Abstract
However Kilka is a valuable fish in nutritional point of view, but a large part of it used in poultry feed. The main reason is the undesirable odor. In this study Kilka oil was blended with milk at 1% and 2% level and then the mixture spray dried. These encapsulated Kilka oil were added to cheese as a fortificant materials at 5% level. Cheese without encapsulated Kilka oil was as a control treatment. Results showed that there was no significant difference (p>0.05) between color of fortificated cheese with control cheese. There was no significant difference (p>0.05) between odor of cheese with 5% encapsulated Kilka oil that contain 1% Kilka oil (A) with control cheese. There was no significant difference (p>0.05) between flavor of cheese with 5% encapsulated Kilka oil that contain 1% Kilka oil (A) with cheese with 5% encapsulated Kilka oil that contain 2% Kilka oil (B) but there was significant difference (p<0.05) between these two treatments with control cheese. Also, the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content of fortified cheeses had significant difference (p<0.05) with control cheese.

Keywords: cheese, encapsulated, kilka oil, fortification, EPA, DHA

1. Introduction
Cheese is one of the most ancient forms of manufactured food (Fox et al., 1995). Nowadays, cheese consumption is widely spread throughout the world. Technology is needed for gentle processing to retain or even accumulate desired nutrients and to remove undesired compounds (Walther et al., 2008).

The fatty acid profile can be altered by fortification of the dairy product. Oils with more favorable fatty acid composition can be added to the dairy products. For instance, fish oil fortification has a potential to increase the intake of n-3 PUFAs (Astrup et al., 2011; Baum et al., 2012).

Experts recommend that saturated fat should be replaced by unsaturated fat (Erkkilä et al., 2008), either by all kinds of polyunsaturated fatty acids (PUFAs) (Astrup et al., 2011) or n-3 PUFAs (Kris-Etherton & Innis, 2007).

Fish oil represents a functional food ingredient, which contains important components for maintenance good health and prevention of a range of human diseases via its beneficial effects on the heart, brain and nervous system (Wu et al., 2009).

Thus fish oil is a significant source of PUFA. Fish oil is specially known for its high long-chain polyunsaturated fatty acids (LCPUFA) content.

Generally, fish oil in its natural state has a taste and smell that makes it less attractive to consumers. Processing technology to mask the smell and taste in food systems is facing a great challenge.

Interest in the encapsulation of long-chain polyunsaturated omega-3 oils is rapidly growing due to its beneficial health effect. Encapsulation is a rapidly expanding technology with a lot of potentials such as pharmaceutical and food industries (Ritvanen, 2013).

The specific aims were to study the fatty acid profiles of the Kilka oil, sensory characteristics and the chemical composition of cheese which fortified with encapsulated Kilka oil.
2. Materials and Methods

2.1 Fatty Acids Composition Analysis

For fatty acid analysis, oil was trans-esterified to fatty acid methyl esters and then the gas-chromatographic resolution of fatty acid methyl esters was done by gas chromatograph (Agilent 6890N, USA). The characteristic of capillary column was 60 m length, 0.25 mm inner diameter and 0.25 mm film thickness. The injector and detector port temperature was 180 °C and 250 °C. The detector type was flame ionization.

The oven temperature program was initially set at 180 °C for the first 30 minutes, and then increased at a rate of 4 °C/min to 220 °C, where it remained for the last 15 minutes. The total running time of each sample was 55 min. The carrier gas was nitrogen (flow rate: 0.6 ml/min and average velocity: 18 cm/sec). The injector was operated in split mode (100:1 split ratio).

2.2 Emulsion Preparation

Milk was used as the base material for making emulsion. Two treatments of Kilka oil-in-milk emulsions were prepared containing 1 and 2 wt% Kilka oil, 0.07 wt% lecithin and 1.93 wt% pectin.

Refined Kilka oil was supplied from an industrial oil factory (Noshdaroo company) in north of Iran. Emulsions were homogenized at 60 °C and 18 MPa using a Rannie Laboratory Homogenizer (Albertslund, Denmark).

