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Chemical, sensory and microbial quality changes of breaded kilka (*Clupeonella cultriventris*) with tempura batter in production stage and during frozen storage

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Abstract

Considering abundant resources of common Kilka (*Clupeonella cultriventris*) in Caspian Sea, the aim of this study was to produce a new product from Kilka with tempura batter in order to increase human consumption and evaluation of proximate composition, chemical, microbial and sensory quality changes in production stage and during storage (at -18°C for 4 months). Nutritional composition of breaded kilka changed during process. In fried breaded Kilka, moisture content reduced while lipid content increased significantly in comparison with raw breaded Kilka after flash frying ($P < 0.05$). All the chemical parameters (PV, TBA, TVB-N and FFA) in breaded Kilka experienced significant differences after frying in comparison with raw breaded Kilka ($P < 0.05$). A significant increase was seen in PV, TBA and TVB-N in fried breaded Kilka ($P < 0.05$). FFA reduced significantly during frying ($P < 0.05$). All microbial counts reduced during frying and frozen storage ($P < 0.05$). PV and TBA value increased throughout the frozen storage ($P < 0.05$). Although the lipid oxidation occurred during frozen storage, PV and TBA value were lower than acceptable limits for human consumption. TVN-B slightly increased within the 3 months of frozen storage thereafter, it increased sharply up to 4 month ($P < 0.05$). TVB-N exceeded from highest permissible limit of it at the end of the fourth month. The sensory parameters, in breaded kilka with tempura batter declined significantly throughout the frozen storage ($P < 0.05$). According to organoleptic result and reduction of total acceptability score, shelf life of breaded kilka with tempura batter was estimated to be 3 months at -18°C.

Introduction

The most abundant fishes of Caspian Sea are the small clupeids known as Kilka including the common Kilka (*Clupeonella cultriventris caspia*), anchovi (*C. engrauliformes*) and larged eyed Kilka (*C. grimi*) (Svetovidov, 1963). It is native species of Caspian Sea and found in all parts of it especially in coastal line. Kilka belongs to pelagic fish and they feed on zooplankton. From 2002 onwards, the frequently of common Kilka increased and reached 24,400 mt in 2009 (Fazli, 2011). The common Kilka (*Clupeonella cultriventris caspia*) is the predominant species of the southern Caspian Sea comprising 97% of total catches of clupeids in south of the sea in 2009. Only stock of common Kilka remains stable, following the severe impacts of Menemiopsis (Fazli, 2011). Unfortunately, a small portion of the Kilka catch is used by human (4%) due to its small size, easy deterioration, impossibility of gutting right away after catching, difficulties in hygienic preservation, packaging, and supply and the rest is used as fish meal for poultry and in aquaculture (Shabanpour *et al*., 2007; Khoshkhoo *et al*., 2010). It is noteworthy that clupeids present a few positive qualities as well, such as abundance in Caspian Sea, low finished cost of it, easy scaling without scaling equipments; therefore, clupeids can be utilized as raw materials in conversion industry (Khoshkhoo *et al*., 2010). Kilka can be processed into salted Kilka, smoked, pickled, sausages, canned, dried and frozen fish. In Iran, Generally, kilka is only freshly consumed. There is not many processing techniques except canned.

The growing tendency to spend less time on food preparation has led to a great demand for time-saving "ready-to-heat" frozen products. Thus breaded foods are very popular today, and their consumption has increased in recent years both in high convenience consumer societies and in developing countries (Varela *et al*., 2008).The main purpose for applying breaded and batter coating on fried foods is to produce high-quality products in terms of visual appeal, crispness, low fat content, flavor and favorable consumer satisfaction (Loewe, 1993; Fiszman and Salvador, 2003).

Batters are defined as liquid mixture composed of

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water, flour, starch, and seasonings, into which food products are dipped prior to cooking. Batters are of two types, adhesive and tempura. The traditional adhesive batter is a fluid, basically consisting of flour and water. Tempura batter is the puff-type specially batter. Tempura–type batters form a crisp, continuous, uniform layer over the food. The tempuras are used at very high viscosity levels and always contain raising/ leavening agents (Venugopal, 2006). Breading was defined as a dry mixture of flour starch, and seasonings, coarse composition, and applied to moistened or battered food products prior to cooking (Suderman, 1983). Then they should prefry for a few seconds in order to make the batter coagulate, and then freezes them. In this form they reach the consumer, who in turn fries them for a few minutes in order to cook them before eating them (Fiszman and Salvador, 2003). Coating was referred as the batter and/or breading adhering to a food product after cooking (Suderman, 1983).

