The influence of addition of papain enzyme and Carboxyl Methyl Cellulose on the textural properties of Tofu

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Abstract. Papain enzyme and carboxyl methyl cellulosa was used in tofu production as coagulant and thickener. Papain enzyme is a protease enzyme that can break proteins. Papain enzyme useful as coagulant to replace acid and base coagulant. The goal of this study is to observe papain enzyme as coagulant and carboxyl methyl cellulose as thickener to increase characteristic of tofu. Tofu is from soy milk has been pasteurized at 70 °C with the addition of papain enzyme and carboxyl methyl cellulose. The concentration of papain enzyme is varied such as 200, 400, 800, and 1000 ppm. After Temperature reaches at 90 °C, carboxyl methyl cellulosa is added in soy milk to produce tofu. This study focuses on introducing papain enzyme as coagulant as well as investigating its potential in improving tofu making process productivity. Further the present work attempts to determine the synergistic effect of combining-CMC/enzyme in tofu characteristic. This research was conducted with soy milk pasteurized at 70 °C with increasing papain enzyme. Protein from tofu was determined by using Spectrophotometer UV-VIS Shimadzu UV-1800 type. The highest protein concentration of the papain enzyme was found in 1000 ppm with CMC concentration of 0.6% w/v and based on organoleptic tests that the adding CMC and enzyme papain does not effect the taste, smell, texture and color of tofu. The taste of tofu produced is similar to the taste of tofu in the market.

1. Introduction

Tofu production is usually made by using a coagulant such as base, acid and enzyme coagulants. Many coagulants were used in tofu production. The use of coagulant ingredients in the process of tofu production will affect the high protein level and also influence the acidic, slightly bitter, taste and the crumbly texture of tofu [1,2,3].

Papain enzyme is a protease (proteolytic) that can break down proteins. Papain enzyme is able to agglomerate protein, resistant to high temperatures and able to function properly at low concentrations, it is also free of chemicals, accessible, easily own-made, non-toxic, and no side reactions. Papain enzyme can agglomerate milk protein in the manufacture of cottage cheese [4]. The papain enzyme obtained from the latex of the papaya fruit. In order to observe the activity of the enzyme papain as a coagulant in the process of tofu production, a research is need to be conducted to

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find the optimum conditions of the enzyme. The use of coagulant in tofu production will affect the level of protein held out, which resulted different characteristic such as sense of knowing that acid, a little bitter and easy destroyed [4].

This research aimed to observe the use of papaya latex as papain enzyme source, which used as a coagulant in the process of tofu production. In addition, this study also examined the addition of carboxyl methyl cellulose in tofu production. Carboxyl methyl cellulose is a derivative of cellulose and is often used in the food industry or used in foodstuffs to prevent retrograde or inverse of the gelatinization process, where starch crystals converge to form a particular form which can affect the texture.

2. Material and Method

The latex of papaya fruit, which is used as an ingredient in the process of enzyme papain. The enzyme papain obtained with the addition of 0.7% NaHSO₃. Before papain enzyme was used in the processing of tofu, the MCU of the enzyme was calculated. MCU used for to determine proteolytic activity of the papain enzyme. Here, papain enzyme derived from the latex of papaya fruit tested to observe proteolytic enzyme. Next step is the process of making soy milk from soybeans. Soybeans used as a source of protein, weighed 500 grams. The soybeans then were soaked for one night to tenderize and enlarge the size. After that, the soybeans husk were cleaned, and then the soaked and cleaned soybeans were weighed and added to the hot water with a ratio 1: 3 of soy beans and water. The soybeans were blended until smooth and then filtered with a white cloth or gauze to separate the residue. After getting soymilk, the process of making papain enzyme derived from the sap of the papaya fruit performed by adding the carboxyl methyl cellulose. Soymilk fed into a 500-ml beaker glass. The milk was pasteurized up to 70°C – boiling point. Pipette enzyme papain with 200, 400, 600, 800, 1000 ppm repeated treatments with soy milk. Following conducted clots at temperatures of 90 °C with the addition of carboxyl methyl cellulose with concentrations of 0; 0.2; 0.3; 0.4; 0.5% w/v. The tofu molded on a silk mold for thirty minutes. Then the protein and organoleptic test analysis were tested and using statistic to determine on the organoleptic parameter.

