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Microencapsulation Stability of Stevia Leaf Extracts of *Stevia rebaudiana* Bert Using Inulin-Chitosan

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Abstract

Extract of Stevia leaf of rebaudiana Bert has a content that is easily oxidized by the presence of light and heating so it is not stable during storage. A microencapsulation process is carried out which aims to protect the contents in the extract. This study aims to determine the effect of storage temperature on the physical stability of the microencapsulation of Stevia leaf of rebaudiana Bert with a combination of encapsulants inulin chitosan. A study was conducted to determine the effect of temperature on the microencapsulation stability of Stevia leaf of rebaudiana Bert over a period of 1 month. Tests carried out on the microencapsulation of Stevia leaf of rebaudiana Bert include the examination of the physical parameters of the microcapsules, namely the microencapsulation yield, moisture content, solubility, flow rate, non-encapsulated and encapsulated extracts content and determination of particle distribution and morphological form. The test results show that the storage temperature does not affect the microencapsulation stability of Stevia leaf of rebaudiana Bert, because the SEM test results did not change *i.e.* the particle size distribution of 1 - 1000 μm. The average yield obtained from making microencapsulation of Stevia leaf extract of rebaudiana Bert was 98.20%. Storage temperatures of 5˚C, 25˚C and 40˚C affect the physical stability of the microencapsulation of Stevia leaf extract of rebaudiana Bert due to changes in the profile of physical characteristics during storage.

Subject Areas

Nutrition

Keywords

Stevia Leaf Extract, Microencapsulation, Physical Stability, Inulin, Chitosan

1. Introduction

Stevia leaf contains several sweetening compounds such as steviosida, rebaudiosida A, B, C, D, E and dulcoside A and B. Steviosida has no teratogenic effect [1], mutagenic [2], and carcinogenic [3]. Stevia leaf (Stevia rebaudiana Bertoni) contains various substances such as alkaloids, tannins and flavonoids [4]. Flavonoids are very easily oxidized by light, air and heating so they are unstable when storing [5]. Encapsulation techniques are needed to protect the antioxidant activity in Stevia leaf of *rebaudiana* Bert [6].

This study aims to determine the effect of temperature (5˚C, 25˚C, 40˚C) on the microencapsulation stability of Stevia leaf of rebaudiana Bert conducted over a period of 1 month with physical characteristics as parameters tested. The temperature selection represents the storage conditions of cold temperature, room temperature and hot temperature. Storage conditions in the refrigerator range between temperature 5˚C ± 3˚C [7]. The temperature of 40˚C is chosen based on the drug stability test guidelines *i.e.* an accelerated stability test is carried out at temperature 40˚C ± 2˚C. The temperature of 25˚C used at room temperature usually ranges between 20˚C - 25˚C [8].

The choice of encapsulation is also very influential on the success of nanoencapsulation. Carbohydrates are widely used in encapsulation, but research on inulin as encapsulation is still limited. The use of inulin as encapsulant in nanoencapsulation also has limitations such as large particle size, besides that inulin also has a sticky character and hygroscopic thus limiting their use as encapsulants. To produce nanoscale particle size in the encapsulation of stevia leaf extract, the presence of inulin can be partially replaced by chitosan. Chitosan is a natural polymer that can be linked by crosslink when a crosslinked agent is added (Sarah, 2001).

2. Materials and Methods

2.1. Tools and Materials Used

The tools used are spray dryer (Buchi Mini Spray Dryer B-29-), rotary vacuum evaporator (Buchi R-114), Scanning Electron Microscope (JEOL JSM-5310LV-20 kV), analytical balance, homogenizer (Ultra Turrax® T50 Basic), and glassware. The materials used in this study consisted of stevia leaves obtained from Solo. Inulin, chitosan, 1% acetic acid, Tween 80 1%.

