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THE DEVELOPMENT, EVALUATION, AND ANTIOXIDANT ACTIVITY ANALYSIS OF CHITOSAN MICROCAPSULES CONTAINING RED GINGER OLEORESIN WITH SODIUM TRIPOLYPHOSPHATE PREPARED BY EMULSION CROSS-LINKING TECHNIQUE

Jayanudin[1, 2], Mohammad Fahrurrozi[1], Sang Kompiang Wirawan[1], Rochmadi[1, ✉]

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Abstract. In this study, chitosan-based carrier of red ginger oleoresin was prepared using the emulsion cross-linking technique with sodium tripolyphosphate (TPP) as a cross-linking agent. The effect of chitosan and TPP concentration, as well as pH on the encapsulation efficiency, particle size and characterization of chitosan microcapsule was determined. The antioxidant activity of microcapsules was analyzed. Chitosan microcapsules containing red ginger oleoresin were produced although with non-smooth surfaces.

Keywords: antioxidant activity, red ginger oleoresin, chitosan microcapsule, emulsion cross-linking, sodium tripolyphosphate.

1. Introduction

Modern drug delivery systems use polymers as carriers of drugs to control the drug release. The advantages of the controlled release include increased efficacy, reduced toxicity, and improved patient compliance and comfort [1]. Chitosan is a biocompatible, biodegradable and non-toxic biopolymer. The high nature of chitosan bioadhesive properties has more advantages compared to other natural polymers such as cellulose, xanthan gum and starch [2]. This nature has made chitosan as a very suitable drug carrier. Chitosan microcapsules are a form of drug delivery system that can be prepared by physical methods such as spray drying, extrusion, fluidized bed, and others. In additon to the physical methods, there are chemical methods which include coacervation, *in situ* polymerization, emulsion cross-linking, and interfacial polymerization. The method used in this study was an emulsion cross-linking with sodium tripolyphosphate as a cross-linking agent. Emulsion cross-linking method was developed using glutaraldehyde or glutaraldehyde saturated toluene (GST) as a cross-linking agent. The microcapsules produced by this method have good spherical geometry with a smooth surface and have been successfully carried out by some authors [3-5]. These studies used glutaraldehyde which might have side effects if used for drug delivery. Although Campos *et al.* [6] concluded that there are no adverse effects on cell viability due to cross-reactions which caused toxicity levels to be minimal, a safe cross-linking agent to replace glutaraldehyde is also necessary.

Sodium tripolyphosphate (TPP) is the most widely used cross-linking agent because it is non-toxic and multivalent to prevent possible damage to drugs [7]. TPP may be used by two different ways. First, TPP is added dropwise to a mixture of polymers and bioactive substances. This way has been reported by Jarudilokkul *et al.* [8] to encapsulate protein and by Alqahtani *et al.* [9] to prepare diclofenac-containing chitosan nanoparticles. The second way is the mixture of polymers and bioactive substances is added dropwise to the TPP solution as practiced in the research conducted by Csaba *et al.* [10] for the delivery of oligonucleotide and plasmid DNA and encapsulation of curcumin loaded in chitosan as a drug delivery carrier as reported in [7]. Ionic gelation/ionotropic gelation methods using TPP as a cross-linking agent produce microspheres/nanospheres chitosan, where the bioactive is more dispersed over the carrier material. The matrix type of encapsulate has a disadvantage as there is a possibility of an active agent found on the particle surface, so it is not effective for the active agent that issensitive to environmental influences. This method is not suitable for encapsulation of red ginger oleoresins which are sensitive to changes in heat, oxygen, and microorganisms. The emulsion cross-linking technique makes the red ginger oleoresin coated in a chitosan

[1] Chemical Engineering Department, Faculty of Engineering, Universitas Gadjah Mada
Jl. Grafika No. 2, Yogyakarta, Indonesia
[2] Chemical Engineering Department, Faculty of Engineering, Universitas Sultan Ageng Tirtayasa
Jl. Jenderal Sudirman km.3, Cilegon, Indonesia
✉ *rochmadi@ugm.ac.id*
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solution because the first step is to form an oil-in-water emulsion (red ginger oleoresin coated by chitosan solution) and then the emulsion is added to the vegetable oil to form an oil-in-water-in-oil emulsion. The use of vegetable oils aims to stabilize the emulsion even without using an emulsifier. The final product is microcapsules where the red ginger oleoresin is inside the core which is protected by chitosan as a wall material of microcapsules (red ginger oleoresin encapsulated by chitosan).

