Lipid Changes in Bonito Meat in the Katsuobushi Processing and Quality Assessment of the Commercial Product Based on Lipid Composition

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Lipid changes during "katsuobushi" processing were investigated and the lipids of several commercially produced katsuobushi were analyzed for quality assessment. Triacylglycerol (TG) decreased from 75.2 % in initial content to 68.3 % in "hadakabushi" and free fatty acid (FFA) increased to 53.0 %. TG further decreased to 25.4 % after mold treatment. Increase in FFA was due to hydrolysis of TG by lipase produced from the mold. Katsuobushi properly mold showed high FFA % and docosahexaenoic acid (DHA) %. Thus, commercial katsuobushi with low FFA (7.1 %) and low DHA (19.1 %) content was judged to be poorly mold treated, with consequent low quality. Smoking caused major loss of phosphatidylethanolamine from 22.5 % to 6.6 %, although phosphatidylcholine content change little. Analysis in lipid of katsuobushi indicated improved product quality and whether katsuobushi had been processed.

1 Introduction

Katsuobushi is a well known product made by Japanese traditional processing and its flavor is related to the lipid. The manufacture of katsuobushi is carried out by various steps such as boiling, drying, smoking, and molding. Smoking and mold treatment are repeated several times to obtain the adequate dryness and flavor, which is a unique procedure since it is not commonly used in the manufacturing of other foods1). Treatment with mold is one of the essential procedures that decreases the lipid content and gives a pleasant flavor of the katsuobushi2),3). Aspergillus repens and A. ruber are well known as excellent molds for katsuobushi processing. “Shirata-fushi”, which is as an oxidized product of katsuobushi due to its high lipid content, has been compared with normal katsuobushi4) and the antioxidative properties of katsuobushi extract reported5). However, little information is available on the lipid change during katsuobushi processing.

This study was conducted to determine changes in lipids during katsuobushi processing. Furthermore, commercial katsuobushi products were examined in order to assess the quality of the products based on their lipid composition. In the first part of this study, samples taken during the manufacture of katsuobushi were analyzed using fresh bonito as a reference sample. In the second part, 8 different samples of katsuobushi obtained commercially were analyzed comparing them with the sample taken from the final katsuobushi processing stage and the quality of the each product was evaluated.

2 Materials and Methods

2.1 Preparation of Samples

Bonito(Euthynnus pelamis) samples were
taken at various stages during katsuobushi processing (boiled fillets, smoked fillets, “hadakabushi” and the final product of katsuobushi), were obtained from a katsuobushi manufacturing company in Makurazaki, Kagoshima. In katsuobushi processing as shown in Fig.-1, after cutting the head and eviscerating of the internal organs and central bone, the fillets (sample 1) of bonito are boiled (sample 2), dried and the fillets are smoked in a smoking chamber (sample 3), then the blackened crust of the fillet is scraped off and the residual dried fillet, “hadakabushi” (sample 4), is then treated by mold over a 2 months period. Katsuobushi, is then produced as the final product (sample 5). All samples were transported to the laboratory in Tokyo by ice box and analyzed immediately.

In addition of the samples taken from manufacturing stages, katsuobushi and sliced product of “katsuokezuribushi” were purchased from the market in Tokyo; sliced katsuobushi packed in polyethylene bags filled with nitrogen gas (samples 6, 7, 8), sliced katsuobushi without packaging (samples 9, 10) and “obushi” without any packaging (sample 11), “mebushi” (sample 12) and “obushi” (sample 13) with vacuum and wrapped by plastic. Quality of each sample was designated by the appearance of color, flavor, and price as shown in Table-2. Samples were sliced, cut into small pieces and thoroughly hand—mixed prior to lipid extraction.

2-2 Assessment of Lipid Oxidation

Lipids were extracted from the samples according to the method of Bligh and Dyer. Lipid oxidation of the samples were assessed by the scores of the thiobarbituric acid (TBA) and peroxide value (PV), which were determined according to the methods of Sinnhuber and Yu, and Fleisher and Pucker, respectively.

