ORIGINAL RESEARCH

Loss of available lysine during processing of different *dulce de leche* formulations

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Dulce de leche *is a dairy-based confectionery product prepared by heat concentration of milk and sugar, which can be partially replaced by other sugars. The effect of different formulations commonly used in the manufacture of this product on the rate of available lysine loss by Maillard reactions was examined. The replacement of 10% of sucrose by glucose increased by 90% the rate of lysine glycation, while the rise with fructose was only 30%. The use of skim milk led to a low fat product with the same available lysine content as the traditional one.* Dulce de leche *prepared with lactose-hydrolysed milk had a very low available lysine value.*

Keywords Available lysine, *Dulce de leche*, Maillard reaction.

INTRODUCTION

Dulce de leche, also called milk caramel, is a soft cream dairy-based confectionery product of a nice brown colour, similar in many ways to sweetened condensed milk. Widely consumed in Argentina and other Latin American countries for more than a century, *dulce de leche* is now also appreciated in other countries for household and industrial uses. It can be eaten alone, spread on bread or toast, as filling for crepes, cookies and cakes, or as topping for ice cream and fruits. Traditionally, it is prepared by heat concentration of whole milk with sucrose until a thick, creamy product with 70% (w/w) total solids is obtained. Argentine food regulations (Secretaría de Agriculture, Ganadería, Pesca y Alimentos, Ministerio de Economía y Producción (2005)) for *dulce de leche* allow up to 40% replacement of sucrose by other sugars such as glucose, fructose or corn syrups, to prevent sucrose crystallization. These sweeteners can be added at the beginning or at the final stage of the process. Sodium bicarbonate is also added during heating to prevent casein coagulation as the pH decreases during the process, and the isoelectric pH of this protein increases with temperature. *Dulce de leche* can also be prepared with skim milk or with lactose-hydrolysed milk to obtain products reduced in fat content and for lactose intolerant people, respectively.

During heat treatment, Maillard reactions occur between reducing sugars and milk proteins. This leads to the development of a brown coloured product with a characteristic and pleasant flavour. However, this nonenzymatic browning reaction can also lessen the nutritive value of *dulce de leche* by damaging essential amino acids (Mauron 1981). Though other essential amino acids are also involved, particularly in the advanced stages of Maillard reaction, lysine is the most affected amino acid as its free ε-amino group can react with carbonyl groups (Hurrell 1990; Mauron 1990). Thus, the loss of available lysine can give a good estimation of the reduction in protein quality (Mauron 1981; Erbersdobler 1989).

Milk proteins are an excellent source of lysine, but the conditions of temperature–time, pH and the presence of lactose during the manufacture of *dulce de leche*, would decrease the available lysine. Moreover, if sucrose is partially replaced in the formulation by reducing sugars, it is expected that lysine damage would be greater, and the extent of glycation would depend on the nature of the sugar added (Naranjo *et al*. 1998).

Despite its popularity in Latin American countries, there are not many studies about *dulce de leche*. Few of them estimate different parameters related to Maillard reactions such as colour development (Hough *et al*. 1991; Pavlovic *et al*. 1994; Pauletti *et al*. 1998; Pauletti *et al.* 1999; Garitta *et al*. 2004), and available lysine content (Pavlovic *et al*. 1994; Malec *et al*. 1999).

This work evaluated the rate of available lysine loss during the processing of *dulce de leche* and compared the effect of different formulations commonly used in its manufacture on this rate. Lysine loss rate during the processing of *dulce de leche* prepared with skim milk and lactose-hydrolysed milk was also analysed.

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Table 1 Composition of milk powders

	Whole milk (% d.b.)	*Skim milk (% d.b.)*	*Lactose-hydrolysed milk (% d.b.)*
Protein	25.6	35.7	26.8
Fat	26.5	1.0	26.8
Lactose	39.3	52.5	10.3
Glucose	—	—	14.4
Galactose	—	—	14.4
Ash	5.9	8.4	6.2

Table 2 Formulations of *dulce de leche*

Formulations	*1*	*2*	*3*	*4*	*5*	*6*	*7*
WMP (g)	12.5	12.5	12.5	12.5	12.5	—	—
SMP (g)	—	—	—	—	—	12.5	—
HLMP (g)	—	—	—	—	—	—	12.5
Sucrose (g)	20.0	18.0	18.0	18.0	18.0	20.0	20.0
Glucose (g)	—	2.0	2.0	—	—	—	—
Fructose (g)	—	—	—	2.0	2.0	—	—
Time of addition of reducing sugar (min)	—	0	90	0	90	—	—

