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Development of Probiotic Apple Juice using Encapsulated Probiotics in Xanthan-Chitosan Based Hydrogels

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Abstract

Background and Objective: Non-dairy probiotic beverages such as fruit juices have been popular for all age groups due to their vitamins, minerals, antioxidants and bioactive components with no allergens such as milk proteins and lactose. To exert their health benefits, probiotics should survive during food processing and storage as well as gastrointestinal tract. Incorporation of probiotics into fruit juices is more challenging than that in dairy products because of the low pH. Therefore, encapsulation of probiotics using various hydrocolloids and appropriate methods can protect probiotics from detrimental factors, improving their viability.

Material and Methods: In the present study, free and encapsulated *Lactiplantibacillus plantarum* and *Bifidobacterium bifidum* were incorporated into apple juice and physicochemical characteristics of the fruit juice (pH, acidity, °Brix and color), viability of the probiotics and sensory evaluation of apple juices were investigated during 60-d storage at 4 °C.

Results and Conclusion: Results showed encapsulation efficiencies of 90.21 and 85.54% for *Lactiplantibacillus plantarum* and *Bifidobacterium bifidum* in xanthan-chitosan hydrogels, respectively. *Lactiplantibacillus plantarum* and *Bifidobacterium bifidum* populations showed logarithmic decreases of 2.21 and 2.54 in free and 0.93 and 1.02 in encapsulated forms, respectively. However, the two bacteria survived in encapsulated form until the end of storage. In apple juices with free *Lactiplantibacillus plantarum* and *Bifidobacterium bifidum*, pH decreased from 3.7 to 3.11 and 3.3, respectively. In encapsulated probiotics, no significant differences were seen in pH of the samples. Moreover, apple juice samples with free probiotics included lower °Brix and higher acidity compared to the samples with encapsulated bacteria. Sensory evaluation of samples revealed that apple juices with encapsulated probiotics received higher scores than that apple juices with free bacteria did. The highest (3.95) and the lowest (2.48) overall acceptability scores after 60 d of storage were observed in samples containing encapsulated *Bifidobacterium bifidum* and free *Lactiplantibacillus plantarum*, respectively. It can be induced that use of xanthan-chitosan hydrogels can be used for the efficient encapsulation of probiotics and improve their survival during storage without adverse effects on sensory evaluation.

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1. Introduction

Recently, consumption of functional foods containing probiotics is increasing worldwide. Probiotics exert various health benefits, including decrease of lactose intolerance, decrease of blood cholesterol, stimulation of the immune system, relief from constipation and increase of mineral absorption as well as anti-mutagenic, anti-carcinogenic and

antihypertensive effects [1-3]. Dairy products are usually considered as appropriate vehicles for probiotic delivery; however, these products are not appropriate for consumption in people suffering from allergy to milk-based products due to the presence of lactose, casein and whey proteins [4,5]. Therefore, other food matrices such as fruit juices can be ideal carriers for probiotics as they contain nutrients including minerals, vitamins and antioxidants, include taste profiles that are pleasant to all age groups and are realized as healthy agents [6] *Lactobacillus* and *Bifidobacterium* genera are the most important probiotic microorganisms commonly incorporated into beverages and food products. *Lactiplantibacillus* (*L.*) *plantarum* has been associated with increasing bioactive compounds such as anthocyanin, phenolics, flavonoids and antioxidants of fruit juices. This species is commonly used for the fermentation of plant matrices as its metabolism can be further adapted to these substrates [7]. In addition to lactobacilli, *Bifidobacterium* (*B.*) *bifidum* is a Gram-positive probiotic with human origin that prevent and treat gastrointestinal disorders including intestinal infections and cancers as well as lowering blood cholesterol and stimulating the immune system [8].

