

## **Determination of optimum level of omega-3 fish oil plus vitamin E and their effects on oxidative and sensory shelf stability in a traditional Persian ice cream formulation using a computer-aided statistical programme**

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### **Abstract**

Fishery products are the richest dietary sources of polyunsaturated fatty acids  $\omega$ -3. These functional ingredients can be applied for fortification of food products. A computer-assisted programme was applied for the product development and to investigate the influence of the different ratios of  $\omega$ -3 fish oil on overall acceptance and stability of the ice cream. Consequently, ice cream with 2.5% fish oil was selected as an optimum prototype. Sensory changes and the oxidative stability (FFA, peroxide, anisidine, and TOTOX values) of the control and fortified samples were investigated during 4 months storage at  $-18^{\circ}\text{C}$ . The same level of moisture (73-74%), and protein (3.0-3.2%) contents, acidity (0.08-0.09), solid-non-fat (2.60-2.70%) and pH (6.7-6.8) was found in fortified ice cream and the control samples. The fat content of the fortified sample (3.3%) was higher than what found in the control (2.1%). Both prototypes were chemically stable and had similar sensory attributes after the production and after 4 months of storage. FFA (0.08-0.15%), peroxides (0.21-0.51 meq  $\text{kg}^{-1}$ ), anisidine (4.61-8.12) and TOTOX (5.14-8.75) values of the control and fortifies samples were within the acceptable levels indicating the stability of the prototypes during frozen storage. According to the results, it could be concluded that nutritional values of ice cream can be enhanced by the fortification with  $\omega$ -3 fish oil. However, to commercialize such product consumers' acceptance and market research should be investigated.

**Keywords:** Fortified ice cream,  $\omega$ -3 fish oil, Oxidative stability, Sensory quality

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## Introduction

The benefits of polyunsaturated fatty acids  $\omega$ -3 (PUFAs) to human health have been widely reported (Ruxton *et al.*, 2004). Fishery products are the richest dietary sources of these fatty acids (Belitz *et al.*, 2009). Therefore, the increasing consumption of  $\omega$ -3 fish oil has been recommended by the health authorities (Gidding *et al.*, 2005; Bakar *et al.*, 2010). In many developing countries due to a low acceptance of fish odor and flavor, the average fish intake is currently far below the recommended 2–3 fish servings per week (Shaviklo, 2011). Therefore, fortification of existing products with  $\omega$ -3 fish oil, which is an innovative way to increase the  $\omega$ -3 PUFA intake, can improve the level and profile of PUFA in the diet and in the tissues of the human body and may provide nutritive foods for growing health-conscious consumers especially children (Kolanowski and Laufenberg, 2006; Ganesan *et al.*, 2014; Shaviklo *et al.*, 2014, 2015).

Despite the health benefits of  $\omega$ -3 PUFA intake, the  $\omega$ -3 PUFA can easily be formed into peroxides and other lipid oxidation by-products which are unhealthy for human (Belitz *et al.*, 2009; Ganesan *et al.*, 2014). Therefore, fortification of food products with  $\omega$ -3 fish oil may negatively impact sensory properties of the prototypes, depending on the amount of added fish oil (Ganesan *et al.*, 2014; Shaviklo *et al.*, 2014, 2015). Generally, the fishy odor and flavor of fresh fish is a desirable characteristic for its palatability but fishy flavor is not a desirable attribute

in the other food products (Shaviklo, 2011). Fish oil has a taste and odor that most people hate it, and this has limited its application in food product development (Kolanowski and Weibrodt, 2007). Therefore, it has been largely distributed within capsules (Ganesan *et al.*, 2014). Unacceptable off-flavor of the product incorporated with fish oil is gradually accelerated during storage due to oxidative deterioration. However, food products fortified with fish oil should be strongly protected against oxidation during processing, packaging, storage and distribution to eliminate all factors promoting this process (Kolanowski and Laufenberg, 2006; Shaviklo *et al.*, 2014; Zhong *et al.*, 2018). Applying unhydrogenated, well-refined and stabilized fish oil for food fortification has been recommended for food fortification (Kolanowski and Weibrodt, 2007).

