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Characterization and Functionality of Raw and Fermented Chia Seeds in Food Products

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ABSTRACT

Due to the increasing demand for functional foods that offer health benefits for special groups suffering from various nutrient deficiencies (such as patients with celiac disease, kwashiorkor, and malnutrition), this study compares raw black chia seeds (Salvia hispanica L.) with yeast-fermented (Saccharomyces cerevisiae var. boulardii) chia seeds. The fermentation process was found to increase the crude protein content from 16.91% to 21.64% and total carbohydrates from 45.02% to 46.64%, while the values of ash, caloric content, crude fiber, and crude ether extract decreased. Except for magnesium, the concentration of minerals (Ca, Fe, P, Mn, and Zn) significantly increased. The DPPH scavenging activity also rose from 93.20% to 95.80% due to fermentation. Additionally, the levels of essential and non-essential amino acids increased, as did the percentage of omega-3 fatty acid (C18:3, n-3) and the n-6 to n-3 ratio (from 1:2.74 to 1:5.15). The study further demonstrated that fermentation enhanced the amounts of several phenolic compounds, including caffeic acid, methyl gallate, quercetin, naringenin, and syringic acid. These volatile compounds influence the aroma of the fermented sample, contributing to a taste and odor that some consumers find desirable. Finally, the authors developed functional products such as jam, cereal bars, chocolate spread, fermented juice, fermented weaning food, and a fermented milk beverage. These products underwent sensory evaluation and were found to be within acceptable limits by panelists.

1. Introduction

In recent years, the pseudo-cereals showed a new trend in the food technology and nutrition fields. One of these grains is chia seed (*Salvia hispanica L.*), an annual plant in the Lamiaceae family. Crude protein (15-20%), ether extract (30-33%), total carbohydrates (26-41%), crude fiber (18-30%), and ash (4-5%) make up the chemical composition of these seeds. It also contains a lot of fiber and minerals, particularly phosphorus, iron, and calcium. Fortunately, α-linolenic acid (n3), which makes up around 60% of its lipid content, has demonstrated preventive effects against obesity and cardiovascular illnesses. On the other hand, chia seeds are rich in naturally occurring antioxidants such as carotenoids, phytosterols, tocopherols, chlorogenic acid, and many phenolic compounds. In addition to having anti-oxidative qualities, chia seeds have from 5 to 6% crude fiber, the dietary fiber represents around one-fourth of chia seeds. Also, the seed contains a high soluble fiber content, which forms a highly hydrophilic mucilage that makes it highly intriguing for use in industry and medicine purposes. That makes the soluble fiber of chia seed a great thickening agent. Then, chia seed does not contain any poisons or allergies, so, it is considered an important functional ingredient used safely in various food products. Furthermore, the chia seed protein is free from the gluten fraction and contains prolamins and other easily digestible source of amino

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Acids (Bochicchio et al., 2015; Coelho and Salas-Mellado, 2015, and Svec and Hrušková, 2017).
Basinskiene and Cizeikiene (2020) illustrated that the bioactive components have positive effect on the public health, thus the chia seed became the cornerstone in our diet. Moreover, consuming whole grains constantly protects us against several diseases, where the cereals have attracted significant attention recently, particularly for medical purposes such as food allergy and food intolerance, as well as due to lifestyle preferences. Beverages that formulated based cereals are gaining prominence. Fermentation represents a good method to improve the chemical composition, sensory characteristics, and nutritional benefits of these formulations. In addition, it is a method of addressing deficiencies in key components like amino acids. Raungrusmee et al. (2022) showed that there is a growing trend towards the development and creation fortified beverages as part of a broader shift towards health-conscious nutrition. Functional foods, particularly those containing probiotics, prebiotics, and synbiotics to support gastrointestinal health, are in high demand. In food fermentation, probiotics yeasts, bacteria alongside with fungi are identified the health-giving microorganisms, because the public human health depends on the balance between good and bad microorganism in the gastrointestinal tract. The probiotics are capable to survive and thrive in the colon to play their vitals benefits. On the other side, these probiotics affect the characteristics of fermented products, such as aroma, digestibility, and stability. Hence, Bekatorou et al. (2006) found that Saccharomyces cerevisiae, a type of yeast, exhibits probiotic properties and demonstrates anti-pathogenic abilities against microorganisms like Shigella spp., Salmonella spp., and Escherichia coli. Yeasts are widely known as feed supplements microorganisms offering many medical effects such as regulating the immune responses, maintaining a healthy microbiome flora, especially after diarrhea, playing as anti-oxidative and anti-pathogens, and alleviating symptoms of lactose intolerance. Controlling blood sugar levels by delaying the rate at which complex sugars convert to their simpler form. Research supports the studies on the fermented cereals because of its multiple health advantages especially, the beverages and highlighting their potential to enhance overall well-being. This is in agreement with what was mentioned by Blandino et al. (2003), they viewed that there many benefits outcome of bioscience activity in food, It improves the appearance of the food and alters positively the biosynthesis of fermented foods, it also prevent growth of the bad microorganisms that contribute to its spoilage and alteration. Jams often fall short as nutritional options, particularly when they have a high glycemic load and lack essential nutrients. The sugar content in some jams can exceed 77.5%, while protein levels are as low as 1% and fiber content is limited to a maximum of 2%. This has led to a growing demand for healthier and more beneficial food alternatives. Research suggests that incorporating novel ingredients, such as pseudo-cereals, can help create nutraceutical jams. One strategy to enhance the nutritional value of jams is to introduce unique, nutrient-rich seeds. Several studies have explored the use of various beneficial ingredients in jams, including dietary fiber. For example, chia seeds can be used to create jams with enhanced nutritional properties (Saleem et al., 2024). Similarly, chocolate has been considered a suitable choice for enhancing nutritional value. Recent initiatives have focused on adding various beneficial substances to chocolate, such as phytosterol esters, eicosapentaenoic-acid (EPA), docosa - hexaenoic - acid (DHA), and other bioactive compounds. Additionally, the addition of chia seeds to chocolate spread can provide consumers with antioxidants, phenols, and flavonoids (Razavizadeh 2021). Due to their high carbohydrate, crude protein, crude lipid, and mineral content, cereal bars are a great snack option with positive sensory and nutritional characteristics. Pregnant and lactating women can benefit from this product as it provides energy and contains minerals like calcium and iron. Given the increasing consumer demand for natural, convenient, and healthy food options, there is a need to innovate and improve the nutritional content of food bars to offer better health benefits (Singh et al., 2020).

