Chocolate with probiotic microcapsules is a new product of functional nutrition

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Abstract. Probiotics can help improve digestion, strengthen the immune system and even fight diseases. But until now, there was no easy way to introduce enough probiotics into our body. Microencapsulated probiotics offer a solution to this problem. They are small enough to be easily digested, and are protected from the aggressive environment of the stomach. Chocolate represents a ubiquitous and popular consumable, yet probiotics within food matrices necessitate strategies for stress mitigation and enhancement of viability. One such method involves the encapsulation of probiotic cells within alginate microspheres, amenable for incorporation into chocolate matrices. In this regard, the aim of study is to explore the effect of microencapsulation on the survival of probiotics in chocolate. Conventional microbiological methods were used: growing bacteria in liquid and solid media, determining the titer of bacteria using the Koch method. The results demonstrated the successful production of chocolate containing microcapsules of probiotics with favourable organoleptic properties. Enhanced viability preservation of Lactobacillus rhamnosus GG in microcapsules compared to free cells was observed.

1 Introduction

In the concept of 21st century nutrition, aimed not only at satisfying basic nutritional needs but also at maintaining health, probiotics play a leading role, probiotics play a leading role.

Probiotics can be defined as "live microorganisms that, when administered in sufficient quantities, bestow advantageous effects on the host organism [1]. What is being referred to in this context is that the presence of a "sufficient quantity" of them in comestible items ought to be substantial, surpassing no less than one or even ten million per gram [2]. However, incorporating live probiotic microorganisms into food and then maintaining their viability throughout storage is challenging. Probiotics exhibit a remarkable susceptibility to a multitude of factors, including but not limited to temperature, oxygen levels, water activity, osmotic pressure, mechanical stress, and pH levels [3].

It is also important to note that probiotics "work" inside the human large intestine. Bacteria need to pass through this entire lengthy transit of the gastrointestinal tract alive and healthy. However, this does not always occur since hydrochloric acid and digestive
enzymes have a detrimental effect on them. As a result, only 5-10% of these bacteria reach the "site of their action" [4].

One way to protect beneficial probiotic bacteria from the environment is through microencapsulation. This method involves separating bacterial cells from the external environment by incorporating them into a polymer network, from which the contents are then released under certain conditions and at a controlled rate [4, 5]. The range of biopolymers for microencapsulation is wide, including alginate, chitosan, gelatin, xanthan and gellan gum, carrageenan, and pectin [6]. Among natural polymers, sodium alginate is the preferred material due to several advantages:

- It immediately forms granules in the presence of polyvalent cations, such as Ca ++;
- It is biodegradable and non-toxic;
- It is abundant in the cell walls of brown algae, ensuring its affordability and availability [7].

Sodium alginate capsules in the stomach transform into a soft, gel-like solid that remains stable in an acidic environment for 6–8 hours. In the small intestine, alginate microcapsules inhibit digestive enzymes, reduce the absorption of cholesterol and glucose, and control fat digestion [8]. Lactobacillus casei in sodium alginate capsules exhibited increased viability under gastrointestinal tract conditions (pH 1.5 and high bile concentration) and during heat treatment (55-65°C) compared to free cells [9]. This indicates their potential not only for probiotic protection but also as a delivery system to the large intestine.

Probiotic bacteria are frequently present in fermented dairy products and cheeses, and they frequently contribute significantly to their manufacturing processes. However, the consumption of dairy products, particularly for health-conscious individuals, is associated with major drawbacks such as lactose intolerance, allergenic milk proteins, and a high fat content. Dairy products' relatively short shelf life and need for refrigeration also place restrictions on their usefulness. The creation of a novel probiotic product may be very significant in this situation. One appropriate food matrix for this kind of product could be chocolate. Because of its abundance of polyphenols, which function as antioxidants, it may have heart-healthy and even antidepressant effects [9]. Furthermore, bacterial counts were four times higher in chocolate-consuming individuals than in dairy product-consuming individuals, suggesting that chocolate is a better intestinal delivery system for probiotics [10]. This is attributed to chocolate's hard texture, low moisture content, and high fat content, ensuring a good shelf life. The presence of fat in a food matrix like chocolate can significantly protect probiotics during manufacturing and passage through the stomach, as fat provides protection against harmful external influences [11]. The usage of a compacted food matrix with an increased quantity of defensive elements presents supplementary advantages in comparison to standard probiotic dairy products.