On the other hand, the market for functional foods enriched with  $\omega$ -3 fish oil is growing, especially in the developed countries (Ganesan *et al.*, 2014; Shaviklo *et al.*, 2015). Only a few  $\omega$ -3 fish-enriched food products including dairy products have been placed in the market. The most suitable foods for fortification with fishery derived ingredients are milk and dairy products that are consumed and stored for a short time at low temperature in packages with no permeability of air and light. Among dairy food products, ice cream and frozen desserts are the most suitable excising foods for fortification with  $\omega$ -3 fish oil because they are produced and stored in cold

and frozen conditions (Nielsen *et al.*, 2009; Shaviklo *et al.*, 2011a; Ullah *et al.*, 2017).

Successful production of some food prototypes (bread, spread, biscuits, cake, ice cream, milkshake powder, milk, yoghurt, cheese, butter, drinking yoghurt, meat, sausages, egg, popcorns, corn snacks) fortified with  $\omega$ -3 fish oil was already reported (Kolanowski and Weibrodt, 2007; Martini *et al.*, 2009; Ganesan *et al.*, 2014; Shaviklo *et al.*, 2014, 2015; Hejazian *et al.*, 2016; Renuka *et al.*, 2016; Ullah *et al.*, 2017; Zhong *et al.*, 2018). The researchers noted negative sensory effects of fish oil on the sensory attributes of the prototypes if the fish oil is incorporated in inappropriate levels. However, this defect is correlated to the product types (Nielsen *et al.*, 2009; Shaviklo *et al.*, 2011b; Nawas *et al.*, 2017).

The amount of fishery derived ingredients in food formulation usually ranged from 1 up to 10% depending on the type of food and the form of the fish oil (Kolanowski and Weibrodt, 2007; Ganesan *et al.*, 2014; Shaviklo *et al.*, 2014, 2015; Renuka *et al.*, 2016). However, the highest fortification level, which does not influence the sensory quality of dairy products, depends on their solidity, fat content and presence of flavoring agent. Moreover, the level of fortification can be increased by using the addition of flavorings or seasonings into fortified foods (Martini *et al.*, 2009; Ganesan *et al.*, 2014). However, the taste of fish oil can be masked partially with flavoring agents such as citrus, peppermint, vanillin, rose flower extract and so on (Shaviklo

*et al.*, 2011a,b). They can effectively mask the off-flavor during processing and storage of the product, also in the presence of oxygen. Flavoring makes sensory attributes of fish oil-fortified food stable during long term storage (Shaviklo *et al.*, 2014, 2015; Zhong *et al.*, 2018).

A major target group for ice cream products are children (Kilaram and Chandan, 2007; Mahrous and Abd-El-Salam, 2014). Therefore, the fortification with omega-3 fish oil may provide a healthy product for them and could be an option for increasing children's nutritional intake (Shaviklo *et al.*, 2014). However, there is still a lack of published scientific works, evaluating the optimum level of fish oil in ice cream and sensory shelf stability of the prototypes. Therefore, the objectives of this work were to determine the optimum level of  $\omega$ -3 fish oil in a traditional Persian ice cream and evaluating its influence on sensory quality and stability of the product during 4 months frozen storage.

## Materials and methods

### Materials

Omega-3 fish oil flavored with lemon (Vitabiotics, Norway) containing 968 mg EPA, 878 mg DHA and 11 mg vitamins E (per 10 ml) was purchased locally. Pasteurized cow milk with 3.4% protein, 2.5% fat and other ingredients such as saffron, sugar, stabilizer, crow-foot (*Orchis mavulata*) and rose flower extract were provided locally (Rasht, Guilan, Iran).

### *Experimental design*

A computer-aided statistical programme (Design-Expert, Version 6.0.2, State-Ease, Minneapolis, MN) and related experimental model (D-optimal Mixture Design) was used for experimental design. The statistical programme optimized the level of  $\omega$ -3 fish oil in ice cream formulation through constructing as well as analyzing the design. The suggestion of low level and high level of  $\omega$ -3 fish oil in the ice cream formula (0-5%) was based on the pretest assessments. The odor, flavor, and overall acceptance were also determined as the responses. Accordingly, 10 formulations have been developed. The upper and lower levels of the fish oil and milk cream were 0–5%. The same amount of the other ingredients was applied for products development. Ice cream mixes including all ingredients except  $\omega$ -3 fish oil was pasteurized at  $73\pm 1$  °C for 10 min, homogenized through a blender (Model MK7OH, Koppens Tetra Laval Food, Netherlands) for 2 min, cooled to 4 °C and stored for 4 h for aging. Omega-3 fish oil was added to the mix and the mixture was fed to a batch ice cream maker (Carpigiani 191 SA, Bologna, Italy) and then blended for 25-30 min (Shaviklo *et al.*, 2011a). The ice cream was packaged in 50 g polyethylene cups for sensory evaluation and analytical tests. Each cup with lid was then kept frozen at -18°C. During the storage time the fortified ice cream and the control prototypes were removed randomly from the freezer for monthly

physicochemical and sensory measurements.

