PAPER

MICROENCAPSULATION OF *ELAEAGNUS MOLLIS* OIL TO ENHANCE THE OXIDATION STABILITY OF POLYUNSATURATED FATTY ACIDS: THE INTERACTION BETWEEN FATTY ACIDS AND WALL MATERIALS

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ABSTRACT

Our study deals with the protective effect of gelatin-acacia as a wall material on *Elaeagnus mollis* oil (EMO), the core material with over 45% polyunsaturated fatty acids. We found that 2.0% of the gelatin-acacia complex was an ideal concentration for facilitating the microencapsulation of EMO. The ideal ratio of EMO/wall materials would be 1:1. As a result, the microencapsulated EMO successfully underwent the spray-drying treatment because of close hydrogen bonding between EMO and the gelatin-acacia complex. Moreover, no spoilage was observed for the heated EMO microcapsules during storage.

Keywords: polyunsaturated fatty acids, storage stability, appropriate ratio, hydrogen bonds

1. INTRODUCTION

The seeds of *Elaeagnus mollis*, a tree species naturally distributed in northern China, are annually harvested to produce edible woody oil by local people (WANG *et al*., 2012). *Elaeagnus mollis* oil (EMO) is reported to contain higher levels of polyunsaturated fatty acids (PUFAs), especially linoleic acid, which can be helpful in preventing heart diseases (ZHANG, 2008; LIU, Low & Nickerson, 2015; KAN *et al*., 2017). The local population not only utilizes the EMO but also sells it for monetary benefits. However, one hurdle for the EMO storage and usage is its shorter shelf life owing to the presence of higher PUFAs levels (TEIXEIRA *et al*., 2004).

It is known in general, that essential PUFAs such as omega-3 and omega-6 are more susceptible to oxidation and subsequently produce undesirable flavors (AUGUSTIN *et al*., 2010; JOHNSON and DECKER, 2015). Hydrogenation is the most feasible means to settle the problem of high PUFA-containing oils, but the process of oil hydrogenation yields more trans fatty acids (ZHAO *et al*., 2018).

Microencapsulation is an alternative technology adapted in the food industry because of its safety (AZIZ *et al*., 2014; GOMEZ *et al*., 2016; SHAMAEI *et al*., 2017; ZHOU *et al*., 2017). Thus, they might provide the effective solution of preventing PUFA oil-mediated oxidation spoilage. Gelatin and acacia are usually selected as preparation material for microcapsules due to their excellent emulsifying and film-forming properties (UMESHA *et al*., 2013; LIU *et al*., 2015; ANVARI *et al*., 2015; HCF *et al*., 2013). However, the difference in PUFA content strongly influences the microencapsulation efficiency of the wall materials (SHAMAEI *et al*., 2017; ZHOU *et al*., 2017). So design the appropriate wall material concentration and wall material/core material ratio to improve the microencapsulation efficiency of EMO as a representative of PUFA content over 45% should be significant.

Therefore, the objectives of this study were to (1) examine the effect of gelatin-acacia in EMO protection and deduce the optimal concentrations of gelatin-acacia, as well as the EMO/gelatin-acacia ratio; (2) investigate the interaction between EMO and gelatin-acacia; and (3) check the tolerance of EMO microcapsules towards heat treatment and their storage stability.

2. MATERIALS AND METHODS

2.1. EMO preparation

Supercritical carbon dioxide extraction (SPE-ED, Applied Separations, Allentown, USA) was used to produce EMO. Seeds of *Elaeagnus mollis* were dried in an electro-thermostatic drying oven (GHG-9420A, Yi-heng Science, Shanghai, China). Then, 200 g seed powder *of Elaeagnus mollis* (d = 0.42 mm) was fed to a 300-ml extraction vessel. The extraction of EMO by supercritical carbon dioxide technique was carried out at 38°C for 6 h with a pressure of 30 Mpa and solvent flow rate of 3.0 L/min.

2.2. Concentration design of gelatin-acacia

Gelatin and acacia (Jinchun Biochemical Technology Ltd, Shanghai, China) were dissolved in distilled water and heated to 50°C until complete dissolution. Gelatin and acacia were mixed in a ratio of 1:1 (v/v). Gelatin-acacia mixtures of 1.0%, 1.5%, 2.0%, 2.5%, and 3.0% were blended with EMO. The ratio of each of the gelatin-acacia complex and EMO sample

was 3:2. Each combination of gelatin-acacia and EMO was emulsified using Ultra-Turrax homogenizer (FA 25, Fluko Ltd, Shanghai, China) at 12000 r/min for 4 min. The emulsions, kept at 40°C, were adjusted to pH of 4.0 and then stirred for 20 min until coacervation. Next, the pH of the emulsions was adjusted to 6.0 after their temperatures dropped within the range of 10°C and 20°C. At this moment, 0.25 g transglutaminase (calculated as gelatin per gram) (Leveking Bio-Engineering Ltd, Shenzhen, China) was added to the emulsions. These emulsions were stirred for 3 h to complete the cross-linking and form microcapsules. Those microcapsules were kept for a while to remove some of the supernatant, and then filtered with a vacuum pump, and washed with an appropriate amount of distilled water to obtain the wet capsules, which were then freeze-dried to obtain the microcapsule products. The dry microcapsules obtained were used to determine the optimal concentration of gelatin-acacia.

