The Effect of Salt-GDL Coagulant and Temperature on Tofu Quality

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Abstract. Soymilk tofu can be coagulated with salts, acid and lactones. The objectives of this investigation was to study the effect of temperature and different coagulant to the quality of tofu; such as protein content, texture, mass and moisture. Soybean was oaked and grinded to produced soymilk. The soymilk was coagulated with two type of mixed coagulant; CaSO₄-Glucono delta-lactone (GDL) and MgSO₄-GDL, the variation of composition were 0:4, 3:1, 2:2, 1:3, 0:4 at temperature of 70℃ and 80℃. The highest protein content was obtained from the tofu with CaSO₄-GDL (2:2) and 70℃. The results showed that the different in coagulation temperature affect the protein content of tofu. Mass and texture of the tofu increased as the composition of salt coagulant increased. General factorial method was used in this experiment, the result supports that the various composition of coagulant increased significantly (P<0.05) from 111-135 gram and 241-642 gram, respectively for tofu mass and texture. Temperature has significant result to protein content and mass of the tofu.

Keywords: coagulation, glucono delta-lactone, salt, tofu

1. INTRODUCTION

Malnutrition in Indonesia is still relatively high. Based on Riset Kesehatan Dasar (Riskesdas) and Pemantauan Status Gizi (PSG) from the Indonesian’s ministry of Health, it was found that the level of malnutrition continues to increase in Indonesia [1]. According to the result of research by Rizqa Widiasti in 2019, protein deficiency reached 37% in Indonesia [2]. Protein can be classified from its source; vegetable and meat source. In this case, there are tofu and tempe made from soybeans (vegetable based food) that can be improved, especially to their high protein content. Its various nutrition; isoflavones also can reduce the risk of cardiovascular problem. Due to the inexpensive price and its high nutrition, tofu might solve the protein malnutrition in Indonesia.

Tofu is one of vegetable based food that has high nutrition content. The preparation of tofu generally includes soybean soaking and grinding in water, filtering, boiling and coagulation of soymilk, molding and pressing. There are several factors that affect the quality of tofu; such as temperature, type of coagulant, soybean variety, pH, and pressing [3]. Some researchers conducted experiments to compare the quality and the result were diverse, but most of the research stated salt coagulant produces the highest protein content with hard texture. According to Shurtleff and Ayogi (1984) in order to give a good texture, some of the coagulant are mixed with GDL; the texture produced is soft and smooth [4]. There are still few studies that examine the effect of mixed coagulant. This prompted researcher to study the effect of variations in the composition and different type of
mixed coagulants (salts and GDL) to the quality of tofu.

2. MATERIAL AND METHOD

2.1 Chemicals
The main materials to conduct this experiments were soybeans that was purchased from the local market (Bandung, Indonesia). Calcium sulphate (CaSO₄·2H₂O), Magnesium sulphate (MgSO₄·xH₂O), magnesium chloride (MgCl₂·xH₂O) and glucono delta lactone (GDL) as coagulant. Sulphuric acid (H₂SO₄), natirum hydroxide (NaOH), boric Acid, hydrochloric acid (HCl), pottasium sulphate (K₂SO₄) and copper sulphate (CuSO₄·xH₂O) as the chemicals to support analysis.

2.2 Preparation of soymilk
100 grams soyben were soaked in water with a ratio of 1:10 in a room temperature for 12 hours. The soaked bean which had doubled in weight was mixed and blend with 800 ml of water. Then the soaked bean was grinded by a laboratory blender and raw soymilk was obtained. The slurry in the raw soymilk then was filtered by cotton tack rag cloth to seperate the solid contaminant. The raw soymilk was heated to its boiling point, then cooled into the temperature of the experiment.

2.3 Tofu Processing
Tofu was prepared by coagulating the previous prepared soymilk using salts coagulant for the preliminary experiment and salt – GDL mixed coagulant for the main experiment. The type of salt coagulant used for the preliminary experiment were calcium sulphate, magnesium sulphate and magnesium chloride. The concentration were also varied into 0,3%, 0,5% and 1,0% w/v in a temperature condition of 80℃. The variations of salts and its concentration were chosen based on previous researcher’s experiment result. The main experiment was proceeded by using the best coagulant and concentration from the preliminary experiment and mixed it with glucono delta lactone. The mixture coagulant of salt and GDL were varied into 4:0, 3:1, 2:2, 1:3 and 0:4.  For each experiment the coagulant was dissolved in 20 mL of water then poured into the soymilk. The soymilk was equipped by an impeller in order to make the coagulants dispersed well and heating plate with temperature of 70℃ and 80℃. The curds thus formed after a 10 minutes mixing the soymilk and coagulant. Moreover, the curds was obtained by seperating the whey with cotton rag. The curds was pressed for 30 minutes using a weigth of 1,5 kg in a container, tofu samples were weighed and stored in water at 5-7℃ before analysis.

