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MICROENCAPSULATED MIXTURE OF FISH OIL AND FORTIFIED IN ICE CREAM

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ABSTRACT

In recent years there has been considerable efforts to rearrange fatty acids composition of dairy products to improve the long-term health of consumers. One of the efforts is to fortify essential fatty acids in ice cream. The objective of this study was to find out the fatty acids profile of either Selaroides spp, Clarias sp and Thunnus sp fish oil, microencapsulated fish oil and ice cream fortified with this fish oil mixture. Selaroides spp, Clarias sp and Thunnus sp used as raw material were obtained from Palu, Central Sulawesi. The study was caried out using Nested Block Design as the experimental design. The microencapsulated fish oil was prepared using freeze drying and spray drying method. The variables measured were fatty acids profile, microencapsulated fish oil and ice cream fortified with microencapsulated fish oil. The research results showed that SFA, MUFA and PUFA contents of fish oil mixture of Selaroides spp and Clarias sp using freeze drying method were 53.74%, 32.20% and 1.91%, while using spray drying method were 61.30%, 38.70% and 0% (not detected), respectively. It can be concluded that freeze drying method from the point of view of total PUFA is the best method for mincroencapsulation process of this fish oil mixture. Fortification of 15 g (w/w) microencapsulated fish oil in ice cream could reduced SFA and increased the MUFA and PUFA.

Key words*: fish oil, microencapsulation, spray drying, freeze drying, ice cream.*

INTRODUCTION

Ice cream is a dairy product made by the process of freezing and agitation on materials consisting of milk and dairy products, sweeteners, stabilizers, emulsifiers, as well as a flavor enhancer. Currently ice cream is a food product that is popular in the community and it usually consumed as a snack (dessert), but the ice cream fortified with essential fatty acids like omega 3, omega 6 and omega 9 are still rare. Essential fatty acids are fats that are needed by the body and have the function to support the cardiovascular system, reproductive, immune and nervous system. Another function of essential fatty acids also can produce prostaglandin that can regulate the body functions such as heart rate, blood pressure, blood clotting, fertility, conception and plays a role in immune function by regulating inflammation and encouraging the body to fight infection. Essential fatty acids can not be synthesized by the body and must be obtained from food. Deficiency of essential fatty acid can affect the health conditions such as heart disease, cancer, insulin resistance, lupus,

schizophrenia, depression, postpartum depression, premature aging, stroke, obesity, diabetes, *arthritis*, and *alzhemeir* diseases. One source of essential fatty acids is fish oil. Fish oil is the main source of long chain polyunsaturated fatty acids (LC PUFA) omega-3, especially EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) which important for health. The positive influence of omega-3 fatty acids on health can prevent heart disease and cancer, and can improve the function of the brain and retina of the eye (Banning, 2005). According to the mixture between trevally fish oil and catfish can produce omega-3, omega-6, and omega-9 were 9.7, 0.06, and 42.81%, respectively (Gobel, 2012). Fatty acids are highly susceptible to oxidation, so it takes a process before fatty acid fortified to food products, and alternative processes that can be used is microencapsulation.

Milk fortified with fish oil at the level of 15 g/kg did not affect the smell of it (Let *et al.*, 2003). Microencapsulation of 2% salmon oil which added to the strawberry yogurt before pasteurization and homogenization can improve eicosapentaenoic acid and docosahexaenoic acid (Estrada, 2011). The addition of lemuru fish oil in yogurt of 0.5%, 1%, 1.5% and 2% can improve the nutritional quality of the product, especially omega 3 and omega 6 fatty acids (Astuti dan Setyawardani, 2006). The research aimed to study the fatty acid profile of ice cream has been fortified with microencapsulated mixture between fish oil of trevally (*Selaroides spp*) and catfish (*Clarias sp*).

