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Standardization on Fermentation of Chia (*Salvia hispanica* L.)

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Authors' contributions

This work was carried out in collaboration between both authors. Author JJDS conceptualized the study, executed and carried out the research work, investigated the result and interpretation, did statistical analysis, wrote, revised and edited the original draft of the manuscript. Author UR conceptualized the research work, administered the project, supervised the study and edited the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the fermentation parameters physico-chemical characteristics and to conduct sensory evaluation to assess the acceptability of the fermented chia seeds.

Study Design: Experimental study involving the fermentation of chia seeds with varying combinations of water, curd, and honey. Assessment of fermentation parameters and physico-chemical characteristics. Microbiological analysis, LAB isolation, and measurement of TSS, acidity, and pH. Sensory evaluation to determine the acceptability of the fermented chia seeds.

Place and Duration of Study: The study undertaken during 2020-23 in the Department of Food Science and Nutrition, University of Agricultural Sciences, GKVK, Bangalore, Karnataka.

Methodology: Chia seeds subjected to different fermentation treatments involving combinations of water, curd, and honey. Microbiological analysis conducted to assess microbial populations. Isolation of lactic acid bacteria (LAB) to study their presence. Measurement of total soluble solids (TSS), acidity, and pH to evaluate physico-chemical changes during fermentation. Sensory evaluation performed to determine consumer acceptability.

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Results: Fermentation treatments showed significant differences in soluble solids ranged (1.63 to 8.10), pH (1.87 to 3.87), and acidity, showcasing organic acid content, notably lactic acid (0.04 to 0.23), oxalic acids (0.04 to 0.23), citric acid (0.09 to 0.50), maleic acid (0.06 to 0.35) and succinic acid (0.05 to 0.30. Fermented white chia with distilled water + curd+ honey (FWC3) and Fermented black chia with distilled water + curd+ honey (FBC3) had the statistically higher mean sensory scores.

Keywords: Chia; fermentation; lactic acid bacteria (LAB); TSS; pH; acidity; fermented black chia; fermentation treatments.

1. INTRODUCTION

Chia seeds (*Salvia hispanica* L.) have gained significant attention in recent years due to their nutritional composition and potential health benefits. Fermentation is a traditional food processing technique that can improve the digestibility, flavour and bioavailability of nutrients in various food products. However, limited research has been conducted on the fermentation of chia seeds and its impact on their physico-chemical properties. Therefore, this study aimed to evaluate the fermentation parameters and physico-chemical characteristics of chia seeds under different fermentation conditions.

