THE EFFECTS OF LUPIN *(Lupinus angustifolius)* AS SOYBEAN SUBSTITUTE AND COAGULANT AGENT TO PROTEIN PRECIPITATION IN TOFU PREPARATION

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ABSTRACT

Lupinus angustifolius is legume that has high protein. Most of this plant growth in Australia. Another name of L. angustifolius is Australian sweet lupin. L. angustifolius is potentially use as the source of food. Tofu is one of the traditional foods in Indonesia. Tofu is made from the coagulation of soybean protein. L. angustifolius has globulin protein that has a role in the production of tofu. The price of L. angustifolius is cheaper than soybean. In this research, L. angustifolius has been used as the substitution in the tofu production. The purpose of this research is to know the nutrition and potential of L. angustifolius as the substitute in tofu. This research is also monitoring the effect of coagulant agent that is used in tofu.

The research result shows that the optimum concentration substitution of lupin that is used in tofu is 30% with $CaCl_2$ coagulant agent. The tofu that is made from 30% lupin with $CaCl_2$ contained 17.22% protein, 5.23 fat, and 1.14% carbohydrate. The observation shows that tofu that is made from 30% substitution of L. angustifolius has higher protein than tofu which is without substitution of L. angustifolius.

Keywords: *Lupinus angustifolius, coagulant agent, tofu, protein*
References: 33 (1981-2006)

INTISARI

Lupinus angustifolius adalah kacang-kacangan yang memiliki kandungan protein yang tinggi. Tanaman ini sebagian besar ditanam di Australia. Nama lainnya adalah Lupin manis Australia. L. angustifolius berpotensi digunakan sebagai sumber makanan. Tahu adalah salah satu makanan tradisional di Indonesia. Tahu dibuat dari koagulasi protein kedelai. L. angustifolius protein globulin yang memiliki peran dalam produksi tahu. Harga L. angustifolius lebih murah daripada kedelai. Dalam penelitian ini, L. angustifolius telah digunakan sebagai substitusi dalam produksi tahu. Tujuan dari penelitian ini adalah untuk mengetahui kandungan gizi dan potensi L. angustifolius sebagai bahan substitusi dalam pembuatan tahu. Penelitian ini juga memonitor efek dari agen koagulan yang digunakan dalam tahu. Hasil penelitian menunjukkan bahwa konsentrasi optimal substitusi Lupin yang digunakan dalam tahu adalah 30% dengan $CaCl_2$ sebagai agen koagulan. Tahu yang terbuat dari 30% Lupin dengan $CaCl_2$ memiliki kandungan protein17,22%, lemak 5,23, dan karbohidrat 1,14%. Pengamatan menunjukkan bahwa tahu yang terbuat dari substitusi L. angustifolius 30% memiliki protein lebih tinggi daripada tahu yang tanpa substitusi L. angustifolius.

Kata kunci: *Lupinus angustifolius, agen koagulan, tahu, protein*

INTRODUCTION

Tofu is one of the types of foods which have a high protein and many of them consumed by Indonesian population. Besides its delicious taste, tofu can be turned into a main dish as well as its low price. Tofu is made from the precipitation of

globulin protein which can be found in the soybean (**Glycine max**).

L. angustifolius is actually legume which has a high protein. *Lupinus angustifolius* is mostly traditioned in Australia and recently Indonesia starts to plant them. There is limited usage of *L. angustifolius* although it has a huge potential to be used as food as it has a high protein.

The research focuses on the substitution of lupin *(L. angustifolius)* for the production of tofu. Lupin is used as the substitute for the making of tofu because lupin has a higher quantity of protein than that of soybean. It has no beany flavour unlike soybean and it is cheaper then the soybean. This research is also focusing on analyzing the effects of the types of coagulant agent towards the tofu that is produced. Coagulant agent takes part in the coagulation of protein. The different types of coagulant will result in different tofu as there are different characteristics from the types of coagulant that is used.