A small spray dryer (Behsozan company, Iran) was used to convert emulsion into encapsulated powder. The inlet temperature was 142 °C and outlet temperature was 70 °C. Powders were collected and stored in glass jars at room temperature for further analysis.

2.3 Production of Fortified Cheese with Encapsulated Kilka Oil

Cheese made using pasteurized milk. Rennet (1%) added to the milk at 40 °C in addition to calcium chloride (0.2%). After curd formation, it cut and whey removed. After salt addition (1.5%), encapsulated Kilka oil was added (5%). Treatments were as follows:

1- Cheese without encapsulated Kilka oil (blank cheese)
2- Cheese with 5% encapsulated Kilka oil that contain 1% Kilka oil (A)
3- Cheese with 5% encapsulated Kilka oil that contain 2% Kilka oil (B)

The ingredients were mixed and after final pressing, cheeses were packed and stored in refrigerator for further analysis.

2.4 Chemical Analysis

The pH values of the samples were measured by means of a glass pH electrode (Metrohm model 744, Switzerland).

For solid matter content, approximately 3 g of cheese samples was placed in an aluminum pan and dried for 3 h at 103±2 °C in oven (Heraeus, Germany) (ISIRI 6629).

Micro kjeldahl (N × 6.37) (Gerhard, Germany) was used for protein content and soxhlet extractor (Gerhard, Germany) applied for fat content.

2.5 Sensory Evaluation

The panel consisted of 20 panelists from the standard organization staff trained to recognize cheeses taste, odor and color. For sensory evaluation, 20 g of each fortified cheese with encapsulated Kilka oil and blank cheese (without encapsulated Kilka oil) were weighed into 3 cup with three-digit coded numbers.

The panelists were asked to score cheeses according to five descriptive terms: extremely dislike, dislike, neither like nor dislike, like, and extremely like (extremely dislike = 1 and extremely like = 5) (Lawless and Hymann, 1998). The assessments were written down using paper forms. The samples were evaluated at room temperature. Sensory evaluation was done after 1, 15 and 30 days after production.

2.6 Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) using the SPSS version 16 to compare the mean values of each treatment. Significant differences between the means of parameters were determined using Duncan’s test (p<0.05). For comparison of sensory attributes concerning ordinal data, Kruskal-Wallis test was used.
3. Results and Discussion

Spray drying was used in this study for encapsulation of Kilka oil. Among the different techniques available, spray drying is the most important technique for the encapsulation of bioactive food ingredients (Desai & Park, 2005). Spray drying is a well-known technology in the food industry and the most commonly used microencapsulation technique. In this study Kilka oil encapsulated with spray dryer. Fatty acids’ profile of Kilka oil was shown in fig 1. Abundant fatty acids of Kilka oil are unsaturated that most of them are fatty acids with one double bond. Another specialty, is omega-3 fatty acids like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Hejazian, 2008).

![Figure 1. Typical Chromatogram of Kilka Oil](image)

Milk and meat fatty acid profiles can be modified as early as in the formation stage, by feed selection and feed fortification. The aim is usually to decrease SFA content and increase the content of PUFAs or n-3 fatty acids. But, large amounts of unsaturated fat in feed may cause adverse effects such as reduced milk yield, fat and protein concentration, shortened shelf life of the end product and off-flavors (Woods and Fearon, 2009).

