# The influence of sodium chloride replacement with potassium chloride on quality changes of hot smoked Kilka (*Clupeonella cultriventris caspia*) during storage at ± 4°C

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**Abstract**

The aim of this work was to study the effect of sodium chloride replacement with potassium chloride on quality changes of hot smoked Kilka during storage at ± 4°C. To achieve this, after initial preparation, samples were exposed to brining process in two salts. This study was designed in two stages: in the first stage, different levels of salt replacement were used to select the best smoked treatment based on sensory test. In this stage, treatments were as: control (100% of NaCl), treatment 1 (75%NaCl/25%KCl), treatment 2 (50% NaCl/50%KCl), treatment 3 (25%NaCl/75%KCl), treatment 4 (100% of KCl). Samples were processed at slow and fast speed smoking for about 4 h, cooled and then tested by test panel. In the second stage, biochemical changes of selected treatment (treatment 1) were compared with control samples during storage at ± 4°C for 15 days. There were no differences ($p$>0.05) in content of fat, moisture, ash and TBA indices between control and treatment1. Protein levels and salt intake were significantly ($p$<0.05) increased while the TVB-N and PV indices decreased in treatment1. The results indicated that 25% sodium replacement with potassium is desirable for this product.

**Keywords:** Hot smoking, Kilka fish, Sodium replacement, Lipid oxidation, Potassium chloride.

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## Introduction

Smoking is one of the oldest methods of food preservation and is still widely used in fish processing (Goulas and Kontominas, 2005; Stolyhwo and Sikorski, 2005). The ability of the smoking process to preserve fish is due to the synergistic action of salt incorporation, the preservative effect of smoke compounds, and dehydration (Karaskova *et al*., 2011). Salt is a powerful depressor of water activity ($a_w$) of food and it is convenient to use as an inhibitor of microbial growth (Turan *et al*., 2007). Salting is generally aimed at reducing $a_w$ to inhibit growth of spoilage microorganisms as well as inactivate autolytic enzymes (Doe and Olley, 1990; Ashie *et al*., 1996; Horner, 1997). In addition to extending the shelf life of fresh fish, salting also provides desirable sensory changes (Andres *et al.*, 2005). Nowadays, salting is considered to give specific sensory characteristics to the final product (Esaiassen *et al.*, 2004; Andres *et al*., 2005; Boudhrioua *et al.*, 2009) and in advanced countries the main reason for adding salt to food is only to improve the taste of the product (Razavie Shirazi, 2007). While sodium is essential for normal human functioning, current sodium intakes far exceed recommendations for good health (Brown *et al*., 2009). Excessive sodium intake is associated with an increase in blood pressure, which is a major cause of cardiovascular diseases. It has been estimated that 62% of stroke and 49% of coronary heart disease is caused by high blood pressure (He and MacGregor, 2010). Excess sodium consumption has also been associated with numerous other negative health effects, including gastric cancer (Tsugane *et al*., 2004), decreased bone mineral density (Devine *et al*., 1995) and possibly obesity (He and MacGregor, 2008). Dietary sodium intake is higher than recommended in developed countries (Fuenttes *et al*., 2010). For these reasons, national and international organizations have set targets for a reduction in the sodium consumed in the diet (WHO/FAO, 2003; WHO, 2004). Numerous attempts have been made to study the feasibility of the reduction of sodium, mainly in meat products (Gimeno *et al*., 2001; Gelabert *et al*., 2003; Muguerza *et al*., 2004; Ruusunen and Puolanne, 2005), in cheese (Katsiari *et al.*, 1997a,b), and fish sauces (Sanceda *et al*., 2003). However, sodium replacement related to fish products has only been studied for a few salted products: such as salted cod (Martínez-Alvarez *et al*., 2005; Rodrigues *et al.*, 2005) and smoked sea bass (Fuenttes *et al.*, 2010, 2011). According to the previous researches the partial substitution of NaCl by KCl appears to be the best alternative to reduce sodium content. Indeed both salts have similar properties and potassium intake has not been linked to the development of hypertension and cardiovascular diseases (Kimura *et al*., 2004; Geleijnse *et al*., 2007). Increasing potassium intake leads to a decrease in urinary calcium excretion and

