EVALUATION OF ANTIOXIDATIVE EFFECTS OF DRIED BONITO STOCK (*KATSUO-DASHI*) ON MEAT FAT IN COOKING AND STORAGE

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Abstract—**Lipids in fish and meat are oxidized during thermal cooking, processing and storage. The oxidation of lipids causes deterioration in the quality of meat and directly affects many quality characteristics such as flavor, color, nutritional value and taste of dishes. *Katsuo-dashi* (dried bonito stock) which is prepared by extraction from *katsuobushi* (dried bonito), is used as a seasoning for various kinds of Japanese dishes because of its unique flavor and taste. Though it has been reported that *katsuo-dashi* has antioxidative activity, very few published reports are available on the antioxidative effect of *katsuo-dashi* used for fish and meat dishes, despite the fact that these are frequently eaten in huge quantities among nations worldwide. In this study, the changes in the degree of oxidation of fish and meat dishes using *katsuo-dashi* in cooking and storage were studied. Sensory evaluation was carried out regarding the odor of fish and meat. The changes in thiobarbituric acid (TBA) values relating to thermal cooking and the peroxide values (POV) seen during storage were investigated. Furthermore, the odor generated during thermal cooking was analyzed by GC-MS. In addition, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of *katsuo-dashi* was measured as an indicator of antioxidative capacity. A sensory evaluation showed that fish and meat dishes cooked with *katsuo-dashi* suppressed the odor. TBA value and POV of the dishes cooked with *katsuo-dashi* were lower than those cooked with water. GC-MS showed that off-flavor contents in the volatiles of dishes cooked with *katsuo-dashi* were also reduced. These results indicated that *katsuo-dashi* was effective in suppressing lipid oxidation caused by thermal cooking, refrigerated and frozen storage. (264/300)**

Index Terms—**antioxidative activity, cooking and storage, *katsuo-dashi* (dried bonito stock), lipid oxidation.**

I. INTRODUCTION

Meat plays a very important role in the diet by contributing quality protein, essential minerals and a range of B vitamins. In addition to its nutritive value, meat has other important attributes, including its attractive sensory properties. The main factors governing the eating quality of meat are taste, color and flavor. Modern trends toward convenience foods have resulted in an increase in the production of pre-cooked meat products. However, such foods are highly susceptible to lipid oxidation and off-flavor development. Lipid oxidation is the primary process by which quality loss of meat based foods occurs (Buckley, Morrissey and Gray, 1995).

Katsuobushi (Dried bonito) is a well-known flavoring agent of traditional Japanese foods. The process of making *katsuobushi* is that the cut up bonito is boiled and the meat is smoked and dried. *katsuo-dashi* (dried bonito stock) is prepared by extraction from *katsuobushi*. *Katsuo-dashi* is used as a seasoning for various kinds of Japanese dishes because of its unique flavor and taste. Many researchers have studied about the flavor and taste compounds in *katsuo-dashi*. For example, phenolic compounds were identified as the important components contributed to the characteristic aroma of *katsuobushi* (Kim, Yamanishi, Nakatani and Matsui, 1971). As for the taste compounds, it was reported that the active taste components identified by the omission test of *katsuo-dashi* were glutamic acid, histidine, lysine, carnosine, IMP, inosine + hypoxanthine, creatinine, lactic acid, sodium, potassium and chloride ions (Fuke, Watanabe, Sakai and Konosu, 1989). Furthermore, it was reported that *katsuo-dashi* scavenged DPPH radical to show antioxidative activity (Yamada, Igarashi and Matsuda, 2008). The purpose in this study is to investigate the antioxidative effect of *katuo-dashi* on lipid peroxidation in fish and meat caused by actual cooking, refrigerated and frozen storage.

II. MATERIALS AND METHODS

Preparation for katsuo-dashi. Fifty g and 100 g of thin sliced (approximately 1 mm) *katsuobushi* (called "*Atsukezuri*" in Japanese) was soaked into 20-times equivalent boiling water (1,000 ml) and extracted for 10 min. Extracts were filtered by No.2 paper filter, giving a final 5% and 10% of *katsuo-dashi*.

