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Nanoencapsulation of Anthocyanin Extract from Fermented Black Garlic (FBG) Based on Biocompatible Polymeric Materials

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ABSTRACT

Garlic contains various polyphenolic compounds such as anthocyanin, a bioactive, water-soluble compound generally known for its remarkable health-enhancing properties. However, it is chemically unstable and easily degrades due to various environmental conditions (temperature, pH, presence of oxygen and light, etc.) in addition to its low bioavailability due to fast metabolization and low absorption in the body. Therefore, a nanoencapsulation strategy is essential to address these limitations. In this work, anthocyanin extraction from FBG (Ilocos variety) with 85% acidified ethanol and its encapsulation using the chitosan-alginate nanoparticle system via pre-gelation and polyelectrolyte complex formation were demonstrated. Anthocyanin-loaded chitosan-alginate nanocapsules were characterized in terms of structural features, particle size, morphology, encapsulation efficiency, total phenolic content (TPC), and radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The obtained anthocyanin-loaded nanocapsules have small particle sizes ranging from 50.7 nm to 92.0 nm with high encapsulation efficiency (T3: 78.82%, T2: 68.18%, T1: 65.77%). Results showed that a higher initial concentration of anthocyanin extract promotes higher encapsulation efficiency. Antioxidant activity of the nanocapsules showed low phenolic content (0.11 mg GAE/g) but high DPPH scavenging activity (14.02 mg AAE/g). The chitosan-alginate complex has successfully encapsulated the anthocyanin from fermented black garlic.

1. INTRODUCTION

Garlic (*Allium sativum L.*) has long been used as a natural remedy and a portion of human medicinal food [1]. Many of garlic's medicinal properties have been documented, including anticancer, antibacterial, antiviral, anti-diabetic, anti-hypertensive, cardioprotective, hepatoprotective, hypolipidemic, and antioxidant effects, as well as an immune enhancement [2]. Despite garlic's numerous health benefits, some people are averse to eating raw garlic because of its pungent odor and flavor [3]. Some bioactive sulfur components produce a strong, pungent odor when raw garlic is crushed or destroyed, related to consumers' unpleasant body and breath odor [4]. Raw garlic could also cause gastrointestinal discomfort to other people [5]. Because of this, food scientists developed a way of preparation of garlic to reduce these discomforts [6]. Fermenting to transform into black garlic could reduce the pungent odor while enhancing its nutritional value [7].

Fermented black garlic (FBG) has a sweet fruit-like taste, no pungent odor, and is directly edible after peeling off the outer coat [8]. It is produced from fresh garlic fermented with controlled temperature and humidity for a certain period. Due to the lower allicin content converted into antioxidant chemicals such as bioactive alkaloids and flavonoid compounds during the aging process, FBG does not have a significant off-odor or flavor compared to raw garlic [9]. Further, FBG contains anthocyanin, a water-soluble flavonoid known for its antimicrobial, anticancer, and anti-hyperglycemic properties [10].

Anthocyanin is a bioactive compound known for its tremendous health-enhancing property [11]. However, most known bioactive compounds are easily degraded when exposed to heat, light, oxygen, and other environmental conditions. Encapsulation is one of the most promising technologies to entrap bioactive compounds while extending the shelf life against degradation during storage [12]. Bioactive compounds' stability, solubility, regulation, and release can be improved [13]. The use of green nanotechnology is a more environmentally friendly method of minimizing hazardous chemicals while encapsulating the material [14, 15]. This study demonstrated the encapsulation of anthocyanin extract from fermented black garlic using a chitosan-alginate nanoparticle system via pre-gelation and polyelectrolyte complex formations. Different analytical techniques were also employed to characterize the synthesized

nanocapsules.

2. MATERIALS AND METHODS

2.1 Garlic, chemicals, and reagents

The raw garlic was purchased from Mariano Marcos State University, Batac, Ilocos Norte, Philippines. Chitosan and other chemicals were purchased from Sigma-Aldrich. Central Luzon State University Nanotechnology Research and Development Facility provided other chemicals and reagents. These chemicals and reagents were used with no further modifications and maintained at a high purity level.

