Optimization of morphology and geometry of encapsulated *Hypophthalmichthys molitrix* oil

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Abstract

In the present study, the effect of stirring speed and the type of cross linking agent on the size and formation of microencapsulated Silver carp *(Hypophthalmichthys molitrix)* oil were investigated. The gelatin/gum Arabic was used for encapsulating and the capsules were prepared by complex coacervation. Microcapsules were analyzed by optical microscopy technique and particle size analyzer. Results suggested the use of glutaraldehyde as the cross-linking agent instead of formaldehyde can caused the Microcapsules become spherical shape, smooth surface with no obvious dents and narrower particle size distribution. The average particle sizes were 537.2±0.8 µm, 84.4±0.5 µm, 12.98±0.4 µm, 8.24±0.5 µm, and 4±0.7 µm at the homogenization stirring speed of 100, 300, 500, 750 and 1000 rpm respectively. The best conditions of experiment were with 25% glutaraldehyde at 1000 rpm of stirring speed.

Keyword: gelatin, gum Arabic, cross linking agent, microcapsules, stirring speed, complex coacervation

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Introduction

Microencapsulation offers the possibility to protect sensitive food ingredients like omega-3 fatty acids against adverse environmental conditions and to control their release (Kolanowski *et al.*, 2004). The wall materials of the microcapsules protect the core substance against influence of oxygen light and humidity (Loksuwan, 2007). Stability and shelf life of fish oil can be improved by microencapsulation (Loksuwan, 2007). Encapsulating agents are for food and pharmaceuticals application. Gum Arabic hydrolyzed starches gelatin chitosan and alginate used as wall materials (Loksuwan, 2007) .autoxidation is particularly important when supplementing foods with omega-3 fatty acids especially the long chain polyunsaturated fatty acids such as docosahexanoeic and eicosapentanoeic acid were applied due to the high degree of unsaturated fatty acids (Drusch *et al.* , 2008) . Microencapsulation is defined as a technology of packaging solids, liquids or gaseous materials in capsules (Drusch *et al*., 2006). Coacervation is a colloidal phenomenon. Neutralization of the overall positive charges on one of the colloids (by the negative charges) is used to separate the polymer-rich complex coacervate phase (Kolanowski *et al*., 2004). Traditionally, gelatin is used in combination with gum Arabic in numerous studies (Drusch *et al*., 2008). Complex coacervation is a phase separation of one or many hydrocolloids from the initial solution and the subsequent deposition of the newly formed coacervate phase around the active ingredient suspended or emulsified in the same reaction media(Drusch *et al.* , 2008) .The coacervate can be hardened by cross-linking agents (Drusch *et al.* , 2008). Complex coacervation is possible being run only at the pH values below the isoelectric point of gelatin. It is at these pH values that gelatin becomes positively charged, but gum Arabic continues to be negatively charged (Kolanowski *et al*., 2004). Microcapsulation can be accomplished by different techniques spray drying, spray chilling, spray cooling, extrusion coacervation and co-crystallization (Venskutonis & Bylaite, 2001). The process of complex coacervation microencapsulation comprises four basic steps: emulsification, coacervation, gelation and hardening (Dong *et al*., 2008). A cross-linking agent is used to harden the microcapsule walls and stabilizing the structure during this process (Dong *et al*., 2008). The changes in size and distribution of microcapsules produced by changing emulsification stirring speed appear as expected (Chang *et al*., 2006). In this study fish oil are encapsulated for several reasons: (1) to retain them in a food product during storage and increase their shelf life; (2) to protect the oil from undesirable interaction with the food; (3) to guard against light, heat, moisture or air induced reactions or oxidation; (4) to provide the controlled or delayed release of flavor and (5) to mask objectionable flavors.

