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Original research

The investigation of probiotics viability, physicochemical and rheological properties of a probiotic dessert based on sesame paste (Tahini)

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ABSTRACT

This study investigated the possibility of developing a novel functional sesame paste based dessert bearing probiotic bacteria (*Lactobacillus paracasei* and *Bifidobacterium lactis*). Probiotics viability in the dessert as well as pH, acidity, texture and microstructure modifications were investigated during 28 days of cold storage. Cell viability investigation showed that the medium preserved cell viability above 10^6 CFU/mL during cold storage. The pH and acidity of inoculated desserts have been modified through storage. Flow behavior and texture analysis showed that desserts inoculated with *B. lactis* showed higher yield stresses and creep elasticity, due to the exertion of polysaccharides by the bacteria. *L. paracasei* exhibited lower pH values and reduced the creep viscosity and elasticity, due to changes in molecular interactions. Microstructure analysis of the samples showed that the oil was in the form of emulsion and proteins were dispersed in the continuous aqueous phase. According to the results of this study, it is concluded that the exploitation of mixed cultures in the dessert formulation could guarantee acceptable probiotic viability and provide better texture attributes.

Keywords: Sesame paste; Probiotics; Cell viability; Dessert; Rheology

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1. Introduction

Functional foods are defined as whole foods, enriched, enhanced and fortified foods or dietary compounds, in addition to traditional nutrient contents possess healthy and physiological benefits (Bellisle et al., 1998; Kwak & Jukes, 2001; Spence, 2006). Food products containing probiotics compromise most functional food markets worldwide (Salmerón et al., 2015). Probiotics include live microorganisms and when consumed sufficiently (at least 10^6_10^7 CFU/ml), increase the health of the host (Meira et al., 2015; Reid et al., 2003). Probiotic bacteria affect human health by recovering the intestines microbiota balance (Kalliomäki et al., 2001; Meira et al., 2015).

Various studies have shown that probiotic microorganisms can be successfully incorporated in different dairy-based food products including yogurt, cheese, ice cream, beverages, desserts, and others (Buriti et al., 2016; Cardarelli et al., 2008; Mousavi et al., 2011; Mousavi & Mousavi, 2019; Mousavi et al., 2013; Nikmaram et al., 2016). Dairy desserts have emerged as interesting options for the incorporation of probiotics, bioactive ingredients and alternative sources of thickeners (Estevam et al., 2017). However, allergic effects of dairy foods and consumer awareness have led to the demand of new formulations based on vegetables and plants. Therefore, there is a major interest among producers to develop new formulations with improved functionality (Ares et al., 2008; Bogue et al., 2009). The use of functional ingredients with the incorporation of probiotic bacteria is an interesting option to promote the functionality of plant-based desserts (Buriti et al., 2016; Fasihnia et al., 2018).

In the Middle East region, Tahini, which is produced from dehulled sesame seeds is used to prepare local foods such as salad and dessert (Abu-Jdayil et al., 2002; Razavi et al., 2007; Torlak et al., 2013). Tahini is rich in protein, lipids (especially omega-6 fatty acids), carbohydrates, thiamin, niacin, calcium, manganese and amino acid methionine (Al-Nabulsi et al., 2014; Torlak et al., 2013). In addition, since Tahini is a natural product of sesame seed, it has antioxidant and anticancer properties and can stimulate liver function (Gharby et al., 2017) Tahini is consumed as a dessert at breakfast with grape molasses (Abu-Jdayil et al., 2002). Recently, the consumption of ready to eat food based on Tahini has increased worldwide (Al-Nabulsi et al., 2014). Therefore, it is

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an opportunity to investigate the application of Tahini as a new matrix in the formulation of potentially probiotic desserts.

This study aims to develop a probiotic dessert containing tahini with the addition of *Lactobacillus paracasei* and *Bifidobacterium lactis*, and to investigate the physicochemical properties of the dessert and viability of bacteria during cold storage. In addition, the rheological characteristics and microstructure of the dessert were evaluated.

