Microbial and Sensory Assessment of Sand Smelt Fish Burger and Finger during Frozen Storage

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Abstract: The current study aimed to assess the microbiological safety and sensory quality of fish burger and finger made from sand smelt fish *(Atherinahepsetus)* which was found unacceptable from consumers. The substitution material (soybean flour (SF) and minced boiled potatoes (MBP)) were used by different levels in this investigation to improve the sensory evaluation of produced fish products. Total bacterial count (TBC) values for burger and finger samples gradually declined till the day 45th then increased in the later period of frozen storage. Yeast and Molds growth were not detected during the frozen storage over the entire period of 90 days storage, except for zero time the score values of the quality attributes: color, taste, odor and texture, as well as overall acceptability of sand smelt fish burgers and fingers, slightly declined during frozen storage. The filling materials SF and MBP used in the production of fish products showed an observed effect in maintaining the sensory qualities of the products for 90 days of frozen storage. It can be concluded that sand smelt fish products maintained good microbial and sensory quality at the end of 90 storage period in the freezer, opening an opportunity for this good nutritious protein source to be used as a daily food.

Keywords: Microbiology, Sensory evaluation, Sand smelt, Fish burger and fish finger.

INTRODUCTION

Fish and fishery products have been recognized as very important sources for human nutrition. Fishes are characterized by their high contents of high quality, easily digestible protein and essential amino acids. Moreover, they are low in the saturated fatty acids and contain considerable amounts of the unsaturated fatty acids, especially omega-3 fatty acids, which are regarded as oxidation preventive compounds, among other health benefits. The oil-soluble vitamins are known to be present in fish muscle. Also, they are a good source of several minerals, particularly fluorine and iodine [1].

In Egypt, the total quantity of catch fish recorded 1,706,273 tones, while the total quantity of Exports and Imports of fish were 47,812 and 311,068 tones, respectively. Annual average share per capita of fish was 21.64kg [2]. Sand smelt (*Atherinaboyeri*) is a common species in the Mediterranean Sea and has shown distribution from north-east Atlantic to northwest coast of Scotland. Also, this species lives in the Black Sea, Aegean Sea, Marmara Sea, Aral and Caspian Sea [3]. Global changes in consumer lifestyle, marked by increasing demand for nutritional and healthy food products, have spurred the continuing rise in demand for fresh and convenient ready-to-cook fish and fishery products. This rising demand for ready to eat fish meals, instead of plane frozen fish, requires the use of preservation procedures that can add value and reduce fishery losses [4]. Freezing preservation of food has been used for thousands of years as it results in high product quality with a huge increase on shelf life, as long as frozen temperatures are kept along the delivery chain. It is a usual method to preserve commercial fish since it preserves freshness inhibiting chemical and microbiological degradation, and is an excellent method of preserving the sensory attributes of fish flesh during long periods of time [5]. Microbiological quality of fresh and frozen fish can be judged using several criteria [6]. For high quality fresh fish, the number of bacteria present in the surface varies from 3 to 4 log cfu\ g. On gills, counts are normally one or two orders higher, and intestinal counts can reach 9 log cfu/ g. [7-8] reported that the reduction in microorganisms by the TVC (Total Viable Count) during freezing may be due to the damage of bacteria cells caused by grown crystals. About 10^4–10^6 TVC/g is considered an acceptable figure in the Australian meat industry [9]. Therefore, the overall objective of this study was to:

1. Examine the microbial safety of sand smelt fish burger and finger during frozen storage at-18ºC for 90 days.

2. 2- Determine the changes in sensory evaluation of sand smelt frozen fish products stored at -18ºC for a 3 month period.

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MATERIALS AND METHODS

Fish

Fresh sand smelt (bassaria) fish *(Atherinahepsstia)* was obtained from Qarun Lake at Fayom Governorate, Egypt during April, 2017. About 25 kg of fish were transported in ice to the laboratory of Fish Research Station (at Shakshouk, Fayoum), National Institute of Oceanography and Fisheries (NIOF).

Burgers and Fish Fingers

Filling Materials

Soybean flour (SF) and Minced boiled potatoes (MBP) were used as filling materials in the processing of fish products. SF was obtained from Food Technology Research Institute, Agriculture Research Center at Giza Governorate, Egypt. MBP was prepared by boiling potatoes for 15 min, then peeled and minced.

Ingredients

The ingredients included sunflower oil, starch, wheat flour, sugar, salt, onion, garlic and spices were obtained from the local market.

