



Survival Survey of *Lactobacillus acidophilus* In Additional Probiotic Bread

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ARTICLE INFO	ABSTRACT
<p><i>Research Article</i></p> <p>Received : 03/10/2018 Accepted : 19/02/2019</p> <p>Keywords: Alginate <i>Lactobacillus acidophilus</i> Mmaltodextrin Xanthan gum Probiotic bread</p>	<p>Bread is a popular food in the world because of its variety and convenience. Currently, studies on the adding probiotics to bread are limited due to the adverse effects of processing, such as baking temperature, aerobic environment to the probiotic bacteria. The objective of this study was to produce probiotic cream bread, in which <i>Lactobacillus acidophilus</i> was microencapsulated with Alginate 2% (A); Alginate 2% + maltodextrin 1% (AM); Alginate 2% + xanthan gum 0.1% (AX); and Alginate 2% + maltodextrin 1% + xanthan gum 0.1% (AMX). Microcapsules were added to the kernel, conducting encapsulation yield investigations, survival in baking, preservation of bread, and in simulated gastric fluid and simulated intestinal fluid conditions after 8 days of storage. The results showed that the addition of xanthan gum enhanced the encapsulation yield, it reached 92.9% and 92.37% in AMX and AX samples, respectively. The viability of <i>L. acidophilus</i> during baking was decreased by 3.64 and 3.75 Log (CFU/bread) in AMX and AM samples, compared to A and AX which were decreased by 4.75 and 4.44 Log (CFU/ bread). In SGF (Simulated Gastric Fluid) and SIF (Simulated Intestinal Fluid) conditions, the AMX microcapsules provide the best probiotic protection among the four tested carriers. The combination of xanthan gum and maltodextrin in alginate matrix, eventually leading to having dual efficiency: First, xanthan gum would act as buffers that reduce acid activity; Second, maltodextrin acting as a protective agent of <i>L. acidophilus</i> against high temperature as well as potential prebiotic that improve the viability of probiotic.</p>

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Introduction

FAO/WHO defines probiotics as “Live microorganisms (bacteria or yeasts), which when ingested or locally applied in sufficient numbers confer one or more specified demonstrated health benefits for the host” (Hill et al., 2014). They contribute significantly to improve human health and safety such as improving digestion and nutrition, preventing the development of harmful organisms, preventing diarrhea, preventing or reducing food allergies, helping to create antibodies to improve immunity, treatment of infectious diseases (Hatakka et al., 2001). However, to ensure the benefits of probiotics, the number of probiotic bacteria must exceed 10⁶ CFU/mL at products’ consumption time (Agrawal, 2005).

Bread is the main food in many countries because it is a source of carbohydrates, proteins, vitamins and minerals. The development of bread products including both providing nutrition and bringing health benefits which are main concerns nowadays. The addition of probiotic to bread is considered an effective way to introduce these beneficial effects into human meals (Kailasapathy and

Chin, 2000). However, the processing process becomes a major barrier of adding probiotics. The effects of probiotics bacteria on food depend on the type of food, the dosage used, and the existence of air (Homayouni et al., 2007). Their viability must be ensured throughout the life of the product and in the digestion system (Kailasapathy and Chin, 2000).

Microencapsulation technology with calcium alginate proves the survival potential of probiotic. Alginate is a food additive, non-toxic, and it bonds with calcium chloride to fix and protect fundamental cells with low cost (Sultana et al., 2000). In contrast, the use of alginate is restricted due to instability in low pH conditions. The combination of alginate with prebiotic such as maltodextrin improves the viability of probiotics and microcapsules (Ding and Shah, 2007). Recent studies have also reported that xanthan gum combined with alginate improves probiotics protection in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) (Chen et al., 2017). However, the combination of these three compounds to protect probiotic bacteria during

bread production and preservation has not been published in previous studies. This study carried out experiments to evaluate the microbial performance, viability of microbial probiotics by carriers Alginate 2%, Alginate 2% + Maltodextrin 1%, Alginate 2% + Xanthan gum 0.1%, Alginate 2% + Maltodextrin 1% + Xanthan gum 0.1% and supplementation in bread with adverse factors which are baking temperature, storage, and survival of *L. acidophilus* *in vitro* and in gastric and bile salts. From these data, the best type of carrier is identified to supplement the probiotic microcapsules with bread.

