## **Research Article**



<u>APPLIED FOOD BIOTECHNOLOGY, 2018, 5 (2):79-86</u> Journal homepage: www.journals.sbmu.ac.ir/afb pISSN: 2345-5357 eISSN: 2423-4214

# Physicochemical Properties of Probiotic Soy milk Chocolate Mousse During Refrigerated Storage

Golnoush Taghizadeh, Mahshid Jahadi <sup>\*</sup>, Hajar Abbasi

Department of Food Science and Technology, Faculty of Agriculture, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.

## Abstract

**Background and Objective:** Recently, several researchers have shown the benefits deriving from probiotic products containing lactobacilli and bifidobacteria in their formulation. The purpose of this study was to develop a probiotic chocolate mousse using milk and soy milk in formulation with regard to survival of probiotic bacteria and sensorial acceptance during 21 days.

**Material and Methods:** Nine functional probiotic chocolate mousse formulations were produced by milk, milk/soy milk and soy milk and 3 probiotic strains (*Lactobacillus acidophilus*; *Lactobacillus paracasei*; *Bifidobacterium lactis*). The pH, acidity, survival of microbial strains, rheological and sensory properties of all treatments were monitored during 21 days refrigerated storage (4°C).

**Results and Conclusion:** The pH drop rate and acidity increase rate of all samples were significant during 21 days of storage ( $p\leq0.05$ ). There was a significant increase in the probiotic bacteria of all samples during 21 days of storage at 4°C ( $p\leq0.05$ ). However, the rate of probiotics growth was accelerated in formulation prepared with soy milk and milk/soy milk (1:1) in the chocolate mousse at day 7 and 14. Rheological experiment demonstrated that all samples known as viscoelastic solid dessert had shear-thinning behavior. In conclusion, chocolate dessert including soy milk as well as milk was shown to be more effective vehicle for delivery of probiotics, including *Lactobacillus acidophilus*, *Lactobacillus paracasei*, *Bifidobacterium lactis*.

Conflict of interest: The authors declare no conflict of interest.

### **1. Introduction**

Probiotics are beneficial to the host when consumed in appropriate quantities every day. A number of health benefits of probiotic bacteria include anti-mutagenic effects, anti-carcinogenic properties, improvement in lactose metabolism, reduction in serum cholesterol level, and immune system stimulation [1,2]. Prebiotics are not easily digested compounds that stimulate the growth and/or the activity of probiotics in the human digestive system [1-3]. Production of functional foods has recently received particular attention. Functional foods mainly aim at the introduction of microorganisms or beneficial compounds into the organism by means of their daily intake. Also, the dairy industry has found probiotic cultures to be a tool for the development of new functional products [4].

In 2002, foods containing soy milk were listed among functional foods because they contain dietary fibers such as prebiotic oligosaccharides (raffinose and stachyose);

#### **Article Information**

#### Article history:

14 Nov2017
7 Jan2018
21 Feb2018

#### **Keywords:**

- Bifidobacterium lactis
- Chocolate dessert
- Lactobacillus acidophilus
- Lactobacillus paracasei
- Probiotic Soy milk
- boy mik

#### \*Corresponding author: Mahshid Jahadi,

Department of Food Science and Technology, Faculty of Agriculture, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.

Tel: +98-34-35342211 E-mail: m.jahadi@khuisf.ac.ir

isoflavone aglycones; as well as essential amino acids; minerals and vitamins that enrich the composition of intestinal flora, decrease triacylglycerol, blood cholesterol levels, and additionally prevent some types of cancer [5]. Fermentation of soy milk by probiotics eliminates unpleasant tastes, improves antioxidant properties, and adds to the nutritional value of the milk [6]. Existence of oligosaccharides in soy milk contributes to the viability of probiotic cultures [7] such as *Bifidobacterium* spp, *Lactobacillus* (*L*.) *acidophilus*, and *L. casei* during storage time due to protecting effects towards probiotic bacteria in food product [8]. Previous studies proved that fermentation with Bifidobacteria makes the proteins contained more digestible and reduces the soy oligosaccharide contents, which can result in digestive problems [9,10].

Several studies have shown that probiotics and prebiotics may be successfully employed in different milk

based food matrices, such as different kinds of yogurt, cheese, ice cream, beverages and so on [4,11,12]. Aerated dairy desserts such as mousse have found a wide potential market because of their practical sensory properties and high nutritional value which are supplied today on an industrial scale, and people consume it in all walks of life [13]. The most popular mousse flavor is chocolate [14]. Bifidobacterium (B.) lactis and L. acidophilus are good candidates for application in soy milk based ice cream because of their more stable networks that resemble a gel structure and high total solid content of soy milk [15,16]. On the other hand, rheological properties of these products may directly affect their appeal, boom and industrial production. Also some of their qualitative characteristics like oral perception and digestibility make it more appealing and more marketable [17]. Aboulfazli et al. improved the quality of probiotic ice cream in a study that found adding soy milk to ice cream results in the lowest flow behavior index and the highest consistency index. Moreover, L. acidophilus and B. lactis did not have any significant effects on apparent viscosity of ice cream [15]. Applying probiotics in soy milk products resolves the unfavorable taste and flatulence and improves its nutritional value. So, as sensory properties of functional desserts are important for consumers, by using this beneficial substance in sweet probiotic desserts this aim will be provided [10].