Despite the highlighted beneficial characteristics, low pH can be a detrimental factor on the viability of probiotics in fruit juices. It has been described that probiotics should be present at a minimum level of 10^6 CFU.g^{-1} food products [9], 10^7 CFU.g^{-1} at point of delivery or consumed in sufficient quantities to achieve a daily intake of 10^8 CFU.g^{-1} [10] to exhibit their health benefits. Furthermore, probiotic bacteria should not affect sensory evaluation of the juices through processing and storage of the products [11]. Therefore, encapsulation of probiotics can be addressed as a promising process for the protection of bacterial cells from environmental factors in food products and during gastrointestinal transition [12]. Various natural polymers such as alginate, starch, gelatin, carrageenan, xanthan and chitosan have been used for the encapsulation of probiotics. From these biopolymer-based encapsulation systems, xanthan-chitosan mixture has been considered as a hydrogel system with high potential for targeted delivery and controlled release of encapsulated compounds [13]. Xanthan is an anionic polysaccharide composed of a cellulose backbone with two mannose and one glucuronic acid side chains per glucose residue [14]. Chitosan is composed of (1,4)-linked 2-amino-deoxy-β-D-glucan and carries positive charges below pH 6.5 due to the presence of amino groups [15]. Therefore, the ionic interaction of the amino groups of chitosan and the carboxyl groups of xanthan gum leads to the formation of a three-dimensional network with low toxicity, high resistance to enzyme and pH-sensitive swelling [16]. Xanthan-chitosan polyelectrolyte complexes have mostly been used in encapsulation of enzymes and there is a little information on its uses for probiotics [17,18]. Therefore, the aim of this study was to assess viability, physicochemical characteristics and sensory evaluation of apple juice as a non-dairy probiotic carrier in free and encapsulated forms in xanthan-chitosan based hydrogels.

2. Materials and Methods

2.1. Materials

Two probiotic strains including *L. plantarum* PTCC1058 and *B. bifidum* PTCC1644 were provided by the Persian Type Culture Collection, Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. Xanthan gum with a molecular weight of 1.02 mDa was purchased from Zhongxuan Biological Chemistry, China. Chitosan (medium molecular weight, 75-85% degree of deacetylation) and L-cysteine hydrochloride were purchased from Sigma-Aldrich, USA. Furthermore, MRS agar and broth media were supplied from Merck, Germany. Apple juice concentrate was purchased from Takdaneh, Iran.

2.2. Preparation of xanthan and chitosan solutions

Xanthan gum powder was dissolved in deionized water with heat and agitation to a concentration of 0.7% (w v^{-1}). Chitosan was dissolved in 1% v v^{-1} acetic acid to a concentration of 0.7% w v^{-1} and pH was 4.5. Solutions were autoclaved at 110 ºC for 10 min before use.

2.3. Probiotic culture preparation

Freeze-dried cultures were grown in MRS agar based on the method of Rajaie Azarkhavarani et al. [19]. Briefly, recovered cultures (each 1 ml) were inoculated into 100 ml of MRS broth and incubated at 37 °C for 18 h. Freshly prepared cultures were mixed with 50% (v v^{-1}) sterile glycerol (ratio of 2:1, respectively). To prepare bacterial inocula, a single colony from MRS agar plate was transferred into 10 ml of MRS broth under sterile condition and incubated at 37 °C for 24 h under aerobic condition using shaker incubator at 150 r/min. *B. bifidum* was cultured in similar media with 0.05% (w v^{-1}) of L-cysteine at 37 °C for 24 h using anaerobic incubator. Then, culture was transferred into 100 ml of MRS broth and incubated under similar conditions. Bacterial cells were separated by centrifugation at 1500× g for 10 min at 25 °C, washed with sterile saline solution and centrifuged under similar conditions. The achieved cells were resuspended in 5 ml of sterile saline solution and then divided into two parts for use as free and encapsulated cells. Viability of the probiotic bacteria was assessed using pour plate technique in MRS agar.

2.4. Microencapsulation process

Extrusion technique was used for the preparation of probiotic microcapsules [20]. Bacterial culture was washed and resuspended in xanthan solution (1: 10). Then, mixture was extruded dropwise into sterile chitosan solution using 0.1-mm needle. To complete gelation process and hardening, beads were set in chitosan solution for 30 min. Probiotic-loaded microcapsules were filtered and washed with sterile distilled water (DW) and set in 0.1% sterile peptone solution at 4 °C.

2.5. Preparation of the probiotic apple juice

An apple juice concentrate (Brix 70) was diluted to Brix 14 by adding distilled water and then pasteurized at 85 ºC for 60 s. Four treatments including juices including free *L. plantarum* and *B. bifidum* and juices including encapsulated *L. plantarum* and *B. bifidum* were prepared and stored at 4 ºC for 60 d.