### *Physicochemical and microbial analysis*

Protein, fat, moisture, total solids-not-fat, acidity and pH were determined according to national standard methods (ISIRI, 2008). Free fatty acids, peroxide value (PV) and anisidine value (AV) were determined according to the AOCS methods (AOCS, 1995). The total oxidation value (TOTOX) was calculated as:  $TOTOX\ value = 2(PV) + AV$  (Shahidi and Wanasundara, 2002). Total plate count, Coliforms, *E-coli*, and *Salmonella spp.* were measured in 3 samples based on national standard methods (ISIRI, 2007).

### *Sensory analysis*

An expert sensory panel including 8 (4 females) trained and experienced experts was used for selecting the optimized fortified ice cream. The evaluation and selection of the prototypes were based upon the highest scores of sensory liking (odor, flavor and overall acceptance). To assess each sensory attribute a 15-cm unstructured line scale was used (Meilgaard *et al.*, 2007).

Sensory evaluation of the fortified ice cream and control during 4 months frozen storage was done by 8 panelists (4 females) with the average age of 25 years. Panelist's selection and evaluation of their performance were based on ISO Standard (ISO 1993, 2007, 2012). Panelists were trained during 2 sessions to evaluate ice cream prototypes using Quantitative

Descriptive Analysis (QDA) method (Meilgaard *et al.*, 2007). Differences in sensory attributes of the prototypes were detected by the panelists ( $p < 0.05$ ). A list of sensory vocabulary (Table 1) to describe the intensity of each sensory attribute was adapted from Shaviklo *et al.* (2011a). Test samples were

numbered with 3-digit random numbers and presented to the panelists in individual booths. The panelists evaluated the ice creams without information about the formula using score sheets.

**Table 1: Sensory vocabulary for control and ice cream fortified with omega-3 fish oil\***

Sensory	Scale (0-100)	Definition
<b>Odor</b>		
Additives	None   Much	Odor of additives used in ice cream formulation (saffron, rose flower extract)
Fish	None   Much	Fish odor
Rancid	None   Much	Rancidity odor
Off odor	None   Much	Unusual odor
<b>Appearance</b>		
Color	Little   Much	Golden yellow color
<b>Flavor</b>		
Additives	None   Much	Flavor of additives used in ice cream formulation (saffron, rose flower extract)
Rancid	None   Much	Rancidity flavor
Fish	None   Much	Cooked fish flavor
Off flavor	None   Much	Unusual flavor

\*adapted from Shaviklo *et al.* (2011a).

### Statistical analysis

The statistical program NCSS 2007 (NCSS, Statistical Software, Kaysville, UT) was also used for the statistical analysis of physicochemical and microbial results. Student's t-test was used to determine whether there was a difference in physicochemical properties of the control and fortified ice cream. The results were shown as a mean  $\pm$  standard deviation. The program was used to calculate multiple comparisons using Duncan's test to determine if the prototypes were different. Significance of difference was defined at the 5% level. Panel Check software (version V1.3.2, Matforsk, Ås, Norway) was applied to

monitor panelists' performance and to analyze sensory data using principal component analysis (PCA).

### Results

The experimental design with independent variables and the related observed responses of the ice cream samples are given in Table 2. The changes in selected responses for both control and fortified ice cream as given by the two component design are shown in mixture response surface contour plots (Figure 1). The responses of these models can be plotted as a function of two components in the mixture, keeping the total as 5%.

