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Effect of chitosan on gelling properties, lipid oxidation, and microbial load of surimi gel made from African catfish (*Clarias gariepinus*)

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Abstract

The effect of the addition of different concentrations of chitosan (0–2.0% w/w) on the gelling properties of surimi gels made from African catfish (*Clarias gariepinus*) was tested. Lipid oxidation, total volatile basic nitrogen (TVB-N), and aerobic plate count (APC) changes during 20 days of storage at 4°C also were evaluated. Surimi gels with 1.5% (w/w) chitosan added exhibited the highest improvement in gel strength (58.92%), whiteness (13.18%), and water holding capacity (36.8%). Incorporation of 2.0% (w/w) chitosan in gels resulted in the lowest TVB-N value (36.63 mg N/100 g) at the end of the 20 days storage period. Both the peroxide values and the 2-thiobarbituric acid values increased more slowly in the chitosan-treated gels than in the control gel during the storage period. Chitosan at concentrations of 1.75% and 2.0% (w/w) conferred the best antioxidant effect on catfish surimi gels and resulted in a significant reduction in APC. Based on the microbiological acceptability limit (10^6 cfu/g), the shelf life of surimi gels with 1.75% and 2.0% (w/w) chitosan was extended to 12 days in refrigerated storage at 4°C, whereas the other samples lasted only 8 days. Hence, the addition of 1.5–2.0% (w/w) chitosan is a promising approach for the preparation of catfish surimi gels, as it improves texture, prevents lipid oxidation, and inhibits microbial growth.

Introduction

World demand for surimi continues to grow due to its unique textural properties and high nutritional value (Eymard *et al*., 2005). The decreasing supply of white flesh fish, such as Alaska Pollock, as the raw material for surimi has led to a new trend in surimi manufacturing (Tina *et al.*, 2010). New species, such as freshwater and pelagic fish, now are being used in increasing quantities to produce surimi.

African catfish (*Clarias gariepinus*) is the most important freshwater fish in Malaysia due to its fast growth rate, easy cultivation, and good market potential. Due to its abundant supply and low price, this species has great potential to be further processed into surimi-based products with high commercial value and demand. However, the gelling properties of catfish surimi are not ideal (Nasution *et al*., 2011). Moreover, this species has a high polyunsaturated lipid content, which makes it fairly susceptible to oxidation and may lead to the development of off flavor during storage (Endinkeau and Tan, 1993; Rahman *et al*., 1994; Ghassem *et al*., 2009).

Various food ingredients have been used to enhance the mechanical properties of surimi. However, the addition of some ingredients has adverse effects, particularly off flavor and off color of surimi gels (Rawdkuen and Benjakul, 2008). The addition of ingredients such as egg white and bovine plasma protein may even cause health problems to consumers (Balange and Benjakul, 2009) and be against religious laws. Therefore, finding an alternative to these preservatives may increase consumer acceptance of surimi-based products. Chitosan, a deacetylated form of chitin, is a natural polysaccharide derived from crustacean shells and the cell walls of fungi (Soultos *et al*., 2008; Aranaz *et al.*, 2009). Currently, chitosan is of interest in food science due to its special functional characteristics, such as antioxidative activity (Darmadji and Izumimoto, 1994), excellent gelling ability (Mao and Wu, 2007), and antimicrobial ability (Georgantelis *et al.*, 2007; Soultos *et al.*, 2008).

A number of studies have been conducted to examine the gelling properties of chitosan in food products, such as meat products (Lin and Chao, 2001; Kachanechai *et al.*, 2007), tofu (Chang *et al*., 2002) and fish muscle (Benjakul *et al*., 2002). Several studies also reported that chitosan could significantly inhibit lipid oxidation and reduce microbial load in fish products (López-Caballero *et al*., 2004; Mao and Wu, 2007). Mao and Wu (2007) reported that low molecular weight chitosan is more effective than high molecular weight chitosan at inhibiting lipid

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oxidation in grass carp surimi.

Since chitosan has been reported to have such beneficial functional properties in certain surimi products, it also may improve the gelling properties and preservation quality of catfish surimi. Thus, the objective of this study was to determine the effect of adding different chitosan concentrations on the gelling properties, lipid oxidation, and microbial load of surimi gel prepared from African catfish.

Material and Methods

Material

Sixty five kilograms of fresh African catfish were purchased from a local supplier and brought alive to the laboratory for further processing. Whole catfish were filleted and processed into surimi. Low molecular weight food grade chitosan (MW 10 kDa, deacetylation degree 95%; viscosity 50–500 cP) was purchased from Qingdao Dacon Trading CO., Ltd (Shandong, China). Commercial surimi blocks (Kinmedai, Grade SA) were purchased from a local supplier for use as a reference. Egg white, starch, and acetic acid were purchased from SIM Company Sdn. Bhd, Penang, Malaysia. All other chemicals used were of analytical grade.

Surimi preparation

Preparation of surimi was based on Chotpradit (2003), Nasution *et al.* (2011) and Department of Fisheries Malaysia (2011) with some modification. Fresh catfish were first washed and soaked in tap water containing 6 kg coarse salt and 6 kg tamarind for 10 min to minimize soil odor and slime. Next, the catfish were degutted, beheaded, and washed in cold water. Deboning was carried out using a deboner machine (Model No.: W-250, Jianlian, China). The process was repeated three to four times to ensure that all of the flesh on the fish was completely removed.

The fish mince was then washed four times at a temperature below 10°C for 20 min for each cycle. During the initial 10 min of washing, the fish mince was continuously stirred, whereas during the last 10 min the mince was allowed to settle. The first two washing cycles used a 0.2% NaCl water solution, the third used a 0.3% NaCl solution, and the final cycle used plain water. The ratio of fish mince to water was 1:5 (w/w) for all washing cycles. Dewatering was conducted by placing the washed mince in muslin cloth and pressing it by using a hydraulic press (Thai Sakaya-11, Thailand). The catfish mince then was mixed with 4% (w/w) sugar and 0.2% (w/w) polyphosphate as cryoprotectants using a bowl chopper. Surimi was packed into polyethylene bags, labeled, and kept frozen at -20°C in freezer until needed.

