# EVALUATION OF LABLAB BEAN (*Lablab purpureus* (L.) *Sweet*) SPROUT MILK FORTIFICATED WITH EGGSHELL EXTRACTED CALCIUM

**David Tjandra Nugraha**
Faculty of Agricultural Technology, Widya Mandala Catholic University Surabaya
60265, Dinoyo Street 42-44, Surabaya, Indonesia
Email : dtdavidtjandra@gmail.com

**ABSTRACT**

*Lablab purpureus* (L.) *Sweet* is a common bean in Asia. High protein content and similar amino acid composition with soybean makes good substitutes against dependency on import products. One example of bean product is vegetable milk. Fortification is executed to create product, which compels our diet. Many European undergo low protein and mineral intake from food. That is the reason of fortification utilizing food waste, namely eggshell waste. Milk created using beans is processed immediately. However, physiological process (germination) is capable of increasing its nutrition quality. This research focuses on variation of germination time: 0, 12, 24, 36, and 48 hours. Protein digestibility is selected as the main parameter to consider the time. Protein, water, and mineral content, pH, and total soluble solid content of the milk is analyzed. Germination time of 36 hours establishes sprout with digestible protein of 13.36±0.59 g/100g, protein content of 7.21 g/100g, pH of 6.74±0.17, and total soluble solid content of 19.0. The addition of eggshell extracted calcium as calcium fortification is 2% w/v, which resulted in mineral content of 276±0.13 mg/100g and water content of 80.87%.

**Key Words**: Vegetable milk, fortification, germination

---

***Introduction***

Lablab bean (*Lablab purpureus* (L.) *Sweet*) is a kind of legumes that is commonly found in Asia and possesses complete nutritional value (Somaatmadja, 1993). The aged seed from this bean is often dried and consumed (Salunke, 1985). Lablab bean has a high productivity rate with 6-10 ton per hectare yield, far more superior than the likes of soybean, which only yields 1,3 ton per hectare (Purseglove, 1981). Lablab bean contains high amount of carbohydrate, fiber, fat, amino acids, and trace minerals. The protein content of this bean is high (20-28%) and one of the best source for iron mineral (155 mg/ 100 g bean) (Kay, 1979). The amount of protein in this bean does not differ too much with other high-protein beans such as soybean. Lablab bean has very similar amino acid composition with soybean, poor in sulfur containing essential amino acid (methionine and cysteine), but rich in lysine (Yulia, 2007).

**Table 1.** Chemical Composition of 100 gr Lablab Bean

| Component | Amount |
|---|---|
| Protein (g) | 21.5 |
| Fat (g) | 1.2 |
| Carbohydrate (g) | 61.4 |
| Fiber (g) | 6.8 |
| Ca (mg) | 98 |
| Fe (mg) | 149 |

Source: Duke (1983)

Germination of beans are a physicochemical process due to metabolism processes (Winarno, 1980). When water penetrates the seed, respiration, protein synthesis, and other metabolic process will be initiated. After a certain period, the embryo will emerge from the seed, signed with the appearance of radicle or hypocotyl in the surface of the seed. The sprout of the seed will emerge because of the elongation of hypocotyl as it pushes cotyledon to the surface (Bewley, 1983). During the germination process, the starch content is converted into smaller parts of glucose and maltose. Glucose and fructose content increases by tenfold and sucrose by twofold. Protein molecule will break down into amino acids, which are lysine (24%), threonine (19%), and phenylalanine (7%). The larger fat molecules will also be hydrolyzed into shorter fatty acids, which are easily digestible. Some minerals such as Ca and Fe that was in the bounded state will be released increasing the bioavailability of the mineral (Winarno, 1980). Overall germination of lablab bean will significantly increases the value of protein due to activation of hydrolytic enzyme. Germination will also reduce the complex carbohydrate content and inactivate trypsin inhibitor enzyme, resulting in better digestibility of nutrient (Osman, 2007).