The nutritional needs of infants who have just started eating solid food require adding protein-rich source to their diet. The infant weaning foods are typically make from cereals and are fermented using lactic acid. The process of creating these weaning foods through fermentation should aim to be as natural as possible and practie in a very hygienic environment. This approach can help lower rates of diarrhea, infections, kwashiorkor, and high infant death rates. Additionally, making these foods is affordable, simple, and offers numerous health benefits and probiotic properties. The food mixture can also be as effective as commercially available infant food (Adeyemo and Onilude, 2018). This study was included two parts. The first one was the examination of the chia seeds (*Salvia hispanica L.*), such chia seeds sample and the yeast fermented chia seeds sample, for nutrient profile (chemical composition, minerals, antioxidant activity, the composition of fatty acid, amino acid and polyphenols, and the volatile compounds). That parallel with the second part of the study, which tried to create some functional food products from the non-fermented and fermented chia seeds and evaluated them for sensory characteristics.

2. Materials and Methods

Materials

The black chia seeds (800g) were purchased from the Abo-Ouf Company, Alexandria, Egypt. Strawberry fruits, honey, sugar, glucose syrup, lemon, olive oil, milk powder, cocoa, sunflower oil, lecithin, vanilla, sodium chloride salt, and whey milk were purchased from the local market, Alexandria government. All the chemicals were of analytical reagent grade, purchased from Sigma-Aldrich Chemical Company, USA. The culture media was purchased from Oxoid, USA.

Methods

Raw chia seeds preparation

Raw chia seeds were prepared according to Ali et al. (2022) by pulverizing in a laboratory mill. To achieve an equal particle size of ground seeds, they were passed through a 60 µm sieve. Half the amount of ground chia seeds was autoclaved for 20 min at 121°C and 15 psi. Finally, the sample was kept in airtight, sterilized glass containers at 4 °C until the fermentation process was completed. Finally, the second half amount of raw ground seeds used in the chemical analysis.

Microorganisms and inoculum preparation

The *Saccharomyces cerevisiae* var *bouladrii* strain was obtained from Scientific Elsadat City, Menoufia Governorate, Egypt. *S. cerevisiae* var *bouladrii* was cultured on Sabouraud Dextrose Agar (SDA) (Oxoid, USA). Then a colony of *S. cerevisiae* var *bouladrii* was separately inoculated into Sabouraud Dextrose Broth (SDB), and it was incubated at 30°C for 72 h, and the resulting inoculum was centrifuged at 6000 rpm/ 15 min. The supernatant was discarded, and the pellet was washed twice with saline water and re-suspended in distilled water with vortexed for 5 min, then optical density (OD 600 nm) was maintained at 0.5 OD (Ali et al., 2022).

Fermentation of autoclaved ground chia seeds

The autoclaved ground chia seeds were carried out in a sterilized glass jar and inoculated with 40 ml of inoculum/100 g sample. The substrate was incubated at 37°C for 72 h. After incubation, the sample was freeze dried by Panch Top Virtis SP Scientific Spain apparatus at the Central Laboratory of the Faculty of Agriculture, Alexandria University. The fermented sample was kept in sterilized airtight glass containers at 4°C (Ali et al., 2022).

Proximate composition of chia seeds samples

The moisture and ash content of the non-fermented and fermented chia seed samples were determined according to AOAC methods. The protein content (N x 6.38) was determined using the Semi-Micro-Kjeldahl Method as described by AOAC (2016). Meanwhile, the crude fat content was determined by the Soxhlet method (AOAC, 2016). On the other hand, after digesting the samples with concentrated sulfuric acid and potassium hydroxide, the fraction remaining was used in the determination of the fiber content (Al-Abdulkarim et al., 2013). Finally, the total carbohydrate content of the samples was calculated by the arithmetic difference equation: Total carbohydrate % = 100 - (moisture % + Ash% + protein% + fat%) according to Ihemeje et al. (2015), and the caloric value of the samples was estimated using the Atwater general factor system according to Kamel et al. (2023).

