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SENSORY, MELTING AND TEXTURAL PROPERTIES OF FAT-REDUCED ICE CREAM INOCULATED WITH THERMOTOLERANT LACTIC ACID BACTERIA

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ABSTRACT
A fat reduced ice cream with inulin as fat replacer was employed as prebiotic to inoculate thermotolerant probiotic lactic acid bacteria. Inoculation of thermotolerant lactic acid bacteria resulted in a softer and more adhesive ice cream texture, probably due to the exopolysaccharide production of the employed strain. In same manner, melting rate of inoculated samples was reduced, enhancing melting properties due lactic acid bacteria inoculation. Inoculated samples presented lower pH values and relatively higher titratable acid content due lactic acid growth during frozen storage. There was no presence of lactic acid bacteria in control samples. In sensory evaluation, although the growth and adaptation of thermotolerant lactic acid bacteria in ice cream, there was no difference between control and inoculated sample, in relation to texture and acceptation. In this view, thermotolerant probiotic lactic acid bacteria in fat reduced ice cream with inulin as fat replacer can be employed as a good alternative to produce symbiotic foods, with inulin as prebiotic ingredient.

1.Introduction

Ice cream is a complex food system with a disperse phase consist in three main structural components (air bubbles, ice crystals and emulsified fat globules) immersed in a continuous liquid phase (unfrozen water with dissolved sugar, proteins and hydrocolloids) (Marshall et al., 2003; Clarke, 2004). During ice cream process, air incorporation during frozen implies a great number of physical changes that are favored by the proteins and emulsifiers stabilizing both emulsion and foam formed. The capacity of the ingredients to interact with each other maintain the physical and sensory properties of the frozen ice cream base during and after the frozen process, cold-chain storage and finally, when ice cream is consumed (Pintor y Totosaus, 2013). Fat is a very important ingredient in the disperse phase. Fat is related to melting, air stabilization and ice crystals formation (Bolliger et al., 2000; Chung et al., 2003; Clarke, 2004; Granger et al., 2005; Goff, 1997). During frozen mixing dispersed and emulsified fat globules avoid coalescence forming a film around air bubbles, stabilizing the system (Chung et al., 2003). Fat interactions like partial coalescence of ice crystals or protein induced flocculation affect ice cream texture (Méndez-Velasco and Goff, 2012). Fat reduction can be compensated with other ingredients. Pintor et al. (2014) replaced both butyric and vegetable fat in ice cream employing inulin.

Ice cream with probiotic has been proposed as a suitable vehicle for beneficial microorganisms' delivery if they remain viable during manufacture and frozen storage (Hekmat and McMahon, 1992; Cruz et al., 2009; Di Criscio et al., 2010; Mohammadi et al., 2011; Ayaz Javed and Nadeem, 2011). The technology to transform ice cream into a vehicle for probiotoic microorganisms must be well understood and studied simultaneously with the knowledge of the metabolism of the added cultures (Cruz et al., 2009). There are two plausible options to enhance probiotic survive during ice cream production. These are to protect bacteria by encapsulation or freeze dry, or to employ thermal shock proteins producing bacteria. Encapsulation has been proposed like an alternative to improve probiotic survival during ice cream manufacture and frozen storage (Mohammadi et al., 2011; Soukoulis et al., 2014), employing resistant starch as prebiotic (Homayouni et al., 2008) or alginate (Ahmadi et al., 2014; El-Sayed et al., 2015), or the use of freeze dry cultures (Nousia et al., 2011). Lactic acid bacteria with probiotic characteristics like adherence to epithelial cells, gastric conditions survive and pathogens inhibition (Ramirez-Chavarín et al., 2010, 2013) has been employed as started culture in cooked sausages (Pérez-Chabela et al., 2013; Diaz-Vela et al., 2015). When lactic acid bacteria survive thermal stress condition due the over-expression of thermal shock proteins, the adaptation to other stress condition is feasible. In this view, the objective of this work was to evaluate the use of a thermotolerant lactic acid bacteria to elaborate a fat reduce ice cream with inulin a prebiotic, in order to obtain a symbiotic ice cream.

