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Changes in the Carbohydrate Fraction of Milk during Heating Processes

A. Olano, M. M. Calvo & N. Corzo

Instituto de Fermentaciones Industriales (CSIC),
Juan de la Cierva, 3, 28006 Madrid, Spain

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ABSTRACT

Carbohydrate composition of commercial pasteurized, UHT, sterilized and dried milk samples, and laboratory heat-treated milks was determined by gas–liquid chromatography. Glucose, galactose and lactulose were present in all types of commercial milk samples. The concentrations of galactose and lactulose increase with the severity of the heating process. The amount of glucose present in commercial milks was similar to that found in unheated milk. Epilactose was only detected in sterilized milk samples. UHT and sterilized milks can be differentiated by their lactulose, galactose or epilactose content.

INTRODUCTION

It has long been known that an in-container process causes a greater chemical change in milk than an ultra-high temperature (UHT) process for the same sterilizing effect; thus, the two types of commercial milks with long shelf life, UHT milk and 'sterilized' milk, are considered to be different.

In spite of the work carried out on the study of changes that occur in milk during heating processes (Burton, 1983) the distinction between milks submitted to different thermal treatments is still unresolved. Among the proposed tests, the most promising are those based on the determination of compounds formed during heat treatment, which are either not present in raw milk or are present in insignificant quantities (Burton, 1983).

Lactulose, which is absent in unheated milk, is formed by isomerization of lactose during the thermal process (Adachi, 1959). Since it was reported that the extent of heat treatment undergone by milk can be followed by the amount of lactulose formed (Martinez-Castro & Olano, 1980), a number of papers have been published studying the formation of lactulose during heat treatment (Andrews, 1986; Corzo *et al.*, 1986). However, during thermal processes other sugars, such as galactose and epilactose, can be formed but their usefulness for the classification of commercial milks has not been evaluated.

It has been proposed that the formation of free lactulose in heated milk proceeds exclusively by the Lobry de Bruyn-Alberda van Ekenstein transformation, catalyzed by the milk salt system (Martinez-Castro *et al.*, 1986; Andrews & Prasad, 1987). Nevertheless, the effect of free ε-amino groups on the formation of other carbohydrates during thermal processes has not been reported.

Although there are some notable differences between model systems and milk, model milk salt systems are of great value in elucidating mechanisms of reactions and also in predicting changes taking place during heating.

The main object of the present work has been the study of the effect of thermal treatments on the composition of the free carbohydrate fraction of milk as well as the classification of commercial milk samples according to that composition.

MATERIALS AND METHODS

Pasteurized, UHT, sterilized and dried milk samples were purchased in various parts of Spain. Dried milk was reconstituted with deionized water to 10% total solids. Bulk samples of fresh cow's milk from a herd in the central region of Spain were used.

Buffer solution, consisting of 3·7 mM Tripotassium citrate, 7·2 mM trisodium citrate, 11·5 mM KH_2PO_4, 8·0 mM KCl, 1·0 mM K_2SO_4 and 50 g lactose hydrate/litre was used with variable amounts of *N*-α-acetyl-L-lysine plus NaOH to adjust to pH 6·6.

Heat treatments

Portions (10 ml) of milk or buffer solution were heated at 63 or 120°C in a silicone oil bath for a stated period in tightly stoppered pyrex glass tubes (16 × 162 mm). An indirect UHT process was carried out using an Alfa Laval pilot plant.

Preparation of derivatives

One millilitre of milk or buffer solution was mixed with 1 ml 0·5% phenyl-β-glucoside in 60% methanol. The mixture was diluted to 10 ml with methanol, kept for 1 h at room temperature and filtered. 1 ml of the filtrate was evaporated under vacuum at room temperature and converted to trimethylsilyl (TMS) derivatives using trimethylsilylmidazole as reported (Martinez-Castro & Olano, 1980).

Gas chromatography

The gas chromatographic analyses were performed on a Sigma 3B gas chromatograph (Perkin Elmer) equipped with a 3 m × 1·0 mm id stainless steel column (Chrompack) packed with 2% OV-17 on non-silanized 120/140 Volaspher A-2 (Merck). The temperature of the injector and of the detector was 300°C. The analysis was performed using temperature programming from 200°C to 270°C at a heating rate of 15°C/min with an initial holding at 200°C for 2 min.