Although sensory characteristics and viability of probiotic bacteria in functional chocolate desserts have been investigated [4,14], there is no research on functional properties and viability of *L. acidophilus*; *L. paracasei*; *B. lactis* during refrigerated storage in milk, milk/soy milk and soy milk formulation of chocolate mousse. Therefore, the aim of the current study was the production of functional probiotic chocolate mousse by adding *L. acidophilus*, *L. paracasei*, *B. lactis* in milk, milk/soy milk and soy milk formulation of probiotic chocolate mousse by adding *L. acidophilus*, *L. paracasei*, *B. lactis* in milk, milk/soy milk and soy milk formulation of probiotic chocolate mousse as a dessert and to determine the pH, acidity, survival of microbial strains and sensory properties of all treatments during refrigerated storage (4°C) were assessed.

### **2. Materials and Methods**

#### 2.1. Materials

The following ingredients were employed for the production of potentially probiotic and synbiotic chocolate mousse; Milk powder (1.5% fat) (Pak Co., Iran), soy milk (Soy milk Co., Iran), sugar (Naghshejahan Co., Iran), cocoa powder (Farmand Co., Iran), gelatin powder (Gelita Co., Germany) and *L. acidophilus* (LAFTI L10,), *L. paracasei* (LAFTI L26)and *B. lactis* (LAFTI B94) (Delvo-Product, A-ustralia).

#### 2.2 Production of probiotic chocolate mousse

The formulations were individually pasteurized at  $74\pm2^{\circ}$ C for 15S and then mixed simultaneously. The content of components used in chocolate mousse formulation is presented in Table 1. The probiotic microorganisms including *L. acidophilus*, *L. paracasei* and *B. lactis* were added with 0.05 % (w w<sup>-1</sup>) at 38-40°C. Then, all samples were stored at  $4\pm1^{\circ}$ C [18]. The chocolate dessert trials were produced in triplicate.

**Table 1.** Ingredient and respective quantities  $(g \ 100 \ g^{-1})$ employed for the production of chocolate mousse formulations

Ingredients (%)	Formulations						
ingredients (%)	Milk	Milk/Soy milk 1:1	Soy milk				
Milk	83.00	41.50	-				
Soy milk	-	41.50	83.00				
Sugar	13.65	13.65	13.65				
Cocoa powder	2.00	2.00	2.00				
Gelatin powder	1.30	1.30	1.30				
Probiotic <sup>a</sup>	0.05	0.05	0.05				
Total	100.0	100.0	100.0				

<sup>a</sup>L. acidophilus, L. paracaseiand B. lactiswere added as probiotic.

### 2.3. Acidity and pH analysis

The pH of samples in each day of storage was measured by a digital pH-meter (Elmentron pH-meter CP-501, Netherlands). Titratable acidity was determined by titrating 10 g of sample in 100 ml of distilled water with NaOH 0.1 N to an endpoint of pH 8.3 according to AOAC [19]. All pH and acidity measurements were carried out in triplicate.

#### 2.4. Enumeration of probiotics

Chocolate desserts were decimally diluted in sterile NaCl solution (0.9%), and 1 ml aliquots were poured into plates of the MRS agar (Ibersco, Iran) in triplicate. *B. lactis* was incubated under anaerobic and *L. acidophius* and *L. paracasei* were incubated aerobic condition at 37°C for 72 h. The results were reported as colony-forming units per gram (log CFU g<sup>-1</sup>) per day of 1, 7, 14 and 21 of storage [20].

#### 2.5. Rheological measurements

The apparent viscosity of the desserts was measured in three shear rates (0.01, 0.1 and 1 S<sup>-1</sup>) by means of a rotational viscometer (Anton PaarPhysica MCR30, Austria) at 10°C 7 days after production [21]. Dynamic oscillatory measurements were done by Cone & Plate rheometer [22]. In Oscillatory shear tests, frequency sweeps were fulfilled in 0.1-50 Hz and rheological parameters of samples were reported at 0.2, 2 and 20 Hz. Elastic modulus (G'), loss modulus (G'') and complex modulus (G\*) are determined. Herschel-Bulkley and Power Low model are used to find an appropriate model for studying the rheological behavior of samples. The equation of this models are as Eq. 1 and Eq. 2.

Power low model: $\tau = K(\gamma)^n$	Eq.1
Herschel-Bulkley model: $\tau = \tau_0 + K(\gamma)^n$	Eq.2

Where *K* is consistency index (Pa S<sup>-1</sup>), *n* is flow behavior index,  $\tau_0$  is Initial shear stress (Pa) and  $\tau$  is shear stress (Pa).

#### 2.6. Sensory evaluation

Thirty panelists tasted the chocolate mousse samples in random order for the assessment of organoleptic properties, and the Hedonic test (eight-point scale) was used for scoring flavor acceptability and overall impression with 8 as the most desirable. Samples were distributed among panelist at 23°C, and the panelists were asked to evaluate the three-digit coded samples of the three different trials of mousse. Samples were tasted by the panel 7 days after the chocolate mousse production [14].

### 2.7. Statistical analysis

Chemical and microbial studies were carried out in 3 replications. Data was analyzed using SPSS version 17 (SPSS Inc., Chicago, IL, USA). Analysis of variance followed by Duncan's multiple range tests used to distinguish significant differences in treatments (viability of microorganisms, pH, titratable acidity and sensory properties) at  $p \le 0.05$ .

