IJESRT: 8(6), June, 2019 ISSN: 2277-9655

International Journal of Engineering Sciences &Research Technology

(A Peer Reviewed Online Journal)
Impact Factor: 5.164

IJESRT

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

IJESRT

INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH TECHNOLOGY

STUDY OF SYNTHESIS AND CHARACTERISTICS OF TOTAL POLYPHENOLS, TOTAL FLAVONOIDSAND S-ALLYL CYSTEINE-LOADED ALGINATE NANOPARTICLES WITH VARIOUS BLACK GARLIC EXTRACTS AND ALGINATE RATIOS

Nguyen Ai Thach[*1,2] & Nguyen Minh Thuy[1]
[1]Department of Food Technology, College of Agriculture, Can Tho University, Viet Nam
[2]Department of Food Technology and Postharvest Technology, Faculty of Agriculture and Food Technology, Tien Giang University, Viet Nam

DOI: 10.5281/zenodo.3256165

ABSTRACT

Black garlic exerts metabolic and cardiovascular beneficial effects. The extraction of bioactive compounds from aged garlic and their quantitative and qualitative estimation is important for exploration of new technique to be used by pharmaceutical/agrochemical industry directly. In order to improve the bioavailability of black garlic, nanotechnology has been applied in this study. Black garlic nanoparticles were synthesized by polymeric alginate with the aged garlic extract/alginate solution rates were 1/1, 2/1, 3/1, 1/2, and 1/3 (v/v). Surface morphology and particle size of nanoparticles were investigated by Field Emission Scanning Electron Microscopy. In addition, zeta potential were measured by zeta potential meter and particle's size measured by using Dynamic Light Scattering. The research results showed that black garlic nanoparticles had a particles size of 60-80 nm and are distributed in water with particle size about 109-178 nm. Black garlic nanoparticles well dispersed stability with zeta potential about -11.0 ÷ -22.5 mV. The FT-IR spectra also showed that no new substances were formed during nanoparticle creating.

KEYWORDS: bioactive compound, black garlic, characteristic, nanoparticle, polymer, synthesis

1. INTRODUCTION

Black garlic is a newly processed food prepared by subjecting whole raw garlic to thermal peocessing for about a month under controlled high temperature and high humidity. The browning process occurred during aging. The pH of black garlic also decreased from about 6.0 in fresh garlic to less than 3.8 in black garlic. This explains the longer preservation of black garlic [1]. An important change during aging process was the increase in polyphenol content [2] and thus increased antioxidant capacity [3]. On the other hand, the variability of some unstable ingredients and the smell of fresh garlic became stable and odorless compounds during aging, predominantly of sulfur organic compounds such as S-allyl cysteine (SAC), also increase the antioxidant activity [4]. SAC is known as a water soluble bioactive compound for its extremely high antioxidant capacity [5]. SAC is formed during hydrolysis of γ-glutamyl-S-allylcysteine by the enzyme γ-glutamyl transpeptidase (γ-GTP, EC 2.3.2.2). SAC content in fresh garlic is about 20-30 µg/g and increases about 6 times during aging [6].
Nanomaterials are materials with a physico-chemical structure that is larger than the atomic/molecular's dimension but smaller than 100 nm (showing the physical, chemical and/or biological properties related to nanostructures of nanomaterials). In fact, nanotechnology involves substances that are special characteristics because of their size, and if based on natural molecules (for example, molecular manufacturing), the function is different with the intention in nature [7, 8].

Alginate has been widely used by special physical and chemical properties. They are often used to create particles (nano or micro scale) and solid gel [9, 10, 11, 12]. Alginate is non-toxic, biodegradable, low cost and available in the market. Alginate is also considered a drug that can adhere to the intestinal wall and release drugs (mucoadhesion), biocompatibility, and non-immunogen. Alginate is a negatively charged polymer, produced by brown algae and bacteria, containing α-L-guluronic acid and β-D-mannuronic acid of reducing form, linearly linked together by 1, 4-glycosidic bonds. The composition and sequence of reduced α-L-guluronic acid and β-D-

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

mannuronic acid depend on the origin of the algae used and they affect the properties of alginate. Alginate may also be chemically modified to regulate its characteristic properties [13, 14]. Also, nanoaggregates, nanocapsules and nanospheres are nanoparticle systems with a particle diameter is about 10 to 100 nm. These systems can contain enzymes, drugs and various compounds by dissolving or locking them inside or attaching (clamping) them to the nanoparticles matrix.

This research was done for studying the impact of various black garlic extracts/alginate ratios on bioactive compounds (total polyphenolics content (TPC), total flavonoid content (TFC) and SAC) encapsulation efficacy, morphology and size of loaded polymeric alginate nanoparticles.

2. MATERIALS AND METHODS

2.1. Materials

Fresh garlics were harvested at Van Hai ward, Phan Rang-Thap Cham city, Ninh Thuan province, Viet Nam. Black garlic was processed at Can Tho University, Viet Nam by using the aging method of whole garlic bulbs. Bionanoparticles were synthesized at Institute of Materials Science, Vietnam Academy of Science and Technology, Ha Noi, Vietnam.

2.2. Methods

2.2.1. Preparation of bioactive compounds loaded alginate polymeric nanoparticles

Black garlic is extracted with a material/solvent ratio of 1/10 in 50% ethanol at 60°C for 90 minutes. Drop slowly the extracted black garlic into alginate (Sigma-Aldrich Co. LLC) solution (1 mg/mL) in an ultrasonic vibrator with a frequency of 40 KHz with the extract/alginate solution rate of 1/1, 2/1, 3/1, 1/2, and 1/3 (v/v). The mixture is sealed and stirred at room temperature for 48 hours. Then, evaporated ethanol in a rotary evaporator at 35°C for 1 hour with a rate of 50 rpm. After the ethanol has been expelled, the mixture was centrifuged at a rate of 5000 rpm for 5 minutes, collecting the supernatants which were kept at 5°C before using.