Red ginger oleoresin contains many active components which are useful for antioxidant, antimicrobial, and anti-inflammatory [11], but has weaknesses that are sensitive to heat, light, and oxygen [12]. Other disadvantages are poor solubility, and low bioavailability. The chitosan-based red ginger oleoresin delivery carrier can improve stability, solubility and bioavailability. In this study, chitosan containing the red ginger oleoresin as a carrier was prepared by the emulsion cross-linking method with sodium tripolyphosphate (TPP) as a cross-linking agent. One of the the aims of this study was to determine the effect of chitosan concentration, pH, and concentration of TPP solution on encapsulation efficiency, particle size, characterization of microcapsules and release profile of red ginger oleoresin. Another aim was to conduct an analysis of the antioxidant activity of red ginger oleoresin microcapsules.

2. Experimental

2.1. Materials

Red ginger oleoresin was obtained from Lansida group Ltd. Sodium tripolyphosphate was gained from Sigma-Aldrich. These materials were then used as cross-linking agents. Glacial acetic acid was purchased from Merck, and toluene technical grade was provided byTri Jaya Dinamika Ltd. Other materials such as petroleum ether, *n*-hexane, and methanol were supplied by Labora Ltd. Chitosan with 87.2% degree of deacetylation (DD) was provided by Biotech Surindo Ltd., and corn oil was produced by Surya Agung Ltd.

2.2. Preparation of Chitosan Microcapsules Containing Red Ginger Oleoresin

Emulsion cross-linking technique used to prepare red ginger oleoresin containing chitosan microsphere was a modification from works [13] and [8]. Chitosan was dissolved with 1% (v/v) glacial acetic acid to generate 1%, 2%, 3%, and 4% (w/v) chitosan concentration. 4 g of red ginger oleoresin were added to 40 ml of chitosan solution, and then stirred using IKA-Werk Ultra-Turrax for 30 min to prepare an oil-in-water (O/W) emulsion. The first emulsion was added to corn oil and stirred again for 1 h to get oil-in-water-in-oil (O/W/O) emulsion. Sodium tripolyphosphate (TPP) solution was added dropwise to the emulsion. After TPP was added, the pH of the mixture was adjusted to 5 by adding 2% acetic acid and the mixture was still stirred for 3 h. Red ginger oleoresin loaded in chitosan microcapsules was separated with a centrifuge and then washed using petroleum ether followed by hexane. The final stage was the drying of chitosan microcapsules in an oven at 348 K.

2.3. Encapsulation Efficiency

Encapsulation efficiency was determined based on the method reported in [3] and [14]. Surface oil was determined by adding 1 g of dried microcapsules into hexane and stirring for 1 min. Then it was filtered, dried, and weighed. Total oil was determined by extracting 1 g of microcapsules in 200 ml of methanol for 6 h in a Soxhlet extractor. After completion, the microcapsules were dried and weighed. The encapsulation efficiency was calculated using Eqs. (1)-(3).

$$\text{Surface oil } (S_O) = w_i - w_f \quad (1)$$

$$\text{Total oil } (T_O) = w_i - w_{extr} \quad (2)$$

$$\%\ \text{Encapsulation efficiency } (EE) = \frac{T_O - S_O}{T_O} \cdot 100 \quad (3)$$

where w_i, w_f and w_{extr} are the microcapsules initial weight, final weight and weight after extracted in a Soxhlet, respectively, g.

