Effects of Cholesterol Extraction Process and Fat and Whey Protein Additions on Ice Cream Mixes

María Eugenia Hidalgo[1,2,*]; Juliana Bordino[1]; Giuliana Acciarri[1]; Juan Manuel Fernández[3]; Sergio Rozycki[3] and Patricia Hilda Risso[1, 2, 4]

[1] Facultad de Ciencias Bioquímicas y Farmacéuticas-CONICET, Universidad Nacional de Rosario (UNR), Rosario 2000, Argentina

[2] Instituto de Física de Rosario (IFIR-CONICET), Rosario 2000, Argentina

[3] Instituto de Tecnología de Alimentos, Facultad de Ingeniería Química, Universidad Nacional del Litoral (UNL), Santa Fe 3000, Argentina

[4] Facultad de Ciencias Veterinarias, UNR, Casilda 2170, Argentina

[*] Corresponding author: maruhidalgo80@yahoo.com.ar

ABSTRACT

The aim of this work was to evaluate the cholesterol extraction process in ice cream mixes (ICM) by using β-cyclodextrin and to analyse the effect of this extraction on the ICM rheological, stability, and sensory characteristics. The effects of fat and whey protein (WP) additions on ICM stability were also evaluated. The maximum percentage obtained for cholesterol extraction was 93.6 %. The flow curves indicated that ICM showed a thixotropic behaviour before and after cholesterol extraction, which was enhanced when the fat content and/or % of β−cyclodextrin increased. The stability of ICM with cholesterol-reduced content (RCho-ICM) was influenced by the fat content and/or the presence of WP. The RCho-ICM with the highest fat and/or WP addition showed less tendency to melt and had the smallest amount of accumulated molten liquid. These latter ICMs presented the slowest melting rates. Also, RCho-ICMs proved to be more stables than ICMs. RCho-ICM samples obtained with a ratio of βCD/fat content of 1 % w/w were evaluated by a trained sensory panel. In addition, an acceptability test of the sample with better sensory attributes was conducted.

Practical Application

The effects of a cholesterol extraction process and fat and whey protein additions on the rheological and stability characteristics of ice cream mixes were evaluated. The extraction of cholesterol from an ice cream mix is interesting from a nutritional point of view and the extraction process of cholesterol itself may also help to improve the mix stability by controlling the fat and/or whey protein contents. These findings may prove useful as a starting point for the rational design of new functional ice cream mixes.

Keywords: cholesterol extraction; β-cyclodextrin; rheological behaviour; ice cream mix stability

INTRODUCTION

Milk and dairy products, which are foods of high nutritional value and constitute an important part of the western style diet, are the foundation of human nutrition. Therefore, the use of milk as a source of active ingredients and raw materials for the elaboration of functional foods has increased in recent years.

Milk fat provides flavour and texture, making food palatable and creamy, which are highly appreciated characteristics among consumers. However, whole milk contains a relatively high level of cholesterol (Cho), depending on the type of cow, its diet and the season of the year (Precht, 2001). Gautam et al. (2018) have reported that the concentration of Cho measured in whole milk was 23.2 mg %. On the other hand, it has been reported that high human blood Cho levels represent one of the major risk factors for developing coronary heart diseases (Peters, Singhateh, Mackay, Huxley, & Woodward, 2016). Excessively high levels of Cho intake, along with other nutritional problems such as obesity, have led to an increase in the trend of consuming specific modified products, called "functional foods". Functional foods are those that have been either enhanced with specific components which are known to render proven therapeutic effect, or modified by the total or partial removal of elements with potentially harmful side effects (Olagnero, Genevois, Irei, Marcenado, & Bendersky, 2007).

Cholesterol reduction in dairy products is an interesting way to avoid the risk of different diseases while keeping the rest of the milk fat unaltered, as it is an excellent source of energy, vitamins (A, D, and E), antioxidants, and essential fatty acids (conjugated linoleic acid or CLA) (Rehm, Drewnowski, & Monsivais, 2015). On the other hand, the extraction or deletion of milk fat from food has led to products which present lower consumer acceptance, as well as fewer nutritional benefits and less commercial value. Thus, it is convenient to avoid the excess of Cho in dairy products without altering their sensory, textural, and nutritional characteristics (Dias, Berbicz, Pedrochi, Baesso, & Matioli, 2010; Galante et al., 2017; Kim, Hong, Ahn, & Kwak, 2009; Pavón, Lazzaroni, Sabbag, & Rozycki, 2014).

Beta-cyclodextrin (βCD) is a nontoxic substance capable of protecting food flavours, vitamins, and natural colours. This is possible because it has a cavity at the centre of its molecular arrangement which can form an inclusion complex with various compounds, including Cho (dos Santos, Buera, & Mazzobre, 2017; Szejtli, 1982). It is edible, crystalline, homogeneous, non-hygroscopic, chemically and thermally stable, and easy to separate from the rest of the sample. As an additional advantage, βCD is an inexpensive compound which is not absorbed in the upper gastrointestinal tract and is completely metabolized by the colon microflora. It is also considered a GRAS (generally recognized as safe) compound (Dias et al., 2010). The use of βCD to encapsulate undesirable components, such as Cho in dairy products, is an effective strategy to improve their nutritional characteristics without altering the remainder of the matrix such as milk fat (Astray, Gonzalez-Barreiro, Mejuto, Rial-Otero, & Simal-Gándara, 2009; Galante et al., 2017; Pavón et al., 2014).

Homogenized dairy emulsions, such as ice cream mix, are colloids containing fat droplets coated with a protein-emulsifier layer as the dispersed phase. Ice cream is a complex food colloid where the mix emulsion is subsequently foamed, creating a dispersed phase of air bubbles, and then it is frozen, forming another dispersed phase of ice crystals (Goff, 1997; Homayouni et al., 2018). Ice cream processing operations can be divided into two distinct stages, mix manufacture and freezing operations. Ice cream mix manufacture consists of several unit operations: combination and blending of ingredients, batch or continuous pasteurization, homogenization, cooling, and mix aging (Marshall & Arbuckle, 1996).

In the ice cream mix, the fat droplets are emulsified by adsorbed proteins, including micellar and nonmicellar casein and whey proteins, and by emulsifiers (Costa, Resende, Abreu, & Goff, 2008). A change in fat globule size during the manufacturing process is known to be related to the ice cream melting resistance (Chavez-Montes, Choplin, & Schaer, 2003).

According to ice cream consumption trends reported, New Zealand leads the world in per capita ice cream consumption (23 kg/person annually) while the United States consumes 15 kg/person annually. In Argentina, ice cream is a favourite dessert, whose consumption has doubled over the last 18 years. On average, Argentinians annually consume about 7 kg of ice cream per capita, according to statistics published by the Asociación de Fabricantes Artesanales de Helados y Afines (AFADHYA) in 2018. During the summer, 53 % of the population consumes ice cream, at least, once a month while 23 % does so once a week (AFADHYA, 2018; Lopez, 2018). On the other hand, the Cho intake via ice cream is ~45 mg/100 g edible portion (Arbuckle, 1986).

