Semina: Ciências Agrárias
ISSN: 1676-546X
semina.agrarias@uel.br
Universidade Estadual de Londrina
Brasil

Tsuchiya, Ana Claudia; da Graça Monteiro da Silva, Ana; Brandt, Daniela; Lahis Kalschne, Daneysa; Drunkler, Deisy Alessandra; Colla, Eliane
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Semina: Ciências Agrárias, vol. 38, núm. 2, marzo-abril, 2017, pp. 749-758
Universidade Estadual de Londrina
Londrina, Brasil

DOI: 10.5433/1679-0359.2017v38n2p749

Lactose-reduced ice cream enriched with whey powder

Sorvete enriquecido com soro em pó com lactose reduzida

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Abstract

Ice cream is a food product that pleases the palate of consumers worldwide. Whey powder (WP) has various technological and functional properties. However, WP increases the lactose content of the final products in which it is incorporated and causes grittiness and intolerance in lactose-sensitive individuals. This study aimed to produce ice cream with milk powder (MP) replaced by WP (MP/WP), decrease the lactose content by enzymatic hydrolysis and verify the physicochemical and microbiological parameters of the final product. Initially, the variables β-galactosidase concentration and reaction time were studied for the response of the percentage of lactose hydrolysis in a milk ice cream base, using a full 2^2 factorial design (FFD). With the reaction conditions defined (0.5 g L^{-1} of β-galactosidase at 37 °C for 4 h) the sucrose concentration and MP/WP replacement variables were then studied in the ice cream formulation for the percentage of lactose hydrolysis and overrun responses using a 2^2 FFD. The lactose hydrolysis, which ranged between 86.59-97.97%, was not affected by the MP/WP replacement in the ice cream, whilst the overrun was increased by the MP/WP replacement. The physicochemical and microbiological parameters of the ice cream were either not influenced or positively influenced by lactose hydrolysis and MP/WP replacement.

Key words: Enzymatic hydrolysis. Lactose hydrolysis. Lactose intolerance. Overrun.

Resumo

O sorvete é um alimento que agrada o paladar dos consumidores mundialmente. O soro em pó (WP) tem várias propriedades tecnológicas e funcionais. No entanto, sua adição em alimentos contribui para aumentar o teor de lactose no produto final, trazendo como consequências a arenosidade e a impossibilidade de consumo por pessoas intolerantes à lactose. O objetivo deste trabalho foi elaborar sorvetes com substituição do leite em pó (MP) por WP (MP/WP), reduzir o teor de lactose por hidrólise enzimática e determinar os parâmetros físico-químicos e microbiológicos do produto final. Inicialmente, as variáveis concentração de β-galactosidase e tempo de reação foram estudadas tendo como resposta o percentual de lactose hidrolisada na base láctea, aplicando um planejamento fatorial completo (FFD) 2^2. Com as condições de reação definidas (0,5 g L^{-1} de β-galactosidase a 37 °C por 4 h), as variáveis concentração de sacarose e substituição de MP/WP foram estudadas na formulação de sorvete tendo como resposta o percentual de lactose hidrolisada e *overrun,* pela aplicação de um FFD 2^2. O percentual de hidrólise da lactose variou entre 86,59 e 97,97% e a substituição MP/WP não afetou a hidrólise da lactose no sorvete, enquanto o *overrun* aumentou com a substituição MP/WP. Os parâmetros físico-químicos e microbiológicos dos sorvetes não foram influenciados ou foram influenciados positivamente pelas variáveis hidrólise da lactose e substituição MP/WP.

Palavras-chave: Hidrólise enzimática. Hidrólise da lactose. Intolerância a lactose. *Overrun.*

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Received: Apr. 14, 2016 – Approved: Sept. 14, 2016

Introduction

Ice cream is a food product that attracts consumers worldwide. It is defined as a frozen mass, which can contain whole milk, skim milk products, cream or butter, sugar, vegetable oil, egg products, fruit and fruit ingredients, coffee, cocoa, aroma substances and approved food colours (BELITZ et al., 2009). Structurally, ice cream is a type of foam in which the air bubbles are covered by ice crystals, individually or partially melted fat globules, and lactose crystals (ARBUCKLE, 1986).

