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# THE EFFECT OF CHITOSAN CONCENTRATION AND STORAGE TIME ON THE QUALITY OF SALTED-DRIED ANCHOVY (*Stolephorus heterolobus*)

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## ABSTRACT

*The use of chitosan as preservative agent in fish processing had been conducted for some reasons for example its usage as food additive that can prevent microbial growth, as antioxidative agent, and, to some extent to produce safe food. This research studied the application of chitosan on salted-dried anchovy (S. heterolobus) preservation during storage at room temperature.*

*The aims of this research were to know the effect of treatment (chitosan concentration and storage time) on the quality of salted-dried anchovy (bacterial count and organoleptic test). The experimental design used was Split plot in time design and using Randomized Complete Block with two factors. The first factor was chitosan concentrations (0,0%; 0,5%; 1,0%) while the second factor storage time (0; 2; 4; 6; 8 weeks).*

*The results indicated that chitosan concentration and storage time significantly reduced the total bacterial count ($p<0,01$) but not significantly different ($p>0,05$) for organoleptic test. The interaction of chitosan concentration and storage time significantly influenced the total bacterial count ($p<0,01$).*

**Key words**: chitosan concentration, salted-dried anchovy, storage time, total bacteria count, organoleptic test

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## INTRODUCTION

Generally traditional processing of salted-dried anchovy lack in sanitation and hygiene from the point of preparation, processing and storage of the product. Consequently, the product of salted-dried anchovy is very susceptible for microbiological, chemical and sensory deterioration. To overcome this problem, fish processors sometime take the easiest way by using some chemical substances such as formalin in order to preserve the product.

Based on research conducted by Drug and Food Inspection Bureau DKI – Jakarta (2005), the use of formalin was positively proofed on most of processed fisheries products (57.14%) in Jakarta bay especially for salted fish products such as

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salted-dried squid (6.77 ppm), salted-dried anchovy of Medan (40.18 ppm), salted-dried ray fish (91.41 ppm) and salted-dried anchovy (2.88 ppm). Based on the fact of general using formalin for fisheries products, it is necessary to find out the way out to produce safe product with high quality without implementing danger chemical substances like formalin. Brzeski (1987) and Robert (1992), said that chitosan has antifungi and antibacterial agen that can be used for general substances. Chitosan compound is safe for human and it is derivate of polymer chitin. Chitin has the form similar to cellulose, but hydroxy C-2 chitin is replaced with amino (NH2). This substance has amino group with positive charge so that it can act as preservative agent.

In Indonesia, study on chitosan application has been conducted for salted-dried ray fish at Muara Angke, at which, chitosan concentration of 1.5% on salted-dried ray fish can retard shelf life. At room temperature, the product preserved with formalin can stand for 3 months and 2 weeks; for the same product preserved with chitosan can stand for 3 months; and product without chitosan only stands for 2 months (Suseno, 2006).

This research was carried out to observe the application of chitosan on salted-dried anchovy by dipping the product in chitosan solution to form edible coating. As generally known, traditionally fishery products including dried fish has short shelf life and low quality and safety due to its low quality control, lack of sanitation and hygiene, practical abuse related to the utilization of chemical substances (formalin, textile coloring agent etc). So it is necessary to conduct research on the utilization of chitosan as edible coating on traditionally fisheries products to prolong the shelf life. In addition, the chitosan is considered as safe, non toxic, edible substance. From this treatment it expected that traditionally fisheries products especially dried fish products have high quality, safe and optimally protect from microbiological and sensory deterioration.

## MATERIALSAND METHODS

1. Source and sampling of anchovy
2. Preparation of fish
   Salted-dried Anchovy was processed using wet salting, by dipping the fish into brine solution (10%) for 3 hours. The salted fish was then dried for 2 days by solar drying and covered with plastic net. The product was dipped in chitosan solution after 1 day drying process and continued for drying
3. Storage treatment
   Dried product was then packed in plastic polyethylene PE (thickness 0.025mm) and stored at room temperature for 8 weeks.
4. Experimental design
   The experimental design used was Factorial Design with Block Randomized Design and was done in duplo. First factor (Factor A) was chitosan concentration solution with 3 taraf of 0, 0.5 and 1.0%. Factor B (storage time) consist of 5 taraf, which are 0,2, 4, 6, and 8 weeks.
5. Laboratory analysis
   - Total Plate Count (TPC)
   - Organoleptic test
6. Data Analysis
   In order to understand the general effect of treatments, it is necessary to conduct analysis of variance and regression analysis using SPSS (Santosa, 2004; Gozali, 2005) to observed variables. The organoleptic data was analyzed using non parametric Kruskal-Wallis method because the data obtained was scoring with 1 (lowest value) and 9 (highest value) (Santosa, 2001).

