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Research Article

Effect of Chia (*Salvia hispanica*) Flour Addition on the Quality and Shelf-Life of Common Carp (*Cyprinus carpio*) Fish Fingers Stored at -18 °C

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Abstract: The objective of this study was to investigate the impact of adding whole chia seed flour on the quality attributes and shelf life of common carp fish fingers stored at a temperature of -18 °C. The samples were categorized into four groups containing 0%, 5%, 10%, and 20% whole chia flour, and were evaluated for various parameters, including moisture, ash, crude protein, crude lipid, peroxide index, total volatile nitrogen bases, thiobarbituric acid, and sensory assessment at 30-day intervals over 90 days. The results indicated that incorporating whole chia flour into fish fingers and preserving them at -18 °C led to an increase in moisture and ash content, while simultaneously decreasing the levels of protein, fat, and peroxide index. Additionally, a higher percentage of flour resulted in an increase in volatile nitrogen bases throughout the 90-day storage period, with the peak observed on day 90. The levels of thiobarbituric acid also generally showed an upward trend. The sensory evaluation findings suggested that there was no significant difference in smell, taste, color, and texture between the control sample and those containing 5% and 10% chia flour. Conversely, fish fingers with 20% whole chia flour received the lowest sensory score after 90 days of storage. In conclusion, the findings of this research suggest that the inclusion of 10% chia flour can positively influence the characteristics of carp fish fingers during a 90-day storage period at -18 °C.

***Keywords*:** antioxidant, chia (*Salvia hispanica*), fish finger, common carp (*Cyprinus carpio*), whole flour

1. Introduction

Between 2014 and 2019, the global average annual per capita seafood consumption increased slightly from 19.9 Kg to 20.5 Kg. Nevertheless, in 2022, seafood consumption fell to its lowest levels in several years, hitting 19.7 Kg per capita. Fish is a vital source of high-quality protein, essential fatty acids (particularly omega-3 fatty acids), vitamins, and minerals, all of which are important for maintaining overall health, improving brain function, supporting cardiovascular health, and promoting growth and development. Additionally, it plays a role in preventing chronic diseases such as heart disease, diabetes, and cognitive decline. Furthermore, fish are crucial for food security and sustainable nutrition, especially in coastal and low-income regions. The composition of raw fish includes 0%-0.5% carbohydrates, 16%-21% protein, 1.2%-1.5% minerals, 0.2%-25% fat, and 66%-81% water, along with Essential Amino Acids (EAA) and fatty acids [1, 2]. In 2023, global food consumption reached an impressive total of 2,651.08

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million metric tons, with fish and seafood accounting for 36.85 million metric tons. This results in a ratio of total food consumption to fish and seafood products of 71.94 [3]. The farming of Cyprinidae has seen significant growth globally, attributed to their rapid growth rate, ease of breeding, and high productivity. However, it is unfortunate that the demand and consumption of these fish do not increase proportionately to their production in many nations. In this context, focusing on value-added products presents a viable path forward. By producing new semi-ready and ready-to-eat products, a consistent supply of these items can be ensured throughout the year. Consequently, this approach will help mitigate the issues of one-time supply of farmed fish, prevent sudden price drops, and ultimately protect the interests of fish farmers [4].

Food oxidation, resulting from the generation of free radicals, hydroperoxides, and other compounds, contributes to spoilage and degradation of product quality; this phenomenon is particularly significant in meat-based products [5, 6]. The interaction of various oxygen species, including hydroxy radical ($HO^{\cdot}$), superoxide anion ($^{-}O_2^{\cdot}$), and aryloxyl radicals ($ROO^{\cdot}$), can oxidize lipids and proteins [7]. The volatile compounds produced from the degradation, oxidation, and hydrolytic reactions of fats (such as hydroperoxides, aldehydes, ketones, fatty acids, etc.) alter the sensory characteristics, nutritional value, and overall quality of the product, leading to consumer dissatisfaction. Measures to prevent or slow down the spoilage of fish and its derivatives have been documented, among which the incorporation of antioxidants is noteworthy [8]. Antioxidants have been utilized as food additives for an extended period; in response to consumer demands for the prohibition of chemical food preservatives and their preference for natural and organic alternatives, food industry manufacturers and researchers are exploring the potential of natural substances to enhance shelf life and preserve food quality [9, 10]. Given the negative impacts of synthetic antioxidants, including mutagenicity, toxicity, and carcinogenicity, there is an urgent need for safer alternatives. Currently, the use of natural antioxidants, such as extracts from spices and plants, is highly recommended as a substitute for artificial antioxidants in food preservation [11, 12]. Chia, scientifically known as *Salvia hispanica* L., is an herbaceous annual plant belonging to the Marigold genus and the mint family. It is cultivated commercially in various South American nations, such as Argentina and Mexico, as well as in Australia and India, for both nutritional and medicinal purposes. This plant is commonly referred to as chia, Mexican chia, and black chia [13]. Chia seeds have been incorporated into human diets for more than 3,500 years and are currently acknowledged as a functional food due to their nutritional profile. They consist of roughly 5% moisture, 16% protein, 30% fat, 34% dietary fiber, and 42% total carbohydrates, along with significant amounts of calcium, phosphorus, potassium, and magnesium, and trace levels of sodium, iron, and zinc. Additionally, they serve as a valuable source of niacin and antioxidants, which help safeguard unsaturated fats from oxidative damage caused by free radicals, a factor that can lead to various diseases and hasten the aging process [14]. Whole Chia Flour (WCF) possesses the same composition as the original chia seed, as it is produced by directly grinding the chia seed. The chemical composition of WCF can vary based on factors such as the seed's origin, soil quality, and climate. WCF exhibits a significant ability to retain and absorb water, making it suitable for use as an emulsifying agent, thickener, stabilizer, or antifreeze agent within the food industry [15]. WCF has been employed as a replacement in various meat formulations. For instance, de Souza Paglarini et al. utilized whole chia flour (2.5%) as a gelling agent to create soybean oil emulsion gels intended to substitute 10% and 20% of pork back fat in Bologna sausages [16]. Similarly, Pires et al. developed Bologna sausages by replacing pork back fat with Echium oil and partially substituting 10% of lean beef with whole chia flour [17]. Furthermore, chicken sausage that incorporated chia seeds at levels of 2%, 4%, and 6% evaluated during a 21-day refrigerated storage period at 4 ± 1 °C, as reported by Limam and Mohamed [18]. Additionally, Fernandez-Lopez et al. assessed the addition of chia seeds at a level of 3% in the form of whole seeds in frankfurters [19]. This research aimed to explore the impact of incorporating whole chia seed flour into common carp fingerlings to enhance their qualitative attributes and extend their shelf life, serving as a natural antioxidant at a temperature of -18 °C.