Battered and breaded product, are commonly stored and marketed in the frozen state. However, fish and fishery products can undergo undesirable changes during frozen storage and deterioration may limit the storage time. These undesirable changes result from protein denaturation (Fijuwara *et al*., 1998; Benjakul *et al.*, 2005) and lipid oxidation (Kurade *et al*., 1987; Tokur et al., 2006). Considering abundant resources of Kilka fish in Caspian Sea, the aim of this study was to produce a new product from Kilka with different sensory properties in industrial scale with tempura batter in order to increase human consumption and evaluation of proximate composition, chemical (pH, PV, TBA, TVB-N, FFA), microbial and sensory quality changes in production stage and during frozen storage at -18°C.

Materials and Methods

Preparation of raw fish

Fresh Kilka fish were bought from special Kilka fishing boats with lantern net in Anzali quay, Gillan province, Iran and then they were transferred to National Research Seafood Processing Center in CSW tanks with 60% fish, 25% ice and 15% marine water. The fish were immediately deheaded and gutted by two skilled technicians and then rinsed with cold hygienic water. Then, the fish were submerged in diluted brine at 4°C for 10 min afterwards, the fish were rinsed for in order to remove the surface salt.

Tempura Batter ingredients and formulations

Tempura batter contained wheat flour (16%), tapioca starch (3%), corn flour (5%), soy flour (5%), dried egg albumin (7%), powdered milk (3%), baking powder (2%), sunflower liquid oil (6%), salt (0.4%), pepper (0.3%), lime juice (0.3%), guar gum (1%), and 14°C water (51%) (Venugopal, 2006) with 100 ppm thyme and 50 ppm rosemary extracts as natural preservatives. All dry ingredients were mixed at low speed for 1 min in a stainless-steel bowl. Ingredients were then mixed with water for 2 min, and then stored in an ice bath to maintain the temperature during batter application.

Preparation of breaded Kilka

Pre-dusting was performed with wheat flour using automated machinery. Then, the common Kilka fish were battered in the tempura batter, the fish were subjected to batter shower by conveyor and the batter were sprayed by a pump from a tank and then, the fish were breaded with conventional breading crumbs and then pre-fried at 170°C for 30 sec in sunflower oil (Tokur *et al*., 2006). The fried samples were immediately frozen through continuous method at -40°C in a spiral freezer and they were packaged with polyethylene coating with 20 fish in each pack then stored at -18°C for four months.

Analyses

Analysis for the determination of proximate composition, initial chemical quality parameters (pH, PV, TBA, TVB-N, FFA) and microbiological counts of fresh kilka and raw breaded kilka were performed on the production day. All the analysis was carried out triplicate. For investigation of sensory, chemical and microbial quality of breaded kilka during frozen storage, 3 package of breaded kilka were taken out randomly from frozen storage and thawing in ambient temperature.

Whole breaded kilka were analyzed for microbiological, chemical and sensory attributes at one day after production and periodically at the end of every month (numbered as 0, 1 to 4 phases).

Chemical analysis

pH was measured for the homogeneous mixtures of breaded fish and distilled water (1:10, w:v), using a Metrohm model pH meter (Switzerland) (Santos *et al*., 1981). peroxide value (PV) was determined according to a method followed by Egan et al. (1998). Thiobarbituric acid value (TBA, mg malonaldehyde/ kg) was determined using a spectrophotometric method (Natseba *et al.*, 2005). Total volatile base nitrogen (TVB-N, mgN/100g) was determined according to Pearson (2006). Free fatty acid (FFA,) was determined according to Natseba *et al.* (2005).

Proximate composition

The moisture content was determined by using

oven at 103°C (AOAC, 2002). The amount of ash was measured by drying the sample in an electrical kiln at 550°C (AOAC, 2002).The amount of crude protein was determined by Kjeldahl Method (AOAC, 2002) and total lipid was measured using Suxele method (AOAC, 2002).

Microbial analysis

Total Bacterial Count (TBC) and Total Coliform (TC) and psychrotrophic microorganisms, Total Combined Yeast And Mold Count (TYMC) were determined according to the Iranian Institute of Standard and Industrial Studies 997 (1995), 2629 (2003), 5272-1 (2003), and 11166 (2008), respectively.