Mineral content analysis

The samples of non-fermented and fermented chia seeds samples were taken to the ICP-OES Lab at the High Institute of Public Health, Alexandria University, for analysis of the mineral composition of calcium (Ca), iron (Fe), phosphorus (P), manganese (Mn), magnesium (Mg), and zinc (Zn). Following standard procedure, US EPA Method 200.7 and US EPA Method. Operating conditions are controlled (temperature 21.9 °C and relative humidity 46 %), Agilent ICP-OES 5100 VDV (Gülfen and Özdemir, 2016).

Radical Scavenging assay for DPPH

The antioxidant activity (DPPH scavenging activity) was estimated for the non-fermented and fermented chia seeds samples. The ethanol extracts were evaluated based on their radical scavenging ability in reacting with 0.005 g of DPPH in 200 ml of methanol, according to Brand-Williams et al. (1995). This mixture was well stirred and left in the dark at 27°C for 20 min. The absorbance of this mixture (A_{sample}) and that of a control sample (A_0) with only methanol, was then measured at 515 nm. Finally, the antioxidant activity of non-fermented and fermented chia seeds could be determined by applying the radical scavenging activity (RSA) equation (1):

$$\text{RSA (\%)} = 100\ (A_0 - A_{sample})\ /A_0 * 100 \qquad (1)$$

Amino acids composition

The lipid fraction of samples was extracted from the non-fermented and fermented chia seeds samples, and then the protein fraction was precipitated using trichloroacetic acid (TCA). The obtained protein fraction was carried out to estimate its amino acids composition using an amino acids analyzer according to AOAC (2016).

Fatty acids composition

The determination of fatty acids composition for the non-fermented and fermented chia seeds samples was estimated as follow;- extraction were performed, and the samples were analyzed using a Trace 1300 GC Ultra/Mass Spectrophotometer ISQ QD (Thermo Scientific) instrument, X-calibur 2.2 software (Thermo X-calibur). GC-MS spectrophotometer equipped with a MS detector using the same column and chromatographic conditions as for GC-FID. The fatty acids composition of non-fermented and fermented chia seeds samples was expressed as a percentage and calculated from the total peak areas (Tavaand and Avato, 2014).

Polyphenol compounds identification

According to the method of El-Hawary et al. (2022), the quantitative determination of polyphenol compounds of non-fermented and fermented chia seeds samples was analyzed using HPLC apparatus, Agilent Series 1200 apparatus (Agilent, USA), 1100 software, and UV detector (280 nm).

Volatile compounds analyzed

The volatile compounds (VC) were analyzed using gas chromatography-mass spectrometry GC-MS (Trace 1300 GC Ultra/Mass Spectrophotometer ISQ QD (Thermo Scientific) instrument and X-calibur 2.2 software (Thermo X-calibur). The non-fermented and fermented chia seeds samples were prepared and analyzed under the following conditions: The injector temperature was 250°C, ion source temperature was 220°C, and interface temperature was 260°C. Helium was used as a carrier gas at a 0.65 ml/min flow rate. The gradient temperature was defined from 40°C for starting to 220°C with increasing 5°C each 3min and from 220°C to 310°C with increasing 15°C each 6min. The volatile compounds were identified according to mass spectrum libraries, which have spectrum and retention index of compounds. Finally, the retention index and the spectrum were used to match with the retention time of the obtained volatile compounds. Alkane mix (C8-C20) was also analyzed to know the retention time of unknown compounds (Bartkiene et al., 2023).

Formulations of non-fermented food products

Strawberry jam

The fresh strawberries were sorted, properly cleaned, and cut into two halves before making jam samples. For every one kg of strawberry fruit, 400 g of sugar, 200 g of glucose syrup, and 125 g of water were added. After preparing the mixture to a boil and adding 50 g of powdered non-fermented chia seeds, the soluble solids were concentrated to around 68%, achieving the desired consistency. Lastly, 1% fresh lemon juice was added. The jam was poured into glass bottles that had been previously sterilized. Prior to sensory evaluation, the glass bottles were sealed and kept in a dry and cool place (Saleem et al., 2024).

Cereal bar

Cereal bar was manufactured according to Concenço et al. (2019) with some modifications. First, the non-fermented chia seeds powder (322 g) was added with a mixture of honey (50 g), sugar (200 g), glucose syrup (100 g), and water (26 g) and subjected to heating until the temperature reached 116°C. The seeds were mixed with the hot honey as a binding agent, which helps hold the bar together and improves their taste at 95°C. The mixture was put into silicon molds, previously laminated with a thin layer of olive oil, pressed and chilled until they formed bars or we shaped it into balls. Samples were stored at room temperature until the sensory evaluation.

Chocolate spread

Chocolate spread was manufactured using 42% sugar powder, 15% milk powder (3.5% fat), 10% cocoa, 27% sunflower oil, 0.7% lecithin, 0.015% vanilla, 0.018% sodium chloride salt, and 5% non-fermented chia seeds powder. Where, the dry ingredients (cocoa, milk powder, sugar powder, salt, lecithin, and vanilla) were mixed well and then placed in an electric mixer. And later the oil was added with a steady flow until the desired consistency of chocolate was spread was obtained and kept until sensory evaluation (Tlay and Baidhani-Al, 2023).

Formulations of fermented food products

Fermented juice

According to D'Souza et al. (2017) with some modifications, strawberry fruits were collected from the local market. 500 g of juice was extracted from the strawberry fruits, then 130 g of sugar was mixed (26°Brix), then the mixture was heated at 95°C for 30 min and cooled to 40°C. Then 50 g of the fermented chia seeds sample was added, and they mixed vigorously, and the juice was kept in a refrigerator until sensory evaluation.