Whey is the liquid remaining after clotting of milk for cheese or casein production (BELITZ et al., 2009). It can be classified as sweet whey when the coagulation of casein during the cheese production process occurs by enzymatic action; or acid whey, obtained from the coagulation of casein in an acid medium, containing either added acid compounds or microorganisms that produce the acid compounds. One tonne of whey is equivalent to the pollutant power of waste produced by 10,000 people (ANTUNES, 2003). The use of whey in the food industry prevents a pollution potential being released into water bodies. Furthermore, whey has excellent chemical, functional and nutritional characteristics (BRANDELLI et al., 2015), particularly due to its bioactive peptides, which have been associated with antioxidant, antimicrobial, anti-hypertensive and anti-diabetic activities (PEÑA-RAMOS; XIONG, 2001; FITZGERALD et al., 2004; JAKUBOWICZ; FROY, 2013; BRANDELLI et al., 2015).

Whey is composed of water (93%), lactose (4.5%), protein (0.8%), ash (0.5%) and lipids (0.5%) (ANTUNES, 2003). After drying, lactose is the major component (73%), followed by protein (13%), minerals (8.2%), moisture (4.6%) and lipids (1.1%) (BELITZ et al., 2009). The high lactose content in whey restricts its use in food because of technological issues, such as grittiness, and nutritional disorders, such as lactose intolerance.

Lactose is a disaccharide with low water solubility and, when present in combination with sucrose, which is a common practice in dairy products, it crystallises faster than sucrose (SORMOLI et al., 2013), leading to the defect known as grittiness (ABBASI; SAEEDABADIAN, 2015). Lactose stimulates the intestinal absorption and retention of calcium. It is digested in the small intestine, where the enzyme, β-galactosidase (lactase), catalyses lactose hydrolysis into glucose and galactose. In lactose intolerant individuals, the ingested lactose is only partially hydrolysed, or it is not hydrolysed at all (SWAGERTY JUNIOR et al., 2002; BEMILLER; HUBER, 2010). There are three ways to overcome the effects of lactose intolerance, including the removal of lactose from milk by the action of β-galactosidase, or by fermentative microorganisms that use lactose as a substrate, or by combining meals with exogenous β-galactosidase (BEMILLER; HUBER, 2010).

Lactose is the main carbohydrate in milk and is present at approximately 7.3% in ice cream (KARAMAN et al., 2014). Milk powder (MP) is a premium product, whereas whey is a by-product of cheese manufacturing, hence, replacing MP by WP (MP/WP) in ice cream preparation would decrease the cost of ice cream production. However, the natural content of lactose in ice cream is high and with WP addition, the lactose content is further increased. This could be overcome by hydrolysing the lactose into glucose and galactose. Compared to lactose, glucose and galactose are more interesting carbohydrates from an industrial perspective because they are sweeter and more water-soluble (NOVALIN et al., 2005). Based on these considerations, this study aimed to evaluate the physicochemical and microbiological quality of chocolate ice cream produced with MP/WP replacement and decreased lactose content by β-galactosidase activity.

Materials and Methods

Materials

Whole ultra-high temperature (UHT) milk (pH = 6.5; acidity = 16 °D; lipid = 3.2 g 100 g^{-1}; defatted

dry extract = 8.65 g 100 g^{-1} and lactose = 4.5 g 100 g^{-1}) (Frimesa, Medianeira, Brazil), β-galactosidase (β-D-galactoside-galactohydrolase, EC 3.2.1.23) obtained from the yeast *Kluyveromyces lactis* (Prozyn Biosolutions, São Paulo, Brazil), WP (lactose = 57.0 g 100 g^{-1}) (Alibra Ingredients, Marechal Cândido Rondon, PR, Brazil), MP (lactose = 30.9 g 100 g^{-1}) (Ilolay, Rafaela, Argentina), sucrose (Alto Alegre, Presidente Prudente, Brazil), stabiliser (Duas Rodas, Jaraguá do Sul, Brazil), chocolate flavouring (Duas Rodas), liquid glucose (Marvi, Ourinhos, São Paulo, Brazil), vegetable fat (Mesa, São Caetano do Sul, Brazil), and emulsifier (Duas Rodas) were used.

