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IDENTIFICATION OF VOLATILE FLAVOUR COMPOUNDS OF HOKI (*Macruronus novaezelandiae*) AND ORANGE ROUGHY (*Hoplostethus atlanticus)* OILS

Identifikasi Komponen Flavor Volatil Minyak Ikan Hoki (Macruronus novaezelandiae) dan Orange Roughy (Hoplostethus atlanticus)

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ABSTRACT

Identification of volatile flavour compounds of hoki (*Macruronus novaezelandiae*) and orange roughy (*Hoplostethus atlanticus)* oils has been carried out. Flavour compounds were extracted by a purging system and collected using a porous polymer Tenax TA trap. The gas chromatography-mass spectrometry (GC-MS) was used to identify the volatile flavour compounds. The predominant compounds contributing to the volatile flavour of hoki oil were methyl ethyl benzoate, ethyl benzoate and 1,1-dimethylethyl-2-propionic acid. Meanwhile, the main volatile flavour components of orange roughy oil were toluene, cyclohexane, 1,1-dimethylethyl-2-methyl propionic acid and tetrachloroethane.

Keywords: hoki oil, orange roughy oil, purging system, volatile flavour compounds

ABSTRAK

Identifikasi senyawa-senyawa flavor volatil dari minyak ikan hoki (*Macruronus novaezelandiae*) dan minyak ikan orange roughy (*Hoplostethus atlanticus)* telah dilakukan. Senyawa-senyawa flavor diekstrak dengan menggunakan *purging system* dan dikumpulkan menggunakan perangkap Tenax TA. Gas chromatography-mass spectrometry (GC-MS) digunakan untuk mengidentifikasi senyawa-senyawa flavour yang volatil. Senyawa-senyawa dominan yang berkontribusi pada flavor volatil dari minyak hoki adalah methyl ethyl benzoate, ethyl benzoate and 1,1-dimethylethyl-2-propionic acid. Sedangkan senyawa-senyawa utama flavor volatil minyak orange roughy adalah toluene, cyclohexane, 1,1-dimethylethyl-2-methyl propionic acid and tetrachloroethane.

Kata Kunci: minyak ikan hoki, minyak ikan orange roughy, purging system, senyawa flavour volatil

1. Introduction

Fish oils containing the bioactive omega-3 (n-3) fatty acids have been known to have health benefits, particularly in reducing hearth attack risk and other degenerative diseases. One of the problems in consuming fish oil is its undesirable flavour, particularly fishy odour. So far, fish oil is produced as by-products of fish meal processing and fish canning. Due to that fact, fish oil producers do not really concern about keeping the quality of fish oil, then bringing about that the flavour problems of fish oil is not only dealing with fishy flavour, but also off-flavour which probably appears because of improper handling, storaging, packing and marketing. Undesirable flavour is often restricting in utilizing fish oil, including for hoki and orange roughy oils as the main fish oil produced in New Zealand.

Hoki (*Macruronus novaezelandiae*) is a commercial demersal fish species in New Zealand, in which their proximate composition was 71.2% moisture, 19.5% protein and 6.8 % lipid. Relative composition of hoki oil consisted of 35.33 % saturated fatty acids (SAFA), 43.21 % monounsaturated fatty acids (MUFA) and 21.45 % polyunsaturated fatty acids (PUFA). The fatty acid composition of hoki was dominated by the saturated fatty acid, palmitic acid C16:0 (24.53%), the monounsaturated fatty acid oleic acid C18:1n-9

(19.34%), and polyunsaturated fatty acid docosahexanoic acid 22:6n-3 (12.2%) (Meynier, Morel, Mackenzie, Macgibbon, Chilvers & Duignan, 2008). Hoki oil has been processed as an omega-3 concentrate through ethyl ester (EE) preparation (Rozaina, 2013), but actually the oil can also be used for various purposes, i.e. food, feed, pharmaceutical and other industrial uses.