WMP, whole milk powder; SMP, skim milk powder; HLMP, lactose-hydrolysed milk powder

MATERIALS AND METHODS

Materials

Whole (WP), skim (SMP) and lactose-hydrolysed milk powders (HLMP) were purchased at the local market. Their proximate compositions (%) are shown in Table 1. Glucose and fructose were from BDH Chemicals Ltd (Poole, Parkstone, England) and sucrose was commercial grade. O-phthaldialdehyde (OPA) was from Sigma Chemical Co. (St. Louis, Missouri, USA). Purified casein was from Difco Laboratories (Detroit, Michigan, USA).

Preparation of *dulce de leche* formulations

Seven formulations of *dulce de leche* were prepared (Table 2). Formulation 1 was that of the traditional *dulce de leche*. Milk powders were reconstituted with distilled water (125 g/l). Sucrose was added to 100 ml of reconstituted milk and the mixture was heated in an open vessel with constant stirring (0.39 ***g***) using an oil bath. The temperature reached 102–103°C at 11 min, remaining constant during the rest of the process. The pH was adjusted to 7.5 with 10% (w/v) $NaHCO_3$ solution after 30 min heating. Monosaccharides were added at the beginning of the process (formulations 2 and 4) or at 90 min of heating (formulations 3 and 5), a common industrial practice. After 120 min of heating, the soluble solids content was 70 ± 2% and the products had the sensory characteristics of *dulce de leche*. In order to evaluate the rate of available lysine loss in each formulation, samples were removed from the oil bath at 15, 30, 45, 60, 90, 105 and 120 min, stored in sealed glass flasks, immediately cooled to 4°C and analysed within 48 h. Each sample was prepared in triplicate. Three unheated samples of each formulation were analysed.

Analytical methods

Water activity (a_w) was measured using a Vaisala Humicap electric hygrometer (Helsinki, Finland). Total nitrogen was determined in duplicate by the Kjeldahl method with a Büchi assembly of a 430 digester and a 320 nitrogen distillation unit (Flawil, Switzerland). The pH was measured with a Mettler 320 pH meter (Mettler–Toledo, Halstead, UK). Soluble solids of *dulce de leche* samples were determined at 20°C with a Carl Zeiss Abbé-type refractometer (Oberkochen, Germany).

Available lysine was determined by the o-phthaldialdehyde (OPA) spectrophotometric method as modified by Vigo *et al*. (1992) to be used in milk products and related systems. Duplicate solutions of each sample were prepared by stirring 0.5 g for 2 h in 10 ml of sodium dodecyl sulphate solution (150 g/l). The absorbance was measured at 340 nm with a Hewlett Packard spectrophotometer mod. 8453 (Avondale, Pennsylvania, USA). Six replicate measurements of each sample were done. The coefficient of variation for this assay was < 3%. The available lysine content was obtained from a standard curve plotted using casein dissolved in pH 9.0 sodium tetraborate buffer solution

in the range of 1.0–10.0 mg/ml. Available lysine content of casein was determined by 1-fluoro-2,4-dinitrobenzene (FDNB) method (Booth 1971). The slope was calculated by linear regression analysis with the least square method ($r^2 = 0.9957$). The possible interference of the free amino groups of amino acids, small peptides and amines was checked in the supernatant of samples dissolved in pH 9.0 sodium tetraborate buffer solution after precipitation of the protein with 10% trichloroacetic acid solution (Goodno *et al*. 1981) being always negligible.

Statistical analysis

Linear regression curves were analysed by the minimum square method. Analysis of variance (ANOVA) was performed to check if the slopes were significantly different from zero. The correlation coefficient value (r^2) was considered as a measure of fitness with a straight line (Labuza 1984). The confidence intervals of the slopes were estimated for a significance level of 95% by means of the Student's *t*-test.

RESULTS AND DISCUSSION

Available lysine losses during preparation of *dulce de leche* with the traditional formulation (formulation 1) and with the addition of reducing sugars (formulations 2 to 5) are shown in Figure 1. Most researchers have reported a first-order reaction for the decrease of lysine by Maillard reaction (Labuza and Saltmarch 1981; Baisier and Labuza 1992; Naranjo *et al*. 1998; Malec *et al*. 2002). In this study, the extent of available lysine loss in formulations with glucose added, reached 50% at the end of the process, but in the other cases it was less than 40%. For this reason, it was not possible to distinguish between zero and first-order reaction (Labuza 1984), and a linear regression was considered for all curves. In all cases, r^2 was higher than 0.94.

