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)

Hari Eko Irianto1,2*, Carmen C. Fernandez3 and G.J. Shaw4

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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 dan 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 dan 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
system for collection of volatile flavour compounds of
described by Irianto (1992). An all glass-purging
compound were carried out using a method as

2.2. Methods
in a chilling room until required for experimentation.
Palmerton North, New Zealand. The oils were stored
bottles and then send to Massey University,
Nelson, New Zealand. The oils were packed in plastic
atlanticus
2.1. Materials
flavour of hoki and orange roughy oils.
identify volatile flavour compounds contributing to the
flavour in fish oil is required prior to certain treatments
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
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 (N₂) 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 N₂ 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 (N₂) at 85 ml/minute through the purging tube.
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 rougthy 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, & Stubre (1983), Vejaphan, Hsieh
& Williams (1988), Karahadian & Lindsay (1989),
Tanchotikul & Hsieh (1989), Hsieh, Williams, Vejaphan,
& Meyers (1989), Sugisawa, Nakamura, & Tamura
(1990), Josephson, Lindsay, & Stubre (1991),
Hcgnaëttir (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 diethylphthalate. Aldehyde comprised 2-methyl-4-pentanal and 1-nonanal. Alcohol and acid traced were 1-penten-3-ol and 1,1 dimethylethyl-2-methyl propionic 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 (Etivvant, Guichard, & Issanchow, 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-methyl 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 propionic 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

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Volatile Flavour Compounds</th>
<th>Relative Amount (%)</th>
<th>Previously Identified in</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>heptane</td>
<td>1.0</td>
<td>Haddock flesh&lt;sup&gt;1&lt;/sup&gt;, tuna eyes oil&lt;sup&gt;11&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>toluene (methyl benzene)</td>
<td>1.7</td>
<td>Crayfish waste&lt;sup&gt;4&lt;/sup&gt;, haddock flesh&lt;sup&gt;4&lt;/sup&gt;, salmon&lt;sup&gt;4&lt;/sup&gt;, mediterranean fish&lt;sup&gt;5&lt;/sup&gt;, krill oil&lt;sup&gt;5&lt;/sup&gt;, tuna eyes oil&lt;sup&gt;5&lt;/sup&gt;, Crayfish waste&lt;sup&gt;4&lt;/sup&gt;, haddock flesh&lt;sup&gt;4&lt;/sup&gt;, crayfish tail&lt;sup&gt;6&lt;/sup&gt;, marine green algae&lt;sup&gt;6&lt;/sup&gt;, Crayfish tail&lt;sup&gt;6&lt;/sup&gt;, marine green algae&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>octane</td>
<td>3.1</td>
<td>tuna oil&lt;sup&gt;5&lt;/sup&gt;, whitefish&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>ethyl benzene</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1,3-dimethyl benzene (m-xylene)</td>
<td>0.9</td>
<td>tuna oil&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>ethyl 1 benzene</td>
<td>1.8</td>
<td>salmon&lt;sup&gt;8&lt;/sup&gt;, tuna oil&lt;sup&gt;9&lt;/sup&gt;</td>
</tr>
<tr>
<td>9</td>
<td>1-nonene</td>
<td>2.9</td>
<td>menhaden oil&lt;sup&gt;10&lt;/sup&gt;, cod liver oil&lt;sup&gt;10&lt;/sup&gt;, tuna oil&lt;sup&gt;10&lt;/sup&gt;</td>
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<tr>
<td>10</td>
<td>nonane</td>
<td>4.7</td>
<td>crayfish waste&lt;sup&gt;4&lt;/sup&gt;, crayfish tail&lt;sup&gt;6&lt;/sup&gt;, marine green algae&lt;sup&gt;4&lt;/sup&gt;</td>
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<tr>
<td>12</td>
<td>1-decene</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>decane</td>
<td>3.6</td>
<td>menhaden oil&lt;sup&gt;12&lt;/sup&gt;</td>
</tr>
<tr>
<td>14</td>
<td>Limonene</td>
<td>3.8</td>
<td>menhaden oil&lt;sup&gt;12&lt;/sup&gt;, whitefish&lt;sup&gt;13&lt;/sup&gt;, cod liver oil&lt;sup&gt;12&lt;/sup&gt;, tuna eyes oil&lt;sup&gt;12&lt;/sup&gt;, tuna oil&lt;sup&gt;12&lt;/sup&gt;</td>
</tr>
<tr>
<td>16</td>
<td>1-undecene</td>
<td>1.5</td>
<td>whitefish&lt;sup&gt;13&lt;/sup&gt;</td>
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<tr>
<td>17</td>
<td>undecane</td>
<td>3.8</td>
<td>menhaden oil&lt;sup&gt;12&lt;/sup&gt;, salmon&lt;sup&gt;14&lt;/sup&gt;, budu&lt;sup&gt;16&lt;/sup&gt;, tuna eyes oil&lt;sup&gt;12&lt;/sup&gt;</td>
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<tr>
<td>21</td>
<td>1-dodecene</td>
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<td>crayfish tail&lt;sup&gt;6&lt;/sup&gt;, marine green algae&lt;sup&gt;6&lt;/sup&gt;, menhaden oil&lt;sup&gt;12&lt;/sup&gt;, budu&lt;sup&gt;16&lt;/sup&gt;</td>
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<tr>
<td>22</td>
<td>dodecane</td>
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<tr>
<td>23</td>
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<tr>
<td>28</td>
<td>hexadecane</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>1-penten-3-ol</td>
<td>2.5</td>
<td>Menhaden oil&lt;sup&gt;12&lt;/sup&gt;, whitefish&lt;sup&gt;13&lt;/sup&gt;, cod liver oil&lt;sup&gt;12&lt;/sup&gt;, tuna oil&lt;sup&gt;12&lt;/sup&gt;, mediterranean fish&lt;sup&gt;5&lt;/sup&gt;, bigeye tuna&lt;sup&gt;12&lt;/sup&gt;, tuna eyes oil&lt;sup&gt;12&lt;/sup&gt;</td>
</tr>
<tr>
<td>11</td>
<td>Butyl ester-2-hydroxy propionate</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Ethyl hexyl acetate</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Ethyl benzoate</td>
<td>10.4</td>
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</tr>
<tr>
<td>20</td>
<td>Methyl ethyl benzoate</td>
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<td>26</td>
<td>diethyl phthalate</td>
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</tr>
<tr>
<td>4</td>
<td>2-methyl-1,4-pentanal</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>1-nonanal</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>1,1 dimethyl ethyl-1,2-dimethyl propionate</td>
<td>12.4</td>
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</tr>
</tbody>
</table>