2.2.2. Analysis and measurement methods

Encapsulation efficiency (EE%): The encapsulation efficiency of the nanoparticles system is calculated by the formulation: EE% = (M_1/M_2)x100. Where, M_1: mass of bioactive compounds in nanoparticle system; M_2 : mass of initial bioactive compounds. M_1 and M_2 were calculated by dry weight.

Morphology and particle size: were determined by Scanning Electron Microscopy, Hitachi S-4800, Singapore.
Zeta potential: were measured by zeta potential meter and particle's size measured by using dynamic light scattering (DLS) method Zetasizer Nano 7.03 (Malvern Instruments Ltd, Malvern, UK). The zeta potential of the black garlic nanoparticles system was determined at 25°C.

Fourier transform infrared spectroscopy (FT-IR): using potassium bromide tablets and infrared spectrophotometer Perkin Elmer (Waltham, Massachusetts, USA) to identify specific functional groups between 4000 and 400 cm^{-1} (wavenumbers).

Total polyphenols content (TPC) (mg gallic acid equivalents (GAE) per g of dry weight (d.w)) was determined by Folin-Ciocalteu method [15]. Phenolic reacts with phosphomolybdic acid in the Folin-Ciocalteau reagent which appears to be blue color in the alkaline medium. The absorbance was recorded at 765 nm by spectrophotometer. Gallic acid was used as a standard for the calibration curve. The phenolic content was reported as gallic acid equivalents (mg) using the following linear equation (mg GAE/g d.w).

Total flavonoids content (TFC): total flavonoids content was determined by colorimetric method with AlCl3 solution in the an alkaline-photometric medium [16]. The absorbance of reaction solution was measured at 415 nm. Based on the quercetin calibration curve to determine total flavonoids content of samples. The results are expressed in mg of quercetin equivalent (QE) per g of dry weight (mg QE/g d.w).

Determination of S-allyl cysteine by high performance liquid chromatography (HPLC). Sample preparation: the extracts of aged black garlic were filtered through a 0.45 µm syringe filter (Sartorius AG, Goettingen, Germany)

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

and the solution was used to analyze. S-allyl-L-cysteine (≥ 98%, Sigma-Aldrich Co. LLC) was used as a standard for the calibration curve.

HPLC analysis conditions: HPLC-UVD system (Shimadzu, Shimadzu Corporation, Japan) was used to analyze S-allyl cysteine content in extract of aged black garlic samples including: an LC-10AD pump, a SPD-10A UV/Vis detector, a CTO-10AC column thermostat and a manual sample injector. Separation of the analyte was carried out using an LiChroCART® column (250x4 mm, 5 µm, Merck Millipore, Merck Millipore Corporation, Germany) at room temperature, and SAC was detected at 210 nm. The mobile phase consists of 0.1% H_3PO_4 solution and acetonitrile solution (Sigma-Aldrich Co. LLC) with isocratic elution. Flow rate of 0.5 mL/min and injection volume of 10 µL.

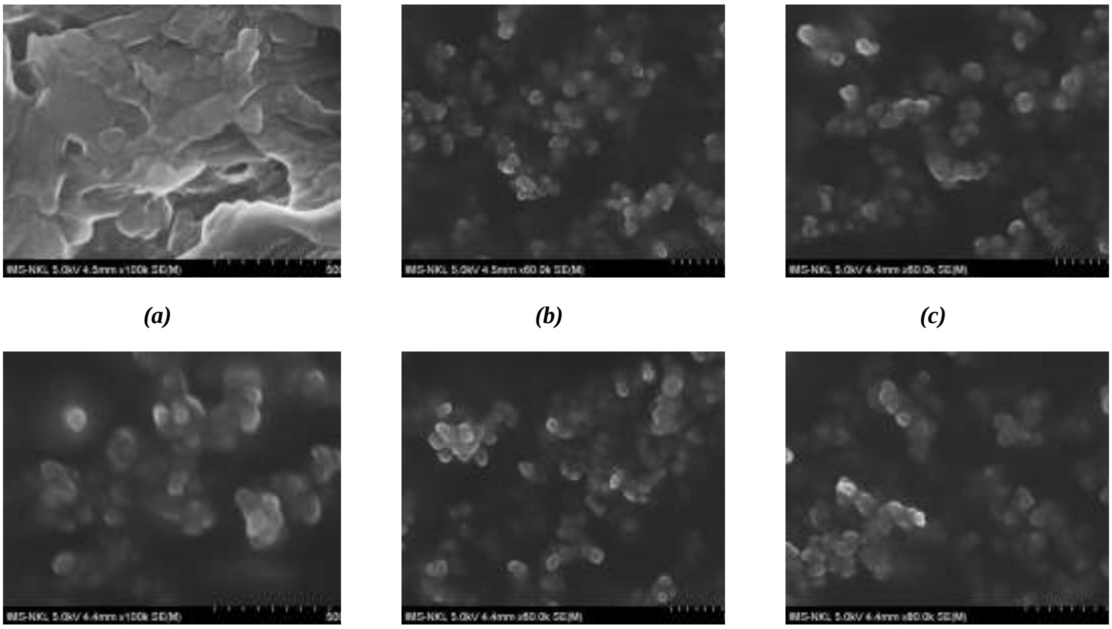
2.2.3. Data analysis

The experiment was repeated three times. The means and standard deviations were also caculated by using Microsoft Excel software.

3. RESULTS AND DISCUSSION

3.1. Morphology and size

FE-SEM images showed that black garlic nanoparticles were synthesized in different proportions which have a shape close to the sphere, the size was between 60-80 nm and less agglomeration (Figure 1b-f). Particularly, Figure 1a expressed the appearance of black garlic has not yet nano-sized, the compounds made up the blocks that have no defined shape and rather large size. The diameter measured in DLS was the hydrodynamic diameter, so the results were a larger size than the particles measured by a scanning electron microscope. Small particles size (< 200 nm) can ensure increased permeability through the vascular wall, lower level of absorption of the endothelial system and improved the rate of used encapsulation materials [17].



