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STUDY ON THE EFFECT OF CHITOSAN CONCENTRATION ON QUALITY AND SELF-LIFE OF TOFU

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Abstract:

The purpose of this study was to evaluate the effect of chitosan concentration on the quality and shelf life of tofu. Tofu is treated by soaking in a chitosan solution with a concentration of 0.75 - 1.5%. The results showed that chitosan concentration 1% give the most stable pH value during storage, pH value ranged from 5.9 to 6.28, chitosan concentration of 0.75%, 1% and 1.25 % for the least reduced protein value. Total acid increased the least in the treatment with chitosan 1% followed by 0.75%, 1.25%, 1.5% and the highest in the control treatment. The results of analysis of total microorganisms, yeasts, and molds showed no significant differences in the chitosan treatments, the control formula with the highest amount of total microorganisms, yeasts and molds. Analysis results of Coliform and *E. Coli* did not detect the presence of *E.coli* and Coliform during storage. Chitosan 1% gave the highest sensory score of 16.2 points.

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NGHIÊN CỨU ẢNH HƯỞNG CỦA NỒNG ĐỘ CHITOSAN ĐẾN CHẤT LƯỢNG VÀ THỜI GIAN BẢO QUẢN ĐẬU PHỤ

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Đậu phụ, chitosan, protein, vi sinh vật tổng số, coliform, E. coli,..

Tóm tắt

Mục đích của nghiên cứu này là đánh giá ảnh hưởng của nồng độ chitosan đến chất lượng và thời gian bảo quản đậu phụ. Đậu phụ được xử lý bằng dung dịch chitosan có nồng độ từ 0,75 – 1,5%, được bảo quản ở nhiệt độ từ 2 - 4^0 C. Kết quả cho thấy chitosan nồng độ 1% cho giá trị pH ổn định nhất trong suốt thời gian bảo quản (giao động từ 5,9 – 6,28), chitosan có nồng độ 0.75%, 1% và 1,25% cho giá trị protein giảm ít nhất. Acid tổng số tăng ít nhất ở công thức xử lý bằng chitosan 1% tiếp theo là 0,75%, 1,25%, 1,5% và cao nhất là công thức đối chứng. Kết quả phân tích vi sinh vật tổng số, nấm men, nấm mốc không có sự khác biệt có ý nghĩa ở các công thức xử lý chitosan, công thức đối chứng có lượng vi sinh vật tổng số, nấm men nấm mốc cao nhất. Kết quả phân tích *Coliform* và *E. Coli* không phát hiện thấy sự có mặt của *E.coli* và *Coliform* trong suốt quá trình bảo quản. Chitosan 1% cho điểm cảm quan cao nhất là 16,2 điểm.

1. Introduction

Chitosan, a deacetylated form of chitin, is a natural polysaccharide delived from crustacean shells and the cell walls of fungi. Currently chitosan is of interest in food science due to its special functional characteristics, such as antioxidative activity, excellent gelling ability and antimicrobial ability [2]. Chitosan has the ability to form a semi-permeable film on the surface of food that helps to partially block CO_2, O_2 and water vapor, thereby preventing oxidation and reducing the loss of nutrients in food. A number of studies have been conducted to examine the gelling properties of chitosan in food products, such as meat product, tofu, fish ball,...Several studies also reported that chitosan could significantly inhibit lipid oxidation and reduce microbial load in fish product [1], [2], [10].

Tofu, a very popular food in the Orient particularly Far Eastern countries, is traditionally made by curding soybean milk using different coagulants. Its is used as a meat substitute due to its high protein contents with good balance of amino acid and better degestibility [6]. Tofu contains about 8% of total proteins, 4 - 5% lipids and about 2% of carbohydrates on fresh weight basis. Tofu has a special nutritional value due to a presence of dietary fibers (about 1%) and the absence of cholesterol, as well as a very low energetic value. The high content of vitamins and minerals also contribute to the physiological value of the tofu [8].

