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Stabilizing Biotechnology of Double Matrix Capsule Containing Lactobacillus Bacteria in Healthy Food Industry

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

[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, 462-807 Republic of Korea
(Corresponding author: Dae-Seop Kim[1], email: danny960404@naver.com, In-Young Kim[2†], email: bbtrd1@biobeautech.com & iykim200@naver.com)*

Abstract

This study is to develop the double matrix capsulation biotechnology in order to increase the conservativeness and stability of lactobacillus bacteria, to be deliciously flavored and give it visually differential effect in healthy food industries, were described. In order to eat easy for wellbeing life science, 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 the first capsule containing lactobacillus bacteria. Nutrient agents were used with botanical squalane, camellia japonica seed 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 2.0~5.0mm (mean diameter: 3.8mm). Activity of lactobacillus bacteria containing sweet cream for depending on various pH variations showed that alkalinity (pH=10.8±0.8) condition was higher than acidity (pH=4.2±0.3) and neutrality (pH=713±0.2) conditions. After a month, it also was certified to the activity of lactobacillus bacteria in incubated at 37±3℃ in culture medium. As application of food industry, we developed the containing lactobacillus bacteria capsule and 4 colored kinds of double matrix capsulation in commercial yogurt cream. As for above mentioned those of results, one of tool to stabilize the living lactobacillus bacteria, doubled matrix capsulation greatly be expected to contribute to food industry. Furthermore, it can be expected to apply the drug delivery system (DDS) having good healthy food with active ingredients of stabilizing technologies at drugs, pharmaceutical divisions and skin care cosmetic industry.

Keywords: *Well-being, lactobacillus bacteria, capsule, stability, fermentation, food industry*

1. Introduction

In recent years, as the aging society continues to be settled well-being culture. Moreover, good health, increase the digestibility, nutrient supply easily absorbed in the human body, in order to have it fermented food containing lactobacillus bacteria as well as yeast being developed. Health foods in Korea are popular with fermented product following four types they introduce as Figure 1.

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Figure 1. Major fermented healthy foods of Korea with lactobacillus bacteria (a): Kimchi (fermented vegetable food), (b): Makkori (fermented rice alcohol), (c) Yogurt (Fermented milk), and (d) Meju (dried soybean during fermented)

Figure 1 shows that is major fermented healthy foods of Korea with lactobacillus bacteria (a): Kimchi (fermented vegetable food), (b): Makkori (fermented rice alcohol), (c) Yogurt (Fermented milk), and (d) Meju (dried soybean during fermented). These products have well known in the worldwide as healthy food for body. Those of major products were contained with fermented healthy food due to lactobacillus bacteria having nutrient, anti-aging efficacy. In particular, these products are not only in Korea but also in China, Japan, U.S., EU, including in the health food world is gaining popularity.

What is the lactobacillus? The other expression is called as lactic acid bacteria [1, 2]. Lactobacillus bacteria is achieved to a large amount of energy by fermenting natural sugars to generate a lactic acid bacteria will be herein after referred to as lactobacillus is just called (Figure 2) [3-5].

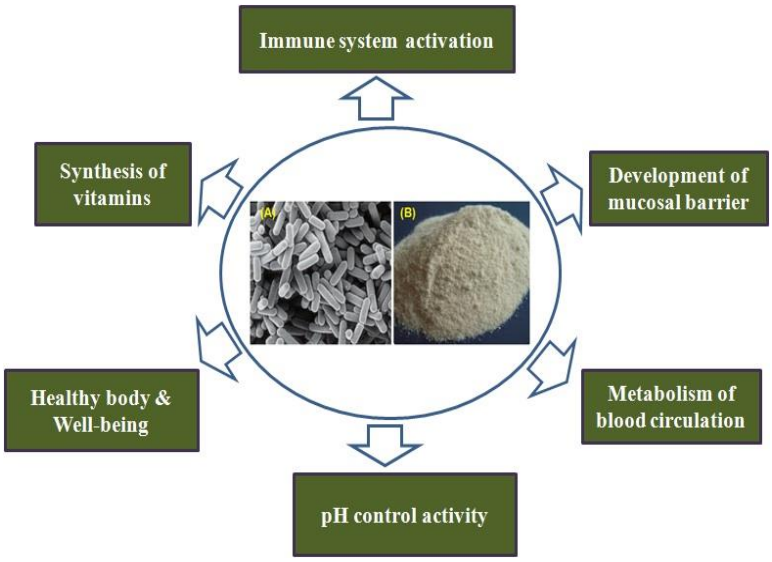

Figure 2. Six efficacy functions of lactobacillus bacteria (A): origin of lactobacillus bacteria, (B): lactobacillus bacteria powder. Nutrient activities are as follows such as immune system action, synthesis of vitamins in the body, to make healthy body, pH controlling effect, metabolism effect of blood circulation, and strengthening mucosal barrier