### **3. Results and Discussion**

#### 3.1. Titratable acidity and pH

The mean value and standard deviation of pH and titratable acidity of different trials of the chocolate mousse evaluated during refrigerated storage are shown in Table 2 and Table 3. The results showed that there was significant reduction in the pH during storage ( $p \le 0.05$ ) and also there was considerable increase in the acidity of all samples during the storage period ( $p \le 0.05$ ). The rise in acidity and decreased pH of the chocolate mousse with *L. paracasei* was significant. However, the rate of acidity increase and pH drop rate of samples including *B. lactis* was slower, when compared to the trails including *L. acidophilus* and *L. paracasei*.

**Table 2.** Effect of *L. acidophilus*, *L. paracasei* and *B. lactis* and different formulations (milk, milk/soy milk and soy milk) on pH of chocolate mousse during the refrigerated storage.

]	Freatment		Time (d	lays)	
Probiotic	Formulation	1	7	14	21
L. acidophilus	Milk	6.77±0.02 °	$6.67 \pm 0.02$ f	5.57±0.03 <sup>q</sup>	4.45±0.03 <sup>y</sup>
L. acidophilus	Milk/Soy milk	6.94±0.03 <sup>a</sup>	6.33±0.04 <sup>ij</sup>	4.76±0.02 <sup>w</sup>	4.48±0.02 <sup>y</sup>
L. acidophilus	Soy milk	6.90±0.02 bc	5.99±0.02 <sup>m</sup>	5.82±0.03 <sup>n</sup>	5.35±0.02 s
L. paracasei	Milk	6.63±0.04 <sup>gh</sup>	6.12±0.03 k	5.51±0.03 r	4.64±0.04 x
L. paracasei	Milk/Soy milk	6.67±0.01 <sup>f</sup>	5.56±0.01 <sup>q</sup>	4.29±0.02 <sup>z</sup>	3.98±0.03 á
L. paracasei	Soy milk	$6.04\pm0.04^{-1}$	5.24±0.03 t	5.15±0.03 <sup>u</sup>	5.05±0.01 v
B. lactis	Milk	6.82±0.03 de	6.65±0.02 fg	6.59±0.02 <sup>h</sup>	5.67±0.03 <sup>p</sup>
B. lactis	Milk/Soy milk	6.85±0.03 <sup>cd</sup>	6.29±0.01 <sup>j</sup>	5.75±0.02 °	5.07±0.03 <sup>v</sup>
B. lactis	Soy milk	6.92±0.03 ab	6.37±0.01 <sup>i</sup>	$6.07 \pm 0.06^{1}$	5.74±0.01 °

Values with different letters are significantly different ( $p \le 0.05$ )

**Table 3.** Effect of *L. acidophilus*, *L. paracasei* and *B. lactis* and different formulations (milk, milk/soy milk and soy milk) on titratable acidity (%) of chocolate mousse during the refrigerated storage.

]	Treatment		Time (o	lays)	
Probiotic	Formulation	1	7	14	21
L. acidophilus	Milk	0.07±0.00 <sup>p</sup>	0.09±0.00 <sup>op</sup>	$0.25 \pm 0.02^{ij}$	0.68±0.03 °
L. acidophilus	Milk/Soy milk	0.07±0.01 <sup>p</sup>	$0.28 \pm 0.05$ hi	$0.59 \pm 0.04^{\text{ d}}$	0.89±0.02 <sup>b</sup>
L. acidophilus	Soy milk	0.13±0.00 <sup>no</sup>	$0.14{\pm}0.00^{mn}$	$0.22{\pm}0.03^{jkl}$	$0.24{\pm}0.02^{ijk}$
L. paracasei	Milk	$0.10 \pm 0.02^{nop}$	$0.25 \pm 0.03^{ij}$	$0.35 \pm 0.02^{\rm f}$	0.65±0.03 °
L. paracasei	Milk/Soy milk	0.13±0.00 <sup>no</sup>	$0.25 \pm 0.00^{ij}$	1.24±0.02 <sup>a</sup>	1.27±0.02 <sup>a</sup>
L. paracasei	Soy milk	$0.18 \pm 0.04$ lm	$0.23 \pm 0.01^{jkl}$	$0.31{\pm}0.02^{fgh}$	$0.35 \pm 0.02^{fg}$
B. lactis	Milk	$0.07 \pm 0.00^{\text{ p}}$	$0.09 \pm 0.00^{\text{ op}}$	$0.10 \pm 0.00^{nop}$	$0.28 \pm 0.02^{hi}$
B. lactis	Milk/Soy milk	0.08±0.00 <sup>p</sup>	$0.30{\pm}0.01^{gh}$	$0.32{\pm}0.07^{fgh}$	0.43±0.03 °
B. lactis	Soy milk	0.10±0.00 <sup>nop</sup>	$0.11 {\pm} 0.00^{nop}$	$0.20{\pm}0.01$ kl	$0.23 \pm 0.01^{jkl}$

Values with different letters are significantly different (p ≤ 0.05)