Talking about the benefits of tofu, many researchers believe that tofu, as well as soybean products, can reduce the number of chronic diseases such as cancer, heart disease, osteoporosis. Soybean protein contains isoplavolesterol and isoflavones, which have anti-atherosclerotic effects [9]. It has also been suggested that consumption of soy protein is more effective in reducing serum total cholesterol, cholesterol, LDL, and triglycerides than animal

proteins. Isoflavones, aglycones, and proteins present in tofu have antioxidant properties that protect against lipid oxidation [8], [10].

Tofu sold in the retail market is produces mostly by the small food processors. It is normally packed in water to prevent weight loss due to evaporation and breakage during handling. Under conditions, freshly made tofu can be kept for only 1 -2 days before spoilage occurs. Temperature fluctuations during transporation and the initial microbial loads cause variations in the storage life of tofu. This short self-life not only result in wastage but also limits the area of distribution [5], [10]. Therefore, finding the right preservative to prolong the shelf life of tofu is very necessary, and meaningful.

2. Material and Method

2.1. Material

Chitosan was used is in the form of powder, with acetilation DA >85%, molecular weight 300.000 DA. It was provided by MTD company Binh Duong branch.

Tofu was made in Faculty of Biotechnology and Food Technology, Thai Nguyen University of Agriculture and Forestry, used lactic fermented soy milk as a coagulant. Tofu has an ivory white color, has a characteristic aroma of cooked soybeans, smooth surface, no cracks, smooth cuts, lightly pressed by hand, has elasticity. Tofu was treated with different concentrations of chitosan and packed by PE bags and stored at 2 - 4^{o} C.

2.2.Method

2.2.1. Experimental set-up method

The experiment consisted of 5 formulas, repeated 3 times

Control: No chitosan treatment

Fomular 1: Chitosan 0,75%

Fomular 2: Chitosan 1%

Fomular 3: Chitosan 1,25%

Formular 4: Chitosan 1,5%

2.2.2. Methods of analysis of research indicators

Determination pH of tofu

Five grams of tofu and 25 ml of deionized water were homogenized using a mortar and pestle, then filtered through a singlr layer of cheesecloth to measure pH (pH mater).

Quantification of protein in tolfu by the Kjeldahl method

Put 1 g tofu sample in digestion flash and then add 3g catalyst compound at the ratio $K_2SO_4/CuSO_4$ (3:1) then put 10ml H_2SO_4 98%. Then put the chemical in the tube placing the Kjeldahl flash in a diagonal position. The attack occurs in 3 stages: the stage 1take place in 30 minutes at temperature of 250^0 C, stage 2: 30 minutes at 300^0 C, stage: 60 minutes at 420^0 C, until the solution in the Kjeldahl tube turn to blue color. At this time, the sample changes from organic nitrogen to inorganic form $(NH4)_2SO_4$. Cool the sample around 10 minutes then transfer to UDK 142 Kjedahl machine. Use BaOH 40% to push NH_3 out of ammonium salt, NH_3 affter being released will be follwed hot steam. After being cooled, it will be absorbed into the H_2SO_4 solution in the collecting vessel to create ammonium sulfate salt with a clear green color. The running time of an analytical sample is 6 minutes. To determine the amount of NH_3 released during distillation, we titrate it with 0,1 N H_2SO_4 acid until solution turns to pink color. From the amount of 0,1 N H_2SO_4 acid consumed in the titration process, we can calculate the amount of protein present in the sample. The calculation formula is below

$$N\ \% = \frac{(V_2 - V_1) \times 1{,}42 \times 100 \times f}{w}$$

Whereas:

N: Protein content in the sample (%)

V_1: The volume of 0,1 N NaOH consumed when titrating the analytical sample (ml)

V_2: The volume of H_2SO_4 0,1N (ml) in the colleting vessel

f: Acid titration coefficient

w: Weight of sample (gram)

1,42: the amount of nitrogen corresponding to 1 ml of 0,1 N H_2SO_4 solution

6.25: the protein – nitrogen conversion

Determination of total organic Acid Content

Grin 2 grams of sample in a ceramic mortar, then transfer to a 250ml conical flask, add water to a volume of 150ml. Heat for 30 minutes in a water bath at the temperature of 80 – 90^0 C, sharking occasionally. Then the solution has cooled, fillter it into a 250 ml volumetric flask and make the volume with disstilled water. Transfer 50 ml solution from volmetric flask into a conical flash, add 1 -2 drops of phenolphtalein and titrate with NaOH 0,1 M until the pink color appears.

