Effects of Different Physical States of Chitosan on the Quality of Chilled Minced Snakehead Fish (*Channa striata*)

Shanti Dwita Lestari, and Sandy Kurnia Nanisa

Abstract**—**Research on chitosan activity in preserving the quality of minced snakehead fish (*Channa striata*) has been conducted with three different physical states, in dry powder, gel and soluble form, all using the concentration of 1.5% chitosan/100g minced fish. Color (whiteness index), biochemical indices (pH and TVBN), total psychrophilic viable counts and sensory evaluation were determined during eight-day chilled storage at 4°C. The results showed that soluble chitosan and chitosan gel were more effective in prolonging the shelf life and maintaining the quality of minced fish than it was in the powder form and the control (no chitosan added). The total psychrophilic viable count (TPVC) for soluble chitosan and chitosan gel stayed lower than 10^5 CFU/g at day 8 while for dry powder chitosan and control the psychrotrophic load were almost ten fold higher. The TPVC result was in agreement with TVBN, with the value at day 8 of 16.10mg N/100g, 17.23mg N/100g, 28.81mg N/100g and 28.08mg N/100g respectively.

Keywords**—**Chitosan, preservatives, shelf life, snakehead, minced fish

I. Introduction

IN South Sumatera, snakehead fish (*Channa striata*) has long been considered as a high-grade raw material for several traditional Palembangnese dishes such as *empek-empek* and *tekwan* for its white flesh, delicate flavor, chewy texture and high gel-forming ability. This lean fish species is mostly marketed as a minced fish. The nutrient composition and the soft texture of minced fish make it an ideal environment for the growth and propagation of spoilage microorganisms as well as foodborne pathogens. Microbial activity is responsible for spoilage of most fresh fish products, including minced fish. The shelf life of fish products, therefore, is markedly extended when products are stored at low temperatures. The shelf life of fish products at chill temperature (5°C) is 2.7 days [1], however, the use of low temperature only suppresses the growth of mesophiles and thermophiles microrganism, whereas the psychrophiles stays alive. It is therefore essential that adequate preservation techniques are applied to maintain its safety and quality, one of which is by using chitosan.

Chitosan is a cationic polysaccharide prepared by deacetylation of chitin, a natural polysaccharide that is abundantly present as a structural compound in shells of crustaceans such as crab and shrimp. It is a copolymer of β-1,4 linked to 2-deoxy-2-amino-D-glucopyranose, and 2-deoxy-2-acetamido-D-glucopyranose (Vernazza *et al*., 2005) *in* [2]. It appears as an amorphous solid, is soluble in the diluted acids but shows insolubility at pH values above 6.3 [3]. Chitosan was no longer bactericidal at pH 7 due to two major reasons, namely presence of a significant proportion of uncharged amino groups and poor solubility of chitosan [4]. The concentrated chitosan dispersion will result on the chitosan gel formation.

The antimicrobial effect of chitosan on the growth of lactic acid bacteria strains responsible for major beer spoilage had been investigated [5]. The authors used 1% acetic acid as a control to make sure that the observed antimicrobial effect is not due to acetic acid but chitosan. They found that 1% acetic acid might have some minor role in inhibiting the production of lactic acid, whereas, the chitosan concentration of 0.5g/L (equal to 0.05%) was able to decrease the accumulation of lactic acid in all of the twelve lactic acid bacteria strains.

Potential applications of chitosan as a biopreservative have also been investigated in various fish products, such as fish sausages [6], surimi [7] and kamaboko gel [8]. The addition of chitosan into food matrices should be done in a precise technique in order to have the best preservation effect. Different physical states of chitosan are the crucial factor influencing antimicrobial activity [9]. However, to our knowledge, attention has not yet been paid to the physical state of chitosan itself. Therefore, the present study was designed to investigate three different physical states of chitosan, in dry powder, gel and soluble form and their effects on the quality and shelf life of a minced snakehead fish during eight-day storage at 4°C.

II. Materials And Methods

A. Sample Preparation

The snakehead fish were purchased alive from local market (Indralaya, South Sumatera) and transported to our laboratory within 30min. Whole snakehead fish were filleted and processed into minced fish immediately upon receipt using Philips HR 7627 Food Processor. Ice was used to maintain low temperature. Food grade chitosan from shrimp shell was purchased from Vitalhouse Indonesia, Ltd. All other chemicals used were of analytical grade.