2.6. Assessments of the bacterial survival and encapsulation efficiency

Microencapsulated probiotics (5 g) and free cells (5 ml) were separately added to apple juice (100 ml) under sterile condition and then stored at 4 °C. Enumeration of viable cells was carried out at 0, 15, 30, 45 and 60 d of storage. To enumerate encapsulated bacteria in apple juice, beads were resuspended in 50 ml of sodium citrate solution (1 M, pH 7) under vigorous agitation for 5 min to break the capsules and release the bacteria. Then, serial dilutions were made in 0.1% (w v^{-1}) peptone water followed by pour plating in MRS agar. Plates were incubated at 37 °C for 72 h using anaerobic jar and Anaerogen (Oxoid, England), with enumeration of the probiotic microorganisms [21]. Encapsulation efficiency (EE%) was expressed as number of colony forming unit (CFU. ml^{-1}) and EE was calculated based on the Eq 1:

$$EE\% = \frac{Log\ N1}{Log\ N0} \times 100 \qquad \text{Eq.1}$$

Where, N_0 was the number of entrapped bacterial cells inside the beads and N_1 was the number of free bacterial cells added into the biopolymer mixture during the preparation process.

2.7. Physicochemical assessments

Briefly, pH changes in juice containing free and encapsulated probiotics were assessed through storage time. Titratable acidity was assessed using titration with 0.1 N sodium hydroxide solution and expressed as citric acid per 100 g of sample. Total soluble solid (ºBrix) was assessed using refractometer (CETi; ABBE, Belgium). HunterLab Colorflex EZ colorimeter, USA, was used to analyze color of the fruit juice using CieLab system with illuminant C. Assessments of L*, a* and b* were carried out through the storage and results were expressed as the total color difference (ΔE_{ab}*) between each stored sample and the freshly-processed sample at a fixed storage time using the Eq. 2:

$$\Delta E_{ab}^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \qquad \text{Eq.2}$$

Where, Δ included differences in the color parameter between the stored and freshly-processed samples.

2.8. Sensory evaluation

A panel of 30 assessors assessed sensory evaluation of the samples including free, encapsulated and control samples. Sensory evaluation included appearance, flavor, color and overall acceptability parameters. Each of these parameters was scored on a five-point scale of 0 = not consumable, 1 = unacceptable, 2 = acceptable, 3= satisfactory, and 4 = excellent. Juice samples were given to the panelists at 4 °C in 3-digit coded containers (30 ml) and mineral water was served for rinsing mouths between the treatments. Sensory analysis was carried out on days 1 and 60 of storage.

2.9. Statistical analysis

All experiments were carried out in triplicate and results were expressed as mean ±SD (standard deviation). Differences between the mean values were analyzed using ANOVA and Duncan multiple range tests. Moreover, SPSS software v.23.0 (IBM, USA) was used to analyze the experimental data. Differences at $p \leq 0.05$ were reported as significant.

3. Results and Discussion

3.1. Survival of bacteria during storage and encapsulation efficiency

In this study, encapsulation efficiency of *L. plantarum* and *B. bifidum* in xanthan-chitosan hydrogels included 90.21 ±0.03 and 85.54 ±0.05, respectively. In a study by Chen et al. [16], encapsulation yield of *B. bifidum* BB01 in xanthan-chitosan hydrogels was reported as 92.46%. Shu et al. [22] encapsulated *L. acidophilus* in xanthan-chitosan hydrogels and achieved optimized microcapsules with an encapsulation efficiency of 86%. It is clear that encapsulation efficiency in xanthan-chitosan based hydrogels depends on various factors including xanthan and chitosan concentrations, xanthan/chitosan ratios, xanthan-bacterial suspension ratios and stirring times [23,24]. As present in Table 1, *L. plantarum* and *B. bifidum* populations showed decreases of 2.21 and 2.54 in free and 0.93 and 1.02 in encapsulated forms, respectively. Furthermore, *L. plantarum* showed higher viability due to its higher tolerance to low pH as well as increasing in bioaccessibility to phenolic compounds with prebiotic functions [25].

For the two strains, final cell count of the encapsulated probiotics in apple juice after 60 d of storage was significantly higher than that of the free bacteria ($p \leq 0.05$).

Table 1. Viabilities of free and encapsulated probiotic bacteria (log CFU.ml^{-1}) in apple juices during 60 d of storage at 4 °C

Probiotic	Time (days)						
	Type	0	15	30	45	60	Log reduction
L. plantarum	Free	9.34±0.05	9.09±0.04	8.22±0.06	7.48±0.05	7.13±0.02	2.21[a]
	Encapsulated	9.66±0.01	9.43±0.04	9.22±0.03	9.09±0.01	8.73±0.07	0.93[b]
B. bifidum	Free	9.65±0.03	9.25±0.02	8.46±0.06	7.35±0.06	7.09±0.04	2.54[a]
	Encapsulated	9.52±0.05	9.32±0.05	9.18±0.05	9.04±0.05	8.50±0.05	1.01[b]

Means with different letters indicate significant difference ($p \leq 0.05$).

