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Development of an omega- 3 fortified ice cream by using ground flaxseeds and microencapsulated fish oil as the source of omega-3

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Abstract
This study was conducted to develop omega-3 fortified ice cream by using ground flaxseed and micro encapsulated fish oil (MEFO) as the source of omega-3. From each omega–3 source, nine formulations were developed by changing the percentages of omega-3 (15% of daily intake (DI), 25% of DI, 35% of DI) and the percentage of Stabilizer/Emulsifier (S/E) system (0.2, 0.3 and 0.5%) and were compared with a commercial ice cream via sensory/chemical evaluations. From viscosity and flow rate of the aged mixes from each stabilizer system and omega-3 source, 6 samples were selected and from sensory evaluation it was found, 0.3% S/E and 25% of DI of omega-3 containing ice cream mixes from flaxseed and MEFO had the highest preference. From the added flavors, the most accepted flavored ice cream mix from flaxseed and MEFO incorporated ice cream was the mango flavor. All the physiochemical parameters of the selected ice cream mixes complied with the SLS 223:2017 standard and it were concluded that flaxseed and MEFO can be incorporated for ice cream successfully.

Keywords: omega-3, Ice cream, flaxseed, micro encapsulated fish oil

1. Introduction

Ice cream is one of the most widely consumed foods in the frozen dairy dessert category [1]. Over the last 2 decades, ice cream science and technology has undergone a remarkable progress exploring and understanding structure-texture-storage stability interactions on a mechanistic basis. This has not been limited for the textural and sensory property development, but for the improvement of health related and nutritional aspects [2]. The favorable effects of conjugated linoleic acid (CLA), omega-3 fatty acids, dietary fiber, and antioxidants on human health may prompt the increase of their concentrations in frozen desserts [1].

Omega-3 contains special types of Poly Unsaturated Fatty Acids such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and α-linolenic acid (ALA). Omega-3 fatty acids are essential nutrients that promote a healthy lifestyle. Human consumption of omega-3 fatty acid is proven to decrease the incidence of cardiovascular disease, reduce inflammation and prevent certain chronic diseases such as diabetes, hypertension, cancer, autoimmune diseases and arthritis [3, 4, 5]. Recently, several applications of omega-3 rich-oils such as fish, flaxseed, rice bran, and algal oil in frozen dairy desserts were launched [6, 7, 8, 9].

Flaxseed oil contains mostly of omega-3 and partly of omega-6 fatty acids [10]. Flaxseed oil which is rich in nutraceutical omega-3 fatty acid has been reported to be a beneficial substitute for milk fat in ice cream [11]. Sea fish including herring, tuna, cod, menhaden, etc., especially fish oils is the richest dietary source of omega-3 Long Chain Poly Unsaturated Fatty Acids [12]. But due to the high degree of unsaturation, when incorporated in to food, with time unacceptable fishy off flavors may tend to develop due to oxidative deterioration [13]. Micro encapsulation is believed to protect fish oil against oxidation by limiting undesirable influences of the environment, i.e., oxygen, light, humidity, etc., [14, 15]. Products which are stored under frozen (-18 ^{o}C) condition are not affected by fatty acid oxidation during storage. Therefore, the low temperature storage condition of ice cream is ideal for omega-3 incorporation as it stabilizes the oil [16].

This study was conducted to formulate omega-3 fortified ice cream for the health conscious ice cream market. Hence, this study aims to determine the physicochemical and sensory effects of omega-3 fortified ice creams, identification of the optimum levels of flaxseeds and microencapsulated fish oil incorporation in terms of sensory properties and structural properties of an ice cream. Apart from that, the chemical, physical and microbiological characters of ice cream and their stability after being incorporated in each formulated ice cream were analyzed for a period of 24 weeks.

2. Materials and Methods

2.1 Ice cream processing

The ice cream mix consist 9% (w/w) fat (milk fat from Abewela, Srilanka), ground flaxseed (Fabrique aux, Canada) and microencapsulated fish oil (MEFO) (BASF Corporation, USA), 10.6% (w/w) milk solids non fat (MSNF) ,16% (w/w) sugar, Stabilizer/Emulsifier mix (Cremodan), Vanilla, Mango and Strawberry essence (Sri Lanka) and water were used for the preparation of 500 g of each formulation based in the Figure 1.