Another method is fortification of final products with encapsulated fish oil. Long-chain PUFAs are typical in fish. Their aroma is often characterized as fishy. In this study the cheese samples were fortified with 5% encapsulated Kilka oil at two level of 1% and 2% Kilka oil. The fatty acids of blank cheese, cheese fortified with (A) powder and cheese fortified with (B) powder is given in table 1.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Blank cheese</th>
<th>Cheese fortified with (A) powder</th>
<th>Cheese fortified with (B) powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Butyric acid) C4:0</td>
<td>1.65 ± 0.028</td>
<td>1.57 ± 0.014</td>
<td>1.6 ± 0.056</td>
</tr>
<tr>
<td>(Caproic acid) C6:0</td>
<td>1.38 ± 0.014</td>
<td>1.28 ± 0.028</td>
<td>1.34 ± 0.042</td>
</tr>
<tr>
<td>(Caprylic acid) C8:0</td>
<td>1.06 ± 0.056</td>
<td>0.98 ± 0.014</td>
<td>1.30 ± 0.42</td>
</tr>
<tr>
<td>(Capric acid) C10:0</td>
<td>2.97 ± 0.028</td>
<td>2.85 ± 0.042</td>
<td>2.87 ± 0.014</td>
</tr>
<tr>
<td>(Lauric acid) C12:0</td>
<td>3.85 ± 0.014</td>
<td>3.73 ± 0.028</td>
<td>3.70 ± 0.056</td>
</tr>
<tr>
<td>(Myristic acid) C14:0</td>
<td>12.87 ± 0.028</td>
<td>12.65 ± 0.042</td>
<td>12.57 ± 0.014</td>
</tr>
<tr>
<td>(Myristoleic acid) C14:1</td>
<td>1.45 ± 0.028</td>
<td>1.38 ± 0.014</td>
<td>1.41 ± 0.056</td>
</tr>
<tr>
<td>(Palmitic acid) C16:0</td>
<td>32.45 ± 0.014</td>
<td>32.18 ± 0.042</td>
<td>32.02 ± 0.056</td>
</tr>
<tr>
<td>(Palmitoleic acid) C16:1</td>
<td>1.64 ± 0.028</td>
<td>1.65 ± 0.042</td>
<td>1.75 ± 0.056</td>
</tr>
<tr>
<td>(Oleic acid) C18:0</td>
<td>10.34 ± 0.014</td>
<td>10.10 ± 0.042</td>
<td>10.14 ± 0.028</td>
</tr>
<tr>
<td>(Stearic acid) C18:1</td>
<td>23.52 ± 0.028</td>
<td>23.43 ± 0.056</td>
<td>23.46 ± 0.042</td>
</tr>
<tr>
<td>(Linoleic acid) C18:2</td>
<td>3.33 ± 0.028</td>
<td>3.22 ± 0.042</td>
<td>3.39 ± 0.014</td>
</tr>
<tr>
<td>(Linoleic acid) C18:3</td>
<td>0.1 ± 0.028</td>
<td>0.13 ± 0.042</td>
<td>0.17 ± 0.07</td>
</tr>
<tr>
<td>(Arachidic acid) C20:0</td>
<td>0.78 ± 0.014</td>
<td>0.68 ± 0.028</td>
<td>0.77 ± 0.056</td>
</tr>
<tr>
<td>(Eicosenoic acid) C20:1</td>
<td>0 ± 0</td>
<td>0.13 ± 0.014</td>
<td>0.18 ± 0.28</td>
</tr>
<tr>
<td>(Eicosapentaenoic acid) C20:5</td>
<td>0 ± 0</td>
<td>0.2 ± 0.042</td>
<td>0.32 ± 0.056</td>
</tr>
<tr>
<td>(Docosapentaenoic acid) C22:6</td>
<td>0 ± 0</td>
<td>0.37 ± 0.028</td>
<td>0.66 ± 0.042</td>
</tr>
</tbody>
</table>
As it clear from table 1, at the 95% confidence level, there was no significant difference (P-value>0.05) between the mean values of C4:0, C6:0, C8:0, C10:0, C12:0, C14:1, C16:1, C18:1, C18:3, C20:0 fatty acids in the 3 groups of treatments (Blank cheese, Cheese fortified with (A) powder and cheese fortified with (B) powder). The average of fatty acids that had significant difference in 3 treatments, were compared separately.

