

PREPARATION OF FISH BURGER and KOFTA FROM BASA
FISH (*Pangasius bocurta*) FILLET AND EVALUATION OF
QUALITY AND SHELF LIFE DURING FROZEN STORAGE
CONDITIONS

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ABSTRACT

Fish is considered one of the most sought-after foods around the world due to its high nutritional value, good sensory qualities, and the wide variety of ways to prepare and serve it. Basa fish is not common in the Egyptian market and is unknown to a large segment of consumers. This research aimed to produce fish burgers and kofta from basa fish fillets treated with a mixture of rosemary oil extract, citric acid, and pepsin enzyme, and then evaluate the quality of the products stored frozen at -20°C, for 4 months. The results showed significant differences in the chemical composition between treated basa fillets and treated burgers and kofta, whether treated or untreated, as treatment reduced the moisture, protein, and fat content while increasing the carbohydrate and ash content. A noticeable increase in the peroxide value, TBA, was recorded as the storage period progressed, especially for untreated products, but these increases were within permissible limits. Although there were no significant differences between the values of water holding capacity throughout the storage period, a noticeable increase was recorded in the values of cooking loss and shrinkage with increasing storage period, and this increase was at a lower rate in the treated and untreated burgers compared to the treated and untreated kofta. The physical color properties were significantly affected by

frozen storage, while all products were sensory acceptable during all storage periods until the end of the experiment. The results of microbiological analyses showed that all treated and untreated products were completely free of Count Coliforms, *Escherichia coli*, *Salmonella spp*, and Coagulase (positive) Staphylococci, and no significant differences were recorded between the treated and untreated products throughout the storage period. It is clear that high-quality products can be prepared from basa fish fillets and can be kept frozen without affecting their quality standards. This is considered a good start for using basa fish in the Egyptian market.

KEYWORDS: Basa fillet, Marination, Fish burger, Fish kofta, Chemical composition, Sensory and Physicochemical properties

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INTRODUCTION

Fish is highly regarded as a valuable protein source in the human diet, primarily due to its naturally abundant content of essential n-3 polyunsaturated fatty acids (PSUFAs). These fatty acids play a crucial role in preventing coronary heart disease (**Mozaffarian *et al.*, 2005**). Fish constitutes a significant portion of animal protein consumption in various parts of the world. Globally, approximately 100 million tons of fish are harvested annually, but only around 70 million tons are utilized for human consumption (**Huss *et al.*, 2000**). Of this quantity, roughly 27% is consumed fresh, while the rest undergoes various food preservation methods such as freezing, salting, drying, smoking, or canning.

Omega-3 fatty acids offer unique health benefits not found in other foods. They are known to reduce the risk of blood clots leading to heart attacks and strokes, as well as improve blood circulation. Omega-3 fatty acids may be particularly advantageous for overweight individuals with hypertension who are on weight loss diets. The

American Diabetes Association (ADA) and the American Heart Association (AHA) endorse the consumption of fatty fish as a safe and effective means to obtain the heart-healthy advantages of omega-3 fatty acids. Regular consumption of fatty fish is a valuable strategy for enhancing the health of individuals with diabetes (**Nettleton, 1995**). With growing consumer awareness of health-related issues, the consumption of fish and fishery products is on the rise. In the current context, value-added minced fish products hold the potential to bring immediate benefits to the existing fish processing industries in the country (**Nowsad *et al.*, 2000**).

Fish and fish products are recognized as excellent sources of protein, boasting high digestibility, nutritional quality, and biological value. They also serve as rich sources of essential unsaturated fatty acids, various vitamins, and minerals, including phosphorus, magnesium, calcium, iron, and selenium (**Gomma, 2005**). Fish products are widely accepted globally and are commonly consumed as ready-to-eat or pre-cooked items. Regarding the cooking process, heat is applied to food to enhance its flavor, deactivate pathogenic microorganisms, and prolong its shelf life (**Bognar, 1998**). Numerous studies have been conducted on the production and quality stability of fishery fast food products like fish cakes, fish crackers, fish balls, and fish burgers. The basa catfish (*Pangasius bocourti*), belonging to the Pangasiidae family, is native to the Lower Mekong River. It has gained popularity due to its favorable traits, including rapid growth, high yield, and the absence of horizontal bones. This catfish has been extensively cultivated as a promising economic fish in freshwater systems of the Mekong Delta, particularly in Vietnam and Thailand (**Jiwyam, 2010**). Furthermore, the white flesh, low-fat content, and delightful taste make it an appealing source of fish for consumption (**Thammapat *et al.*, 2010**). Pangasius fish production reached an estimated 800,000 tons per year in 2013, supplying over 100 countries worldwide with an export value of USD 1.5 billion (**Van Doan *et al.*, 2016**).

Burgers, fish kofta, and meatballs are popular ready-made products in many countries. However, they are primarily made from red meat,

which is linked to cardiovascular and various other health issues. Concerns about high cholesterol in red meat have led to fish becoming an alternative source for these products (**Vicente and Torres, 2007**). Fish burgers are among the most widely accepted food products globally and are commonly consumed as ready-to-eat or pre-cooked items. Fish and fish products are typically consumed after freezing, a method employed for long-term preservation that minimizes quality loss over extended periods. While undesirable changes are controlled during frozen storage, alterations occur in the quality of myofibrillar proteins and lipids(**Khoshnoudi-Nia et al., 2018**).

Seafood is highly perishable, with its shelf life limited by enzymatic and microbiological spoilage. Numerous quality control methods have been developed to assess fish spoilage (**Metin et al., 2002**). One significant quality issue is lipid oxidation, which is more prevalent in fish than in meat due to the high unsaturation of fish lipids. The application of antioxidants is an economical and effective approach to combat lipid oxidation (**Karpinska et al., 2001**). Various synthetic and natural antioxidants are employed to prevent lipid oxidation in foods. Natural antioxidants have gained considerable interest due to their potential to extend the shelf life of food products by inhibiting and delaying lipid oxidation (**Sanchez-Alonso et al., 2008**). Given the carcinogenic activity of synthetic antioxidants like tert-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), and butylatedhydroxyanisole (BHA), natural antioxidants have been extensively researched. Many herbs and spices have demonstrated antioxidant properties in various food systems, with rosemary being notable for its high antioxidant activity.