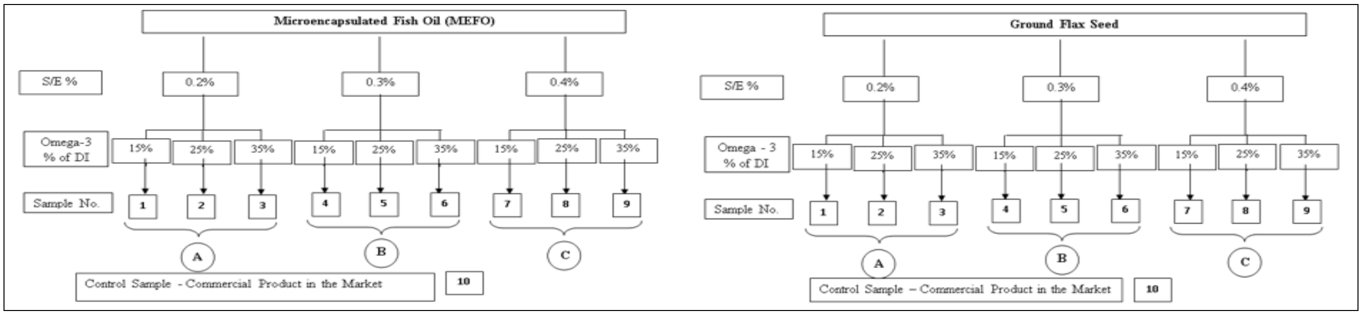

Fig 1: Statistical Design of the Experiment

The combination of different proportions of omega-3 source was given as % DI: % DI with the following order; 15%, 25%, 35% and Stabilizer/Emulsifier (S/E) mix was given as % (w/w): % S/E with the following order; 0.2%, 0.3% and 0.4%. All ingredients were mixed and pasturized at 83°C for 25s. And hormogenization was carried out using digital homogenizer (FJ-200, China) with 3000 rpm for 5 min, followed by 2000 rpm for 5 min. The ice cream mix was cooled and stored overnight at 4°C. The mixes were whipped in an ice cream maker (Gelato, ICM 15) for 30 min. The whipped mixes were stored in the freezer at -18 °C for 24 h.

2.2 Physical analyses to select best formulations

Ice-cream samples were analyzed in triplicate. The best formulation based on the % of S/E systems from each omega-3 sources were determined by analyzing the viscosity and the flow rate. The viscosity was determined using DV-E Brook Field viscometer where 200 ml of ice cream mixture at 25 °C was allowed to stabilize and at 50 rpm with spindle No.02. The flow rate was measured by time taken to collect 100 ml of the aged ice cream mix.

The selected formulation from each S/E system from both omega-3 sources were organoleptically examined by twelve trained panelists and eighteen untrained panelists through consumer hedonic test methods. The samples were stored overnight in refrigerator at -18°C prior to sensory testing and were served at room temperature (~25°C). Each panelist was provided with a set of three ice cream samples from each category (MEFO incorporated and flaxseeds incorporated) in two phases.

The products were assessed for smoothness, degree of mouth coating, degree of melting before consumption and overall acceptability to select best formulation based on the structural properties of the ice cream. For the three selected ice cream mixes from each omega-3 sources**,** mango, vanilla and strawberry flavor were added separately to determine the best flavor profile to be used.

The flavored samples were also subjected to sensory evaluation by the same panelist, for taste, odor, appearance and overall acceptability. The best flavored ice cream mix from both omega-3 sources were selected and analyzed for the physiochemical, proximate composition and microbiological properties along with the commercial ice cream in the market.

2.3 Physiochemical properties

Meltdown test was carried out based on a modified method of Roland *et al.* [17] and Goh *et al.* [16]. Over run % (SLS 223:2017), the titratable acidity (SLS 735: Part 2), total solids (SLS 735: Part 5), MSNF (SLS 223:2017) were determined according to SLS 223:2017 standard for Ice cream [18]. Protein content (Kjeldahl method), fat content (AOAC 2000.18, Gerber Method), crude fiber, total ash and mineral content (Ca, Mg, K) of ice cream was determined by standard AOAC Official Methods [19].