After analysing the present scenario and the growth prospects of the global ice cream market for 2017-2021, analysts forecast that the global ice cream market will grow at annual growth rate of 4.67% during the period 2017-2021 (TechNavio, 2017). Therefore, the improvement of ice cream mix processing is an interesting field of research.

This research focused on the study of the first stage of the ice cream processing operations: the ice cream mix manufacture. The aim of this work was to evaluate the effect of cholesterol content reduction and fat and whey protein concentration on the rheological, stability, and sensory properties of ice cream mixes.

MATERIAL AND METHODS

Materials

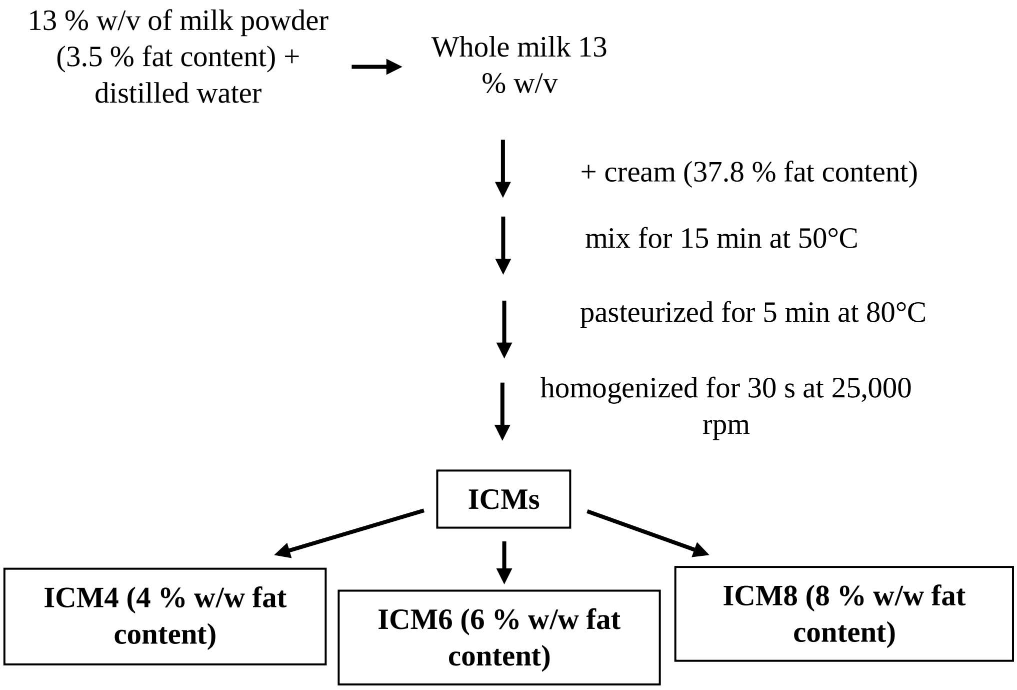
The cream (37.8 % fat content) and the whole milk (3.5 % fat content) used to obtain the ice cream mix (ICM) were purchased from La Cabaña (Santa Fe, Argentina) and Milkaut (Santa Fe, Argentina), respectively. Commercial βCD (Kleptose®, Roquette, France) was the extracting agent used to remove Cho from the ICM. The additive whey protein concentrate (WPC) was purchased from Milkaut (Santa Fe, Argentina).

Methods

Ice cream mix (ICM) preparation

Whole milk was prepared by dissolving 13 % w/v of milk powder (Milkaut, Santa Fe, Argentina) in distilled water. The quantities of cream (37.8 %) and whole milk (3.5 %) required to obtain the different ICMs (4, 6, and 8 % w/w fat content) were mixed for 15 min at 50°C and pasteurized for 5 min at 80°C. Then, the ICMs were homogenized for

30 s at 25,000 rpm using a mechanic homogenizer (OMNI GLH, Kennesaw, Georgia, USA) and stored at 4°C. The ICMs were named ICM4, ICM6, and ICM8, according to their total fat content levels (4, 6, and 8 % w/w fat, respectively).



Cho-reduced content ice cream mix (RCho-ICM) preparation

The influence of the fat content level (4, 6, and 8 % w/w fat content) and the amount of βCD added (0.5, 1, and 1.5 %/3.5 % w/w fat content) on the percentage of Cho extraction (% ChoExt) was evaluated using a factorial experimental design of two variables at three levels. The removal of the Cho was carried out by the inclusion complexation method using βCD in the different proportions mentioned above (Hedges, 1998). Solid βCD was added at a ratio of 0.5, 1 or 1.5 %. The final mixture was kept under mechanic agitation at room temperature for 20 min and at constant rate to favour interaction with the ligand. The system was centrifuged at 2,200 rpm (Sigma Laboratory Centrifuges 3-18 KH, UK) and 15°C for 15 min to precipitate the βCD/Cho complex, obtaining an ICM with cholesterol-reduced content (RCho-ICM) in the supernatant. The RCho-ICMs were named RCho-ICM4, RCho-ICM6, and RCho-ICM8, according to the total fat content contained (4, 6, and 8 % respectively).

Quantification of Cho

According to the technique published by Pavón, Lazzaroni, Sabbag, & Rozycki (2014), samples of ICM were saponified, prior to Cho extraction using n-hexane (Cicarelli, Santa Fe, Argentina). The quantification of Cho was performed by an enzymatic-colorimetric technique using a commercial kit (Colestat®, Wiener Lab., Rosario, Argentina). The Cho content of the samples was determined by absorption at 505 nm using a UV–vis spectrophotometer (Spekol® 2000, Jena, Turingia, Alemania) and was compared to the Cho standard solution. The Cho content of the ICM, before and after βCD treatment, was determined in triplicate. The percentage of Cho extraction (% ChoExt) was calculated as follows:

$$\%\ \text{ChoExt} = \left[\frac{(\text{amount of Cho in untreated ICM-amount of Cho in }\beta\text{CD treated ICM})}{(\text{amount of Cho in untreated ICM})} \right] \text{x } 100 \qquad (1)$$

ICM and RCho-ICM rheological behaviour

ICM and RCho-ICM rheological characteristics were obtained using a rotational viscometer (LVDV-III Brookfield Engineering Laboratories, Stoughton, USA) equipped with a cone-plate geometry (CP-42) at (24 ± 1) °C. Shear stress values (τ) were determined while increasing and decreasing the shear rate (γ •) at a rate range of 1-50 s^{-1} and 50-1 s^{-1}. Graphs of τ vs. γ• and viscosity (η) vs. γ• were analysed in order to characterize the rheological behaviour of the samples. The thixotropic behaviour was evaluated by analysing the hysteresis loop area between the upward and downward flow curves.

ICM and RCho-ICM stability evaluation

A factorial experimental design of two variables in three levels was applied to study the influence of the fat content (4, 6, and 8 % w/w) and WP addition (0, 1, and 2 % w/w) on the ICM stability characteristics. The ratio βCD/fat content remained constant in an optimal value (1 % w/w), in the absence and the presence of different amounts of WP at (23 ± 1) °C. The ICM and RCho-ICM drip time (t_d), the amount of accumulated mass (Ac), and the melting rate (k) were determined in the following way: 15 g of each sample was frozen at -20 °C and the frozen samples were then placed on a funnel (previously cooled to -20 °C). Both the time elapsed until the first drop (t_d) was released (Fritz & Timm, 1989) and the weight of the fluid which was released every 5 min (Ac) for 70 min were analysed. The experimental data of Ac as a function of the exposure time (t) at (23 ± 1) °C were adjusted with the following integral kinetic model, and the specific global melting rate constant (k, $g^{n-1}min^{-1}$) was determined (Muse & Hartel, 2004):

$$Ac = [k\ t\ (1-n)]^{1/_{1-n}} \qquad (2)$$

where t is the exposure time (min) and n is the global order of the fusion process.