(a) *(b)* *(c)*

(d) *(e)* *(f)*

Figure 1. FE-SEM images of the black garlic system (a) and black garlic nanoparticle system with extract/alginate solution ratio were 1/1 (b), 2/1 (c), 3/1 (d), 1/2 (e), 1/3 (f)

3.2. Nanoparticle distribution and zeta potential

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

When the charge of the same particles would repel each other, alginate of small enough size and low density in the suspension system, the high zeta potential would stabilize because the dispersion would resist agglomeration [18]. The results presented in Table 1 which showed that the size distribution of the black garlic nanoparticle system with the average particles size was 109-178 nm, all dispersed well (100%). The zeta potential distribution of the black garlic nanoparticles produced in the different proportions (Figure 2, 3, 4, 5, and 6) showed that zeta potential values in the range were -11.0 ÷ -22.5 mV. From these results, it can be deduced that the suspension of black garlic nanoparticles was negatively charged with small particle size and relatively high zeta potential as a relatively stable dispersion.

Table 1. Particles size distribution and zeta potential of the black garlic nanoparticles system synthesized at different rates of extract/alginate solution

Rate of black garlic extract/alginate solution (v/v)	1/1	2/1	3/1	1/2	1/3
Particle size distribution by intensity (%)	100 (≈165nm)	100 (≈109nm)	100 (≈137nm)	100 (≈178nm)	100 (≈144nm)
Particle size distribution by quantity	100 (≈165nm)	100 (≈109nm)	100 (≈137nm)	100 (≈178nm)	100 (≈144nm)
Particle size distribution by volume (%)	100 (≈165nm)	100 (≈109nm)	100 (≈137nm)	100 (≈178nm)	100 (≈144nm)
Zeta potential (mV)	-12.1	-22.5	-15.1	-11.0	-14.4

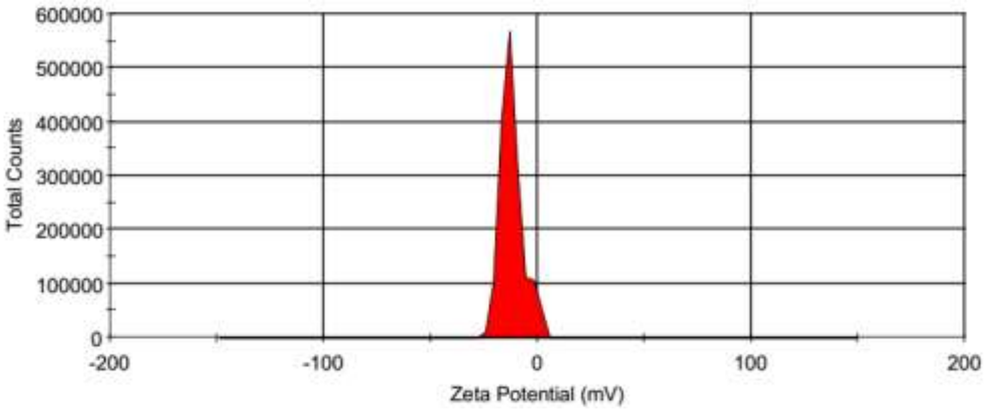


Figure 2. The zeta potential of the black garlic nanoparticles system was synthesized at the ratio of extract/alginate solution is 1/1

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

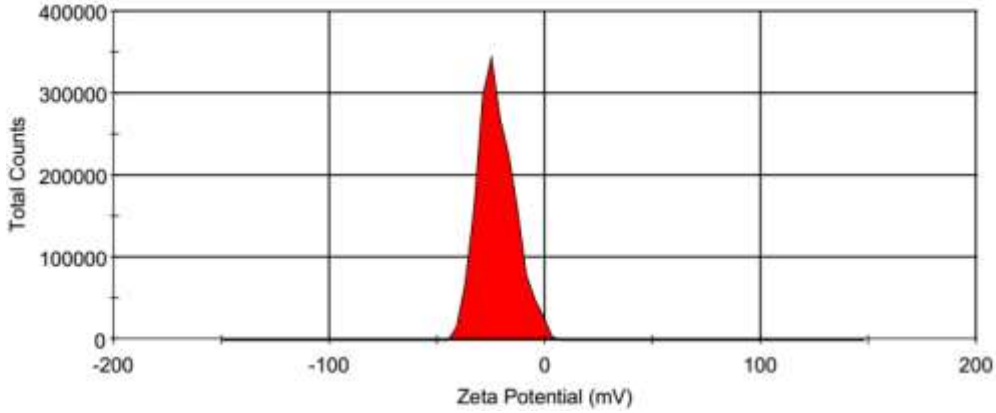


Figure 3. The zeta potential of the black garlic nanoparticles system was synthesized at the ratio of extract/alginate solution is 2/1

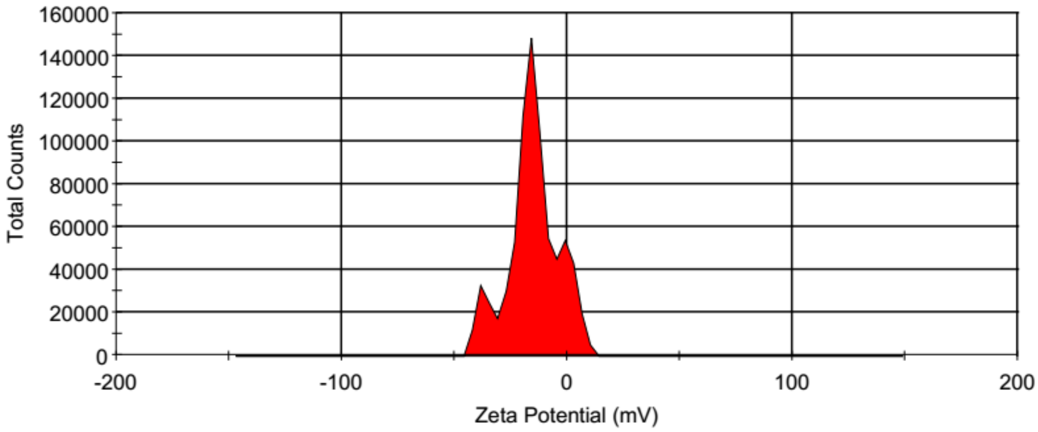


Figure 4. The zeta potential of the black garlic nanoparticles system was synthesized at the ratio of extract/alginate solution is 3/1