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Materials and methods

Gelatin solution (12.5weight percent, wt) and gum Arabic (12.5% wt) were mixed at 40 ºC for 30 min at 100 rpm (Dong *et al*., 2007). 4.2 g of purified *Hypophthalmichthys molitrix* oil was mixed with 0.5 g of polyvinyl alcohol at 40 ºC for 30 min at 100 rpm, during mixing, 200 ml of distilled water, was added to mixture. At 40 ºC the pH of the emulsion was adjusted to 4.5-5 by adding 10% (w/w) aqueous acetic acid solution at 100 rpm. Then 200 ml of distilled water added to the above mixture at 0 ºC and mixed for 60 min at 100 rpm (Dong *et al*., 2008). After 60 min, the pH was adjusted to 9.5-9.7 through the addition of 10% (w/w) aqueous solution of sodium hydroxide. The 10% aqueous solution of formaldehyde was added to start the cross linking in the microcapsules for 30 min at 3-5 ºC and the stirring rate of 100 rpm . The pH was adjusted to 9.5 once more through addition of 10% (w/w) aqueous solution of sodium hydroxide. The solution under this condition agitated for 22 h. Finally the microcapsules were filtered, washed and recovered (Drusch *et al*., 2008). The same method was applied for glutaraldehyde at the stirring speed of 100, 300, 500, 750 and 1000 rpm, respectively. The morphology and particle size distribution of microcapsules were analyzed by optical microscopy (Carl Zeiss Germany) connected to a digital video camera (Canon PC 1089 Japan) and particle size analyzer (LEICADMLB Germany).The effect of glutaraldehyde and polyvinyl alcohol were investigated in microcapsules formation and their results were compared.

Results

The appearance of emulsions structure was checked in optical microscopy under 3 and 10 × magnifications. The size of microcapsules particle were from 537.2±0.8 µm (n: 20) to 4±0.7 µm (n: 20) at the homogenization stirring speed range of 100 to 1000 rpm, respectively. Figures (1 to 17) showed morphology and particle size distribution of microcapsules after reticulation versus stirring speed.

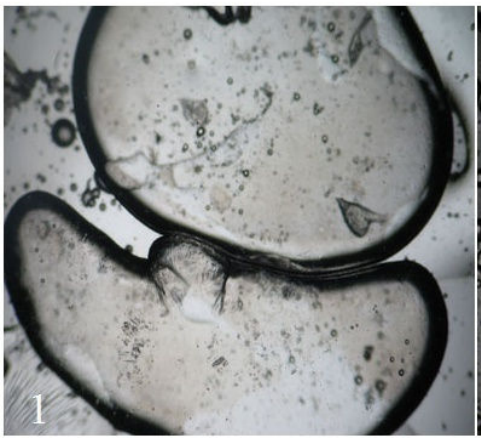

Figure1:The microcapsules at 100 rpm

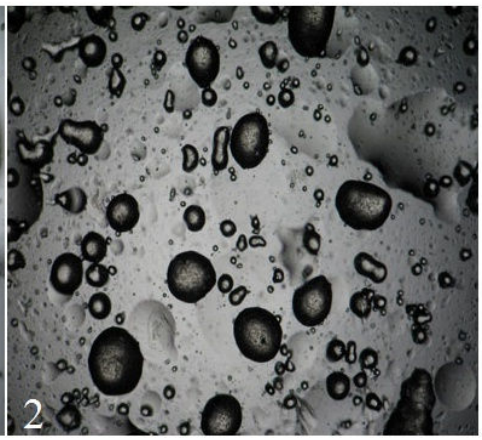

Figure 2: The microcapsules at 300 rpm

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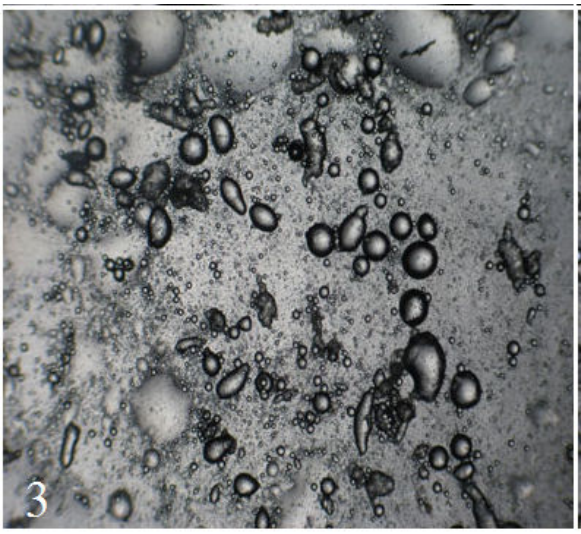