This research has two aims. They are general aim and specific aim. The general aim of this research is to be able to identify the quantity of the nutrition and the potential of the *L. angustifolius* as the substitute in the tofu production. On the other hand, the specific aim of this research is to be able to identify the optimum concentration substitution of lupin that is used in tofu and the best coagulant agent that used in that concentration in which the consumer is going to get.

MATERIALS AND METHODS

Equipments and Materials

The equipments are analytical balance, stove, National branded blender, thermometer, pan, pressure equipment, forming equipment, stopwatch, deep fryer, wash-basin, trays, 2000 mL measuring cup, spoons, pH meter, measuring cup, Kjeldahl flask, Soxhlet, porselen cup, erlenmeyer, oven, desicator, chromameter, and texture analyzer TAXT-Plus.

The materials that are soybean *(G. max)*, *L. angustifolius* (dehulled), water, Filma cooking oil, $CaCl_2$ (food grade), GDL (food grade), petroleum benzene, HCl, K_2SO_4, $CuSO_4$, H_2SO_4, H_3BO_3, and pp indicator.

Research Procedure

Concentration Substitution of Lupin

Decide percentage ratio of the concentration of soybean and lupin can produce curd, which can be done from the tofu production using different types of the concentration of the lupin substitution. The concentration of the lupin substitution which can produce curd is 30%. Therefore, the percentage ratio of soybean and lupin in the production of tofu are 0%, 10%, 20% and 30%.

Tofu Production Process

The production of tofu is done using four concentration of lupin substitution (0%, 10%, 20%, and 30%) and two types of coagulant ($CaCl_2$ and GDL). The total weight of the combination of soybean and lupin that is used is 200 gram.

