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Effect of Frozen Storage on Nutritional, Microbial and Sensorial Quality of Fish Balls and Fish Fingers Produced From Indian Mackerel

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Abstract

Fish quality is important in the food industry. Studies on the nutritional, microbial and minerals in Indian mackerel fish are limited. Therefore, this study was carried out to assess the quality and production of fish products (balls and fingers). Additionally, the effect of frozen storage for six months on the microbial, nutritional and sensory evaluation of fish balls and fingers was studied. The obtained results showed that the estimated minerals (zinc, cadmium, chromium, copper, lead and mercury) contents in Indian mackerel muscles were lower than the maximum permissible limits for human consumption. The levels of total bacterial counts and total yeast counts in Indian mackerel purchased from three different stores varied. After 6 months of storage, the microbial content decreased in Indian mackerel fish balls and fingers to less than 2.0×10^{2} CFU/g, which was due to the effect of freezing on the growth and activity of microorganisms, while the carbohydrate, fat and energy contents increased, and the ash, protein and moisture contents decreased; however, Indian mackerel fish fingers had elevated ash, carbohydrate, fat, protein and energy contents and a reduced moisture content after freezing. Sensory evaluation of Indian mackerel balls and fingers at the start and end of the storage period (6 months) revealed good scores for appearance, odor, texture, taste and acceptability. These results provide insights into the benefits of good-quality Indian mackerel fish in the fish product industry and their availability after storage for six months.

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Introduction

Indian mackerel fish are important fishery resources that contribute to a nutritious diet, are commonly consumed in Southeast Asian countries, and are distributed in the East China Sea, Pacific coast of Japan and zones of India.[1] Indian mackerel has important characteristics, such as a delicious taste, global availability and a relatively low cost. Mackerel has high nutritional value and contains high amounts of unsaturated fatty acids, amino acids and essential minerals, which are needed for human health.[2,3] However, heavy minerals can accumulate in fish from food intake, water, and sediments, which may affect human health. Heavy minerals can cause heart disease, liver damage and renal failure.[4-7] The concentration of heavy minerals in fish has been studied in many regions worldwide. Most of these studies concentrated on heavy minerals in fish muscles.[8] According to these studies, there are many factors that affect the absorption of minerals in fish, such as size, age, sex, reproductive cycle, feeding behavior, and geographic location.[5,9] Therefore, international standards have been established to assess fish quality for human consumption.[10]

As expected, deterioration of the sensory quality and nutritional value of fish is a result of various microbiological spoilage and biochemical reactions as the protein and lipid fractions change.[11] Mackerel are fatty fish and contain high amounts of hemoglobin, so they are highly susceptible to lipid oxidation and rancidity, yielding elevated total volatile base nitrogen and biogenic amine contents. Microbiological proliferation of Indian mackerel is commonly caused by bacteria such as *Pseudomonas, Vibrio, Serratia, Listeria and Micrococcus,* resulting in fish spoilage and reduced nutritional value and sensory scores.[12-14]

Fish spoilage occurs rapidly. Spoilage of fish immediately occurs due to endogenous flesh enzymes. However, the activity of these enzymes decreases at low temperatures.[15] Preservation of food is essential to increase its shelf life and conserve its nutritional value, flavor and texture. Therefore, optimized methods for food preservation must prevent damage to food by microorganisms while maintaining its quality and nutritional value.[12] The shelf life of fish products can be increased by freezing, which creates unfavorable conditions that retard microbial growth and the biochemical decomposition of fish muscle.[15]

However, the preservation of fish freshness becomes problematic during freezing, and most consumers prefer fish products with an increased shelf life and nutritional value.[1,12]

The main goal of the present study was to estimate the minerals and microbial quality of Indian mackerel purchased from three different stores in Riyadh to determine a safe source to prepare mackerel fish products as fish balls and fingers. Additionally, we investigated the effect of storage for six months on the microbial content, nutritional values and sensory evaluation of these products.

Materials and Methods

Indian Mackerel Fish

Fresh Indian mackerel (*Rastrelliger kanagurta*) was obtained from three different stores in Riyadh and transported to the laboratory in an icebox. Fish were washed and eviscerated, and the skin, head, and bones were removed to obtain three samples of Indian mackerel muscle for minerals analysis and microbial analyses.

Estimation of Minerals

By following the standard methods in, 16 some minerals, i.e., zinc (Zn), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and mercury (Hg), were determined in the Indian mackerel muscle samples from different sources.

