Effects of Washing Media and Storage Condition on the Color of Milkfish Meat Paste

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Fresh samples of milkfish were stored in ice for up to two weeks or frozen at -20°C for up to two months. At due time intervals, the dorsal part of ordinary muscle excised was subjected to washing by various media (water, 40 mM phosphate buffers of pH 5.6–8.0, alkaline wash), and the effects of washing media on the extracting efficiency of pigment (myoglobin) and the tristimulus color values (L, *a* and *b*) of the extract and the meat paste (washed mince) were investigated. The results obtained showed that the pigment extracting efficiency decreased during ice or frozen storage. On the other hand, as the pH values of washing media were raised, the L values of both the extract and washed mince decreased, while *a* and *b* values became higher for the extract and tended to be lower for the washed mince.

Key words: milkfish, kamaboko, washing, storage, color, meat paste

Introduction

The milkfish *Chanos chanos* Forskal is one of the tropical fishes, and has traditionally been cultivated in Taiwan, but the recent establishment of cultivating techniques in deep freshwater has expanded the production of milkfish greatly. The harvest of milkfish, which used to be around 20–30 thousand tons, increased to 90 thousand tons in 1990.¹⁾ Such a rapid increase in production, however, caused a drop in its commercial value.²⁾ Therefore, it is necessary to develop new techniques in order to raise the evaluation of milkfish products.

Generally, milkfish is not accepted by consumers as it is, because there are many tiny bones in the muscle. Thus, only the belly portion is usually used, while the bony dorsal portion is removed of the skin, bones and connective tissue, and ground into paste (surimi) for fish balls. Since the color of milkfish flesh is light pink due to the presence of myoglobin, the products turn grayish when heated. Even though the milkfish products show high gel strength, the dark color is actually a serious deterrent for human consumption.

Concerning the characteristics of milkfish muscle from biochemical aspects, several reports deal with the properties and denaturation profiles of myosin and actomyosin.^{3–7)} However, little information is available on milkfish kamaboko. Recently, Ko and Liou⁸⁾ reported that the gel strength of milkfish kamaboko was increased by washing up to about 2.5 times.

For milkfish, the most urgent problem is to remove the pigment (mostly myoglobin) from the flesh as much as possible so that the products become acceptable to con-

sumers. We therefore studied the effects of washing on the efficiency of pigment extraction and the color of meat paste (washed mince) from fresh, ice- and frozen-stored milkfish to search for a way to improve the quality (mainly the color) of milkfish kamaboko. Attempts were also made to elucidate the effect of washing media on the quality of kamaboko from fresh fish.

Materials and Methods

Materials

Fresh specimens of milkfish (in rigor mortis) were purchased from the Nan-Tzu Wholesale Market, Kaohsiung, Taiwan. The body weight and total length of the specimens were in the range of 0.6–0.9 kg and 30–40 cm, respectively. The specimens were divided into three groups; the first group was used immediately (“fresh” fish), the second group was stored in ice for up to 2 weeks (ice-stored fish), and the third group was frozen at -60°C for 24 h and then stored at -20°C for up to 2 months until used (frozen-stored fish). The fish was stored in plastic bags without any processing. Just before use, the frozen samples in bags were thawed by tap water as quickly as possible. In the following experiments, only the ordinary muscle of the dorsal portion was used.

Extraction of Pigment

Pigment (myoglobin) was extracted according to the methods of Warriss⁹⁾ and Chow *et al.*¹⁰⁾ Minced muscle was homogenized with a Nihonseiki AM-5 homogenizer (1,000 rpm for 5 min) once with 10 volumes of the following ice-cold washing media: deionized water; 40 mM Na-phosphate buffers (pH 5.6, 6.0, 6.4, 6.8, 7.2, 7.6, and 8.0);

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and alkaline wash (0.2% NaHCO_3 -0.15% NaCl). The homogenates were centrifuged at 7,000 rpm (*ca.* $5,000 \times g$) for 15 min with a Hitachi CR20B2 centrifuge, and the supernatants (referred to as "extract") obtained were used for the measurement of pigment content and color. The moisture of the precipitate (meat paste, referred to as "washed mince") was determined, and was adjusted to 82% by adding appropriate volumes of deionized water, and prior to the color measurement, was mixed by a silent cutter as described later.