Sensory analysis

RCho-ICM samples obtained with a ratio of βCD/fat content of 1 % w/w were evaluated by a trained sensory panel composed of researchers and professors of ITA (Instituto de Tecnología de Alimentos, Facultad de Ingeniería Química, Universidad Nacional del Litoral, Santa Fe, Argentina). The sensory panel has been pre-selected for many years (almost 20 years) and, in each case, the best ones are chosen according to their responses in commercial products evaluations, selecting a subgroup out of the general group (10 members, 5 females and 5 males). The evaluators had previously been trained to carry out the sensory analysis for 25 h (in sessions of 3 h, 2 times a week, for 1 month), using similar commercial products in the Argentine market and samples prepared with various formulations including fat, whey proteins, and βCD. The sessions of the panel were carried out individually in a room set illuminated with fluorescent light. The samples were coded with three-digit random numbers and presented in thermal vessels in order to preserve the evaluation temperature.

Different texture, taste, and flavour descriptors on a 10 cm unstructured line scale anchored with appropriate terms at the left and right ends were evaluated. For texture attributes (creaminess, roughness, and graininess, and the astringency trigeminal sensation), the anchor points were as follows: 1 = "almost nothing", 9 = "a lot". The flavour descriptors analysed were creamy, whey, powdered milk, cooked milk, rancid, rusty, metallic, foreign, acid, old, sweet, and other tastes. The references for the discrete scale were: 1 ("barely perceptible"), 3 ("little perceptible"), 5 ("moderately perceptible"), 7 ("very perceptible") and 9 ("extremely perceptible"). During the tasting, each panel member marked the perceived intensity of every attribute in such scale. Afterwards, the intensities of each descriptor were measured in each scale in order to assign a numeric value for statistical analysis (Pavón et al., 2014). In addition, we calculated the perceived percentage (PP) and the weighted average (WA), by summing up the scores assigned to each opinion and multiplying this result by the number of panellists who chose that opinion. Finally, this result was divided by the total number of panellists. This allowed us to select a sample and classify it as having the best sensory characteristics. Finally, a panel composed of 97 consumers of different ages and of both sexes carried out an acceptability test with the best sample. The sample was evaluated by using a hedonic scale of 9 points, with degrees of taste ranging from "I like it very much" to "I dislike it very much", in order to determine the number of consumers for each verbal expression.

Statistical analysis

All the determinations were performed at least in duplicate. Statistical analysis was performed with Sigma Plot 10.0 and Design Expert 6 software. ANOVA test was used in all experimental determinations, and the significance of the results was analysed by the least significant difference test. Differences of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Quantification of Cho

Table 1 shows the Cho (mg %) in ICM before βCD treatment for ICM4, ICM6, and ICM8, respectively. These results indicate that as fat content increases, the amount of Cho increases proportionally.

Table 1: ICM cholesterol content before βCD treatment

ICM cholesterol content before βCD treatment (mg %)	
ICM4	15.4 ± 0.1
ICM6	20.7 ± 0.1
ICM8	29.4 ± 0.1

The maximum % ChoExt obtained was 93.6 ± 0.5 % for the ICM6 treated with 1 % of βCD. On the other hand, when 0.5 % of βCD was used, the average % ChoExt obtained was 45.9 ± 0.5 %. According to the Código Alimentario Argentino (CAA), a food is considered as "without Cho" when the product has a maximum Cho content of 5 mg % but also has a maximum content of 1.5 g of saturated fat/100 g of product (ANMAT, 2018). Therefore, the use of 0.5 % of βCD as a Cho-complexing agent is not enough to reduce the Cho content to less than 5 mg %. On the other hand, even though the use of 1 % βCD decreased the Cho content to 1.33 ± 0.1 mg %, the content of saturated fat was higher than 1.5 g %. Therefore, in this work, the nomenclature adopted is "with Cho-reduced content".

When 1.5 % of βCD was used, the average % ChoExt obtained was 84.02 ± 0.5 %. Therefore, an addition of 1 % βCD is enough to obtain a % ChoExt greater than 80 %, and hence, it has proved to be useful to obtain ICM with Cho-reduced content (RCho-ICMs).

Other authors have reported the use of βCD as a Cho-complexing agent in other dairy products. Dias et al. (2010) reported that the mean Cho content in butter was 215.1 ± 10 mg %. These authors confirmed that the treatment of butter with βCD is an appropriate process used for Cho removal (% ChoExt = 90.74 ± 4.15 %) without modifying the fatty acid content. Pavón et al. (2014) reported that the Cho content before and after βCD treatment was 14.391 ± 1.063 mg % and 1.971 ± 0.539 mg %, respectively. In this case, the average % ChoExt obtained was 86.405 ± 2.736 %. Meanwhile, Galante et al. (2017) reported that the Cho content of zinc-fortified soft cheese was 10 ± 2 and 0.7 ± 0.1 mg % before and after βCD treatment, respectively. Therefore, the average % ChoExt obtained was 93 ± 1%. In general, all these dairy products presented similar sensorial characteristics to the products without βCD treatment.

On the other hand, Table 2 shows the nutrition information for the RCho-ICM samples obtained with a ratio βCD/fat content of 1 % w/w, in order to highlight the difference in the final percentage of fat and protein. The total fat, total energy, and Cho content increase as % fat levels in samples increase. For ICMs (without βCD treatment), only the Cho content shows differences. These values are 9.24 ± 0.1, 12.41 ± 0.1, and 17.64 ± 0.1 mg/ 60 g ice cream mix for ICM4, ICM6, and ICM8, respectively.

Table 2: Nutrition facts of the different RCho-ICMs. Ingredients: whole milk, sugar, milk cream and whey protein. **CONTAINS MILK**

RCho-ICM4			**RCho-ICM6**		**RCho-ICM8**	
1 portion – 60 g (1 unit)		**% VD (*)**		**% VD (*)**		**% VD (*)**
Energy (kcal)	73	4	80	4	90	5
Carbohydrates (g)	10	4	10	3	10	3
Proteins (g)	1.8	2	1.8	2	1.8	2
Total Fat (g)	2.4	4	3.5	7	4.9	9
Saturated Fat (g)	1.7	8	2.5	11	3.4	15
Mono-Unsaturated Fat (g)	0.6	**	0.9	**	1.3	**
Poly-Unsaturated Fat (g)	0.1	**	0.1	**	0.2	**
Trans Fat (g)	0	**	0	**	0	**
Cholesterol (mg)	1.4	**	2.1	**	2.8	**
Sodium (mg)	36	2	36	2	36	2
Does not provide significant amounts of dietary fiber						

(*) Daily values based on a diet of 2000 kcal = 8400 kJ. Your daily values may be higher or lower depending on your energy needs.