Total organic acid content was calculated according to the formular:

$$X = \frac{a.0{,}0067.V.T.100}{v.c}$$

Whereas:

X: acid content (%)

a: Number of ml of NaOH 0,1N need for titration

0,0067: Number of gram of acid corresponding to 1 ml NaOH 0,1N

T: Correction factor for NaOH 0,1N

V: Total volume of extraction solution

v: Number of ml of solution taken for titration

c: Weight of sample (gam)

Microbiological method

Determination of total aerobic microorganisms according to TCVN 4884:2005

Prepare medium: The medium for determing total microorganism is TGA (Tripton – Glucos – Agar). 1 lit midium includes (5g pepton, 4g glucose, 2,5g yeast extract, 15g agar, 1 lit distilled water), sterilization for 20 minutes ats temperature 121^0 C.

Sample dilution: take one peace of tofu sample in each treatment, crush them with a ceramic mortar, mix well, weight one gram of sample, dilute to a concentration of 10^{-1}, 10^{-2}, 10^{-3},..

Inoculation: take 100 µl of diluted sample and cultivate on petri dishes with TGA medium, dilute 3 plates for each concentration, the incubate at 37^0 C after 48 – 72 hours, count all colonies appearing on the agar plate (number of colonies in each dish should range from 30 to 300). The average number of microorganisms in 1 g sample is calculated by the formula.

$$\text{N colonies / g or ml} = \frac{\sum C}{(n_1 + n_2) f1V}$$

Whereas:

∑C: total number of colonies counted on all plates

n plate count at the first dilution concentration

n2: plate count at the 2nd dilution concentration

f1: The dilution coefficient is in the first count plate

V: volume of the inoculum in each petri dish

Note: If the pure (liquid) or stock suspension has a number of colonies less than 30, still take the result

Determination of Yeast anf molds according to TCVN 4993 – 89

Principle: culture media containing bacteria inhibitors (antibiotics such as Oxytetracylin or Cholpramphenicol) cultured at 30 ± 1^0 C in aerobic conditions after 48 – 72 hours. Count the number of colonies on the petri dish from which the total number of yeasts, and molds was determined

Prepare medium: the medium used form determining yeast and molds is YGC (Yeast – glucose – Chloramphenicol) 1 lit medium contain (20g glucose, 5g yeast extract, 0,1 g chloramphenicol, 20g agar, 1 lit distilled water). Sterilize at 20 minutes at temperature 121^0 C.

Method of preparing and diluting samples, and carrying out the determination of the number of cells as for the determination of total microorganisms.

Method of determing coliform and *E.coli* according to MPN method

The MPN method is based on the principle of statistical probably distribution of microorganisms in different dilution concentrations. The dilution is cultured repeatedly (3 – 10 times). Dilutions are chosen such that in the replicates there are a number of positive, some of negative. The number of possitive times is recorded and compared with statistics deduce the estimated value of the number of microorganisms in the sample.

Coliforms are a group of bacteria including a number of Gram-negative, non-sporeforming, aerobic or anaerobic bacteria that are capable of fermenting lactose, producing steam within 48 hours at the appropriate culture temperature like *E.coli*, *Citrobacter*, *Klebsiella* and *Enterobacter*.

Principle: Endo medium containing sodium and fucshin is capable of inhibiting Gram positive bacteria during growth on this medium, Coliforms ferment lactose to form aldehydes and acids, aldehydes affect the Funcsin-sulfite complex, and release of fucshin, which then stains colonies from pink to red lotus petals, round, evenly uniform, which is may or may not be iridescent.