Radical scavenging activity of Katsuo-dashi. Radical scavenging activity was evaluated by colorimetric method using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as previously reported with minor modifications (Kitagaki and Tsugawa, 1999). Eight hundred µl of sample was mixed with 200 µl of 0.5 M Tris-HCl buffer (pH 7.4) and then added 1 ml of 500 µM DPPH in ethanol. The mixture was shaken vigorously and incubated at 50°C for 20 min in the dark. The absorbance at 517 nm was measured. Distilled water was used as a blank instead of the sample. Radical scavenging

ratio was calculated using the equation; ($A_{blank} - A_{sample}/ A_{blank}$) x 100. A_{sample} and A_{blank} were the absorbances of the sample and the blank. The half-inhibition concentration (IC_{50}) values were expressed as umol of trolox equivalent (TE) per 100 ml of each sample because trolox was a stable antioxidant and is widely used as an index of antioxidative activity.

Preparation for dishes. Five % and 10% *katsuo-dashi* were used for preparing all experimental dishes. Distilled water was used as a control instead of *katsuo-dashi*. Boiled sardine was selected as a representative fish dish. Four sardines with their head and internal organs removed were boiled at 90°C for 30 min in water or *katsuo-dashi*. Chicken meatball was chosen as a representative meat dish because of its susceptibility to lipid peroxidation. One hundred g of minced chicken thigh was mixed with 5 g of potato starch, 1 g of salt and 15 ml of *katsuo-dashi* by a food processor. 10 g of the mixture was ovally shaped and roasted in an oven for 7 min. The sardine fishball was cooked as follows. One hundred g of sardine fillet, 7 g of potato starch, 2 g of salt and 15 ml of *katsuo-dashi* was mixed in a food processor. Ten g of mixture was ovally shaped and boiled for 3 min. After packing in a polyethylene bag and cooling in water, the sardine fishball was refrigerated at 5°C and frozen at -30°C respectively.

Sensory evaluation. A trained panel (n=8-15) evaluated cooked samples of boiled sardine and chicken meatball. At each panel session, panelists were served 3 types of sample dishes treated with 5%, 10% *katsuo-dashi* and water as a control. They evaluated undesirable fish or meat odor intensity using scoring method with a scale of 0 (not detectable) to 5 (extremely intense).

Thiobarbituric acid (TBA) assay. The degree of lipid oxidation was determined by TBA methods reported by Kikugawa, Kojima, Yamaki and Kosugi (1992). Briefly, 3g of sample was homogenized with 10 ml of 1.15% KCl solution. One hundred µl of homogenate, 200 µl of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid solution adjusted at pH3.5, 50 µl of 0.8% dibutylhydroxytoluene (BHT), 1.5 ml of 0.8% TBA solution and 700 µl of 5 mM ethylenediaminetetra acetic acid (EDTA) disodium salt were added in this order and mixed well. The mixture was kept on the ice for 60 min and then heated in boiling water for 60 min. The reaction was stopped by adding 1 ml of water and n-butanol : pyridine (15:1, v/v). After centrifuging for 3,000 rpm for 10 min, absorbance at 532 nm of upper layer was measured. Distilled water was used for blank instead of the homogenate. TBA value (µmol/ g wet) was calculated using the equation as follows; $(A_{sample} - A_{blank})/ 156{,}000 \times 5.8/ 10^3 \times 10^2/ 3 \times 10^6$.

GC-MS analysis. Volatile components of boiled sardine and chicken meatball were analyzed by GC-MS. The GC-MS conditions were as follows. GC: Agilent Technologies 6890N, MS: Agilent Technologies 5975, Column: DB-WAXTER (0.25 mm x 60 m). Carrier gas: He 1.0 ml/ min. The column temperature was programmed from 40°C (for 5 min) to 260°C (for 5 min). These two steps were connected via a 6°C/ min gradient. The injector temperature was held at 220°C. The MSD detecter's ionizing voltage was set at 70 eV and the ion source temperature was set at 230°C.