Soluble chitosan was prepared by dispersing 3.75% (w/v) chitosan in a 1.0% (v/v) acetic acid solution, whereas chitosan

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gel was prepared by dissolving 7.5% (w/v) chitosan also in a 1.0% (v/v) acetic acid solution. In both cases, the pH was adjusted to 5.8 with 1M NaOH. After 30 min stirring, both chitosan preparations were autoclaved at 120°C for 15 min. Subsequently, 1.5g of chitosan powder (C1), 20g chitosan gel (C2) and 40g of soluble chitosan (C3) were added separately into the minced fish to form a final concentration of 1.5% chitosan/100g minced fish followed by homogenization using Philips HR 7627 Food Processor. Minced fish without any chitosan addition served as a control (C0). After treatment, 100g of each sample was packed in sterile styrofoam tray, covered with plastic wrap and kept in a chiller, the temperature was maintained at 4°C for 8 days. The samples were withdrawn at certain intervals for microbiological, chemical and sensory evaluation.

B. Microbiological Anayses

Microbiological analyses were carried out according to Indonesian National Standards (SNI) 01-2332.3-2006. As much as 25g sample from each part of minced fish were aseptically weighed and homogenized in 225 ml of peptone water for two min in a stomacher at room temperature. Decimal dilutions were prepared in the same solution, and aliquots of 1 ml of the appropriate dilutions were plated in triplicate on plate count agar (PCA; Oxoid) and incubated aerobically at 21°C for 72h for the enumeration psychrotrophic bacteria.

C. Physicochemical analyses

The methodology for total volatile base nitrogen (TVBN) determination was done according to Indonesian National Standard (SNI) 2354.8-2009. The determination of pH was conducted according to [10], using 5 g of minced fish and 45 mL of distilled water. Whiteness of the minced fish was measured using a chroma meter (Minolta, Osaka, Japan). The measurement data of L*, a* and b* was recorded and the whiteness was calculated using the equation: whiteness = $100-[(100-L^*)^2+a^{*2}+b^{*2}]^{1/2}$.

D. Sensory evaluation

Twenty five semi-trained panelists experienced in fish product evaluation carried out the sensory analysis. The panel included twelve males and thirteen females in age group of 20 to 30 years. The products were coded with three random digits and presented as raw, except for the taste evaluation steaming was applied before presenting to the panelists. The sensory attributes evaluated were appearance, color, odor, texture and taste. The scores given by the panel varied from 1 to 9 where 9 corresponded to a fresh sample and 1 corresponding to a deteriorate sample (Table 1). A sample was considered as unacceptable for a sensorial characteristic if the score was lower than 5.

E. Data analysis

The statistical analysis of the microbiological and physicochemical data was performed using the statistical one-way analysis of variance (ANOVA), followed by the Tukey test to determine significant difference between experimental responses. Statistical significance was indicated at 95% confidence level. For the sensory assessment, data were subjected to the Kruskal–Wallis test.

III. Results and Discussion

A. Total Psychrotrophic ViableCcount (TPVC)

Quality of the minced snakehead fish was evaluated upon storage. The total psychrotrophic viable count (TPVC) was found to contain less than 10^5 colony forming units (CFU)/g after 8 days storage at 4°C for both C2 and C3, while the psychrotrophic load of C0 and C1 were almost ten fold higher (Fig. 1).

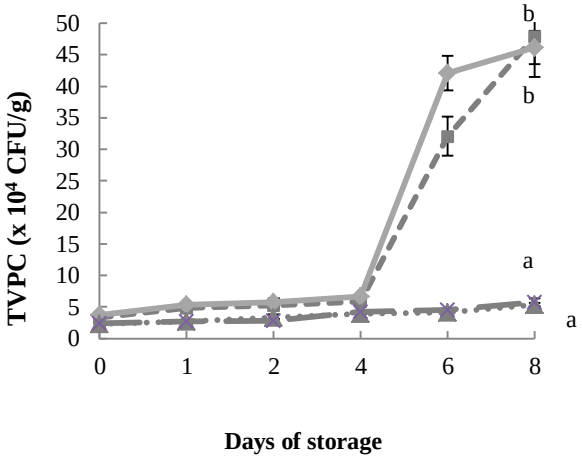

Fig. 1 Total psychrotrophic viable counts (x 10^4 CFU/g) of minced snakehead fish containing 1.5% chitosan stored at chilled temperature (4 ± 1 °C). (—◆—) = control; (- ○ -) = chitosan powder; (···▲···) = chitosan gel; (- × -) = soluble chitosan. Different letters (a,b) indicate significant differences (P<0.05) on each lot as a function of chitosan physical states.