Microbial Analysis

The microbial parameters of bacteria in the Indian mackerel samples were analyzed by using standard methods of the FDA/CFSAN.[17] Total aerobic plate counts were determined using nutrient agar, while *E. coli and fecal Coliform* bacteria were counted through the most probable number (MPN) method using EC broth for seawater and shellfish. *Listeria monocytogenes* was counted on agar (Difco), while *Staphylococcus aureus* was counted on Baird-Parker medium. The quantification of *Vibrio parahaemolyticus* was performed through the MPN technique using thiosulfate citrate bile salt sucrose agar (TCBS). Salmonella was also detected using lactose broth for enrichment.[17] In addition, yeasts and molds were analyzed using potato dextrose agar as described previously.[18] This analysis was performed in triplicate for different Indian mackerel muscle sources.

Preparation of Indian Mackerel Balls

The selection of mackerel muscle source was mainly based on the minerals and microbial quality of the fish. Samples were prepared for microbial, nutritional and sensory analyses at the beginning of storage and after six months. The following components were thoroughly mixed and carefully minced: 500 g of boneless Indian mackerel muscle, 400 g of boiled rice, 70 g of green vegetables, and 30 g of natural flavors.[19] The mixture was left to cool in a fridge for two hours, shaped into balls, packed in polyethylene bags and stored at a temperature of -18 °C for six months.

Preparation of Indian Mackerel Fingers

Five hundred grams of boneless Indian mackerel muscle was carefully minced with 400 g of boiled potatoes, 70 g of green vegetables, and 30 g of natural flavors. The mixture was left to cool in a fridge for two hours, shaped as fingers, covered with bread crumbs,[19] packed in polyethylene bags and stored at a temperature of 18°C.

Nutritional Evaluation of Mackerel Balls and Fingers

Mackerel balls and fingers were investigated for moisture, protein, lipid and ash contents. The moisture content was analyzed by a hot air oven. The protein, lipid and ash contents of the samples were estimated by methods reported previously.[20-22] The carbohydrate and energy contents were calculated by reported previously methods.[23]

Sensory Analysis

Sensory evaluation was performed by a panel of 10 members using a ten-point hedonic scale for fish ball and finger product acceptability immediately after production (start) and after storage for six months at -18°C.[24] The samples were presented randomly and coded by different capital letters. Panelists evaluated the appearance, odor, texture, taste, and overall acceptability of the products. An overall acceptability score was calculated by the sum of scores and divided by the number of attributes examined. The scores were determined as follows: ten, excellent; nine, very good; eight, good; six to seven, average; and five or below, bad and refused.

Statistical Analysis

Statistical analyses were carried out using the SPSS program version 22.0 (SPSS Inc., Chicago, Illinois, U.S.A.) (SPSS., 2018. All tests were performed in triplicate. The significance of the difference between means was determined by Duncan's multiple range tests ($p < 0.05$). The value was expressed as the means of three determinations ± the standard deviation.

Results

Minerals in Indian Mackerel

Some minerals in fish, such as copper and zinc, are essential nutrients, while others, such as cadmium, mercury, and lead, are toxic.[6] In our study, elemental analysis of mackerel muscle samples collected from three different stores in Riyadh showed the presence of zinc, cadmium, chromium, copper, lead and mercury. The edible mackerel muscle showed safe and accepted levels of nonessential minerals, such as cadmium, lead and mercury, with values of 0.03 ± 0.001, 0.002 ± 0.001 and 0.001± 0.001 ppm, respectively. Zinc, chromium and copper are essential minerals and were present below the maximum allowable limit by Saudi and international legislations for the human consumption of fish and had concentrations of 20.1143 ±2.889, 0.213 ± 0.201 and 1.04 ± 0.006 ppm, respectively, as shown in Table I.

Table 1: Minerals in Indian mackerel

Zn (ppm)	Cd (ppm)	Cr (ppm)	Cu (ppm)	Pb (ppm)	Hg (ppm)
20.143±2.889^{a}	0.03±0.001^{b}	0.213±0.201^{b}	1.04±0.006^{a}	0.002±0.001^{b}	0.001 b±0.001^{b}

The results are presented as the mean ± SD (n=3). Different lower case letters indicate Values are significantly different ($p<0.05$) between different minerals.