2.4. Methods of Analysis

2.4.1. Scanning electron microscopy (SEM)

Morphological analysis of red ginger oleoresin containing chitosan microcapsules was conducted using a scanning electron microscope (SEM) of JSM 6510LA type. Resolution: high vacuum mode 3.0 nm (30 kV) and low vacuum mode 4.0 nm (30 kV); acceleration voltage is 0.5–30 kV. Chitosan microcapsules were coated with platinum.

2.4.2. Thermal gravimetric analysis (TGA)

Thermal gravimetric analysis (TGA) with DTG-60 Shimadzu type was used to analyze the thermal stability using DTG-60 detector. The samples were heated from 303 to 573 K with a rate of 10 K/min; nitrogen flow rate was 30 ml/min.

2.4.3. Fourier-transform infrared spectroscopy (FTIR)

Interaction analysis between components in microcapsules was carried out *via* Fourier transform infrared spectroscopy (FTIR) using KBr pellets in a Shimadzu IR spectrophotometer, which operated between 500 and 4000 cm^{-1}.

2.4.4. Analysis of particle size

The diameter of microcapsules was determined by a digital microscope with 500× magnification referring to the study reported by Jayanudin *et al.* [15]. Microcapsule size calibration was done by comparing the real diameter size of the wire fibers with the size of wire fibers using a digital microscope, and then made as a correction factor. The diameter size of microcapsules measured by a digital microscope was multiplied by a correction factor to obtain the real diameter size of microcapsules. The average diameter was calculated by observing 100 microcapsules. The average diameter of microcapsule was determined in accordance with Eq. (4):

$$\bar{d} = \sum_{i=n}^{n} \frac{d_i}{N} \quad (4)$$

where $\bar{d}$ is an average diameter, m; d_i is a droplet diameter, m; and N is the total number of calculated droplets.

2.4.5. Analysis of antioxidant activity

Antioxidant activity of red ginger oleoresins in chitosan microspheres could be determined using DPPH assay. This method was modified from the works [16] and [17]. The reaction mixture consisted of the sample (1 ml), ethanol (6 ml) and 0.5mM DPPH solution in ethanol (0.6 ml). After 30 min a reaction between DPPH and the antioxidant compound occurred causing a color change which was recorded by GENESYS 10S UV/VIS spectrophotometer with a wavelength of 517 nm was used. Determination of blank absorbance was determined by the ethanol mixture with samples. The percentage of antioxidant activity was calculated by Eq. (5):

$$AA\% = 100 - \left[\frac{\left(Abs_{control} - Abs_{sample} \right) \cdot 100}{Abs_{control}} \right] \quad (5)$$

where $Abs_{control}$ is the control reaction of absorbance (only absorbance of DPPH) and $Abs_{tsample}$ is an absorbance in the presence of a sample (absorbance of DPPH along with concentrations of sample).

3. Results and Discussion

3.1. Encapsulation Efficiency

Encapsulation efficiency determines the effectiveness of the encapsulation process of red ginger oleoresin using the emulsion cross-linking with TPP as a cross-linking agent. Table 1 shows the effect of chitosan solution concentration, TPP solution concentration and pH on the encapsulation efficiency of red ginger oleoresin microcapsules. The value of the encapsulation efficiency was found to be from 83.25±0.04 to 91.64±0.02 %. Minimum encapsulation efficiency was generated at 4 % chitosan concentration and pH 4. While, the maximum value of encapsulation efficiency was obtained at 4% chitosan concentration, and pH 5.

At low pH, the most amino group and TPP molecules are protonated. The result is a lower charging density of the molecule. The chitosan molecules cannot be adequately cross-linked by TPP to form stable particles [18] and this causes lower encapsulation efficiency at pH 4 than that at pH 5. Meanwhile the encapsulation efficiency decreases again at pH 6 because this value of pH approaches the isoelectric point of chitosan (pKa = = 6.3). So, the deprotonation process could occur and cause aggregation by decreasing the repulsion force between particles [19]. The aggregation process between particles allows a small portion of red ginger oleoresin to be diffused out during the process of chitosan microcapsules compaction. Similar results were reported by Patil *et al.* [20], when increasing pH caused a decrease in encapsulation efficiency.