Preparation of Fish

On arrival at the laboratory, fish was beheaded, gutted and washed gently with tap water. The edible portion of fish was soaked in 1% salt solution contained 0.5% acetic acid for 5 min to remove the fishery odor and taste, then drained off and minced using an electric meat mincer (Braun plus 1300). A portion of the minced fish was used in the chemical analysis and the microbial examination of the raw material (sand smelt fish). The remaining quantity was divided into two portions, kept to process fish products into burger and finger.

Fish Burger Formulation and Processing

Control fish burger (no filling material) was composed of 75g of minced fish and 25g of the ingredients mixture for 100g batter as shown in Table (**1**) which indicates the recipe of sand smelt burger as mentioned by [10]. The experimental burgers were formulated by using SF and MBP as filling materials. Each of the two filling materials was used at levels of 10, 15 and 20% of weight of the minced fish used. The experimental formulated samples were made by replacing the minced fish with the filling material at the desired level as illustrated in Table (**1**). All the formulations were needed by hand until homogeneous dough was obtained. Portions of 50g were shaped (8.5 cm diameter and 1.0 cm thickness) by manually operated forming machine (NOAW- Affetacrane, Italy). Burger samples were packed in polyethylene bags and stored at -18ºC until required for analysis.

Fish Fingers Formulation and Processing

Fish fingers were prepared as described by [11-12]. Control fish fingers (without filling material) were composed of 93.5g of minced sand smelt fish and 6.5g of the ingredients mixture as shown in Table (**2**). The experimental fingers were formulated by using the filling materials *i.e*. SF and MBP at levels of 10, 15 and 20% of the minced fish as described in burger formulation. The formulated samples were made by replacing the minced fish with the filling materials at the tested levels (Table **1**). The minced fish was mixed with the ingredients and the mixture was homogenized until smooth dough was obtained. The dough was shaped into fingers and frozen at -18C for 2h before battering. The frozen fish fingers were rapidly coated with batter solution (3 parts of water plus 2 parts contained 94% maize flour, 2% skim milk, 2% egg yolk and 2% salt) and then they were rubbed with ground crumb.

Table 1: Substitution Levels of the Filling Materials (SF and MBP) used in the Preparation of Sand Smelt Fish Burger and Fingers

Fish Burger		Fish Fingers		Substitution Level (%)
Minced Fish (g)	(SF) or (MBP)(g)	Minced fish (g)	(SF) or (MBP) (g)	
75.00	-	93.50	-	0
67.50	7.50	84.15	9.35	10
63.75	11.25	79.47	14.03	15
60.00	15.00	74.80	18.70	20

SF: Soybean flour, MBP: Minced boiled potato.

Table 2: Recipes of Sand Smelt Fish Burger and Fingers

Fish Burger		Fish Fingers	
Ingredients	%	Ingredients	%
Fish mince	75.00	Fish mince	93.50
Vegetable oil	9.00	Salt	1.50
Starch	8.00	Sugar	1.00
Salt	2.30	Wheat flour	3.00
Sodium bicarbonate	0.40	Cumin	0.24
Onion	2.50	Onion	0.24
Garlic	0.50	Garlic powder	0.24
Polyphosphate	0.30	Black pepper	0.24
*Spices mixture	2.00	Thyme	0.04

*Spices mixture composed of 32% black pepper, 22.5% coriander, 15% cumin, 10% cardamom, 9% red pepper, 7.5% cubeb and 4% clove.

Microbiological Analysis

10 g of fish sample were aseptically weighted and homogenized with 90 ml of sterile saline water for 1 min for each treatment. The homogenized samples were serially diluted using 9 ml sterile saline for bacteriological analysis. Total bacterial count (TBC), Yeasts and Molds count were examined along the 90 days storage period.

Total Viable Count (TBC)

TBC was determined by using nutrient agar medium [13].

Yeasts and Molds Count

Yeasts and molds counts were enumerated on malt agar as mentioned by [14].

Sensory Evaluation

Sensory evaluation of raw, smoked and Mullet fish and fried tilapia was performed by ten panelists chosen from the staff members of Shakshouk Research Station (NIOF). The organoleptic properties of raw, processed products were tested according the scale described by [15] as follows:

1-2 rejected	3-4 accepted	5-6 good
7-8 very good	9-10 excellent	

The characteristics of color, odor, taste, texture and overall acceptability were tested.