**Table 2: Design of experiment for optimizing 2 main components of ice creams (omega-3 fish oil and milk cream) and related responses.**

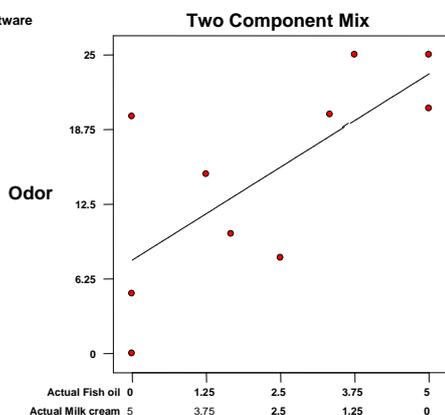
Run	Component 1: fish oil (%)	Component 2: milk cream (%)	Response 1: Odor	Response 2: Flavor	Response 6: Overall acceptance
1	5.00	0.00	20.5	45.6	60.8
2	1.25	3.75	21.6	65.4	85.6
3	3.75	1.25	65.1	26.9	67.7
4	0.00	5.00	42.3	46.6	78.5
5	1.70	3.30	19.9	56.4	76.5
6	2.50	2.50	78.9	78.8	98.1
7	3.30	1.70	69.7	64.3	75.5
8	0.00	5.00	87.1	77.4	79.7
9	5.00	0.00	76.5	43.5	57.5
10	0.00	5.00	60.4	80.3	67.3

Fig. 1, displays that the mixture of fish oil and milk cream, affected sensory attributes of the ice cream significantly. It revealed that fish oil played an important role in ice cream odor, flavor and the overall acceptance. As the level of fish oil increased, the acceptance of the ice cream decreased. In sensory-based optimization process, acceptance scores indicate the degree of dislike to liking and can be applied to decision making (Kristbergsson, 2001). However, applying such data for new food product development and selection of the optimized product depend on the specifications of the product and comments on expert panel (Shaviklo *et al.*, 2013).

The desirable maximization of the fortified ice cream was carried out by numerical techniques using mathematical optimization procedure of the Design Expert Software Package. Optimization criterion was based on the highest level of sensory scores, including acceptance which is thought to be the most important parameter in food fortification studies (Varghese and Pandey, 2015). The solution was obtained using the software, which sought to maximize the desirability function by being at random starting points and proceeding on a path of the steepest slope to a maximum. The best among them was taken as optimum.

Design-Expert® Software

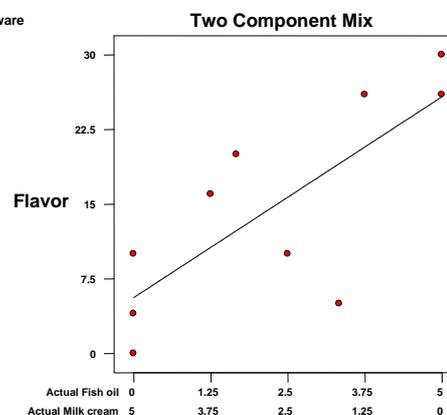
Odor  
 ● Design Points  
 X1 = A: Fish oil  
 X2 = B: Milk cream



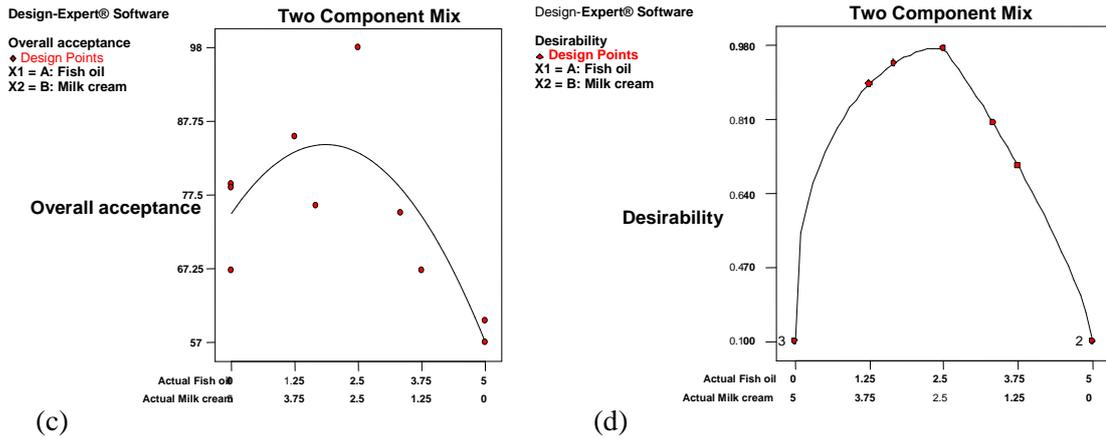
(a)

Design-Expert® Software

Flavor  
 ● Design Points  
 X1 = A: Fish oil  
 X2 = B: Milk cream



(b)