2.3. Ratio design of EMO/gelatin-acacia

The ratios of EMO to the mixtures of gelatin-acacia (used at a concentration of 1.5%) were designed according to the ratios of 1:2, 2:3, 1:1, 3:2, and 2:1. The combinations of EMO and gelatin-acacia were emulsified using Ultra-Turrax homogenizer (FA 25, Fluko Ltd, Shanghai, China) at 12000 r/min for 4 min. They were then kept at 40°C, were adjusted to a pH of 4.0, and stirred for 20 min until coacervation. Moreover, the emulsions' pH values were adjusted to 6.0 after their temperatures lowered between 10°C and 20°C. Next, 0.25 g transglutaminase (calculated in gelatin per g) was added to the emulsions to complete the cross-linking process. The microcapsules after cross-linking were then freeze-dried to obtain the dry microcapsule products, which were used to determine the optimal ratio of EMO/gelatin-acacia.

2.4. Morphological observation

The shapes of the EMO microcapsules were observed by a polarizing microscope (XSP-1813, Shanghai optical instrument factory, Shanghai, China), and their images were captured using a digital camera (DSC-W800, Sony, Japan).

2.5. Microencapsulation efficiency

One gram of EMO microcapsules were dissolved in 50 ml of petroleum ether for 1 min and repeated the operation for two times then went through filtration. The filtrate was then evaporated and dried at 60°C (KAN *et al*., 2017) until constant weight was achieved to obtain the surface oil. The total oil referred to amount of the oil that was used to prepare encapsulation products. The Microencapsulation efficiency (ME) was calculated as follows (XIAO *et al*., 2014):

$$ME = (1 - (\text{surface oil} / \text{total oil})) \times 100\ \% \qquad (1)$$

2.6. Microencapsulation yield

Microencapsulation yield was calculated according to the formula adapted from previously published literatures follows XIAO *et al*. (2014):

$$MY = (W_1/W_2) \times 100\ \% \quad (2)$$

Where W_1 represents the weight (g) of the freeze-dried microcapsules products, and W_2 stands for the initial weight (g) of core and wall materials used.

2.7. Thermal stability analysis of EMO microcapsules

2.7.1 Spray-drying treatments

Gelatin-acacia encapsulated EMO and non-encapsulated EMO were treated via spray dryer (B290, Buchi Ltd, Switzerland) for the evaluation of heat resistance. Non-encapsulated EMO treated with spray-drying was segregation but not as powder (MAURER *et al*., 2016). Spray-drying parameters were carried out using the following condition settings: feed rate of 7.5 ml/min, inlet temperature of 140°C, and outlet temperature of 80°C.

2.7.2 Polyunsaturated fatty acid analysis of EMO and EMO microcapsules

The PUFA composition and content of EMO and EMO microcapsules after spray-drying were performed by Gas Chromatography and Mass Spectrometry (GC-MS) (GC-MS-2010, Shimadzu corporation, Shimadzu, Japan) with the help of silica capillary column Rtx-5MS (0.25 mm × 30 m × 0.25 μm). The operation conditions were as follows: injection temperature of 280°C, sample quantity of 1 μl, Split ratio of 40:1, He as carrier gas, and speed of 1 ml/min. The PUFAs in EMO were identified by more than 95% matching with the retention time of MS database from a commercial library (NIST ver. 2011 library & NIST11s ver. 2011 library). The concentration of PUFAs was calculated by area ratio method. The test of PUFAs in spray-dried EMO microcapsules referred to the EMO extracted from the spray-dried microcapsules. The EMO was extracted from the spray-dried powder by petroleum ether. The extraction process was repeated until no EMO was leached then the extracted EMO went through filtration. The filtrate was then evaporated to remove the petroleum ether.

2.7.3 Micro-structure observation

Micro-structure of spray-drying EMO microcapsules were visualized and obtained via scanning electron microscopy (SEM). The powder of spray-dried EMO microcapsules was pasted onto a piece of double sided tape, and then a thin gold layer was sprayed onto it. SEM (S-3400N II, Hitachi High technologies, Tokyo, Japan) was operated at 5 kV. The imaging visuals of representative samples were chosen for taking pictures.

2.7.4 Analysis of vitrification temperature of EMO microcapsules

DSC (DSC100, TA instruments, New Castle, USA) was employed to determine the vitrification temperature of spray-dried EMO microcapsules. Samples, placed into DSC100, were heat-treated from 25°C up to 90°C at an increasing rate of temperature i.e. 5°C/min for DSC analysis.

2.7.5 Interaction between gelatin-acacia and EMO

All samples from gelatin, acacia, spray-dried EMO microcapsules, EMO, and the blank were characterized by FTIR spectroscopy (Spectrum100D, PerkinElmer Company, America) in the wavelength range of 0 to 4000 cm^{-1}. The experimental data were explained according to the published references to determine the interaction between gelatin-acacia and EMO (XIAO *et al*., 2014; BUĞRA, 2012; MAJI and HUSSAIN, 2010).

2.7.6 Computational docking

All computational work was performed by Discovery Studio 2017R2 software (Accelrys Software Inc., USA). The simulation model of interaction between gelatin-acacia and EMO was built using the "Receptor-Ligand Interaction" module. The structural details of gelatin were uncertain but it shared similarity to collagen (HONGO *et al*., 2001; JIRAVANICHANUN *et al*., 2006; ZONG *et al*., 2005). Therefore, the structure of collagen adhesin from *S. aureus*was used as an alternative template in this simulation. Linoleic acid, being the most abundant PUFA in EMO, was used during simulation while alpha-L-arabinopyranose was chosen as the representative of acacia. The structure of linoleic acid (Pubchem CID: 5280450), alpha-L-arabinopyranose (Pubchem CID: 439731), and collagen adhesin from *S. aureus* (PDB ID: 2F68) were downloaded from Pubchem database.