** Value not established

Rheological behaviour of ICM before and after βCD treatment: Flow curves

To evaluate the effect of the Cho extraction process on the ICM rheological characteristics, ICM flow curves were obtained at 24 ± 1 °C before and after βCD treatment (Figure 1).

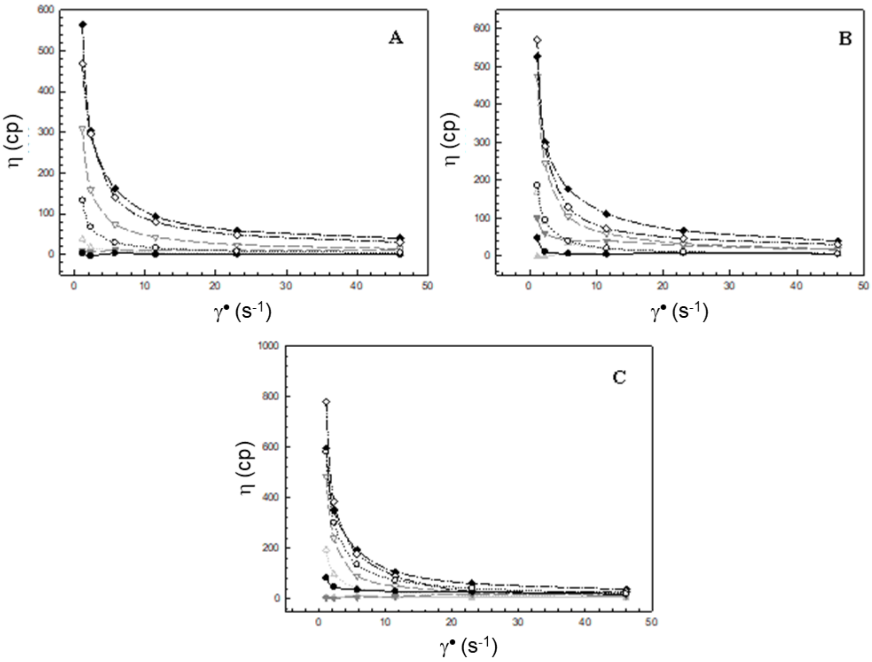


Figure 1. ICM and RCho-ICM flow curves: **(A)** ICM4 (4 % w/w fat content), **(B)** ICM6 (6 % w/w fat content), and **(C)** ICM8 (8 % w/w fat content). Filled symbols correspond to a shear rate (γ•) increment and empty symbols correspond to a γ• decrease. (Circle) ICM without βCD treatment, (Triangle up) ICM treated with 0.5 % of βCD, (Triangle down) ICM treated with 1 % of βCD, and (Diamond) ICM treated with 1.5 % of βCD. T = (24 ± 1) °C.

Except for RCho-ICM8 (treated with 1.5 % βCD), which showed a rheopectic behaviour, all the remaining ICM treated and untreated with βCD showed a thixotropic flow behaviour, showing the typical η decrease when γ• increased due to a progressive breakdown of the structure. This behaviour was more evident with the increase of fat content in the samples and the amount of βCD used for the Cho extraction process. Therefore, when a shear stress is applied, particles are oriented towards the flow and offer less resistance to it (a decrease in η). When this effort ceases, the behaviour is reversed (an increase in η), and the aggregates or agglomerates of fat globules are re-formed. The size and/or amount of these agglomerates may increase as % βCD increases.

On the other hand, the viscosity (η) initial values observed for all RCho-ICM samples were higher than the values observed for ICM samples. This behaviour may confirm the existence of fat globules agglomerates. In addition, the η observed at the end of the hysteresis curve was higher than the initial one. This behaviour was particularly observed for RCho-ICM treated with 1 % βCD, which was the RCho-ICM with the highest % ChoExt. It could be hypothesized that Cho molecules may be linked to fat agglomerates which, in turn, may impact on the ICM viscosity. Therefore, the presence of Cho would hinder fat agglomerate formation, causing a dramatic decrease in viscosity. These phenomena were previously reported by Tölle, Meier, Rüdiger, Hofmann, & Rüstow (2002) for Cho addition to phospholipid mixtures.

On the other hand, Koxholt, Eisenmann, & Hinrichs (2001) reported that when fat globules and their aggregates present a critical diameter (by partial coalescence), the serum draining is avoided . In contrast, when fat globules and their aggregates are smaller than this critical diameter, they may flow out of the ice cream matrix along with the serum, promoting ice cream instability and melting. Therefore, the Cho extraction process may help to avoid serum draining by inducing the formation of fat aggregates.

ICM and RCho-ICM stability characteristics

The network of fat globules plays an important role in the melting rate, i.e., the higher the level of destabilized fat content, the lower the melting rate. On the other hand, protein addition stabilizes the lipid emulsion after homogenization by forming a dense adsorbed layer on the fat globule interface, which prevents the coalescence of droplets by steric repulsion (Dickinson, 2003). Therefore, the influence of fat content (% fat) and whey protein concentration (% WP) on the t_d and Ac of ICMs and RCho-ICMs obtained after adding a constant amount of βCD (optimum value: 1 % w/w), was evaluated by using a factorial experimental design of two variables in three levels.

Table 3 shows the t_d and the Ac values obtained for ICMs and RCho-ICMs. Table 4 shows the relative k and n parameters obtained from the adjustment of the experimental data with the integral kinetic model (equation 2).

Table 3: ICM and RCho-ICM drip time (t_d) and accumulated amount (Ac) values obtained at T = 23 ± 1 ° C after 70 min of exposure.

		ICM		RCho-ICM	
% fat	**% WP**	**t_d ± 0.01 (min)**	**A_c ± 0.01 (g)**	**t_d ± 0.01 (min)**	**A_c ± 0.01 (g)**
4 (-1) *	0 (-1)	18.26	12.23	14.80	14.10
4 (-1)	1 (0)	15.75	12.49	16.23	13.96
4 (-1)	2 (+1)	18.23	12.50	19.39	14.18
6 (0)	0 (-1)	18.27	11.45	17.19	14.03
6 (0)	1 (0)	20.02	10.74	21.16	12.26
6 (0)	2 (+1)	20.19	12.13	19.81	13.47
8 (+1)	0 (-1)	19.14	11.19	16.80	11.56
8 (+1)	1 (0)	19.45	10.66	20.80	11.62
8 (+1)	2 (+1)	20.06	11.10	23.55	9.78

*Values between parentheses correspond to the coded values of the analysed variables

Table 4: ICM and RCho-ICM relative k and n parameters obtained at controlled T = 23 ± 1 °C

