

## EFFECT OF COMPLEXATION CONDITIONS ON MICROCAPSULATION OF *LACTOBACILLUS ACIDOPHILUS* IN XANTHAN-CHITOSAN POLYELECTROLYTE COMPLEX GELS\*

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### ABSTRACT

**Background.** *Lactobacillus acidophilus* has become increasingly popular because of their beneficial effects on health of their host, and are called proboscis. In order to exert beneficial effects for probiotics, they must be able to tolerate the acidic conditions of the stomach environment and the bile in the small intestine. Micro-encapsulated form has received reasonable attention, since it can protect probiotic organisms against an unfavourable environment, and to allow their release in a viable and metabolically active state in the intestine. The aim of this study was to investigate some factors, such as chitosan solution pH and concentration, xanthan concentration, cell suspension-xanthan ratio, mixed bacteria glue liquid-chitosan ratio, which impacted the process of microencapsulation of *L. acidophilus*.

**Material and methods.** In this study, *L. acidophilus* was immobilized with xanthan/chitosan gel using extrusion method. The viable counts and encapsulation yield of *L. acidophilus* encapsulated in different chitosan solution pH (4.5, 5, 5.5 and 6), in different chitosan concentration (0.5%, 0.7%, 0.9% and 1.1%), in different xanthan concentration (0.5%, 0.7%, 0.9% and 1.1%), in different cell suspension-xanthan ratios (1:5, 1:10, 1:15 and 1:20), in different mixed bacteria glue liquid-chitosan ratios (1:3, 1:4, 1:5 and 1:6), have been investigated by single factor experiment method.

**Results.** The optimum conditions of microencapsulated *L. acidophilus* have been observed. The optimum chitosan solution pH for *L. acidophilus* was 5.5; the optimum chitosan concentration was 0.9%; the optimum xanthan concentration was 0.7%; the optimum cell suspension-xanthan ratio was 1:10; the optimum mixed bacteria glue liquid-chitosan ratio was 1:3.

**Conclusions.** These results will be helpful to further optimize the process of *L. acidophilus* microencapsulation, and provide reference for obtaining higher viable counts and entrapped yield of *L. acidophilus* microcapsules.

**Key words:** xanthangum, chitosan, *Lactobacillus acidophilus*, microencapsulation, extrusion

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## INTRODUCTION

Considering the increasing demand for functional foods, probiotics have become one of the most important health promoting food enhancement in recent years (Agrawal, 2005). Probiotics are viable microorganisms which are beneficial to the host when administered in adequate amounts (Xiao et al., 2011).

*Lactobacillus acidophilus* have become increasingly popular because of their beneficial effects on the health of their host, and are called proboscis (Fuller, 1989; Motohiro Shima et al., 2009). In order to exert beneficial effects for probiotics, they must be able to tolerate the acidic conditions of the stomach environment and the bile in the small intestine (Doleyres et al., 2004; Gardiner et al., 2000). The acidic environment of the stomach and the bile salts secreted into the duodenum are the main obstacles for the survival of the ingested bacteria. Unluckily, most of the probiotics including *L. acidophilus* lack the ability to survive in the harsh conditions of acidity and bile concentration commonly encountered in the gastrointestinal tract of humans (Krasaekoopt et al., 2003; Xiao Yan Li et al., 2011). As a result, the viable counts of *L. acidophilus* probiotic dairy products often have a exponential curve downward trend, which leads to the fact that those products have a difficulty to achieve the healthy effect (Kimoto et al., 2000; Vinderola and Reinheimer, 2003).

Microencapsulated form has received reasonable attention, since it can protect probiotic organisms against an unfavourable environment, and to allow their release in a viable and metabolically active state in the intestine (Heidebach et al., 2009; Sandoval-Castilla et al., 2010). In the process of microencapsulation of probiotics, coatings and mixtures of suitable biopolymers, such as alginate, k-carrageenan, gellan-gum are applied (Champagne et al., 1994; Picot and Lacroix, 2004). However, those embedding materials have some defects in the process of microencapsulated probiotics, e.g. alginate is not stable in acidic conditions.

Chitosan is a natural biological macromolecule, which has excellent biological properties such as biocompatibility, biodegradability, lack of toxicity, and so on (Dutta et al., 2004; Felse and Panda, 1999). Xanthan gum is stable over a wide range of temperatures and pH, which finds many applications in food (Garcia-Ochoa et al., 2000; Meyer et al., 1993).

