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Stabilizing Technology of Lactobacillus with Double Matrix Capsulation

Dae-Seop Kim[1*], In-Young Kim[2†], Mi-Ey Park[1], and So-Yeun Yoo[1]

[1] Dept. of natural science, Hankuk Academy of Forign Studies, Wangsan-ri, Yongin-si,Gyeonggi-do, 449-854 Republic of Korea
[2†] Dept. of material study, Research & Development Center, Biobeautech Co., Ltd.,Sagimakgol, Junwon-gu, Seongnam-si, Gyeonggi-do, 462-721 Republic of Korea
(Corresponding author: Dae-Seop Kim[*1], email: danny960404@naver.com,
In-Young Kim[2†], email: bbtrd1@biobeautech.com)

Abstract: This study is to develop the double capsulation technology in order to increase the conservativeness and stability of lactobacillus, to be deliciously flavored and give it visual differential effect in food industries, were described. As the first capsulation with o/w (oil-in-water) emulsifying system, our study group was especially made to soft and moisture cream using 5wt% of sucrose ester emulsifier as first capsulation. Nutrient agents are squalane, camellia oil. Double matrix capsulation was formed with the best stabilized bead type capsules when it blended 1 : 3 ratio of chitosan and sodium alginate. The bead diameter size was about 2.0~4.5mm (mean diameter: 3.2mm). Activity of lactobacillus containing cream for depending on various pH variations showed that alkalinity (pH=10.8±0.5) condition was higher than acidity (pH=4.2±0.2) and neutrality (pH=7.1±0.3) conditions. After a month, it also was certified to the activity of lactobacillus in incubated at 37±1°C in culture medium. As application of food industry, we developed the containing lactobacillus capsule and 4 colored kinds of double matrix capsulation in yogurt cream. As for above mentioned those results, one of tool to stabilize the living lactobacillus, doubled matrix capsulation greatly be expected to contribute to food industry. Furthermore, it can be expected to apply the drug delivery system (DDS) to active ingredients of stabilizing technologies at drug, pharmaceutical division and cosmetic industry, etc.

Keywords: Lactobacillus, capsule, stability, fermentation, food industry

1 Introduction

What is the lactobacillus? The other expression is called as lactic acid bacteria [1, 2]. Lactobacillus is achieved to a large amount of energy by fermenting natural sugars to generate a lactic acid bacteria will be hereinafter referred to as lactobacillus is just called (**Fig. 1**) [3-5]. **Fig. 1** shows various functions of lactobacillus [2]. Fig. 1 (A) is lactic acid bacteria. Fig. 1 (B) is lactobacillus po**w**der which is Probiotics [1, 4]. There are a lot of activities such as immune system activation, synthesis of vitamins, healthy

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body, pH control activity, metabolism of blood circulation, and development of mucosal barrier, etc [5-7]. You can eat the best food in the world, but if your body can't digest and absorb it there's no nutritional value [1, 3, 8]. As mentioned before, lactobacillus and digestive enzymes help with digestion and absorption, two of the most critical priorities of the human body [9, 10]. Not only do lactobicillus help with the absorption of nutrients, they actually produce vitamins themselves. Many of the important B vitamins, vitamin A and vitamin K are manufactured in our intestines by the little buggers. Lactobacillus may also help make you stronger by breaking down proteins into amino acids to be used to build muscle and other tissues [5, 7]. Generally one of the specious lactobacillus such as *lactococcus*, *leuconostoc*, *pediococcus*, *bifidobacterium* is involved in bacteria lactobacillus. Definition of lactobacillus is morphologically divided as a micrococcus such as *lactococcus*, *pediococcus*, *leuconostoc* and bacillus such as *lactobacillus*, *bifidobacterim*, *stainability*, these specious is gram positive [11]. Lactobacillus is well produced various lactic acid from fermenting sugar although where is less oxygen [12-15]. Acid-resistant characteristics indicate a very complex nutritional need, various kinds of amino acids, sugars and vitamins in addition to the demand. Species, depending on the strain growth micronutrients may not be possible, and do not be. Meanwhile, yogurt of the food industry, there are many products that contain a variety of lactic acid bacteria being released [16]. However, dairy products have disadvantages due to the perishable, sweet and sour all products were bitters. Dairy products are generally divided into eating and drinking types. These products are guaranteed to period for shelf life during sealed package. These products are not good for long-term preservation once you open the lid, and the taste is easily turned into long-term storage [17].

