

Control of Lactose Crystallization in "Dulce de Leche" by *Kluyveromyces lactis* Fermentation

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ABSTRACT

Kluyveromyces lactis was an inoculum in milk to hydrolyze lactose. Five mixtures of lactose-hydrolyzed: normal milk were prepared (0:1, 1:2, 1:1.5, 1:1, and 1.5:1) for comparisons of lactose crystallization in "dulce de leche". "Dulce de leche" was produced, stored at room temperature, and tested by sensory evaluation for lactose crystallization and preference immediately and after 30, 60, 90, and 120 days storage. Statistical analysis of sensory evaluation showed no significant differences among treatments. There was no crystallization in any treatment after 120 days storage, except for the "dulce" produced with the standard (0:1, control) milk.

INTRODUCTION

"Dulce de leche" is defined by Brazilian legislation as a product made from concentrated milk mixed with sugar (sucrose) and partially caramelized. Other food materials such as coconut, peanuts, chocolate, and Brazil nuts may be added. Up to 2.0% starch may be used as a stabilizer.

Physical and chemical standards for traditional paste dulce de leche are: moisture, maximum 30.0%; nonreducing carbohydrates as sucrose, maximum 60.0%; fat, minimum 2.0%; protein, minimum 6.0%; and ash, maximum 2.0% (3).

This product typically is made in Latin America with Argentina, Brazil, and Uruguay as major producers. It is used as a dessert or as a confection ingredient. It is considered to be a potential product for the international market (5).

The most relevant technological problem in dulce de leche production concerns its physical stability as related to prevention of lactose crystallization. Crystallization causes a sandy texture and lowers product acceptability (4, 10, 11).

Milk is concentrated during dulce de leche production to a ratio of 2.5:1. This results in a saturated lactose solution which progressively crystallizes (4, 5, 10).

Many papers have been published by Brazilian researchers reporting possible methods to minimize or avoid this problem (2, 5, 10, 11, 12, 14).

Lactose hydrolysis with beta-D-galactosidase (E.C. 3.2.1.23) prevents crystallization, but current cost of enzyme makes the treatment uneconomical (5, 11).

Forced crystallization or "seeding" by inoculating the product with lactose microcrystals has been used by the condensed milk industry with good results (7, 10). Dulce de leche industries face certain technical difficulties in application of forced crystallization such as controlled cooling of the product and proper "seeding" procedures (10). In addition, "seeding" increases total operation time and induces air bubble formation in the product because of agitation (5).

Hydrolysis of lactose by microorganisms (8, 9, 10) can be an alternative process to control crystallization in dulce de leche.

The objective of this work was to study the feasibility of controlling crystallization by lactose hydrolysis resulting from *Kluyveromyces lactis* growth in milk to be used in dulce de leche production.

Received September 2, 1983.

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MATERIALS AND METHODS

Microorganism and Media

Kluyveromyces lactis was provided by the Department of Food Science, University of California, Davis. It was maintained under refrigeration at 4°C, in a culture medium containing 10 g peptone, 20 g lactose, 1 g yeast extract, 15 g agar, and 1000 ml water. The pH was adjusted to 7.0 and the medium sterilized at 121°C for 20 min.

Growth of Yeast in Milk and Consumption of Lactose

Yeast viability in sterilized skim milk was followed during 24 h by a plating-counting method using the same medium. For this purpose 200 ml of sterilized skim milk was inoculated in 500-ml Erlenmeyer flask with 10 ml of the culture obtained from 100 ml of the same skim milk and incubated for 24 h at 28°C and agitated with a magnetic stirrer. Simultaneously with sampling for counting, 5 ml of the culture was transferred to test tubes and immediately heated to 80°C for 5 min to stop yeast growth. The nonmetabolized lactose was determined by the method of Teles et al. (13).