2-3 Moisture Content

Moisture contents were determined by Official Method 1.1.3.2 a–71 of the Japan Oil Chemists’ Society.

2-4 Analysis of Lipid Class

Lipid class composition of each sample was analyzed by thin layer chromatography (TLC). The TLC developer for neutral lipid was a mixture of petroleum ether, diethyl ether, and acetic acid (80:20:1, vol/vol/vol). The solvent system for polar lipid was chloroform, methanol, water, and acetic acid (65:45:2:1, vol/vol/vol/vol). TLC plate (Silicagel G) used was obtained from E. Merck.

Table-1 Change in the lipid content of bonito muscle during katsuobushi processing.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Lipid content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wet wt</td>
</tr>
<tr>
<td>1*1</td>
<td>2.7*2</td>
</tr>
<tr>
<td>2</td>
<td>1.6</td>
</tr>
<tr>
<td>3</td>
<td>4.4</td>
</tr>
<tr>
<td>4</td>
<td>4.6</td>
</tr>
<tr>
<td>5</td>
<td>4.2</td>
</tr>
</tbody>
</table>

*1 : See text in Fig.-1.
*2 : g/100 g of muscle.

Fig.−1 Flow chart of katsuobushi processsing and samples at different stages.
AG., and the thickness of the coating was 0.25 mm. After the sample was spotted and developed, a liquid of saturated potassium dichromate in 75 % sulfuric acid was sprayed on the TLC plate and was activated at 130°C for 5 min. The spot sample was analyzed using a Shimadzu high speed TLC scanner CS-9200 to determine the lipid composition quantitatively. The compounds of the separated bands were identified by comparison of standard lipids. In this study, no further separation was carried out between monoacylglycerols (MG) and phospholipids (PL) due to overlap of the bands on the TLC.

2.5 Analysis of Fatty Acid Composition

Extracted lipids were converted to their fatty acid methyl esters by heating the lipids in a mixture of benzene and boron trifluoride methanol complex solution at 80°C for 30 min. Methyl esters were analyzed by gas liquid chromatography (GLC) equipped with a Supelcowax-10 fused silica open tubular column (30×0.25mm i.d. Supelco LTD., Japan. The chromatographic conditions were as follows; oven temperature conducted from 170°C to 225°C with a program rate of 1°C/min, injection port temperature of 250°C, flame ionization detector at temperature of 270°C. Nitrogen was used as the carrier gas at an inlet pressure of 2 kg/cm². Chromatographic peaks of the fatty acids esters were identified by comparison of their retention times with available standards and log-plots against the number of carbon atoms in the chain and reported equivalent chain length (ECL) values. All values are the mean of three determinations, except the lipid extraction.

3 Results

Table 1 gives the lipid contents (wet and dry weight) of bonito muscle during katsuobushi processing. In the raw muscle, the lipid content was 2.7 g/100 g (wet weight). The initial lipid content of raw muscle 9.0 g/100 g (dry weight) decreased to 4.7 g/100 g during the processing.

Changes in the PV and TBA value during the katsuobushi processing and for the samples purchased from the market are given in Table 2. In the boiled bonito sample, PV was the highest 24.4, and decreased during the processing to 9.8 in the mold treated final product. After the boiling treatment, the TBA number was 3.9 and increased to 8.5 in the smoked bonito muscles. The highest TBA number was detected in the mold treated final product. PV and TBA values of the samples purchased from the market varied between 12.3~26.3 and 4.9~14.6, respectively.

Figures 2 and 3 show the fatty acid compositions of the samples taken from the katsuobushi processing stages and that of commercial katsuobushi products, respectively. The predominant fatty acids in the raw muscle were DHA (22 : 6) (28.0 %), 16 : 0 (24.4 %), 18 : 1 (15.9 %), 18 : 0 (8.9 %),

Table 2 PV and TBA in the katsuobushi samples at different stages of processing and the commercial katsuobushi products.

