2025年臺灣國際科學展覽會 優勝作品專輯

作品編號 080015

參展科別 生物化學

作品名稱 Exploring the Potential of Pachyrizus

Erosus-Derived Calcium as an Affordable

Nutritional Solution for Lactose

Intolerance

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關鍵詞 <u>Pachyrhizus erosus, plant-based milk, calcium, lactose intolerance</u>

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Exploring the Potential of Pachyrhizus Erosus-Derived Calcium as an Affordable Nutritional Solution for Lactose Intolerance

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ABSTRACT

An exploration of Pachyrhizus erosus as an option for plant-based milk in the Indonesian market for people with lactose intolerance. With its prevalence in tropical climates, Pachyrhizus erosus is an affordable crop in Indonesia. Pachyrhizus erosus is a root vegetable containing a calcium content of 15.6 mg per cup (130q) in its unprocessed form, concentrated in its white flesh. Based on its affordability and calcium content, Pachyrhizus erosus can be transformed into a beverage product with nutritional qualities on par with existing plant-based milk, therefore being a solution for calcium sustenance that is more accessible due to its high capability to be locally grown in Indonesia. However, this may be a partial case as Pachyrhizus erosus only thrives in regions with long warm seasons. In this research, three trials of Pachyrhizus erosus-based milk recipe have been conducted Trial 1 consists of a 1:1 ratio of Pachyrhizus erosus to water, Trial 2 consists of a 2:1 ratio of Pachyrhizus erosus to water, and Trial 3 consisting of a 10:10:1 ratio of Pachyrhizus erosus to water, and to a small amount of soybean. Based on the results of 14 organoleptic test respondents, it is concluded that the best ratio of ingredients is 10:10:1 (water: Pachyrhizus erosus: soybean) due to an overall preference of the third trial with this ratio, in terms of taste, aroma, color, and consistency. Pachyrhizus erosus is the dominant ingredient in developing alternate plant-based milk. However, findings from the most preferred trial in the organoleptic test suggest that implementing a minor amount of soybean would stabilize the milk-like consistency and flavor. Pachyrhizus erosus' ability to retain calcium in its water content has been additionally proven in a calcium test using a reagent solution of ammonium oxalate, as even with the trials' step of straining the liquid content of Pachyrhizus erosus that had been blended with added water, all three trials tested positive based on the high level of the precipitate. Other tests that tackle the quality of each trial include In Silico Testing, biuret protein test, alcohol test, COB test, and pH level testing.

Keywords: Pachyrhizus erosus, plant-based milk, calcium, lactose intolerance

1. BACKGROUND

Indonesian community-dwelling insufficient older individuals consume energy and nutrients, and their milk consumption is notably the lowest globally, as $\frac{2}{3}$ of the elderly population in Indonesia are lactose intolerant. One of the reasons for low milk consumption is due to lactose intolerance. Lactose intolerance condition in which a person is unable to digest or absorb lactose, where this condition is also known the as

pathophysiology of lactase resistance or damage to the epithelial cell layer of the digestive tract. More than 70% of people worldwide are revealed to be lactose intolerant to some level. Numerous attempts have been made to create lactose-free food items that are nutritionally enhanced. One of them is lactose-free milk, which benefits those who are unable to digest lactose and lets them enjoy milk's flavor without experiencing the uncomfortable digestive side effects of lactose consumption.

Lactose intolerance is prevalent in Indonesia in children aged 3-5 years at 21.3%, children aged 6-11 years at 57.8%, and children aged 12-14 years at 73%. Lactose malabsorption is also high in children who drink milk regularly and irregularly, at 56.2% and 52.1% respectively (Hegar and Widodo A, 2015). Around 3.6 million people, or 19.7% of parents in Indonesia, suffer from lactose intolerance and avoid animal products. However, little is known about the prevalence of this issue and how it impacts nutritional intake (e.g. levels of calcium in the body). The lack of information and access among Indonesian consumers regarding lactose-free milk milk) emphasizes (plant-based how essential it is to increase public awareness understanding of this beneficial substitute/alternative for those who are lactose intolerant to make sensible and wise choices for their dietary requirements.

