Clarification and encapsulation of *Stevia rebaudiana* Bertoni water extract

Y Martono1*, F E R Pranawati1, C A Riyanto1,3, J Muninggar2

1Chemistry Department, Faculty of Science and Mathematics, Universitas Kristen Satya Wacana Salatiga 50711, Indonesia
2Physics Department, Faculty of Science and Mathematics, Universitas Kristen Satya Wacana Salatiga 50711, Indonesia
3Study Center for Multidisciplinary Applied Research and Technology (SeMARTy)

*yohanes.martono@uksw.edu

Abstract. This study is carried out for clarification and encapsulation of water extract from *Stevia rebaudiana* Bertoni. Clarification was performed using adsorption method of acid activated kaolin. Encapsulation of *S. rebaudiana* extract was carried out using spray drying method. Product evaluation was performed based on concentration of stevioside and rebaudioside A, moisture content, hygroscopicity, and sensory. The content of stevioside and rebaudioside A was determined by HPLC. Clarification of *S. rebaudiana* water extract by using acid activated kaolin was successfully reduce 95% green pigment and 65% yellow pigment. The content of stevioside and rebaudioside A in the optimal encapsulation products is 0.83% and 2.25%, respectively. Water content and hygroscopicity of sweetening products obtained were 9.8% and 12.91%, respectively. Sweetener products still have a lower level of sweetness based on organoleptic test results.

1. Introduction
Sweetener is one of the most commonly used ingredients in processed food products such as food and beverages available from household to industrial scale. In general, the sweetener is divided into two types, natural and artificial sweetener. Natural sweetening agents commonly used are cane sugar, while popular artificial sweeteners (synthetic) include aspartame and saccharin. Natural sweeteners and artificial sweeteners have their respective advantages and disadvantages. Consumption of excessive natural sweetening agents can lead to obesity and diabetes. While the artificial sweetener which is consumed in longer consumption will increase the risk of cancer [1].

Society tends to look for a natural sweetener that efficacious antidiabetic and safe for health. Stevia rebaudiana Bertoni is a plant herb that is potential to be developed as natural sweetener source. The major compounds in *S. rebaudiana* plants are stevioside (4–10%) and rebaudioside A (2–4%) [2]. The chemical structure of the two compounds is shown in figure 1 and figure 2. The advantages of this compound in addition to having a sweet taste with a sweetness level of 250-300 times sucrose is as a non-caloric sweetener, has a high blood sugar lowering activity, and can improve the function of β-pancreatic cells broken. In addition, steviosida can also increase the amount and sensitivity of insulin and do not lower blood sugar at normal levels and do not cause desensitization β-pancreatic cells. So, it
is safe to maintain blood sugar levels and is very potential for type 2 Diabetes Mellitus therapy in the long term [3].

Previous research has succeeded in making stevia functional drinks using pH adjustment treatment and analyzed stevioside content, total phenolic, and flavonoid compounds. In addition, the resulting functional beverage had a blood sugar decrease activity of 64% and 57% antioxidant activity to reduce DPPH radicals [4]. However, the beverages developed have not been optimized in easier to use and stable dosage forms. One of the advantages of the powder dosage form is that it is more practical in presenting and maintaining product stability. Purified steviol glycosides can be encapsulated using maltodextrin and inulin using spray drying method [5]. Study on encapsulation of *S. rebaudiana* water extracts into a natural sweetening powder using spray drying method still limited. Based on this background, this study aims to perform clarification and encapsulation *S. rebaudiana* water extract and evaluate the product in terms of water content, solubility, hygroscopicity, encapsulation efficiency, and sensory evaluation.

![Stevioside structure](image1)

**Figure 1.** Stevioside structure.

![Rebaudioside A structure](image2)

**Figure 2.** Rebaudioside A structure.

2. Materials and method

2.1. Materials
*S. rebaudiana* leaves were obtained from Bandungan farm, Semarang, Central Java, Indonesia. Stevioside and rebaudioside A standard were purchased from WAKO, Japan with a purity of 99.8% by
HPLC. Methanol and acetonitrile were HPLC grade (E-Merck, Germany) and ethanol for sample preparation prior HPLC was a pro-analysis grade (E-Merck, Germany). Maltodextrin was obtained from chemical store Indrajaya, Semarang, Central Java.

2.2. Sample preparation
All parts of the *S. rebaudiana* Bert plant which have been cleaned from the soil were dried using cabinet dryer for 24 hours at ± 50 °C. The dried sample is then ground into powder. Samples were sieved with 20 mesh sieves.

2.3. Steviol glycoside extraction from *S. rebaudiana*
A total of 50.00 g of powder was dissolved in 1 liter of distilled water. *S. rebaudiana* leaves were extracted in batch extraction (4 × 250 mL) using sonication at 40 °C for 15 minutes each batch. A solution was filtered and filtrates were collected. pH solution was adjusted using citric acid solution into 4.00 and increased to pH 10.00 using CaCO3. Next, the pH solution was adjusted to 5.00-7.00. This solution is called the water extract of *S. rebaudiana*.