Milk is an oil in water emulsion that includes soluble content. Water content in the milk is very high (87,5%) with specific milk sugar (lactose) of 5%, protein of 3,5%, and fat around 3-5%. Milk is a very good source of calcium, phosphor, and vitamin A (Wardlaw, 2009). Milk from animals contains lactose that cause allergic reactions to people with lactose intolerance condition. As an alternative, vegetable milk is created by extracting the milk from vegetables products, mixing it with water and continuing with filtration and pasteurization. Commonly used materials are legumes due to high protein content and similar amino acid composition as the animal milk; making it a good substitute for some people (Astawan, 2004).

Eggshell is a common food waste that is produced by poultry, household, and industry (King, 2011). Eggshell contains 98,2% calcium carbonate and trace components such as boron, copper, iron, manganese, molybdenum, sulfur, silicon, and zinc (Romanoff *et. al.*, 1949). Calcium carbonate from the eggshell is a great natural source for calcium and far better than the limestone extracted calcium. One eggshell from medium-sized egg can be converted into one teaspoon of eggshell powder that contains 750-800 mg of calcium. The calcium composition of eggshell is very similar to those in the human body such as in teeth and bones (Bee, 2011). The requirements for this mineral for 4-9 year old is 1.000 mg/day, and 1.200 mg/day for those with the age of 10-18. This amount is less for people older than 30, which is 1.000 mg/day. Consumption of eggshell calcium can be one of the alternative solution for calcium deficiency.

$$2HCl_{(aq)} + CaCO_{3(s)} \rightarrow CaCl_{2(aq)} + CO_{2(g)} + H_2O_{(l)}$$

**Figure 1.** Eggshell Calcium Chloride Production
Source : Garnjanagoonchorn (2007)

The main problem of eggshell based calcium is that calcium carbonate is hard to absorb due to its low solubility. The extraction of calcium will utilize the reaction between salt and acid (Garnjanaggonchorn, 2007).

***Methods***

Utensils that were used are digital weigh terminal (Mettler Toledo PG603-6), plastic bowls, plates, spoon, kitchen stove (Rinnai RI-522C), blender (Phillips HR2106), filter, wooden stirrer, and oven (Nayati). Analysis utensils that were used are analytic weigh terminal (Mettler Toledo AT200M), beaker glass (Pyrex), measurement flask (Iwaki), glass burrete, Erlenmeyer flask (Iwaki), hand refractometer (ATC), and pH meter (Extech). The materials that are used were lablab bean from the local Surabaya Market, sugar (Gulaku), UHT milk (Ultramilk, Ultrajaya Brand), and aquadest. The materials that were used for analysis were EDTA 0,05M, Salmiac Buffer (pH 10,0), EBT (1:200), concentrate $H_2SO_4$, NaOH 10N, Zn powder, $H_2C_2O_4$ 0,1N (PA), NaOH 0,1 N, HCl 0,1N, kjeldahl tablet, MR:MB (1:2) indicator, and phenolphthalein indicator. The research was done in the laboratory of food processing and laboratory of chemistry, Widya Mandala Catholic University Surabaya.

The experiment design was created to find the best germination time in order to create the optimal product. The variable that is chosen is protein digestibility. Random experiment design is used with one factor (germination time) and five repetitions. Five germination times were used (0, 12, 24, 36, 48 hours). The data from the experiment are tabulated and analysed with ANAVA at $\alpha$=5% and DMRT (Duncan Multiple Range Test) with the same confidence level. Extraction of calcium was done by reacting the calcium carbonate with HCl 2,5% (1:25 w/v) and stirred occasionally to reduce the production of foam. The solution is heated at 100-

115°C until it is dried and the resulting product will be the calcium chloride powder (Garnjanagoonchorn, 2007).

