Encapsulation of Soursop Leaf Extract (*Annona Muricata* L) with Xanthan Gum and Whey Protein Isolate Using Foam Mat Drying Method

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Abstract. Soursop leaves contain polyphenolic compounds, including quercetin, procyanidins, kaempferol, and catechins. These compounds have antioxidant properties that are easily oxidised and sensitive to heat and light. Encapsulation is the process of protecting the core material with a coating. Encapsulation using the foam mat drying method can be an alternative for preserving the compounds in soursop leaves. This study aims to determine the effect of xanthan gum and whey protein isolate concentration on the encapsulation characteristics of soursop leaf extract using the foam mat drying method. The experimental design was a completely randomised design (RAL) with one factor: the ratio of xanthan gum to whey protein isolate (3:1). The results showed an effect of increasing the concentration of the dressing material. The results showed that treatment P5 (xanthan gum 3.75 g: whey protein isolate 1.25 g) was the best result with 5.58% moisture content, 16.55% ash content, 90.03% solubility, 9.36% yield, 4.28 mg GAE/g encapsulated phenol, 77.79% encapsulation efficiency, and 41.24 μm diameter.

1. INTRODUCTION

Soursop (*Annona muricata* L.) is a plant whose all parts can be utilized, one of which is soursop leaves. Soursop leaves contain polyphenolic compounds, including quercetin, procyanidins, kaempferol, and catechins [1]. Phenolic compounds are secondary metabolites that are beneficial as a source of antioxidants, anti-inflammatories, anticancer, antimicrobials, and others. Antioxidants are compounds that can absorb or neutralize free radicals in the body. However, the processing of soursop leaves must be considered, as the antioxidant compounds it contains are sensitive to processing. One processing method that can maintain the compound content is encapsulation, which can extend its shelf life [2].

Encapsulation is the process of protecting the core material using a dressing material. Encapsulation is achieved by coating a core material with a dressing material. The coating material in encapsulation envelops the core material, masks unpleasant tastes and odors, and protects it from light, heat, and oxidation. Encapsulation of agricultural products has been widely applied, including dragon fruit, purple sweet potato, temulawak, rosella flowers, noni, and others [3].

Based on the above statement, encapsulation aims to control the release of core materials, such as bioactive components, cells, and enzymes derived from carbohydrates and proteins. Polysaccharides used as coatings include maltodextrin, gum arabic, pectin, carrageenan,

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alginate, chitosan, and xanthan gum. Xanthan gum is an extracellular polysaccharide produced from the secretion of the gram-negative bacterium *Xanthomonas* campestris. This is also support, which states that xanthan gum is one of the hydrocolloids used as a binder because it has good viscosity at low concentrations. The ability to bind xanthan gum to the core material will protect the compound or core material [4]. In addition to carbohydrates, proteins also have an important role as a coating in the encapsulation process. Protein acts as an emulsifier and promotes good gel formation. Whey protein isolate (WPI) is widely used in the food industry to improve food quality due to its good gelation, emulsifying, and foaming properties. Whey protein has a high encapsulation efficiency (89.6%) compared to soy protein (75.9%). Proteins and phenolic compounds can interact and improve protein solubility, thermal stability, digestibility, antioxidant activity, foaming, and emulsifying [5]. The combination of xanthan gum and WPI is expected to provide better encapsulation than either ingredient alone.

The encapsulation process can be done by coacervation, co-crystallization, spray drying, spray cooling, fluidized bed coating, extrusion, and freeze drying. Foam mat drying can be an option for the encapsulation method that will be used in this study. Foam mat drying is a method of drying a sample by foaming it with a foaming agent. The materials dried with foam mat drying retain compounds better than those that are not. This is also in accordance with the purpose of encapsulation, which is to protect the bioactive components of soursop leaves. stated that bioactive components such as flavonoids, tannins, and phenols are damaged above 50 ^{o}C [6].

Several studies on the encapsulation of soursop leaf extract have been conducted by [7] using gum arabic and maltodextrin via spray drying, and using dextrin and casein via vacuum drying. The novelty of this research lies in the encapsulation of soursop leaf extract (*Annona muricata L.*) using the foam mat drying method with xanthan gum and whey protein. The purpose of this study is to determine the effect of the concentrations of gum xanthan and whey protein isolate on the characteristics of soursop leaf extract encapsulated using the foam mat drying method.