The hydrogel network formed through the ionic interactions between the amino groups of chitosan and carboxyl groups of xanthan shows pH-sensitive swelling characteristics, which enable the controlled release of entrapped materials such as therapeutic agents, enzymes and bacteria (Chellat et al., 2000; Chu et al., 1996). Therefore, xanthan-chitosan hydrogels are recognised as promising candidates for targeted delivery and controlled release of encapsulated products for oral administration.

In this study, *L. acidophilus* was immobilized with xanthan/chitosan gel using extrusion method. Some factors, such as chitosan solution pH and concentration, xanthan concentration, cell suspension-xanthan ratio, mixed bacteria glue liquid-chitosan ratio have been investigated. The optimum conditions of microencapsulated *L. acidophilus* will be further observed. The obtained results will be helpful to optimize the process of *L. acidophilus* microencapsulation, and provide reference for obtaining higher viable counts and entrapped yield of *L. acidophilus* microcapsules.

## MATERIAL AND METHODS

### Preparation of chitosan and xanthan solutions

Chitosan with a minimum of 80~95% deacetylation and a molecular weight of 370 000 was purchased from Sigma-Aldrich Chemicals (St. Louis, MO). The chitosan was dissolved in 1N HCl by agitating, and adjusting the different chitosan concentration to 0.5%, 0.7%, 0.9% and 1.1%. The desired solution pH was adjusted by 1M NaOH and deionized (DI) water was added to bring it to the final volume, and the pH was adjusted to 4.5, 5, 5.5 and 6. Xanthan gum with a molecular weight of 1.02 million was kindly supplied by Zibo Zhongxuan biological chemistry company. Xanthan gum was dissolved in DI water under heating and agitation, and the xanthan concentration was adjusted to 0.5%, 0.7%, 0.9% and 1.1%. It was autoclaved (110°C, 15 min) before use.

### Microorganism

*L. acidophilus* was obtained from College of Life Science and Engineering, Shaanxi University of Science and Technology. One milliliter of hydrated *L. acidophilus* cells was inoculated into 18 mL MRS broth and incubated at 35°C for 24 h. Actively growing cells

were recovered from the MRS broth by centrifuging at 10 000 rpm for 15 min. DI water was added to the cell pellet and vortexed.

### Microencapsulation

*L. acidophilus* cells were mixed with xanthan gum solution (1:10) and encapsulation was achieved by dropwise addition of this mixture (11 mL) into the chitosan solution (66 mL) by using a manually operated syringe with 0.7-mm cannula (BectoneDickinson, Franklin Lakes, NJ). The chitosan solution was agitated continuously for 40 min to allow crosslinking and to avoid coalescence of the capsules. The capsules were filtered through a 160- $\mu$ m, washed twice with DI water.

### Viable counts

The sample to be tested with sterile saline solution into the bacterial suspension, was next diluted at 10 times, taking the dilution of  $10^{-7}$  to  $10^{-8}$  of the suspension inoculation of 0.1 mL to the solid culture medium. After the bacteria were cultured for 48 h at 37°C, we could observe and count the average values, and investigate the various factors on the microencapsulation of *L. acidophilus* viable counts. The viable counts of microcapsules were weighed through a formula according to eq. (1):

$$VC = N \cdot T \cdot 10 \quad (1)$$

where:

$VC$  – viable counts of the original suspension a per milliliter,  $CFU \cdot mL^{-1}$ ,

$N$  – average colony number of 3 repeat solid culture in the same dilution,  $CFU \cdot mL^{-1}$ ,

$T$  – times of dilution.

### Encapsulation yield (EY)

1 g capsules were subjected to the simulated gastric fluid (10 mL) at pH 2.0 for 40 min at 37°C under 210 rpm.

$$EY = N_1 \cdot M / (N_0 \cdot V_0) \cdot 100\% \quad (2)$$

$$N_1 = G_1 \cdot V_1 \quad (3)$$

where:

$G_1$  – the number of viable counts released from the simulated gastric fluid, 1 mL,

$V_1$  – the volume of simulated gastric fluid, mL,

$N$  – the living bacteria number, in wet capsule,  $CFU \cdot g^{-1}$ ,

$N_0$  – the viable counts of the original suspension on a per milliliter,  $CFU \cdot mL^{-1}$ ,

$V_0$  – the volume of original bacteria liquid which used by preparing microcapsule, mL,

$M$  – the total weight of the wet capsule, g.

