Sugar formulation effect on available lysine content of dulce de leche

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Dulce de leche is a dairy-based confectionery product, widely consumed in Argentina and other Latin American countries as filling or topping for desserts, cakes and biscuits (Hansen, 1978; Bouzas, 1997). It is prepared by heat concentration of whole milk with added sucrose until it reaches 700 g total solids/kg dulce de leche. Sucrose is usually partly replaced by other sugars (not more than 400 g/kg of total sugars added according to Normas Mercosur, 1996) to avoid crystallization. Sodium bicarbonate is added to prevent coagulation of proteins as the pH of the mixture decreases and the isoelectric pH of proteins increases during heating.

Maillard browning is the main reaction of milk proteins during the manufacture of dulce de leche. This results in an attractive flavour and colour, but also leads to less desirable changes, such as loss of nutritional value and formation of toxic compounds (Rizzi, 1994; Friedman, 1996*a*). The natural reactants are free amino groups of milk proteins and lactose. However, as the addition of other reducing sugars is an accepted practice, the consequences may be quite different depending on the formulation used. In addition, other reactions leading to crosslinkages between protein chains contribute to the reduction in nutritional value (Mauron, 1990; Friedman, 1996*b*; Finot, 1997). The reduction of nutritional quality of milk proteins is attributed to the lower digestibility and the biological inactivation or destruction of essential amino acids. Lysine is the essential amino acid most affected as its free ϵ-amino group is very reactive (Hurrell, 1990; Mauron, 1990; Finot, 1997).

Several studies have investigated the physical and rheological characteristics (Pauletti *et al*. 1984, 1996), sensory properties (Sabioni *et al*. 1984; Buera *et al*. 1990; Hough *et al*. 1990), sugar composition and microbiological stability (Ferramondo *et al*. 1984) of dulce de leche. However, there is little information about the loss in available lysine during manufacture (Pavlovic *et al*. 1994).

The aim of this study was to examine the effects of the nature and quantity of various sugars commonly used in the manufacture of dulce de leche on the loss of available lysine.

MATERIALS AND METHODS

Materials

Whole milk powder (containing (g/kg) moisture 17, proteins 256, fat 260, lactose 386 and minerals 58) and refined sugar were purchased at local markets. Milk powder was reconstituted with distilled water (125 g/l). Anhydrous glucose ACS reagent was from Sigma (St Louis, MO 63178, USA) and fructose GPR from BDH (Poole BH12 4NN, UK). Corn [maize] syrup 42 DE was obtained from Corn Products Corporation

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(1001 Buenos Aires, Argentina). Its sugar fraction consisted of (g/kg) glucose 22, maltose 18 and oligosaccharides 60.

Preparation of dulce de leche

Basic formulation. Refined sugar (200 g) dissolved in 1 l reconstituted milk was heated with constant stirring for 120 min in an open vessel using an oil bath (boiling temperature 102–103 °C). After 30 min heating, the pH was adjusted to 7·5 with $NaHCO_3$ solution (100 g/l). Samples of dulce de leche were immediately cooled to 60 °C and stored at 4 °C until the analysis was performed.

Other formulations. A total of 27 other formulations of dulce de leche were prepared using the same procedure but with different initial sugar composition. Six were prepared with 0, 100, 150, 200, 250 and 300 g sucrose/l reconstituted milk. The other 21 samples were manufactured by replacing between 10 and 400 g/kg of the sucrose in the basic formulation with glucose, fructose or corn syrup (2–80 g reducing sugar/l milk). The molar ratio of reducing sugar added : available lysine was between 0·6 : 1 and 26 : 1. The concentration of reducing sugars in corn syrup was expressed as glucose. The reducing sugars were added to the initial mixture or, in some cases, during manufacture after 90 min heating, a common industrial practice. The initial available lysine content was the same in all formulations. Each sample of dulce de leche was prepared in triplicate. In all of them, the soluble solids content was 700 ± 20 g/kg, the pH $6{\cdot}7 \pm 0{\cdot}01$ and the water activity $0{\cdot}85 \pm 0{\cdot}01$.

Three model systems consisting of casein (I), casein–sucrose (II) and casein–lactose (III) in the same proportions as those in the basic formulation were processed in the same way.