Sensory analysis

In order to assess sensory properties, the breaded Kilka fish were fried in sunflower oil for 3 min after thawing in ambient temperature. The assessment was performed through Hedonic Method using 10 educated trained persons in standard chambers (ASTM, 1969). The panelists scored odor, flavor, texture, crispness, cohesiveness of the batters and general acceptability using a five-point hedonic scale (1, Dislike extremely to 5, like extremely). In order to avoid interference of odor and flavor during the assessment, the panelist smelled coffee before odor assessments and they washed their mouth cavities before flavor assessments.

Statistical analysis

Data were analyzed by One-way analysis of variance (ANOVA) using SPSS Software version 15. Duncans multiple range Test for chemical quality and Kruskal–Wallis H for sensory quality were used to find significant differences between storage periods.

Results

Production stage

Chemical composition

Moisture, total lipid, crude protein, and crude ash in fresh, raw breaded kilka and fried breaded kilka in production stage (phase zero) were shown in Table 1. It can be seen that there were significant differences between raw and fried breaded Kilka in terms of moisture, fat, and crude protein contents ($P<0.05$). Moisture content in fried breaded Kilka reduced significantly while lipid content in fried breaded Kilka significantly increased in comparison with raw breaded Kilka ($P < 0.05$).

Table 1. Chemical composition (gr/100 gr) of fresh, raw breaded and fried breaded kilka by use of tempura batter

Chemical composition	Fresh kilka	Raw breaded kilka	Fried breaded kilka
Moisture	75/00±0/28 a	62/02±0/03 b	48/45±0/07 c
Total lipid	6/5±1/14 a	5/40±0/42 a	20/10±0/48 b
Crude protein	15/50±0/28 a	15/90±0/56 a	17/65±0/07 b
Crude ash	3/00±0/28 a	3/30±0/00 ab	3/55±0/07 b

Data are expressed as means ± standard deviation (n = 3)

Means within the same row having different superscripts are significantly ($P < 0.05$) different

Table 2. Total Bacteria Count, Total Coliforms, Psychrotrophic Bacteria and yeast – mold (log cfu/gr) in fresh, raw breaded and fried breaded Kilka with tempura batter.

Analysis	Fresh kilka	Raw breaded kilka	Fried breaded kilka
TBC (log cfu/g)	3/29±0/77 b	5/50±0/56 c	2/69±0/83 a
TC (log cfu/g)	3/23±0/77 b	2/65±0/00 b	1/15±0/05 a
Psychrotrophic (log cfu/g)	4/10±0/04 a	4/20±0/03 a	3/01±0/23 b
Yeast – mold (log cfu/gr)	0/78±0/23 a	0/98±0/05 a	0

Data are expressed as means ± standard deviation (n = 3)

Means within the same row having different superscripts are significantly ($P < 0.05$) different

Table 3. Chemical quality parameters in fresh and breaded Kilka with tempura batter during processing and frying

Analysis	Fresh kilka	Raw breaded kilka	Fried breaded kilka
PV (meq O_2/kg)	2/01±0/45 a	2/4±0/78 a	3/40±0/14 b
TBA (mg malonaldehyde/kg)	0/26±0/02 a	0/28±0/08 ab	0/33±0/06 b
FFA (gr/100 gr lipid)	1/40±0/28 a	1/50±0/56 a	0/74±0/07 b
TVN-B (mg N/100 gr)	14/20±0/18 a	14/00±0/40 a	15/20±0/29 b
pH	6/80±0/04 a	6/78±0/01 a	6/72±0/03 a

Data are expressed as means ± standard deviation (n = 3)

Means within the same row having different superscripts are significantly ($P < 0.05$) different

Microbial quality

Microbial analysis of fresh fish, raw breaded and fried breaded Kilka in production stage was demonstrated in Table 2. The contamination rate during production of breaded Kilka may be estimated through comparing microbial load of fresh Kilka and raw breaded Kilka. In the raw breaded Kilka produced with tempura batter, total bacteria count increased from 3.29 to 5.50 log cfu/gr ($P < 0.05$). it was not significant increase in Psychrotrophic bacteria, molds and yeast counts in raw breaded Kilka in comparison with fresh kilka ($P > 0.05$).All microbiological counts (TBC, TC, and total Psychrotrophic count) were decreased significantly in fried breaded Kilka compared to raw breaded Kilka by frying and freezing at -18°C ($P < 0.05$) and no yeast and mold were detected in fried products.

