Journal of Food Biosciences and Technology,
Islamic Azad University, Science and Research Branch, Vol. 13, No. 2, 27-38, 2023
DOI:10.30495/jfbt.2022.69606.10298 https://dorl.net/dor/20.1001.1.22287086.2023.13.2.3.1

Changes in Microbial, Rheological, and Sensory Characteristics of Probiotic Yogurt Sauce Containing *Lactobacillus rhamnosus* During Cold Storage

H. Pourjavid [a], ***M. Ataee*** [b], ***R. Pourahmad*** [c*], ***A. A. Anvar*** [d], ***H. Behmadi*** [e]

[a] PhD student of the Department of Food Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[b] Assistant Professor of the Department of Food Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[c*] Professor of the Department of Food Science & Technology, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran.
[d] Associate Professor of the Department of Food Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[e] Assistant Professor of the Agricultural Engineering Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.

Received: 19 September 2022 Accepted: 10 October 2022

ABSTRACT: Nowadays, there is a great interest in using yogurt sauce as a flavored dressing for salads and foods. The current study aimed to determine the possibility of producing probiotic yogurt sauce and evaluate its physicochemical, microbiological, sensory and rheological properties throughout refrigeration storage. *Lactobacillus rhamnosus* was encapsulated with sodium alginate and resistant starch using the emulsion method. The survival of free and microencapsulated *L. rhamnosus* was studied in simulated gastrointestinal conditions. Two forms (free and microencapsulated) of *L. rhamnosus* were added to the yogurt sauce. The samples were kept for 30 days at 4 ˚C and evaluated on interval days (1st, 10th, 20th, and 30th) for the above mentioned properties. Survival improvement was demonstrated in microencapsulated *L. rhamnosus* compared to free *L. rhamnosus*. After two hours, the free form of *L. rhamnosus* showed five logarithmic cycles decreasing in cell viability, while microencapsulated *L. rhamnosus* presented only one logarithmic cycle reduction. Similarly, on day 30 of storage, the number of viable microencapsulated *L. rhamnosus* cells in the probiotic yogurt sauce was 6.61 log CFU/g, while the viable count for a sample containing free *L. rhamnosus* was 5.00 log CFU/g. The produced probiotic yogurt sauce was considered a pseudo-plastic fluid and presented mayonnaise behaviour. Moreover, samples containing microencapsulated probiotic bacteria displayed lower post-acidification values than samples containing free bacteria. The microencapsulation of probiotic bacteria improved the sensory quality of the produced probiotic yogurt sauce. Hence, producing a probiotic yogurt sauce with desirable properties is possible.

Keywords: *Lactobacillus rhamnosus*, Microencapsulation, Probiotic Yogurt Sauce.

Introduction

Probiotics are living microorganisms that, when employed in suitable quantities provide health benefits to the host (Kaur Sidhu *et al*., 2020). Probiotics are usually added to foodstuffs as a supplement to offer benefits to consumers such as regulation of immune response or use to treat gastrointestinal illness (Mora-Villalobos *et al*., 2020). Probiotic bacteria usually belong to two genera: *Lactobacillus* and *Bifidobacterium* (Afzaal

*Corresponding Author: rjpourahmad@yahoo.com, rezvanpourahmad@iauvaramin.ac.ir

et al., 2019), Lactobacilli are Gram-positive rods and members of the lactic acid bacteria (characterized by the formation of lactic acid as a product of carbohydrate metabolism) and are found in the normal gastrointestinal flora (Tannock, 2004). Among them, *L. rhamnosus* is a probiotic with curative activity and is primarily used in dairy products such as fermented milk and its derivatives (Afzaal *et al*., 2019). To be considered probiotics, they must be metabolically stable in foods, survive during storage, and survive passage through the human gastrointestinal system (Afzaal *et al*., 2019).

Several investigations have shown that microencapsulation can be used to protect probiotics from harsh conditions in the gastrointestinal tract and throughout production procedures (Afzaal *et al*., 2019; Liu *et al*., 2020; Qi *et al*., 2019). Microencapsulation is a method by which a substance (e.g., a probiotic) is coated and protected with wall materials (e.g., sodium alginate and resistant starch) (Krasaekoopt *et al*., 2003, 2006).

Sodium alginate has been used as a microencapsulation material owing to its remarkable characteristics such as simplicity of the process, biocompatibility, and no toxic effect on probiotic strains (de Araújo Etchepare *et al*., 2016). Moreover, this polymer can protect probiotics against process factors and storage circumstances including, dissolved oxygen and heat (de Araújo Etchepare *et al*., 2016). Apart from all the good features of sodium alginate for microencapsulation, its produced gel is sensitive to pH, which can affect the protection of the encapsulated material. Adding resistant starch in the formulation of the micro-particles is a way to overcome this problem (de Araújo Etchepare *et al*., 2016). Resistant starch, a trivial portion of starch resistant to digestion, can be fermented by the healthy microflora in the large intestine of humans (de Araújo Etchepare *et al*., 2016). The favourable properties of resistant starch such as white color, slight taste, little water-holding capacity, and having a natural source, make it a valuable material to use in the microencapsulation of probiotics in foodstuffs (Homayouni *et al*., 2014). In addition, concurrent application of resistant starch with sodium alginate provides more protection to probiotic bacteria, which is due to the synergistic effect of the two mentioned materials (de Araújo Etchepare *et al*., 2016; Mirzaei *et al*., 2012).