Comparison of the average content of C14:0 in treatments shown that at the 95% confidence level, there is significant differences between the mean values of fatty acids in treatments. The highest amount of C14:0 was in blank cheese. The amount of C14:0 groups, cheese fortified with (A) powder and cheese fortified with (B) powder were in the second place without significant difference. Also, there is significant difference between the mean values of C16:0 in treatments. The amount of C16:0 in blank cheese had the highest value. The amount of C16:0 in cheese fortified with (A) powder was in the second place and cheese fortified with (B) powder was in third place.

As well, the amount of C18:0 in blank cheese had the highest value. Also, the amount of C18:0 in cheese fortified with (A) powder and cheese fortified with (B) powder were in the second place without any significant difference.

The amount of C18:2 in blank cheese and cheese fortified with (B) powder had the highest value. Also, the amount of C18:2 in cheese fortified with (A) powder located in the second place with significant difference. The amount of C20:1 in cheese fortified with (A) powder and cheese fortified with (B) powder had the highest value with significant difference with blank cheese.

Comparison of the average content of C20:5 and C22:6 in treatments shown that there is significant differences between the mean values of fatty acids in the 3 groups of treatments. The amount of C20:5 in cheese fortified with (A) powder and cheese fortified with (B) powder had the highest value with significant difference with blank cheese. The amount of C22:6 in cheese fortified with (B) powder were the highest.

3.1 Chemical Analysis

Chemical characteristics of produced cheeses were analyzed (table 2). Results show that there wasn’t significant difference between treatments.

Table 2. Chemical characteristics of cheese samples

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Solid matter (%)</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank cheese</td>
<td>35.5 ± 0.30</td>
<td>16 ± 0.2</td>
<td>14.4 ± 0.5</td>
<td>4.8 ± 0.03</td>
</tr>
<tr>
<td>Cheese fortified with (A) powder</td>
<td>35.2 ± 0.33</td>
<td>15.8 ± 0.2</td>
<td>14.4 ± 0.4</td>
<td>4.72 ± 0.03</td>
</tr>
<tr>
<td>Cheese fortified with (B) powder</td>
<td>35.4 ± 0.25</td>
<td>16.4 ± 0.4</td>
<td>14.2 ± 0.3</td>
<td>4.78 ± 0.03</td>
</tr>
</tbody>
</table>

3.2 Sensory Evaluation

The sensory evaluation was performed by using a 5-point hedonic scale. The assessed attributes were color, odor and flavor. Point 1 meant that the subject extremely disliked the sample; point 2 represented dislike of the sample, point 3 meant that the subject neither liking nor disliking the sample, point 4 and 5 represented liked and extremely liked the sample, respectively. Descriptive statistics of taste, color and odor characteristics of treatments was shown in Table 3.

Table 3. Panelists scores for organoleptic assay (taste, color and odor)

<table>
<thead>
<tr>
<th>Cheese</th>
<th>Flavor</th>
<th>Color</th>
<th>Odor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheese fortified with (A) powder</td>
<td>4.6 ± 0.59</td>
<td>5±0</td>
<td>5±0</td>
</tr>
<tr>
<td>Cheese fortified with (B) powder</td>
<td>4.5±0.60</td>
<td>5±0</td>
<td>4.85±0.36</td>
</tr>
<tr>
<td>Blank cheese</td>
<td>5±0</td>
<td>5±0</td>
<td>5±0</td>
</tr>
</tbody>
</table>

As is clear from table 3, from panelists’ point of view, the blank cheese as a control treatment had the best taste. The color of the three groups of treatments was in the same position and the cheese fortified with (A) powder and blank cheese had the best odor without any significant difference.

For comparison of flavor, color and odor, separately, and because of the qualifying data, the Kruskal-Wallis test was used (data was not shown).
There is no significant difference between the colors of three types of cheeses. In other words, the color of the three types of cheese is the same. There is significant difference between flavors of three types of cheese. Given the significance of Kruskal-Wallis test, followed by Mann-Whitney test was used to form homogeneous groups.