Statistical Analysis

The statistical analysis of the results obtained was carried out according to SPSS version 16 software program 2007. Means and standard deviation (SD) measured by L.S.D at 5% level of significant.

RESULTS AND DISCUSSION

Microbiological Quality

The microbiological changes during storage of fish and fishery products are responsible for progressive decline of organoleptic quality caused mainly by the microbial growth and metabolism [16]. Bacteria and fungi spoilage have an important role in the quality and safety of fishery products.

Sand smelt fish burgers and fingers were examined microbiologically for total bacterial count, as well as mold and yeast count, immediately after processing and during frozen storage at – 18 C for 90 days. As shown in Table (**3**) the initial total bacterial counts for control, 15% SF and 15% MBP burger samples before storage were found to be 3.25±.115, 3.71±.150 and 4±.087cfu/g, respectively. Also, the initial values of TBC for finger samples were found to be slightly higher than in burger samples and determined by 3.61±.115, 4.11±.063 and 4.44±.196cfu/g for control, 20% SF and 15% MBP finger samples, respectively. These findings indicated the microbiological good quality of the fresh samples of Sand smelt product since the value of TBC is considerably lower than the maximum limit (7 log cfu/g) of microbiological criteria for fresh fish given by the International Commission Microbiological Specification for Food [17]. These results could be attributed to the good freshness of sand smelt fish used in the production, the good quality of the ingredients and the sanitary conditions applied during preparation, processing and handling the products.

The results presented in Table (**3**) show the microbiological changes in respect of TBC of sand smelt fish burgers and fingers during frozen storage. It was observed that TBC values for burger and finger samples gradually declined till the day 45 th then increased in the later period of frozen storage. At the end of 90 days storage TBC values reported for burger and finger samples ranged from 3.89±.080 to 4.74±.080cfu/g. The increase of TBC may be due to the multiplication of microbial counts that can able to grow under freezing conditions [18]. While the reduction in TBC may be due to the damage of bacterial cells caused by grown ice crystals [19]. [20] reported that freezing generally causes a reduction in bacterial count and the number will continue, in most cases, to fall during frozen storage. Our data indicate that even after 90 days of frozen storage the total bacteria counts in sand smelt fish products did not exceed the recommended limit**.** Similar findings were reported by [12-21].

Data given in Table (**4**) show mold and yeast counts in Sand smelt fish products during frozen storage. The initial counts of mold and yeast in the different samples ranged from 2.01±.092 to 2.84±.196cfu/g at zero time storage. During the frozen storage over the entire period of 90 days no mold and yeast could be found by microbiological analysis. Similarly**,** [21] found no growth of microorganisms during frozen storage of some fish products at temperature below -18ºC.

Sensory Quality Attributes

Sensory quality attributes of fish and fishery products may undergo some changes during storage, which will considerably affect the consumer acceptability of such products. Therefore, fish products made from sand smelt fish were evaluated for their organoleptic characteristics at intervals of 15 days during frozen storage for 90 days, to assess the storage stability of such products. Burger and fingers

Table 3: Changes in Total Bacterial Count (TBC) of Sand Smelt Fish Products during Frozen Storage at – 18 ºC

Storage Period (day)	Fish Burger			Fish Finger		
	Control	SF (15%)	MBP (15%)	Control	SF (20%)	MBP (15%)
0	3.25±.115	3.71±.150	4±.087	3.61±.115	4.11±.063	4.44±.196
15	3.19±.086	3.65±.086	3.94±.115	3.54±.138	4.06±.034	4.49±.109
30	3.11±.063	3.49±.051	3.85±0.86	3.33±.190	4.00±.115	4.34±.196
45	3.00±.057	3.35±.202	3.79±.086	3.15±.086	3.91±.178	4.11±.063
60	3.21±.121	3.39±.086	3.84±.138	3.54±.138	3.9±7.213	4.17±.098
75	3.74±.089	3.44±.127	3.94±.138	3.95±.103	4.14±.080	4.29±.167
90	4.24±.138	3.89±.080	4.11±.063	4.74±.080	4.34±.115	4.64±.196
Sig.	.000	.065	.428	.000	.360	.276

Data are presented as mean ±SE of 3 replicates. –SE: standard error. -Significant difference at P < 0.05.SF: Soybean flour, MBP: Minced boiled potato

Table 4: Changes in Yeast and Mold Count of Sand Smelt Fish Products during Frozen Storage at – 18ºC