<table>
<thead>
<tr>
<th>Sample number*1</th>
<th>PV (meq/kg)*2</th>
<th>TBA (mg/kg)*3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.2±0.5*4</td>
<td>2.6±0.4</td>
</tr>
<tr>
<td>2</td>
<td>24.4±0.6</td>
<td>3.9±0.7</td>
</tr>
<tr>
<td>3</td>
<td>15.0±0.9</td>
<td>8.5±0.9</td>
</tr>
<tr>
<td>4</td>
<td>8.4±1.0</td>
<td>7.0±1.6</td>
</tr>
<tr>
<td>5</td>
<td>9.8±1.2</td>
<td>19.9±0.6</td>
</tr>
<tr>
<td>6</td>
<td>12.3±1.5</td>
<td>5.2±0.2</td>
</tr>
<tr>
<td>7</td>
<td>26.3±1.2</td>
<td>14.6±0.2</td>
</tr>
<tr>
<td>8</td>
<td>17.0±0.8</td>
<td>13.9±0.7</td>
</tr>
<tr>
<td>9</td>
<td>19.4±0.7</td>
<td>4.9±0.0</td>
</tr>
<tr>
<td>10</td>
<td>16.5±0.5</td>
<td>13.4±0.2</td>
</tr>
<tr>
<td>11</td>
<td>16.3±0.6</td>
<td>10.7±0.6</td>
</tr>
<tr>
<td>12</td>
<td>20.5±1.3</td>
<td>9.3±0.4</td>
</tr>
<tr>
<td>13</td>
<td>23.8±0.6</td>
<td>7.6±0.9</td>
</tr>
</tbody>
</table>

*1: Samples No. 1~5, see text in Fig. 1: No. 6: High quality, sliced, and packed in polyethylene bags with nitrogen : No. 7, 8 : Good quality, sliced, and packed in polyethylene bags with nitrogen : No. 9, 10 : Low quality, sliced, and unpacked ; No. 11: Low quality, shiratabushi ; No. 12 : High quality, mebushi, vacuum packed ; No. 13 : High quality, obushi, vacuum packed

*2: Peroxide value

*3: Thiobarbituric acid

*4: Mean±SD (n : 3)
icosapentaenoic acid (EPA) (20:5) (5.0 %), 16:1 (4.0 %), 14:0 (3.9 %), and 20:4 n−6 (2.1 %). Through the boiling and smoking stages, no significant changes were observed in the fatty acid compositions of the bonito muscles. In hadakabushi, DHA drastically decreased to 20.7 % while 16:0 increased to 30.8 %. After the mold treatment, the DHA content increased to a level of 24.8 % due to the decreases in 16:0 and 18:1.

In the purchased katsuokezuribushi (sample numbers 6~10), and the whole (non-sliced fillets) katsuobushi (sample numbers 11~13), fatty acids of 14:0, 16:0, 16:1, 18:0, 18:1, 20:4 n−6, EPA, and DHA content varied in the range of 1.5~3.9, 21.2~31.1, 2.0~4.3, 8.0~12.6, 11.7~15.0, 2.3~4.3, 4.0~5.3, and 19.1~30.9 %, respectively. In samples 10 and 11, DHA content was low at 19.1 and 21.4 %, respectively.

Figure-4 shows the lipid class composition of katsuobushi samples taken from the processing stages. In the raw muscle, TG and PL+MG were the major component at 75.2 and 21.6 %, respectively. FFA, sterol (ST), and 1,2-diacylglycerol (DG) contents
were 1.6, 0.9 and 0.4 %, respectively. In the boiled sample, TG decreased to 19.0 % while PL+ MG, FFA, and ST increased to 54.4, 18.5, and 8.0 %, respectively. In the smoked sample, TG showed a relative increase to 58.6 %, because of the sharp decrease in PL+MG down to 10.0 %. FFA, 1,3-DG, ST, and 1,2-DG contents were 23.0, 4.8, 2.1, and 1.2 %, respectively. In the hadakabushi, TG and FFA were 68.3 and 11.7 %, respectively. After the mold treatment, FFA significantly increased to 53.0 % while the TG decreased to 25.4 %. Lipid class composition of the commercial katsuobushi samples is shown in Fig.-5. In sample numbers 9 and 10, PL+MG was the highest, 61.2, 54.8 %, respectively, while FFA was the lowest, 7.1 % in sample number 10. In all samples, ST varied between 3.3–9.9%.