2.8. Storage stability of EMO microcapsules

2.8.1 Effect of temperature on the oxidation of EMO microcapsules

Spray-dried EMO microcapsules and EMO (as control) were stored without light at 4°C, 20°C, and 37°C. The peroxide value (PV) of all samples was determined each week for 5weeks. PV was determined according to AOAC (1995) method. First of all, the EMO from the spray-dried EMO microcapsules was extracted by petroleum ether. Next, 3.0g EMO and EMO from the spray-dried EMO microcapsules were placed into Erlenmeyer flasks. Then, 30 ml chloroform-acetic acid mixture (2:3 v/v) and 1.0 ml KI solution, each, were added to the flasks to mix with non-encapsulated EMO or extracted EMO from spray-dried microcapsules. The mixtures, after being left for 5 min in a dark place, were then blended with 100 ml distilled water. The solutions were titrated with sodium thiosulfate and starch indicator. PV was calculated using the following formula:

$$PV = (A \times N \times 1000)/E \quad (3)$$

Where A represents the volume (ml) of sodium thiosulfate solution required for the samples, N represents the normality of thiosulfate, and E means the mass (g) of the EMO or EMO which extracted from the spray-dried EMO microcapsules weighed.

2.8.2 Effect of light on the oxidation of EMO microcapsules

Spray-dried EMO microcapsules and EMO (as control), which were stored at room temperature, were treated with the presence and absence of light (darkness). Each week, the PV of all samples was evaluated until the 5th week. PV was determined as described in the previous section.

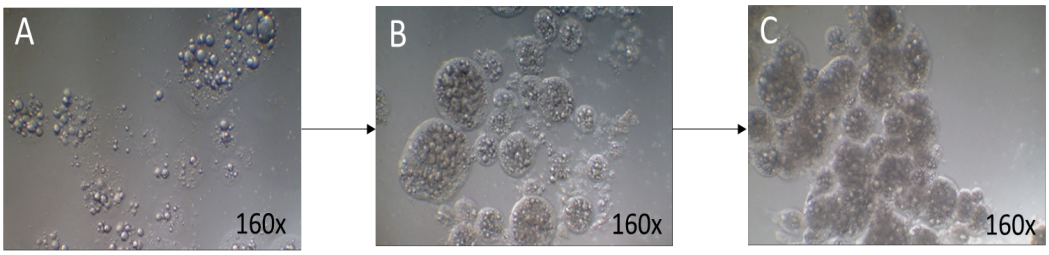
2.9. Statistical analysis

All experiments and analyses were carried out in triplicate, and the values were represented as the mean values. Microsoft Office Excel 2010 and SPSS (17.0) system software were used for data analysis.

3. RESULTS AND DISCUSSION

3.1. The optimal ratio of gelatin-acacia on EMO

Fig. 1 shows the morphological properties of EMO microcapsules in the presence of various gelatin-acacia concentration complexes. It was seen that if the concentration of gelatin-acacia was 1.0% (Fig. 1 A), the EMO microcapsules were irregularly shaped, and the microencapsulation efficiency (ME) and microencapsulation yield (MY) of EMO were 56.43% and 44.94%, respectively (Fig. 1 D). Use of 1.5% gelatin-acacia concentration raised the ME and MY values of EMO (Fig. 1 D). Besides, the EMO microcapsules formed a globular structure. The maximal ME (69.85%) and MY (67.19%) of EMO occurred when the gelatin-acacia concentration was 2% (Fig. 1-D). In this case, EMO microcapsules were homogeneous and multinuclear in shape. Higher amount of EMO was encapsulated and no adhesion took place between EMO microcapsules (Fig. 1 B). However, ME and MY of EMO decreased if gelatin-acacia concentration was further increased to 2.5% (Fig. 1 D). Use of 3.0% gelatin-acacia concentration caused the ME of EMO to quickly fall to 51.39% (Fig. 1 D). The appearance of EMO microcapsules was highly irregular and they adhered strongly to each other (Fig. 1 C).
Fig. 2 deals with the effects of EMO/gelatin-acacia ratio on the morphological properties of EMO microcapsules. It was seen that 1:2 of EMO/gelatin-acacia achieved a good ME and MY score 70.74% and 45.12%, respectively (Fig. 2 D). However, this was accompanied by an irregular shape, and no sufficient EMO was encapsulated (Fig. 2 A). A 2:3 ratio of EMO/gelatin-acacia enabled ME of EMO to reach the maximum (72.92%) (Fig. 2 D). Nevertheless, the EMO microcapsules were unable to achieve the morphological spherical structure. Interestingly, when the ratio was 1:1, we observed a multinuclear outer-shape of EMO microcapsules with homogeneous shell thickness and appropriate oil content (Fig.2-B). The ME and MY of EMO were 72.01% and 49.67% respectively (Fig. 2 D). Upon using 3:2 of EMO/gelatin-acacia, the shape became irregular, and excessive oil was entrapped. When this ratio was 2:1, the highest MY of EMO occurred but the oil secreting from the shell caused the microcapsules to get aggregated (Fig. 2 C-D).