For the analysis of shelf life, Aerobic Plate Count (APC) SLS 516: Part 1, total *E. coli* / Coliform (SLS 516 Part 3/ Section 1) [20] and the peroxide value (AOAC 965.33) were determined weekly for a period of 24 weeks.

2.4 Statistical analysis

Sensory evaluation non-parametric data were statistically analyzed using Kruskal-Wallis test at 95% confidence interval by using Minitab-17 software and all the parametric measurements were taken in triplicates and reported as mean ± Standard deviation.

3. Results and Discussion

3.1 Selection of the best formulation based on the viscosity and the flow rate.

3.1.1 MEFO incorporated Ice cream

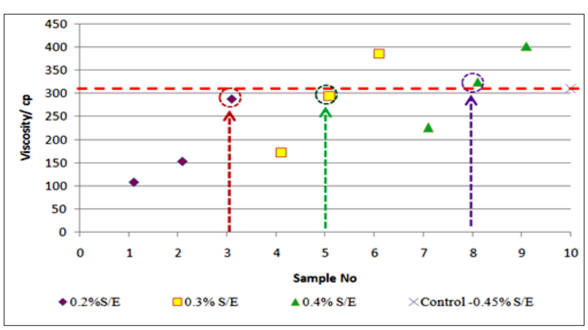

Fig 2: Variation of Viscosity with the Sample

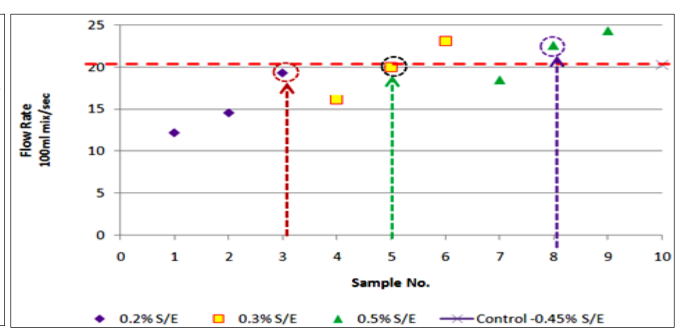

Fig 3: Variation of flow rate with the Sample

As indicated in figure 2 and 3, according to the results obtained from viscosity and flow rate measurements, sample 3 (0.2% S/E and 35% of DI of omega-3), Sample 5 (0.3% S/E and 25% of DI of omega-3) and sample 8 (0.4% S/E and 25% of DI of omega-3) of MEFO incorporated ice cream mixtures showed values similar to the commercial ice cream sample (Sample 10). A certain level of viscosity is essential for proper whipping and retention of air, and for good body and texture in ice cream [1].