The PL composition of the katsuobushi samples taken from the processing stages is

1: PL, phospholipid including monoacylglycerol; DG, diacylglycerol; ST, sterol; FFA, free fatty acid; TG, triacylglycerol.
2: See text in Fig.-1.

Fig.-4 Change in the lipid class during katsuobushi processing.

Fig.-5 Lipid classes in sliced katsuobushi of commercial products.
shown in Fig. 6. In the raw muscle, the major PLs were phosphatidylcholine (PC) (48.3 %), phosphatidylethanolamine (PE) (30.3 %), lysophosphatidylcholine (LPC) (9.5 %), phosphatidylserine (PS) (4.3 %), and lysophosphatidylethanolamine (LPE) (3.0 %). After the boiling process, PE decreased to 22.5 % and LPE increased to 8.1 %. In smoked bonito muscles, PC, LPC, and PE were found at 46.7, 24.8, and 6.7 %, respectively. In the hadakabushi, PS and PE were slightly higher at 11.3 and 11.7 %, respectively. After the mold treatment, LPC, PE, PS, and LPE slightly decreased to 20.9, 9.9, 8.1, and 2.0 %, respectively. The PL composition of commercial samples is shown in Fig. 7. In all samples, the PC (40.7–65.7 %), LPC (15.0–16.0 %), and PE (8.4–20.6 %) were predominant. In samples 9, 10, and 11, PE was 12.8, 20.6, and 19.3 %,

Fig. 6 Changes in lipid classes of PL fractions during katsuobushi processing.

Fig. 7 Lipid classes in PL fractions of commercial katsuobushi products.
respectively, with these values being higher than in other samples.

4 Discussion

The lipid content of 2.7 g/100 g muscle was in the range of the report by Balogun et al. on the bonito that the lipid content varied from 1.4 to 3.6 g/100 g over a one-year period\textsuperscript{13}. During the katsuobushi processing, lipid content of raw muscles sharply decreased after the boiling process. In katsuobushi processing, the lipid content and initial freshness of the fish markedly effects the quality of the final product. It is known that in katsuobushi manufacturing the preferable lipid content of bonito is about 2\textperthousand 3%. In the high lipid content bonito, the catalytic effect of ferric hemes on the oxidation of unsaturated lipid, intermediates the peroxide that can decompose hemes, resulting in a loss of color which decreases the quality of products\textsuperscript{4}.

In this study, PV increased to a maximum after the boiling stage. This result complements the sharp rise and fall in PV found by Suyama\textsuperscript{3}. The PV and TBA value indicate that boiling and smoking processes do not oxidize the unsaturated fatty acids of the samples. Chandrasekhar et al. reported that in smoked oil sardine a PV below 20 was quite acceptable\textsuperscript{14}. Peroxide values as high as 184.6 meq/kg lipid have been reported for edible, salted, and sun-dried Indian mackerel (\textit{Rastrelliger kenagarta})\textsuperscript{15}. High PV in the 8 different commercial katsuobushi products might be due to the long storage. In the previous research, aliphatic aldehydes and aliphatic ketones were thought to have been formed by the oxidation of lipids and fatty acids and by the degradation of amino acids during the katsuobushi manufacturing process\textsuperscript{16}.

