Food Technology Research Journal, Vol. 4, issue 2, 92-106, 2024

FTRJ

Nutritional, Physical and Microbiological Properties of Gluten Free Bread with Chia Seed Flour as Alternative Thickening Agent

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Original Article

Article information

Received 16/05/2024
Revised 08/06/2024
Accepted 10/06/2024
Published 15/06/2024
Available online 15/06/2024

Keywords:
pan-bread, microbiology, nutritive properties, additives; thickener

ABSTRACT

This study investigated the impact of substituting xanthan gum with chia (*Salvia hispanica* L.) on the nutritional and microbiological quality of gluten-free pan bread. A gluten-free pan bread base using composite flour made from millet, corn, potato, and chickpea flour was developed. chia seeds were then incorporated at three levels (1%, 2%, and 3%) as a potential replacement for xanthan gum. The composite flour analysis revealed a composition of 8.94% protein, 1.63% fat, 2.15% crude fiber, 3.38% ash, and 83.90% carbohydrate. Gluten-free pan bread samples were analyzed for their nutritional properties and microbiological quality over 12 days of storage (room temperature). Our results indicated that pan bread made with chia was superior in many nutritional aspects compared to the control and the xanthan treatment. Chia inclusion in pan bread significantly increased protein, fat, fiber, and minerals like iron, calcium, zinc, and potassium. In all gluten-free pan bread samples and the composite flour, the content of insoluble dietary fiber (IDF) was higher than soluble dietary fiber (SDF). Compared to the control and 2% xanthan samples, adding chia increased the levels of most essential amino acids (isoleucine, lysine, cystine, methionine, threonine, and valine) and most fatty acids. Microbiological analysis indicated that chia inclusion improved the shelf-life of the gluten-free pan bread by protecting it from microbial spoilage. Overall, chia seeds demonstrate promise as a thickening ingredient for gluten-free pan bread, offering significant nutritional benefits and improved shelf life.

1. Introduction

Celiac disease is an autoimmune condition caused by lifetime sensitivity to gluten proteins present in wheat, rye, and barley. Since celiac patients' diets must be fully gluten-free, all wheat, rye, barley, and oat items must be substituted with maize, rice, millet equivalents, and different forms of starch (corn, rice, and potato) or suitable mixes (Quiñones et al., 2015). While rice and soy flour are popular replacements for cereal-based flour in gluten -free baking, they frequently lack structure and fail to create a cohesive dough phase. This significantly impacts the nutritional value, texture, and overall sensory experience of the final product, resulting in lower-quality pan bread (Abdi et al., 2023). When making gluten-free baked goods, several non-gluten flour substitutes could be used in place of wheat flour, however, these substitutes' functional qualities could be insufficient to create a firm dough structure (Hoand Nurul, 2024). The absence of gluten has a significant impact on dough qualities, the bread making process, the final quality, and the shelf life of gluten-free bread (Fratelli et al., 2021). Gluten helps to build cohesive and elastic dough by holding gas throughout the fermentation process, resulting in bread with a spongy texture. As a result, various scientific attempts have been made to overcome the challenges of producing gluten-free bread by using hydrocolloids that can compensate for the lack of gluten and give bread its spongy structure (Abdel-Gawad et al., 2023). Numerous researches have examined the physicochemical and sensory characteristics of gluten-free baked goods made with diverse flours and gums. Xanthan gum is a hetero-polysaccharide formed by

Journal website: https://ftrj.journals.ekb.eg/

Published by Food Technology Research Institute, ARC

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the strains of *Xanthomonas* spp. and has been used to improve texture and moisture retention in cake batters and bread doughs. Also, extend cereal shelf life by preventing starch retrogradation, and improve eating quality and attractiveness (Hagras et al., 2023). However, its nutritious is low. Chia seeds have emerged as a popular health food due to their impressive nutritional profile and versatility. Beyond their nutritional value, chia seeds offer valuable functional properties for food applications. Their high water absorption capacity makes them an attractive ingredient. Furthermore, their high content of phenolics, fiber, protein, and omega-3s suggests potential health benefits. Additionally, the presence of mucilage and gum allows them to absorb up to 12 times their weight in water. These properties make chia a versatile tool in food manufacturing, acting as an absorber, chelator, emulsifier, gel-forming agent, foam enhancer, suspension former, clarifying agent, and dehydrating agent (Sadeek et al., 2021). To enhance gluten-free pan bread quality, emulsifiers and gums are frequently added but there's a growing interest in utilizing highly nutritious, naturally gluten-free ingredients that also enhance the physical properties of baked goods. Chia flour, for example, is a promising option due to its gluten-free nature, rich nutrient profile, and presence of mucilage (Sharma et al., 2020). The present study aimed to investigate the usage of chia seed flour (*Salvia hispanica* L.) as an additive and thickening agent in gluten-free pan bread and to evaluate the nutritional, physical, sensorial, and microbial characteristics of the produced pan bread.

