APPLICATION OF MIXING INDEX (*IP*) FOR THE EVALUATION OF GEL-FORMING ABILITY IN MYOFIBRIL-PROTEIN GELS OF FISH PASTES

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ABSTRACT

This study was aimed to evaluate the mixing process effectiveness of fish meat using a mixing index (Ip). Samples used were Tilapia, Oreochromis niloticus, Walleye pollack surimi, and a mixture of both. The ability of fish meat paste myofibril-protein to form gel was evaluated with a rigidity test and MHC distribution with SDS-PAGE. The results indicated that the mixing process effectiveness with salt was 3 minutes with an Ip close to 0. This was reflected by a rigidity value >MHC. Hence, this study concluded that mixing index could be used to evaluate the ability of fish meat gel formation.

***Key words** : Mixing index , rigidity, myofibril-protein, myosin heavy chain, surimi.*

[1] INTRODUCTION

The production of fish jelly, such as kamaboko (fish cake) gel, involves mincing of fish muscle, washing with water, mixing with salt, and heating to form the gel (Okada,1992; Hashimoto, 2001 and Hagihara *et al*., 2006). It was reported that in comminuted fish muscle, 2-3% of NaCl is usually used to solubilize myofibrillar proteins for gel formation (Cheng *et al.,* 1979). The use of different mixers to blend the fish meat with salt on the gel formation was carried out as well (Babbit *et al.,* 1988). However, information on the time effectiveness required to result in the gel formation of fish muscle is still very little. Beside that, there were several different techniques to evaluate gel properties, such as tensile strength (TS) (Onibala, *et al*.1997), penetrating strength (PS) (*Miki et al*., 1988), and etc. This study used the mixing index to evaluate the gel-forming ability in myofibrillar protein gels prepared from fish pastes in order to obtain the effectiveness of mixing process in fish jelly production. This method is expected to be able to optimize the mixing process efficiency in order to produce the best quality gel.

MATERIALS AND METHODS

Materials

Frozen Walleye pollack surimi, Tilapia, *Oreachromis niloticus* and mixtures (surimi and tilapia) with 1:1 ratio were used as samples for the experiments. These samples were collected from fish market, Kagoshima, Japan.

Preparation of fish pastes

The skinless meat blocks of approximately 10 mm^3 were made from all samples and rinsed 5 times with 2°-4°C cold water. To prepare the fish pastes, each 150 g of sanple was ground with 3% NaCl for 1, 2, 3, 4, and 5 minutes mixing time at 4°C using a speed cutter (National,MK-K3), then the moisture content of 80% and the pH value of 6.8 were adjusted to prepare the fish paste.

Preparation of myofibrillar-proteins

The myofibrillar proteins were prepared following the method of Hashimoto *et al.,*(1979). The fish paste (20 g) was homogenized with 40 mM boric acid of buffered pH 7.0 as much as 5 times the paste weight in homogenizer for 1 min. The homogenization was repeated 3 times.

The precipitate was collected by centrifugation of homogenate at 3000 x g for 10 min. To collect the myofibril-protein solution, the precipitate was dissolved in 0.1 M potassium chloride-40mM boric acid of buffered pH 7.0 and centrifuged at 3000 xg for 10 min. This procedure was repeated 3 times.

The preparation of myofibril-protein sol was decribed below. For the purpose of dehydration, the precipitate was collected by centrifugation at 20,000 x g for 30 min after the dilution. The precipitate was then dissolved for 3 hours in a final concentration of 0.1 M potassium chloride-40mM boric acid of buffered pH 7.0, and myofibril-protein sol was obtained. The dehydration of myofibril-protein was calculated as the following equation (Equation 1):

$$Swo + Wi/S + Wi = 0.92 \quad \text{. . . . Equation 1.}$$

Where,

S : weight of myofibrillar protein after centrifuged at 20,000 x g,for 30 min.

Wo : water content of myofibrillar protein after centrifuged at 20,000 x g, for 30 min.