Figure 3: The microcapsules at 500 rpm

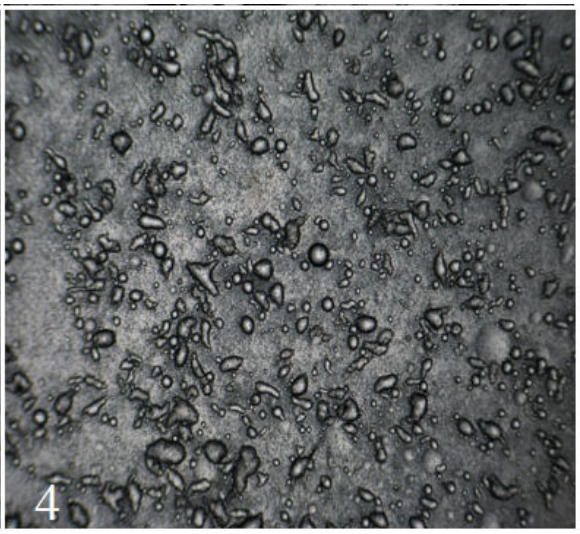


Figure 4: The microcapsules at 750 rpm

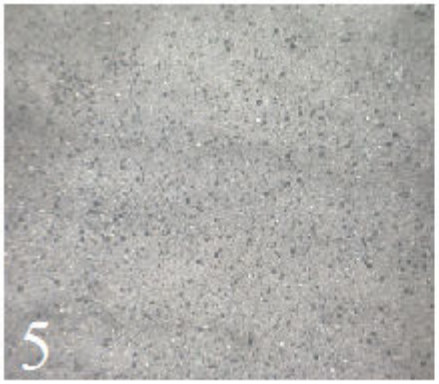


Figure 5: The microcapsules at 1000 rpm

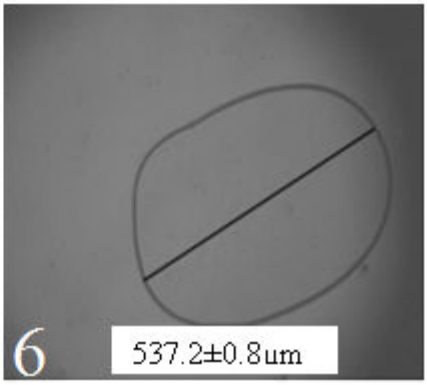
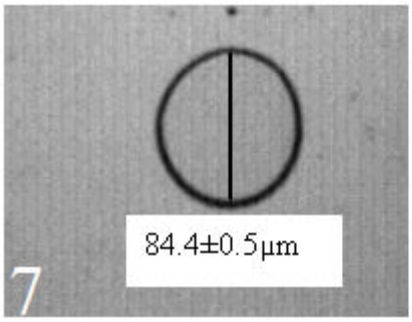