Analytical methods

All analyses were performed at least in duplicate. Milk acidity was measured by titration with NaOH. Total nitrogen was determined by the Kjeldahl method in a Büchi assembly with 430 digestor and 320 distillation unit (CH-9230 Flawil, Switzerland). The pH was measured with a Mettler 320 pH meter (Mettler-Toledo, Halstead CO9 2DX, UK) and soluble solids were determined with a Carl Zeiss Abbé type refractometer (D-73446 Oberkochen, Germany). Water activity was measured at 25 ± 1 °C using a Vaisala HMK 11 humidity calibrator (FIN-00421 Helsinki, Finland).

Available lysine was determined by the spectrophotometric method of Švedas *et al.* (1980) and Goodno *et al.* (1981) with *o*-phthaldialdehyde as modified by Vigo *et al.* (1992) for use in dairy products. Duplicate solutions of each dulce de leche were prepared by dissolving 0·5 g in 10 ml sodium dodecyl sulphate solution (150 g/l). The solutions were stored overnight to allow solubilization of the proteins of the fat globule membrane and promote coalescence of the fat globules (Anderson *et al.* 1974) in order to reduce the intrinsic absorbance. Six replicate spectrophotometric measurements of each solution were carried out with a Hewlett Packard HP 8451 spectrophotometer (Avondale, PA 19311, USA).

Statistical analysis

Results were subjected to analysis of variance. The average CV for available lysine determinations was 2·8%.

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RESULTS AND DISCUSSION

The loss of available lysine in model system I after heat treatment was significant: 15 % ($P < 0{\cdot}01$) even in the absence of sugars. This is most probably due to reactions of the ϵ-amino group with dehydroalanine and carboxyl groups of protein side chains involving the formation of lysino alanine, ϵ-(β-aspartyl)-lysine and ϵ-(γ-glutamyl)-lysine (Walstra & Jenness, 1987; Finot, 1997). The addition of sucrose (model system II) did not modify this loss. This was to be expected as sucrose is not a reducing sugar and can become involved in the Maillard reaction only after hydrolysis to its constituent reducing sugars (Hurrell & Carpenter, 1977; Smith & Friedman, 1984). Such hydrolysis is likely to be negligible under the conditions used in preparing these samples. However, the presence of lactose (model system III) increased the loss significantly: 38 % ($P < 0{\cdot}01$) owing to the Maillard reaction.

The loss of available lysine in the six dulces de leche prepared with different quantities of sucrose was $\sim 30\,\%$, regardless of the amount of sugar added, in agreement with the results obtained with the model systems.

The available lysine values of dulce de leche are related to the initial molar ratios of glucose, fructose and corn syrup to available lysine (Fig. 1). The partial replacement of sucrose by glucose significantly ($P < 0{\cdot}01$) increased the loss of available lysine even with an initial glucose:available lysine molar ratio as low as 0·6:1. The availability of lysine decreased from 57 to 25 mg/g protein as the molar ratio increased in the range 0·6:1–16:1. No significant difference between available lysine values of dulce de leche was found when initial molar ratios were 16:1 and 26:1. Therefore, it can be inferred that, under the conditions of this study, the available lysine value levelled off at ~ 25 mg/g protein above a certain amount of glucose, possibly when the lysine became limiting. Lea & Hannan (1950) found that the loss of available lysine was maximum at a glucose:lysine molar ratio of 3:1. However, Warmbier *et al.* (1976) and Wolf *et al.* (1981) reported that the loss continues to increase at higher ratios. The discrepancies among the results of these studies may be explained by the different composition of the systems and conditions of heat treatment. However, these authors investigated glucose:available lysine ratios much lower than those in the present study.

The available lysine values of dulce de leche manufactured with glucose added to the initial mixture and after 90 min were not significantly different (Fig. 1).

The effect of fructose addition was similar to that of adding glucose, but much smaller (Fig. 1). The lower loss of available lysine of dulce de leche with fructose compared with that made with glucose indicated a slower reaction rate with the former. This has been reported in other studies (Lewis & Lea, 1950; Baxter, 1995; Naranjo *et al.* 1998) and is attributed to differences in the electrophilicity of the carbonyl groups of the two sugars (Bunn & Higgins, 1981). It is clear from the Figure that the lysine availability did not reach the limiting value in dulce de leche with fructose. Unlike the case with glucose, the available lysine values of dulces de leche prepared with the same quantities of fructose added at the beginning of heating and after 90 min were significantly different ($P < 0{\cdot}01$). The loss of available lysine was 12 % higher in the former. Kinetic studies now under way may explain this different behaviour.