3.1.2 Flaxseed incorporated ice cream

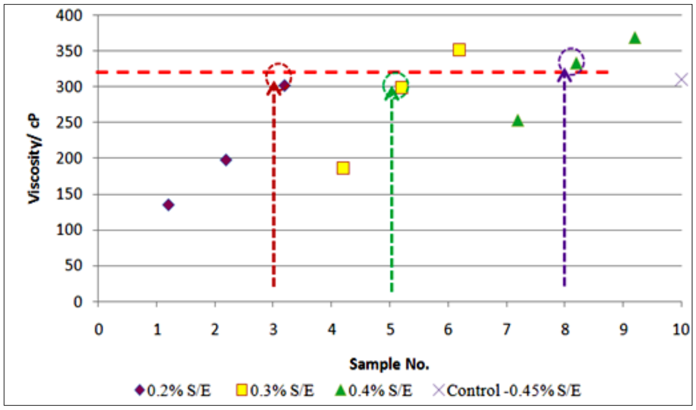

Fig 4: Variation of Viscosity with the Sample

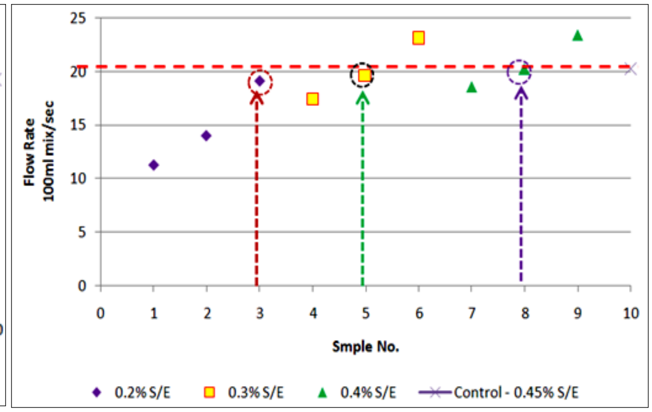

Fig 5: Variation of flow rate with the Sample

Simillar to MEFO incorporated ice cream, sample 3 (0.2% S/E and 35% of DI of omega-3), sample 5 (0.3% S/E and 25% of DI of omega-3) and sample 8 (0.4% S/E and 25% of DI of omega-3) of the flaxseed incorporated ice cream mixtures showed similar viscosity and flow rate values to the commercial ice cream (Sample 10).

Therefore 3rd, 5th and 8th formulations from both MEFO incorporated and flaxseed incorporated ice creams were selected for the sensory evaluation to select best formulation for the addition of the flavours.

3.2 Sensory Evaluation

3.2.1 MEFO incorporated ice cream

Table 1: Sensory attribute comparison for MEFO incorporated ice cream as the source of omega-3

Sample code	Average Rank				
	Viscosity	Smoothness	Mouth coating	Melting before consumption	Overall acceptability
521 (Sample 3)	43.90^{b}	50.7^{a}	41.70^{b}	40.80^{b}	43.40^{b}
684 (Sample 5)	31.50^{b}	27.7^{b}	30.20^{b}	29.70^{b}	24.60^{c}
712 (Sample 8)	61.20^{a}	58.2^{a}	64.70^{a}	65.90^{a}	68.50^{a}

Values in the columns with different letters are significantly different from each other ($p<0.05$).

From the results shown in Table 1, the outcome of consumer hedonic test had suggested that there was significant difference ($p<0.05$) in term of each attributes among all ice cream samples. The highest average rank for all the sensory attributes was obtained for the sample 5 (0.3% S/E and 25% of DI of omega-3) showing consumers high acceptability. The evaluation revealed that the amount of microencapsulated fish oil substitution was positively correlated to the function of emulsifier stabilizer system and also sample coded 521 and 684 in poor acceptability due to inadequate of emulsifier stabilizer function to retain final structure of the product.

3.2.2 Flaxseed incorporated ice cream

Table 2: Sensory attribute comparison for flaxseeds incorporated ice cream as the source of omega-3.

Sample code	Average Rank				
	Viscosity	Smoothness	Mouth coating	Melting before consumption	Overall acceptability
562 (Sample 3)	33.00^{b}	25.30^{c}	30.00^{b}	54.90^{a}	42.60^{b}
621 (Sample 5)	62.00^{a}	63.80^{a}	65.10^{a}	61.00^{a}	66.20^{a}
792 (Sample 8)	41.50^{b}	47.40^{b}	41.40^{b}	20.60^{b}	27.80^{b}

Values in the columns with different letters are significantly different from each other ($p<0.05$).

According to Table 2, for the faxseed incorporated ice cream, the outcome of consumer hedonic test had suggested that there was significant difference ($p<0.05$) in term of each attributes among all ice cream samples. Sample 5 (0.3% S/E and 25% of

DI of omega-3) has obtained the highest average rank values for all sensory attributes. It can be assumed that the Sample 3 (0.2% S/E) is low in acceptability due to inadequate of emulsifier stabilizer function to retain final structure of the product. And sample 8 (05% S/E System) is also low in acceptability due to high emulsifier/stabilizer level.

3.3 Selection of the best flavor profile

For the selection of the most suitable flavor to be added to the selected omega-3 incorporated ice cream mixes, (MEFO - 0.3% S/E and 25% of DI of omega-3 and Flaxseed - 0.3% S/E and 25% of DI of omega-3), samples were prepared separately by adding mango, vanilla and strawberry flavors.

3.3.1 MEFO incorporated ice cream

Table 3: Sensory attribute comparison for MEFO incorporated ice cream to select the best flavor profile

Sample code	Average Rank			
	Taste	Odor	Appearance	Overall acceptability
452	25.20 [b]	28.40 [b]	27.70 [b]	24.90 [c]
582	63.50 [a]	58.60 [a]	60.90 [a]	67.50 [a]
627	47.90 [a]	49.50 [a]	48.00 [a]	44.10 [b]

Values in the columns with different letters are significantly different from each other ($p<0.05$).