2.2 Processing of fermented black garlic

Samples of fermented black garlic were produced following the described method by Najman et al. [16], with minimal modifications. In this study, the fermentation process was shortened up to 35 days instead of 45 days on the published method. Briefly, the raw materials were cleaned and kept in a refrigerator for 24 h at 10℃ and 85% relative humidity. The garlic was fermented without removing its outer covering by thermal treatment in a ripening chamber at 70-80℃ for 35 days without any additional treatments and additives. Two varieties of garlic were used in this study: the first one is native garlic (*Ilocos variety*), and the other one is imported (*Taiwan variety*).

2.3 Screening of fermented black garlic samples

The anthocyanin content of black garlic samples was determined using the modified method of Francavilla and Joye [17]. In a 15 ml conical centrifuge tube, 300 mg of powdered black garlic samples and 10 ml of acidified ethanol (85% v/v ethanol in 1 M HCl, pH 1) were homogenized using a vortex mixer. The solutions were agitated for 1 h before being filtered via regular filter paper. The supernatant was transferred to a 50 ml volumetric flask, and acidified ethanol was used to dilute it to the mark. The volume of supernatant collected was less than 10 ml, considering the molar volume of ethanol and the extraction process before dilution. Furthermore, the amount of liquid added will be greater than 40 mL to dilute the supernatant in 50-mL via a volumetric flask. The absorbance was measured at 535 nm using a UV-Vis Spectrophotometer (UH5300, Hitachi).

Based on Lambert-Beer's Law, the total anthocyanin content (TAC) was calculated using the following formula:

$$TAC = \frac{A \times VE \times MW \times 10^6}{1000 \times \varepsilon \times mass} \quad (1)$$

2.4 Nanocapsules preparation

Based on a modified approach of De and Robinson [18], chitosan-alginate nanocapsules were made via ionic pre-gelation and polyelectrolyte complex formation. Preparation of emulsified anthocyanin was done first to avoid the leaching of anthocyanin during pre-ionic gelation and polyelectrolyte complex formation and to promote higher encapsulation efficiency. Tween-20 was used as an emulsifier for the anthocyanin to avoid leaching during ionic pre-gelation and polyelectrolyte complex formation. It reacts with anthocyanin molecules in such a way that they will not get attracted to water. Since anthocyanins are water-soluble, they tend to leach out or get attached to the water molecules then get entrapped inside the egg-box type formation through the pre-gel process. With the emulsification process, we can resolve the issue of leaching as per anthocyanin molecules are concerned.

The crude anthocyanin extract (CAE) was mixed with Tween 20 (concentrations were based on the treatments shown in Table 1) using a magnetic stirrer for a few minutes at room temperature. The mixtures were stored in vials prior to encapsulation.

Exactly 40 ml of sodium alginate solution (2.25 mg/ml, pH 4.9) was diluted with 15 ml distilled water, then 0.03 M of calcium chloride ($CaCl_2 \cdot H_2O$) was mixed with either CAE (in mg/ml of Tween-20) or distilled water with a volume ratio of 1:1. Using a burette, slowly add 10 ml of $CaCl_2$: CAE mixture while mixing in a magnetic stirrer for ionic pre-gelation. For 5 min, the resulting pre-gel was constantly stirred. Then 10 ml of chitosan solution (6.3 mg/ml, pH 5.5) was transferred into the burette and gradually dispensed with constant stirring to promote the polyelectrolyte complex. The mixture was constantly stirred for another 10 min and equilibrated overnight at 4℃ to attain uniform particle size. The supernatant was collected for encapsulation efficiency study after the mixture was filtered. The collected pellets were stored in the freezer at -5℃ and were lyophilized using a freeze-dryer for approximately 24 h to obtain the anthocyanin-loaded nanocapsules. For the anthocyanin-loaded chitosan-alginate nanocapsules, the anthocyanin concentration to be included in the $CaCl_2$ solution was varied, as shown in Table 1. The rest of the procedure was the same as the blank chitosan-alginate for each treatment.