This may be attributed to the lower speed of growth in B. lactis than L. acidophilus, L. paracasei. The optimum pH growth for most bacteria is something around neutral and if pH falls below 5, the growth of the bacteria is hampered [14]. In the current experiment, the rise in acidity caused, hampered the growth of the bacteria at day 21. L. paracasei is considered to be an optional heterofermentative lactobacillus that consumes lactose and converts it into lactic acid, acetic acid and carbon dioxide, which are responsible for acidification of the medium [22]. L. paracasei subsp. paracasei has acid-producing ability and has reduced the pH after 28 days of refrigerated storage in synbiotic chocolate mousse [14]. Regarding the effect of formulation (milk, milk/soy milk and soy milk) of chocolate mousse, at first day of storage, the samples containing soy milk showed higher titratable acidity due to lower pH buffering capacity of soy milk than milk [23]. However, at the end of the storage, samples containing milk/soy milk (1:1) showed lower pH and higher acidity most of the time. In chocolate mousse produced by milk/soy milk (1:1), microorganisms could use lactose of milk and also prebiotic content of soy milk like oligosaccharides [6].

#### 3.2. Viability of probiotic in chocolate desserts

The viability of *L. acidophilus, L. paracasei* and *B. lactis* in chocolate mousse during 21 days storage at 4°C is illustrated in Table 4. The counts of *L. acidophilus, L. paracasei* and *B. lactis* in chocolate mousse were higher than 7 log CFU g<sup>-1</sup> by the end of storage period. It shows that it is sufficient for a standard probiotic product [17]. The results showed that there was a significant increase in

all the mentioned probiotics bacteria (L. acidophilus, L. paracasei, B.lactis) of all the probiotic chocolate mousse during 21 days of storage 4°C. However, chocolate mousse prepared with soy milk and L. paracasei, showed a decrease in survival from day 14 to 21 (Table 4), because high activation of lactobacilli and high production of lactic acid and decreasing pH prevented the growth of lactobacilli [13]. Viability and lactic acid production of L. paracasei was higher than other sample (Table 4). There was a significant increase in population of probiotics bacteria (L. acidophilus, L. paracasei, B. lactis) in the samples including soy milk and milk/soy milk (1:1) in the formulation of chocolate mousse at day 7 and 14. However, at the end of storage, population of probiotic bacteria in samples including milk was higher due to the restriction effect of higher acidity on viability of probiotics (Table 4). Chocolate mousse containing L. paracasei, showed the most and *B. lactis* showed the least population of during 21 days storage at  $4^{\circ}$ C (Table 4) (p $\leq 0.05$ ). Vinderola et al. Showed that the survival of L. pap-racasei subsp paracasei was suitable (above 8 log CFU g<sup>-1</sup>) in creamy milk dessert and symbiotic formulation during 28 storages at 5°C [8]. Similar results on survival of L. acidophilus, L. casei, L. paracasei, L. rhamnosus, B. lactis which incorporated into milk [24], soy milk/ milk [6], cheese [8], ice cream [25], chocolate mousse [14] and creamy milk chocolate dessert [22] were found in the literature. These studies demonstrated that all dairy products mentioned can be used as a vehicle for probiotic bacteria and these probiotic bacteria survived satisfactory during storage.

Table 4. Viability (CFU g <sup>-1</sup> ) of L. acidophilus, L. paracasei and B. lactis during the refrigerated storage of different
formulation of chocolate mousse (milk, milk/soy milk and soy milk).

Trea	tment		Time (d	lays)	
Probiotic	Formulation	1	7	14	21
L. acidophilus	Milk	7.24±0.12 <sup>qrs</sup>	7.40±1.14 <sup>qr</sup>	9.13±0.02 <sup>ij</sup>	9.41±0.03 <sup>def</sup>
L. acidophilus	Milk/Soy milk	7.17±0.02 <sup>rst</sup>	$8.90 \pm 0.30^{kl}$	$9.24{\pm}0.79^{\mathrm{fi}}$	$9.36 \pm 0.02^{efg}$
L. acidophilus	Soy milk	7.54±0.06 <sup>p</sup>	$8.72 \pm 0.07^{lm}$	9.06±0.01 <sup>jk</sup>	$9.34 \pm 0.04^{eh}$
L. paracasei	Milk	$8.90 \pm 0.01^{kl}$	$9.22 \pm 0.10^{gi}$	9.34±0.70 <sup>eh</sup>	$9.74 \pm 0.08^{b}$
L. paracasei	Milk/Soy milk	8.10±0.06°	9.34±0.11 <sup>eh</sup>	$9.58 \pm 0.43^{bcd}$	$9.62 \pm 0.02^{bc}$
L. paracasei	Soy milk	8.02±0.01°	9.43±0.62 <sup>de</sup>	10.01±0.01 <sup>a</sup>	9.47±0.08 <sup>cde</sup>
B. lactis	Milk	7.10±0.05 <sup>st</sup>	$7.35 \pm 0.05^{q}$	$7.31 \pm 0.08^{qr}$	9.31±0.73 <sup>ei</sup>
B. lactis	Milk/Soy milk	7.62±0.15 <sup>p</sup>	$8.67 \pm 0.08^{m}$	8.37±0.11 <sup>n</sup>	$8.69 \pm 0.03^{m}$
B. lactis	Soy milk	$7.00 \pm 0.00^{t}$	$8.13 \pm 0.02^{\circ}$	9.31±0.72 <sup>ei</sup>	9.17±0.11 <sup>hij</sup>

Values with different letters are significantly different ( $p \le 0.05$ )