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potentially protects skeletal mass (Lemann *et al*., 1993). Too much potassium in the body could lead to muscle weakness and slow heart rate (Hemmatkhah, 2005). The use of KCl is mainly limited by its bitter taste (Reddy and Marth, 1991). Replacement of sodium chloride with more than 50% of potassium chloride can detract from flavor intensity and produce bitter tastes (Hand *et al*., 1982); however, the replacement level can vary depending on the type of food product. To this end, several researchers have attempted in recent decades to develop acceptable low salt products using NaCl/KCl mixtures. (Fuenttes *et al*., 2010; Fuenttes *et al*., 2011).

Sodium chloride in smoked fish not only contributes to increasing its shelf-life, but also influences its water holding capacity (WHC), fat binding, colour, flavour, and texture; so total or partial substitution of NaCl might lead to changes in these attributes. For this reason, it is important to know how Na replacement affects product quality, in order to establish the feasibility of NaCl reduction. The aim of this work was to study the effect of sodium chloride replacement with potassium chloride on quality changes of hot smoked Kilka during storage at ± 4°C**.**

## Materials and methods

*Raw material*

45 kg of fresh early morning caught Kilka (*C. cultriventris caspia*) was purchased from Bandar Anzali (Guilan Province, Iran) in November 2012. Fish were placed in chilled sea water (C.S.W) boxes (containing 25 % ice powder, 15 % sea water and 60 % fish) and transported to National Fish Processing Research Center (Anzali, Iran) within 2 h. Then they were promptly washed with fresh and cool water to eliminate surface mucus and probable pollutants. Upon arrival in the laboratory they were headed, gutted, and washed.

*Brining and smoking process*

This study was designed in two stages: in the first stage, different levels of salt replacement were used to select best smoked treatment based on sensory tests. Kilka (*C. cultriventris caspia*) were randomly divided into five groups before the salting stage. Treatments were as: control (100% of NaCl), treatment 1 (75%NaCl and 25%KCl), treatment 2 (50%NaCl and 50%KCl), treatment 3 (25%NaCl and 75%KCl), treatment 4 (100% of KCl). The fish were submerged in brine (15 % salt solution) and kept at 5ºC for 3 h. After salting, fishes were rinsed under tap water and placed in the smoker. Treatments were processed at slow and fast speed smoking. The processing time in the kiln was divided into two stages: (1) a preliminary drying (1h) at 40ºC; (2) a smoking and cooking period at 60ºC, 75ºC and 85ºC (3 h). After cooking and cooling, all samples were tested by sensory analysis to select the nominated treatments for the future and immediately packed in polyethylene bags. In the second stage, biochemical changes of the selected treatment (treatment 1) were compared with

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control samples during storage at ± 4°C for 15 days. Chemical analyses were performed at 3 day intervals during storage.

*Chemical composition*

*Determination of proximate:*

The moisture content of flesh was determined by drying to constant weight at 105°C for 24 h according to the AOAC standard method (AOAC, 2005). Crude ash was determined after heating the sample overnight at 550°C (AOAC, 2005). Crude protein content was determined by the Kjeldahl method (AOAC, 2005), employing the 6.25 conversion factor. The fat content of Kilka was determined by a solvent extraction (Submersion) method for fat (crude) in meat and meat products (AOAC, 2005). Salt content in fish muscle was determined by the volumetric method of Volhard (AOAC, 1995). PV value was determined using the method of (AOAC, 2000), PV value was expressed as mmol $O_2$/kg lipid sample. TBA value was determined using the method of (Namaulema *et al*., 1999), and was expressed as mg malondialdehyde/kg sample. Total volatile base nitrogen (TVB-N, mg N/100g) was determined according to AOAC (2005).