Microbial Quality of Indian Mackerel

The Indian mackerel fish muscles from three different stores in Riyadh were subjected to microbial investigation. The second sample of Indian mackerel muscle showed the lowest total aerobic plate count ($2.0×10^4$ CFU/g) compared with those of the first and third samples, which had values of 6.0×104 and $1.8×10^5$ CFU/g, respectively. The results showed no detected levels of *E. coli* (CFU/g), *Listeria monocytogenes, Salmonella* and *Vibrio parahaemolyticus* (CFU/25 g); less than 10 CFU/25 g fecal *Coliform* bacteria and *Staphylococcus aureus*; and less than 100 CFU/g mold in the three samples. The yeast count in the first sample was $14× 10^2$ CFU/g, while those in the second and third samples were $19×10^2$ and $34×10^2$ CFU/g, respectively. The obtained results showed different levels of total bacterial counts and yeast counts in Indian mackerel obtained from three different stores, as recorded in Table 2. From the results of this table, the second sample was chosen for subsequent production of mackerel balls and fingers.

Table 2: Microbial analyses of Indian mackerel purchased from three different stores in Riyadh

Samples Variables	Indian mackerel fish samples		
	First*	Second**	Third***
Total aerobic plate count (CFU/g)	$6.0×10^{4a}$	$2.0×10^{4b}$	$1.8×10^{5a}$
E. coli (CFU/g)	ND	ND	ND
Fecal Coliform bacteria (CFU/g)	$<10^{a}$	$<10^{a}$	$<10^{a}$
Listeria monocytogenes (CFU/25 g)	ND	ND	ND
Staphylococcus aureus (CFU/g)	$<10^{a}$	$<10^{a}$	$<10^{a}$
Salmonella (CFU/25 g)	ND	ND	ND
Vibrio parahaemolyticus (CFU/25 g)	ND	ND	ND
Yeasts count (CFU/g)	$14×10^{2a}$	$19×10^{2a}$	$34×10^{2a}$
Mold count (CFU/g)	$<100^{a}$	$<100^{a}$	$<100^{a}$

ND= Not detected, the results are presented as the mean (n=3). Different lower case letters indicate significantly different ($p<0.05$) between different stores, *= (Carrefour) **= (Danube) ***= (Panda)

Table 3: Microbial analysis of Indian mackerel balls and fingers

Samples Variables	Indian mackerel fish balls		Indian mackerel fish fingers	
	Baseline	After 6 months of storage	Baseline	After 6 months of storage
Total aerobic plate count (CFU/g)	$4.0×104^{a}$	$2.0×10^{2b}$	$3.0×104^{a}$	$<100^{b}$
Fecal coliform bacteria (CFU/g)	$<10^{a}$	ND	$<10^{a}$	ND
Staphylococcus aureus (CFU/g)	<10	ND	<10	ND
Yeast count (CFU/g)	$24×10^{2a}$	<100	$21×10^{2a}$	$<100^{b}$
Mold count (CFU/g)	$<100^{a}$	$<10^{b}$	$<100^{a}$	$<10^{b}$

ND= Not detected, the results are presented as the mean (n=3). Different lower case letters indicate significantly different ($p<0.05$).

Microbial Quality of Mackerel Balls and Fingers

Table 3 shows the effect of storage on the microbial investigation of homemade Indian mackerel fish products (ball and fingers). After six months of storage, the total aerobic plate count, fecal *coliform* bacterial count, *Staphylococcus aureus* count, and yeast and mold counts decreased.

Table 4: Chemical composition of Indian mackerel balls and fingers

Samples Variables	Indian mackerel fish balls		Indian mackerel fish fingers	
	Baseline	After 6 months of storage	Baseline	After 6 months of storage
Ash (g/100 g)	1.73±0.014[a]	1.56±0.048[a]	1.65± 0.024[a]	2.01±0.013[a]
Carbohydrates (g/100 g)	11.06±0.024[a]	12.91± 0.082[b]	12.56± 0.029[a]	20.88±0.014[a]
Fats (g/100 g)	0.43±0.020[a]	1.23±0.072[b]	1.03±0.004[a]	3.91± 0.020[a]
Protein (g/100 g)	12.94±0.049[a]	11.61±0.028[a]	7.22 ±0.008	8.70±0.014[a]
Moisture (g/100 g)	73.84±0.036[a]	72.69±0.053[b]	77.54±0.032[a]	64.50±0.249[b]
Energy (Kcal)	99.87±0.138[b]	109.15±0.113[b]	88.39±0.024[a]	153.51±0.032[a]

The results are presented as the mean ± SD (n=3). Different lower case letters indicate significantly different (p<0.05) between different periods.