		ICM			RCho-ICM		
% fat	**% WP**	**k**	**n**	**r^2**	**k**	**n**	**r^2**
4 (-1) *	0 (-1)	0.196±0.002	0.14±0.01	0.999	0.1187±0.0007	0.13±0.02	0.997
4 (-1)	1 (0)	0.189±0.002	0.14±0.01	0.999	0.1069±0.0004	0.32±0.02	0.996
4 (-1)	2 (+1)	0.202±0.003	0.13±0.01	0.999	0.1065 ±0.0005	0.30±0.03	0.995
6 (0)	0 (-1)	0.172±0.002	0.009±0.009	0.999	0.1153 ±0.0009	0.11±0.03	0.996
6 (0)	1 (0)	0.172±0.002	0.18±0.01	0.999	0.0991 ±0.0002	0.31±0.02	0.998
6 (0)	2 (+1)	0.219±0.003	0.075±0.012	0.999	0.1065±0.0008	0.21±0.03	0.994
8 (+1)	0 (-1)	0.179±0.002	0.156±0.009	0.999	0.1096±0.0001	--	0.994
8 (+1)	1 (0)	0.173±0.002	0.19±0.01	0.999	0.0973 ±0.0003	0.31±0.02	0.997
8 (+1)	2 (+1)	0.1708±0.0009	0.207±0.006	0.999	0.091±0.004	0.19±0.03	0.997

* Values between parentheses correspond to the coded values of the analysed variables

The adjustment by multiple regression of t_d and the Ac values obtained for ICMs did not allow us to obtain an adequate mathematical model as statistical values were nonsignificantly different (P = 0.116). Besides, the fat and/or WP contents did not affect significantly the melting rate (k, P = 0.1709).

Table 5 shows the effects and p-values obtained for the response variables t_d, Ac, and k for RCho-ICMs.

Table 5: Analysis of the effects and p-values of the response variables t_d, Ac, and k for RCho-ICMs

	t_d		Ac		k	
Factor	Coef	p-value	Coef	p-value	Coef	p-value
Constant	19.23	— [b]	12.88	— [b]	0.100	— [b]
% fat (L)	1.74	— [b]	-1.25	— [b]	-0.0057	— [b]
% WP (L)	2.27	— [b]	-0.082	— [b]	-0.0066	— [b]
% fat * % fat (Q)	— [a]		— [a]		— [a]	
% WP * % WP (Q)	— [a]		— [a]		0.007976	— [b]
% fat * % WP	— [a]		— [a]		— [a]	
	r^2 = 62.13%		r^2 = 76.06%		r^2 = 97.07%	

L = linear effect
Q = quadratic effect
[a] Not significant (P > 0.05)
[b] Significant (P < 0.05)

When fat and/or WP content of RCho-ICMs increased, the time taken by the first drop of the liquid mixture to fall was longer (higher t_d value). Therefore, an increase in any of these variables may be related to a delay in the fusion of RCho-ICM samples.

Model equations for codified variables were obtained by multiple regression of experimental data and are shown in equations 3 (P = 0.0054) and 4 (P = 0.0027) for t_d and Ac parameters, respectively:

$$t_d = 19.23 + 1.74\ \%\ \text{fat} + 2.27\ \%\ \text{WP} \quad (3)$$

It is known that in gelled systems, fat generates a more packed microstructure with smaller pores. This hinders the flow of liquid from the food matrix to the outside and causes a higher liquid retention (higher t_d) (Dalgleish, 1990; Xiong, Aguilera, & Kinsella, 1991). Also, interactions between fat and protein gel occur, more specifically between beta-lactoglobulin (βLG) adsorbed on the fat globules surface and kappa-casein (κ-CN) by disulphide bonds formed during the heating stage (Dalgleish, 1990). In the case of RCho-ICM samples, due to heating at 80 °C during pasteurization, such interactions may be present and may explain the t_d increase when the fat content increases. On the other hand, WP is used as an additive in food products that are prone to serum release (syneresis) because of their high water absorption capacity (Spreer, 1991). When milk is heated, βLG is denatured and interacts with κ-CN, forming an insoluble complex. When WP is added to RCho-ICM samples, the presence of βLG molecules may exceed the presence of κ-CN ones and, as a result, other protein complexes involving only WP may be formed. Therefore, after heating, RCho-ICMs exhibit higher viscosity and better properties to retain water (Hugunin, 2008). A higher WP content would provide a greater number of molecules with high water uptake capacity, which would slow the passage of liquid from the food matrix to the outside, causing an increase in t_d.

On the other hand, it was observed that RCho-ICM4 (4 % w/w fat content) did not show significant differences ($P = 0.110$) in Ac when the % WP increased. The Ac observed as % WP increased was 14.10 ± 0.01 g, 13.96 ± 0.01 g, and 14.18 ± 0.01 g, respectively. In contrast, in RCho-ICM6 and RCho-ICM8, the Ac decreased significantly as the % WP increased ($P = 0.042$). The lowest Ac was observed for the RCho-ICM8 with the highest fat and WP content (9.78 ± 0.01 g). This latter sample also showed the highest value of t_d (23.55 ± 0.01 min).

$$Ac = 12.88 - 1.25\ \%\ fat - 0.082\ \%\ WP \qquad (4)$$

According to equation 4, and consistent with the observed effect on t_d, Ac may decrease when the fat and/or WP content increase, the effect of fat content prevailing.

Figure 2 shows the response surfaces obtained for t_d and Ac of RCho-ICM samples vs. fat and WP content.

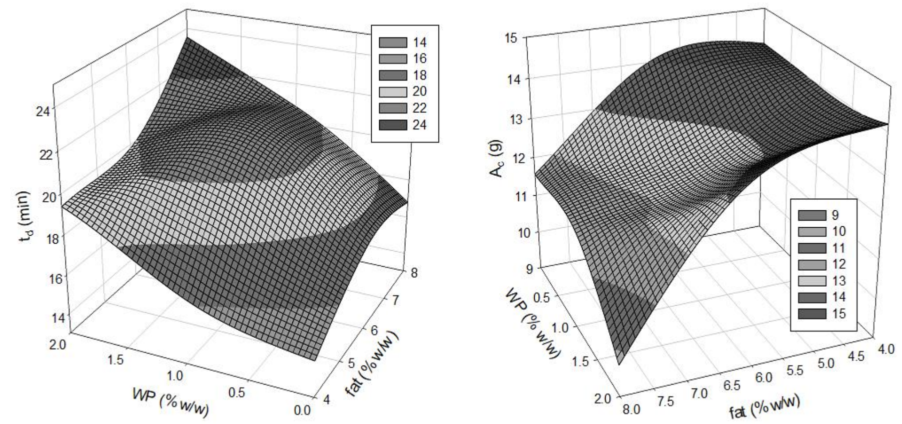


Figure 2. Response surfaces obtained for t_d and Ac of RCho-ICM samples vs. fat and WP content (% w/w).

In summary, RCho-ICM samples formulated with higher fat and/or WP content (P = 0.00405) presented higher values of t_d (23.55 ± 0.01 min) and lower values of Ac (9.78 ± 0.01 g), i.e., they would take longer to release the first drop of molten liquid (higher t_d) and the amount of accumulated molten liquid, after the corresponding exposure time, would be smaller (lower Ac). Therefore, an increase in fat and/or WP content may increase RCho-ICMs stability.

Equation 5 shows the model equation for codified variables that adequately fitted the experimental data of k (P = 0.0158):

$$k = 0.100 - 0.0057\ \%\ fat - 0.0066\ \%\ WP + 0.007976\ \%\ WP^2 \qquad (5)$$

According to equation 5 and Figure 3, % fat and % WP influenced the melting rate (k) of samples negatively. Therefore, as fat and WP added increase, k of samples decreases.

