Quality Maintenance of Mackerel Using Honey

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***Abstract*—In recent years, the popularity and consumption of mackerel has increased worldwide. However, it is important to maintain the quality of mackerel since it deteriorates rapidly. In this study, we investigated the effects of honey on the number of microorganisms, freshness, and lipid oxidation, which are related to the quality of chub mackerel. As an influence on the number of microorganisms, we isolated microorganisms from fish meat of chub mackerel and investigated the propagation inhibition effect of honey. We investigated the effect of honey on IMPase activity, which influences freshness. Also, we measured the degree of lipid oxidation in the presence of fish oil and honey or water. As a result, quality retention was improved in all three evaluation methods.**

***Index Terms*—freshness, honey, lipid oxidation, mackerel, microorganisms, quality**

I. Introduction

Mackerel is a very common edible sea fish predominantly found along the western Pacific and Atlantic coastal regions [1]. Mackerel is rich in lipids and is considered very delicious, thus it is popular in many areas. Mackerel is a fast growing fish species that was not previously restricted. However, as a result of fishers ignoring resource maintenance guidelines, the amount of chub mackerel in the sea near Japan decreased from about 4.8 million tons in 1978 to about 200,000 tons around 1990 [2]. Therefore, the total allowable catch (TAC) system was established in 1996 and the fishing of chub mackerel became restricted, along with saury and bluefin tuna [3]. On the other hand, the main fish species caught in the Atlantic Ocean is Atlantic mackerel, and the stock amount has fluctuated greatly since 1985; also, there has not been a remarkable increase or decrease and the numbers have gradually recovered from 2006 to 2010 [4]. However, the amount of exports to Asia, including Japan, has increased since 1990 and it is also important to maintain resources. Therefore, the TAC system has been applied to Atlantic mackerel as well and the catch is determined by international negotiations ever year [5]. In this way, chub mackerel is caught while maintaining resources, and maintaining quality after catching is important in order to not waste limited resources.

Since the quality of mackerel deteriorates relatively rapidly, it is currently generally frozen prior to distribution. In the case of refrigeration, it is often preserved by soaking it with seasonings such as salt, vinegar, and mirin to prevent deterioration in quality. The salt in the seasonings has microbial growth inhibitory effects [6] and enzyme activity inhibitory effects [7], so it is used to preserve many foods. On the other hand, sugar reduces water activity by keeping the sugar content above a certain level and suppressing the growth of many microorganisms; thus, it is mainly used to preserve foods such as jam and marron grace [8]. Honey has a high sugar content like sugar.

Honey contains more than 150 ingredients such as vitamins B1, B2, and folic acid; 27 minerals such as Na, K, Ca, and Fe; 22 amino acids; and 80 enzymes [9], so the nutritional value is higher than that of caster sugar. In addition, when heated with disodium hydrogen phosphate or sodium hydrogen carbonate, honey improves quality retention by suppressing the growth of *Escherichia coli* and promoting the growth of lactic acid bacteria [10]. The effect of honey in delaying the lipid oxidation of beef [11] and the protective effects on rat antioxidant enzymes [12] have also been reported. Therefore, it is hypothesized that quality can be maintained by using honey.

In addition, honey has good effects on cooking and health. For example, it can be used to deodorize fish, it improves the taste of some foods, helps in recovery from fatigue [13], and improves the intestinal environment by isomaltooligosaccharide [14]. Therefore, it has an effect superior to that of sugar, and the use of honey can be expected to increase the added value in addition to quality preservation of mackerel.

In general, the quality of marine products is evaluated based on the sensory evaluation of the number of microorganisms, freshness, lipid oxidation, appearance, and odor [15]. However, as mackerel deteriorates quickly, the K value, which is an indicator of freshness, rises relatively quickly and the number of microorganisms tends to increase. In addition, because it is a fish species rich in lipids, the oxidation of lipids also greatly affects quality. The K value representing freshness is the ratio of the decomposition products of ATP (adenosine triphosphate) and is expressed by the following formula [16],

$$\text{K value (\%)} = (\text{HxR}+\text{Hx})/(\text{ATP}+\text{ADP}+\text{AMP}+\text{IMP}+\text{HxR}+\text{Hx}) \times 100$$

ADP, adenosine diphosphate; AMP, adenosine monophosphate; IMP, inosine monophosphate; HxR, inosine; Hx, hypoxanthine

Since the degradation from ATP to IMP proceeds relatively quickly, IMP accumulates in the muscle.