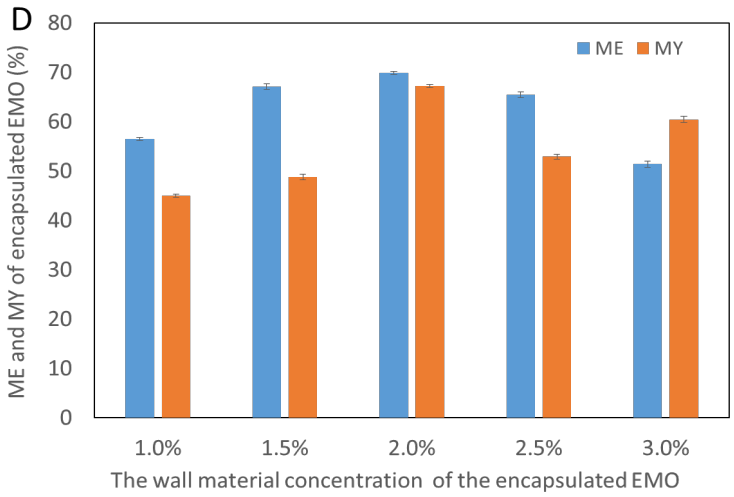


Figure 1. Gelatin-acacia microcapsules observed by polarizing microscope (160×magnification) as a function to determine the optimal concentration: (A) 1.0% of gelatin-acacia, (B) 2.0% of gelatin-acacia, (C) 3.0% of gelatin-acacia, (D) shows the microencapsulation efficiency (ME) and microencapsulation yield (MY) of *Elaeagnus mollis* oil (EMO) microcapsules prepared with different concentration of gelatin-acacia.

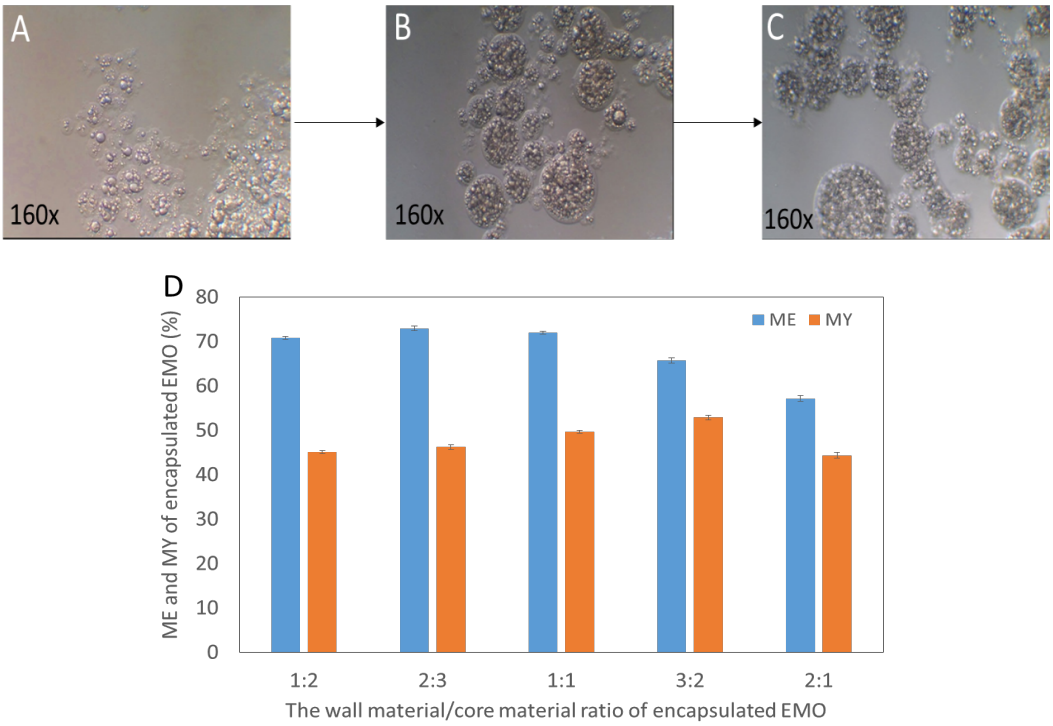


Figure 2. Gelatin-acacia microcapsules observed by polarizing microscope (160×magnification) as a function to determine the optimal ratio of EMO/gelatin-acacia: (A) EMO/gelatin-acacia in 1:2, (B) EMO/gelatin-acacia in 1:1, (C) EMO/gelatin-acacia in 2:1, (D) shows the microencapsulation efficiency (ME) and microencapsulation yield (MY) of *Elaeagnus mollis* oil (EMO) microcapsules prepared with different ratio of EMO/gelatin-acacia.

An appropriate gelatin-acacia combination and proper EMO/gelatin-acacia proportions are clearly required for successful encapsulation. In case of the oil containing more than 45% of PUFAs, the experimental data showed that high ME and MY of EMO could hardly be achieved with lower wall material concentration (Fig. 2 D). The resulting wall would be quite weak, reflecting poor storage ability. If gelatin-acacia concentration was too high, globular shape was hardly formed due to strong adherence between microcapsules (Fig.1-C). Researchers have demonstrated that increase in the wall materials' concentration leads to enhanced MY and ME of oil, though the spherical multinuclear microcapsules would gain more irregularity (XIAO *et al*., 2014; DONG *et al*., 2007). Of course, large amount of oil existing in emulsion also results in aggregation among microcapsules because of high surface oil content. AZIZ *et al*. (2014) confirmed this finding and proposed/speculated that the ratio of oil/wall influences the internal structure of microcapsules. The experiment results demonstrated that increase in EMO/gelatin-acacia ratio for a given wall material concentration caused the EMO microcapsules to enlarge with weaker walls (Fig. 2 C). Therefore, 2.0% gelatin-acacia complex as a carrier plus 1:1 ratio of EMO/gelatin-acacia significantly minimizes the loss of the woody oil.