Orange roughy (*Hoplostethus atlanticus*) is a slow-growing fish that can live for up to 130 years. It is found in deep water around New Zealand at depths of 750 to 1,500 meters. Orange roughy grow to 50 centimeters long and weigh about three and a half kilograms when fully grown (Anon., 2011). Orange roughy has 75.90% moisture, 14.70% protein, 7.00% lipid and 0.90% ash (Silva & Chamul, 2000). In terms of fatty acid profile, orange roughy oil consisted of 4% SAFA, 92.9% MUFA and 0.9% PUFA. Monounsaturated fatty acids dominating fatty acids in orange roughy lipids were oleic acid (43.7%), gondoic acid (26.0%) and erucic acid (12.3%) (Vlieg & Body, 1988). Orange roughy oil spreads extremely well so it is used widely such as in cosmetics for milky lotion, creams, lipsticks, foundations, shampoos, and rinses. It is also used as a raw material for industrial oil preparations (Anon., 2007).

The above explanation showed that both hoki and orange roughy oils have a prospect to be processed into various developed products to improve its added value and to widen its utilization. However, the fishy flavour is probably to be the main constrain to realize that idea. Further treatments are probably demanded to upgrade its acceptability. Understanding of important compounds contributing to the undesirable flavour in fish oil is required prior to certain treatments to be applied. Therefore, this study was aimed to identify volatile flavour compounds contributing to the flavour of hoki and orange roughy oils.

2. Material and Methods

2.1. Materials

Hoki (*M. novaezelandiae*) and orange roughy (*H. atlanticus*) oils were supplied by Sealord Product Ltd., Nelson, New Zealand. The oils were packed in plastic bottles and then send to Massey University, Palmerston North, New Zealand. The oils were stored in a chilling room until required for experimentation.

2.2. Methods

Identification and quantification of volatile flavour compound were carried out using a method as described by Irianto (1992). An all glass-purging system for collection of volatile flavour compounds of both hoki and orange roughy oils was constructed as shown in Figure 1. The size of the tube was 20 cm in length and 2,3 cm in diameter. The length of the purge tube was 15 cm, and was terminated with fine nozzles at one end. A 15 ml fish oil sample was sparged with Nitrogen (N_2) at 85 ml/minute through the purging tube. The purging unit was placed in a constant temperature water bath held at 30 °C. Fish oil samples were prepurged for two minutes to remove oxygen from the tube to avoid oxidation during sample extraction. The volatile components were entrained and concentrated onto a porous polymer Tenax TA trap (60/80 mesh size, Alltech Association, Illinois, USA) attached to the exit port of the purging unit. Purging time was four hours. To desorb the volatile flavour components, the tenax polymer was washed with 7.5 ml of triply glass distilled diethyl ether, and then concentrated by passing a fine jet of N_2 over the surface of the solution to evaporate most of diethyl ether.

The volatile flavour concentrate was analyzed using a gas chromatography-mass spectrometry (GC-MS). Volatile compounds separated on a Hewlett-Packard 5890 Series II equipped with a DB-wax capillary column (30 m x 0.25 mm ID). The carrier gas was nitrogen (N_2) at 10psi. The detector was kept at 260 °C and the injection port at 220 °C. This equipment was connected to the Hewlett-Packard 3393 integrator. The detector was kept at 260 °C and the injection port at 220 °C. The column temperature was programmed from 40 °C to 280 °C at a rate of 5 °C/ minute and held at the upper temperature for 25 minutes. GC peak identification and quantification was carried out using a VG70-250S high resolution mass spectrometer.

3. Results and Discussion

Traces of volatile flavour compounds of hoki and orange rounghy oils are shown in Figure 2 and Figure 3, while their relative amounts are presented in Table 1 and Table 2.

Hoki and orange roughy oil had different compounds responsible for their volatile flavour. As shown in Table 1 and Table 2, the compounds identified in both oils have been identified in fish, fish oil, marine green algae and fishery products by Angeline & Merritt (1975), Crawford, Kretdh, & Guadagni (1976), Josephson, Lindsay, & Stuber (1983), Vejaphan, Hsieh & Williams (1988), Karahadian & Lindsay (1989), Tanchotikul & Hsieh (1989), Hsieh, William, Vejaphan, & Meyers (1989), Sugisawa, Nakamura, & Tamura (1990), Josephson, Lindsay, & Stuber (1991), Hçgnadüttir (1999), Muhamed, Man, Mustafa, & Manap (2012), Giogios, Kalogeropoulos, & Grigorakis (2013), and Sun, Wang, Huang, Hou, Chen, & Su (2013).