Figure 2 shows various functions of lactobacillus [2]. Figure 2(A) is lactic acid bacteria. Figure 2(B) is lactobacillus powder which is Probiotics of commercial grade [1, 4]. Lactobacillus bacteria, there are a lots of activities such as immune system activation,

synthesis of vitamins in body, to make healthy body, pH controlling activity, metabolism of blood circulation, and strengthening mucosal barrier, *etc.* [5-7]. You can eat the best food in the body, but if your body can't digest and absorb it there is no nutritional value [1, 3, 8]. As mentioned before, lactobacillus bacteria and digestive enzymes help with digestion and absorption, two of the most critical priorities of the human body [9, 10]. Not only to do lactobacillus help with the absorption of nutrients, actually produce the vitamins themselves. Many of the important vitamin B, 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 bacteria 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 bacteria 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 action, all products were bitters such as sweet and sour. 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]. 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 our 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, cosmetic and pharmaceutical industries. Especially we expect that triple matrix capsule collaborated cubosome containing active ingredients to deep penetrate in skin care cosmetics in the future.

2. Experimental and Method

2.1 Materials

In this experiment the main component is used in the Lactobacillus bacteria (Probiotics, Sigma Aldrich) as reagent grade, which is contained in the sucrose ester raw material for making the ester-type cream sucrose esters (Sigma Aldrich), Camellia japonica seed oil (Biobeautech Co., Ltd., Korea) originated from Jeju of Korea, botanical squalane (Neossance, USA), tocopherol (Sigma Aldrich), stearic acid (Sigma Aldrich) was used as a food grade. In addition, in order to make the double capsule matrix, the raw materials were used the chitosan and sodium alginate by Sigma Aldrich Corporation. All ingredients are used with food grade without any purification.

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 especially produced form R&D center of Biobeautech Co., Ltd. of South Korea.

2.3 Preparing Method of the 1st Capsule

To make the first capsule sucrose ester cream with lactobacillus bacteria, it was described in Table 1. The composition of the cream containing lactobacillus bacteria using 5wt% of a sucrose ester as an emulsifier, 3wt% of stearic acid, 5wt% of camellia japonica seed oil, 2wt% of botanical squalane, 0.5wt% of the tocopherol was used. And also, to make a doubled matrix capsule applied the glycerol, chitosan, sodium alginate as Table 1.

Table 1. Composition of 1st Capsule Sucrose Ester O/W (Oil-in-Water) Emulsion Cream with Lactobacillus Powder; Type of Soft Cream

Phase	Ingredients	Formula (wt%)	Function
A	Sucrose Fatty Ester	5.0	Emulsifier
	Stearic Acid	3.0	Emollient
	Camellia Japonica Seed Oil	5.0	Emollient
	Botanical squalane	2.0	Emollient
	Tocopherol	0.5	Anti-oxidant
B	Glycerin	10.0	Moisturizer
	Chitosan (5% Solution)	10.0	Matrix polymer
	Sodium Alginate (5% Solution)	10.0	Matrix polymer
	D.I. Water	49.5	Solvent
C	Lactobacillus Bacteria Powder	5.0	Active ingredient
	Total	100.0	-

Method for manufacturing a raw material as shown in Figure 3, as Table 1, it separated by exactly weighing, after phase A was warmed to 70~85°C dissolving. Put here a mixture of phase B as 3,000 rpm using a homo-mixer for 3 minutes after stirring, add phase C at 50°C, and it stirs for 1 min, homo-mixing 3,000rpm, until to 25°C cooled to complete the manufacture. This is encapsulated in the primary, so that the double matrix of very good stability and safety was well encapsulated.

