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Formulation of Petroleum Ether Fraction Microencapsulation and Isolate *Bixa orellana* L.

Mutmainah, Annisa Nanda Fatina, Yuvianti Dwi Franyoto, Lia Kusmita, Ika Puspitaningrum
Departmen of Pharmaceutical Science, Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi Semarang
Semarang, Indonesia
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ABSTRACT: The production of free radicals in excess of the need can trigger degenerative diseases, so antioxidant compounds are needed that inhibit damage caused by free radical oxidation. *Bixa orellana* L. contains the compound bixin which is known to have antioxidant properties. To increase the stability and acceptability of bixin compounds, the microencapsulation method using gum arabic and maltodextrin was used. This study aims to determine the comparison of the formula of the petroleum ether fraction and bixin isolate with gum arabic and maltodextrin encapsulants. The concentrations of gum arabic and maltodextrin used were 2.78% and 17.22%, respectively. The formulation was made with petroleum ether fraction (F1) and isolate bixin (F2). Evaluations carried out on the results were microcapsule yield, microcapsule moisture, microcapsule solubility, flow rate, and microcapsule efficiency. The results of the T-test showed that the results of the solubility test and flow rate were significantly different, while the microcapsule efficiency test was not significantly different..
KEY WORDS: *Bixa orellana* L., petroleum ether fraction, bixin isolate, microencapsulation.

I. INTRODUCTION

Free radicals are a group of atoms, molecules or ions with unpaired electrons that are very unstable and active against chemical reactions with other molecules/compounds [1]. Antioxidants are molecules that are able to inhibit the oxidation of other molecular compounds [2]. The use of natural antioxidants from plants is used as an alternative to overcome the oxidation of a compound because the price is relatively low, compatible, and does not cause harmful effects in the human body.

Bixa orellana L. is a plant from the Bixaceae family with the genus Bixa [3]. *Bixa orellana* L. widely used as a traditional natural food coloring, especially in Asian countries [4]. Other benefits of *Bixa orellana* L. are as antidiabetic, antioxidant, anti-inflammatory, and anticarcinogenic [5]. One of the main color pigments in *Bixa orellana* L. seeds is bixin [6]. The best known properties of bixin compounds are antioxidants [7].

In its use, bixin compounds have limitations, namely low solubility, tend to oxidize under light and high temperatures [8]. One of the efforts that can be used to increase the solubility and protect bixin from degradation is the microencapsulation method. Microencapsulation is a method used to protect compounds from the influence of temperature and light sensitivity, so as to increase bioavailability, solubility, and control the release of an active compound [8]. The microencapsulation consists of a core and a shell, where the core contains the active compound and the shell is a polymer [9]. The use of the microencapsulation method depends on the application and specific parameters, such as particle size, physicochemical properties of the core and coating, release mechanism, process cost, etc. [10].

Freeze drying is a method that applies low temperatures in drying for compounds that are sensitive to high temperatures [11]. The principle of freeze drying is sublimation, where the water vapor is directly converted from the liquid phase to the gas phase, and finally a powder is formed [12]. Selection of coating material for microencapsulation of bixin isolate *Bixa orellana* L. based on the optimization results of previous studies on microencapsulations of the petroleum ether fraction *Bixa orellana* L. which uses a combination of gum arabic and maltodextrin for microencapsulation.

Use of maltodextrin coating material and gum arabic is expected to form stable microcapsules and protect bixin compounds due to the film-forming properties of gum arabic and maltodextrin ability to protect microcapsules from oxidation [10].

II. RESEARCH METHODS

2.1 Object

The object of this research is the physical characteristics of the bixin isolate microencapsula *Bixa orellana* L. . Physical characteristics include organoleptic test, microencapsulated yield, microencapsulated efficiency, moisture content, solubility, flow rate.

2.2 Tools

Separating funnel, rotary evaporator (Heidolph), silica plate GF254, chamber, cuvette, volume pipette, filler, measuring flask, spectrophotometer (UV-1280 Shimadzu), Freeze Dryer (Thermo Scientific), funnel, Moisture Meter (Shimadzu).