Chemical quality parameters

Table 3 shows chemical quality parameters such as PV, TBA value, FFA, TVN-B, and pH in fresh fish and raw breaded Kilka and fried breaded kilka. All the mentioned parameters in breaded Kilka experienced significant differences after frying in comparison with raw breaded Kilka ($P < 0.05$). Lipid oxidation in breaded Kilka with tempura batter was detected via measurement of PV and TBA during frying. In the present study, a significant increase was seen in PV and TBA value in fried breaded Kilka compared to raw samples ($P < 0.05$). FFA reduced significantly during frying ($P < 0.05$); however, TVN-B increased

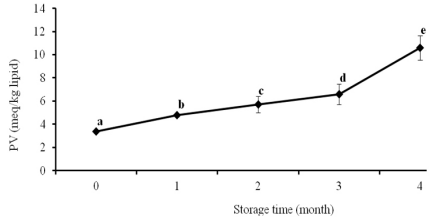

Figure 1. Changes in Peroxide values (meq O_2/kg lipid) in breaded Kilka with tempura batter during frozen storage at -18°C

Different lowercase letters indicate significant difference at different times.

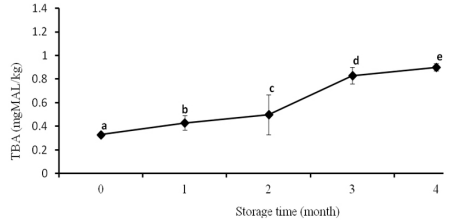

Figure 2. Changes in TBA (mg Malonealdehyde /kg tissue) in breaded Kilka with tempura batter during frozen storage at -18°C

Different lowercase letters indicate significant difference at different times.

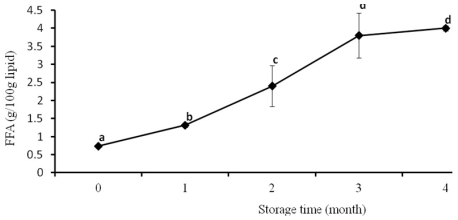

Figure 3. Changes in FFA (g/100 gr lipid) in breaded Kilka with tempura batter during frozen storage at -18°C

Different lowercase letters indicate significant difference at different times.

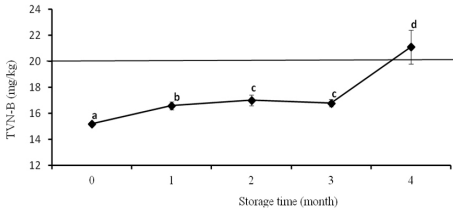

Figure 4. Changes of TVN-B (mg N/100 gr) in breaded Kilka with tempura batter during frozen storage at -18°C

Different lowercase letters indicate significant difference at different times.

significantly after frying ($P < 0.05$). Raw and fried breaded Kilka had lower pH than fresh fish although it was not significant ($P < 0.05$).

Frozen storage

Microbial quality

Table 4 depicts the results obtained from TBC, TC, Psychrotrophic Bacteria and yeast – mold counts in breaded Kilka with tempura batter during frozen storage. All microbial counts reduced during frozen storage at -18°C ($P < 0.05$). Yeast and Mold count was not performed during cold storage.

Chemical quality parameters

The lowering of quality during frozen storage could also be caused by lipid changes. Oxidative

Table 4. TBC, TC, Psychrotrophic Bacteria and yeast – mold (log cfu/gr) in breaded Kilka with tempura batter during frozen storage at -18°C

Analysis	Storage time(month)				
	0	1	2	3	4
TBC (log cfu/g)	2/69±0/83 b	2/68±0/06 b	2/55±0/11 b	2/12±0/09 a	2/14±0/02a
TC (log cfu/g)	1/60±0/05 b	1/12±0/03 a	1/05±0/08 a	1/02±0/3 a	1/00±0/30a
Psychrotrophic (log cfu/g)	3/01±0/23 b	2/73±0/03 b	2/73±0/07 b	2/38±0/05 b	1/90±0/24 a
Yeast – mold (log cfu/gr)	0	0	0	0	0

Data are expressed as means ± standard deviation (n = 3)
Means within the same row having different superscripts are significantly ($P < 0.05$) different