# RESULTS AND DISCUSSION

The effectiveness of chitosan as food preservative depends on chitosan quality itself. There is a quality standard of chitosan in the global market. Chitosan used for this experiment has characteristics which has been described in detail in Table 1. The chitosan comply with international standard of commercial chitosan. Purity of chitosan can be assessed from low in water and ash content, but has high degree of deacetylation (Bustaman, 1989). The higher deacetylation degree, the more amino group contained in chitosan molecule consequently the chitosan become more reactive. This polysaccharide coating can be used as a good barrier, because this kind of coating material can act as a strong matrix and compact that can be protect the food from suffering deterioration. Chitosan can dissolve in organic acid solution and has strong positive charge that can combine and attract negative charge from other compounds.

Microbiological analysis (Total Plate Count) and organoleptic test (appearance, odor, taste and consistency) of the sample can be seen on Table 2. Furthermore, the change of TPC and sensory value of the sample during storage for 8 days are expressed on Figure 1, 2, 3, 4 and 5.

**Table 1.** Characteristic of Chitosan used in the research and International standard

| Parameters | Characteristic of chitosan | |
|---|---|---|
| | Chitosan used in research* | Chitosan based on International standard** |
| Particle size | Granule/powder < 2 mm | Granule/powder < 2 mm |
| Water content | 7.54% | < 10% |
| Ash content | 0.75% | < 2% |
| Protein | – | – |
| Deacetylation degree | 75.42% | 70% (minimal) |
| Odor | Odorless | Odorless |
| Color (solution) | Transparan/white | Transparan/white |
| Viscosity | 300 cps | 200 - 799 cps |

Source: *Suseno (2006)
**Protan (in Bustaman, 1989)

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**Table 2.** TPC Value (colony/g) and Organoleptic test of salted-dried anchovy