Measurement of Pigment Content

In order to estimate the extractable amount of pigment, the myoglobin content in the extract was measured according to the method of Chow *et al.*¹⁰ The extract was filtered through a Toyo 5C filter paper, further filtered through a Millipore membrane filter (pore size $0.2 \mu\text{m}$). Two milliliters of the filtrate thus obtained was mixed with 2 ml of 1 M Na, K-phosphate buffer (pH 7.0) and $50 \mu\text{l}$ of 5% NaNO_2 , allowed to stand for 1 min, and the absorbance was measured at 540 nm (A_{540}) after the addition of $50 \mu\text{l}$ of 1% KCN. The molar extinction coefficient used for cyanmetmyoglobin was 11,300.¹¹ The pigment concentration was determined by the following equation, using 15,900 daltons as the molecular weight of myoglobin (W.-L. Chen, unpublished data),

$$\text{Pigment content (mg/ml)} = \frac{A_{540} \times 2}{11,300} \times 15,900$$

Color Measurement

The color of the extract and washed mince was measured with a Tokyo Denshoku TC-3600 colorimeter. The instrument was calibrated beforehand with a white standard disc (X, 98.1; Y, 100; Z, 118.2). The results were expressed by the tristimulus values of the Hunter's color difference scale, L (lightness), a (+, red; -, green) and b (+, yellow; -, blue). Transmittance was measured for the extract in a cubic glass cell, while the reflectance was measured for the washed mince using a round glass cell. Measurements were carried out four times for each kamaboko gel and in duplicate for the washed mince, and only the average values were given. Standard deviations were negligible: less than 0.7 for L value, and less than 0.4 for a and b values.

Measurement of Metmyoglobin to Total Myoglobin Ratio (MetMb%)

MetMb% was measured according to the method of Sano and Hashimoto¹² and Chow *et al.*¹⁰ To the filtrate prepared as above was added an equal volume of 1 M Na, K-phosphate buffer (pH 7.0), then it was divided into three portions. To the first portion was added a trace amount of sodium hydrosulfite, bubbled with CO gas, and the absorbance at 568 nm was measured (designated as A_1). The second portion was directly bubbled with CO gas and the absorbance at the same wavelength was measured (A_2). To the third portion was added 0.05 ml of 2% potassium ferricyanide and the absorbance at 568 nm was measured (A_3). MetMb% was calculated as follows:

$$\text{MetMb\%} = \frac{A_1 - A_2}{A_1 - A_3} \times 100$$

Measurements were performed in triplicate, and the mean values were given.

Preparation of Kamaboko

After washing once with 10 volumes of each medium, the moisture was adjusted to 82%. The washed mince was then ground by a silent cutter (Ching-Sheng-Hao Metal Factory, Model K-3) with sequential addition of 2.2% NaCl , 0.6% monosodium glutamate, 3% sugar and 5% sweet potato starch. The ground mince was then transferred into the cubes ($21 \times 30 \times 3 \text{ cm}$) and incubated at 40°C for 1 h for setting, and subsequently cut into cubes ($3 \times 3 \times 3 \text{ cm}$) and heated in a water bath at 90°C for 15 min. These setting and heating conditions were determined based on preliminary experiments to maximize the gel strength of kamaboko. The kamaboko thus prepared was cooled in ice immediately, contained in a plastic bag, and stored at 4°C overnight prior to the textural analyses as follows.¹³

Measurement of the Gel Strength of Kamaboko

Gel strength of kamaboko was measured according to the method of Chow.¹⁴ Kamaboko, cut into cubes ($2 \times 2 \times 2 \text{ cm}$), was subjected to a rheometer (CR-200D, Sun Scientific Co.) equipped with a globular plunger (7 mm in diameter). The gel strength was expressed by maximum stress (g) and deformation (cm) at the breaking point. Determination was carried out 4 to 6 times for each sample, and the mean values and standard deviations were given.

Folding Test

The folding test of kamaboko was performed according to the method of Fujii,¹⁵ and the results were graded as follows:

- AA : no cracks when folded into quadrants;
- A : no cracks when folded into semicircle;
- B : cracks when folded into semicircle;
- C : broken into pieces when folded into semicircle.