Fig. 1. Various functions of lactobacillus (A): lactic acid bacteria, (B): lactobacillus powder. Activities are as follows such as immune system activation, synthesis of vitamins, healthy body, pH control, metabolism of blood circulation, and development of mucosal barrier.

Optimum ways to increase the shelf life of lactobacillus, they have been studied in mono-capsulation method and doubled capsulation method until nowadays. But these

technologies are not easy to find industrialized process in food industry [15]. Depending on the type of the normal lactobacillus fermentation (homo) and hetero fermentation, are divided by lactic acid fermentation [2,5,8-10]. Lactobacillus widely distributed in nature such as food, farm, human, and animal body. However, the development of lactococcus 45°C at 10°C, the optimum temperature is 30°C without growth before and after the entry into force of normal aureus. There are many species are used in food processing as dairy starter (starter). Fermentation with Pediococcus leuconostoc most bacteria, is independent of the animal's biological [17],. Streptococcal fermentation of Leuconostoc party is over degradation, growth, growth by pH and can be classified into four types of species [18].

In this study, we tried to study regarding the new method maintaining stability of lactobacillus with improving chewy texture, to make the long-term preservation through the doubled encapsulation. Firstly, as the first capsule, we tried to develop the incorporated in nutritive cream containing lactobacillus, then we make a doubled matrix capsulation using chitosan and sodium alginate natural polymers in order to increase the long-term stability even though room temperature. To make a doubled matrix capsulation, depend on various pH changes, was examined. And also, the various encapsulation of a variety of colors as the application were carried out. Therefore, this study can be contributed to the development of new tools applying advanced unique stabilizing technique with drug delivery system (DDS) in food and pharmaceutical industries.

2 Experimental and Method

2.1 Materials

In this experiment the main component is used in the Lactobacillus (Probiotics, Sigma Aldrich) as reagent grade, which is contained in the sucrose raw material for making the ester-type cream sucrose esters (Sigma Aldrich), Camellia oil (Biobeautech Co., Ltd., Korea) originated from Jeju of Korea, squalane (Kishimoto, Japan), tocopherol (Sigma Aldrich), stearic acid (Sigma Aldrich) was used as a food grade. In addition, in order to make the double capsule of chitosan matrix, the raw materials were used the chitosan and sodium alginate by Sigma Aldrich Corporation.

2.2 Equipments

As the equipments are used to as follows, Homomixer (Hanyang industry, Korea), an optical microscope (Olympus, Japan) with CCD camera, and a particle size distribution measuring device of laser light scattering system (Melvern UK, Model Mastersizer 2000), Matrix Capsule Machine (Biobeautech Co., Ltd., Korea).

Table 1. Formula of 1st Capsule Sucrose Ester Cream with Lactobacillus Powder; Formation of Soft Creamy Emulsion

Phase	Ingredients	Formula (wt%)
A	Sucrose Fatty Ester	5.0
	Stearic Acid	3.0
	Camellia Japonica Seed Oil	5.0
	Squalane	2.0
	Tocopherol	0.5
B	Glycerin Chitosan (5% Solution) Sodium	10.0
	Alginate (5% Solution) D.I. Water	10.0
		10.0
		49.5
C	Lactobacillus Powder	5.0
	Total	100.0

2.3 Preparing Method of the 1st Capsule

To make the first capsule Sucrose ester cream was described in Table 1. The composition of the cream containing lactobacillus containing 5wt% of a sucrose ester, 3wt% of stearic acid, 5wt% of camellia oil, 2wt% of squalane, 0.5wt% of the tocopherol was used, and also, to make a matrix capsule applied the glycerol, chitosan, sodium alginate as Table 1.