2.2. Microencapsulation of Stevia Leaf Extract

The microencapsulation of Stevia leaf extract made an emulsion system with the ratio of inulin encapsulation: chitosan is 25:75b/b. Chitosan is dissolved in 1% acetic acid and then Tween 80 1% emulsifiers are added. Furthermore, inulin is put into chitosan solution and homogenized using Homogenizer Ultra Turrax® T50 Basic with a speed of 5000 rpm for 5 minutes. Furthermore, the microencapsulation process of stevia leaf extract is carried out using the spray drying method at a feed rate of 15 ml/minutes and inlet temperature 120˚C. The result-

ing powder is a microcapsule whose properties are analyzed. Microencapsulation preparations that have been made are then divided into 3, then stored at temperature of 5˚C, 25˚C and 40˚C. Next, the physical parameters of the microcapsules were analyzed, namely the microencapsulation yield, the moisture content, solubility, flow velocity, the level of the non-encapsulated and encapsulated extracts and the determination of particle distribution and morphological form.

2.3. Microencapsulation Yield

Microencapsulation yield was evaluated by comparing the weight of the material after processing it with the material before processed [9].

2.4. Moist Content

Microparticles are measured using a moisture meter (moisture balance) at temperature of 105˚C. Then the moisture content of the constant is calculated [10].

2.5. Solubility Test

Solubility is evaluated by dissolving 1 gram of sample in 25 ml distilled water and then filtered, filtered paper to obtain residual weight, the results obtained are then calculated against the sample weight to determine the percent of sample that can be dissolved [11].

2.6. Flow Speed

Flow speedy is evaluated by inserting a few samples in the flow funnel and then determining the time required for the entire sample to flow [12].

2.7. Unencapsulated Extract Levels

Determined by 1 gram of sample was immersed in 20 ml of ethanol, then filtered and the filtrate was measured using a spectrophotometer.

2.8. Determination of Particle Distribution and Morphological Shape

Determination of the size distribution of microparticles is done using a light microscope and *Scanning Electron Microscope* (SEM) [13]. Observation using SEM was done by coating the microcapsules with gold and seen at magnification 5000 times (**Table 1**).

3. Results and Discussion

The average yield obtained from making microencapsulation of Stevia leaf extract of rebaudiana Bert is 98.20%. The yield obtained is quite high, possibly due to the process temperature of low freeze dry so that there is no sublimated material other than water. From the SEM analysis results in **Figure 1**, it can be seen that the microencapsulation of the Stevia leaf extract of rebaudiana Bert is spherical, uneven, non-porous and micro-sized. Generally the size of a microcapsule product ranges 1 - 1000 μm [14].

Table 1. Microencapsules formula of Stevia extract.

Materials	F1
Stevia leaf extract(ml)	100
Inulin (g)	2.075
Chitosan (g)	6.225
Acetic acid 1% (ml)	8.3
Tween 80 1% (ml)	8.3

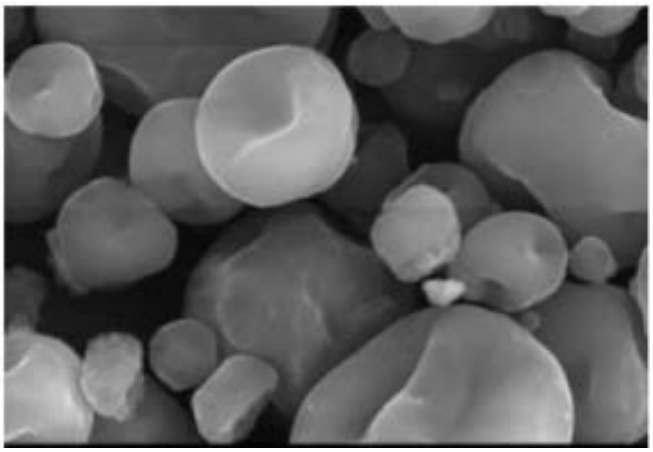

Figure 1. The morphological form of microcapsules of Stevia leaf extract at 5000 times magnification.

The results of microencapsulation physical stability test of Stevia leaf extract of rebaudiana Bert can be seen in **Table 2**. Storage at temperature of 5˚C, 25˚C and 40˚C cause the moist content of microencapsulation of Stevia leaf extract of rebaudiana Bert to decrease. This can be caused by the water content in the preparation evaporating and causing the preparation to become dry [15]. Testing the flow rate of microencapsulation of Stevia leaf extract of rebaudiana Bert. Storage at 5˚C, 25˚C and 40˚C shows that the flow rate of microencapsulation of Stevia leaf extract of rebaudiana Bert is increasing, this is due to a decrease in moisture content. Flow character is affected by humidity, the higher the humidity, the flow characteristics will be worse because the cohesiveness (bonding between particles) is getting stronger so that the microencapsulation ability of Stevia leaf extract rebaudiana Bert to flow becomes more difficult.