2. Material and Methods

2.1. Dessert ingredients

Desserts containing 20% milk powder, 50% water, 20% Tahini, 7% maltodextrin and 3% inulin were formulated. The following ingredients were used to develop the Tahini-based dessert: 20% milk powder (Pak dairy Co., Iran), 6% maltodextrin (DE=5, Kaman Co., Iran), 1% cocoa powder (Barsam Co., Iran), 20% tahini (Barsam Co., Iran), and 3% inulin (long chain, Sigma, Germany). *Lactobacillus paracasei* (LBC82, Takgene, Iran), *Bifidobacterium lactis* (BBO4, Persian Type Culture Collection, Iran) and their combination were added as probiotic cultures.

2.2. Preparation of cultures

The lyophilized microorganisms were activated in MRS (De Man, Rogosa and Sharpe broth, Merck, Germany) and maintained at −80 °C, in tubes containing MRS broth with the addition of glycerol (80:20). In each experiment, the cultures were activated and sub-cultured twice in 10-mL TSB (Triptic Soy broth, Sigma, USA) and incubated at 37 °C for 24 h.

2.3. Production of probiotic tahini -based dessert

Initially, each ingredient was weighted individually and the ingredients were dissolved in hot water (75 °C). The final mixture was subsequently cooled to 37 ° C. As soon as the mixture reached the desired temperature, *L. paracasei* and *B. lactis* were added to the dessert to obtain concentrations of approximately 7 log cfu /g. The final products were packed in individual plastic cups, sealed with a metallic cover, and stored at 4 °C.

2.4. Analysis of probiotic viability and growth

Dessert samples were decimally diluted in sterile NaCl solution (0.9%). Then, 1 ml aliquots were poured into plates on MRS agar (Qlab, China) for *L. paracasei* and MRS agar supplemented with 0.05% (w/v) L-Cys-Hcl (Merck, Germany) for *B. lactis* and were incubated under aerobic and anaerobic conditions, respectively, at 37°C for 72h. The results were reported as colony-forming units per gram (log CFU/g). The pH of all samples was examined by a digital pH meter (METROHM 744, Germany). Titratable acidity of the samples was analyzed by titrating 10-g sample in 100 ml of distilled water with NaOH 0.1 N to pH 8.3 and expressed as percentage lactic acid.

2.5. Rheological measurements

A controlled stress R/S plus rheometer (Brookfield Engineering, US) was used to achieve the flow characteristics of the samples at 25 °C. The viscosity dependence of the apparent shear rate was investigated in different stages (production start, 7 and 28 days after dessert production). In addition, the controlled stress behavior of the samples was analyzed by recording the shear rate at different shear stress values.

To study the viscoelastic properties of the probiotic dessert samples, creep test was performed by the R/S plus rheometer device at the stress value of 2 Pascal. Herschel-Bulkley (Eq. 1) and Burger's (Eq. 2) models were used to analyze the rheological behavior of the samples.

$$\tau = K(\gamma)^n + \tau_0 \quad (1)$$

$$\frac{\sigma_0}{E_0} + \frac{\sigma_0}{\mu_0} + \frac{\sigma_0}{E_1}\left(1 - e^{-\frac{t}{t^*}}\right) \quad (2)$$

where K is consistency index, n is the flow behavior index, τ is the shear stress (Pa), E represents the general deformation, σ_0/E_0 and σ_0/E_1 are instantaneous deformation and viscoelastic (or delayed) deformation, respectively, μ_0 is the viscosity at zero shear stress.

2.6. Statistical analysis

Analysis of variance for repeated measures (ANOVA) and Duncan test was used to determine significant differences ($p < 0.05$) between means.

Table 1. Mean values of pH, titratable acidity and water activity of tahini-based probiotic dessert containing different strains during 7, 14, 21, 28 days of cold storage.