MATERIALS AND METHODS

Sample

Three kind of non-economical fish such as Trevally (*Selaroides spp*), Catfish (*Clarias sp)* and Tuna (*Thunnus sp*) were obtained from sea water of Palu, Central Sulawesi Province. The chemical agents used for the analysis were hexane, acetone, ethanol, diethyl ether, acetic acid, formic acid, petroleum ether, KOH and Na_2SO_4 anhydrous (Sigma – Aldrich).

Experimental Design

This research was design using Nested Block Design with two treatments of microencapsulation method, namely freeze drying method (F1) and spray drying method (F2), respectively. The microcapsules of fish oil were obtained from a mixture of trevally and catfish oil (M1) and tuna fish oil (M2). The omega 3, 6, and 9 from the microcapsules of fish oil was determined using Gas Chromatography (GC) (SHIMADZU-FID) (AOAC, 2000).

Fatty Acid, SFA, MUFA, and PUFA Profile from Trevally, Catfish, and Tuna Oil

Trevally and catfish oil used in this study was extracted by wet rendering method, while tuna fish oil using solvent method (AOCS, 1990). Subsequently, the fatty acids profiles from extracted fish oil were analyzed using Gas Chromatography (GC) (SHIMADZU-FID) (AOAC, 2000). According to the fish oil analysis the content of PUFA and MUFA, a mixture of trevally and catfish oil with ratio 1:1 were adopted for the next step of this research.

Microencapsulation of Fish Oils and Fatty Acid Profile Analysis, SFA, MUFA and PUFA of Microcapsules Fish Oil

One encapsulating material used in this research was sodium caseinate with ratio 6:1 of sodium caseinate and fish oil (Estiasih dkk., 2008). Freeze drying and spray drying (Buchi Mini Spray Drier B-290) method were obtained to encapsulate trevally fish oil mixed with catfish oil and tuna fish oil. Spray drying was achieved by preparation of emulsion material, homogenizing of the ingredient and spraying the emulsion into the chamber.

The emulsion preparation, coatings is dissolved into the water, then coating materials and ingredients were mixed up into a homogeneous emulsion. The homogenized emulsions were spray dried using inlet and outlet temperature of 130 and 72^0C, with the flow rate of 5 ml/sec, respectively. For microencapsulation by freeze drying method was employed using freezer dryer scanvaccoolsave. Samples were closed with aluminium foil cover to protect samples against light and stored at freeze temperature for 24 hours, then were kept at freeze dryer for 40 hours. Moreover, the fatty acid profile, SFA, MUFA, and PUFA in microcapsules was characterized using Gas Chromatography (AOAC, 2000).

Analysis of Fatty Acid Profile, SFA, MUFA, and PUFA of Ice Cream Fortified with Microencapsulated Fish Oil

The cow's milk for ice cream was obtained from Agricultural Training Center, Batu Malang. Further, ice cream was characterized for fatty acid profile, SFA, MUFA, and PUFA using GC (AOAC, 2000). Ice cream was then fortified with microencapsulated fish oil using three treatments, i.e 15 g/kg; 20 g/kg and 25 g/kg. The best treatment was then selected (De Garmo, 1984) and analyzed the fatty acids profile, SFA, MUFA and PUFA using GC, as well as organoleptic testing.

Statistical Analysis

The descriptive analysis was applied to the data obtained from the profile of fatty acid, PUFA, MUFA, and SFA. Analysis of variance was performed using SPSS software to evaluate the effect of the parameters studied. Differences among mean values were examined using Test Honestly Significant Difference (HSD) at the $p<0.05$ significance level. The best treatment was determined. Mathematic model was used to analyze the effect of microencapsulation methods and parameters studied (omega 3, 6, and 9): $Y_{ijk} = \mu + t_i + \beta_{(ij)} + E_{ijk}$.