Wi : volume of 0.1 M KCl-40mM H_3BO_3 buffer pH 7.0

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Measurement of gel rigidity

The measurement of gel rigidity was achieved following the method of Miki *et al.,*1988, (Figure1). The myofibril-protein sol was put into a catridge type-sample tube and heated at 80°C for 20 min. The myofibril-protein gels were cooled in 4°C cold water for 20 min and set on the holder with cappilary tube. The gel was kept at 20°C

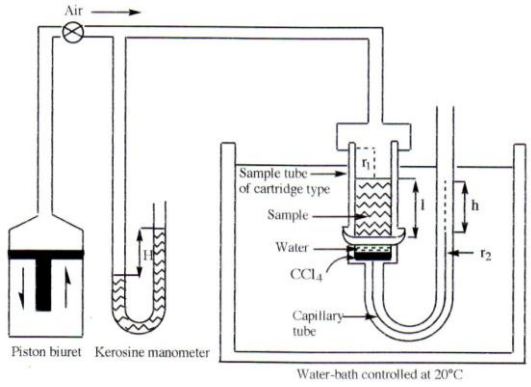

Figure 1. Schematic diagram of apparatus used for the rigidity measurement of heat-iduced gels.

for 10 min and measured. The gel rigidity was calculated from the equation (Equation 2):

$R = P \times r_1^4 / 8 \times l \times r_2^2 \times h$ . . . . Equation 2.

Where,

R = The rigidity of heat-induced gel (dyne/cm^2)
l = Length of sample (cm)
r_1 = Inside radius of sample (cm)
r_2 = Inside radius of capillary tube (cm)
h = Recovered displacement index (cm)
P = H x 980 x g (dyne/cm^2)
H = Differencial distance (cm)
G = Density of kerosine, 0.8 (g/cm^3)

SDS-PAGE analysis

The muscle gel (0.4 g of each sample) was solubilized by heating at 100°C for 2 min in 7 ml solution containing 2% SDS, 8 M urea, 2% mercaptoethanol, and 20 mM Tris-HCl of buffered pH 8.0 and shaken for 24 hours at 25°C, and was then centrifuged at 20,000 x g for 20 min. A 30 µl aliquot of each solubilized sample was subjected to SDS-PAGE using 5% polyacrylamide disk gel according to the method of Weber and Osborn, (1972). The distribution of myosin heavy chain (MHC) in heat-induced gel was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 5% slab gel using the method of Weber and Osborn (1969).

Densitometry analysis.

Stained gels with coomassie brillient blue R-250 were scanned at 570 nm with a densitometer (Shimadzu CS-930). Peak fractions were separated automatically and relative amount of proteins were calculated. Each protein content was expressed as relative density (%) on the basis of total density on SDS-gels (Numakura *et al*., 1985). The band of higher molecular weight than that of MHC was estimated as polymer of MHC and the band of lower molecular weight than that of MHC was estimated as proteolysis of MHC (Kim *et al*.,1993). The callibration proteins for molecular weight in the range of 45,000 to 200,000 dalton were used. They were myosin (200,000),ß-galactosidase (116,250), phosphorilase (97,400), serum albumin (66,200), and ovalbumin (45,000), respectively.

Calculation of Mixing Index (*Ip*) in fish pastes

To obtain the effectiveness of mixing process in fish pastes, the fish muscle of about 150 g was ground with 3% NaCl for 1, 2, 3, 4, and 5 min using a speed cutter. The distribution of MHC in heat-induced gels and in the fish paste (as a control) was evaluated with SDS-PAGE.

The effectiveness of mixing process in fish pastes was calculated using a statistical procedure of Waren *et al*.,(1976). For easy analysis of MHC, there are several notations as follows: relative content of MHC before mixing process is μ; relative content of MHC after mixing process is *Xi*; average value of MHC is $\overline{X}$, and number of sample is *N*. If the paste was perfectly mixed (and each analysis was perfectly accurate), the measure value of *Xi* would equal to $\overline{X}$. If the mixing is not complete, the measured values of *Xi* differ from $\overline{X}$ and their standard deviation about the average value of $\overline{X}$ is a measure of the mixing quality. This standard deviation is estimated from the analytical results as follow :

$$S = \sqrt{\frac{\sum_{i=1}^{N}(Xi-\overline{X})^2}{N-1}} \quad \text{. . . . Equation 3.}$$

The value of *S* is a relative measure of mixing, valid only for a set of tests with a specific mixer. A more general measure is the ratio of *S* to the standard deviation at zero mixing σo. Before the mixing began, the fish paste in the mixer had existed as two layers, one of which contained no MHC and one of which did MHC only. Samples of the first layer would have the analysis *Xi* = 0 and in the other layer *Xi* = 1. Under these conditions, the standard deviation is given as follow :

$$\sigma_o = \sqrt{\mu\,(1-\mu)} \quad \text{. . . . Equation 4.}$$

The mixing index for fish pastes *Ip* is, then, from equation 3 and 4,

$$Ip = \frac{S}{\sigma_o} = \sqrt{\frac{\sum_{i=1}^{N}(Xi-\overline{X})^2}{(N-1)\mu(1-\mu)}} \quad \text{. . . .Equation 5.}$$