Formulations	Time (days)	pH	Titratable acidity (%)
L. paracasei	7	5.30^{Ca*}	0.12^{Cc}
	14	4.45^{Cb}	0.12^{Cc}
	21	4.28^{Cc}	0.14^{Cb}
	28	4.29^{Cc}	0.17^{Ca}
B. lactis	7	6.19^{Aa}	0.06^{Dc}
	14	6.02^{Bb}	0.08^{Db}
	21	5.66^{Bc}	0.085^{Db}
	28	5.20^{Bd}	0.129^{Da}
Mix	7	4.64^{Ba}	0.14^{Bc}
	14	4.30^{Db}	0.21^{Bb}
	21	4.00^{Dc}	0.24^{Ba}
	28	3.91^{Dd}	0.25^{Ba}
Control	7	6.19^{Aa}	0.42^{Aa}
	14	6.19^{Aa}	0.03^{Aa}
	21	6.08^{Ab}	0.03^{Aa}
	28	6.08^{Ab}	0.68^{Ab}

*Values with different letters are significantly different ($p < 0.05$). Capital letters represent statistical differences between treatments and small letters represent statistical differences between times of storage.

3. Results and Discussion

3.1. Physicochemical properties

The mean values and standard deviation of pH and titratable acidity of different Tahini-based probiotic dessert formulations are shown in Table 1. As shown in Table 1, the pH of all samples decreased significantly during 28 days of cold storage, and a significant increase in acidity values of all samples was observed.

Table 2. Flow behaviour and creep analysis parameters of probiotic tahini dessert.

Treatment	Day	Creep analysis				Flow behavior		
		$\mu 1$*	μ_0	E_1	E_0	n	k	τ_0
L. paracasei	7	-	0.000014	-	-	0.29	0.81	0.59
	28	392.85	62.59	2.88	5.47	0.35	4.40	0.83
B. Lactis	7	47494.65	6638.37	606.24	281.53	0.18	76.74	2.46
	28	53475.93	8703.89	702.49	467.39	0.19	2.51	6.34
Mix	7	-	0.1530	-	-	0.29	2.78	0.80
	28	53.19	15.72	6.52	3.57	0.11	8.98	0.84

*Creep viscosity (μ_1), initial creep viscosity (μ_0), creep elasticity (E_1), initial creep elasticity (E_0), Consistency coefficient (K), flow behavior index (n), yield shear stress (τ_0).

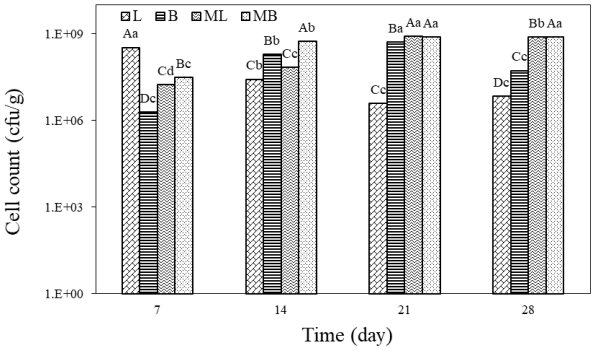

Fig.1. The viability of cells (CFU.g^{-1}) in dessert samples containing individual and mix cultures *L. paracasei* (L) and *B. lactis* during 28 days of cold storage. (L): *L. paracasei*, (B): *B. lactis*, (ML), *L. paracasei* in mixed culture, (BL): *B.lactis* in mixed culture. Values with different letters are significantly different ($p \leq 0.05$).

