Journal of Food Processing & Technology
ISSN: 2157-7110

Siow and Ong, J Food Process Technol 2013, 4:1
DOI: 10.4172/2157-7110.1000199

Research Article Open Access

Effect of pH on Garlic Oil Encapsulation by Complex Coacervation

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Abstract

Microencapsulation of garlic oil was performed via complex coacervation using type A or type B gelatin and gum acacia as wall materials. Effect of pH on garlic oil encapsulation yield and loading efficiency was investigated. Results showed that the highest garlic oil encapsulation yield and loading efficiency for type A or B gelatin and gum acacia occurred at pH 4.5 and 3.5, respectively. Scanning electron microscopy (SEM) observation showed that coacervates made from type A or B gelatin-gum acacia were spherical with smooth topography. Both cross-linked type A and B gelatin-gum acacia exhibited controlled release of garlic oil during five hours of incubation in pH 2 pepsin solution. Coacervates produced from type A or B gelatin-gum acacia protected garlic oil from primary and secondary oxidation during 12 days storage at 45°C.

Keywords: Controlled-release; Oxidative stability; Gelatin; Encapsulation; Gum acacia

Introduction

Coacervation is known to be true microencapsulation because the core material is completely surrounded by a continuous coating of wall material. Coacervation involves interaction between one or many hydrocolloids, particularly between oppositely charged protein and polysaccharide in the same reaction media [1]. The interaction relies on the charges carried by the biopolymers, pH of the coacervation mixture, ionic strength, ratio between the two biopolymers, accessibility of the charges for interaction and other mechanical and physical factors. pH plays an important role in complex coacervation because it affects the formation of protein-carbohydrate complexes by influencing the degree of ionization of the functional group of protein (amino group) and carbohydrate (carboxyl group) [2]. In a mixture containing an anionic polysaccharide and a protein, adjustment of pH below the isoelectric point (pI) or the electrical equivalence pH (IEP) would result in maximum electrostatic attraction as the two biopolymers are carrying oppositely charge. Polymer systems that have been reported for microencapsulation via complex coacervation include gelatin-gum acacia [3-5], whey protein-gum acacia [6] and soybean protein isolate-gum acacia [7].

Coacervation has gained much interest in the microencapsulation literatures. It is used in agriculture chemicals, pharmaceuticals and food products [8] due to its unique high encapsulation load of a maximum of 99% active ingredients. Microcapsules produced by coacervation allow specific targeted and controlled-release of core material based on temperature, prolonged release and mechanical stress [1]. Comparing to other microencapsulation technologies such as spray drying, coacervation is a gentle process because no high heat is involved. Hence, flavour degradation or evaporation can be avoided during the coating process. Coacervate microcapsules are impervious to oxygen as the core is completely surrounded by the continuous layer of wall material. Hence, core material can be 65 protected against oxidation or evaporation [8].

Gelatin is a water soluble protein derived primarily from controlled partial hydrolysis of mammalian collagen [9]. Type A gelatin, is produced through acid hydrolysis of porcine skin whilst Type B gelatin is produced by lime hydrolysis of bone and animal skin of bovine source [10]. Garlic oil is of particular interest due to the presence of organosulfur compounds such as diallyl sulfide (DS), diallyl disulfide (DADS), diallyl trisulfide (DATS), diallyl tetrasulfide (DATTS) and many other garlic sulphides, which contribute to its antimicrobial and antioxidant properties [11]. These organosulfur compounds in garlic oil are highly volatile, low solubility in water, prone to oxidation and emit unpleasant smell.

Since limited literatures are available on the microencapsulation of garlic oil by complex coacervation, the objectives of this study were to investigate the effect of pH on the formation of garlic oil coacervates using type A or type B gelatin with gum acacia. Controlled release of garlic oil from non-cross-linked and cross-linked coacervates in pepsin and the oxidative stability of garlic oil were determined.