2.Materials ad Methods

Ice cream elaboration

Fat-reduced ice cream was elaborated according the formulation described by Pintor et al. (2014). Solid ingredients like sugar (15% w/v), non-fat dry milk (8% w/v, DILAC, Cuautitlan Izcalli, Mexico), whey protein concentrate (4.0% w/v, DILAC, Cuautitlan Izcalli, Mexico), chicory inulin (3% w/v, Nano Nutrition, Naucalpan, México, Viscarin GP209 lambda carrageenan (0.25% w/v, FMC Biopolymers, Philadelphia), carboxymethylcellulose (0.25% w/v, FMC Biopolymers, Philadelphia), emulsifiers (sorbitan and glyceryl monostearates, 0.25% w/v, ARCY, Ecatepec, Mexico) were hydrated in water (ca. 58% v/v) at 60 °C, to disperse with a Oster homogenizer both butyric fat (7.0 % w/v, ARCY, Ecatepec, Mexico) and vegetal fat (3.5 % w/v, La Mixteca, Ecatepec, Mexico). The homogenized mixture was kept in refrigeration (2-4 °C) until processing.

A previously reported thermotolerant probiotic lactic acid bacteria, *Pediococcus pentosaceus* UAM22 (Ramirez-Chavarín et al., 2010, 2013) was reactivated in 10 mL Man, Rogosa, and Sharpe (MRS) broth, incubating at 37 °C for 24 h, until an optical density close to one (λ = 600 nm), containing approximately 10^8 CFU/mL. After centrifugation at 2,000 $\times$ g during 10 min, the cellular pellet was rinsed with distilled water and centrifuge at same condition again. The washed cellular pellet was dispersed in 5 mL of ice cream base and mixed with the rest of ice cream base before pasteurization. Control treatment was non-inoculated.

Ice cream base mixture was pasteurized at 70 °C during 30 min, ice cooled and stored overnight at 4 °C. Ice cream was elaborated in a 2 quarters Frozen Ice Cream CIM-50RSA machine (Cuisinart, East Windsor), mixing during 30 min until obtain a uniform frozen paste. Ice cream was immediately distributed in 250 mL containers and kept in frozen storage at -23 °C until further analysis after at least 24 h.

2.1.Texture and melting properties

Ice cream textural properties were determined adapting the methodology reported by Soukoulis et al. (2008). Ice cream samples were tempered at room temperature for 10 min. before being penetrated with a 10 mm ∅ acrylic probe at a constant speed of 1 mm/s in a Brookfield LFRA 4500 texturometer (Brookfield Engineering Lab, Middleboro). From force-time curves, ice cream hardness (maximum force during penetration), penetration work (energy required to beak ice cream structure during penetration), and adhesiveness (negative force to separate the probe form sample after penetration) were reported.

Melting properties were determined removing the ice cream samples from their containers after 10 min of tempered at room temperature. Samples were placed in stainless steel mesh sieve size 14 (1.4 mm opening) registering the time for the first drop of melted ice cream. The weight of melted ice cream was recorded each 5 min during one h to calculate melting rate (weight change versus time, in g/min) (Soukoulis et al., 2008).

2.2.Titratable Acidity, pH and bacterial count

Lactic acid bacteria count was determined during ice cream frozen storage at 1, 6, 9, 13, 16 and 21 days. Ten g of both inoculated and control samples were diluted with 90 mL of a sterilized serological solution (0.85% NaCl). From this dilution, aliquot of 1 mL was diluted with 9 mL of same solution in order to be inoculated in bacteriological agar plates. Plates were incubated at 37 °C during 24 h, reporting CFU/g of ice cream (Akin et al., 2007).

The changes in acidity as a putative result of lactic acid bacteria growth in ice cream were determined measuring titratable acidity and pH of the samples. 20 mL of melted ice cream was mixed with 40 mL of distilled water adding six drops of phenolphthalein indicator solution. Samples were titrating with NaOH standard solution (0.1 N) while stirring constantly until a faint pink color appearance. Titration continues until a persistent pink coloration. Acidity in g of lactic acid per liter was reported. The pH was measured to melted ice cream samples (approx. 20 mL) with a Beckman Φ 500 Benchtop Meter (Beckman Coulter, Fullerton).