Fermented weaning food

According to Adeyemo, (2012) with some modifications, the sample of fermented chia seeds powder (100 g) was mixed in the ratio 1:3 with hot distilled water (40°C) until thickening. The formulation was done when the weaning food was ready for sensory evaluation.

Fermented milk beverage

According to Yadav et al. (2016) with some modifications, 100 ml of pasteurized whey milk was mixed with 8% sucrose and 0.5% vanilla, then the mixture was heated to 80°C until the sugar dissolves completely then allow the mixture to cool until it reached 40°C. Then the fermented chia seeds powder (10%) was added and mixed well with the previous mixture, then filled into sterilized glass container at 4°C until sensory evaluation.

Sensory evaluation of fermented and non-fermented products

Sensory evaluation for prepared non-fermented and fermented chia seeds products was measured according to Sneha and Vijayakumar, (2019). The products were conducted by ten semi-trained panelists from the students of Home Economics department of the Agriculture Faculty, Alexandria University. Each panelist was provided with the sample and asked to evaluate the samples for the color, taste, odor, texture and overall acceptability according to a numerical hedonic scale ranging from 1 to 9 (where 1 is extremely dislike and 9 is extremely like).

Statistical Analysis

The experiment was carried out in triplicate, and the data were statistically analyzed using one-way ANOVA at a significance level of 5%. The data were transferred to the SPSS 16.0 software (SPSS, 2007).

3. Results and Discussion

Results of chemical composition, minerals, and antioxidant activity are shown in Table 1. These results illustrated that there were differences in moisture (3.29 and 5.08%), crude protein (21.64 and 16.91%), crude ether extract (24.35 and 28.08%), ash (4.08 and 4.91%), crude fiber (20.71 and 22.06%), carbohydrate (46.64 and 45.02%), caloric value (492.27 and 500.44%), calcium (400.0 and 254.50 mg/100g), phosphorus (660.89 and 589.61 mg/100 g), iron (9.22 and 7.68 mg/100g), manganese (5.66 and 2.17 mg/100 g), magnesium (232.0 and 313.0 mg/100 g), zinc (4.50 and 2.88 mg/100 g) and DPPH scavenging% (95.8 and 93.20%) for fermented chia seeds and non-fermented chia seeds, respectively. Kibui et al., (2018) evaluated that the proximate analysis of chia seeds showed a closed content of moisture (5.16%) and was rather lower than the other literature studies (6.3%). This may due to the difference in conditions of cultivation and the degree of drying after harvest. As well, they found a high content of the crude protein (20.90%) and they reported that this was the higher content than some previous studies (16.54% and 19.6%) and this study (16.91%). Overall, they showed closed crude ether extract content with this study result (29.06%), and it ranged from 29.4% to 34.4 % in other literature results. That may refer to the method of the extraction or/and the condition and cultivation area. As for the level of ash, which reflects the percentage of minerals present in the chia seeds, it was 4.45%, which was in line with the current value and other proportions established before. Similarly, the percentage of crude fiber was nearly the ratio determined in this research (21.14%) and lower than other research which reported values ranging from 34% to 40%. This percentage of crude fiber meets a large percentage of the daily needs of adults, as determined by the nutritionists who recommended an intake of crude fiber ranging from 18 to 38 grams, which may vary depending on age and sex and is varied from 8 to 20 g per 1000 k cal (Ioniţã-Mindrican et al., 2022). On the other hand, Ikumi et al. (2023) and Kib,ui et al. (2018) referred to the ratios of the minerals such as calcium (1293.15mg/kg100g versus 254.50 mg/100 g in this research and 557-770 mg/100 g and 478-544 mg/100g in other literature, respectively) and phosphorus (116.30mg/kg versus 589.61mg/100 g in this research and 533-888mg/100 g in recent research), both elements are good for bone health among humans. However, the iron content was 9.14 mg/ kg versus 7.68mg/100g in this research and 6.3 -9.9 mg/ kg and 7.08-9.05 mg/100g in other studies, respectively. Also, magnesium content was 225.93 mg/kg versus 313.0 mg/100 g in current results and 325-390mg/kg and 363-440mg/100 g in various researches, respectively. Then, manganese was 7.56 mg/kg versus 2.17 mg/100 g in this paper and 2.9-5.42mg/100 g in similar research, respectively. Finally, zinc content was 6.68 mg/kg versus 2.88 mg/100 g in current results and <0.1-4.95 mg/kg and 2.48-5.47 mg/100g in such investigations. The fermented chia seeds were increased in protein, total carbohydrate, calcium, iron, phosphorus, zinc, and DPPH scavenging%, and decreased in crude ether extract, crude fiber, and ash. That agree with the results of Kamel et al. 2023, who found that the fermentation of the chia seeds leads to an increase in protein content from 25.29% to 28.10% and a decrease in fat content from 32.35% to 25.88%, crude fiber from 32.94% to 32.67%, and ash from 6.05% to 6.0%, respectively. In addition, they found appreciable increases in calcium content from 609.7 mg/100g to 744.2mg/100g, phosphorus from 160.25 mg/100g to 167.25mg/100g, iron from 11.2 mg/100 g to 12.8 mg/100g, and zinc from 7.9 mg/100g to 8.55 mg/100g. Unlike the chia seeds content of manganese, it was decreased from 5.05 mg/100g to 4.8 mg/100g by fermentation. On the other side, the antioxidant activities of chia seeds were analyzed using the DPPH method, and the results recorded non-significant changes between raw and fermented samples, respectively (87.17 and 60.67%).