Table 1. Nanocapsules treatment and their corresponding compositions

Treatment	Composition
Treatment 0	Chitosan-alginate
Treatment 1	Chitosan-alginate + 5 mg/ml anthocyanin extract
Treatment 2	Chitosan-alginate + 10 mg/ml anthocyanin extract
Treatment 3	Chitosan-alginate + 15 mg/ml anthocyanin extract

2.5 Total phenolic content determination

The total phenolic content of anthocyanin extracts, both crude and encapsulated, was determined utilizing the method of Singleton et al. [19] with minor modifications. The concentration range of the calibration curve was modified so that the values for the sample would fit in the linear model. About 400 µL of the acidified methanolic extracts were mixed with 800 µL of distilled water and 1000 µL of Folin-Ciocalteu phenol reagent that was diluted by distilled water (1:10). Two ml of 7.0% sodium carbonate (w/v) was added after 5 min of incubation. It was allowed to stand for 2 h before reading. Using a UV-Vis Spectrophotometer (UH5300, Hitachi), the absorbance of the solution was measured at 765 nm against a blank (methanol). The reference standard was gallic acid (GA). Based on the Gallic acid standard calibration curve, the TPC was computed and represented as mg GA equivalent (GAE) per gram of sample. The calculation method was based on the equation of the line obtained using calibration standards. Using the slope-intercept form, y=mx+b, the absorbance (y) can be determined in the analysis and thus concentration (x) can be calculated. The Total Phenolic Content was calculated as follows:

$$TPC = c\frac{V}{M} \quad (2)$$

2.6 DPPH radical scavenging assay

The protocol modified by Mahdi-Pour et al. [20] was adopted for the antioxidant scavenging activity of samples on 2,2-diphenyl-1-picryl hydrazyl (DPPH) radicals. One and a half milliliters of different methanolic extracts were mixed with 2.5 ml DPPH solution (in different concentrations). The control was made by substituting methanol for the extract. Positive control was employed, which was ascorbic acid. The mixture was shaken vigorously and left to stand in the dark for 30 min. The samples' absorbance was measured using a UV-Vis Spectrophotometer (UH5300, Hitachi) at 517 nm. Trolox was used as a reference standard. The DPPH radical scavenging activity (RSA) was calculated using the following formula:

$$DPPH\ RSA\ (\%) = \frac{A_b - A_s}{A_b} \times 100 \quad (3)$$

The extracts' antioxidant activity was measured using EC_{50} values, which are used to compute the amount of Ascorbic acid equivalent (AAE) per gram of sample.

2.7 Anthocyanin-loaded nanocapsules' encapsulation efficiency

Ong et al. [21] modified approach was used to assess encapsulation efficiency (EE). About 5 ml of supernatant and anthocyanin extract (in different concentrations) was diluted into a 25-ml volumetric flask using 85% acidified ethanol. The concentration of unencapsulated anthocyanin was obtained from its absorbance at 535 nm using the UV-Vis Spectrophotometer (UH5300, Hitachi). Lambert-Beer's formula was used to calculate the concentration of the unencapsulated anthocyanin using the absorbance of the original anthocyanin extract. The Total Anthocyanin Content formula was based on Lambert-Beer's formula that was used to calculate the concentration for encapsulation efficiency. The calculation method is indirect since the value obtained does not represent the actual content inside the capsules but what remained in the solution compared to the initial concentration. The following formula was used to compute Encapsulation Efficiency (%).

$$\%EE = \frac{Conc._{original} - Conc._{unencapsulated}}{Conc._{original}} \times 100 \quad (4)$$

2.8 Characterization of anthocyanin-rich chitosan-alginate nanocapsules

The anthocyanin extract and its encapsulated form were subjected to various instrumental analyses to determine their chemical characteristics and behaviors. Scanning Electron Microscopy (SEM) (SU3800, Hitachi) was used to observe the morphological structure of blank and anthocyanin-loaded nanocapsules (with the highest encapsulation efficiency). The different functional groups present in chitosan, sodium alginate, blank chitosan-alginate nanocapsules, anthocyanin-loaded chitosan-alginate nanocapsules, crude anthocyanin extract was determined using FTIR (Fourier Transform Infrared) spectroscopy (Spectrum Two, PerkinElmer) in the mid-IR region (4000-500 cm^{-1}). Nanopartica analyzer (SZ-100, Horiba) was used to determine the particle size of the blank and anthocyanin-loaded chitosan-alginate nanocapsules using dynamic light scattering (DLS) technique at a scattering angle of 173°.