| Sample | TPC (colony/g) | Organoleptic Test | | | |
|---|---|---|---|---|---|
| | | Appearance | Odor | Taste | Consistency |
| A1B1 | 250±0$^{ab}$ | 7,0 ± 0,0$^{a}$ | 7,0 ± 0,0$^{a}$ | 6,9 ± 0,2$^{a}$ | 7,2 ± 0,2$^{a}$ |
| A2B1 | 105±21$^{de}$ | 7,0 ± 0,0$^{a}$ | 7,0 ± 0,0$^{a}$ | 6,7 ± 0,0$^{a}$ | 7,0 ± 0,0$^{a}$ |
| A3B1 | 135±21$^{cd}$ | 7,0 ± 0,0$^{a}$ | 6,7 ± 0,0$^{a}$ | 7,0 ± 0,0$^{a}$ | 7,0 ± 0,0$^{a}$ |
| A1B2 | 90±0$^{de}$ | 6,9 ± 0,2$^{a}$ | 6,9 ± 0,2$^{a}$ | 6,7 ± 0,0$^{a}$ | 7,0 ± 0,0$^{a}$ |
| A2B2 | 25±7$^{e}$ | 6,9 ± 0,2$^{a}$ | 7,0 ± 0,0$^{a}$ | 6,9 ± 0,2$^{a}$ | 7,3 ± 0,0$^{a}$ |
| A3B2 | 45±7$^{de}$ | 7,0 ± 0,0$^{a}$ | 7,0 ± 0,0$^{a}$ | 7,0 ± 0,0$^{a}$ | 6,9 ± 0,2$^{a}$ |
| A1B3 | 75±7$^{de}$ | 6,8 ± 0,2$^{a}$ | 7,0 ± 0,0$^{a}$ | 7,0 ± 0,0$^{a}$ | 6,8 ± 0,3$^{a}$ |
| A2B3 | 45±21$^{de}$ | 7,0 ± 0,0$^{a}$ | 7,2 ± 0,2$^{a}$ | 7,0 ± 0,0$^{a}$ | 7,3 ± 0,0$^{a}$ |
| A3B3 | 55±21$^{de}$ | 7,0 ± 0,0$^{a}$ | 7,0 ± 0,0$^{a}$ | 7,0 ± 0,0$^{a}$ | 7,0 ± 0,0$^{a}$ |
| A1B4 | 145±35$^{bcd}$ | 6,9 ± 0,2$^{a}$ | 6,9 ± 0,2$^{a}$ | 7,0 ± 0,0$^{a}$ | 6,9 ± 0,2$^{a}$ |
| A2B4 | 105±21$^{de}$ | 7,0 ± 0,0$^{a}$ | 7,0 ± 0,0$^{a}$ | 6,9 ± 0,2$^{a}$ | 7,0 ± 0,4$^{a}$ |
| A3B4 | 70±0$^{de}$ | 6,9 ± 0,2$^{a}$ | 6,9 ± 0,2$^{a}$ | 6,9 ± 0,2$^{a}$ | 7,0 ± 0,0$^{a}$ |
| A1B5 | 330±28$^{a}$ | 6,7 ± 0,0$^{a}$ | 7,0 ± 0,0$^{a}$ | 7,0 ± 0,0$^{a}$ | 6,9 ± 0,1$^{a}$ |
| A2B5 | 160±42$^{bc}$ | 6,7 ± 0,0$^{a}$ | 7,0 ± 0,0$^{a}$ | 6,7 ± 0,0$^{a}$ | 6,9 ± 0,2$^{a}$ |
| A3B5 | 155±35$^{bc}$ | 6,7 ± 0,0$^{a}$ | 6,7 ± 0,0$^{a}$ | 7,0 ± 0,0$^{a}$ | 7,0 ± 0,0$^{a}$ |

Note: data was taken from average of two replication
Number followed by the same letter show there is no significant different (p>0,05)
A= chitosan consentration (1=0,0%; 2=0,5%; 3=1,0%)
B = length of storage (1=0 week ; 2=2 weeks; 3= 4 weeks; 4= 6 weeks; 5= 8 weeks)

The use of chitosan on salted-dried anchovy processing is acted as antimicrobial agent. Generally antimicrobial is determined as any component that can retard or destroy microbial growth and its metabolism. According to Tsai et.al (2002), antimicrobial activity of chitosan will increase with increasing deacelylation degree. Chitosan is more effective to attack microbe than fungi. Chitosan with high degree of deacetylation (95-98%) in concentration of 50 – 200 ppm effective to protect the food from *Bacillus cereus, Escherichia coli, Listeria monocytogenes, Pseudomonas aeroginosa, Shygella dysenteriae, Staphylococcus aureus, Vibrio cholera, Vibrio parahaemoliticus*.

There are some factors affecting microbial killing by antimicrobial substances. All should be considered on effectiveness of application practical methods iin microbiological control on fisheries products. For example the use of chitosan solution on dried-salted anchovy processing. Concentration and amount of antimicrobial substances are the most important factors. (Pelczar and Chan, 1988).

The change of total microorganism on samples subjected to chitosan treatment during storage can be seen on Figure 1. Salting and drying process could not completely kill all microorganism present in fish. Degradative bacteria generally do not tolerant to salt, but halophilic bacteria do. Some xerophyllic bacteria can also stay at low water activity (Aw). Bacteria that commonly find on dried-salted fish product are *Alcaligenes*, *Pseudomonas*, *Flavobacterium*, and *Corinebacterium* (Hadiwiyoto, 1993). Microorganism can be killed during salting and drying due to the reduction of water activity up to 0.63 – 0.65.