3.2 Thermal stability analysis of EMO microcapsules

3.2.1 Polyunsaturated fatty acids change before and after heat stress

To evaluate the tolerance of the EMO microcapsules towards elevated temperature, firstly spray-drying was used to process them. The fatty acids of EMO and spray-dried EMO microcapsules are illustrated in Table 1. 11 kinds of fatty acids had been identified in EMO and EMO microcapsules. 4 kinds of those fatty acids were PUFAs, and their relative content was up to 46.10%. Omega-6 was the main PUFA in EMO, and its relative content was 45.09%. The content of Omega-6 of EMO microcapsules was 44.99% upon heating. The PUFA content showed no significant different between EMO and EMO microcapsules (p>0.05). Data from PUFAs composition change proved that 2% of the gelatin-acacia and 1:1 of EMO/gelatin-acacia ratio ensured that the composition and content of PUFAs of EMO were unaffected upon heating.

3.2.2 Micro-structure observation

Micro-structure observation of EMO microcapsules has been shown in Fig. 3. It was seen that the shape of EMO microcapsules was spherical. Moreover, there were visible wrinkles over their surface, indicating that wall materials perfectly protected core materials. The wrinkles appeared due to the high temperature in the drying chamber, wherein the very fine atomized droplets are subjected to a maximal drying rate to withdrawal of water from them (RAJABI *et al*., 2015; QV *et al*., 2011). This keeps a very low water level, protecting the oil from quick oxidation.

3.2.3 Vitrification of EMO microcapsules

The glass transition refers to the phenomena where a supercooled, malleable liquid or rubbery material changes into a disordered solid glass upon cooling, or vice versa (RAJABI *et al*., 2015). DSC analysis revealed that the vitrification temperature of EMO microcapsules was around 62°C (Fig. 4). Previous report by LAINE *et al.* (2010) also proposed that the vitrification temperature of gelatin was 62°C with Aw of 0.24. This

confirmed the protective effect of gelatin. Normally, the storage temperature of PUFA-enriched products ranges between 0°C and 30°C (QV *et al*., 2011). Thus, the EMO encapsulated by gelatin-acacia, which was able to resist harsh heat-treatment such as spray drying, possesses good stability during longer storage periods.

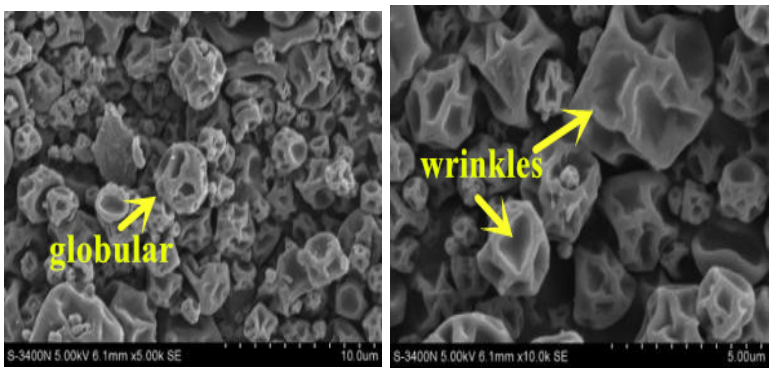


Figure 3. Scanning electron microscopy micrographs of *Elaeagnus mollis* oil microcapsules after heat treatment was observed at 5000 and 10000 magnification.