2.3.Sensory evaluation

The sensory evaluation was carried out in the Universidad Autonoma Metropolitana Iztapalapa Sensorial Laboratory facilities, in special individual booths. A total of 54 untrained panelists (students and staffs) were asked to participate in the sensory evaluation. In order to determinate the “affective status” or how well the ice cream inoculated with thermotolerant lactic acid bacteria is liked by consumers, an acceptance test was performed, comparing the inoculated samples with the non-inoculated control (Meilgaard et al., 1999) was carried out in two sessions, one for the expectation about the color, creaminess and acidity employing a 5 point hedonic scale (much more than expected/much less than expected). In another session, texture and taste acceptance were evaluated with a 7 point hedonic scale (dislike extremely/like extremely). Both ice cream samples (inoculated and non-inoculated control) were presented directly from freezer (around -18 °C) in plastic containers (ca. 10 g), coded with three digit random numbers. Panelists were asked to rinse their mouth between samples. Ice creams samples had 13 days of frozen storage, time enough to allow lactic acid bacteria development.

2.4.Experimental design and data analysis

The effect of inoculate lactic acid bacteria in a fat reduced ice cream with inulin was determined according to the model:

$$yij = \mu + \alpha i + \beta j + \in ij \quad (1)$$

where yij represents the variable response for the i-th treatment (inoculated or non-inoculated) at the j-th time of storage (1, 6, 9, 13, 16 and 21 days); μ is the overall mean; αi and βj are the main effects of inoculation and storage time; and $\in ij$ is the residual or error terms assumed to be normally distributed with zero mean and variance σ^2 (Der and Everitt, 2002). Results were analyzed with the PROC ANOVA procedure in SAS Software v 8.0 (SAS System, Cary). Significant differences between means were determined by the Duncan means test.

For sensory evaluation, data of each analyzed attribute for both ice creams were compared using the t-test, PROC TTEST in SAS Software, to establish significantly ($P<0.05$) difference between the two ice cream samples.

3.Results and discussions

Ice cream texture and melting properties

The inoculation of thermotolerant lactic acid bacteria resulted in a significantly ($p<0.05$) softer texture, where ice cream hardness decreased significantly ($p<0.05$) during frozen storage. Same tendency was observed for penetration work, this is, control samples were significantly ($p<0.05$) harder than the inoculated ones. Energy to penetrate the ice cream also decreased significantly ($p<0.05$) during frozen storage. In contrast, ice cream tackiness was significantly ($p<0.05$) higher in inoculated samples, and this textural property increased significantly ($p<0.05$) during storage (Table 1).

The time to start ice cream melting, first drop, was not significantly ($p<0.05$) different for both ice cream samples, and the time for first drop decreased significantly ($p<0.05$) during storage. Melting rate was significantly ($p<0.05$) lower in inoculated samples. Ice cream melting rate decreased significantly ($p<0.05$) as well during storage time (Table 2).

The inoculation of thermotolerant lactic acid bacteria resulted in a softer and tackier texture. Although it has been reported that inulin improved texture and melting of probiotic ice cream (Akalin et al., 2008), in the ice cream formulations employed the only variable was the thermotolerant lactic acid bacteria inoculation. In this view, changes in ice cream texture must be attributed to thermotolerant lactic acid bacteria development at the experimental conditions employed. Pérez-Chabela et al. (2013) and Diaz-Vela et al. (2015) reported that this thermotolerant lactic acid strain, *P. pentosaceus* UAM22, produced exopolysaccharide in cooked meat batters, enhancing texture and moisture retention. *In situ* applications of exopolysaccharides are traditionally yogurt or kefir. Nonetheless, ropy cultures improved texture increasing viscosity in the cryo-concentrated serum phase during freezing, resulting in a more firm ice cream, replacing stabilizers (Goh et al., 2008).