Endo medium: 1 lit Endo medium contain (10g pepton, 10g lactose, 2.5g K_2HPO_4, 3,3g natrisulfite, 0,3 g fucshin alkaline, 20g agar, water 1 lit, pH 7.5) sterilize for 20 minutes at 121^0 C. all colonies of pink to red lotus petals, round, uniform, with or without iridescence.

Sensory evaluation (according to TCVN 3215 - 79).

The sensory quality of tofu was evaluated by point system (from 1 to 5). Estimate was done by a panel consisting of 10 members. They give the average mark of tofu quality and average corrected mark of sensory

characteristics. The average marks of tofu quality is the average value of 4 quality parameters (color, smell, taste, general appearence of tofu). Average mark corrected for quality parameter significance was obtained by dividing the corrected mark with the number of panel members. The corrected mark was obtained by multiplying marks for single tofu quality parameter with the appropriate coefficient of important (for the appearance, consistency, cut of tofu, – coefficient is 2; for color and smell – coefficient is 3, for taste – coefficient is 8). Then sume of the results was divided with 20 (sum of coefficient). The mark for smell and taste is an average sum of marks for smell and sum of marks for taste. Samples for sensory evaluation were stored at 4^0 C and warmed to room temperature before evaluation. Tofu was cut in to cubic samples (about 6 cm x 6cm x 4,5 cm) and placed on a plastic plate.

2.3. Statistical analysis method

Using SPSS software (version 20), the data was analyzed using a one – factor ANOVA with a significant $P \leq 0.05$

3. Result and Discussion

3.1. Effect of chitosan concentration on tofu pH

pH is one of the factors that greatly affacts the sensory quality of tofu during storage. The result of studying the effect of chitosan on the change of pH during storage are presented in figure 1.

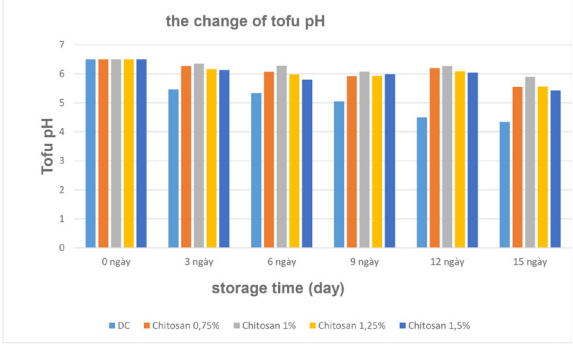

Figure 1. The bar chart shows the effect of chitosan concentration on the pH value

The results shown that the pH value in preserved tofu samples treated with chitosan at different concentration decreased significantly during storage. The formula treated with chitosan 1% had the least decrease in pH, the rest of the formulas had no significant difference in level $\alpha < 0,05$, the control fomula has the most decrease in pH value. It can be said that the pH of tofu treated with chitosan 1% is relatively stable in the range of 5.9 to 6.28. Tofu is a product rich in proten, with high water content, nutrition in tofu is a suitable emvironment for many microorganisms to infect and grow. The cause of thr decrease in the pH value of tofu during storage may be due to the lactic acid bacteria present in the sour water nd other fermenting bacteria during product storage. Chitosan has the effect of reducing the number as well as inhibiting the growth microorganisms, thereby preventing the fermentation process caused by mocroorganisms, resulting in the pH value of the product is less changed compared to the control formula.

3.2. The effect of chitosan concentrations on the protein content

The effect of chitosan concentration on the protein content was shown in the table 1.

Results of the table 1, it can be seen that the protein content in tofu decreased gradually during the storage time. The tofu samples treated with chitosa were significantly better than the control. After 15 days of storage, the protein content in the control decreased more than 2 times (only 4,12%). Meanwhile, the fomulas treated with chitosan had higher protein content ranging from 4.97 to 5.92% than the control. The formulas treated with chitosan concentrations 1%, 1.25% and 1.5% had no significant different at level $\alpha < 0,05$. These results show that chitosan has antibacterial ability, resists invasion of microorganisms, controls gas exchange of chitosan, prevents oxygenation of organic compounds in the product, especially protein, and helps tofu keep better quality and less loss of protein content. So the formula of chitosan concentration equal to 1% is the best, the protein content decreased the least.

