IJBPAS, August, 2015, 4(8): 268-276

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

SURVIVAL OF ENCAPSULATED LATOBACILLUS *ACIDOPHILUS* LA5, AND *LACTOBACILLUS CASEI* 431 ENCAPSULATED IN ORANGE JUICE STORED IN REFRIGERATOR TEMPERATURE

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ABSTRACT

The objective of this study is to evaluate the survival of probiotic *Lactobacillus casei* 431 and *Lactobacillus acidophilus* La-5 in orange juice in a 4-week storage in refrigerator temperature. First, 12 °Bx orange juice was produced and then pasteurized for 4 minutes in 80 °C. After inculcating the bacteria into the juice (approximately 5-6 × 10^9 bacteria per one milliliter of juice), the juice was initially fermented for 3 days in 37 °C. Then, the sample was stored for 4 weeks in 4 °C. Bacteria's survival in intervals of 1, 2, 3, and 4 weeks were measured. The number of *lactobacillus* and *acidophilus* bacteria after 4 weeks of storage in 4 °C was more than 6.5 × 10^8 per juice milliliter. This suggests that the capsules containing *lactobacillus* and *acidophilus* could preserve the required durability during their containment in orange juice. Similar results were found for *lactobacillus casei*; encapsulated bacteria could preserve their durability during storage in refrigerator temperature with more than 10^7 bacteria per milliliter

after the fourth week. Results from this study showed that orange juice is a suitable product for producing probiotic natural juice by adding lactobacillus acidophilus and lactobacillus casei bacteria in encapsulated form.

Keywords: Orange juice, Lactobacillus acidophilus, *Lactobacillus casei*, Probiotic

INTRODUCTION

Foods that promote health and provide nutrients for the body are called Functional Foods. This implies consuming foods rich in active compounds such as prebiotics, probiotics, vitamins, minerals, diet fibers, fish oil and plant Sterols [13]. According to the definition, probiotic bacteria are living microorganisms that settle in the intestines after consumption and positively affect human health by improving the natural gut micro-flora. The survival capabilities of the selected bacteria in this product during production and storage are important for health. In this regard, the number of probiotic bacteria surviving in the food should be at least between 10^6 and 10^7 bacteria per gr or ml for them to be beneficial to health [13].

Currently, most of the probiotic products in the market are probiotic dairy products, but in recent years demand for nondairy probiotic products has increased. The reasons for reduced consumption of dairy products are the presence of cholesterol in dairy products and the fact that some people are lactose intolerant. If there is no suitable replacement for dairy-based probiotic products for vegetarians, demand for probiotics with their medicinal potentials will decrease. Since they are rich in anti-oxidants, vitamins, diet fibers, and minerals, vegetables and fruits are classified as healthy food. In addition, vegetables and fruits do not have the disadvantages mentioned for dairy products including allergic reactions [8], [24]. In recent years consumer demand for nondairy probiotic products has been on the rise. Therefore, using probiotics in nondairy drinks has drawn a lot of attention. Fruit juices that are highly demanded are the perfect environment for the growth of probiotics.

Fruit juice can provide important opportunities for world health because they are nutrient rich for all ages and are considered healthy and energizing. Furthermore they are suitable for consumers who are not willing to use to dairy products. Fruit juices are rich in minerals, vitamins, dietary fibers, and antioxidants [18]. Probiotic fruit juice is one of the latest opportunities for innovation in healthy drinks business throughout the world [22].

In recent years, some research has been done on using probiotics in fruit juices [8], [24].

Using different strains of probiotic bacteria in tomato juice (Lactobacillus acidophilus LA39, lactobacillus Plantarum C3, lactobacillus casei A4, lactobacillus Delbrueckii D7) [24], producing probiotic drinks using malt, barley and a combination of both (Lactobacillus plantarum and lactobacillus acidophilus) [19], orange juice and pineapple juice (lactobacillus casei, lactobacillus rhamnosus, lactobacillus paracasei) [23], carrot juice (bifidobacterium lactis and bifidobacterium bifidium) [12], beet juice (lactobacillus acidophilus, lactobacillus casei, lactobacillus delbrueckii, lactobacillus plantarum) [25], orange juice and tomato juice (lactobacillus rhamnosus GG, lactobacillus casei & lactobacillus fermentum), pomegranate juice, grapefruit juice and strawberry juice (bifidobacterium langum) [17] and apple juice (lactobacillus casei) [18]have been studied.