2. Materials and Methods

Materials

Millet and whole meal-chickpea flour were obtained from the Agricultural Research Center. (ARC). Potato flour and xanthan gum were procured from Sky Live Co. for the food industry, located in Giza, Egypt. Chia seeds were purchased from Abu Auf Company, Cairo, Egypt. Instant yeast containing natural dough yeast *Saccharomyces cerevisiae*), corn flour, sunflower oil, eggs, salt, sugar, and powdered milk (4%) were purchased from the local market. Pre-gelatinized corn starch and sodium sterol lactate were purchased from Sigma-Aldrich.

Methods

Composite gluten-free flour preparation and pan bread formulation

This study used composite flour formulated by mixing millet flour (40%), corn flour (30%), potato flour (20%), and chickpea flour (10%). Five formulations of gluten-free pan bread were prepared during the study. The formulations employed for making the gluten-free pan bread are depicted in Table 1. The first group served as control composed of 100% composite flour without any additive and gum-free (C), the second group (X) contained 2% xanthan gum as thickening material; the groups T1, T2, and T3 were supplemented with powdered chia seeds (as thickening material instead of xanthan gum) at levels of 1,2 and 3%, respectively.

Yeast, sugar, and warm water were combined and activated for two minutes at 37°C.Dry ingredients (flour mixture) were then mixed in a bowl for 10 minutes using a hand mixer. 200g of the dough were put into aluminum pans of 18 × 7.5 × 7.0 cm and allowed to ferment for 60 min at 35°C. After proofing, the pan bread samples were baked at 250° C, for 30 min. Once cooled, the pan bread samples were stored in polyethylene packages at room temperature for further analysis.

Nutritional assessment

Proximate analysis

Protein, fat (ether extractable), fiber, and ash contents of composite flour and pan bread samples were determined according to methods described by the official methods of analysis (AOAC 2016). Moisture content was also determined by AOAC (2016).Additionally, total carbohydrates were estimated according to the method outlined by Egan et al., (1981).

Fiber analysis

Soluble dietary fiber (SDF), insoluble dietary fiber (IDF), and total dietary fiber (TDF) were determined according to the official methods of analysis (AOAC 2016).

Table 1. The formula of gluten-free pan bread's ingredients

Ingredients	C	X	T1	T2	T3
	Ingredient weight (gm)				
Composite flour*	100	100	100	100	100
Pre-gelatinized corn starch	3.33	3.33	3.33	3.33	3.33
Sunflower oil	3.33	3.33	3.33	3.33	3.33
Fresh white egg	8.37	8.37	8.37	8.37	8.37
Salt	1.56	1.56	1.56	1.56	1.56
Sugar	4.0	4.0	4.0	4.0	4.0
Instant dry yeast	1.50	1.50	1.50	1.50	1.50
Milk powder	4.00	4.00	4.00	4.00	4.00
Sodium stearol lactate	0.42	0.42	0.42	0.42	0.42
Xanthan	-	2.0	-	-	-
Chia seed flour	-	-	1.0	2.0	3.0

* Composite flour= 40% millet flour + 30% corn flour + 20% potato flour +10% chickpea flour

Amino acids analysis

Amino acids determination was performed according to AOAC, (2016) using an Amino Acids Analyzer Eppemdorf LC 3000 EZChrom. An essential amino acid index (EAAI) is then calculated using the equation provided by Ijarotimi and Keshinro (2013).

$$EAAI = \sqrt[n]{\frac{100a \times 100b \ldots\ldots.100h}{a_v \times b_v \ldots\ldots.h_v}} \qquad (1)$$

where:

n = number of essential amino acids

a1, a2, ..., an = quantities of the respective essential amino acids in the test sample

av1, av2, ..., avn = corresponding reference values for the essential amino acids in a standard protein (casein)

The nutritional index (NI) was calculated using Equation (2).

$$NI\ (\%) = EAAI \times protein / 100 \qquad (2)$$

The Biological value (BV) was calculated using Equation (3) (Ijarotimi and Keshinro, 2013; Oser, 1959)

$$BV = 1.09 \times EAAI - 11.7 \qquad (3)$$

The Predicted protein efficiency ratio (P-PER) was calculated using the regression equation, Equation (4) (Nieman, 1992 and Aremu et al., 2011):

$$P\text{-}PER = -0.468 + 0.454(LEU) - 0.105\ (TYR) \qquad (4)$$

Fatty acids determination

Saturated and unsaturated fatty acids in the oil were determined using the methyl esters boron trifluoride outlined in AOAC (2016).

Minerals determination

Minerals were analyzed by inductively coupled plasma ICP-OES Optima 2000 DV Perkin-Elmer following the methods described in AOAC (2016).

Shelf-life determination and microbiological evaluation of pan bread samples

Pan bread samples were stored at room temperature (26°C-33°C) for 12 days and observations of spoilage were recorded throughout the storage period. To assess the microbial quality and shelf life, the total bacterial count and fungal count (yeast and mold) of gluten-free pan bread samples were determined at various storage times (0, 3, 6, 9, and 12 days). The analyses followed guidelines established by the American Public Health Association (APHA 2001). Pan bread samples were prepared by aseptically crushing and homogenizing them with peptone water. To enumerate aerobic bacteria and fungi, decimal dilutions were prepared, and 0.1 mL aliquots were spread plated onto nutrient agar (NA) and potato dextrose agar (PDA). PDA plates were incubated at room temperature (28 ± 2 °C) for 3-5 days, while NA plates were incubated at 37 °C for 24-48 hours. After incubation, colonies were counted and reported as colony-forming units (CFU) per gram (CFU/g) of the sample.