Unencapsulated extracts are more susceptible to oxidation and thus reduce the quality of microencapsules [16]. Storage over at temperature of 5˚C, 25˚C and 40˚C causes the extract encapsulated levels in the microencapsulated preparations of Stevia leaf extract of rebaudiana Bert to decrease. Storage at this temperature causes the solubility of microencapsulation of Stevia leaf extract of rebaudiana Bert to decrease, this can be due to the influence of the moist content will affect the weighing of the sample and show the results more easily dissolved (**Figure 2**).

Table 2. The solubility of Stevia extract of Microencapsule.

Treatment.	Solubility (%)
1	97.96 ± 0.01
2	97.94 ± 0.01
3	97.96 ± 0.11
4	97.95 ± 0.01
5	97.96 ± 0.20
6	97.96 ± 0.01
7	97.93 ± 0.01
8	97.96 ± 0.01
9	97.92 ± 0.02
10	97.96 ± 0.01
11	97.94 ± 0.01
12	97.96 ± 0.02
13	97.96 ± 0.01

Information: Treatment 1: 0 week storage; Treatment 2: 1 week storage at a temperature of 5˚C; Treatment 3: 1 week storage at a temperature of 25˚C; Treatment 4: 1 week storage at a temperature of 40˚C; Treatment 5: 2 week storage at a temperature of 5˚C; Treatment 6: 2 week storage at a temperature of 25˚C; Treatment 7: 2 week storage at a temperature of 40˚C; Treatment 8: 3 week storage at a temperature of 5˚C; Treatment 9: 3 week storage at a temperature of 25˚C; Treatment 10: 3 week storage at a temperature of 40˚C; Treatment 11: 4 week storage at a temperature of 5˚C; Treatment 12: 4 week storage at a temperature of 25˚C; Treatment 12: 4 week storage at a temperature of 40˚C.

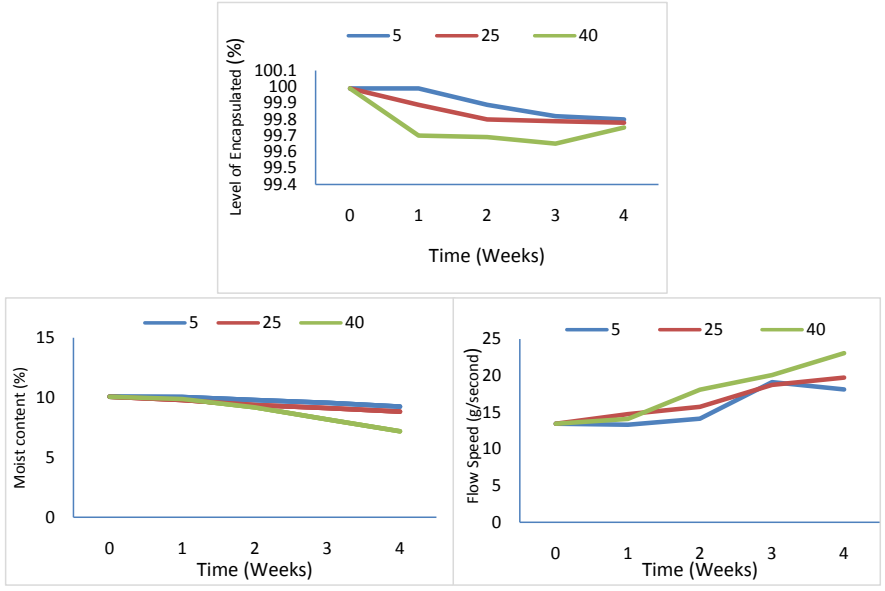

Figure 2. The physical characteristic results of Stevia extract of Microencapsule.

4. Conclusion

Storage temperatures of 5˚C, 25˚C and 40˚C affect the physical stability of the microencapsulation of Stevia rebaudiana Bert leaf extract.

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Conflicts of Interest

The authors declare no conflict of interests.

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