Encapsulation of structured phenolic lipids by complex coacervation method

Aziz, S., Neufeld, R.J., and Kermasha, S*.
Dep. Food Sc. & Agric. Chem., McGill University, Quebec, Canada (sarya.aziz@mail.mcgill.ca)

INTRODUCTION AND OBJECTIVE

Due to its important biomass (Massrieh 2008), krill oil (KO) offers a new abundant source of ω-3-polyunsaturated fatty acids (ω-3-PUFAs), in particular eicosapentaenoic acid (EPA, $C_{20:5}$ *n*-3) and docosahexaenoic acid (DHA, $C_{22:6}$ *n*-3). The enzymatic transesterification of krill oil with the phenolic acid 3,4-dihydroxyphenylacetic acid (DHPA) resulted in the synthesis of novel bio-molecules, phenolic lipids (PLs), with enhanced anti-oxidative and solubility properties (Aziz et al. 2012). The entrapment of PLs within micron-sized particles could be an effective approach for their protection and delivery into the food system. As compared to other technologies, the complex coacervation, which involves the electrostatic attraction between two biopolymers of opposing ranges, offers several advantages including, higher payload, very low amount of surface oil and a relatively thick outer shell (Barrow et al. 2007). The objective of this study was to optimize the encapsulation of PLs, obtained by the transesterification of KO with DHPA, via complex coacervation.

MATERIALS AND METHODS

Materials: Kosher-certified Beef-hide gelatin (GE) was obtained from Vyse Gelatin Co. Gum arabic (GA) was purchased from ACP Chemicals Inc. High-Potency krill oil, extracted from *Euphausia superba*, was generously obtained from Enzymotec Ltd. Commercial immobilized lipase, Novozym 435, from *Candida antartica* with an activity of 10,000 propyl laurate units per g solid enzyme, was purchased from Novozymes A/S. The 3,4-dihydroxyphenylacetic acid (DHPA) was purchased from Sigma Chemical Co. Sodium hydroxide, ammonium hydroxide, ethanol, glacial acetic acid and organic solvents of high-performance liquid chromatography (HPLC) grade were purchased from Fisher Scientific.

Microencapsulation: The coacervated particles were produced according to a modification of the method of Liu et al. (2010). The microencapsulation was carried out in a double-jacketed reactor, linked to a circulator-bath. Stock solutions of gelatin (1%, w/v) were prepared and their pH was adjusted, from 5.8 up to 8.5, by the addition of 0.25 N sodium hydroxide. A defined amount of the synthetized PLs was emulsified into GE, using a homogenizer as well as a sonicator ultrasonic liquid processor at a ratio of core material to wall (RCW) of 1.25:1. The GA (1%, w/v) was then added to the GE-stabilized emulsion and the mixture was stirred for an additional 5 min, followed by an acidification to pH 4.0 by the dropwise addition of 10% (v/v) acetic acid to induce the complex coacervation. The heating unit of the circulator-bath was then turned off and the mixture was allowed to slowly cool from 45±3°C to room temperature over time under constant mechanical stirring at 400 rpm. The particle suspension was then slowly cooled, with continuous stirring, to 10°C using an ice-bath. The coacervate rich phase was then recovered and stored at 4°C for further analyses.

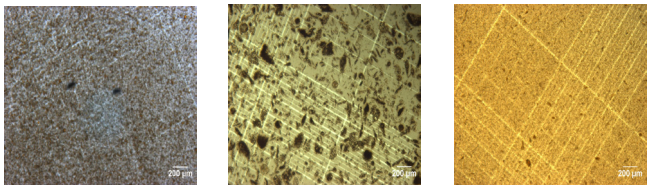
Determination of encapsulation efficiency & morphology of microcapsules: The total and surface oils were determined according to a modification of the method of Liu et al. (2010) and Drusch et al. (2006), respectively. The encapsulation efficiency (% EE) was calculated following the equation: ((total oil - surface oil)/total oil x100). The morphology of the microcapsules was observed on a hemacytometer, using a stereomicroscope equipped with a camera. The diameters of the microcapsules were measured using ImageJ Analysis Software.

Statistical analysis: One way Anova statistical analysis was used to determine the difference among several groups, followed by the Holm-Sidak test for pairwise comparisons.