2.9 Statistical analysis

The screening of the anthocyanin content of the varieties of garlic and the determination of total phenolic content (TPC) and DPPH radical scavenging activity of the treatments were carried out in triplicates. To determine the significant difference in each treatment and compare means between replicates, a one-way analysis of variance (ANOVA) was employed. For the one-way ANOVA, the mean and variance of each treatment were calculated. The within-group variance was then obtained by getting the average of the variance of the treatments. Consequently, the between-group variance was determined by getting the variance of the means of each treatment and multiplying by the number of replicates. F-statistic was done by calculating the ratio of between-group variance versus within-group variance and was compared against the F-critical value at a 95% level of significance. At a 5% level of significance ($p<0.05$), Tukey's HSD test was used to compare the means of the encapsulation efficiency of the prepared nanocapsules, while the least significant difference (LSD) test was employed to compare the treatment means at $p<0.05$ of the two varieties of the fermented black garlic. The non-linear fit of exponential decay was used in the DPPH scavenging assay to compute the EC_{50} values. The statistical analysis of the TPC, DPPH, and to determine the differences in anthocyanin content of a different variety of garlic, the Statistical Tool for Agricultural Research (STAR) was used.

3. RESULTS AND DISCUSSIONS

3.1 Synthesis and characterization of fermented black garlic

Effect and changes of color during the fermentation process are shown in Figure 1. The color changes are mainly due to the Maillard reaction between a reduced carbonyl sugar group and an amino group, forming an amadori non-enzymatic browning reaction.

Figure 1. The maturing process of fermented black garlic

3.2 Anthocyanin content of fermented black garlic

Between the two varieties of garlic shown in Table 2, the *Ilocos variety* (0.21 mg/g) showed higher anthocyanin content than the *Taiwan variety* (0.16 mg/g). The anthocyanin from fermented black garlic was made through the Maillard reaction. The garlic bulbs were heated for 35 days without any additional treatments and additives. The produced fermented black garlic has no pungent odor compared to fresh garlic. It also has a sweet and sour taste and is directly edible after peeling the outer coat. The variety with the highest

anthocyanin content was chosen as a sample for making crude anthocyanin extract (CAE) for encapsulation. Thus, the *Ilocos variety* was chosen.

Table 2. Anthocyanin content of fermented black garlic samples

Fermented black garlic sample	Anthocyanin content (mg/g)
Ilocos variety	0.21 ± 0.02^{a}
Taiwan variety	0.16 ± 0.00^{b}

Note: Similar letter of superscript of means signifies no significant difference at 5% level of significance as evaluated using Least Significant Difference (LSD) test.

3.3 Encapsulation efficiency of the nanocapsules

The crude anthocyanin extract from the *Ilocos variety* was selected to undergo the encapsulation process using chitosan-alginate polyelectrolyte complex formation. The lyophilized blank nanocapsules have white coloration, while the anthocyanin-loaded chitosan-alginate nanocapsules were brownish to black. Encapsulation was done using the modified process of Ong et al. [21] with modifications.

As seen in Table 3, T_3 has the highest encapsulation efficiency (78.82%) compared to T_1 (65.11%) and T_2 (68.18%). The use of Tween-20 as a surfactant has promoted higher encapsulation efficiency. Furthermore, since anthocyanin is a water-soluble compound, there is a high probability of sample leaching, resulting in low encapsulation efficiency. Encapsulation efficiency increases when samples are prepared involving water-in-oil in emulsion rather than in aqueous media. This method prevented the anthocyanin-leaching during the nanocapsules preparation. The blank nanocapsules and the treatment with the highest encapsulation efficiency were subjected to further analysis and characterizations. Shown in Table 3 is the encapsulation efficiency of the prepared nanocapsules.