Statistical test used by Q Cochran test method, this test used three or more sets of scores (proportions or frequencies) paired significantly. The scale is used the ordinal scale. If the value of Q cohcran ≥ the value of Chie squared at a significant level of 5% then Ho rejected and Ha accepted. Conversely, if the value of Q Cochran ≤ the value of Chie squared on 5% significant level then Ho accepted and Ha rejected. The formula is

$$Q = \left[ \left( k - 1 \right) k \sum_{i=1}^{k} c_i^2 - \left( \sum_{i=1}^{k} c_i \right) \right]$$

Where :
Q approaches X² with db = k-1
Test rule: Reject H₀ if Q≥X² -tables with db = k-1
K = number of samples (treatment)
N = number of replications
Ci = number of successes in each treatment (1 to k)
Li = the number of successes in each repetition (1 to n)

Hypothesis:
Ho: There is no effect of adding papain enzyme and carboxyl methyl cellulose for tofu production to organoleptic quality and favorability.
Ha: There is an effect of adding papain enzyme and carboxyl methyl cellulose for tofu production in organoleptic quality and favorite level

3. Result and Discussion

3.1 Test of Enzyme Activity Papain from the latex of Papaya Fruit

This research has been conducted to observe the use of crude extract papain from papaya latex as a coagulant in the tofu production by looking at the carboxyl methyl cellulose as a thickening agent. The papain enzyme which is a rough extract latex papain from papaya fruit is used as a coagulant the activity of the enzyme was tested. The activity of papain enzyme has conducted by using the MCU method. The table 1. shown the activity of the papain enzyme.

| Temperature °C | Agglomeration Time Minute | Enzyme Activities MCU   |
|----------------|---------------------------|-------------------------|
| 40             | 2.28                      | 439.28                  |
| 45             | 2.61                      | 385.92                  |
| 50             | 1.75                      | 574.47                  |
| 55             | 2.97                      | 354.66                  |
| 60             | 3.54                      | 283.80                  |
| 65             | 4.63                      | 216.95                  |
| 70             | 3.29                      | 305.69                  |
| 75             | 3.60                      | 280.10                  |

Papain enzyme produced from the latex of the papaya fruit. Before this enzyme used as the ingredient in Tofu production, its activity tested against the temperature. Before papain enzyme used as coagulant in tofu production, the papain enzyme tested its activity. The activity enzyme was used MCU method. With the variation of temperatures, it reviewed that the coagulant formed in the process of enzyme activity by using milk. The selected papaya fruits were unripe papaya fruits aged 2-3 months as they have a lot of sap, The papain enzyme used in this study were taken from the plants grown in marshland. The papaya sap tapped for ± 2 hours. The reason for using 2 hour tapping was because papaya latex will be contaminated with air and the color will be brown if it was left for too long and it can affect the outcome of papain flour made. Papain from papaya latex is a substance which easily damaged by air oxidation occurring either during manufacturing or storage, for this reason, the process of making the papain enzyme used sodium bisulfite which functions as a preservative. The best concentration of sodium bisulfite is 0.7%. This definition includes microbial inhibitors, antioxidants, marinade ingredients and binder. The solution mixed homogeneously dried in an oven using temperature of 70 °C because principally papain enzyme will work well at low temperatures, 55-70 °C. The drying step was done for 3 hours until the papain enzyme was completely dry, the previous research used period of 6 hours to dry the enzyme papain. The reason for using 3 hours drying time because the papain solution that has been pipetted is homogeneous 10 ml per petri dish while the previous research used 6 hours with 30 ml. Once the flour papain processing was finished, the next step was to see the level of enzyme activity. The activity of papain enzyme is using MCU (Milk clotting Units) method against the influence of several factors; time, temperature of drying, and the addition of sodium bisulfite (NaHSO₃) to determine the quality of the enzyme papain generated. MCU were best contained in the leaves and the rod was 200 MCU while the fruit was 400 MCU.

3.2 Test of functional groups for the enzyme papain

Papain enzyme produced from the latex of the fruit papaya. In this study, it was compared
functional groups of enzyme papain between enzyme commercial with enzyme papain from latex papaya. To compare functional group was used FTIR. FTIR analysis performed to measure the functional groups in papain enzymes.

This analysis aims to identify organic compounds based on a reading group functions held in the form of the spectrum. Here, FTIR analysis was conducted on a commercial papain enzyme and papain enzyme derived from the sap of papaya fruit. FTIR analysis conducted for both of these enzymes; the results of the test obtained at Figure 5.1 and 5.2. From the pictures shown in the figure 5.1 and 5.2, it shows that the wave spectrum of the papain enzyme and papain enzyme commercial were same, it addressed that the papain enzyme derived from the sap of the papaya has the same structure spectrum as enzymes commercial papain. Wave spectrum at the peak was seen in the range of 2300 cm⁻¹ showed a C≡C group, in the range of 3750 cm⁻¹ it showed the appearance of the wave peaks that showed the presence of O-H groups which are pure cellulose main functional groups. C-H group is the group-forming cellulose detected at wave number 300 cm⁻¹. Carbonyl group C = O as cellulose binding was detected at wave number 1650 cm⁻¹.