Figure 6: The size distribution of the microcapsules at 100 rpm



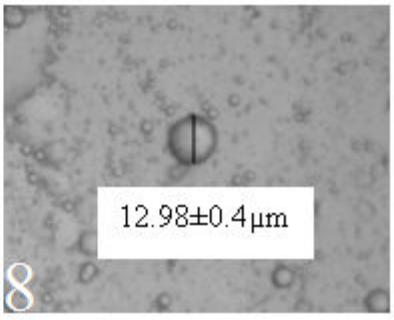


Figure 7: The size distribution of the microcapsules at 300 rpm

Figure 8: The size distribution of the microcapsules at 500 rpm

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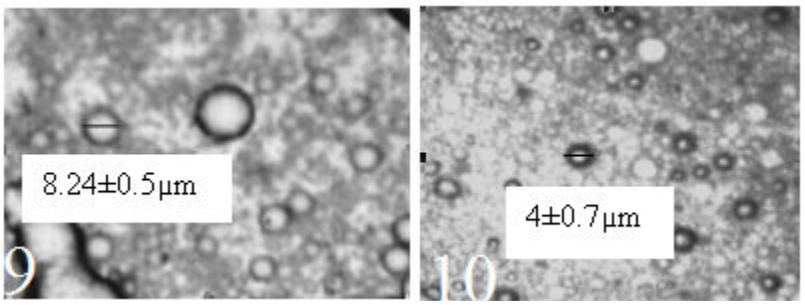

Figure 9: The size distribution of the microcapsules at 750 rpm
Figure 10: The size distribution of the microcapsules at 1000 rpm

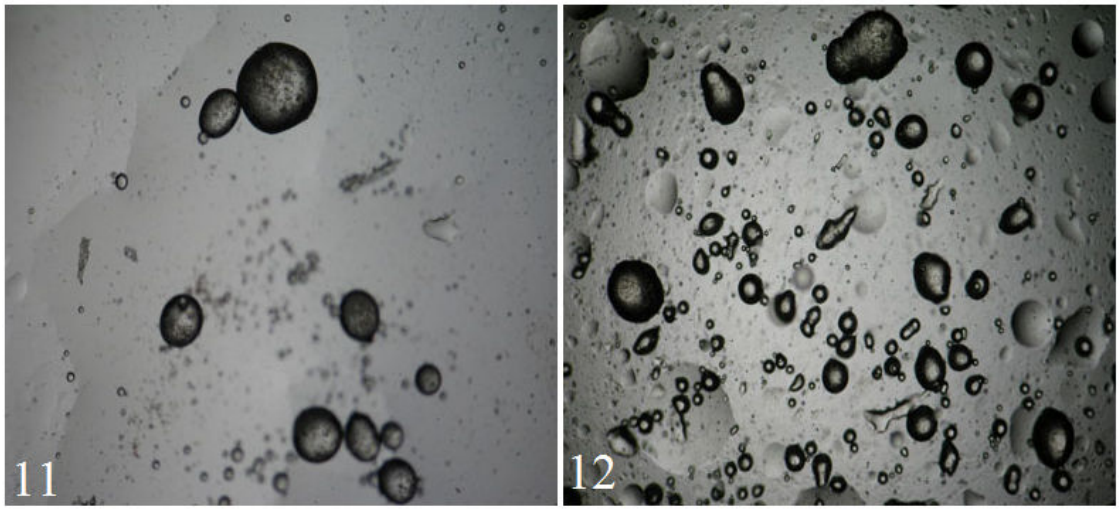

Figure 11: The microcapsules with formaldehyde
Figure 12: The microcapsules with glutaraldehyde

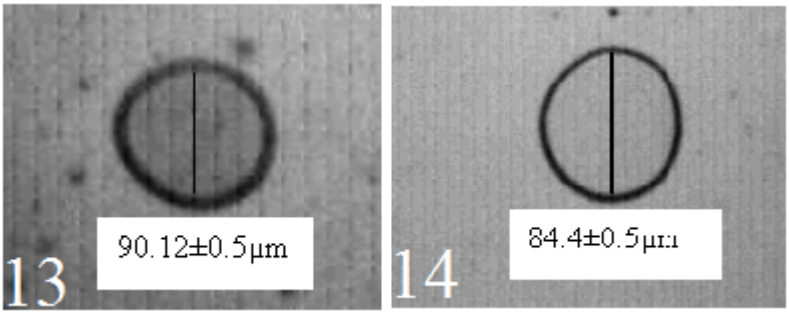

Figure 13: The size distribution of the microcapsules with formaldehyde at 300 rpm
Figure 14: The size distribution of the microcapsules with glutaraldehyde at 300 rpm