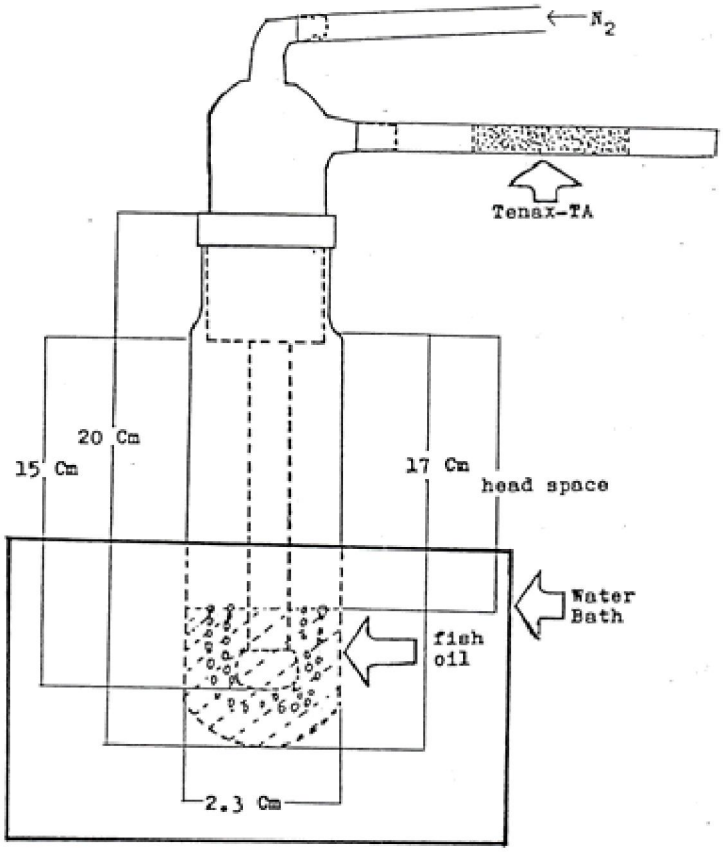

Figure 1. Purging system for collection of volatile flavour compounds.

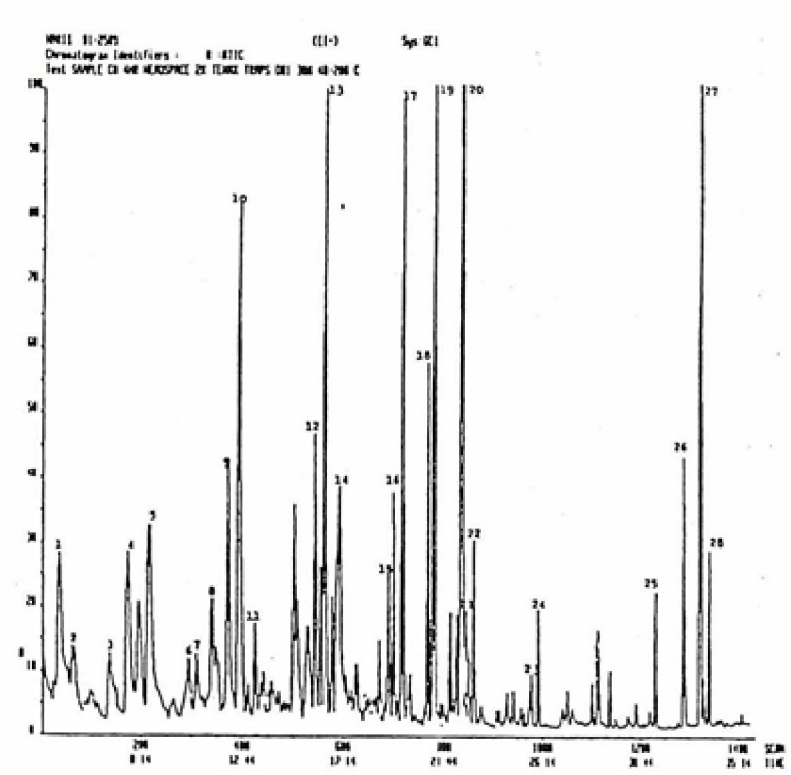

Figure 2. Traces of volatile flavour compounds of hoki (*M. novaezelandiae*) oil.