Defining the conditions for lactose hydrolysis

The ice cream with MP/WP replacement was initially prepared by lactose hydrolysis with β-galactosidase. The enzyme concentration and reaction time were determined by evaluating the enzyme activity in the formulation with 100% MP/WP replacement because it had the highest lactose content. Hydrolysis was performed only on the milk base (whole UHT milk and WP) because the other components used in the ice cream formulation did not contain lactose. In these experiments, the β-galactosidase was used according to the recommendations of the supplier that were based on its performance in liquid milk. Also, the lactose hydrolysis was performed at 37 °C. The variables, β-galactosidase concentration (0.5-0.9 g L^{-1}) and reaction time (2-4 h), were studied using a full 2^2 (3 centre points, a total of 7 runs) factorial design (FFD) and the response was the percentage of lactose hydrolysis (Table 1).

After mixing UHT milk and WP, β-galactosidase was added at the concentrations specified in Table 1, and the mixture then incubated at 37 °C in a water bath for the stipulated reaction time (Table 1). After, pasteurisation was performed at 70 °C for 30 min to inactivate the enzyme.

Table 1. FFD (2^2) matrix with coded and real values for the variables and response of percentage of lactose hydrolysis on milk base.

Run	x_1 [a]	x_2 [b]	Lactose hydrolysis (%)
1	-1 (0.5)	-1 (2)	70.72
2	+1 (0.9)	-1 (2)	78.76
3	-1 (0.5)	+1 (4)	86.63
4	+1 (0.9)	+1 (4)	95.10
5	0 (0.7)	0 (3)	91.20
6	0 (0.7)	0 (3)	91.32
7	0 (0.7)	0 (3)	91.25

[a] β-galactosidase concentration (g L^{-1}); [b] reaction time (h).

The glucose content was determined before (initial glucose) and after hydrolysis to measure the percentage of lactose hydrolysis, based on the formation of one molecule each of glucose and galactose from each lactose molecule degraded. The glucose release was determined using an enzymatic kit for glucose oxidase (Laborlab, Guarulhos, Brazil), and the concentration was calculated using a glucose standard curve.

MP/WP replacement in formulations

A standard formulation (based on whole UHT milk) included sucrose (140-200 g L^{-1}), liquid glucose (70 g L^{-1}), milk powder (100 g L^{-1}), vegetable fat (50 g L^{-1}), chocolate flavouring (50 g L^{-1}), stabiliser (10 g L^{-1}) and emulsifier (10 g L^{-1}).

The effects of sucrose concentration and MP/WP replacement on the percentage of lactose hydrolysis and overrun was evaluated in various

formulations using a 2^2 (3 centre points, a total of 7 runs) FFD. The sucrose amount ranged from 14-20% and the MP/WP replacement from 0-100%. A control formulation using 100% MP without lactose hydrolysis treatment, was prepared for comparison (Table 2).

Table 2. FFD (2^2) matrix with coded and real values for the variables and responses of percentage of lactose hydrolysis and overrun.

Run	x_1 [a]	x_2 [b]	Lactose hydrolysis (%)	Overrun (%)
1	-1 (14)	-1 (0)	86.59	37.26
2	+1 (20)	-1 (0)	86.82	38.57
3	-1 (14)	+1 (100)	91.75	48.88
4	+1 (20)	+1 (100)	91.32	45.26
5	0 (17)	0 (50)	97.36	44.85
6	0 (17)	0 (50)	96.68	44.40
7	0 (17)	0 (50)	97.97	45.36
8[c]	0 (17)	-1 (0)	Without hydrolysis	39.94

[a] Sucrose concentration added (%); [b] MP/WP replacement (%); [c] control, without addition of sucrose, without MP/WP replacement and without lactose hydrolysis.