2. Material & methods

2.1 *Sample preparation*

The research involved the procurement of common carp from a fish market, each averaging 500 g in weight. The carp were transported to the laboratory in a Unilite box filled with ice, subsequently washed, and processed using a meat grinder featuring a pore diameter of 3 mm. The resulting ground fish meat was combined with various additives to form a uniform paste, which was then shaped into fish fingers measuring 40 mm in diameter and 4 × 8 cm in size (as detailed in Table 1) before being glazed. Following this, the fish fingers were fried with sunflower oil at 180 °C for 30 sec and allowed to cool to ambient temperature prior to being packaged in polyethylene covers and stored in a freezer at -18 °C. Sensory evaluations and chemical analyses of the fish fingers were performed on days 0, 30, 60, and 90 post-production.

Table 1. Formulation of common crap fish finger samples containing chia flour

Ingredients	Control (C)	Fish fingers with 5% whole chia flour (T1)	Fish fingers with 10% whole chia flour (T2)	Fish fingers with 20% whole chia flour (T3)
Minced fish	93.5	86.5	83.5	73.5
Chia seed flour	0	5	10	20
Salt	1.5	1.5	1.5	1.5
Sugar	1	1	1	1
Wheat flour	3	3	3	3
Onion	0.24	0.24	0.24	0.24
Garlic powder	0.24	0.24	0.24	0.24
Black pepper	0.24	0.24	0.24	0.24
Cumin	0.24	0.24	0.24	0.24
Thyme	0.04	0.04	0.04	0.04

2.2 *Compositional analysis*

The moisture content of the sample was assessed utilizing the Association of Official Analytical Chemists (AOAC) method (2000), which involved subjecting the sample to a temperature of 103 ± 2 °C for 2 h. A digital balance was employed to weigh 10 g homogenized fish finger samples, which were subsequently placed in a muffle furnace at 550 °C for 16-18 h until it was converted into white ash. The ash content was then calculated as a percentage using the AOAC method (2000). The Kjeldahl method, involving sample digestion with sulfuric acid, was implemented to evaluate the crude protein content [20]. The measurement of crude fat was conducted using the Soxhlet method [20]. Following this, the peroxide level was computed in terms of milliequivalents of active oxygen (meq O_2) per Kg of sample, in accordance with Egan and Sawyer [21]. To quantify the Total Volatile Basic Nitrogen (TVB-N), both the Kjeldahl set and the AOAC method (2000) were utilized. The measurement of Thiobarbituric Acid (TBA) was performed using butanol as solvent and in conjunction with the thiobarbituric acid reagent at a wavelength of 530 nm, as per the American Oil Chemists' Society (AOCS, 2009). The sensory characteristics were assessed using a five-point hedonic scale divided into three sections. Initially, the samples were assigned codes, followed by the preparation of a requisite questionnaire, which was distributed to 25 individuals interested in consuming marine products. The evaluators rated the samples based on appearance and color, aroma and scent, texture, and overall acceptance, selecting a score between 1 and 5 points (where 1 indicates very poor quality and 5 signifies excellent quality), with a minor adjustment [22].

2.3 *Statistical analysis*

All experiments were conducted in triplicate. The outcomes were represented as mean values along with Standard Deviation (SD). The differences among the sample values were assessed using one-way Analysis of Variance (ANOVA) and Duncan's post hoc test at a significance level of $p < 0.05$. These analyses were performed using SPSS V.18.0 software. Regarding sensory evaluation, the results obtained from the evaluators' completed forms were compared across the experimental groups utilizing the Chi-square test at a significance level of $p < 0.05$.

3. Results

The results of the humidity measurement are presented in Table 2. It was observed that increasing the percentage of chia seed flour in fish fingers resulted in a decrease in moisture level. Specifically, adding 20% chia flour on the first day led to a significant difference in moisture content ($p < 0.05$). After 30 days of storage, there was no significant difference between the control sample and the 5% and 10% treatment ($p < 0.05$). However, the moisture content was significantly different from other samples ($p < 0.05$) with the addition of 20% chia flour. After 60 days of storing fish fingers in the freezer, the moisture content of the control sample increased and reached 75.8. The addition of 5% chia seed flour did not show a significant difference from the control sample on day 60. Three months after storage, there was a significant difference between the control sample and the 20% chia flour treatment ($p < 0.05$). In general, the highest moisture content, 77.6%, was measured in the control group after 90 days of storage. The lowest moisture content, 68.7%, was measured in the treatment containing 20% chia flour on the preparation day.