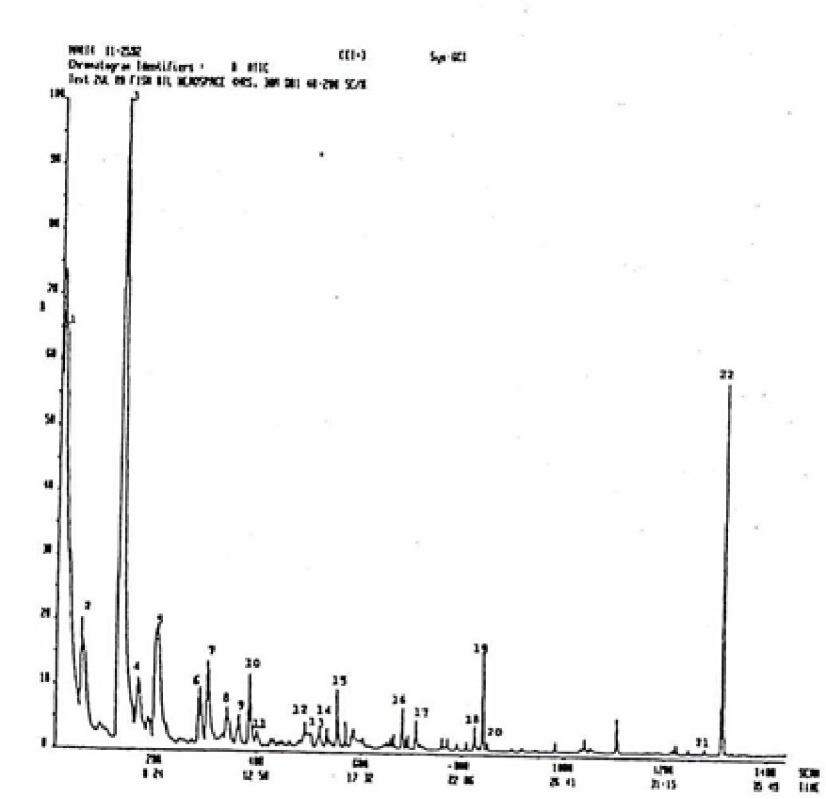

Figure 3. Traces of volatile flavour compounds of orange roughy (*H. atlanticus)* oil.

The volatile flavour compounds identified in hoki oil consisted of 19 hydrocarbons, 1 alcohol, 5 esters, 2 aldehydes and 1 acid. Hydrocarbons detected were heptane, toluene (methyl benzene), octane, ethyl benzene, 1,3-dimethyl benzene (m-xylene), ethynyl benzene, 1-nonene, nonane, 1-decene, decane, limonene, 1-undecene, undecane, 1-dodecene, dodecane, 1-tridecene, tridecane, pentadecane, and hexadecane. Five esters identified were butyl ester-2-hydroxy propionate, ethyl hexyl acetate, ethyl benzoate, methyl ethyl benzoate, and diethylphtalate. Aldehyde comprised 2-methyl-4-pentanal and 1-nonanal. Alcohol and acid traced were 1-penten-3-ol and 1,1 dimethylethyl-2-methyl propionc acid respectively.