Table 1. The effect of chitosan concentration on the protein content (unit %)

Chitosan concentration	0 day	3 days	6 days	9 days	12 days	15 days
Control						
Chitosan 0,75%						
Chitosan 1%						
Chitosan 1,25%						
Chitosan 1,5%						

Note: The letters in the same column represent the statistically significant diference at level α <0,05

3.3. The effect of chitosan concentrations on total organic acid

Table 2. Effect of chitosan concentratons on tofu acid (% unit)

Chitosan concentration	0 day	3 days	6 days	9 days	12 days	15 days
Control						
Chitosan 0,75%						
Chitosan 1%						
Chitosan 1,25%						
Chitosan 1,5%						

Note: The letters in the same column represent the statistically significant diference at level α <0,05

The longer the storage time, the higher the total organic acid content due to the growth of microorganisms, the physicochemical changes of tofu and the soaking solution. From the results of Table 2, it shows that the control tofu sample had the highest total organic acid content of 2.51%. Due to the development of microorganisms during storage, organic acids such as lactic acid and acetic acid are produced, the pH of the tofu decreases, the tofu has a sour taste, and the total organic acid content in the beans tends to increase. After 15 days of storage, the total organic acid content in the sample treated with chitosan 1% was 1.39%, followed by the chitosan treatment formula 0.75%, 1.25% and 1.5%, the formula with the highest increase in total organic acid content was the control formula 2.51%

3.4. The efect of chitosan concentrations on the Total Aerobic Microorganisms and Yeast and Mold

The content of bacteria in a product is one microbiology patameter in determining the appropriateness of the product consumed, it is necessary for analysis of a product and determine microbial growth (indicator can be total aerobic microorganism and yeast and mold) in the product during storage. Microorganisms are one of the main causes of food spoilage during storage. The invasion of microorganisms in food changes color, flavor, spoils food, causes food poisoning cases [7]. The results of the analysis of total aerobic microorganism and yeast and mold in the sample of tofu application of chitosan on observation day 3 to day 15 are presented in the table 3 and 4 below.

Table 3. Efect of chitosan concentrations on total aerobic microorganisms (cells /g 10^3)

Chitosan concentrations	3 days	6 days	9 days	12 days	15 days
Control	$0,64^{b}$	$1,1^{b}$	$1,635^{b}$	$1,73^{c}$	$1,995^{b}$
Chitosan 0,75%	$0,045^{a}$	$0,558^{a}$	$0,72^{a}$	$0,935^{ab}$	$1,245^{a}$
Chitosan 1%	0,015	$0,293^{a}$	$0,31^{a}$	$0,615^{a}$	$1,085^{a}$
Chitosan 1,25%	0.021	$0,303^{a}$	$0,4^{a}$	$1,075^{b}$	$1,2^{a}$
Chitosan 1,5%	$0,2^{a}$	$0,508^{a}$	$0,86^{a}$	$1,163^{b}$	$1,27^{a}$

Note: The letters in the same column represent the statistically significant diference at level α <0,05

Table 4. Effect of chitosan concentrations on Yeast and Mold (cells /g 10^3)

Chitosan concentrations	3 days	6 days	9 days	12 days	15 days
Control	$0,08^{b}$	$0,188^{c}$	$0,233^{c}$	$0,475^{b}$	$0,705^{b}$
Chitosan 0,75%	$0,013^{a}$	$0,028^{a}$	$0,035^{a}$	$0,063^{a}$	$0,105^{a}$
Chitosan 1%	$0,005^{a}$	$0,018^{a}$	$0,033^{a}$	$0,05^{a}$	$0,073^{a}$
Chitosan 1,25%	$0,008^{a}$	$0,035^{a}$	$0,073^{ab}$	$0,1^{a}$	$0,133^{a}$
Chitosan 1,5%	$0,01^{a}$	$0,073^{b}$	$0,088^{ab}$	$0,108^{a}$	$0,193^{a}$