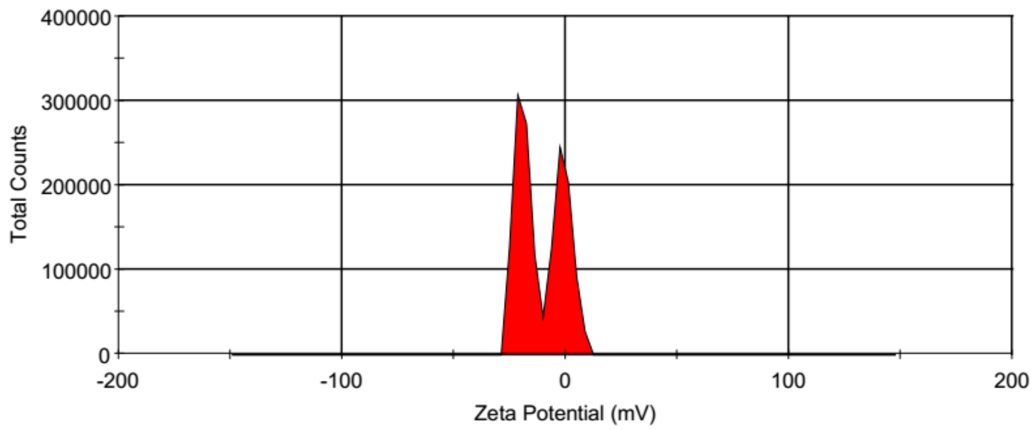


Figure 5. The zeta potential of the black garlic nanoparticles system was synthesized at the ratio of extract/alginate solution is 1/2

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

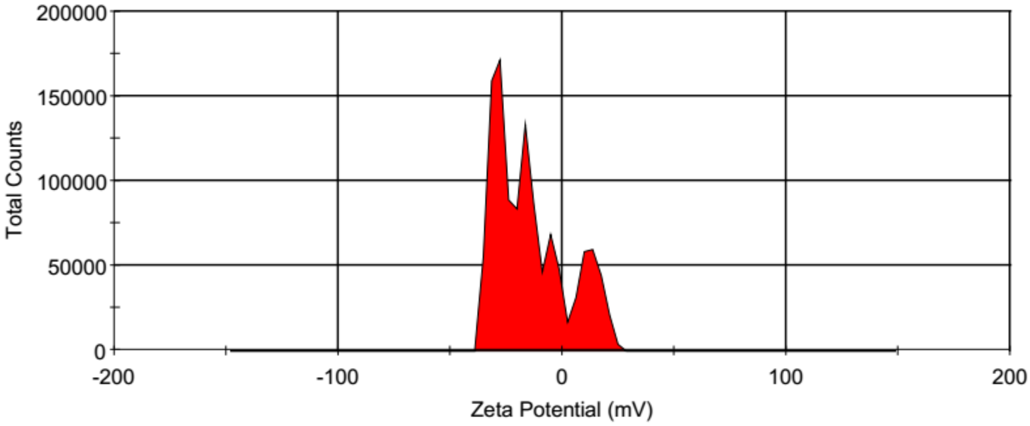


Figure 6. The zeta potential of the black garlic nanoparticles system was synthesized at the ratio of extract/alginate solution is 1/3

3.3. FT-IR spectrum

Determining bioactive compounds by using FT-IR based on the existence of the characteristic frequency of the functional group, they have the same value nearly the same regardless of which other functional groups appear in it. Their differences in infrared spectra are mainly due to differences in absorption intensity. The results of infrared spectra of black garlic, alginate and black garlic nanoparticles system were shown in Figure 7, 8, and 9. Based on spectral peaks, new bonds were formed in new compounds (black garlic nanoparticles system) that all were presented in the original compounds (black garlic and alginate). Therefore, the nano-processing of creating black garlic nanoparticles has not appeared new oscillation peaks but only a shift in the wavenumbers associated with those specific bonds. This demonstrated that no new chemical bonds were formed, further asserting that alginate compounds and materials are combined through physical interactions rather than chemical reactions.

The results presented in Figure 7, 8, and 9 showed that a wide range of hydrogen bonds in the –OH group appearing at 3,200-3,500 cm^{-1}. The main bands (such as the representative group for the –OH group) at 3,395-3,417 cm^{-1} due to the oxygen-containing function. The –CH group was shown at the peak of 2,927-2,936 cm^{-1}. Typical absorption bands between 4,000 and 2,500 cm^{-1} were 3,480-3,440, 3,260-3270, and 2,960-2,878 cm^{-1}, which characterize the extended area of the –OH, –NH, and –CH group, respectively. The absorption bands around 1,636-1,640, 1,413-1,431, 1,153-1,155, 1,048-1,080, and 1,013-1,024 cm^{-1} were the presence of C=C, CH, CN, CO, C-O-C, and C-C groups, respectively. Other characteristic absorption bands that occur at 915-931, 852-859, 762-770 and 566-578 cm^{-1} due to the extensive oscillation of the whole anhydroglucose ring. This indicated that melanoidins were formed in black garlic consisting of –OH, –CH, amide I, and amide III groups.

