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The impact Encapsulation Exerted by Tragacanth Gum on Viability and Staling of *Lactobacillus Plantarum and Lactobacillus acidophilus* During Baking and Storing Gluten-free Sorghum Bread

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KEYWORDS

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ABSTRACT: Supplementing bread probiotics is considered to be challenging because of the high baking temperatures. In this study the influence of encapsulation by tragacanth gum on the validity of *Lactobacillus Plantarum* and *Lactobacillus acidophilus* during baking and storing gluten-free sorghum bread for three days. Moreover, the effect of probiotics encapsulation on moisture content and hardness as two major factors of bread staling were investigated. The process of baking process reduced the observed validity of *L. Plantarum* and *L. acidophilus* by about 3 logs CFU/g in gluten-free sorghum bread significantly. Additionally, we found that *Plantarum*, *L. acidophilus, and* encapsulated *L.* during baking and storing processes strongly depend on matrix composition to survive. Encapsulation of probiotic cells by tragacanth gum can improve the viability of probiotic cells can be improved by encapsulating them by more than 2 log cycles in gluten-free sorghum bread during the storing process. The tragacanth gum showed a good protecting impact on L. Plantarum and L. acidophilus cells during 72 h storage. Overall, what the findings suggest is that encapsulating probiotics by tragacanth gum is a strategy promising to promote the survival of bacteria and delay staling of gluten-free sorghum bread.

INTRODUCTION

Bread is considered to be a nutritious food that is non-dairy based providing a large portion of the nutrients required for growth, health maintenance, and well-being. It is deemed a good source of proteins, vitamins, minerals, carbohydrates, and fibers[1]. Although decades ago and glutenins, wheat gliadins, barley, and related proteins in rye were shown to be toxic to those suffering from celiac disease. Currently, celiac disease is deemed to be a long-term autoimmune disorder occurring in those who are genetically predisposed where gluten indigestion leads to damage in the small intestine [2]. Sorghum (*Sorghum bicolor* (L.) Moench) is considered a significant cereal and has been often recommended as a safe food for celiac patients. Therefore sorghum can be a good source of gluten-free bread [3].

Further, when consumed in adequate amounts probiotics such as the yeast that is life have been boosted owing to providing the host with some health benefits. Additionally, microbes must be able to survive during the processing and storing of food because of the presence of unstressed microbial cells and high numbers of viable during their usage that is bound to provide

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some health benefits. The counts of probiotics between 10^6 to 10^9 CFU g^{-1} are generally recognized in the food [4]. A field of innovation in the sector of probiotic food is that of bread which has gained increasing interest in research. However, adding probiotics to bread is considered to be challenging because the high temperatures at the time of baking negatively affect the rate of surviving bacteria and lead to further loss of bacterial viability when stored at room temperatures subsequently [5].

Encapsulating the bacterial cells in a protective powder is deemed to be a promising strategy to promote the capability of probiotic bacteria to survive. The matrix composition influences the viability of the probiotic over the heat process in some solid matrices [5]. The current investigation seeks to create some novel functional food, to enhance *Lactobacillus Plantarum* and *Lactobacillus acidophilus* validity during baking and storing gluten-free sorghum bread using encapsulation into tragacanth gum. Moreover, the effect of adding freeze-dried encapsulated probiotic strains in tragacanth gum on staling and physicochemical properties of gluten-free sorghum bread has been assessed.

MATERIALS AND METHODS

Probiotic strains and bacteria culture

Agricultural Biotechnology Research Institute of Iran Culture Collection (Karaj, Iran) provided *Lactobacillus acidophilus* (NRRL B-4495) and *Lactobacillus Plantarum* P8 (ATCC-14917). Probiotic cultures were typically prepared through the growth medium of MRS broth (Neogen Corporation, Lansing). One colony of each microorganism was injected singularly in 10 mL sterile MRS broth and pre-cultured at 37°C for 12 h. Afterward, 1% v/v inoculum of *L. Plantarum* and *L. acidophilus* were sub-cultured in 100mL MRS broth at 37°C for 24 h not involving any agitation. Sterile 0.9 % saline solution through centrifugation at 10000 × g for 10 min at 4°C was used to wash and harvest *L. acidophilus and L. Plantarum* cell cultures (Model J2-HC, Beckman Coulter, Inc., CA). The wall material solution of tragacanth gum (10% w/v) was prepared and sterilized for 10 minutes at 75°C [6]. The solution of wall material was then mixed with cell cultures of harvested probiotics (~10^9 CFU mL^{-1}) to produce some different solutions.