Note: The letters in the same column represent the statistically significant diference at level α <0,05

The total aerobic microorganism and yeast and mold affter application of chitosan, it can be said that the number of microorganism on the product know tends to increase with the leng of storage in chitosan at concentration of 0,75%, 1%, 1,25%, 1,5%. Result of laboratory tests on the value of total aerobic microorganism on tofu application of chitosan on the day 3 averaging around 10^1 to 10^2 colonies for all concentrations. On the day15 the lowest value of total aerobic microorganism of tofu was 1.085 x 10^3 colonies found in 1% chitosan, yeast and mold was 0.073 x 10^3 colonies where the sum of total aerobic microorganism value is allowed to TCVN 4884. 2005 tofu of 10^5. The formulas treated with chitosan concentrations 0,75%, 1%, 1.25% and 1.5% had no significant different at level $\alpha < 0,05$ in both total aerobic microorganism and yeast and mold. This condition showed that the used of chitosan at 0,75% to 1,5% to maintain the quality of tofu until 15 day and still suitable for consumption.

3.5. The effect of chitosan concentrations on E. Coli and coliform.

In food *E.coli* and coliform are two types of microorganisms commonly found in preserved foods. Is the cause of many cases of food poisoning due to *E.coli* and coliform contamination. Research results on the effect of chitosan with different concentrations on preserved tofu samples showed. No presence of *E.coli* and coliform was detected in the chitosan-treated tofu samples as well as in the control sample.

3.6. Effect of chitosan concentration on sensory quality of tofu

The evaluation of overall sensory quality is important in determining how well a product is accepted by consumers. Although a non specific indication of the reasons, it is a good indication of the potential consumer demand of the product. The sensory quality of tofu was treated with different chitosan concentrations was shown in table 5

Table 5. The effect of chitosan concentrations on tofu sensory quality

Chitosan concentration		Average mark with the coefficient of important				
	0 day	3 days	6 days	9 days	12 days	15 says
Control	18.5	15,2	11,6	10,8	10.6	10.4
Chitosan 0,75%	18,3	18,3	15,5	14,0	13,5	14,1
Chitosan 1%	18,2	18,2	17,9	16,2	17,4	16,2
Chitosan 1,25%	18.4	15,2	15,3	13,6	11,8	12,8
Chitosan 1,5%	18.1	15,2	15,6	13,6	12,6	13,9

The results for sensory evaluation are presented in table 5. The scores for colour and appearance, which are the first deciding factors that determine the acceptance or rejection of a product, reflected that the tofu samples were treated with difference chitosan concentrations had a good acceptability. The colour and appearence were not affected by the use of different chitosan concentrations and has a slightly change during the storage. All the samples had similar creamy white colour which is the acceptable colour of tofu [4]. Flavour, a combination of both taste and smell, was a concern in tofu samples with were treated by different chitonsan concentrations have a natural beany flavour. However, the results indicated that all the sample had acceptable flavour score. In our study the different chitosan concentrations had a significant effect on the flavour of tofu, it is possible that high chitosan concentration could get less mark because of high viscosity, ans bad smell of chitosan. Result on the table 5 show that sensory scores tend to decrease during storage. Chitosan with a concentration of 1% gave the best sensory quality score of 16.2 points, which was rated as good.

Conclusion

Chitosan has a concentration of 1% for the best quality tofu preservation. The pH and protein content decreased the least, the total organic acid content increased the least, the total microorganisms, yeasts and molds appeared in the lowest samples and were assessed as safe for users. Analysis results of *E. Coli* and coliform showed that there was no appearance of *E coli* and coliform in the product during the storage period. Sensory quality is rated as good. Chitosan treatments with different concentrations showed that chitosan had a significant effect on biochemical as well as microbiological changes in the sample due to its antibacterial, antifungal, and antioxidant capacity.

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