Preparation of surimi gels

Nine surimi gels formulations were prepared by incorporating different concentrations of low molecular weight chitosan (0%, 0.25%, 0.5%, 0.75%, 1.0%, 1.25%, 1.5%, 1.75%, and 2.0%). All formulations were prepared in duplicates. A reference surimi gel was prepared using commercial surimi (Kinmedai). Surimi gels were subjected to various analyses, including color, texture, and water holding capacity (WHC). Changes in peroxide value (PV; meq/kg sample), 2-thiobarbituric acid (TBA) value (mg malondialdehyde (MDA)/kg sample), total volatile basic nitrogen (TVB-N; mg N/100 g sample), and aerobic plate count (APC; log cfu/g) during 20 days of refrigerated storage at 4°C were measured as well.

The formulations of the catfish surimi gels were modified from Mao and Wu (2007) as shown in Table 1. First, frozen surimi was thawed overnight in a refrigerator at 4°C. The thawed surimi was cut into small cubes and blended for 1 min using a food processor. Salt was added to the surimi and blended for 1.5 min. Ice water then was added and blended for another 1 min to form a viscous and tacky paste. Next, starch and egg white were added and the mixture was blended for 3 min. Low molecular weight chitosan was dispersed with 1% (w/w) acetic acid and then added into the mixture. During preparation of the surimi gels, the minced fish was kept in an ice water bath to keep the temperature below 10°C. All formulations were standardized at 78% moisture (using iced water) (Park and Lin, 2005). An infrared moisture analyzer was used to measure the moisture content.

Table 1. Formulations of surimi gels made from African catfish with different concentration of chitosan added

Ingredients (g)	Commercial Surimi	0%	0.25%	0.5%	0.75%	1.0%	1.25%	1.5%	1.75%	2.0%
Surimi	675	675	667.5	660	652.5	645	637.5	630	622.5	615
Ice water	255	255	260	265	270	275	280	285	290	295
Chitosan	0	0	2.5	5	7.5	10	12.5	15	17.5	20
NaCl	20	20	20	20	20	20	20	20	20	20
Egg White	10	10	10	10	10	10	10	10	10	10
Starch	40	40	40	40	40	40	40	40	40	40
Total (g)	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

All formulations were standardized at 78% water and 22% solids.
(Source: Modified from Mao & Wu, 2007).

Each surimi gel was stuffed into a cellulose casing (30 mm diameter) using a sausage stuffer (Mainca, UK Limited). Both ends of the cellulose casing were knotted with strings and the gels were subjected to the setting process at 25°C for 3 h before being cooked in a water bath at 90°C for 20 min. After heating, the surimi gels were cooled immediately by immersing them in ice water at 4°C for 30 min. The gels were

stored at 4°C in polyethylene bags prior to further analyses.

Color analysis

The L^*, a^*, b^* values of of each surimi gel (subsample of each gels with 30 mm x 25 mm dimensions) was determined following Shie and Park (1999) using a Konica Minolta colorimeter (Konica Minolta Inc, Tokyo, Japan) at ambient temperature. The surimi gels were allowed to equilibrate at ambient temperature (28–30°C) before being subjected to color measurement. The colorimeter was calibrated with a Minolta standard white reflection plate (Y = 93.8, x = 0.3133, and y = 0.3196). Whiteness was also calculated for each surimi gels samples as $W = L^* - 3b^*$.

Gelling properties

The strength of catfish surimi gels was measured according to Panpipat *et al.* (2010) using a TA-XT Plus Texture Analyzer (Stable Micro Systems, Surrey, UK). A cylindrical surimi gel sample (30 mm in diameter and 25 mm in height) was equilibrated and tested at ambient temperature (28–30°C). Breaking force (gel strength) and deformation (elasticity/deformability) were measured using a P 0.25S spherical plunger at a test speed of 1.1 mm sec^{-1}. Gel strength was expressed as the breaking force (g) multiplied by the deformation distance (mm).

Water holding capacity (WHC)

The WHC of the surimi gel for each treatment was measured following Huda *et al.* (2011) with some modification. Samples (2 g ± 0.2 g) of fish gels were homogenized for 5 min with 8 ml of distilled water using a Waring blender (Waring Laboratory®, Torrington, connecticut, USA). An aliquot of the homogenate was placed in a 12 ml centrifuge tube. The homogenate was centrifuged at 2000 rpm for 5 min (Model Hettich Universal 32, Germany). The supernatant was filtered through Whatman filter paper No. 1. The volume of the supernatant was subtracted from the original 8 ml, and the result was reported in terms of the volume of water held (ml) per g of protein (Miller & Groninger, 1976).

Changes during 20 days of refrigerated storage

The effect of 20 days of refrigerated storage on lipid oxidation, TBA, TVB-N, and APC of surimi gels also was examined. PVs were measured following Mao and Wu (2007). The TBA (2-thiobarbituric acid) test was carried out according to Gomes *et al.* (2003). The TVB-N test was carried out following the method of Goulas and Kontominas (2005).

For APC, a 10 g gel sample from each formulation was aseptically transferred to a sterile bag. Next, 90 ml of buffered peptone water were added and the contents was homogenized using a stomacher machine for 1 min. Total plate counts were determined using the spread-plating method by spreading 0.1 ml of the sample onto plate count agar. Duplicate plates were used for each dilution. All plates were incubated at 37°C for 48 h (Murcia *et al.*, 2003).

Statistical analysis

Results were reported as mean values ± standard deviation (SD). Analyses of variances were performed using one-way ANOVA (Minitab version 16.0). Differences among the mean values of the various treatments and storage periods were determined using Tukey's test, and a significance difference was defined as $p < 0.05$.