521 – Vanilla flavored MEFO ice cream, 684 - Mango flavored MEFO ice cream, 712 - Strawberry flavored MEFO ice cream

From the results shown in Table 3, the outcome of consumer hedonic test had suggested that there was significant difference ($p<0.05$) between the overall acceptability of the 3 flavors. But for taste odour and appearance there was no significant difference between the mango and strawberry flavored ice cream mixes and there was a significant difference between mango and vanilla and strawberry and vanilla flavored ice cream mixes. By considering the overall acceptability parameter, mango flavored ice cream mix had the highest average value and it can be concluded that for the MEFO incorporated ice cream mix, the most suitable flavor to be added is the mango flavor. This must be due to mango flavor can effectively mask that fishy odor given by MEFO. The fishy flavor from MEFO was prominent in vanilla flavored ice cream, and which was not commonly accepted by majority of the panelists and was least preferred by the panelists.

3.3. Flaxseed incorporated ice cream

Table 4: Sensory attribute comparison for flax seeds incorporated ice cream to select the best flavor profile.

Sample code	Average Rank			
	Taste	Odor	Appearance	Overall acceptability
487	52.30 [a]	59.90[a]	58.20[a]	51.00[a]
507	54.20 [a]	42.30[b]	53.00[ab]	49.50[b]
692	30.00 [b]	34.30[b]	25.40[b]	36.00[c]

Values in the columns with different letters are significantly different from each other ($p<0.05$).

487 – Vanilla flavored Flaxseed incorporated ice cream, 507 - Mango flavored, Flaxseed incorporated ice cream, 692 - Strawberry flavored, and Flaxseed incorporated ice cream

From the results shown in Table 4, there is no significant difference between the taste of vanilla and mango flavored ice cream mixes but there is a significant difference between vanilla and strawberry and mango and strawberry flavored ice cream. The highest average rank for the taste was recorded in Mango flavored ice cream and for the odor, appearance and over all acceptability, the highest average rank was recorded for the vanilla flavored ice cream mix. Therefore by considering all the sensory parameters, it can also be concluded that for the flaxseed incorporated ice cream, mango flavor is the best flavor to be added for the consumer acceptance.

3.4 Physicochemical properties of the selected flavored omega-3 incorporated ice cream

Table 5: Physicochemical attributes of final products versus commercial mango flavored Ice Cream (IC)

Parameter	Commercial IC	MEFO added IC	Flaxseed added IC
Total solids % (by mass)	35.15 ± 1.0	40.54 ± 0.07	39.24 ± 0.10
MSNF % (by mass)	10.39 ± 1.0	10.90 ± 0.15	10.96 ± 0.17
Acidity % (as lactic acid)	0.24 ± 0.02	0.19 ± 0.00	0.20 ± 0.01
Overrun % (By mass)	100 ± 1.0	98.64 ± 0.70	85.65 ± 1.65

According to the SLSI standard for ice cream (SLS 223:2017), the minimum requirement of total solids, fat and MSNF by mass are 32%, 08% and 08% respectively. Also the acidity as lactic acid should not be higher than 0.25% by mass. According to analyzed results the developed products were in acceptable levels in compliance with SLSI standards. When comparing the developed products with commercial sample there is no much difference in total solids, MSNF, fat, protein and ash content.

As expected the total solids content for ice-cream samples in this study has increased slightly in developed ice creams than control sample. In addition, results showed that total solids were slightly higher in MEFO added ice cream compared to the flaxseeds added ice cream. This could be due to the requirement of higher mass of MEFO than flaxseeds requirement to complete 25% of DI value. But both developed ice creams comply with the SLS standards.

The apparent acidity of ice cream mix was due to the milk proteins, mineral salts and dissolved carbon dioxide [21]. The acidity was highest in the commercial ice cream and lowest in the MEFO incorporated ice cream.

Overrun of ice cream mainly depends upon fatty acid composition of fat used in ice cream [6]. The overrun % in both flax seed and MEFO incorporated sample was lower than commercial sample. Addition of unsaturated fatty acids in ice cream results in lower overrun of ice cream [22]. The overrun% was lowest in the flaxseeds incorporated sample and the reason may be due to the flaxseed particles.