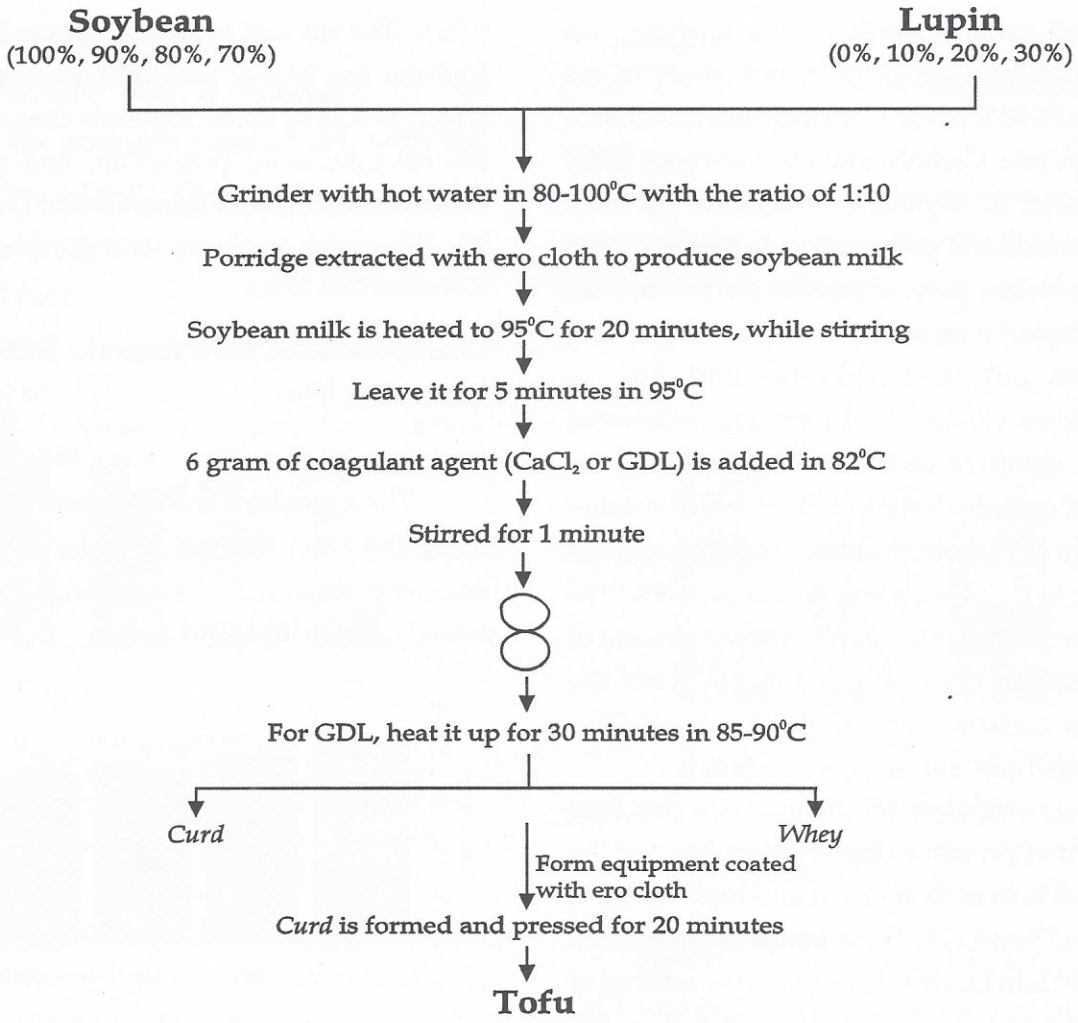

Figure 1. Steps in the production of tofu with lupin as a substitute

Analysis

Analysis that is made in the research is organoleptic analysis, texture, pH, yield, and water level. For the materials of soybean and lupin is done using proximate analysis (according to AOAC 1999). For the best tofu, proximate analysis is done, shelf life, and colour.

RESULT AND DISCUSSION

Proximate Analysis of the Raw Materials

In the research, proximate analysis is done towards raw materials (soybean and lupin) to identify the quantity of each of the raw material's nutrition. The result of the raw material's proximate analysis is shown in **Table 1.**

Table 1. Result of the Soybean and Lupin's Proximate Analysis

Component	Soybean		L. Angustifolius	
	Quantity (%)	Standard (%) according to Suprapto (1995)	Quantity (%)	Standard (%) according to Lqari et al (2002)
Water (wr basis)	9.66	7.5	9.96	-
Ash	4.54	4.8	1.18	1.5
Protein	34.43	34.9	40.27	38 - 52
Fat	16.49	18.1	7.24	5,4 - 10
Carbon	34.88	34.8	41.35	41

Based on the result of the analysis, we observe that the amount of carbohydrate in the soybean is 34.88% while *L. angustifolius* has 41.35% of carbohydrate. Carbohydrate that are both in the soybean and *L. angustifolius* consists of the oligosaccharide and polysaccharide that is hard to be digested. The polysaccharide in the soybean will not dissolve in water and alcohol *(Koswara 1992)*. Few polysaccharide (around 1%) in *L. angustifolius* will dissolve in water *(Caballero et al, 2003)*. The result of the analysis shows that the amount of carbohydrate in the soybean matches the amount of carbohydrate that is in the soybean according to *(Suprapto, 1995)*, which is 34.9%. The result of the analysis also shows that the amount of carbohydrate in the *L. angustifolius* matches the amount of carbohydrate in the *L. angustifolius* according to *(Lqari et al, 2002)*, which is 41%.

The amount of protein in lupin is higher than the amount of protein in the soybean. Most of the protein that is in both soybean and lupin consists of globulin 7S and 11S. The amount of globulin 7S and 11S that is in Lupin is lower than the amount of globulin 7S and 11S in the soy bean *(Caballero et al, 2003)*. The result of the analysis shows that the amount of protein in *L. angustifolius* matches the amount of protein in *L. angustifolius* according to (Lqari et al, 2002), which is 38 - 52%. The result of the analysis also shows that the amount of protein in soybean is lower compared to *(Suprapto, 1995)*, which is 34.9%.