RESULTS AND DISCUSSION

Fatty Acids Composition of the Trevally, Catfish, and Tuna Fish Oil

Table 1 shows the composition of fatty acids in trevally, catfish and tuna fish oil. The fatty acids produced by these fish oil were saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFAs). Further, result shows that the dominant polyunsaturated fatty acids found are eicosatrionic, linoleic and docosahecsanoic acid, while the predominant saturated fatty acid in trevally, catfish and tuna fish oilis palmitic acid of which 33.03, 24.99 and 26.53%, respectively. Palmitic and palmitoleic acids are the fatty acid dominant in *Clariasgariepinus*, *Tilapia zillii*, *Pentanemusquinquarius*, *Pseudotolithustypus* in the sea water of Lagos, Nigeria (Adesola, 2011). Several factors affecting of fatty acid composition are climate, temperature, rain fall, species, sex, weight, size and feed (Adesola, 2011; Nowsad *et al.*, 2012).

Furthermore, the omega 3, 6 and 9 of trevally, catfish and tuna fish oil are shown in Table 2. In all of the fishs oil, trevally fish produced omega 3, 6 and 9 of 13.48, 11.77 and 15.50%, respectively. For the later the mixture of travelly and catfish oil were chosen for further research studyto increase the accumulation of omega 3, 6 and 9. The mixture of trevally and catfish at ratio 1:1, led to the omega 3, 6 and 9, approximately 5.25, 20.61 and 35.52%, respectively (Gobel, 2012).

Table 1. Fatty Acids Composition of Trevally *(Selaroides spp*), Catfish (*Clarias sp*), and Tuna Fish Oil (*Thunnus sp)*

Fatty Acid	*Selaroidesspp*	*Clariassp*	*Thunnussp*
Caproic	0.007 ± 0.006	0 0	0 0
Caprilic	0.003 ± 0.001	0.046 ± 0.003	0.124 ± 0.002
Lauric	0.106 ± 0.035	1.963 ± 0.047	0 0
Tridecanoic	0.093 ± 0.014	0.009 ± 0.002	0.080 ± 0.010
Myristoleic	0.030 ± 0.004	0.053 ± 0.001	0 0
Myristic	5.916 ± 0.943	1.999 ± 0.038	4.483 ± 0.072
Pentadecanoic	1.689 ± 0.287	0.210 ± 0.006	1.348 ± 0.022
Palmitoleic	8.183 ± 0.581	4.956 ± 0.072	7.884 ± 0.079
Palmitic	33.026 ± 0.865	24.999 ± 0.416	26.528 ± 0.470
Heptadekanoic	0.150 ± 0.020	0 0	0.888 ± 0.010
Cis-	± 0.419	0	± 0.027
heptadekanoic	2.361	0	1.447
Oleic	15.502 ± 0.569	34.967 ± 0.488	19.746 ± 0.038
Stearic	3.013 ± 0.528	22.955 ± 1.351	2.844 ± 0.039
Linoleic	10.241 ± 0.499	6.294 ± 0.102	6.756 ± 0.158
Arakidat	6.304 ± 1.025	0 0	4.571 ± 0.072
Linolenic	1.525 ± 0.2;30	0.866 ± 0.728	0.759 ± 0.012
Heneicosanoic	0.316 ± 0.047	0 0	21.771 ± 0.095
Euric	0.261 ± 0.041	0.321 ± 0.516	0 ± 0
Eicosatrinoic	11.383 ± 0.712	0.118 ± 0.041	0.128 ± 0.013
Arachidonic	1.530 ± 0.257	0 0	0.500 ± 0.011
Tricosanoic	0.584 ± 0.096	0.002 ± 0.002	0 ± 0
Eicopentanoic	0.231 ± 0.037	0.004 ± 0.004	0.237 ± 0.018
Nervonic	0.689 ± 0.151	0.037 ± 0.037	0 ± 0
Docohexsanoic	0.336 ± 0.055	0.003 ± 0.003	0 ± 0
SFA	48.259	49.953	38.426
MUFA	23.685	39.923	27.630
PUFA	25.246	7.285	8.380

Table 2. The Composition of omega 3, omega 6 and omega 9 from Trevally *(Selaroides spp*), Catfish (*Clarias sp*), and Tuna Fish Oil (*Thunnus sp)*