Table 5. Sensory quality changes of breaded kilka with tempura batter during frozen storage at -18°C

Analysis	Storage time (month)				
	0	1	2	3	4
Odor	4/73±0/48 a	4/78±0/24 a	4/30±0/41 a	3/73±0/73 b	2/78±0/13c
Taste	4/27±0/78 a	4/34±0/48 a	4/04±0/65 a	3/27±0/05 b	2/27±0/76c
Texture	4/18±0/75 a	4/23±0/73 a	4/00±0/23 a	3/18±0/31 b	2/53±0/25c
Crispness	3/81±0/75 a	3/92±0/75 a	3/70±0/34 a	3/74±0/21 a	3/00±0/38b
Cohesiveness of batter	4/54±0/52 a	4/72±0/32 a	4/55±0/42 a	4/00±0/92 a	3/20±0/32 b
General acceptability	4/64±0/50 a	4/30±0/32 a	4/35±0/23 a	3/54±0/09 ab	2/97±0/82b

Data are expressed as means ± standard deviation (n = 3)
Means within the same row having different superscripts are significantly ($P < 0.05$) different

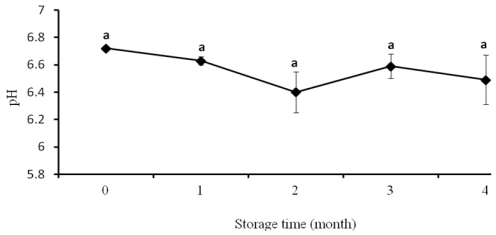

Figure 5. Changes of pH in breaded Kilka with tempura batter during frozen storage at -18°C

Different lowercase letters indicate significant difference at different times.

rancidity during frozen storage at -18°C was evaluated by determining the peroxide value (Figure 1) and TBA (Figure 2). PV (meq O_2/kg lipid) increased throughout the frozen storage so that the highest was for the fourth month; it should be noted that the increase was significant in different months ($P < 0.05$). In the present study, the TBA value (mg Malonealdehyde/kg tissue) in fried breaded Kilka with tempura batter significantly increased ($P < 0.05$) and the highest value was seen in the fourth month. FFA measurement is a favorable index in order to define effect of lipolytic enzymes on lipid content in fish and other meat products. Gradual increase in FFA content was found up to month 3 and the highest FFA content was obtained on mouth 4 in breaded kilka with tempura batter (Figure 3). The results obtained from the chemical tests showed that FFA in different months of sampling increased significantly ($P < 0.05$).

Changes in TVN-B of breaded Kilka with tempura batter during frozen storage were shown in Figure 4. TVN-B in breaded Kilka increased as the storage time increased ($P < 0.05$). From this result, TVN-B slightly increased within the 3 months of frozen storage thereafter, it increased sharply up to 4 month and the highest rate was seen in the fourth month of sampling.There were no significant differences between the initial value and those at the storage period for pH in Breaded Kilka with tempura

batter (P > 0.05). No significantly fluctuations in pH of samples were observed throughout frozen storage (P > 0.05) (Figure 5).

Sensory quality

The sensory quality of breaded kilka with tempura batter were evaluated in terms of odor, taste, texture, crispness, cohesiveness of batter and general acceptability (Table 5). The sensory scores, in breaded kilka with tempura batter declined significantly throughout the 4 months of frozen storage (P < 0.05). However, breaded kilka remained quite fresh after three months.

Discussion

Chemical composition of breaded Kilka in production stage

Frying affected significantly total lipid and moisture content of breaded Kilka so that moisture content decreased while total lipid increased significantly in fried breaded Kilka (P < 0.05). One of the important mechanisms of oil uptakes during deep-fat frying is water replacement (Gamble *et al.*, 1987; Mellema, 2003; Dana and Saguy, 2006). When the food is exposed to frying temperatures, water evaporates rapidly, the outer surface becomes dry and a crust forms. Moisture within the product is converted to steam, creating a positive pressure gradient. Thus steam escapes through cracks, defects, open capillaries and channels in the cellular structure and membranes. As the process progresses, oil adheres to the food, entering into the food through the large pores formed by the frying and evaporation of water (Dana and Saguy, 2006). Here, there is a linear relation and high correlation (r = -0.80) between reduction of moisture and fat uptake (r = -0.80) (Krokida *et al.*, 2002). Similar results were found about reduction of moisture content and elevation of fat content in breaded fish products by Ihm *et al.* (1992), Taşkaya *et al.* (2003), Yazdan *et al.* (2009), Elyasi *et al.* (2010) and Moradi *et al.* (2010).