Many microencapsulation methods have been used to produce microcapsules for protecting probiotics, in food products for instance, spray drying, extrusion, high-voltage electrostatics, and emulsion (Afzaal *et al*., 2019; Prasanna and Charalampopoulos, 2018). In the case of the spray drying technique, irrespective of its desirable features (e.g., economical and continuous operation), there are some disadvantages such as complexity of the tools, uncontrollable particle size, and the drying chamber's unstable condition (Nedovic *et al*., 2011). Furthermore, the bad shape of alginate beads and their large diameter produced by the extrusion technique are negative characteristics that can affect the encapsulated material. The high-voltage electrostatics method's small yield is not appropriate for high production scales (Qi *et al*., 2019).Thus, an emulsification technique was utilized as an alternative in this study to produce microcapsules due to its good properties such as smaller size of produced alginate capsules on a large scale, simple reactive settings, and simple process (Qi *et al*., 2019).

Yogurt is one of the best-flavored dairy products, which provides health benefits to customers because of its lactic acid

bacteria content (Afzaal *et al*., 2019). Nowadays, yogurt sauce has gained more popularity due to its pleasant taste, resulting from adding some seasonings such as vinegar, parsley, thyme, tarragon, olive oil, salt, pepper, and garlic to the pure yogurt. Considering the presence of the abovementioned bacteria in yogurt products, the production of novel yogurt derivatives would be of great interest.

This study aimed to produce a probiotic yogurt sauce containing *L. rhamnosus* and evaluate its physicochemical, microbiological, sensory, and rheological properties throughout 30 days of storage.

Materials and Methods

–*Preparation of probiotic bacterial culture*

The lyophilized culture of *L. rhamnosus* (ATCC 53103) was obtained from the Iranian Research Organization for Science and Technology. Then, it was transferred to Man-Rogosa Sharp broth (MRS) and incubated at 37 ˚C for 24 h under anaerobic circumstances (Golestani and Pourahmad, 2017). Subsequently, *L. rhamnosus* cells were centrifuged at 4000 rpm, at 4 ˚C for 15 min and washed in sterile saline solution 9% with the same centrifugation process. Later, the achieved probiotic cells were used in the microencapsulation procedure (Afzaal *et al*., 2019).

–*Microencapsulation of L. rhamnosus*

Every glassware and solutions used in the microencapsulation technique was decontaminated at 121 ˚C for 15 min. The emulsion procedure was set for the microencapsulation method. 2 g of sodium alginate (Sigma Aldrich 71238) together with 2 g of resistant starch (Hi maize 260, starch UK) were gradually added to 100 ml of distilled water up to completely dissolved. Then, 0.1% *L. rhamnosus* cultures were transferred into the mentioned solution while stirred for 5 min. The final solution was then suspended in 200 ml if corn oil containing 0.2% tween 80, (Merck, Germany) and mixed using stirrer with 350 rpm for 20 min to produce an emulsion. Subsequently, add 200 ml calcium chloride 0.1 M into the produced emulsion to create the alginate capsules as a result of phase separation. Following the rest of the mixture for 30 min, the alginate capsules were created in the bottom of the bottle. Finally, the alginate capsules were separated from the mixture by centrifugation (350 rpm, 15 min), and the capsules were washed with 1% peptone water and stored at 4 ˚C (Sultana *et al*., 2000).

–*Survival of free and microencapsulated L. rhamnosus in simulated gastric fluid (SGF) and simulated intestine fluid (SIF)*

Sodium chloride (0.2% w/v, pH=2) was used for the preparation of SGF followed by adding pepsin into the above solution to reach final concentration of 3.2 gr/L. To sterilise, the solution was filtered through a 0.22 µm membrane. In the 9.5 ml SGF, 0.5 ml of free bacteria and 0.5 g of microencapsulated bacteria were added and incubated at 37 ˚C (shaking incubator and 150 rpm). At the end of the incubation time, the SGF was neutralized by phosphate buffer (0.2 m/L, pH=7, at 4 ˚C). The viable number of free and microencapsulated *L. rhamnosus* cells was counted at determined interval times (0, 30, 60, 90 and 120 min) (Chen *et al*., 2017).

To prepare SIF, potassium hydrogen phosphate (K_2HPO_4) was dissolved into the distilled water to a final concentration of 6.8 g/L, pH=7.4. This was followed by adding pepsin into the above solution to reach a final concentration of 10 g/L. To

sterilise, the solution was filtered through a 0.22 µm membrane. In the 4.5 ml SIF, 0.5 ml of free bacteria and 0.5 g of microencapsulated bacteria were added and incubated at 37 C (shaking incubator and 150 rpm). At determined interval times (0, 30, 60, 90, and 120 min), the survival of free and microencapsulated *L. rhamnosus* was counted by a pour plate technique using Nutrient Yeast Extract Salt (NYSM) agar (Chen *et al*., 2017).