Storage Period (day)	Fish Burger			Fish Finger		
	Control	SF (15%)	MBP (15%)	Control	SF (20%)	MBP (15%)
0	2.01±.092	2.34±.138	2.61±.115	2.56±.150	2.71±.173	2.84±.196
15	ND	ND	ND	ND	ND	ND
30	ND	ND	ND	ND	ND	ND
45	ND	ND	ND	ND	ND	ND
60	ND	ND	ND	ND	ND	ND
75	ND	ND	ND	ND	ND	ND
90	ND	ND	ND	ND	ND	ND
Sig.	.000	.000	.000	.000	.000	.000

Data are presented as mean ±SE of 3 replicates. –SE: standard error. -Significant difference at P < 0.05.ND: not detected. SF: Soybean flour, MBP: Minced boiled potato.

samples were cooked by frying in vegetable oil (sunflower oil) before carrying out the evaluation. The panelists were asked to evaluate the sensory properties of the product in terms of color, taste, odor, texture and overall acceptability. Data collected from the evaluation was statistically analyzed and the results obtained are tabulated in Figures **1**, **2**, **3**, **4** and **5**.

Color

Color score values of sand smelt fish products during frozen storage are shown in Figure **(1**) The results indicated that color score values of the fresh control burger sample and those prepared by incorporation of 15% SF and 15% MBP were 6.2, 9.0 and 8.9, respectively. Also, control finger sample and the samples formulated with adding 20% SF and 15% MBP scored color values of 6.6, 7.9 and 9.0, respectively. These results clearly show that colors of the formulated sand smelt products were significantly preferred by panelists.

The results given in Figure (**1**) showed no significant difference between SF and MBP formulated burger samples but in the case of fish fingers, MBP sample was significantly ($P<0.05$) better. During frozen storage of sand smelt fish products color score values slightly declined. Meanwhile, the 90 days storage period showed no significant effect on the color of the different samples. The formulated burgers and fingers samples retained their good colors without undesirable changes over the entire period of frozen storage.

Odor and Taste

Data given in Figure (**2**, **3**) show the changes in the odor and taste of sand smelt fish products during frozen storage. The result indicated that score values

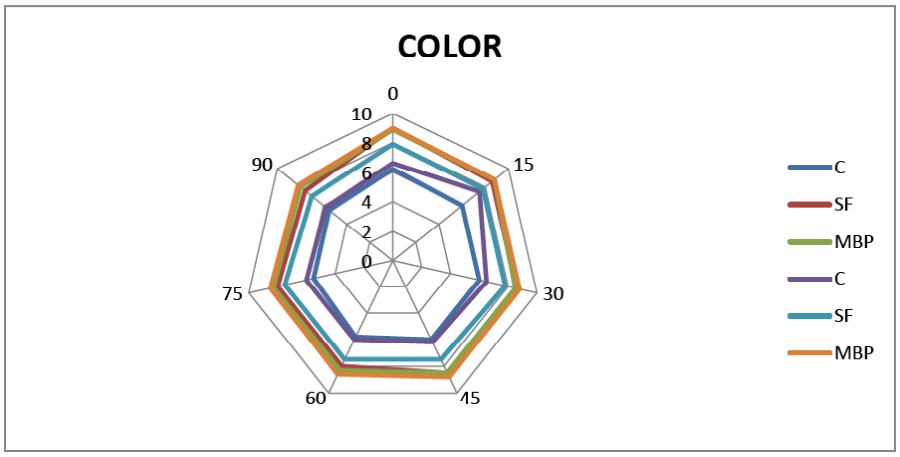

Figure 1: Changes in color of sand smelt fish products** during storage at - 18±1°C.
**Samples were cooked by frying before sensory evaluation.