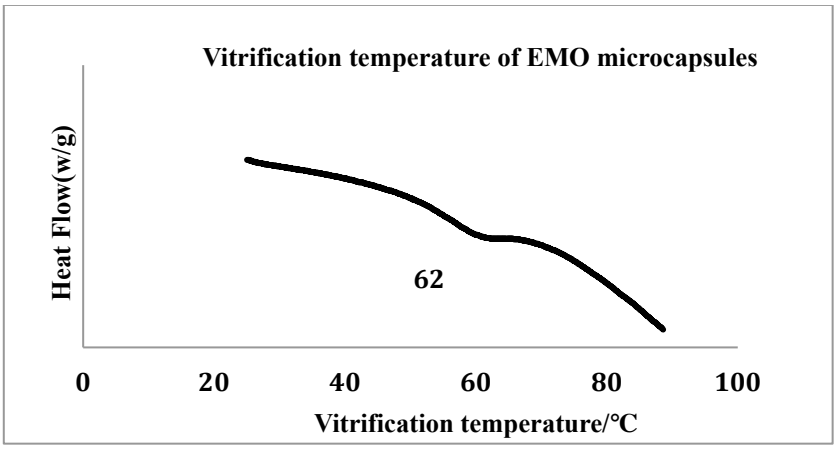
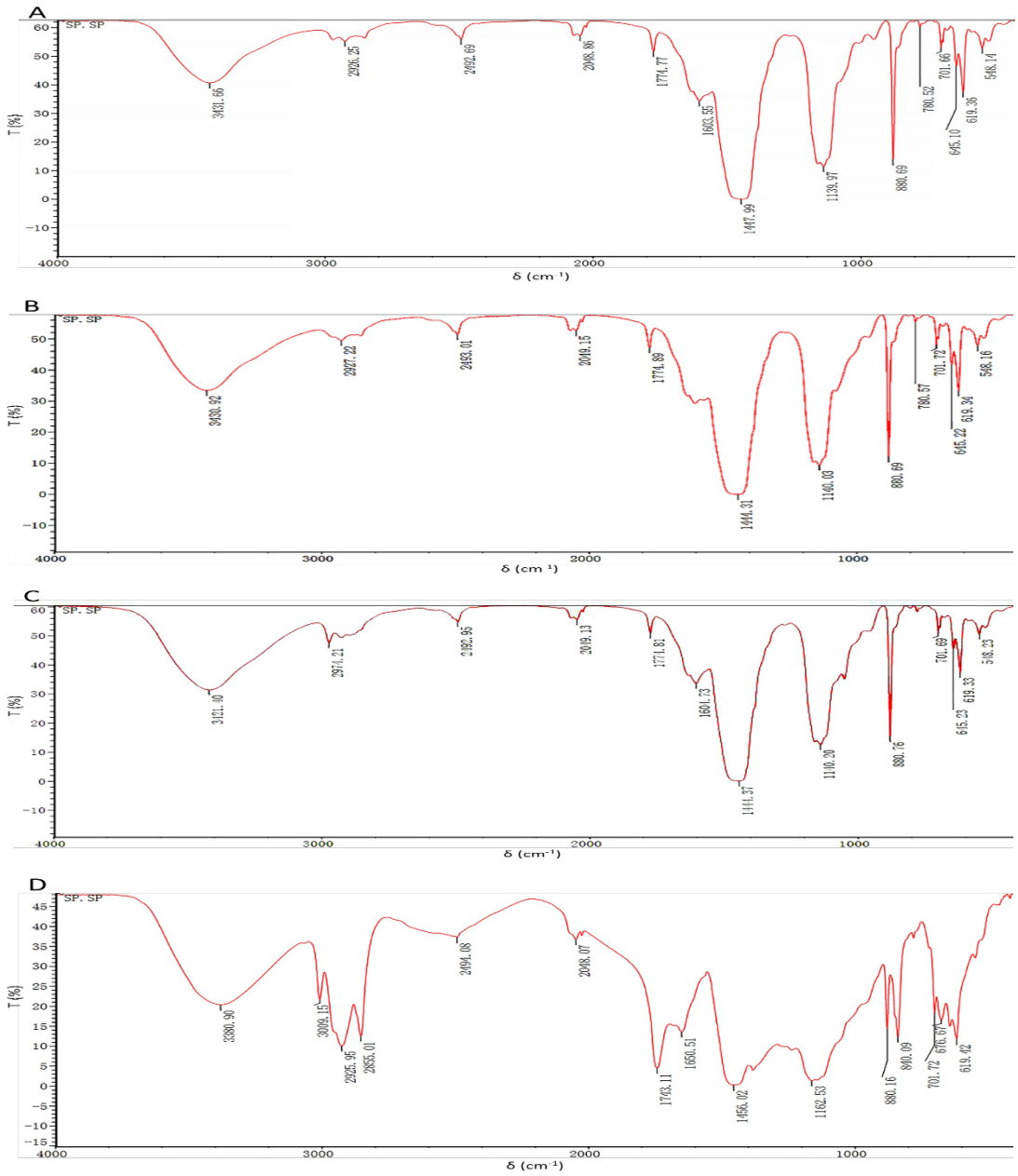


Figure 4. The vitrification temperature of spray-dried *Elaeagnus mollis* oil microcapsules which determined by DSC.

3.2.4 The interaction between gelatin-acacia and EMO

An appropriate ratio of gelatin-acacia complex and EMO/gelatin-acacia enabled EMO with 45% of PUFAs to have a heat-resistant property. The interplay between gelatin-acacia and EMO is noteworthy and requires adequate discussion. Gelatin, acacia, EMO, EMO microcapsules, and the blank microcapsules were detected by FTIR (Fig.5). The strong absorption peak of EMO, found at wavelength of 2925 cm^{-1}, 2855 cm^{-1} was due to CH_2 asymmetric stretching vibration and CH_2 symmetric stretching vibration. In the microcapsules, the carbonyl stretching bands shifted to 2974 cm^{-1}, indicating that no chemical changes occurred between EMO and gelatin-acacia (Fig. 5 A-B-C). The FTIR spectrum at 1740 cm^{-1} to 1755 cm^{-1} comprised the absorption peaks of characteristic molecule

groups such as -COOR and C=O (BUĞRA, 2012; MAJI and HUSSAIN, 2010). The wavelength of 1744 cm^{-1} in the FTIR spectrum of gelatin, acacia, EMO microcapsules, and EMO remained unchanged (Fig. 5 A-B-C-D). Therefore, EMO was encapsulated successfully by gelatin-acacia coupled with physical interaction.



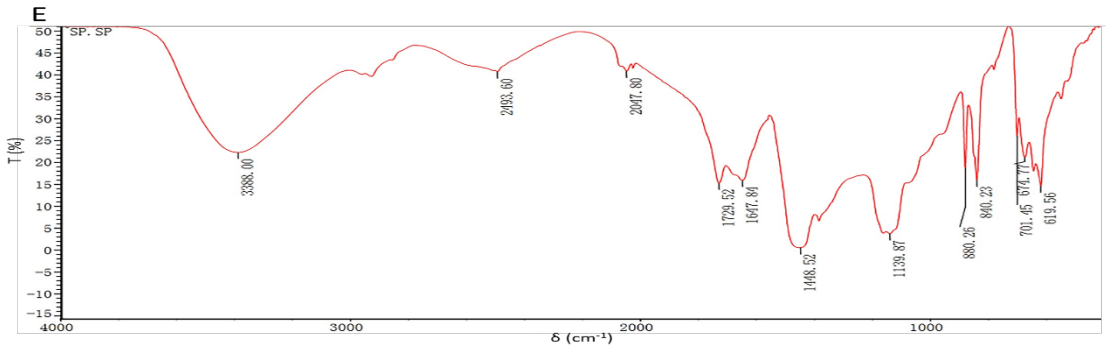


Figure 5. Comparison analysis of infrared spectroscopy of (A) acacia, (B) gelatin, (C) *Elaeagnus mollis* oil microcapsules, (D) *Elaeagnus mollis* oil, and (E) blankmicrocapsules as a function to indicate the interaction between gelatin-acacia and *Elaeagnus mollis* oil.