Ice cream preparation

The mixtures of UHT milk, MP and WP proposed in Table 2 were treated with 0.5 g L^{-1} β-galactosidase for 4 h and the percentage of lactose hydrolysis determined as above mentioned. The sugar and stabiliser were added and the mixture homogenised, pasteurised at 70 °C for 30 min, and then cooled immediately to 5 °C in a refrigerating chamber. The emulsifier, vegetable fat and chocolate flavouring were added and the mixture homogenised for 2-3 min using a domestic blender. The syrup was aerated in an ice cream maker (GGSA 1300 model, Gelopar, Araucária, Brazil) at –6 ± 1 °C. The frozen formulations were stored at –18 °C. The overrun response was based on the volume of the aerated sample and the melted volume of the sample (SOLER; VEIGA, 2001).

Physicochemical and microbiological parameters of ice cream

The ice creams were analysed for melting (SOLER;VEIGA, 2001), moisture, lipid, protein, ash (AOAC, 1995), pH, *Salmonella* sp., and coliforms (at 35 and 45 °C), coagulase-positive *Staphylococcus*, mesophilic and psychrotrophic bacteria, and moulds and yeasts (SILVA et al., 2010) enumerations. All analyses were performed in duplicate.

Statistical analysis

All data were analysed using Statistica 8.0 software. The suitability of linear models to the FFD results was evaluated by analysis of variance (ANOVA). The physicochemical parameters were analysed by one-way ANOVA, Tukey's test and Pearson's correlation. A significance level of 10% was considered due to the inherent variability of enzymatic processes (RODRIGUES; IEMMA, 2014)

Results and Discussion

Conditions for lactose hydrolysis

The lactose hydrolysis ranged between 70.72-95.10% (Table 1). The effects of β-galactosidase concentration (0.5-0.9 g L^{-1}) and reaction time (2-4 h) on the extent of lactose hydrolysis, are presented in Table 3.

Table 3. Effects estimates about factors studied for response of percentage of lactose hydrolysis.

Factor	Effect (%)	Standard error	t(3)	p-value
Mean	86.43	2.42	35.78	<0.00*
x_1 [a]	8.26	6.39	1.29	0.29
x_2 [b]	16.12	6.39	2.52	0.09*
x_1 by x_2	0.21	6.39	0.03	0.98

[a] β-galactosidase concentration (g L^{-1}); [b] reaction time (h); * $p < 0.10$.

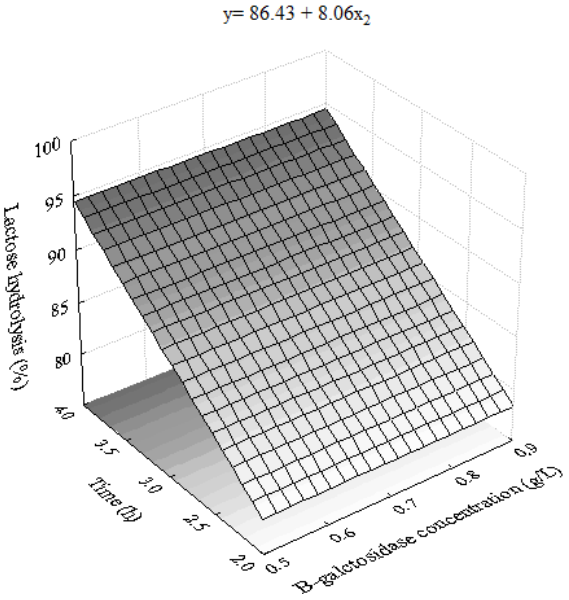
There was no significant effect of β-galactosidase concentration ($p > 0.10$) on the percentage of lactose hydrolysis. Therefore, increasing the β-galactosidase concentration from 0.5 to 0.9 g L^{-1} did not increase the percentage of lactose hydrolysis of the milk base. In contrast, Klein et al. (2010) demonstrated that the β-galactosidase concentration (from *K. lactis*) and the percentage of lactose hydrolysis were proportional. The lactose content decreased by 41.4% when 0.4 g L^{-1} of β-galactosidase was added to pasteurised milk and reacted at 6 °C, for 5 h (KLEIN et al., 2010). These authors used a lower temperature (6 °C) compared to the present study (37 °C); however, their aim was to partially hydrolyse the lactose to avoid grittiness in milk jam.