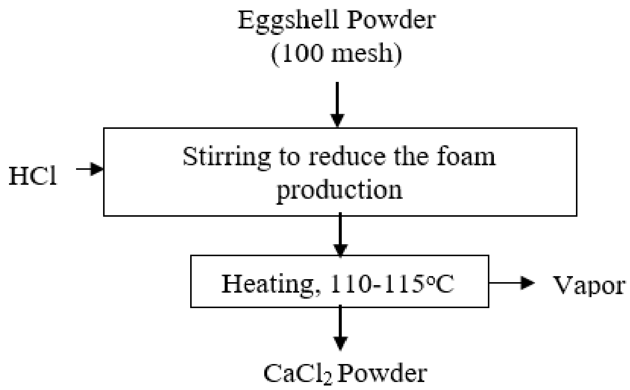


**Figure 2.** Flow Diagram of Calcium Chloride Powder
Source : Garnjanagoonchorn (2007)

The production of vegetable milk started with the pretreatment of the lablab bean sprout. The sprouts were taken manually and the remaining bean are soaked in $NaHCO_3$ 1% solution with the ratio of solution : bean = 4:1. The soaking was done around 15 minutes in order to remove cyanide content of the bean. The extraction was done with the blender by the help of water 4 times of the bean's weigh. This process was done to get the filtrate of the bean. Mixing of 10% w/v sugar and $CaCl_2$ powder followed by heating at 80-90°C for 15 minutes was done to get the final product.

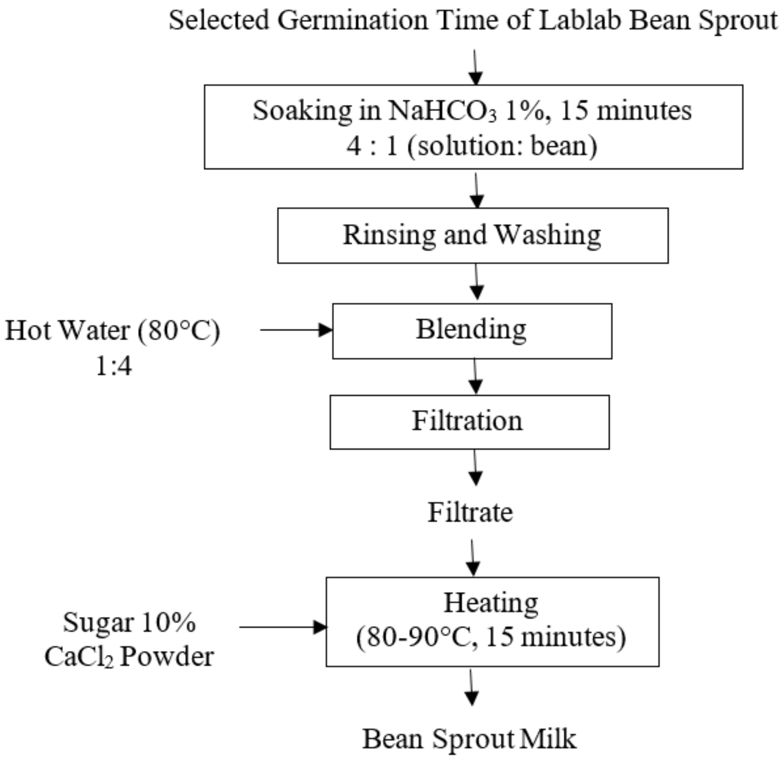


**Figure 3.** Flow Diagram of Lablab Bean Sprout Milk Production

The proximate analysis method for the protein digestibility used the kjeldahl principle (AOAC, 2005). This is also the same method that was used to calculate the total protein content. Protein digestibility was measured by putting 1 gram of sprouted bean inside aquadest with 1:5 ratio w/v, and the solution was analyzed of protein content. Mineral content determination was done with titrimetric method of complexometry. Bivalent ion can be determined with the reaction with Ethylene Diamine Tetra Acetate (EDTA). The reaction was done at pH 10-11 for better selectivity of Ca content. The reaction was done with Eriochrome Black T. (EBT 1:200) indicator by titrating 10 mL of sample with EDTA (Basset, 1994). Water content was done with thermogravimetric method based on AOAC (2005). pH value of samples were measured with pH meter that was standardized with buffer of 4,0 pH and 10,0 pH. Total soluble solid content was measured with hand refractometer and the unit of measurement is % brix.