Measurement of the Expressible Water of Kamaboko

In order to estimate the water-holding capacity of kamaboko, the amount of expressible water was determined according to the method of Jauregui *et al.*¹⁶ Kamaboko was cut into a slice (0.5 g, 1 mm thick) and packed in a Toyo 4A filter paper, and centrifuged at 16,000 rpm (*ca.* $15,000 \times g$) for 15 min with a Hitachi CR20B2 centrifuge. The percentage of the weight loss was expressed as the amount of expressible water. Measurements were carried out 4 to 6 times, and the mean values and standard deviations were given.

Determination of Moisture Content

Moisture content was determined according to the AOAC method.¹⁷ Determinations were done in triplicate.

Measurement of pH

The pH value of the extract obtained by washing was directly measured with a Corning pH meter 220 equipped

Fig. 1. Effects of washing media and storage period on the pigment content and Hunter's tristimulus values (L, a and b) of the extracts from ice-stored milkfish.

Table 1. Final pH of the extracts obtained by washing the fresh, ice-stored and frozen-stored milkfish

Washing media	Fresh	Ice-stored		Frozen-stored		
		1 week	2 weeks	2 weeks	1 month	2 months
Deionized water	5.72	5.99	5.64	5.44	5.32	5.56
40 mM phosphate buffer						
pH 5.6	5.67	5.73	5.69	5.61	5.56	5.63
6.0	5.84	5.88	5.99	5.89	5.84	5.85
6.4	6.11	6.14	6.32	6.26	6.17	6.19
6.8	6.45	6.48	6.67	6.61	6.53	6.51
7.2	6.78	6.77	6.93	6.85	6.81	6.80
7.6	7.02	6.98	7.10	6.98	6.97	6.95
8.0	7.15	7.01	7.03	6.81	6.79	6.96
Alkaline wash*	7.07	7.01	7.03	6.81	6.79	6.96

* 0.2% NaHCO₃-0.15% NaCl.

with a complex glass electrode.

Results and Discussion

Effect of Washing Media on Ice-Stored Fish

Muscles from the fresh and ice-stored fish were washed with various media, and the pigment contents and tristimulus values of the extract were examined (Fig. 1). The pH values of the extract from each sample after washing are shown in Table 1. Preliminary experiments revealed that the pH values of washed mince were almost the same as the respective values for the extract (the differences were generally less than 0.03). Even using pH 5.6–8.0 phosphate

buffers for washing, the pH values of the extract fell in the range of pH 5.7–7.2. This suggests that the milkfish muscle used had already turned acidic due to the accumulation of lactic acid and inorganic phosphate, which were produced through glycolysis and breakdown of ATP, respectively.

The extractable amount of pigment from fresh muscle was 150–280 mg/l. Myoglobin is a highly water-soluble protein, and the extracting efficiency from fresh sample (by washing at pH 7.6 in this case) is considered to be close to the maximum. Taking the dilution ratio of the extract into consideration, myoglobin concentration in milkfish muscle is estimated to be around 300 mg/100 g, which is quite a reasonable value for milkfish. On the other hand, it is quite difficult to determine the amount of insolubilized pigment in the washed mince.

The extracting efficiency was the lowest for washing by water. The usage of alkaline wash (0.2% NaHCO₃-0.15% NaCl) or phosphate buffers of pH 6.4–8.0 resulted in the higher efficiency of extraction. The extractable amount of pigment, however, decreased during the two weeks of ice storage (below 150 mg/l), suggesting that myoglobin was insolubilized during ice storage. The values of met-myoglobin formation ratio (metMb%) were 19.1, 24.6 and 37.9% for the samples stored in ice for 0, 1 and 2 weeks, respectively. Such an increase in metMb% means that myoglobin oxidation proceeded in the muscle of milkfish during the ice storage. As reported previously,¹⁸⁾ autoxidation of myoglobin is accompanied by insolubilization of the protein. It follows that insolubilization of myoglobin also

steadily proceeded during ice storage of milkfish. This was also true for the case of round herring.¹⁰⁾

The *L* value (lightness) tended to increase as the pigment content decreased. The *L* value turned out to be the highest in the case of washing by water. Washing by pH 5.6 buffer resulted in a similar *L* value (>94), whereas, using phosphate buffers (pH 6.0–8.0) and alkaline wash, *L* values were decreased to around 90. It is clear that acidic media seem to have contributed to the lightening (whitening) of washed mince. On the other hand, *a* and *b* values of the extract from fresh fish were higher for alkaline media than for acidic media. The *a* value decreased moderately after one week of ice storage, but remained almost unchanged thereafter. The *b* value changed in a similar way to the *a* value, but under acidic pH (6.0 and 6.4), the values for the one-week stored sample were higher than those for the two-week stored one.