Materials and Methods

Materials

Type A gelatin from porcine source, type B gelatin from bovine source and p-anisidine (2-methoxyaniline) were purchased from Sigma Alrich (St. Louis, Mo, USA). Gum acacia was purchased from Euro Chemo Pharma Sdn. Bhd. (Penang, Malaysia). Garlic oil (UPHA Pharmaceutical Manufacturer, Bangi, Malaysia) was purchased from a local pharmacy. Formaldehyde (~37%) was purchased from R&M Chemicals (Essex, UK) whereas pepsin powder (from porcine gastric mucosa) was purchased from Merck KgaA (Darmstadt, Germany).

Preparation of coacervates by complex coacervation

Gelatin-gum acacia coacervates were prepared according to Junyaprasert et al. [12] with some modifications. One percent (w/v) of type A gelatin or gum acacia was prepared at 40°C. The solutions were maintained at 40°C (WNB 14, Memmert GmbH & Co. KG, Germany) throughout the experiment.

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Received September 25, 2012; **Accepted** November 28, 2012; **Published** December 07, 2012

Citation: Siow LF, Ong CS (2013) Effect of pH on Garlic Oil Encapsulation by Complex Coacervation. J Food Process Technol 4: 199. doi:10.4172/2157-7110.1000199

Twelve gram of garlic oil were added to 1% (w/v) gelatin followed by emulsification using an overhead stirrer (RW20 digital, IKA Works, NC, USA) at 200 rpm for 30 minutes. One hundred ml of 1% (w/v) gum acacia solution was gradually added into the system to make a total biopolymer weight of 2 g in the final volume of 200 mL. Stirring was maintained at 200 rpm using the overhead stirrer (RW20 digital, IKA Works, NC, USA).

pH of the emulsion was adjusted to 4.0 (SG2-ELK, Mettler-Toledo® International Inc., Switzerland) by dropwise addition of 10% (v/v) acetic acid to induce complex coacervation. The mixture was cooled down to 5°C while agitation was maintained at 200 rpm (RW20 digital overhead stirrer, IKA Works, NC, USA). Two mL of formaldehyde was added into the mixture and pH of the mixture was adjusted to 9.0 (SG2-ELK, Mettler-Toledo® International Inc., Switzerland) by dropwise addition of 20% (w/v) sodium hydroxide to induce cross- linking of wall materials. The mixture was stirred at 200 rpm (RW20 digital, IKA Works, NC, USA) for an hour. Stirring was terminated when there was a coacervate-rich layer at the top of the mixture.

The coacervate-rich layer was washed with approximately 50 mL of 2-propanol and then filtered under vacuum (Oil-less vacuum pump, Rocker 300, Taiwan) using filter paper (Whatman Limited, No. 1, UK). The washing step was stop until a clear filtrate was obtained. Microcapsules were kept in a desiccator to remove traces of moisture until a constant weight was achieved. Microcapsules with type B gelatin-gum acacia were prepared in a similar manner.

Effect of pH on complex coacervation: pH of the mixture was adjusted to pH 3.0, 3.5, 4.0, 4.5 or 5.0, respectively, to form complex coacervate with gum acacia. Other process variables (1:1 gelatin-to-gum acacia ratio and 6:1 of core-to-wall ratio) were remained constant.

Effect of gelatin-to-gum acacia ratio on complex coacervation: Gelatin-to-gum acacia ratios of 1:1, 3:2, 2:1 or 5:2, respectively, were used to make a total biopolymer weight of 2 g in the final volume of 200 mL sample. Other process variables (pH 3.5 for type B gelatin-gum acacia/ pH 4.0 for type A gelatin-gum acacia and ratio of 6:1 of core-to-wall) were remained constant.

Effect of core-to-wall ratio on complex coacervation: The core-to-wall ratios used in the preparation of coacervates were 2:1, 4:1, 6:1 and 8:1. Other process variables (pH 3.5 for type B gelatin-gum acacia/ pH 4.0 for type A gelatin-gum acacia and 1:1 gelatin-to-gum acacia ratio) were remained constant.

