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Article

Effect of Whey Protein Isolate (WPI)–Pectin Ratio on Phenolic Content Stability of Propolis Microcapsules

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Abstract: Propolis is known to have many benefits, especially in the health sector. Due to the sharp aroma & taste, and the phenolic compounds contained in propolis are easily oxidized & hydrolyzed at high temperatures, the application of propolis in the food sector is still lacking. Microencapsulation with a coacervation complex is used to overcome these problems. The thickness of the coating material is an important factor in the microencapsulation of complex coacervation. This study aimed to determine the coating ratio used to produce propolis microcapsules with the highest phenolic stability during storage and heating. This study consisted of three treatment ratios of whey protein isolate (WPI) & pectin coating materials 1:2, 1:1, and 2:1. The results showed that the yield value ranged from 37.279–40.116%, aw 0.375–0.529, and encapsulation efficiency 85.45–96.44%. The color of the microcapsules produced was bright ($L^* = 94.28\text{--}95.35\%$), tended to be reddish ($a^* = 0.07\text{--}0.11$) & yellowish ($b^* = 4.36\text{--}4.83$). The difference in the ratios treatment significantly affected the storage stability, while the heating stability ratio only significantly affected the 2:1 treatment. The best total phenolic stability of propolis microcapsules during storage & heating was obtained by 1:1 treatment with retention values of 97.31% and 63.86%.

Keywords: WPI; pectin; coacervation; microcapsules; propolis

1. Introduction

Propolis is a natural resin material commonly used as a glue or adhesive for beehives and is collected from various parts of plants from sprouts to buds [1]. Propolis is known to have many benefits, especially in the health sector such as preventing and treating flu, scars, diabetes, dental caries, and others. This is motivated by propolis's content, which have more than 300 bioactive compounds, including flavonoids, terpenoids, phenolics, hydrocarbons, minerals, coumarins, and other compounds [2].

The application of propolis in the food industry has disadvantages, such as sharp aroma and taste, and propolis extraction can only be soluble in alcohol solvents [3]. In addition, propolis's phenolic compounds are highly sensitive, such as easily oxidized and easily hydrolyzed at high temperatures [4], and have low physicochemical stability and are limited in bioaccessibility and bioavailability [5]. Therefore, further processing is needed to maintain propolis's phenolic content and expand propolis products' application in the food industry.

Propolis extract can be encapsulated by complex coacervation methods and spray drying techniques; it can be an alternative to reduce some of the shortcomings mentioned. Microencapsulation of propolis with the spray drying technique can reduce unwanted taste and aroma and protect the bioactive compounds contained in propolis [1]. The complex coacervation method produces microcapsules with high encapsulation efficiency values [6]. Coating materials commonly used in encapsulation methods come from polymers of carbohydrates, proteins, lipids, gums, or others. The coating materials used are whey protein isolate (WPI) and high methoxyl pectin (HMP). The use of ingredients of protein and polysaccharides to complement each other as coating

materials, where polysaccharides have low emulsifying properties while proteins have good emulsion stability [7]. The proper mixing ratio and the total concentration of biopolymers resulted in a stable charge in the solution so that the encapsulation results were maximized and had a good encapsulation efficiency value [8].

The study was conducted regarding the effect of the ratio of whey protein isolate (WPI) - pectin on the total phenolic stability of propolis microcapsules to determine the best ratio in optimizing the use of heating and storage temperatures to maintain the quality of phenolic in propolis microcapsules.

2. Materials and Methods

2.1. Materials

Propolis microcapsules were made using propolis extract (12.5% wb water content), commercial high methoxyl pectin, commercial whey protein isolate, maltodextrin DE 18, 1M HCL 37%, 0.1M NaOH, and distilled water. The propolis used comes from bees (*Trigona sp.*) South Sulawesi. Sodium Chloride (NaCl) for testing water activity. Glacial acetic acid, gallic acid, ethanol 96%, methanol, Na₂CO₃ 7.5%, and Folin-Ciocalteu reagent for the encapsulation efficiency.

2.2. Preparation of Propolis Extracts

The propolis extraction process is prepared by following the modification method [1]. 168 grams of crude propolis was added with 1200 mL of 70% ethanol solution and macerated for 48 hours. The solutions were filtered and stored in the freezer at -20 °C for 10 hours. After that, centrifugation (Hermle Z306) was carried out for 2 x 10 minutes at a temperature of 10 °C at a speed of 4500 rpm. The centrifuged supernatant was then evaporated using a rotary vacuum evaporator (IKA RV 10) at a temperature of 60 °C.