Sensory analyses were done, on smoked kilka salted with salt mixture KCl:NaCl and control NaCl including color, odor, taste, texture and total acceptability using an 0-7 point hedonic scale according to ASTM, (1969). In this test, 50 assessors (25 females and 25 males) compared the quality of samples. Less point in each index indicates lower quality of samples.

*Statistical analysis*

Statistical tests were performed using the SPSS computer program (SPSS Statistical Software 16.0). Nonparametric Kruskal-Wallis statistics used to analyze sensory the data. One-way ANOVA analysis of variance was carried out. The difference of means between pairs was resolved by means of confidence intervals using a Tukey test at a level of significance of $p<0.05$.

**Results**

Proximate composition and PV, TBA and TVB-N content of raw kilka are shown in Table 1. The results of the sensory evaluation of all the attributes examined for the new products are shown in Table 2. The mean scores for color of smoked kilka salted with the different salt mixtures were not significantly different among samples ($p>0.05$). However, odor, taste, texture and total acceptability scores were significantly ($p<0.05$) lower for samples with less than 75% NaCl content (treatment 2, 3, 4). There were significant differences in all studied attributes between treatments 2, 3 and 4 with treatment 1 and control samples (Table 2). This result indicates that replacing NaCl with up to 25% KCl does not affect the sensory attributes of smoked Kilka.

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**Table 1: Proximate composition of raw Kilka.**

| Proximate composition (%) | Mean±SD |
|---|---|
| **Moisture** | 75.00 ± 0.28 |
| **Fat** | 6.50 ± 1.14 |
| **Ash** | 3.00 ± 0.48 |
| **Salt** | 0.01 ± 0.00 |
| **Protein** | 15.05 ±0.28 |
| **PV** | 0.10 ±0.00 |
| **TVB-N** | 10.45 ± 0.60 |
| **TBA** | 0.01 ± 0.00 |

**All values are the mean ± standard deviation (n =3).**

**Table 2: Analysis of sensory assessment samples smoked Kilka.**

| Treatments | Overeall acceptable | Texture | Taste | Odour | Colour |
|---|---|---|---|---|---|
| 100% NaCl | $7.00 \pm 0.00^{a}$ | $7.00 \pm 0.00^{a}$ | $7.00 \pm 0.00^{a}$ | $7.00 \pm 0.00^{a}$ | $7.00 \pm 0.00^{a}$ |
| 75%/25%NaCl/KCl | $7.00 \pm 0.00^{a}$ | $7.00 \pm 0.00^{a}$ | $7.00 \pm 0.00^{a}$ | $7.00 \pm 0.00^{a}$ | $7.00 \pm 0.00^{a}$ |
| 50%/50%NaCl/KCl | $3.57 \pm 0.53^{b}$ | $5.20 \pm 0.00^{b}$ | $3.40 \pm 0.73^{b}$ | $7.00 \pm 0.00^{a}$ | $7.00 \pm 0.00^{a}$ |
| 25%/75%NaCl/KCl | $2.80 \pm 0.09^{c}$ | $4.23 \pm 0.13^{c}$ | $1.80 \pm 0.39^{c}$ | $5.37 \pm 0.03^{b}$ | $7.00 \pm 0.00^{a}$ |
| 100%KCl | $0.00 \pm 0.00^{d}$ | $3.58 \pm 0.23^{d}$ | $0.00 \pm 0.00^{d}$ | $4.05 \pm 0.10^{c}$ | $7.00 \pm 0.00^{a}$ |

- Data is expressed as mean ± SD (n=50).
- Means with different lowercase superscripts in each column are significantly different ($p$<0.05).

Therefore, according to the results of sensory analysis, treatment 1 was accepted as the chosen treatment and its chemical changes during the refrigerated storage of control samples were tested during 15 days.