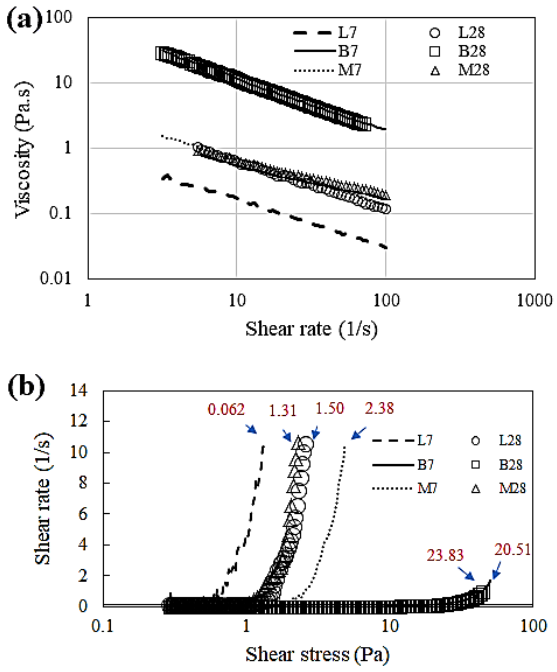

Fig. 2. Dynamic viscosity (a) and controlled stress analysis (b) and dessert samples containing individual and mixed cultures *L. paracasei* and *B. lactis* after 7 and 28 days of cold storage. Static yield stress values are shown for each curve (b). (L): Samples containing *L. paracasei*, (B): Samples containing *B. lactis*, (M): Samples containing a mixture of strains.

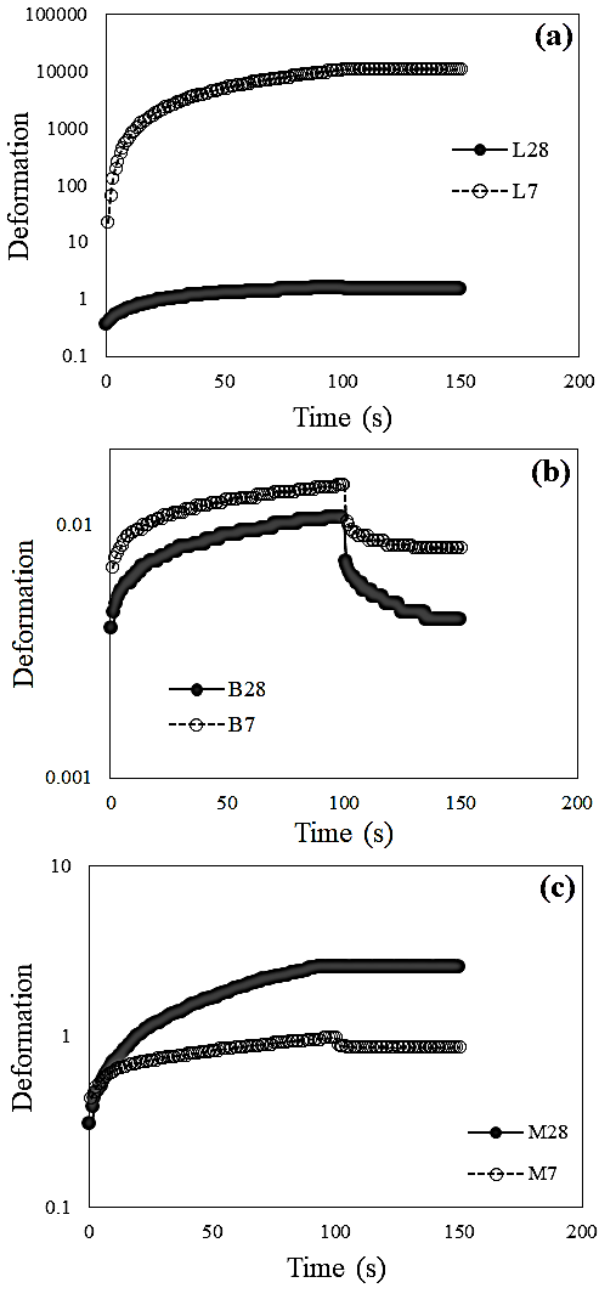

Fig. 3. Creep behavior of tahini-based dessert samples: a) Samples containing *B. lactis*; b) Samples containing *L. paracasei*, c): Samples containing mixed cultures of *B. lactis* and *L. paracasei*.