Peroxide value (POV) assay. The POV method was based on previous report (Ishida, Fukui, Matsuda and Matoba, 2005). Briefly, a 3g sample of dish was homogenized with 1.5 ml of distilled water, 5 ml of 90% methanol and 2.5 ml of chloroform. The homogenate was extracted with 2.5 ml of distilled water, 2.5 ml of chloroform and 10 ml of hexane. The extract was shaken and centrifuged at 2,000 rpm for 5 min. In a 25 ml of volumetric flask, chloroform was added to 1 to 2 ml of the upper layer to give a final volume of 5 ml. Next, 10 ml of acetic acid and 1 ml of 50% (w/v) potassium iodide solution were added followed by deaeration with nitrogen gas. The mixture was shaken vigorously and allowed to stand for 5 min at room temperature. Subsequently, 2% cadmium acetate was added to make a final volume of 25 ml, and the sample was shaken and allowed to stand for more 5 min at room temperature. The mixture was heated at 40°C for 1 min for separation. And the absorbance of the upper layer at 410 nm was measured. POV (meq/ kg) was calculated by the following equation; $\{(A_{sample} - A_{blank}) \times 60.14 + 0.69\}/ (0.008 \times W)$. A_{sample} and A_{blank} mean the absorbances of sample and blank. W means the weight of the lipid of the extract. For measurement of the weight of the lipid, 1 ml of the upper layer of the chloroform/ hexane extract was exposed to nitrogen gas to remove the solvent, and the residue was weighed.

III. RESULTS AND DISCUSSION

DPPH radical scavenging activity of katsuo-dashi. The radical scavenging activity increased in a concentration-dependent manner. The activity of 5% and 10% *katsuo-dashi* was 70.3 µmol TE/ 100ml and 130.3 µmol TE/ 100ml respectively. The scavenging activity was maintained during thermal cooking (data not shown). We have previously identified phenol compounds and creatinine as the antioxidative components in *katsuo-dashi* (Yamada, Akahori and Matsuda, 2009).

Sensory evaluation of undesirable fish and meat odors of dishes. In the boiled sardine, the fish odor of dishes boiled with 5% and 10% *katsuo-dashi* was scored 3.14 and 2.25 respectively (Table 1). As for the chicken meatball, all panelists evaluated the meat odor of the meatball cooked with water strongest with a score of 5. The meat odor of the meatball cooked with 5% and 10% *katsuo-dashi* was rated 2.38 and 1.75 respectively (Table 2) confirming that both experimental dishes with 5% and 10% *katsuo-dashi* had statistically significant differences. From these results, the suppressive effect of *katsuo-dashi* on the fish odor of the boiled sardine and the meat odor of the chicken meatball can be seen. In addition, the suppressive effect of *katsuo-dashi* followed a concentration-dependent manner.

Table 1. Sensory evaluation of boiled sardine.

Boiled with	Intensity of fish odor
water	4.61
5% *Katsuo-dashi*	3.14*
10% *Katsuo-dashi*	2.25*

* within the table is significantly different from water ($p < 0.05$).

Table 2. Sensory evaluation of chicken meatball.

Cooked with	Intensity of meat odor
water	5.00
5% *Katsuo-dashi*	2.43*
10% *Katsuo-dashi*	1.43*

* within the table is significantly different from water ($p < 0.05$).

TBA value of dishes. Lipid peroxides generate a variety of secondary reaction compounds including cleavage to form aldehydes. In meat and meat products, an evaluation of TBA value is the most commonly accepted index for detecting lipid peroxidation (Melton, 1983). Figure 1 shows the TBA value of sardine boiled with water and *katsuo-dashi*. Five % and 10% *katsuo-dashi* decreased the TBA value by approximately 20% and 55% as compared with water. In the chicken meatball, concentrations of the 5% and 10% *katsuo-dashi* were 23% and 28% lower than that with water, which was significantly different (Figure 2). The higher the scavenging activity of *katsuo-dashi* was, the more the TBA value of boiled sardine and chicken meatball was suppressed. These results suggested that the activity was retained and was responsible for the suppression of lipid oxidation during thermal cooking.