Moreover, the simulation among linoleic acid, collagen adhesin from *S. aureus*, and alpha-L-arabinopyranose demonstrated a possible interaction among EMO, gelatin and acacia in their original states. The hydrogen and oxygen atoms of C18 side chain of linoleic acid and *S. aureus* collagen adhesin were attracted to each other with hydrogen bonds (Fig. 6 A). The hydrogen bonding was also found between hydrogen side chain of alpha-L-arabinopyranose and *S. aureus* collagen adhesin (Fig. 6 B). Bumps were found at many sites between the same side chains of linoleic acid and alpha-L-arabinopyranose, indicating strong hydrogen bonding (Fig. 6 C). The carbonyl stretching and vibration in the microcapsules may be due to the hydrogen bonding between EMO and acacia rather than chemical changes (Fig. 6 D). The simulation results confirm the interaction between EMO and gelatin-acacia to be physical, and the PUFAs of EMO remained unchanged even after heating.

3.3. Storage stability of EMO microcapsules

3.3.1 Effect of temperature and light on EMO and EMO microcapsules

Temperature and light are selected to study the protective effect of microcapsules on EMO during the storage in the present research, because the temperature and exposure to light are usually considered as main factors affecting the storage stability of PUFAs (ZHOU *et al*., 2017). Data from Fig. 6 demonstrated that the PV of EMO increased significantly at 37°C, indicating that temperature had a significant influence on EMO storage stability. On the contrary, the PV of EMO protected by gelatin-acacia increased gradually. When stored at 4°C and 20°C, both the PVs of the encapsulated EMO relatively held stability for the first 3 weeks (Fig. 7 A-B). The PV only increased to 15 meq/kg oil after the encapsulated EMO was incubated at 37°C for 5 weeks (Fig. 7 C). Clearly, use of gelatin-acacia to entrap EMO retarded the PUFAs from quick oxidation, weakening the influence of storage temperature on the oil quality.
The fast growth in the PV of EMO indicated that light was another factor affecting the stability of the PUFAs (Fig. 7 D-E). The change in the PV of EMO protected by gelatin-acacia was very low, even when it was exposed to light. Therefore, the light-resistant ability was enhanced in EMO microcapsules. Therefore, the EMO microcapsules are able

to maintain a stable status after being exposed to 4°C, 20°C, 37°C, and light during their storage.

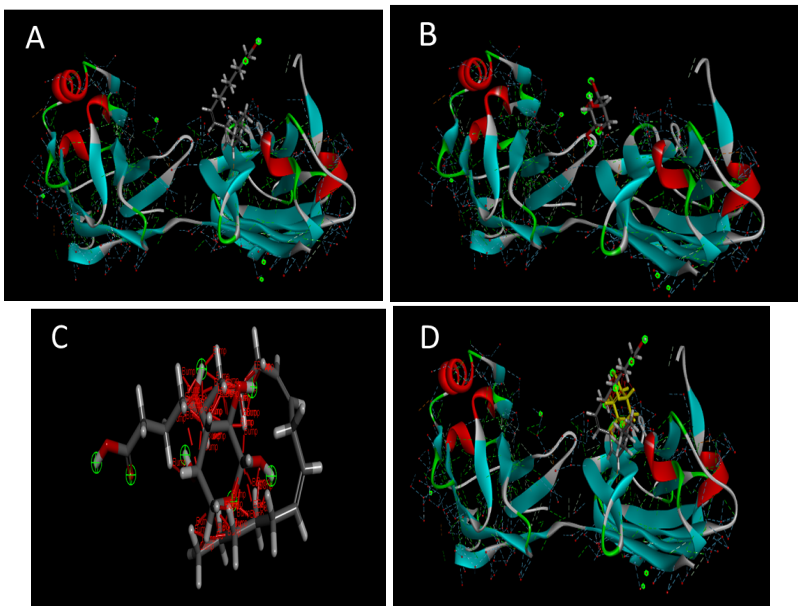
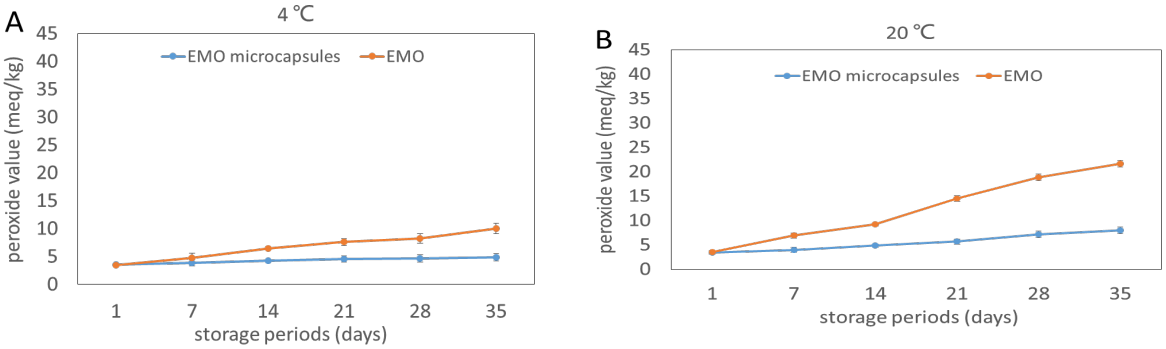