Probiotic solutions' freeze-drying

We transferred *Lactobacillus Plantarum* and *Lactobacillus acidophilus* cell suspensions to pre-frozen glass tubes that are sterile and indifferent solutions at -20°C over in a freeze dryer 12 h before the major vacuum-freeze-drying step (Cryodos-50/230 V-50 Hz, Telstar, Madrid, Spain) for 50 h and in this case, we set the temperature at -50°C. Then, we fully ground the lyophilized matrices into some fine powders using a tool consisting of mortar and a pestle, and we stored the powders were stored at 4°C in some sealed bottles in a certain desiccator.

Bread making

The following recipe was used to make the bread samples: sugar (4 g), salt (1.5 g), instant yeast (1 g), sorghum flour (100 g), UHT skim milk (65g), and butter (3 g) with probiotics were added separately. The bread was made with the addition of non-encapsulated bacteria as the control. We mixed the dry ingredients at 40 rpm for 1 min and mixed it at 80 rpm for 7 min after adding the milk (Hauswirt® HM730, China). We divided the dough into balls of 50 respectively after resting for 5 min. Then, we proofed the dough at 40°C, 85% RH in a climate chamber (Yiheng Scientific Instruments Co., Ltd., China) for 1 h. Subsequently, we baked the bread samples in an electric oven for 8 min (Changdi® CRTF30W, China) at 180 °C for 8 min. Bread samples weighing 30g initially were then sealed in some polyethylene bags and then they were stored for three days in the climate chamber at 25°C, 55% RH.

Viability of probiotics

To measure the viable counts of probiotics for both dough and bread, we homogenized 5 g of the sample using 45 mL peptone water that was sterile (0.1 % w/w) and using a stomacher (400, Seward, USA). We made the suspensions serial dilutions (100 μL) in 900 μL sterile peptone water, it was a 100 μL solution that was plated onto the given MRS agar broth (Neogen, Corporation, Lansing, MI) that was supplemented using

200 mg L^{-1} natamycin (Antai®, China). To inhibit the growth of the yeast on the MRS agar plate we added natamycin intending to inhibit yeast growth in the MRS agar plate exerting no effect on the growth of the probiotics [7]. The plates were injected at 37°C and enumerated after 48 h where the presented results were in the form of per gram sample as units forming colonies (CFU g^{-1}).

Determination of moisture content

Moreover, we measured bread content after applying AACC Approved Method 44-15.02 [8]. We dried the samples at 105°C till reaching a certain constant weight. Subsequently, the moisture content was measured as the water weight that was removed during the drying process divided by the initial weight of powder. In addition, the moisture content of fresh bread was measured after being stored for 24, 48, and 72h.

Determination of hardness

We measured the hardness of the crumb in some QTS 25 texture meters (Brookfield). In addition, we compressed A 2.5 cm thick slice by a 40% deformation using an acrylic probe measuring 38.1 mm, 60 seconds of hold time, and at 120 mm min^{-1} speed. Furthermore, six analyses were carried out for each sample.

Physicochemical characteristics

Physicochemical properties of fresh bread include oven spring (loaf volume increased after baking), specific volume index, and pH by a digital pH meter. Subsequently, we weighed the bread after cooling them and determined its volume (cm^3) using the rapeseed displacement method. We also calculated the specific volume (cm^3 g^{-1}) as bread weight/loaf volume [9].

Statistical analysis

We analyzed the obtained data using a certain one-way analysis of variance test performed through the SPSS software (version 23.0, IBM, Chicago, USA). Then, we also used Duncan's Multiple Range Test to calculate the significance of differences existing among results ($p<0.05$).