Rosemary (***Rosmarinus officinalis L.***) extract, a common spice, is widely used in the food industry (**Gonzalez-Trujano et al., 2007**). Its antioxidant efficacy is attributed to its rich content of phenolic compounds, including monoterpenes (e.g., eucalyptol), diterpenophenols (carnosic acid, carnosol, rosmanol, epirosmanol, isorosmanol, methylcarnosate), phenolic acids (rosmarinic acid), flavonols, and triterpene acids (ursolic acid, oleanolic acid, butilinic

acid) (**Riznar *et al.*, 2006**). These compounds break free radical chain reactions by donating hydrogen atoms.

Pepsin, derived mainly from porcine, bovine, and microbial sources, is a crucial industrial enzyme accounting for approximately 60% of commercially marketed enzymes (**Dheyauldeen *et al.*, 2021**). Pepsin finds applications in various industries, including leather tanning, detergents, agrochemicals, food, and pharmaceuticals. It is also used in producing peptides with significant antioxidant, antihypertensive, antimicrobial, and other biological activities. Pepsin treatment has been observed to reduce the allergenicity of certain proteins, an essential function in the human digestive system (**Zhang *et al.*, 2020**). Pepsin can thus be employed to produce hydrolysates or peptides with reduced allergenicity and enhanced biological activities.

Bioactive peptides can be produced using various proteases, such as Alcalase, papain, flavourzyme, and neutrase (**Huang *et al.*, 2022**). Pepsin is advantageous due to its selectivity, as it cleaves bonds involving hydrophobic amino acids. Consequently, the released peptides have hydrophobic amino acids at their ends (leucine, isoleucine, or valine), leading to superior antioxidant and ACE-inhibitory activities (**Lee and Hur, 2017**). The citric acid (CA) is a primary food acidulant used to prevent oxidative deterioration of taste and color in products like jellies, sweets, and soft drinks. To be used as a food additive, CA must have a purity of 99.5% (**United States Pharmacopeial Convention, 2014**). CA's antioxidant properties stem from its ability to chelate metal ions, such as calcium, iron, and copper, which are present in significant amounts in various food systems. CA is also employed to mask unpleasant flavors and enhance palatability (**Ghorpade *et al.*, 2018**).

Considering these factors, this research aimed to produce fish burgers and kofta from basa (*Pangasius bocourti*) and assess their shelf life and quality when treated with natural additives. Additionally, various objective tests were employed to evaluate the quality and spoilage degree of fish burgers and kofta during frozen storage.



Figure (1). (*Pangasius bocourti*) Basa fish

MATERIALS AND METHODS

Materials

Fish samples: Frozon Basa filets (*Pangasius bocourti*) were purchased from Carrefour Cairo markets, Egypt (average weight 800-850 g).

Chemicals: pepsin enzyme, rosemary oil and citric acid crystals were purchased from Sigma-Aldrich (St. Louis, MO).

Ingredients: powdered spices, edible salts, smashed potato, vegetable oil, sunflower oil, rusk, wheat flour and food additives were obtained from the local market.

Marination Process

Firstly, frozen basa fillet samples were thawed in a refrigerator at 4 °C for 12 hours. The treated group was prepared by soaking basa fillets in a marinade solution containing 0.1% pepsin enzyme + 0.5% rosemary EOs + 0.5% citric acid (E +R +CA). In addition to the control (C) group soaked in distilled water. Treated and control groups were packed in sterile polyethylene bags and stored in a refrigerator over night, then removed from the solution and the control and treated fillets were cut and minced by a kitchen meat mincer using a 3 mm diameter holes plate. Minced fish were divided into two batches to obtain different basa products,i.e. fish burger and kofta.

Preparation of the fish burger

The first batch of minced fish obtained from the pangus fish muscle was ground with 2% NaCl, 8% oil, , 2% spices (onion, garlic, ginger, and hot spices), and 18% of boiled potato (**Table 1**). The mixing was done generally for 5-7 minutes. The whole dough was stuffed into a steel frame. The size of each burger patty was (10 × 9.5 × 1.00cm). Burgers were formulated by Deemax Burger Machine (WF- A130) , China. Then the patties were separated from the steel frame and dipped in a batter formulation(**Table 2**). Then it was fried in dip-oil. After cooling, the burger patties were packaged in an air-tight polyethylene bag for quality tests.

Table (1): Recipe of basa burger patties*

| Ingredients | % | *Spices mix | % |
|--------------------|----------|--------------------|----------|
| Fish mince | 65.0 | Black Pepper | 5.0 |
| Boiled Potato | 18.0 | Garlic Powder | 20.0 |
| Table Salt | 2.0 | Onion Powder | 20.0 |
| Vegetable Oil | 8.0 | Ginger | 15.0 |
| Rusk | 5.0 | Red Pepper | 5.0 |
| Spices mix | 2.0 | | |

*from: El-Sherif *et al.*, 2012

Table 2. Ingredients and their percentages used for the batter formulation for burger and kofta.

| Ingredients | % |
|--|----------|
| Wheat Flour | 34.0 |
| Table Salt | 1.0 |
| Spices (green pepper, ginger, garlic, onion) | 1.0 |
| Egg | 20.0 |
| Water | 44.0 |

Preparation of the fish kofta

The second batch of fish mince, boiled potato and other ingredients were well mixed (**Table 3**), and then rusk was added to the minced mixture. Then it was shaped manually and dipped in a batter formation as in a burger (**Table 2**). Samples from each formula took place once after (0, 2, and 4) months under frozen storage at -20°C.

Table (3): Recipe of basa kofta*

| Ingredients | % | Spices mix | % |
|--------------------|----------|-------------------|----------|
| Fish minced | 72.0 | Black Pepper | 1.0 |
| Boiled potato | 18.0 | Cinnamon | 1.0 |
| Rusk | 2.5 | Cumin | 1.0 |
| Salt | 2.0 | Laurel Leaves | 0.50 |
| Sugar | 2.5 | Cardamon | 0.50 |
| Garlic and Onion | 1.5 | Thyme | 0.50 |
| Spices mix | 1.5 | Cloves | 0.50 |
| | | Cubeb | 0.50 |
| | | Red Pepper | 0.50 |

*from: El-Sherif *et al.*, 2012

Analytical methods

Proximate analysis

Proximate chemical analysis of all ingredients was analyzed for dry matter total solids (DM), crude protein (CP), ether extract (fat) and ash content according to **AOAC (1995)**. The total carbohydrates (CHO) were calculated by subtracting the summation percentages of CP, EE and ash from total solids.