Nevertheless, a fresh commodity with operational characteristics must satisfy the organoleptic quality demands, as this is fundamental for consumer approval and prosperous market positioning. Flavor and texture are two sensory properties for which chocolate is renowned and are the most susceptible to alteration by the addition of probiotics. Improper use of microencapsulated probiotic cells may result in off-flavors. The importance of the sensory properties of chocolate cannot be overstated, and it is crucial that the introduction of probiotic bacteria does not in any way change these characteristics.

Therefore, the technique of microencapsulation of probiotics serves to improve their survival rate during their journey through the digestive system, as well as during the various stages of food production and storage. Several studies have investigated the inclusion of probiotics in chocolate [12–15].

In this regard, the present investigation aimed to ascertain the impact of microencapsulation on the viability of probiotics in chocolate that has been stored at room
temperature for 1–2 months. Additionally, an evaluation was conducted on the sensory parameters of the resultant product.

2 Materials and Methods

Alginate microcapsules containing probiotic *Lactobacillus rhamnosus* GG ATCC 53103 (LGG). Samples of "Kazakhstan" chocolate with/ without probiotic microcapsules.

2.1 Culture preparation

Inoculate LGG strain into 20 ml of MRS broth and incubate at 37°C for 24 hours. Centrifuge the culture liquid at 4°C, 6000 rpm for 15 minutes. Wash the cells twice with distilled water and resuspend in 3 ml of sodium chloride solution.

Determine cell concentration by serial dilution until reaching a dilution factor of 10^-7 CFU/ml, followed by plating on Petri dishes at 37°C for 24 hours.

The grown colonies were counted and the CFU value was calculated using the following formula (1):

\[ CFU = \frac{n \times V_{total}}{V_{pl} \times X} \]  

where, CFU – colony forming units,

n – average number of grown colonies,

V_{total} – the total volume from which the material for sowing was taken,

V_{pl} – volume of material plated onto Petri dishes,

X – dilution factor.

2.2 Microcapsules preparation

Mix the cell suspension with 2% sodium alginate solution in a 1:5 ratio. The mixture extruded through a needle with a diameter of 0.6 mm into a sterile solution of 0.1 M calcium chloride. This extrusion process performed using an Armed MP-2003(China) syringe dispenser. Throughout the extrusion process, the distance between the needle and the calcium chloride solution remains constant at 25 cm. When the mixture comes into contact with the calcium chloride solution, gel spheres are formed instantly. To ensure complete solidification, the gel spheres are left undisturbed for a period of 30 minutes. Afterward, they are dried using a Kitfort KT-1906 dehydrator at a temperature of 40°C.

2.3 Chocolate Preparation

Chocolate melted in water bath, divided into three stages: melting (50°C); cooling to crystallization point (32°C); crystallization (27°C).

Microcapsules added at 3% weight of finished product after tempering (32 ± 2°C). Mass poured into molds. Sample Preparation: control sample (no microcapsules); sample with free probiotics; sample with microencapsulated probiotics.

2.4 Sensory Evaluation

Conducted by 20 volunteers. Evaluated characteristics such as appearance, taste, odour, surface properties, hardness, chewiness, mechanical properties and structure. Scores ranged from 1 to 3.
2.5 Survival Assessment

Viable lactobacilli cells counted immediately after drying, next day after microcapsule addition and after 30 and 60 days of storage. Capsules suspended in phosphate buffer, shaken for 60 minutes, then plated on MRS agar. Chocolate samples homogenized in phosphate buffer, plated on MRS agar. Statistical analysis performed using SPSS version 25.

3 Results and discussion

3.1 Obtaining Probiotic Microcapsules

The resulting capsules appeared as translucent white balls (Fig. 1). These balls ranged in size from 2500 to 3000 microns. Such large microcapsules are unsuitable for the food industry. Additionally, chocolate being a solid product with low moisture content, cannot accommodate wet ingredients. To address this, the capsules were dehydrated in a gentle dehydrator at 40°C. Upon moisture loss, the size of the microcapsules decreased to 800 microns.

Fig. 1. Method of microencapsulation(A); appearance of wet microcapsules (B) and dried microcapsules (C).