Table 2. Effects of adding different percentages of chia seed flour on the moisture content of carp fish finger (%)

Experimental groups	Storage duration			
	0 day	30 days	60 days	90 days
C	72.02 ± 1.55^{Aa}	74.4 ± 2.06^{Aa}	75.8 ± 2.25^{Aba}	77.6 ± 2.04^{Ba}
T1	71.6 ± 1.41^{Aa}	73.5 ± 2.18^{Aa}	74.7 ± 2.38^{ABab}	76.1 ± 2.13^{Bab}
T2	70.41 ± 1.47^{Aab}	72.6 ± 2.44^{Aab}	73.9 ± 2.43^{ABab}	75.2 ± 2.15^{Bab}
T3	68.7 ± 1.65^{Ab}	69.3 ± 2.10^{Ab}	70.2 ± 2.12^{Abb}	71.8 ± 2.57^{Bb}

*Different uppercase letters in each row and lowercase letters in each column indicate significant differences in the means ($p < 0.05$). C: Control, T1: Fish fingers with 5% whole chia flour, T2: Fish fingers with 10% whole chia flour, T3: Fish fingers with 20% whole chia flour

Based on the data presented in Table 3, it is evident that the ash content of fish fingers increased as the percentage of chia seed flour increased. Specifically, the addition of 20% chia flour on the first day of the experiment resulted in an ash content of 2.68%, which was significantly higher than the other samples, and the same increasing pattern was recorded after 30 days of storage ($p < 0.05$). On the 60 day of preservation, the control sample showed a decrease in ash content, while the sample with 20% chia seed flour still had a significantly higher content ($p < 0.05$) compared to the other experimental groups. Finally, on the 90 day, the ash content ranged from 2.08% to 2.39%, and the sample with 20% chia flour had the highest percentage of ash compared to the other samples ($p < 0.05$).

Table 3. Effects of adding different percentages of chia seed flour to the ash content (%) of carp fish fingers

Experimental groups	Storage duration			
	0 day	30 days	60 days	90 days
C	2.45 ± 0.05^{Aa}	2.32 ± 0.03^{Aba}	2.21 ± 0.05^{BCa}	2.08 ± 0.03^{Ca}
T1	2.51 ± 0.06^{Aa}	2.41 ± 0.07^{Aba}	2.31 ± 0.04^{ABa}	2.21 ± 0.03^{Ba}
T2	2.57 ± 0.02^{Aa}	2.49 ± 0.05^{Aa}	2.4 ± 0.02^{Aba}	2.35 ± 0.04^{Ba}
T3	2.68 ± 0.08^{Aa}	2.56 ± 0.06^{Aba}	2.48 ± 0.07^{Aba}	2.39 ± 0.05^{Ba}

*Different uppercase letters in each row and lowercase letters in each column indicate significant differences in the means ($p < 0.05$). C: Control, T1: Fish fingers with 5% whole chia flour, T2: Fish fingers with 10% whole chia flour, T3: Fish fingers with 20% whole chia flour

By increasing the proportion of chia seed flour in fish fingers, the protein content was found to increase. Table 4 shows that on the first day, there was a significant difference in protein levels between C and T1 (containing 5% flour) and the group containing the highest amount of chia flour ($p < 0.05$). During storage, the protein content of fish fingers in both the control group and the group containing 20% chia flour showed an increasing trend. Specifically, on the 60 and 90 days of storage, the protein content of fish fingers increased in the control group and T3 from 17.95% to 17.46%

and 20.25% to 20.06%, respectively.

Table 4. Effects of adding different percentages of chia seed flour on the protein content (%) in carp fish fingers

Experimental groups	Storage duration			
	0 day	30 days	60 days	90 days
C	18.58 ± 0.8^{Ab}	18.27 ± 0.74^{Ab}	17.95 ± 0.48^{Abb}	17.46 ± 0.27^{Bb}
T1	18.91 ± 0.75^{Ab}	18.63 ± 0.59^{Ab}	18.25 ± 0.39^{Abb}	17.93 ± 0.65^{Bb}
T2	19.52 ± 0.95^{Aab}	19.36 ± 0.67^{Aab}	19.08 ± 0.84^{ABab}	18.82 ± 0.55^{Bab}
T3	20.74 ± 0.88^{Aa}	20.48 ± 0.92^{Aa}	20.25 ± 0.62^{Aba}	20.06 ± 0.93^{Ba}

*Different uppercase letters in each row and lowercase letters in each column indicate significant differences in the means ($p < 0.05$). C: Control, T1: Fish fingers with 5% whole chia flour, T2: Fish fingers with 10% whole chia flour, T3: Fish fingers with 20% whole chia flour

After adding chia seed flour with varying percentages to fish fingers and storing them in a freezer for 90 days, a general decrease in crude fat content was observed. The findings in Table 5 indicate that the sample with 20% chia flour has a significant difference ($p < 0.05$) in fat content compared to other samples. However, there was no significant difference ($p > 0.05$) between the control samples containing 5% and 10% chia flour. Based on the results, it can be concluded that the sample with 20% chia flour had a significantly lower fat content than the other samples ($p < 0.05$).