In terms of relative amount, the main compounds contributing to the volatile flavour of hoki oil were methyl ethyl benzoate (29.5%), ethyl benzoate (10.4%) and 1,1-dimethylethyl-2-propionic acid (12.4%). These compounds were probably responsible for the strong fishy odour and taste in hoki oil. Ethyl benzoate was also found as a volatile flavour in plums (Dirninger, 1989), providing aromatic odour (Stecher, Windholz, Leahy, Bolton & Eaton, 1968). Alkane compounds of heptane, undecane and dodecane found in hoki oil were encountered in vanilla aroma (Vidal, Fort, Aulsier, & Richard, 1989). Nonane, tridecane, pentadecane and hexadecane detected in this fish oil were found in plums (Etilvant, Guichard, & Issanchowl, 1986; Dirninger, Schaeffer, & Humbert, 1989). Octane and dodecane traced in hoki oil also contributed to the aroma of strawberries (Belitz & Grosch, 1987). Limonene, as analyzed in hoki oil was also detected in plums (Dirninger et al., 1989).

The volatile flavour compounds identified in orange roughy oil were 15 hydrocarbons, 1 alcohol, 2 esters, 1 aldehyde, 2 acids and 1 halogen. Those hydrocarbons cyclohexane, 3-methyl hexane, toluene (methyl benzene), heptane, ethyl benzene, 1,3-dimethyl benzene (m-xylene), 1,4-dimethyl benzene (p-xylene), nonane, 1-methylethyl benzene, 1-ethyl-3-mthyl benzene, 1,3,5-trimethyl benzene, 1,2,4-trimethyl benzene, decane, undecane and dodecane. Two esters identified were octyl acetate and diethyl phtalate. Two acids comprised 1-methylethyl ester benzoic acid and 1,1 dimethylethyl-2-methyl propionc acid. Alcohol, aldehyde and halogen detected were 2-butoxy ethanol, 1-nonanal and tetrachloroethene respectively.

According to relative amount, toluene (51,5%) was noted as the most volatile flavour compounds in orange roughy oil. Other compounds encountered at significant level were cyclohexane (6.4%), 1,1-dimethylethyl-2-methyl propionic acid (5.7%) and

Table 1. Relative amounts of volatile flavour compounds of hoki (*M. novaezelandiae*) oil

Peak No.	Volatile Flavour Compounds	Relative Amount (%)	Previously Identified in
	Hydrocarbons		
2	heptane	1.0	Haddock flesh[b], tuna eyes oil[o]
3	toluene (methyl benzene)	1.7	Crayfish waste [a], haddock flesh[b], salmon[h], mediterranean fish[l], krill oil[n], tuna eyes oil[o]
			Crayfish waste [a], haddock flesh [b], crayfish tail [c], marine green algae[d]
5	octane	3.1	tuna oil[l]
			whitefish[f]
6	ethyl benzene	0.4	
7	1,3-dimethyl benzene (m-xylene)	0.9	tuna oil [i]
8	ethyny l benzene	1.8	salmon[h], tuna oil[l]
9	1-nonene	2.9	menhaden oil[e], cod liver oil[g], tuna oil[l]
10	nonane	4.7	crayfish waste[a], crayfish tail[c], marine green algae[d]
12	1-decene	2.2	
13	decane	3.8	menhaden oil[e]
14	Limonene	3.8	menhaden oil[e], whitefish[f], cod liver oil[g], tuna eyes oil[o]
			tuna oil[l]
16	1-undecene	1.5	whitefish[f]
17	undecane	3.8	menhaden oil[e], salmon[h], budu[j], tuna eyes oil[o]
21	1-dodecene	0.6	crayfish tail[c], marine green algae[d], menhaden oil[e], budu[j]
22	dodecane	1.4	
			crayfish waste[a], marine green algae[d], whitefish[f], cod liver oil[g], tuna oil[l], budu[j], fresh fish[k], mediterranean fish[l], bigeye tuna[m], tuna eyes oil[o]
23	1-tridecene	0.4	
24	tridecane	0.7	
25	pentadecane	0.8	
28	hexadecane	1.1	
	Alcohols		
1	1-penten-3-ol	2.5	
			Menhaden oil[e], whitefish[f], cod liver oil[g], tuna oil[i], mediterranean fish[l], bigeye tuna[m], tuna eyes oil[o]
	Esters		
11	Butyl ester-2-hydroxy propionate	0.8	
18	Ethyl hexyl acetate	1.8	
19	Ethyl benzoate	10.4	
20	Methyl ethyl benzoate	29.5	
26	diethylphtalate	2.4	
	Aldehydes		
4	2-methyl-4-pentanal	2.4	
15	1-nonanal	0.8	
	Acids		
27	1,1 dimethylethyl-2-methyl propionc	12.4	