Manuscript received September 26, 2020; revised November 28, 2020.

doi: 10.18178/ijfe.6.2.35-39

However, IMP is degraded to HxR and Hx over time by IMPase. Therefore, it is necessary to suppress the activity of IMPase in order to suppress the increase in K value.

Therefore, in this study, we first investigated the effects of honey on microorganisms isolated from mackerel, followed by mackerel IMPase activity, and finally fish oil oxidation. In this manner, the purpose of this study was to investigate the quality retention effect of honey on mackerel.

II. Materials and Methods

A. Materials

Mackerel (*Scomber japonicus*) was purchased at a retail store near our university (Tamagawa University). The honey used was "Tamagawa Flower Field Hundred Flower Honey (Made by Ou Bee Garden)". The fish oil used for lipid oxidation measurement was DHA oil.

B. Bacterial Count in Honey

To confirm the presence of halophilic microorganisms in the honey used, 100 μL of honey diluted 2, 5, 10 times with sterile water was smeared on 0.9 % saline medium and incubated at 20 ℃ for 1 week.

C. Isolation of Some Bacteria from Mackerel Muscle

Mackerel was minced into 5 g pieces, collected in a 50 mL centrifuge tube, and the volume was brought to 50 mL with 0.9 % saline. After mixing well, the supernatant was appropriately diluted, and 100 μL of the diluted solution was smeared on a standard agar medium containing 0.9% sodium chloride. After culturing at 20 ℃ for 168 h, colonies that appeared were confirmed, and 10 types of bacteria with different colony properties and colors were isolated [17].

D. Effect of Honey in Bacterial Count in Chub Mackerel

Of the 10 species of bacteria isolated, the bacterial species with the highest number of bacteria was suspended in 10 mL of 0.9 % sterilized saline to obtain a bacterial solution. To confirm the effect of honey on the bacteria, 0.1 mL of the bacterial solution, 0.5 mL of appropriately diluted honey, or 0.5 mL of 41 % glucose, and 0.4 mL of saline were placed in an Eppendorf tube and cultured at 20 ℃ for 2-3 weeks. After incubation, 100 μL of the diluted solution was smeared on a standard agar medium containing 0.9 % NaCl and cultured at 20℃ for 1 week.

E. Effect of Honey in IMP-degrading Enzyme Activity in Chub Mackerel

The mackerel was homogenized with three times the amount of RO water and the resulting homogenate was dialyzed against cold water at 4 ℃ for 2 days. The filtrate obtained by filtering the dialyzed solution with a 0.45 μm filter was used as the crude enzyme solution. To measure the IMPase activity, 0.5 mL of 25 mM IMP, 2 mL of Buffer (50 mM Maleic acid / Tris / NaOH: pH 6), honey or water diluted 2-fold, 5-fold, or 10-fold in 0.5 mL of crude enzyme solution 0.5 mL was added to prepare a reaction solution. The reaction was allowed to react for 1 day at 30 ℃, and the enzyme reaction was stopped by adding 1.5 mL of 10% $HClO_4$. This was centrifuged at 12,000 rpm at 25 ℃ for 5 minutes, and the phosphate released in the supernatant was measured by the molybdenum blue method (750 nm).

F. Effect of Honey in Lipid Oxidation in Chub Mackerel

The degree of lipid oxidation was analyzed by the iron thiocyanate method [18]. Honey or water (10 mL) diluted in appropriately 2 mL of fish oil was added, and the mixture was oxidized for 0 to 30 hours using an incubator at 60 ℃. After oxidation, 10 μL of fish oil was added to 10 mL of ethanol / diethyl ether, which was mixed well at a 6:4 ratio. Furthermore, 0.1 mL of 30 % ammonium thiocyanate aqueous solution was added and mixed well, and then 100 μL of 0.02 M iron (II) chloride-3.5 % hydrochloric acid aqueous solution was added and mixed well. After 10 minutes of reaction, absorbance at a wavelength of 490 nm was measured with a plate reader.

G. Statistical Analyses

Data were subjected to one-way ANOVA using the least significant difference method and the Student's t-test. Differences were considered statistically significant at a p value < 0.05.

III. Results and Discussion

As a result of culturing honey diluted 2, 5, 10 times on a standard agar medium containing 0.9% NaCl, colonies were not confirmed at any dilution factor, and it was found that halophilic microorganisms were not present in the honey. Ten species of bacteria with different colony properties and colors were isolated from chub mackerel.