Materials and methods

Microorganisms and Micro-Encapsulation

Lactobacillus acidophilus ATCC 4356 was obtained from stain collection of Faculty of food technology of the Ho Chi Minh University of food industry. *Lactobacillus acidophilus* was harvested from 100 ml of a 22-h culture (late log phase) by centrifugation at 5000 rpm. The cells were then washed twice and resuspended in 10 ml of sterile saline and used in the microencapsulation process.

Prepare Microcapsule Preparation and Test Encapsulation Yield

Microcapsule preparation was made by an emulsifying method which was described in the study by Sultana *et al.*, (2000) with slight modification. Briefly, 20ml of alginate 2% (A); alginate 2% + maltodextrin 1% (AM); alginate 2% + xanthan gum 0.1% (AX) and alginate 2% + Maltodextrin 1% + 0.1% xanthan gum (AMX) were mixed with 5 ml of *L. acidophilus*. The mixture was stirred by a magnetic stirrer and dispersed for 15 minutes in 100 ml of oil containing tween 80 1.5% (v/v) at a speed of 900 rotation per minutes. After 5 minutes, added slowly 150 mL of CaCl₂ 0.05 M solution to break the emulsion. Collect post-emulsion and preserve at 4°C to conduct experiments. Examined encapsulation yield in the microcapsule preparation and calculated according to the following formula:

$$\text{Encapsulation yield (\%)} = \frac{\sum \log \text{CFU}_{\text{after}}}{\sum \log \text{CFU}_{\text{before}}} \times 100\%$$

Prepare Bread, Check the Survival of Bacteria During Baking and Storage

The kernel of bread was prepared in the following main proportions: Wheat flour (10% w/v), fresh milk (40% v/v), egg yolk (10% w/v), sugar (22% w/v) with the proper ratio, then, cook it and leave it cool. Then, mix microcapsule preparation with the ratio of 4:1, and divide it into 5 grams pieces and storage at -18°C for 20 min. Then add to the prepared cake. The bread crust was prepared in the following main proportions: wheat flour (45% w/v), yeast (5% w/v), sugar (6% w/v), milk (20% v/v), eggs (5% w/v), butter (20% w/v)... mixed with water in appropriate proportion, divide into smaller pieces, each piece was 50 grams. After, incubated them at 32°C within 1 hour 30 minutes. Conduct baking at 185°C for 13 minutes. Bread was cooled down and stored at 4°C. Samples contain free cells as samples to confront. The viability of *L. acidophilus* was tested according to the following formula:

$$\text{Survival (\%)} = \frac{\sum \log \text{CFU}_{\text{after baking}}}{\sum \log \text{CFU}_{\text{before baking}}} \times 100\%$$

Bread samples were preserved at 4°C and tested for the viability of *L. acidophilus* were examined indirectly at time intervals of 0, 2, 4, 6, 8 days of storage.

Effects of Simulated Gastric Fluid (SGF) And Intestinal Fluid (SIF) On *L. Acidophilus* After 8 Days of Storage

Five grams of bread kernel after 8 days of storage were incubated in 45 ml of SGF solution (9 g/l NaCl + 3 g/l pepsin (Himedia) adjusted to pH 2.5 with 5N HCl), at 37°C for 120 minutes. After incubation in SGF, the samples were collected and transferred to 45 ml of SIF (simulated intestinal fluid) (0.85% NaCl, 0.3% bile salts, pH 6.5) incubated at 37°C for the next 3 hours. The viability of *L. acidophilus* from microspheres was immediately assayed by plating on MRS media.

Statistical Analysis

All the experiments were repeated three times, and the results were presented as average ± SD. Analysis of variance ANOVA (P<0.05) was used for the mean of average comparison. All statistical calculations are done using STATGRAPHICS software

Results and Discussion

Encapsulation Yield of Carrier Matrix with *L. acidophilus*

Encapsulation yield of *L. acidophilus* of carrier matrix was demonstrated in Fig. 1. The results indicated that the used carrier matrix brought effectiveness more (80%). Carriers that had the highest encapsulation yield was AMX which has the same result of carrier AX (P>0.05). The results were approximately 92.9% and 92.37%. The lower efficiency carriers were AM and A which was 86.7% and 86.32%.