Table 5. Effects of adding different percentages of chia seed flour on the fat content (%) of carp fish fingers

Experimental groups	Storage duration			
	0 day	30 days	60 days	90 days
C	3.45 ± 0.05^{Aa}	3.12 ± 0.02^{Ba}	2.85 ± 0.08^{Ca}	2.52 ± 0.07^{Da}
T1	3.40 ± 0.07^{Aa}	3.08 ± 0.05^{Ba}	2.80 ± 0.02^{Ca}	2.48 ± 0.03^{Da}
T2	3.35 ± 0.04^{Aa}	3.01 ± 0.08^{Ba}	2.73 ± 0.06^{Ca}	2.42 ± 0.05^{Da}
T3	3.05 ± 0.05^{Ab}	2.78 ± 0.03^{Bb}	2.34 ± 0.05^{Cb}	2.21 ± 0.02^{Cb}

*Different uppercase letters in each row and lowercase letters in each column indicate a significant differences in the means ($p < 0.05$). C: Control, T1: Fish fingers with 5% whole chia flour, T2: Fish fingers with 10% whole chia flour, T3: Fish fingers with 20% whole chia flour

Below are the results of our investigation on the impact of chia seed flour on peroxide levels in fish fingers. Table 6 shows the peroxide values from day 0 to 90 for four experimental groups. Our findings indicate that adding different percentages of chia seed flour to fish fingers and storing them in a freezer for 90 days resulted in a decrease in peroxide levels. On day 90, the sample with 20% chia flour had the lowest peroxide value at 0.41 meq O_2/kg. We observed a significant difference ($p < 0.05$) in peroxide index between the control sample and the treatments containing 10% and 20% chia flour.

Table 6. Effects of adding different percentages of chia seed flour on the peroxide index (meq O_2/kg) of carp fish fingers

Experimental groups	Storage duration			
	0 day	30 days	60 days	90 days
C	1.15 ± 0.05^{Aa}	1.37 ± 0.08^{Ba}	0.98 ± 0.005^{Ca}	0.64 ± 0.002^{Da}
T1	1.14 ± 0.06^{Aa}	1.32 ± 0.05^{Bab}	0.85 ± 0.003^{Cab}	0.57 ± 0.005^{Dab}
T2	1.15 ± 0.02^{Aa}	1.27 ± 0.06^{Aab}	0.75 ± 0.005^{Bb}	0.52 ± 0.004^{Cb}
T3	1.13 ± 0.03^{Aa}	1.17 ± 0.09^{Ab}	0.58 ± 0.008^{Bc}	0.41 ± 0.007^{Cc}

*Different uppercase letters in each row and lowercase letters in each column indicate a significant difference in the means ($p < 0.05$). C: Control, T1: Fish fingers with 5% whole chia flour, T2: Fish fingers with 10% whole chia flour, T3: Fish fingers with 20% whole chia flou

According to the study, the addition of chia seed flour in fish fingers has the potential to decrease the amount of volatile nitrogenous bases. According to Table 7, the control group and treatments with different percentages of chia seed flour had similar levels of this factor on the first day of the experiment. However, after 30 days of storage, the amount of TVB-N reduced in T3 compared to the control sample. On the 60 day, there was no significant difference between the control sample and T1, but there was a significant difference between the control group and T2 and T3. After three months, there was a significant difference in the TVB-N content of the control and treatment groups. However, there was no significant difference between the treatment containing 10% and the treatment containing 20% chia flour.

Table 7. Effects of adding different percentages of chia seed flour on the volatile nitrogen bases content (mg/100 g) in carp fish fingers

Experimental groups	Storage duration			
	0 day	30 days	60 days	90 days
C	12.21 ± 0.15^{Aa}	13.35 ± 0.62^{Ba}	14.68 ± 0.25^{Ca}	16.87 ± 0.45^{Da}
T1	12.20 ± 0.12^{Aa}	13.19 ± 0.57^{Bab}	14.20 ± 0.46^{Cab}	15.71 ± 0.38^{Db}
T2	12.22 ± 0.17^{Aa}	12.85 ± 0.73^{Bb}	13.73 ± 0.64^{Cb}	14.63 ± 0.52^{Dc}
T3	12.21 ± 0.19^{Aa}	12.58 ± 0.69^{ABc}	12.89 ± 0.81^{Bc}	13.20 ± 0.18^{Cc}

*Different uppercase letters in each row and lowercase letters in each column indicate a significant difference in the means ($p < 0.05$). C: Control, T1: Fish fingers with 5% whole chia flour, T2: Fish fingers with 10% whole chia flour, T3: Fish fingers with 20% whole chia flou

According to the study, the initial amount of thiobarbituric acid reactive substances was consistent at 0.4 mg/100 g across all samples on the first day of measurements. After a month of storage, all treatments showed an increase in thiobarbituric acid levels compared to previous records. The control group exhibited the highest amount of 1.26 mg/100 g. As the percentage of chia flour in fish fingers increased, the level of thiobarbituric acid decreased. On the 60 day of storage, Thiobarbituric Acid Reactive Substances (TBARS) showed significant differences ($p < 0.05$) between the control group and samples containing 5%, 10%, and 20% chia flour. At the end of the experiment, the level of TBARS index decreased, with the lowest amount found in the sample containing 20% chia flour, which was 1.91 mg/100 g. However, as shown in Table 8, there was no significant difference ($p < 0.05$) between the control group and the 5% chia flour treatment.