TABLE I. Number of Microorganisms in Mackerel when Dilute Honey is Added

$\log_{10}$(CFU/mL)	1-week	2-week
2x diluted honey	0	0
5x diluted honey	1.3 (2.5)	0
10x diluted honey	5.9 (0.27)	3.9 (0.34)

Values represent the mean of four independent determinations (n =4). Standard deviations are given in parentheses

Table I shows the results of cultured bacteria isolated from mackerel muscle with diluted honey. In 1-week cultures, the number of bacteria was not confirmed when 2x diluted honey was added, but the number of bacteria was 1.3 and 5.9 $\log_{10}$CFU/mL when 5x and 10x diluted honey was added to each sample, and the number of bacteria was suppressed (p<0.05). In 2-week cultures, the number of bacteria was not confirmed when 2x and 5x dilution honey was added, but the number of bacteria was 3.9 $\log_{10}$CFU/mL only when 10x diluted honey was added. Therefore, as in 1-week cultures, the higher the honey concentration, the more the bacteria were suppressed (p<0.05). Furthermore, when comparing the 1-week culture and the 2-week culture with the 5x, 10x diluted honey additions, there was no significant difference in the 5x honey dilution addition (p> 0.05).

But the number of bacteria in the samples that were cultured for 2 weeks with 10x dilution honey was more suppressed than the 1 week samples (p<0.05).

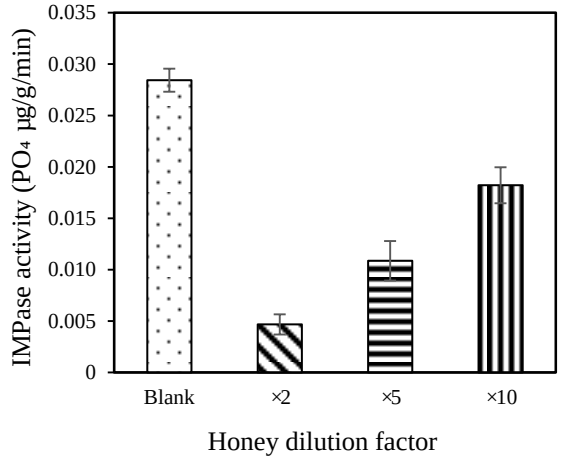

Figure 1. The effect of honey on mackerel IMPase. Error bars denote standard deviations of the mean (n=3).

Fig. 1 shows the effect of honey on IMPase activity. The IMPase activity of honey-free mackerel was 0.028 PO_4 μg/g/min; the addition of 2x, 5x, and 10x diluted honey produced the results of 0.0047, 0.011, and 0.18 PO_4 μg/g/min, respectively. The addition of 10x dilution honey showed no significant difference from honey-free (p>0.05), but the addition of 2x and 5x dilution honey showed lower values (p<0.05). Also, the IMPase activity decreased with increasing honey concentration (p<0.05), indicating that honey inhibits mackerel IMPase activity.

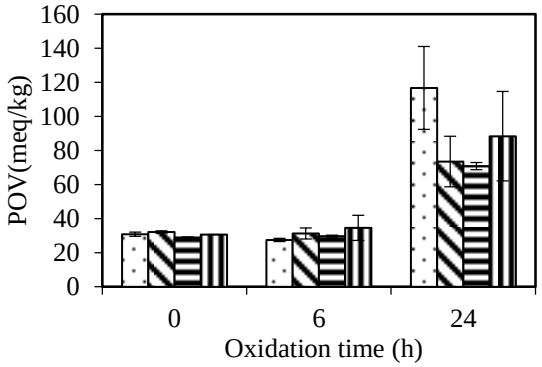

Figure 2. Effect of honey on lipid oxidation in fish oil. Error bars denote standard deviations of the mean (n=3).
Blank, Diluted honey(×2),
Diluted honey(×3), Diluted honey(×10)

Fig. 2 shows the result of the changes in the degree of lipid oxidation in fish oil by adding diluted honey. Immediately after adding honey, the lipid oxidation degree of fish oil oxidized was 29 to 32 meq/kg in each test condition, with no significant effect on lipid oxidation was observed with the addition of honey (p>0.05). The lipid oxidation degree of fish oil oxidized for 6 hours was 27 to 35 meq/kg in each test condition, and no effect on lipid oxidation by adding honey was observed (p>0.05). In addition, there were no significant changes in lipid oxidation from 0 to 6 hours in each test condition (p>0.05). On the other hand, when oxidized for 24 hours, honey-free mackerel was 117 meq/kg and it was not significantly different from the 10x dilution honey added sample, which was 88 meq/kg (p>0.05). However, when 2x and 5x diluted honey was added, it showed significantly lower values at 73 meq/kg and 71 meq/kg each (p<0.05). Although, there was no significant difference in the effect of the addition 2x and 5x diluted honey on lipid oxidation (p>0.05), the degree of lipid oxidation decreased with increasing honey concentration.