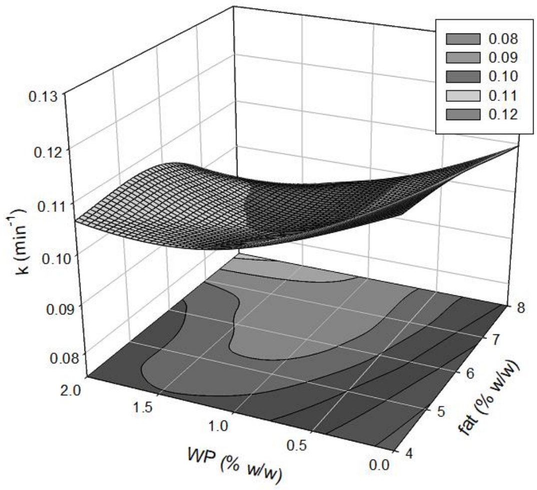


Figure 3. Response surface obtained for k of RCho-ICM samples vs. fat and WP content (% w/w).

These results are in agreement with those reported by Roland, Phillips, & Boor (1999). They observed that an increase in fat content from 7 to 10 % in ice cream caused an increase in its half-life. Tiwari, Sharma, Kumar, & Kaur (2015) also reported that melting rates increased when fat content was reduced by substituting fat with inulin. These authors indicated that this effect was probably attributed to the fact that milk fat presents a lower heat transfer coefficient than the aqueous phase.

When WP content increased from 0 to 2 % a significant decrease (P = 0.016) of the melting rate was observed (Table 4). This behaviour was opposed to that reported by Daw & Hartel (2015). These authors indicate that WP content increases the ICM melting rate due to a decrease in the level of partially-coalesced fat. WP are capable of absorbing in the oil-water interface, forming a thin layer, and hence, hindering the partial coalescence of fat globules. This effect was observed at WP concentrations between 4 and 10 %. As mentioned above, fat globules and their aggregates should have a critical diameter (by partial coalescence) to avoid the melting process. In this context, the level of WP assayed in ICM samples (0 to 2 %) was not enough to hinder the partial coalescence of fat globules.

In conclusion, the RCho-ICM samples formulated with a higher percentage of WP additions presented higher stability characteristics because they began to melt later (longer t_d) and, after an exposure time of 70 min, the accumulated liquid mass was lower (lower Ac) due to a slower melting rate (k).

Comparing the results obtained from the ICM and the RCho-ICM samples, it was observed that, except for ICM without WP, the extraction of Cho increased the t_d and Ac. This may indicate that the Cho extraction process delayed the beginning of the melting process. However, Cho extraction promoted a higher amount of accumulated molten liquid (Ac) in comparison with its untreated counterpart.

On the other hand, results indicated that there were no significant changes among k values obtained for the ICM and the RCho-ICM samples. This may indicate that the melting rate of the ICM samples was not affected by the Cho extraction process. Thus, the kinetics of the destabilization process may depend only on the fat and/or WP content used in the formulations.

Sensory analysis

The average values and the multiple regression analysis of texture descriptors are shown in Tables 6 and 7, respectively.

Table 6: Average values of texture descriptors of RCho-ICM samples obtained at a ratio βCD/fat content of 1%

% fat	% WP	Creaminess	Roughness	Astringency	Graininess
4 (-1)*	0 (-1)	1.43	2.30	1.53	8.43
4 (-1)	1 (0)	2.39	1.38	1.36	5.59
4 (-1)	2 (+1)	2.18	1.18	1.29	7.57
6 (0)	0 (-1)	3.90	1.62	1.68	5.49
6 (0)	1 (0)	3.71	1.42	1.41	5.50
6 (0)	2 (+1)	3.78	1.57	1.58	5.96
8 (+1)	0 (-1)	6.61	1.30	1.89	4.09
8 (+1)	1 (0)	6.81	1.26	1.78	3.11
8 (+1)	2 (+1)	5.85	1.20	1.44	5.21

* Values between parentheses correspond to the coded values of the analysed variables

Table 7: Multiple regression analysis for texture descriptors RCho-ICM samples obtained at a ratio βCD/fat content of 1% (coded variables)

	Creaminess		Roughness		Astringency		Graininess	
Factor	Coef	p-value	Coef	p-value	Coef	p-value	Coef	p-value
Constant	4.423	<0.0001	1.476	0.0001	1.569	<0.0001	4.350	0.0002
% fat (L)	2.212	<0.0001	— [a]		0.155	0.0134	- 1,531	0.0013
% WP (L)	— [a]		— [a]		- 0.132	0.0246	— [a]	
% fat * % fat (Q)	0.178	0.0250	— [a]		— [a]		— [a]	
% WP * % WP (Q)	- 0.583	0.0008	— [a]		— [a]		1.775	0.0094
% fat * % WP	- 0.378	0.0006	— [a]		— [a]		— [a]	
	r^2 = 99.90 %		r^2 = 81.00 %		r^2 = 82.80 %		r^2 = 95.80 %	

L = linear effect
Q = quadratic effect
[a] Not significant (P > 0.05)
[b] Significant (P < 0.05)

Creaminess is a parameter that describes the greater or lesser ease of a sample to be spread over a surface or fall uniformly or in blocks. According to Table 7, an increase in % fat causes an increase in the creaminess of RCho-ICM samples. It is known that fat content influences texture by producing a lubricating effect, increasing the creaminess as reported by Buyck et al. (2011). These authors observed an increase in creaminess in full-fat ice cream with respect to light ice cream. Sonne et al. (2014) reported an increase in creaminess as friction processes are decreased and they demonstrated a decrease in the effects of friction when increasing fat level. On the other hand, the analysis revealed an interaction between % fat and % WP, and a decrease in creaminess at the highest % WP. Despite the fact that an increase in protein content produces more viscous ice cream mixes, high % WP may generate protein aggregates which increase friction processes. These authors observed that friction effects decreased with increasing fat level and these friction effects were larger for high-fat yoghurt with high protein level.

Roughness is the sensation of difficulty for the displacement of the tongue on the palate. Neither the % fat nor the % WP had significant effects on this sensory parameter.

Astringency is the sensation resulting from a contraction of the oral mucosa that culminates in dryness. A direct relation of % fat and an inverse relation of % WP on astringency was observed. Kelly et al. (2010) have reported that the astringency of WP is a complex process determined by the extent of WP aggregation occurring in the mouth and the saliva flow rate, where both processes increased with the increase in protein concentrations.

Graininess is the sensation produced in the mouth by the presence of frozen water. As with creaminess, graininess is related to friction effects. As the percentage of fat increases, friction decreases and, therefore, the granular sensation also does so.

As regards taste and flavour attributes of RCho-ICMs, PP and WA were summarized in Table 8. Some of the attributes of flavour were classified as defects: whey, rancid, rusty, metallic, old, among other tastes. Other characteristics, such as sweet, cream, milk powder, cooked tastes, were described as desirable.

Table 8: Perceived percentage (PP) and weighted average (WA) calculated of taste and flavour attributes of RCho-ICMs obtained at a ratio βCD/fat content of 1 %.