Figure 6. Model interaction among linoleic acid, collagen adhesin from *Staphylococcus aureus*, and alpha-L-arabinopyranose; (A) hydrogen bonds between linoleic acid and collagen adhesin from *S. aureus*; (B) hydrogen bonds between alpha-L-arabinopyranose and collagen adhesin from *S. aureus*; (C) hydrogen bonds and bumps between linoleic acid and alpha-L-arabinopyranose; (D) interaction between linoleic acid, collagen adhesin from *S. aureus* and alpha-L-arabinopyranose. Hydrogen bonds are shown as green colour in side chain of linoleic acid and side chain of alpha-L-arabinopyranose and surface of collagen adhesin from *S. aureus*. Bumps are coloured as red between linoleic acid and alpha-L-arabinopyranose.



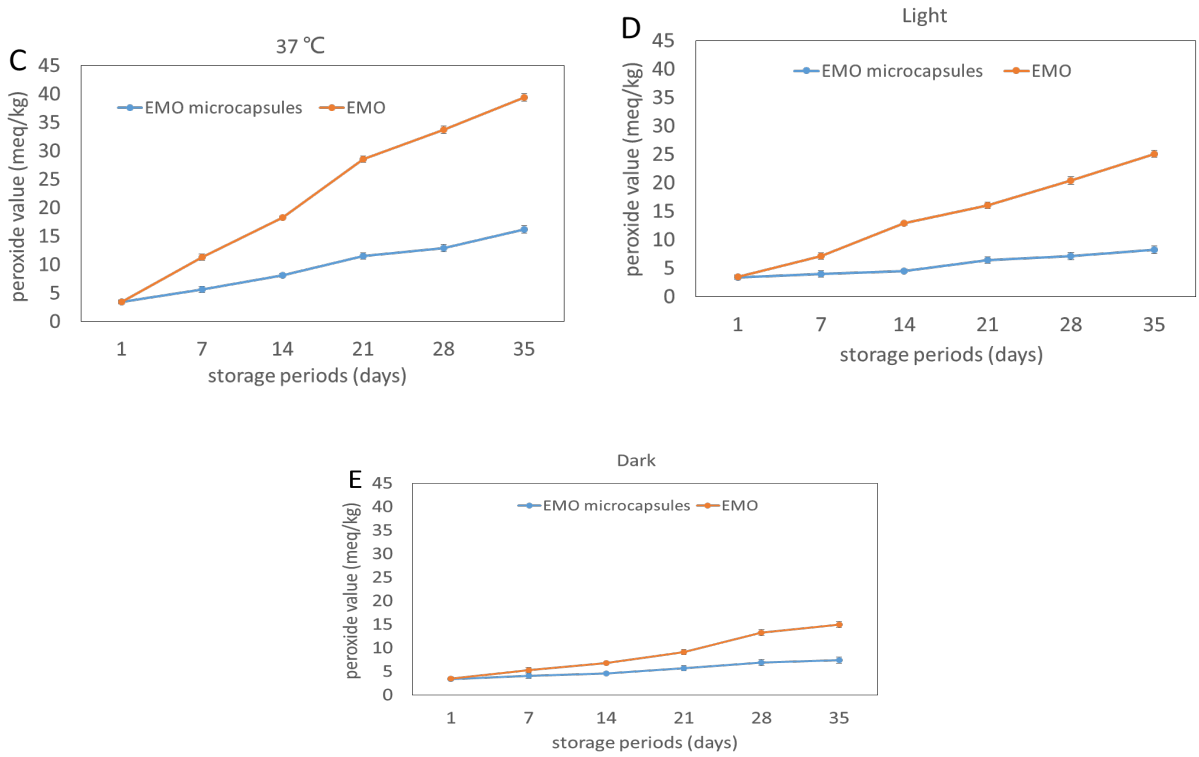


Figure 7. The stability assessment of spray-dried *Elaeagnus mollis* oil microcapsules with different temperatures as influential factors: (A) storage at 4°C, (B) storage at 20°C, and (C) storage at 37°C; The stability assessment of spray-dried *Elaeagnus mollis* oil microcapsules with light as influential factor: (D) storage in light and (E) storage in dark.

4. CONCLUSIONS

In summary, a gelatin-acacia combination could be applied in preventing PUFAs from oxidation in the food industry. The microencapsulated EMO with 45% PUFAs was able to tolerate heat stress and maintain long-term storage. However, either more wall material or higher oil concentrations would cause the aggregation of microcapsules, reducing the microencapsulation yield of EMO. Thus, the optimal concentration of the gelatin-acacia combination was 2.0%, and a 1:1 ratio of EMO to gelatin-acacia would guarantee the maximal EMO to be encapsulated. Moreover, the microencapsulation of EMO with gelatin-acacia combination involves only hydrogen bonding and no chemical bonding. The microencapsulation of EMO with gelatin-acacia combination might provide a safe way for the manufacturing of PUFA-containing products.

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