2. METHODOLOGY

2.1 Tools and Materials

The tools used for soursop leaf extraction were analytical scales, *Buchner*, *rotary evaporator*, *mixer*, *food dehydrator*, oven, analytical balance (Ohaus), furnace, desiccator, Whatman No. one filter paper, centrifuge, measuring pipette, pipette filler, vortex, cuvette, UV-VIS spectrophotometer (BEL Photonic), and light digital microscope (Jenco).

The materials needed for soursop leaf extraction are soursop leaf powder (UPT. Materia Medica Batu), and 85% methanol. Materials required for encapsulation were xanthan gum, whey protein isolate. The materials needed for analysis were Folin-Ciocalteu solution, gallic acid, distilled water, and Na_2CO_3.

2.2 Research Methods

This study used a completely randomized design (CRD) with 1 factor. The factor used was the percentage of encapsulant addition. The sample formulation is shown in Table 1. After that, the analysis of water content, ash content, yield, solubility, phenolic content of the soursop leaf extract, phenolic content of the product, encapsulation efficiency, and diameter measurement will be conducted. The data obtained were processed using one-way ANOVA (analysis of variance), and the results were followed by Duncan's Multiple Range Test (DMRT) at a significance level of $p = 0.05$.

The formulation of encapsulated ingredients with a dressing material ratio of 3:1 (xanthan gum and whey protein isolate) is shown in Table 1.

Table 1. Formulation of soursop leaf extract encapsulate ingredients

Treatment	Gum Xanthan (g)	Whey Protein Isolate (g)
P1 (1%)	0,75	0,25
P2 (2%)	1,50	0,50
P3 (3%)	2,25	0,75
P4 (4%)	3,00	1,00
P5 (5%)	3,75	1,25

2.3 Research Procedures

2.3.1 Preparation of Soursop Leaf Extract

Soursop leaf powder was sieved, then mixed with 85% methanol (1000 ml) at a 1:10 ratio. After homogenization, the cells were macerated for 24 hours at room temperature. The obtained macerate was concentrated using a rotary evaporator at 40 ℃, 200 mbar, and 70 rpm [25], with modifications.

2.3.2 Encapsulation of Soursop Leaf Extract

100 ml Soursop leaf extract was mixed with xanthan gum and whey protein isolate with concentrations of 1%, 2%, 3%, 4%, and 5% using a mixer for 5 minutes. After that, it was dried with a food dehydrator at 40 ºC for 24 hours. Then, it was mashed with a mortar and pestle and sifted through a 50-mesh sieve [8], with modifications.

2.4 Testing Analysis

In this study, there were six tests. These tests include testing water content [9], ash content [10], yield [11], solubility [12], phenol content of soursop leaf extract [13], encapsulated phenol content [14], encapsulation efficiency [15], and diameter measurement [15].

3. RESULTS AND DISCUSSION

3.1 Phenols of Soursop Leaf Extract

Based on raw material testing, soursop leaf extract with methanol solvent contains 5.51 mg GAE/g of phenol. The phenol content in this study differs, which found that the methanol extract of soursop leaves was 7.179 mg GAE/g. The difference in phenol content may be due to differences in leaf age at harvest. Soursop leaf tea with a younger age contains higher phenol levels than those in older soursop leaf tea. Phenolic compounds are metabolite products in plants that act as plant self-defense mechanisms against the environment. The production of new secondary metabolites in plants decreases or stops as leaves age [16].

3.2 Water Content

Based on the analysis of variance, the addition of xanthan gum concentration and whey protein isolate significantly affected the encapsulated water content. The results of the water content analysis are shown in Table 2.

Table 2. Water Content Analysis Results Encapsulate

Treatment	Moisture Content (%)
P1 (XG 0,75:WPI 0,25)	$16,38 \pm 0,62^{e}$
P2 (XG 1,50:WPI 0,50)	$13,45 \pm 0,84^{d}$
P3 (XG 2,25:WPI 0,75)	$10,59 \pm 1,11^{c}$
P4 (XG 3,00:WPI 1,00)	$8,20 \pm 0,74^{b}$
P5 (XG 3,75:WPI 1,25)	$5,58 \pm 0,25^{a}$

Notes: Numbers followed by the same letter in the same column indicate results that are not significantly different ($p>0.05$).