To people's fulfillment, the essential proteins and nutrients included in lactose (regular) milk, such as calcium, vitamin A, vitamin D, and so on, may also be efficiently replaced by plant-based milk (e.g. oat milk, almond milk, etc). As worldwide demand and awareness for plant-based goods grow (Suhartanto et al., 2022), the plant-based milk market is expected to expand, assisted by the development of the vegan lifestyle. Based on a 2018 Euromonitor International poll, Indonesia currently ranks 3rd in terms of vegetarianism growth (Dewi, Kurniati, & Dalilah, 2022).

Pachyrhizus erosus L, moreover known as Pachyrhizus erosus or Mexican yam bean (Pachyrhizus erosus), could be a root vegetable local to Mexico and widely developed in Southeast Asia. It features a circular, tuberous root with pale brown skin and fresh white tissue that's gently sweet and refreshing, comparable in surface to a

water chestnut. Low in calories and tall in fiber, Pachyrhizus erosus L may be a nutritious alternative, also rich in vitamin C, potassium, and antioxidants. It is commonly eaten raw in salads, such as the Indonesian dish rujak, or included in stir-fries and spring rolls for its crunchy surface. Its tall water substance makes it а hydrating nourishment, beneficial for absorption and general wellbeing. Flourishing in tropical climates, Pachyrhizus erosus L requires well-drained soils to develop. Whereas the root is consumable, the plant's seeds and clears out contain poisons and ought to be dodged.

Α possible concern in the development of milk from plant-based foods is the lack of calcium bioavailability due to many of its kind having a high composition of oxalates (Hodges et al, 2019). USDA's FoodData Central database has additionally recorded that in every 130 grams (or 'cup') of raw Pachyrhizus erosus, 15.6 mg of calcium can be derived. To develop a milk product, the calcium content would be maximized when drained of its water content (González-Vázquez et al.) which had made up 87% of its total composition. Other than the aspect of calcium absorption ability toward the body, 130 grams of raw Pachyrhizus erosus would already fulfill the carbohydrate minimum of replacing cow milk (Katoch et al, 2021), at a calculation of 11.5 grams. Raw Pachyrhizus erosus of the same amount is also not too far off in energy, at 38 kcal compared to the 64 kcal in regular cow milk. Draining its raw water content would ensure that Pachyrhizus erosus's protein content would also be comparable to the 3.28 grams in cow milk. Which becomes suitable а substitute/alternative for lactose intolerance, due to the high potential of calcium absorption from Pachyrhizus erosus.

To ensure optimal health, it is important to maintain adequate calcium intake throughout life, particularly during periods such as childhood. adolescence, and pregnancy, as well as in older adults. A calcium deficiency can lead to a range of health issues, including weakened bones, muscle cramps, and an increased risk of fractures. Prolonged severe deficiency may contribute to the development of conditions like osteoporosis and osteopenia. Therefore, incorporating calcium-rich foods, such as Pachyrhizus erosus L, into one's diet is beneficial for sustaining long-term health and preventing age-related health complications.

2. RESEARCH PROBLEMS

- How is Pachyrhizus erosus an effective alternative or substitute for conventional plant-based milk in terms of taste, texture, and nutritional value?
- 2. How do we ensure the calcium properties of Pachyrhizus erosus are effective in substituting calcium in regular (lactose) milk?
- 3. In what ways does the active compound in Pachyrhizus erosus-based milk contribute to essential nutritional requirements, such as calcium intake, for those with lactose intolerance?

3. RESEARCH PURPOSES

 To develop a sustainable and affordable plant-based milk alternative using accessible ingredients that support individuals with lactose intolerance in increasing their milk consumption.

- Many people face limited options in plant-based milk, as well as fluctuating and inconsistent pricing, which can affect their purchasing decisions.
- 3. Exploring the benefits of Pachyrhizus the erosus as primary ingredient, combined with as an active compound, will contribute to future research aimed at transforming these materials into а functional. cost-effective product that provides essential nutrients.