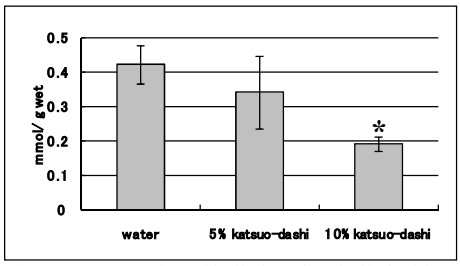

Figure 1. TBA value of boiled sardine boiled with water or *Katsuo-dashi*.
* within the column is significantly different from water ($p < 0.05$).

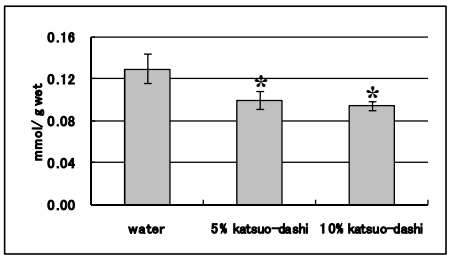

Figure 2. TBA value of chicken meatball cooked with water or *Katsuo-dashi*.
* within the column is significantly different from water ($p < 0.05$).

GC-MS analysis of volatiles from boiled sardine and chicken meatball. It has been reported that aldehydes and ketones generated by lipid peroxidation cause fishy odor of sardine (Yoshiwa, 1992), and in particular, hexanal, heptanal, octanal, 2,4-octandienal, 2,3-pentandion have a low threshold of smell and are responsible for fishy undesirable odor (Takamura, 2006). In this study, 14 kinds of aldehydes and 4 kinds of ketones are observed in the volatiles of boiled sardine. The peak area ratios of aldehydes and ketones in samples cooked in 10% *katsuo-dashi* decreased between 0.57 and 0.84, 0.65 and 0.77 when compared with samples cooked in water alone (Table 3). This result was consistent with the report that lipid oxidation causes degradation of fatty acids to generate undesirable fish odor (Takamura, 2006). As for the chicken meatball, it has been reported that hexanal is the characteristic component causing undesirable chicken meat odor (Kerler and Grosch, 1997). Figure 3 shows the GC-MS chromatograms of chicken meatball before and after cooking with water and 10% *katsuo-dashi*. Hexanal content increased 4.7 fold during thermal cooking and was suppressed 15% in the *katsuo-dashi* sample.

Table 3. The comparison of volatile compounds in the 10% *katsuo-dashi* and water samples.

Aldehydes	
Compounds	Ratio
Pentanal	0.81
Hexanal	0.82
2-Pentenal	0.72
Heptanal	0.84
2-Hexenal	0.84
4-Heptenal	0.78
Octanal	0.68
2-Heptanal	0.70
Nonanal	0.64
2-Octanal, (E)-	0.57
2,4-Hptadienal, (E, E)-	0.67
Benzaldehyde	0.74
2,6-Nonadienal, (E, E)-	0.72
2,4-Octadienal, (E, E)-	0.70
Ketones	
Compounds	Ratio
2,3-Pntanedione	0.73
2,5-Octanedione	0.71
2-Nonanone	0.77
3,5-Octadien-2-on	0.65