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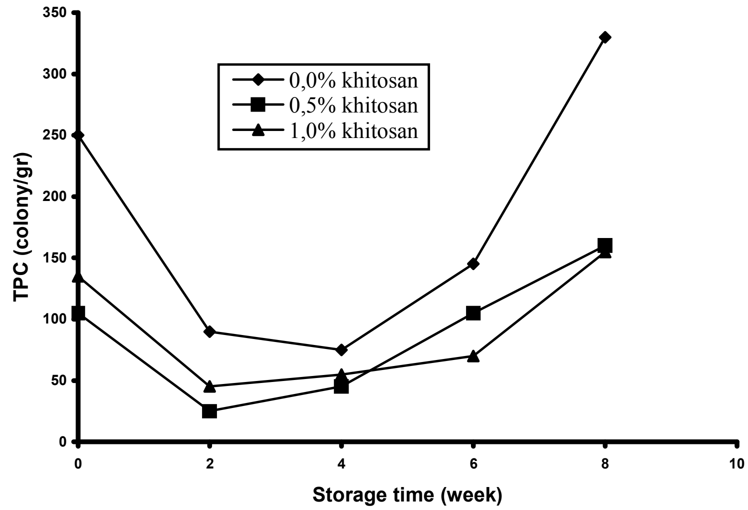


**Fig1.** TPC value (colony / gram) of salted-dried anchovy

Based on analysis of variance, it was found that chitosan concentration and length of storage gave significant effect ($p<0.01$) to total bacterial count (TPC) of dried-salted anchovy. Based on Tukey test, there is significant different between chitosan concentration treatment of 0.0% compared to chitosan concentration treatment of 0.5% and 1%, but there is no significant different between chitosan concentration treatment of 0.5% and 1% ($P>0.05$). Based on these results, the use of chitosan concentration of 0.5% can retard the growth of microorganism on the product. The use higher concentration of chitosan (1%) would not give significant different on the product stability. Therefore, it is recommended to use chitosan concentration of 0.5% instead of 1% to minimize production cost with same results of product stability.

Tukey test was also performed on the TPC data during storage. Length of storage of 0 week gave significant different ($P<0.05$) compared to the length of storage of 2, 4, 6, and 8 weeks. The length of 2 weeks storage has significant different to the length of 0, 6, and 8 weeks but not different to 4 weeks storage. During 2 – 4 weeks storage, an increase in number of microorganism relatively low. Whereas, during 4 – 8 weeks storage, there was significant increase in microbial number. When antimicrobial substance is added to food containing bacteria, the substance will not kill all bacteria cell at the same time, but will kill during certain period following the exponential rate. The rate of death on microorganism is actually reciprocal of rate of exponential microorganism growth (Pelczar and Chan, 1988). The number of bacteria survive will then grow if the environment compatible for their growth.

On 0% chitosan concentration treatment, death of microorganism was due to salting and drying process. These treatments will result in plasmolisis of bacteria or decrease in water activity that cause bacterial activity. Whereas for 0.5% and 1.0% chitosan concentration treatments, apart from salting and drying effect also the present of chitosan as coating material that prevent the salted-dried fish from deterioration. This substance can reduce total bacteria on salted-dried fish product compared to that of control (without chitosan) during storage of salted-dried fish at room temperature.

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Mechanism of chitosan as antibacterial agent can be expressed to some ways. Chitosan can act as chelating agent that chelate some metal ions needed by bacterial enzim. (Muzzarelli, 1977 in Nicholas, 2003). It is known that cation – NH3+ can interrupt metabolism by interaction with other negatif ions present at bacterial cell membrane (Chen, et al. 1998 in Nicholas, 2003). Microorganism/bacteria who survive at adapted environment will further growth and multiply.

Linear model that can be used to predict the total bacteria count (TPC) was: Y= 14.375 – 78.750 $X_1$ + 26.667 $X_2$; where $X_1$ is the concentration of chitosan and $X_2$ is the storage time. The interaction between two treatments applied showed by decreasing cithosan concentration and increasing storage time will increase in total bacteria count (TPC) of salted-dried anchovy product. From the data, it was found that regression coefficient obtained was 0.694. It means that 69.4% variation in total bacteria count of salted-dried anchovy was due to variation of chitosan concentration and storage time variables. The rest of 30.6% was due to other factors excluded model. According to Pelczar and Chan (1988), there are many factors affecting the bacterial activity apart from concentration are temperature, initial bacterial number, species and the present of other organic compounds that can react with antibacterial substances.