While, Coelho and Salas-Mellado (2014) reported that the phenolic chia seeds extract was successful in getting rid of 70% of the free radicals due to its content of many bioactive compounds, which played an important role as anti-oxidant compounds, and they have effective physiological roles against cancer and genetic mutations.

Table 1. Chemical composition (%), mineral contents (mg/100 g) and DPPH scavenging (%) of non-fermented and fermented chia seeds.

Components	Samples	
	Non-fermented chia seeds	Fermented chia seeds
*Moisture %	5.08±0.13	3.29±0.365
*Crude protein (N×6.25) %	16.91±1.49	21.64±1.72
*Crude ether extract %	28.08±3.06	24.35±1.69
*Crude fiber %	22.06±0.94	20.71±0.77
*Ash %	4.91±0.11	4.08±0.07
*Total carbohydrate %	45.02±1.70	46.64±2.74
*Caloric value (Kcal/100g)	500.44±14.83	492.27±8.71
Calcium (mg/100 g)	254.50	400.00
Iron (mg/100 g)	7.68	9.22
Phosphorus (mg/100 g)	589.61	660.89
Manganese (mg/100 g)	2.17	5.66
Magnesium (mg/100 g)	313.00	232.00
Zinc (mg/100g)	2.88	4.50
DPPH scavenging %	93.20 ± 0.005	95.80 ± 0.008

*The values are represented in mean ±standard deviation derived for triplicate experiments (n= 3).

The results of amino acids in non-fermented and fermented chia seeds samples are tabulated in Table 2. The seeds samples contained 17 of 22 amino acids, and the total amount of nine essential amino acids was 7.27 and 10.58g/100 g for non-fermented and fermented chia seeds, respectively. Whereas, both samples contained eight non-essential amino acids with a total amount of 9.09 and 12.88g/100g for non-fermented and fermented chia seeds, respectively. It is worth mentioning that the amount of amino acids increased in the fermented sample as a result of the increase in the amount of crude protein by the fermentation process. Some results of chia seeds's amino acids composition were 18 amino acids in the black chia seeds included tryptophan (10 essential and 8 non-essential amino acids), the content of glutamic acid, a non-essential amino acid that regulates the immune system and provides the physical capacity, was 3.50 g/100 g versus 3.33 and 4.44 g/100 g for non-fermented and fernented chia seeds samples in current research (Kamel et al., 2023). The amount of arginine amino acid, which has a necessary function to reduce heart failure, was 2.14 g/100 g versus 1.30 and 2.32 g/100 g in non-fermented and fermented chia seeds in current results. And we noticed that all values of the amino acids were in agreement with the amino acids composition of chia seeds (Suri et al., 2016).

The composition of fatty acids in non-fermented and fermented chia seeds is listed in Table 3. Both samples were varied in the fatty acids composition since some fatty acids only appeared in non-fermentd sample such as caproic, tridecanoic, myristic, pentadecanoic, palmitic, heptadecanoic, stearic, oleic, and eicosanoic. It is worth noting that the viable cell of yeast in the fermenting sample may consume some fatty acids during the fermentation process, and the omega-3 fatty acid (α-linolenic) was increased from 52.37 to 69.32%. whereas, linoleic acid , α-linolenic acid and docosanoic fatty acids appeared in both, but non-adecanoic fatty acid only appeared in the fermented one, which could be a result of the metabolism of viable yeast cells. The fermentation process improved the composition of fat in chia seeds and it became healthier than the fat of non-fermented seeds. As a result of the increased ratio of the omega -6 to omega-3 ratio from 1:2.74 to 1: 5.15, which is useful in regulating the hypertension and blood lipids. Kamel et al. (2023) found that the ratio of n6: n3 was 1:2.89 and the ratio of n9 was 10.24% in the black chia seeds. Bartkiene et al. (2023) found that the ɑ-linolenic acid (C18:3, n3) was the main fatty acid in non-fermented and fermented chia seeds and it was increased by 6.50%, then the cis-linoleic acid (C18:2, n6) was decreased by 3.88% during the fermentation. On the other hand, the stearic (C18:0), oliec (C18:1), and eicosanoic acid (C20:0) fatty acids were decreased by fermentation.

Table 2. Amino acids composition (g/100g) of non-fermented and fermented chia seeds

Essential amino acids (g/100g)	Samples				
	Non-fermented chia seeds	Fermented chia seeds	Non-essential amino acids (g/100g)	Non-fermented chia seeds	Fermented chia seeds
Arginine	1.30	2.32	Glutamic	3.33	4.44
Leucine	1.34	1.67	Aspartic	1.64	2.00
Lysine	0.97	1.23	Alanine	0.85	1.47
Phenylalanine	0.88	1.39	Serine	0.82	1.31
Valine	0.79	1.03	Glycine	0.64	1.27
Isoleucine	0.67	0.91	Proline	0.88	0.98
Threonine	0.60	0.89	Tyrosine	0.67	0.97
Histidine	0.45	0.65	Cysteine	0.26	0.44
Methionine	0.27	0.49	Total	9.09	12.88
Total	7.27	10.58	-	-	-

Table 3. Fatty acids composition (%) of non-fermented and fermented chia seeds.