Table 6: Proximate composition of Ice cream formulations

Parameter	Commercial IC	MEFO added IC	Flaxseed added IC
Fat- Gerber % (by mass)	8.93 ± 0.16	9.30 ± 0.10	9.03 ± 0.16
Total Fat % (by mass)	8.99 ±0.1	9.0 ± 0.1	9.03 ± 0.07
Fibre % (by mass)	0.0	0.0	0.58 ± 0.01
Protein % (by mass)	3.76 ± 0.1	3.89 ± 0.2	4.08 ± 0.1
Ash % (by mass)	0.91 ± 0.02	0.96 ± 0.06	0.96 ± 0.03
Minerals (mg/100g)			
Ca	35.98	43.09	40.69
K	214.03	297.32	285.30
Mg	1.32	3.32	1.49

Based on the data in Table 6, incorporation of Omega-3 sources has increased the protein and the total fat content of the omega-3 incorporated ice creams. Fiber was not present in the commercial and MEFO incorporated ice creams but was present in flaxseed incorporated ice cream as ground flaxseeds were added. This can be considered as a value addition other than omega-3 fortification in developed omega-3 fortified ice cream. But this can negatively affect the consumer preference as well as the textural characters compared to the commercially available ice cream. And also the mineral content was higher in the omega-3 fortified ice cream samples than the commercial sample.

3.5 Meltdown characteristics

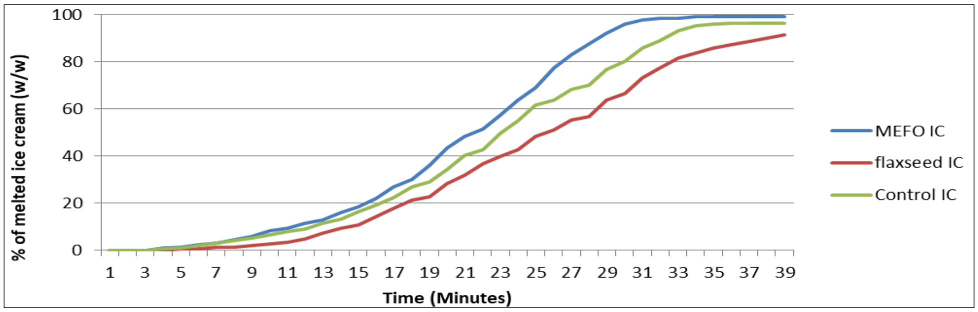

Fig 6: Graphical representation of meltdown characteristics of ice cream at 20°C.

Melting rate has the greatest significance to the consumer. A fast-melting product is undesirable as it is readily susceptible to heat shocks. Slow melting is often desirable as it causes slow release of delicate flavors [1]. According to the Figure 6, MEFO incorporated ice cream has the highest meltdown rate while the Flaxseed incorporated ice cream has the lowest meltdown rate. According to the proximate composition analysis, the highest protein content (4.08 ± 0.1 %) was recorded for the Flaxseed incorporated ice cream. The water holding capacity of proteins leads to enhanced viscosity in the mix and increases the meltdown time of ice cream [1].