Chia seeds offer versatility and can be utilized either in their raw state or after undergoing various processing techniques. Processing not only improves the bioavailability of nutrients but also enhances consumer acceptability. Techniques such as drying, roasting, soaking, popping, germination, fermentation, among others are employed to make the product more functional, catering to diverse preferences and nutritional needs [1,2]. The rheological and sensory properties of fermented milk drinks enriched with chia flour at three different concentrations: without chia flour, with 0.5 per cent chia flour, and with 1.0 per cent chia flour. Rheological analysis revealed a pseudoplastic non-Newtonian behavior, with the 0.5 per cent chia flour treatment exhibiting higher shearing tension rates and slight viscosity changes compared to the 1.0 per cent chia flour treatment. Sensory evaluation indicated that the milk drink without chia flour received the highest ratings across all attributes (appearance, taste, texture, viscosity, and overall impression), suggesting better sensory acceptance compared to the chia-enriched counterparts [3]. Fortification with 3 per cent chia seeds showed promising potential for creating dairy products with enhanced nutraceutical properties and favourable sensory characteristics. Additionally, the preventive effects of chia seeds on lipid levels were assessed in rats with induced myocardial infarction, revealing significant improvements in lipid profiles, particularly with higher doses of chia seed supplementation, suggesting a potential cardioprotective role through its hypolipidemic properties [4]. In the study by Maidana *et al*. [5] addressed the challenge of gluten-free baked goods by utilizing lactic acid bacteria (LAB) from chia flour and spontaneously fermented sourdough. LAB strains, including Weissella cibaria CH28, Lactobacillus plantarum FUA3171, and Lactobacillus fermentum FUA3165, were employed to ferment chia and flaxseed sourdoughs for sorghum gluten-free bread. Compared to chemically acidified controls, fermentation increased lactate, xylose, arabinose, free amino acids, and hydrogen peroxide while reducing glucose. Sorghum-based breads with 30-40 per cent oilseed sourdoughs showed improved volume and appearance, with sensory evaluations favouring 40 per cent replacement. The approach enhances functionality and health aspects of gluten-free baked foods. Calvo-Lerma *et al*. [6] conducted a study on solid-state fermentation of chia and sesame seeds using *Pleurotus ostreatus*, leading to increased lipid and protein content and notable alterations in fatty acid profiles, especially elevated polyunsaturated fatty acids. *In-vitro* digestion revealed higher lipolysis in fermented sesame seeds compared to chia seeds, with both fermented variations showing increased lipolysis compared to controls. Physical changes such as reduced particle size, enhanced matrix degradation, and decreased viscosity correlated with increased lipolysis. In the study by Teichert and chudy, 2022 fermented mare’s milk beverages enriched with chia seeds and inoculated with mesophilic LAB containing probiotic bacteria were prepared. Mare’s milk, recognized for its nutraceutical properties, boasts a unique protein profile and low-fat content, making it more easily digestible than cow’s milk. The addition of chia seeds not only enhanced the nutritional value by increasing unsaturated fatty acids but also sustained lactase enzyme activity and probiotic bacteria levels during storage. This study highlights the potential of fermented mare's milk with chia as a functional dairy product with improved nutritional attributes. Bartkiene *et al*. [7] investigated the impact of lactic acid bacteria (LAB) on chia seed fermentation, observing lower pH values and increased viable LAB counts in samples subjected to submerged fermentation compared to solid-state fermentation, attributed to differences in solid-to-liquid ratio. The study investigated the impact of chia seeds on blood glucose and lipids in Streptozotocin-induced diabetic rats [8]. Rats were fed traditional kishk supplemented with 10 per cent raw, germinated, or fermented chia seeds. Results showed reduced blood glucose, triglyceride, and total cholesterol levels with chia seed supplementation, with fermented chia seeds exhibiting the strongest effect. However, histopathological alterations were observed in liver, kidney, and pancreatic tissues of rats fed chia seed-supplemented diets. In the study by Wang *et al*., [9] protein content increased post-extraction, yielding protein concentrates with high protein content (88.32-89.20 g/100 g dw). The main protein fractions were globulins and albumins, with essential amino acid indices showing a 2-fold increase compared to reference protein. *In vitro* digestibility increased post-extraction but decreased after fractionation, while antinutrient levels varied depending on processing methods. The study aimed to assess the potential use of Chia (*Salvia hispanica* L.) plant in silage production by examining four different applications (control, 2.5% molasses, 1% salt, 2.5% molasses + 1% salt) on silage quality. Results indicated that silages with 2.5% molasses + 1% salt demonstrated higher quality compared to control samples, with an RFV value of 164.58±4.73, suggesting its suitability for high-quality silage production [10]. The study evaluates microbial fuel cells (MFCs) for household organic waste treatment and energy generation. Initial parameters of cauliflower leaf waste were measured, followed by treatment in MFCs under optimized conditions. Significant reductions in various chemical parameters were observed after eight days, along with a maximum power density of 10.1 W/m3. Scanning electron microscopy (SEM) showed bacterial adherence on the graphite electrode, and molecular characterization confirmed the presence of *Bacillus* sps via 16S rRNA sequencing [11]. The study explores the genetic connections of *Saccharum spontaneum* L., a versatile plant with applications in food, fodder, and medicine. Utilizing genomic material from nuclear ribosomal DNA internal transcribed spacer (nrDNA-ITS) and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) sequences, eight samples from Tamil Nadu were analyzed. Results revealed a close phylogenetic relationship among samples based on conserved sites in the rbcL region, aiding molecular identification. Additionally, ITS markers effectively delineate phylogeny, showcasing close relationships among four collected specimens and two commercial samples, while indicating recent evolutionary divergence in two other populations [12]. In recent years, seeds and pseudocereals like chia, sesame, flax, or quinoa have gained popularity as nutritional enhancers in various food products, offering health benefits due to their nutrient composition. However, their incorporation into new food formulations can also increase exposure to potentially harmful compounds like chemical process contaminants or allergens. The composition of these ingredients, weighing the benefits and risks of their inclusion in bakery products, and evaluating their effects on sensory characteristics in these novel food formulations [13].

