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Research Article

MICROENCAPSULATION OF STEVIA LEAF EXTRACTS *STEVIA REBAUDIANA* BERT IN DIFFERENT INULIN-CHITOSAN VARIATIONS

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ABSTRACT

Stevia plant (Stevia rebaudiana) is a type of plant which contains the dominant compound of diterpen glycoside as stevioside and rebaudioside A. In the industry, purified extracts of stevioside and rebaudioside A are widely used as sweeteners for low-calorie food and beverage products or as a sugar substitute for diabetics. Plants are microencapsulated to facilitate product handling and packaging. This study aims to evaluate the effect of inulin and chitosan mixture encapsulants with various concentrations on physical properties, chemical nanocapsules of stevia leaf extract using spray drying.In microencapsulation, inulin is added chitosan with several variations, namely 25:75, 50:50 and 75:25 to get the best encapsulation.The results showed that microencapsulation of stevia leaf extract could be produced using the spray dry method. In some variations showed that in the ratio of inulin: chitosan (25:75) showed the best activity as encapsulants because they had the highest chemical content of microcapsules when compared to other concentrations of 5.94% stevioside and 3.52% rebaudioside A. Microcapsules at these concentrations have recoveries of 6.61%; moist content 30.31%; flow velocity 0.24 g/s, encapsulation efficiency 10.37%; particle size distribution 1 - 500 μm.

KEYWORDS: *Stevia Leaf Extract, Microencapsulation, Inulin, Chitosan.*

INTRODUCTION

Today, the presence of sugar is a fact that sweeteners have an important function related to the fulfillment of human food needs.Low-calorie sweeteners can be a sweetener alternative for diabetics to reduce blood sugar levels in people with diabetes.Among the various types of sweeteners, there are glycoside compounds extracted from herbal plants with the species *Stevia rebaudiana* (Bert.).

Stevia leaves contain several sweetening compounds such as stevioside, rebaudioside A, B, C, D, E and dulcoside A and B. Stevioside does not have mutagenic effect [1]. The use of stevioside from *Stevia rebaudiana* (Bert) is one of the new breakthroughs in the field of food as a low calorie sweetener. In addition to its potential as a sweetener (200-300 times sucrose), *Stevia rebaudiana* Bert is harmless, contains low calories up to zero calories so it is safe for diabetics [2]. Rebaudioside A, which has an extra glucose unit relative to stevioside, is superior in terms of both sweetness and quality of taste.

Microencapsulation needs to be done to maintain the active components in stevia leaf extract and to cover all forms of deficiency from stevia, one of which is bitter. The source of bitter taste in stevia is caused by tannins, flavonoids, etc [3].

This study aims to determine the microencapsulation potential of stevia leaf extract by spray drying method. Spray drying method produces a more homogeneous microencapsulation and a better solubility rate than other methods, namely drying, drum drying. The choice of encapsulants greatly influences the success of nanoencapsulation. Carbohydrates are widely used in encapsulation, but research on inulin as encapsulants is still limited. The highest vanillin encapsulation efficiency results are by using inulin encapsulant compared to alginate and HPMC.

The use of inulin as encapsulant in nanoencapsulation also has limitations such as large particle size, besides that, inulin also has sticky and hygroscopic properties which limits its use as encapsulants. To produce nanoscale particle size in encapsulation of stevia leaf extract, the presence of inulin can be replaced in part by chitosan. Chitosan is a natural polymer that can bind crosslinked when crosslinked agents are added [4].

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MATERIAL AND METHODS

Materials:

This research 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 glass tools for analysis needs. The materials used in this study consisted of stevia leaves which is obtained from Solo; Inulin, chitosan, 1% acetic acid, 1% Tween 80.

Microencapsulation of Stevia Leaf Extract:

Microencapsulation of Stevia leaf extract was made by an emulsion system with a variation of inulin encapsulant ratio: chitosan was 25:75, 50:50 and 75:25 b / b. Chitosan was dissolved in 1% acetic acid then added with Tween 80 emulsifiers 1%.Furthermore, inulin was 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 was carried out using spray drying method at a feed rate of 15 ml / min and an inlet temperature of 120°C. The resulting powder is a microcapsule whose properties are analyzed. Furthermore, physical parameters of microcapsules were examined, namely recovery, moisture content, flow rate, particle distribution and morphological form.

Recovery test:

The recovery factor is determined by comparing the total microparticles obtained to the total active substance with the polymer used in making microparticles. To determine the recovery factor used is as a following formula [5]:

$$\% \text{ Recovery} = \frac{Wm}{Wt} x100\%$$

Description:
% Recovery = recovery factor (%),
Wm = weight of the microparticles obtained (g),
Wt = weight of the material forming the microparticles (g)

Determination of moisture content:

Microparticles are measured using a moisture level gauge (moisture balance) at a temperature of 105˚C. Then the constant moist content is calculated [6].