–*Production of probiotic yogurt sauce*

In order to prepare the yogurt sauce, the exact portion of ingredients such as citric acid (0.0314%), sugar (5%), mustard (0.3%), and salt (1.2%) were added to the water (12%) and entirely mixed. This was followed by adding an appropriate amount of yogurt (35%) to the prepared solution. Later, carrageenan fine particles were slowly added to the above prepared solution, followed by adding egg (9%) to the other ingredients and mixed up to achieve an even material. Oil drops (30%) were added to the final prepared solution, and at the end of this process yogurt sauce samples were made. Now, *L. rhamnosus* in two different forms (free or microencapsulated) were added to the yogurt sauce samples to produce the probiotic yogurt sauce. All yogurt sauce samples were placed at 4˚C for 30 days while they were tested for microbiological, physicochemical, and sensory analysis at intervals of days (0, 10, 20, and 30 day).

–*Measurement of pH and acidity*

A digital pH meter (Metrohn, Switzerland) at 20 ˚C was utilized to measure the pH of probiotic yogurt sauce samples. To measure acidity, distilled water was used (up to 100 mL) for dissolve 10 g of each probiotic yogurt sauce sample and mix completely. At that time, 1ml of phenolphthalein indicator was added into 25 ml of the prepared solution and it was titrated alongside standard sodium hydroxide solution until a pink colour appeared (Mokarram *et al*., 2009).

–*Rheological features*

The viscosity of probiotic yogurt sauce samples was measured using a Brookfield viscometer (Brookfield Engineering Lab Inc, Stoughton, MA) by spindle No.5, RPM 25, at 25 ˚C.

–*Probiotic bacterial count*

The colonies of *L. rhamnosus* were counted by plating on MRS-bile agar (Merck, Co. Germany) and incubating for 3 days at 37 °C under anaerobic circumstances (Afjeh *et al*., 2019).

–*Sensory evaluation*

Sensory evaluation (taste, color, texture, odor, and overall acceptance) was performed by a panel of 12 trained members. Ranking method was used. The quantified scores were chosen as follows: 5: delightful, 4: good, 3: acceptable, 2: bad, and 1: unacceptable.

–*Statistical analysis*

A completely randomized block was performed via the Duncan test statistical analysis system. Data were analyzed by SPSS 25 (SPSS Inc., Chicago, IL) software. Differences were considered significant when $p<0.05$. All data were stated as mean ± SD. The presented values were the mean of the triplicate analysis ± standard deviation.

Results and Discussion

–*Survival of Microencapsulated and Free L. rhamnosus in SGF and SIF*

According to Figure 1a, a significant difference ($p< 0.05$) was observed between viable probiotic cells in SGF after 2 hours. The number of *L. rhamnosus* cells in both

forms (microencapsulated and free) decreased during the 2 h. However, *L. rhamnosus* cells in microencapsulated form were more resistant to the harsh gastric conditions compared to those in free form. After 2 h, the free form of *L. rhamnosus* showed five logarithmic cycles decreasing in cell viability, while microencapsulated *L. rhamnosus* presented only one logarithmic cycle reduction. The results of this study confirmed the protective effect of microencapsulation for probiotic cells in SGF conditions. Similar findings have shown that microencapsulation of probiotic cells provides a proper protection against harsh SGF conditions (3, 6, 12). A recent investigation found that sodium alginate microcapsules enhanced the *L. acidophilus* survival in simulated gastrointestinal conditions and yogurt samples (Afzaal *et al*., 2019). Another study demonstrated increased viability of *Enterococcus faecium* and *Saccharomyces boulardii* in SGF conditions when probiotic cells were microencapsulated in alginate polymers (Qi *et al*., 2019). Moreover, Prasanna *et al.* reported that microencapsulation with various sodium alginate milk(cow and goat) matrices provided better protection for *B. longum* subsp. *infantis* cells in SGF conditions compared to free probiotic cells (Prasanna *et al*., 2018).

As it is shown in Figure 1b, the number of *L. rhamnosus* cells in both forms (microencapsulated and free) in SIF decreased during the 2 h. In comparison between two forms, microencapsulation provided a significant ($p< 0.05$) protection for probiotic cells in SIF through 2 hours. The viable count of microencapsulated *L. rhamnosus* showed less than one logarithmic cycle reduction, while at the same time and condition the viable count of free *L. rhamnosus* was reduced to four logarithmic cycles. The results of the present study confirmed the protective effect of microencapsulation for probiotic cells in SIF conditions. This may be because of the interaction of free probiotic cells with the bile salt and consequently loss of the cell walls integrity, leakage of intracellular materials, and cell death. Similarly, calcium alginate and sodium alginate matrices were shown to have an increased effect on the protection of microencapsulated *Lactobacillus acidophilus* and *L. rhamnosus* in SIF (Zanjani *et al*., 2012). Furthermore, a good protective effect of microencapsulated *Enterococcus faecium* and *Saccharomyces boulardii* was detected in SIF condition (Qi *et al*., 2019). In addition, the protective effect of microencapsulation with calcium alginate-whey protein for *L. paracasei* and *L. bulgaricus* cells was confirmed in SIF (Han *et al*., 2020).