Results and Discussion

Gel color

Color is one of the most important attributes that determines the quality of a surimi product. Generally, gels with high lightness (L^*), low yellowness (b^*), and high whiteness (W) are preferred by consumers (Hsu and Chiang, 2002). Table 2 shows the color profiles of catfish surimi gels containing different concentrations of chitosan.

Table 2. Color profiles of catfish surimi gels with different concentration of chitosan added as compared to the commercial surimi gel

Treatments	Whiteness (W)	Yellowness (b*)	Lightness (L*)
0% chitosan	55.73 ± 0.72^{b}	6.27 ± 0.07^{a}	74.54 ± 0.93^{b}
0.25% chitosan	56.35 ± 1.29^{b}	6.23 ± 0.03^{ab}	75.05 ± 1.21^{ab}
0.50% chitosan	59.05 ± 0.94^{ab}	6.21 ± 0.04^{abc}	77.67 ± 1.07^{ab}
0.75% chitosan	59.68 ± 0.76^{ab}	6.07 ± 0.14^{abc}	77.88 ± 0.35^{ab}
1.0% chitosan	60.44 ± 2.03^{ab}	5.96 ± 0.21^{abc}	78.32 ± 1.40^{ab}
1.25% chitosan	61.15 ± 0.92^{a}	5.92 ± 0.29^{abc}	78.91 ± 1.80^{ab}
1.50% chitosan	63.08 ± 1.66^{a}	5.74 ± 0.15^{abc}	80.30 ± 2.11^{a}
1.75% chitosan	62.01 ± 0.92^{a}	5.64 ± 0.14^{bc}	78.93 ± 1.33^{ab}
2.0% chitosan	61.64 ± 0.71^{a}	5.60 ± 0.12^{c}	78.45 ± 1.09^{ab}
Commercial surimi (0% chitosan)	71.49 ± 0.73	3.94 ± 0.17	83.31 ± 1.75

Different superscript letters in the same column represent significant difference between the means of treatments ($p < 0.05$)

The L^* values of the catfish surimi gels increased with increasing chitosan concentration until a maximum L^* value was reached at 1.5% chitosan, and then they began to decrease. However, only the L^* value of the control surimi gel was significantly lower than that of the 1.5% chitosan gel ($p < 0.05$). Mohan *et al.* (2011) also reported a higher L^* value in the chitosan-coated double filleted Indian oil sardine compared to the control sample.

In general, b^* values of the catfish surimi gels decreased as chitosan concentration increased. However, only the b^* values of gels containing 1.75–2.0% chitosan were significantly lower than that of the control sample ($p < 0.05$). This result shows that 1.75% chitosan is the minimum concentration needed to lower the yellowness of catfish surimi gels

significantly. The b^* values in this study differed from those reported by Mao and Wu (2007), who found that the concentration of chitosan had no marked effect on the b^* values of grass carp surimi gels. Mohan *et al.* (2011) also found no significant changes in b^* values between chitosan-coated double filleted Indian oil sardine and the untreated sample.

Whiteness of catfish surimi gels were increased with the increased of chitosan concentration until a maximum whiteness was reached at 1.5% chitosan, and then it started to decrease. The W value of catfish surimi gels with 1.5% chitosan added was 13.19% higher than that of the control sample ($p < 0.05$). The W values of the control gel and the gel with 0.25% chitosan added were significantly lower than those of gels containing 1.25–2.0% chitosan ($p < 0.05$). This finding shows that the effective chitosan concentration for improving whiteness of catfish surimi gel was 1.25%. Overall, the addition of 1.25–2.0% chitosan improved the color attributes of catfish surimi gels by increasing the W and L^* values and decreasing the b^* value.

Compared to the commercial surimi, catfish surimi gel with 0% chitosan added was high in yellowness but low in lightness and whiteness. The differences in color attributes between the control catfish gel and the commercial gel might be due to the presence of more dark muscle fibers in dark-fleshed fish species such as mackerel, tuna, and catfish as compared to the white-fleshed fish used to make the commercial surimi (Panpipat *et al.*, 2010). Dark muscle fibers are usually difficult to remove during processing and therefore are retained in the final fish product. Dark-fleshed fish also contain more lipids and myoglobin than lighter-fleshed fish. Myoglobin plays an essential role in determining the degree of whiteness in surimi gels; fishes with higher myoglobin content tend to have lower whiteness (Jin *et al*., 2007).

The improvement of color in chitosan-treated gels relative to the control gel might be due to the rearrangement and interaction of water molecules, proteins, and polysaccharide molecules that occurs when chitosan is added. The chitosan–chitosan interaction and protein–chitosan cross-linking are presumed to alter the surimi gel network, causing the gel to exhibit a more lustrous and translucent appearance, which indirectly helps to modify the lightness of fish gels (Mao and Wu, 2007).

Gelling properties

The gelling properties of catfish surimi gels with different concentrations of chitosan added are shown in Table 3. In general, the addition of chitosan up to 1.5% increased the breaking force of catfish surimi gels; at higher concentrations the breaking force started to decline. The breaking force values of catfish gels containing 1.0–2.0% chitosan were significantly higher than those of the control gel and the gels containing 0.25–0.50% chitosan ($p < 0.05$).