***Results***

**Table 2.** Soluble Protein of Lablab Bean Sprout with Various Germination Time

| Germination Time (hours) | Protein Content (g/100 g) |
|---|---|
| 0 | $10.09 \pm 0.62^{a}$ |
| 12 | $12.45 \pm 0.45^{b}$ |
| 24 | $12.49 \pm 0.51^{b}$ |
| 36 | $13.36 \pm 0.59^{c}$ |
| 48 | $13.39 \pm 0.66^{c}$ |

Notes: The value with the same notation does not differ in DMRT ($p \leq 0.05$)

**Table 3.** Protein Content of Lablab Bean Milk and Lablab Bean Sprout Milk

| Germination Time (hours) | Protein Content (g/100g) |
|---|---|
| 0 | 6.49 |
| 36 | 7.21 |

**Table 4.** Calcium Content of Various Kinds of Milk

| Variety | Calcium (mg/100g) |
|---|---|
| UHT | $299 \pm 0,46^{a}$ |
| Lablab Bean Sprout Milk + $CaCl_2$ | $276 \pm 0,13^{a}$ |
| Lablab Bean Sprout Milk | $85 \pm 0,17^{b}$ |
| Soymilk | $77 \pm 0,18^{b}$ |

Notes: The value with the same notation does not differ in DMRT ($p \leq 0.05$)

**Table 5.** Water Content of Lablab Bean Sprout Milk

| Sample | Initial Weight (g) | Final Weight (g) | Water Content (%) |
|---|---|---|---|
| Before Ca addition | 1.0312 | 0.1753 | 83.01 |
| After Ca addition | 1.0621 | 0.2031 | 80.87 |

**Table 6.** pH Value of Various Kind of Milk

| Variety | pH |
|---|---|
| UHT | 6.43±0.10[a] |
| Lablab Bean Sprout Milk + $CaCl_2$ | 6.69±0.13[a] |
| Lablab Bean Sprout Milk | 6.74±0.17[a] |
| Soymilk | 6.77±0.18[a] |

Notes: The value with the same notation does not differ in DMRT ($p \leq 0.05$)

**Table 7.** Total Soluble Solid Content of Various Kind of Milk

| Variety | % brix |
|---|---|
| UHT | 12.5[a] |
| Lablab Bean Sprout Milk + $CaCl_2$ | 12.5[a] |
| Lablab Bean Sprout Milk | 19.0[b] |
| Soymilk | 18.8[b] |

Notes: The value with the same notation does not differ in DMRT ($p \leq 0.05$)

***Discussion***

Protein digestibility indicates the amount of soluble protein inside the material. High amount of easily soluble protein is favorable. Germination process breaks down larger protein molecules and increasing its digestibility. From the experiment result, there is a definite increase in protein digestibility during germination process. The germination time of 36 hours was selected because it does not differ with the longer germination time (48 hours) but will be more optimal in terms of time.

Protein content of the milk is then analyzed by comparing the protein content from normal vegetable lablab bean milk and lablab bean sprout milk with 36 hours of germination time. It can be seen that germination process still exhibits increase in the protein content due to protein synthesis of germination. The protein content is lower than the raw bean sprout because the milk undergo heating process (pasteurization) and some protein was denatured.

Calcium content of lablab bean sprout milk was compared to other variants of milk, namely the UHT Milk, lablab bean sprout milk with the addition of $CaCl_2$, lablab bean sprout milk without the addition of $CaCl_2$, and soymilk. It can be seen from the experiment that the addition of $CaCl_2$ by 2% w/v is optimal to get the similar calcium content as the more familiar high-calcium containing UHT milk.

Water content was analyzed because of the addition of salt ($CaCl_2$). Due to the sample being liquid, the thermogravimetry method utilizes vacuum oven. There are many short chain fatty acid (SCFA), which are volatile and might hinder the water content analysis. Water content of milk is normally read at 80-90% (Santoso, 2009). Addition of calcium powder did not change the properties of the milk to undesirable level.

pH level was measured because of the high phenol, flavonoid, and alkaloid content of the lablab bean. Lablab bean indicates more alkaline pH than the other kind of milks. The addition of $CaCl_2$ powder did not change the pH drastically because $CaCl_2$ is a salt derives from strong acid and strong base and possess neutral pH.