Table 3. Encapsulation efficiency of the prepared nanocapsules

Fermented black garlic sample	Anthocyanin content (mg/g)
T_1 - Chitosan-alginate + 5 mg/ml anthocyanin extract	65.77 ± 5.01^{b}
T_2 - Chitosan-alginate + 10 mg/ml anthocyanin extract	68.18 ± 2.86^{b}
T_3 - Chitosan-alginate + 15 mg/ml anthocyanin extract	78.82 ± 5.10^{a}

Note: Similar letter of superscript of means signifies no significant difference at 5% level of significance as evaluated using Tukey's Honest Significant Difference (HSD) test.

3.4 Phytochemical properties of the encapsulated anthocyanin

The TPC and DPPH radical scavenging activity of the crude anthocyanin extract (CAE) and the anthocyanin-loaded nanocapsules (ALN) with the highest encapsulation efficiency were evaluated further for their phytochemical properties. The anthocyanin extraction from the powdered nanocapsules was conducted using 85% acidified methanol agitated for 8 h. The supernatant was collected by filtering before phytochemical analyses.

The TPC of the CAE and ALN were expressed as mg GAE per g sample plotted against the Gallic acid standard calibration curve. The TPC of the 100 mg CAE was found to be 32.64 mg GAE/g, while the ALN was found to be 0.11 mg GAE/g.

Table 4. TPC, DPPH values, and radical scavenging activity of the crude anthocyanin extract (CAE) and anthocyanin-loaded nanocapsules (ALN)

Phytochemical analyses	Units	CAE	ALN
TPC value	mg GAE/g sample	32.64	0.11
DPPH value	mg AAE/g sample	36.05	14.02
Radical scavenging activity	%	74.49	86.38

The TPC value of the CAE implies a high antioxidant property while the ALN has a low antioxidant property which its scavenging activity can further assess through DPPH assay. The amount of phenolics present in a sample correlates with its antioxidant property.

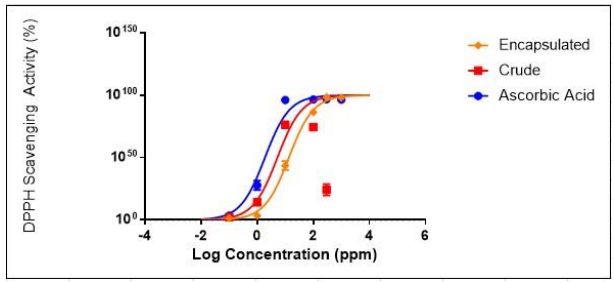

Figure 2. Log concentration vs. scavenging activity curve of ascorbic acid, crude anthocyanin extract, and anthocyanin-loaded nanocapsules

The scavenging activity of the CAE curve, as shown in Figure 2, is much closer to the scavenging activity curve of ascorbic acid than the scavenging activity curve of ALN, which means it has high antioxidant activity. Furthermore, CAE showed extreme discoloration (74.49%) of DPPH, while ALN showed an 86.38% inhibitory effect (Table 4). The DPPH values were plotted against the Ascorbic acid calibration curve and expressed as mg AAE/g sample. The antioxidant property of CAE was found to be 36.05 mg AAE/g, while ALN has 14.02 mg GAE/g. Anthocyanins are known to have high antioxidant activity; this explains the high antioxidant property of the CAE. However, the high antioxidant property of the ALN might be due to the solvent (85 % acidified methanol) used in the extraction and the high encapsulation efficiency. More anthocyanin is present in the capsule that can be readily available for scavenging with the DPPH radicals since the ALN has been efficiently extracted and encapsulated. Evaluating the anthocyanin-loaded nanocapsules encapsulation efficiency is crucial, as shown by its variable phytochemical properties.