RCho-ICM4							RCho-ICM6						RCho-ICM8					
Taste Attributes	% WP = 0		% WP = 1		% WP = 2		% WP = 0		% WP = 1		% WP = 2		% WP = 0		% WP = 1		% WP = 2	
	PP	WA	PP	WA	PP	WA	PP	WA	PP	WA	PP	WA	PP	WA	PP	WA	PP	WA
Creamy	17.86	1.8	10.7	3.7	10.71	2.3	28.6	5.5	28.57	2.5	25.0	3.3	75.00	7.9	14.29	4.0	39.29	4.8
Whey	3.57	5.0	21.4	3.7	10.71	5.0	14.3	3.0	17.86	4.2	17.9	1.0	17.86	3.4	10.71	2.3	10.71	3.7
Powdered milk	85.71	6.0	25.0	3.8	17.86	4.2	35.7	3.4	28.57	3.0	53.6	2.9	32.14	3.2	17.86	3.4	28.57	3.0
Cooked milk	60.71	5.0	14.3	4.0	17.86	5.0	35.7	3.4	25.00	4.4	21.4	2.3	17.86	6.6	7.14	7.0	14.29	2.0
Rancid	0.00	0.0	7.1	5.0	3.57	1.0	3.6	5.0	7.14	1.0	3.6	1.0	0.00	0.0	3.57	1.0	0.00	0.0
Rusty	21.43	2.3	28.6	4.0	0.00	0.0	17.9	1.8	39.29	3.5	28.6	2.5	3.57	5.0	10.71	5.0	7.14	1.0
Metallic	0.00	0.0	14.3	3.0	7.14	1.0	17.9	1.0	10.71	2.3	21.4	1.7	3.57	5.0	7.14	5.0	3.57	1.0
Foreign	3.57	1.0	0.0	0.0	3.57	1.0	0.0	0.0	0.00	0.0	0.0	0.0	0.00	0.0	3.57	5.0	0.00	0.0
Acid	0.00	0.0	0.0	0.0	3.57	1.0	0.0	0.0	0.00	0.0	3.6	1.0	0.00	0.0	3.57	5.0	0.00	0.0
Old	0.00	0.0	7.1	1.0	3.57	1.0	3.6	1.0	10.71	3.7	0.0	0.0	7.14	5.0	10.71	3.7	0.00	0.0
Sweet	57.14	5.7	53.6	5.5	10.71	1.0	82.1	5.3	67.86	5.6	89.3	5.8	64.29	5.4	46.43	5.3	50.00	7.0
Others	3.57	7.0	0.0	0.0	0.00	0.0	0.0	0.0	3.57	5.0	0.0	0.0	32.14	1.4	0.00	0.00	0.00	0.0

By means of the WA, the samples are considered to present defects classified as "little" and "moderate". These defects occur, to a lesser extent, as the % fat enhances, increasing, in turn, the desirable characteristics to "moderate" and "a lot". From these results, the RCho-ICM8 sample with 2 % WP was selected to perform the acceptability test, since it presented the highest WA for the desirable taste attributes and no undesirable attributes. The results of the acceptability test are shown in Figure 4.

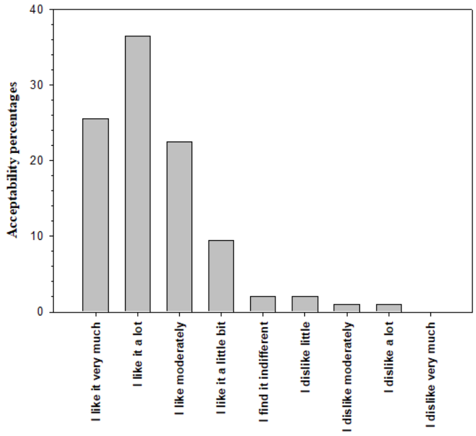


Figure 4. Acceptability test of RCho-ICM8 with 2 % WP

it was observed that the sample evaluated had very good acceptability, receiving scores with the descriptor "I dislike it", in any of its levels, in a percentage less than or equal to 2 %.

CONCLUSIONS

In conclusion, these results demonstrate that the extraction of Cho from the ice cream mixes is interesting from a nutritional point of view and the extraction process of Cho itself may also help to improve the mix stability by controlling the fat and/or WP contents and with very good acceptability by a panel of consumers. This may prove useful as a starting point for the rational design of new functional ice cream mixes.

ACKNOWLEDGMENTS

María Eugenia Hidalgo would like to thank Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) for the fellowships. The authors would like to thank the staff from the English Department (Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR) for the language correction of the manuscript, to Victor Rozycki for collaborated in the performed of the nutrition table, and to Universidad Nacional de Rosario (UNR) who provided the financial support (1BIO389).

AUTHOR CONTRIBUTIONS

María Eugenia Hidalgo performed the detailed experiments in the manuscript, wrote the manuscript, and contributed to the discussion of the obtained results.

Juliana Bordino and Giuliana Acciarri carried out the preparation of ice cream mix, preparation of Cho-reduced content ice cream mix and quantification of Cho in all the samples.

Rheological behaviour and stability characteristic of ice cream mix and Cho-reduced content ice cream mix were carried out by Juliana Bordino.

Juan Manuel Fernandez performed the sensory and acceptability analysis of the RCho-ICM samples.

Sergio Rozycki made contributions in the statistical analysis and in the discussion of the results obtained.

Patricia Risso participated in the writing of the manuscript and collaborated in the discussion.

REFERENCES

AFADHYA. (2018). http://www.afadhya.com.ar. *Asociación de Fabricantes Artesanales de Helados y Afines.*

ANMAT. (2018). *Código Alimentario Argentino*. Buenos Aires, Argentina: Ministerio de Salud de la Nación.

Arbuckle, W. S. (1986). *Ice cream* (4° ed.): Springer Science + Business Media.

Astray, G., Gonzalez-Barreiro, C., Mejuto, J. C., Rial-Otero, R., & Simal-Gándara, J. (2009). A review on the use of cyclodextrins in foods. *Food Hydrocolloids, 23*(7), 1631-1640.

Buyck, J. R., Baer, R. J., & Choi, J. (2011). Effect of storage temperature on quality of light and full-fat ice cream. *Journal of Dairy Science, 94*(5), 2213-2219. doi: https://doi.org/10.3168/jds.2010-3897

Costa, F. F., Resende, J. V., Abreu, L. R., & Goff, H. D. (2008). Effect of Calcium Chloride Addition on Ice Cream Structure and Quality. *Journal of Dairy Science, 91*(6), 2165-2174. doi: http://dx.doi.org/10.3168/jds.2007-0932

Chavez-Montes, B. E., Choplin, L., & Schaer, E. (2003). Rheo-reactor for studying the processing and formulation effects on structural and rheological properties of ice cream mix, aerated mix and ice cream. *Polymer International, 52*(4), 572-575. doi: 10.1002/pi.986

Dalgleish, D. G. (1990). Denaturation and aggregation of serum proteins and caseins in heated milk. *Journal of Agricultural and Food Chemistry*, *38*, 1995.