The salt, moisture, protein, fat and ash contents of smoked kilka during 15 days storage at refrigerator are given in Table 3. According to statistical findings, there were significant ($p$<0.05) differences in the protein and salt content between treatment1 and control but no significant ($p$>0.05) differences were found in the fat, ash and moisture content of both smoked kilka during storage.

The TVB-N content significantly increased during storage ($p$<0.05). As it can be seen from Fig. 1, the TVB-N values between treatment1 and control were different significantly ($p$<0.05) from beginning to the end of the storage period.

There was a significant difference ($p$<0.05) in peroxide values (PV) between samples in smoked fish during storage. As it can be seen from Fig. 2, there was a general increase with the storage time for both groups.

Secondary lipid oxidation products measured by the TBA values provided a general and gradual increase with the storage time for both groups. Fig. 3 represents the TBA values. A strong effect of the presence of salt can be concluded since the treatment1 showed in most cases higher TBA values than the other group ($p$<0.05).

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**Table 3: Chemical analysis parameters during storage at 4ºC**.

| Analysis | Group | Storage time (day) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 3 | 6 | 9 | 12 | 15 |
| **Salt** | **A** | 4.12±0.04$^{Ab}$ | 3.44±0.08$^{Bc}$ | 4.64±0.06$^{Ba}$ | 4.80±0.00$^{Aa}$ | 4.09±0.00$^{Ab}$ | 4.01±0.00$^{Ab}$ |
| | **B** | 4.49±0.57$^{Abc}$ | 5.36±0.03$^{Ab}$ | 6.47±0.09$^{Aa}$ | 4.32±0.08$^{Ac}$ | 4.23±0.20$^{Ac}$ | 4.09±0.13$^{Ac}$ |
| **Moisture** | **A** | 49.79±1.45$^{Aa}$ | 49.75±1.48$^{Aa}$ | 49.35±1.49$^{Aa}$ | 49.10±1.70$^{Aa}$ | 47.90±0.56$^{Aa}$ | 47.70±0.56$^{Aa}$ |
| | **B** | 48.04±0.11$^{Aa}$ | 47.30±0.28$^{Aab}$ | 46.96±0.49$^{Ab}$ | 46.52±0.41$^{Abc}$ | 46.32±0.35$^{Abc}$ | 46.05±0.70$^{Ac}$ |
| **Protein** | **A** | 26.20±0.85$^{Bb}$ | 26.29±0.00$^{Bb}$ | 27.21±0.13$^{Ab}$ | 27.30±0.11$^{Ab}$ | 31.19±0.13$^{Aa}$ | 30.35±0.92$^{Ba}$ |
| | **B** | 29.04±0.20$^{Abc}$ | 29.22±0.31$^{Ab}$ | 28.05±0.07$^{Ac}$ | 28.14±0.06$^{Ac}$ | 32.05±0.20$^{Aa}$ | 32.75±0.49$^{Aa}$ |
| **Ash** | **A** | 9.14±0.07$^{Aa}$ | 9.00±0.00$^{Aa}$ | 7.40±0.42$^{Aa}$ | 7.01±0.40$^{Ab}$ | 4.86±0.13$^{Ac}$ | 4.40±0.14$^{Ac}$ |
| | **B** | 10.08±0.32$^{Aa}$ | 9.90±0.28$^{Aab}$ | 7.80±0.99$^{Ab}$ | 6.64±0.40$^{Abc}$ | 5.83±0.66$^{Abc}$ | 5.50±0.42$^{Ac}$ |
| **Fat** | **A** | 10.65±0.77$^{Aa}$ | 11.50±1.55$^{Aa}$ | 11.07±0.39$^{Aa}$ | 11.75±1.20$^{Aa}$ | 11.93±0.52$^{Aa}$ | 13.54±0.21$^{Aa}$ |
| | **B** | 9.05±0.21$^{Aa}$ | 9.25±0.35$^{Aa}$ | 10.65±0.91$^{Aa}$ | 11.30±0.85$^{Aa}$ | 11.55±1.04$^{Aa}$ | 11.29±1.41$^{Aa}$ |

- Data are expressed as means± standard deviation (n=3).
- Means with different uppercase superscripts in each column are significantly different ($p$<0.05).
- Means with different lowercase superscripts in each raw are significantly different ($p$<0.05).
- group A: Control (100% NaCl) group B: treatment1 (25%KCl/ 75% NaCl).