In this study the encapsulation efficiency has higher values (from 87.81±0.03 to 91.64±0.02 %) in comparison with those using ionic gelation methods (45.77±1.25 % [21], 31.1±3.1 % [9], and 54.72 ± 1.342 % [22]).

Table 1

Encapsulation efficiency and particle size of chitosan microcapsules filled with red ginger oleoresin depending on chitosan and TPP concentrations and pH

Parameter				
Chitosan concentration, % (w/v)	TPP concentration, % (w/v)	pH	[a,b]*EE*, %	[a,c]Particle size, μm
1	5	5	87.81±0.03	35.71±6.6
2			89.40±0.07	36.95±5.4
3			90.18±0.05	38.52±4.9
4			91.64±0.02	41.43±8.3
4	5	4	83.25±0.04	47.35±9.1
		5	91.64±0.02	41.43±8.3
		6	91.42±0.01	48.11±9.1

Notes: [a]Mean±SD, [b]n = 3, [c]n = 100

3.2. Particle Size

The particle size was determined by observing a hundred particles using a digital microscope before the average diameter was defined. The effect of the parameters on the average particle size can be seen in Table 1. The average particle size was found to be from 35.71±6.6 to 63.95±7.5 μm. The particle size of chitosan microcapsules containing red ginger oleoresin increased with the increase in chitosan concentration. The lower viscosity of the chitosan solution had a better solubility for the efficiency of the gelation process that caused the particle size to become smaller. The lower concentration of chitosan solution results in the smaller particle size and *vice versa*. The lower molecular weight of chitosan could reduce particle size because it has a shorter polymer chain that supports dissolution and interaction with TPP [23]. Higher concentrations of chitosan solution make the walls of the microcapsules more rigid and compact [24], the walls become thicker, so the diameter of the microcapsules becomes larger.

Particle size was also influenced by pH. Table 1 showed that larger particle size was obtained at pH 4 than that at pH 5, but the increase of the pH value to 6 again increases the particle size, even greater than those at pH 4 and 5. This increase is caused by the reduction in cross-linking due to the predominance of deprotonation. The decrease in pH causes quite strong charge interaction between two molecules, thus the sizes of microcapsules become larger [18].

The chitosan concentration and pH are the important parameters to determine the particle size of chitosan microcapsules. The highest average particle size in this study is 48.11±9.1 μm obtained at 4% (w/v) concentration of chitosan solution and pH 6. Meanwhile, the smallest particle size is 35.71±6.6 μm which was obtained at 1% (w/v) concentration of chitosan solution and pH 5. If we compare the particle size obtained using the ionic gelation method (from 22.45±0.90 to 1287.70±0.60 nm [21] and from 295.33±3.01 to 336± 22.1 nm [9]), the particle size in this study is larger.

3.3. Characterization of Red Ginger Oleoresin Containing Chitosan Microcapsules

3.3.1. FTIR analysis

FTIR analysis for chitosan and chitosan-TPP microcapsules is shown in Fig. 1. FTIR analysis shows that the absorption bands for chitosan and chitosan-TPP microcapsules are at 3425.58 and 3387 cm^{-1}, assigned to N–H and O–H stretching vibrations, respectively.