Determination of microencapsulation yield and loading efficiency

Microencapsulation yield defines the recovered dry coacervate's weight using the following equation [13]:

$$Microencapsulation\ yield\,(\%) = \frac{Weight\ of\ the\ microcapsules\ recovered}{Total\ raw\ materials\ added} \times 100 \quad (1)$$

Microencapsulation loading efficiency defines core material that was encapsulated in the microcapsules to the total amount of core material added. The extracted oil was determined using Soxhlet extraction method [14] and calculated using the following equation [15]:

$$Microencapsulation\ loading\ efficiency\,(\%) = \frac{Weight\ of\ garlic\ oil\ loaded\ into\ the\ coacervates}{Amount\ of\ garlic\ oil\ initially\ added\ into\ emulsion} \times 100 \quad (2)$$

Observation under Scanning Electron Microscope (SEM)

Morphology of coacervates was examined under the variable pressure scanning electron microscope (VPSEM) (LEO 1455, Leo® Ltd, UK). Coacervates were first mounted onto a circular aluminum stubs with double-sided sticky tape and then observed under the VPSEM (LEO 1455, Leo® Ltd., UK) at an accelerating voltage of 10 kV (low vacuum mode).

Observation under light microscope and size estimation

Coacervates were put on a micrometer microscope slide (100mm) and observed using a binocular light microscope (BA200, MoticTM Group, Hong Kong) under objective magnification of 10×. Image was captured using a digital camera (Lumix DMC-FP8, 136 Panasonic Inc, Japan). Scale bar was added onto each image and coacervates sizes (diameters) were estimated using the ImageJ software (National Institute of Health, USA) based on the average of 10 coacervate particles.

Controlled-release of garlic oil-containing coacervates

The controlled-release study was conducted according to Cho et al. [16], with some modifications. Coacervates (0.1 g) were placed into five separate test tubes, each of which containing 2 mL of pepsin solution (2 mg/mL of 0.1M citric acid, pH 2.0). The five mixtures were then incubated at 37°C in a water bath for 5 hours (WNB 14, Memmert GmbH & Co. KG, Germany). Oil released from coacervates was carefully pipette out at 1 hour interval for a total of 5 hours and placed on a Whatman No. 1 filter paper to absorb the released oil. Subsequently, the filter paper was dried in an oven at 50°C (UFE-400, Memmert GmbH & Co. KG, Germany). The filter paper was weighed before and after oil absorption to determine the amount of oil released at each interval. Controlled-release of garlic oil was calculated based on the following equation:

$$Core-release\,(\%) = \frac{Final\ weight\ of\ filter\ paper - initial\ weight\ of\ filter\ paper}{Total\ oil\ in\ the\ 0.1g\ microcapsules} \times 100 \quad (1)$$

Oxidative stability of garlic oil-containing coacervates during storage

Oxidative stability of garlic oil before and after encapsulation and during storage was determined by the primary oxidation products estimated using peroxide value (PV) [17]. Secondary oxidation products were estimated usingz p-anisidine value (p-AV) [18]. Garlic oil-containing coacervates and bulk oil were stored in a separate universal bottle and then incubated in a hot air oven (UFE-400, Memmert GmbH & Co. KG, Germany) at 45°C for 12 days. Determination of PV and p-AV were calculated using the following equations:

$$PV = \frac{[(S-B)\times N\times 1000]}{W} \quad (4)$$

where S represents the volume (mL) of sodium thiosulfate used to titrate the oil, B is the volume (mL) of sodium thiosulfate required for blank, N is the normality of sodium thiosulfate solution used for titration and W is the weight of oil (g).

$$p-AV = \frac{[25\times(1.2As-Ab)]}{m} \quad (5)$$

where As is the absorbance of test solution after reacting with p-anisidine, Ab is the absorbance of the sample with addition of p-anisidine and m is the mass of the garlic oil containing microcapsules in gram.

Statistical analysis

Experiments were performed in triplicates (n=3). Results were tested for one-way analysis of variance (ANOVA) and Tukey's post-hoc test. The statistical analysis was performed using Systat software (SPSS Inc., Ver. 16.0, 2007, Chicago, IL, USA). Significant difference was defined at $p \leq 0.05$.