RESULTS AND DISCUSSION

Figure 2 illustrates the relationship between mixing time and rigidity of myofibril-proteins heated at 80°C for 20 min. As a result, the rigidity values of myofibril-protein gels increased about 41, 31, and 53% at 3 min of mixing time, and then prolonged to 5 min, while the rigidity of the gels decreased slowly about 11, 10, and 24% prepared from tilapia, mixture, and walleye pollack surimi, respectively. It was found that a 3 min of the mixing time in fish muscle with salt could produce the

highest gel-forming ability. It has been assumed that the gelation of fish paste during heating at 80°C is due to the formation of network structure or polymerization of MHC, a main component of muscle proteins. Initial studies performed by Itoh *et al*., (1979) on carp actomyosin solution demonstrated a concomitant increase in the level of oxidized sulfhydril groups (estimated from reduction in SH content) with increase in temperature up to 80°C. This implies that S-S interchanges between protein molecule were in the development of gel structure. The addition of cysteine and cystine to actomyosin solutions and assorted fish meat pastes, was observed to improve the gel-strength (Itoh *et al*., 1980). Futhermore, the gel forming ability of myofibril-protein gel decreased with prolonged mixing time. Such a decrease in gel-forming ability was due to the changes in protein functionality resulting from protein-protein interactions (Deng *et al*., 1976).

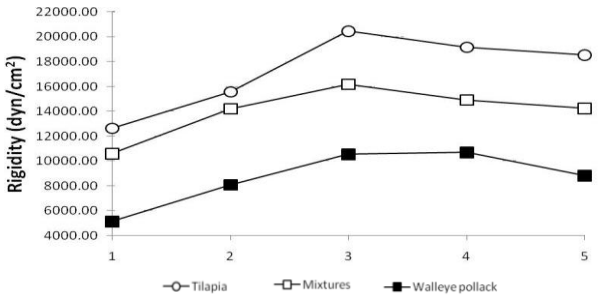

Figure 2. Relation between mixing time and rigidity of myofibril-proteins heated at 80°C for 20 min.

Based on the results, it was concluded that the 3 min of mixing time in fish muscle with salt could produce the highest gel-forming ability. Beyond this time, the level of gel-forming ability decreased after extended mixing time. Samples of solubilized protein gels were subjected to electrophoresis to monitor protein subunits changes in the gelation process (Numakura *et al.,* 1985, Three main bands appeared in the disk gel, the first band at the top of the disk gel was assigned to the polymerization of myosin heavy chain (>MHC), the second at around 200,000 Dalton to MHC, and the third to actin (Ac). Another weak band appeared between MHC and Ac was lower molecular weight than MHC (<MHC).

As a result, the densitometry analysis showed quantitatively that the relative amount of MHC and >MHC in the gels of all fish samples was changes during mixing process, but lower molecular weight than MHC and actin remained almost constant. The relative amount of MHC in solubilized protein gels at 3 min of mixing time decreased about 32, 46, and 36% compared to that of control (unheated gel), and then, the prolonged mixing time to 5 min caused an increase in MHC relative amount in the gels prepared from tilapia (Figure 3), mixture (Figure 4), and walleye pollack surimi (Figure 5), respectively.

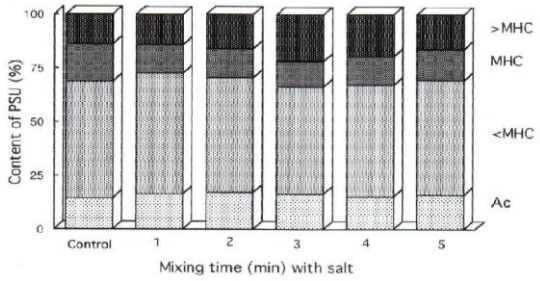

Figure 3. SDS-PAGE analysis of the mixing time effect of the tilapia pastes on the polymerization of MHC (>MHC) in heat-induced gels at 80°C for 20 min.