The reaction time had a positive and statistically significant effect ($p < 0.10$) on the percentage of lactose hydrolysis (Table 3). When the hydrolysis time was increased from 2 to 4 h, a higher percentage of lactose was hydrolysed.

The insignificant parameters (x_1, x_1 by x_2) were incorporated into the residue of the model, generating new regression coefficients. Using regression analysis, the optimal conditions for lactose hydrolysis as a function of reaction time ($p < 0.10$) generated a model with a first-order equation (Figure 1). The $F_{calculated}$ (6.82) was greater than the $F_{tabulated}$ (4.06). Thus, the response surface can be generated, considering the inherent variability of the enzymatic processes (RODRIGUES; IEMMA, 2014).

Similar behaviour, regarding the effect of reaction time on lactose hydrolysis, was described by Campos et al. (2009), whereby a 90% lactose hydrolysis was achieved using 0.24 g of β-galactosidase in 300 mL of UHT milk under equivalent conditions of 3.5 h at 40 °C, 4 h at 30 °C, and 7 h at 20 °C. However, at 4 and 10°C, only a 75% lactose hydrolysis was attained after 24 h. Thus, the reaction time is an important parameter that must be controlled during the enzymatic reaction to optimise the extent of hydrolysis.

Figure 1. Response surface of percentage of lactose hydrolysis as a function of β-galactosidase concentration and time of reaction.



Replacement of MP/WP in formulations

The lactose hydrolysis ranged between 86.59-97.97% in the ice cream formulations (Table 2). The percentage of lactose hydrolysis was greatest (≈ 97%) at the centre points. Sucrose concentration had

a negative effect on lactose hydrolysis. Although the effect of MP/WP replacement was positive, it was not significant ($p > 0.10$) over the range studied (Table 4). Decreasing the sucrose concentration from 20 to 14%, and increasing the MP/WP replacement from 0 to 100%, did not increase the percentage of lactose hydrolysed.

Table 4. Effects estimates of factors studied for responses of percentage of lactose hydrolysis and overrun.

Factor	Effect (%)	Standard error	t(3)	p-value	Effect (%)	Standard error	t(3)	p-value
	Lactose hydrolysis (%)				Overrun (%)			
Mean	92.64	2.36	39.32	<0.00*	43.51	0.70	62.58	<0.00*
x_1[a]	-0.10	6.23	-0.02	0.99	-1.16	1.84	-0.63	0.57
x_2[b]	4.83	6.23	0.77	0.49	9.15	1.84	4.98	0.02*
x_1 by x_2	-0.33	6.23	-0.05	0.96	-2.47	1.84	-1.34	0.27

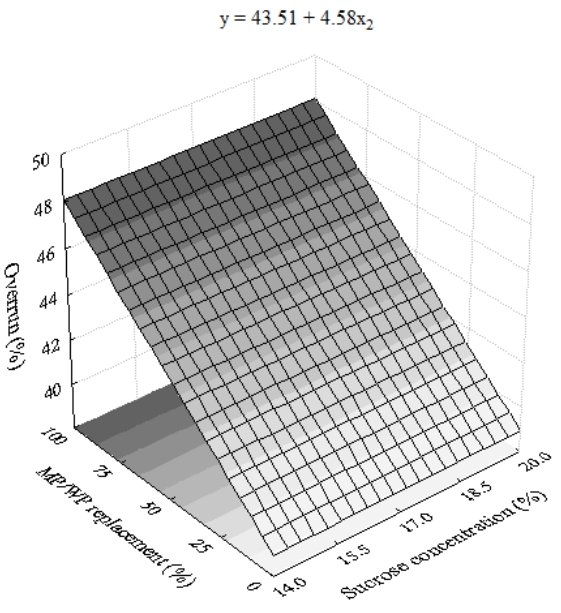
[a] Sucrose concentration (%); [b] MP/WP replacement (%); * $p < 0.10$.