2.4. Kaolin activation
Activation was done using an acid solution. As 25.00 g of kaolin are refluxed in H2SO4 10 M, 500 mL. Then the solution is decanted. The solution is neutralized with distilled water and decanted again until the H2SO4 concentration decreases and then filtered. Sediment was rinsed with distilled water several times, and then was dried and ground into powder. Kaolin powder calcination was performed at 600 °C for 6 hours.

2.5. Stevia extract dechlorophyllation
Dechlorophyllation was done to clarify *S. rebaudiana* water extract into a clear solution. Stevia extract clarification was carried out using adsorption method. Adsorption was done using acid-activated kaolin. The ratio of kaolin to *S. rebaudiana* water extract was 1: 10 (w/v). Adsorption was done in three continuous batch adsorption. The clarified solution was filtered and used for the next process.

2.6. Encapsulation *S. rebaudiana* extract using spray dryer [5]
Clarified *S. rebaudiana* water extracted was added with maltodextrin powder material using a concentration variation of 10% and 20% (w/v). The mixture was encapsulated using spray dryer which was operated under the following conditions; inlet air temperature (Tin): 160 °C, water temperature outlet (Tout): 88 °C, feed temperature: 60 °C, air pressure: 5 bar, aspirator: 90%, pump: 40% and compressed air flow rate: 500 L/h. The solid powder obtained was used for further tests.

2.7. Encapsulated *S. rebaudiana* water extract analysis

2.7.1. Determination of stevioside and rebaudioside A. Dominant steviol glycoside of stevioside and rebaudioside A was determined using High-Performance Liquid Chromatography (HPLC) according to [6]. A number of 20 μL samples injected in HPLC under chromatographic conditions used. Operational conditions of HPLC were stationary phase of Eurosphere C-18 columns (250 x 4.6 mm, 5 μm). The mobile phase used was a mixture of water solvents: methanol (90: 10, v / v) as solvent (A) and acetonitrile (B) at a ratio of 65: 35 (A: B, v / v). The flow rate of the mobile phase is 0.6 mL/min. Detection was performed using a UV detector at a wavelength of 210 nm.

2.7.2. Determination of hygroscopicity. Assay of hygroscopicity was determined to be a gram of water in 100 g of sample. Hygroscopicity measurement was performed according to [7]. A total of 1.0000 g of each sample was placed in an impermeable camber, containing saturated sodium sulphate solution (RH = 81%), and weighed again after 7 days. The camber was stored at 25 °C in an incubator, with a controlled temperature.
2.7.3. Encapsulation Efficiency assay. Encapsulation efficiency was determined according to [8]. The encapsulation efficiency (EE%) of the final product is based on stevioside and rebaudioside A (RA) content which is determined using HPLC as shown in the equation:

\[
EE\% = \frac{\text{stevioside RA content in product}}{\text{stevioside RA content of } S.\text{rebaudiana extract}} \times 100
\]  

2.7.4. Organoleptic assay of product. The organoleptic assay was done using the Hedonic test. The sweetness level was tested by 25 panelists. The scale used is 1 = very not sweet, 2 = not sweet, 3 = medium sweet, 4 = sweet, 5 = very sweet.

3. Results and discussion

3.1. Extraction and Clarification process control

Encapsulated product was optimized from the production process including extraction and clarification. Every step was standardized based on stevioside and rebaudioside A content using HPLC method. Clarification process using adsorption method produced clearly solution. Clarification step is critical for producing the white powder of encapsulated S. rebaudiana water extract. Adsorption using activated kaolin is more effectively to eliminate green pigment than yellow pigment. Percentage of clarification at 410 nm (yellow pigment) was 65% and at 665 nm (green pigment) was 95%. Adsorption was effectively adsorbed chlorophyll a. Activated acid-Kaolin have high amorphous silica which is effective to adsorbed pigments such as chlorophyll a [9]. Unfortunately, activated kaolin also adsorbed stevioside and rebaudioside A. Profile of stevioside and rebaudioside A content in each step of extraction and adsorption process is presented in table 1.

| Sample                 | Content (µg/ml) | Percentage of adsorption (%) |
|------------------------|----------------|------------------------------|
|                        | Reb A          | Stevioside                   | Reb A | Stevioside |
| Water extract          | 5212.02        | 2111.69                      |
| adsorption phase I     | 4465.32        | 1277.43                      | 14.33 | 39.51      |
| adsorption phase II    | 3956.06        | 1497.65                      | 24.10 | 29.08      |
| adsorption phase III   | 1825.65        | 607.19                       | 64.97 | 71.25      |

Continuous batch adsorption could clarify S. rebaudiana water extract effectively by removing green dark colour in solution. Triplicate batch adsorption could produce a fine yellowish solution as presented in figure 3. Triplicate continuous batch adsorption revealed adsorb rebaudioside A of 64.97% and stevioside of 71.25%. More batch adsorption, higher adsored stevioside and rebaudioside A. Activated kaolin is more adsorb stevioside than rebaudioside A. Acid-activated kaolin was carried out by pre-treatment using heat or high temperature. Calcination of acid activated kaolin using high temperature will remove Al\(^{3+}\) and increase porosity and surface area of adsorbent [10]. It could occur that porous size of kaolin after calcination is more appropriate to stevioside molecule than rebaudioside A. Stevioside and rebaudioside A have a molecular weight of 804.87 g/mole and 967.01 g/mol.