Results and Discussion

Effect of pH on complex coacervation

pH 4.5 and 3.5 were found to give the highest microencapsulation yield and loading efficiency for coacervates of type A or type B gelatin-gum acacia, respectively, (Table 1). Both microencapsulation yield and loading efficiency were found to be significantly reduced ($p \leq 0.05$) for both gelatin-gum acacia systems when pH of the gelatin-gum acacia mixture was adjusted to above or below the optimum pH. Similar observation was reported by Leclercq et al. [19] for coacervates of type A gelatin and Burgess [20] for coacervates of type B gelatin. Difference in the optimum pH between the two gelatin-gum acacia systems was probably due to the different nature of gelatin productions, which resulted in different number of NH_2 and COOH groups on the gelatin molecules. As such, type A gelatin has a higher pI (6-8) compared to type B gelatin (4-5) [21].

The amount of garlic oil in coacervates (loading efficiency) was also noted to be proportional to the yield of coacervates (Table 1). This could be due to more coacervates are available to encapsulate the garlic oil with increasing coacervates yield, thus leading to the high loading efficiency. The size of coacervates was in the range of 100µm (Table 1).

Effect of gelatin-to-gum acacia ratio on complex coacervation

The highest microencapsulation yield and loading efficiency for both type A gelatin-gum acacia and type B gelatin-gum acacia was at the mixing ratio of 1:1 gelatin-to-gum acacia (Table 2). Similar experimental results have been also previously reported [14,23]. Further increasing the gelatin: acacia ratio (3:2, 2:1 or 5:2) significantly decreased the coacervates yield and loading efficiency ($p \leq 0.05$). When one of the colloids (gelatin or gum acacia) is in excess, the presence of non-neutralized charges would result in the formation of more soluble complexes (ionic aggregates) than complex coacervates [3]. The addition of unequal amount of the two colloids into the coacervation system could result in imbalance charges in the coacervation mixture. This may result in reduction of the attraction between the two colloids and subsequently reducing the coacervates yield [23]. Gelatin-gum acacia ratio did not have a significant effect on the size of the coacervates made from both gelatin-gum acacia systems ($p > 0.05$) (Table 2).

Effect of core-to-wall ratio on complex coacervation

The yield of coacervates showed significant differences ($p \leq 0.05$) when the core-to-wall ratio increased from 2:1 to 6:1 or 8:1 (Table 3). The lower coacervates yield at the core to wall ratio of 2:1 or 4:1 was probably due to insufficient core material to be coated by gelatin and gum acacia, thus resulting in the biopolymers remained in the coacervation mixture. The size of coacervates were not significantly different from one another ($p>0.05$) (Table 3).

Scanning electron microscopy observation

The outer topography of the coacervates produced using the ideal parameters for both gelatin-gum acacia systems are presented in Figure 1 (a-d). Coacervates made from both type A or B gelatin-gum acacia systems were spherical in shape with smooth surface (Figure 1a and 1c).

	pH	3.0	3.5	4.0	4.5	5.0
Type A gelatin-acacia	Yield (%)	66.1 ± 0.5^{e}	76.7 ± 0.8^{c}	83.0 ± 1.1^{b}	86.8 ± 1.7^{a}	69.8 ± 1.4^{d}
	Core loading (%)	66.7 ± 0.7^{e}	84.4 ± 0.9^{c}	95.9 ± 0.9^{b}	98.6 ± 0.2^{a}	73.4 ± 0.5^{d}
	Particle Size (µm)	106.2 ± 31.3^{a}	120.1 ± 26.8^{a}	105.8 ± 28.7^{a}	101.6 ± 12.4^{a}	108.9 ± 18.0^{a}
Type B gelatin-acacia	Yield (%)	66.1 ± 0.5^{d}	85.0 ± 0.8^{a}	76.8 ± 0.5^{b}	64.9 ± 0.8^{c}	N/A
	Core loading (%)	67.0 ± 0.7^{c}	96.2 ± 1.5^{a}	84.3 ± 0.3^{b}	62.5 ± 1.1^{d}	N/A
	Particle Size (µm)	106.2 ± 31.3^{a}	94.5 ± 29.8^{a}	110.2 ± 24.4^{a}	90.1 ± 32.2^{a}	N/A

$^{a, b, c, d, e}$ Means within the same row with different letters are significantly different ($p \leq 0.05$). N/A: No data is available

Table 1: Coacervates yield, loading of core material and particle size of coacervates prepared as a function of pH for the complex coacervation 370 between Type A gelatin and gum acacia and between Type B gelatin and gum acacia. Mean ± S.D., n = 3.