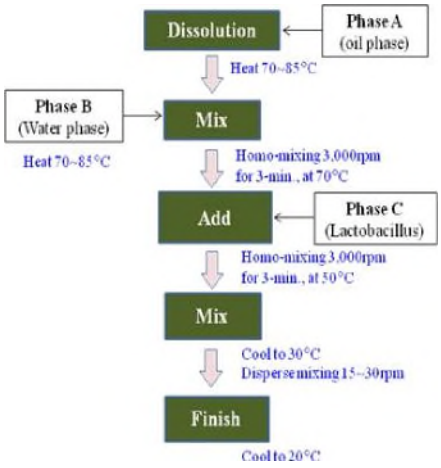

Fig. 2. Preparing method of 1st capsule oil in water type cream with sucrose fatty acid ester containing lactobacillus powder.

Method for manufacturing a raw material as shown in Fig. 2, separated by exactly weighing After A phase was warmed to 70~85 °C dissolved. Here a mixture of phase B 3,000 rpm using a homo-mixer for 3 minutes after stirring, phase C, and it 1 min stirring, 25°C cooled to complete the manufacture. This is encapsulated in the primary, so that the matrix was well encapsulated.

2.4 Preparing Method of the 2nd Matrix Capsule

Double matrix capsules were prepared by the following procedure. The composition of Table 1 was prepared as a cream containing lactobacillus that passes through a device, such as Fig. 3 and was able to make a stable matrix capsules. Fig. 3 of primary encapsulated in a soft cream, put the device moved to a closed vessel, and then, injected air into the low pressure through a small nozzle with a needle to fall drop by drop through. At this time, the prepared solution of 1% of calcium, a 5% of ethanol solution dropped on a hard capsule is formed.

2.5 Measuring method of Stability of Lactobacillus

In order to measure the stability of lactobacillus, 50-time diluted solution of lactobacillus as the control, and have a dual matrix encapsulating the sample loaded on an agar medium, put in a 37°C incubator and related to whether the living lactic acid bacteria was confirmed by microscope analysis.

3 Results and Discussion

3.1 Merits of Double Matrix Capsule

There are various merits and benefits through stabilizing system of double matrix capsule of lactobacillus. Applying chitosan and sodium alginate were merit that is possible to eat because of natural food grade. Chitosan is good stable although it is acidic and alkaline conditions. Chitosan has known that is a good safety, stable material which has excellent in moisture-retaining effect and excellent anti-oxidative activity. As the physical properties of chitosan, they are generally insoluble in water. But it can be dissolved maintaining above pH=10.8 in citric acid solution, acetic acid solution, hydrochloric acid solution of small content due to form a salt.

Fig. 3. Pictures of preparing equipment; (a) manufacturing equipment, (b) magnified needles (diameter: 0.5mm)

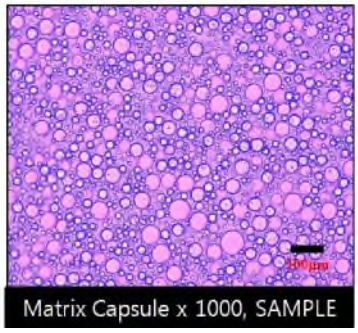

Fig. 4. Microscope of 1st sucrose ester cream in corporate with lactobacillus; observed magnification x 1,000 times, photo-light microscope.

Chitosan is formed with gelation at alkali condition (above pH10.80±0.5) as well as is formed with the gel with polymer. In addition, sodium alginate extracted from the natural algae, it is also water-insoluble material but it can be formed the gel due to absorb in water. Generally, alginic acid is used to be applied as gelling agent due to condense ethanol and Na, K, Ca is substituted to give a water-soluble. In this study, we used with sodium alginate. The optimum condition of chitosan and sodium alginate is 1: 3 (mixed ratio). The alginate and chitosan were mixed in an optimized ratio of 1:3, 0.8~1.0wt% of those materials should be dissolved, finally we could manufactured the stabled double matrix capsule. The matrix film at low concentration was not formed with capsule due to not stable. And also, at high concentration was too hard formed the stiff capsule due to making a hard gelation. In case of hard gel could not pass through inside nozzle. Further, surface of the capsule had smooth feel. The particle shape was a round-shaped granule. The biggest reason for using two mixtures because they have various activities having anti-bacterial effect, we fixed two compounds. So it can stably preserve without any preservatives for long-term.