Soybean has higher amount of fats compared to lupin. Fats in soybean consist of saturated fatty acid and unsaturated fatty acid. The result of the analysis shows that the amount of fats in soybean is lower compared to *(Suprapto, 1995)*, which is 18.1%, while the amount of fats in *L. angustifolius* matches the ones according to *(Lqari et al, 2002)*, which is 5.4 - 10%.

The result of the amount of water in soybean is 9.66%, while lupin has higher amount, which is 9.96%. The amount of ash in lupin is 1.18%, while soybean has higher amount compared to lupin, which is 4.54%. Some minerals that soybean has are calcium, iron, potassium, and phosphorus *(Matthews, 1989)*. Some minerals that *L. angustifolius* has are calcium, iron, manganese, and zinc *(Caballero et al, 2003)*.

Characteristic of Tofu from the Substitution of *L. angustifolius*

Water Level

The water level measurement of tofu is done using the oven method in order to identify the amount of water that remains inside curd tofu. The result is shown in **Figure 1.**

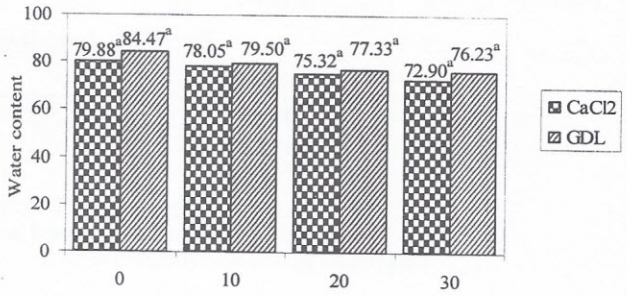

Figure 1. Effects of the Water Level Experiment
Notes: superscript with the same letters shows no real difference to $\alpha = 0.05$

In **Figure 1** can be shown that the water level of tofu without lupin substitution has no real difference with lupin substitution of 10%, 20% and 30%. Tofu that is produced with coagulant $CaCl_2$ has water level which has no real difference with GDL. Based on the statistic analysis, the factor that affects the significance towards the water level of tofu is the concentration of lupin substitution. Water level of tofu is influenced by globulin 11s that acts in the stability of three dimension network forming the structure of gel. The raise of the concentration of lupin substitution affects the ratio of globulin 7S and 11S which has different gel characteristic *(Mori et al, 1986)*.

Texture (Gel Strength)

The texture that is analyzed in the research is the strength level of gel, with the measurement g/cm^2. Texture measurement (gel strength) of tofu is done using TAXT texture analyzer.

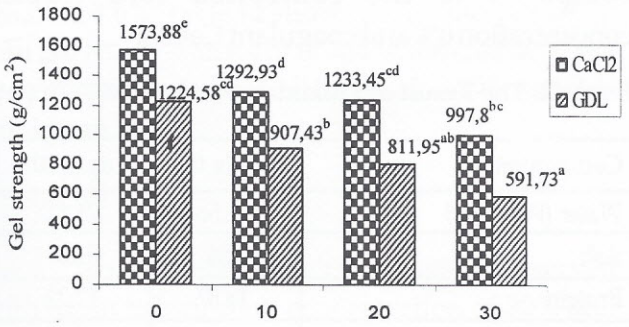

Figure 2. Effects of Different Kinds of Treatment towards Gel Strength
Notes: superscript with the same letters shows no real difference to α= 0.05

Figure 2. shows that the gel strength of tofu without lupin substitution with coagulant $CaCl_2$ has no real difference with the concentration of lupin substitution of 10%, 20%, and 30%. Gel strength of tofu without lupin substitution with coagulant GDL is also different with the concentration of lupin substitution of 10%, 20%, and 30%.

Based on the statistic analysis, the factor that affects the significance towards tofu texture is the concentration of lupin substitution and coagulant type. The concentration of lupin substitution has significant effects towards gel strength of tofu because of the difference globulin 7S and 11S in soybean and lupin. Types of coagulant have significant effects towards gel strength of tofu because there is a different working mechanism of that particular coagulant. $CaCl_2$ can produce a strong texture (gel strength increases) because of the existence of Ca^{2+} ion, Ca^{2+} ion will form ionic bonding with protein molecule *(Kinsella, 1979)*.