Bacteria contaminated on salted-dried anchovy not always pathogenic, but can be bacteria that can deteriorate food and responsible for producing some substances affected appearance, odor, taste and consistency that finally the food is considered not suitable for consumption.

Organoleptic of salted-dried anchovy is summarized on Table 2 and Figure 2,3,4 and 5. The average of organoleptic value was in the range of 6.7 – 7.3. Based on National Standardization Agency (NSA, 1992), the limit for fresh fish determined by National Standard Indonesia 01-2708-1992 for salted-dried anchovy is 7.

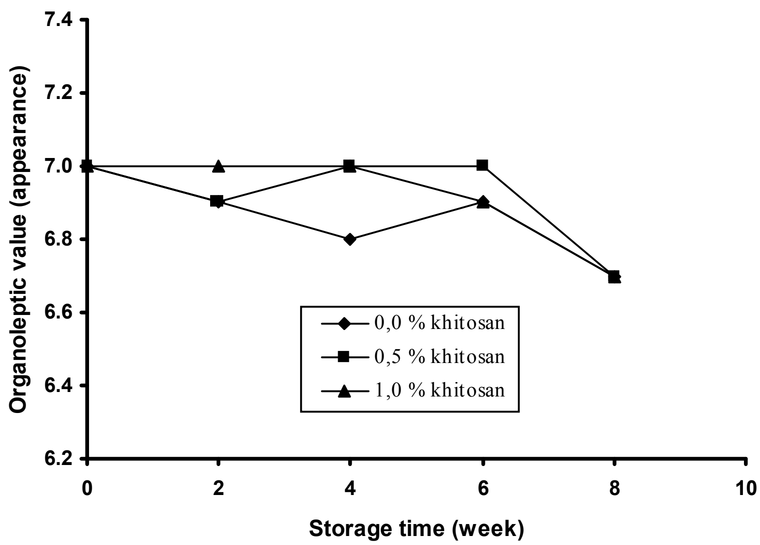


**Fig 2.** The change organoleptic value of appearance of salted-dried anchovy during storage

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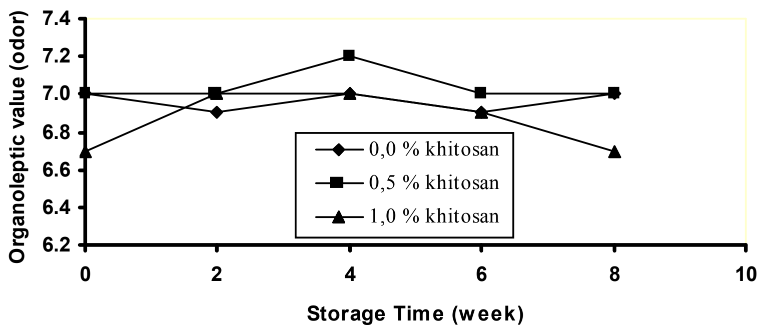


**Fig 3.** The change of organoleptic value of odor of salted-dried anchovy during storage

During 8 weeks of storage the change of organoleptic was relatively small on all treatments. Based on statistical analysis of Kruskal Wallis, there was no significant effect of chitosan concentration and storage time treatments ($p>0.05$) to organoleptic value (appearance, odor, taste and consistency) of salted-dried anchovy. The use of chitosan concentration on salted-dried anchovy did not give negative effect during storage for 8 days. There was relatively small change on appearance during storage of the product for all treatment. This was related to total bacteria number present in the product which is relatively small and far below the standard limit for salted-dried fish determined by Indonesia National Standard of 1x105 colony/gr (NSA, 1992). Low in bacteria number can reduce the rate of product deterioration.

In addition low value of Aw occurred in slated-dried anchovy (0.63 – 0.65) minimize the browning reaction of the product. During drying there is heat transferred to product that can affect browning reaction (Maillard) between amino compound and reduction sugar. The browning reaction can also initiated by reaction between protein, peptide or amino acid with fat decomposition product. (Lee. 1983). Maillard reaction can easily occur on food containing water more than 2% (Jay, 1992).