4. Methods and Experimental Details

4.1 Research Steps

4.1.1 Material

- Pachyrhizus erosus L
- Water
- Salt

4.1.2 Tools

- Blender
- Stove
- Saucepan
- Measuring scale
- Measuring cup
- Spatula
- Fine-mesh sieve/cheesecloth
- Air-tight container/bottle
- Knife
- Vegetable Peeler

4.1.3 Procedures for making

a. Preparation of Pachyrhizus erosus L:

- Choose ripe, firm Pachyrhizus erosus L with smooth skin. Avoid those with blemishes or soft spots.
- 2. Wash the Pachyrhizus erosus L thoroughly under

- running water to remove any dirt or residue.
- Peel the skin using a vegetable peeler.
- 4. Cut the Pachyrhizus erosus L into smaller, manageable pieces.

b. Blending and Filtering:

- 1. Place the cut Pachyrhizus erosus L pieces into a blender. Add a small amount of water (about 1/4 cup) to help in blending. Blend until the mixture is smooth and fine, adjust more water as needed.
- Strain the blended mixture through a fine-mesh sieve or cheesecloth to remove any remaining fibers.

c. Heating and Flavoring:

- Transfer the filtered Pachyrhizus erosus L liquid to a saucepan. Heat over medium heat, stirring occasionally, until the liquid thickens.
- Add cinnamon sticks or cinnamon powder to the heated liquid. Simmer for a few minutes to allow the flavors to infuse.

d. Cooling and Storage:

- Remove the saucepan from the heat and let the liquid cool completely.
- 2. Add 1 sachet of stevia to each trial.
- 3. Transfer the cooled milk to a clean, airtight container.

Store in the refrigerator for up to a week.

4.1.4 Ratio & Ingredients for each trial

Ratio & Ingredients of each trial				
No.	Ratio (water: Pachyrh izus Erosus)	Water	Pachyrhiz us Erosus	Additi onal ingred ient (soyb ean)
Trial 1	1:1	500ml	500g	-
Trial 2	1:2	250ml	500g	-
Trial 3	10:10:1	500ml	500g	50g

4.2 In Silico Testing

Silico Testing refers to a method of evaluating bioactive compounds via simulation software that incorporates computation modeling for toxicity prediction, based on existing databases regarding the compound. This test is a viable alternative due to the ethical concern of animal testing and provides the convenience of up-to-date results.

4.3 Calcium content test

A calcium content test is important to determine the calcium content of the liquid (or drinking water) which directly helps consumers accurately track their calcium content and ensure the quality of drinking water/beverages. In the case of this product, this test is crucial to determine whether or not Pachyrhizus erosus can be used as an alternative to plant-based milk. When precipitation is formed, the substance tests positive for calcium. The higher the intensity of precipitation, the higher the calcium content is contained.

4.3.1 Procedure

- Create the reagent solution by mixing 1.24 grams of ammonium oxalate powder with 100 ml of distilled water (the reason for the high distilled water volume is for the sake of an effective mass of the powder). Be very careful in handling and wearing gloves as ammonium oxalate is an irritant and corrosive.
- Pour 5ml of each trial into separate cylinders, then pour 5ml worth of reagent solution per cylinder.
- Mix each cylinder's contents thoroughly. After that, either apply heat or have the cylinders sit at room temperature for an hour to enable the reaction of precipitation to fully take place
- Once done, dispose of the remaining reagent solution in a designated sink at the lab.

4.4 Boiling Test

A test used to examine the thermal stability of milk after processing is called the "clot on boiling test" (COB). A test used to examine the thermal stability of milk after processing is called the "clot on boiling test" (COB). The COB Test evaluates milk's ability to coagulate or clot when heated to determine if it is appropriate for processing. This makes it less likely that the milk will go through undesirable processing steps like pasteurization or ultra-high temperature (UHT) treatment. Therefore, COB is required to determine if pasteurized food will remain stable for subsequent processing.

4.4.1 Procedure

 Prepare a Bunsen burner/hotplate, 3 test tubes, and 50 ml of water in a beaker.

- 2. Take approximately 5 mL of milk into a test tube.
- Heating: Place the test tube in a boiling water bath for about 5 minutes.
- Observation: Remove the test tube from the water bath and gently shake it.
- Examine the contents for signs of coagulation or clotting. Normal, fresh milk should remain clear and liquid without showing any evidence of clotting.

4.5 Protein Test with Biuret

Proteins in the sample can be found using the Biuret test. A purple-violet complex is created when protein peptide linkages react with CuSO4 in an alkaline environment. Proteins in the sample can be found using the Biuret test. A purple-violet complex is created when protein peptide linkages react with CuSO4 in an alkaline environment. When combined with proteins, the reaction produces a pale purple color, however, when the reagent is pure, it is pale blue.