Table 2 shows that the overrun ranged between 37.26-48.88%. The sucrose concentration did not significantly ($p > 0.10$) affect the overrun response, over the range studied (Table 4). Thus, decreasing the sucrose concentration from 20 to 14% did not increase the overrun. Although sucrose usually impairs foamability, it increases foam stability (DAMODARAN, 2010). The MP/WP replacement demonstrated a significant and positive effect ($p < 0.10$) on the overrun (Table 4). Therefore, when MP/WP replacement was increased from 0 to 100%, more air was incorporated into the ice cream. Similarly, Rodrigues et al. (2006) reported an increase in ice cream overrun with MP/WP replacement. The overrun increase can be attributed to the functionality of whey proteins. In particular, α-lactalbumin has a good foaming capacity (ANTUNES, 2003).

A higher percentage of lactose hydrolysis and overrun were observed for formulations 3-7. El-Neshawy et al. (1988) reported that ice cream mixes containing 50 and 75% lactose hydrolysis had higher viscosity, whippability and yielded ice cream with higher overrun and organoleptic properties than the control. Furthermore, 75% lactose hydrolysis was more effective than 50% lactose hydrolysis in these properties.

The insignificant parameters (x_1, x_1 by x_2) were incorporated into the residue of the model, generating new regression coefficients. Using regression analysis, the response of overrun as a function of MP/WP replacement ($p < 0.10$), generated a first-order model ($R^2 = 0.83$) (Figure 2). The $F_{calculated}$ (23.86) was greater than the $F_{tabulated}$ (4.06). Thus, the response surface can be generated (RODRIGUES; IEMMA, 2014).

Figure 2. Response surface of overrun as a function of sucrose concentration and MP/WP replacement.



Physicochemical and microbiological parameters of ice cream

The ice cream completely melted during the analysis period. However, formulations 1, 2 and 8 (without WP) melted faster than the others. Similar results were reported by El-Neshawy et al. (1988). In contrast, Abbasi and Saeedabadian (2015) reported that an increase in the percentage of hydrolysed lactose accelerated the melting rate. However, they did not use WP, which may reinforce the results of Soler and Veiga (2001) that found whey proteins help to make softer and more compact ice cream, preventing the formation of a brittle compound, and raising the viscosity and melt resistance.

The pH ranged between 6.65-7.01. Formulations 1, 2 and 8 (without MP/WP replacement) had a higher pH compared to the other formulations studied (Table 5). Similarly, Rodrigues et al. (2006) reported a higher pH in ice cream formulations without MP/WP replacement. The moisture, lipid and protein content ranged between 61.15-65.8, 5.13-7.60 and 3.54-6.95 g 100 g^{-1}, respectively (Table 5). The ash content ranged between 1.09-1.24 g 100 g^{-1}. Formulations 1 and 2 (without MP/WP replacement) presented a lower ash content than formulations 3 and 4 (with 100% MP/WP replacement). Similarly, Rodrigues et al. (2006) reported that increased levels of MP/WP replacement generated an increase in ash content.

Table 5. Physicochemical parameters of ice cream formulations.