3.2 Physical properties of sucrose ester cream

Table 1 showed composition of the primary cream emulsion using sucrose ester emulsifier (Fig. 4). The appearance was whitish color. Texture was softness and moisture feel having viscous gel. The pH of the cream (diluted 10% water solution) was 5.8 measured by pH meter. The viscosity of soft cream was 7,000~15,000 cps. Specific gravity with Brookfield viscometer was 0.95 to 1.05 (spindle number: No.4, for 30rpm, for 1min). Fig. 4 shows the picture observed by optical microscopy. The particle size of the cream was about 5~50μm (Fig. 4). Average particle size was about 20m in stable condition could know that. The specific gravity was 0.95~1.05 in water and had a slightly lower specific gravity. The taste was a little sour without any additional stabilizer, it was used to create a secondary matrix capsules.

3.3 2nd matrix capsulation

Preparing method of double matrix capsule was carried by the apparatus shown in Fig. 3. In order to make a capsule applied our specific order made device from Biobeautech co., ltd. Fig. 3 (A) is a picture of the capsule device. The needles were specifically produced in order to get look good shape and various color. Fig. 3 (B) showed a small diameter needle. First, the size of millet circular in order to make the beautiful capsule, the inner diameter of the needle diameter was produced about 0.5mm size. In order to form a stable double matrix membrane capsule, we made 500mL with containing a 1% solution of calcium chloride, a 5% ethanol solution and sodium citrate. The pH was pH=10.8 ± 0.3.

Fig. 5. Various capsulations of four colored matrix capsulations incorporated with lactobacillus with chitosan and alginate. (A): white color, (B): green color, (C): red color, (D): Wellow color.

In order to make the cream, and the contents were placed in vessel. Manufacturing methods are explained as follows. Cream is filled by injecting the air pressure in the vessel, the contents are discharged through a syringe needle. Dropping the prepared solution containing lactobacillus capsule was formed a hard and stable.

This is taken out, washed 2-3 times with clear water out of the capsule was able to make a clean grain. This capsule is shown in Fig. 5. As shown in the figure, the size is made from millet shape, the size of the capsule was formed with a particle size of 3mm diameter. In order to improve the capsule, put food coloring in various colors improved. Fig. 5 (A) of the capsule was white. Fig. 5 (B) was green. Fig. 5 (C) was encapsulated in the red. Fig. 5 (D) was able to make capsules of the yellow.

The capsules were contained in all of lactic acid bacteria. The freeze-dried lactic acid bacteria was Probiotics that cream was placed in a 20-time dilution. Chitosan and alginate in a mixture in a ratio of 1:3 was dissolved in 0.8 ~ 1.0wt% as the capsule is made from what most stable. The surface of the capsule was also smooth state, the round shape of the particle, respectively. This plain yogurt, bread, etc., or by mixing with drinking water used, the more unique formulation of functional foods expected to be developed.

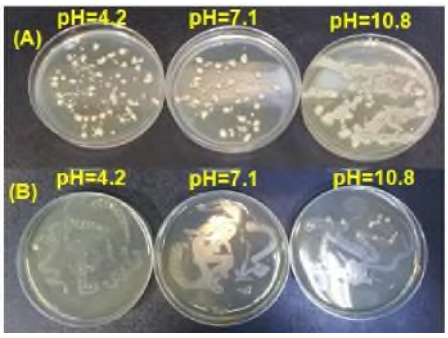

Fig. 6. Activity of lactobacillus compared control with doubled matrix capsulation in agar badge incubated at 37°C. (A): Doubled matrix capsule storage for one month in refrigerator (at 4°C), (B) Control group storage for one month in refrigerator (at 4°C).

3.4 Activity of lactobacillus depend on pH change

If the capsule is made with lactic acid to the matrix, was tested whether the stably retained. The 1st sucrose type cream to regulate the pH, the acid area is controlled to use citric acid solution. Adjusting the alkalinity of the sodium citrate solution, cream was produced. Thus the activity of the lactic acid bacteria was confirmed. The three kinds of samples were pH=4.2±0.3, pH=7.1±0.2, pH=10.8±0.3 whish are adjusted to make pH conditions from acid to alkalinity.