–*Survival of probiotic bacteria in yogurt sauce*

The count of *L. rhamnosus* cells in refrigerated samples during 30-day storage is shown in Table 1. A decreasing trend in the number of viable *L. rhamnosus* cells was observed from day one to day 30. As expected, on day 30, the minimum viability (5.00 ± 0.01) was obtained by sample containing free *L. rhamnosus.* In addition, on the first day, the highest viability (7.88 ± 0.00) of probiotic cells was gained by sample containing microencapsulated *L. rhamnosus.* Our results also showed that on the day 30, the highest viability (6.61 ± 0.00) belonged to the sample containing microencapsulated *L. rhamnosus.* Microencapsulation increased the viability of *L. rhamnosus* in the probiotic yogurt sauce during storage. This finding is in accordance with the results of another study in which a decreasing trend was reported in the mortality of microencapsulated

Bifidobacterium bifidum cells compared to free probiotic cells, while sodium alginate and chitosan were used for coating the probiotic bacteria (Iqbal *et al*., 2019). Moreover, another investigation used microencapsulation with resistant starch and calcium alginate for *Bifidobacterium bifidum* and *Lactobacillus casei* cells. The result revealed an increased number of viable cells in microencapsulated form compared to the free form of probiotic bacteria in mayonnaise sauce samples (Fahimdanesh *et al*., 2013).

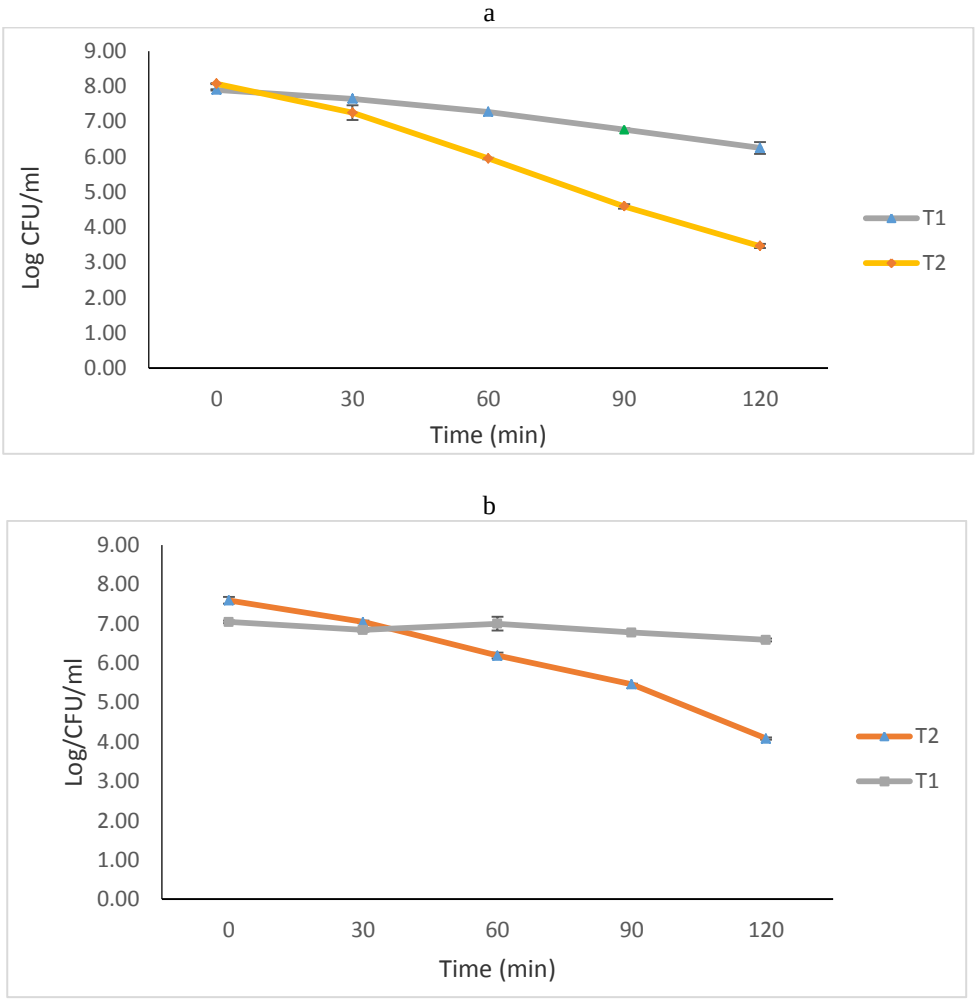

Fig. 1. Survival of microencapsulated and free *L. rhamnosus* probiotic bacteria in simulated gastric fluid (SGF) (a) and simulated intestine fluid (SIF) (b)
T1: *L. rhamnosus* (microencapsulated), T2: *L. rhamnosus* (free).

Table 1. Viable counts of *L. rhamnosus* in probiotic yogurt sauce samples during storage

sample	Day 1	Day 10	Day 20	Day 30
		Log/CFU/g		
T1	$7.85 \pm 0.00^{A,b}$	$6.85 \pm 0.06^{B,b}$	$5.90 \pm 0.05^{C,b}$	$5.00 \pm 0.01^{D,b}$
T2	$7.88 \pm 0.00^{A,a}$	$7.26 \pm 0.02^{B,a}$	$6.77 \pm 0.04^{C,a}$	$6.61 \pm 0.00^{D,a}$

T1: *L. rhamnosus* (free), T2: *L. rhamnosus* (microencapsulated),
Data are presented as a mean ± standard deviation. Different small letters represent the statistical difference ($p < 0.05$) within a column. Different capital letters represent the statistical difference ($p < 0.05$) within a row.