3.5 Characterization of encapsulated anthocyanin-based nanoparticles from fermented black garlic

3.5.1 Scanning electron microscopy

The surface morphology of the blank chitosan-alginate nanocapsules and anthocyanin-loaded chitosan-alginate nanocapsules was characterized using SEM (SU3500, Hitachi). Figure 3 shows the surface morphology at a scale bar of 10 µm

and 50 µm. At a scale bar of 10 µm (a), the blank nanocapsules have a surface area that is slightly smooth with irregular round crystal-like shapes. While the anthocyanin-loaded nanocapsules, at a scale bar of 50 µm (b), showed a rough surface with some distinct elongated shapes with irregular round shapes.

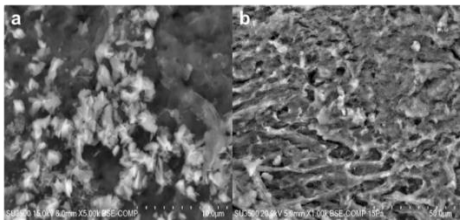

Figure 3. SEM images of a) blank chitosan-alginate nanocapsules (T0) and b) anthocyanin-loaded nanocapsules (T3)

The clumping observed on the two nanocapsules may be due to the absence of cryoprotectants in the freeze-drying process. Cryoprotectants like sucrose or polyvinyl pyrrolidone (PVP) can preserve the integrity of the nanocapsules. The obtained particle size of the nanocapsules is in the range of nano dimension; thus, it pertains to the increase of the bioavailability of the produced encapsulated drug.

3.5.2 FTIR analysis

Figure 4 shows the spectra of chitosan, alginate, blank chitosan-alginate nanocapsules, anthocyanin-loaded chitosan-alginate nanocapsules, and crude anthocyanin extract from fermented black garlic. In the chitosan spectrum, as indicated in Figure 4, stretching vibrations of O-H bonds and N-H bonds can be found in the range of 3600-3000 cm^{-1}, corresponding to the leading functional group of chitosan. The bending of the secondary amide and the C-H (sp3 hybridization) stretching were also observed at 1653 cm^{-1} and 2873 cm^{-1}, respectively. Moreover, a weak absorption band observed at 1385 cm^{-1} was attributed to C-N stretching vibrations. The stretching vibrations of C-O were also observed at 1069 cm^{-1}, 1031 cm^{-1}, and 902 cm^{-1}, indicating the presence of secondary (characteristic peak of -CH-OH in cyclic alcohols, C-O stretch) and primary hydroxyl (distinct peak of $-CH_2-OH$ in primary alcohol, C-O stretch), and asymmetric stretch of C-O-C respectively. In the alginate spectrum, stretching vibrations of O-H bonds were also observed centered at 3336 cm^{-1}. The observed peak at the 2964-2953 cm^{-1} range was attributed to the stretching vibrations of aliphatic C-H (sp^3 hybridization). The C-O stretching vibration of the pyranosyl ring was found at 1026 cm^{-1}. For the blank chitosan-alginate spectrum, the absorption peak range of O-H and N-H was also observed at the range of 3600-3000 cm^{-1}. The bending of the N-H bond was observed at 1599 cm^{-1}. The significant peaks of chitosan and alginate were also observed in the blank chitosan-alginate nanocapsules spectra.

On the other hand, it can be distinguished that the major peaks of the blank chitosan-alginate nanocapsules were also observed in anthocyanin-loaded nanocapsules. These results implied good interaction of chitosan and alginates. It can also be observed that the intensity of the transmittance peak of the O-H groups of blank chitosan-alginate nanocapsules and anthocyanin-loaded nanocapsules tends to diminish due to the enhanced O-H groups in the nanocapsules because of the OH-groups present in the anthocyanin molecular structure. These findings indicated a connection between the functional groups of chitosan-alginate nanocapsules and anthocyanin, indicating that the anthocyanin was successfully encapsulated in the nanocapsules.