Therefore, the present study aims to investigate the survival of encapsulated probiotic lactobacillus acidophilus and lactobacillus casei bacteria by extruding sodium alginate in orange juice and studying the role of this bacterium in the chemical-physical and organoleptic characteristics of the final product.

MATERIALS AND METHODS

Producing Fruit Juice and Bacteria Inculcation

Fruit juice concentrate was stored in -15 °C before use. The concentrate with 65 °Brix was diluted using distilled water up to 12 °Brix and then pasteurized for 4 minutes in 80 °C. 40 ml of pasteurized orange juice was injected in ach sample test tube and all the encapsulated bacteria were inculcated with calcium alginate. The inculcation dosage for both bacteria in encapsulated form was approximately 5-6 × 10^9 per milliliter.

Preparing the Probiotic Bacteria

The probiotic bacteria used in this study were purchased from the Danish Christian Hansen Company. The packages containing the bacteria were opened carefully in sterile conditions and one gram of bacteria was uniformly mixed in 100 ml of MRS broth and was stored for 24 hours in 37 °C so that bacteria absorb water and start their logarithmic growth phase. Then, 1 ml of the obtained culture was added to 99 ml of the new culture environment and put in 37 °C incubator for 24 hours. Finally, bacteria suspension was centrifuged for 5 minutes with 4500 revolutions per minute; then the sediment produced in centrifuge was separated and washed with 0.1 % peptone water and ether solution and bacteria

sediment was solved in 0.1 % peptone water. Then, bacteria's light absorption was determined using spectrophotometer with a wavelength of 595 nm Bacteria counting was done using surface plate counting [7].

After bacteria inculcation, samples were stored for 72 hours in 37 °C . After 72 hours, the samples were stored for 4 weeks in 4 °C temperature. In this stage, microbial analyses were performed in 1, 2, 3, and 4 week intervals.

Encapsulation

Encapsulation of the probiotics was done using extrusion method. First, 0.4 gr of sodium alginate was added to 20 ml of distilled water and then sterilized. Then, the alginate solution was stored in the refrigerator for one night so that alginate particles could absorb as much as water. The next day, the alginate solution was transferred from the refrigerator to the laboratory to become isothermal with the room temperature and then 5 cc of the bacteria suspension was mixed with 0.1 peptone water with 20 ml of 2% (volume/mass) sterile alginate sodium solution (for 15 minutes in 121 °C temperature). The cellular suspension was injected using a sterile syringe with 0.2mm-diameter to a dish containing 0.05 molar sterile calcium chloride solution. The capsules were kept in the above solution for 30 minutes to be solid and then rinsed and stored until use in 0.1 % terile peptone water in 4 °C [10].

Bacterial Counting

For decomposition of the capsules and freeing of its content, the first dilution of samples was prepared in 2 % trisodium citrate solution. In order to prepare that, 10 gr of the homogenized sample was distributed in sterile zipped bags containing 90 ml of 2 % sterile trisodium citrate and then homogenized for 5 minutes using a stomacher with 260 revolutions per minute so that capsules were fully decomposed (in samples containing free bacteria, this stage was skipped). Next series of dilutions were obtained by adding on milliliter of each dilution to 9 ml of 0.1 % sterile peptone water [9].

Then, using surface plate counting, lactobacillus acidophilus was cultivated in Sorbitol agar –MRS environment (10 ml of 10 % sorbitol solution had been added to 9 ml of the culture environment prior to pouring onto the plates). The plates were incubated for 72 hours in 37 °C by type C gas-pak under anaerobic conditions [10]. The case of lactobacillus casei was also similar to acidophilus with the difference that plates were directly put in the incubator. Also,

sorbitol was not added to its cultivation environment.

Microbiological Experiments

In 1, 2, 3, and 4 week intervals, 1 ml of the sample was homogenized in 9 ml of 0.1 % peptone water and subsequent dilutions were prepared in pipes containing 0.1 peptone water. In order to count, lactobacillus acidophilus was cultivated in Sorbitol agar – MRS environment (10 ml of 10 % sorbitol solution had been added to 9 ml of the culture environment prior to pouring onto the plates). The plates were incubated for 72 hours in 37 ℃ by type C gas-pak in anaerobic jars. The procedure for cultivating lactobacillus casei was similar to lactobacillus acidophilus with the difference that plates were directly put in the incubator and no sorbitol was added to its cultivation environment [10] [4].

Survival Capability of Probiotic Bacteria Encapsulated in Orange Juice

In order to make sure that probiotic orange juice is produce, lactobacillus acidophilus and lactobacillus casei bacteria were added to the fruit juice in encapsulated form. The amount of inculcated bacteria was approximately 5-6 × 10^9 bacteria per ml of fruit juice. Considering Fig 1, the results indicate a significant difference between the subject bacteria for all storage days.