–***pH, and acidity of the probiotic yogurt sauce samples***

As it is presented in Table 2, the pH value of all probiotic yogurt sauce samples was reduced during 30-day storage. However, microencapsulated *L. rhamnosus* showed a higher pH compared to free *L. rhamnosus.* On day 30, the highest and the second highest pH values were observed for control (4.13 ± 0.01) and microencapsulated *L. rhamnosus* (4.08 ± 0.01) samples, respectively. According to Table 2, acidity values of all probiotic yogurt sauce samples were increased during 30-day storage. On day 30, the lowest acidity belonged to the control (without probiotic bacteria) sample (0.61±0.01) and after that to the microencapsulated *L. rhamnosus* (0.65 ± 0.005). Samples containing microencapsulated probiotic bacteria displayed lower post-acidification values than samples containing free bacteria. Similar results were reported by Picot *et al*. Who found a higher pH encapsulated *Bifidobacterium breve* in whey protein compared to the unencapsulated form of probiotic bacteria (Picot and Lacroix, 2004). Furthermore, some researchers demonstrated a decreasing trend in the pH of yogurt samples during 28 days of refrigeration, while they observed a higher pH in microencapsulated *L. acidophilus* than in free *L. acidophilus* cells (Afzaal *et al*., 2019).

–***Rheological characteristics of the probiotic yogurt sauce samples***

The results of measuring viscosity in refrigerated probiotic yogurt sauce samples during 30-day storage are shown in Figure 2. The viscosity of all samples was reduced during storage, however, the sample containing free *L. rhamnosus* showed more decreased compared to microencapsulated and control samples. Furthermore, on day 30, the highest (211.3 ± 1.52) and second highest (145.6 ± 0.57) viscosities belonged to the control and microencapsulated samples, respectively. The produced probiotic yogurt sauce was considered a pseudo-plastic fluid and presented a mayonnaise behavior. The viscosity of all samples was reduced during storage. However, a sample containing free *L. rhamnosus* showed a higher viscosity decrease than microencapsulated and control samples. This may be because of the lactic acid bacteria's activity and acid production. Microencapsulation is suggested to offer an appropriate way to control the viscosity decrease in the produced probiotic yogurt sauce. A similar finding was reported earlier which indicated an increased viscosity in full-fat mayonnaise samples

Table 2. The values of pH and acidity (% in terms of acetic acid) of probiotic yogurt sauce samples during cold storage

Parameters	Samples	First day	10th day	20th day	30th day
pH	T1	4.28 ± 0.02 ^{A,b}	4.11 ± 0.01^{B,c}	4.04 ± 0.02^{C,b}	4.01 ± 0.01 ^{C,c}
	T2	4.34 ± 0.01 ^{A,a}	4.28 ± 0.00^{B,a}	4.15 ± 0.01^{C,a}	4.08 ± 0.01^{D,b}
	T3	4.28 ± 0.01 ^{A,b}	4.22 ± 0.02^{B,b}	4.15 ± 0.01 ^{C,a}	4.13 ± 0.01 ^{C,a}
Acidity	T1	0.60 ± 0.02 ^{D,a}	0.62 ± 0.02 ^{C,a}	0.63 ± 0.02 ^{B,a}	0.67 ± 0.01 ^{A,a}
	T2	0.59± 0.01 ^{B,a}	0.60 ± 0.01 ^{B,a}	0.61 ± 0.00 ^{B,a}	0.65 ±0.00^{A,b}
	T3	0.59 ± 0.01 ^{A,a}	0.60 ± 0.01 ^{A,a}	0.61 ± 0.01 ^{A,a}	0.61 ± 0.01 ^{A,c}

T1: *L. rhamnosus* (free), T2: *L. rhamnosus* (microencapsulated), T3: Control.
Data are presented as a mean ± standard deviation. Different small letters represent the statistical difference ($p < 0.05$) within a column. Different capital letters represent the statistical difference ($p < 0.05$) within a row.

containing olive leaf extract (OLE) encapsulated by alginate and pectin materials. Moreover, all mayonnaise samples exhibited non-Newtonian shear behavior at the same shear rate (Flamminii *et al*., 2020). The study aligns with two other investigations using low-fat mayonnaise samples containing fish oil. Both studies pointed to an increase in the viscosity of mayonnaise samples when fish oil was microencapsulated by Zein (Flamminii *et al*., 2020) and dextran and glucose syrup materials (Miguel *et al*., 2019).

–***Sensory quality***

The result of taste analysis was indicated in Table 3, and showed that on day 30 there was a minor difference between samples. The highest taste score belonged to the control sample (3.00± 0.11) while both samples containing *L. rhamnosus* (with and without microcapsules) received 3.00 ± 0.00 scores. According to Table 3, there is a steady trend in the odor value of control sample during storage while at the same time the scores obtained by the samples containing free and microencapsulated *L. rhamnosus* were reduced. On the first day, the highest score was obtained by the control sample (5.00±0.00) and the lowest score (3.67 ± 0.57) was given to the sample containing free *L. rhamnosus*. On day 30, the lowest (3.00 ± 0.00) and second lowest (3.33 ± 0.57) scores belong to the samples containing free and microencapsulated *L. rhamnosus* respectively. According to Table 3, on the first day, the highest (4.00 ± 0.10) and second highest (4.00 ± 0.00) texture scores were obtained by samples containing microencapsulated *L. rhamnosus* and control correspondingly. On day 30, the given scores for the sample containing free *L. rhamnosus* remained constant to the first day, while the sample containing microencapsulated *L. rhamnosus* showed a trivial increase (4.00 ± 0.10). However, no difference was observed in the texture of the control sample during storage. Table 3 shows the results of color scores, which show that time has a slightly negative effect on the color of the samples, which