Table 3. Gelling properties of catfish surimi gel with different concentration of chitosan added as compared to the commercial surimi gel

Treatments	Breaking force (g)	Breaking deformation (mm)	Gel strength (g.mm)
0% chitosan	238.13 ± 13.25^{c}	8.37 ± 0.07^{e}	1993.03 ± 126.84^{c}
0.25% chitosan	234.30 ± 19.32^{c}	8.68 ± 0.21^{de}	2031.05 ± 216.82^{c}
0.50% chitosan	238.38 ± 7.42^{c}	8.65 ± 0.14^{de}	2061.73 ± 97.10^{c}
0.75% chitosan	264.67 ± 7.26^{bc}	9.16 ± 0.14^{cd}	2435.18 ± 103.07^{bc}
1.0% chitosan	285.46 ± 3.74^{ab}	9.35 ± 0.07^{bc}	2653.20 ± 54.99^{b}
1.25% chitosan	291.65 ± 16.33^{ab}	9.34 ± 0.22^{bc}	2770.87 ± 155.18^{ab}
1.50% chitosan	311.35 ± 7.66^{a}	10.17 ± 0.00^{a}	3167.50 ± 79.44^{a}
1.75% chitosan	290.47 ± 6.93^{ab}	9.86 ± 0.10^{ab}	2867.22 ± 97.08^{ab}
2.0% chitosan	282.58 ± 8.08^{ab}	9.47 ± 0.13^{bc}	2682.72 ± 113.41^{ab}
Commercial surimi (0% chitosan)	665.08 ± 5.87	10.67 ± 0.11	7089.81 ± 85.41

Different superscript letters in the same column represent significant difference between the means of treatments ($p < 0.05$)

The breaking deformation of catfish surimi gels also increased with the increased of chitosan concentration up to 1.5%, before declined (Table 3). The breaking deformation values of catfish gels containing 0.75–2.0% chitosan were significantly higher than those of the control gel and the gels with 0.25–0.50% chitosan added ($p < 0.05$).

In general, gel strength of catfish gels were increased as the concentration of chitosan increases, until the maximum gel strength was reached at 1.5% chitosan. Catfish gels containing 1.5% chitosan had significantly higher gel strength compared to the other samples. The gel strength of these gels was 58.92% higher than that of the control gel. Kataoka *et al.* (1998) found that the gel strength of low-quality walleye pollock gels was nearly doubled by the addition of 1.5% chitosan when setting occurred below 20°C. Benjakul *et al.* (2000) reported that 1.0% 7B chitosan with calcium chloride was the optimal concentration for increasing the gel strength of barred garfish surimi.

Overall, 1.5% chitosan was the optimum concentration for improving the three texture parameters of catfish surimi gels tested in this study. At concentrations higher than 1.5%, the breaking force, breaking deformation, and gel strength of catfish surimi began to decline. Kataoka *et al.* (1998) reported a similar result for low-quality walleye pollock surimi. A higher concentration of chitosan might disrupt polymerization and aggregation of fish myofibrils during the setting or heating process. Aggregation of proteins inhibits gel formation and results in reduced gel strength of surimi (Sun and Holley, 2010). The addition of a higher amount of chitosan also dilutes the concentration of myofibril protein in surimi. Thus, a less-ordered gel matrix forms between myofibrils and results in reduced

hardness of the surimi gel (Benjakul *et al.*, 2000).

The gelling properties of the control catfish gel (0% chitosan) were low compared to those of the commercial surimi (Table 3). The gel strengthening effect of the addition of chitosan likely is related to the presence of a reactive amino group at the C-2 position of the glucosamine unit (Kataoka et al., 1998). Benjakul *et al.* (2000) found that the setting process during surimi processing is associated with the cross-linking of proteins (myosin head chain) through glutamyl-lysine linkages mediated by endogenous transglutaminase (TGase). This cross-linking of proteins during the setting process can contribute to more elastic and rigid gels after cooking at high temperatures. TGase is the enzyme in fish muscle that catalyzes the acyl transfer reaction between the γ-carboxamide groups of a glutamine residue and the primary amino groups. Thus, in the presence of chitosan, protein-polysaccharide conjugates would be formed between the reactive amino group of glucosamine in chitosan (as the acyl acceptor) and the glutaminyl residue of the myofibrillar proteins in fish mince (Mao and Wu, 2007). The amino groups of the chitosan molecule that partially cross-link with myofibrils also act as a filler in the gel matrix and thus result in a stronger gel (Benjakul *et al.*, 2000; Benjakul *et al*., 2003).

Water holding capacity (WHC)

Figure 1 shows the WHC values of catfish surimi gels containing different concentrations of chitosan. The addition of chitosan at concentrations up to 1.25% did not result in a significant increase in the WHC of catfish surimi gels ($p > 0.05$). However, when 1.5% chitosan was added, the WHC (0.93 ml/g surimi) was increased significantly. This value was 36.76% higher than that of the control sample. Mao and Wu (2007) also reported that the addition of 1% chitosan improved the WHC of surimi products. WHC values measured in different studies might differ for several reasons, such as differences in centrifugal force and duration of centrifugation. Differences in sample size, temperature, and salt content of the surimi product might also affect the WHC values obtained (Zhang *et al*., 1995).

Mahawanich (2008) reported that there is a relationship between WHC and gel strength of surimi gels. It is presumed that surimi gels with higher gel strength generally possess higher WHC. In this study, the WHC values of catfish surimi gels followed a trend similar to that of gel strength; both increased in gels with up to 1.5% chitosan added, and then they began to decline. Kataoka *et al.* (1998) postulated that a higher concentration of chitosan will disrupt

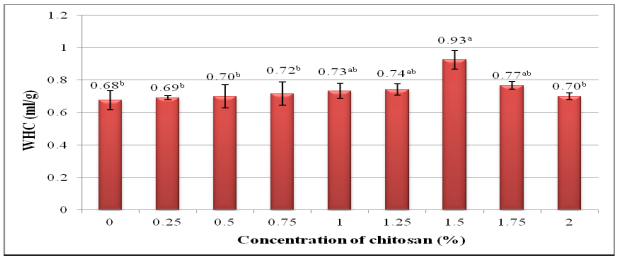

Figure 1. Water holding capacity of catfish surimi gel with different concentration of chitosan added. Different superscript letters in figure indicate significant differences between treatments ($p < 0.05$).

polymerization and aggregation of fish myofibrils in surimi. As a result, a less structured gel matrix will form between myofibrils, thus resulting in reduced hardness of the gel.