Fish oil	Omega 3 (%)	Omega 6 (%)	Omega 9 (%)
Trevally(*Selaroides*spp)	13.48 ± 0.34	11.77 ± 0.72	15.50 ± 0.48
Catfish(*Clarias*sp)	0.99 ± 0.17	6.29 ± 0.10	34.96 ± 0.49
Tuna Fish Oil (*Thunnus*sp)	1.12 ± 0.03	7.25 ± 0.15	19.75 ± 0.04

Microencapsulation of Fish Oil on the Fatty Acids, Omega 3, 6 and 9 Composition

Results of polyunsaturated (PUFA), monounsaturated (MUFA), and saturated (SFA) fatty acids from microencapsulation of trevally mixed with catfish oil and tuna fish oil are presented in Table 3. As shown in Table 3, the predominant SFA produced by microencapsulation of trevally mixed with catfish oil were palmitic and stearic acid of 41.05 and 13.90% using freeze drying method, respectively. Then a slower rate was noted when spray drying was achieved for microencapsulation method of 40.72 and 12.49%, respectively, whereas palmitic acid of 47.87 and 44.97%, respectively were achieved when tuna fish oil was microencapsulated using freeze drying and spray drying method. Therefore, the spray drying method could effectively be employed as a microencapsulation method and led to higher oleic acid of trevally mixed with catfish oil and tuna fish oil approximately 38.70 and 18.86%, respectively. The highest SFA production was obtained in tuna fish oil using freeze and spray drying microencapsulation method, while the highest MUFA occurred in trevally mixed with catfish oil using spray drying microencapsulation method. Therefore, it is could employed as a microencapsulation method for tuna fish oil since most of fatty acid present are monounsaturated fatty acid (MUFA).

Additionally, spray drying is one of the most commonly used for fish oil microencapsulation and drying technologies because the process is flexible and produces good quality powder. The disadvantage of this technology is the high temperature conditions necessary for drying and access to air (Heinzelmann *et al*., 2000). Moreover, parts of the product during drying may adhere to the surface of the capsules, which presents potential for oxidation and changes in the flavor balance of the finished food products.

Otherwise microencapsulation using freeze drying method could effectively led to increase the concentration of PUFA i.e linoleic, linolenic, arachidonic and eikosatrionic acid in trevally mixed with catfish oil and tuna fish oil from undetectable to 1.19% and from 1.65% to 2.29%, respectively. Therefore, drying processes at low temperature such as freeze drying could be an alternative for microencapsulation of fish oil, since it is essential to protect fish oil against oxidation due to the high susceptibility to oxidation of the highly unsaturated PUFA. PUFA plays an important role in human health body and recognized as essential in the diet (Kolanowski *et al*., 2006; Drusch *et al*., 2008*;* Beindoeff *et al*., 2010).

In Figure 1 A, 21 peaks were detected in trevally fish oil mixed with catfish oil. In comparison with chromatograms obtained using standard, it was found that these oil contained saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) with the concentrations were found to be 53.74, 32.20 and 1.19%, respectively. Additionally, results showed that PUFA component in these fish oil were linoleic, linolenic, and arachidic and eicosatrionic acids. The results are in good agreement with that reported by literature, who found that the fatty acid detected in *Clariasgariepinus*fish oil was MUFA approximately 26%, respectively (Adesola, 2011). The major component of fatty acid in menheden and salmon fish oil were MUFA and SFA, approximately 24-29% and 21-34%, respectively (Beindoeff *et al*., 2010).

Moreover, the fatty acids compositions in tuna fish oil using freeze drying method are shown in Figure 1 B. According to Figure 1 B, total 16 fatty acids compound were identified. It was observed that the major fatty acids produced by tuna fish oil were palmitic acid (47.87%), stearic acid (15.57%) and oleic acid (15.59%).