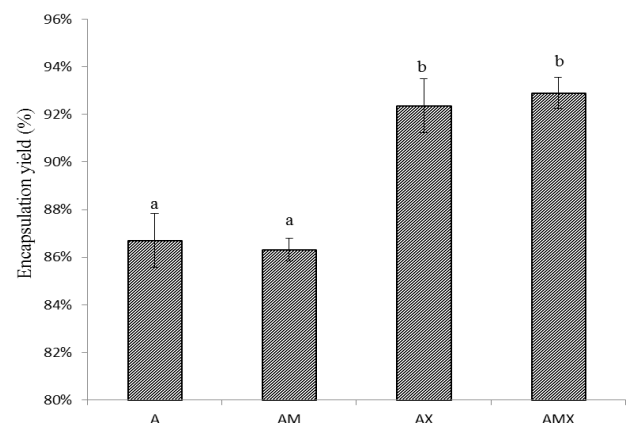


Fig 1. Encapsulation yield of carriers (A: alginate 2%; AM: alginate 2% + maltodextrin 1%; AX: alginate 2% + xanthan gum 1%; AMX: alginate 2% + maltodextrin 1% + xanthan gum 0,1%). ^{a-b} represents for the significant difference (P<0.05) in each experience.

Encapsulation yield of probiotics is necessary for considering in adding probiotic into bread products. The higher the yield, the higher the number of microorganisms in products, resulting in reducing the number of microcapsules needed to be added to the bread kernel, thereby reducing the sensory value of bread products with probiotic. Trabelsi et al. (2014) conducted a test on the encapsulation yield of *L. plantarum* with alginate at different concentrations (1%, 2% and 3% w/v) showed that 2% alginate concentration gave the best results (Trabelsi et al., 2014). The study by Fareez et al. (2015) showed that microparticles were emulsified with alginate carriers; Alginate - Xanthan gum; Alginate - Xanthan gum - Chitosan; Alginate - Chitosan, the result states that the encapsulation yield has the existence of alginate and xanthan gum achieve more than 90% (Fareez et al., 2015).

In the present study showed that the different carrier components significantly affected the encapsulation yield of the *L. acidophilus*, in which the complex microcapsule containing the xanthan gum component (AX and AMX) gave encapsulation yield higher than others in the same research ($P < 0.05$) (Fig 1). This suggests that xanthan gum contributes significantly to the encapsulation yield of the carrier that adds xanthan gum.

Survival Ability of *L. acidophilus* During Baking Process and Storage

The viability of *L. acidophilus* before and after baking was demonstrated in Fig. 2. The results showed that, after baking, the different carriers provided the ability to protect *L. acidophilus* under the effect of temperature was different. The viability of *L. acidophilus* in the AMX and the AM samples was equivalent ($P > 0.05$) of about 3.64 and 3.75 Log (CFU/bread), corresponding to 66.5% and 63.2%, AX and A had a greater loss of *L. acidophilus* during the baking, more than 4 Log (CFU/bread) (Fig. 2). The viability of *L. acidophilus* in the control sample had a significant loss, reaching 3.11 Log (CFU/bread) compared to 11.45 Log (CFU/bread) before baking, corresponding to the survival rate of only 32.90%, this was much lower than microcapsules, proving microencapsulation help to protect *L. acidophilus* against high temperature.

A study by Zhang et al. (2014) on the viability of *Bifidobacterium lactis* Bb12, proving that probiotics exist during baking at 205°C for 12 minutes (Zhang et al., 2014). This proves the feasibility of developing probiotic bread lines. In addition, during baking, the temperature inside biscuits are lower than the baking temperature, which increases the probiotic surviving inside biscuits (Reid et al., 2007). The study of Lieu et al. (2017) showed that maltodextrin provided the ability to protect *L. casei* against the high-temperature influence during the spray-drying process (Lieu et al., 2017). In the present study, the presence of maltodextrin in AMX and AM improved the ability of *L. acidophilus* to protect against the high temperatures of the baking process (Fig. 2). This suggested that maltodextrin is necessary to protect probiotic against the high-temperature.