The dessert containing *L. paracasei* exhibited lower pH values and higher acidity at the end of storage compared to the dessert containing *B. lactis*. This difference is be attributed to the ability of *L. paracasei* to produce higher levels of lactic acid during 28 days of cold storage, increasing total acidity from 0.12 to 0.17 at the end of storage period (Fasihnia et al., 2018; Taghizadeh et al., 2018). The most considerable drop in pH occurred in the dessert containing the mixture of *L. paracasei* and *B. lactis* (3.91). *L. paracasei* is a homofermentative *Lactobacilli* strain, which can metabolize carbohydrates and provide glucose and lactic acid for the proper growth of *B. lactis*. In addition, the proteolytic activity of *L. paracasei* triggers the production of *kappa* casein derivative of protein originated from milk. *Kappa* casein is recognized as a bifidogenic compound, simulating the growth of *B. lactis* (Azuma et al., 1984). Therefore, a high decrease in pH was anticipated in the formulated dessert containing both probiotic strains. In addition, the acidity in this dessert formula increased to its highest level (0.25). In contrast to the inoculated dessert, pH and acidity changes in the non-inoculated sample were not considerable. The significant decrease in pH and rise in acidity eventually hamper the growth of unwanted microorganism in the product in the probiotic dessert.

The viability of *L. paracasei* and *B. lactis* in Tahini-based probiotic desserts during 28 days of storage at 4°C is depicted in Fig. 1. The data showed that both *L. paracasei* and *B. lactis* maintained their viability above 10^6 CFU/mL during cold storage. Therefore, the medium provided proper conditions for the growth of both strains. The results were in agreement with previous similar works (Aragon-Alegro et al., 2007; Taghizadeh et al., 2018).

The viability of *B. lactis* increased significantly ($p < 0.05$) during 21st days of storage in the *B. lactis* inoculated desserts and reached its highest value of 5.2×10^8 CFU / g. However, the cell viability dropped to 5.4×10^7 CFU/g at the end of storage. Compared to *B. lactis*, the *L. paracasei* cells decreased thoroughly during cold maintenance. However, the final number of viable cells was above the recommended level for improving health (10^6 CFU/g) (Aragon-Alegro et al., 2007).

The viability analysis of strains in different formulations indicated that the application of c0-culture of probiotic strains in the dessert maintained the viability of both probiotic above 8 Log until the end of 28th days of storage. In a similar work performed by Vasconcelos et al. (2014), the number of viable cells of *L. acidophilus* and *B. lactis* in co-cultured probiotic desserts was considerable compared to individually inoculated desserts. These findings approved that the cells could properly maintain their maximum viability point in co-culture medium of the probiotic desserts.

3.2. Rheological measurements

The dynamic flow behavior parameters of dessert samples are shown in Table 2. This table presents the yield stress (τ_0), consistency coefficient (K) and the flow behavior index (n) of the samples according to the model parameters that best fitted the curves. The coefficients of determination for the fitted data to the model were higher than 0.97.

Dessert samples showed flow indices (n) ranging from 0.11 to 0.35. The flow index in all samples showed values less than one, indicating that all samples exhibited a non-Newtonian behavior. The viscosity curve of the samples shown in Fig. 2a, also confirmed that the samples were pseudoplastic material in dynamic shear experiments. Similar results have been observed for other probiotic dairy dessert samples (Taghizadeh et al., 2018; Yang et al., 2012). The viscosity of all samples was decreased by increasing the shear rate. When the shear rate was increased sufficiently to overcome the Brownian motion, the dessert emulsion droplets were arranged along the flow and exhibited less resistance to flow, decreasing the viscosity of the samples. The variation in the flow index was related to the type of bacteria inoculated and the storage time. The highest index was observed in the sample of chocolate dessert inoculated with *L. paracasei* after 28 days of storage and the smallest amount of flow index is for the inoculated dessert with blended *L. paracasei* and *B.lactis* after 28 days of storage.