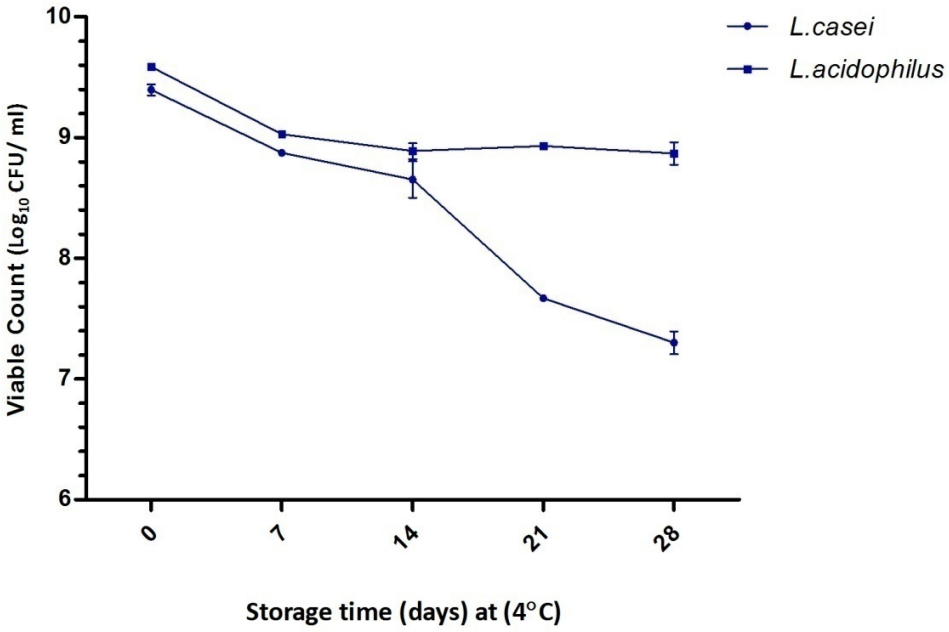

Figure 1: Durability and Variation in Encapsulate Bacteria Count in Orange Juice Samples Containing Lactobacillus Casei and Lactobacillus Acidophilus Bacteria during 4-week Storage in 4 °C

According to Fig 1, results obtained in this study indicate a significant difference between the two subject bacteria for all storage days. The number of lactobacillus acidophilus bacteria encapsulated in orange juice was more than 6.5×10^8 per every ml of fruit juice after 4 weeks of storage in 4 °C. This implies that the capsules containing lactobacillus acidophilus could preserve the required durability in orange juice during the storage.

Similar results were also found for lactobacillus casei: the encapsulated bacteria were able to preserve their durability during storage in refrigerator temperature so much that their number exceeded 10^7 bacteria per every milliliter. However, considering the fact that this bacterium was inherently a weaker bacterium than lactobacillus acidophilus in terms of durability in red grapes, at the end of the 4-week storage in 4 °C, the final number of the lactobacillus casei bacterium was a logarithmic cycle lower than lactobacillus acidophilus.

In a study, Ding and Shah (2008) studied the survival of 8 strains of probiotic bacteria in their free and encapsulated forms in orange juice and showed that the number of probiotics had rapidly reduced up to 4 weeks of storage in 4 °C in orange and apple juices and had lost their ability to survive after 5 weeks of storage, whereas bacteria encapsulated in fruit juice had survived after 6 weeks of storage. They reported that probiotics encapsulated in orange juice and apple juice are more durable than the free bacteria in fruit juice. Results from our study agree with those obtained in that study.

They also stated that lactobacillus acidophilus in its free form is more durable (about 4 weeks) than other bacteria in orange juice which is due to its high tolerance of acidic conditions.

Studies suggest that only encapsulated probiotic bacteria were able to survive in highly acidic fruit juice for 2 weeks [14].

CONCLUSION

Considering the numerous studies carried out on the benefits of probiotic bacteria in nondairy food products, results from the present study revealed that it is possible to produce orange juice using lactobacillus acidophilus and casei. The number of lactobacillus acidophilus and casei bacteria encapsulated in orange juice after 4 weeks of storage in 4 °C was more than 6.5×10^8 and 10^7 per ml of fruit juice, respectively. This means that the capsules containing lactobacillus acidophilus and casei were able to preserve the necessary durability during storage in orange juice.

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