2. MATERIALS AND METHODS

2.1 Standardization of Fermentation of Chia

The fermentation parameters of chia seeds were investigated using three different fermentation treatments: water (control), water with curd, and water with curd plus honey at 37℃ for 36 hours (3 days). The fermentation process was conducted for various time intervals, and samples was analyzed for microbiological composition, LAB isolation, TSS, acidity, and pH. Sensory evaluation was also performed to assess the acceptability of the fermented chia seeds.

2.2 Total Soluble Solids (TSS) [14]

The Total soluble solids (TSS) was measured by using a digital hand refractometer and expressed in degree Brix (°Brix). Erma hand refractometer was used in the range of 0 to 32 °Brix. Distilled water was used to standardize the refractometer. A drop of the sample was placed on the prism and reading was recorded in °Brix at 20ºC.

2.3 pH [15]

The pH was determined using pH 700 Digital meter at room temperature. The pH meter was standardized using pH buffer of 4.0, 7.0 and 9.2.

Ten g of powdered chia seeds sample in a 100 ml standard flask was taken and made upto the mark with distilled water. Mixed well and centrifuge to obtain clear solution. 25.0 ml of the supernatant was taken and pH determined.

2.4 Determination of Titratable Acidity [16]

The acidity of the chia sample was determined by titrating with 0.1N NaOH adding phenolphthalein indicator to determine the end point.

Ten g of powdered chia sample was added with 30.0 ml warm water and the mixture was shaken well. The mixture was allowed to stand for 5 min, centrifuged at 3000 rpm for 10 minutes, supernatant collected, added with 1.0 drop of phenolphthalein indicator and titrated with 0.1N NaOH solution. The titration was completed in 20 seconds and titer value in ml noted. A blank of 10.0 ml of supernatant was taken in another flask for comparison of colour.

Calculation:

$$\text{Titratable acidity (\% of LA)} = 9 \times A \times N / W$$

Where, A is Volume of standard NaOH required for titration in ml, N is Normality of standard NaOH solution and W is Weight of the sample taken in g.

2.5 Colony Formation of Lactic Acid Bacteria (LAB)

About 10 ml of each chia fermentation samples was measured and 90 ml of sterilized water was added into a sterilized conical flask and they were mixed well. Media, ten gram of Lactobacillus agar in 100 ml distilled water separately into the flask and covered with a cotton plug. It was autoclaved at 121℃ for 30 min.

The technique used was poured plate method and microbial population was recorded in log CFU/ml. The samples obtained were decimally diluted in prepared dilution series. All processes were carried in laminar air flow cabinet or aseptic condition. The plates were rotated twice in clockwise and anticlockwise direction for uniform distribution of the inoculums. After solidification of the media, plates were kept for incubation in an inverted position at 30 ± 1ºC in biological oxygen demand (BOD) incubator for 2-4 days and emerged colonies were counted [17].

2.6 Sensory Evaluation of Processed Chia

Sensory evaluation of fermented chia was carried out using nine-point hedonic scale. The score card was developed for evaluation of products and was based mainly on the appearance, colour, texture/consistency, flavour, taste, after-taste and overall acceptability. Twenty-one semi trained judges experienced in quality testing, processing, good health status and interested in sensory evaluation were selected from Food Science and Nutrition Department, UAS, Bangalore. Prior to the presentation of the fermented chia, each variation was coded and was placed along with a glass of water to rinse the mouth [18].