Mokhtari et al. [26] added *L. acidophilus* and *B. bifidum* in the form of free and encapsulated alginate beads to pasteurized grape juice. At the end of storage, encapsulated probiotics demonstrated higher survival rates compared to free probiotics due to the protective layers around the bacterial cells. Similarly, *L. plantarum* in free form and encapsulated in alginate-soy protein isolate-based hydrogel beads was added to mango juice after pasteurization. Free cells were killed in mango juice after exposure to pasteurization temperature (72 °C, 90 s). In mango juice containing encapsulated probiotics, 1 log CFU.ml^{-1} decrease was observed in the population of *L. plantarum.* Viability of the encapsulated *L. plantarum* in mango juice decreased from 8.56 to 5.83 log CFU.ml^{-1} during 28 d of storage, while no free probiotic cells were detected in pasteurized mango juice during the storage [27]. Shu et al. [22] reported 1.2 and 0.7 log CFU.ml^{-1} losses in *L. acidophilus* numbers entrapped in xanthan-chitosan and xanthan-chitosan-xanthan hydrogels respectively during 21 d of storage at 4 °C. Although probiotic populations decreased during storage in the present study, the final cell count was still in the necessary level (10^6 CFU.ml^{-1}) for probiotic products [28].

3.2. Physicochemical analysis

The initial pH of control samples without bacteria was 3.7 on Day 0 and decreased in all treatments during storage (Figure 1). In juice samples containing free *L. plantarum* and *B. bifidum*, pH decreased from 3.7 to 3.11 and 3.3, respectively and the decrease was higher, compared to the juice samples containing encapsulated probiotics. This could be attributed to the utilization of carbohydrates in fruit juice and generation of organic acids, thus decreases of pH [29]. Similar results have been reported by Shah et al. [30] who reported decreases in pH of model fruit juice containing free *L. rhamnosus*, *B. lactis* and *L. paracasei* after 42 d of storage at 4 ºC. In another study, orange juice containing encapsulated *L. rhamnosus* GG and *L. acidophilus* NCFM showed higher pH compared to juice samples with free probiotics after 12 d of storage at 25 ºC or 35 d of storage at 4 ºC [31].

Gandomi et al. [32] reported decreases in pH of apple juice containing free *L. rhamnosus* from 3.82 to 3.46 during 90 d of storage. In contrast, Antunes et al. [33] stated no significant differences in pH and acidity values of aleo nectars containing free and encapsulated *B. animalis* subsp. *lacti*s BB-12. Furthermore, no changes were recorded in pH of orange juice containing free and encapsulated *L. rhamnosus* GG during storage [34]. Lower pH in juice samples containing free *L. plantarum* compared to *B. bifidum* could be linked to the higher acidifying activity of *Lactobacillus* spp. than *Bifidobacterium* spp. Naturally, the optimum pH of *B. bifidum* is 6.5-7.3 while *L. plantarum* survives in pH as low as 3.2 [35]. In samples with encapsulated probiotics, no significant differences were seen between the juices containing *L. plantarum* and *B. bifidum*. However, this was lower for pH decreases compared to other samples. Xanthan-chitosan coating created a barrier and limited the diffusion of sugars into the microcapsules [36]. This report was similar to the reports of Mokhatri et al. [26] and Shah et al. [30].

Based on Figure 2, the initial acidity of apple juice was 4.5 g 100 ml^{-1}. During 60 d of storage, acidity increased in all samples except the control. The highest acidity was observed in juice samples containing free bacteria due to accessibility and fermentation of sugars by the bacteria. No significant difference was observed between the control and samples containing *B. bifidum*; however, higher acidity was recorded in samples containing *L. plantarum* due to a higher fermentation activity, similar to the results by Antunes et al. [33] who reported the mean titratable acidity values of 0.2, 0.29 and 0.28 g/100 g for the control sample and for those with probiotics added in the form of microparticles and free cells respectively through 35 d of storage with no significant differences between the treatments. Similarly, Afzaal et al. [37] reported the highest and the lowest increases in acidity of grape juices containing free and encapsulated probiotics in sodium alginate, respectively. Accessibility of free bacteria to utilize sugars in fruit juice and production of acids was reported as the reason of the achieved results. Moreover, it has been reported that the dead and/or degraded probiotic cells without capsules could release enzymes to hydrolyze sugars in the fruit juice, affecting two parameters of pH and acidity over extended cold storage times [38].