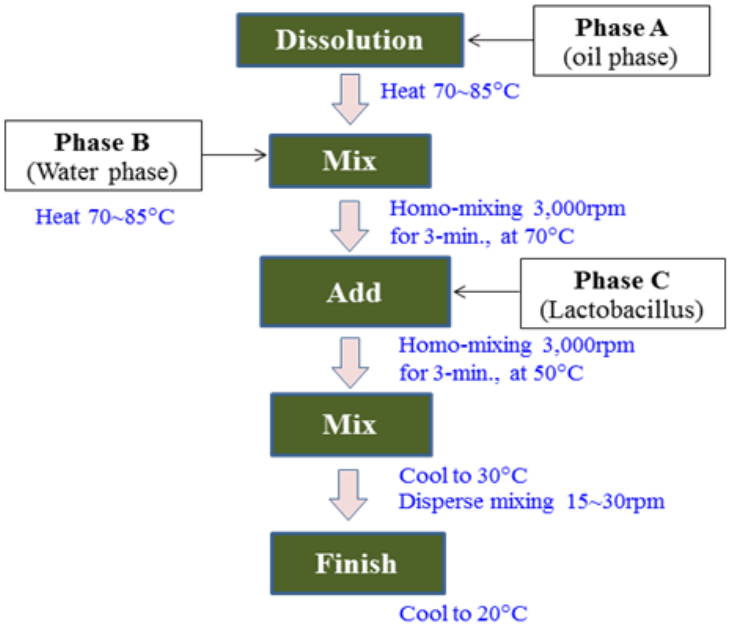

Figure 3. Preparing method of 1st capsule O/W (oil-in-water) type soft cream with sucrose fatty acid ester containing lactobacillus bacteria powder

2.4 Preparing Method of the 2nd Matrix Capsule

Double matrix capsules were prepared by the following procedure as Figure 4. The composition of Table 1 was prepared as a cream containing lactobacillus bacteria. In order to make doubled matrix capsule, specific formula showed as Table 2. First of all, we prepared the 100 grams of 1st matrix capsule soft cream, after to make a hard matrix gelling balls it prepared the calcium chloride 5.0g solution containing 10 g of ethanol with 300 g of D. I. water. Preparing procedure of double matrix capsule showed as follows Figure 4. 1st capsule soft cream put in the reservoir. Then inject the air in tank using air compressor. Pass through the nozzle. Finally we could make the double matrix capsules due to exchange from sodium alginate to calcium chloride ion each other. And also, chitosan matrix pH change from alkalinity (pH=10.7±0.7) to acid (pH=4.5±0.5).

Table 2. Double Matrix Capsule Composition to Make Small Size Balls

Part	Samples	Weight (g)	Remarks
A	1st Capsule O/W Emulsion Soft Cream	100.0	1st capsule with lactobacillus
B	Calcium Chloride	5.0	Metal
	Ethanol	10.0	Solvent
	D.I. Water	300.0	Solvent

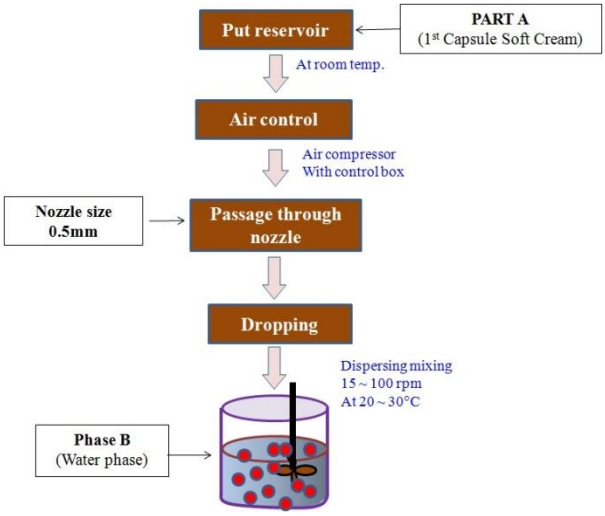

Figure 4. Preparing method of double matrix capsule containing lactobacillus bacteria powder as visible size about 0.5~ 5mm

The capsule device as Figure 5 and was able to easy make a stable doubled matrix capsules. Figure 5 of primary encapsulated in a soft cream, then put the device moved to a closed vessel, and then, injected air into the low pressure (1~5Kg) through a small nozzle (diameter size = 0.5mm) with a needle to fall drop by drop through capsule machine. We could achieve the hard gelling small balls whose size was about 0.5mm~ 5.0mm depend on the nozzle size.