Table 2 presents the results of the water content analysis, which indicate a significant difference. The higher concentration of xanthan gum and whey protein isolate will reduce the water content. This can occur because xanthan gum and whey protein isolate bind water, so the water content decreases as concentration increases. This is higher than the research by [17], which reported water content of 6.26% to 7.88%. The moisture content of the encapsulate is influenced by the raw material and the dressing material (xanthan gum and whey protein isolate). The water content of soursop leaves in this study was 6.87%. The moisture content contained in xanthan gum is 13-15%, while the moisture content in whey protein isolate is 7.81%. The decrease in water content in this study was influenced by the nature of xanthan gum, which binds water. The ability of xanthan gum to bind water is $32,300 \pm 1,100$ g H 2O/100 g solid [41]. Xanthan gum can retain up to 19.2 g water/100 g hydrocolloid. The ability of xanthan gum to bind water will reduce the volume and evaporation of water content, thereby reducing the amount of free water that evaporates. The water-holding capacity of whey protein isolate reached 3.83 g water/g protein. With the water-binding ability of whey protein isolate, the more whey protein that is added, the more water is bound by the protein, thus reducing the water content [18].

3.3 Ash content

Based on the results of analysis of variance, the addition of xanthan gum concentration and whey protein isolate significantly affected the ash content of the encapsulate. The results of the ash content analysis are shown in Table 3.

Table 3. Results of Ash Content Analysis Encapsulate

Treatment	**Ash Content (%)**
P1 (XG 0,75:WPI 0,25)	$7,55 \pm 0,01^{a}$
P2 (XG 1,50:WPI 0,50)	$9,96 \pm 0,07^{b}$
P3 (XG 2,25:WPI 0,75)	$12,04 \pm 0,06^{c}$
P4 (XG 3,00:WPI 1,00)	$14,22 \pm 0,04^{d}$
P5 (XG 3,75:WPI 1,25)	16.55 ± 0.15^{e}

Notes: Numbers followed by the same letter in the same column indicate results that are not significantly different ($p>0.05$).

Ash content indicates the total mineral content of food ingredients [19]. Table 3 shows the results of the water content analysis: the P5 treatment had the highest ash content of 16.55%, while the P1 treatment had the lowest ash content of 7.55%. The higher the concentration of xanthan gum and whey protein isolate, the higher the ash content. The ash content contained in soursop leaf powder is 5.60%. In addition to the raw materials, the dressing materials (xanthan gum and whey protein isolate) also affect the ash content of the encapsulate. The greater the addition of xanthan gum, the greater the ash content. The increase in ash content is directly proportional to the concentration of added xanthan gum. According to [20], the ash

content of xanthan gum is 7-12%. Meanwhile, the ash content contained in whey protein isolate is 4.5-6%.

3.4 Solubility

Based on the results of analysis of variance, the addition of xanthan gum concentration and whey protein isolate significantly affected the solubility of the encapsulate. Solubility analysis results are shown in Table 4.

Table 4. Solubility Analysis Results Encapsulate

Treatment	Solubility (%)
P1 (XG 0,75:WPI 0,25)	42,26 ± 1,663[a]
P2 (XG 1,50:WPI 0,50)	53,15 ± 6,483[b]
P3 (XG 2,25:WPI 0,75)	66,73 ± 2,383[c]
P4 (XG 3,00:WPI 1,00)	76,85 ± 5,252[d]
P5 (XG 3,75:WPI 1,25)	90,03 ± 2,153[e]

Notes: Numbers followed by the same letter in the same column indicate results that are not significantly different (p>0.05).