The WHC of the commercial surimi gel was 1.34 ml/g, whereas that of the control catfish gel was only 0.68 ml/g. The lower WHC of the control catfish gel as compared to the commercial surimi gel likely was because catfish has weak gelling properties in nature. As a result, catfish surimi gels lose water more easily than the stronger commercial gel. The improvement in WHC of gels with chitosan added might be due to protein-carbohydrate cross-linking that is mediated by the endogenous TGase during the setting process (Kataoka *et al*., 1998; Benjakul *et al*., 2000; Benjakul *et al*., 2003). The bonds formed between chitosan and myofibrillar fish protein result in a stronger final structure of the surimi gel and hence contribute to the higher WHC.

PV, TBA and TVN-N during refrigerated storage

Table 4 shows the changes in PV values, TBA values and TVB-N that occurred in catfish surimi gels during 20 days of storage at 4°C. The PV values of the control and chitosan-treated samples with concentrations up to 1.0% increased as the storage time increased up to Day 12 ($p > 0.05$) and then started to decline. For the other chitosan-treated samples (1.25–1.75%), the PV value continued to increase until Day 16 before starting to decrease. Only samples containing 2% chitosan exhibited a continuous increase in PV value throughout the 20 days of storage.

According to Maqsood and Benjakul (2010), an increase in PV shows that hydroperoxide was first formed as the primary oxidation product; a decrease in the later stage of the PV graph might be due to the decomposition of unstable hydroperoxide into secondary oxidation products. The formation of secondary oxidation products is undesirable, as it may result in the development of off flavor of fish products. Interactions of lipid hydroperoxides and malonaldehyde (MA) with proteins or amino acids

Table 4. Peroxide value, TBA value and TVB-N of catfish surimi gels with different concentrations of chitosan during refrigerated storage as compared to the commercial surimi gel

	Treatments	Storage days					
		0	4	8	12	16	20
Peroxide value (meq peroxide/ kg sample)	0% chitosan	2.34 ± 0.46^{a}	4.33 ± 0.59^{a}	6.52 ± 0.14^{a}	11.26 ± 0.41^{a}	9.07 ± 0.14^{c}	7.53 ± 0.27^{c}
	0.25% chitosan	1.98 ± 0.05^{a}	3.96 ± 0.14^{ab}	6.48 ± 0.10^{a}	11.23 ± 0.24^{a}	9.17 ± 0.08^{c}	7.78 ± 0.18^{c}
	0.5% chitosan	1.96 ± 0.04^{a}	3.93 ± 0.09^{ab}	5.89 ± 0.16^{b}	10.50 ± 0.28^{ab}	9.39 ± 0.11^{c}	7.81 ± 0.16^{c}
	0.75% chitosan	1.96 ± 0.04^{a}	3.91 ± 0.08^{ab}	5.90 ± 0.17^{b}	10.36 ± 0.14^{ab}	9.46 ± 0.08^{c}	7.91 ± 0.29^{c}
	1.0% chitosan	1.95 ± 0.02^{a}	3.25 ± 0.05^{bc}	5.89 ± 0.18^{b}	10.34 ± 0.11^{ab}	10.12 ± 0.25^{bc}	8.87 ± 0.29^{b}
	1.25% chitosan	1.94 ± 0.03^{a}	3.24 ± 0.04^{bc}	5.82 ± 0.04^{bc}	9.89 ± 0.42^{b}	11.20 ± 0.18^{a}	9.06 ± 0.09^{b}
	1.5% chitosan	1.94 ± 0.03^{a}	3.24 ± 0.04^{bc}	5.27 ± 0.18^{c}	8.44 ± 0.07^{c}	10.90 ± 0.40^{ab}	9.14 ± 0.23^{b}
	1.75% chitosan	1.93 ± 0.03^{a}	3.23 ± 0.03^{bc}	5.27 ± 0.17^{c}	8.40 ± 0.07^{c}	10.54 ± 0.38^{ab}	9.21 ± 0.35^{b}
	2.0% chitosan	1.93 ± 0.06^{a}	2.58 ± 0.04^{c}	4.54 ± 0.08^{d}	7.81 ± 0.14^{c}	9.13 ± 0.09^{c}	11.16 ± 0.12^{a}
	Commercial surimi (0% chitosan)	1.93 ± 0.06	3.88 ± 0.08	5.89 ± 0.08	9.69 ± 0.10	11.54 ± 0.20	9.43 ± 0.30
TBA (mg MDA/ kg sample)	0% chitosan	0.13 ± 0.01^{a}	1.60 ± 0.04^{a}	2.00 ± 0.18^{a}	2.23 ± 0.06^{a}	1.82 ± 0.05^{a}	1.58 ± 0.08^{a}
	0.25% chitosan	0.13 ± 0.03^{a}	1.59 ± 0.07^{a}	1.99 ± 0.07^{a}	2.23 ± 0.07^{a}	1.83 ± 0.04^{a}	1.59 ± 0.04^{ab}
	0.50% chitosan	0.13 ± 0.01^{a}	1.59 ± 0.13^{a}	1.97 ± 0.08^{a}	2.23 ± 0.04^{a}	1.87 ± 0.06^{ab}	1.60 ± 0.06^{ab}
	0.75% chitosan	0.13 ± 0.01^{a}	1.57 ± 0.03^{ab}	1.94 ± 0.08^{a}	2.22 ± 0.05^{a}	1.87 ± 0.06^{ab}	1.62 ± 0.02^{bc}
	1.0% chitosan	0.12 ± 0.03^{a}	1.33 ± 0.21^{abc}	1.71 ± 0.05^{a}	2.12 ± 0.06^{a}	2.04 ± 0.11^{ab}	1.74 ± 0.03^{bc}
	1.25% chitosan	0.12 ± 0.00^{a}	1.32 ± 0.03^{abc}	1.67 ± 0.12^{ab}	2.08 ± 0.05^{a}	2.00 ± 0.04^{ab}	1.77 ± 0.05^{c}
	1.50% chitosan	0.12 ± 0.02^{a}	1.17 ± 0.10^{bc}	1.29 ± 0.11^{bc}	1.97 ± 0.10^{ab}	1.94 ± 0.03^{ab}	1.88 ± 0.04^{c}
	1.75% chitosan	0.12 ± 0.02^{a}	1.07 ± 0.11^{c}	1.24 ± 0.10^{c}	1.70 ± 0.16^{bc}	1.83 ± 0.07^{ab}	1.90 ± 0.07^{c}
	2.0% chitosan	0.12 ± 0.02^{a}	0.96 ± 0.09^{c}	1.12 ± 0.13^{c}	1.61 ± 0.06^{c}	1.75 ± 0.05^{b}	2.06 ± 0.08^{c}
	Commercial surimi (0% chitosan)	0.06 ± 0.02	1.40 ± 0.13	1.79 ± 0.08	2.09 ± 0.05	1.96 ± 0.06	1.85 ± 0.04
TVB-N (mg N/100 g sample)	0% chitosan	3.50 ± 0.33^{a}	10.03 ± 0.33^{a}	17.03 ± 0.99^{a}	28.00 ± 1.98^{a}	37.10 ± 2.31^{a}	43.87 ± 1.98^{a}
	0.25% chitosan	3.03 ± 0.99^{a}	9.57 ± 0.99^{ab}	17.03 ± 0.33^{a}	28.00 ± 0.66^{a}	36.87 ± 1.32^{a}	43.63 ± 0.99^{a}
	0.50% chitosan	3.03 ± 0.33^{a}	9.57 ± 0.33^{ab}	16.80 ± 0.66^{ab}	27.77 ± 0.99^{a}	36.17 ± 2.97^{a}	42.23 ± 0.99^{ab}
	0.75% chitosan	3.03 ± 0.99^{a}	9.10 ± 0.33^{ab}	16.33 ± 0.66^{ab}	27.07 ± 1.98^{ab}	35.93 ± 1.32^{a}	41.53 ± 2.64^{abc}
	1.0% chitosan	3.03 ± 0.99^{a}	8.17 ± 1.65^{ab}	15.87 ± 0.66^{ab}	25.20 ± 1.98^{abc}	32.43 ± 0.33^{ab}	39.90 ± 0.33^{abc}
	1.25% chitosan	3.03 ± 0.33^{a}	8.17 ± 0.33^{ab}	15.17 ± 0.33^{ab}	24.97 ± 0.33^{abc}	31.97 ± 0.33^{ab}	39.20 ± 1.98^{abc}
	1.50% chitosan	2.57 ± 0.99^{a}	7.70 ± 0.33^{ab}	14.47 ± 1.98^{ab}	22.63 ± 0.99^{abc}	30.10 ± 0.33^{b}	37.80 ± 0.66^{bc}
	1.75% chitosan	2.57 ± 0.33^{a}	7.47 ± 0.66^{ab}	13.77 ± 0.99^{ab}	21.70 ± 1.65^{bc}	29.87 ± 0.66^{b}	37.57 ± 0.33^{bc}
	2.0% chitosan	2.57 ± 0.99^{a}	7.00 ± 0.66^{b}	13.30 ± 0.33^{b}	20.77 ± 0.33^{c}	28.00 ± 0.66^{b}	36.63 ± 0.33^{c}
	Commercial surimi (0% chitosan)	1.63 ± 0.33	8.17 ± 0.33^{c}	15.17 ± 0.33^{c}	23.10 ± 0.99^{c}	**30.80** ± 0.33^{c}	38.50 ± 0.66^{c}