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

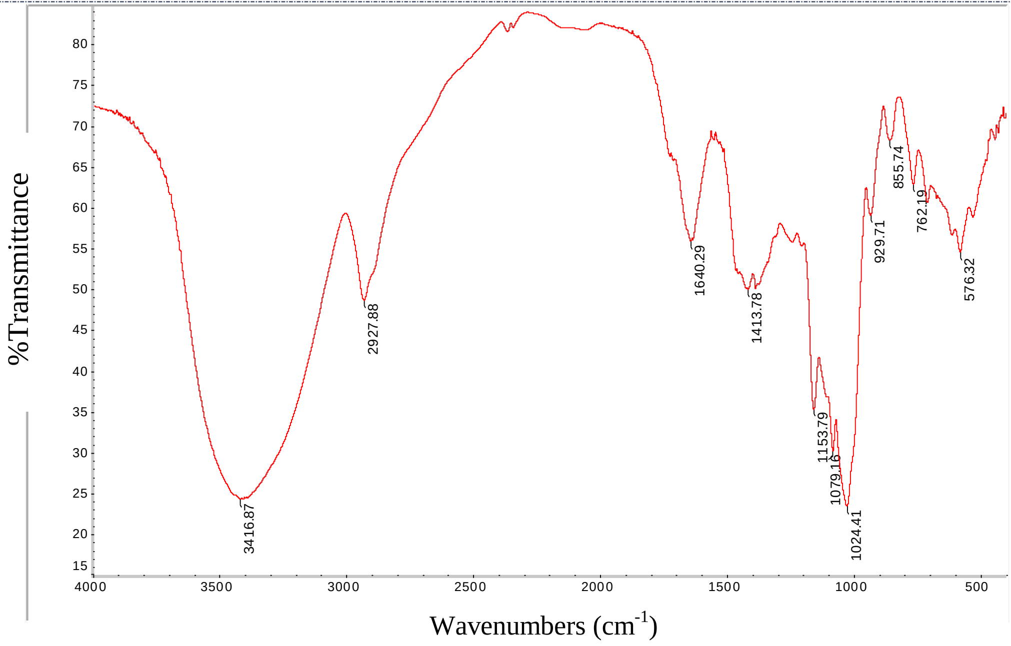


Figure 7. FT-IR spectrum of black garlic

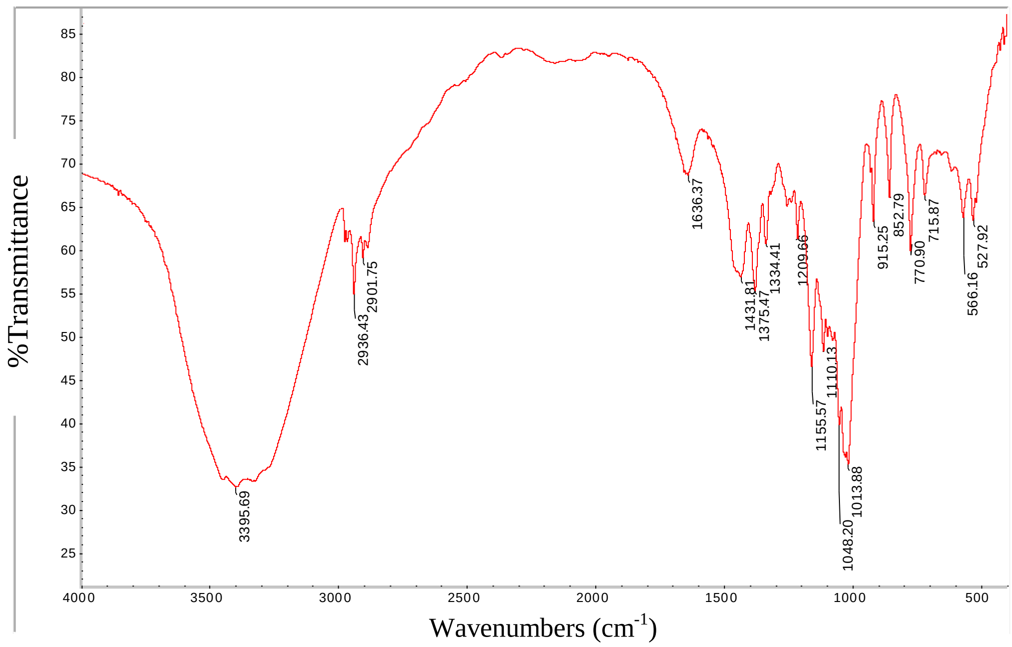


Figure 8. FT-IR spectrum of alginate

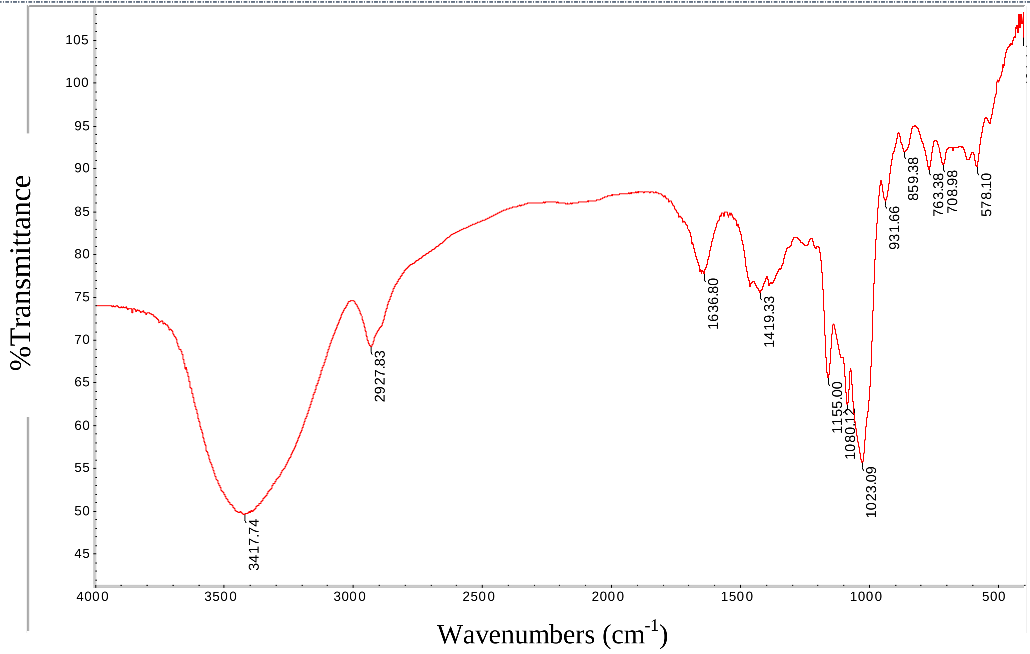


Figure 9. FT-IR spectrum of black garlic nanoparticles system

Beside that, FT-IR spectrum of black garlic nanoparticles system was synthesized at the rate of extract/alginate solution were 1/1, 2/1, 3/1, 1/2, and 1/3 (Figure 10, 11, 12, 13, and 14) that there is no significantly difference and no strange peaks were formed.