	Gelatin:acacia ratio	1:1	3:2	2:1	5:2
Type A gelatin-acacia	Yield (%)	86.8 ± 1.7^{a}	80.1 ± 1.2^{b}	77.9 ± 1.0^{b}	65.6 ± 0.3^{c}
	Core loading (%)	98.6 ± 0.2^{a}	92.2±1.1^{b}	84.5 ± 0.7^{c}	67.3 ± 0.6^{d}
	Particle Size(µm)	101.6 ± 12.4^{a}	92.3 ±10.6^{a}	83.3 ± 19.5^{a}	93.8 ± 14.8^{a}
Type B gelatin-acacia	Yield (%)	101.6 ± 12.4^{a}	80.2 ± 0.7^{b}	73.0 ± 2.5^{c}	64.6 ± 2.1^{d}
	Core loading (%)	96.2 ± 1.5^{a}	90.1 ± 0.8^{b}	81.1 ± 1.1^{c}	64.4 ± 1.7^{d}
	Particle Size(µm)	94.5 ± 29.8^{a}	93.4 ± 15.5^{a}	92.7 ± 24.7^{a}	97.2 ± 18.0^{a}

$^{a, b, c, d}$ Means within the same row with different letters are significantly different ($p \leq 0.05$)N/A: No data is available

Table 2: Coacervates yield, loading of core material and particle size of coacervates prepared as a function of various gelatin-to-gum acacia ratio for the complex coacervation between Type A gelatin and gum acacia and between Type B gelatin and gum acacia. Mean ± S.D., n =3.

	Gelatin:acacia ratio	2:1	4:1	6:1	8:1
Type A gelatin- gum	Yield (%)	63.7 ± 2.8^{c}	73.8 ± 1.2^{b}	86.6 ± 1.7^{a}	86.7 ± 1.9^{a}
	Core loading (%)	92.3 ± 1.2^{b}	93.3±1.1^{b}	98.6 ± 0.2^{a}	94.9 ± 2.4^{ab}
	Particle Size(µm)	100.1 ± 25.8^{a}	81.2 ±24.4^{a}	101.6 ± 12.4^{a}	89.0 ± 17.8^{a}
Type B gelatin- gum	Yield (%)	61.1 ± 1.3^{c}	65.7 ± 1.9^{b}	85.0 ± 0.9^{a}	86.3 ± 1.2^{a}
	Core loading (%)	92.3 ± 1.0^{b}	94.0 ± 1.1^{ab}	96.2 ± 1.5^{a}	95.7 ± 1.1^{b}
	Particle Size(µm)	109.1 ± 21.1^{a}	111.2 ± 32.8^{a}	94.5 ± 29.8^{a}	94.5 ± 29.8^{a}

$^{a, b, c}$ Means within the same row with different letters are significantly different ($p \leq 0.05$)

Table 3: Coacervates yield, loading of core material and particle size of coacervates prepared as a function of core-to-wall ratio for the complex coacervation between Type A gelatin and gum acacia and between Type B gelatin and gum acacia. Mean ± S.D., n = 3.

