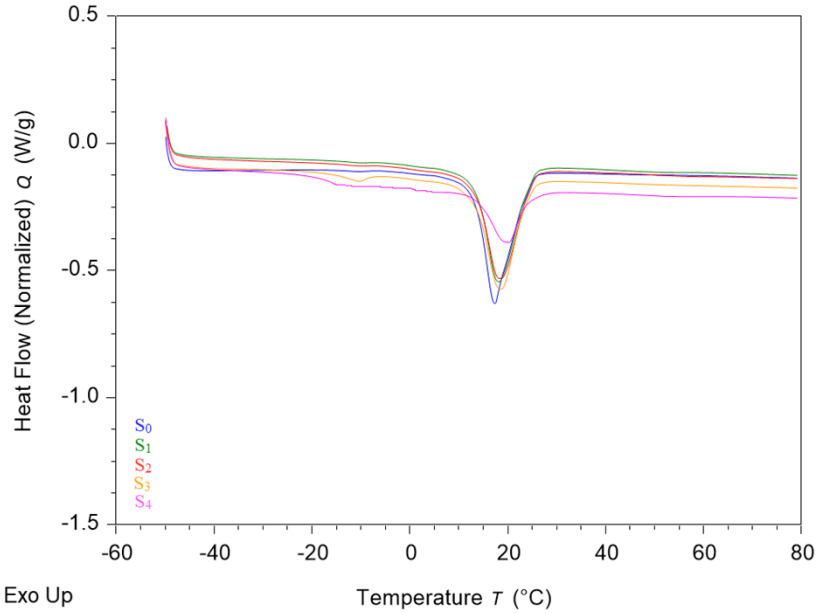

Fig. 3. The melting profile of the chocolate and the analyzed samples at 20°C. S_0 represents the control sample consisting of only dark chocolate, S_1 represents the sample with 95% dark chocolate and 5% amaranth seeds, S_2 represents the sample with 90% dark chocolate and 10% amaranth seeds, S_3 represents the sample with 80% dark chocolate and 20% amaranth seeds, and S_4 represents the sample with 50% dark chocolate and 50% amaranth seeds

Table 2.

DSC analysis of chocolate bars

Sample	Parameters					
	Onset (°C)	Midpoint (°C)	Endset (°C)	Enthalpy (J/g)	Melting point (°C)	ΔCp (J/g · °C)
S0	13.22	13.25	14.52	41.21	17.34	1.723
S1	13.49	13.54	14.48	41.48	18.22	0.056
S2	13.25	13.31	14.36	39.95	18.51	1.094
S3	13.29	13.35	14.29	38.90	18.66	0.071
S4	15.25	16.20	17.19	44.21	20.18	0.910

This increase may be due to the mixture with amaranth seeds, which alters the crystal formation process. Similarly to the maximum melting temperatures, where significant differences were recorded between sample 4, consisting of 50% dark chocolate and 50% amaranth seeds, and the other samples, the enthalpy value was ≤ 5.31. The melting profiles of the control sample and sample 1 were stable and did not change throughout the analysis.

3.8. Texture Analysis

Figure 4 shows the texture parameter hardness for both the control sample, which consists of plain chocolate, and the other samples containing various percentages of amaranth seeds. The hardness parameter was determined at room temperature, which was 20°C. This temperature represents the optimal condition for the chocolate samples to form crystals for a good texture and an optimal melting profile [13]. As observed in Figure 2, the hardness of sample 3, containing 20% amaranth seeds, and sample 4, containing 50% amaranth seeds, was lower than that of the control sample. A possible explanation could be that the samples with higher amaranth seed content have more β-type crystals compared to the control sample, which consists only of dark chocolate and has a lower melting point than β-type crystals [19]. At 20°C, there were not significant differences in hardness between sample 1 with 5% amaranth seeds and sample 2 with 10% amaranth seeds. This is due to the small percentage of amaranth seeds in the composition of these two samples, which does not notably affect the hardness.