Note: a) Tanchotikul & Hsieh (1989); b) Angeline & Merritt (1975); c) Vejaphan et al. (1988); d) Sugisawa et al. (1990); e) Hsieh et al. (1989); f) Josephson et al. (1983); g) Karahadian & Lindsay (1989); h) Josephson et al. (1991); i) Crawford et al. (1976); j) Muhamed et al. (2012), k) Hçgnadüttir (1999), l) Giogios et al. (2013), m) Sun et al. (2013), n) Haque et al. (2014), o) Shin et al. (2003)

Table 2. Relative amounts of volatile flavour compounds of orange roughy (*H. atlanticus)* oil

Peak No.	Volatile Flavour Compounds	Relative Amount (%)	Previously Identified in
	Hydrocarbons		
1	Cyclohexane	6.4	tuna oil [i)]
2	3-methyl hexane	1.9	
3	toluene (methyl benzene)	51.5	Crayfish waste [a)], haddock flesh[b)], salmon[h)], mediterranean fish[j)], krill oil[l)], tuna eyes oil[o)] Haddock flesh [b)], tuna eyes oil[o)] crayfish tail [c)], marine green algae[d)]
4	heptane	4.0	tuna oil [i)]
6	ethyl benzene	3.3	tuna oil [i)]
7	1,3-dimethyl benzene (m-xylene)	5.2	tuna oil[i)]
8	1,4-dimethyl benzene (p-xylene)	2.2	tuna oil [i)]
10	Nonane	2.2	
11	1-methylethyl benzene	0.8	Crayfish waste [a)], crayfish tail [c)], menhaden oil[e)]
12	1-ethyl-3-mthyl benzene	0.2	Crayfish waste [a)], crayfish tail[c)], menhaden oil[e)]
13	1,3,5-trimethyl benzene	1.4	menhaden oil[e)], tuna oil [i)] menhaden oil[e)], tuna oil [i)]
14	1,2,4-trimethyl benzene	1.0	menhaden oil[e)], whitefish[f)], tuna oil [i)], tuna eyes oil[o)]
15	decane	1.2	crayfish tail[c)]
17	undecane	0.5	
20	dodecane	0.3	tuna oil [i)]
	Alcohols		
9	2-butoxy ethanol	2.0	
	Esters		menhaden oil[e)], whitefish[f)], cod liver oil[g)], tuna oil[i)], mediterranean fish[j)], bigeye tuna[k)], tuna eyes oil[m)]
19	octyl acetate	1.6	
21	diethyl phtalate	0.3	
	Aldehydes		
16	1-nonanal	0.9	
	Acids		
18	1-methylethyl ester benzoic acid	0.7	
22	1,1 dimethylethyl-2-methyl propionc acid	5.7	
	Halogen		
5	tetrachloroethene	6.8	

Note: a) Tanchotikul & Hsieh (1989); b) Angeline & Merritt (1975); c) Vejaphan et al. (1988); d) Sugisawa et al. (1990); e) Hsieh et al. (1989); f) Josephson et al. (1983); g) Karahadian & Lindsay (1989); h) Josephson et al. (1991); i) Crawford et al. (1976), j) Giogios et al. (2013), k) Sun et al. (2013), l) Haque et al. (2014), m) Shin et al. (2003)

tetrachloroethane (6.8%). Toluene was traced in the aroma of vanilla as well (Vidal et al, 1989) imparting a benzene like odour (Stecher, 1968) or a plastic like odour (Tanchotikul & Hsieh, 1989). Both m-xylene and p-xylene detected in orange roughy oil were also found in plums (Dirninger et al., 1989). Octyl acetate in volatile flavour of orange roughy oil also contributed to strawberry aroma (Belitz & Grosch (1987). Tetrachloroethene, giving a chloroform like odour (Stecher et al., 1968), was encountered in the volatile flavour compounds of orange roughy oil while other halogen compounds such as dichloromethane and trichloromehane are also reported contribute to the volatile flavour of fish (Van Straten & Maarse, 1983).