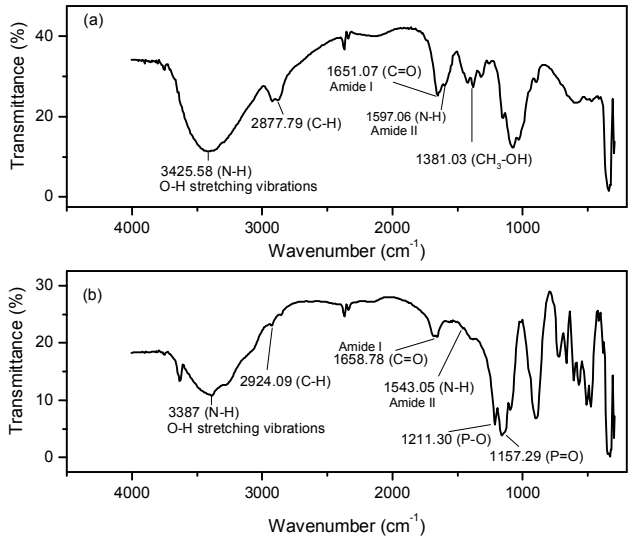

Fig. 1. FTIR spectra of chitosan (a) and chitosan/TPP (b). Chitosan microcapsules used in FTIR analysis was without red ginger oleoresin

Fig. 1 shows that there are differences in the peak between chitosan (a) and chitosan-TPP microcapsules (b). The differences are the peak of 1211.30 cm^{-1} (P–O) and 1157.29 cm^{-1} (P=O)

3.3.2. TGA and DTA analyses

Thermogravimetric analysis (TGA) is a type of test carried out to determine a weight loss in a sample due to temperature changes. TGA is used to characterize materials such as polymers for determining the degradation of temperature, decomposition of organic and inorganic materials, and solvent residues. TGA analysis is usually simultaneous with DTA analysis, which is used to analyze material changes as a temperature function. DTA is used to study thermal properties and phase changes due to enthalpy changes from the material. Fig. 2 shows the results of TGA/DTA analysis for chitosan microcapsules containing red ginger oleoresins based on changes in chitosan concentration.

TGA analysis was carried out within the temperature range of 303–573 K. The weight loss occurred in two stages. The first stage took place within 303–385 K and the second stage 385–573 K. The weight loss in the first stage occurred due to evaporation of water in chitosan microcapsules. After that, chitosan microsphere melted and decomposed at 385–573 K. At this stage, the weight of chitosan microsphere decreased drastically because of decomposition. The acetylated and deacetylated units of the polymer could activate depolymerization and decomposition. The saccharide rings could also dehydrate at the temperatures of 463–603 K [25]. The weight losses were 24.158, 18.168, 19.564 and 28.273 % (Fig. 2).

The results of DTA analysis are also shown in Fig. 2. It can be seen that chitosan microcapsules have different endothermic peaks at different concentrations. The melting point (endothermic peak) for 1% (w/v) concentration of chitosan solution occurred at 401.34 K with enthalpy ΔH = -126.49 J/g; for 2% (w/v) concentration – at 396.73 K with ΔH = -105.26 J/g; for 3% (w/v) concentration – at 397.30 K with ΔH = -129.15 J/g; and for 4% (w/v) concentration – at 396.49 K with ΔH = -239.63 J/g.

3.3.3. SEM analysis

The SEM analysis was used to determine the surface morphology and shape of chitosan microcapsules. Fig. 3 shows the surface morphology of chitosan microcapsules cross-linked with TPP at various magnifications.