2.5 Measuring Method of Stability of Lactobacillus Bacteria

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.

Figure 5. Picture of preparing equipment; (A) manufacturing equipment, (B) magnified needles (diameter: 0.5mm)

3. Results and Discussion

3.1 Merits of Double Matrix Capsule

In term of double matrix capsule, there are various merits and benefits through stabilizing system of double matrix capsule of lactobacillus. Applying chitosan and sodium alginate, they have 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.

Chitosan is formed with gelation at alkali condition (above pH=10.80±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 and Particle Analysis of Sucrose Ester Cream

3.2.1 Results of Physical Properties

Table 1 showed composition of the primary cream emulsion using sucrose ester emulsifier. 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 with Brookfield viscometer (spindle number: No.4, for 30rpm, for 1min) was 5,000~20,000 cps. The specific gravity was 0.95~1.05 in water and had a slightly lower specific gravity.

3.2.2 Observation of Particle Size using Microscopic Analysis

Particle size of 1st capsule soft cream was measured with microscopic analysis set-up the polarized light microscope. Figure 6 showed the two pictures observed by optical and polarized light microscopy. The particle size of the cream was about 1~90μm (Figure 6 (a), mag. x 1000). Average particle size was about 20m in stable condition could know that. Figure 6 (b) showed that droplets were formed oil-in-water structure with lactobacillus bacteria. Around on the droplet was formed with multi-lamellar structure due to use sucrose diester surfactant. The taste was a little sour. Cream was stabilized at room temperature and at 45°C incubating condition without any additional stabilizer. So, we can expect that its cream is possible to make double matrix capsule.

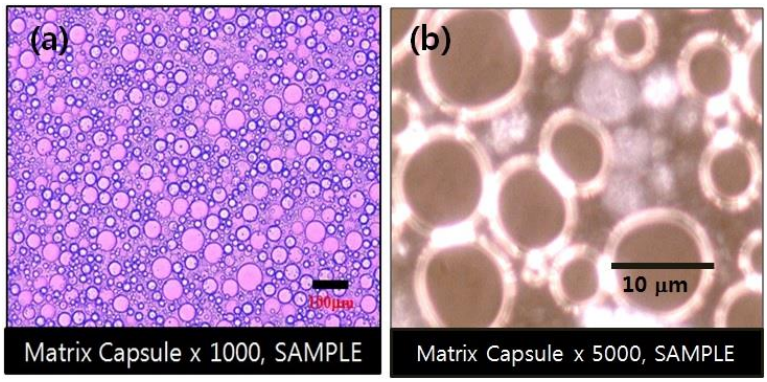

Figure 6. Microscope of 1st sucrose ester cream in corporate with lactobacillus; observed magnification x 1,000 times, photo-light microscope

3.3 Development of Double Matrix Capsulation

Preparing method of double matrix capsule was carried by the apparatus shown in Figure 5. In order to make a capsule applied our specific order made device from Biobeautech co., Ltd. Figure 5(A) is a picture of the capsule device. The needles (Figure 5(B)) were specifically produced in order to get look good shape and various color. Figure 5(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 had to make 500mL with containing a 1% solution of calcium chloride, a 5% ethanol solution and sodium citrate. The pH was pH=10.8 ± 0.3. 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 Figure 7. 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. Figure 7(A) of the capsule was white. Figure 7(B) was green. Figure 7(C) was encapsulated in the red. Figure 7(D) was able to make capsules of the yellow.

Figure 7. Various capsulations of four colored matrix capsulations incorporated with lactobacillus with chitosan and alginate. (A): white color, (B): green color, (C): red color, (D): yellow color

The capsules were contained in all of lactic acid bacteria. The freeze-dried lactic acid bacteria was Probiotics of commercial grade that cream was placed in a 20-time dilution.

Chitosan and sodium 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.