Table 8. Effects of adding different percentages of chia seed flour on the TBA content (mg/100 g) of carp fish fingers

Experimental groups	Storage duration			
	0 day	30 days	60 days	90 days
C	0.4 ± 0.05^{Aa}	1.26 ± 0.05^{Ba}	1.85 ± 0.05^{Ca}	2.35 ± 0.05^{Da}
T1	0.4 ± 0.05^{Aa}	1.15 ± 0.05^{Bab}	1.71 ± 0.05^{Cab}	2.16 ± 0.05^{Dab}
T2	0.4 ± 0.05^{Aa}	1.03 ± 0.05^{Bb}	1.59 ± 0.05^{Cb}	2.08 ± 0.05^{Db}
T3	0.4 ± 0.05^{Aa}	0.85 ± 0.05^{Bc}	1.35 ± 0.05^{Cc}	1.91 ± 0.05^{Dc}

*Different uppercase letters in each row and lowercase letters in each column indicate a significant difference in the means ($p < 0.05$). C: Control, T1: Fish fingers with 5% whole chia flour, T2: Fish fingers with 10% whole chia flour, T3: Fish fingers with 20% whole chia flour

Figures 1a-2b showing the results of sensory evaluation of fish finger carp with the addition of chia flour at the three-month storage condition at -18 °C. According to the results, the smell and taste of the samples have had a downward trend over time. There was no significant difference ($p > 0.05$) between the samples in terms of smell and taste. The lowest sensory score was reported for fish fingers containing 20% chia flour after 90 days of storage. From the point of view of color and texture, the sample containing 20% whole wheat flour received the lowest score, but the samples containing 5% and 10% whole wheat flour, as well as the control, received similar scores from the evaluators.

Note that the samples in 5% and 10% concentrations were not significantly different ($p > 0.05$) from the control sample in terms of color and texture. The fish finger sample containing 20% chia flour was significantly different ($p < 0.05$) from other samples. Regarding taste, smell, color, and texture, the sample containing 20% whole wheat flour received the lowest score, while the samples containing 5% and 10% whole wheat flour, as well as the control, obtained similar scores from the evaluators.

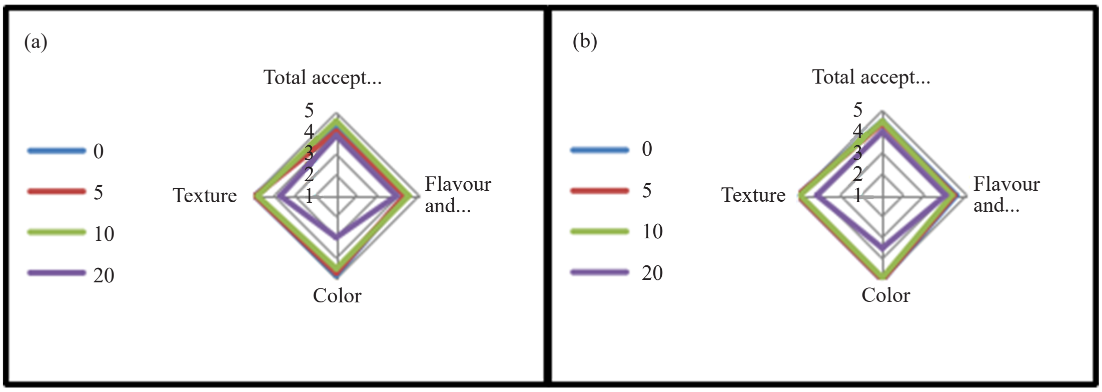

Figure 1. Effects of adding different percentages of chia seed flour on the sensory evaluation of carp fish fingers at (a) day 0 and (b) day 30

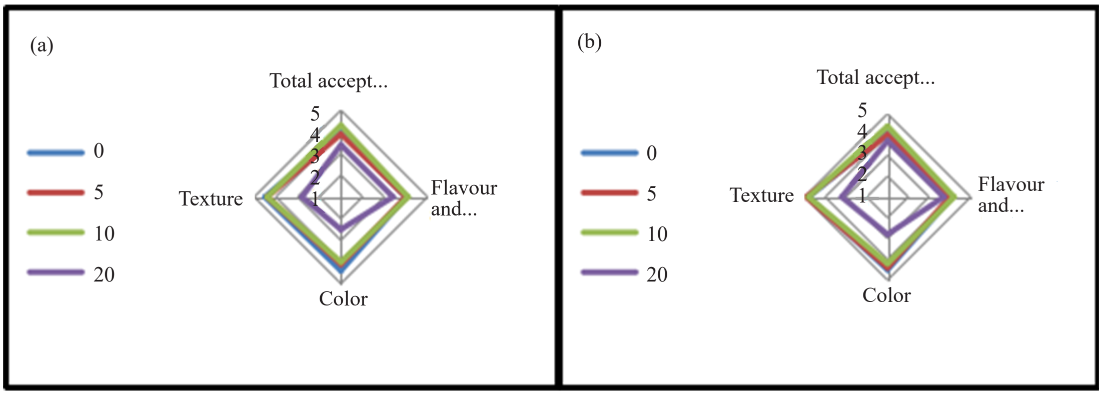

Figure 2. Effects of adding different percentages of chia seed flour on the sensory evaluation of carp fish fingers at (a) day 60 and (b) day 90