Determination of gluten-free pan bread physical characteristics

The specific volume (cm^3/g) was obtained by dividing the volume (cm^3) by the pan bread's weight (g).

Texture profile analysis for pan bread samples including firmness, gumminess, chewiness adhesiveness, cohesiveness; springiness, and resilience was also performed. The water activity of the pan breadcrumb and crust was also determined as described by Gebreil and Mohamed (2023).

Determination of sensorial properties and acceptability

Ten customers from the Food Technology Research Institute were recruited to evaluate the sensory quality and acceptability of the pan bread samples. Before testing, all participants confirmed they had no allergies or intolerances to the pan bread's ingredients. A nine-point structured hedonic scale was used to assess the acceptability of the pan bread's' color, taste, odor, texture, and overall acceptability. The scale ranged from 1 = "disliked extremely" to 9 = "liked extremely" (adapted from Gebriel and Mohamed, 2023).

Statistical analysis

Analysis of variance (ANOVA) was performed using CoStat software version 6.4 (USA) to assess the significance of differences between samples for each measured parameter. Duncan's multiple range test was employed at a significance level of α = 0.05 to identify statistically significant differences between sample means (Gomez and Gomez, 1984).

3. Results and discussion

Proximate and minerals contents of composite flour and chia seeds

Table 2 shows the proximate (protein, ash, lipids, fiber, carbohydrates) and mineral (calcium, phosphorus, iron, zinc, magnesium) content of the composite flour and its ingredients. The results revealed that the prepared composite flour contains the potential amount of protein, ash, lipids, fiber, and carbohydrates as well as important minerals including calcium, phosphorus, iron, zinc, and magnesium. The results align with previous studies reporting protein content in composite flour from various millet types ranged between8.06% and11.23 %. Similarly, ash content fell within the reported range of 1.20% to 2.9%, and crude fiber content ranged from 2.45% to 2.62% (Anberbir et al., 2024). Flour with elevated levels of crude fiber is essential for cakes, pan bread , cookies, pastries, and pizza crusts because it aids digestion and lowers the hazard of chronic illnesses including stroke, heart disease, and type 2 diabetes (Ramashia et al., 2021). The high carbohydrate content observed in composite flour is crucial for baked products as it provides the body with energy for proper functioning (Feyera et al., 2020).

Table 2. Proximate and minerals composition of composite flour and chia seeds

	g/100g dry weight					Minerals (mg/100g dry weight)					
	Protein	Fiber	lipids	Total carbohydrates	Ash	P	K	Ca	Mg	Fe	Zn
Composite flour	8.94	2.15	1.63	83.90	3.38	384.0	308.5	143.7	101.3	3.99	2.5
Chia seeds	24.11	4.46	35.13	31.7	4.60	833.3	659.0	837.3	309.6	7.73	5.1

In the present work, chia seeds used in pan bread formulation were also analyzed and their composition is presented in Table 2. The advantages of chia seeds as a nutritional supplement and nutraceutical food cannot be overstated. As expected, chia seeds proved to be a valuable source of nutrients, boasting high levels of protein (24.11%) and lipids (35.13%), along with appreciable amounts of minerals. These results are consistent with previous studies reporting chia seeds to contain considerable levels of lipids, ranging from (25 to 40%), protein from (15 to 25%), ash percentage between 4 and 5%, and carbohydrates from (26 to 40%) (Cardenas et al., 2018). The minerals analysis of chia seeds revealed a rich composition, including high amounts of phosphorus, calcium, potassium, and magnesium, with lower iron contents. This aligns well with previous findings by Kulczyński et al., (2019) who reported similar mineral profiles in chia seeds. In their study, magnesium, potassium, calcium, and phosphorus content ranged from 335-449 mg/100g, 407–726 mg/100g, 456–631mg/100g, and 860–919mg/100g, respectively.

Nutritional assessment of gluten-free pan bread

Proximate composition and minerals

The proximate composition and mineral content of gluten-free pan bread are presented in Table 3. The results demonstrate that incorporating chia significantly improved the nutritional value compared to the control bread (gum-free) and the bread with xanthan gum. The inclusion of chia resulted in a significant increase in the protein content of free gluten-free pan bread samples as compared to the control. Conversely, xanthan gum significantly decreased protein content.

The fat content of gluten-free pan bread significantly increased by 4.20, 8.40 and 12.44% for gluten-free pan bread samples with 1, 2, and 3% of chia flour, respectively, compared to the control in contrast, xanthan gum resulted in a slight decrease (1.85%) in fat content. There were no significant differences in ash content between gluten-free pan bread control and samples with 1, 2, and 3% of chia flour. However, xanthan gum addition caused a small increase (1.83%) in ash content. All bread samples, including those with xanthan gum and chia flour, showed a significant decrease in total carbohydrate content compared to the control pan bread. These findings align with previous studies da Costa Borges et al., (2021) who reported that while wheat pan bread typically offers higher protein content than gluten-free pan bread , chia seed, and flour inclusion can significantly enhance the nutritional value. Additionally, Fernandes et al. (2019) found that increasing the amount of chia flour or seeds in pan bread leads to a rise in lipid content due to the high oil content (around 30%) of chia seeds. Because of the minerals included in the seed, the ash content of the bread rose as the concentration of the seed or chia flour increased. Carbohydrate content dropped as protein and fat levels increased (da Costa Borges et al., 2021).