Monounsaturated fatty acids (MUFA) were oil and tench fish oil of 35.9, 35.8 and 38.3%, respectively (Luczynska *et al*., 2012). Monounsaturated fatty acids (MUFA) were present in Malabar Red Snapper fish oil, trevally fish oil and Spain Mackarel at the concentrations of 141.8, 286.8, 70.6 mg/100 g, while for polyunsaturated fatty acids (PUFA) at the concentrations of 724.7, 869.6, and 322.8 mg/100 g, respectively (Nurnaida *et al*., 2013). The differences of fatty acids composition are greatly affected by species, season, and water condition (Steffens *et al*., 2005).

Table 3. The Composition of Fatty Acids, Omega 3, 6 and 9 in Trevally fish *(Selaroides* spp*)* Mixed with Catfish (*Clarias* sp) Oil and Tuna Fish Oil (*Thunnus* sp*)* (% Total Fatty Acids)

Fatty Acid	*Freeze Drying*Trevally fish mixed with Catfish	*Freeze Drying*Tuna Fish Oil	*Spray Drying*Trevally fish mixed with Catfish	*Spray Drying*Tuna Fish Oil
Caproic	0.001 ± 0.004	0 0	0 0	0 0
Caprilic	0.024 ± 0.013	0 0	0 0	0.430 ± 0.042
Capric	0.008 ± 0.001	0 0	0 0	0 ± 0
Lauric	0.238 ± 0.048	0.070 ± 0.001	0 0	0.411 ± 0.194
Myristoleic	0.037 ± 0.006	0.032 ± 0.006	0 0	0 0
Myristic	4.565 ± 0.124	6.079 ± 0.182	3.958 ± 0.401	6.350 ± 0.155
Pentadecanoic	1.362 ± 0.018	2.084 ± 0.069	0 0	1.588 ± 0.063
Palmitoleic	6.238 ± 0.303	6.119 ± 0.037	2.890 ± 0.084	5.874 ± 0.880
Palmitic	41.052 ± 0.484	47.874 ± 1.032	40.724 ± 1.065	44.972 ± 1.163
Heptadecanoic	1.836 ± 0.014	2.966 ± 0.023	0 0	2.145 ± 0.088
Oleic	25.965 ± 0.711	15.585 ± 0.990	38.701 ± 0.383	18.863 ± 0.870
Stearic	13.900 ± 0.240	15.566 ± 0.002	12.492 ± 0.451	15.475 ± 0.346
Linoleic	0.032 ± 0.007	0.048 ± 0.008	0 0	0 0
Arachidonic	1.886 ± 0.006	0.842 ± 0.233	1.242 ± 0.354	0.709 ± 0.019
Linolenic	0.554 ± 0.010	1.037 ± 0.006	0 0	0.826 ± 0.031
Behenic	0.021 ± 0.001	0.022 ± 0.001	0.610 ± 0.030	0.012 ± 0.001
Eicosatrinoic	0.176 ± 0.001	0.422 ± 0.032	0 0	0.317 ± 0.014
Euric	0.176 ± 0.001	0 0	0 0	2.887 ± 0.207
Arachidonic	0.429 ± 0.003	0.787 ± 0.025	0 0	0.509 ± 0.010
Lignoceric	0.165 ± 0.001	0.342 ± 0.008	0 0	0.013 ± 0.013
Nervonic	0.280 ± 0.002	0.750 ± 0.002	0 0	0.051 ± 0.051
SFA	53.741	75.338	61.306	67.909
MUFA	32.203	21.704	41.510	24.737
PUFA	1.191	2.294	0	1.652

Freeze drying is proved to be the best drying process to produce microcapsules, since it contained the highest omega 3 and 6. It is also obvious that omega 3 and 6 in tuna fish oil were considered statistically significant ($p<0.01$) compared with travelly mixed with catfish oil using freeze drying and spray drying method. Typical fish oil microencapsulation is usually based on the formation of ordinary emulsion in which fish oil droplets are emulsified using combination of matrices

and then spray dried ir freeze dried to produce microcapsules. Encapsulated materials are generally used for microencapsulation is sodium caseinate. Sodium caseinate could be employed as encapsulan to produce stable microcapsules (Estiasih dkk. 2008). Sodium caseinate is a protein-based coating that is able to protect against oxidation reaction, extreme storage conditions, and has a fairly high efficiency of the microcapsules (Hogan *et al*., 2001; Day *et al*., 2007).