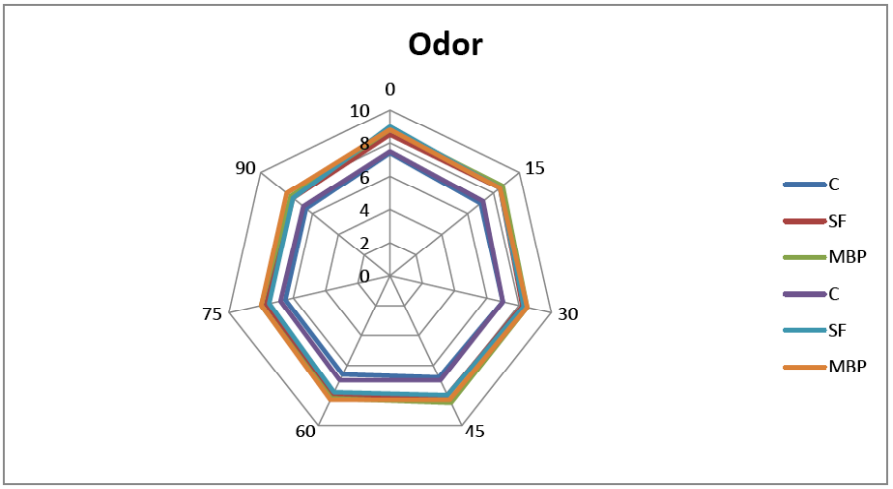

Figure 2: Changes in odor of sand smelt fish products** during storage at - 18±1°C.
**Samples were cooked by frying before sensory evaluation.

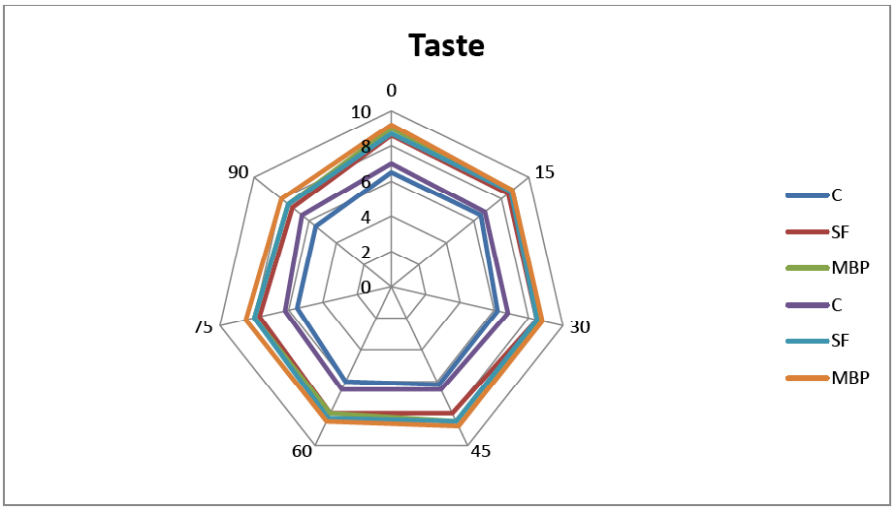

Figure 3: Changes in taste of sand smelt fish products** during storage at - 18±1°C.
**Samples were cooked by frying before sensory evaluation.

for taste and odor of burger and fingers decreased as the storage period prolonged and the changes were more pronounced in control sample prepared without adding the filling materials. Odor and taste of burger control sample showed significant changes after 45- 60 days of frozen storage. Almost similar changes were recorded for control finger sample. On the other hand burger and finger samples formulated by incorporation of filling materials maintained their high good qualities for odor and taste over the entire 90 days of frozen storage.

Texture

Data given in Figure (**4**) Showed score values of texture of fish products made from sand smelt fish and their changes during frozen storage. Significant differences ($p < 05$) were found between the texture of burger and finger samples formulated with SF and MBP fillings and their control samples. Based on the score values, panelists evaluated texture of the formulated burger and fingers as excellent while textures of the control samples were fairly good.

It is important to consider the freeze – thaw stability of mince based fish products such as fish burgers and fingers during frozen storage. Data presented in Figure **X D** showed that texture score values of sand smelt fish products slightly decreased during frozen storage. However, the formulated samples of burger and finger maintained their stable and good texture over the entire storage period, while control samples particularly for finger product showed an observed change during frozen storage. The common problem with frozen