**Figure 1: Variations in odor (a), flavor (b), overall acceptance (c) and desirability (d) of ice cream with different levels of ω-3 fish oil and milk cream in the mixture.**

The desirability model (Figure 1d) was obtained from the software (Design-Expert) calculation. It has been recommended a mixture containing 2.5% fish oil, 2.5% milk cream and 95% other ingredients. The proposed mixture was developed for reconfirmation. No significant differences were observed between the predicted response values and sensory evaluation scores provided by the

panelists (Table 3) indicating the capability of 2-mixture design in food product development. Therefore, fortified ice cream and the control samples were prepared based on the optimized mixture, packed and stored 4 months in the freezer (-18°C) to investigate the stability and quality changes of the products.

**Table 3: Predicted values (suggested by the software) and actual values obtained from the panelists for sensory responses of ice cream fortified with 2.5% fish oil.**

Response	Predicted values	Actual obtained values	P value
Odor	57.51	52.89	NS
Flavor	66.63	63.02	NS
Overall acceptance	83.46	80.98	NS

NS: not statistically significant differences ( $p>0.05$ )

All products had the same levels ( $p>0.05$ ) of acidity (0.08-0.09) and pH (6.7-6.8), protein (3.0-3.2%) and solid-non-fat (2.60-2.70%) contents. The fortified ice cream had significantly higher fat content (3.3%) than that found in the control (2.1%). No significant differences were found for pH of both control and fortified ice cream samples within frozen storage.

Results of oxidative stability of ice cream fortified with fish oil are presented in Table 4. In this study, FFAs of the fortified ice cream and control samples were 0.10 and 0.08%, just after production, respectively. FFAs of fortified ice cream and control slowly increased to 0.15 and 0.14%, respectively, after 4 months storage but were not significant.

**Table 4: Oxidative stability of ice cream samples.**

Prototypes	Storage time (month)	Free fatty acids (%Oleic Acid)	Peroxide value (meq kg <sup>-1</sup> )	Anisidine value	TOTOX value
Control*	0	0.08 ± 0.01 <sup>d</sup>	0.21 ± 0.03 <sup>b</sup>	4.72 ± 0.19 <sup>c</sup>	5.14 ± 0.11 <sup>c</sup>
	1	0.11 ± 0.02 <sup>c</sup>	0.33 ± 0.05 <sup>b</sup>	4.82 ± 0.24 <sup>c</sup>	5.48 ± 0.21 <sup>c</sup>
	2	0.11 ± 0.06 <sup>c</sup>	0.41 ± 0.07 <sup>a</sup>	6.11 ± 0.52 <sup>b</sup>	6.93 ± 0.42 <sup>b</sup>
	4	0.14 ± 0.04 <sup>a</sup>	0.34 ± 0.02 <sup>b</sup>	7.39 ± 0.31 <sup>a</sup>	8.07 ± 0.39 <sup>a</sup>
Fortified**	0	0.10 ± 0.02 <sup>c</sup>	0.25 ± 0.04 <sup>b</sup>	4.75 ± 0.08 <sup>c</sup>	5.25 ± 0.13 <sup>c</sup>
	1	0.12 ± 0.03 <sup>b</sup>	0.27 ± 0.07 <sup>b</sup>	4.61 ± 0.21 <sup>c</sup>	5.15 ± 0.15 <sup>c</sup>
	2	0.14 ± 0.01 <sup>a</sup>	0.51 ± 0.09 <sup>a</sup>	7.23 ± 0.29 <sup>a</sup>	8.25 ± 0.10 <sup>a</sup>
	4	0.15 ± 0.01 <sup>a</sup>	0.33 ± 0.09 <sup>b</sup>	8.12 ± 0.29 <sup>a</sup>	8.75 ± 0.19 <sup>a</sup>

Different lowercase superscript letters in the same column indicate significant differences among products. \*Ice cream with 5% milk cream, \*\* Ice cream with 2.5% fish oil, 2.5% milk cream

The peroxide value of control and fortified ice cream were 0.21 and 0.25 (meq kg<sup>-1</sup>) after production respectively, but increased during the storage time to 0.33-0.34 (meq kg<sup>-1</sup>) which was not significant. The anisidine values of control and fortified samples were 4.72-4.75 at the beginning, but increased slightly to 7.39-8.12 at the end of the study. No significant differences were detected in anisidine values between control and fortified ice cream after 4 month storage.