Daw, E., & Hartel, R. W. (2015). Fat destabilization and melt-down of ice creams with increased protein content. *International Dairy Journal, 43*, 33-41. doi: http://dx.doi.org/10.1016/j.idairyj.2014.12.001

Dias, H. M. A. M., Berbicz, F., Pedrochi, F., Baesso, M. L., & Matioli, G. (2010). Butter cholesterol removal using different complexation methods with beta-cyclodextrin, and the contribution of photoacoustic spectroscopy to the evaluation of the complex. *Food Research International, 43*(4), 1104-1110. doi: https://doi.org/10.1016/j.foodres.2010.02.002

Dickinson, E. (2003). Interfacial, emulsifying, and foaming properties of milk proteins. In P. F. Fox & P. L. H. McSweeney (Eds.), *Advanced dairy chemistry: Proteins part B* (3rd ed., Vol. 1, pp. 1229-1260). New York, NY, USA: Springer.

dos Santos, C., Buera, P., & Mazzobre, F. (2017). Novel trends in cyclodextrins encapsulation. Applications in food science. *Current Opinion in Food Science, 16*, 106-113. doi: https://doi.org/10.1016/j.cofs.2017.09.002

Fritz, & Timm. (1989). *Fabricación de Helados*. Zaragoza (España): Editorial Acribia, S.A.

Galante, M., Pavón, Y., Lazzaroni, S., Soazo, M., Costa, S., Boeris, V., Rozycki, S. (2017). Effect of cholesterol-reduced and zinc fortification treatments on physicochemical, functional, textural, microstructural, and sensory properties of soft cheese. *International Journal of Dairy Technology, 70*(4), 533-541. doi: doi:10.1111/1471-0307.12361

Gautam, V., Singh, K., & Yadav, V. L. (2018). Polyaniline/MWCNTs/starch modified carbon paste electrode for non-enzymatic detection of cholesterol: application to real sample (cow milk). *Analytical and Bioanalytical Chemistry, 410*. doi: 10.1007/s00216-018-0880-6

Goff, H. D. (1997). Colloidal aspects of ice cream—A review. *International Dairy Journal, 7*(6–7), 363-373. doi: http://dx.doi.org/10.1016/S0958-6946(97)00040-X

Hedges, A. R. (1998). Industrial Applications of Cyclodextrins. *Chemical Reviews, 98*(5), 2035-2044.

Homayouni, A., Javadi, M., Ansari, F., Pourjafar, H., Jafarzadeh, M., & Barzegar, A. (2018). Advanced Methods in Ice Cream Analysis: a Review. *Food Analytical Methods, 11*(11), 3224-3234. doi: 10.1007/s12161-018-1292-0

Hugunin, A. (2008). Productos de Suero de Leche en Yogurt y Productos Lácteos Fermentados. *Mundo lácteo y cárnico*, 4-8.

Kelly, M., Vardhanabhuti, B., Luck, P., Drake, M. A., Osborne, J., & Foegeding, E. A. (2010). Role of protein concentration and protein–saliva interactions in the astringency of whey proteins at low pH. *Journal of Dairy Science, 93*(5), 1900-1909. doi: https://doi.org/10.3168/jds.2009-2853

Kim, S.-Y., Hong, E.-K., Ahn, J., & Kwak, H.-S. (2009). Chemical and sensory properties of cholesterol-reduced processed cheese spread. *International Journal of Dairy Technology, 62*(3), 348-353. doi: 10.1111/j.1471-0307.2009.00500.x

Koxholt, M. M. R., Eisenmann, B., & Hinrichs, J. (2001). Effect of the Fat Globule Sizes on the Meltdown of Ice Cream. *Journal of Dairy Science, 84*, 31–37.

Lopez, M. A. (2018). Estudios: Aumenta el consumo de helado en Argentina. *América Retail*.

Marshall, R. T., & Arbuckle, W. S. (1996). *Ice Cream* (5th ed.). New York, USA: Chapman and Hall.

Muse, M. R., & Hartel, R. W. (2004). Ice Cream Structural Elements that Affect Melting Rate and Hardness. *Journal of Dairy Science, 87*(1), 1-10. doi: 10.3168/jds.S0022-0302(04)73135-5

Olagnero, G., Genevois, C., Irei, V., Marcenado, J., & Bendersky, S. (2007). Alimentos funcionales: Conceptos, definiciones y marco legal global. *Diaeta, 25*(119), 33-41.

Pavón, Y. L., Lazzaroni, S. M., Sabbag, N. G., & Rozycki, S. D. (2014). Simultaneous effects of gelatin and espina corona gum on rheological, physical and sensory properties of cholesterol-reduced probiotic yoghurts. *Int. J. of Food Sci. & Tech., 49*(10), 2245-2251. doi: 10.1111/ijfs.12538

Peters, S. A. E., Singhateh, Y., Mackay, D., Huxley, R. R., & Woodward, M. (2016). Total cholesterol as a risk factor for coronary heart disease and stroke in women compared with men: A systematic review and meta-analysis. *Atherosclerosis, 248*, 123-131. doi: https://doi.org/10.1016/j.atherosclerosis.2016.03.016

Precht, D. (2001). Cholesterol content in European bovine milk fats. *Nahrung Food, 45*(1), 2–8.

Rehm, C. D., Drewnowski, A., & Monsivais, P. (2015). Potential Population-Level Nutritional Impact of Replacing Whole and Reduced-Fat Milk With Low-Fat and Skim Milk Among US Children Aged 2–19 Years. *Journal of Nutrition Education and Behavior, 47*(1), 61-68.e61. doi: http://dx.doi.org/10.1016/j.jneb.2014.11.001

Roland, A. M., Phillips, L. G., & Boor, K. J. (1999). Effects of Fat Content on the Sensory Properties, Melting, Color, and Hardness of Ice Cream¹. *Journal of Dairy Science, 82*(1), 32-38. doi: 10.3168/jds.S0022-0302(99)75205-7

Sonne, A., Busch-Stockfisch, M., Weiss, J., & Hinrichs, J. (2014). Improved mapping of in-mouth creaminess of semi-solid dairy products by combining rheology, particle size, and tribology data. *LWT - Food Science and Technology, 59*(1), 342-347. doi: https://doi.org/10.1016/j.lwt.2014.05.047

Spreer, E. (1991). *Lactologia industrial* Zaragoza: Acribia S. A.

Szejtli, J. (1982). Chemistry and separation of cyclodextrins *Cyclodextrins and Their Inclusion Complexes* (pp. 17–43). Budapest, Hungary: Akademiai Kiado.

TechNavio. (2017). Global Ice Cream Market 2017-2021 (pp. 70). Kent, United Kingdom

Tiwari, A., Sharma, H. K., Kumar, N., & Kaur, M. (2015). The effect of inulin as a fat replacer on the quality of low-fat ice cream. *International Journal of Dairy Technology, 68*(3), 374-380. doi: doi:10.1111/1471-0307.12176

Tölle, A., Meier, W., Rüdiger, M., Hofmann, K. P., & Rüstow, B. (2002). Effect of cholesterol and surfactant protein B on the viscosity of phospholipid mixtures. *Chemistry and Physics of Lipids, 114*(2), 159-168. doi: https://doi.org/10.1016/S0009-3084(01)00204-3

Xiong, Y. L., Aguilera, J. M., & Kinsella, J. E. (1991). Emulsified milkfat effects on rheology of acid-induced milk gels. *Journal of Food Science, 56* 920-925.