## RESULTS AND DISCUSSION

### Effect of chitosan solution pH on encapsulation of *L. acidophilus*

According to the initial preparation conditions of microcapsulation, chitosan solution pH was adjusted to 4.5, 5.0, 5.5 and 6, the results as shown in Figure 1.

According to Figure 1, with increasing of the chitosan solution pH, the viable counts and entrapped yield of *L. acidophilus* microcapsules increased at first, and then decreased. When the chitosan solution pH was 5.5, the viable counts and entrapped yield of *L. acidophilus* microcapsules up to  $1.21 \cdot 10^{10} CFU \cdot g^{-1}$  and 78%, respectively. The reason of this tendency on figure was that the change of the chitosan solution pH, since chitosan solution pH continually increased, the amino groups became less charged, which lead to fewer ionic linkages would occur between the two polymers. As a result, the crosslinking densities of xanthan-chitosan hydrogel capsules decreased, diffusion

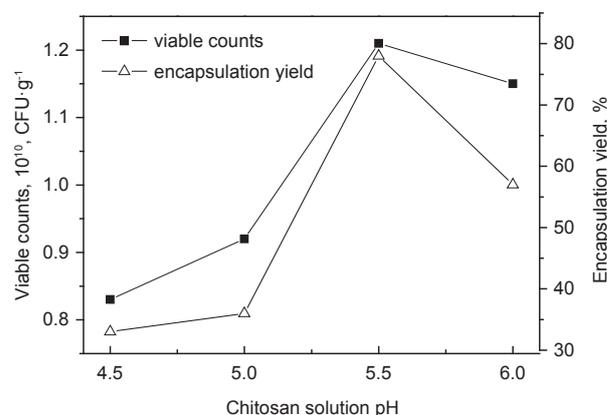


Fig. 1. Effect of chitosan solution pH on viable counts and entrapped yield of *L. acidophilus* of microcapsules

coefficient became higher for chitosan chains, so amounts of bacteria spread out from xanthan-chitosan hydrogel capsules, viable counts of encapsulation and encapsulation yield of *L. acidophilus* microcapsules will be reduced, correspondingly.

As a result, there is a preliminary determination about the chitosan solution pH for *L. acidophilus* microencapsulated. The optimum chitosan solution pH was 5.5, which corresponds to viable counts and entrapped yield were  $1.21 \cdot 10^{10}$  CFU·g<sup>-1</sup> and 78%, respectively.

### Effect of chitosan concentration on encapsulation of *L. acidophilus*

According to the initial preparation conditions of microcapsulation, chitosan concentration was adjusted to 0.5%, 0.7%, 0.9% and 1.1%, the results as shown in Figure 2.

According to Figure 2, with increasing of the chitosan concentration, the viable counts and entrapped yield of *L. acidophilus* microcapsules increased at first, and then decreased. When the chitosan concentration was 0.9%, the viable counts and entrapped yield of *L. acidophilus* microcapsules up to  $1.1 \cdot 10^{10}$  CFU·g<sup>-1</sup> and 73%, respectively. Since chitosan concentration increased, the amino groups became more charged, which lead to more ionic linkages would occur between the two polymers. As a result, the crosslinking densities of xanthan-chitosan hydrogel capsules increased, amounts of bacteria would be embedded

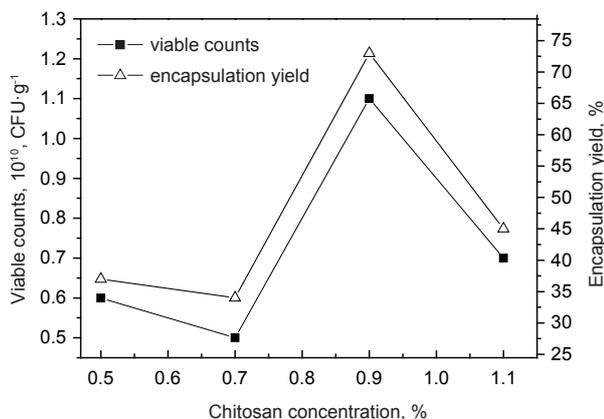
completely, viable counts of encapsulation and encapsulation yield of *L. acidophilus* microcapsules would be increased, correspondingly. However, when the chitosan concentration continually increased, the viscosity of chitosan solution correspondingly increased, which impeded the positive and negative charges to combine, resulting in the crosslinking densities of xanthan-chitosan hydrogel capsules decreased, amounts of bacteria would be embedded incompletely, viable counts of encapsulation and encapsulation yield of *L. acidophilus* microcapsules would be decreased, correspondingly.