Praepanitchai et al. [27] indicated that mango juice containing encapsulated *L. plantarum* TISTR 050 in calcium-alginate-soy protein isolate-based hydrogel beads included constant acidity values during 35 d of storage. Additionally, encapsulation of the probiotics was able to preserve pH and acidity of the fruit juice during storage. For total soluble solids (Figure 3), no significant changes were recorded in apple juices with encapsulated probiotics whereas significant decreases were seen in ºBrix of samples containing free probiotics due to consumption of sugars.

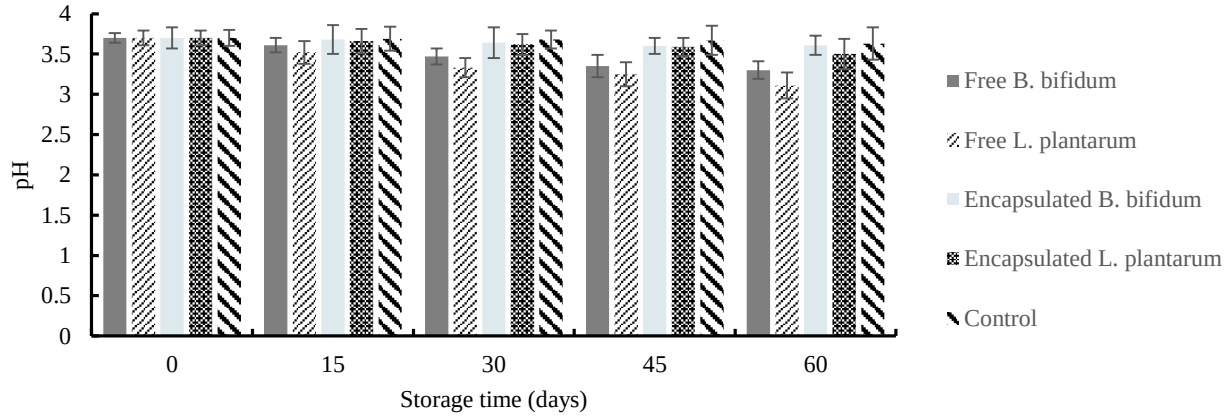

Figure 1. The pH changes in apple juices containing free and encapsulated probiotics during 60 d of storage at 4 °C [vertical bars show standard error of the mean (±SD)]

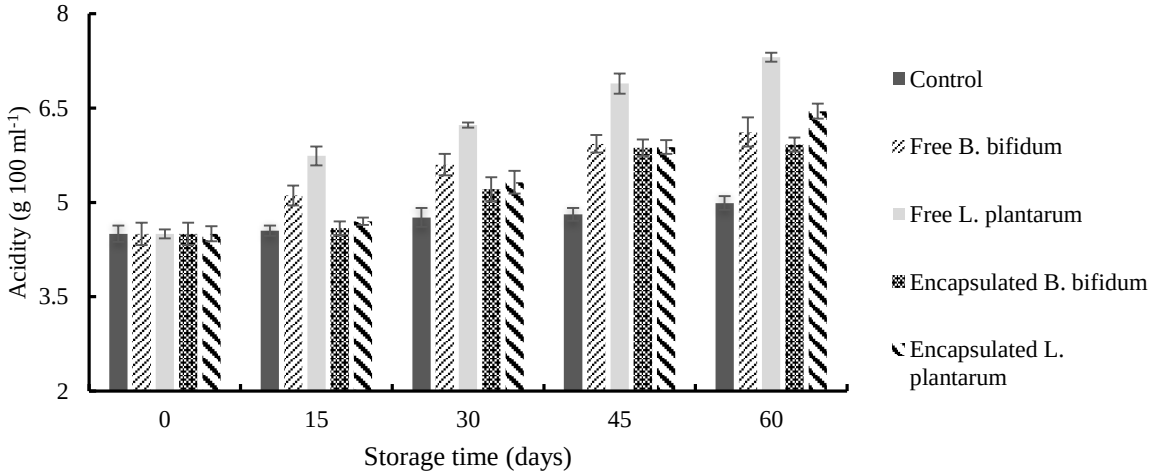

Figure 2. Acidity changes in apple juices containing free and encapsulated probiotics during 60 d of storage at 4 °C [vertical bars show standard error of the mean (±SD)]