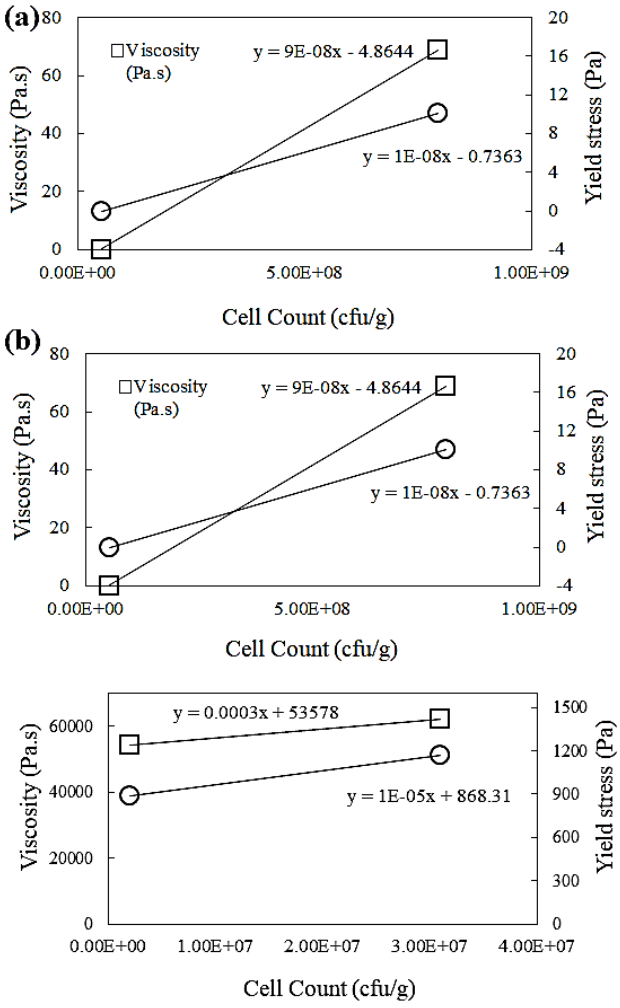

Fig. 4. The effect of *B. Lactis* population on the viscosity and yield stress of tahini-based dessert samples. a) mixed culture of *B. lactis* and *L. paracasei* ; b) pure culture of *B. lactis*.

Consistency coefficient of dessert samples varied with different probiotics, and the highest consistency of the samples of inoculated dessert with *B. lactis* on day 28 and the lowest of the samples of inoculated desserts with *L. paracasei* on day 7, respectively ($p < 0.05$). The data were in agreement with the viscosity curves of the samples, with the highest viscosity values for the samples containing *B. lactis* and the lowest values for the samples containing *L. paracasei*. The dessert bearing mixed culture exhibited viscosity curves in the middle of the two pure cultures.