3.5.3 Particle size analysis

The nanocapsules' particle size was determined using the dynamic light scattering technique at a scattering angle of 173°. The particle size of the synthesized nanocapsules is presented in Figure 5. As shown, T_0 has a smaller mean particle size of 53.7 ± 0.2 nm than T3 and T2; this is because T0 has no encapsulated bioactive compound. While on the anthocyanin-loaded nanocapsules, T_3 has the largest particle size with the value of 92.0 ± 6.1 nm, followed by T_2 (88.7 ± 3.5 nm) and T_1 (50.7 ± 3.3 nm). It was also observed that anthocyanin concentrations directly affect the average particle size of the anthocyanin-loaded nanocapsules. Higher anthocyanin concentration promotes the high encapsulation efficiency in the nanocapsules, thereby increasing their particle size.

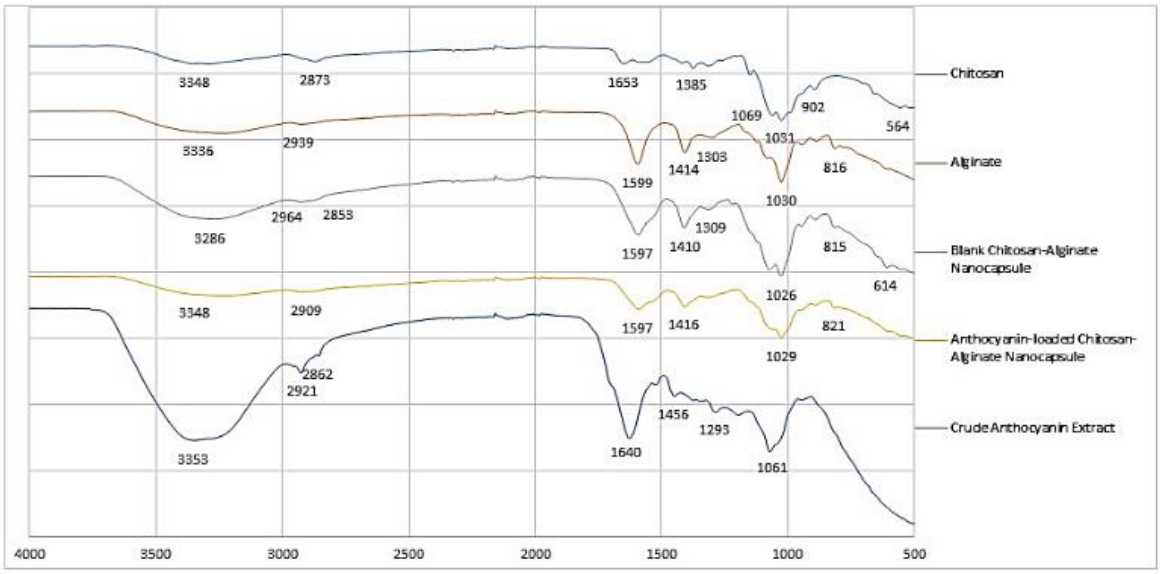

Figure 4. FTIR spectra of chitosan, alginate, blank chitosan-alginate nanocapsules (Blank CAN), anthocyanin-loaded chitosan-alginate nanocapsules (ALCAN), and crude anthocyanin extract (CAE) from fermented black garlic