Microbial quality of breaded kilka with tempura batter in production stage

TBC is an important factor for evaluation of microbial quality assessment in food products. In the present study, TBC in fresh kilka was found to be 3.293 log cfu/gr, which was lower than maximum permissible rate reported by ICMSF. Moreover, Iranian National Standard Organization (no. 5625) reported the maximum recommended TBC in fresh Kilka to be 5 log cfu/gr. Therefore, the kilka introduced to the production line in the present study were in a proper range in terms of TBC. Contamination load of breaded kilka with tempura batter increased during different steps of production so that TBC increased to 4.65 log cfu/gr in raw breaded Kilka with tempura batter. Some products like breaded fish steaks, fish burger, and fish finger have very different microbial load from fresh fish because of additives, extra processes, exposure to machinery, conveyors, workers' hands, and also environmental contaminations and packaging (Frazier and Westhoff, 2003). TBC reduced in fried breaded kilka compared to raw ones (P < 0.05). Heat during frying devastated the microorganisms in the fried breaded kilka. Reduction of TBC in fried fish finger after frying at 170-180°C were detected in several studies (Cakli *et al*., 2005; Tokur *et al*., 2006; Elyasi *et al.*, 2010; Izci *et al.*, 2011). According to the standard of ICMSF, the maximum recommended bacterial counts for good quality products and maximum recommended bacterial counts for marginally acceptable quality products of precooked breaded fish are 5×10^5 and 10^7, respectively. Thus, the breaded kilka with tempura batter in the present study can be considered high quality as its TBC was 2/69 log cfu/gr.

TC is a hygienic index in food products. TC decreased in raw breaded kilka with tempura batter compared to fresh fish; this can be attributed to antibacterial effect of thyme and rosemary concentrates in tempura batter. Antibacterial effect of thyme and rosemary extracts is due to the presence of hydroxyl groups in their phenolic compounds (Shahidi and Wanasundara, 1992; Del campo *et al.*, 2000; Karamanoli *et al.*, 2000). Antibacterial and antioxidant properties of thyme extract have been reported in coated semi fried fillets of mullet fish containing 2.5 and 5 percent thyme (Yasin *et al.*, 2007). TC during frying encountered a decreasing trend like TBC. Reduction of TC in fried fish finger compared to raw fish finger after frying has been documented by several studies (Cakli *et al.*, 2005; Elyasi *et al*., 2010; Izci *et al*., 2011). Maximum level of TC in fish products was given as 400 cfu/g by ICMSE (1986). Consequently, the products in the present study with tempura batter are approved in terms of hygienic criteria. No yeast and mold were seen in fried breaded kilka. Heat during frying eliminated molds and yeasts. Furthermore, elimination of mold and yeasts in the fish fingers produced from Atheria boyeri after flash frying at 180°C has been documented by Izci *et al.* (2011). These microbial data indicate that the processing of breaded kilka with tempura batter until frozen storage has been done under good sanitary conditions.

Chemical quality of breaded kilka with tempura batter in production stage

In the present study, Frying significantly increased PV and TBA value in fried breaded kilka compared to raw samples ($P < 0.05$) indicated that lipid oxidation took place during frying. It has reported that unstable primary oxidation products, hydroperoxides, are decomposed rapidly into secondary oxidation products such as aldehydes and ketons (Aubourg and Medina, 1999). Cooking marine products via different methods affects lipid hydrolysis and oxidation. During cooking, lipids are affected by thermal oxidation which is swifter than oxidation in raw samples and as a result, some changes occur in functional properties (Fogerty *et al.*, 1990). The changes are more visible during frying (Zakipour Rahimabadi and Baker, 2011). Increase in PV and TBA values of fried samples also reported by Nikoo *et al.* (2010) and Zakipour Rahimabadi and Baker (2011). As reported by Al-Saghir *et al.* (2004), in addition of heat treatment, the kind of cooking oil also can alter the peroxide value. The upper acceptability recommended rate for PV and TBA values in fish are 10-20 meq O_2/kg lipid (Huss, 1995) and 1-2 mg malonedialdehyde/kg of fish sample (Lakshmanan, 2000), respectively. The changes in chemical composition of lipid during processing and frying the breaded kilka with tempura batter in the phase zero were in permissible ranges. FFA reduced significantly during frying breaded kilka with tempura batter ($P < 0.05$). Loss of volatile FFA during heating in high temperatures as well as inactivation of enzymes may be attributed to reduction of FFA (Al-Saghir *et al*., 2004).