4. Discussion

Investors in the food sector are presently concentrating on the development of innovative and advantageous food items, including aquatic products like minced meat burgers, which serve as a substantial protein source for consumers. Nonetheless, it is crucial to exercise proper care for cooked stored meat products and their processed derivatives due to their inherent sensitivity. Various techniques suggested in numerous articles may assist in the advancement of oxidation processes; however, they may also affect the physical and chemical quality of proteins, the bioavailability of amino acids, and the lipid composition of meat, consequently diminishing its nutritional value. Furthermore, the intensity and duration of heat treatment, along with the intrinsic characteristics of meat products, may lead to losses in weight, water, and fat. As a result, there is an increasing necessity to employ various additives to counteract or lessen these adverse effects when utilizing thermal methods [23]. Chia seed, recognized as a type of pseudocereal, contains between 30 and 33.5 g of oil per 100 g, characterized by a high concentration of Polyunsaturated Fatty Acids (PUFA), with Alpha-Linolenic Acid (ALA) being the predominant essential fatty acid (57-65 g/100 g), which has garnered the interest of numerous researchers [24]. Another benefit of chia seed is its high both soluble and insoluble fiber content, coupled with its remarkable technological properties as a thickener and stabilizer, which contribute to gel formation and play a direct role in its capacity to retain water and fat [14, 25-27], as well as antioxidants such as cinnamic, chlorogenic, and caffeic acids, along with flavonoids like myricetin, quercetin, and kaempferol [28]. Consequently, chia seeds serve as an excellent ingredient for those on a diet due to their beneficial effects, which include lowering blood cholesterol and glucose levels, as well as influencing intestinal function and enhancing antioxidant activity. In terms of the specific functional properties of chia, it can significantly contribute as an additive in meat-based products, as demonstrated by various authors [29-32], and it has been shown to effectively increase the nutritional and functional value of the products.

Scapin et al. and Caudillo et al. found that chia seeds are rich in polyphenolic compounds, including flavonoids such as quercetin, kaempferol, and myristin, which demonstrate significant antioxidant properties [30, 33]. Mesias et al. state that chia seeds are safe for incorporation into food products due to their absence of toxic substances and gluten [34]. While earlier research has concentrated on the botanical characteristics of chia seeds, more recent studies have investigated their application in various products [35-38]. Carbonera et al. and Riernersman and Maria, in their review of chia flour in fish and meat products, recognized the enhancement of the nutritional profile of processed food items [23, 39]. In the current study, the findings from the chemical analysis indicated that the incorporation of chia flour into fish fingers and their subsequent storage in the freezer resulted in an increase in moisture and ash content, while protein and fat levels decreased. The rise in ash content associated with the addition of chia flour may be linked to the increase in dry matter [11, 40]. The results of the present study were consistent with the results of other studies [41, 42]. Moisture includes the amount of free and continuous water in the texture of the food matrix, so it could be mentioned as an important indicator in crispness and shelf life of the product. The reason for the increase in moisture content can be attributed to the hydrocolloid property of chia seeds, which reduces the amount of product moisture loss during storage [43]. The research conducted by Riernersman and Maria [23], which assessed the alterations in nutritional value of control hamburgers (lacking chia flour) and those containing 5.92 g of whole chia flour per 100 g (optimal) over a 90-day storage period at -18 degrees Celsius, yielded results that were not in agreement with the current study. They noted a reduction in moisture content from 74.05 ± 0.01 to 70.36 ± 0.34 g/100 g, alongside a decrease in protein content from 18.57 ± 0.13 to 17.47 ± 0.77 g/100 g, respectively. The results of the present study suggest that the optimization of the product has led to negligible water loss and no alteration in protein content post-cooking. A comparable result was reported by Gök et al. [44] in their investigation of meatballs, where fat was substituted with 20 g of poppy seeds per 100 g in comparison to the control. The findings of the current study align with those of other research on moisture parameters [36, 45, 46].

The observed increase in protein content corresponding with the rise in the proportion of whole chia flour aligns with findings from previous research, which indicates a higher fiber and protein content in chia seeds [42, 47, 48]. Furthermore, the decline in protein percentage observed in the treatments throughout the storage duration can be attributed to the liberation of amino compounds. In essence, as the duration of storage extends, the rate at which nitrogen-containing compounds are released also escalates over time, resulting in their removal from the primary components of the protein chain, which ultimately contributes to a reduction in protein levels in the treatments during the storage period. Generally, freezing fish products at -18 °C does not automatically lead to the degradation of proteins and nucleic acids. This temperature is widely utilized for food preservation as it effectively inhibits enzymatic reactions and microbial proliferation, thereby averting spoilage [49]. Although extremely low temperatures may potentially denature proteins over extended durations due to phenomena such as ice crystal formation (which can physically damage cells), this is not the predominant effect observed at -18 °C. The primary objective of freezing is to preserve the quality of the food for future consumption. Wang et al. [50] demonstrated that the water-holding capacity, salt-soluble protein, and moisture content of tuna diminished with prolonged storage duration. In comparison to a frozen storage temperature of -18 °C, superior quality bluefin tuna was achieved at a frozen storage temperature of -55 °C. Given that there was minimal alteration in quality during brief periods of frozen storage, a temperature of -18 °C may be appropriate for short-term frozen storage intended for sales. Additionally, another distinctive characteristic of tropical fish, such as the common carp, was believed to be attributed to its remarkable thermal stability. However, the processes of freezing and thawing can cause irreversible harm to proteins, resulting in a loss of structural integrity, conformation, and biological function. According to Zhang et al. [51], repeated freeze-thaw cycles lead to the reorganization of ice crystals and accelerated oxidation of proteins, which modifies their secondary and tertiary structures and impacts the mobility of internal water. Zhao et al. [52] suggested that these cycles trigger protein denaturation, which increases surface hydrophobicity. This denaturation may enhance the emulsifying properties of proteins, thereby affecting the taste and quality of food products that contain these proteins. Fish proteins show a notable reduction in solubility during freezing [53]. Protein molecules are characterized by their spatial conformation, which is maintained through covalent and non-covalent bonds. Denaturation mainly involves alterations in secondary and tertiary structures due to the disruption of these bonds [51]. The freezing process, combined with extended storage periods, alters the physical environment around proteins. This environment is characterized by high crystallization concentrations and increased salt ion levels, resulting in protein unfolding and instability [54]. This instability is further intensified by the concentration of solutes in the unfrozen aqueous phase, especially during slow freezing processes [55].