As a result, there is a preliminary determination about the chitosan concentration for *L. acidophilus* microencapsulated. The optimum chitosan concentration was 0.9%, which corresponds to viable counts and entrapped yield were  $1.1 \cdot 10^{10}$  CFU·g<sup>-1</sup> and 73%, respectively.

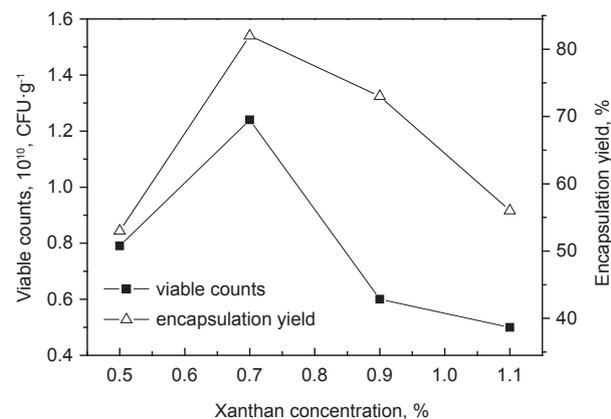
### Effect of xanthan concentration on encapsulation of *L. acidophilus*

According to the initial preparation conditions of microcapsulation, xanthan concentration was adjusted to 0.5%, 0.7%, 0.9% and 1.1%, the results as shown in Figure 3.

According to Figure 3, with increasing of the xanthan concentration, the viable counts and entrapped yield of *L. acidophilus* microcapsules increased at first, and then decreased. This phenomenon may be due to



**Fig. 2.** Effect of chitosan concentration on viable counts and entrapped yield of *L. acidophilus* of microcapsules



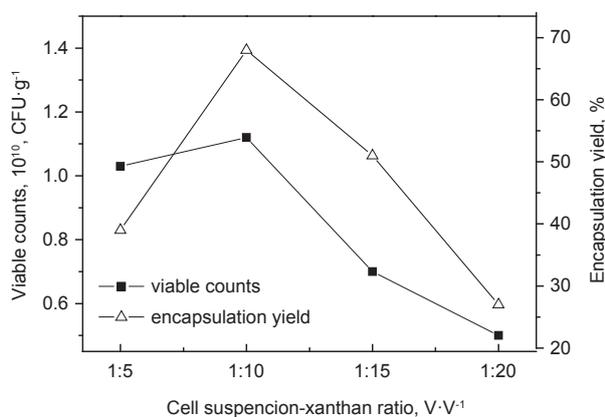
**Fig. 3.** Effect of xanthan concentration on viable counts and entrapped yield of *L. acidophilus* 1 of microcapsules

the degree of swelling decreased when increasing the concentration of xanthan solution. When the concentration of xanthan solution increasing from 0.5% to 0.7%, the crosslink density of xanthan-chitosan hydrogel capsules become increased. When the xanthan concentration was 0.7%, the viable counts and entrapped yield of *L. acidophilus* microcapsules up to  $1.24 \cdot 10^{10}$  CFU·g<sup>-1</sup> and 82%, respectively. However, we count the number of the bacteria in a certain period of time, increasing xanthan concentration resulted in significantly greater the crosslink density of xanthan-chitosan hydrogel capsules and lower the dissolution rate. As a result, it takes a lot of time to release probiotic cells under the degradation media, the viable counts and entrapped yield of *L. acidophilus* microcapsules will be decreased, correspondingly.

As a result, there is a preliminary determination about the xanthan concentration for *L. acidophilus* microencapsulated. The optimum xanthan concentration was 0.7%, which corresponds to viable counts and entrapped yield were  $1.24 \cdot 10^{10}$  CFU·g<sup>-1</sup> and 82%, respectively.