Total plate count was less than 10<sup>2</sup> log cfu g<sup>-1</sup> in the samples. *Coliforms* were less than 10 cfu g<sup>-1</sup> and *E. coli* was 0 cfu g<sup>-1</sup> and *Salmonella* spp. was 0 cfu g<sup>-1</sup>.

Results from the analysis of variance (ANOVA) of sensory attributes of fortified ice cream and control during 4 months storage are presented in Table 5. All sample groups had the same sensory properties after production and within 4 months frozen storage.

**Table 5: Average flavor scores (scale: 0-100) for fortified ice cream with 2.5% fish oil omega-3 fish oil during 0-4 months storage at -18°C.**

Sample	O. additives	O. rancid	O. fish	Off-odor	Color	F. additives	F. rancid	F. fish	Off-flavor
C0	13.21	0.0	0.0 <sup>d</sup>	0.0 <sup>c</sup>	46.21	47.28 <sup>a</sup>	0.0	0.0 <sup>c</sup>	0.0 <sup>c</sup>
C1	13.12	0.0	0.0 <sup>d</sup>	0.0 <sup>c</sup>	45.12	45.32 <sup>a</sup>	0.0	0.0 <sup>c</sup>	0.0 <sup>c</sup>
C2	19.35	0.0	0.0 <sup>d</sup>	0.0 <sup>c</sup>	46.02	38.41 <sup>a</sup>	0.0	0.0 <sup>c</sup>	2.01 <sup>c</sup>
C4	15.21	0.0	0.0 <sup>d</sup>	0.0 <sup>c</sup>	50.31	35.26 <sup>ab</sup>	0.0	0.0 <sup>c</sup>	3.25 <sup>c</sup>
F0	11.01	0.0	0.0 <sup>d</sup>	7.35 <sup>b</sup>	52.56	11.25 <sup>c</sup>	0.0	13.02 <sup>b</sup>	17.56 <sup>b</sup>
F1	13.25	0.0	5.21 <sup>bc</sup>	8.65 <sup>b</sup>	53.02	12.36 <sup>c</sup>	0.0	14.12 <sup>b</sup>	18.60 <sup>b</sup>
F2	11.41	0.0	10.28 <sup>b</sup>	17.21 <sup>a</sup>	53.71	14.50 <sup>c</sup>	0.0	16.56 <sup>b</sup>	24.57 <sup>b</sup>
F4	18.65	0.0	37.56 <sup>a</sup>	35.14 <sup>a</sup>	53.50	18.20 <sup>c</sup>	0.0	50.40 <sup>a</sup>	49.45 <sup>a</sup>
Sig.	NS	NS	p<0.05	p<0.05	NS	p<0.05	NS	p<0.05	p<0.05

Values are means of 16 evaluations.

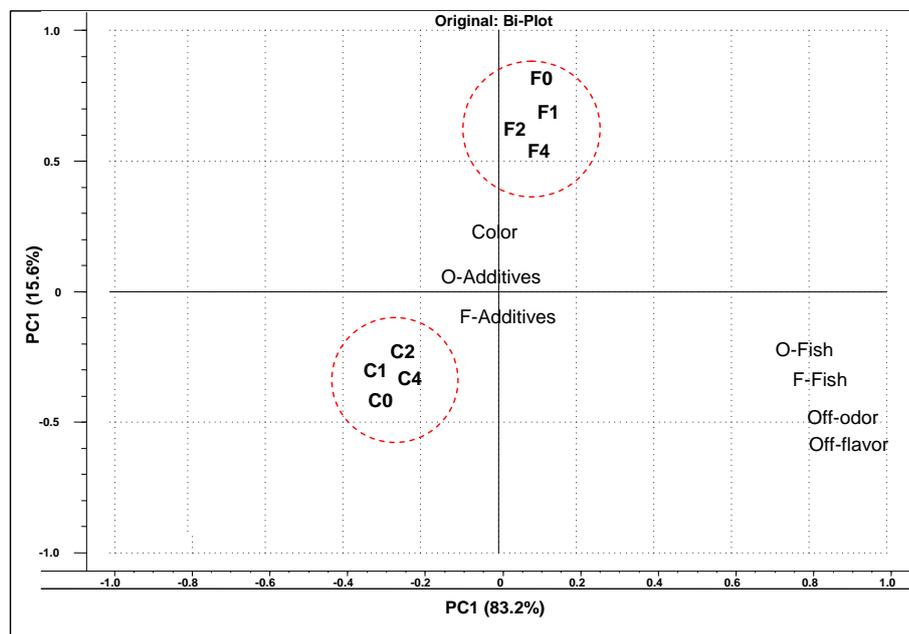
Different lowercase superscript letters in the same column indicate significant differences among products. (C) Ice cream without omega-3 fish oil (control

sample). (F) Fortified ice cream with 2.5 % fish oil. O: odor; F: flavor. Numbers 0 to 4 represent storage time

(month). Sig. Significant. NS=Not significant ( $p>0.05$ ).