3. RESULTS AND DISCUSSION

3.1 Parameters of Fermented Chia

The total soluble solids, pH and titratable acidity are important fermentation parameters. Total soluble solids (TSS) comprises mainly sucrose which was added in the form honey to chia fermentation. Higher titratable acidity of organic acids and lower pH reduces the risk of pathogen and important for beneficial microbial growth. TSS of fermented chia seeds ranged from 1.63 to 8.10 (Table 1). The highest TSS was found in Fermented white chia with distilled water + curd+ honey (FWC3) among white and Fermented black chia with distilled water + curd+ honey (FBC3) among black having 2.80 and 8.10 respectively. The pH of ranged from 1.87 to 4.77. pH was more acidic for FWC3 and FBC3 obtaining 3.57 and 1.87 respectively.

Fermentation makes the food slightly acidic. Oxalic acid in the form of oxalates are present in chia and act as anti-nutrient. Statistically significant results in the fermented chia was found highest in FBC3 for lactic acid (0.23), oxalic acid (0.23), citric acid (0.50), malic acid (0.35) as well as succinic acid (0.30) than the rest of the treatments. Majority of other treatments were on par with each other. The result showed significant difference for all parameters except for malic acid, there is non-significant difference.

Both Bustos *et al*. [19] and Maidana *et al*. (2020) observed slightly elevated pH values (4.3 and 5.4) alongside comparable LAB viable counts (9.2 log CFU/g and 7.98 log CFU/mL) in chia flour sourdough fermented for 24 hours. These sourdoughs were prepared using *Lactiplantibacillus* (Lp) *plantarum* C8 and Lp. *plantarum* FUA3165, respectively. Discrepancies in these parameters could stem from variations in fermentation techniques, as well as the enzymatic activity and metabolic processes of LAB on different carbohydrates and plant-derived polysaccharides. Similarly, Costa *et al*. [20] conducted a study to create a fermented dairy beverage using whey as the dairy base supplemented with chia seed (*Salvia hispanica* L.) and acerola syrup (*Malpighia emarginata*). They assessed various physicochemical parameters including acidity, pH, soluble solids, water activity, and syneresis. The results showed acidity ranging from 1 to 1.27 percent, pH from 3.86 to 4.11, soluble solids from 15.67 to 21.6, water activity from 0.93 to 0.99, and syneresis from 46.67 to 68.08.

3.2 Lactic Acid Bacteria (LAB) Analysis of Fermented Chia Seeds

Table 2 provides information on the analysis of lactic acid bacteria (LAB) in fermented chia seeds based on different dilutions. Various dilutions of the fermented chia seed sample used for the LAB analysis. Average colony forming units per milliliter (CFU/mL) of the LAB observed in each dilution. The analysis demonstrates the presence of lactic acid bacteria in the fermented chia seed samples. At higher dilutions (10^{-5} and 10^{-6}), where the concentration was lower, measurable counts of LAB was observed and quantified in terms of CFU/mL. The data suggests that the LAB count was significant, especially at more diluted concentrations, indicating a potentially high presence of lactic acid bacteria in the fermented chia seed

Table 1. Assessment of physico-chemical parameters of fermented chia

Treatments	TSS (ºBrix)	pH	LA	OA	CA	MA	SA
FWC1	2.16^{d}	3.87^{b}	0.05^{b}	0.05^{b}	0.11^{bc}	0.32^{a}	0.07^{b}
FWC2	1.63^{e}	4.77^{a}	0.04^{b}	0.04^{b}	0.09^{c}	0.06^{a}	0.05^{b}
FWC3	2.80^{c}	3.57^{c}	0.06^{b}	0.06^{b}	0.14^{bc}	0.10^{a}	0.09^{b}
FBC1	2.06^{d}	3.57^{c}	0.06^{b}	0.06^{b}	0.12^{bc}	0.08^{a}	0.07^{b}
FBC2	7.06^{b}	2.04^{a}	0.08^{b}	0.08^{b}	0.17^{b}	0.12^{a}	0.10^{b}
FBC3	8.10^{a}	1.87^{e}	0.23^{a}	0.23^{a}	0.50^{a}	0.35^{a}	0.30^{a}
Mean ± SD	3.97±0.061	3.28±0.010	0.09±0.003	0.09±0.003	0.19±0.006	0.17±0.139	0.11±0.003
F Test	*	*	*	*	*	NS	*
SEm ±	0.043	0.007	0.002	0.002	0.004	0.098	0.002
CD (P≤0.05)	0.134	0.023	0.007	0.007	0.014	-	0.007