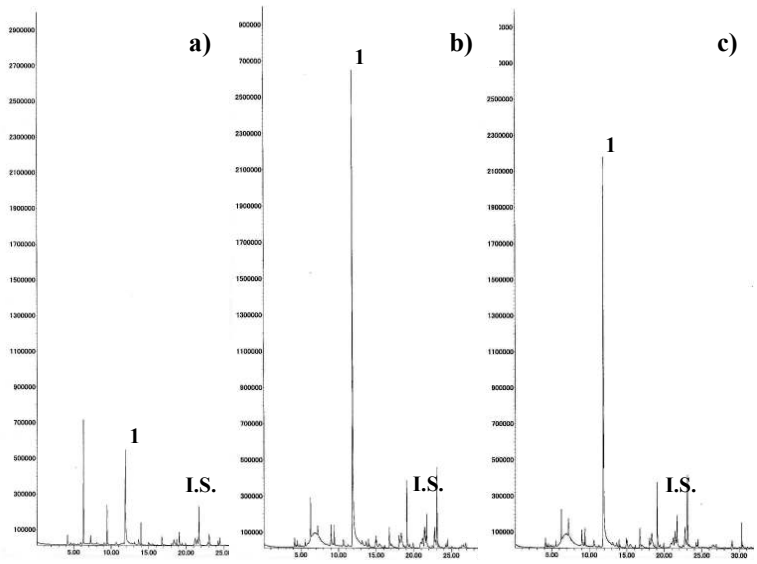

Figure 3. GC-MS chromatograms of a chicken meatball before or after thermal cooking (cooked with water or 10% *katsuo-dashi*).
a): before cooking, b): cooked with water, c): cooked with 10% *katsuo-dashi*
1: hexanal, I.S.: cyclohexanal

POV of sardine fishball during refrigerated and frozen storage. Figure 1 shows the change of POV of sardine fishballs stored at 5°C for 1 week. Although there weren't any significant differences among the three sample dishes just after cooking, a rise in the value was seen after two days. This value was suppressed with *katsuo-dashi*. In particular, on the fifth and seventh day, the values with 5% and 10% *katsuo-dashi* were significantly lower when compared with water. Figure 2 indicates the change of POV at -30°C for 3 months. There weren't any significant differences among the three sample dishes just after cooking. After the first and second months, the values in the *katsuo-dashi* processed samples were lower than those of water. As for 10% *katsuo-dashi*, the values were significantly lower for the entire three months time period. These results suggested the antioxidative activity of *katsuo-dashi* was maintained not only in thermal cooking but also during storage.

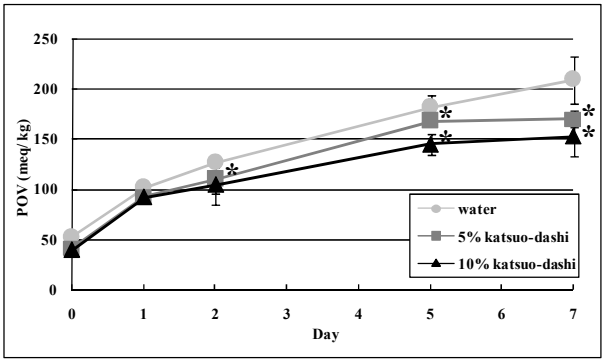

Figure 1. The change in POV of sardine fishball during refrigerated storage. * within the graph is significantly different from water ($p<0.05$).

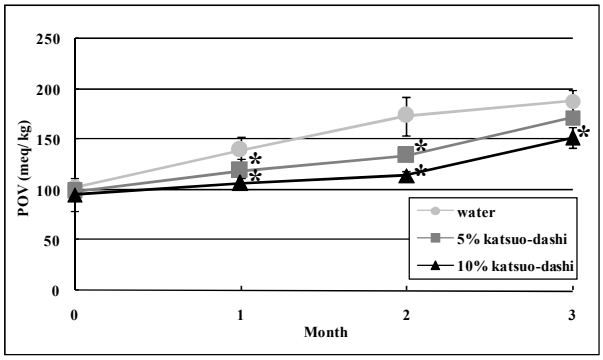

Figure 2. The change in POV of sardine fishball during frozen storage. * within the graph is significantly different from water ($p<0.05$).

IV. CONCLUSION

We investigated the antioxidative effect of *katsuo-dashi* on lipid oxidation in fish and meat caused by actual cooking, refrigerated and frozen storage. In this study, *katsuo-dashi* suppressed the lipid oxidation of the dishes after thermal cooking, refrigerated and frozen storage, and showed usefulness in a variety of food processing. These results show how to prepare foods with significantly lower amount of lipid oxidation. It was found that *katsuo-dashi,* which is traditionally used as a seasoning for various kinds of Japanese dishes, would be useful for thermal cooking and improving the preservation of processed foods.

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