The study conducted by Coates [24] demonstrated that incorporating 5.92 g of whole chia flour into the burger formulation resulted in an increase in total fat content from 9.42 ± 0.03 to 10.19 ± 0.10 g/100 g. The n-3 fatty acid concentration in the optimal burger was found to be double that of the control treatment, attributable to the substantial fat content in chia flour (approximately 30%), which constitutes around 60% alpha-linolenic acid of the total fatty acids. The observed decrease in total fat levels in the samples analyzed in this study is likely due to fat oxidation and the influence of enzymes that facilitate hydrolytic spoilage, leading to the conversion into free fatty acids and other primary and secondary oxidation products [40, 56]. The fat percentage findings from this investigation were in agreement with the research conducted by Yüncü et al. [42]. Furthermore, the enhanced fat and moisture retention in the optimal burgers can be linked to the adhesive and stabilizing properties of the fibrous fraction of whole chia flour [27]. According to Anderson and Berry [57], the mechanism behind water and fat absorption is primarily physical, and in addition to absorbing some fat, fiber can interact with minced meat protein to create a matrix that inhibits the integration and migration of fat from the product.

In the current investigation, the incorporation of chia seed flour at varying percentages into fish fingers and its storage for 90 days at -18 °C resulted in a general decrease in the peroxide index. In other words, the inclusion of chia seed flour curtailed the peroxide production process. A study carried out by researchers examined the application of chia seed extract to prevent lipid oxidation in fresh pork sausages; the findings suggested that utilizing chia extract as a natural antioxidant is advisable [33]. Research conducted by Álvarez-Chávez et al. [58] revealed that chia seeds and oil are rich in natural antioxidant compounds, including tocopherols, phytosterols, carotenoids, and polyphenolic compounds. These compounds have been demonstrated to lower the risk of chronic diseases such as cancer and heart disease, and they also provide protective effects against various disorders, including arteriosclerosis, stroke, diabetes, Alzheimer's, and Parkinson's [59-62]. Furthermore, several studies identified a significant presence of polyphenolic compounds in chia seeds [63, 64].

The research carried out by Teets and Were [65] revealed that hamburgers made with chia flour kept peroxide levels under 2 milliequivalents/kg of fat for the entire duration of the 90-day study. In a similar vein, the peroxide index of nuggets that were treated with different amounts of chia flour in the current investigation did not surpass the allowable threshold of 10 meq O_2/kg of fat throughout all days of storage. These findings align with the results reported by Yüncü et al. and Fernández-López et al. [19, 42]. Moreover, the current investigation noted a reduction in the levels of volatile nitrogenous bases as the proportion of chia seed flour in fish fingers increased. Although the levels of volatile nitrogenous bases rose during the 90-day storage duration, the incorporation of chia seed flour mitigated this rise. The peak concentration of volatile nitrogenous bases was recorded on the 90 day of storage. Reports indicate that fish and their derivatives with TVB-N levels below 20-30 mg/100 g of the sample are deemed acceptable, whereas those surpassing 30 mg are considered unfit for consumption [66, 67]. In light of this, the shelf life of fish fingers on the day of storage, which exhibited the highest TVB-N levels, remained within the usable range. These results were also in agreement with the findings of Coban and Coban [68].

The TBA test serves as a technique for assessing the quantity of secondary oxidation products, particularly aldehydes, present in fats. Malonaldehyde, which arises from hydroperoxides, is utilized as a marker for evaluating fat oxidation. An increase in this index is attributed to the presence of free iron and other peroxides within muscle tissue, alongside the generation of aldehydes from secondary products that emerge from the breakdown of hydroperoxides. Although free radicals are recognized as a significant contributor to fat oxidation in meat, the quantity of fat and the composition of fatty acids also significantly influence the oxidation of meat fat during storage. A study by Riernersman and Marí [23] revealed that incorporating whole chia flour into baked fish burgers enhanced yield, water and fat absorption, n-3 fatty acid content, and oxidative stability. Specifically, the addition of 5.92 g of whole chia flour and