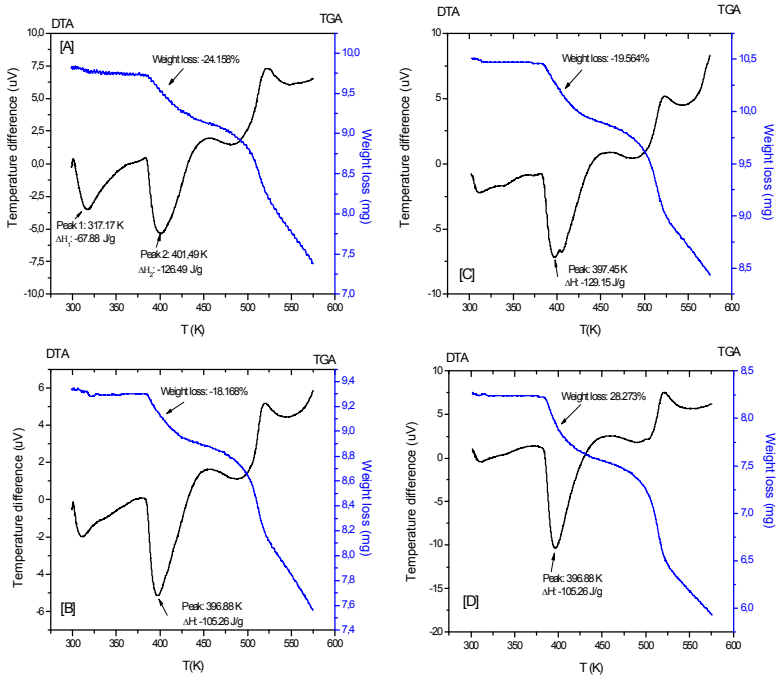

Fig. 2. TGA and DTA analyses of chitosan microcapsules for different concentrations (w/v) of chitosan solution: 1% (a), 2% (b), 3% (c), and 4% (d). The chitosan microcapsules containing red ginger oleoresin were obtained at 5% TPP concentration and pH 5.

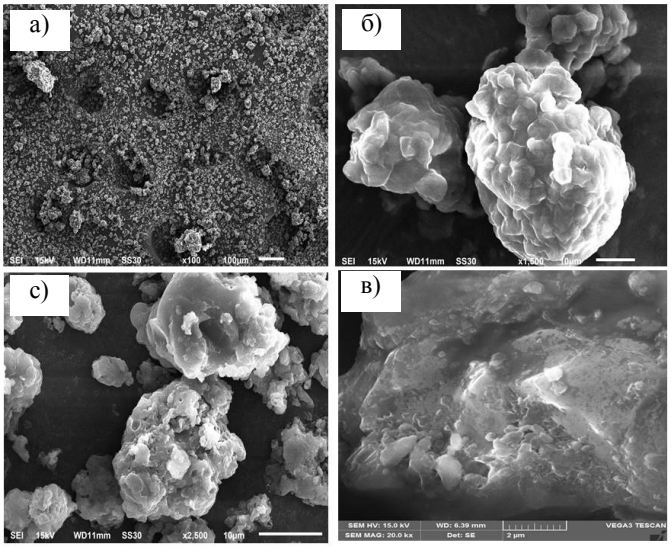

Fig. 3. Surface morphology analysis of chitosan microcapsules containing red ginger oleoresin with magnification of 100× (a); 1,500× (b); 2,500× (c) and 20,000× (d). This form of microcapsules was made at 4% (w/v) concentration of chitosan solution, 5% (w/v) concentration of TPP solution, and pH 5

Fig. 3 shows that the surface shape of chitosan microcapsules cross-linked with TPP generates a non-smooth surface and does not exhibit good spherical geometry. Many chitosan flakes are attached to the microcapsules surface. At the initial process of solidification, the microcapsule wall layer is a gel-shaped one because it was cross-linked with TPP. Then, it was dissolved due to the addition of acetic acid to adjust the pH. The chitosan solution was hardened again during the cross-linking process. This phenomenon was very likely caused by the number of chitosan flakes attached to the surface of the microcapsules, as shown in Figs. 3c and d. The uneven surface shape did not exhibit good spherical geometry and might be caused by non-uniform cross-linking reaction of chitosan-TPP as shown in Fig. 3b. Meanwhile, the solid surface is seen with 20,000× magnification (Fig. 3d), even though many flakes of chitosan are attached to the chitosan microcapsule surface.

3.3.4. Antioxidant activity of red ginger oleoresin containing chitosan microsphere

DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was used to analyse the antioxidant activity of chitosan microcapsules containing red ginger oleoresin. This method was used to predict antioxidant activity by the mechanisms in which antioxidants in a substance inhibit lipid oxidation by a DPPH scavenging radical. DPPH can be a stable diamagnetic molecule because it can accept electrons or hydrogen radicals. It can be oxidized with difficulty and then irreversibly [26]. The results of the antioxidant activity of red ginger oleoresin containing chitosan microsphere can be seen in Table 2.