In *dulce de leche* prepared with sucrose as the only sugar added (formulation 1), remaining available lysine was about 70% at the end of processing. Most researchers consider that sucrose participates in Maillard reactions exclusively through its hydrolysis products (Hurrell and Carpenter 1977; Smith and Friedman 1984). During preparation of *dulce de leche*, the conditions are not favourable for sucrose hydrolysis, therefore it is very feasible that in this formulation, lactose was the only sugar involved in the blockage of available lysine.

The replacement of 10% of sucrose with reducing sugars augmented significantly ($P < 0.05$) the rate of available lysine loss in all cases. The addition of glucose at the beginning of the process (formulation 2) increased the reaction speed by 90%, whereas when the sugar added was fructose (formulation 4), the increase was 30%. The greater reactivity showed by glucose in *dulce de leche* relative to that of fructose agrees with most reports about their influence on available lysine loss (Lewis and Lea 1950; Baxter 1995; Naranjo *et al*. 1998; Malec *et al*. 1999).

When available lysine loss rate in the traditional formulation is compared with that of the formulations with the addition of monosaccharides, it must be taken into account that, besides the reducing sugar added, there was lactose in the media. Lactose : lysine molar ratio in formulation 1 was 8:1, and the addition of the monosaccharides increased the reducing sugar : lysine molar ratio in the formulations to 15:1. Malec *et al*. (1999) found in a similar system that the reducing sugar : lysine molar ratio influenced the decrease of available lysine up to relationships even higher than this, this effect being much more pronounced with glucose than with fructose. However, the increment in lysine loss rate caused by the addition of reducing sugars was too high to be explained only by this fact. A more important cause of the increase in available lysine loss rate seemed to be the greater reactivity of the monosaccharides added in comparison with lactose. In the case of glucose, this assumption was supported by many authors (Lewis and Lea 1950; Baxter 1995; Naranjo *et al*. 1998) who observed a higher reactivity of this sugar with respect to that of lactose. The influence of fructose reactivity was not so evident. Reports about its effect on available lysine loss compared with that of lactose were not always concordant (Lewis and Lea 1950; Baxter 1995; Naranjo *et al*. 1998). However, it must be borne in mind that the systems and conditions in these experiments were quite different. Naranjo *et al*. (1998) observed in a model system of casein and reducing sugars, that the reactivity of fructose was more influenced by temperature than that of the other sugars and estimated that it might become more reactive than lactose at temperatures above 80°C. Hence, during manufacture of *dulce de leche*, the high temperature could enhance the reactivity of fructose exceeding that of lactose.

The assumption that the reaction rate is more influenced by the sugar reactivities than by the reducing sugar : lysine molar ratio is supported by the difference observed between the slopes of formulations 2 and 4 containing glucose and fructose, respectively. The quantity of reducing sugar added was the same in both cases, but in formulation 2, the slope was 50% higher than in formulation 4, agreeing with the greater reactivity of glucose as generally reported (Lewis and Lea 1950; Baxter 1995; Naranjo *et al*. 1998; Malec *et al*. 1999).

The curves corresponding to formulations 3 and 5 (Figure 1) showed a significant increase ($P < 0.05$) in the slope at 90 min, the moment when reducing sugars were added. The composition

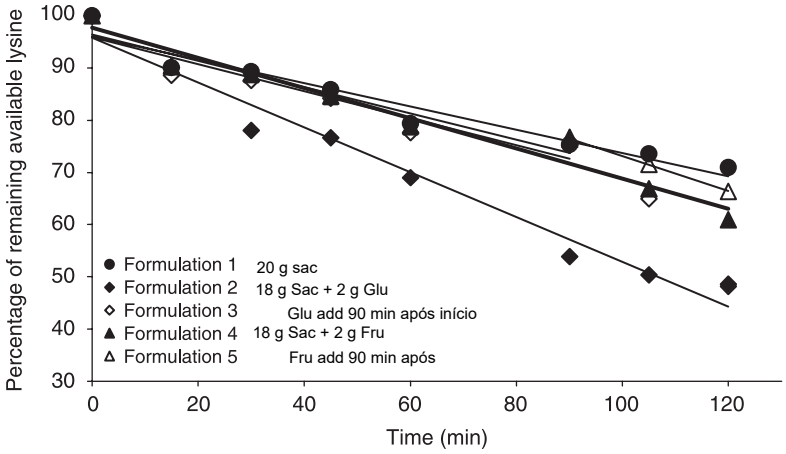

Figure 1 Available lysine loss during manufacture of *dulce de leche* with formulations 1–5.