Milk Selection and Treatment

Raw milk was provided by the Dairy Plant of the Federal University of Viçosa — MG (Brazil). For each replication 300 liter samples of whole milk were skimmed by centrifugal separation, vat pasteurized at 90°C for 2 min, cooled to 25°C, and stored at 2°C until used for further treatments. Cream received the same treatment given to the milk and was used for fat standardization.

Milk Preparation with Low Lactose

From each 300 liters of milk, 100 liters of skim milk was used for complete lactose hydrolysis by yeast, as follows: .5 liters of inoculum as described was added to 7.5 liters sterile skim milk in a 10-liter stainless steel fermentor. The fermentor (Biasinox Industria e Comércio Ltda., Lambari MG Brazil) was provided with aeration and agitation systems. The 8 liters of skim milk was incubated for 12 h at 30°C while agitating at 170 rpm. Aeration was equivalent to 1 volume of air per volume of media per minute (1 vol/vol/min). After in-

cubation, 8 liters of culture was transferred to a 200 liter stainless steel tank that contained 92 liters of skim milk heated to 90°C and cooled to 30°C. The tank was provided with an aeration system, connected to an air compressor (Douat, 40 liters) for producing filtered air. To minimize bacterial growth in the milk in the open tanks, 5 µg/ml each of streptomycin and chloramphenicol was added.

Yeast growth continued until all of the lactose was metabolized. Nonmetabolized lactose was determined by the method of Teles et al. (13).

The delactosed milk was standardized to 2.5% fat and divided into 17, 20, 25, and 30-liter portions. To each of these portions 34, 30, 25, and 20 liters normal milk containing 2.5% fat was added, resulting in five mixtures of lactose-hydrolyzed: normal milk as follows: 0:1, 1:2, 1:1.5, 1:1, and 1.5:1. These were identified as treatments 1, 2, 3, 4, and 5. Fat and percent lactic acid (6) and lactose (13) were determined on each mixture.

Manufacture of Dulce de Leche

Milk acidity in each lot was neutralized to .13% lactic acid by addition of sodium bicarbonate. Dulce was made by addition of 8 kg of sucrose (commercial grade) to 50 liters of milk and then concentrated in an open concentrator to 65 to 70° Brix, as determined by a densitometer. The product was placed in 250-g glass cups, sealed, and heated in steam for 20 min, cooled in water to 50°C, and stored at room temperature. Moisture was determined by the method of Lara et al. (6).

Sensory Evaluation for Flavor and Crystallization

The effect of removal of lactose from milk by yeast on flavor preference of dulce was tested by a 20-member sensory panel, according to ASTM (American Society for Testing and Materials) mentioned by Silva (11). To avoid possible interference of lactose crystallization, the initial observation was within 1 wk after dulce manufacture. Evaluation was with a 9-point hedonic scale: 1) horrible; 2) bad; 3) objectionable; 4) less than acceptable; 5) acceptable; 6) more than acceptable; 7) good; 8) very good; 9) excellent.

Sensory evaluation of crystallization was after 30, 60, 90, and 120-day storage. This

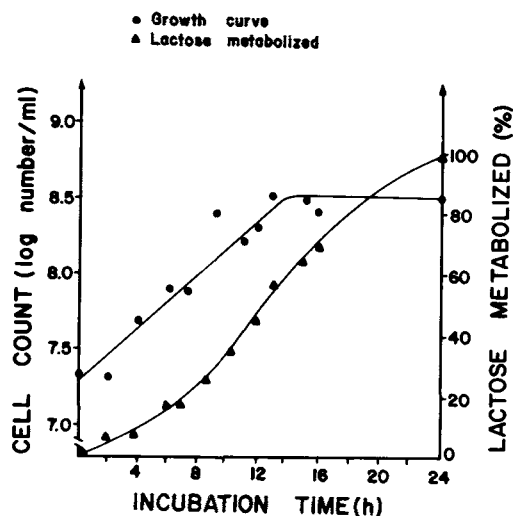


Figure 1. Growth of *Kluyveromyces lactis* in skim milk incubated at 28°C and lactose metabolized at various times.

group of panelists was five trained, experienced persons. The scale was: 1) without perceptible crystallization; 2) minor or fine crystallization; 3) sandy; 4) very sandy; 5) excessively sandy.