![Figure 1. The FTIR for enzyme papain from enzyme papain form the latex of papaya fruit.](image1)

![Figure 2. The FTIR for enzyme papain commercial.](image2)

3.3 Effect of Enzyme Papain enzyme, CMC and their combination on Protein production

Papain enzyme derived from the papaya fruit sap used in the manufacturing process. From the research that has been done previously, it can be concluded that the enzyme papain can be used as a coagulant. Clotting that occurred in the manufacturing process carried out at temperature of 70 °C. At this temperature, the enzyme papain added in the process of tofu production as in this temperature the papain enzyme is still active. The coagulation of tofu that carried out the papain enzyme is very
influential in forming protein, where the papain enzyme as coagulant material served as a catalyst that accelerates the reaction, in addition to the papain enzyme used to sever the bond of peptide soymilk into acid amino acids.

Spectrophotometry was used to analyze the protein. The method used is Lowry method to examine the levels of soluble protein found in tofu. From Figure 5.3, it showned that the higher the concentration of carboxyl methyl cellulose the higher protein produced. From the graph in Figure 5.3, it also can be seen that the higher the concentration of enzyme protein produced also the highest protein content in tofu, here the highest protein concentration of the enzyme papain was found in 1000 ppm with carboxyl methyl cellulose concentration of 0.6% w/v. This range showed the influence of the addition of the enzyme papain that increase the protein content. While the lowest solubility protein, soy milk without the addition of carboxyl methyl cellulose was found in the addition of a concentration of 200 ppm.

3.4 Organoleptic test

In this research, organoleptic test did to taste, color, texture, aroma and favorite level. The addition of papain enzyme and carboxyl methyl cellulose were made in various concentrations and tested on a rather well trained by panelist. Panelists argue that the concentration of papain enzyme 200 ppm with the increment of carboxyl methyl cellulose produces tofu which with a very soft texture.

| Papain Enzyme Concentration (ppm) | Q Cochran | Chi square |
|-----------------------------------|-----------|------------|
| 200                               | 9,7       | 10,8       |
| 400                               | 0,7       | 5,7        |
| 600                               | 1,8       | 2,4        |
| 800                               | 2,05      | 18         |
| 1000                              | 4,2       | 8,9        |

Figure 3. The effect of CMC addition and enzyme papain toward protein in tofu.
According to the data collected, it is known that the resulting soy-scented soybean like tofu market and the addition of carboxyl methyl cellulose with a concentration of 0.15% b/v, 0.30% b/v, and 0.45% w/v the resulting flavor is still the same as soy-scented with a very soft texture. The panelists like tofu very much with the addition of papain enzyme and carboxyl methyl cellulose. Due to the bitter taste contained in papaya sap is not felt in the tofu, the texture of tofu is very soft and chewy also become a separate reference in the panelist assessment. This table show that the statistic data which use chochran equatio to determine the organoleptic level from panelists.

Based on taste levels of tofu was determined with using panelist. The results from panelist is calculated by Cochran equation and Chi square (table 2). Table 2 shows that panelists like tofu by adding papain enzyme and carboxyl methyl cellulose. Papain enzyme and carboxyl methyl cellulose addition does not effect for taste, aroma, texture, color and favorite level. It can be describe from the value of Q Cochran and Chi Square. This calculation to know the level of the organoleptic parameter. It can be shown that The value of Q cochran is lower than Chi square for organoleptic parameter. It means that organoleptic parameter from tofu was illustrating that panelists like tofu with adding papain enzyme and carboxyl methyl cellulose. For example, 400 ppm of papain enzyme addition has Q chochran around 0.75 and Chi square around 15.2. The value of Q chochran was lower than Chi square. It mean that Ho is accepted and Ha is rejected. There is no effect of adding papain enzyme and carboxyl methyl cellulose for tofu production to organoleptic quality and favorability. The hypotesis explans that the papain enzyme and cmc addition do not effect for the quality and favorability of organoleptic test for various concentration of papain enzyme and carboxyl methyl cellulose.

4. Conclusions
From this research, it concluded that the enzyme papain from papaya latex used as a material for coagulant in tofu production. It found that the best concentration was 1000 ppm and 0.6% carboxyl methyl cellulose and the result of organoleptic test show that it does not affect for organoleptic test for taste, aroma, texture and favorite level from tofu with adding papain enzyme and carboxyl methyl cellulose.

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