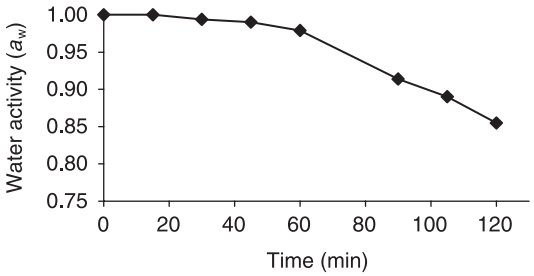

Figure 2 Water activity (a_w) variation during manufacture of *dulce de leche* with formulation 1.

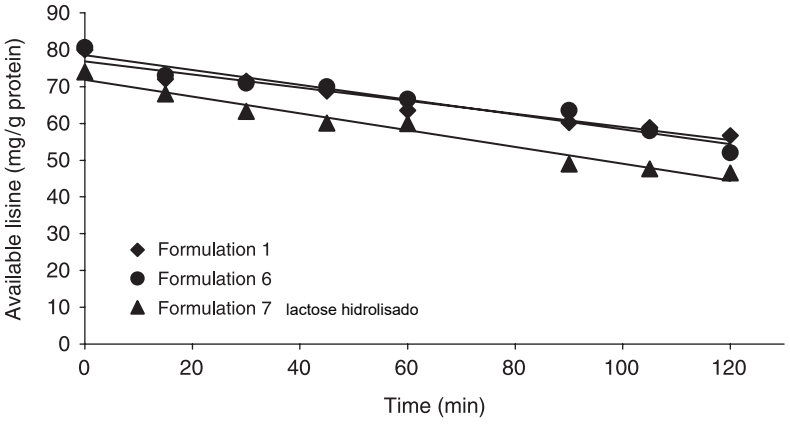

Figure 3 Available lysine loss during the manufacture of *dulce de leche* with whole milk powder (formulation 1), skim milk powder (formulation 6) and lactose-hydrolyzed milk powder (formulation 7).

of these formulations during the first 90 min, was the same as that of formulation 1. Thus, as expected, the slopes of the curves for the first part of the process of formulations 3 and 5 were not significantly different ($P < 0.05$) from that of formulation 1. When glucose was added at 90 min, the slope increased four times with respect to that of the first part of the process. Furthermore, it was twice as great as that of formulation 2, attaining the same available lysine content at 120 min in both cases. This significant increment in the reaction rate was attributed to the lower a_w value at the moment of the addition. Figure 2 shows the evolution of a_w during the preparation of *dulce de leche*. The shape was quite similar for all formulations. During the first 60 min, a_w remained practically unchanged at a very high value. Then, it fell to around 0.9, a more favourable value for Maillard reactions. When fructose was added at 90 min (formulation 5), the slope increased by 32% compared with that of the first part of the process. The available lysine content at the end of the process did not reach that of formulation 4, as happened with glucose, due to its lower reactivity.

Figure 3 shows available lysine in *dulce de leche* prepared with the traditional formulation and with skim milk (formulation 6) and lactose-hydrolysed milk (formulation 7). The curves corresponding to formulations 1 and 6 were not significantly different ($P > 0.05$). Hence, the quantity of milk fat seemed to have no effect on Maillard reactions. The sugar composition of formulation 7 was more complex than those of the other formulations. Lactose-hydrolysed milk contained less lactose than whole milk and skim milk, but two other reducing sugars, glucose and galactose, are present. This resulted in a slope of the curve 30% higher than that of formulation 1. Besides, the available lysine value before heating was lower than in the other formulations, as it can be seen in Figure 3, probably due to a previous loss during the drying of milk because of the high monosaccharide content. This *dulce de leche* had an available lysine value as low as that prepared with the addition of glucose.

According to the results obtained, it can be concluded that the *dulce de leche* manufactured with the traditional formulation had the lowest decrease in available lysine content. Nevertheless, in order to avoid sucrose crystallization during storage with minimum loss of available lysine, the best option would be the addition of fructose at the final stage of the process. The replacement of whole milk by skim milk led to a low fat product with the same available lysine content as the traditional one. Instead, *dulce de leche* prepared for lactose-intolerant people had a very low available lysine value.

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