Fatty acids (%)	Samples		Fatty acids (%)	Samples	
	Non-fermented chia seeds	Fermented chia seeds		Non-fermented chia seeds	Fermented chia seeds
Caproic acid (C6:0)	0.35	-	Stearic acid (C18:0)	4.52	-
Tridecanoic acid (C13:0)	0.37	-	Cis-Oleic acid (C18:1)	8.51	-
Myristic acid (C14:0)	0.36	-	Cis-Linoleic acid (C18:2,n6)	19.13	13.47
Pentadecanoic acid (C15:0)	0.52	-	ɑ-Linolenic acid (C18:3,n3)	52.37	69.32
Cis-10- Pentadecanoic acid (C15:1)	0.24	-	Nonadecanoic acid (C19:0)	-	2.25
Palmitic acid (C16:0)	9.11	-	Eicosanoic acid (C20:0)	0.49	-
Heptadecanoic acid (C17:0)	-	4.17	Docosanoic acid (C22:0)	4.03	10.79
Total unsaturated fatty acids	80.01	82.79	Total saturated fatty acids	19.75	14.96
n6:n3	1: 2.74	1: 5.15	-	-	-

Table 4 represents the polyphenols in non-fermented and fermented chia seeds as 17 compounds, all these compounds existed in both except for coumaric acid, catechin, and rutin, which were produced due to the fermentation process, but cinnamic acid and ferulic acid disappeared after fermentation. Some compounds were increased, such as caffeic acid, querectin, syringic acid, daidzein, methyl gallate, and naringenin. Others, were decreased, such as rosmarinic acid, chlorogenic acid, gallic acid, vanillin, pyrocatechol, and ellagic acid. Kamel et al. (2023) reported that naringin amount was increased by fermentation from 10.76 mg/100 g to 12.41 mg/g in non-fermented and fermented black chia seeds, respectively. Other polyphenol compounds such as 4,amino benzoic, caffeic acid, vanillin, coumaric acid, catechin, and pyrogallol were increased by the fermentation treatment. This does not agree with this study, and this may be due to the species of microorganism used in the fermentation process. Coelho and Salas-Mellado (2014) stated that the main phenolic component in chia seeds was caffeic acid (30.89µg/g) and in previous studies it was chlorogenic acid (45.9-102 µg/g) then caffeic acid (3-6.8 µg/ g) but in this study the predominant phenolic compounds were rosmarinic acid (1127.77µg/g) followed by chlorogenic acid (132.77µg/g) then gallic acid (72.48µg/g) compared to (82.29µg/g), (51.35µg/g), and (53.58µg/g) for non-fermented and fermented chia seeds, respectively. Which means that the phenolic profile may be due to the cultivation region, conditions of cultivation, the efficiency of the extraction, or the treatment that may be performed on the seeds, such as fermentation.

Table 4. Polyphenol compounds (µg/g) of non-fermented and fermented chia seeds

Polyphenol Compounds (µg/g)	Samples		Polyphenol Compounds (µg/g)	Samples	
	Non-fermented chia seeds	fermented chia seeds		Non-fermented chia seeds	fermented chia seeds
Rosmarinic acid	1127.77	82.29	Syringic acid	4.41	11.03
Chlorogenic acid	132.77	51.35	Ferulic acid	3.33	0.00
Gallic acid	72.48	53.58	Daidzein	2.94	6.99
Vanillin	21.77	9.43	Methyl gallate	2.57	57.45
Caffeic acid	10.72	18.84	Naringenin	1.84	18.99
Pyrocatechol	14.00	11.21	Coumaric acid	0.00	5.08
Ellagic acid	6.86	2.31	Catechin	0.00	5.03
Querectin	5.09	38.00	Rutin	0.00	2.93
Cinnamic acid	5.02	0.00	-	-	-

In comparisons between the non-fermented and fermented chia seeds samples, Table 5 illustrates changes in volatile compounds percentages in non-fermented and fermented chia seeds. The main volatile compounds in both samples were butanoic acid, butyl ester, n-hexadecanoic acid, butyl 9,12,15-octadecatrienoate, 9,12,15-octadecatrienoic acid, methyl ester (Z,Z,Z), butanoic acid, ethyl ester, 9,12 -octadecadienoic acid (Z,Z), methyl ester, and 2-hexenal, 2-ethyl, as mentioned in Table 5. On the other hand, there were some volatile compounds that appeared in the non-fermented sample such as 9,12,15-octadecatrienoic acid (Z,Z,Z), gamma-sitosterol, phenol, 2,4-bis (1,1-dimethylethyl), phosphite 7,9-di-tert-butyl-1-oxaspiro, deca-6,9-diene-2,8-dione, acetic acid, hexyl ester, gamma-tocopherol, 7,10,13-eicosatrienoic acid, methyl ester, decane, 2-methyl, 1-heptatriacotanol, campesterol, pentadecane, pentadecanoic acid, 14-methyl, methyl ester, eicosane, styrene, 2,4-di-tert-butylphenol,stigmasta-5,22-dien-3-ol, (3.beta.,22E), beta-sitosterol, nonadecane and myristic acid, TMS derivative. After fermentation process, there were volatile compounds increased as butyl 9,12,15-octadecatrienoate (moreover 7.24 times), 9,12,15-octadecatrienoic acid, ethyl ester (Z,Z,Z) (moreover 5.4 times), 9,12,15-octadecatrienoic acid, methyl ester (Z,Z,Z) (moreover 1.64 times), hexanoic acid, butyl ester, and 9,12-octadecadienoic acid (Z,Z), methyl ester. Also, other volatile compounds were decreased as butanoic acid, butyl ester (moreover 8.67 times), n-hexadecanoic acid (moreover 6.09 times), butanoic acid, ethyl ester, and 2-hexenal, 2-ethyl. Finally, there were new volatile compounds that appeared after fermentation: 9,12,15-octadecatrienoic acid, 1-methylethyl ester (Z,Z,Z), and ethyl 9.cis.,11.trans.-octadecadienoate.