2.3 Materials

PE fraction, bixin isolate, acetone, hexane, H2SO4, KOH, FeCl3 (*Merck*), NaCl, etanol 70%, Reagen Mayer, Dragendorff's reagent, and filter paper, gum arabic, maltodextrin (DE=11), gelatin, CMC, tween 80, aquadest.

2.4 Research procedure

Making microencapsulates by mixing maltodextrin, gum arabic, gelatin and water was homogenized for 1 minute, then the mixture of bixin and tween 80 isolates was added and homogenized for 10 minutes [13]. The solution was frozen in the freezer for 24 hours, and dried by freeze drying for 72 hours. The dry samples were ground and sieved using a 24 mesh sieve. Microencapsulates are stored in tightly closed containers and protected from light. The design of the microcapsule formula is presented in table 1.

Table 1. Design Formula of Microencapsules of *Bixa orellana. L.*

Bahan	F1 % (b/v)	F2 % (b/v)
PE Fraction	0,03	-
Isolate bixin	-	0.03
Arabic gum	2,78	2.78
Maltodextrin	17,22	17.22
Tween 80	0,5	0.5
Gelatin	1	1
CMC	0,4	0.4
Aquadest	ad 100	ad 100

a. Encapsulation efficiency testing

This test was carried out with 2 grams of sample dissolved in 100 mL of acetone and then the absorbance was measured using a spectrophotometer at λ 502 nm [14].

$$\textbf{Total uncapsulated bixin} = \frac{A \times 100000 \times 100}{2870 \quad \text{sample weight (mg)}} \quad (1)$$

$$\textbf{Total encapsulated bixin} = 100\% - \text{total uncapsulated bixin} \quad (2)$$

$$\textbf{Microencapsulation Efficiency} = \frac{\text{total encapsulated bixin} - \text{total unencapsulated bixin} \times 100}{\text{Total encapsulated bixin}} \quad (3)$$

b. Moisture testing

The test is carried out with 0.5 grams of microcapsules and measured in a moisture meter, the start button is pressed and then the resulting number is recorded when the notification sound is heard [10].

c. Solubility test

Test by dissolving 1 gram of microcapsules in 25 ml of distilled water, the resulting solution is filtered using Whatman paper No. 42, Then the filter paper and residue were dried in an oven at 105°C for three hours and then cooled and weighed [10].

$$\textbf{Percent solubility} = 100\% - \text{percent residue}$$

$$\textbf{Percent residue} = \frac{\text{filter paper weight and residue} - \text{filter paper weight} \times 100\%}{\text{Sample weight}} \quad (4)$$

d. Flow speed test

Test with 10 grams of microcapsules inserted into a closed funnel. The cover at the end of the funnel was then opened and the microcapsules were allowed to flow until no microcapsules remained in the funnel, and the flow time was recorded. [10].

III. DISCUSSION

The results of testing the physical characteristics of microcapsules are presented in table 2. Determination of the concentration of microcapsule coating materials of bixin isolates of *Bixa orellana* L. with concentrations of gum arabic and maltodextrin on microencapsulated petroleum ether fraction of *Bixa orellana* L. by 2.781% and 17.219%. The test result data can be seen in table 2.

Table 2. The results of testing the physical characteristics and antioxidant activity of microcapsules

Testing	F1	F2
Organoleptic Form Smell Color flavor	powder sweet orange tasteless	powder sweet orange tasteless
Yield (%)	83,27 ± 0,62	85,88 ± 3,60
Microencapsulation Efficiency (%)	99,10 ± 0,13	98,89 ± 0,13
Moist content (%)	2,62 ± 0,12	2,42 ± 0,09
Flow Rate (grams/second)	4,38 ± 0,20	4,15 ± 0,19
Solubility (%)	85,81 ± 0,37	85,60 ± 0,27

(*Description: F1 = micro encapsulation of PE fraction; F2 = microencapsulation of bixin isolate*)

The yield of microcapsules showed the amount of material lost during the drying process, the yield was good, which was close to 100%. Table 2 shows that the yield of microencapsulated PE fractions and isolates is close to good yields. One of the factors that can affect the yield, including maltodextrin as an encapsulant is known to have an effect on increasing the yield because the higher the concentration of maltodextrin used, the more solids will be and the yield also increases. In contrast to gum arabic which has a branched structure of short chains and high hydrophilic properties, it results in the attachment of particles to the drying wall so that the amount of yield produced does not reach 100%. [15].