Yield

In the research, measurement towards the weight of tofu is done to identify the yield that is produced from tofu with lupin substitution. The yield of Tofu can be seen in *Figure 3.*

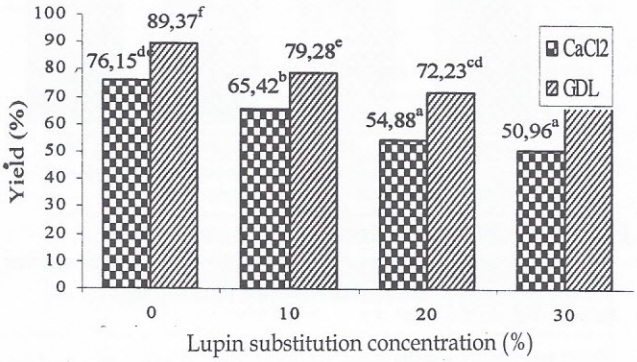

Figure 3. Effects of Types of Treatment towards Yield
Notes: superscript with the same letters shows no real difference to α= 0.05

Figure 3. shows tofu's yield with coagulant $CaCl_2$ and lupin substitution concentration is 0%, which is different with 10%, 20%, and 30% lupin substitution. Tofu with lupin substitution concentration 10% and coagulant GDL is different with 20% and 30% of lupin. Based on the statistic analysis, the concentration of lupin substitution and the types of coagulant has significant effects towards tofu's yield.

Tofu that is done from soy bean has high yield because soy bean contains globulin 7S and 11S which is higher compared to lupin. Tofu with coagulant GDL produce higher yield compared to $CaCl_2$. The production of tofu with coagulant GDL is heated for 30 minutes to transform GDL to gluconate acid. Heating treatmen will also cause precipitation of protein increases until the tofu's yield that is produced increases as well *(Shurtleff and Aoyagi, 1984)*.

pH

Measurement of pH is done towards curd tofu with the aim to identify the level of acidity of tofu. The result of tofu from pH can be seen in **Figure 4.**

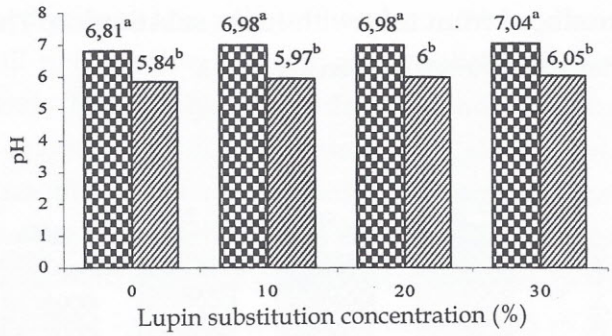

Figure 4. Effects of Treatment towards pH
Notes: superscript with the same letters shows no real difference to $\alpha = 0.05$

Figure 4. shows that tofu with coagulant $CaCl_2$ has pH 6 - 7, while tofu with coagulant GDL has pH 5 - 6. Based on the statistic analysis, the factor that significantly affects pH of tofu is the types of coagulant. The different working mechanism of coagulant causes different outcome of pH of tofu. Tofu that is produced using coagulant GDL has the pH lower compared to the tofu produced using coagulant $CaCl_2$. This is caused by GDL mechanism that produces gluconate acid that is able to make the pH of soybean milk lower ***(Shurtleff and Aoyagi, 1984).***

Choosing the Best Formulation of Tofu

Choosing the best tofu's formulation is done by analyzing every parameter, which are water level, yield, pH, texture, and organoleptic. The result of the organoleptic analysis is made priority in choosing the best tofu's formulation because the aim is really specific, which is to identify the level of how the consumen accept tofu with lupin substitution. Based on the organoleptic analysis, tofu with the best formulation is 30% lupin with coagulant $CaCl_2$.