Encapsulation Efficiency Test:

This test is determined by calculating the active substance content that is absorbed in the microparticles using a formula [5]:

$$\% \text{ EE} = \frac{\text{The fraction of active subtance in microcapsules}}{\text{The theoretical fraction of active subtances in microencapsules}} x\ 100\%$$

Determination of the distribution of particles and morphological forms:

The morphology of the microencapsulated samples was evaluated utilizing a scanning electron microscope (SEM) shown in table 1. The samples were systematically observed with 5000 magnification [7].

Table No. 1: Stevia Extract Microencapsule Formula

Material	F1	F2	F3
Stevia leaf extract (ml)	100	100	100
Inulin (g)	2,075	4,15	6,225
Chitosan (g)	6,225	4,15	2,075
1% acetic acid (ml)	8,3	8,3	8,3
Tween 80 1% (ml)	8,3	8,3	8,3

Description:
F1 = microencapsulation of stevia extract with inulin: chitosan (25: 75)
F2 = microencapsulation of stevia extract with inulin: chitosan (50 : 50)
F3 = microencapsulation of stevia extract with inulin: chitosan (75 : 25)

RESULTS

Test of physical characteristics including microencapsule recoveries, moisture content, flow rate, particle size, particle distribution and morphological form and determination of stevioside and rebaudioside A levels. Microencapsulation physical parameters test results can be seen in table 2.

Table No. 2: Test results of physical parameters of Stevia Extract microcapsules

Physical Parameter Test	F1	F2	F3
Microcapsule recovery (%)	6,61 ± 0,09	15,84 ± 0,11	15,81 ± 0,29
The content of moisture (%)	10,31 ± 1,21	10,95 ± 0,22	9,56 ± 0,27
Flow rate (g/s)	0,24 ± 0,03	0,53 ± 0,01	2,40 ± 0,48
Encapsulation Efficiency (%)	30,31	27,38	16,98
Distribution of particle sizes (µm)	1 – 500	1 - 500	1 - 500

Stevia extract microcapsules with chitosan inulin encapsulation produced are powder, smooth and yellowish white (Figure 1). The microcapsules formed are homogeneous alloys. Homogeneous mixture is achieved if there is no visible difference between the constituent components, both in shape and color, because all components have been mixed evenly[8]. The results obtained from all formulas enter the microcapsule particle size range. The difference in particle size distribution can be influenced by the number of coatings used as forming microcapsule walls.

Form of microcapsule morphology of stevia leaf extract with scanning electron microscope shown in figure 2. This results in the content being more difficult to enter and causing less coated stevioside and rebaudioside A level shown in Figure 3.

Fig. 1: Stevia extract microencapsule

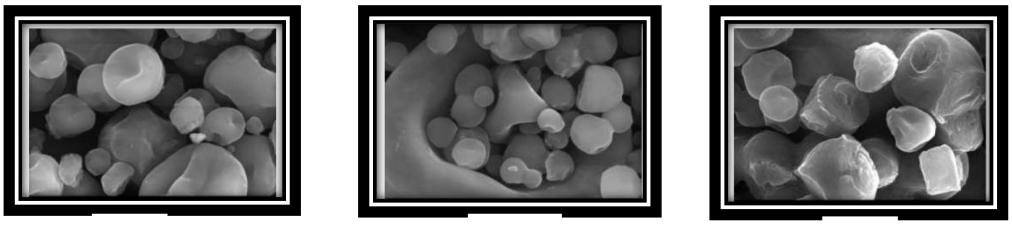

Fig. 2: Forms of microcapsule morphology of stevia leaf extract at 5000 times magnification

Description: (a) = formula 1 inulin chitosan (25:75), (b) = formula 2 inulin chitosan (50: 50), (c) = formula 3 inulin chitosan (75: 25)
This results in the content being more difficult to enter and causing less coated stevioside and rebaudioside A level.