Determination of oxidative stability indices

Peroxide value (PV)

The PV was determined according to the **AOAC (1995)**. Two g from the tested samples were weighed in a flask with a ground-glass cap. 250–300 ml, 10 ml of chloroform were added and shaken for 10 min, 15 ml of glacial acetic acid, and 2 g of sodium bicarbonate NaHCO₃ were added, after stirring, 1 ml of a saturated solution of KI was added and shaken for 1 min and kept in dark place for 5 min, immediately. After the specified time, 75 ml of distilled water, then 0.5 ml of starch solution were added, the resulting solution was titrated with a solution of Na₂S₂O₃ (0.01 N) to the disappearance of the blue color, and the blank was carried on the same procedures but without sample. A peroxide value of the tested oil is given by the equation:

$$PV [\text{meq.O}_2/\text{kg}] = [(V_1 - V_2) \times N \times 1000] / W$$

Where V₁ volume of sodium thiosulfate solution consumed in the titration of the sample (ml), V₂ volume of sodium thiosulfate consumed in the titration of the blank (ml), N the normality of sodium thiosulfate, W weight of fat taken to denote (g).

Thiobarbituric Acid reactive substances (TBAR) assay

Oxidation products (e.g., malonaldehyde) were analyzed spectrophotometrically using a TBA test (**Hekmat, and McMahon, 1997**). One gram of sample was weighed into a glass screw-top test tube, and 9 ml of 15% (wt/vol) TBA, 0.375% (wt/vol) 4,6-dihydroxypyrimidine-2-thiol, and 0.25N HCl solution were added,

mixed well, and heated in a boiling water bath for 15 min. Samples were then cooled to room temperature (20°C) and centrifuged at 7000 × g for 15 min at 20°C; absorbance was measured at 535 nm.

Acid value (AV)

The method used was adapted from **AOAC (1995)**. A mixture of absolute ethanol and diethyl ether (1:1 v/v) was carefully neutralized with 0.10 N potassium hydroxide solution using 1% phenolphthalein indicator. About 5 g of the tested samples were dissolved in 50 ml neutralized ethanol-diethyl ether solvent and titrated with 0.10 M potassium hydroxide with constant shaking until a pink color persisted for 15 s. The acid value of the tested samples is given by the equation:

$$\text{Acid value (mg/g)} = [\text{mL of KOH} * \text{N} * 56.11] / \text{mass of sample}$$

Where N is the normality of KOH.

Measurement of pH value

Five grams of each of the prepared samples were homogenized with 20 ml distilled water for 10- 15 seconds. The pH was measured using a pH meter (Suntex-Ts-1) with a probe type -P combined electrode (Inglod) as the method described by **Abdel-Naeem et al. (2021)**, where 3 readings were obtained and the mean was recorded.

Physical properties:

Cooking Loss

The weight of each sample was measured before and after cooking using the method of **Orozvari and Tornberg (2004)** with some modifications. Each kind of sample was cooked for 30 min on aluminium foil at 150°C. After cooking, the samples were allowed to cool for 30 min. The percentage of cooking loss was determined for each sample by the following equation:

$$\% \text{ Cooking Loss} = \frac{W_b - W_a}{W_b} \times 100$$

W_b=Weight of the raw sample, W_a=Weight of the cooked sample.

Diameter shrinkage

The percentage of shrinkage in diameter during cooking was determined by measuring the diameter of the burger at six points. The average was then calculated by the following equation (**Orozvari and Tornberg, 2004**),

$$\% \text{ Diameter shrinkage} = \frac{D_b - D_a}{D_b} \times 100$$

D_b=diameter before cooking, D_a=diameter after cooking.

Water holding capacity (WHC)

Triplicate samples (about 62 and 32gm) from the burger and kofta samples were used. Each sample was placed on humidified filter paper (Whatman No.4 in desiccators over saturated KCl solution) and pressed between two Plexiglas plates for 1 minute at 25 kg/cm² load. The meat filter area was traced with a ball pen and the filter paper was allowed to dry. Meat and moisture areas were measured with a compensating planimeter. The resulting area covered by the meat was divided into the moisture area to give a ratio expressed as the water holding capacity of meat. A large ratio indicates an increase in the watery condition of the muscle or a decrease in the water-holding capacity (**Orozvari and Tornberg, 2004**).

$$\% \text{ WHC} = \frac{\text{AM-8.4B}}{M} \times 100$$

Where,

A= percentage of moisture content in sample.

M= sample weight (mg).

B= wet area of filter paper.

Objective color measurement

The color attributes of fish samples were measured using a spectrophotometer with the CIE color scale (Hunter, Lab scan XE) according to Commission Internationale de l'Eclairage (CIE) (1976). This instrument was standardized against the white tile of Hunter Lab color standard (LX No.16379): X= 77.26, Y= 81.94, and Z= 88.14. The L*, a*, and b* values were reported. Total color difference (ΔE) was calculated as: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$

Sensory Evaluation

Sensory evaluation was presented to an eight-member panel on an 8-point scale, 8 being extremely desirable and 1 being extremely undesirable (Chytiri, *et al.*, 2004).

Microbiological tests

Representative samples (10 g) were taken and homogenized in 90 ml of 0.9% NaCl for 30 s. Serial 10-fold dilutions were prepared in saline tubes, and 1 ml of solution was used for microbial counting. Microbial enumeration was performed using the pour plate method according to FDA (2002).

Coliforms and *Escherichia coli*

Coliforms and *Escherichia coli* were enumerated using violet red bile agar medium method recommended by the FDA (Feng, *et al.*, 2002). Exactly, 1 mL aliquot of each dilution was transferred to Petri

dishes, and the medium was poured, followed by 2nd agar layer after solidification. Plates were incubated at 18-24 h at 35°C. Purple-red colonies that are 0.5 mm or larger in diameter and surrounded by a zone of precipitated bile acids were considered typical colonies. To perform the completed test for *E. coli*, carefully remove typical colonies and streak for isolation on a L-EMB agar plate and incubate for 18-24 h at 35°C ± 0.5°C. Examine plates for suspicious *E. coli* colonies, i.e., dark centered and flat, with or without metallic sheen. Transfer up to 5 suspicious colonies from each L-EMB plate to PCA slants, incubate them for 18-24 h at 35°C ± 0.5°C and use for further testing. Further biochemical behavior was confirmed by Catalase (+) and H₂S (-), IMViC test, and microscopic examination (short rods, gram negative, non-spore forming).