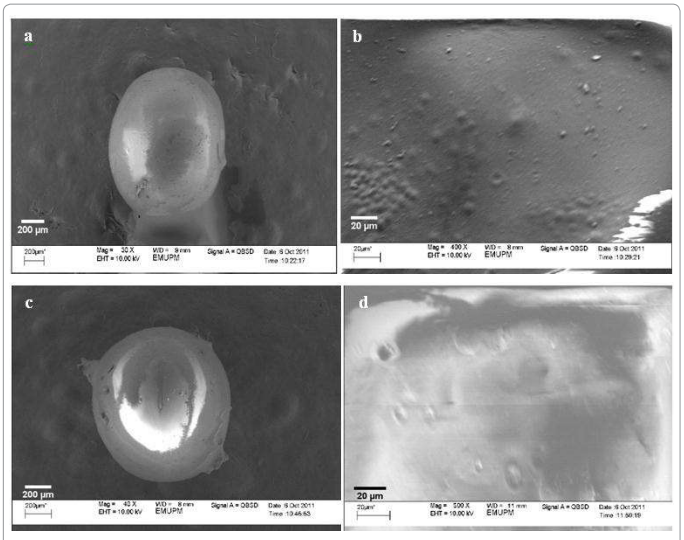

Figure 1: Scanning electron microscope micrographs of coacervates containing garlic oil. (a): coacervates made from Type A gelatin-gum acacia 419 produced at pH 4.5, gelatin-to-gum acacia ratio at 1:1 and core-to-wall ratio at 6:1 at magnification of 30×. (b): surface morphology of Type A 420 gelatin-gum acacia coacervates at magnification of 400×. (c): coacervates made from Type B gelatin-gum acacia produced at pH 3.5, gelatin-to-421 gum acacia ratio at 1:1 and core-to-wall ratio at 6:1 at magnification of 40×. (d) surface morphology of Type B gelatin-gum acacia coacervates at 422 magnification of 400× and 500× respectively.

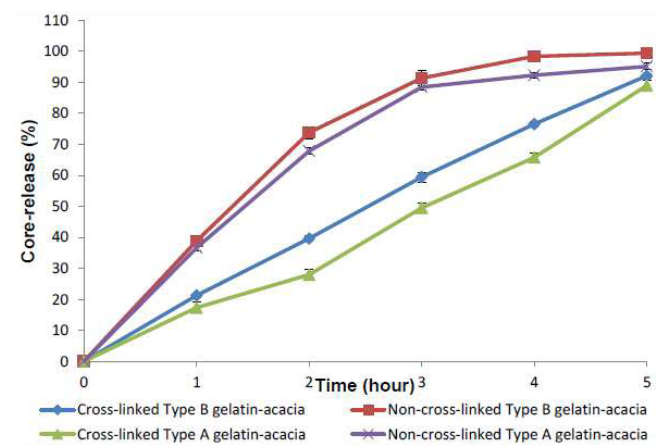

Figure 2: The controlled-released profile of garlic oil from non-cross-linked and cross-linked coacervates during the incubation at 37°C in pepsin solution for 5 hours. Mean ± S.D., n = 3.

At higher magnification, small domes and craters-like structures were observed on the surface of the coacervates for both type A or B gelatin-gum acacia systems but no holes were found on the surface (Figure 1b and d).

Controlled-release study of garlic oil-containing coacervates

Controlled-release mechanism of garlic oil between the cross-linked coacervates and non-cross-linked coacervates was investigated in a pepsin solution at pH 2.0 (resembling gastrointestinal tract) at 37°C for 5 hours (Figure 2). Non-cross-linked coacervates showed higher oil leakage compared to cross-linked coacervates for both gelatin types ($p \leq 0.05$) (Figure 2). A slower release of garlic oil from cross-linked coacervates was observed in comparison to non-cross-linked coacervates ($p \leq 0.05$) (Figure 2). Sustained released of microalgal oil from cross-linked gelatin-gum acacia coacervates has been recently reported by Zhang et al. [22]. Similar observation on cross-linked isolate soybean coacervates was also reported by Cho et al. [16].

Cross-linked type A gelatin-acacia coacervates showed a relatively slow release of garlic oil compared to type B between the 2nd to 4th hour ($p \leq 0.05$) (Figure 2). A similar trend was observed for the non-cross-linked coacervates ($p \leq 0.05$). This phenomenon could probably be explained by the presence of a higher amount of tyrosine and serine found in the amino acid of type A gelatin compared to type B gelatin. The presence of these additional amino acid groups contribute to the formation of hydrogen bonds between water molecules and the hydroxyl groups of the amino acids, leading to a higher gel strength [23].