2.4 Protein analysis
The protein content were determined by kjedahl method using a protein conversion factor of 5.75 method. The process consist of three stages; digestion, distillation and titration of the sample.

2.5 Texture analysis
Texture analysis of tofu was carried out using an instrument; texture analyzer. A part from the central of the tofu were cut x2x2 cm and used for texture evaluation. A test speed of 4 mm/s was used with a probe of a cylindrical metal with 6 mm diameter. The sample was compressed to 19 mm of its height. There were two samples carried out for each variation. The hardness of tofu was determined by the peak of the force from the diagram shown by the software.

2.6 Moisture analysis
The water content of tofu were obtained by drying a weighed amount of samples to moisture analyzer. The moisture analyzer operates at 105℃ in order to evaporate the water inside the tofu.

2.7 Mass analysis
The mass of samples were obtained by measuring the mass of the tofu in gram. The tofu always came from same amount of soymilk and pressed with the same amount of mass. The measurements were replicated two times and the mean value was obtained.
3. RESULTS AND DISCUSSION

Coagulation is the most important step in the tofu-making process. Coagulation occurs due to the cross-linking of protein molecules in soymilk with salt’s cation. There was a preliminary experiment to determine the best salt coagulant (CaSO$_4$, MgSO$_4$, MgCl$_2$) and concentration (0.3%, 0.5%, 1.0% w/v). The result shown in Table 1.1, tofu sample from 0.5% w/v CaSO$_4$ and MgSO$_4$ produced high protein content with a value of 13.1% and 11.2% respectively. MgCl$_2$ coagulant produced the lowest protein content. Due to their high protein content, CaSO$_4$ and MgSO$_4$ were mixed with GDL as the coagulating agent for further preparation of tofu.

Table 1. Tofu protein content from preliminary experiment

| Concentration (w/v) | CaSO$_4$ | MgSO$_4$ | MgCl$_2$ |
|---------------------|----------|----------|----------|
| 0.3%                | 12.9     | 10.9     | 1.5      |
| 0.5%                | 13.1     | 11.2     | 1.5      |
| 1.0%                | 12.6     | 9.8      | 1.2      |

The variation result of protein content is due to the differences of cations and anions which affect the solubility of protein content in the soymilk. Cl$^-$ and SO$_4^{2-}$ ions have the ability to reduce the protein solubility. Based from Hofmeister order, Cl$^-$ can make the protein become more soluble (salting in) rather than SO$_4^{2-}$ [5]. The following figure shows the hofmeister order:

3.1. Composition Variation of Salt and GDL

The purpose of the main experiment was to determine the effect of variation composition in salt and GDL and coagulation temperature on the quality of the tofu produced. The tofu was analyzed based on protein content, mass, moisture and texture of the sample. The protein content was summarized in the following Fig.2

The protein content from figure 2 showed that there is a trend in protein content regarding to the salt and GDL composition; most of the data from all variation in the experiment showed the highest protein content at composition 2:2. The result of protein content with CaSO$_4$-GDL as coagulant at temperature of 70°C and 80°C are 14.1-15.4% and 13.1-13.6% respectively. While MgSO$_4$ – GDL relatively have the same protein content at temperatures of 70°C and 80°C; with a range of 14.2-15.3% and 13.1-14.5%. The difference of protein content caused by the composition
of coagulant that also manipulated the pH of soymilk. Table 1.2 showed the pH of soymilk caused by variations on the coagulant composition.