Detection of Salmonella

Salmonella detection was performed according to **FDA (2002)**. As 25 g of properly homogenized samples were added to 225 ml of sterile buffered peptone water for pre-enrichment. After incubation at 37°C/24 h, 10 ml of growth suspension was transferred to 90 ml boiling sterilized Selenite broth supplemented with 4 g l-1 sodium biselenite (Oxoid) and incubated at 37°C/24 h. After incubation, XLD plates were streaked from selenite broth (**McCarthy, 1966**). Colonies were detected as Salmonella when appeared red with black centres, or Shigella when appeared reddish. The presence of Salmonella was confirmed by recording Catalase-positive; urease-negative; gram-negative short rods. Further confirmation was made through Triple Sugar Iron test. The positive isolate showed Glucose fermentation (yellow button), and H₂S positive (Blackening), but negative gas production.

Staphylococci count

Staphylococci count was examined according to **Ollis et al. (1995)**, Baird-Parker agar (Oxoid). Exactly 0.1 ml of sample dilution was inoculated on the surface of the Baird-Parker agar plate using a sterile glass spreader. After incubation at 37°C for 24 hours, colonies

characterized by a grey-black color with shiny appearance, were enumerated as *Staphylococcus* sp. Typical colonies were further confirmed by the presence of coagulase positive; Catalase positive; and Gram-positive clustered cocci (FDA, 2002).

Statistical Analysis

All experiments were repeated three times. Conventional statistical methods were used to calculate means and standard deviations. Data analysis was performed based on Analysis of Variance (ANOVA). Significant differences were ascertained using Duncan's Multiple Range Test ($p > 0.05$). All statistical analyses were conducted by SPSS statistical package (SPSS 15, SPSS Inc., Chicago, IL, USA). Comparison between two kinds of basa fish products was performed by the student's test t method.

RESULTS AND DISCUSSIONS

Proximate analysis

Proximate compositions of treated basa fish fillet, control and treated fish meat products are given in **Table (4)**. Although significant differences were found ($P < 0.05$) in the proximate compositions among different samples, this could be due to the results of sample-to-sample differences. Moisture content ranged between 68.77% (CK) to 70.22% (CB). There were significant differences between CK and CB treatments but there were no significant differences among TK, CB and TB treatment. Overall, the moisture content in fish kofta and burger in all samples compared with minced fillet was decreased; this might have resulted from the release of water-drip during cooking (Ejaz *et al.*, 2009). The lowest value of protein content was in CB treatment (7.53%). On the other hand, there were no differences among CK, TK and TB treatments. Overall, the protein content in fish kofta and burger on all samples compared with minced fillet was reduced might be due to excessive heat generated during cooking that denatured the protein and/or burned it (Ejaz *et al.*, 2009). TK treatment had the lowest value in fat content (2.83%), while the CK

treatment had the highest value. Ash content ranged between 5.55% (TK) to 7.00% (CB). The higher ash content of fish kofta and burger was due to the use of additives. The remaining percentages of the total proximate analyses are thought to be due to carbohydrates. In general, fish are known to have low amounts of carbohydrates in their muscle. However, the higher amount of carbohydrates in burgers might be derived from coating materials which contain carbohydrate-rich ingredients, such as flour and sugar (Parvizi and Moosavi-Nasab, 2020).

Table (4):Chemical Composition of basa fish fillet, control and treatment kofta and burger*

| Sample | Moisture | Protein | Fat | Ash | Carbohydrate |
|------------------|--------------------------|--------------------------|--------------------------|-------------------------|--------------------|
| Basa fish fillet | 86.86±0.16 ^a | 11.39±0.17 ^a | 1.03±0.03 ^{de} | 0.70± 0.10 ^c | 0.00 |
| CK | 68.77±0.21 ^b | 7.81± 0.10 ^a | 3.01± 0.11 ^a | 6.88± 0.11 ^a | 13.53 ^b |
| TK | 69.46±0.15 ^{ab} | 7.67± 0.22 ^{ab} | 2.83± 0.05 ^c | 5.44± 0.23 ^b | 14.60 ^a |
| CB | 70.22± 0.18 ^a | 7.53± 0.30 ^b | 2.98± 0.05 ^{ab} | 7.00± 0.26 ^a | 12.27 ^c |
| TB | 70.00±0.24 ^{ab} | 7.80± 0.11 ^a | 2.88± 0.02 ^{bc} | 5.57± 0.21 ^b | 13.75 ^b |

CK: Control Kofta, **TK:** Treatment Kofta, **CB:** Control Burger, **TB:** Treatment Burger. *Means in a column did not share the same superscript and were significantly different at P<0.05

Lipid oxidation is another important factor indicating spoilage in frozen fish and meat products (Khoshnoudi-Nia and Moosavi-Nasab, 2019a). Table(5) showed the oxidative stability indices on fish kofta and burger products during a storage period of four months at -20° C. Overall, there was a slight and statistically insignificant rise in the acid value parameter observed for all controlled and treated samples throughout the four months of storage, but the values of treated kofta and burger products were lower than the controlled ones.