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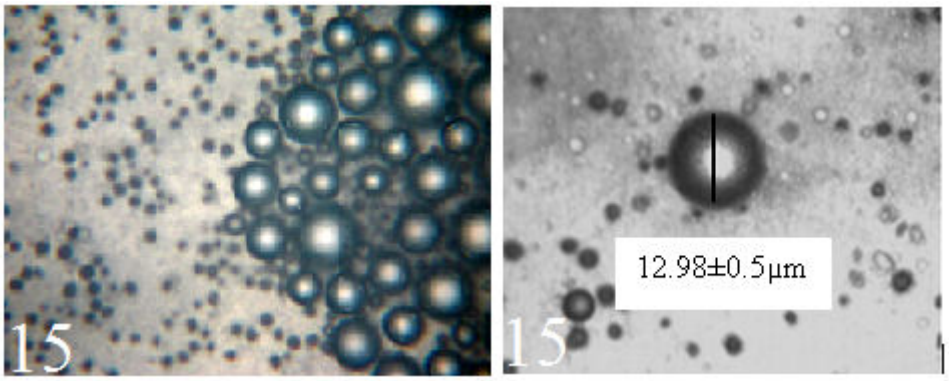
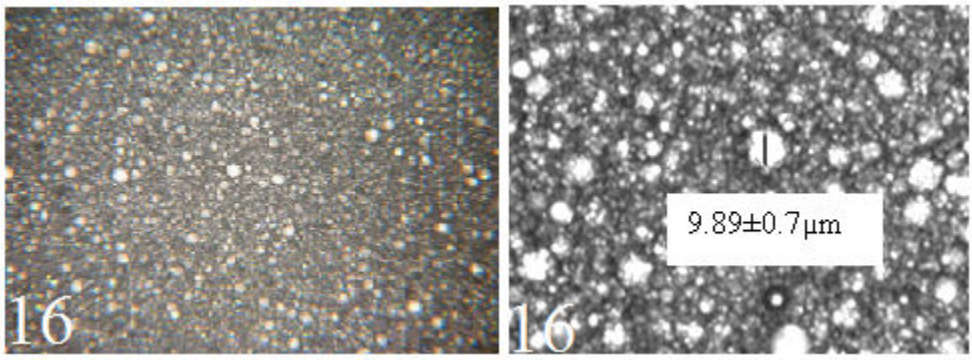


Figs 15: The morphology and the particle size distribution of the microcapsules with polyvinyl alcohol and without glutaraldehyde at the 1000 rpm



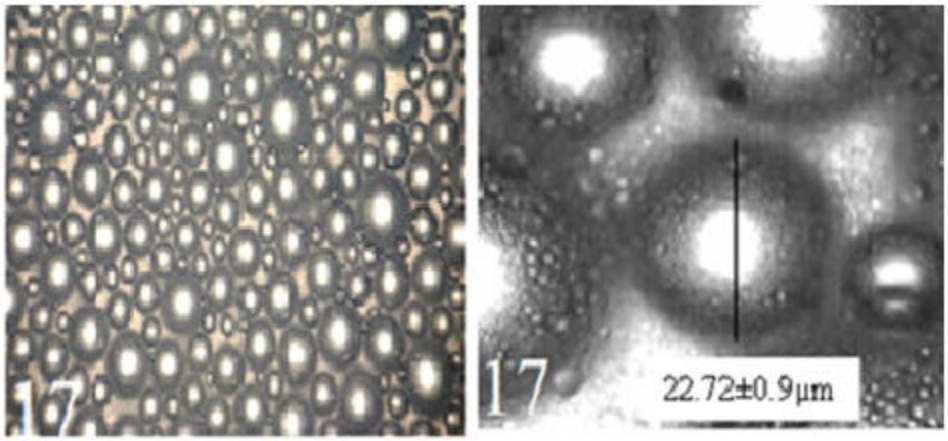


Figure 16: The morphology and the particle size distribution of the microcapsules with glutaraldehyde and without polyvinyl alcohol at the 1000 rpm

Figure 17: The morphology and the particle size distribution of the microcapsules Without polyvinyl alcohol and glutaraldehyde at the 1000 rpm