Table 1. Ice cream textural properties during frozen storage

Time (days)	Hardness (N)		Penetration work (N s)		Tackiness (N)	
	Control	Inoculated	Control	Inoculated	Control	Inoculated
1	13.85±1.08 A, a	12.40±1.66 B, a	113.64±12.37 A, a	82.26±13.76 B, a	-4.28±0.63 B, d	-6.93±3.08 A, d
6	12.96±4.46 A, ab	11.75±1.29 B, ab	118.84±59.15 A, a	80.60±9.95 B, a	-4.64±2.54 B, c	-7.11±0.36 A, c
9	12.85±2.85 A, bc	11.55±3.06 B, bc	108.66±35.95 A, ab	75.21±7.96 B, ab	-5.01±1.33 B, bc	-7.47±0.71 A, bc
13	12.05±4.72 A, bc	11.23±0.88 B, bc	110.39±27.82 A, bc	75.13±10.30 B, bc	-5.85±0.00 B, b	-7.80±1.65 A, b
16	11.95±3.78 A, cd	10.75±0.72 B, cd	102.55±40.03 A, c	69.17±5.89 B, c	-6.12±1.12 B, a	-8.55±1.19 A, a
21	11.00±3.63 A, d	10.40±0.51 B, d	95.97±22.34 A, d	52.63±4.69 B, d	-6.99±2.66 B, a	-8.74±0.11 A, a

A, B Means with same letter are not significantly (P>0.05) different for control or inoculated sample.
a, b, c, d Means with same letter are not significantly (P>0.05) different for storage time.

Table 2. Ice cream melting properties during frozen storage

Time (days)	First drop (min)		Melting rate (g/min)	
	Control	Inoculated	Control	Inoculated
1	25.00±0.00 B, a	26.50±6.74 A, a	1.20±0.16 A, a	1.04±0.24 B, a
6	26.50±3.63 B, a	27.00±2.07 A, a	1.14±0.21 A, ab	0.98±0.17 B, b
9	25.10±0.00 B, b	26.00±2.07 A, b	0.98±0.18 A, bc	0.94±0.24 B, bc
13	25.00±2.07 B, b	25.00±0.00 A, b	0.92±0.04 A, bc	0.92±0.16 B, bc
16	22.00±2.07 B, bc	23.50±0.51 A, bc	0.86±0.18 A, c	0.82±0.13 B, c
21	21.50±1.55 B, c	23.60±3.11 A, c	0.83±0.11 A, c	0.81±0.10 B, c

A, B Means with same letter are not significantly (P>0.05) different for control or inoculated sample.
a, b, c, d Means with same letter are not significantly (P>0.05) different for storage time.

Table 3. Ice cream lactic acid bacteria (CFU), tritatable acidity and pH

Time (days)	Log CFU/mL		Tritatable acidity (lactic acid g/L)		pH	
	Control	Inoculated	Control	Inoculated	Control	Inoculated
1	0.0±0.0 B, c	7.28±0.53 A, c	2.30±0.67 B, d	2.69±0.72 A, d	6.10±0.23 A, c	6.14±0.08 B, c
6	0.0±0.0 B, b	8.23±0.10 A, b	2.32±0.65 B, d	2.58±0.83 A, d	6.24±0.11 A, ab	6.22±0.00 B, ab
9	0.0±0.0 B, a	8.60±0.01 A, a	2.93±0.77 B, b	3.56±0.63 A, b	6.28±0.07 A, a	6.21±0.03 B, a
13	0.0±0.0 B, a	8.65±0.51 A, a	3.09±1.18 B, a	3.92±0.76 A, a	6.27±0.09 A, b	6.15±0.22 B, b
16	0.0±0.0 B, b	8.26±0.05 A, b	2.58±0.39 B, c	2.69±0.72 A, c	6.34±0.04 A, ab	6.20±0.15 B, ab
21	0.0±0.0 B, bc	8.17±0.62 A, bc	2.52±0.68 B, c	2.90±0.50 A, c	6.41±0.08 A, b	6.13±0.04 B, b

A, B Means with same letter are not significantly (P>0.05) different for control or inoculated sample.
a, b, c, d Means with same letter are not significantly (P>0.05) different for storage time.