Different superscript letters in the same column represent significant difference between the means of treatments ($p < 0.05$)

may also cause modification of textural properties in surimi gel products during storage (Huang and Weng, 1998; Medina *et al*., 2009). The results show that catfish gels containing 2% chitosan had the best antioxidant effect relative to both the commercial gel and the control catfish gel. Comparison of the PV values of all samples for the first 12 days of storage revealed that the 2% chitosan catfish gel had the lowest PV value. This result illustrates the effectiveness of chitosan treatment, especially at the level of 2%, on the retardation of lipid oxidation in catfish surimi gels stored at 4°C.

According to the Fisheries and Aquaculture Organization (2011), the maximum acceptable limit for peroxide in fish is 10–20 meq/ kg fish. On Day 12, the PV of the control catfish gel had exceeded the acceptable limit. In contrast, the PV of all chitosan-treated samples were still within the acceptable limit on Day 12. Shahidi *et al*. (1999) reported that the antioxidant mechanism of different concentrations of chitosan in food products might be attributed to their metal-binding capacities. Several sources of protein-bound iron, such as myoglobin, hemoglobin, ferret, and transferrin exist in fish tissues. Although fish mince usually is subjected to several washing cycles before being processed into surimi blocks, some iron substances still remain in the fish mince. Thus, home proteins, which are strong pro-oxidant substances, are not completely removed during processing (Eymard *et al*., 2009). As a result, these protein- bound irons may be released during product storage or cooking, which will catalyze the oxidative reaction in products. Chitosan can chelate these protein-bound irons and thus eliminate their pro-oxidant activity or their conversion to the ferric ion that initiates lipid oxidation (Kamil *et al*., 2002; Kim and Thomas, 2006).

Besides lipid properties, the inhibition of lipid oxidation by chitosan is highly dependent on the concentration and the types of chitosan used (i.e., different viscosity or molecular weight) (Darmadji and Izumimoto, 1994). Mao and Wu (2007) reported that the application of chitosan at a level of 1% (w/w) significantly inhibited lipid oxidation in grass carp surimi gels. Kamil *et al.* (2002) found that the PV of herring (*Clupea harengus*) samples treated with 14 cP chitosan at 200 ppm were 61% lower than those of the control sample after 8 days of storage.