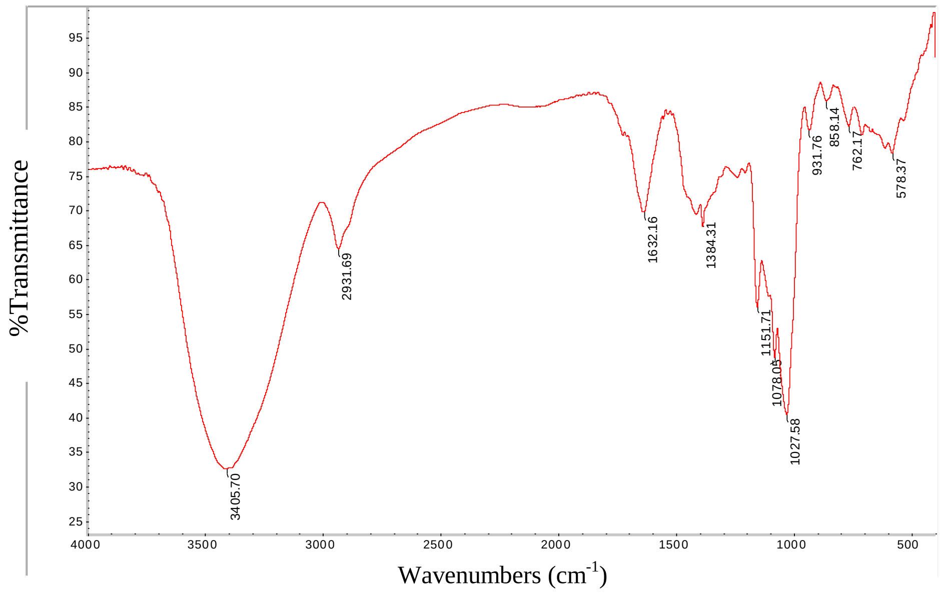


Figure 10. FT-IR spectrum of the black garlic nanoparticles system was synthesized at rate 1/1

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

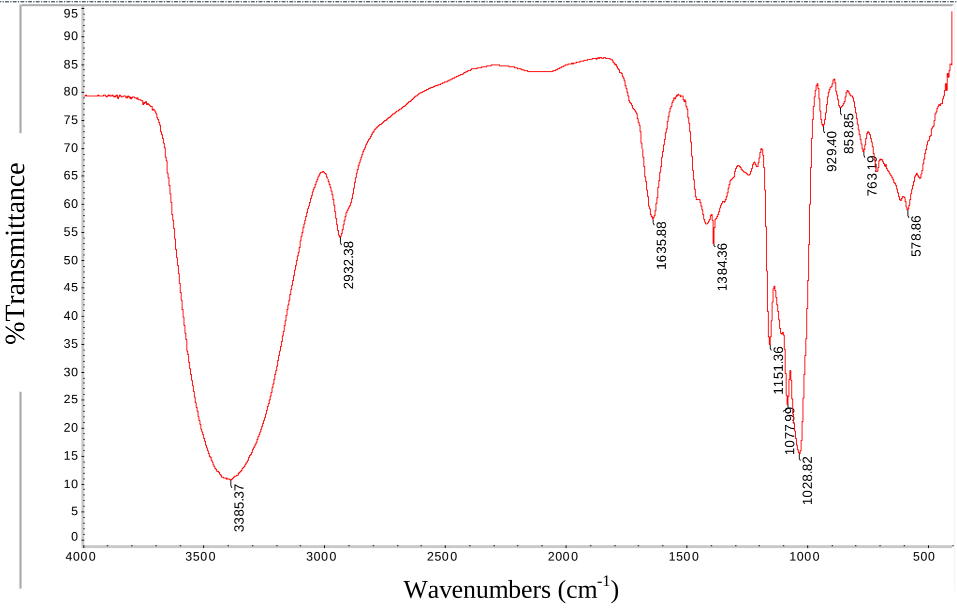


Figure 11. FT-IR spectrum of the black garlic nanoparticles system was synthesized at rate 2/1