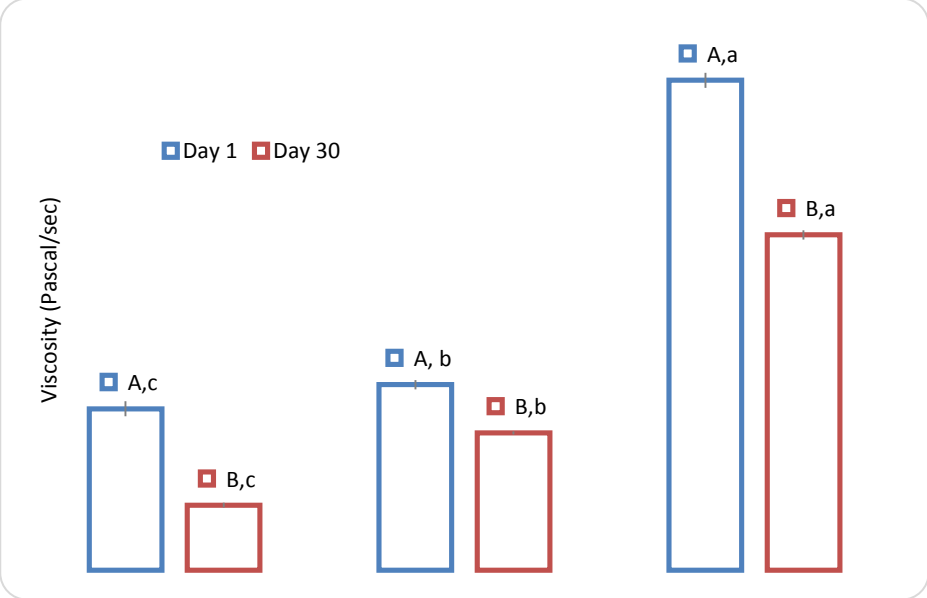

Fig. 2. Viscosity (Pascal/sec) of the probiotic yogurt sauce samples during storage
T1: *L. rhamnosus* (free), T2: *L. rhamnosus* (microencapsulated), T3: Control

was noticeable by consumers. On day 1, the sample containing microencapsulated *L. rhamnosus* along with the control sample showed the highest score (3.66 ± 0.57), while the lowest score (3.33 ± 0.57) belonged to the sample containing free *L. rhamnosus*. On the other hand, on day 30, the sample containing microencapsulated *L. rhamnosus* obtained the highest score (3.33 ± 0.57). On the same time, the control sample together with the sample containing free *L. rhamnosus* achieved the minimum scores (3.00 ± 0.00). According to Table 3, on day 1**,** no significant difference was observed in terms of overall acceptance between sample containing microencapsulated *L. rhamnosus* and control sample whereas differences were detected throughout storage time. On day 30, the highest score (3.66 ± 0.57) was given to the sample containing microencapsulated *L. rhamnosus,* whereas the sample containing free *L. rhamnosus* obtained the lowest score (3.00 ± 0.00). There were no significant differences ($p>0.05$) between the samples regarding texture, taste, and color. Nevertheless, samples containing free *L. rhamnosus* received the minimum scores regarding odor and total acceptability. Therefore, the addition of probiotic bacteria in the form of microencapsulation to the probiotic yogurt sauce had a negative effect on the aforementioned sensory properties. As was expected, the addition of resistant starch in cooperation with sodium alginate showed no adverse effect on the texture, taste, and color of probiotic yogurt sauce samples. This could be because resistant starch presents a white color and has a trivial taste. The study also agrees with a recent investigation by some researchers who used mayonnaise samples containing OLE in two forms: free and encapsulated. They reported no significant differences between the samples regarding texture, taste, and color, but detected lower total acceptability of mayonnaise samples containing OLE microencapsulated in alginate and pectin compared to samples

Table 3. Sensory parameters score of probiotic yogurt sauce samples during cold storage