RESULTS AND DISCUSSION

Effect of encapsulation on the viability of probiotic

Table 1 presents the survival process of both encapsulated and free *L. acidophilus and L. Plantarum* cells in tragacanth gum during baking and 72 h of storage at room temperature of gluten-free sorghum bread are presented in Table 1. In addition, we might expect *L. acidophilus and L. Plantarum viability* samples of bread to decrease eventually as a result of high baking temperature [10]. As results indicate, the baking process decreased the *L. acidophilus and L. Plantarum* viable cell count concerning free cells by 6.09 and 6.55 log CFU g^{-1}, respectively. However, the observed reductions in viable cell count were significantly lower in the encapsulated cells into tragacanth gum during baking. Zhang et al. [1] reported that the baking process reduced *L. Plantarum* viability to $10^{4\sim5}$ CFU g^{-1} in bread significantly. That the bread bacteria survive during baking was dependent on the adopted approach to involve both probiotics and physical properties of encapsulating the materials connected with the moist-heat exposure that the bacterial cells underwent. In addition, it seemed that the moisture content particularly had a major impact on the bacteria as their survival after the exposure [11]. Our results are consistent with those obtained by Thang et al. [12] indicating that *L. acidophilus* viability encapsulated when combined with xanthan gum and alginate was decreased by 3.64 log CFU g^{-1} over the process of baking concerning the non-capsulated one decreased by about 5 log CFU g^{-1}. Encapsulating probiotic cells using tragacanth gum could increase the viability of the cells in gluten-free sorghum bread during storage by more than 2 log cycles, therefore, the tragacanth gum showed a high protecting impact on *L. Plantarum* and *L. acidophilus* cells during 72 h storage. The study of Trabelsi et al. [13] showed that the protecting impact of alginate as combined with polymer compounds on the *L. Plantarum* viability indicated some results than using alginate alone over storing it at 4°C over 35 days [13].

Table 1. Impact of being encapsulated by tragacanth gum on the viability of *Lactobacillus Plantarum* and *Lactobacillus acidophilus* over the processes of baking and storing bread (log CFU/g).

Sample	Storage (h)			
	0	24	48	72
***L. Plan* - Dough**	9.21± 0.25^{A,a}	8.35 ± 0.38^{B,a}	7.87 ± 0.25^{BC,a}	7.59 ± 0.18^{C,a}
***L. Acid* - Dough**	9.44 ± 0.18^{A,a}	8.21 ± 0.27^{B,a}	7.65 ± 0.14^{C,a}	7.46 ± 0.35^{C,a}
***L. Plan* - Control**	3.12 ± 0.12^{A,c}	2.65 ± 0.08^{B,c}	2.42 ± 0.07^{C,c}	2.22 ± 0.16^{C,c}
***L. Acid* - Control**	2.89 ± 0.15^{A,c}	2.54 ± 0.14^{B,c}	2.45 ± 0.16^{B,c}	2.08 ± 0.11^{C,c}
Encapsulated *L. Plan*	4.78 ± 0.33^{A,b}	4.11 ± 0.17^{B,b}	4.02 ± 0.28^{B,b}	4.35 ± 0.14^{AB,b}
Encapsulated *L. Acid*	4.95 ± 0.18^{A,b}	4.36 ± 0.26^{B,b}	4.18 ± 0.25^{B,b}	4.44 ± 0.22^{B,b}

* Means within a column with different letters are significantly different (P<0.05).

Effect of encapsulation on bread staling

Moisture content during storage

The process of bread staling is so complex that cannot be explained by moisture content loss, the existence of a single impact, distribution of water content between the crystalline zones where the hardness is bound to participate in the bread staling process [14]. Table 2 presents the moisture content of probiotic gluten-free sorghum bread over the processes of baking and storing. In addition, the moisture content of the probiotic dough with *L. Plantarum* and *L. acidophilus* was decreased from 46.5 and 48.1% to 32.5 and 33% during baking in fresh bread. The moisture content of the probiotic bread containing tragacanth as a capsulate coating was considerably (P<0.05) greater compared to the control after baking (0 days). The moisture content of these samples on the first day of experiments was in the range of 36.82-37.22% and reached 34.53-34.77% on the last day of storage. These findings were attributed to the capacity of the tragacanth to hold water. Tragacanth gum has a mucilaginous jelly texture, which provides its ability to hold the water with its matrix while also holding a greater amount of water during the heating process. Tragacanth shows its barrier capabilities through which it is capable of preventing the removal of water over the process of baking by forming bonds of water molecular hydrogen thus making them more influential in avoiding the loss of weight in the final product [15]. Guarda et al. [16] have found similarly that xanthan gum has increased the initial moisture content in the case of fresh white bread while decreasing the moisture loss over the storing process.