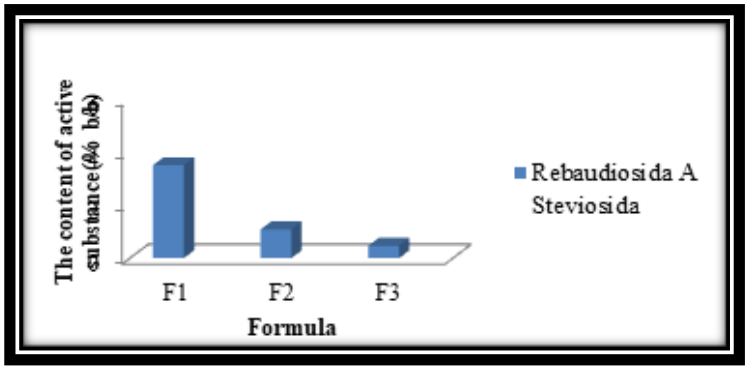

Fig. 3: Results of measurement of content in Stevia Leaf Extract Microcapsules

DISCUSSION

Microencapsule recovery:

Recovery parameters are useful in determining the effectiveness and efficiency of coating materials and the micro-encapsulation method used. Based on Table 2, the highest recovery value in formula 2 is 15.84%. Small results may occur because in the manufacturing process not all chitosan polymers have interacted with inulin to form microparticles. This is most likely due to the fact that there are still many chitosan in the supernatant part of the emulsion which is not cross connected. In addition, it can also be influenced by the way of making microparticles which can cause shrinking microparticle size after the manufacturing process, resulting in loss of moisture from the polymer. Reduced moisture from the polymer will reduce the weight of the microparticles produced.

The content of moisture:

From the results of the moisture content test, formula 2 has the highest moisture content (10.95%), while the formula 3 has the lowest moisture content (9.56%). Moist content is a parameter that determines the quality of microcapsules. Low water levels can inhibit the growth of harmful microbe microencapsulation. Complex molecular forms are one of the things that can cause bonding with water molecules to be stronger so that when the drying process takes place water molecules are rather difficult to evaporate and require greater evaporation energy. Powders with low moisture content have a great capacity to absorb humidity from the environment, which is related to the high water concentration gradient between the product and the surrounding air [9].

Encapsulation Efficiency Test:

Encapsulation efficiency (EE) shows the percentage of active substances coated on the three microencapsulation formulas. The result of determining the EE value shown that formula 1 with inulin encapsulant has the highest result among the other formulas. Encapsulation efficiency shows the percentage of total active substance encapsulated compared to the number of initial active substances. Encapsulation efficiency is influenced by several factors including the nature of the coating material (viscosity and solubility), the ratio of the core to the coating, and the inlet air temperature [10].

Flow rate:

The microcapsule flow rate will affect weight uniformity if microcapsule products are processed into other dosage forms such as tablets or filled into capsule and sachet shells. In table 2 shows that formula 1 has the smallest flow rate of 0.24 g / s, while formula 3 has the largest flow rate of 2.40 g/s. This shows that the flow rate test with inulin encapsulants gives a greater influence on the flow test than chitosan does. Reduced inulin levels in the mixture will reduce the flow velocity response. The flow rate requirement is not more than 10 g / sec. From the results of the evaluation carried out on the three formulas, they have fulfilled the requirements.

Particle distribution and form of microcapsule morphology:

The surface structure of microcapsules not only affects the microencapsulation effect, but also is closely related to physicochemical properties of microcapsules, such as fluidity and dispersibility[11]. The results of particle measurements showed that microcapsule showed a microcapsule morphology that was round but not perfectly spherical or concave on its surface for each formula. In all formulas, the microcapsules obtained have various shapes with smooth microcapsule surfaces. The results of the microcapsule shape and morphology examination were performed using a scanning electron microscope (SEM) with a magnification of 5000 times which can be seen in Figure 2. Through the spray-drying process, solvent evaporation occurs, so the matrix that has absorbed water will lose water content. As a result, this results in the formation of hollows on the outer surface of the microcapsules. This is common in coating polymers derived from polysaccharides. In addition, surface smoothness is also affected by the polymer used. Polymers that are more able to cover the holes and fibers found on the surface of the microcapsules. Generally the size of microcapsule products ranges from 1 - 1000 µm.

Determination of the microencapsulation content of Stevia leaf extract:

After the microencapsulation process, the results obtained for the content of stevioside and rebaudiosideA in formula 1 with the chitosan inulin encapsulation ratio (25: 75) had the highest content of 5.94% and 3.52%. This shows that in this condition steviosides and rebaudiosides A can be coated well. The decrease in the levels of steviosides and rebaudiosides A in other formulas is thought to be due to the greater composition of chitosan, which causes the density of the coating material to be tighter [12].

CONCLUSION

The microencapsulation formula of stevia extract using inulin and chitosan coating material at a ratio of 25: 75 (formula 1) is the best microencapsule formula indicated by the measurement results of physical parameters (recovery, moisture content, flow rate, particle size and shape) and chemical parameters (content stevioside and rebaudioside A) in microencapsules.

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