#### 3.3. Rheological measurement

As shown in Figure 1, the apparent viscosity of probiotic chocolate mousse decreased with an increasing shear rate. All samples showed shear-thinning behavior in three shear rates. This decrease may be due to the reduction in the size of colloidal aggregates and gel disruption as the shear rate increased. With increasing shear rate, these molecules aligned in more similar directions and consequently intermolecular friction and viscosity values decreased. This phenomenon could be illustrated by the ability of soy protein existed in dessert formulation to form a stable network similar to a gel structure [26]. Table 5 presents the yield stress ( $\tau_0$ ), consistency coefficient (K) and the flow behavior index (n)of the sample according to the model parameters that best fit the curves. In order to evaluate the rheological behavior of probiotic chocolate mousse, the data were fitted to the rheological models such as Herschel-Bulkley and Power law. Average coefficient determination (r<sup>2</sup>) of Herschel-Bulkley and Power law models were 0.984 vs. 0.977, respectively. Therefore, both models presented high values of coefficient determination which are suitable for explanation of flow behavior of all samples. The flow behavior index (n) of all probiotic chocolate mousse presented a shear thinning behavior (0 < n < 1). The consistency coefficient increased in all samples by increasing the soy milk content in formulation duo to improvements in resistance of chocolate mousse against structural damaging. Soy proteins create a constant gel

structure because of their molecular properties so it could be strengthening the structure of probiotic chocolate dessert against various fractures and therefore increase viscosity [15].

Therefore, the *K* value of samples prepared by soy milk were the highest. The difference between viscosity of samples were due to difference between quantity of proteins of soy milk [27]. The consistency coefficient of chocolate mousse including *B. lactis* and *L. paracasei* were the most and least respectively ( $p \le 0.05$ ). This could be related to lower viability and acid production of *B. lactic* and higher viability and acid production of *L. paracasei* in all chocolate mousse samples (Tables 3 and 4).

As shown in Table 6, a visco-elastic behavior observed in probiotic chocolate mousse samples through the smallamplitude oscillatory test, which measures the elastic (G'), viscous (G") and complex (G\*) modulus. In Oscillatory shear test, frequency sweep was performed in frequency range of 0.1-50 (Hz) and rheological parameters of samples were reported at 0.2, 2 and 20 (Hz). According to Table 6, probiotic chocolate mousse showed visco-elastic solid like behavior because all ranges of frequency elastic modulus (G') was above loss modulus (G"). Complex modulus (G\*), representing the strength of foods, showed the most values of G\* observed in the soy milk formulation thanks to the firmness of soy proteins creation [27,28]. Visco-elastic behavior of probiotic chocolate mousse including B. lactis and L. paracasei illustrated the most and least elastic modulus (G').

Table 5. Output measurements	of Herschel-bulkely and	power law models
------------------------------	-------------------------	------------------

Treat	tment		Herschel H	Bulkley			Power law	
Probiotic	Formulation	K	n	$\tau_0$	r	Κ	n	r
L. acidophilus	Milk	26.5	0.388	7.04	0.99	28.6	0.299	0.98
L. acidophilus	Milk/Soy milk	31.4	0.442	8.62	0.98	29.9	0.306	0.99
L. acidophilus	Soy milk	19.1	0.381	6.61	0.97	25.2	0.222	0.97
L. paracasei	Milk	31.9	0.355	10	0.99	28.9	0.189	0.97
L. paracasei	Milk/Soy milk	33.5	0.326	11.7	0.98	35.5	0.336	0.99
L. paracasei	Soy milk	27.7	0.319	10.5	0.98	23.7	0.184	0.95
B. lactis	Milk	39.2	0.340	12	0.99	50.1	0.235	0.98
B. lactis	Milk/Soy milk	41.5	0.342	11.5	0.99	53.9	0.261	0.98
B. lactis	Soy milk	37.4	0.320	9.64	0.99	48.1	0.275	0.99

Consistency coefficient (K), flow behavior index (n), yield shear stress ( $\tau_0$ ) and coefficient determination (R)

Table 6. Values of modulus	$(G', G'' \text{ and } G^*)$	) in frequency sweep test	in 0.2, 2 and 20 Hz
----------------------------	------------------------------	---------------------------	---------------------

Trac	tment				1	Frequency	/ (Hz)			
Ilea	tinent		0.2			2			20	
Probiotic	Formulation	G'	G"	G*	G'	G"	G*	G'	G"	G*
L. acidophilus	Milk	90.5	5.76	90.7	100	10.9	101	108	12.5	108
L. acidophilus	Milk/Soy milk	91.3	6.58	91.6	101	7.98	101	112	11.7	113
L. acidophilus	Soy milk	88.9	4.27	89	91.1	17.9	92.9	97.3	6.67	97.5
L. paracasei	Milk	108	5.8	108	120	8.97	121	187	15.9	188
L. paracasei	Milk/Soy milk	121	6.7	121	134	9.31	134	192	15.1	193
L. paracasei	Soy milk	61.7	5.34	61.9	69.7	9.82	70.4	82	17.6	83.9
B. lactis	Milk	156	12.5	157	184	18.6	185	189	29	192
B. lactis	Milk/Soy milk	162	12.1	162	199	18.4	200	245	30.3	246
B. lactis	Soy milk	112	11.7	113	177	17.6	178	185	25	188