Generally, chilled storage at 4°C was able to inhibit bacterial growth which is showed in Fig. 1 as a logarithmic growth delay until day 4. After 4 days, minced fish treated with C2 and C3 showed extended lag phase while C0 and C1 started their logarithmic phase. The acidic conditions produced by the acetic acid medium in which the chitosan had been dissolved were also contributed in suppressing the growth of the microorganisms, even though according to [5], the antimicrobial activity of acetic acid is considered low when being compared to chitosan. These results indicated that the effectiveness of chitosan in inhibiting bacterial growth turned out to be influenced by its solubility. As a protein, chitosan requires solvents to activate the functional groups which play major roles in antimicrobial and antioxidant effect. This agreed with [9] who stated that chitosan's water-solubility casts important impact on its particular antimicrobial activities. Chitosan powder was unable to be dissolved in minced fish due to its neutral pH, so the reactive charged groups were unformed.

Based on several previous studies it is known that the main mechanism of chitosan in inhibiting the microbial growth is through the interaction between positively charged chitosan molecules and negatively charged microbial cell membranes which leads to the leakage of proteinaceous and other

intracellular constituents (Sudharshan *et al*. 1992 *in* [11]). Polycations of chitosan crosslink with anions on the bacterial surface and this changes the membrane permeability [12]. Other proposed mechanisms are binding of chitosan with bacterial DNA and penetration of chitosan into the nucleus which will interfere with the synthesis of mRNA and proteins (Sudharshan *et al*. 1992, Hadwiger *et al*. 1992 in [11]). Research findings from the studies conducted by Papineu *et al*. (1991) in [11] suggested that chitosan acted mainly on the outer surface of the bacteria. At a lower concentration, the polycationic chitosan binds to the negatively charged bacterial surface to cause agglutination, while at higher concentrations the larger number of positive charges may have imparted a net positive charge to the bacterial surfaces to keep them in suspension.

B. TVBN, pH and Whiteness Index

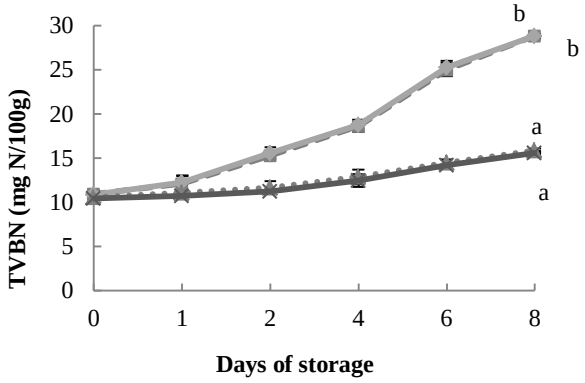

Fig. 2 Total volatile bases nitrogen (mg N/100g) of minced snakehead fish containing 1.5% chitosan stored at chilled temperature (4 ± 1 °C). (—◆—) = control; (- O -) = chitosan powder; (···▲···) = chitosan gel; (- × -) = soluble chitosan. Different letters (a,b) indicate significant differences (P<0.05) on each lot as a function of chitosan physical states.

Fig. 2 shows that the inhibition of spoilage activities of psychrotrophic bacteria by chitosan gel and soluble chitosan resulted in low TVBN values. Psychrotrophic bacteria are capable of growing in minced fish and releasing undesired metabolic compounds mainly arising from nutrient degradation at low temperatures. In case of protein degradation, simpler compounds such as trimethylamine, dimethylamine, ammonia and other volatile nitrogenous compounds are formed as a results of spoilage bacteria and enzymatic activity. The level of TVBN for white fish is generally considered to be fresh if the TVB is less 20 mg N/100 g sample. If the TVB reaches 30 mg N/100 g most authorities consider the fish to be stale, whilst at level of 40 mg N/100 g the fish is regarded as unfit for consumption (Egan *et al.*, 1981 *in* [13]).