Table (5): Effect of frozen storage on oxidative stability of basa fish kofta and burger*

| Samples | Storage Periods (months) | Oxidative Stability indices | | | |
|---------|--------------------------|-----------------------------|--|--------------------------|-----------------------|
| | | Acid Value (mg KOH/ g) | Peroxide Value (meq.O ₂ / kg) | TBA (A551nm) | pH |
| CK | 0 | 0.925±0.050 ^f | 0.215±0.022 ^h | 0.455±0.010 ^e | 5.85±0.0 ^d |
| | 2 | 1.625±0.033 ^c | 0.413±0.015 ^f | 0.667±0.033 ^d | 6.00±0.0 ^c |
| | 4 | 2.088±0.050 ^a | 0.597±0.022 ^e | 0.835±0.030 ^c | 6.12±0.0 ^c |
| TK | 0 | 0.766±0.033 ^g | 0.115±0.025 ⁱ | 0.333±0.014 ^f | 6.55±0.0 ^b |
| | 2 | 1.285±0.040 ^e | 0.305±0.033 ^g | 0.515±0.021 ^e | 6.67±0.0 ^b |
| | 4 | 1.775±0.040 ^b | 0.455±0.022 ^f | 0.715±0.033 ^d | 6.67±0.0 ^b |
| CB | 0 | 0.515±0.043 ^h | 0.820±0.024 ^c | 0.515±0.013 ^e | 6.80±0.0 ^a |
| | 2 | 0.955±0.040 ^f | 1.225±0.024 ^b | 0.860±0.033 ^c | 6.80±0.0 ^a |
| | 4 | 1.725±0.023 ^b | 1.515±0.024 ^a | 1.163±0.040 ^a | 6.68±0.0 ^b |
| TB | 0 | 0.445±0.025 ^h | 0.333±0.013 ^g | 0.325±0.021 ^f | 6.80±0.0 ^a |
| | 2 | 0.775±0.033 ^g | 0.475±0.030 ^f | 0.628±0.021 ^d | 6.80±0.0 ^a |
| | 4 | 1.400±0.020 ^d | 0.728±0.033 ^d | 1.005±0.035 ^b | 6.87±0.0 ^a |

CK: Control Kofta, TK: Treatment Kofta, CB: Control Burger, TB: Treatment Burger, *Means in a column did not share the same superscript and were significantly different at P<0.05.

For concentrations of primary oxidation products (PV) during 120 days of frozen storage are presented in **Table (5)** PV showed an increasing trend (p<0.05). However, there were significant differences among all samples during frozen storage Furthermore, PV of treated fish kofta and burger were lower than that of control kofta and burger samples(p<0.05). The increasing trend of PV value during storage time could be due to an increase in free heme and/or other prooxidants in myofibrils of fish muscle after death (**Khoshnoudi-Nia and Moosavi-Nasab, 2019b**). In agreement with our finding, **Bavitha et**

al. (2016) on fish burgers produced from catla (Catlacatla) showed that PV increased to 4.98meq.O₂/kg fat after 17 days of storage at 4±1°C. In this study, the PV values of all products were in the standard range during storage. Therefore, a rancid odor did not develop in the fish burger and kofta during 120 days of frozen storage and desired quality was maintained.

Thiobarbituric acid (TBA) is a widely used indicator for the assessment of the degree of lipid oxidation (Ojagh *et al.*, 2010). The consumability limit value of the TBA content was between 7 and 8 mg MDA kg⁻¹ In food suitable for consumption, the TBA values might reach the upper limit of 7 to 8 mg of MDA kg⁻¹; in “perfect material,” the TBA value should be less than 3 mg of MDA/kg, and in “good material,” the TBA value should be no more than 5 mg of MDA kg⁻¹. The TBA values indicate the degree of rancidity of products, and values greater than 3-4 mg of MDA kg⁻¹ indicate a loss of product quality (Frangos, *et al.*, 2010). The TBARS values of the basa fish kofta and burger products were observed in **Table (5)**. TBARS values increased in the duration of storage time in all samples. The lowest TBARS values were obtained from the treated samples. These results showed the antioxidant characteristics of essential oils. A similar pattern of the increase in TBARS has been reported in rainbow trout (Can and Ersan, 2013). Also, the results showed that the CB sample had the highest TBA value during all storage periods. However, it is worth noting that despite the difference in the values recorded for all samples, in general, until the end of the frozen storage period, no sample exceeded the value of 1 MDA kg⁻¹, which indicates the high quality of the products, especially the treated ones.

The pH of fresh fish flesh is approximately neutral in the post-mortem period, the decomposition of nitrogenous compounds leads to an increase in the pH of the fish flesh, which indicates a loss of quality. The pH values of the samples (**Table 5**) ranged between 5.85 for CK and 6.80 for CB and TB at zero storage time, while ranged between 6.12 for CK and 6.87 for TB at the end of the storage period. pH values of marinated samples decrease after the marination process. Marinades have a low pH due to acetic acid content. During the

storage of marinades ,heterofermentative lactic acid bacteria can grow and cause the amino acids to degrade. Thus, the formation of carbon dioxide and other decarboxylation products is observed. Due to these degradation products the pH of the marinade increases (Topuz *et al.*, 2014). Similar findings were reported by other researchers. It is not hidden that the plant sources used in the preparation of products as fillers and dipping materials all limit the increase in the pH number, as well as the very low temperature during freezing preservation, and all of them limit the acid taste of the products.

Table (6) Effect of frozen storage periods on physical properties of fish kofta and burger basa fillet*

| Samples | Storage Periods (months) | Physical properties | | |
|---------|--------------------------|----------------------------|---------------------------|---------------------------|
| | | WHC (%) | Shrinkage (%) | Cooking loss (%) |
| CK | 0 | 70.167±0.205 ^a | 7.653±0.055 ^c | 17.875±0.125 ^d |
| | 2 | 70.188±0.333 ^a | 10.414±0.071 ^b | 20.054±0.086 ^c |
| | 4 | 70.215±0.425 ^a | 16.162±0.035 ^a | 23.348±0.334 ^a |
| TK | 0 | 69.945±0.117 ^a | 5.128±0.005 ^d | 8.345±0.550 ⁱ |
| | 2 | 69.966±0.188 ^a | 5.405±0.015 ^d | 12.111±0.258 ^g |
| | 4 | 69.988±0.221 ^a | 5.908±0.019 ^d | 16.129±0.334 ^e |
| CB | 0 | 68.733±0.120 ^b | 2.564±0.011 ^f | 10.155±0.550 ^h |
| | 2 | 68.805±0.127 ^b | 7.333±0.035 ^c | 15.244±0.322 ^f |
| | 4 | 68.760±0.205 ^b | 10.216±0.054 ^b | 21.338±0.567 ^b |
| TB | 0 | 69.426±0.133 ^{ab} | 2.564±0.015 ^f | 6.451±0.095 ^j |
| | 2 | 69.420±0.167 ^{ab} | 4.005±0.027 ^e | 10.644±0.115 ^h |
| | 4 | 69.447±0.133 ^{ab} | 5.504±0.009 ^d | 15.838±0.412 ^c |

CK: Control Kofta, **TK:** Treatment Kofta, **CB:** Control Burger, **TB:** treatment Burger. *Means in a column did not share the same superscript and were significantly different at $P < 0.05$.