Results with corn syrup (Fig. 1) were similar to those with fructose, and were linear over the whole range tested ($r^2 = 0{\cdot}9707$). Presumably in neither case did the available lysine content become limiting. These results seem logical bearing in mind that the reducing sugars in corn syrup comprise not only glucose, a very reactive

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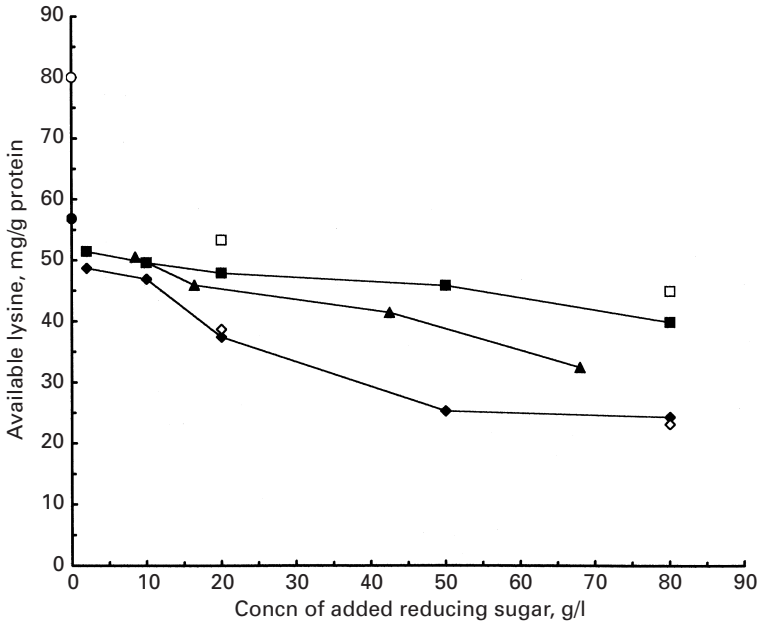


Fig. 1. Effect of added reducing sugars on the available lysine content of dulce de leche: ◆, glucose added to the initial mixture; ◇, glucose added after 90 min heating; ■, fructose added to the initial mixture; □, fructose added after 90 min heating; ▲, corn syrup added to the initial mixture. Values on the vertical axis: ●, basic dulce de leche formulation (see text); ○, milk.

sugar, but also maltose, much less reactive (Naranjo *et al.* 1998) and oligosaccharides of very low reactivity.

For small amounts of reducing sugars, up to an initial reducing sugar:available lysine molar ratio of ~ 5:1, the effect of the nature of the sugar on available lysine loss was not significant. Nevertheless, it became relevant when larger quantities of reducing sugars were used. For example, when the initial molar ratio was 0·6:1 the available lysine contents in dulce de leche with fructose, corn syrup and glucose were 51, 50 and 49 mg/g protein respectively. However, when the molar ratio increased to 26:1 the values decreased to 40, 32 and 25 mg/g protein respectively.

Pavlovic *et al.* (1994) reported 56 and 32 mg available lysine/g protein by the 2,4,6-trinitrobenzenesulphonic acid and 1-fluoro-2,4-dinitrobenzene methods respectively for dulce de leche made without added reducing sugars. The first value agrees with that obtained with our basic formulation (56·8 mg available lysine/g protein) in spite of the different conditions of processing and methods used for the determination of available lysine in the two studies.

From the nutritional standpoint it must be taken into account that dulce de leche is a product widely consumed by children. Dulce de leche manufactured with sucrose alone retained an available lysine content very close to the value suggested by FAO/WHO for preschool children (58 mg/g protein; Friedman, 1996*b*). It is usually necessary to replace part of the sucrose by reducing sugars to avoid sucrose crystallization. Fructose would be the best choice since its reactivity and concentration effect on lysine availability were the lowest, particularly when the addition was made towards the end of heating. Nevertheless organoleptic, technological and economic aspects also have to be taken into account in order to obtain pleasant dulce de leche with the least possible loss of nutritional value.

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