#### 3.4. Sensory properties

Table 7 presents the results of the sensory evaluation of the chocolate mousse. Regarding the effect of chocolate mousse formulation (milk, milk/soy milk and soy milk), the addition of soy milk, negatively affected chocolate mousse flavor, aroma and overall acceptance ( $p \le 0.05$ ). The chocolate mousse containing milk, exhibited the highest score for these attributes in comparison with other treatments. On the other hand, by increasing the soy milk level in chocolate mousse, the score of consumer acceptance decreased. Although, formulation of chocolate mousse containing milk and soy milk showed the highest and lowest overall acceptance score respectively, the consumer acceptance of formulation including milk/soy milk (1:1) was relatively acceptable. This result is in line with those of Wang et al. which reported that addition of soy milk decreases the palatability of fermented milk duo to the existence of 2-isopropyle-3-methoxy pyrazine [6,29], which produce sulfur compound with roasted aroma during sterilization of soy milk and decrease concentration of lactose [30]. To consider the effect of probiotics on sensory properties of chocolate mousse, B. lactis and L. acidophillus obtained more scores on flavor, odor and general acceptance in comparison with L. paracasei (Table 7). Panelists did not recognize any significant differences in the preference of aroma and flavor between all formulations including L. acidophilus and B. lactis (p≤0.05).

**Table 7.** Sensory properties of probiotics chocolate mousse formulation

Trea	Sensory attribute			
Probiotic	Formulation	Flavor	Aroma	Overall acceptance
L. acidophilus	Milk	6.03 <sup>a</sup>	6.27 <sup>ab</sup>	6.50 <sup>a</sup>
L. acidophilus	Milk/Soy milk	5.07 bc	5.17 <sup>cd</sup>	5.10 bc
L. acidophilus	Soy milk	4.77 °	4.63 de	4.67 <sup>cd</sup>
L. paracasei	Milk	5.07 bc	5.70 <sup>bc</sup>	5.27 <sup>bc</sup>
L. paracasei	Milk/Soy milk	3.73 <sup>d</sup>	$3.70^{\text{ fg}}$	3.70 °
L. paracasei	Soy milk	3.97 <sup>d</sup>	3.33 <sup>g</sup>	3.60 °
B. lactis	Milk	6.10 <sup>a</sup>	6.57 <sup>a</sup>	6.73 <sup>a</sup>
B. lactis	Milk/Soy milk	5.43 <sup>b</sup>	5.80 <sup>b</sup>	5.37 <sup>b</sup>
B. lactis	Soy milk	4.73 °	4.07 <sup>ef</sup>	4.40 <sup>d</sup>

Values with different letters in the same column are significantly different (p $\!\leq\!0.05)$ 

# 4. Conclusion

In the present study, applying soy milk in probiotic chocolate mousse can produce a multi-functional dessert which improves the viability of three probiotic bacteria (*L. acidophilus, L paracasei, B. lactis*) as well as milk thanks to the use prebiotic properties of soy milk. Although soy milk had negative effects on sensory evaluation but using milk/soy milk (1:1) improves organoleptic properties of chocolate mousse in an acceptable level. According to dynamic mechanical analysis, the probiotic chocolate

mousse known as viscoelastic solid desserts also showed shear-thinning behavior, but this property was more impressive in soy milk desserts. In conclusion, this study demonstrates that chocolate mousse may be used as a vehicle for probiotic bacteria which survived satisfactory during 21 days of cold storage.

# 5. Acknowledgements

Authors would like to thank laboratory of department of food Science and Technology, Faculty of Agriculture, Isfahan (Khorasgan) Branch, Isfahan, Islamic Azad University, Isfahan, Iran.

# 6. Conflict of Interest

The authors declare that they do not have any conflict of interest.

### References

- 1. Rad A, Akbarzadeh F, Mehrabany EV. Which are more important: Prebiotics or probiotics? Nutr. 2012; 28(11-12): 1196-1197. doi: 10.1016/j.nut.2012.03.017.
- Rad A, Torab R, Mortazavian AM, Mehrabany EV, Mehrabany, LV. Can probiotics prevent or improve common cold and influenza? Nutr. 2013; 29(5): 805-806. doi: 10.1016/j.nut.2012.10.009
- Pandey KR, Naik SR, Vakil BV. Probiotics, prebiotics and synbiotics-Review. J Food Sci Technol. 2015; 52(12): 7577-7587. doi: 10.1007/s13197-015-1921-1.
- Granato D, Branco GF, Cruz AG, Faria JAF, Shah NP. Probiotic dairy products as functional foods. Comp Rev Food Sci Food Safety. 2010; 9(5): 455-470. doi: 10.1111/j.1541-4337.2010.00120.x.
- Brouns F. Soya isoflavones: A new and promising ingredient for the health foods sector. Food Res Int. 2002; 187-193.doi: 10.1016/S0963-9969(01)00182-X
- Wang YC1, Yu RC, Chou CC. Antioxidative activities of soy milk fermented with lactic acid bacteria and bifidobacteria. Food Microbiol. 2006; 23(2): 128-35. doi: 10.101-6/j.fm.2-005.01.020.
- Kazemi A, Mazloomi S, HassanzadehRostami Z, Akhlaghi M. Effect of adding soy milk on physicochemical, microbial and sensory characteristics of probiotic fermented milk containing *Lactobacillus acidophilus*. Iran J Vet Res. 2013; 48: 206-210.
- Vinderola CG, Prosello W, Ghiberto D, Reinheimer JA. Viability of probiotic (Bifidobacterium, *Lactobacillus acidophilus* and *Lactobacillus casei*) and nonprobiotic microflora in Argentinian fresco cheese. J Dairy Sci. 2000; 83: 1905-1911.
- Chen T, Wei Q, Chi Z. Effect of oligosaccharides and isoflavonesaglycones in defatted soy meal fermented by *Lactobacillus paracasei* and *bifidobacteriumlongum*. Afr J Microbiol Res. 2011; 5(15): 1-9. doi: 10.5897/AJMR10.553
- Tsangalis D, Shah NP. Metabolism of oligosaccharides and aldehydes and production of organic acids in soy milk by probiotic bifidobacteria. Int J Food Sci Technol. 2004; 39(5): 541-554. doi: 10.1111/j.1365-2621.2004.00814.x.