Storage time increased additives and fish odors, off-odor and rancid, fish flavors and off-flavors after 4 month storage. No Significant differences ( $p>0.05$ ) were detected between products during storage. Multivariate analysis of the sensory data presented that 99% of variations between the samples were explained by the first 2

principal components. Identified zones on the PCA Bi-Plot describe 2 groups of products with specific attributes (Fig. 2). All products stored for 0 to 4 months were grouped in the central part of the plot with similarities in color and additives odor and flavor. Negative attributes "i.e." fish odor and flavor and off-odor/flavor was grouped in lower right side of the plot.



**Figure 2: Principal Component Analysis (PCA) describing sensory characteristics of ice cream stored 4 months at  $-18^{\circ}\text{C}$  as evaluated by trained sensory panelists. (C) Control (F) Fortified ice cream with 2.5% omega-3 fish oil. O: odor, F: flavor. Numbers 0 to 4 indicate storage months.**

## Discussion

FFAs are developed because of fat hydrolysis and some factors such as moisture, storage time and temperature, lipases, and metal ions (Shahidi, 2005; Rahman *et al.*, 2014). The low content of FFAs in fortified ice creams was due to the lower free fatty acids in fish oil. Similar results were reported for FFA content in ice cream fortified with omega-3 fatty acids reported by Ullah

*et al.* (2017). It has been reported that FFA values greater than 3% are inedible and may develop oxidation products and can influence the sensory attributes of the foods (Özyurt *et al.*, 2013).

Hydroperoxides which are the products of primary oxidation process of food, are measured by the PV (Belitz *et al.*, 2009). It has been reported that food products with PV below  $5 \text{ meq kg}^{-1}$

<sup>1</sup> may still be odorless if secondary oxidation has not been started. If oxidation is more advanced (PV between 5-10 meq kg<sup>-1</sup>) the PV may be relatively low but the food will be obviously rancid (Health Canada, 2009). The secondary stage of oxidation occurs when the hydroperoxides decompose to form carbonyls and other components, in particular aldehydes. They develop a rancid smell in the food and they are measured by anisidine value (AV) (Shahidi and Wanasundara, 2002; Belitz *et al.*, 2009). Fish oil as recommended by Health Canada (2009) should have an AV below 20.

On the other hand, peroxide is decomposed and their levels decrease after reaching a stable concentration. This can result in misleading interpretation of lipid oxidation data. Therefore, assessment of PV, AV and calculation of total oxidation (TOTIOX) values are important for a precise measurement of lipid oxidation (Shahidi and Wanasundara, 2002). The TOTOX value describes the lipid oxidation products and provides adequate information about the oxidation state of a product (Wai *et al.*, 2009). According to Health Canada (2009) a TOTOX value of 26 for fish oil has been recommended as a maximum limit. This result may be important for food product development because sensory quality of the food is considered acceptable when TOTOX value is below 26. Therefore, antioxidant and/or cold storage may be necessary to slow oxidative deterioration in food systems (Zhong *et al.*, 2018).

Increasing of peroxide and other products from the secondary and tertiary stages of auto-oxidation values in non-frozen dairy products fortified with fish oil such as yoghurt, milk, cream cheese, and drinking yoghurt during storage has been reported previously (Gonzalez *et al.*, 2003; Timm-Heinrich *et al.*, 2004; Let *et al.*, 2005; Jacobsen *et al.*, 2006; Estrada *et al.*, 2011; Horn *et al.*, 2012; Ghorbanzade *et al.*, 2017; Nawas *et al.*, 2017; Ullah *et al.*, 2017; Zhong *et al.*, 2018). PV, AV and TOTOX values of fortified ice cream were below the maximum recommendations limit (Health Canada, 2009). Similar results were reported by Ullah *et al.* (2017) for ice cream fortified with omega-3 fatty acids, by Ghorbanzade *et al.*, (2017) for fortified yogurt with fish oil and by Özyurt *et al.* (2013) for fish oil products.