The tristimulus values of the washed mince from the ice-stored fish were clearly different from those of unwashed mince: *a* and *b* values of the washed mince were lower and the *L* value was higher (Fig. 2). It was thus suggested that the color of the mince could be improved by washing. It was observed that washing under higher pH resulted in lower *L* values. The *L* value tended to increase slightly during storage, though the extracting efficiency of pigment decreased during ice storage (Fig. 1). In the case of the washed mince, the *a* value increased after 1 week, but decreased after 2 weeks. The reason is not clear. The *b*

value showed similar changes to those for a value (except at pH 7.2 and 7.6), but generally, the higher the pH value of the washing media, the lower the *b* values became. Chow *et al.*¹⁹⁾ found that, during ice storage of tuna meat, *L* and *b* values increased and the *a* value decreased along with the increase in metMb%. They also suggested that myoglobin autoxidation was accelerated under acidic pH, causing the increase in *L* and *b* values and decrease in the *a* value.¹⁰⁾ In the case of milkfish, however, the changes in *a* and *b* values were far from those reported for tuna meat. The reasons for such differences between the two fish species could be ascribed to those in the minimum muscle pH after death and myoglobin content.

Effect of Washing Media on Frozen-Stored Fish

The pH values of the respective extract obtained by washing the frozen-stored milkfish are shown in Table 1. The values were slightly lower than those for fresh and ice-stored samples. This is supposed to be due to thaw rigor, which accelerated the breakdown of ATP.

The pigment content in the extract from the frozen-stored fish tended to decrease during storage (Fig. 3). Washing by water and acidic buffers resulted in a lower efficiency of pigment extraction. However, as the pH of the wash was raised, the extractability was increased. Though the pigment content sharply decreased only after 2 weeks of frozen storage, there were practically no further changes during the subsequent storage. Such a sharp

Fig. 2. Effects of washing media and storage period on the Hunter's tristimulus values (*L*, *a* and *b*) of the washed mince of ice-stored milkfish. Moisture content of the washed mince was adjusted to 82% after centrifugation. Control: unwashed mince.

Fig. 3. Effects of washing media and storage period on the pigment content and Hunter's tristimulus values (L, a and b) of the extracts from frozen-stored milkfish.

decrease could be due to the insolubilization of myoglobin as described above. Chow *et al.*¹⁸⁻²⁰ found that myoglobin from bluefin tuna was partly insolubilized when stored frozen, due to the structural changes of myoglobin by freezing and thawing. Incidentally, the values of metMb% were 19.1, 29.2 and 32.9% for the samples stored frozen for 0, 2 weeks and 1 month, respectively. It follows that myoglobin oxidation proceeded even during the frozen storage as was the case with the ice storage.

Except in the case of water washing, L values tended to be slightly higher compared to those of fresh fish, while a and b values were lower than those of the fresh one. Among the washing media tested, the phosphate buffers of higher pH and alkaline wash could extract a larger amount of pigment, and as a result, a and b values increased and the L value decreased.

The L values of the washed mince from the frozen-stored fish were higher than those of unwashed mince, irrespective of the washing media used (Fig. 4). However, as the pH value of washing media was raised, the L value tended to decrease. Such a tendency is quite similar to that observed for the ice-stored fish (Fig. 2). When the mince was washed by water or pH 5.6–6.0 buffers, L values were higher than 70, indicating that the washing by the acidic media resulted in lighter colored mince. Though the efficiency of pigment extraction decreased during storage as shown in Fig. 3, the effect of washing on the color of

washed mince was not obvious. The lightness of the washed mince from frozen-stored fish was roughly comparable to that of the ice-stored fish. There were no significant differences in the a value between the fresh and frozen samples. The a values of washed mince from the ice-stored fish showed a temporary increase regardless of the pH values of washing media (Fig. 2), but the extent of the fluctuation in the a value was much less in the case of frozen-stored fish. The b value decreased gradually during prolonged frozen storage.