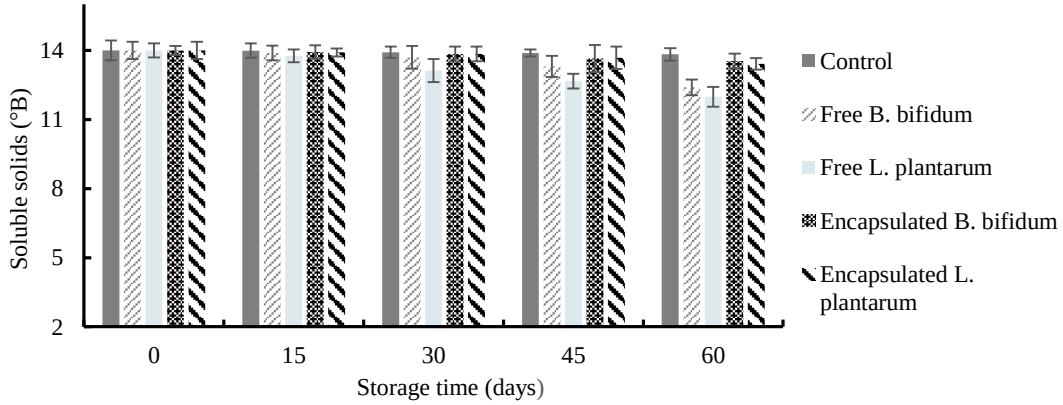

Figure 3. Brix changes in apple juices containing free and encapsulated probiotics during 60 d of storage at 4 °C [vertical bars show standard error of the mean (±SD)]

In a study by Nami et al. [39], Brix value of orange juice with unencapsulated probiotic cells significantly decreased from 11 to 3.1% compared to encapsulated probiotics in alginate-Persian gum. Results demonstrated that free probiotic cells used higher quantities of sugars in orange juice than those microencapsulated probiotics did. These results were similar to the results by Afzaal et al. [37] who reported further gradual decreases of Brix in grape juices containing encapsulated probiotics compared to those with free probiotics due to indirect accesses to soluble sugars.

Differences between the colors of the sample and control is interpreted as ΔE. On Day 1 of storage, differences in color of all samples were recorded compared to the control (Table 2). Color variation was higher in samples with encapsulated probiotics compared to that in free bacteria. This could be associated to the existence of colorless xanthan-chitosan microcapsules in yellow background of the apple juice which led to decreases in the color. During storage, no significant differences were recorded in the color of samples with free probiotics. Antunes et al. [33] described that addition of probiotics in free or encapsulated form did not affect the color of apple juices.

3.3. Sensory evaluation

Sensory stability during storage is an important characteristic that should be considered in formulated probiotic food products [32]. As shown in Table 3, no significant differences were seen between appearance and color of the apple juices containing free *L. plantarum* and *B. bifidum* and the control samples on Day 1 of storage while juice samples containing microcapsules received the lowest scores. This lower acceptability by the consumers could be attributed to the presence of white microcapsules in the juices. In fruit juices containing probiotic beads (1-2 mm), less than 20% of the consumers did not like the product because the microcapsules remained in the mouth and induced unpleasant feeling in the throat [40]. For the Flavor, apple juices containing encapsulated probiotics received higher scores than those with free probiotics due to the fermentative activity of bacteria and production of various metabolites into the juices. Regarding overall acceptability after 60 d of storage, the highest score was attributed to the control with no significant differences ($p \leq 0.05$) between the scores of control and juices containing probiotic microcapsules.

Gandomi et al. [32] reported that apple juice samples containing *L. rhamnosus* GG included improvements in sensory evaluation (color, flavor, transparency, odor and texture). Similarly, Krasaekoopt and Kitsawad [40] showed that the presence of chitosan coated alginate beads in fruit juices positively affected their sensory evaluation. Moreover, apple juices with free probiotic bacteria were found as undesirable and sour with buttery flavor that can be ascribed to the fermentation metabolites released from the free cells [41] while the encapsulation limited acidification [31]. It has been suggested that acidification occurred due to the release of enzymes from the dead bacteria and hydrolysis of sugars in the fruit juice [42]. These results were similar to those by Rajaie Azarkhavarani et al. [19] who explained that incorporation of free probiotic cells into the sour cherry juice changed color of the fruit juice at the end of storage.