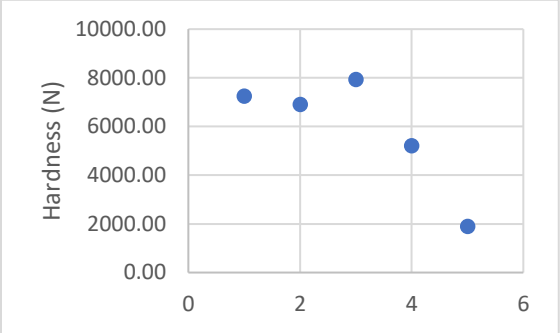

Fig. 4. Chocolate bars hardness

3.9. Sensory Analysis

The sensory properties of the dark chocolate samples mixed with amaranth seeds, which were thermally treated using the sous-vide method, are presented in Figure 5.

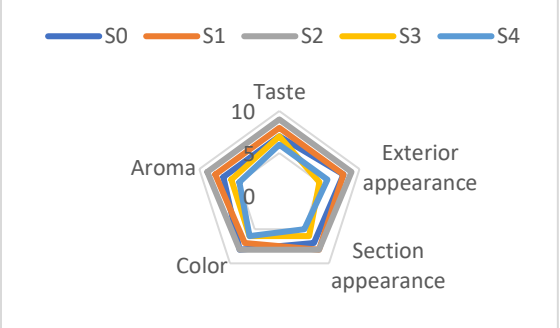

Fig. 5. Sensory analysis

Sample 2, consisting of dark chocolate and amaranth seeds, did not vary significantly in the evaluations by study participants and received the highest score in all assessment criteria. Sample 4, composed of 50% dark

chocolate and 50% amaranth seeds, was rated with the lowest score in all criteria and, along with sample 3, exhibited the greatest fluctuations in participants' preferences. Aroma and external appearance received the highest scores among all evaluation parameters.