RESULTS AND DISCUSSION

Effects of the phenolic acid and phenolic lipids, present in the esterified KO, and the emulsification device on the emulsion: The experimental findings showed (Figure 1a) that a stable homogenous emulsion was obtained, when KO was emulsified into GE, at a homogenisation rate of 20,500 rpm. However, when the esterified KO (EKO) was emulsified into GE, under the same conditions, small particles of irregular shapes were formed at the emulsification stage (Figure 1b). These findings could be due to the fact that the homogenizer created shearing by applying a tangential force to the mixture which could have favored the interactions between the PLs and the phenolic acids in the oil with the gelatin (Burden 2008). These results are in agreement with those of Zhang et al. (2010), who confirmed that the phenolic compounds do react with the amino groups in GE leading hence to the formation of crosslinks. In order to improve the stability of the emulsion, the effect of using the ultrasonic liquid processor was investigated. Figure 1c shows that the emulsion,

obtained with the ultrasonic liquid processor was homogenous, stable and free of particles. These findings could be due to the fact that the ultrasonication may have resulted in an emulsion by applying shock waves using differential pressure, which prevented the formation of particles between the GE and the EKO (Burden 2008).



a b c

Figure 1: Emulsions of gelatin-krill oil (a) & gelatin-phenolic lipids (b & c), as observed by a stereomicroscope, using as the emulsification device, the homogenizer (a & b) and the ultrasonic liquid processor (c).

Effects of pH of gelatin and emulsification devices on the EE and the size of the capsules: Using the homogenizer at pH 5.8, which corresponds to that of the beef GE, the formed capsules were susceptible to breakage, which made the determination of EE difficult (Figure 2). In addition, the formation of these capsules, at the emulsification stage, had an influence on the size of the microcapsules. Zhang et al. (2010) reported that the cross-linking of GE with phenolic acids was obtained under alkaline conditions.

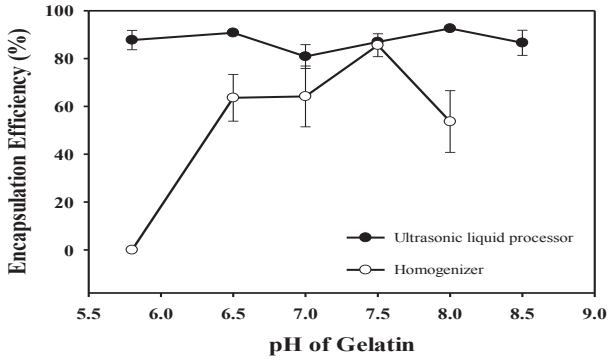


Figure 2: Effects of pH of gelatin & emulsification device on the encapsulation efficiency of phenolic lipids.

Using two emulsification devices, the effects of the pH of GE on the EE and the size of the microcapsules were investigated. The experimental findings indicated that using the homogenizer and varying the pH of GE from 5.8 to 8.0, there was a statistically significant difference ($P < 0.05$) in the EE of PLs (Figure 2), but statistically insignificant difference ($P > 0.05$) in the size of the PLs microcapsules (Table 1). On the other hand, using ultrasound and varying the pH of GE from 5.8 to 8.5, there were no statistically significant differences in the EE and in the size ($P > 0.05$) of the PLs microcapsules (Figure 2, Table 1).

Table 1: Effects of pH of gelatin & emulsification device on the size of the capsules.

	Homogenizer	Ultrasonic liquid Processor
pH	**Size of capsules (µm)**	
5.8	626 ±127[a]	608 ±121
6.5	708 ±16	457 ±22
7.0	705 ±72	477 ±47
7.5	594 ±10	417 ±3
8.0	677 ±12	408 ±6
8.5	ND[b]	515 ±12

[a]Standard deviation; [b]Not determined.

CONCLUSION

Overall, the ultrasonic liquid processor was found to be a more appropriate device for the emulsification of the EKO in GE, as compared to the homogenizer. The EE, size and stability of the EKO microcapsules were dependent on the nature of the emulsion. Unlike the homogenizer, using the ultrasound, the pH did not have any significant effect on the EE of microcapsules, with an EE ranging from 80 to 92%.

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