Microbial quality of breaded kilka with tempura batter during four months of frozen storage at -18°C

All microbial counts in breaded kilka with tempura batter during cold storage at -18°C experienced reduction. Comparison of microbial indices in raw and fried breaded kilka in different phases showed that the highest reducing effect was seen in the breaded kilka in the phase zero. i.e. after deep frying and continuous freezing at -40°C. The effect may be due to high thermal shock followed by cooling shock as a result of continuous freezing.

Chemical changes in breaded kilka with tempura batter during four months of frozen storage at -18°C

The lowering of quality during frozen storage could also be caused by lipid changes. Peroxide value is widely employed for determining the formation of hydroperoxides, which are primary products of oxidative reactions (Simic and Taylor, 1987). Increased amount of PV in frozen samples of breaded kilka defines development of lipid oxidation during frozen storage. In general, increased frozen storage time resulted in acceleration of lipid oxidation and hydroperoxide rate. When hydroperoxides in fish muscle are low, their formation becomes faster than their breakdown, following a monomolecular mechanism, resulting in hydroperoxide accumulation (Vidya and Sriker, 1996). When the hydroperoxide concentration increases, their decomposition would follow a bimolecular model and would become faster than their formation with a consequent reduction in their level (Ben-Gigirey *et al*., 1999).The results obtained from the present study showed that PV in breaded kilka with tempura batter was lower than the permissible rate recommended by Huss (1995) at the end of the fourth month.

The TBA value is widely used as an indicator of the degree of lipid oxidation (Tokur *et al.*, 2006). In the present study, TBA value significantly increased in the breaded kilka with tempura batter during frozen storage ($P < 0.05$). The increasing trend during storage may be owing to increased free iron and other peroxidants in fish muscle. Tokur *et al*. (2006) reported hemoglobin (Hb) can show strong pro-oxidant activity for some species between pH 6 and pH 7 and it can retard oxidation at pH values above 7. It has been reported that the maximum level of TBA value indicating good quality of the fish during storage period is 1-2 mg malondialdehyde/kg fish sample (Lakshmanan, 2002). In the current study, TBA values was much lower than such proposed limits throughout the 4 mouths storage period. However, Aubourg (1999) has reported that TBA values may not give the actual rate of lipid oxidation, since malondialdehyde can interact with other components of fish such as nucleosides, nucleic acids, proteins, amino acids, phospholipids and other aldehydes that are end-products of lipid oxidation. Also, in the present study, thyme and rosemary concentrates may decrease lipid oxidation significantly due to their antioxidant and antimicrobial effects. Antioxidant and antibacterial effects of the mentioned concentrates are attributed to polar phenolic compounds (Del campo *et al.*, 2000, Karamanoli *et al.*, 2000). Antibacterial and antioxidant effects of thyme concentrate have been reported in semi-fried fillets of mullet with edible coating containing 2.5% and 5% thyme (Yasin *et al*., 2007).

During frozen storage, the increase in FFA indicated hydrolysis of lipids for breaded kilka with tempura batter. Relation between FFA release and loss of freshness has been reported by Ozogul *et al*. (2007). Lipid hydrolysis development strongly depends on the hydrolytic enzyme content, this being highly

influenced by different external and internal factors (Aubourg *et al.*, 2007). The release of FFA form a triacylglycerol matrix may accelerate the rate of lipid oxidation and generation of off-flavors (Ozogul *et al*., 2007). FFAs attach themselves hydrophobically or hydrophilically to the appropriate sites on the protein surface, creating a hydrophobic environment which results in a decrease in protein solubility (Sarma *et al.*, 2000). The pro-oxidant effect has been explained as a catalytic effect of the carboxyl group on the formation of free radicals by the decomposition of hydroperoxides (Aubourg, 1999).