Table 2. Impact of encapsulation of *Lactobacillus Plantarum* and *Lactobacillus acidophilus* by tragacanth gum on the moisture of bread over baking and storing (log CFU/g).

Sample	Storage (h)			
	0	24	48	72
***L. Plan* - Dough**	46.53 ± 0.72^{a}	-	-	-
***L. Acid*-Dough**	46.11 ± 0.53^{a}	-	-	-
***L. Plan* - Control**	32.50 ± 0.44^{A,c}	32.32 ± 0.39^{A,b}	31.43 ± 0.43^{B,b}	30.01 ± 0.49^{C,b}
***L. Acid* - Control**	33.04 ± 0.53^{A,c}	32.85 ± 0.61^{A,b}	31.71 ± 0.42^{B,b}	29.80 ± 0.51^{C,b}
Encapsulated *L. Plan*	36.82 ± 0.49^{A,b}	36.01 ± 0.50^{AB,a}	35.24 ± 0.48^{BC,a}	34.53 ± 0.38^{C,a}
Encapsulated *L. Acid*	37.22 ± 0.32^{A,b}	36.13 ± 0.61^{BC,a}	35.50 ± 0.54^{CD,a}	34.77 ± 0.44^{D,a}

* Means within a column with different letters are significantly different (P<0.05).

Hardness during storage

Table 3 shows the impacts of encapsulated and ALS-free *L. Plantarum* and *L. acidophilus* in tragacanth gum on the hardness of gluten-free sorghum bread during the 72-h storage process at ambient temperature. The hardness of all bread samples eventually may be expected to increase during storage time. Encapsulation probiotics by tragacanth gum decreased the hardness, considering the softer texture than that of the control. It appears that hydrocolloids exert some effect on the structure of starch that is a weakening effect provoking better water retention and distribution while provoking a decrease in the crumb resistance also. Some study similar to this one has demonstrated that some hydrocolloids could also decrease the hardness of the process of storing bread [16]. As for hydrocolloids, mainly HPMC, we should ascribe the softening impact of their capacity to retain water, and also the possibility of inhibition of the amylopectin retrogradation, because hydrocolloid preferential binds to starch [17]. Xanthan gum reduced the hardness observed in both fresh and stored bread, to achieve some reduction required from whole wheat bread [18].

Table 3. Impact of encapsulation of *Lactobacillus Plantarum* and *Lactobacillus acidophilus* by tragacanth gum on the hardness of bread over the processes of baking and storing (log CFU/g).

Sample	Storage (h)			
	0	24	48	72
L. Plan **- Control**	1.75 ± 0.01^{D,a}	1.89 ± 0.05^{C,a}	2.51 ± 0.07^{B,a}	2.79 ± 0.09^{A,a}
L. Acid **- Control**	1.72 ± 0.07^{D,a}	1.84 ± 0.04^{C,a}	2.58 ± 0.02^{B,a}	2.72 ± 0.05^{A,a}
Encapsulated *L. Plan*	1.32 ± 0.04^{C,b}	1.40 ± 0.05^{BC,b}	1.52 ± 0.08^{B,b}	1.85 ± 0.08^{A,b}
Encapsulated *L. Acid*	1.36 ± 0.02^{C,b}	1.42 ± 0.06^{C,b}	1.56 ± 0.05^{B,b}	1.82 ± 0.04^{A,b}

* Means within a column with different letters are significantly different $(P<0.05)$.

Effect of encapsulation on physicochemical properties of bread

Figure 1a illustrates the effect of the addition of encapsulated probiotics on the pH of bread. The results indicated that the pH of all samples ranged from 5.74 to 5.91. So they were in the suitable range for the survival of probiotic bacteria. As shown in Figure 1a, Adding encapsulated and non-capsulated probiotics to bread did not significantly impact bread pH. The values of specific volume and oven spring of bread were significantly $(P > 0.05)$ influenced by encapsulation by tragacanth gum. Bread with encapsulated probiotics by tragacanth gum showed the higher oven spring and specific volume values than the control samples (Figure 1b, c). Some increases in bread volume with gums for refined wheat bread have been reported by several studies. The volume increase could be due to the increases in fermentation stability, the strength of dough strength, gas retention capacity, and also stability of the gluten-starch network [14, 19 and 20].