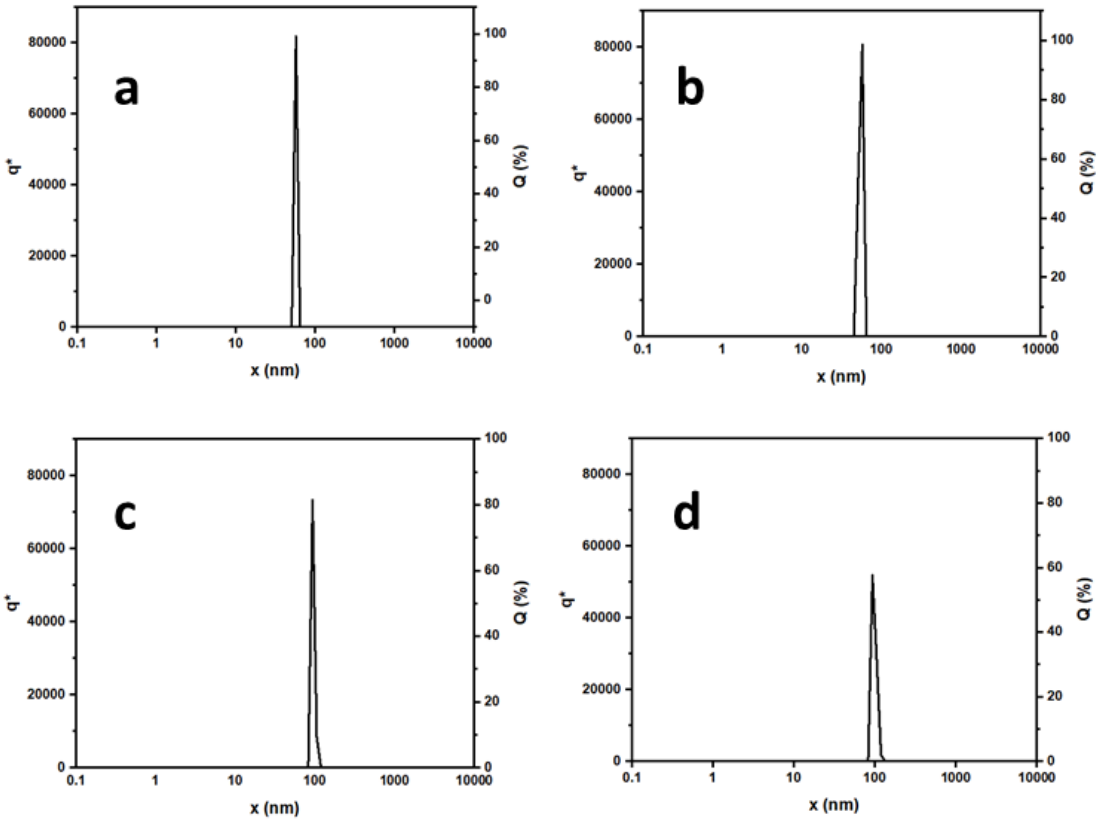

Figure 5. Particle size analysis of chitosan-alginate nanocapsules, a) T0, b) T1, c) T2, d) T3

4. CONCLUSION

In summary, this study has demonstrated the successful extraction of anthocyanin from fermented black garlic and its encapsulation in chitosan-alginate nanocapsules. When fermented into black garlic, the results showed that *Ilocos variety* contained 0.21 mg/g anthocyanin; brown to black powder was the final product of the encapsulated anthocyanin extract from fermented black garlic. FTIR analysis confirmed that the anthocyanin content from the fermented black garlic showed a well-incorporated framework with the chitosan-alginate nanocapsules. Also, SEM analysis showed the surface morphology of the blank nanocapsules and anthocyanin-loaded nanocapsules to have round, crystal-like shaped morphology and larger irregular shapes, respectively. Furthermore, particle size analysis using the dynamic light scattering technique showed that anthocyanin-loaded nanocapsules were nanoparticles with particle sizes ranging from 50.7 ± 3.3 nm to 92.0 ± 6.1 nm. The encapsulation efficiency of the treatments was 65.11% (T_1), 68.18% (T_2), and 78.82% (T_3). Surfactants further promote the encapsulation of a water-soluble compound like anthocyanin. In this work, the highest encapsulation efficiency of the anthocyanin extract in the chitosan-alginate polyelectrolyte complex can be obtained using the highest initial concentration of anthocyanin extract from fermented black garlic (15 mg/ml).

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NOMENCLATURE

A	Absorbance
VE	Volume of extract
MW	Molecular weight of cyanidin-3-glucoside (449.2 g/mol)
c	Concentration of gallic acid obtained from calibration curve in mg/ml
V	Volume of extract in ml
M	Mass of extract in grams
A_b	Absorbance of the blank
A_s	Absorbance of the sample
EC_{50}	Concentration required to obtain a 50% antioxidant effect

Greek symbol

ε	molar absorptivity of cyanidin-3-glucoside (25,965 L/cm mol)