Moreover, freeze drying method showed the best result for producing encapsulated fish oil in the form of microcapsules (Heinzelmann *et al*., 2000). While the stability of omega 3 in fish oil encapsulated was influenced by the best combination of material and the type of encapsulan drying method in use (Anwar *et al*., 2011).

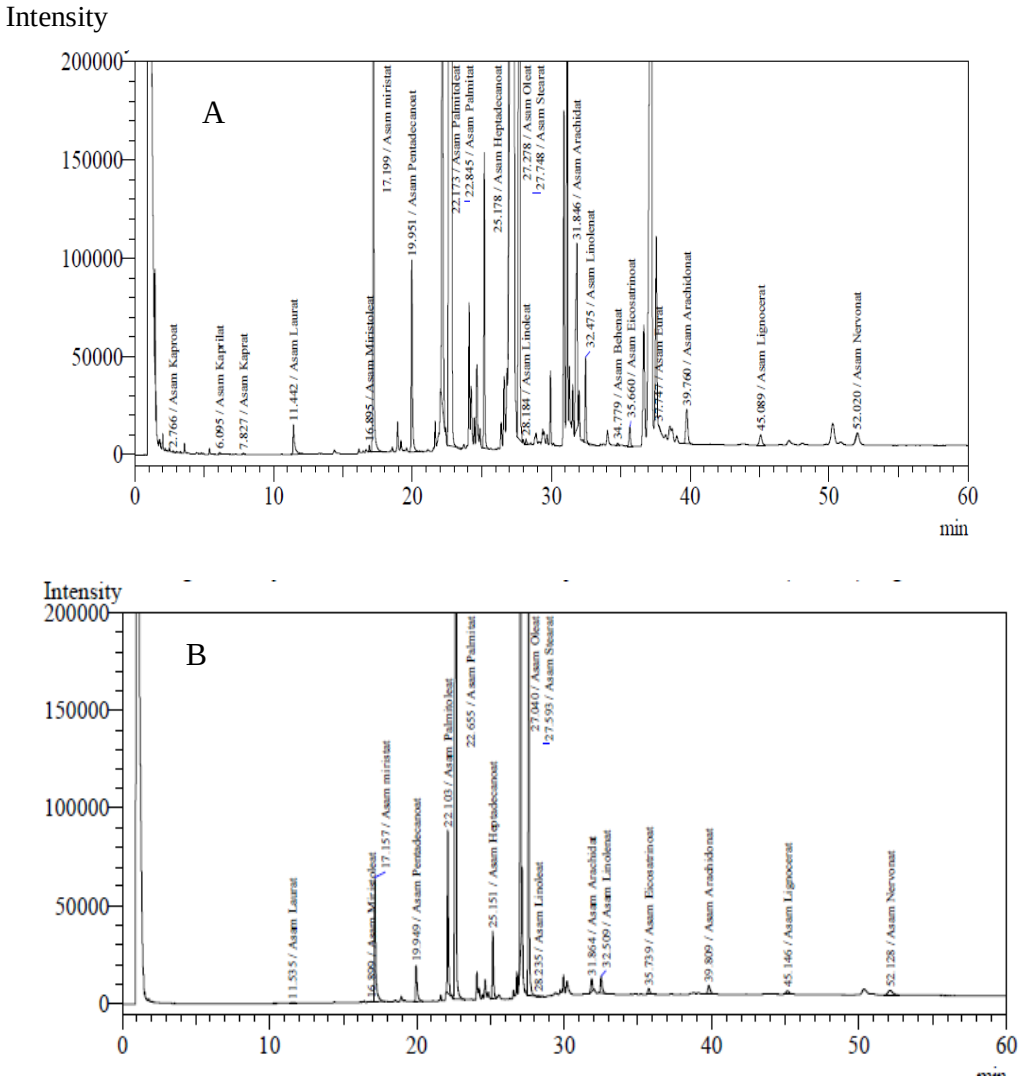

Figure 1. Chromatogram of Fatty Acids Composition in Microencapsulation of Trevally Fish Oil Mixed with Catfish Oil (A); Tuna Fish Oil (B) using Freeze Drying Method