Oxidative stability of garlic oil-containing coacervates

Peroxide value (PV) of the unencapsulated garlic oil significantly increased at day 4 in type A or type B gelatin-gum acacia based coacervates ($p \leq 0.05$) (Figure 3a), indicating the primary oxidation products started to appear in the unencapsulated garlic oil. Type A or B gelatin-gum acacia coacervates were found to be similarly effective against primary oxidation as the PV were similar throughout the study ($p>0.05$) (Figure 3a).

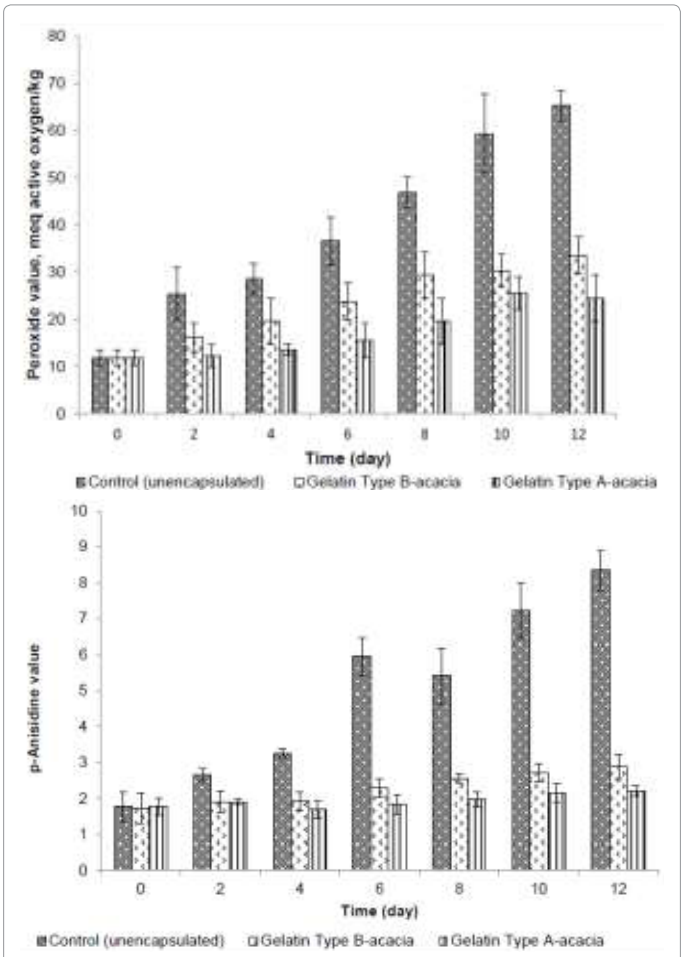

Figure 3: Changes in the (a) peroxide value (PV) (b) *p*-anisidine values (*p*-AV) for encapsulated and uncapsulated garlic oil in Type A gelatin- gum acacia and Type B gelatin-gum acacia coacervates under storage for 12 days at 45°C. Mean ± S.D., n = 3.

Secondary oxidation products as shown in p-anisidine values increased in the unencapsulated garlic oil starting from second day ($p \leq 0.05$) (Figure 3b). The increase of secondary oxidation products in garlic oil was relatively slow and minimal when garlic oil is encapsulated in the gelatin-gum acacia coacervates. Hence, type A or B gelatin-gum acacia systems were equally effective in slowing down the rate of secondary oxidation in the garlic oil over 12 days at 45°C.

Conclusion

The highest microencapsulation yield and loading efficiency for type A or B gelatin- gum acacia were pH 4.5 1:1 gelatin-to-gum acacia ratio and 6:1 core-to-wall ratio whilst for type B gelatin-gum acacia were pH 3.5, 1:1 gelatin-to-gum acacia ratio and 6:1 core-to-wall ratio. The cross-linked garlic oil-containing coacervates allowed a controlled release of garlic oil in pepsin solution. Coacervates produced using both type A or B gelatin-gum acacia were found to be equally stable and effective in the protection of garlic oil against primary and secondary oxidation during the 12 days storage at 45°C compared to unencapsulated garlic oil.

Acknowledgement

We would like to thank and appreciate for the funding provided by Monash University Sunway Campus, Malaysia, and the authorities from Bioscience Institute, University Putra Malaysia (UPM) for their permission to use the scanning electron microscope.

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