Run	pH	Moisture (g 100 g^{-1})	Lipid (g 100 g^{-1})	Protein (g 100 g^{-1})	Ash (g 100 g^{-1})
1	$7.01^{a} \pm 0.05$	$65.80^{a} \pm 0.14$	$5.13^{e} \pm 0.10$	$6.95^{a} \pm 0.35$	$1.09^{b} \pm 0.01$
2	$6.94^{ab} \pm 0.03$	$64.79^{ab} \pm 0.35$	$6.46^{d} \pm 0.22$	$4.81^{bc} \pm 0.05$	$1.10^{b} \pm 0.02$
3	$6.71^{c} \pm 0.03$	$64.61^{b} \pm 0.30$	$7.60^{a} \pm 0.11$	$5.83^{ab} \pm 0.18$	$1.23^{a} \pm 0.02$
4	$6.73^{cd} \pm 0.04$	$61.15^{d} \pm 0.22$	$6.90^{c} \pm 0.05$	$3.54^{d} \pm 0.71$	$1.24^{a} \pm 0.04$
5	$6.65^{c} \pm 0.05$	$62.93^{c} \pm 0.33$	$7.49^{ab} \pm 0.06$	$4.96^{bc} \pm 0.10$	$1.18^{ab} \pm 0.04$
6	$6.72^{c} \pm 0.03$	$62.45^{c} \pm 0.35$	$7.48^{ab} \pm 0.12$	$4.35^{cd} \pm 0.29$	$1.18^{ab} \pm 0.03$
7	$6.74^{cd} \pm 0.03$	$63.46^{c} \pm 0.50$	$7.22^{abc} \pm 0.09$	$5.45^{bc} \pm 0.36$	$1.17^{ab} \pm 0.04$
8^{f}	$6.86^{bd} \pm 0.04$	$61.25^{d} \pm 0.21$	$7.21^{bc} \pm 0.03$	$5.61^{b} \pm 0.04$	$1.15^{ab} \pm 0.03$

Means with different superscript letters on the same column have significant difference ($p < 0.10$) (n=2); ^{f} Control.

Multiple correlation analysis showed the lipid content, ash content and overrun were negatively correlated with pH (Table 6). The negative correlation between the lipid content and pH can be attributed to hydrolysis of the milk lipids into fatty acids, which may have contributed to the decrease in pH. WP has a higher ash content (10.6-11.8 g 100 g^{-1}) (MIZUBUTI, 1994) than MP (5.35-5.48 g 100 g^{-1}) (KAJAL et al., 2012), which justifies the negative correlation between the ash content and pH in the presence of WP. The negative correlation between overrun and pH is associated with the isoelectric point (pI) of the whey proteins (pI = 5.2 for β-lactoglobulin, the main protein fraction) (CAPITANI et al., 2005), which is much greater than that of casein (pI = 4.6) (SWAISGOOD, 2010). Several studies have shown that protein-stabilized foams are more stable at pH ≈ pI of the proteins, provided there is no protein insolublisation at its pI (DAMODARAN, 2010).

The lipid content was negatively correlated with protein content but positively correlated with ash content and overrun (Table 6). As above mentioned, a higher ash content is expected in the formulations with 100% MP/WP replacement, due to the composition of WP. However, MP has a higher lipid content than WP, yet, formulations with no MP (100% MP/WP replacement) had a higher lipid content than those with MP. The hydrolysis

of lactose by water diminishes the amount of free water in the milk (TREVISAN, 2008). This could cause a slight increase in lipid content due to the increased concentration of the medium. The positive correlation between lipid content and overrun is controversial in that lipids markedly impair the foaming properties of proteins because they readily adsorb at the air-water interface and inhibit the adsorption of proteins during foam formation (DAMODARAN, 2010).

The ash content was positively correlated with the overrun (Table 6). As above mentioned, a higher ash content in the ice cream formulations was associated with the addition of the WP, which generated a greater overrun, explaining the positive correlation between the ash content and overrun.

Table 6. Multiple correlation matrix of physicochemical parameters and FFD responses.

Parameters	pH	Moisture	Lipids	Protein	Ash	Lactose hydrolysis	Overrun
pH	1.00						
Moisture	0.50	1.00					
Lipids	-0.86*	-0.57	1.00				
Protein	0.53	0.68	-0.49*	1.00			
Ash	-0.83*	-0.57	0.70*	-0.55	1.00		
Lactose hydrolysis	-0.32	0.42	-0.03	-0.21	0.18	1.00	
Overrun	-0.90*	-0.28	0.78*	-0.37	0.89*	0.40	1.00

* $p < 0.10$.