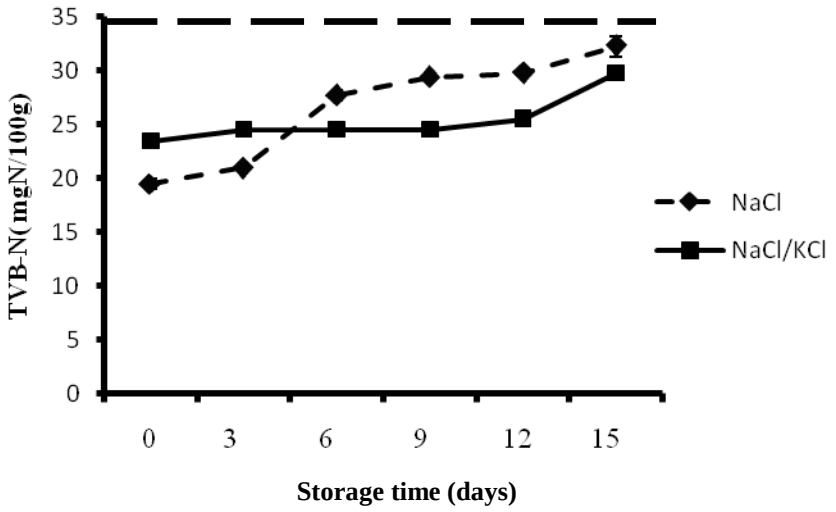


**Figure 1: TVB-N content of smoked kilka brined in different salt compositions**.

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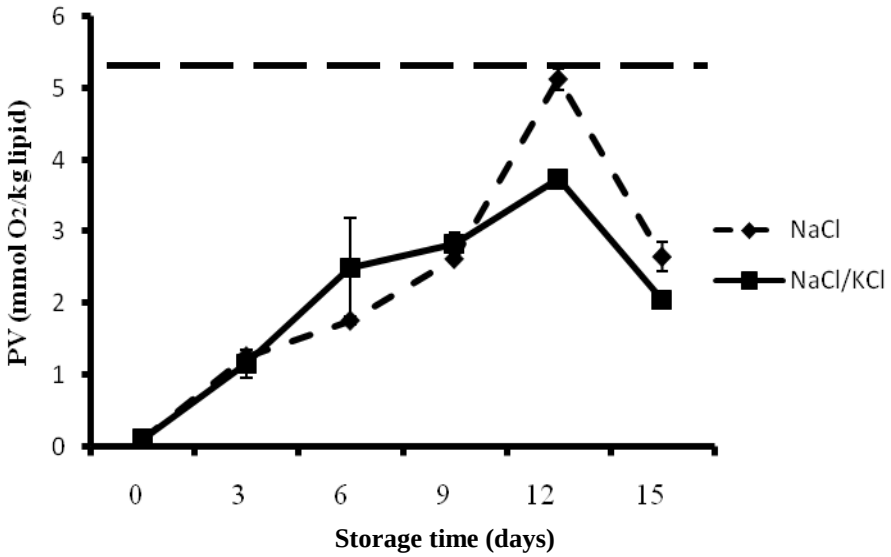