Regarding, the analysis of the mineral content significantly increases with adding 1, 2, and 3% chia seed flour compared to control and xanthan treatments. This aligns with our results, of higher mineral content in raw chia flour compared to wheat flour, which translates to higher mineral levels in the final pan bread. It has been previously reported that there were two to four times as much zinc, calcium, and iron in the chia-based pan bread as in the wheat flour substitute. Considering the recommended daily allowances (RDAs) and assuming an average daily pan bread intake of 100 grams, incorporating chia seeds into pan bread could significantly increase the percentage contribution of essential minerals, provided there are no mineral absorption inhibitors present in the diet (Miranda-Ramos et al., 2020).

Table 3. Chemical composition (on a dry weight basis) of gluten free pan-bread made from composite flour

Micronutrients / Treatments	Protein %	Fat %	Crude fiber %	Ash %	Total carbohydrate %	Fe mg/100g	Ca mg/100g	Zn mg/100g	K mg/100g
C	9.95^{b}	5.95^{d}	2.81^{d}	4.92^{a}	76.37^{a}	5.10^{c}	66.70^{d}	2.47^{c}	124.5^{b}
X	9.77^{c}	5.84^{e}	4.57^{a}	4.83^{c}	73.99^{d}	4.81^{d}	65.53^{e}	1.89^{d}	122.4^{b}
T1	10.07^{ab}	6.20^{c}	3.04^{c}	4.91^{a}	75.78^{b}	5.20^{c}	67.92^{c}	2.82^{b}	131.8^{ab}
T2	10.18^{a}	6.45^{b}	3.27^{b}	4.92^{a}	75.17^{b}	5.51^{b}	71.72^{b}	2.85^{b}	138.0^{a}
T3	10.31^{a}	6.69^{a}	3.49^{b}	4.91^{a}	74.60^{c}	7.43^{a}	83.96^{a}	3.64^{a}	141.0^{a}

Small letters (a,b,c..) in the superscripts signify that data within the same column are significantly different ($p<0.05$)
C: bread made of 100% composite flour (without gums), X: bread made of 100% composite flour with xanthan gum,
T1: breads made of 100% composite flour with 1% chia seed flour,
T2: breads made of 100% composite flour with 2% chia seed flour and
T3: breads made of 100% composite flour with 3% chia seed flour

Amino acids content and protein quality of pan bread samples

Amino acids composition of gluten-free pan bread samples produced from composite flour (no additives and gum-free), 2% xanthan gum, 1, 2, and 3% chia seed flour are shown in Table 4. The addition of chia seed flour generally increased the concentration of most essential amino acids (isoleucine, cystine, lysine, threonine, methionine, and valine), compared to the control group. The essential amino acid index (EAAI) of all gluten-free pan bread samples at 1, 2, and 3% chia seed flour were the highest value (from 80.52 to 86.45) followed by the control gluten-free pan bread sample (80.01). In contrast, the gluten-free pan bread sample with 2% xanthan gum had a slightly lower EAAI than the control. In previous research by Miranda-Ramos et al., (2020) who reported that pan bread formulations containing chia had significantly higher amino acid levels compared to wheat pan bread, this is likely due to the high protein content of chia seeds. The nutritional value of vegetable proteins to human health is determined by their biological quality, which is defined as the existence of all necessary amino acids (Vera-Cespedes et al., 2023).

Table 4. Amino acid profile (g/16gN) and protein quality of gluten-free pan bread samples

Amino acids	C	X	T1	T2	T3
Essential amino acids					
Therionine	3.86	3.91	3.98	3.66	4.10
Valine	5.34	5.38	5.31	5.44	5.65
Isoleucine	4.16	4.10	4.28	4.00	4.41
Leucine	7.72	7.63	7.76	7.55	8.12
Phenylealanine	5.15	5.08	5.07	5.22	5.34
Histidine	1.98	2.06	1.99	2.00	2.06
Lysine	4.56	4.40	4.58	4.77	4.93
Methionine	1.58	1.66	1.59	2.00	1.65
Total E.A.A	34.35	34.22	34.56	34.64	36.26
Non-Essential amino acids					
Aspartic acid	11.45	11.84	11.58	11.55	12.42
Serine	4.60	4.58	4.66	4.55	4.73
Glutamic	17.90	17.90	17.92	17.11	18.59
Glycine	3.52	3.39	3.47	3.00	3.49
Alanine	4.79	4.58	4.85	6.22	4.93
Tyrosine	3.62	3.49	3.57	3.55	3.69
Argnine	5.77	5.57	5.65	5.88	5.75
Proline	3.91	4.08	3.96	5.33	3.90
Cystine	2.74	2.79	2.77	3.33	3.18
Total non-E.A.A	58.30	58.22	58.43	60.52	60.68
Total amino acids	92.65	92.44	93.39	95.16	96.94
Protein quality parameters of gluten-free					
EAAI $_a$	80.01	78.71	80.52	84.82	86.45
PER $_b$	3.98	3.88	4.02	4.03	4.20
BV $_c$	91.78	90.72	92.23	92.34	94.16
NI $_d$	7.961	7.690	8.108	8.635	8.913