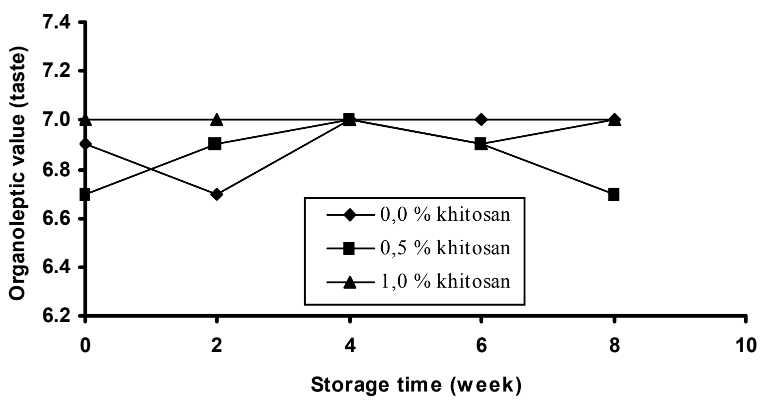


**Fig 4**. The change of organoleptic value of taste of salted-dried anchovy during storage

Indriati et al. (1991) stated that browning reaction of salted-dried fish in Indonesia mainly occur on product containing salt 7.7% - 16.9% with Aw between 0.7 – 0.78. In order to prevent the product from deterioration, the concentration of salt and Aw should strictly control during processing. The flavor of salted-dried anchovy is affected by changing of macromolecules compound that result in some unpleasant materials on food. During storage from 0 – 8 weeks there was no significant change on flavor (taste) of the products subjected to chitosan treatment. This occurrence was considered due to small number of bacteria present in the product so only small macromolecules compound broken down and did not affect taste of the product. Consistency of food is closely related to water content.

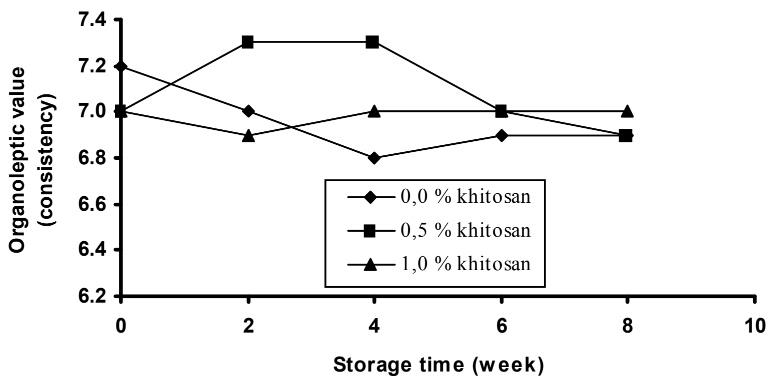


**Fig 5.** The change of organoleptic value of consistency of salted-dried anchovy during storage

The smaller the water content of food, more brittle of product resulted. (Winarno, 1991). Sensory analysis of salted-dried anchovy on all combination treatments range between 6.9 – 7.3. Score 7 on sensory analysis of consistency has criteria of : very hard and not brittle. Salted-dried anchovy resulted with water content of 16.7% - 17.8% is very hard and this was due to drying process taken very long time.

Packaging applied on salted-dried anchovy was proposed to protect the product from ambient humidity during storage. If the product was not packed, high humidity of atmosfer can increase water content of the product and becoming soft. Increasing water content of salted-dried anchovy will significantly decrease consistency of product.

## CONCLUSION

Chitosan concentration, length of storage and their interaction have very significant effect ($p < 0.01$) to the total bacterial count of slated-dried anchovy. Multiple regreasion model suitable for the prediction of total bacterial count of the product was $Y = 14.375 - 78.750 X_1 + 26.667 X_2$. Decrease in chitosan concentration and prolong of storage time would increase total bacteria count of the product. In addition, the treatment of chitosan concentration and length of storage time did not have significant effect ($p > 0.05$) to sensory analysis / organoleptic value of salted-dried anchovy (including appearance, odor, taste and consistency).

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