Nutritional Evaluation of Mackerel Balls and Fingers

Table 4 shows the effect of storage on the chemical composition that reflects nutritional values of Indian mackerel fish products (ball and fingers). After six months of storage, the carbohydrate, fat and energy contents of Indian mackerel fish balls increased, and the ash, protein and moisture contents decreased compared to those in the fresh products; however, the Indian mackerel fish fingers had increased ash, carbohydrate, fat, protein and energy contents and reduced a moisture content after six months of storage.

Table 5: Sensory evaluation analysis of Indian mackerel balls and fingers

Properties	Indian mackerel balls		Indian mackerel fingers	
	Baseline	After 6 months of storage	Baseline	After 6 months of storage
Appearance	8.47±0.06[a]	8.63±0.09[a]	8.77±0.12[a]	8.53±0.06[a]
Odor	8.12±0.11[a]	8.37±0.12[a]	8.52±0.17[a]	8.43±0.12[a]
Texture	8.53±0.21[a]	8.33±0.15[a]	8.62±0.10[a]	8.27±0.15[a]
Taste	8.31±0.10[a]	8.47±0.06[a]	8.67±0.15[a]	8.57±0.15[a]
Acceptability	8.36 ±0.12[a]	8.45±0.11[a]	8. 65±0.14[a]	8.45 ±0.12[a]

The results are presented as the mean ± SD (n=3). Different lower case letters indicate significantly different (p<0.05) between different periods.

Sensory Evaluation of Mackerel Balls and Fingers

Sensory evaluation of the products was performed, and the results are shown in Table 5. Evaluation of Indian mackerel balls at the start of storage showed good scores for appearance, odor, texture, taste and acceptability. At the end of the storage period, the fish balls (6 months) also showed good scores for all attributes that did not significantly differ from those at the start of storage. Sensory evaluation of the mackerel fish fingers at the start of storage had a good score for all attributes, and this score was maintained after storage, exhibiting non significantly lower scores than those observed before storage.

Discussion

Although fish contain protein, essential minerals, vitamins, and unsaturated fatty acids, it is important to assure that the minerals and microbes in fish are within safe limits. It has been reported that minerals in fish food, water, and sediments normally accumulate in fish in a manner dependent on minerals uptake factors, such as age, sex, size, reproductive cycle, and feeding behavior, in addition to geographical location.[9,5] According to international standards, the maximum allowable levels on fish of zinc, mercury, lead, cadmium, chromium, and copper are 30 ppm. 1.0 ppm, 0.3 ppm, 1.0 ppm, 1.0 ppm and 30 ppm, respectively.[25,26,27] Several serious threats from the intake of minerals, including cardiovascular disease, hepatorenal failure and death, have been extensively reported.[6] Zinc is an essential element and is considered safe when taken below the maximum permissible limits.[28] Excessive intake of copper results in acute symptoms such as gastrointestinal distress, vomiting and even internal bleeding. Additionally, lead toxicity in children has permanent adverse health effects, particularly on brain and nervous system development; however, in adults, lead toxicity could increase blood pressure, cause renal damage, and have adverse neurological effects. Ingestion of cadmium over the maximum permissible limits causes immediate poisoning symptoms and damage to the kidneys and liver.[29,7] Table (1) shows the safe limits of the investigated minerals, the levels of which appeared below the permissible limits. These results agreed with those of Windom *et al.,*[30] who found that fish (*Coryphaenoides armatus* sp.) from the Atlantic and Pacific Oceans had cadmium levels ranging from 0.025- 0.027 ppm, copper ranging from 0.034-0.086 ppm and lead ranging from 0.012-0.016 ppm dry weight. Romeo[31] reported relatively low levels of copper (2.3 ppm dw), zinc (142 ppm dw), cadmium (<0.1 ppm dw) and lead (<0.5 ppm dw) in the muscle of *Mugilcephalus* from the northern coast of Mauritania in the Atlantic Ocean. Additionally, the obtained results agreed with the results recorded by Ashraf and Jaffar,[32] who estimated copper, lead, cadmium and chromium levels in six marine fish muscles from the Arabian Sea. Our results were also consistent with those of studies reported previously.[29, 33]