In our study, all TVBN values remained below this limit of acceptability throughout the entire storage period, with the values ranged from 10.94 to 28.08 mg N/100 g flesh for C0, 10.83–28.8 mg N/100 g for C1, 10.57–17.23 mg N/100 g for C2 and 10.4–16.21 mg N/100 g for C3during the eight-day period of storage at 4°C. Since TVBN is produced mainly by bacterial decomposition of fish flesh, the higher values of total psychrotrophic viable counts of C0 and C1 also accounted for the higher TVBN values. The addition of chitosan in the form of dry powder failed to inhibit bacterial growth due to its insolubility and the presence of uncharged amino groups.

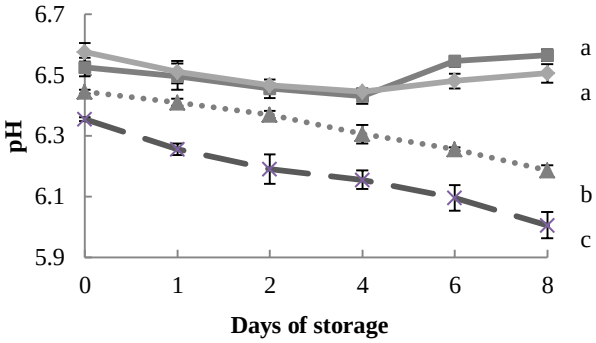

Fig. 3 pH of minced snakehead fish containing 1.5% chitosan stored at chilled temperature (4 ± 1 °C). (—◆—) = control; (- O -) = chitosan powder; (···▲···) = chitosan gel; (- × -) = soluble chitosan. Different letters (a,b) indicate significant differences (P<0.05) on each lot as a function of chitosan physical states.

Fig. 3 shows that the addition of chitosan gel and soluble chitosan lowered the pH values of minced snakehead fish due to the acetic acid residues used to dissolve chitosan. The pH decrease in the beginning of storage time was probably due to the formation of lactic acid from glycolysis process. The ranges of pH values during the study were 6.52-6.6 (C0), 6.57-6.50 (C1), 6.44-6.18 (C2) and 6.35-6.00 (C3). The antibacterial properties of chitosan will inhibit the degradation of protein molecules by bacteria into nitrogen bases compounds and maintained the lower pH. Accumulation of nitrogen bases resulted in pH increase in C0 and C1 after day 4. The rise in the pH value will also facilitate the development of other microorganisms that are not acid resistant.

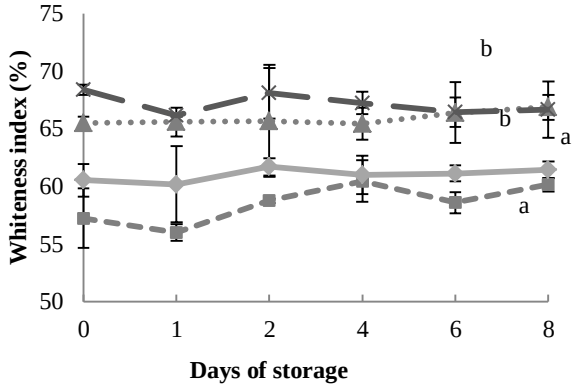

Fig. 4 Whiteness index of minced snakehead fish containing 1.5% chitosan stored at chilled temperature (4 ± 1 °C). (—◆—) = control; (- O -) = chitosan powder; (···▲···) = chitosan gel; (- × -) = soluble chitosan. Different letters (a,b) indicate significant differences (P<0.05) on each lot as a function of chitosan physical states.

It is unavoidable that the addition of chitosan might interfere with the color of minced snakehead fish. Color is one of the important attributes that will lead to the acceptance or rejection of a product. The whiteness index of the minced fish

was slightly fluctuated during the 8-day storage, ranged from 57.24%-60.13% (C0), 60.52%-61.43% (C1), 65.48%-66.87% (C2) and 68.37%-66.67% (C3) as presented in Fig. 4.

The addition of chitosan tended to increase the whiteness index. One of the chitosan functionalities is its ability to act as a clarifying agent. Chitosan is able to bind the colored compounds such as remnants of blood (myoglobin), and negative radicals such as phosphate, sulfite, nitrate or chloride [14].