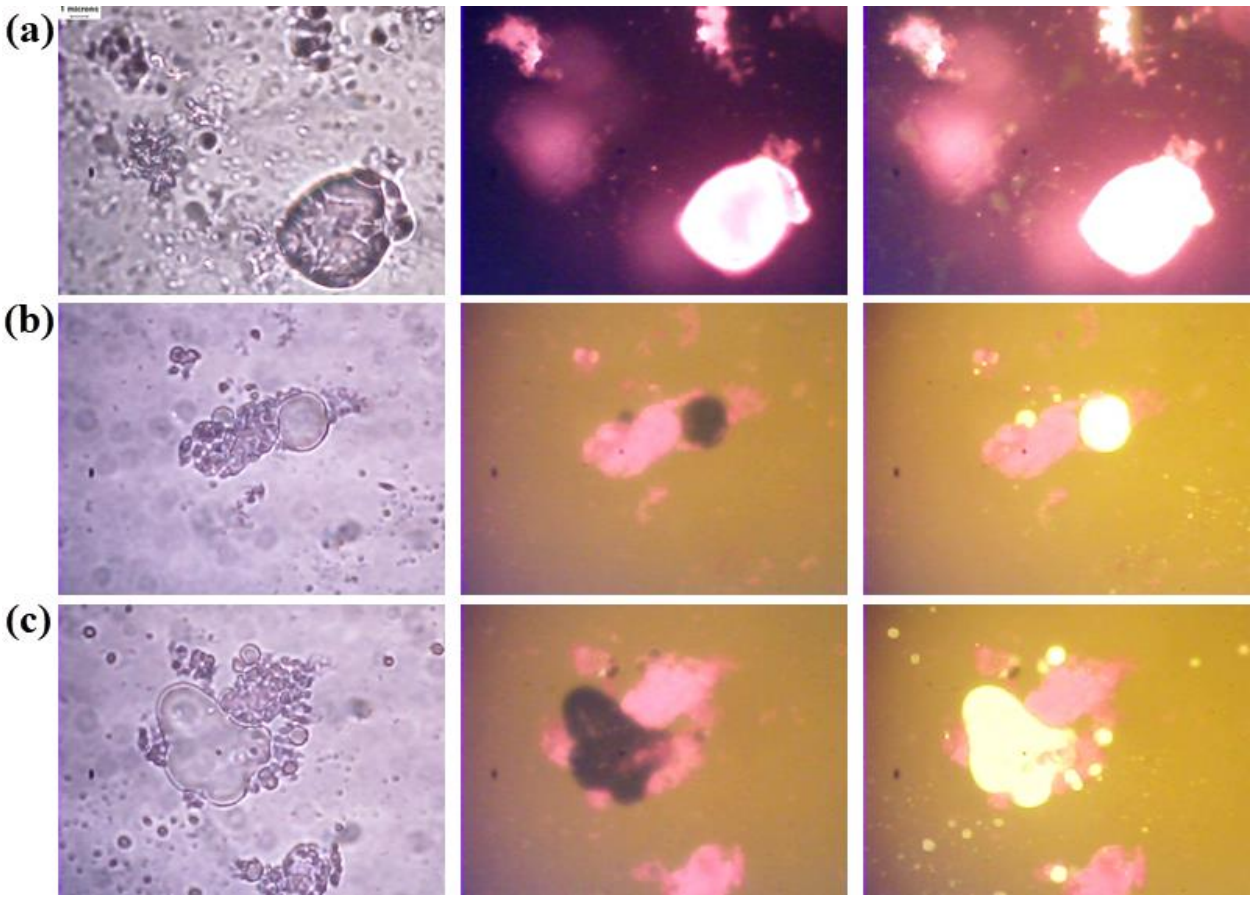

Fig. 5. Microstructure of tahini-based dessert samples (a: culture of *B. lactis* ; b: culture of *L. paracasei* ; c: mix culture of *B. lactis* and *L. paracasei*). Left: phase contrast image, centre: fluorescent under violet light filter image, right: fluorescent under blue light filter image.

The dynamic yield stress values (obtained from the Herschel-Bulkley model) shown in Table 2 varied from 0.6 to 6.5, and the highest value was observed for samples inoculated with *B. lactis* after 7 days of storage.

During the storage period of the probiotic bacteria containing samples at 4 °C, the rheological behavior showed significant changes. This behavior is attributed to the changes in the inter-molecular bonds by adding probiotic bacteria, changes in the pH value of the products and production of macromolecules by the bacteria present in the samples. The presence of Bifidobacteria decreased the value of n, increased the value of k and resulted in higher yield stress values.