Long-term storage if the activity of the living lactobacillus for the test results were shown in Fig. 6(A). First, the activity of the initial state is not specifically indicated. Both control and doubled matrix capsule group, the activity of lactic acid bacteria to obtain very good results. On the other hand, keeping them for 1 month in the refrigerator, the same sample by using a sample, the active was observed. As shown in Fig. 6(B), the control medium is a non-encapsulated acidic, neutral, basic, but was not very high, the activity of lactic acid bacteria. In particular, we know that they had high activity when these capsules were stabilized at acidic or alkaline condition.

3.5 Application of drug delivery system

Based on the results obtained through the study, the stabilization of a lactic acid bacterium which is used as a new tool is expected to be possible. Matrix capsules using equipment that can make the purpose, in accordance with the size of the capsule can be variously adjusted. This technology has a variety of applications in different industries can be expected. In addition, the lactic acid bacteria against which absorbs most of the above, if the results are used in the above section, some degradation can reach up to the new capsule is expected to be developed. Among them, Fig. 7 is shown, yogurt, put in a capsule looks nice, you can preserve the taste, formulated examples demonstrated that it is possible.

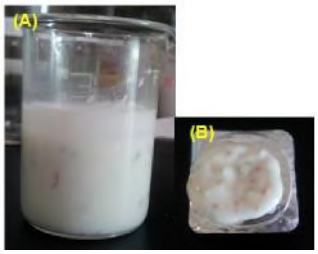

Fig. 7. Application of yogurt containing doubled matrix capsules in food industry.

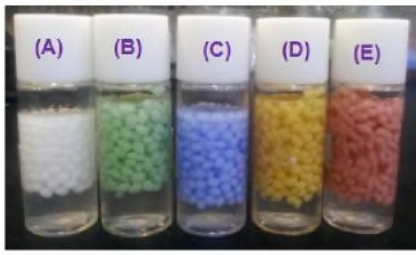

Fig. 8. Various doubled matrix capsulations containing active ingredients; (A) insulin, (B) alpha-tocopherol, (C) squalene, (D) Korean ginseng extract, (E) alpha lipoic acid

Also, Fig. 8 is applied to a double matrix of five kinds of health food in a variety of known active ingredients for use according to the application that it was prepared

by coating five colors. These are shown in Fig. 7 to put the capsule in yogurt and delicious preserve is that it is possible for example in the proposed formulation. Fig. 8 is also applied to the double matrix capsule of five types of functional health food containing active ingredients. The five colors were produced by coating capsules. (A) of Fig. 8 is insulin, (B) is alpha-tocopherol, (C) is a squalane, (D) is the ginseng extract originated from Korea, (E) is alpha-lipoic acid respectively. For the stability of the active ingredient of these, rather than not encapsulated discoloration as well as odor was significantly improved stability. This double encapsulation removed by contact with air and therefore, would be evidence that the stability of the capsule could be investigated.

4 Conclusion

In this study, lactobacillus was used in food preservation and stability to enhance the flavor and visual way to differentiate and give way to a double matrix, suggesting encapsulation. First, the primary method of encapsulating 5wt% of sucrose ester using the cream was able to make soft and moist. Second, the secondary matrix Capsule chitosan and alginate is a mixture in a ratio of 1:3 most stable when encapsulated in the capsule is formed. The size of the capsule matrix 3mm to form a bead surface is smooth and could make solid. Third, changes in the activity of lactic acid bacteria is acidic pH than in alkaline showed a more excellent activity, even after one month of live lactic acid bacteria was confirmed activity. Fourth, the food industry in the application of yogurt containing capsule formulations coated with 5 different colors to introduce the capsule matrix, can be utilized in various fields ever presented. These results suggested that, in the method for stabilizing Lactobacillus as a tool, double matrix capsule is expected to greatly contribute to the food industry, pharmaceutical industry, furthermore through the encapsulation of insulin drug delivery systems (DDS), and the active ingredients in the cosmetic industry in the application of this stabilization technique are expected to be possible.

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