The Result of the Tofu Proximate Analysis

Tofu with the lupin substitution concentration of 30% and coagulant $CaCl_2$ goes through proximate analysis to identify the amount of protein, fats, carbohydrate, water, and ash. The result of the proximate analysis of tofu with lupin concentration 30% and coagulant $CaCl_2$ is compared to the controlled tofu (lupin concentration 0% and coagulant $CaCl_2$).

Table 2. The Result of Proximate Analysis of Tofu (%)

Component	Lupin 0%	Lupin 30%
Water *(Wet basis)*	76.56	75.61
Ash	0.84	0.80
Protein	13.87	17.22
Fat	6.54	5.23
Carbohydrate *(by difference)*	2.19	1.14

The water level of tofu with lupin substitution concentration of 30% is lower compared with the lupin substitution concentration of 0%. This is because soy bean has globulin 11S more than lupin, so that it can retain more water). ***(Shurtleff and Aoyagi, 1984)***

The amount of protein in tofu with lupin substitution concentration of 30% is higher compared with tofu, which is without lupin as a substitute. This is because lupin has more protein than soybean, which is 38 - 52% ***(Lqari et al, 2002).*** With the existence of lupin substitution in the production of tofu, the amount of protein in tofu increases. The amount of protein with lupin substitution concentration of 30% in the requirement based on SNI 01 - 3142 - 1998 is minimum 9%.

The amount of fats with lupin substitution concentration of 30% is lower compared to tofu, which is without lupin substitution (soy bean 100%). This is because lupin has less fat than soybean, which is 5.4 - 10% ***(Lqari et al, 2002).*** The amount of fats in tofu with lupin substitution concentration of 30% in the requirement based on

The amount of carbohydrate with lupin substitution concentration of 30% is lower compared to tofu, which is without lupin substitution. Tofu is a food product with high protein and has low amount of carbohydrate. The amount of carbohydrate with lupin substitution concentration of 30% is lower compared with the amount of carbohydrate according to Health and Nutrition Department Republic Indonesia (1981), which is 1.6%. **Table 3.2** shows the amount of ash in tofu is 0.8%. The amount of ash in tofu matches the requirement based on SNI 01 - 3142 - 1998, which is minimum 1%.

Shelf Life

In the research, shelf life of tofu is observed with lupin substitution concentration of 30% and is compared with controlled (tofu without lupin substitution). Tofu is kept at room temperature and refrigerator temperature.

Table 3. Result of Tofu Observation at Room Temperature

Days	Lupin Substitution Concentration (%)	Color	Aroma	Texture
0	0	normal white	normal tofu	solid
	30	normal white	normal tofu	solid
1	0	yellowish white	acid	brittle
	30	yellowish white	acid	brittle, less of mucus
2	0	yellowish white	acid	brittle, less of mucus
	30	yellowish white	acid	brittle, less of mucus

Tofu that is kept at room temperature shows colour changes, aroma, and texture after keeping it for one day. Changes that take place are caused by proteolitic bacteria that break protein to form amino acid. Some examples of proteolitic bacteria are *Pseudomonas, Bacillus,* and *Clostridium* **(*Fardiaz, 1992*)**. Besides that, the existence of bacteria that forming mucus can cause mucus to form in tofu. Some bacteria that are often found in tofu are *Alcaligenes, E. coli, Lactobacillus, Streptococcrus,* and *coliform* **(*Fardiaz, 1992*)**.