Total volatile basic nitrogen (TVB-N) that is mainly composed of ammonia trimethylamine (TMA) and dimethylamine (DMA), is widely used as an indicator of meat deterioration (Fan *et al*., 2008). In the present dtudy, the value for TVB-N increased as storage time progressed ($P < 0.05$). The increasing of TVB-N value during storage is related to bacterial spoilage and activity of endogenous enzymes (Chomnawang *et al*., 2007). TVB-N content of fish is in general an indicator of the freshness. A rejection limit of 25 mg/100 gr TVB-N has been proposed for fish products (Gimenez *et al*., 2002; Arashisara *et al*., 2004). Nevertheless, Iranian National Standard No. 5849 (2003) reported the highest permissible limit of TVB-N for semi-cooked breaded fish fingers to be 20 mg/100 gr product. Therefore, TVN-B could be used as an indicator of breaded kilka quality as TVB-N exceeded from 20 mg N/100 gr of flesh at the end of the fourth month in the present study. On this basis, the maximum shelf life for breaded kilka with tempura batter at -18°C is recommended to be three months.

pH is not recommended as a suitable index for measuring spoilage and it is only suggested as a guideline to assess fish and fish products qualities (Ruiz-Capillas and Moral, 2001). This factor is affected by chemical, sensory, and microbial factors (Ersoy *et al*., 2008). In the present study, no significant difference was detected in pH value in the beginning and end of the storage period. pH fluctuated in different months while it was not significant ($P < 0.05$). Increase of pH might be attributed to the formation of basic decomposition products, such as ammonia and trimethylamine. These compounds are produced by endogenous enzymes and bacterial spoilage (Ruiz-Capillas and Moral, 2001). Reduced pH may be caused by reduction or cessation of microbial growth (Widayaka *et al*., 2001). Eun *et al.* (1994) reported Catfish muscle pH after frozen storage for various times were not significantly different from fresh muscle pH. Tokur *et al.* (2006), also, reported such fluctuations in pH rates of the fish fingers made from mirror carp with out any significant difference in pH value in the beginning and end of 6 months storage at -18°C.

Sensory evaluation of breaded kilka with tempura batter during four months of storage at -18°C

Sensory evaluation is used as one of the indices for measurement of fish quality during storage. Assessment of organoleptic indices along with chemical and microbial tests (as a complementary method) is necessary for evaluation of spoilage rate and shelf life of fish and fish products. Battered products have better flavor, texture, and appearance and battering is considered a protection against moisture and natural concentrate losses as it contrasts to effect of freezing or reheating. Therefore, battering makes the inside of products juicy while the outside looks crispy (Fiszman and Salvador, 2003). Freezing and frozen storage are important methods for the preservation of fish products (Vidya Sager Reddy and Sriker, 1996). However, undesirable reactions associated with lipids and proteins occurred that leads to detrimental changes in nutritional and sensory properties (Sikorski and Kolakowska, 1994; Erickson, 1997). Throughout the storage period of breaded kilka there were decreases and significant changes ($P < 0.05$) in all sensorial criteria. Reduction of organoleptic scores may have occurred as a result of lipid oxidation and decomposition of hydroperoxides into aldehyde and ketone compounds and subsequently, formation of rancid flavor, formation of non-soluble lipid-protein compounds, formation of volatile compounds such as sulphureted compounds, aldehydes, ketones, esters, hypoxanthine, and molecules with low molecular weight caused by lipid oxidation, various amines (Tokur *et al*., 2006), and denaturation of myofibril proteins (Pons-Sanchez *et al*., 2006). Changing trend of sensory properties in breaded kilka with tempura batter during frozen storage is coordinated with changing trend of lipid oxidation (PV, TBA) which cause reduction of sensory properties and also elevation of lipid hydrolysis; also, accumulation of FFA cause reduction of some acceptability indices. FFA affects protein stability and decomposes texture through reduction of reaction with protein (Kolakowska *et al*., 2006; Rodriguez *et al.*, 2008). According to organoleptic result from the present study and reduction of total acceptability score, shelf life of breaded kilka with tempura batter in frozen storage at -18°C was estimated to be 3 months.

Conclusion

According to the results of present study, breaded

kilka with tempura batter, as an alternative product, were found within the acceptable limits during frozen storage for 3 months without undesirable changes of sensory and chemical quality. However, it is suggested that breaded kilka and the effects of frozen storage on chemical and sensory qualities should be further investigated, in a larger scale study, preferably as a socio-economic evaluation, a production feasibility report, and an evaluation of long term frozen storage on quality changes.

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