**Figure 2: PV content of smoked kilka brined in different salt compositions**.

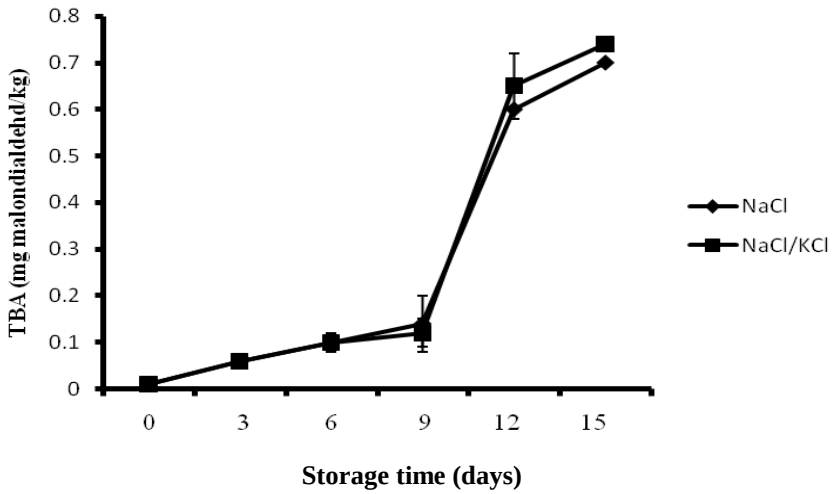


**Figure 3: TBA content of smoked kilka brined in different salt compositions.**

At the end of the experiment, a higher TBA value (0.74 mg malondialdehyde/kg sample) was obtained from samples in treatment 1 ($p$<0.05).

## Discussion

When salt solution or dry salt are used as salting agent, two main simultaneous flows are usually generated; water loss and salt uptake. Diffusion is said to be the most important mass transfer

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mechanism responsible for sodium, potassium and chloride transport. Solutes diffuse from the salting agent and water diffuses out of the fish, due to the differences in concentration and osmotic pressures among inter-cells and salting agent (Raoult-Wack, 1994; Yao and Le Maguer, 1996). A significantly higher salt content was found in the treatment 1versus the control. The higher permeability of K into fish muscle could be related to its lower charge density compared to Na (Blesa *et al*., 2008). Fish salting leads to a reduction in moisture (Jittinandana *et al*., 2002). Moisture content of treatment 1 was less than that of control, however, moisture content of control and treatment1 was not different ($p$>0.05). The salting induces changes in the muscle proteins resulting in changes in texture and water holding capacity (Thorarinsdottir *et al*., 2002; Sannaveerappa *et al*., 2004). Nketsia-Tabiri and Sefa-Dedeh (1995) indicated that denaturation of muscle protein facilitated diffusion of water from fish. Ash content of treatment 1 was much higher than that of control. Water losses associated with brining resulted in increased ash content (Jittinandana *et al*., 2002). Fat content of samples are shown in Table 3. As indicated, fat content in both samples increased by increasing the storage time. Maximum value of fat (13.54%) was observed in control, after 15 days of storage. Most of the water within muscles is located in the myofibrils in the spaces between thin and thick filaments, and the swelling of myofibrils and the WHC is primarily determined by alterations in this interfilament spacing (Offer and Trinick, 1983). During immersion, as salt diffuses into muscle fibers, swelling occurs due to increased electrostatic repulsion, depolymerization of thick filaments and weakening of structural linkages. This indicates that the same mechanisms that give rise to fiber swelling are also responsible for reduced drip loss. Protein content showed an increase during 15 days. Protein content of treatment 1 was higher than that of control samples. Na+ and K+ ion concentrations, which correlate inversely with each one other, mean that when the sodium content was reduced and the potassium content increased, the overall tendency was for the product to be less dehydrated; in other words, moisture was higher but hardness and water-extractable protein (WEP) were lower. Loss of hardness could be a direct consequence of increased moisture, while reduced WEP could be associated with higher K+ content (Martinez-Alvarez *et al*., 2005). KCl has been shown to decrease protein solubility (Grishchenko, 1958; Kolodziejska and Sikorski, 1980), possibly because KCl has a greater capacity to aggregate myosin (Thorarinsdottir *et al*., 2002).