3.2. Encapsulation of S. rebaudiana water extract

S. rebaudiana water extract was encapsulated using a spray drying method by maltodextrin mixture of 10 and 20% (w/v). According to Wuryantoro (2014), the use of maltodextrin for the drying of S. rebaudiana sweetener can increase the total of dried solids. It makes higher in yield obtained. The content of Rebaudioside A and Stevioside of the encapsulated product were determined using HPLC.
Rebaudioside A and stevioside content in encapsulated product is presented in table 2.

![Figure 3](image)

**Figure 3.** Clarification result by triplicate adsorption, from left to right: crude extract of *S. rebaudiana*-first adsorption by acid activated-kaolin 20%-second adsorption by CaCO$_3$-third adsorption by acid activated-kaolin 20%.

| Sample          | Concentration (%) |  |
|-----------------|-------------------|--|
|                 | Rebaudioside A    | Stevioside |
| 10%             | 2.25              | 0.83       |
| 20%             | 1.63              | 0.56       |
| Commercial      | 2.25              | 0.10       |

Table 2. The content of Rebaudioside A and Stevioside of encapsulated product.

According to table 2, encapsulated product using 10% maltodextrin shows similar rebaudioside A content comparing to the commercial product of stevia natural sweetener. Higher maltodextrin content lowered rebaudioside A and stevioside content of the encapsulated product. Encapsulated product from water extract of *S. rebaudiana* by spry drying method is shown in figure 4.

![Figure 4](image)

**Figure 4.** Encapsulated product of *S. rebaudiana* water extract by spray drying method using maltodextrin as filler of [A] 10% and [B] 20%.

3.3. **Physic properties assay of encapsulated product**

Hygroscopicity indicates its capacity to adsorb water content in the air. Water will bound to the product by hydrogen interaction. Higher hygroscopicity will reduce the stability of the product. Higher content
of maltodextrin in the encapsulated product will increase water interaction to encapsulated product (Table 3). This is in accordance with the statement of [11] which states that the nature of hygroscopic maltodextrins (the ability to absorb water) makes the levels of hygroscopicity to increase along with the addition of maltodextrin. Hygroscopicity profile of encapsulated S. rebaudiana water extract is shown in table 3.

**Table 3.** Average hygroscopicity of encapsulated product obtained.

| Maltodextrin | 10%     | 20%     | Maltodextrin : inulin (60:40) [5] |
|--------------|---------|---------|----------------------------------|
| hygroscopicity (%) | 12.91±0.09 | 14.80±0.13 | 20.26±0.12                      |

The encapsulation efficiency states how much of the active compounds can be coated during the microencapsulation process. The result of encapsulation efficiency is still quite low (Table 4). It can be due to method analysis used for encapsulated failed to break the encapsulated system for extracting active compounds targeted inner encapsulated system. Furthermore, stevioside and rebaudioside A detected may be were the outer encapsulated system. So, it needs to optimize sample preparation for extracting targeted compounds in the encapsulated system.

**Table 4.** Efficiency Encapsulation (EE) of Rebaudioside A and Stevioside using 10% and 20% (w/v) maltodextrin ratio.

| Sample | EE (%) | Rebaudioside A | Stevioside |
|--------|--------|----------------|------------|
| 10%    | 5.79%  | 5.64%          |
| 20%    | 4.14%  | 3.78%          |

3.4. **Organoleptic assay**

The sweetness level of the encapsulated product was obtained from the organoleptic assay of 25 panelists (table 5).

**Table 5.** Organoleptic assay of the encapsulated product compare to sugar cane and market product of stevia sweetener.

| sample | 10%     | 20%     | 100%    | market product sweetener | sugar cane | water |
|--------|---------|---------|---------|--------------------------|------------|-------|
| W = 0.1470 | 2.92±0.19 | 2.76±0.20 | 2.28±0.16 | 4.16±0.21 | 3.20±0.87 | 2.04±0.15 |

Market product sweetener is superior to the encapsulated product obtained based on sweetness level. The market product also includes sorbitol and erythritol which are also sweetener. The encapsulated product also has a lower sweetness level compared to sugar cane. It is caused by lower encapsulation efficiency of stevioside and rebaudioside A using maltodextrin as filler. It is necessary to find out another filler that appropriate to coat stevioside and rebaudioside A beside to optimized encapsulation process.
4. Conclusion
Water extract of *S. rebaudiana* could be clarified using adsorption method and encapsulated by maltodextrin using spray drying method. Encapsulated product has good hygroscopicity properties but still lower in sweetness level.

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