Table 1.2. pH observed with different composition of salts and GDL.

| Composition (Salt : GDL) | pH Ca 70°C-80°C | pH Mg 70°C-80°C |
|-------------------------|----------------|-----------------|
| 4 : 0                   | 5.4 - 5.5      | 5.5 - 5.6       |
| 3 : 1                   | 4.9 - 5.2      | 4.9             |
| 2 : 2                   | 4.6 - 4.7      | 4.4 - 4.5       |
| 1 : 3                   | 4.3 - 4.5      | 4.3             |
| 0 : 4                   | 4.3 - 4.4      | 4.3             |

Dzikunoo (2015) and Li (2015) stated that soy protein contains two main types of proteins; β-conglycinin (7S) and glycinen (11S) [6][7]. Li (2015) also stated that acidic coagulants coagulate high β-conglycinin (7S) and less glycinen (11S), whereas salt coagulant coagulate higher glycinen (11S) protein than β-conglycinin (7S). Proteins can coagulate themselves at their isoelectric pH (4.5-4.8). GDL is a weak organic acid, so it can reduce the pH of the solution. The highest protein is obtained when the composition of salt-GDL is 2:2. It is predicted due to the mixed salt-GDL coagulant produced solution that reached the isoelectric point. Data from salt:GDL at 3:1 and 4:0 showed the system has a pH above isoelectric point which probably does not coagulate much of the 7S, due to the bonds that occur in the 7S are hydrogen and hydrophobic bonds which does not attracted to salt’s ions yet it does attract to other protein when the system reach neutral point (isoelectric point). When the composition of CaSO₄: GDL was at 0:4 and 1:3 the system is below the isoelectric point, the system charge becomes positive and the salt coagulant does not work well when the system below the electrical point.

The experiment showed that variation in composition of coagulant also affects the mass of the tofu. The figure 3 represent that there was an increment of tofu mass along with the increment of salt composition.

![Figure 3. Mass of the tofu profile](image)

11S protein has a greater molecular weight of 350 kDa and 7S has a molecular weight smaller than 11S, which is 170 kDa [4]. Larger molecular weight is proportional to the tofu mass. As it was stated before, salt coagulants coagulate more 11S and acid coagulant coagulate more 7S protein. The result shows the tofu with high salt composition gave higher mass than tofu with high composition of GDL. The highest mass of tofu was obtained from CaSO₄:GDL at 4:0 with a value of 135 gram. The data showed corresponding result to the theory which have been stated before.

Most of the data showed an increment of hardness in tofu’s texture due to increment of salt coagulant. The following figure 4 showed tofu’s hardness data toward the salt and GDL composition.
Figure 4a showed an increment of hardness is corresponding to the salt composition. In variation of CaSO$_4$:GDL at 0:4 showed the lowest hardness (241 gram) and CaSO$_4$:GDL at 4:0 showed the highest hardness (621 gram). MgSO$_4$:GDL showed the same pattern, whereas the hardness at 0:4 was the lowest (275 gram) and at 4:0 was the highest (642 gram). Li (2015) stated tofu with high GDL levels has high water content, it is caused by the increase production of 7S protein [7]. It was suspected that the water content is increasing because 7S protein has many hydrogen bonds that can bind water molecules with it better than 11S can bind with. According to the Syah (2014) and Li (2015), 11S protein has covalent - disulfide bonds between proteins, which are stronger than proteins and water [8][7]. The moisture content from the data were not significance towards the composition variation of coagulants and temperature. According to data from fig.4b, the water content at CaSO$_4$: GDL and MgSO$_4$:GDL at 0:4 has range values of 61-66% and 55-65%.

3.2. Temperature variation of Salt and GDL

The figure 2 observed the effect of different temperature on the protein content. The data shows coagulation at 70℃ are higher than 80℃. According general factorial method (p<0.05), the deviation were significantly different to coagulant CaSO$_4$:GDL and MgSO$_4$:GDL. The protein can be denatured if the temperature of the system surpasses the temperature of denaturation. Denatured temperature for 7S protein is 70℃ and for 11S is 86℃ [9]. Therefore, the protein content in 70℃ relatively higher than 80℃.

The other quality of tofu; mass was also significantly affected by the temperature. Figure 3 shows that mass at 80℃ are relatively higher than 70℃, because the temperature affect the aggregation of protein. Tofu protein has the ability to hold water, fat, polysaccharide compounds and components from other bean milk [10]. An increase of coagulation temperature may increase the vibration energy and the rotation of dissolved proteins. These events result in the possibility of proteins colliding and aggregating faster and bind with fat, polysaccharide and other. Therefore, the weight of tofu will be increased [11]. Whereas, the variation in temperature did not affect significantly to the data of moisture content and the hardness of the tofu.

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