In this study, when honey was cultured in a medium supplemented with 0.9% sodium chloride, the growth of the bacteria was not confirmed. Yamazaki [19] investigated 17 bacteria in honey samples and reported that bacteria were detected in 11 of them. The reported bacterial species were *Ascosphaera apis*, *Eurotium amstelodami*, *Aspergillus*, *Cladosporium*, *Paecilomyces*, *Penicillium*, and *Scopulariopsis*, but no halophilic character has been reported for these species. Thus, it was considered that no colonies were confirmed at each dilution rate.

In this study, 10 types of bacteria with different colony properties and colors were isolated. It is said that in marine fish, including frozen and stored chub mackerel, *Pseudomonas* III/IV-H is the most common, and *Vibrio*, *Pseudomonas* III/IV-NH and *Moraxella* are the more common following it. In addition, it is reported that there are a few *Acinetobacter*, *Flavobacterium-Cytophaga*, cocci, etc. [20]. Also, *Streptococcus iniae* has been isolated from chub mackerel [21], and *Pseudomonas fragi*, *Pseudomonas veronii* or *Pseudomonas extremaustralis* has been isolated from jack mackerel [22]. We used mackerel in this study. It can be considered that 10 types of bacteria with different colony properties and colors we isolated were close to these bacterial spices.

Table I shows that honey inhibits the growth of bacteria isolated from chub mackerel. It is reported that the main antibacterial action of honey is through H_2O_2 produced from glucose oxidase [23], [24]. In 39 types of Nordic single flower honey, it was reported that the concentration of honey was 15%-30%, and as the concentration increased, the growth of *P. aeruginosa* and *S. aureus* was suppressed by many types of honey [25]. Since these bacteria are halophilic [26], it is considered that the growth-inhibiting effect of marine bacteria in this study were also confirmed. On the other hand, high sugar content also lowers water activity, and thus has an effect of inhibiting bacterial growth. Since honey and jam contain more than 36 % sugar [27] and, in general, bacteria are inhibited from growing, growth may be inhibited by high sugar content.

Fig. 1 shows that honey inhibits IMPase activity in chub mackerel. As for the effect of sugar on IMPase, it was reported that when sucrose was added to young yellowtail fish meat, the 5'-NTase activity of IMP-degrading enzyme was suppressed as the sucrose concentration increased [28]. Since honey is used in this study, the sugar component is glucose, but the activity was inhibited as the concentration increased; thus, it can be said that sugar has an inhibitory effect on IMPase activity. As a result, we identified that the amount of IMP decomposition decreased with increasing concentration of honey.

Fig. 2 shows that there was no difference in the lipid oxidation degree of the fish oil which oxidized for 6 hours, and before the start of oxidation; however, in the case of the oil oxidized for 24 hours, the degree of lipid oxidation decreased in the honey added sample. Therefore, it can be said that honey had a lipid oxidation inhibitory effect.

Honey has antioxidant effects on various foods; the effect of delaying beef lipid oxidation [11] and preventing turkey lipid oxidation have been reported [29]. Also, there are reports that honey is used to prevent lipid oxidation in meat as a natural food antioxidant [30]. The lipid antioxidant effect of honey is due to the propolis contained in honey, and the antioxidative effect of beef patties from propolis extract [11] and the lipid antioxidant effect of butter have been reported [31]. Hence, it is conceivable that the effect of the antioxidant for fish oil by propolis in honey was confirmed in this study too.

We investigated the effects of honey on microbial counts, freshness, and lipid oxidation, which are items that affect chub mackerel quality. As a result, regarding microbial counts, the number of bacteria decreased with increasing honey concentration against halophilic microorganisms. Regarding maintaining freshness, the IMPase activity was suppressed as the honey concentration increased. Regarding lipid oxidation, as the honey concentration increased, the effect of inhibit fish oil oxidation was confirmed. Therefore, it can be said that honey is effective in maintaining the quality of chub mackerel.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Hiroko Seki conducted the research; all authors analyzed the data and wrote the paper; all authors had approved the final version.

Acknowledgment

The authors wish to thank Prof. Jun Nakamura from Tamagawa University.

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