15 g of water per 100 g of burger resulted in a yield percentage of 94%, a water absorption percentage of 92%, and a fat absorption percentage of 97%. The n-3 fat content increased from 704.57 ± 21.66 mg to 1,551.71 ± 47.71 mg/100 g of cooked burgers with the inclusion of chia flour. Melo et al. [69] observed that the TBARS value rose in all hamburger samples treated with chia seed extract compared to the control during frozen storage at -18 °C. Pork sausage treated with varying concentrations of chia seed extract exhibited an increase in TBA value during the initial 7 days of storage at 4 °C, while TBA values decreased from 14 days onward until 28 days of storage [33]. Camel burgers that incorporated chia seed demonstrated significantly lower TBA values compared to the control sample during 12 days of storage at 4 °C [70]. Kulczynski et al. reported that chia seeds possess high antioxidant properties [71]. The oxidative stability of the final product remained adequate throughout the storage duration, with only a minimal formation of hydroperoxides and reactive thiobarbituric acid substances observed during the 30 days of frozen storage. This stability is likely attributed to the presence of polyphenolic compounds exhibiting antiradical activity and reducing power within the fibrous component of the flour. The current investigation revealed that incorporating chia seed flour into fish fingers at varying percentages and storing them at freezing temperatures for 90 days increased thiobarbituric acid levels. Nevertheless, the alterations in this parameter were constrained by the inclusion of chia seed flour. The research demonstrated that chia flour provides a significant protective effect against lipid oxidation in cooked fish fingers, which can be ascribed to the polyphenolic compounds that possess anti-radical activity and reducing capabilities.

According to the current study, the sensory assessment, including smell and taste, of the samples exhibited a deterioration over time. After a storage period of 90 days, the fish fingers received the lowest sensory rating, with the sample containing 20% chia flour recording the lowest score among all samples on that day. Researchers have identified that the flavor in seafood arises from specific compounds generated during the cooking of fish meat, akin to the Maillard reaction, as well as from the breakdown of cysteine and methionine [72]. Ding et al. indicated that the incorporation of chia seeds into reconstituted ham enhanced its nutritional, physicochemical, and sensory attributes [73]. Nevertheless, in the study conducted by Heck et al., the addition of chia flour resulted in a decline in sensory quality compared to the control treatment, while the hardness remained unchanged, and the fat absorption was improved [74]. The research carried out by Pintado et al. examined the influence of chia emulsion gels on fresh sausages [75]. The findings indicated a reduction in waste generated during the cooking process as well as an improvement in the firmness of the product's texture. Despite some alterations in sensory properties, the overall acceptance rate of these products surpassed that of the control group. Similarly, Santillan-Alvarez et al. explored the reconstituted meat of common carp that was enhanced with up to 8% chia flour. Their study revealed enhancements in both the cooking characteristics of the final product and its nutritional value when compared to the control treatment [28]. The outcomes of the current study align with the findings of previous studies [33, 70]. Nonetheless, it is crucial to recognize that the composition and concentration of bioactive compounds present in chia seeds are influenced by several factors, including climatic conditions, geographical origin, and extraction techniques [76-78]. According to Coelho & de las Mercedes Salas-Mellado, chia seeds serve as a valuable raw material and food supplement [37].

Moreover, the present study has shown that chia flour improves moisture retention and protein levels, not only delaying signs of spoilage but also maintaining sensory qualities throughout storage. The increase in protein content may be attributed to molecular modeling and the formation of van der Waals and hydrogen bonds within a multicomponent system, which could provide insights for future investigations aimed at identifying binding sites, as noted in [79]. Consequently, it is recommended to combine natural additives such as chia flour with an emphasis on biomedical applications or material science related to biointerfaces. This may involve the integration of formulations and the synthesized PEGylation of liposomes to develop advanced, safe, and effective food preservation methods that extend shelf life while maintaining product quality. As illustrated by Rahnama [80], formulations indicate that the careful incorporation of bioactive compounds into carriers like PEGylation and folate targeting enhances stability, bioavailability, and therapeutic effectiveness of insulin over time, providing better protection against degradation and improved absorption; a similar approach could be applied to bioactive compounds found in chia seeds. Furthermore, assessing its functionality and the potential increase in absorption, bioavailability, and rapid metabolism of these functional compounds, efforts can be directed towards employing various formulation strategies. According to [81], the formulation of nano-scale carriers combined with different proteins could effectively protect functional compounds from degradation and enhance their stability during storage and transport. The advantages of the biological synthesis method include its eco-friendly characteristics and the generation of more biocompatible nanoparticles compared to conventional chemical synthesis; as [82] demonstrated the efficacy of cellulose nanocrystals derived from Jujube seeds for biomedical applications, which could be evaluated for chia seeds in future studies.

Based on the findings, it appears that the addition of 10% chia flour to the carp fish finger recipe has led to positive product attributes throughout a 90-day storage duration at -18 degrees Celsius. Therefore, it is recommended to identify and apply the ideal proportions of chia flour in various aquatic-based meat products, both in ready-to-eat and semi-prepared formats, while considering the characteristics of this grain for commercial applications.

5. Conclusion

In summary, the addition of chia seed flour to fish products not only boosts their nutritional value by elevating protein and omega-3 fatty acid levels but also enhances oxidative stability and moisture retention throughout storage. These results highlight the potential of chia seeds as a beneficial ingredient in the food sector, encouraging healthier choices while preserving product quality. Furthermore, the integration of chia flour into fish fingers significantly improves nutritional content and oxidative stability during storage, in addition to enhancing moisture retention and protein content. Nevertheless, it is crucial to carefully evaluate sensory characteristics, as increased concentrations may result in a decline in flavor and overall acceptance, emphasizing the necessity for optimal formulation strategies in aquatic-based meat products.

Conflict of interest

The authors declare no competing interests.

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