Note: Values are expressed as mean of three determinations; NS: Non-significant; Different alphabets indicate significant difference; TSS: Total soluble solids; pH: pH is defined as the negative log of the hydrogen ion concentration; LA: Lactic acid; OA: Oxalic acid; CA: Citric acid; MA: Maleic acid and SA: Succinic acid; FWC1: Fermented white chia with distilled water; FWC2: Fermented white chia with distilled water + curd; FWC3: Fermented white chia with distilled water + curd+ honey; FBC1: Fermented black chia seeds with distilled water; FBC2: Fermented black chia with distilled water + curd; FBC3: Fermented black chia with distilled water + curd+ honey

Table 2. Lactic acid bacteria (LAB) analysis of fermented chia

Dilution	Average	CFU
10^{-3}	>300	Too much to count
10^{-4}	>300	Too much to count
10^{-5}	1.66	1.6×10^{7}
10^{-6}	5.5	5.5×10^{7}
10^{-7}	<30	Too less to count

Note: CFU: Colony forming unit; LAB: Lactic acid bacteria

Table 3. Sensory evaluation of fermented chia

Treatments	Appearance	Colour	Texture	Flavour	Taste	After-Taste	Overall-Acceptability
FWC1	6.66^{c}±0.12	6.14^{d}±0.15	6.47^{b}±0.21	6.33^{b}±0.22	6.71^{ab}±0.17	6.33^{b}±0.26	6.67^{b}±0.12
FWC2	6.97^{bc}±0.17	6.78^{c}±0.19	6.52^{b}±0.21	5.92^{b}±0.30	6.47^{ab}±0.19	6.11^{b}±0.38	6.87^{b}±0.19
FWC3	7.81^{a}±0.11	7.71^{a}±0.10	7.42^{a}±0.11	7.33^{a}±0.10	6.92^{a}±0.13	7.28^{a}±0.15	7.65^{a}±0.08
FBC1	6.71^{c}±0.14	6.90^{c}±0.19	6.47^{b}±0.19	6.04^{b}±0.25	6.28^{b}±0.19	6.19^{b}±0.21	6.89^{b}±0.10
FBC2	7.16^{b}±0.17	7.14^{ac}±0.32	6.78^{b}±0.21	6.16^{b}±0.38	6.28^{b}±0.22	6.21^{b}±0.25	6.80^{b}±0.13
FBC3	7.71^{a}±0.14	7.71^{ab}±0.14	7.38^{a}±0.16	7.19^{a}±0.08	6.88^{ab}±0.21	7.12^{a}±0.14	7.62^{a}±0.09
Mean ± SD	7.17±0.206	7.06±0.279	6.84±0.265	6.5±0.350	6.59±0.270	6.54±0.350	7.08±0.184
F Test	*	*	*	*	NS	*	*
SEm ±	0.146	0.198	0.188	0.247	0.191	0.247	0.130
CD (P≤0.05)	0.408	0.554	0.526	0.694	-	0.694	0.364

Note: Values are expressed as mean of twenty-one determinations; NS: Non-significant; Different alphabets indicate significant difference; FWC1: Fermented white chia with distilled water; FWC2: Fermented white chia with distilled water + curd; FWC3: Fermented white chia with distilled water + curd+ honey; FBC1: Fermented black chia with distilled water; FBC2: Fermented black chia with distilled water + curd; FBC3: Fermented black chia with distilled water + curd+ honey samples. Colony formation of Lactic acid bacteria occurred only for fermented white chia treated with distilled water, curd and honey (FWC3) with serial dilution at 10^{-5} and 10^{-6}. This analysis was essential in understanding the microbial composition of fermented chia seeds, specifically regarding the presence and concentration of lactic acid bacteria. These bacteria often contribute to the fermentation process and might have implications for the nutritional quality and shelf-life of the fermented chia seed product.