3.2 Preparation of chocolate with probiotics and determination of organoleptic properties

Commercially available chocolate "Kazakhstan" from JSC "Rakhat" was chosen for the study. The ingredients listed on the packaging include sugar, cocoa mass, cocoa butter, whey powder, and whole milk powder. The cocoa content is stated to be at least 45%. Soy lecithin serves as an emulsifier, while natural vanilla extract is utilized as a flavor enhancer. Nutritional information per 100 grams of the product includes 6.3 grams of protein, 33.4 grams of fat, and 52.7 grams of carbohydrates.

Using illustrated method (Fig. 2) chocolate samples (control, i.e. with LGG cells, and with microcapsules) were prepared.
The chocolate samples, augmented with microcapsules, underwent sensory evaluation by a panel of 20 volunteers familiar with the product to evaluate the effects of the microcapsule addition on the chocolate's organoleptic properties. Based on the collected evaluation results, the resulting samples were determined to possess excellent organoleptic characteristics. Averages for each parameter were computed, indicating that the addition of microcapsules did not significantly alter the taste of the chocolate. To visually compare the different samples of the finished product, a graphical representation (Fig. 3) was generated.

Certain parameters suggest that samples containing microcapsules were rated higher than control samples, indicating potential improvements in specific aspects of chocolate quality with the addition of microencapsulated probiotics. Such comparable results between the two sample types suggest a lack of significant differences, further highlighting the overall positive influence of microencapsulation on the organoleptic attributes of chocolate.

### 3.3 Determination of probiotic bacteria survival rate in chocolate

The storage conditions for the chocolate, as indicated on the packaging, specify a temperature range of 8°C to 23°C, with a relative humidity not exceeding 75%, and a shelf
life of 12 months. To assess the longevity of encapsulated lactobacilli viability within the chocolate, finished samples were stored at room temperature. Two types of samples were prepared: control samples (without microcapsules) and test samples (with microcapsules). Viable lactobacilli counts were examined on the next day, as well as on the 30th and 60th days (Figure 4).

The results show that, compared to control samples, samples with capsules show much greater survival of lactobacilli. The bacterial titer of the tested samples was higher by 2.6 and 5.5 log CFU/g, respectively, on the 30th day and later periods of storage, compared to control samples.

Furthermore, the survivability assessment of probiotic bacteria in chocolate revealed notable differences between the control and test samples during extended storage periods. This observation underscores the efficacy of microencapsulation in enhancing the viability of lactobacilli during prolonged storage under the specified conditions.

All things considered, research indicates that microencapsulation may be an effective way to keep probiotic bacteria alive in chocolate without sacrificing its organoleptic properties. These findings contribute to the growing body of research on functional food development and underscore the potential of incorporating probiotics into chocolate products for enhanced nutritional value and consumer appeal. Further research could explore additional parameters such as the influence of different encapsulation materials and storage conditions on probiotic viability and chocolate quality.

4. Conclusion

The results obtained from our experiments lead us to the following key conclusions:

1. Microcapsules containing probiotic *Lactobacillus rhamnosus* GG cells within an alginate matrix were successfully obtained using the extrusion method. The dimensions of the wet microcapsules ranged from 2500-3000 microns, which reduced to 800 microns upon drying. This technique offers a promising approach for incorporating probiotics into chocolate products.

2. Chocolate enriched with probiotic microcapsules exhibited excellent organoleptic characteristics, with no noticeable changes in taste, smell, colour, or surface compared to conventional chocolate. This suggests that the addition of microcapsules does not compromise the sensory appeal of chocolate, thereby enhancing its consumer acceptance.
3. Study demonstrated an enhanced efficiency in preserving the viability of \textit{Lactobacillus rhamnosus} GG within microcapsules compared to free cells. This finding underscores the effectiveness of microencapsulation in protecting probiotic bacteria from harsh environmental conditions, such as those encountered during chocolate production and storage.

**Authors’ Contribution**

Kistaubayeva A., Savitskaya I. and Khamitkyzy Zh. conceived of the presented idea. Khamitkyzy Zh., Karpenyuk T, Goncharova A. and Savitskaya I. developed the methodology, designed and performed the experiments. All authors discussed the results and contributed to the final manuscript.

**References**