TVB-N content showed increase in both groups during 15 day. This parameter is widely used as an indicator of fish spoilage. The increase in TVB-N content is related to the activity of spoilage bacteria and endogenous enzymes (Özyurt *et al*., 2009). Several authors have reported that this

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parameter increases with the onset of microbial spoilage (Fernández-Segovia *et al*., 2007; Kykkidou *et al*., 2009; Özyurt *et al*., 2009). At the beginning of the storage period, TVB-N values were 19.49 and 23.45 mgN/100 g for control and treatment1 samples, respectively. The TVB-N contents significantly increased during storage ($p$<0.05) in both samples. The TVB-N content of treatment1 remained significantly lower than control samples after the 6th day. This could be associated with lower moisture content, higher salt level in treatment 1 samples than in the corresponding control samples. The TVB-N content of control and treatment1 were significantly lower than the acceptability limit of 35 mg N/100 g of muscle set by the EU (EEC, 1995). The TVB-N values did not exceed 35 mgN/100g after 15 days of storage for treatment1 and control samples.

The stability of lipids in smoked kilka was evaluated by PV and TBA values (Simic and Taylor, 1987). The PV was used to measure the primary lipid oxidation products especially hydroperoxides (Simic and Taylor, 1987). There was a significant difference ($p$<0.05) in peroxide values between control and treatment 1 smoked fish during storage. The PV content of smoked samples in treatment1 remained significantly lower than that in controls of smoked samples at the end of the storage period. NaCl has been shown to catalyze lipid oxidation in muscle tissue including fish (Nambudiry, 1980). Kanner *et al.* (1991) found that the prooxidative activity of NaCl is due to its ability to release iron from heme pigments and other heme binding molecules. Chloride ion can be converted to a radical via a mechanism as observed with myeloperoxidase (Hultin, 1992). It could then be added directly to a double bond or abstract hydrogen (Kanner and Kinsella, 1983). Alternatively, the Na+ may replace the iron from a cellular complex via an ion exchange reaction. The displaced iron may then participate in the initiation of lipid peroxidation (Hultin, 1992). Chaijan *et al*. (2006) reported that the decreased PV was presumed to be due to the decomposition of hydroperoxides. Hydroperoxides break down in several steps, yielding a wide variety of decomposition products including aldehydes (Nawar, 1996). A level of 5 mmol O2/kg lipid has been considered the upper limit above which fishery products are considered unfit for human consumption (Sikorski *et al.*, 1990). Comparison of the different controls and treatment1 showed, in most cases, higher peroxide values for fish samples than other groups ($p$<0.05); a very high value (5.13±0.15 mmol O/kg lipid) was obtained on day 12 for control samples.

The increase in TBARS indicated the formation of secondary lipid oxidation products (Kolakowska, 2002). As can be seen in Fig. 3, there is a trend towards an increase in TBA values up to a certain point during the storage period. The increase in TBA value during the smoking procedure may be attributed to the partial

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dehydration of fish and to the increased oxidation of unsaturated fatty acids as a result of smoking at relatively high temperatures (up to 70ºC) (Yanar *et al*., 2006). Final TBA values of 0.74 and 0.70 mg MDA/kg for smoked fish did not exceed the value of 1–2 mg MDA/kg which is usually regarded as the limit beyond which the fish will normally develop an objectionable odor/taste (Connell, 1990).

The chemical test results of hot smoked kilka had better performance in treatment 1 (lower PV and TVB-N values) compared to the control at the end of storage period. As well as, the results obtained in this research showed no significant effect on ash, fat, moisture and TBA in hot smoked kilka due to replacement with 25% KCl instead of NaCl. Thus, based on the hazards of intake of sodium chloride on human health and to reduce this substance in food, consistent with the results obtained in this research, partial replacement of NaCl with KCl can be done without any negative effect on its chemical properties. As, smoked fish is widely used in European countries and also in north of Iran, hazards can be reduced by reduction of common salt used and replacement of sodium ion with potassium ion to adjust the blood pressure to help consumers.

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