#### Effect of cell suspension-xanthan ratios on encapsulation of *L. acidophilus*

According to the initial preparation conditions of microcapsulation, the difference proportion of prepared bacteria suspension volume (mL) and xanthan solution volume (mL) were investigated, such as 1:5, 1:10,



**Fig. 4.** Effect of cell suspension-xanthan ratios on viable counts and entrapped yield of *L. acidophilus* of microcapsules

1:15, and 1:20. The effect of various cell suspension-xanthan ratios on encapsulation of *L. acidophilus* was shown in Figure 4.

According to Figure 4, with increasing of the proportion of xanthan and bacteria suspension, the viable counts and entrapped yield of *L. acidophilus* of microcapsules continually increased at first, and then decreased, this phenomenon may be due to the high proportion of xanthan. With increasing the ratio of cell suspension-xanthan from 1:5 to 1:10, the crosslink density of xanthan-chitosan hydrogel capsules become increased. However, when the ratio of xanthan continually increased, and the viscosity of xanthan solution correspondingly increased, it is difficult to combine for xanthan and chitosan. As a result, the phenomenon of incomplete embedded would emerge, and most of the cells were not embedded strongly.

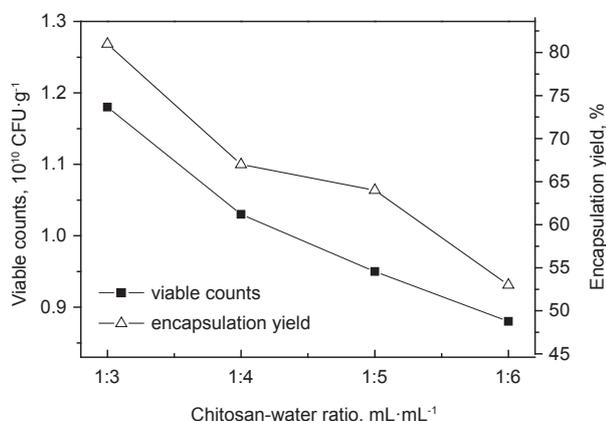
As a result, there is a preliminary determination about the cell suspension-xanthan ratio for *L. acidophilus* microencapsulated. The optimum cell suspension-xanthan ratio was 1:10, which corresponds to viable counts and entrapped yield were  $1.12 \cdot 10^{10}$  CFU·g<sup>-1</sup> and 68%, respectively.

#### Effect of chitosan-mixed bacteria glue ratios on encapsulation of *L. acidophilus*

(bacteria glue liquid-chitosan ratio is the mixtures of Xanthan gum and bacteria liquid and chitosan ratio. the purpose of this section was to study the effects of chitosan volume on the formation of microcapsules)

According to the initial preparation conditions of microcapsulation, the different proportion of mixed bacteria and xanthan volume (mL) and chitosan volume (mL) were adjusted to 1:3, 1:4, 1:5, 1:6, the results as shown in Figure 5.

According to Figure 5, with increasing of the chitosan-water ratio, the viable counts and entrapped yield of *L. acidophilus* microcapsules continually decreased. The reason of this tendency on figure was that the high value about proportion of mixed bacteria glue liquid and chitosan. With increasing of chitosan solution, chitosan solution package was thick, and the mass of microcapsule increased, so the living bacteria number contained in the unit quality of microcapsule and encapsulation yield will decrease gradually.



**Fig. 5.** Effect of e chitosan-water ratios on viable counts and entrapped yield of *L. acidophilus* of microcapsules

As a result, there is a preliminary determination about the cell suspension-xanthan ratio for *L. acidophilus* microencapsulated. The optimum mixed bacteria glue liquid and chitosan ratio was 1:3, which corresponds to viable counts and entrapped yield were  $1.18 \cdot 10^{10}$  CFU·g<sup>-1</sup> and 81%, respectively.

## CONCLUSIONS

According to the above experimental study, several factors, including chitosan solution pH and concentration, xanthan concentration, cell suspension-xanthan ratio, mixed bacteria glue liquid-chitosan ratio, have an important influence on microcapsulation of *L. acidophilus*. The optimum chitosan solution pH for *L. acidophilus* was 5.5; the optimum chitosan concentration was 0.9%; the optimum xanthan concentration was 0.7%; the optimum cell suspension-xanthan ratio was 1:10; the optimum mixed bacteria glue liquid-chitosan ratio was 1:3. These results will be helpful to further optimize the process of *L. acidophilus* microencapsulation, and provide reference for obtaining higher viable counts and entrapped yield of *L. acidophilus* microcapsules.

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