Good powder humidity is in the range of 2-4%. The results obtained that the moisture content of the fatigue fraction was higher than that of the isolates. Humidity is influenced by the absorption of water in the surrounding air by the coating material. Water absorption in maltodextrin is lower because maltodextrin with low DE (Dextrose Equivalent) has a smaller number of hydrophilic groups than gum arabic [16]. The flow rate is related to the uniformity of dosage and particle size of the powder in the preparation, and a good flow rate is greater than 10g/s (Staniforth, 2002 : 207). In addition to the humidity of the microcapsules powder, the flow rate of the powder can also be affected by the humidity in the surrounding environment, high environmental humidity reduces the flowability of the powder because the powder tends to get wet and will stick to the wall and will be difficult to flow. The good solubility of microcapsule powder is up to 100%, it is related to the ability of the powder to release the active substance. In this study, solubility is strongly influenced by ambient humidity because high humidity causes the powder to tend to stick and cause the powder to be difficult to spread in water because there are no pores that are used by water molecules to wet and dissolve the powder, so the solubility does not reach 100% [17].

The efficiency of the microcapsules shows the ability of the coating material to coat/protect the active ingredients, and a good efficiency of the microcapsules is up to 100% [18]. The results showed that the efficiency of microencapsulated both fractions and isolates was close to 100%. The maltodextrin encapsulation used has a high oxidation resistance thereby increasing the efficiency of the microcapsules [19]. Gum arabic in its structure has a protein component that makes gum arabic have good emulsifying properties and can produce microcapsules that have high retention/protection level, so that the efficiency of microcapsules is also high. The morphology of the microcapsules with a magnification of 100 x and 1000 x obtained a size of 100 m with a flake-like shape, the results can be seen in Figure 1.

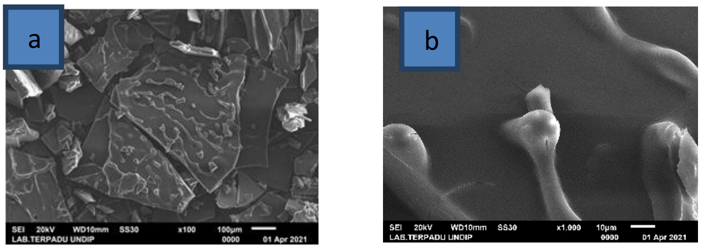

Figure 1. Mikrograf of Mikroencapsul

(Description : a. magnification of 100 x; b. magnification of 1000 x)

The results of the microcapsule test were then analyzed using the One Sample T-Test to compare the results of the PE fraction microcapsule test with the isolate results. The results of the One Sample T-Test test can be presented in table 3. Based on table 3, the experimental results of each parameter of the PE fraction test are compared with the results of the isolate test. The test results of flow rate, solubility, yield, and moisture content of the PE fraction microcapsules formula with isolates were significantly different because the significance value was < 0.05. In contrast to the results of the microcapsule efficiency test, the isolates were not significantly different because the significance value was >0.05. Grinding and sifting (micronization) on microcapsules with freeze drying is unavoidable because after drying the powder is still in the form of agglomerates and the size is not uniform, so a micronization process is needed to reduce its size to micrometer size to make the size uniform [20].

Table 3. One Sample T-Test between F1 and F2

Test	Conclusion
Flow Speed	Significantly different
Solubility	Significantly different
Yield	Significantly different
Moisture content	Significantly different
Microencapsulation efficiency	Not significant difference

IV. CONCLUSION

The results of the physical characteristics test of the PE fraction microencapsulated formulation with bixin isolate of *Bixa orellana* L. shows that the test results The test results of flow rate, solubility, yield, and moisture content are significantly different (significance value <0.05), while the microcapsule efficiency test was not significantly different.

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