As is clear from Table 3, the color does not differ in three types of cheese. The score of fortified cheeses in regard to color was similar to cheese without fish oil (without significant difference).

As is clear from Table 3, the odor of the cheese fortified with (A) powder and blank cheese had the best odor. The odor of cheese fortified with (B) powder is also in second place.

As the flavor of foods fortified with fish oil is the most sensitive indicator of their sensorial quality (Huss, 1988), it investigated by panelists, also. Results showed that the best flavor is related to blank cheese. The taste of cheese fortified with (A) powder and cheese fortified with (B) powder were in second place.

General(total score of odor, color and taste) descriptive statistic of blank cheese, cheese fortified with (A) powder and cheese fortified with (B) powder at 1, 15 and 30 days after production are shown in table 4.

There is significant difference between the general characteristics of three types of cheese. Given the significance of Kruskal-Wallis test, followed by Mann-Whitney test was used to form homogeneous groups. As is clear from table 4, the best general features related to the blank cheese. Also, the general characteristics of cheese fortified with (A) powder and cheese fortified with (B) powder ranked second place without any significant difference.

<table>
<thead>
<tr>
<th>Day</th>
<th>Cheese fortified with (A) powder</th>
<th>Cheese fortified with (B) powder</th>
<th>Blank cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.6 ± 0.59</td>
<td>14.35 ± 0.58</td>
<td>15 ± 0</td>
</tr>
<tr>
<td>15</td>
<td>14.39 ± 0.51</td>
<td>14.15 ± 0.57</td>
<td>15 ± 0</td>
</tr>
<tr>
<td>30</td>
<td>14.11 ± 0.56</td>
<td>14.05 ± 0.58</td>
<td>14.9 ± 0.2</td>
</tr>
</tbody>
</table>

Ye et al. (2009), recorded that the food with even very low fish flavor threshold significantly decreases its sensorial quality but they showed that the difference in the sensory perceptions of processed cheeses containing a low level of fish oil (5g kg-1) and the check sample containing no fish oil was not significant, perhaps because of masking property of fermented dairy products. They reported that no fishy off-flavor was detected based on the used level of fish oil (4 g kg-1) and the sensory results showed that the fortified UF-Feta cheese had more than 70% acceptance and desirability. Kolanowski et al., (2007) reported that in semi-solid dairy products like yogurt and cream, the fish oil level should be restricted between 1-5g kg-1.

Farbod et al., (2013), that studied fortified feta cheese with fish oil, recorded that based on the results of taste-panelists, the texture and flavor of the cheeses were rated favorably after 30th day of storage. Overall, the taste panelists could not detect the fishy, oxidized or rancid flavor in fortified low fat UF-feta cheese samples.

Kolanowski and Weisbrodt (2007) studied the effects of fish oil fortification on several dairy products. In their study the sensory quality of the dairy products (yoghurt, cream, butter and variable cheeses) decreased as the amount of fish oil increased. The storage stability was affected in cheese but not in butter. On the other hand, the effect of fish oil fortification on sensory quality was lowest in process cheeses. Martini et al.(2009) studied cheddar with three levels of DHA and EPA fortification. The fishy off-flavor was detected with the highest fortification level, but decreased when cheese was aged 3 months.

4. Conclusions

Encapsulation of Kilka fish oil masks its unpleasant odor and taste. With adding Kilka oil to milk and then converting it to powder with spray dryer, it is possible to benefit omega-3 fatty acids, EPA and DHA. We can fortify some foods like cheese with encapsulated Kilka oil. Cheese as a dairy product consume daily, via fortification its nutritional value will increase. With this strategy, Kilka oil as a valuable source of omega-3 fatty acids will be utilized for human and not just for feed.

References


Baum, S. J., Kris-Etherton, P. M., Willett, W. C., Lichtenstein, A. H., Rudell, L., Maki, K. C., … Block, R. C.


**Copyrights**

Copyright for this article is retained by the author(s), with first publication rights granted to the journal. This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).