However, in this study PV of ice cream prototypes was lower than what reported by Nielsen *et al.* (2009) for fish oil enriched drinking yogurt and by Nielsen and Jacobsen, (2009) for developing fish-oil-enriched energy bar. PV, AV, and TOTOX values were stable in the fortified ice cream because of the presence of vitamin E in the fish oil and frozen storage conditions of the ice cream samples. The antioxidant potential of the vitamin E is high, and it also protected the product against oxidation processes. Similar results were reported by Mahrous and Abd-El-Salam (2014) for developing a functional frozen yoghurt fortified with  $\omega$ -3 and vitamin E.

On the other hand, lipid oxidation is pH dependent, especially in the presence of proteins, since pH may affect the charge of the proteins (Motalebi Moghanjoghi *et al.*, 2015). This could either lead to increased repulsion or attraction between proteins and metal ions, depending on the pH in the emulsion and the isoelectric point of the proteins as stated by Nielsen *et al.* (2009). In this study pH values of ice cream products were stable due to frozen storage of the prototypes which was previously been suggested by Kilaram and Chandan (2007) and Ullah *et al.* (2017). The ingredients in the fortified ice cream samples did not appear to affect oxidation which has been evidenced previously by Nielsen *et al.* (2009) and Shaviklo *et al.* (2014, 2015).

Therefore, to develop food fortified with acceptable level of fish oil, application of high purity fish oils which are generally low in autoxidation products and also possess less intense fishy flavor (Nielsen *et al.*, 2009; Zhong *et al.*, 2018), applying natural antioxidant such as vitamin E (Mahrous and Abd-El- Salam, 2014) and, using flavoring ingredients (Nielsen *et al.*, 2009; Shaviklo *et al.*, 2014, 2015) are recommended.

The absence of *E. coli* and *Salmonella* spp. in the ice cream samples was approved the prototypes safety for sensory evaluation (ISIRI, 2007). The rancid off-flavors occurred not only because of lipolysis, but also because of proteolysis in ice cream prototypes. Even small amount of compounds resulted from lipolysis and

proteolysis including aldehydes, ketones, and alcohols can affect severely sensory attributes of the food products. Rancid odor or flavor in enriched ice creams at the end of the study was probably caused by hydrolytic rancidity of milk fat or lipid oxidation of  $\omega$ -3 fish oil (Kolanowski and Weibrodt, 2007). Fish off-odor/ flavor note typically results from oxidation of omega-3 PUFAs and is regarded as particularly objectionable in fortified milk and dairy products (Jacobsen *et al.*, 2008). Even though fish oil may not smell when incorporating to foods, it often becomes odorous during storage, and exhibits an extremely deteriorated odor and flavor. Consequently if fortified food with fish oil is not stabilized its quality deteriorates rapidly (Ullah *et al.*, 2017). Even with a good fish oil quality, oxidative deterioration occurred within 2 weeks (Kolanowski and Weibrodt, 2007).

This study revealed that the presence of vitamin E in fish oil could prevent lipid oxidation processes in fortified ice cream. On the other hand applying flavorings agents "*i.e.*" rose flower extract and saffron could masked negative attributes "*i.e.*" fish odor and flavor in  $\omega$ -3 fish oil incorporated fish oil. They can also mask fish oil deterioration flavor in fortified foods (Shaviklo *et al.*, 2015). However, the risk of oxidation in dairy products can be controlled by using natural antioxidant and storage conditions as mentioned before (Jacobsen *et al.*, 2008).

This study noted that it is possible to produce an ice cream fortified with the  $\omega$ -3 PUFA from fish oil with acceptable and shelf stable sensory characteristics. Ice cream fortified with  $\omega$ -3 fish oil can be a novel functional dessert with high nutritional value giving a well accepted traditional product additional health benefits. This could be a way to combine the consumer market for ice cream with the potential health benefits of  $\omega$ -3 fish oil. However, for countries with a low fish intake, an ice cream fortified with  $\omega$ -3 fish oil seems to be a very good vehicle for ensuring adequate intake of  $\omega$ -3 PUFA because the product is stored in the freezer and lipid oxidation in this condition is very low. It can be concluded that fortified ice cream with  $\omega$ -3 fish oil might have much more effect on consumer health especially on children.

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