Table (6) presented the percentages of physical properties (WHC, Shrinkage, and Cooking Loss) for CK, TK, CB, and TB samples during a storage period of four months at -20°C . Overall, there was a slight and statistically insignificant rise in WHC percentages observed for both CK and TK throughout the four months of storage. The WHC percentages of the CB sample were consistently lower than those of the TB sample. The WHC for CB ranged between 68.733% and 68.760%, while for TB, it ranged from 69.426% to 69.447%.

According to the shrinkage properties of all samples, there was a significant increase observed over the storage periods. The CK sample exhibited a varying rate of increase in shrinkage, ranging from 7.653% to 16.162%, while the TK sample showed a comparatively lower rate ranging from 5.128% to 5.908% over the four months. On the other hand, during the four months of storage, the TB sample displayed a lower increase in shrinkage percentage compared to the CB sample. The increase in shrinkage for TB was 2.49%, whereas for CB, it was higher at 7.652%. This suggests that the TB sample experienced a slower rate of shrinkage compared to the CB sample over the storage duration. Overall, shrinkage percentages were lower in control and treated burger products than the control and treated kofta products over the storage periods because there is a positive relationship between fat content and cooking loss in burgers after cooking (**Ueda et al., 2007**). These results agreed with **Serdaroglu and Degirmencioglu (2004)** who reported that fat content affects hamburger patty shrinkage and reducing fat content from 20% to 5% significantly decreases the shrinkage. In addition, they showed that meat balls up shrinking during the cooking process, due to the denaturation of meat proteins which lose water and fat contributing to the shrinkage process.

Regarding the cooking loss percentages, all samples (CK, TK, CB, and TB) showed a significant increase from 0 to four months of storage. In general, the rate of increase in cooking loss percentages

from 0 to 4 months was lower for the CK sample (5.473%) compared to the TK sample (7.784%). while the CB sample exhibited a higher rate of increase in cooking loss (11.183%) compared to the TB sample (9.387%) over the same four-month period. Moreover, storage time has a significant effect on cooking loss ($p < 0.05$). Freshly produced fish products (0th month) showed low cooking loss which increased after 4 months of frozen storage. Overall, the cooking loss percentages were lower in the control and treated burger products than in the control and treated kofta products over the storage periods. This can be due to Keeping fat in the matrix of meat products during processing is necessary for ensuring sensory quality and acceptability. There is a positive relationship between fat content and cooking loss in burgers after cooking. During heating burgers firstly, the fat melts and then collagen, which is a major part of the connective tissue, pressing fat out of the cell . These results agreed with (**Parvizi and Moosavi-Nasab, 2020**) who stated that the cooking loss of fish burger was significantly lower than that of beef burger. Moreover, storage time has a significant effect on cooking loss ($p < 0.05$). Freshly produced fish and beef burgers (0th day) showed low cooking loss values in (8.00 ± 1.00 and $16.5 \pm 0.5\%$) respectively, which increased gradually to 13.23 ± 0.25 and 24.77 ± 0.25 after 3 months of frozen storage.

Changes in L^* (lightness), a^* (redness), and b^* (yellowness) values of the fish basa fillet products during frozen storage at -20°C for 4 months are depicted in **Table (7)**. (L^*) values of the four fish product groups were 57.73 (CB) and 65.40 (TB) at the beginning and at the end of the storage period, respectively. Significant differences ($P < 0.05$) were determined among the different fish meat products and storage periods. (L^*) parameter showed slight changes with storage periods, with a trend towards an increase in values as the storage period increased. Notably, TB displayed the highest value among all products. Regarding the (a^*) parameter, there were a significant differences ($P < 0.05$) among the different fish meat products and storage periods. TB exhibited the lowest values across all storage periods, followed closely by CB and TK. While CK was recorded the highest value at the end of the storage period. There were a significant differences ($P < 0.05$) among the different fish meat products and

storage periods of the b^* except, the value of CK treatment remained relatively consistent across all storage periods (20.98 to 21.10), denoted by the absence of significant variation ($p > 0.05$). On the other hand, the CB recorded as the highest value of b^* for four products at the end of the storage period. Moreover, storage time has a significant effect on objective color measurements whereas, all control and treated products were increased gradually during the storage periods especially, kofta products.

Table (7) Effect of frozen storage periods on objective color measurements of basa fish kofta and burger*

| Samples | Storage Periods (months) | Hunter Color | | | | |
|---------|--------------------------|--------------------------|-------------------------|-------------------------|-------------|-------------|
| | | L^* | a^* | b^* | $\Delta E1$ | $\Delta E2$ |
| CK | 0 | 58.73±0.78 ^c | 8.49±0.35 ^c | 20.98±0.42 ^b | 0.00 | 0.00 |
| | 2 | 61.07±0.55 ^b | 9.05±0.45 ^b | 21.10±0.33 ^b | 2.41 | 2.41 |
| | 4 | 63.40±0.35 ^{ab} | 9.70±0.53 ^a | 21.10±0.42 ^b | 4.84 | 4.84 |
| TK | 0 | 60.97±0.54 ^c | 7.98±0.27 ^e | 18.53±0.30 ^d | 0.00 | 3.06 |
| | 2 | 62.55±0.33 ^{bc} | 8.06±0.57 ^d | 20.00±0.43 ^c | 4.78 | 3.97 |
| | 4 | 65.01±0.29 ^a | 8.28±0.32 ^c | 21.03±0.30 ^b | 5.23 | 2.52 |
| CB | 0 | 57.73±1.52 ^c | 7.36±0.22 ^e | 18.58±0.37 ^d | 0.00 | 0.00 |
| | 2 | 60.55±0.62 ^{bc} | 7.84±0.42 ^d | 19.75±0.45 ^c | 3.09 | 3.09 |
| | 4 | 64.33±0.44 ^{ab} | 8.39±0.29 ^c | 21.95±0.28 ^a | 4.73 | 4.73 |
| TB | 0 | 58.29±0.23 ^e | 7.03±0.15 ^e | 16.32±0.31 ^e | 0.00 | 2.85 |
| | 2 | 62.35±0.41 ^{bc} | 7.18±0.33 ^{ee} | 18.07±0.40 ^d | 4.42 | 4.49 |
| | 4 | 65.40±0.50 ^a | 7.23±0.10 ^e | 20.01±0.33 ^c | 8.02 | 7.80 |

CK: Control Kofta, **TK:** Treatment Kofta, **CB:** Control Burger, **TB:** Treatment Burger, *Means in a column did not share the same superscript and were significantly different at $P < 0.05$.