Table 4 shows the results of the encapsulation solubility analysis. In treatment P5, the highest solubility was 90.03%, and in treatment P1, the lowest was 42.26%. The higher the concentration of xanthan gum and whey protein isolate, the greater the solubility. This happens because the more encapsulant material there is, the greater the solubility. Xanthan gum is hydrophilic and soluble at low and high temperatures. Also, xanthan gum can absorb water quickly, so it readily interacts with water and dissolves readily. In addition to xanthan gum, whey protein isolate helps increase encapsulation solubility. Microcapsules with more whey protein isolate formulations will increase the solubility of microcapsules. Whey protein isolate can accelerate the dissolution of microencapsules because whey protein isolate is a good emulsifier by helping the fusion of substances in suspension in both the water and oil phases. Good microencapsulation results in high water solubility [21].

3.5 Yield

Based on the results of analysis of variance, the addition of xanthan gum concentration and whey protein isolate significantly affected the encapsulate yield. The results of the yield analysis are shown in Table 5.

Table 5. Yield Analysis Results Encapsulate

Treatment	Yield (%)
P1 (XG 0,75:WPI 0,25)	4,03 ± 0,133[a]
P2 (XG 1,50:WPI 0,50)	5,30 ± 0,671[b]
P3 (XG 2,25:WPI 0,75)	6,64 ± 0,285[c]
P4 (XG 3,00:WPI 1,00)	7,96 ± 0,991[d]
P5 (XG 3,75:WPI 1,25)	9,36 ± 0,558[e]

Notes: Numbers followed by the same letter in the same column indicate results that are not significantly different (p>0.05).

Table 5 shows the results of the yield analysis: treatment P5 obtained the highest yield of 9.36%, and P1 the lowest of 4.03%. This can occur because adding the concentration of the dressing material increases the mass and yield produced. This is higher than the yield of 0.35% reported in the research on nanoencapsulates with xanthan gum. The encapsulated yield is influenced by the raw material and the encapsulant material (xanthan gum and whey protein

isolate). The yield of soursop leaf methanol extract is 8.30%. This is corroborated by the statement of [22] that the more total solids in the dried material, the higher the yield.

3.6 Phenols Encapsulate

The analysis of variance showed that the addition of xanthan gum concentration and whey protein isolate had a significant effect on phenol encapsulation. The results of the phenol analysis of the product are shown in Table 6.

Table 6. Phenol Analysis Results Encapsulate

Treatment	Phenol (mg Gae/g)	Reduction (%)
P1 (XG 0,75:WPI 0,25)	$1,03 \pm 0,125^{a}$	80
P2 (XG 1,50:WPI 0,50)	$1,63 \pm 0,020^{b}$	70
P3 (XG 2,25:WPI 0,75)	$2,03 \pm 0,125^{b}$	62
P4 (XG 3,00:WPI 1,00)	$3,35 \pm 0,342^{c}$	38
P5 (XG 3,75:WPI 1,25)	$4,28 \pm 0,528^{d}$	21

Notes: Numbers followed by the same letter in the same column indicate results that are not significantly different ($p>0.05$).

Table 6 shows the highest reduction in treatment P1 at 80%. The reduction of phenol extract could be due to the wind in the food dehydrator. The principle of the food dehydrator is that a heat element distributes hot air through the wind. The reduction in phenol compounds may be due to the heat generated by the wind in the food dehydrator. Based on the phenol analysis results, the P5 treatment showed the highest phenol content of 4.38 mg GAE/g, and the P1 treatment showed the lowest phenol content of 1.03 mg GAE/g. The higher the concentration of xanthan gum and whey protein isolate, the higher the phenol content. This can occur because xanthan gum and whey protein isolate can protect phenols in soursop leaf extract. This is higher than the research by [23], which reported phenol levels of 0.55 to 0.78 mg GAE/g.

The difference in phenol content can be due to differences in the encapsulant material. Xanthan gum has a helical structure formed by mannose and glucoronic acid groups, which protect the main chain of glucose. The helix structure of xanthan gum makes the xanthan gum solution stable at high and low PH and temperature, also resistant to degradation. So that the helix structure of xanthan gum can protect the core material. In addition to xanthan gum, whey protein isolate is also able to protect the core material. Whey protein isolate easily forms a film that will protect the core material and increase emulsion power.

3.7 Encapsulation Efficiency

Based on the results of analysis of variance, the addition of xanthan gum concentration and whey protein isolate significantly influenced the encapsulation efficiency. The results of the encapsulation efficiency analysis are shown in Table 7.