Bartkiene et al. (2023) concluded that when they added non-fermented chia seeds (20%) or solid-state fermented chia seeds (30%) in bread samples, volatile compounds such as 2-methylpyrazine and 1-(2-furanyl)-ethanone were not found. And they attributed this to the amount of chia seeds that were added to the bread. On the other side, the existence of 3-furaldehyde and 3-furanmethanol was not detected in ones that were made with 20% non-fermented chia seeds, this may mean that the type of treatment does not affect the 3-furanmethanol and 5

-5methyl-2-furancarboxaldehyde contents.
However, 5-methyl-2-furancarboxaldehyde was not detected in control, non-fermented chia seeds (10 and 20%) or solid-state fermented chia seeds (30%) bread. Hence, it was found that this volatile compound was affected by the type of treatment carried out on chia seeds and thus may affect the final characteristics of the produced bread. Moreover, the volatile compound as maltol was contained in all bread samples, besides the bread sample free from chia seeds and the non-fermented chia seeds bread sample. The insertion of chia seeds in fermented sausage products showed a noticeable increase in the volatile compounds such as terpenes (Borrajo et al., 2022). There were more than 60 compounds (fatty acids, esters, alcohols, aldehydes, ketones, peroxides, and pyrans) that influenced on the aroma profile of all four cereal media, resulting in distinct volatile profiles for each fermented cereal beverage Salmerón, 2017). The functional non-fermented chia seeds products (Jam, cereal bar, and chocolate spread) and fermented chia seeds products (fermented juice, fermented weaning food, and fermented milk beverage) are shown in Figure 1 and the sensory evaluation of functional non-fermented chia seeds assessed, as mentioned in Figure 2, the color, taste, odor, texture, and overall acceptability as sensory chia seeds properties. The non-fermented chia seeds jam was the highest in color, then chocolate spread, and finally the cereal bar. Meanwhile, the non-fermented chocolate spread was the highest in taste, odor, texture, and overall acceptability, then the jam, and finally, the cereal bar. The means values of jam, cereal bar, and chocolate spread were 7.50±1.17, 6.70±1.25, and 6.80±1.03, for color, respectively. Therefore, 7.10±1.60, 6.50±1.2, and 8.10±1.96 for taste, respectively, then 7.20±1.14, 7.00±0.95, and 7.30±1.41, for odor, respectively, thus, 7.40±1.17, 7.10±1.10, and 7.50±1.27, for texture respectively. Finally, 7.30±0.82, 6.70±1.06, and 7.40±0.70, for overall acceptability, respectively. In contrast, color, taste, odor, texture, and overall acceptability of the fermented chia seed products (fermented juice, fermented weaning food, and fermented milk beverage) were recorded as follows: 7.55±0.98, 6.60±1.43, and 7.11±1.54; 6.81±1.08, 6.70±1.49, and 7.22±1.48; 7.36±0.81, 7.20±1.40, and 7.56±1.01 and 7.64±0.674, 7.30±1.16, and 7.67±1.12, respectively. Previously, the fermented juice was the favorite in color, the fermented milk beverage was the favorite in taste, odor, texture, and over all acceptability, but the fermented weaning food had the lowest degree of preference for all sensory properties except odor. Capriles et al., (2020) found that the overall acceptability score of gluten-free pan bread (which contained from 2 to 3% chia flour) was acceptance. Also, Adeyemo and Onilude (2018) discovered that the nutritional benefits and sensory parameters of weaning food made from fermented cereals were enhanced due to the microbial metabolism products resulted by the fermentation process. In addition, Zaki, (2018) showed that incorporating 3% chia seeds in camel burgers enhanced the sensory qualities of the camel burger compared to the other burger samples. The contract with Bustos et al., (2017), who produced wheat bread made from sourdough chia seeds and chia seeds flour, discovered enhancement in cohesion of the bread in both types. Past research conducted by Salmerón, (2017) indicated that the creation of volatile substances in fermented foods during the fermentation process is essential for defining their sensory characteristics. Many volatile substances are believed to greatly influence the aroma of these foods.