Parameters	Samples	First day	10th day	20th day	30th day
Taste	T1	3.00 ± 0.00 ^{A,b}	3.00 ± 0.00 ^{A,b}	3.00 ± 0.00 ^{A,a}	3.00 ± 0.00 ^{A,a}
	T2	3. 67 ± 0.57 ^{A,a}	3.33 ± 0.57 ^{B,a}	3.00 ± 0.00 ^{C,a}	3.00 ± 0.00 ^{A,a}
	T3	3.00 ± 0.00 ^{A,b}	3.00 ± 0.00 ^{A,b}	3.00 ± 0.00 ^{A,a}	3.00 ± 0.11 ^{A,a}
Odor	T1	3.67 ± 0.57 ^{A,c}	3.66 ± 0.57 ^{A,c}	3.00 ± 0.00 ^{B,c}	3.00 ± 0.00 ^{B,c}
	T2	4. 67 ± 0.57 ^{A,b}	4.00 ± 0.00 ^{B,b}	3.66 ± 0.57 ^{C,b}	3.33 ± 0.57 ^{D,b}
	T3	5.00 ± 0.00 ^{A,a}	5.00 ± 0.00 ^{A,a}	5.00 ± 0.00 ^{A,a}	5.00 ± 0.00 ^{A,a}
Texture	T1	3.00 ± 0.00 ^{A,b}	3.00 ± 0.10 ^{A,b}	3.00 ± 0.20 ^{A,b}	3.00 ± 0.00 ^{A,b}
	T2	4. 00 ± 0.10 ^{A,a}	4. 00 ± 0.20 ^{A,a}	4. 00 ± 0.00 ^{A,a}	4. 00 ± 0.10 ^{A,a}
	T3	4. 00 ± 0.00 ^{A,a}	4. 00 ± 0.00 ^{A,a}	4. 00 ± 0.00 ^{A,a}	4. 00 ± 0.00 ^{A,a}
Color	T1	3.33 ± 0.57 ^{A,b}	3.33 ± 0.57 ^{A,b}	3.00 ± 0.00 ^{B,c}	3.00 ± 0.00 ^{B,b}
	T2	3.66 ± 0.57 ^{A,a}	3.66 ± 0.57 ^{A,a}	3.66 ± 0.57 ^{A,a}	3.33 ± 0.57 ^{B,a}
	T3	3.66 ± 0.57 ^{A,a}	3.66 ± 0.57 ^{A,a}	3.33 ± 0.57 ^{B,b}	3.00 ± 0.00 ^{C,b}
Total acceptability	T1	3.66 ± 0.57 ^{A,b}	3.33 ± 0.57 ^{A,c}	3.00 ± 0.57 ^{B,c}	3.00 ± 0.00 ^{B,b}
	T2	4.00 ± 0.00 ^{A,a}	4.00 ± 0.00 ^{A,a}	4.00 ± 0.00 ^{A,a}	3.66 ± 0.57 ^{A,a}
	T3	4.00 ± 0.00 ^{A,a}	3. 66 ± 0.57 ^{A,b}	3.66 ± 0.57 ^{A,b}	3.33 ± 0.57 ^{A,b}

T1: *L. rhamnosus* (free), T2: *L. rhamnosus* (microencapsulated), T3: Control.

Data are presented as a mean ± standard deviation. Different small letters represent the statistical difference ($p<0.05$) within a column. Different capital letters represent the statistical difference ($p < 0.05$) within a row.

with free OLE (Hermund *et al*., 2019). In another study, the sensorial analysis resultsdemonstrated no differences between the control sample and mayonnaise samples containing microencapsulated oils enriched with omega 3 and omega 6 (Rojas *et al*., 2019).

Conclusion

During 30 days of storage, the microencapsulated *L. rhamnosus* in the sodium alginate and resistant starch matrices showed survival improvement as compared with the free *L. rhamnosus*. Furthermore, due to the protective effect of microencapsulation, probiotic yogurt sauce samples containing microencapsulated *L. rhamnosus* exhibited greater tolerance in gastrointestinal conditions. Accordingly, the number of viable cells in microencapsulated *L. rhamnosus* improved as compared to the free probiotic cells. Microencapsulation using resistant starch/sodium alginate may improve sensory assessment of probiotic yogurt sauce.

References

Afjeh, M.E.A., Pourahmad, R., Akbari-Adergani, B. & Azin, M. (2019). Use of glucose oxidase immobilized on magnetic chitosan nanoparticles in probiotic drinking yogurt. *Food Science of Animal Resources*, 39 (1), 73. http://dx.doi.org/10.5851/kosfa.2019.e5. PMid:30882076.

Afzaal, M., Khan, A.U., Saeed, F., Ahmed, A., Ahmad, M.H. & Maan, A.A. (2019). Functional exploration of free and encapsulated probiotic bacteria in yogurt and simulated gastrointestinal conditions. *Food Science and Nutrition*, 7 (12), 3931-3940.

Chen, H.Y., Li, X.Y., Liu, B.J. & Meng, X.H. (2017). Microencapsulation of Lactobacillus bulgaricus and survival assays under simulated gastrointestinal conditions. *Journal of Functional Foods*, 29, 248-55.

de Araújo Etchepare, M., Raddatz, G.C., Cichoski, A.J., Flores, É.M.M., Barin, J.S. & Zepka, L.Q. (2016). Effect of resistant starch (Hi-maize) on the survival of *Lactobacillus acidophilus* microencapsulated with sodium alginate. *Journal of Functional Foods*, 21, 321-9.

Fahimdanesh, M., Mohammadi, N., Ahari, H., Zanjani, M.A., Hargalani, F.Z. & Behrouznasab, K. (2013). Effect of microencapsulation plus resistant starch on survival of *Lactobacillus casei* and *Bifidobacterium bifidum* in mayonnaise sauce. *African Journal of Microbiology Research*, 6 (40), 6853-8.

Flamminii, F., Di Mattia, C.D., Sacchetti, G., Neri, L., Mastrocola, D. & Pittia, P. (2020). Physical and sensory properties of mayonnaise enriched with encapsulated olive leaf phenolic extracts. *Foods*, 9 (8), 997. 26.

Golestani, M. & Pourahmad, R. (2017). Comparison of three treatments (two fermented treatments and one nonfermented treatment) in production of synbiotic ice cream. *Journal of Food Processing and Preservation*, 41(2), e12839. http://dx.doi.org/10.1111/jfpp.12839.