4.5.1 Procedure

- Copper(II) sulfate (CuSO₄): Provides Cu2+ ions. This is the first component of the Biuret reagent.
- The process requires an alkaline environment, which sodium hydroxide (NaOH) produces.
- Reagent addition: Pour 2 mL of a 1% sodium hydroxide solution into a test tube that already contains 2 mL of the sample solution.
- 4. Add a few drops of a 1% copper(II) sulfate solution to the mixture.
- 5. Observation: Gently stir and let the mixture stand for around five minutes.

4.7 Alcohol Test

4.7.1 Procedure

- Prepare 3 cylinders; 3ml of each trial would be poured into individual cylinders.
- 2. The ratio of the reagent (Alcohol 70%) and the trial would be 1:1. In this case, add 3ml of the reagent per cylinder, and mix thoroughly.
- Observation: Look for visual changes in consistency; the occurrence of clumps stuck to the cylinder would indicate a positive result.

4.8 Organoleptic Test

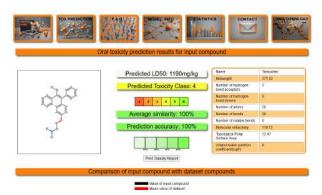
The organoleptic test is a product evaluation method that relies on human sensory perception. Parameters assessed include aroma, taste, color, and texture. Through this test, the quality of a product can be measured and monitored regularly. The results of the organoleptic test can be used as a basis for making improvements if there are discrepancies with the predetermined standards.

4.8.1 Questionnaire

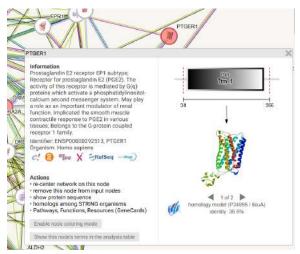
Fourteen (14) respondents were used in the organoleptic test, and they examined each milk experiment and were questioned based on its texture, flavor, color, and aroma from a scale of 1 (strongly disagree) to 5 (strongly agree) and preference from all of the trials. Additional questions regarding the presence of plant-based milk in Indonesian markets were targeted to the respondents.

5. Data and Discussion

5.1 Results of In Silico Test



The predicted toxicity class for isoflavone demonstrated a 4; indicating it's safe for oral consumption but can't be utilized for long-term consumption and average similarity demonstrated 100%; indicating the prediction is accurate since the compound (isoflavone) contains the levels of toxicity that resembles with the compound that is known.



Prostaglandin E2 receptor subtype EP1 (EP1) is one of four receptors for prostaglandin E2 (PGE2), a lipid compound important in various body processes such as inflammation, pain, and temperature regulation. EP1 plays a role in several key biological functions, including enhancing pain perception, regulation of smooth muscle contraction, and blood pressure control. In addition, EP1 also influences digestive tract function and bone metabolism.

Milk made from jicama (*Pachyrhizus erosus*) contains isoflavones, which can support the work of Prostaglandin E2 subtype EP1 receptors in the body. These isoflavones are useful in helping manage pain, supporting digestive tract function, and maintaining healthy blood pressure and bone metabolism. Thus, consuming jicama milk can have a positive effect on overall health.

5.2 Results of the Calcium Test

No.	Photo	Result
Trial 1		Positive
Trial 2		Positive
Trial 3	5	Positive (intensely clumped at the base)

After having the filled cylinders sit at room temperature for an hour, all three trials tested positive for calcium content as there was a precipitate formation that appeared white. Trials 1 and 2 produced precipitates in the form of

small crystals, while Trial 3 noticeably produced the most precipitate to the point of clumping at the base rather than being within the fluid, due to the soybean content's contribution. Overall, all 3 trials resulted in adequate levels of precipitate, especially for only 5ml worth of each being tested but Trial 3 had the highest intensity of precipitation, which indicates the highest calcium content is contained among all trials.

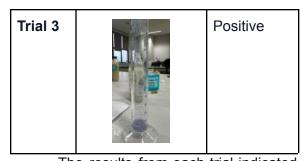
5.3 Results of the Boiling Test

No.	Photo	Result
Trial 1		Little to no clumps
Trial 2		Moderate amount of clumps
Trial 3		Most/ a lot of clumps

If the milk forms clumps or precipitates during the Clot-on-Boiling (COB) Test, it indicates potential quality issues such as increased acidity, enzymatic activity, or contamination. These factors can significantly affect the milk's usability for processing. In Trials 1, 2, and 3, the samples exhibited positive results, showing coagulation after being heated and boiled. This clumping signifies that the milk is unsuitable for pasteurization and further processing, indicating its inability to remain stable over time. In contrast, clear, unclotted milk demonstrates good heat stability and suggests that the sample is safe for processing without adverse reactions. However, among all the 3 trials, Trial 1 showed the least clumps, indicating the milk's potential quality issues such as increased acidity, enzymatic activity, or contamination less compared to the other 3 trials.