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Table1: Comparison of morphology and size distribution of microcapsules at different operational condition by complex coacervation method

Stirring speed(rpm)	Formaldehyde 10%	Glutaraldehyde 25%	Poly vinyl alcohol (0.5g)	Morphology	Size distribution (µm)
100	Without	With	With	Irregular and big	537.2±0.8
300	With	Without	With	Irregular and big	90.12±0.5
300	Without	With	With	More regular	84.4±0.5
500	Without	With	With	Regular and small	12.98±0.4
750	Without	With	With	More regular and smaller	8.24±0.5
1000	Without	With	With	Spherical shape and very small	4 ± 0.7
1000	Without	Without	With	Irregular	12.98±0.5
1000	Without	With	Without	Irregular	9.89±0.7
1000	Without	Without	Without	Irregular and big	22.72±0.9

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Discussion

The emulsion used to form micro particles allowed the preparation of particles with a spherical shape in Figs 1 to 5 and size distribution as shown in Figs 6 to 10. When glutaraldehyde was used as the cross linking agent instead of formaldehyde, the morphology became spherical and the particle size distribution became narrower (Figs 11 to 14).Figure 1 demonstrates the effect of stirring speed on the morphology of microcapsules. When the stirring speed was 100 rpm, the difference of the oil content in different particle size microcapsules was very important. I comparison with the smaller particle size microcapsules (4±0.7, 8.24±0.5 and 12.98±0.4 μm), the bigger ones (84.4±0.5 and 537.2±0.8 μm) had higher oil content (Figs 6 to 10). This was due to the fact that, at lower stirring speed, lots of emulsion droplets floated upward and accumulated into microcapsules with bigger size and higher oil contents (Dong *et al*., 2007). Accordingly, the quantity of coacervates in the bulk was relatively increased which deposited around a small quantity of residual emulsion droplets and formed relatively smaller microcapsules with lower oil content. At the stirring speed of 300 rpm, the difference in microcapsules with different particle size was small (84.4±0.5 μm) (Fig 7). When the stirring speed was increased to 500 rpm, the morphology of microcapsules became irregular due to the violent stirring (Figs. 1 to 5). As the stirring speed decreased the mean particle size of the microcapsules increased and the particle size distribution became wider (Chang *et al*., 2006). When the stirring speed decreased from 500 rpm to 300 rpm, the mean particle size of microcapsules increased from 12.98±0.4 μm to 84.4±0.5 μm. Also when the stirring speed decreased from 300 rpm to 100 rpm, the mean particle size of microcapsules increased from 84.4±0.5 μm to 537.2±0.8 μm. When the stirring speed was below 100 rpm microcapsules could not be formed (Dong *et al*., 2007) . Since the effect of the stirring speed on the particle size of spherical microcapsules was also very important, the spherical microcapsules with desired particle size could be made by modulating the stirring speed. By increasing the stirring speed, the loading of microcapsules was also increased, because the amount of coacervates deposited around emulsion droplets was reduced at the high stirring speed (Dong *et al*., 2007). The spherical microcapsules had narrower particle size distribution and uniform morphology at 1000 rpm which is the optimum stirring speed. When glutaraldehyde was used as a cross-linking agent instead of formaldehyde, the morphology and particle size distribution of microcapsules changed significantly and became narrower (Figs 11 to 14) suggesting that spherical microcapsules can be produced by the glutaraldehyde cross-linking agent instead of formaldehyde .This also has been showed by previous studies (Drusch *et al*., 2008). Using gelatin and gum Arabic as wall material and Fitofague oil as core material, the effect of various processing parameters, including the kind of cross-linking agent and stirring speed on the morphology and particle size distribution

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were investigated. The Optimum conditions for preparing the spherical microcapsules obtained at wall material concentration 12.5% wt core/wall 1:1 pH 4.5-5 and the stirring speed of 1000 rpm by using glutaraldehyde as the cross linking agent instead of formaldehyde Where in such a condition, morphology and particle size distribution became spherical shape, more regular, with smooth surface and narrower size distribution.

Acknowledgments

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