Table 5. The changes in volatile compounds (%) in non-fermented and fermented chia seeds

Samples								
Volatile Compounds (%)	Non-fermented chia seeds	fermented chia seeds	Volatile Compounds (%)	Non-fermented chia seeds	fermented chia seeds	Volatile Compounds (%)	Non-fermented chia seeds	fermented chia seeds
Butanoic acid, butyl ester C8H16O2	11.45	1.32			-	Pentadecanoic acid, 14-methyl-, methyl ester C17H34O2		
n-Hexadecanoic acid C16H32O2	9.2	1.51	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1) C42H63O3P	1.28	-	Eicosane C20H42	0.55	-
Butyl 9,12,15-octadecatrienoate C22H38O2	8.59	62.2	-7,9Di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione C17H24O3	1.18	-	Styrene C8H8	0.52	-
-9,12,15Octadecatrienoic acid, methyl ester, (Z,Z,Z) C19H32O2	3.69	6.06	Acetic acid, hexyl ester C8H16O2	1.07	-	-2,4Di-tert-butylphenol C14H22O	0.51	-
Hexanoic acid, butyl ester C10H20O2	2.44	2.71	Gamma-Tocopherol C28H48O2	1.00	-	Stigmasta-5,22-DIEN-3-OL, (3.beta.,22E) C29H48O	0.51	-
-9,12,15Octadecatrienoic acid, ethyl ester, (Z,Z,Z) C20H34O2	2.26	12.25	-7,10,13Eicosatrienoic acid, methyl ester C21H36O2	0.98	-	Beta-Sitosterol C29H50O	0.49	-
Butanoic acid, ethyl es-ter C6H12O2	1.82	1.3	Decane, 2-methyl C11H24	0.71	-	Nonadecane C19H40	0.39	-
-9,12Octadecadienoic acid (Z,Z), methyl ester C19H34O2	1.17	1.52	-9,12Octadecadienoic acid (Z,Z) C18H32O2	0.71	-	Myristic acid, TMS deriv-ative C17H36O2Si	0.37	-
-2Hexenal, 2-ethyl C8H14O	1.16	0.81	-1Heptatriacotanol C37H76O	0.62	-	Hexadecanoic acid, butyl ester C20H40O2	0.37	-
-9,12,15Octadecatrienoic acid, (Z,Z,Z) C18H30O2	34.28	-	Campesterol C28H48O	0.58	-	-9,12,15Octadecatrienoic acid, 1-methylethyl ester, (Z,Z,Z) C21H36O2	-	4.46
Gamma-Sitosterol C29H50O	4.41	-	Pentadecane C15H32	0.55	-	Ethyl 9.cis.,11.trans.-octadecadienoate C20H36O2	-	3.87

Figure 1. The functional non-fermented and fermented chia seeds products

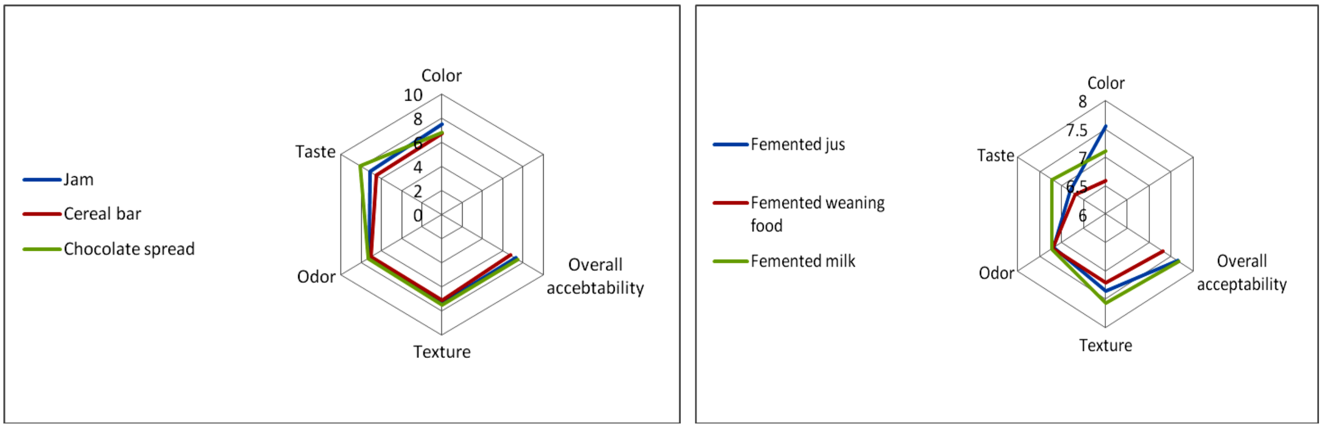

Figure 2. Graphical representation of sensory evaluation of functional non-fermented and fermented chia seeds products.

4. Conclusion

This study compared the non-fermented and fermented chia seeds in different chemical characteristics. It found that the fermentation process by Saccharomyces cerevisiae var *boulardii* increases the percentage of protein and carbohydrate and reduces the percentage of lipid, fiber, and ash, but these changes are not valuable. Although there is a small decrease in the ash content, there are dramatic changes in the mineral composition, especially Ca, Fe, P, Mn, and Zn. As well, there is an increase in the DPPH scavenging percent from 93.20 to 95.80%. The increase in the percentage of protein coincided with an increase in the percentage of all amino acids, both essential and non-essential. As for the composition of fatty acids, the percentage of omega-3 increased by 32.36% in the fermented sample. On the other hand, when studying the composition of phenol and volatile compounds in the samples, there were noticeable changes in these compounds, both quantitatively and qualitatively by the fermentation process. Then, by sensorial evaluation for the produced products that contained both of the non-fermented and fermented chia seed samples, such as jam, cereal bar and chocolate spread, fermented juice, weaning food, and a fermented milk beverage, they scored high values for non-fermented products and lower values for fermented products. Fortunately, all the values for both samples were within the acceptance limits by panelists.

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