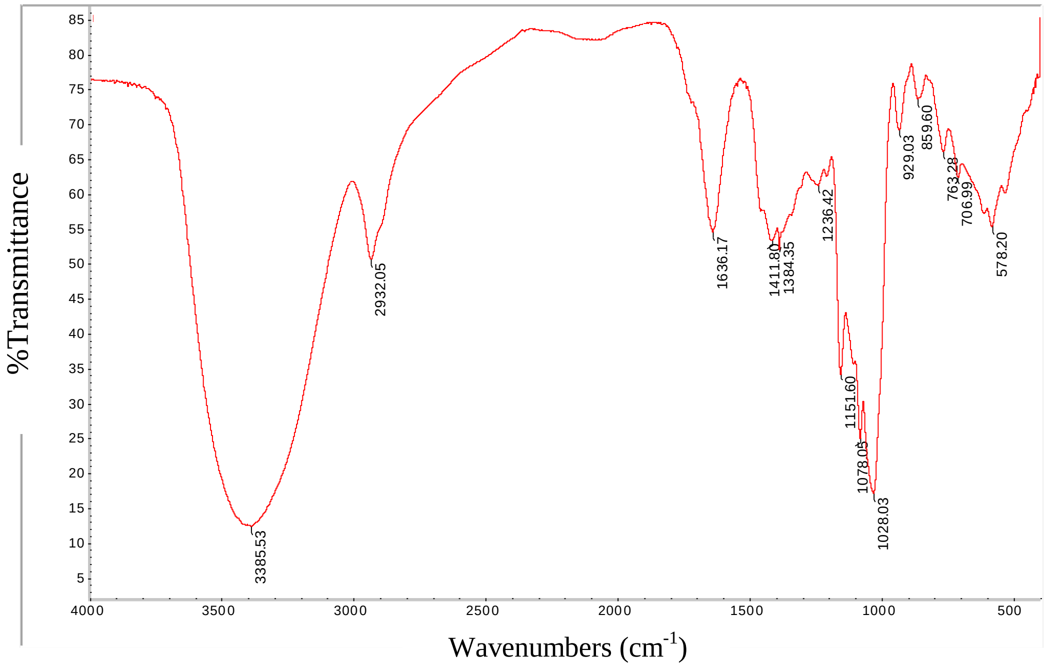


Figure 12. FT-IR spectrum of the black garlic nanoparticles system was synthesized at rate 3/1

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

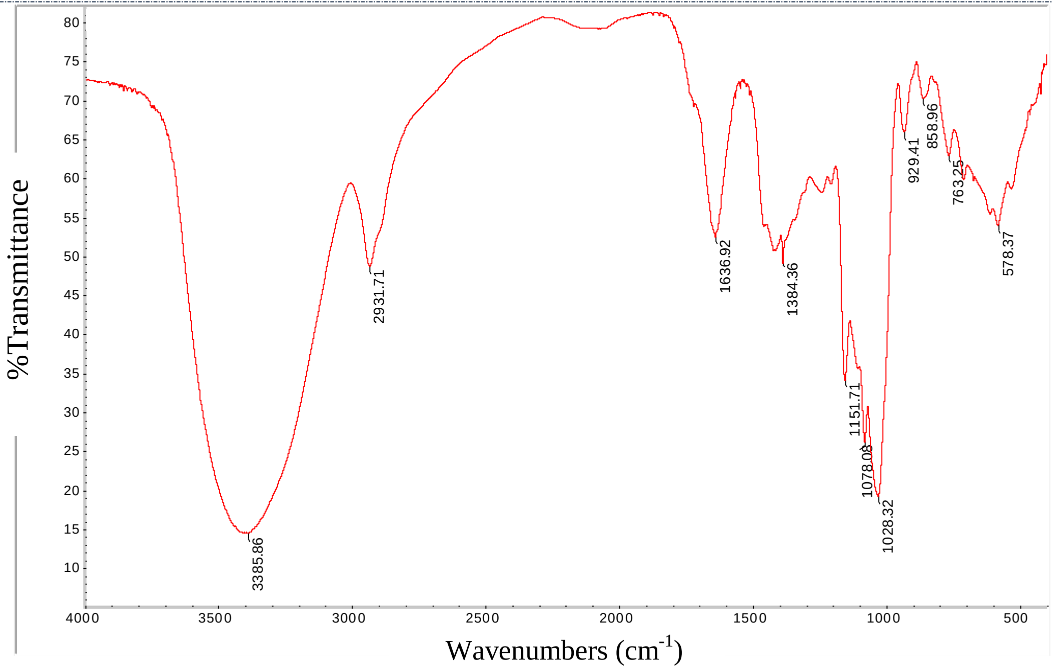


Figure 13. FT-IR spectrum of the black garlic nanoparticles system was synthesized at rate ½

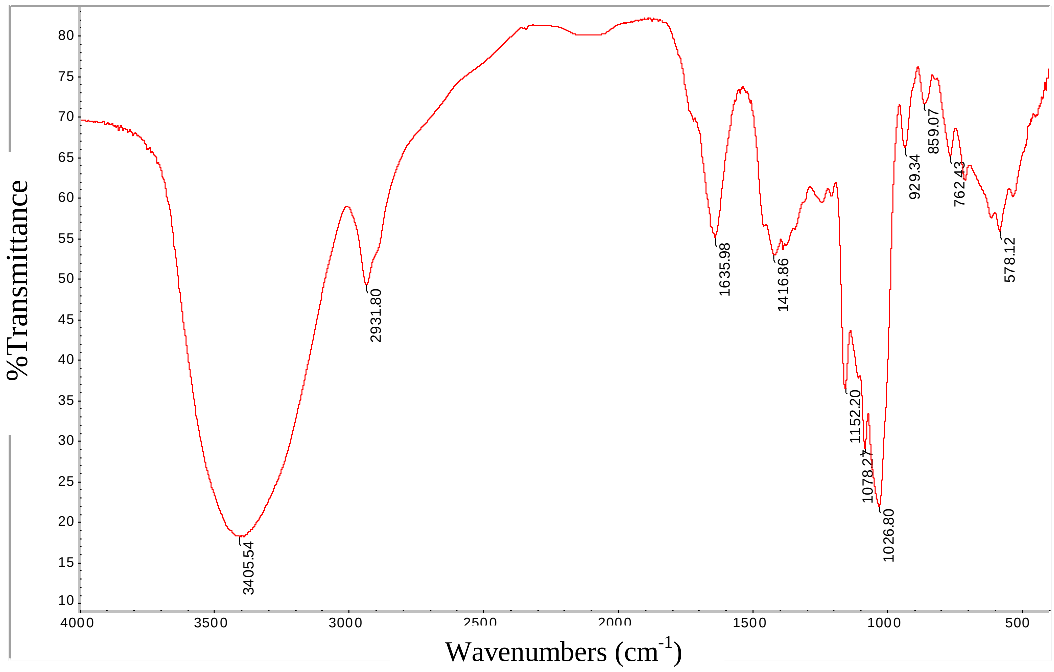


Figure 14. FT-IR spectrum of the black garlic nanoparticles system was synthesized at rate 1/3

3.4. Encapsulation efficiency (EE%)

The data presented in Table 2 shown that the encapsulation efficiency increased from 28.2 to 75.1% and 33.2 to 82.1% for TPC and SAC, respectively, when increasing the ratio of alginate. This might be properly because SAC and TPC are both hydrophilic compounds, so they will interact well with the hydrophilic core of alginate. These lead to an increase in the amount of compounds in the core and increase efficiency. In contrast, TFC contains most of the hydrophobic flavonoids, so the increase in alginate ratio will have to little effect on the

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

flavonoid content (0.234-0.298 mg QE/mL). In addition, the over encapsulation efficiency would increase the particle size, the particle shape was not close to the circle and the zeta potential of the black garlic nanoparticles system was reduced which will lead to aggregation and loss of nanoscale. Therefore, from the above data analysis, the rate of 2/1 would use to synthesize black garlic nanoparticles which was considered to be the most suitable and used to evaluate biological activity.