2.3. Microencapsulation Process

2.3.1. Carrier Preparation

Carrier preparation is prepared by following the modification method [3], the solution of whey protein isolate (0.025 - 0.05%), high methoxyl pectin (0.025 - 0.05%), and maltodextrin (75%) were done by dissolving several concentrations of the material with distilled water and adjusting to the treatment of a predetermined coating ratio. The whey protein isolate (WPI) solution was adjusted to a final pH of 7.8.

2.3.2. Microencapsulation

Propolis microcapsules are prepared by following the modification method [9]. Propolis extract was added to whey protein isolate and then homogenized using a homogenizer (D-Lab 100), then a high methoxyl pectin solution was added. The homogeneous solution was adjusted to the final pH of 3.75, and the maltodextrin solution was added. The final solution was stored at 4 °C for 24 hours.

The powder formulation was carried out using a spray dryer (Buchi Mini Spray Dryer B-290) and set at 150 °C for inlet temperature, and the outlet temperature was monitored at 70-80 °C, aspiration at 90%, and pump rate at 25%.

2.4. Microcapsules Characterization

2.4.1. Encapsulation Yield

Testing the yield of propolis microcapsules prepared by the following method [10]. The propolis extract, the coating material used, and the final product of the propolis microcapsules were weighed, then the yield was calculated using the formula:

$$\text{Yield (\%)} = \frac{\text{weight of encapsulated propolis (g)}}{\text{weight of propolis extract and coating agent (g)}} \times 100 \quad (1)$$

2.4.2. Water Activity (a_w)

The a_w value is determined by the following method [1], using the a_w meter (Aqualab LITE). Before use, the instrument was calibrated using a salt solution of NaCl. The sample was tested at room temperature.

2.4.3. Color Properties

Color properties on propolis microcapsules were prepared by the following method [11], using a colorimeter (Spectrophotometer CM-5). The samples were put into a glass cup, and then the samples were tested. The data results show the values of L^* (transparency), a^* (redness), and b^* (yellowness).

2.4.4. Encapsulation Efficiency

Encapsulation efficiency prepared by the following modification method was carried out by calculating the total phenolic in microcapsules (TPC) and surface phenolic microcapsules (SPC) [12]. The total phenolic test in microcapsules (TPC) was done by dissolving propolis microcapsules with ethanol/acetic acid/aquades (50:8:42) mixed reagent in the same ratio (1:1). Microcapsule phenolic surface (SPC) testing was carried out in the same way with different solvents, namely ethanol/methanol mix reagent (50:50).

Samples of propolis microcapsules (600 mg) were dissolved with a reagent mix. An aliquot of 0.5 mL sample was mixed with 0.5 mL of Folin-Ciocalteu reagent and 4 mL of 7.5% Na_2CO_3 . After 30 minutes of incubation, the absorbances were measured at 760 nm using a UV spectrophotometer (UV-9200). Gallic acid was used as the standard.

The yield encapsulation efficiency is calculated using the formula:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{TPC} - \text{SPC}}{\text{TPC}} \times 100 \quad (2)$$

2.4.5. Scanning Electron Microscopy (SEM)

The morphology of microcapsules was evaluated by scanning electronic microscopy or SEM (Hitachi TM 3000) to identify the surface morphology of the dry solid microcapsules [10]. Microcapsule powder is spread as thin as possible, the sample is put into the chamber, and an image is taken at a particular magnification.

2.5. Stability of Microcapsules

2.5.1. Storage Stability

The storage stability test was prepared by the following modification method [13]. Propolis microcapsules were stored in glass vials at room temperature ($\pm 25^\circ\text{C}$) for 28 days and tested every 7 days (0, 7, 14, 21, 28).

2.5.2. Heating Stability

The heating stability test was prepared by the following modification method [14]. Propolis microcapsules were stored in test tubes and heated in a water bath at 80°C for 3 hours. Tests were carried out at every 1-hour range (0, 1, 2, 3).

2.6. Statistical Analysis

The results of the data were analyzed using SPSS 24 software. The data displayed \pm standard deviation. The hypothesis analysis test used one-way ANOVA (Analysis of Variance) to determine significant differences between treatments with a 95% confidence level ($\alpha = 0.05$). The follow-up test used to see the difference between treatments was carried out by the Tukey test.