5.4 Results of the Biuret Test

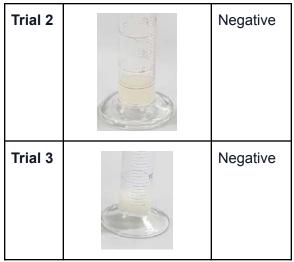
No.	Photos	Results
Trial 1		Negative
Trial 2		Negative



The results from each trial indicated different protein presence based on the color they produced from the reaction. The 1st trial shows a blue color after the biuret test, indicating the absence of proteins (negative). Meanwhile, the 2nd trial shows a pale dark blue color, indicating the absence of proteins as well (negative). After adding the Biuret reagent, Trial 1 & Trial 2 remain blue, indicating that the samples are free of peptide bonds and contain no considerable amounts of proteins peptides. However, the third trial shows a purple color, indicating the presence of proteins but in low concentrations (approx. 5-20 mg/mL). The Biuret test uses colorimetric analysis to offer information about protein content based on color intensity, making it a qualitative and quantitative assay for proteins

5.5 Alcohol Test Result

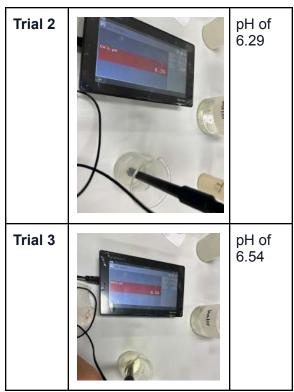
No.	Photos	Results
Trial 1	20	Negative



The alcohol test uses qualitative analysis to check for possible ethanol or similar compounds in liquid samples. The results from all 3 trials indicate a negative presence of alcohol content based on the consistency produced. The consistency of Trials 1, 2, and 3 remain fully liquid with no signs of sticking or any color changes. As all trials are negative for alcohol, they share a similar property to regular bovine milk in this aspect; negative alcohol content also suggests that all trials do not risk inflammation of bodily tissues and do not require moderation such as ones that apply to alcoholic beverages.

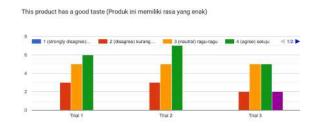
5.6 pH Test Results

No.	Photos	Result s
Trial 1	Cat have	pH of 6.44

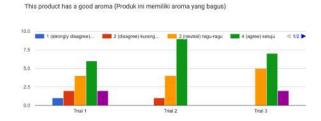


The closer the pH is to 7, the better the quality of a milk product is based on its level of freshness as an acidic pH typically indicates that there has been fermentation as a result of bacterial overgrowth. Out of all, Trial 3 has the best pH results as its pH of 6.54 falls in the ideal range of 6.5-6.9. On the other hand, Trial 1's result is 6.44 while Trial 2's result is 6.29. As a consideration, the lower pH in Trials 1 and 2 is due to the higher dominance of Pachyrhizus Erosus' raw pH of 5≤, compared to Trial 3 which had a minor addition of a soybean ingredient.

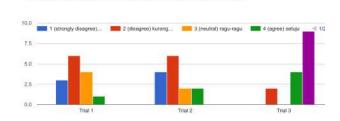
5.7 Results of the Organoleptic test



A strong preference in taste between Trial 2 and Trial 3 is demonstrated as 7/14 (50%) respondents agree with the good taste from Trial 2 and 5/14 (35.7%) agree with the good taste from Trial 3 with an additional 2/14 (14.3%) respondents strongly agree with the good taste in Trial 3. This may be due to the presence of an additional ingredient which is soybean, as they may be familiar with the taste of soybean instead of Jicama (*Pachyrhizus erosus*).