The viability of *L. acidophilus* during 8 days of storage at 4°C was demonstrated in Fig 3. The results showed that the viability of *L. acidophilus* in AX, AM and AMX slightly increased in the first two days of storage which were 0.76; 0.56; 0.75 Log (CFU/bread) respectively and

then decreased in a small amount from day 2 to day 8 of the experiment. The control sample also recorded a slight increase from the first day was 3.11 Log (CFU/bread) and reach 4.02 Log (CFU/bread) after 8 days of storage. *L. acidophilus* concentration in carrier A decreased on the first day (6.33 Log (CFU/bread)) to the day 8 (5.8 Log (CFU/bread)).

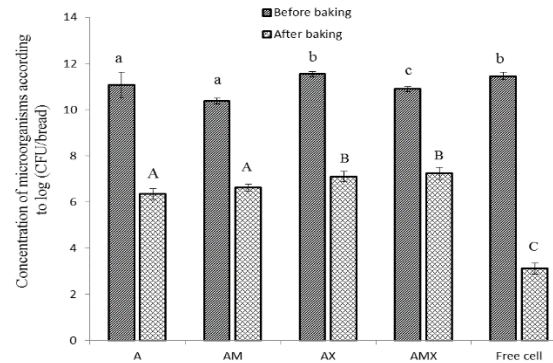


Fig 2. *Lactobacillus acidophilus* bacterial survival with high temperature (A: alginate 2%; AM: alginate 2% + maltodextrin 1%; AX: alginate 2% + xanthan gum 1%; AMX: alginate 2% + maltodextrin 1%+ xanthan gum 0,1%). a-d, A-C represents a significant difference ($P < 0.05$)

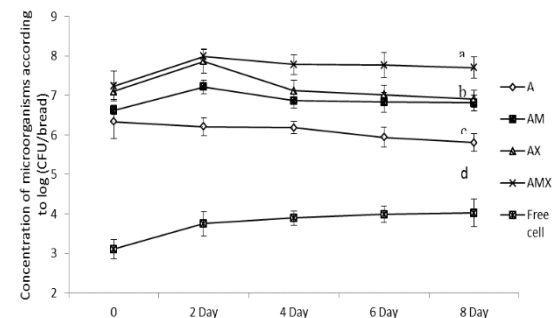


Fig 3. Survival of *L. acidophilus* during bread storage (A: alginate 2%; AM: alginate 2% + maltodextrin 1%; AX: alginate 2% + xanthan gum 1%; AMX: alginate 2% + maltodextrin 1% + xanthan gum 0.1%). a-d representing significant differences ($P < 0.05$) in each experiment.

The slight increase in *L. acidophilus* viability during the first two days of storage due to *L. acidophilus* after baking using the substrate in the bread for further growth, which produced probiotic higher than dying probiotic. This phenomenon leads to the increasing the amount of *L. acidophilus* in the first 2 days of storage. From day 2 onwards, probiotic was decreased the viability which would due to the storage condition influence.

The influence of encapsulated particles on the probiotic viability during storage have been reported in the previous study. The study of Trabelsi et al. (2014) indicated that the protective effect of alginate in combination with polymer compounds on the *L. plantarum* viability showed better than using alginate alone during 35 days of storage at 4°C (Trabelsi et al., 2014). The similar result was observed in the present study, the viability of *L. acidophilus* in the alginate carrier that uses alone had the lowest compared to others (Fig 3). Compared to the preservation of probiotic bread and conventional bread, these two types have similar storage times. However, with the added benefit of probiotic

for consumers, this product will have superiority over traditional bread products. In this study, different carriers will provide the ability to protect *L. acidophilus* bacteria under varying temperatures differently, both AM and AMX carriers providing the viability of *L. acidophilus* more than 80%. Both contain maltodextrin in their constituents.