a: Essential Amino Acid Index , b:Protein Efficiency Ratio , c: Biological Value , d: Nutritional index.
C: breads made of 100% composite flour (without gums), X: breads made of 100% composite flour with xanthan gum,
T1: breads made of 100% composite flour with 1% chia seed flour,
T2: breads made of 100% composite flour with 2% chia seed flour and
T3: breads made of 100% composite flour with 3% chia seed flour

Also, from the results of Table 4, it could be noticed that a 3% chia seed flour addition increased protein value relative to the control group. This likely as a result of improvements in essential amino acids like proline, glutamic acid, lysine, and other vital amino acids. However, the gluten-free pan bread sample with ~~a~~ 3% substitution of chia seed flour exhibited the largest increment in both the computed protein efficiency ratio (PER) and biological value (BV) (4.20 PER and 94.16 BV) compared to all other samples..

Fatty Acid Profile of pan bread samples

The percentage of fatty acids distributions of gluten-free pan bread samples produced by substituting xanthan gum with chia seed flour is shown in Table 5. The addition of chia seed flour to composite flour appeared to increase the concentration of most of the fatty acids especially for palmitic, stearic, oleic, and linoleic acids over control and 2% xanthan samples. Breads with 3% chia seed flour showed a predominance of unsaturated fatty acid linolenic (omega-3). Linoleic and linolenic acid are two essential fatty acids for humans. Because mammalian cells lack to synthesis these fats, these two polyunsaturated fatty acids must be obtained from diet. Omega-3 fatty acids play a crucial role in preventing chronic diseases. Consuming a balanced diet rich in these fats has been linked to positive health outcomes, including reduced risk of cardiovascular disease, inflammation, hyperlipidemia, and even cancer (Tomić et al., 2022). Chia seeds, along with other similar seeds, are well-known sources of omega-3 fatty acids.

Table 5. Fatty acids profile (mg/100 mg fatty acids) of gluten-free pan bread samples

Fatty Acids	Pan bread samples				
	C	X	T1	T2	T3
Capric acid (C10:0)	0.16	0.15	0.26	0.45	0.45
Lauric acid (C12:0)	0.66	0.66	0.89	0.96	0.97
Myristic (C14:0)	1.18	0.82	2.22	2.52	2.82
Pentadecanoic acid (C15:0)	-	-	0.13	0.33	0.40
Palmitic acid (C16:0)	12.88	11.4	13.21	14.52	16.5
Palmitoleic acid (C16:1) ω7	0.24	-	0.27	0.38	0.70
Heptadecanoic acid (C17:0)	0.18	-	0.20	0.24	0.30
Stearic acid (C18:0)	4.65	4.60	4.84	5.26	6.17
Oleic acid (C18:1)ω9	25.44	24.5	25.58	25.78	26.57
Vaccenic acid (C18:1) ω7	0.70	0.68	0.76	0.85	1.05
C18:1 ω5	0.13	-	-	0.17	0.20
C20:1 ω5	-	-	-	-	0.38
Linoleic acid (C18:2) ω6	45.26	37.9	46.6	50.51	52.87
C18:2 ω5	0.42	0.37	0.46	0.86	1.23
Linolenic acid (C18:3) ω3	0.94	0.80	2.15	4.0	4.78
Stearidonic acid (C18:4) ω3	-	-	-	-	0.40
Arachidic acid (C20:0)	0.40	0.35	0.37	0.37	0.40
Behenic acid (C22:0)	0.44	0.51	0.43	0.46	0.40
Non-identified fatty acids	0.14	0.23	0.34	0.04	0.14

C: bread made of 100% composite flour (without gums), X: bread made of 100% composite flour with xanthan gum,
T1: breads made of 100% composite flour with 1% chia seed flour,
T2: breads made of 100% composite flour with 2% chia seed flour and
T3: breads made of 100% composite flour with 3% chia seed flour