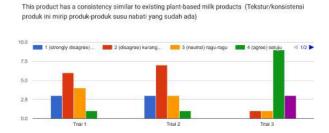
A strong preference for aroma between Trial 2 and Trial 3 is demonstrated as 9/14 (64.3%) respondents agree with the good aroma from Trial 2 and 7/14 (50%) agree with the good aroma from Trial 3 with 7/14 (50%) responding with agree with an additional 2/14 (14.3%) respondents strongly agree with the good aroma in Trial 3. This may be due to the presence of an additional ingredient cinnamon, cinnamon works as the source of aroma for beverages without altering the taste of the milk itself.



This product has a good color (Produk ini memiliki warna yang bagus)

In Trial 3, 9/14 (64.3%) respondents strongly agree with good color in Trial 3. In Trial 3, the color that was produced

resembles the characteristic of dairy milk, which becomes one of the factors that led to the respondents choosing the milk in Trial 3 in terms of color. Besides in Trial 3, the presence of soybean which contains lecithin helps in strengthening the composition of Jicama (*Pachyrhizus erosus*), and water to minimize the presence of residue in plant-based milk.



In trials related to consistency, respondents compared vegetable milk made from Jicama (*Pachyrhizus erosus*) with vegetable milk made from other ingredients, 9/14 (64.3%) respondents agreed, and 3/14 (21.4%) respondents strongly agreed. In Trial 3 the consistency is similar to existing plant-based milk products.

Based on the organoleptic test results, it can be concluded that Trial Composition 3 is the variant that is most popular with respondents, in terms of taste, color, aroma, and consistency. This is due to the addition of the distinctive soybean flavor and cinnamon aroma which provides a unique and pleasant sensation for the sense of smell. The combination of the savory taste of soybeans with a touch of cinnamon aroma provides the right balance between natural taste and warmth, which makes the results of Trial 3 more acceptable and liked by respondents. In addition, the correct consistency and attractive color contributed to the acceptance of the Trial 3

results, making it a superior choice compared to other compositions in the test.

6. CONCLUSIONS

Based on the findings of the trials, Pachyrhizus erosus (yam bean) proves to be an effective alternative to conventional plant-based milk in terms of taste, texture, and nutritional value. The addition of soybean in Trial 3 contributed to a unique and pleasant flavor profile, combining the savory taste of soy with the warm aroma of cinnamon. This combination of flavors enhanced the overall sensory experience, making it a highly acceptable option compared to other plant-based milk alternatives. In terms of texture, the consistency of Trial 3 was found to be suitable for consumption, providing a smooth and enjoyable mouthfeel.

Nutritionally, Pachyrhizus erosus showed promising calcium properties. The results from the precipitation test indicated that Trial 3, which contained Pachyrhizus erosus, demonstrated the highest calcium content, suggesting that this plant can effectively serve as a source of calcium, potentially substituting the calcium found in regular dairy milk. This makes Pachyrhizus erosus a viable option for those seeking non-dairy alternatives to meet their calcium intake, particularly for individuals who are lactose intolerant.

Furthermore, the active compounds in Pachyrhizus erosus contribute to its nutritional value by providing essential nutrients, including calcium. The higher calcium content found in Trial 3 can play a key role in supporting bone health and other vital functions in individuals with lactose intolerance, who may otherwise struggle to meet their calcium needs from traditional

dairy sources. This makes Pachyrhizus erosus a promising ingredient in the development of plant-based milk alternatives that are not only nutritionally adequate but also appealing in terms of flavor and texture.

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Advantages:

Pachyrhizus erosus could be an option for plant-based milk in the Indonesian market for people with lactose intolerance. Pachyrhizus erosus' ability to retain calcium in its water content has been additionally proven.

Comments and suggestions:

- 1. The quality of each trial was tested using biuret protein test, alcohol test, COB test, and pH level testing. However, the results could be further discussed for future modifications of the experimental conditions.
- 2. It takes some more effort for commercial applications.
- 3. Why not just eating Pachyrhizus erosus as a solid food, but not liquid form as milk to obtain a sufficient amount of calcium? If people with lactose intolerance really like to drink milk, they could drink soy bean milk oat milk, almond milk, etc. with supplement of Pachyrhizus erosus to get both plant-based proteins and calcium.
- 4. How were the rations decided? There seem to be no logical reason. The taste, aroma, color, and consistency preferred by the 14 organoleptic test respondents were quite subjective, via the senses.