Effect of Washing Media on the Quality of Kamaboko from Fresh Fish

Kamaboko was tentatively prepared only from fresh samples, and the effects of the washing media on the quality of kamaboko were examined (Table 2). Though the kamaboko prepared from the unwashed mince (control) gave grade AA in the folding test, the maximum stress and deformation were 596 g and 1.2 cm, respectively, and the water holding capacity was also low as judged from the amount of expressible water. Washing by any media improved the quality of kamaboko. Especially, the maximum stress and deformation were increased up to 1,317 g and 1.5 cm by washing with pH 7.2 and 8.0 buffers, respectively, and the amount of expressible water decreased to 23% by washing with pH 7.6 buffer and alkaline wash. On the other hand, the two parameters of gel forming ability

Fig. 4. Effects of washing media and storage period on the Hunter's tristimulus values (L, a and b) of the washed mince of frozen-stored milkfish. Moisture content of the washed mince was adjusted to 82% after centrifugation. Control: unwashed mince.

Table 2. Effect of washing on the quality of kamaboko prepared from fresh milkfish

Washing media	Gel strength		Expressible water (%)	Folding test	Color values		
	Max. stress (g)	Deformation (cm)			L	a	b
Control ^{*1}	596 ± 69	1.2 ± 0.2	30 ± 4	AA	62.5	6.2	4.9
Deionized water	814 ± 93	0.9 ± 0.1	27 ± 2	AA	66.7	4.2	2.3
40 mM phosphate buffer							
pH 5.6	1,100 ± 82	1.1 ± 0.1	32 ± 3	AA	65.9	4.4	3.1
6.0	1,218 ± 71	1.2 ± 0.1	32 ± 3	AA	66.1	4.5	2.8
6.4	1,277 ± 95	1.3 ± 0.1	26 ± 2	AA	64.8	4.2	2.6
6.8	— ^{*2}	1.2 ± 0	26 ± 3	AA	64.0	4.0	2.5
7.2	1,317 ± 63	1.3 ± 0.1	28 ± 3	AA	63.4	3.8	2.8
7.6	1,082 ± 51	1.4 ± 0.1	23 ± 3	AA	62.0	4.0	2.8
8.0	1,149 ± 102	1.5 ± 0.1	24 ± 3	AA	62.0	3.2	2.7
Alkaline wash ^{*3}	1,059 ± 15	1.4 ± 0.1	23 ± 1	AA	63.0	4.1	2.6

^{*1} Prepared from unwashed mince. Moisture content was adjusted to 82%.

^{*2} Data were not available.

^{*3} 0.2% NaHCO₃–0.15% NaCl.

were the lowest when the muscle was washed by distilled water, though the stress was increased by water washing from 596 g to 814 g. The improvement of gel forming ability of milkfish by washing observed here is quite similar to the data reported by Ko and Liou.⁸⁾

The L values were slightly higher for those washed by water and acidic media. Generally, the higher the pH value of the media, the lower the L value became. The L values

of washed mince were dependent on the pH values of washing media and were in the range of 60–75 (Figs. 2 and 4), but the L values of kamaboko prepared from the respective minces fell in quite a narrow range (62.0–66.7). This is probably because the lightness due to myofibrillar proteins in the mince was intensified by thermal denaturation through setting and heating, and this masked the discoloration due to myoglobin. It is also likely that denaturation of

myoglobin itself was involved in this phenomenon. However, the lightness of kamaboko reflected that of the washed mince, though the extent was much less compared to the latter. On the other hand, *a* and *b* values were decreased by washing, irrespective of the media used. No significant differences in these parameters were observed regardless of the washing media tested.

The data in Table 2 demonstrated that the quality of milkfish kamaboko was largely affected by the washing media and pH. For fresh milkfish, washing by pH 7.2 medium gave the highest maximum stress. Washing by pH 8.0 medium was also considered to be effective to maximize the deformation, though the lightness of kamaboko is slightly sacrificed. Further studies are, however, necessary to establish the effects of storage condition on the gel forming ability of milkfish muscle.

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