- 11. Homayouni Rad AH, Mehrabany EV, Alipoor B, Mehrabany LV, Javadi M. Do probiotics act more efficiently in foods than in supplements? Nutr. 2012; 28(7): 733-736. doi: 10.1016/j.nut.2012.01.012.
- Homayoni Rad A, VaghefMehrabany E, Alipoor B, VaghefMehrabany L. The comparison of food and supplement as probiotic delivery vehicles. Crit Rev Food Sci Nutr. 2016; 56(6): 896-909. doi: 10.1080/10408398-.2012.733894.
- Buriti FCA, Bedani R, Saad SMI. Probiotic and prebiotic dairy desserts. In: Watson RR, Preedy VR. Probiotics, prebiotics and synbiotics, Elsevier Inc, 2016; 345-360. doi: 10.1016/B978-0-12-802189-7.00023-X
- Casale Aragon-Alegroa L, Henrique Alarcon Alegrob J, Roberta-Cardarellib H, ChihChiub M, IsaySaadb SM. Potentially probiotic and symbiotic chocolate mousse. LWT-Food Sci Technol. 2007; 40(4): 669-675. doi: 10.1016/j.lwt.2006.02.020
- 15. Aboulfazli F, Salihin baba A, Misran M. Replacement of bovine milk with vegetable milk: Effects on the survival of probiotics and rheological and physicochemical properties of frozen fermented dessert. Int J Dairy Tech. 2014; 13: 1-27. doi: 10.1111/1471-0307.12219
- Homayouni A, Azizi A, Javadi M, Mahdipour S, Ejtahed H. Factors influencing probiotic survival in ice cream: A review. Int J Dairy Sci. 2012; 1-10. doi: 10.3923/ijds.2012.1.10
- Lalicic-Petronijevic J, Popov-Raljic J, Obradovic D, Radulovic Z, Paunovic D, Petrrusic M, Pezoiu L. Viability of probiotic strains *Lactobacillus acidophilus* NCFM and *bifidobacterium lactis* HN019 and their impact on sensory and rheological properties of milk and dark chocolates during storage for 180 days. J Func Foods. 2015; 5: 541-550. doi: 10.1016/j.jff.2015.03.046
- Richter Reis F, Bellarmino de pereira-netto A, Silveira JLM, Haminiuk CWI, Candido LMB. Apparent viscosity of a skim milk based dessert: Optimization through response surface methodology. Food Nutr Sci. 2011; 2(2): 90-95. doi: 10.4236/fns.2011.22012
- 19. AOAC. (2000). Official methods of analysis 17th. AOAC. International, Gaitherburg, M.D,
- Sarvari F, Mortazavian A.M, Fazeli M.R. Biochemical characteristics and viability of Probiotic and yogurt bacteria in Yogurt during the Fermentation and refrigerated storage. Appl Food Biotechnol. 2014; 1(1): 55-61. doi: 10.2203-

7/afb.v1i1.7125.

- Pang Z, Deeth H, Sopade P, Sharma R. Bansal N. Rheology, texture and microstructure of gelatin gels with and without milk proteins. Food Hyrocoll. 2014; 35: 484-493. doi: 10.1016/j.foodhyd.2013.07.007.
- 22. Sarmento Valencia, M. Magalhaes Salgado S, Alvachian Cardoso Andrade S, Montarroyos Padilha V, Souza Livera AV, Montenegro Stamford TL. Development of creamy milk chocolate dessert added with fructo-oligosaccharide and *Lactobacillus paracasei* LBC 81. LWT-Food Sci Technol. 2016; 69, 104-109. doi: 10.1016/j.lwt.2016.01.039
- Wang J, Guo Z, Zhang Q, Yan L, Chen W, Liu XM, Zhang HP. Fermentation characteristics and transit tolerance of *Lactobacillus casei* in soy milk and bovine milk during storage. J Dairy Sci. 2009; 92, 2468-2476. doi: 10.3168/jds.2008-1849
- Oliveria D, Vidal L, Ares G, Walter EHM, Rosenthal A, Deliza R. Sensory, microbiological and physicochemical screening of probiotic cultures for the development of nonfermented probiotic milk. LWT-Food Sci Technol. 2017; 79: 234-241. doi: 10.1016/j.lwt.2017.01.020
- Cruz AG, Antunes AEC, Sousa ALOP, Faria JAF, Saad SMI. Ice-cream as a probiotic food carrier. Food Res Int. 2009; 42(9): 1233-1239. doi: 10.1016/j.foodres.2009.03.020
- Mahdian E, Karazhian R. Effects of fat replacers and stabilizers on rheological, physicochemical and sensory properties of reduced-fat ice cream. J Agric Sci Technol. 2013; 15: 1163-1174.
- Adapa S, Dingeldein H, Schmidt KA. Rheological properties of ice cream mixes and frozen ice creams containing fat and fat replacers. J Dairy Sci. 2000; 83: 2224-2229. doi: 10.3168/jds.S0022-0302(00)75106-X.
- Nishinari K, Fang Y, Guo S, Phillips GO. Soy proteins: A review on composition, aggregation and emulsification. Food Hydrocolloid. 2014; 39: 301-318. doi: 10.1016/j.foodhyd-. 2014.01.013.
- 29. Kaneko S, Kumazava K, Nishimura O. Studies on the key aroma compounds in soy milk made from three different soybean cultivars. J Agric Food Chem, 2011; 59(22): 12204-12209. doi.: 10.1021/jf202942h
- Lozano PR, Drake M, Benitez D, Cadwallader KR. Instrumental and sensory characterization of heat-induced odorants in aseptically packaged soy milk. J Agric Food Chem. 2007; 55(8): 3018-3026. doi: 10.1021/jf0631225