3. Results

3.1. Microencapsulation Characterization

3.1.1. Encapsulation Yield

Based on the results in Figure 1, the average yield of propolis microcapsules ranged from 37.279-40.116%. The yield amount of microcapsule above 50% is considered good [15,16]. Treatment C, or at a higher WPI ratio than pectin, was the best treatment to produce the highest yield value, while treatment B was the lowest treatment to produce yield compared to other treatments. The resulting study is in line where the optimum ratio of WPI and pectin complex formation is at 2:1, along with a decrease in pH from 3 to 4 [17]. The lower the concentration of polysaccharides and proteins used, the lower the yield of coacervate produced [18].

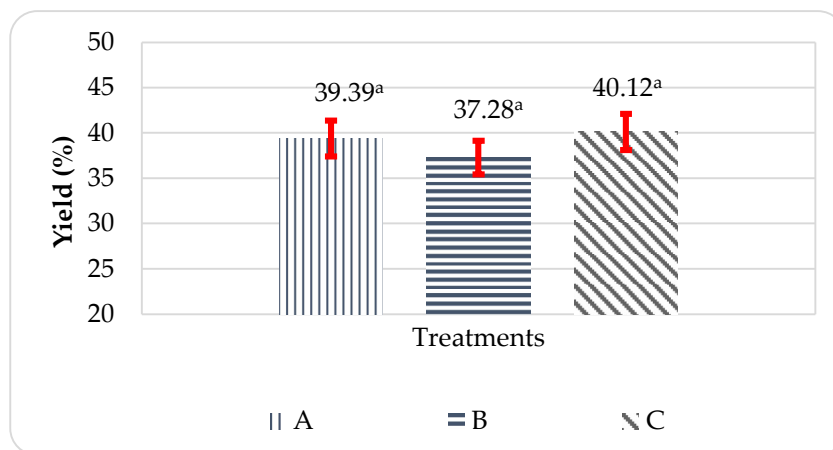


Figure 1. Average Yield of Propolis Microcapsules.

The results of the statistical analysis of variance (ANOVA) showed that the yield of propolis microcapsules had a $P_{\text{value}} > 0.05$, which means that there is no significant difference between the ratio to the yield value of the propolis microcapsules produced.

3.1.2. Water Activity (a_w)

Figure 2 displays the a_w value of propolis microcapsules can be seen in Figure 2, where the average a_w of propolis microcapsules is in the range of 0.375-0.529. The overall a_w value produced by propolis microcapsules can be good because foods with an a_w value < 0.60 have stable microbiological activity [19]. The a_w value of the propolis microcapsules tends to be low due to the presence of hydrocolloid materials such as pectin as a coating material. Gel formation in pectin is caused by hydrogen bonds between free carboxyl groups and between hydroxyl groups with water [20]. It causes a decrease in food in free water, inhibiting microbial growth. The decrease in the a_w value was accompanied by an increase in the concentration of the hydrocolloid used [21]

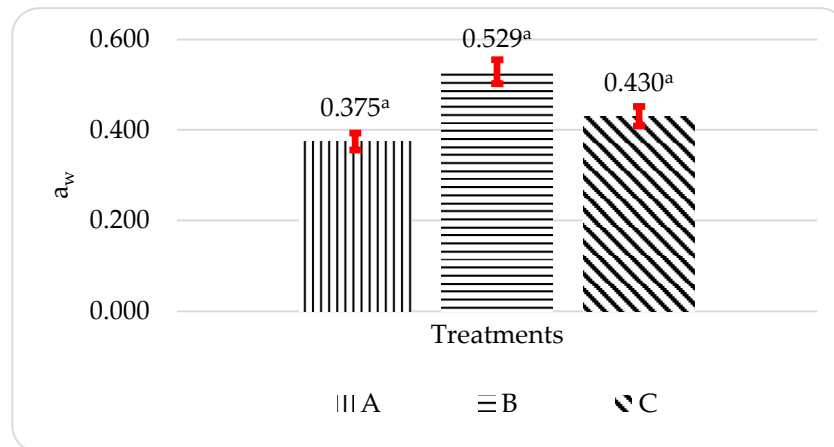


Figure 2. Average Water Activity (a_w) of Propolis Microcapsules.