Dietary fiber of pan bread samples

Dietary fiber intake plays a significant role in overall metabolic health influencing factors like insulin sensitivity. Studies have shown connections between dietary fiber consumption and various diseases, including mortality risk (Barber et al., 2020). Gut microbiota also plays a vital role in mediating the benefits of dietary fiber, impacting appetite management, metabolic activities, and chronic inflammatory pathways. Table 6 presents the dietary fiber analysis results for the developed gluten-free pan bread samples. Concerning the values of insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) it could be noticed that, in all free gluten pan bread samples and composite flour, IDF values were higher than SDF. According to the percentage of IDF/TDF in gluten-free pan bread samples , they could be arranged in descending order as follows: T3 (36.63%), 2% Xanthan (69.70%), T2 (69.66%), control sample (65.49%) and T3 (63.37%), while the percentage of SDF/TDF in gluten-free pan bread samples could be arranged in descending order as follows: T1 (74.83%), control sample (34.5%), T2 (30.34%), 2% Xanthan (30.30%), and T1 (25.17%). For SDF 2% Xanthan and T2 had almost the same content of SDF (30.30 and 30.34%) whereas, other gluten-free pan bread samples were as follows (34.5% for the control sample, 25.17% for T1 and 36.63% for T3). The formulations of gluten-free pan bread with 2% of xanthan gum and 2% of chia seed flour had ratios close to this value (30.30 SDF : 69.70 IDF, and 30.34 SDF :69.66 IDF, respectively). These bakery products could be included in the diet, especially in the diet of people who do not achieve an adequate intake of total dietary fiber, and they could have healthy effects such as reducing cholesterol, preventing constipation, and lowering the risk of developing diabetes or cardiovascular disease (Nohra, and Bochicchio, 2015; Slavin, 2013). Most of the fiber in chia seeds is soluble fiber, owing to the high proportion of mucilage, which can absorb up to 35.2 times its weight in water. This water-holding capacity increases the viscosity of foods and also of the alimentary bolus, which could delay gastric emptying and thus reduce the accessibility of nutrients such as glucose (Lazaro et al., 2018).

Table 6. Dietary fiber contents of gluten-free pan bread samples

Treatments / Dietary fiber	C	X	T1	T2	T3
IDF	7.40^{d}	9.20^{c}	11.30^{b}	12.40^{a}	12.80^{a}
SDF	3.90^{c}	4.00^{c}	3.80^{c}	5.40^{b}	7.40^{a}
TDF	11.30^{e}	13.20^{d}	15.10^{c}	17.80^{b}	20.20^{a}

Small letters (a,b,c..) in the superscripts signify that data within the same row are significantly different ($p<0.05$).
IDF= Insoluble dietary fiber, SDF= soluble dietary fiber, and TDF= total dietary fiber
C: bread made of 100% composite flour (without gums), X: bread made of 100% composite flour with xanthan gum,
T1: breads made of 100% composite flour with 1% chia seed flour,
T2: breads made of 100% composite flour with 2% chia seed flour and
T3: breads made of 100% composite flour with 3% chia seed flour

Assessment of the microbiological quality of gluten-free pan bread

Microbial spoilage is a primary factor that reduces the shelf life of pan bread products, leading to financial losses for both producers and consumers. Numerous specific circumstances, including packaging, hygienic production procedures, storage conditions, and product turnover, might be to blame for these losses (Saranraj 2010). Pan bread spoilage is a major contributor to global food waste, with fungal growth accounting for roughly 60% of pan bread spoilage cases (Hassan et al., 2020). Table 7 presents the microbial load analysis of the pan bread samples (control, xanthan gum, 1% chia flour, 2% chia flour, and 3% chia flour) stored at room temperature for 12 days to assess their shelf life. The analysis focused on total plate count (TPC), total mold, and total yeast. No microbial growth was detected at zero time for any samples, likely due to the high baking temperature eliminating microorganisms. However, bacterial counts increased in all samples starting from day three of storage. These findings align with research by Karaoglu et al., (2005) who reported a significant increase in pan bread microbial counts during storage at room temperature without additional preservatives. However, bacterial counts increased in all samples starting from day three of storage. These findings align with research by Karaoglu et al., (2005) who reported a significant increase in pan bread microbial counts during storage at room temperature without additional preservatives. Interestingly, the results for 2% and 3% chia flour samples are below the WHO standard (1994) for acceptable total plate count in baked goods (2.0×10^5 CFU/g). In contrast, the control pan bread, xanthan gum pan bread, and 1% chia flour pan bread samples exceeded this limit.

Table 7. The microbial load of free-gluten pan bread samples stored for different storage periods

			Total bacterial count CFU/g		
Treatments	0time	3days	6days	9days	12days
C	ND	$20x10^2$	$33x10^4$	$3x10^5$	$28x10^6$
X	ND	$23x10^2$	$20x10^4$	$4x10^5$	$18x10^6$
T1	ND	$13x10^2$	$22x10^3$	$28x10^4$	$27x10^5$
T2	ND	$11x10^2$	$29x10^2$	$8x10^3$	$12x10^4$
T3	ND	$12x10^2$	$27x10^2$	$10x10^3$	$28x10^3$
			Total mould count CFU/g		
C	ND	ND	$8x10^3$	$29x10^3$	$8x10^4$
X	ND	ND	ND	ND	ND
T1	ND	ND	ND	$23x10^3$	$26x10^4$
T2	ND	ND	ND	ND	ND
T3	ND	ND	ND	ND	ND
			Total yeast count CFU/g		
C	ND	$20x10^2$	$17x10^4$	$23x10^5$	$20x10^6$
X	ND	ND	ND	ND	ND
T1	ND	ND	ND	$15x10^2$	$21x10^3$
T2	ND	ND	ND	ND	ND
T3	ND	ND	ND	ND	ND

C: bread made of 100% composite flour (without gums), X: bread made of 100% composite flour with xanthan gum,
T1: breads made of 100% composite flour with 1% chia seed flour,
T2: breads made of 100% composite flour with 2% chia seed flour and
T3: breads made of 100% composite flour with 3% chia seed flour