Table 4. The Profile of Fatty Acids of Fortified Ice Cream (15 g / kg) with Microcapsules Mixture between Trevally and Catfish Fish Oil (% Total Fatty Acids)

Fatty Acid	Not fortified ice cream	Fortified ice cream
Caproic	0.380 ± 0.008	0 ± 0
Caprilic	1.016 ± 0.103	0 ± 0
Capric	0.014 ± 0.003	0.029 ± 0.001
Lauric	2.164 ± 0.021	1.742 ± 0.029
Tridecanoic	0 0	0.041 ± 0.001
Myristoleic	0.789 ± 0.012	0.030 ± 0.001
Myristic	10.159 ± 0.133	3.941 ± 0.050
Pentadecanoic	1.214 ± 0.016	1.009 ± 0.012
Palmitoleic	1.295 ± 1.116	4.633 ± 0.077
Palmitic	36.293 ± 0.443	26.466 ± 0.307
Heptadecanoic	0.687 ± 0.009	1.332 ± 0.015
Oleic	27.228 ± 1.055	33.799 ± 0.382
Stearic	17.182 ± 0.186	9.660 ± 0.111
Linoleic	0.633 ± 0.019	0.153 ± 0.002
Arachidonic	0.251 ± 0.008	1.511 ± 0.021
Linolenic	0.189 ± 0.059	1.164 ± 0.016
Behenic	0.020 ± 0.001	0 0
Euric	0.015 ± 0.001	10.546 ± 0.122
Eicosatrinoic	0.034 ± 0.002	1.620 ± 0.017
Arachidonic	0.077 ± 0.001	0.313 ± 0.003
Lignoceric	0.030 ± 0.001	0 ± 0
Eicopentanoic	0 0	0.090 ± 0.001
Nervonic	0.032 ± 0.024	0.223 ± 0.003
Docohexsanoic	0 0	0.007 ± 0.001
SFA	65.2	46.21
MUFA	28.52	38.43
PUFA	0.933	3.347

The Profile of Fatty Acid Ice Cream that Fortified with Microcapsules Contains of Trevally with Catfish Fish Oil.

The results of this analysis can be used to determine the changes that occur in the fatty acid composition. The Profile of Polyunsaturated Fatty Acids (PUFAs), Monounsaturated Fatty Acids (MUFA) and Saturated Fatty Acids (SFA) of the ice cream that fortified with microcapsules contains of trevally with catfish fish oil are presented in Table 4. The data in Table 4 explained that the ice cream that has been fortified with microcapsules mixture between trevally and catfish fish oil, is dominated by Saturated Fatty Acids (SFA), Monounsaturated Fatty Acids (MUFA) and Polyunsaturated Fatty Acids (PUFAs). After it has been fortified, the Saturated Fatty Acids (SFA) concentration decreased. On the other hand, MUFA and PUFA concentration was increasing. Therefore, from the aspect of health, fortified ice cream using microcapsules mixture between trevally and catfish fish oil, could be consider to give good influence and potential as a functional ice cream.

Reduce the amount of some specific saturated fatty acids, especially myristic acid (C14: 0) and palmitic acid

(C16: 0) might be increasing the amount of oleic acid vasenatacid and rumenatacid is a particular target, which could improves the long-term health for consumers (Smet *et al*., 2010).

CONCLUSION

It can be concluded that freeze drying method from the point of view of total PUFA is the best method for mincroencapsulation process of this fish oil mixture. Fortification of 15 g (w/w) microencapsulated fish oil in ice cream could reduced SFA and increased the MUFA and PUFA.

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