All samples became less red due to the reduced freshness and greyer appearance compared to day 0. An increase in L^* value

indicated the development of lipid oxidation during storage time, leading to a brighter fillet. These results were in agreement with (Ozyurt *et al.*, 2015) who showed that frozen fish fillets soaked with citric acid solution demonstrated effectively increased whiteness (L^*) and yellowness (b^*) compared to the other samples during storage.

Table (8) Effect of frozen storage periods on sensory evaluation of basa fish kofta and burger*

| Sensory Evaluation (out of 10) | | | | | | | |
|--------------------------------|--------------------------|-------------------------|--------------------------|------------------------|-------------------------|-------------------------|-------------------------|
| Samples | Storage Periods (months) | Color | Odor | Taste | Texture | Tenderness | Overall acceptability |
| CK | 0 | 8.06±0.30 ^c | 8.44±0.16 ^{bc} | 8.06±0.21 ^c | 8.36±0.24 ^{bc} | 8.24±0.33 ^c | 8.44±0.24 ^b |
| | 2 | 8.14±0.20 ^c | 8.18±0.33 ^d | 8.14±0.30 ^c | 8.48±0.24 ^b | 8.20±0.33 ^c | 8.38±0.14 ^b |
| | 4 | 8.09±0.30 ^c | 8.27±0.17 ^d | 8.09±0.32 ^c | 8.37±0.20 ^{bc} | 8.36±0.17 ^c | 8.40±0.24 ^b |
| TK | 0 | 8.58±0.21 ^{ab} | 8.56±0.15 ^{bc} | 8.52±0.28 ^b | 8.56±0.24 ^{ab} | 8.54±0.26 ^b | 8.60±0.30 ^b |
| | 2 | 8.44±0.25 ^b | 8.70±0.21 ^{ab} | 8.42±0.32 ^b | 8.53±0.20 ^{ab} | 8.45±0.21 ^{bc} | 8.55±0.18 ^{ab} |
| | 4 | 8.41±0.28 ^b | 8.82±0.21 ^a | 8.46±0.25 ^b | 8.44±0.24 ^b | 8.47±0.21 ^{bc} | 8.48±0.20 ^b |
| CB | 0 | 7.88±0.22 ^b | 8.39±0.25 ^c | 8.86±0.27 ^a | 8.17±0.31 ^a | 8.33±0.15 ^c | 8.70±0.22 ^a |
| | 2 | 8.02±0.22 ^c | 8.48±0.34 ^{ac} | 8.32±0.19 ^b | 8.46±0.22 ^{ab} | 8.46±0.25 ^{bc} | 8.55±0.25 ^{ab} |
| | 4 | 8.14±0.30 ^c | 8.52±0.30 ^{bc} | 8.36±0.12 ^b | 8.40±0.31 ^{ab} | 8.52±0.15 ^b | 8.38±0.25 ^b |
| TB | 0 | 8.58±0.33 ^{ab} | 8.64±0.29 ^{abc} | 8.78±0.21 ^a | 8.52±0.19 ^{ab} | 8.66±0.27 ^{ab} | 8.57±0.27 ^{ab} |
| | 2 | 8.62±0.24 ^a | 8.00±0.18 ^b | 8.69±0.19 ^a | 8.58±0.24 ^{ab} | 8.73±0.20 ^a | 8.64±0.21 ^a |
| | 4 | 8.71±0.33 ^a | 8.79±0.29 ^a | 8.44±0.21 ^b | 8.70±0.22 ^a | 8.81±0.20 ^a | 8.75±0.27 ^a |

CK: Control Kofta, **TK:** Treatment Kofta, **CB:** Control Burger, **TB:** Treatment Burger. *Means in a column did not share the same superscript and were significantly different at $P < 0.05$.

The data presented in **Table (8)** Investigate the effects of four fish meat products (CK, TK, CB, and TB) and three storage periods of 0, 2, and 4 months on various sensory properties, including color, odor, taste, texture, tenderness, and overall acceptability. The data indicated variations in sensory properties among the different fish meat products and storage periods. For color parameters, the scores of CK treatment

remained relatively consistent across all storage periods (8.06 to 8.14), denoted by the absence of significant variation ($p > 0.05$). However, for TK, CB, and TB, the color scores showed slight changes with storage periods, with a trend towards a decline in scores as the storage period increased. Notably, TB displayed the highest color scores among all products, suggesting its superior color retention during storage.

Regarding the odor parameter, the scores for all fish meat products generally remained stable during storage, with no significant differences observed ($p > 0.05$). However, TK exhibited the highest odor scores across all storage periods, followed closely by TB. On the other hand, CB demonstrated comparatively lower odor scores, especially during the initial storage period. The taste scores for all products were relatively consistent during the storage period of 0 and 2 months. However, when stored for 4 months, slight variations were observed. TK consistently achieved the highest taste scores, while CB and TB showed comparable taste scores, and CK displayed slightly lower scores.