This results is in agreement with the study conducted by [15] which exhibited whiter surimi gel by addition of chitosan into common carp surimi. Their data showed that the control treatment had the lowest whiteness (56.81) whereas by addition of different levels of chitosan (0.5%, 1.0% and 1.5%), the whiteness of resultant surimi gel significantly ($p<0.05$) improved by 8.3%, 8.8% and 11%, respectively. The interaction of chitosan-chitosan and cross linking of protein-chitosan covalent apparently could modify the gel network, exhibiting a more gleaming and transparent appearance, and thus modifying the lightness of resultant surimi gels [8].

C. Sensory Analyses

The sensory attributes were scored according to the descriptive terms in Table 1. The effect of various physical state of chitosan on sensory characteristics of snakehead minced fish is shown in Table 2.

TABLE I
DESCRIPTIVE TERMS RELATED TO MINCED SNAKEHEAD FISH SENSORY EVALUATION

Score	Appearance	Color	Odor	Flavor	Texture
9	Bright shining, clean, no slime, very attractive	Translucent, pinkish white	Fresh odor	Strong and specific fish flavor	Firm and elastic
7	Bright shining, clean, no slime, attractive	Translucent, yellowish white	Fresh odor,slight acid aroma	Specific fish flavor, slightly sour	Firm
5	Waxy, clean, still attractive	Yellowish	Fishy odor, slight acidic aroma	Specific fish flavor, sour	Soft
3	Waxy, slightly slimy, less attractive	Grayish	Stale odor, acidic aroma	Non specific fish flavor, sour	Soft, slighly slimy
1	Dull, slimy, unattractive	Gray	Spoiled odor, strong acidic aroma	Off flavor, sour	Very soft and slimy

TABLE II
THE EFFECT OF VARIOUS PHYSICAL STATES OF CHITOSAN ON SENSORY CHARACTERISTICS OF SNAKEHEAD MINCED FISH

	Appearance				Color				Aroma				Flavor				Texture			
	C0	C1	C2	C3	C0	C1	C2	C3	C0	C1	C2	C3	C0	C1	C2	C3	C0	C1	C2	C3
H0	7.8	7.72	8.2	7.96	7.4	7.08	7.16	6.68	7.88	7.64	7.4	7.32	7.08	7.24	7.32	6.92	7.16	7.24	7.48	6.92
H1	7.72	7.24	7.48	7.32	7.24	6.84	6.92	6.52	7.64	7.24	7.32	7.08	6.92	7.16	7.24	6.6	6.84	7.08	7.32	6.2
H2	6.76	6.68	7.16	6.84	6.68	6.36	6.6	6.68	7.4	7.08	7.16	6.84	6.68	6.68	6.92	6.28	6.6	6.68	7.24	6.12
H4	6.36	6.04	6.52	6.44	6.04	5.48	6.44	6.28	6.52	6.68	6.92	6.44	6.52	6.44	6.2	5.96	5.56	5.64	6.68	6.04
H6	5.4	5.24	6.04	5.8	5.48	5.16	6.36	6.04	6.2	6.12	6.52	5.8	6.28	6.12	6.04	5.56	5.16	5.48	6.52	5.32
H8	5.08^a	4.84^a	5.88^b	5.56^b	5.08^a	4.84^a	6.04^b	5.96^b	6.12	6.04	6.36	5.56	6.12^b	5.96^b	5.72^b	4.36^a	4.92^a	4.84^a	6.36^b	5.24^a

Different letters (a,b) indicate significant differences ($P<0.05$) on each lot as a function of chitosan physical states

The data analysis using Kruskal-Wallis test indicated that addition of different physical state of chitosan had no significant difference ($P>0.05$) with the control during the first six days for all sensory attributes. At day 8, significant differences between treatments were noted on the appearance, color, flavor and texture. The addition of chitosan gel (C2) improved the appearance, color and texture. On the other hand, addition of soluble chitosan (C3) gave both positive and negative effects on the sensory attributes, it improved the appearance and color but but also lowered the texture quality ($P<0.05$).

IV. CONCLUSION

This study concluded that the addition of different physical states of chitosan resulted in different preservation effects of the minced snakehead fish during 8-day storage at 4^oC. Since the antimicrobial effects of chitosan are influenced by its solubility, no significant difference was noted between chitosan powder and the control for all related parameters (TPVC, TVBN and pH). The same results were also applied for whiteness index and sensory analysis. Chitosan in the form of gel exhibited the best bacterial inhibition, while in the form of soluble chitosan, best whiteness index and the lowest TVBN value were recorded. The sensory analysis confirmed that there was no significant difference between all treatments in the first six days of evaluation.

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