Statistical Analysis

Sensory evaluations were analyzed according to a casualized block experimental design with five treatments (concentrations of milk with or without lactose) and 20 blocks (panelists). Scores given by panelists were converted to square root for analysis of variance.

RESULTS AND DISCUSSION

Curves for yeast growth and lactose metabolism showed an initial population of 1.9×10^7 cells/ml of *K. lactis* in milk initially containing 4.7% lactose, with almost complete metabolism in 24 h at 28°C (Figure 1). Lactose metabolism by yeast increased positively with time of incubation, independent of the growth phase. Approximately 50% of the lactose was metabolized when the population reached 5.2×10^7 cells/ml, corresponding to 12 h of incubation. Lactose metabolism continued after the maximum stationary growth phase had been reached.

Yeast growth in milk produced a characteristic bread-like fermentation odor during concentration and resulted in alterations of

basic sensory properties of the dulce de leche. However, analysis of variance indicated that yeast growth in milk did not produce significant flavor changes ($P < .05$) in any treatment (Table 1).

Combinations of delactosed milk and standard milk reduced lactose crystallization, because some of this sugar was utilized by the yeast during the fermentation process (Table 2). In treatments 3, 4, and 5, lactose concentrations did not surpass its limit of solubility, which is 17 to 18% at 25°C (1) and did not crystallize. Even though treatment 2 resulted in a lactose concentration higher than saturation, this product did not crystallize after 120 days storage (Table 3). The probable cause for the lack of lactose crystallization in this dulce was not studied. However, Nickerson (9) stated that microbial action in concentrated whey not only reduced lactose concentration but also inhibit crystallization because of liberation of fermentation by-products. According to Webb and Whittier (15), these by-products may inhibit formation of crystallization nuclei and prevent or retard crystallization. It is possible, therefore, that yeast cell components, as well as other fermentation products, accounted for the retardation of crystallization.

The use of delactosed milk inhibited crystallization independent of the proportion used (Table 3). Crystallization occurred only in the control product after 30 days storage. The extent of crystallization was directly related to storage period, as seen in scores by panelists. This agrees with results of Doan (4) and Silva (11).

TABLE 1. Mean sensory evaluation scores for flavor in dulce de leche.

Treatment	Mean sensory evaluation scores ^a
0:1 (Control)	6.79
1:2	6.67
1:1.5	6.79
1:1	6.47
1.5:1	6.02

^a1) Horrible, 2) bad, 3) objectionable, 4) less than acceptable, 5) acceptable, 6) more than acceptable, 7) good, 8) very good, 9) excellent.

TABLE 2. Mean lactose contents of the mixtures, moisture, lactose content in the dulce and lactose in solution in the dulce de leche.

Treatment	Lactose in the original mixture	Moisture	Lactose in the dulce and lactose in solution in the dulce de leche ^b	
			"dulce" ^a	
(%)				
0:1 (Control)	4.68	31.41	12.54	29.15
1:2	3.03	24.44	7.57	20.11
1:1.5	2.57	29.32	6.47	17.86
1:1	2.04	29.98	5.31	14.39
1.5:1	1.61	29.73	3.82	12.13

^aPercent lactose in the dulce = (% lactose in original mixture) × 2.5.

^bPercent lactose in solution in the dulce de leche = % lactose in the dulce/% lactose in the dulce + % moisture × 100.

CONCLUSIONS

Growth of *K. lactis* in milk intended for use in dulce de leche prevented lactose crystallization. Although the product had a slight taste of yeast fermentation, it was not considered significant in a flavor pattern. Disadvantages of the process relate to requirements of inoculum preparation, contamination risks during yeast growth in the milk, and increase of manufacturing time. Some of these disadvantages could be minimized in large industries where facilities would be available for recovery of yeast cell by continuous centrifugation for use in other milk. This could reduce total time required for lactose metabolism and lower contamination risk.