Table 2. Total differences in color (ΔE) of apple juices during 60 d of storage at 4 °C

Apple juice samples	Time (days)				
	0	15	30	45	60
Apple juice+ free *L. plantarum*	4.11 ± 0.06^{bD}	4.19 ± 0.09^{bC}	4.87 ± 0.08^{aD}	5.03 ± 0.03^{aD}	5.11 ± 0.04^{aB}
Apple juice+ free *B. bifidum*	3.99 ± 0.05^{cC}	4.09 ± 0.03^{cD}	4.64 ± 0.07^{bC}	4.82 ± 0.06^{bC}	4.97 ± 0.02^{aB}
Apple juice+ encapsulated *L. plantarum*	7.18 ± 0.09^{cA}	7.23 ± 0.03^{cA}	7.76 ± 0.04^{bA}	7.92 ± 0.05^{aA}	8.03 ± 0.03^{aA}
Apple juice+ encapsulatd *B. bifidum*	6.95 ± 0.10^{eB}	7.11 ± 0.04^{dB}	7.38 ± 0.06^{cB}	7.69 ± 0.02^{bB}	7.94 ± 0.05^{aA}

*Values with different lower case letters in the same row group are significantly different ($p \leq 0.05$).
Values with different uppercase case letters in the same column group are significantly different ($p \leq 0.05$).*L.* = Lactobacillus, *B.* =Bacillus

Table 3. Sensory evaluation of apple juices containing free and encapsulated probiotics during 60 d of storage at 4 °C

Treatments	Appearance		Flavor		Color		Overall acceptability	
	1	60	1	60	1	60	1	60
Control	$4.01\pm0.04^{aA*}$	3.96 ± 0.05^{aA}	4.11 ± 0.05^{aA}	4.05 ± 0.04^{aA}	4.23 ± 0.04^{aA}	4.21 ± 0.02^{aA}	4.11 ± 0.02^{aA}	4.12 ± 0.05^{aA}
Apple juice +free *L. plantarum*	3.94 ± 0.05^{aA}	3.92 ± 0.01^{aA}	2.77 ± 0.06^{aC}	1.63 ± 0.01^{bC}	3.93 ± 0.06^{aB}	3.89 ± 0.01^{aB}	2.53 ± 0.02^{aD}	2.48 ± 0.02^{aD}
Apple juice+ free *B. bifidum*	3.98 ± 0.02^{aA}	3.90 ± 0.03^{aA}	2.84 ± 0.01^{aC}	1.89 ± 0.02^{bC}	4.02 ± 0.01^{aB}	3.98 ± 0.03^{aB}	2.79 ± 0.01^{aC}	2.66 ± 0.04^{bC}
Apple juice+encapsulated *L. plantarum*	3.01 ± 0.01^{aB}	2.79 ± 0.05^{bB}	3.95 ± 0.02^{aB}	3.80 ± 0.03^{bB}	3.32 ± 0.01^{aC}	3.27 ± 0.06^{aC}	3.94 ± 0.05^{aB}	3.91 ± 0.04^{aB}
Apple juice+ encapsulatd *B. bifidum*	2.88 ± 0.03^{aB}	2.80 ± 0.02^{aB}	3.99 ± 0.03^{aB}	3.86 ± 0.06^{bB}	3.29 ± 0.05^{aC}	3.26 ± 0.02^{aC}	3.98 ± 0.02^{aB}	3.95 ± 0.01^{aB}

*Values with different lower case letters in the same row group are significantly different ($p \leq 0.05$).
Values with different uppercase case letters in the same column group are significantly different ($p \leq 0.05$). *L.* = Lactobacillus, *B.* =Bacillus

4. Conclusion

Results of this study revealed that development of apple juice containing *L. plantarum* and *B. bifidum* probiotics using xanthan-chitosan is quite feasible and encapsulated bacteria maintained their viability through the cold storage for 60 d and cell population numbers were higher than the necessary numbers for probiotic products. Moreover, encapsulation did not affect sensory evaluation of the apple juice and samples were acceptable by the consumers. However, size of the microcapsules could be optimized based on the highest efficiency and mouth feel for a better appearance. Moreover, results indicated that apple juice could be an appropriate delivery vehicle for the probiotics. However, further studies are needed to investigate stability of encapsulated probiotics in xanthan-chitosan based hydrogels under simulated gastrointestinal conditions as for other fruit juices. Additionally, it is noteworthy to design studies for the optimization of encapsulation processes and assessment of the effects of various variables such as xanthan and chitosan concentrations, pH and stirring time to achieve the maximum EE% and viability.