3.10. Principal component analysis

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

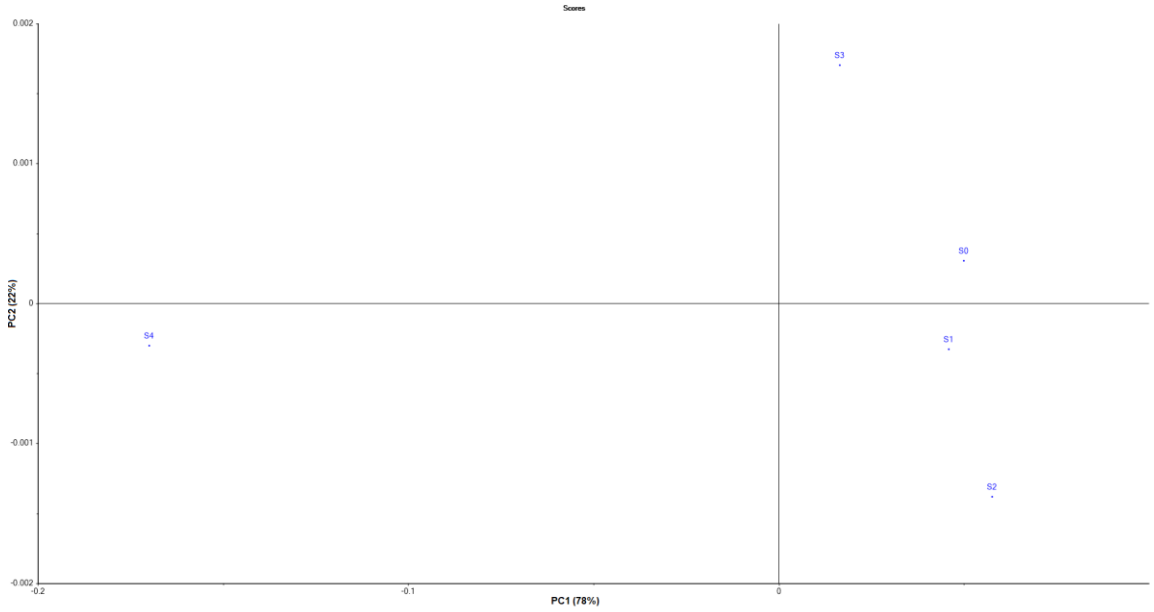

Fig. 6. Principal component analysis of chocolate bars

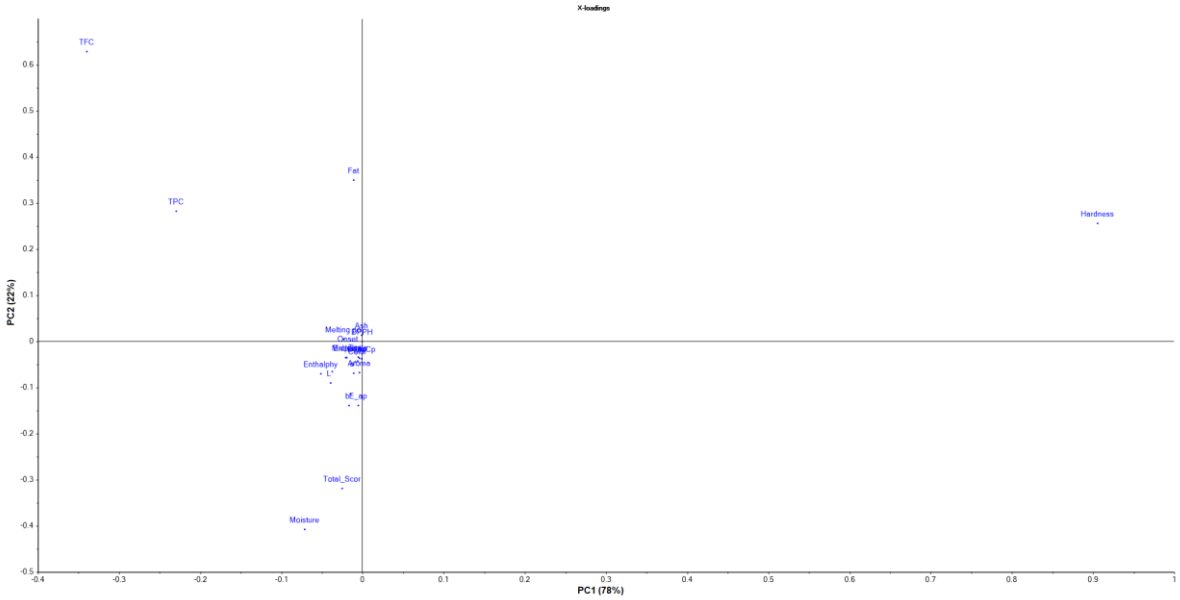

Fig. 7. Principal component analysis of the physicochemical parameters loadings

It was found that the two principal components (PCs) explained 100% of the variations in the dataset. It can be observed that the sample S0, S1, S2 and S3 are grouped, while the S4 is placed in a different region of the projection. 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.

The parameters TFC, TPC, moisture content, and hardness influence strongly the projection. The S4 is influenced by the moisture content and total score, while the S0 and S3 are influenced by hardness.

4. Conclusions

The structural aspects of the samples were evaluated considering the melting profile, crystal formation, and sensory characteristics of the control sample consisting of 100% dark chocolate compared to the other samples with varying percentages of amaranth seeds treated thermally using the sous-vide method. Samples 1 and 2, which had the lowest amounts of amaranth seeds, exhibited the most stable melting profiles and sensory characteristics. In terms of biologically active compounds, samples 3 and 4 stood out with higher values for both phenolic profiles and antioxidant capacity. However, due to their significant water content, these samples do not represent a viable alternative for medium- and long-term storage under normal conditions. All samples had approximately equal nutritional compositions, and samples 1 and 2 demonstrated comparable levels of consumer satisfaction. In conclusion, sample 1 with 5% amaranth seeds is comparable to the control sample consisting of 100% cocoa chocolate in terms of physicochemical properties and sensory attributes. The most appreciated sample is sample 2 with 10% amaranth seeds, which was comparable to the control sample, although some physicochemical characteristics differed.

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