The results of the statistical analysis of variance (ANOVA) showed that the a_w of propolis microcapsules had a $P_{\text{value}} > 0.05$, which means that there is no significant difference between the ratio to the a_w value of the propolis microcapsules produced.

3.1.3. Color Properties

Table 1 describes the color properties of propolis microcapsules. All propolis microcapsules had a high brightness level, as shown by the L^* value ranging from 94.28-95.36. The formation of insoluble complexes can cause a high L^* value during the coacervation of the solution. The insoluble complex shows the solution's charge balance at the coacervation time [22]. The L^* value decreased as the pectin ratio increased, pectin solution causes reduced the L^* value [23]. Propolis microcapsules also tend to be reddish and yellowish, as indicated by the positive a^* and b^* values. The color can be caused by the content of propolis extract in the microcapsules. The positive values of a^* and b^* can also be caused by the high outlet temperature during drying. An increase in outlet temperature can cause an increase in reddish and yellowish color [24].

Table 1. Color Properties of Propolis Microcapsules.

Treatment	Average Color Properties Value of Propolis Microcapsules		
	$L^*(D65)$	$a^*(D65)$	$b^*(D65)$
A	94.28 ± 0.701	0.10 ± 0.081	4.36 ± 0.275
B	95.34 ± 0.237	0.11 ± 0.075	4.83 ± 0.508
C	95.35 ± 0.429	0.07 ± 0.045	4.58 ± 0.232

L^* : Lightness; a^* : redness-greenness; b^* : yellowness-blueness. ¹ Values are means \pm standard deviation.

3.1.4. Encapsulation Efficiency

The average encapsulation efficiency of propolis microcapsules ranged from 85.45-96.44%, as shown in Figure 3. Treatment B, or the ratio of WPI and pectin 1:1 was the best treatment for the value of encapsulation efficiency compared to other treatments. Decreasing the whey protein ratio in some carbohydrate coatings could increase the value of microencapsulated encapsulation efficiency [25]. The high encapsulation efficiency value of the propolis microcapsules was due to the polysaccharide-protein interaction between WPI and pectin. The addition of pectin to form the polysaccharide-protein complexes can increase protection and protect proteins that can be denatured during spray drying [26,27].

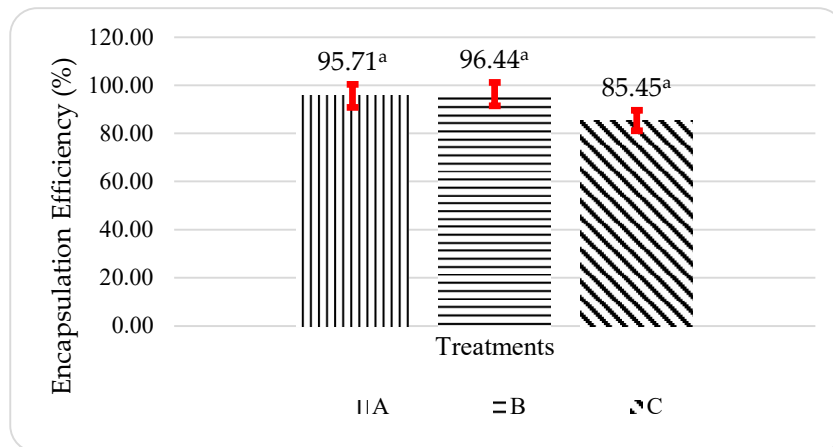


Figure 3. Average Efficiency of Propolis Microcapsule Encapsulation.

The results of the statistical analysis of variance (ANOVA) showed that the encapsulation efficiency of propolis microcapsules had a $P_{\text{value}} > 0.05$, which means that there is no significant difference between the ratio to the encapsulation efficiency value of the propolis microcapsules produced.