The results of controlled stress analysis and of dessert samples are shown in Fig. 2b. All dessert samples exhibited an initial resistance to flow. The static yield stress values measured by this method were significantly higher than the yield stress values obtained from the Herschel-Bulkley model (Table 1). These observations were conducted in the controlled stress mode and precisely detected the low shear behavior of the samples. In addition, the differences detected among samples were larger and this method could accurately differentiate between samples. Desserts cultured by *B. lactis* exhibited high yield stress values with more than 30-fold increase in the values compared to desserts cultured by *L. paracasei*. The mixed cultures showed yield stresses higher than *L. paracasei* but much lower than that *B. lactis* containing samples. A high static yield stress value is related to a strong gel like network in the sample. At the stress values less than the critical stress, the samples behaved similarly to a viscoelastic solid, whereas in higher stresses the material started to flow.

The parameters for creep analysis of dessert samples are shown in Table 2 and the creep-recovery curves for different samples are illustrated in Fig. 3. The highest elastic coefficient was obtained in the formulation of samples of desserts inoculated with *B. lactis* after 28 days of storage. The creep-recovery curves of the samples containing *B. lactis* exhibited solid-like behaviors with exponential creep and a strong recovery. Insignificant deformations were detected for these samples compared to other dessert formulations. Alternatively, the introduction of *L. paracasei* caused fluid like behaviors with higher deformation values accompanied by no or small recovery values (Figs. 3a and 3c). As discussed before, the samples containing *B. lactis* had the highest yield stress values, which was in accordance with the creep test results. A higher elastic coefficient and a higher yield stress were due to a stronger gel network within the sample. The highest viscous coefficient was for the dessert sample inoculated with *B. lactis* after 28 days of storage. Possibility the differences in the viscous coefficients may be due to intra-molecular bonds by adding probiotics. Elasticity-free desserts were viscous and had no elastic state, therefore, only the viscosity of these samples has been reported.

Probiotics indirectly changed rheological properties by changing the physiochemical properties. Exo-polysaccharides produced by the bacteria increased the resistance to flow, and hence viscosity increased over time. In addition, tissue integrity and softness were increased in samples inoculated with the mixture of

L. paracasei and *B. lactis* after 7 days of storage. The presence of protein compounds and sesame oil could also reduce the loosening in the product over time (Kristo et al., 2003; Tafti et al., 2013a; Tafti et al., 2013b).

The rheological observations indicated that Bifidobacteria could produce a stronger gel network within the samples. These results could be attributed to the ability of *B. lactis* to exert exopolysaccharides into the sample. The analysis provided in Fig. 4 indicates a correlation between the cell count of *B. lactis* and viscosity and yield stress in all samples. Fig. 4a reveals that in a mixed culture an increase in the population of *B. lactis* increased the viscosity and yield stress, significantly. A similar trend was observed for a pure culture of *B. lactis* (Fig. 4b).

The presence of organic acids and hydrocolloids produced by probiotics in the dessert can create the optimal texture in the dessert. These results follow previous reports of acid production, growth and metabolism, and a_w by *L. paracas*ei and *B. lactis* in maintenance periods. It is also consistent with reports found that some probiotics could reduce viscosity in the product (Emmons et al., 1972; Kristo et al., 2003).

Fig. 5 depicts the microstructure of tahini-based dessert samples obtained by phase-contrast and fluorescent microscopy. The images indicated that oil bodies are present in the sample covered by protein particles (stained by rhodamine B, pictured in red by fluorescence microscopy). The oil in the desserts was in the form of emulsions and proteins and other ingredients dispersed in the continuous aqueous phase. Protein particles and oil droplets exhibited different shapes and sizes. The interaction between the particles present in the sample shaped the rheological behavior of the dessert. Since the exopolysacharides produced by *B. lactis* were water soluble, they could interact with the suspended particles and form a three-dimensional structure within the dessert samples.

4. Conclusion

In this study, probiotic bacteria were successfully incorporated into a formulated healthy dessert. Tahini provided a favorable environment for the viability of the probiotics. Positive physicochemical and textural modifications of the probiotic dessert during storage confirmed the suitability of probiotic application in Tahini-based desserts. Further studies are necessary to analyze the metabolism of Tahini originated compounds such as dietary fibers, proteins and lipids by probiotics.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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