Table 4. Sensory analysis means scores for the expectation (color, creaminess and acidity) and acceptation (texture and taste) of ice cream

Treatment	Color*	Creaminess*	Acidity*	Texture**	Taste**
Control	3.09±0.83 a	2.48±1.21 a	3.85±0.89 a	3.24±1.31 a	3.36±1.25 a
Inoculated	3.29±0.98 a	2.20±1.07 a	3.83±0.82 a	3.31±1.22 a	3.42±1.61 a

a, b Means with same letter are not significantly (P>0.05) different for each sensory attribute
* Scores were based on a 5 points hedonic scale with 1 as “much less than expected” and 5 as “much more than expected”
** Scores were based on a 7 points hedonic scale with 1 as “dislike extremely” and 7 as “like extremely”

Cultures that produce exopolysaccharides increase cells thermal and physical shock presenting a thermotolerant capacity (Hong and Marshall, 2001). The production of exopolysaccharides affected as well the melting properties of inoculated ice cream. Non-inoculated ice cream melted faster than probiotic ice cream (Salem et al., 2005).

Lactic acid bacteria, titratable acidity and pH

As expected, lactic acid bacteria population was significantly ($p<0.05$) higher in inoculated ice cream samples. There was no detectable presence of lactic acid bacteria in control samples. Bacteria population increased significantly ($p<0.05$) during the first 13 days of storage, and then decline (that is why the sensory evaluation was performed at day 13). As consequence of lactic acid bacteria growth in ice cream, tritatable acidity was significantly ($p<0.05$) higher in inoculated samples, but the amount of titratable acid was not significantly ($p<0.05$) different during storage after the day one, with a tendency to decrease. In same manner, ice cream pH was significantly ($p<0.05$) higher in control samples, although with a significantly ($p<0.05$) tendency to increase as well during storage (Table 3).

The growth of inoculated thermotolerant lactic acid bacteria explains the changes in pH and titratable acidity. The decrease in bacterial counts is attributed to cell damage during ice cream manufacture (freezing plus mechanical stress, osmotic shock and air incorporation) (Akin et al., 2007; Magariños et al., 2007; Akalin et al., 2008; Turgut and Cakmakci, 2009; Ferraz et al., 2012). Cell viability depends on the strain, substrate, and final acidity (Mohammadi et al., 2011). Ice cream inoculated with *B. longum* and *B. lactis* reach a pH around 4.5 and resisted freezing process (Favaro-Trindade et al., 2006). Inulin in ice cream also improved bacteria survival. *L. acidophilus* and *B. lactic* growth in ice cream reached a pH of 5.9 (Akin et al., 2007). *L. casei* and *L. rhamnosus* growth in inoculated ice cream as well (Di Criscio et al. 2010).

As a consequence of lactic acid bacteria metabolism, inoculated ice cream had lower pH and higher acidity (Alamprese et al., 2005; Turgut and Cakmakci, 2009; Abghari et al., 2011; Nousia et al., 2011;) In ice cream elaborated with lactic acid bacteria, pH range was relatively high 6.24-6.42, due to ice cream buffer capacity (Salem et al., 2005). The pH variations in dairy products are influenced by the buffering capacity of milk soluble compounds like phosphate, calcium, citrate, caseins and whey proteins, and their distribution within the aqueous or solid phase of the product, where caseins and inorganic phosphate have maximum buffering capacity at pH between 5 and 6 (Salaün et al, 2005).