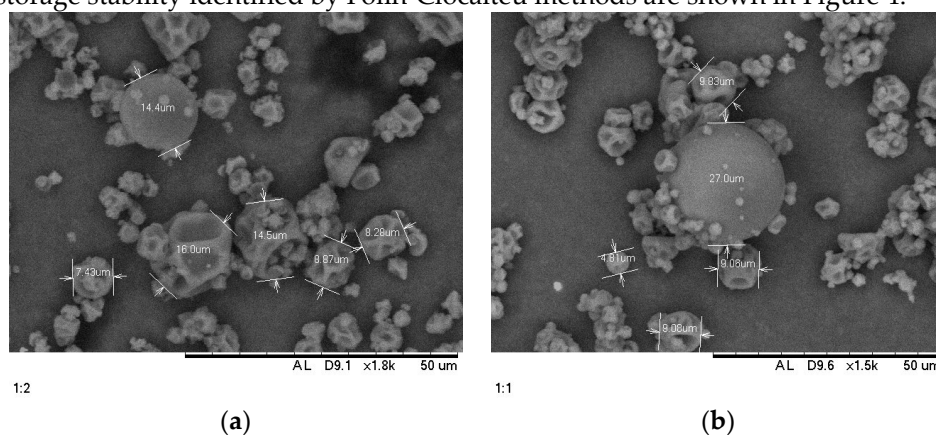
3.1.5. Scanning Electron Microscope (SEM)

The results showed that the microcapsule structure of propolis at several magnifications showed that most of the particles in each ratio treatment were generally round shapes and irregular surfaces with sizes ranging from 30-50 μm . Irregular surfaces can be caused by rapid evaporation during the spray drying process, and the use of high temperatures can also affect the characteristics of the resulting microcapsules [10], [28]. Protein as a wall material using the spray drying technique has the characteristics of a rough surface and aggregates of microcapsules [29]. The interaction between protein and polysaccharides can affect the surface of the microcapsules and can act as a plasticizer to produce a smoother surface [30].

3.2. Stability of Microcapsules

3.2.1. Storage Stability

The storage stability identified by Folin-Ciocalteu methods are shown in Figure 4.



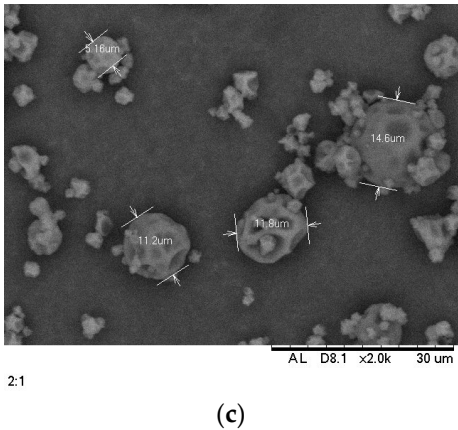


Figure 4. Microphotographs of propolis microcapsules: (a) WPI:HMP (1:2) at 1800x magnification (b) WPI:HMP (1:1) at 1500x magnification (c) WPI:HMP (2:1) at 2000x magnification.

Treatment B showed no drastic decrease in the total phenolic content during the storage period, indicated by the total phenolic retention value of propolis microcapsules which reached 97.31%. The decrease in the total phenolic content in propolis microcapsules could be caused by the degradation of polyphenol compounds [31]. Treatment A showed a decrease in total phenolic content in the first 2 weeks of storage and tended to be stable for the rest of the storage period. Although there was a decrease, treatment A can be categorized as relatively stable compared to treatment C. Adding polysaccharides such as pectin can maintain phenolic compounds such as anthocyanins and increase antioxidant activity [32]. In line with this statement, the total phenolic retention of propolis microcapsules in treatment A reached 82.18%.

The stability of the total phenolic content of propolis microcapsules in treatment C decreased in the first week and then increased until the end of the storage period. The increase in total phenolic compounds could be due to oxidized polyphenol reactions and the formation of new compounds, where these compounds interacted with the Folin-Ciocalteu reagent, which increased total phenolic content. The method of determining the total phenolic content with the Folin-Ciocalteu reagent is not specific because the reagent can be influenced by reducing sugars, ascorbic acid, organic acids, and other compounds [33]. Overall, storage at room temperature did not affect the stability of the propolis microcapsules for treatments A and B.

The results of the statistical analysis of variance (ANOVA) showed that the total phenolic stability parameter of propolis microcapsules during storage had a $P_{value} < 0.05$, which means that there is a significant difference between the ratio to the total phenolic stability during storage.

3.2.2. Heating Stability

The heating stability identified by Folin-Ciocalteu methods are shown in Figure 5.