In addition to fish products, chitosan is an effective antioxidant in other muscle food products. Darmadji and Izumimoto (1994) found that chitosan prevented lipid oxidation in ground beef more effectively when 1% chitosan was added. Georgantelis *et al*. (2007) showed that 1% chitosan, either by itself or in combination with other natural antioxidants (rosemary or alpha-tocopherol), was more effective at reducing lipid oxidation in frozen beef patties compared to rosemary or alpha-tocopherol alone.

The TBA test has been widely used to measure lipid oxidation in meat products due to its speed and simplicity (Gaebler *et al.*, 2002; Gomes *et al*., 2003). There was no significant difference between chitosan-treated samples and the control sample on Day 0 of storage ($p > 0.05$) (Table 4).

In this study, TBA values for both control and chitosan-treated gels with concentrations up to 1.5%

continued to increase until Day 12 ($p < 0.05$). After Day 12, the TBA values decreased in these samples. However, gels containing 1.75% and 2.0% chitosan showed a continuous increase in TBA value until the end of the experiment on Day 20. Maqsood and Benjakul (2010) explained that an initial increase in TBA values likely occurs due to the decomposition of primary oxidation products to secondary oxidation products, whereas a later decrease in TBA values is due to the decomposition of volatile and unstable secondary oxidation products (e.g., MA and other short-chain carbon products of lipid oxidation) to organic alcohols and acids (which are not measured by the TBA test). The decrease in TBA value after a peak point might also be due to the interaction between TBA-reactive substances with protein molecules in the sample, which form tertiary degradation products (Fernandez *et al*., 1997). This explanation is in agreement with Kamil *et al.* (2002), who reported that the TBA value of cooked herring treated with chitosan increased gradually up to a certain point during storage, followed by a decrease in values after that.

Compared to the commercial surimi gel, the gels with 2.0% chitosan added had the lowest TBA value within the first 12 days of storage ($p < 0.05$). This result indicates that 2.0% chitosan was the optimum concentration to retard lipid oxidation of catfish surimi gels during refrigerated storage. According to Sallam *et al.* (2007), a TBA value of 5 mg MDA/kg is indicative of a good quality of fish, whereas 8 mg MDA/kg is the maximum acceptability limit for fish to be consumed safely. The oxidative rancidity of all chitosan-treated samples (0.25–1.75%) was relatively low throughout the entire 20 days of storage at 4°C, and they were within the acceptable limits for fish consumption. Mohan *et al.* (2011) postulated that the antioxidation mechanism of chitosan could be related to the existence of primary amino groups in chitosan, which form a stable fluoresphere with volatile aldehydes such as MA that are derived from the breakdown of fats during oxidation. Hence, the addition of chitosan into catfish surimi gel efficiently prevents the occurrence of lipid oxidation.

Inhibition of lipid oxidation by chitosan is concentration dependent. Darmadji and Izumimoto (1994) investigated the effect of different concentration of chitosan (0%, 0.2%, 0.5%, and 1.0%) on meat preservation and found that 1.0% chitosan had the best antioxidant effect, as it caused a 70% reduction of the TBA value in meat after 3 days of storage at 4°C. Soultos *et al.* (2008) reported that fresh pork sausage containing 1% chitosan showed 80% reduction in MDA values, whereas fresh pork sausage with 0.5% chitosan showed merely 70% reduction in MDA values compared to control. Mohan *et al*. (2011) found that double filleted Indian oil sardine coated with 2.0% chitosan coating film had a better antioxidant effect compared to samples containing 1.0% chitosan as well as control samples.

Measurement of TVB-N is a traditional chemical method that is widely used to evaluate the degree of spoilage in seafood. TVB-N is a general term that includes the measurement of trimethylamine (TMA), dimethylamine (DMA), ammonia, and other volatile basic nitrogenous compounds associated with seafood spoilage (Ocaño-Higuera *et al*., 2011). There was no significant difference in TVB-N content between the control sample and chitosan-treated gels on Day 0 ($p > 0.05$) (Table 4).

As storage time increased, TVB-N content of the control and chitosan-treated gels increased ($p < 0.05$). The TVB-N content of the control sample increased to 43.87 mg N/100 g during storage at 4°C for 20 days, whereas the TVB- N contents of the 1.5–2.0% chitosan-treated gels ranged from 36.63 to 37.80 mg N/100 g and were significantly lower than that of the control sample ($p < 0.05$). The catfish surimi gels containing 2% chitosan showed the greatest reduction (16.50%) in TVB-N value after 20 days of storage. However, there was no significant difference in TVB-N value between gels containing 2% chitosan and those containing 0.75–1.75% chitosan ($p > 0.05$).

The percentage reduction in the TVB-N value during storage in this study was lower than that reported in other studies. Mohan *et al.* (2011) reported a reduction of 14.9% and 32.7% TVB-N for 1% and 2% chitosan-coated double filleted Indian oil sardine compared to the control sample after 9 days of storage. Mao and Wu (2007) reported a 48.33% reduction in the formation of TVB-N for grass carp surimi at the end of a 15 days storage period. Such variations in TVB-N content in different samples might be related to differences in the non-protein nitrogen content of the fish used. Fish non-protein nitrogen content is highly dependent on the type of fish feed, season of catching, size, and sex of fish as well as other environmental factors. The differences in TVB-N content of fish also are directly correlated to the microbial activity in the fish tissue. (Debevere and Boskou, 1996; Ozogul *et al*., 2004).