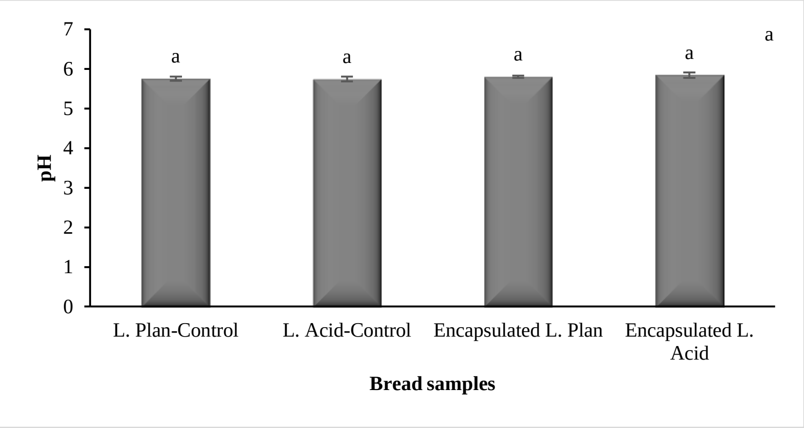
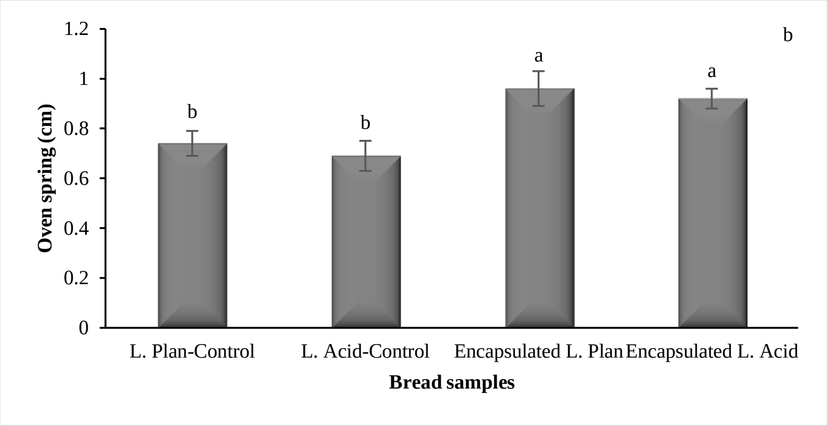


Figure 1. pH (a), oven spring (b), specific volume (c) of bread supplemented with encapsulated probiotics into tragacanth gum



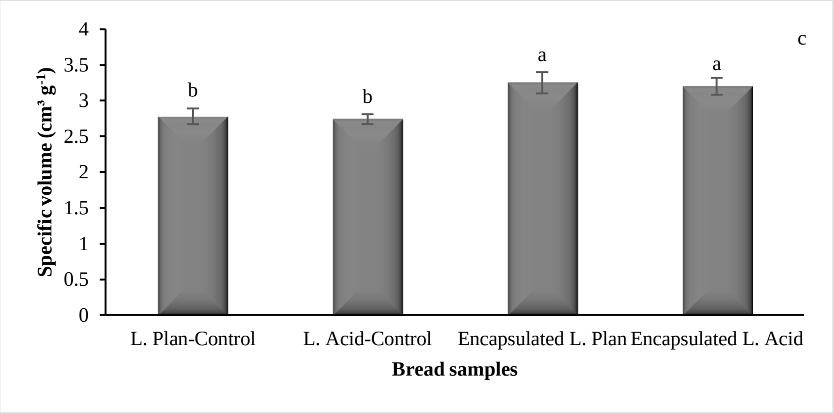


Figure 1. Continued.

CONCLUSIONS

There was a significant decrease in *L. acidophilus and L. Plantarum validity applied by the* baking process i.e. by about 3 log CFU g^{-1} in gluten-free sorghum bread. The use of tragacanth gum strongly influences the survival of encapsulated *L. acidophilus and L. Plantarum* during baking and storing processes indeed strongly influenced, so encapsulating probiotic cells using tragacanth gum could increase their viability to more than 2 log cycles in gluten-free sorghum bread during storage. The tragacanth gum showed a high protective effect on *L. acidophilus and L. Plantarum* cells causing better moisture retention in bread and reducing the hardness changes during 72 h storage.

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Conflict of interests

There is no conflict of interest in the study.

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