The texture scores for CK, TK, and TB remained relatively stable across different storage periods. However, CB displayed a decline in texture scores as the storage period increased. Notably, CK showed the highest texture scores among all products. For tenderness of the fish meat products, no significant variations were observed for CK, TK, and TB during the storage period. However, CB displayed a decline in tenderness scores with prolonged storage. TK consistently exhibited the highest tenderness scores. The overall acceptability scores were generally high for all fish meat products. TK consistently received the highest overall acceptability scores across all storage periods. TB also demonstrated favorable overall acceptability, while CB showed slightly lower scores. CK maintained good overall acceptability throughout the storage period. In general, the results of changes in the scoring of sensory properties in fish burgers showed that the difference between 0 and 120 days of frozen storage was not significant ($p > 0.05$). Furthermore, there was no significant difference between the scoring of color, odor, texture, flavor, and overall

acceptability properties of fish burger during frozen storage ($p > 0.05$) (Parvizi and Moosavi-Nasab, 2020). These results were in disagreement with Hsieh *et al.* (2001) who stated the changes in the sensory attributes of control and treated fish fillets samples during refrigerated storage at $2 \pm 1^\circ\text{C}$. The average scores of sensory attributes (overall acceptability) provided by the panelists at day zero were excellent (20 ± 0.0) for control (C) and thyme-treated (T) samples, while it was very good (18.8 ± 0.06) for rosemary-treated (R) samples. Low scores given to the (R) group could be attributed to yellow discoloration and rosemary odour observed in fillet samples.

Table (9) Effect of frozen storage periods on microbiological properties of basa fish kofta and burger*

| Samples | Storage Periods (months) | Coliforms Count | <i>Escherichia coli</i> | <i>Salmonella spp</i> | Coagulase (positive) Staphylococci |
|---------|--------------------------|-----------------|-------------------------|-----------------------|------------------------------------|
| CK | 0 | Nil | Nil | Negative | Nil |
| | 2 | Nil | Nil | Negative | Nil |
| | 4 | Nil | Nil | Negative | Nil |
| TK | 0 | Nil | Nil | Negative | Nil |
| | 2 | Nil | Nil | Negative | Nil |
| | 4 | Nil | Nil | Negative | Nil |
| CB | 0 | Nil | Nil | Negative | Nil |
| | 2 | Nil | Nil | Negative | Nil |
| | 4 | Nil | Nil | Negative | Nil |
| TB | 0 | Nil | Nil | Negative | Nil |
| | 2 | Nil | Nil | Negative | Nil |
| | 4 | Nil | Nil | Negative | Nil |

CK: Control Kofta, **TK:** Treatment Kofta, **CB:** Control Burger **TB:** Treatment Burger. *Means in a column did not share the same superscript and were significantly different at $P < 0.05$.

Table (9) summarized the results of microbial counts (Log₁₀ CFU/g) of kofta and burger basa fillet fish marinated in (0.1% pepsin

enzyme + 0.5 rosemary+0.5% citric acid) during frozen storage at -20°C for 4 months. The results indicated that all control and treated samples were completely free of Coliforms Count, Escherichia coli, salmonella, and positive staphylococci groups and there were no significant differences among the control and treated samples during all frozen storage. The antimicrobial effect could not be attributed to one or a few active components, because it was significantly affected by the presence of a mixture of different chemical compounds. A number of EOs and some of their components have been reported to have antimicrobial activity against a wide range of spoilage and pathogenic bacteria Thyme contains high concentrations of phenolic compounds including carvacrol, thymol, p-cymene and γ -terpinene (**Komaki *et al.* 2015**). The thyme and rosemary oils can be considered effectively inhibitory on the total aerobic flora. Similar results were observed by several researchers (**Can and Ersan, 2013**).

CONCLUSION

The study was carried out on Basa fish fillets, the fish that the Egyptian consumer has not accepted to eat significantly until now. The study consisted of treating fillets with a mixture of rosemary essential oils extract, citric acid and pepsin enzyme, followed by preparing a fish burger and kofta of it. The chemical, physical, organoleptic and microbiological results showed that these products can be prepared high quality from basa fish fillets and can be preserved frozen without affecting their quality parameters. This is considered a good start for the use of basa fish in the Egyptian market.

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الملخص العربي

تحضير برجر السمك والكفتة من فيليه سمك الباسا وتقييم الجودة والصلاحية أثناء ظروف التخزين المجمد

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تعتبر الأسماك من أهم الأغذية المرغوبة حول العالم؛ نظرًا لقيمتها الغذائية العالية، وصفاتها الحسية الجيدة، والتنوع الكبير في طرق إعدادها وتقديمها. تعتبر سمكة الباسا غير شائعة في السوق المصري، وغير معروفة لشريحة كبيرة من المستهلكين. استهدف هذا البحث إنتاج برجر السمك والكفتة من شرائح سمك الباسا المعالج بخليط مستخلص زيوت إكليل الجبل وحمض الستريك وإنزيم الببسين، ومن ثم تقييم جودة المنتجات المصنعة والمخزنة مجمدة على -20°م، ولمدة 4 شهور. وقد أظهرت النتائج وجود فروق معنوية في التركيب الكيماوي بين فيليه الباسا المعالج وبين البرجر والكفتة المصنعتين سواء المعالجة أو غير المعالجة، حيث قلل التصنيع كلاً من المحتوى الرطوبي، والبروتين، والدهن، بينما زاد محتوى الكربوهيدرات والرماد. سُجّلت زيادة ملحوظة في قيمة البيروكسيد، TBA مع تقدّم فترة التخزين، وخصوصًا للمنتجات غير المعالجة إلا أن هذه الزيادات كانت في الحدود المسموح بها. على الرغم من عدم وجود فروق معنوية بين قيم قدرة مسك الماء طول فترة التخزين فقد سُجّلت زيادة ملحوظة في قيم فقد الطهو، والانكماش بزيادة فترة التخزين، وقد كانت هذه الزيادة كانت بمعدل أقل في البرجر المعالج وغير المعالج مقارنة بالكفتة المعالجة وغير المعالجة. تأثرت خواص اللون الفيزيائية معنويًا بالتخزين المجمد، بينما فقدت جميع المنتجات مقبولة حسيًا في كل فترات التخزين وحتى نهاية التجربة (120 يوم). أوضحت نتائج التحليلات الميكروبيولوجية أن جميع المنتجات المعالجة وغير المعالجة كانت خالية تمامًا من *Salmonella spp* و *Escherichia coli* و *Coliforms Count* و *Coagulase (positive) Staphylococci* ولم تسجّل أية اختلافات معنوية بين المنتجات المعالجة وغير المعالجة طوال فترة التخزين. في ضوء هذه النتائج يتضح أنه يمكن تحضير منتجات ذات جودة عالية من شرائح سمك الباسا ويمكن حفظها مجمدة دون التأثير على معايير جودتها. وتعتبر هذه بداية جيدة لاستخدام سمك الباسا في السوق المصرية.