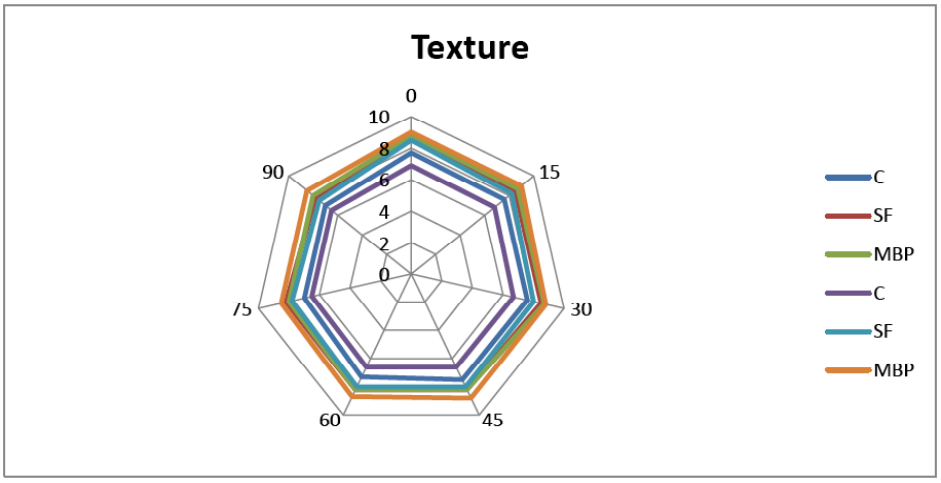

Figure 4: Changes in texture* of sand smelt fish products** during storage at - 18±1°C.
**Samples were cooked by frying before sensory evaluation.

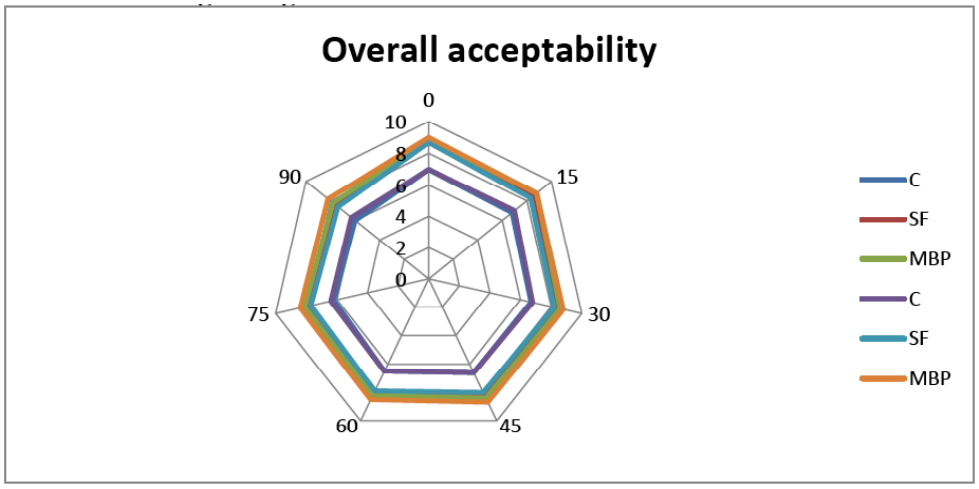

Figure 5: Changes in the overall acceptability* of sand smelt fish products** during storage at - 18±1°C.
** Samples were cooked by frying before sensory evaluation.

storage of fish products is texture hardening and lack of moisture in the finish thawed products. This observation might be attributed to the drip separation accompanied by thawing process of the frozen product.

[22] Reported that ingredients such as soy protein and milk protein were found to have the ability to reduce the high freeze- thaw drip loss and maintaining the texture from hardening. Of course the rate of freezing is also essential to guaranty the small size of the ice crystals. The smaller the better to avoid cell disruption by ice crystals growth, with subsequent loss of cytoplasmic content as the drip loss.

Overall Acceptability

According to the results given in Figure (**5**) Score values of the overall acceptability showed that sand smelt fish products particularly those formulated with adding SF and MBP as filling materials had high good quality during the storage period of 90 days. This is the outcome of maintaining all the sensory quality attributes without undesirable changes during frozen storage. The undesirable quality changes in frozen fish products are associated with discoloration or brown stains, lipid oxidation and fishy odor development, and texture hardening or toughening. Score values for color, odor, taste and texture (Figure **1** to **5**) indicated the high stability of these quality parameters of the formulated products made from sand smelt fish during frozen storage. Consequently, sand smelt fish products were liked by the panelists and they maintained the high acceptability during frozen storage for 90 days. This conclusion is supported by the results of microbiological examination of the products during the storage.

CONCLUSION

In conclusion fish burger and fish finger made from sand smelt fish showed high stability of quality parameters of the formulated products made from sand smelt fish with soy flour and minced potato fillings during frozen storage. Consequently, sand smelt fish products were liked by the panelists and a high acceptability was maintained during frozen storage for 90 days. This conclusion is supported by the good results of microbiological examination of the products during the storage, opening a good perspective for the production of these fish products to improve the sustainable use of fishery and increase the offer of good quality protein foods.

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