Bartkiene *et al*. [7] reinforced these findings by examining the alterations in acidity during chia seed fermentation, which are brought about by LAB. These bacteria produce organic acids, including lactic and acetic acid, through carbohydrate metabolism. The decrease in pH levels and the rise in viable counts of lactic acid bacteria (LAB) in submerged fermentation samples can be attributed to the decreased viscosity of the fermentation medium, resulting from a lower ratio of solids to liquids compared to solid-state fermentation.

Bustos *et al*. [19] conducted a study on lactic acid fermentation to enhance the textural properties, phenolic compounds, and antioxidants in chia (*Salvia hispanica* L.). Autochthonous lactic acid bacteria (LAB) were isolated from chia dough and selected based on their acidification kinetics and proteolytic activity. Specifically, strain no. C8, identified as *Lactobacillus plantarum* C8, was chosen and employed as a starter culture to ferment chia sourdough. During lactic acid fermentation, the mass fractions of organic acids—such as lactic, acetic, and phenyl lactic acids—increased to 12.3 g, 1.0 g, and 23.8 µg per kg of dough, respectively. Additionally, antioxidant activities rose by approximately 33–40 per cent compared to unfermented chia flour dough. The sourdough underwent fermentation at 37°C for 24 hours, resulting in cell counts of 9.2 CFU/g and a pH of 4.3. This decline in pH was attributed to the production of organic acids as a byproduct of fermentation.

3.4 Sensory Evaluation of Fermented Chia

Fermented chia seeds referred to chia seeds that had undergone a fermentation process, where microorganisms break down the carbohydrates and proteins present in the seeds, leading to changes in their properties, taste, and potentially enhancing their nutritional value. Fermentation is a metabolic process conducted by beneficial microorganisms like bacteria, yeast, or molds. The results of the sensory evaluation of fermented chia seeds revealed (Table 3) that Fermented white chia with distilled water + curd+ honey (FWC3) and Fermented black chia with distilled water + curd+ honey (FBC3) had the statistically higher mean scores for appearance (7.81 and 7.71), colour (7.71), texture (7.42 and 7.38), flavour (7.33 and 7.19), taste (6.92 and 6.88), after taste (7.28 and 7.12), and overall acceptability (7.65 and 7.62) respectively. Results indicated that fermentation of chia with distilled water + curd+ honey were most accepted in white and black chia seeds. The F Test confirms significant differences among treatments for most attributes, suggesting that the treatments have distinct effects on the sensory characteristics of fermented chia seeds. The highest sensory score of fermented chia seeds was selected for further physico-chemical and nutritional analysis. Fermented chia seeds were not selected for product development since it has fishy-odour.

The results of the fermentation process revealed significant variations in the physico-chemical properties of the fermented chia seeds. Total soluble solids (TSS), pH, and titratable acidity was important parameters monitored during fermentation. The highest TSS values was observed in samples treated with water, curd, and sugar, indicating enhanced sweetness and flavor development. Moreover, the pH levels were more acidic in samples with added curd and sugar, suggesting increased microbial activity and fermentation efficiency. Lactic acid bacteria (LAB) analysis demonstrated the presence of beneficial microbial populations in the fermented chia seeds, contributing to the fermentation process and potential health benefits.

Sensory evaluation results indicated that chia seeds fermented with water, curd, and sugar was preferred for their appearance, colour, texture, flavour, and overall acceptability. However, some samples exhibited undesirable sensory attributes, such as a fishy odour, which may limit their potential for product development.

flavour, and overall acceptability. However, some samples exhibited undesirable sensory attributes, such as a fishy odour, which may limit their potential for product development.

4. CONCLUSION

In conclusion, the fermentation of chia seeds with water, curd, and honey resulted in significant changes in physico-chemical properties and sensory attributes. The presence of LAB and favourable fermentation parameters suggest the potential for incorporating fermented chia seeds into various food products to enhance their nutritional value and sensory appeal. Further research is needed to optimize fermentation conditions and explore the application of fermented chia seeds in functional foods and beverages.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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