Table 7. Results of Encapsulation Efficiency Analysis

Treatment	Encapsulation Efficiency (%)
P1 (XG 0,75:WPI 0,25)	$18,76 \pm 2,216^{a}$
P2 (XG 1,50:WPI 0,50)	$29,51 \pm 0,340^{b}$
P3 (XG 2,25:WPI 0,75)	$36,89 \pm 2,275^{b}$
P4 (XG 3,00:WPI 1,00)	$60,90 \pm 6,247^{c}$
P5 (XG 3,75:WPI 1,25)	$77,79 \pm 9,588^{d}$

Notes: Numbers followed by the same letter in the same column indicate results that are not significantly different ($p>0.05$).

Encapsulation efficiency is used to assess the success of the encapsulation process. Encapsulation efficiency is the percentage of the compound protected during encapsulation. \This can occur because xanthan gum and whey protein isolate are able to protect phenols in soursop leaf extract. This is in line with the research used xanthan gum dressing material with an encapsulation efficiency of 76.75-93%. Xanthan gum forms a strong gel even in low concentrations, so it is able to act as a protector. Thus, the gel structure will limit compounds with other factors that can degrade. Xanthan gum and whey protein isolate are suitable binders to be combined. The combination of polysaccharides and proteins can improve emulsion stability and better protect the encapsulated core material. Bioactive components can be well encapsulated by proteins. Proteins are able to package or protect compounds due to their gelling properties that will form nanoparticles for bioactive compounds. In addition to gel formation, the ability to form foam by whey protein isolates can also protect the core material. This is in accordance with the research of [24], which states that the foam formed from WPI can protect the core material. The combination of polysaccharides and proteins, proteins act as emulsifiers and film formers, while polysaccharides act as a protective matrix so that the core is not easily degraded.

3.8 Encapsulate Diameter

The results of the diameter measurement analysis showed that increasing the concentration of xanthan gum and whey protein isolate decreased the size of the encapsulate diameter. The results of the diameter analysis can be observed in Table 8.

Table 8. Diameter Analysis Results Encapsulate

Treatment	**Diameter Size** (μm)
P1 (XG 0,75:WPI 0,25)	124,71
P2 (XG 1,50:WPI 0,50)	68,49
P3 (XG 2,25:WPI 0,75)	66,41
P4 (XG 3,00:WPI 1,00)	46,74
P5 (XG 3,75:WPI 1,25)	41,24

Table 8 shows the results of particle measurements in P1 of 124.71 μm, P2 of 68.49 μm, P3 of 66.41 μm, P4 of 46.74 μm, and P5 of 41.24 μm. Visualization of diameter measurements can be seen in Appendix 4. Capsule particles are classified into three sizes: macrocapsules >5,000 μm, microcapsules between 02-5,000 μm, and nanocapsules <0.2 μm. The higher concentration of xanthan gum and whey protein isolate will decrease the diameter. This is inversely proportional to the research of [25] that xanthan gum and whey protein isolate increase the diameter of microencapsulation. This difference could be due to an increase in the concentration of the dressing material. The dressing material wraps the core material more closely, so the resulting diameter decreases. Proteins are able to package or protect compounds due to their gelling properties that will form nanoparticles to protect bioactive compounds. In addition to the dressing material, this difference could be due to the water content of the encapsulate. High water content causes clumping or agglomeration of particles, resulting in microcapsules with larger particles. This is in line with the research of [26] on the microencapsulation of kenikir leaves, which showed a smaller particle size with increased dressing material. In the study, the 1:10 dressing material had a particle size of 1.55 μm, while the 1:20 dressing material had a particle size of 1.45 μm.

4. CONCLUSIONS

The results showed that the addition of gum xanthan and whey protein isolate had a significant effect ($p < 0.05$) on moisture content, ash content, capsule solubility, yield, encapsulated

phenolics, and encapsulation efficiency. The best treatment was encapsulated in P5. The best treatment resulted in 5.58% moisture content, 16.55% ash content, 90.03% solubility, 9.36% yield, 4.28 mg GAE/g encapsulated phenolics, 77.79% encapsulation efficiency, and 41.24 µm diameter. This study achieves better encapsulation results than previous studies, but experiments should be conducted on other encapsulation methods.

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