**Note:**
- a) Tanochotikul & 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)

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

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Volatile Flavour Compounds</th>
<th>Relative Amount (%)</th>
<th>Previously Identified in</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hydrocarbons</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Cyclohexane</td>
<td>6.4</td>
<td>tuna oil</td>
</tr>
<tr>
<td>2</td>
<td>3-methyl hexane</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Toluene (methyl benzene)</td>
<td>51.5</td>
<td>Crayfish waste</td>
</tr>
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<td>4</td>
<td>Heptane</td>
<td>4.0</td>
<td>tuna oil</td>
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<tr>
<td>6</td>
<td>Ethyl benzene</td>
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<td>1,3-dimethyl benzene (m-xylene)</td>
<td>5.2</td>
<td>tuna oil</td>
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<tr>
<td>8</td>
<td>1,4-dimethyl benzene (p-xylene)</td>
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<td>tuna oil</td>
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<tr>
<td>10</td>
<td>Nonane</td>
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<td>Crayfish waste</td>
</tr>
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<td>1-methylethyl benzene</td>
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<td>Crayfish waste</td>
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<td>1-ethyl-3-methyl benzene</td>
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<td>Crayfish waste</td>
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<td>13</td>
<td>1,3,5-trimethyl benzene</td>
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<td>menhaden oil</td>
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<td>14</td>
<td>1,2,4-trimethyl benzene</td>
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<td>menhaden oil</td>
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<td>Decane</td>
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<td>Undecane</td>
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<td>Dodecane</td>
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<td><strong>Alcohols</strong></td>
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<tr>
<td>19</td>
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<td>menhaden oil</td>
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<td>21</td>
<td>Diethyl phthalate</td>
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<td>16</td>
<td>1-nonenal</td>
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<td>22</td>
<td>1,1 dimethylcyclohexyl-2-methyl propionic acid</td>
<td>5.7</td>
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<tr>
<td><strong>Halogen</strong></td>
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</tr>
<tr>
<td>5</td>
<td>Tetrachloroethene</td>
<td>6.8</td>
<td></td>
</tr>
</tbody>
</table>

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 (Dirminger 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. However dimethylethyl-2-propionic acid, while the predominant volatile flavour compounds of hoki oil were dimethylethylbenzoate, 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 tetrachloroethene. Improvement of fish oil flavour is recommended using refining process.

Deodorization process is conducted to remove particularly FFA, aldehydes and ketone which give abjectable 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 °C) 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 180°C, 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).

Creci, 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 outflow. 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.
5. Acknowledgement
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