During the boiling and smoking stages the fatty acid composition of bonito remained almost constant. Thus, the fatty acid composition of the bonito lipids seemed to be stable to the boiling and smoking processes. These results are similar to those of other investigators such as Gall et al. who concluded that the fatty acid composition is not significantly changed by baking, broiling, or microwave processes\textsuperscript{17}. It is interesting that smoking of mackerel also did not to effect the polyunsaturated fatty acid content. Probably a combination of surface hardening and the phenolic antioxidants of smoke were factors enhancing this stability\textsuperscript{18}. After the mold treatment, DHA percentage increased due to decreases in 16 : 0 and 18 : 1 which were possibly consumed by mold. After the boiling treatment, TG sharply decreased with increases in PL+MG and FFA, due to the hydrolysis of TG. In the smoked muscles, PL+MG decreased and this relatively increased the TG and FFA percent. It can be concluded that the heat treatment oxidized the PL in bonito muscle which contains highly unsaturated fatty acids. However, an increase in the PV of the sample was not observed. This may be due to the surface of the smoked fillets being scraped off just before the analyses. The PL fraction was characterised by its relatively high amount of polyunsaturated fatty acids compared to the other lipid fractions. It has been reported that commercial fish oils are generally stripped of their PL content during processing\textsuperscript{19}. In general, lipid oxidation in meats and meat products occurs in PL and TG. However, Igene et al. reported that PL was initially oxidized and then after a prolonged period of time the TG was oxidized\textsuperscript{20}. Principle differences in the neutral lipid composition was in the significant increase in FFA during the mold treatment. In the molding process, it is clear that the mold produces lipase which hydrolyzes the fatty acids from glycerides. In the commercial katsuobushi products, FFA content was high, except for sample 10. The reason for this might be that the product was not properly mold treated which requires considerable time.

The smoking process decreased the PE, and LPC relatively increased in bonito muscles. Decreases in the phospholipid components may be due to either autolipid hydrolysis, hydrolytic decomposition, lipid browning reactions or lipid–protein co-polymerization as outlined by Lea\textsuperscript{21}. He studied oxidation of
egg PE and PC, and found that PE oxidized much faster than the PC fraction. Variations in the fatty acids of PL fraction in the smoked sardine fillets during storage were pronounced. Igene et al. modelled oxidative process and found the PL fraction, in particular PE, to be especially vulnerable to oxidation\(^2\)). It has been pointed out that polyunsaturated fatty acids associated with PE tend to be more labile to heat than those of PC\(^2\)). After the mold treatment, slight changes were observed in the PE and PS. It has been reported that the PLs, PC, PE, SPM, and PS of bonito muscle during ice storage decreased, while LPC, LPE, and FFA increased\(^\text{21}\)). In most of the commercial samples, lipid composition was the same as that of the final katsuobushi product taken from the processing stages. Surprisingly, however, the samples which had different lipid classes were identified as low quality before the analyses.

As a result, variations in the lipid composition and the fatty acid content were observed at each processing stage. Lipid constituents were mostly altered during the mold process, by lipase produced by the mold. By analyzing the lipid of katsuobushi, the quality of the products can be evaluated and whether katsuobushi processing was fully carried out properly or not can be ascertained.

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References
かつお節製造における脂質変化及び脂質組成からの市販製品の品質評価

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かつお節製造における節の脂質変化及び市販かつお節の品質に及ぼす脂質の影響について検討した。脱節脂質のトリアシルグリセリン (TG) の組成比は原料の 75.2％から 68.3％に減少し、かび付き処理後、TG はさらに 25.4％になったのに対し、遊離脂肪酸 (FFA) は 53％に増えた。これはかびからのリパーゼによる TG の加水分解と考えられた。また、ドコサヘキサエン酸 (DHA) の組成比も FFA の増加に伴って増えた。これらのことから、FFA 組成比 (7.1％) と DHA の組成比 (19.1％) がともに低い市販品かつお節はかび付け処理がうまく行われていなかったと判断された。ぱい（焙）乾処理後、ホスファチジルエタノールアミンは 22.5％から 6.6% に減少し、ホスファチジルコリンはほとんど変化しなかった。このようにかつお節の脂質分析でその製品の品質評価及び加工処理の適切さを推察した。

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