Toluene, limonene, xylene and benzene derivates in hoki and orange roughy oils were probably degradation products of carotenoids (Beltz & Grosch, 1987; Tanchotikul & Hsieh, 1989; Josephson et al., 1991). This degradation process may have occurred during the heat cooking stage of fish meal production. Diethylphtalate was traced in both hoki and orange roughy oils. This compound was also detected in the volatile flavour compound of plums (Dirninger et al., 1989). However diethylphtalate is odourless, as reported by Stecher et al. (1968). Some of these volatile flavour compounds were also detected in marine green algae (Sugisawa et al.,1990), indicating that these compounds were probably obtained by fish during feeding. Heptane, toluene, dodecane, pentadecane, 1-penten-3-ol and nonanal were also identified in oxidized tuna eyes oil (Shin, Jang, Lee, An, & Lee, 2003)

Fishy flavour and other undesirable flavour compounds which are probably generated during production and storage of both hoki and orange roughy oils should be removed to improve their sensory acceptability and to widen their utilization for various purposes. The undesirable compound removal can be performed through refining process. According to European Food Safety Authority (2010) that refining operation is to remove undesirable components from the oil regarding human consumption and further processing. Various amounts of materials that may give undesirable flavour and colour, such as small amounts of proteins, water, pigments, free fatty acids (FFA), phospholipids, and lipid oxidation products. The conventional oil refining in industry is usually made by chemical methods, which include several steps such as settling and degumming, de-acidification, bleaching, deodorization, antioxidant addition or winterisation.

Deodorization process is conducted to remove particularly FFA, aldehydes and ketone which give abjectionable smell and flavour characteristics. Oil deodorization is traditionally based on the application of high temperatures. In this step the volatile materials are stripped by means of a stripping gas, normally steam at high temperature (190-210 ºC) and low pressure (2-5 mBar). However, the application of this method to fish oil is problematic because it has been reported that, for temperatures above 180ºC, omega-3 FA degradation occurs, involving the formation of polymers, isomers, cyclic FA monomers and other undesirable compounds. Alternative methods based on vacuum steam distillation at low temperatures followed by a treatment in a silica gel column, adsorption with a resin, or treatment with diatomaceous earth have been put forward for removing odours from fish oil. Antioxidants can be added to the oil to protect it against oxidation (European Food Safety Authority, 2010).

Crexi, Monte, Soares, & Pinto (2010) demonstrated deodorisation of fish oil, in which the oil was loaded in a vessel under vacuum (750 mm Hg) with one opening connected to a condenser, in order to remove the volatiles from the system. This vessel also possessed a steam inlet, provided by a steam boiler, with a valve controlling the outûow. Deodorisation was carried out at 220°C for 60 min with 5% steam.

Undesirable flavour of fish oil can also be overcome by applying microencapsulation technique, in which the oil is surrounded by a matrix typically composed of proteins or carbohydrates. Hannah (2009) has successfully developed fish oil microcapsules using blends of chitosan, high-amylose starch and pullulan. Meanwhile, Norziah , Nuraini, & Lee (2009) produced microencapsulated fish oil using spray drying technique and found that microencapsulation of 50% oil loading with dextrose equivalent of 25 and 10% sodium caseinate blend gave fish oil powder with microencapsulation efficiency of 84.0 ± 0.2%.

4. Conclusions

Some volatile flavour components of both hoki and orange roughy oils were actually compounds normally found in fishery products. Main volatile flavour compounds of hoki oil were different compared to those identified in orange roughy oil, consequently flavour impression showed by both oils is also different. The predominant volatile flavour compounds of hoki oil were methyl ethyl benzoate, ethyl benzoate and 1,1-dimethylethyl-2-propionic acid, while the predominant volatile flavour components of orange roughy oil were toluene, cyclohexane, 1,1-dimethylethyl-2-methyl propionic acid and tetrachloroethane. Improvement of fish oil flavour is recommended using refining process.

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