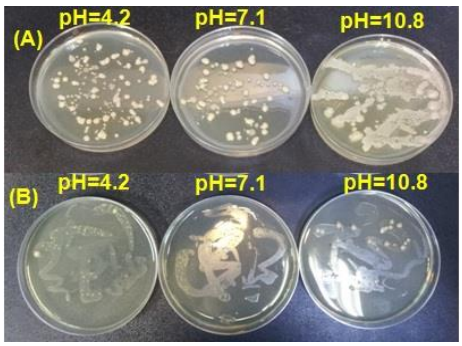

Figure 8. Activity of lactobacillus compared control with doubled matrix capsulation in agar badge incubated at 37°C. (A): Doubled matrix capsule storage for one month in refrigerator (at 4°C), (B) Control group storage for one month in refrigerator (at 4°C)

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 Figure 8(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 Figure 8(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, Figure 9 is shown, yogurt type, put in 4 kinds of capsules such as white, green, red and yellow color containing 1% respectively. Appearance was looks nice, you can preserve the taste for long-term priod, formulated examples demonstrated that it is possible.

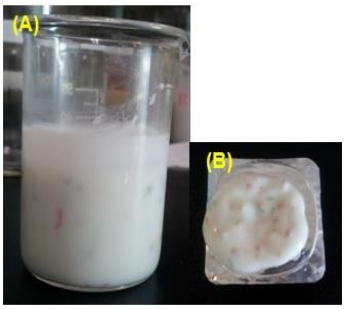

Figure 9. Application of yogurt type containing doubled matrix capsules in food industry. Useage of double matrix capsule: white, green, red and yellow color: containing 1% respectively

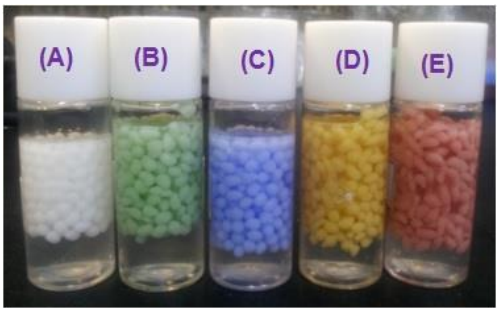

Fig. 10. Various doubled matrix capsulations containing active ingredients; (A) insulin, (B) alpha-tocopherol, (C) idebenone, (D) resveratrol, (E) alpha lipoic acid

Also, Figure 10 is applied to a double matrix of five kinds of health food in a variety of known active ingredients depend on use purpose according to the application that it was prepared by coating five colors. These are shown in Figure 10 to put the capsule in yogurt and delicious preserve is that it is possible for example in the proposed formulation. Figure 10 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. Figure 10 is contained to loaded such as (A) insulin, (B) alpha-tocopherol, (C) idebenone, (D) resveratrol, (E) alpha-lipoic acid, respectively. For the stability of the active ingredients 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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Authors

Dae-Seop Kim; student

Student: Dept. of natural science, Hankuk Academy of Forign Studies

Position: Grade two (natural science)

Major experience: He studied the development of new medicine using bio-fermentation for department of life science at Pasteur R&D Center in Seoul Korea. He has published the unique article regarding Bio-technology at Journal of the Korean Oil Chemist' Society 2013. And also, his specifical patent was already registered from Korea patent office 2014. He announced new article at Asia-Pacific Conference on Computer Science and Electrical Engineering 9-11 Jan 2014. He awarded grand prize of ARC2013 conference in Hankuk Academy of Forign Studies.

In-Young Kim Ph.D

Professor: Dept. of Chemical Engineering, Soongsil University, KOREA

Company: R&D Center of Biobeautech Co., Ltd. KOREA

Position: CTO (Technical director)

He received Ph.D at Dept. of Chemical Engineering, Soongsil University Ph.D. Technical advisor: Korea Kolmar Co., Ltd., Cosmecca Korea Co., Ltd. / Hankook Cosmetic Co., Ltd. R&D Center / CJ group, cosmetic division skin science R&D Center/ NIKKO Chemicals Co., Ltd. Cosmos Technical Center in Tokyo Japan / LOREAL KSP R&D Center in Japan.

Mi-Ey Park; Teacher

She has been a teacher at Biology of natural science in Hankuk Academy of Forign Studies in Gyeonggi-do Korea. She has a lots of article publications in various areas of Biological Science, concentrating on fermentation technologies.

So-Yeun Yoo; Teacher

She has been a teacher at Biology of natural science in Hankuk Academy of Forign Studies in Gyeonggi-do Korea. She has a lots of article publications in various areas of Biological Science and Bio-synthesis, concentrating on bio-synthetic technologies.