The Survival of *L. acidophilus* in Simulated Gastric Fluid (SGF) and Intestinal Fluid (SIF)

The *L. acidophilus* viability in the SGF and SIF condition was demonstrated in Fig 4. After 2 hours of incubation in SGF, the viability of *L. acidophilus* in AMX and AX was reduced respectively from 7.70 and 6.92 Log (CFU/bread) to 3.42 and 2.57 Log (CFU/bread) which corresponds to 44.4% and 37.20% respectively. The viability of *L. acidophilus* was not found in A, AM and the control samples (Fig 4). Continuing testing in the environment of SIF for 3 hours, the viability of *L. acidophilus* in the AMX sample was decreased to 2.25 log (CFU/bread), whereas the *L. acidophilus* viability was not recorded in the AX sample.

Survival ability in SGF and SIF conditions is an important criterion for assessing the effectiveness of probiotics. The study by Fareez et al. (2015) states that the addition of xanthan to the carrier's constituents helped to improve the survival of *L. plantarum* LAB12 under SGF and SIF conditions. The interaction of xanthan gum and the acidic environment of gastric juices have explained the probiotic activity of xanthan gum in combination with alginate. The negative charge structure of Xanthan gum can bind to H⁺ ions, reducing the effect of an acidic environment on the metabolism of bacteria (Jiménez-Pranteda et al., 2012). In addition, maltodextrin would act as potential prebiotic to improve the viability of *L. casei* in SGF condition (Đông et al., 2015). Chen et al. (2017) indicated that xanthan gum was added to the carrier matrix, which leads to the structure of the particles decreases their porosity, as the ion exchange between xanthan gum and simulated intestinal fluid can reduce the porosity of the microencapsulated matrix (Chen et al., 2017). In the present study, xanthan gum in the alginate matrix helped to improve the probiotic viability and was more improved in the present of maltodextrin (Fig. 4).

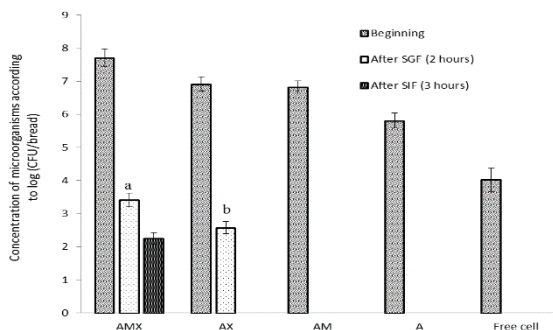


Fig 4. Survival of *L. acidophilus* in SGF and SIF after 8 days (A: alginate 2%; AM: alginate 2% + maltodextrin 1%; AX: alginate 2% + xanthan gum 1%; AMX: alginate 2% + maltodextrin 1% + xanthan gum 0.1%). a-b represents significant differences ($P < 0.05$) in each experiment.

The results obtained suggest that, xanthan gum and maltodextrin is necessary to improve the probiotic viability. The combination of xanthan gum and maltodextrin in alginate matrix, eventually leading to have dual efficiency: First, xanthan gum would act as buffers that reduce acid activity; Second, maltodextrin act as potential prebiotic that improve the viability of probiotic

Conclusion

Application of probiotic micro-encapsulation technology in bread production is an economic research. Selecting the carrier has important meaning in increasing the survival rate of probiotic bacteria in processed, preserved and in the digestive environment. The results indicated that the addition of probiotics to bread was highly feasible. In the tested carrier agents, the best encapsulation yield was achieved with two carrier matrixes were AMX and AX (92.9% and 92.37%), which contained xanthan gum in the carrier matrix. Maltodextrin acting as protective agent of *L. acidophilus* against high temperature, the viability of *L. acidophilus* was improved in the alginate matrix that contains maltodextrin compared to AX and A when baking at 185°C for 13 minutes. The combination of maltodextrin and xanthan gum in the alginate matrix provides the best survivability during storage (7.7 Log CFU/bread), as well as in simulated gastric fluid (SGF) and intestinal fluid (SIF) compared with the three systems carrier A, AM, AX. Xanthan gum shows its potential in protecting *L. acidophilus* in simulated gastric fluid (SGF) and intestinal fluid (SIF). Maltodextrin demonstrates the probiotic benefits of limiting temperature effects during processing. The combination of several carriers in microcapsules helps to take advantage of individual carriers to improve probiotic protection. Production of probiotic bread with AMX carrier gave the best protection against *L. acidophilus* compared to the other carriers

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