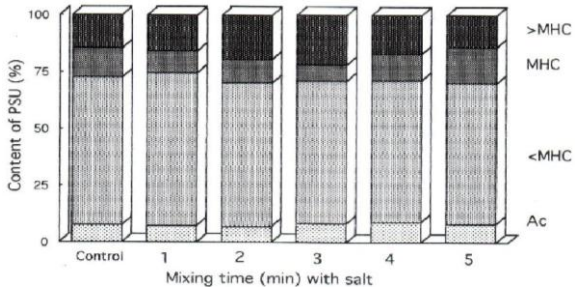

Figure 4. SDS-PAGE analysis of the mixing time effect of the mixtures pastes on the polymerization of MHC (>MHC) in heat-induced gels at 80°C for 20 min.

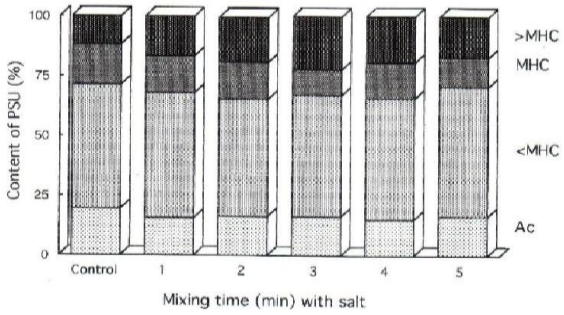

Figure 5. SDS-PAGE analysis of the mixing time effect of the walleye pollack surimi on the polymerization of MHC (>MHC) in heat-induced gels at 80°C for 20 min.

The relative amount of >MHC in the gels at the same mixing time 3 min increased about 37, 34, and 48% but with prolong of mixing time the amount of >MHC decreased in gels prepared from tilapia, mixture, and walleye pollack surimi, respectively. It was found that the changes of MHC and >MHC in myofibril-protein gels affected by the mixing time, and 3 min of the mixing time resulted the maximum amount of >MHC or the minimum for MHC in the gels. Based on these results, it was suggested that the polymerization of the MHC in myofibril-protein gels could be determined from its gel-forming ability by using the rigidity test.

To evaluate the effectiveness of mixing process in fish muscle, the component of myofibril-proteins, such as MHC in gel before and after mixing with salt, was calculated statistically as a *Ip* by Waren et al.,1976. They reported that if the paste is perfectly mixed (*Ip* = 0), every measured value of *Xi* equals to

$\overline{X}$. If mixing is not complete (*Ip* ≠ 0), the measured value of *Xi* differs from $\overline{X}$. It was also found that *Ip* values of fish pastes would approach to zero after 3 min mixing time (Figure 6).

Theoretically, *Ip* would approach zero at long mixing time. In fact, it does not for two reasons : (1) mixing is never quite complete; (2) unless the analitycal methods are extraordinarily precise, the measured value of *Xi* never agree exactly each other or $\overline{X}$ and *Ip* is not found to be zero even with perfectly mixed material. The minimum limit of *Ip* value for completely mixed materials varies with the consistency of the materials processed, the effectiveness of the mixer, and the precision of the analytical method. Typically, it falls in the range between 0.1 and 0.01.

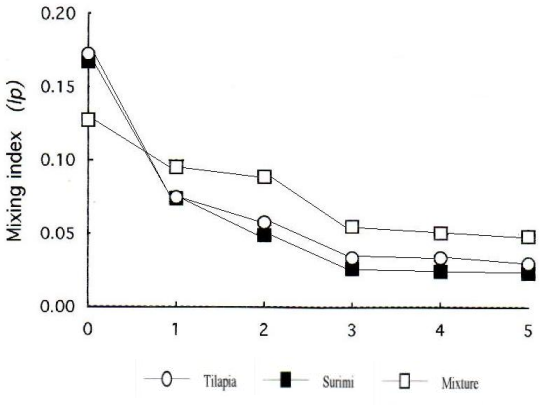

Figure 6. Mixing time versus *mixing index (Ip)* of fish pastes.

It was concluded that the gel formation of myofibril-protein was proved graphically by the formation pattern of high molecular weight MHC in myofibril-protein gels. However, the optimum mixing time which showed the maximum of gel-forming ability was also found out clearly to be 3 min by using *Ip*. These results corresponded to the results of rigidity measurement in myofibril-protein gels.

CONCLUSION

The gel formation of myofibril-protein was proved graphically by the formation pattern of >MHC in solubilized protein gels. However, the optimum mixing time which showed the maximum rigidity was found out more clearly to be 3 min using its mixing index than the SDS-PAGE pattern of MHC. Mixing theory could also be applied to evaluate the gel-forming ability of fish muscle in gel production.

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