The no presence of lactic acid bacteria in control ice cream demonstrated the effectiveness of ice cream base thermal processing. In probiotic ice cream samples, Di Criscio et al. (2010) reported that undesirable microorganisms (enterobacteria, total and fecal coliforms) were detected at a very low numbers and not detectable. Although inulin improved lactic acid bacteria survive in ice cream (Akin, 2005; Akin et al., 2007), the thermotolerant capacity of the employed strain explains their prevalence during frozen storage. Abrupt decrease in temperature induces the over-expression of thermal shock proteins to the optimal adaptation to lower temperature (Hébraud and Potier, 1999). The temperature expressed cold-shock proteins are a response to cold shock and expressed when an exponentially growing culture is shifted from its optimum growth temperature to a lower temperature (Phadtare 2004). Exposure of the microorganisms to temperatures of 4 °C during 24 h resulted in the cryotolerance, capacity to survive freezing conditions, of partially frozen mixture after ageing of ice cream (Abghari et al., 2011). Freezing temperatures do not negatively affect survival of lactic acid bacteria during ice cream storage (Başyiğit et al., 2006; Di Criscio et al., 2010).

Sensory test

Sensory evaluation revealed that color and acidity of inoculated ice cream were "just as expected", with no significantly ($p>0.05$) difference between inoculated and control samples. Ice cream creaminess resulted "less than expected", but with no significantly ($p>0.05$) difference between both ice cream samples as well. This means that the consumers expectation for an ice cream inoculated with lactic acid bacteria was as they expected, where no difference in color and acidity was detected. Consumers asserted that ice cream creaminess was "less than expected", but with no significantly ($p>0.05$) difference between inoculated and control samples (Table 4).

On other hand, the acceptation of texture and taste was in the range of "dislike moderately". Nonetheless, for both sensory attributes there was no significantly ($p>0.05$) difference detected by panelists (Table 4). Again, instrumental texture differences detected were not reflected in sensory appreciation of ice cream texture, although the acceptance was fairly accepted. In same manner, since the amount of lactic acid was not as high as in a fermented food like yogurt, the acceptance for taste had a tendency to dislike, but panelists didn't find difference between both inoculated and control samples.

Although the relatively higher amount of lactic acid bacteria in inoculated samples, the buffering capacity of ice cream formulation not allowed the concentration of acid and pH decrease during ice cream storage. In same manner, while instrumental texture and melting differences were detected, no change in sensory creaminess was appreciated. Inoculated ice cream presented relatively higher scores than non-inoculated ones, presenting a good acceptation (Salem et al., 2005; Favaro-Trindade et al., 2006; Nousia et al., 2011; Ferraz et al., 2012). Ice cream low acidity increased overall consumer acceptation (Hekmat and McMahon, 1992; Cruz et al., 2009). Microorganism addition along inulin had no effect on ice cream consistency or taste (El-Nagar et al. 2002; Akin et al. 2007; Di Criscio et al. 2010; Pandiyan et al, 2012). In general, during sensory evaluation the judges didn't find difference between both inoculated and non-inoculated ice cream samples. Ice cream color was "just as expected". Creaminess expectation was slightly lower, but acidity for the inoculated ice cream was above the expected. For texture and taste, both attributes was close to "neither like or dislike" in the ordinal hedonic scale proposed. The information provided to panelist about an ice cream containing lactic acid bacteria could provoke certain expectancy about texture and flavor, evaluated as creaminess and acidity. Lactic acid bacteria are associated to fermented foods as yogurt, or even yogurt ice cream, with an acid taste, but the overall expectancy was just as expected, although the acceptance was not over the favored or pleased acceptation. Probably the addition of fruit flavor could improve the taste acceptance.

4.Conclusions

The use of thermotolerant probiotic lactic acid bacteria in fat reduced ice cream with inulin as fat replacer and prebiotic demonstrated to be a good alternative to formulate symbiotic foods. Inoculation of lactic acid bacteria resulted in softer texture with longer melting rates. Although growth of lactic acid bacteria resulted in lower pH and more acidity, there was no difference in ice cream acceptation, even the expectation about an inoculated ice cream (associated to a sour flavor, related to ferment dairy products like yogurt). The capacity to survive thermal stress ensures that the lactic acid bacteria employed in this research survived ice cream process before and during frozen step and storage. The use of this strains is recommended to artisanal ice cream retail sold (average batch size 20 L with a shelf life between 10-15 days), since the time for consumption is shorter than industrial ice cream with higher volume of production.

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