Analyses for mold growth during storage at room temperature for 12 days, the control samples showed no mold until day three, but the mold count reached 8 x 10^4 CFU/g by day 12. In contrast, no fungal counts were detected in the pan bread containing xanthan gum, 2% chia, and 3% chia seed flour. Interestingly, adding 1% chia exhibited an initial count of fungi on day nine reaching $23×10^3$ CFU/g and $26×10^4$ CFU/g by 12th day of storage. These findings align with previous research by Ijah et al., (2014) and Khanom et al., (2017) who reported that the total aerobic and fungal counts increased progressively with a longer storage period of pan bread. It was observed that results of xanthan, chia 2%, and chia 3 % remained below the maximum acceptable limit for molds in foods (1000 CFU/g) (Khanom et al., 2017), while the control and 1 % chia exceeded the maximum acceptable limit.

Results of total yeast showed that xanthan, 2% chia or 3% chia samples were free of yeast cells. On the other hand, control and 1% chia recorded $20×10^6$ and $21x10^3$CFU/g respectively. These counts are less than those reported by Tapsoba et al., (2022). Control samples, exceeded the maximum acceptable limit, of 1000 CFU/g for yeast in food (Khanom et al., 2017 and Opondo et al., 2022).

The 1% chia flour sample remained below this limit (Opondo et al., 2022). The microbiological analysis (Table 7) indicated that the pan bread formulations containing xanthan gum, 2% chia flour, and 3% chia flour were safe for consumption as no mold or yeast growth was detected throughout the 12-day storage period at room temperature. This aligns with the findings of Fratelli et al., (2021).

In contrast, the control samples and the 1% chia flour pan bread exceeded the acceptable limits for mold and yeast growth during storage, rendering them unsuitable for consumption due to compromised quality. Shelf life, defined as the period during which a food product remains safe and appealing to consumers (Kumari et al., 2019), is significantly impacted in these cases. Previous results showed that samples with 2% and 3 % chia extended the shelf life of gluten-free pan bread. This could be attributed to the potential antimicrobial properties of chia seeds; this is in agreement with Divyapriya (2016) who found that an ethanolic and aqueous extract of chia seeds has antimicrobial activity against *P. gingivalis, A. actinomycetemcomitans* and *F. nucleatum*. TunçİI and ÇelİK (2019) also found that chia seed extract and oil showed antibacterial efficacy against human infections utilizing agar disc-diffusion and agar well-diffusion tests. Güzel (2020) investigated the antibacterial, antifungal, and anti-proliferative activities of chia seeds, and found that ethanol extract from chia seeds had an inhibitory effect against *Aeromonas hydrophila*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Escherichia coli*, *Candida albicans*, *Bacillus subtilis*, *Candida tropicalis*, and *Candida glabrata* (Güzel 2020).

Chia seeds, when moistened, produce highly hygroscopic mucilage with thickening properties has been proposed as a component of bio-films to extend the shelf life of food products. Additionally, incorporating chia into wheat pan bread has been shown to increase protein, lipid, ash, and dietary fiber content while suppressing amylopectin retrogradation, ultimately extending shelf life (Menga et al., 2017). Chia seeds offer the potential to reduce dietary fat content while increasing fiber, omega-3 fatty acids, and the ratio of polyunsaturated to saturated fats. Overall, chia seeds and their mucilage may enhance the functionality of food products (Menga et al., 2017).

Sensory attributes

The analyses of the mean sensory scores for the gluten free pan bread are shown in Table 8. Sensory properties play a crucial role in consumer acceptance of a food product. Crust and crumb color were significantly affected by using chia flour. Pan breads containing xanthan gum (X) had a lighter crumb and crust compared to other formulations, likely due to the grayish color of chia powder. The use of chia flour significantly improved the aroma and taste of the pan bread compared to the control pan bread, which received low scores (5.0 and 6.2 for taste and odor, respectively). Notably, panelists found pan breads with 2% and 3% chia flour to be the most acceptable. However, chia flour inclusion resulted in improved internal appearance, with panelists noticing greater pan bread expansion, better shape, and characteristics generally preferred by consumers. These findings align with research by Capriles et al., (2020) who suggested that combining ingredients can address common defects in gluten-free pan bread production, such as compact structure, hard texture, and cracked crust. When compared to pan breads made without seed flour, panelists observed that the addition of chia flour improved the pan bread s' overall acceptability. The most widely accepted usage of chia flour was found to be in the range of 2 to 3%.

Physical properties

Physical measurements of gluten-free pan bread samples included weight, volume, and specific volume as shown in Table 9 and Figure 1 presents images of the baked pan bread.