Han, C., Xiao, Y., Liu, E., Su, Z., Meng, X. & Liu, B. (2020). Preparation of Ca-alginate-whey protein isolate microcapsules for protection and delivery of *L. bulgaricus* and *L. paracasei*. *International Journal of Biological Macromolcules*, 163, 1361-8.

Hermund, D., Jacobsen, C., Chronakis, I.S., Pelayo, A., Yu, S. & Busolo, M. (2019). Stabilization of fish oil-loaded electrosprayed capsules with seaweed and commercial natural antioxidants: effect on the oxidative stability of capsule-enriched mayonnaise. *European Journal of Lipid*

Science and Technology, 121(4), 1800396.

Homayouni, A., Amini, A., Keshtiban, A.K., Mortazavian, A.M., Esazadeh, K. & Pourmoradian, S. (2014). Resistant starch in food industry: A changing outlook for consumer and producer. *Starch-Stärke*, 66 (1-2), 102-14.

Iqbal, R., Zahoor, T., Huma, N., Jamil, A. & Ünlü, G. (2019). In-vitro GIT tolerance of microencapsulated *Bifidobacterium bifidum* ATCC 35914 using polysaccharide-protein matrix. *Probiotics and Antimicrobial Proteins*, 11(3), 830-9.

Kaur Sidhu, M., Lyu, F., Sharkie, T.P., Ajlouni, S. & Ranadheera, C.S. (2020). Probiotic Yogurt Fortified with Chickpea Flour: Physico-Chemical Properties and Probiotic Survival during Storage and Simulated Gastrointestinal Transit. *Foods*, 9 (9), 1144.

Krasaekoopt, W., Bhandari, B. & Deeth, H. (2003). Evaluation of encapsulation techniques of probiotics for yoghurt. *International Dairy Journal*, 13 (1), 3-13.

Krasaekoopt, W., Bhandari, B. & Deeth, H.C. (2006). Survival of probiotics encapsulated in chitosan-coated alginate beads in yoghurt from UHT-and conventionally treated milk during storage. *LWT-Food Science and Technology*, 39 (2), 177-83.

Liu, H., Xie, M. & Nie, S. (2020). Recent trends and applications of polysaccharides for microencapsulation of probiotics. *Food Frontiers*, 1 (1), 45-59.

Miguel, G.A., Jacobsen, C., Prieto, C., Kempen, P.J., Lagaron, J.M. & Chronakis, I.S. (2019). Oxidative stability and physical properties of mayonnaise fortified with zein electrosprayed capsules loaded with fish oil. *Journal of Food Engineering*, 263: 348-58.

Mirzaei, H., Pourjafar, H. & Homayouni, A. (2012). Effect of calcium alginate and resistant starch microencapsulation on the survival rate of *Lactobacillus acidophilus* La5 and sensory properties in Iranian white brined cheese. *Food Chemistry*, 132(4), 1966-70.

Mokarram, R., Mortazavi, S., Najafi, M.H. & Shahidi, F. (2009). The influence of multi stage alginate coating on survivability of potential probiotic bacteria in simulated gastric and intestinal juice. *Food Research International*, 42 (8), 1040-5.

Mora-Villalobos, J. A., Montero-Zamora, J., Barboza, N., Rojas-Garbanzo, C., Usaga, J. & Redondo-Solano, M. (2020). Multi-Product Lactic Acid Bacteria Fermentations: A Review. *Fermentation*, 6 (1), 23. https://doi.org/10.3390/fermentation6010023

Nedovic, V., Kalusevic, A., Manojlovic, V., Levic, S. & Bugarski, B. (2011). An overview of encapsulation technologies for food applications. *Procedia Food Science*, 1, 1806-15.

Picot, A. & Lacroix, C. (2004). Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. *International Dairy Journal*, 14 (6), 505-15.

Prasanna, P. & Charalampopoulos, D. (2018). Encapsulation of Bifidobacterium longum in alginate-dairy matrices and survival in simulated gastrointestinal conditions, refrigeration, cow milk and goat milk. *Food Bioscience*, 21, 72-9.

Qi, W., Liang, X., Yun, T. & Guo, W. (2019). Growth and survival of microencapsulated probiotics prepared by emulsion and internal gelation. *Journal of Food Science and Technology*, 56 (3),1398-404.

Rojas, V.M., Marconi, L.F.D.C.B., Guimarães-Inácio, A., Leimann, F.V., Tanamati, A. & Gozzo, Â.M. (2019). Formulation of mayonnaises containing PUFAs by the addition of microencapsulated chia seeds, pumpkin seeds and baru oils. *Food Chemistry*, 274, 220-7.

Sultana, K., Godward, G., Reynolds, N., Arumugaswamy, R., Peiris, P. & Kailasapathy, K. (2000). Encapsulation of probiotic bacteria with alginate–starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. *International Journal of Food Microbiology*, 62(1-2), 47-55.

Tannock, G.W. (2004). A special fondness for lactobacilli. *Applied Environmental Microbiology*, 70 (6), 3189-94.

Zanjani, M.A.K., Tarzi, B.G., Sharifan, A., Mohammadi, N., Bakhoda, H. & Madanipour, M.M. (2012). Microencapsulation of Lactobacillus casei with calcium alginate-resistant starch and evaluation of survival and sensory properties in cream-filled cake. *African Journal of Microbiology Research*, 6 (26), 5511-7.