#### **Research Article**

pISSN: 2345-5357

eISSN: 2423-4214

APPLIED FOOD BIOTECHNOLOGY, 2018, 5 (2):79-86 Journal homepage: www.journals.sbmu.ac.ir/afb





# خواص فیزیکوشیمیایی موس شکلات زیستیار (Probiotic) بر پایه شیر سویا در مدت نگهداری در يخيال

گلنوش تقیزاده، مهشید جهادی\*، هاجر عباسی

گروه علوم و صنايع غذايي، دانشكده كشاورزي، واحد اصفهان (خوراسگان)، دانشگاه آزاد اسلامي، اصفهان، ايران.

# چکیدہ

**سابقه و هدف:** اخیراً، چندین محقق منافع ناشی از محصولات زیستیار حاوی باکتریهای لاکتوباسیلوس و بیفیدوباکتریها در فرمولاسیون هایشان را نشان دادهاند. هدف از این پژوهش بهبود موس شکلاتی زیستیار با استفاده از شیر و شیر سویا به لحاظ زنده مانی باکتریهای زیستیار و پذیرش حسی در مدت ۲۱ روز بود.

**مواد و روشها:** ۹ فرمول موس شکلاتی زیستیار فراسودمند با استفاده از شیر، شیر/شیر سویا و شیر سویا و سه گونه زیستیار (*لاکتوباسیلوس اسیدوفیلوس، لاکتوباسیلوس پاراکازئی و بیفیدوباکتریوم لاکتیس*) تهیه شد. pH، اسیدیته، میزان زندهمانی گونههای میکروبی، خواص رئولوژیکی و حسی تیمارها در مدت ۲۱ روز نگهداری در یخچال (۴°C) پایش شد.

**یافتهها و نتیجه گیری:** میزان افت pH و افزایش میزان اسیدیته تمام تیمارها در مدت ۲۱ روز نگهداری بـه صـورت معنیدار بود (p≤۰/۰۵). در همه نمونهها افزایش معنیداری در تعداد باکتریهای پروبیوتیک در ۲۱ روز نگهـداری در یخچال در درجه حرارت ۲°C وجود داشت (p≤۰/۰۵). با این حال، میزان رشد باکتریهای زیستیار در فرمولهای موس شکلاتی تهیه شده با شیر سویا و شیر/شیر سویا (۱:۱) در روزهای ۷ و ۱۴ شتاب پیدا کرده بود. آزمونهای رئولوژیکی نشان داد که تیمارها، به عنوان دسر جامد ویسکوالاستیک شناسایی می شوند و رفتار رقیقشوندگی با برش از خود نشان میدهند. در نتیجه دسر شکلاتی پروبیوتیک حاوی شیر سویا همانند شیر حاملی موثرتر برای باكترىهاى زيست يار شامل *لاكتوباسيلوس اسيدوفيلوس، لاكتوباسيلوس پاراكازئي و بيفيدوباكتريوم لاكتيس* ميباشد.

**تعارض منافع:** نویسندگان اعلام می کنندکه هیچ تعارض منافعی وجود ندارد.

# تاريخچه مقاله

دریافت ۱۴ نوامبر ۲۰۱۷ داوری ۰۷ ژانویه ۲۰۱۸ پذیرش ۲۱ فوریه ۲۰۱۸

### واژگان کلیدی

- بيفيدوباكتريوم لاكتيس
  - دسر شکلاتی
- لاكتوباسيلوس اسيدوفيلوس
  - •لاكتوباسيلوس پاراكازئي
    - زيستيار
    - شيرسويا

# \*نویسنده مسئول

**مهشید جهادی**،گروه علوم و صنايع غذايي، دانشكده كشاورزي، واحد اصفهان (خوراسگان)، دانشگاه آزاد اسلامی، اصفهان، ايران.

تلفن: ۳۵۳۴۲۲۱۱-۹۸-۹۴+ يست الكترونيك: m.jahadi@khuisf.ac.ir