The specific volume (an indicator of good volume and texture) was higher in pan breads containing 2%, or 3% chia flour compared to the control pan bread. This suggests a positive correlation between specific volume and the level of gum substitution. Researchers have explored various approaches to improve the quality of gluten-free products including the addition of hydrocolloids, starches, or combinations of these (Sarabhar et al., 2021). While Santos et al., (2020) reported that dietary fiber can decrease pan bread volume by reducing gas retention, this study demonstrates that using hydrocolloids like xanthan gum and 2-3% chia flour has a positive effect on the specific volume of gluten-free pan bread. The ability of xanthan gum to absorb water likely contributes to this positive effect. This interaction helps the pan bread dough expand during the early stages of baking, limiting gas loss and promoting pan bread growth (Hager and Arendt, 2013). Water activity also is shown in Table 9. Water activity (a*w*) is a critical indicator of the shelf life of foods, which greatly, affects the microorganism's growth (Singh et al., 2016).The highest water activity was for control pan bread and it decreased significantly with chia treatments. This decrease can be attributed to the high water-binding capacity of chia mucilage, which reduces the amount of free water available for microbial growth.

Table 8. Sensory evaluation of gluten-free pan bread

Treatments	Crust color	Crumb color	Taste	Odor	Texture	Overall acceptability
C	8.9^{a}	8.3^{b}	5.0^{d}	6.2^{e}	5.5^{e}	6.78^{c}
X	8.1^{b}	9.0^{a}	6.5^{c}	7.1^{d}	7.0^{c}	7.54^{b}
T1	7.0^{c}	7.8^{c}	6.0^{c}	7.9^{c}	6.5^{d}	6.82^{c}
T2	7.5^{c}	6.9^{d}	7.7^{b}	9.0^{a}	9.8^{a}	8.02^{a}
T3	5.4^{d}	6.8^{d}	9.0^{a}	9.2^{b}	8.0^{b}	7.48^{a}

Small letters (a,b,c..) in the superscripts signify that data within the same column are significantly different ($p<0.05$).
C: bread made of 100% composite flour (without gums), X: bread made of 100% composite flour with xanthan gum,
T1: breads made of 100% composite flour with 1% chia seed flour,
T2: breads made of 100% composite flour with 2% chia seed flour and
T3: breads made of 100% composite flour with 3% chia seed flour

Table 9. Physical properties of gluten-free pan bread

Treatments	Weight (gm)	Volume (cm3)	Specific volume (cm3/gm)	Water activity aw
C	75.00^{d}	120^{e}	1.60^{c}	1.000^{a}
X	81.00^{b}	156^{c}	1.93^{b}	0.997^{ab}
T1	82.72^{a}	135^{d}	1.65^{c}	0.997^{ab}
T2	79.50^{c}	173^{a}	2.18^{a}	0.996^{b}
T3	80.00^{bc}	165^{b}	2.06^{ab}	0.990^{c}

Small letters (a,b,c..) in the superscripts signify that data within the same column are significantly different ($p<0.05$)
C: bread made of 100% composite flour (without gums), X: bread made of 100% composite flour with xanthan gum,
T1: breads made of 100% composite flour with 1% chia seed flour,
T2: breads made of 100% composite flour with 2% chia seed flour and
T3: breads made of 100% composite flour with 3% chia seed flour

Control

X

T1

T2

T3

Figure 1. Gluten-free pan bread with different formulations

Texture profile analysis of pan breads

The texture analysis of gluten-free pan pan bread (Table 10) revealed that incorporating chia flour (1, 2 or 3%) increased crumb hardness compared to the control pan bread . This is likely due to a decrease in free water content within the dough, resulting in a less smooth texture. Interestingly, both xanthan gum and chia flour treatments increased the chewiness and gumminess of the crumb of pan bread compared to the control. However, all chia flour additions led to higher springiness (mm) compared to the xanthan formulation. This enhanced springiness can be attributed to the larger pan bread volume achieved with chia flour. Springiness essentially reflects the pan bread's ability to regain its shape after being deformed, a quality often associated with a desirable softer texture for consumers (Gomez et al., 2004). The judges showed more admiration for pan bread with softer, springier textures. The cohesiveness and resilience of pan bread increased with xanthan treatment and a minor increment was observed with chia addition at different levels in pan breads.

Table 10. Texture measurements of gluten-free pan bread

Treatments	Hardness (N)	Chewiness (mj)	Gumminess (N)	Springiness (mm)	Cohesiveness	Resilience
C	7.87	13.20	11.39	1.07	1.18	0.65
X	10.32	15.40	13.32	1.11	1.69	0.92
T1	9.15	14.80	12.19	1.16	1.25	0.71
T2	9.24	14.90	12.21	1.22	1.30	0.71
T3	9.67	16.00	12.60	1.26	1.32	0.72

C: bread made of 100% composite flour (without gums), X: bread made of 100% composite flour with xanthan gum,
T1: breads made of 100% composite flour with 1% chia seed flour,
T2: breads made of 100% composite flour with 2% chia seed flour and
T3: breads made of 100% composite flour with 3% chia seed flour

4. Conclusion

Chia is an excellent raw ingredient for gluten-free pan bread production with high nutritional value. Potentially lowering saturated fat intake, increasing omega-3 fatty acids, and providing additional dietary fiber, all of which contribute to a healthy diet. Moreover, chia flour acts as a zero-calorie thickener and hydrocolloid, further enhancing the functionality of gluten-free pan bread products. Overall, incorporating chia flour presents a compelling approach to improve the nutritional value and functionality of gluten-free pan bread.

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