The temperature of lactose hydrolysis increases the efficiency of the enzymatic process but contributes to the development of microorganisms (HARJU et al., 2012). Therefore, the microbiological analysis was an important component of the current study. *Salmonella* sp. were absent from all the ice cream formulations (Table 7). Similarly, Warke et al. (2000) reported the absence of this microorganism in 30 samples of four commercial ice cream brands. The count of coliforms ranged between 2.4 x 10^1-2.4 x 10^2 MPN g^{-1} (most probable number per gram) at 35 °C, and between 0.4-7.5 x 10^1 MPN g^{-1} at 45 °C (Table 7). Tonet et al. (2011) reported coliform counts in chocolate ice cream ranging from < 3-1.1 x 10^3 MPN g^{-1}, and < 3-2.3 x 10^1 MPN g^{-1}, at 35 and 45 °C, respectively. Coagulase-positive *Staphylococcus* was present at < 10^2 CFU g^{-1} (colony forming unit per gram) for all the ice cream formulations. This count was inferior to that previously reported in various flavoured ice creams, which ranged between 7.9 x 10^1-3.2 x 10^3 CFU g^{-1} (WARKE et al., 2000). The mesophilic bacteria count ranged between 3.9 x 10^3-1.8 x 10^5 CFU g^{-1} in the ice cream formulations. In comparison, Kamat et al. (2000) reported a higher mesophilic bacteria count of 5.5 x 10^6 CFU g^{-1}. The psychrotrophic microorganisms ranged between < 10^2-6.2 x 10^3 CFU g^{-1}. The moulds and yeasts count ranged between <10^1-2.0 x 10^1 CFU g^{-1} in the ice cream formulations, which was lower than that reported by Warke et al. (2000), which ranged between 6.5 x 10^1-3.4 x 10^5 CFU g^{-1}. The variations in microorganism counts across studies can be associated with the hygiene conditions used during the ice cream production and the microbial quality of the raw products. However, in the current formulations, the variables sucrose concentration and MP/WP replacement did not affect the microbiological quality of the ice cream.

Table 7. Microbiological parameters of ice cream formulations.

Run	*Salmonella* sp. (in 25 g)	Coliforms at 35 ºC (MPN g^{-1})[b]	Coliforms at 45 ºC (MPN g^{-1})	Coagulase-positive *Staphylococcus* (CFU g^{-1})[c]	Mesophilic bacteria (CFU g^{-1})	Psicrotrofic bacteria (CFU g^{-1})	Moulds and yeasts (CFU g^{-1})
1	Absence	2.4×10^1	2.3×10^1	$<10^2$	2.5×10^4	4.2×10^3	$< 10^1$
2	Absence	2.4×10^1	1.5×10^1	$<10^2$	3.9×10^3	6.5×10^2	$< 10^1$
3	Absence	2.4×10^1	2.3×10^1	$<10^2$	1.5×10^4	5.5×10^3	$< 10^1$
4	Absence	2.4×10^1	2.3×10^1	$<10^2$	5.0×10^3	6.2×10^3	2.0×10^1
5	Absence	2.4×10^1	0.4×10^1	$<10^2$	4.6×10^3	$< 10^2$	$< 10^1$
6	Absence	2.4×10^2	1.1×10^1	$<10^2$	7.4×10^4	$< 10^2$	$< 10^1$
7	Absence	1.1×10^2	7.5×10^1	$<10^2$	4.2×10^4	1.0×10^2	1.0×10^1
8[a]	Absence	2.8×10^1	1.1×10^1	$<10^2$	1.8×10^5	5.7×10^3	$< 10^1$

[a] Control; [b] MPN = most probable number; [c] CFU = colony forming units.

Conclusions

β-Galactosidase effectively hydrolysed the lactose present in the milk base used in ice cream preparation. The percentage of lactose hydrolysis ranged between 86.59-97.97% in the ice cream formulations. The physicochemical and microbiological parameters of the ice creams were either not influenced or positively influenced by lactose hydrolysis and MP/WP replacement. The results obtained suggest that ice cream produced with 100% MP/WP replacement and lactose hydrolysis treatment, is technologically feasible. Under this condition, the lactose content was decreased by 91%, allowing the consumption of this product by lactose intolerant individuals.

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