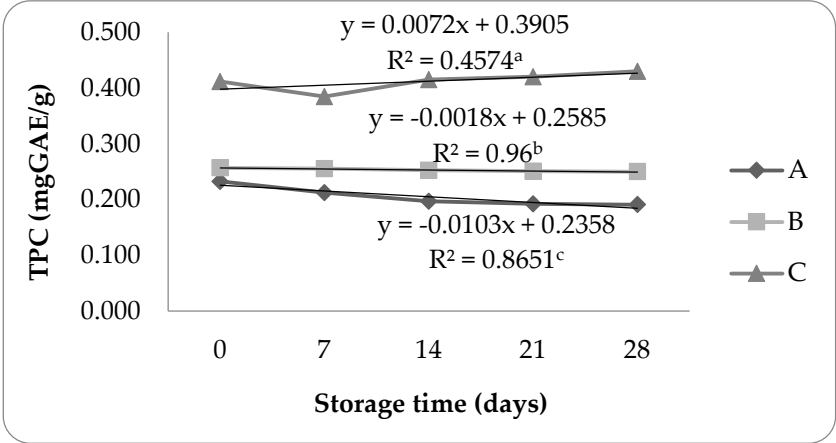
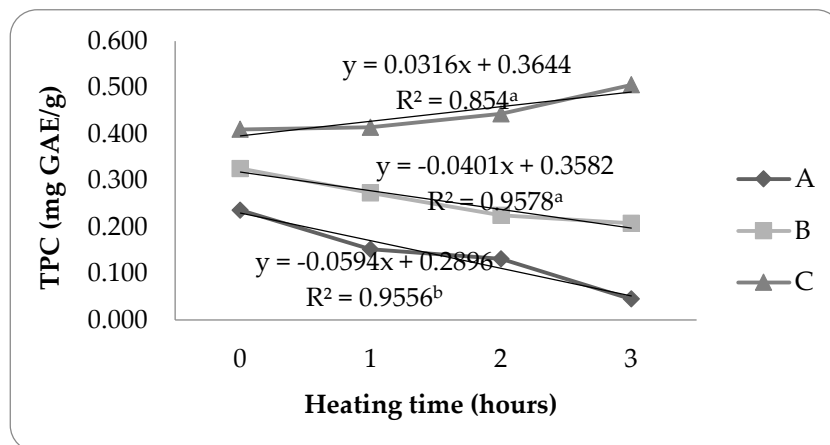


Figure 5. Total Phenolic Stability Rate During Propolis Microcapsule Storage.**Figure 6.** Total Phenolic Stability Rate During Heating Propolis Microcapsules.

Treatment B tends to decrease the total phenolic content during heating, indicated by the total phenolic retention value of propolis microcapsules produced by 63.86%. Treatment B had higher heating stability than treatments A and C, stability increase accompanied by reducing the use of the WPI ratio. The results are in line who stated that increasing the WPI ratio can reduce the total phenolic content due to its easily denatured nature [34].

Treatment A showed a drastic decrease in the total phenolic content during 3 hours of heating. The total phenolic value content drastically decreased, resulting in the total phenolic of propolis microcapsule retention being very low, which only reached 19.07%. Meanwhile, the stability of the total phenolic content of propolis microcapsules in treatment C increased with the length of heating time. Whey protein has the main component of B-lactoglobulin these components can denature at temperatures above 70°C [35].

The results of the statistical analysis of variance (ANOVA) showed that the total phenolic stability parameter of propolis microcapsules during heating had a $P_{\text{value}} < 0.05$, which means that there was a significant difference between the ratio to the total phenolic stability during heating.

4. Conclusions

The ratio of whey protein isolate (WPI) – pectin as wall materials did not significantly affect the physical characteristics of the propolis microcapsules. The different wall materials ratio of propolis microcapsules resulted in high encapsulation efficiency (85.45 – 96.44%), yield values tended to be low (37.279 – 40.116), and microbiology stable aw values (0.375 – 0.529). While on stability, the ratio of whey protein isolate (WPI) – pectin had a significant effect on the physical characteristics of the propolis microcapsules produced. Treatment A resulted in good stability of propolis microcapsules with total phenolic retention of propolis microcapsules during storage for 28 days (97.31%) and heating for 3 hours at 80°C (63.86%).

Author Contributions: Conceptualization, methodology, N.S.; supervision, project administration, N.S, M.J.L., Y.C. and M.H; data curation, writing—original draft preparation, writing—review and editing, N.S and D.S.A. All authors have read and agreed to the published version of the manuscript.

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