In this study, the chitosan-treated samples containing 2.0% chitosan had the lowest TVB-N value within the first 16 days of storage ($p < 0.05$) (Table 4). A value of 30–35 mg N/100 g sample is generally considered to be the maximum limit past which some fish products are regarded by the European Union

as being spoiled and unfit for human consumption (European Economic Community, 1995). In the present study, the TVB-N value of the control sample exceeded the limit of acceptability on Day 16, whereas the chitosan-treated gels approached this limit on Day 20. Therefore, the catfish surimi gels containing 1.75–2.0% chitosan had a longer shelf life (longer by 4 days) compared to the control sample. Darmadji and Izumimoto (1994) postulated that the lower TVB-N values in chitosan-treated samples were due to the antibacterial properties of chitosan. Increased TVB-N content of certain fish products usually is associated with the activity of spoilage bacteria and endogenous enzymes in fish muscle (Ozogul *et al*., 2004). The enzymatic action results in the formation of compounds such as ammonia, monoethylamine, dimethylamine, and trimethylamine, which lead to the development of off flavors in fish products (Debevere and Boskou, 1996).

APC during refrigerated storage

Bacterial growth is the main cause of spoilage of fish and fish products. Therefore, it is advisable to count bacteria numbers as an index to determine the quality of certain products (Suvanich *et al*., 2000). The APC of the catfish surimi gels during the 20 days storage period are presented in Table 5. The surimi gel samples containing 1.5%, 1.75%, and 2.0% chitosan had lower initial microbial counts compared to the other gels. This indicates that the addition of chitosan had a significant effect on reducing the microbial load of catfish surimi gels ($p < 0.05$). The initial microbial loads of chitosan-treated or untreated gels were consistent with previous studies conducted by Singh and Balange (2005). They reported that the initial APC of pink perch surimi ranged from 10^2 to 10^4 cfu/g.

In general, the APC of commercial, control, and chitosan-treated gels increased as storage time increased. The APC test was stopped once the reported value exceeded 10^6 log cfu/g, which is considered to be the maximal limit for fish to be safe for consumption (Institute of Food Science and Technology, 1999). Catfish gels containing 2% chitosan had significantly lower microbial counts (5.87 log cfu/g) than the commercial and control samples ($p < 0.05$). Thus, the addition of 2% chitosan effectively extended the storage life of the gels until Day 12, whereas the other gels lasted for only 8 days in refrigerated storage at 4°C.

Darmadji and Izumimoto (1994) reported that the effect of chitosan on APC was concentration dependent. Among all concentrations tested (0%, 0.2%, 0.5%, and 1%), they found that 1% chitosan had the best antibacterial effect on meat. Mohan *et al.* (2011) also found that the effectiveness of a 1% chitosan coating treatment for double filleted Indian oil sardine was slightly less than it was for the 2% chitosan treatment. In their study, the microbial count for samples treated with 1% chitosan exceeded 7 log cfu/g on Day 7, whereas the 2% chitosan-treated samples did not spoil until Day 9 of storage.

Table 5. Aerobic plate count (log cfu/g) of catfish surimi gel with different concentration chitosan during refrigerated storage as compared to the commercial kinmedai surimi gel

Treatments	Storage days				
	Day-0	Day-4	Day-8	Day-12	Day-16
0% chitosan	4.28 ± 0.09^{a}	4.68 ± 0.31^{a}	5.70 ± 0.30^{a}	7.08 ± 0.12^{a}	-
0.25% chitosan	4.24 ± 0.09^{a}	4.64 ± 0.26^{a}	5.63 ± 0.26^{ab}	7.00 ± 0.04^{a}	-
0.5% chitosan	4.22 ± 0.07^{a}	4.56 ± 0.21^{a}	5.63 ± 0.07^{ab}	6.80 ± 0.47^{ab}	-
0.75% chitosan	4.16 ± 0.12^{a}	4.50 ± 0.06^{a}	5.54 ± 0.13^{ab}	6.75 ± 0.45^{ab}	-
1.0% chitosan	3.98 ± 0.10^{ab}	4.49 ± 0.06^{a}	5.10 ± 0.09^{abc}	6.39 ± 0.12^{ab}	-
1.25% chitosan	3.91 ± 0.07^{ab}	4.15 ± 0.09^{a}	5.04 ± 0.12^{bcd}	6.30 ± 0.13^{ab}	-
1.5% chitosan	3.71 ± 0.03^{b}	4.08 ± 0.14^{a}	4.91 ± 0.05^{cd}	6.16 ± 0.15^{ab}	-
1.75% chitosan	3.70 ± 0.09^{b}	4.06 ± 0.07^{a}	4.91 ± 0.03^{cd}	5.94 ± 0.07^{b}	7.32 ± 0.10^{a}
2.0% chitosan	3.63 ± 0.16^{b}	4.05 ± 0.10^{a}	4.47 ± 0.02^{d}	5.87 ± 0.07^{b}	7.27 ± 0.15^{a}
Commercial surimi (0% chitosan)	3.76 ± 0.05	4.23 ± 0.06	5.49 ± 0.13	**6.73** ± 0.13	7.25 ± 0.10

Different superscript letters in the same column represent significant difference between treatments ($p < 0.05$)

Several mechanisms have been proposed to explain the antimicrobial activity of chitosan. The main antimicrobial mechanism may involve the interaction between the positively charged amino ($–NH^{3+}$) groups of chitosan with the negatively charged carboxylate ($–COO^{–}$) groups that are located at the surface of the bacterial cell membrane (Tsai *et al*., 2002). These ionic interactions result in inhibition of microbial growth by disrupting the lipopolysaccharide layer of the outer membrane of Gram-negative bacteria and by acting as a barrier against nutrient and oxygen transfer (Kong *et al*., 2010). Chitosan may also inhibit mRNA and protein synthesis via permeation into the bacterial cell nucleus (Aranaz *et al.*, 2009). Chitosan also can chelate toxic and other essential metal ions (ferric and zinc ions) that are needed for bacterial growth (No *et al*., 2004).

Conclusion

The addition of 1.5–2.0% (w/w) chitosan is a promising approach to producing catfish surimi gels with improved texture and to preventing lipid oxidation and inhibiting microbial growth in the gels.

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