TABLE 3. Mean sensory evaluation scores for crystallization of lactose in dulce de leche.

Treatment	Days of storage			
	30	60	90	120
Mean sensory scores ^a				
0:1 (Control)	1.67	2.29	3.80	3.95
1:2	1.00	1.00	1.00	1.00
1:1.5	1.00	1.00	1.00	1.00
1:1	1.00	1.00	1.00	1.00
1.5:1	1.00	1.00	1.00	1.00

^a1) Without perceptible crystallization, 2) minor or fine crystallization, 3) sandy, 4) very sandy, and 5) excessively sandy.

ACKNOWLEDGMENT

The authors gratefully acknowledge the careful appraisal and helpful suggestions from J. Warren Stull, Nutrition and Food Science Department, University of Arizona.

REFERENCE

- 1 Aurand, L. W., and A. E. Woods. 1973. Food chemistry. The Avi Publ. Co., Inc., Westport, CT.
- 2 Coelho, E.B.B. 1980. Utilização da beta-D-galactosidase no controle da cristalização do leite. M.S. Thesis, Universidade Federal de Viçosa, Brazil, Imprensa Universitária.
- 3 Comissão Nacional de Normas e Padrões para Alimentos. 1978. Pages vi-251 in Resolução no. 12/78 Min. Saúde, Brazil.
- 4 Doan, F. J. 1958. Problems of lactose crystallization in concentrated milk products. *J. Dairy Sci.* 41:325.
- 5 Hosken, F. S. 1969. Doce de leite - Durabilidade e cristalização. *Rev. Inst. Can. Postes.* 24:19.
- 6 Lara, A.B.W.H., G. Nazário, M.E.W. Almeida, and W. Pregolato. 1976. Normas analíticas do Instituto Adolfo Lutz. 2nd ed. Vol. 1, Sec. Est. Saúde, São Paulo, Brazil.
- 7 Nickerson, T. A. 1965. Lactose. Pages 224-260 in *Fundamentals of dairy chemistry*. B. H. Webb and A. H. Johnson, eds. Avi Publ. Co., Westport, CT.
- 8 Nickerson, T. A. 1970. Lactose. By-products from milk. B. H. Webb and E. O. Whittier. 2nd ed. Avi Publ. Co., Westport, CT.
- 9 Rossi, J., and M. Terence-Jemmet. 1977. Delactosation du lait par cellules de levures, em phase de reproduction, pour la production de yoghurt. *Le Lait.* 567:393.
- 10 Santos, D. M. 1976. Arenosidade no doce de leite. *Rev. Inst. Lat. Cand. Tostes.* 31:3.
- 11 Silva, T.J.P. 1980. Utilização da beta-D-galactosidase no processo contínuo de fabricação do doce de leite homogeneizado. M.S. Thesis, Universidade Federal de Viçosa, Viçosa, Brazil. Imprensa Univ.

- 12 Souza, L.R.P. 1979. Efeito da acidificação e do tratamento térmico do leite na qualidade do doce de leite. M.S. Thesis, Universidade Federal de Viçosa, Viçosa, Brazil. Imprensa Univ.
- 13 Teles, F.F.F., C. K. Young, and J. W. Stull. 1978. A method for rapid determination of lactose. *J. Dairy Sci.* 61:506.
- 14 Vargas, M. M. 1979. Influência da gordura da glucose e do amido na cristalização de doce de leite. M.S. Thesis, Universidade Federal de Viçosa, Viçosa, Brazil. Imprensa Univ.
- 15 Webb, B. H., and E. O. Whittier, ed. 1970. By-products from milk. 2nd ed. Pages 183–212. Avi Publ. Co., Inc. Westport, CT.