All of the fish samples were considered acceptable and of good quality in terms of microbial contents, according to the Saudi Standards, Metrology and Quality Organization (SASO) and the International Commission on Microbiological Specifications for Foods (ICMSF), which specify 1.0 × 107 and 5.0 × 105 CFU/g as the upper (rejectable) and lower (marginal) levels of acceptability, respectively.[34, 35]

It has been reported that the microbiological load in mackerel fish muscle is related to seasonal variation, harvesting conditions and storage.[36] The freshness of fish could decrease after death because of several biochemical reactions, such as protein and lipid fraction changes and microbiological spoilage, resulting in a decrease in sensory quality and nutritional value.[37] Microbiological investigation of Indian mackerel muscle is an important technique in the processing of fish products. After fish die, bacteria and fungi multiply and decompose the muscle, resulting in fish autolysis.[38] The ICMSF stated that the aerobic plate count (APC) is an important factor for the evaluation of microbial quality estimation in food products and is an indicator of the overall degree of microbial contamination of foods.[39] Statistical results showed a high significant difference at the evaluated probability level ($p<0.05$) in the APC in fish balls and fingers before and after storage; that is, the average APC in fish balls and fingers decreased during the storage period because of bacterial cell loss due to the effect of freezing on the growth and activity of microorganisms. Freezing reduces the chances of a material to provide the necessary nutritional requirements for bacterial growth, and the effect of freezing on most microorganisms is a result of the change in bacterial cell protein content, an increase in the concentration of dissolved substances in unrefined water, or physical damage caused by the formation of ice crystals.[40]

Microbial growth can also be inhibited by decreasing the humidity and increasing the percentage of filler material (potatoes, rice), consequently reducing the water activity required for bacterial growth, as well as decreasing the protein percentage with the addition of other components, including spices and garlic, which leads to an increase in their inhibitory effectiveness against the growth of many bacteria. The products were free from fecal *coliform* bacteria and *Staphylococcus aureus*. The yeast count and mold count decreased during storage. This may have been due to the adequate hygiene maintained during the entire process of fish ball and finger preparation. This agreed with the results of Mehta *et al.,*[41] Arulkumar *et al.,*[42] and Cordoba *et al.,*[43]

Changes in the nutritional values of Indian mackerel balls by freezing storage, including reduced ash, protein and moisture contents, could be attributed to dehydration, whereas the decrease in protein content because of frozen storage could decrease the oxidative stability of proteins (sarcoplasmic and myofibrillar), affecting the water holding capacity and protein extractability of mackerel muscle. The chemical composition that reflects the nutritional values of Indian mackerel fingers showed an increase in fat, carbohydrate, and protein contents at the end of the storage period, which was related to the changes in moisture content.[44,45] The increase in fat content and reduction in moisture content in the fish balls and fingers was due to dehydration during frozen storage.[46] The freshness of raw material is important because prolonged storage prior to freezing reduces the product quality and storage stability of mackerel muscle.

Sensory evaluation is a method for forming a significant quality index and is the most dependable test for processed fish products. The variation in sensory attributes is related to panel acceptance. Consumer acceptance is mainly related to the freshness of fish products, and there is strong evidence that freezing is the most effective method to preserve fish quality. In addition, freezing temperatures may affect protein denaturation after prolonged storage, and initial freezing at -20°C did not significantly affect the degree of denaturation of myofibrillar proteins in minced mackerel in studies related storage temperatures.[44,45,47]

Conclusion

In conclusion, due to the availability, low price and nutritional value, Indian mackerel can be introduced into the fish industry. The estimation of minerals and microbial loads of Indian mackerel fish is important in elevating product quality. The different levels of total bacterial counts and yeast counts of Indian mackerel varied according to purchase origin of the obtained fish and were related to the freshness of the fish. The estimated minerals in Indian mackerel muscles appeared safe for consumers. After 6 months of frozen storage at -18°C, Indian mackerel fish balls and fingers had somewhat increased nutritional values and had good scores in sensory evaluation, while the microbial content decreased for both products. The reason for that the decreased microbial content was the loss of bacterial cells due to the effect of freezing on the growth and activity of microorganisms. The obtained results might be useful in offering a basis for the Indian mackerel fish products industry.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

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Conflicts of Interest

The author declares that there are no conflicts of interest.

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