5. Acknowledgements

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6. Conflict of Interest

The authors report no conflict of interest.

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توسعه آب سیب پروبیوتیک حاوی پروبیوتیک‌های ریزپوشانی‌شده در هیدروژل بر پایه زانتان-کیتوزان

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چکیده

سابقه و هدف: تمایل به مصرف نوشیدنی‌های پروبیوتیک غیرلبنی مانند آب‌میوه‌ها به دلیل وجود ویتامین‌ها، مواد معدنی، آنتی‌اکسیدان‌ها و ترکیبات زیست‌فعال و نبود ترکیبات حساسیت‌زا مانند پروتئین‌های شیر و لاکتوز، برای همه گروه‌های سنی افزایش یافته است. زیست‌یارها[۱] باید در طول فرآوری و نگهداری محصولات غذایی و همچنین هنگام گذر از دستگاه گوارش، زنده بمانند تا اثرات سلامت‌بخش خود را نشان دهند. افزودن زیست‌یارها به آب‌میوه‌ها به دلیل pH پایین، دارای چالش‌های بیشتری در مقایسه با محصولات لبنی است. از این‌رو، ریزپوشانی زیست‌یارها با استفاده از هیدروکلوئیدهای گوناگون می‌تواند یکی از راه‌های مناسب برای حفاظت آن‌ها از عوامل نامطلوب محیطی باشد و موجب بهبود زنده‌مانی شود.

مواد و روش ها: در این مطالعه، *لاکتی‌پلانتی‌باسیلوس (ل.) پلانتاروم* و *بیفیدوباکتریوم بیفیدوم* به صورت آزاد و ریزپوشانی‌شده به آب سیب افزوده شدند و ویژگی‌های فیزیکوشیمیایی آب سیب (pH، اسیدیته قابل تیتراسیون، بریکس و رنگ)، زنده‌مانی زیست‌یارها و ویژگی‌های حسی نمونه‌های آب سیب در طول ۶۰ روز نگهداری در دمای ۴°C، بررسی شد.

یافته‌ها و نتیجه‌گیری: نتایج نشان داد که کارایی ریزپوشانی *ل. پلانتاروم* و *ب. بیفیدوم* در هیدروژل زانتان-کیتوزان، به‌ترتیب ۹۰/۲۱ ٪ و ۸۵/۵۴ ٪ بود. میزان کاهش جمعیت *ل. پلانتاروم* و *ب. بیفیدوم* در نمونه‌های آب سیب به شکل آزاد به‌ترتیب ۲/۲۱ و ۲/۵۴ سیکل لگاریتمی و به شکل ریزپوشانی‌شده به‌ترتیب، ۰/۹۳ و ۱/۰۲ سیکل لگاریتمی بود. با این‌حال، هر دو باکتری زیست‌یار به شکل ریزپوشانی‌شده تا انتهای دوره نگهداری زنده ماندند. در نمونه‌های آب سیب حاوی زیست‌یارهای آزاد *ل. پلانتاروم* و *ب. بیفیدوم*، pH به ترتیب از ۳/۷ به ۳/۱۱ و ۳/۳، کاهش یافت. در نمونه‌های حاوی زیست‌یارهای ریزپوشانی‌شده، تفاوت معنی‌داری در pH نمونه‌ها، مشاهده نشد. همچنین، نمونه‌های حاوی زیست‌یار آزاد بریکس کمتر و اسیدیته بالاتری در مقایسه با نمونه‌های حاوی باکتری‌های ریزپوشانی‌شده داشتند. ارزیابی حسی نمونه‌ها نشان داد که نمونه‌های آب سیب حاوی باکتری‌های ریزپوشانی‌شده، امتیاز بالاتری نسبت به نمونه‌های حاوی باکتری آزاد، دریافت کردند. بالاترین نمره پذیرش کلی (۳/۹۵) و کمترین نمره (۲/۴۸) پس از ۶۰ روز نگهداری، به‌ترتیب در نمونه‌های حاوی *ب. بیفیدوم* ریزپوشانی‌شده و *ل. پلانتاروم* آزاد، مشاهده شد. با توجه به نتایج به دست‌آمده، می‌توان نتیجه گرفت که هیدروژل زانتان-کیتوزان می‌تواند به‌طور موثری برای ریزپوشانی زیست‌یارها استفاده شود و زنده‌مانی آن‌ها را در طول نگهداری بدون داشتن اثر نامطلوب بر ویژگی‌های حسی، بهبود دهد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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