Total soluble solid content was analyzed in order to compare the soluble materials in the product. Sugar content of commercial soymilk is around 10-15%, the same proportion used for the production of lablab bean. The UHT milk does not add any sugar, but they contain the milk specific sugar (lactose)

### *Conclusion*

Lablab bean is a prominent bean commonly found in Asia and can be utilized as a value added product. Germination of lablab bean was able to increase the overall nutritional quality of the bean. The germination time of 36 hours was chosen and produce the most optimal product time-wise with the protein content of 13.36±0.59 g/100g. Lablab bean sprout milk germinated for 36 hours contains 7.21 g/100g protein, 19.0 % brix total soluble solid content, and pH value of 6.74±0.17. Lablab bean sprout milk does not contain many calcium, so it was Fortificated with eggshell extracted calcium in the form of calcium chloride powder. The powder was added 2% (w/v) and yields the calcium content rivaling commercial milk 276 ± 0,13 mg/100g, and water content of 80,87%.

### *Recommendations*

The condition for germination such as gas, light, temperature, and other external-internal factors might affect the germination process and the product it yields. Further study is needed to analyze these effects.

### *References*

**AOAC**. (2005) Determination of Moisture, Ash, Protein and Fat. Official Method of Analysis of the Association of Analytical Chemists. 18th Edition, AOAC, Washington DC.

**Astawan, M.** (2004) *Tetap Sehat dengan Produk Makanan Olahan*. Solo: PT. Tiga Serangkai Pustaka Mandiri.

**Basset, J.** (1994) Buku Ajar Vogel:Kimia Analisis Kuantitatif Anorganik. Translation A. Hadyana Pudjaatmaka and L. Setiono. Penerbit Buku Kedokteran EGC. Jakarta.

**Bee, W**. (2011) How to Make Calcium from Egg Shells. www. healingnaturallybybee.com (3rd of March, 2017).

**Duke, J. A.** (1983) Handbook of Legumes of World Economic Importance. Plenum Press, New York.

**Garnjanagoonchorn, W.** (2007) Preparation and Partial Characterization of Eggshell Calcium Chloride. International Journal of Food Properties, 10, 497-503.

**Kay, E. K** . (1979) Food Legumes. London: Tropical Products Institute.

**King, A. M**. (2011) A Review of the Uses of Poultry Eggshells and Shell Membranes. International Journal of Poultry Science, 10 (11), 908-912.

**Osman,M.A**. (2007) Changes in Nutrient Composition, Trypsin Inhibitor, Phytate, Tannins, and Protein Digestibility of Dolichos Lablab Seeds (Lablab purpureus (L.) Sweet) Occuring During Germination. Journal of Food Technology, Vol 5: 294-299.

**Purseglove, J. W., E. G. Brown, C. L. Green, S. R. J. Robins.** (1981) Spices Vol. 1. New York: Longman Inc.

**Romanoff, A. L., dan A. J. Romanoff**. (1949) The Avian Egg. New York: John Wiley & sons, Inc.

**Santoso**. (2009) Susu dan Yoghurt Kedelai. Malang: Laboratorium Kimia Pangan Fakultas Pertanian Universitas Widyagama.

**Salunke, D. K., S. S. Kadam and J. K. Chafan.** (1985) Postharvest Biotechnology of Food Legumes. Florida: CRC Press, Boca Raton.

**Winarno, F.G.** (1993) Pangan Gizi Teknologi dan Konsumen. Jakarta: Gramedia Pustaka Utama.

**Yulia, O.** (2007) Pengujian Kapastias Antioksidan Ekstrak Polar, Non Polar, Fraksi Protein dan Non Protein Kacang Komak (Lablab purpureus (L.) sweet). Bogor: Institut Pertanian Bogor, Indonesia.

***Appendix***

Production Documentation of Lablab Bean Sprout Milk