Table 2. Encapsulation efficiency (%) of black garlic nanoparticles system at different rates of extract/alginate solution

Rate of extract/alginate	Loaded bioactive compounds content			EE (%)		
	TPC (mg GAE/mL)	TFC (mg QE/mL)	SAC (mg/L)			
1/1	1.32±0.021	0.234±0.021	11.2±1.04	28.2	4.51	33.2
2/1	1.40±0.015	0.292±0.020	13.4±1.25	30.5	5.12	35.9
3/1	1.57±0.034	0.245±0.015	17.5±1.56	44.3	4.63	46.8
1/2	2.78±0.098	0.282±0.019	21.3±1.25	67.6	5.01	73.4
1/3	2.89±0.055	0.298±0.021	27.9±1.34	75.1	5.16	82.1

4. CONCLUSION

Research results showed that the black garlic nanoparticles system was synthesized by alginate when black garlic extract/alginate solution ratio increase from 1/1 to 3/1 which had a particle size about 60-80 nm and a domestic distribution was about 109-178 nm. Black garlic nanoparticles disperse quite stable with zeta potential range -11.0 ÷ -22.5 mV. FT-IR spectra images also showed that no formation of new compounds was found in manufacturing process of loaded bioactive compounds nanoparticles.

REFERENCES

[1] Toledano-Medina M.A., J. Perez-Aparicio, R. Moreno-Rojas, and Merinas-Amo, 2016. Evolution of some physicochemical and antioxidant properties of black garlic whole bulbs and peeled cloves. Food Chemistry, 199: 135–139.
[2] Kim I., J.Y. Kim, Y.J. Hwang, K.A. Hwang, A.S. Om, J.H. Kim, and K.J. Cho, 2011. The beneficial effects of aged black garlic extract on obesity and hyperlipidemia in rats fed a high-fat diet. Journal of Medicinal Plant Research, 5: 3159–3168.
[3] [3]. Nencini, C., A. Menchiari, G.G. Franchi, and L. Micheli, 2011. In vitro antioxidant activity of aged extracts of some Italian Allium species. Plant Foods for Human Nutrition, 66(1): 11–16.
[4] Lee, Y.-M., O.C. Gweon, Y.J. Seo, J. Im, M.J. Kang, M.J. Kim, and J.I. Kim, 2009. Antioxidant effect of garlic and aged black garlic in animal model of type 2 diabetes mellitus. Nutrition Research and Practice, 3(2): 156–161.
[5] Bae, S. E., S.Y. Cho, Y.D. Won, S.H. Lee, and H.J.Park, 2012. A comparative study of the different analytical methods for analysis of S-allyl cysteine in black garlic by HPLC. LWT - Food Science and Technology, 46(2): 532–535.
[6] Bae, S.E., S.Y. Cho, Y.D. Won, S.H. Lee, and H.J.Park, 2014. Changes in S-allyl cysteine contents and physicochemical properties of black garlic during heat treatment. LWT - Food Science and Technology, 55(1): 397–402.
[7] Linkov, I. and J. Steevens, 2008. Nanomaterials: Risks and Benefit – NATO Sciencefor Peace and Security Series C: Environmental Security , Dordrecht, Netherlands: Springer.
[8] [8]. Narlikar, A.V. and Y.Y. Fu, 2010. The Oxford Handbook of Nanoscience and Technology, New York: OxfordUniversity Press.
[9] Gombotz, W.R. and S.F. Wee, 1998. Protein release from alginate matrices. Adv Drug Deliv Rev., 31: 267–85.

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

[10] [Murtaza, G., A. Waseem, R. Nisar ur, and I. Hussain, 2011. Alginate microparticles for biodelivery: a review. Afr J Pharm Pharmacol, 5: 2726–37.
[11] Goh, C.H., P.W.S. Heng, and L.W. Chan, 2012. Alginates as a useful natural polymer for microencapsulation and therapeutic applications. Carbohydr Polym, 88: 1–12.
[12] Lee, K.Y. and D.J. Mooney, 2012. Alginate: properties and biomedical applications. Progress in Polymer Science. Elsevier. UK. pp 106–26.
[13] Yang, J.S., Y.J. Xie, and W. He, 2011. Research progress on chemical modification of alginate: a review. Carbohydr Polym, 84: 33–9.
[14] Pawar, S.N. and K.J. Edgar, 2012. Alginate derivatization: a review of chemistry, properties and applications. Biomaterials, 33: 3279–305.
[15] Wolfe K., X. Wu, and L.H. Liu, 2003. Antioxidant activity of apple peels. J Agric Food Chem, 51: 609-614.
[16] Zhu H., Y. Wang, Y. Liu, Y. Xia, and T. Tang, 2010. Analysis of flavonoids in Portulaca oleracea L. by UV–vis spectrophotometry with comparative study on different extraction technologies. Food Analytical Methods, 3(2): 90-97.
[17] Lu, Q., P.M. Lu, J.H. Piao, X.L. Xu, J. Chen, L. Zhu, and J.G. Jiang, 2014. Preparation and physicochemical characteristics of an allicin nanoliposome and its release behavior. LWT - Food Science and Technology, 57(2): 686-695.
[18] Sou, K., 2011. Electrostatics of carboxylated anionic vesicles for improving entrapment capacity. Chemistry and Physics of Lipids, 164(3): 211-215.