Table 2

Antioxidant activity of red ginger oleoresin microcapsules

Chitosan concentration, % (w/v)	Antioxidant activity, %
1	58.65±1.54
2	67.48±1.43
3	64.22±1.29
4	83.63±1.52
pH	
4	54.59±1.49
5	83.63±1.52
6	77.01±1.16

Notes: Mean±SD, $n = 3$

In general, the antioxidant activity increases with the increase in the concentration of chitosan. Although the solution with 2% (w/v) chitosan concentration has higher antioxidant activity than that with 3% (w/v) concentration of chitosan, the difference is not significant. Antioxidant activity at pH 6 is lower than that at pH 5 and higher than at pH 4. This is likely related to the encapsulation efficiency produced, as shown in Table 1. The higher encapsulation efficiency has higher oleoresin content, so the antioxidant activity is also higher. The value of antioxidant activity produced from chitosan microcapsules containing red ginger oleoresins is quite high (from 54.59±1.49 to 83.63±1.52 %. When compared to the results reported by Eleazu *et al.* [27] who represented antioxidant activity from 45 to 75 %, the antioxidant activity values obtained in this study are higher. They are even higher than those for the synthetic antioxidants. As reported in [17], the antioxidant activity of butylatedhydroxyanisole (BHT) and butylatedhydroxytoluene (BHA) is 48–60 % and 50–69 %, respectively. The obtained values are also higher than the antioxidant activity for ginger oleoresin resulting from ginger extraction (40–76 %) [28]. The same is for the antioxidant activity of oleoresin which was calculated using 2,2'-azino-bis (3-ethylbenzenthiazoline-6-sulphonic) acid (ABS) method (from 9.17±0.94% to 50.58±2.86 %) [29]. The high value of the antioxidant activity of red ginger oleoresin containing chitosan microcapsules shows one of the success parameters of the encapsulation process by emulsion cross-linking technique using sodium tripolyphosphate as a cross-linking agent.

4. Conclusions

In summary, this study reveals that the concentration of chitosan, TPP concentration, and pH have an impact on the efficiency, particle size, thermal stability, and antioxidant activity. Based on the parameters studied, chitosan microspheres obtained at 4% chitosan concentration, 5% TPP concentration, and pH 5 show the highest encapsulation efficiency and antioxidant activity: 91.64±0.02 % and 83.63±1.52 %, respectively. The particle size was from 35.71±6.6 to 63.95±7.5 μm. Although the surface was not smooth, chitosan microspheres have been successfully produced. The values of antioxidant activity of red ginger oleoresin microcapsules were from 54.59±1.49 to 83.63±1.52 %.

Acknowledgments

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ОДЕРЖАННЯ, ХАРАКТЕРИСТИКА ТА АНТИОКСИДАЦІЙНА АКТИВНІСТЬ ХІТОЗАНОВИХ МІКРОКАПСУЛ З ЖИВИЦЕЮ ЧЕРВОНОГО ІМБІРЮ ТА ТРИПОЛІФОСФАТОМ НАТРІЮ, ПРИГОТОВЛЕНИХ МЕТОДОМ ЕМУЛЬСІЙНОГО ЗШИВАННЯ

Анотація. *З використанням методу емульсійного зшивання приготовлено хітозанові капсули живиці червоного імбирю з триполіфосфатом натрію (ТПН) як зшиваючого агента. Визначено вплив концентрації хітозану та ТПН, а також величини pH на ефективність інкапсуляції, розмір частинок та властивості мікрокапсул хітозану. Визначено антиоксидантну активність мікрокапсул. Показано, що мікрокапсули можуть бути успішно одержані, не зважаючи на негладку поверхню.*

Ключові слова: *антиоксидаційна активність, живиця червоного імбиру, мікрокапсула хітозану, емульсійне зшивання, триполіфосфат натрію.*