Table 4. Result of Tofu Observation at Refrigerator Temperature

Days	Lupin Substitution Concentration (%)	Colour	Aroma	Texture
0	0	normal white	normal tofu	solid, no mucus
	30	normal white	normal tofu	solid, no mucus
1	0	normal white	normal tofu	solid, no mucus
	30	normal white	normal tofu	solid, no mucus
2	0	normal white	normal tofu	solid, no mucus
	30	normal white	normal tofu	solid, no mucus
3	0	normal white	nofmal tofu	solid, no mucus
	30	normal white	normal tofu	solid, no mucus
4	0	normal white	normal tofu	solid, no mucus
	30	normal white	normal tofu	solid, no mucus
5	0	normal white	normal tofu	solid, no mucus
	30	yellowish white	acid rather	brittle, less of mucus
6	0	yellowish white rather	acid rather	brittle, less of mucus
	30	yellowish white	acid	brittle, less of mucus
7	0	yellowish white	acid	brittle, less of mucus
	30	yellowish white	very acid	brittle, lot of mucus

Table 4. shows that tofu with lupin substitution concentration of 30% that is kept at refrigerator temperature (100°C) will start to have a sign of damaged in day 5, while controlled tofu starts to have a sign of damaged in day 6. Change of colour and aroma is caused by proteolitic bacteria (breaks protein to form amino acid). Tofu texture that is has mucus is caused by the activity of bacteria that forming mucus. Mucus that is formed is caused by the metabolism of that particular bacteria *(Fardiaz, 1992)*.

Tofu that is kept at room temperature will start to have a sign of damaged after keeping it for one day, while tofu that is kept at refrigerator temperature will last for 5 days before there is a sign of damaged. Refrigerator temperature is the suitable temperature to keep tofu because that temperature will prevent the bacteria to grow in the tofu. Tofu is still safe to be consumed if the bacteria that are inside it do not exceed the limit according to SNI.

Colour

In the research, the tofu colour with lupin substitution concentration of 30% compared with controlled tofu colour (without lupin substitution). Colour measurement is done using chromameter. The principle of chromameter is based on the reflection of colour that is produced by the sample.

The sample will be placed in the petri dish. Value L, a, and b will be discussed in every sample. Value L will show lightness. Value +a indicates the direction of red colour. Value -a indicates the direction of green colour. Value +b indicates the direction of yellow colour. Value -b indicates the direction of blue colour. The result of the tofu colour analysis is shown in **Table 5.**

Table 5. Tofu Colour Comparison

Component	Lupin Substitution Concentration (%)	
	0	30
L*	78.91	74.49
a*	-0.92	-0.25
b*	11.44	15.98

In **Table 5**, it can be shown that lightness value (L) of tofu with lupin substitution concentration of 30% is lower compared to tofu without lupin substitution. Based on the value a and b, tofu with lupin substitution concentration of 30% has the colour of more yellowish compared to tofu without lupin substitution. This is caused by the amount of lupin that has more yellowish colour than soybean.

CONCLUSIONS AND SUGGESTION

The statistic analysis shows that lupin substitution concentration significantly affects the water level of tofu. Tofu with lupin substitution concentration of 0% has higher water level, while tofu with lupin substitution concentration of 30% has lower water level.

The texture (gel strength) of tofu depends on the types of coagulant and lupin substitution concentration. Tofu with lupin substitution concentration of 30% has the lowest texture value. Tofu with coagulant $CaCl_2$ has higher texture value compared to GDL.

Types of coagulant, lupin substitution concentration, and interaction between coagulant and lupin substitution concentration significantly affects tofu's yield. Tofu with coagulant GDL has higher yield than $CaCl_2$. Tofu with lupin substitution concentration of 30% has the lowest yield.

Types of coagulant have significant effect toward tofu pH. Tofu with coagulant GDL has lower pH compared to $CaCl_2$. Tofu with coagulant $CaCl_2$ has pH value of 6 - 7, while tofu with coagulant GDL has pH value of 5 - 6.

Based on the organoleptic analysis, panelist prefers tofu with lupin substitution concentration of 30% with coagulant $CaCl_2$. Tofu with lupin substitution can be accepted in organoleptic way with good level of acceptance. Tofu with lupin substitution concentration of 30% has higher amount of protein compared to tofu without lupin substitution.

Based on the research, *L. angustifolius* is legume that has high protein and good potential to be made food product which is useful. Therefore, it is recommended to do analysis to make other types of food products using *L. angustifolius as* the material that is useful for our body.