3.6 Shelf life study
3.6.1 Variation of Peroxide Value

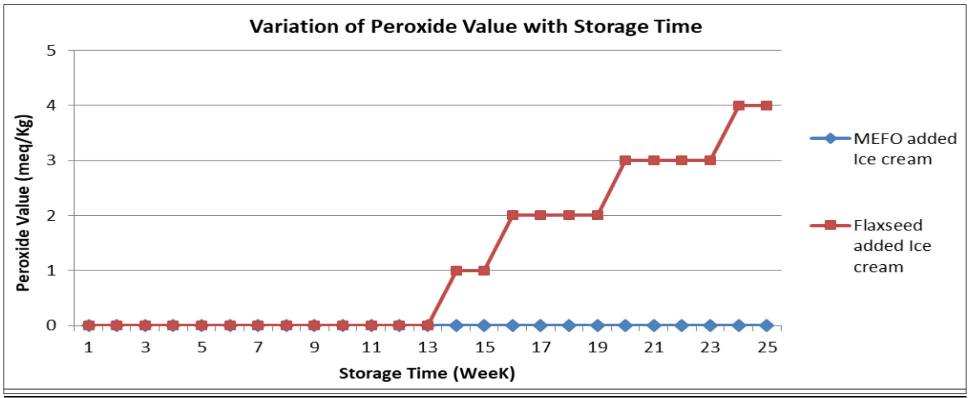

Fig 7: Graphical presentation of Peroxide value Vs storage time of ice cream

The shelf life of a frozen dairy dessert can be evaluated using its microbiological parameters and rancidity development with time. For issues of oxidative rancidity, Peroxide Value (PV) can be used as an indicator. This method measures peroxides and other products of fat oxidation [23]. The PV is an indicator of oxidative rancidity which creates an off-smell of the product. An off-smell caused by rancid taste and smell become noticeable when the peroxide values are between 20 and 40 meq/kg [24]. PV of ice cream increases during the storage period [6]. In the present work, peroxide values of flax seeds incorporated ice cream has slightly increased up to 4 meq/kg after 6 months and the PV was zero for the MEFO incorporated ice cream through-out the period. Gonzalez et al. [6] also reported the PV of control ice cream to be 4.12–8.06 meq/kg fat, whereas modified ice cream containing oleic acid gave a PV of 2.91–7.77 meq/kg fat during the 2 months of storage.

3.6.2 Variation of microbiological parameters

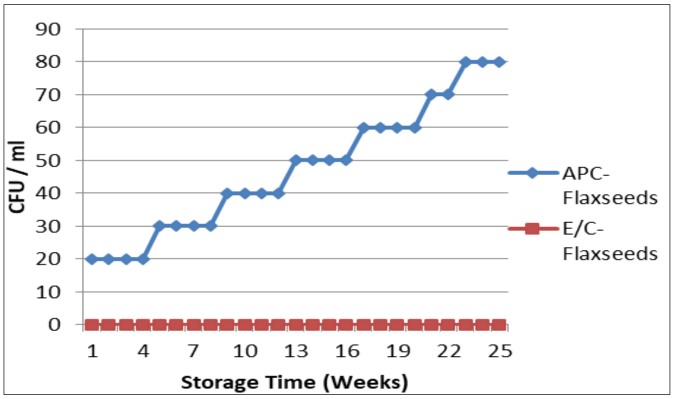

Fig 8: Variation of APC and *E.coil* /Coliform with Time in Flaxseed Incorporated IC

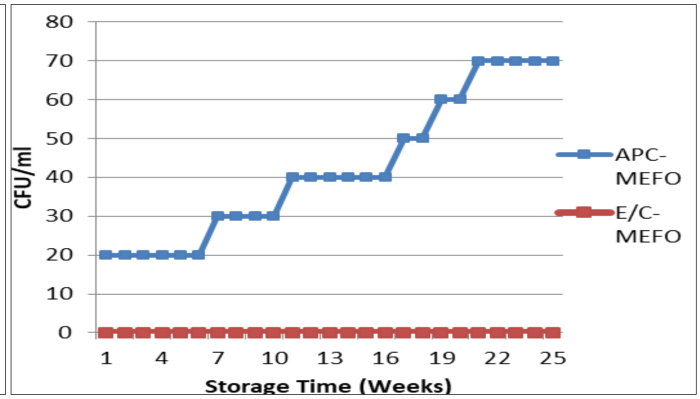

Fig 9: Variation of APC and *E.coil* /Coliform with time in MEFO Incorporated IC

Consumer confidence in the microbiological quality and safety of frozen desserts is one of the main areas to be concerned in frozen dessert industry [1]. According to Figure 8 and 9, very low levels of APCs were observed in both products and *E. coli* was not found in any of the ice cream during the storage period of 6 months and the microbiological counts comply with the SLS 223:2017 standards. Therefore both products are microbiologically safe for the human consumption after a storage period of 24 weeks.

4. Conclusion

Incorporation of ground flaxseeds and MEFO as the sources of omega-3 to ice cream was successful with 0.3% Stabilizer/Emulsifier Systems and omega-3 source to complete 25% of Daily Intake of omega-3. The mango flavor was the most preferred flavor for the flaxseed and MEFO incorporated ice cream. The developed ice creams satisfied the SLSI requirements. By adding micro encapsulated flaxseed oil instead of ground flaxseed, can improve the textural characteristics of the developed ice cream.

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