RESULTS

Table 1 shows the formation of galactose, epilactose and lactulose during heat treatment of lactose in buffer solution containing variable amounts of *N*-α-acetyl-L-lysine. An increase of galactose and epilactose was observed when 240 mg/100 ml of *N*-α-acetyl-L-lysine was added to the buffer system. Further addition of *N*-α-acetyl-L-lysine reduced the amount of carbohydrates formed. Similar results were found by Andrews & Prasad (1987) in a study of the effect of protein concentration on the formation of lactulose during heating of milk. These authors proposed that the decrease of

TABLE 1
Formation of Carbohydrates during Heat Treatment at 120° and 20 min of 5% Lactose–Buffer Solution

N-α-*acetyl*-L-*lysine added (mg/100 ml)*	*Carbohydrates formed (mg/100 ml)*		
	Galactose	*Epilactose*	*Lactulose*
0	38·9	77·2	474·2
240	47·1	94·3	389·4
480	36·8	89·7	331·1
720	36·7	53·9	349·3

lactulose formed with the increase of protein concentration is due to increased formation of lactosyl-amino compounds which would reduce the substrate concentration for lactulose formation or to increased formation of lactulosyl-amino compounds by the condensation of lactulose and an amino group which would remove some of the lactulose formed from free solution, or to a combination of both factors.

During heat-treatment of milk, beside lactulose formation, some chemical changes in the carbohydrate composition take place. As shown in Table 2, the galactose content increased with the severity of heating; glucose remained almost unaltered and epilactose was only formed under in-container sterilization conditions. The low level of glucose present in heated milk suggests that hydrolysis of lactose to give equal amounts of galactose and glucose is practically negligible. Most of the galactose present could be formed through degradation of the reducing moiety of lactose giving rise to saccharinic acids and galactose (Corbett & Kenner, 1953).

Table 3 shows the effect of thermal treatment on the carbohydrate composition of reconstituted dried milk. Pasteurization caused slight variations, mainly on the galactose content. Sterilization gave rise to a considerable increase of galactose and lactulose (up to 31·4 and 154 mg/100 ml, respectively) as well as the formation of epilactose (7·7–11·3 mg/100 ml).

The lactulose content in different types of commercial milks is shown in Fig. 1. Pasteurized and reconstituted dried milks contained less than 9 mg/100 ml and could not be distinguished according to their lactulose contents. UHT milk, processed in directly heated UHT plant, showed a lactulose content in the range of 10–33 mg/100 ml, lower than samples from indirectly heated UHT plant (40–75 mg/100 ml); therefore, both types of milk may be differentiated on the basis of their lactulose content. The maximum concentration of lactulose was found in in-container sterilized milks (75–175 mg/100 ml).

TABLE 2

Effect of Thermal Processing on Carbohydrate Composition of Milk

Thermal treatment	*Carbohydrate composition (mg/100 ml)*					
	Galactose	*Glucose*	*Lactulose*	*Epilactose*	*Lactose*	*Total*
None	7·08	2·21	—	—	4 082	4 091
63°C/30 min	9·42	2·30	0·52	—	4 068	4 080
140°C/4 s	12·5	2·41	23·1	—	4 041	4 079
120°C/20 min	21·2	2·39	108·6	8·32	3 913	4 054
120°C/30 min	31·4	2·27	139·2	13·1	3 878	4 064

TABLE 3
Carbohydrate Composition of Reconstituted Dry Milk Samples after Thermal Processing

Thermal treatment	*Carbohydrate content in milk samples (mg/100 ml)*																			
	Galactose				*Glucose*				*Lactulose*				*Epilactose*				*Lactose*			
	1	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*
None	6·77	13·8	6·67	7·34	2·38	2·32	2·77	2·12	1·31	2·28	tr.	3·79	—	—	—	—	4 164	5 263	4 190	4 235
63°C/30 min	12·1	12·7	14·8	8·18	2·91	2·21	2·71	2·69	1·04	2·79	1·20	3·08	—	—	—	—	4 160	5 247	4 145	4 259
120°C/30 min	19·4	31·4	19·5	24·1	2·65	2·22	2·56	3·09	93·6	98·2	114·4	154·0	7·71	9·01	9·18	11·3	4 078	5 191	4 120	4 169

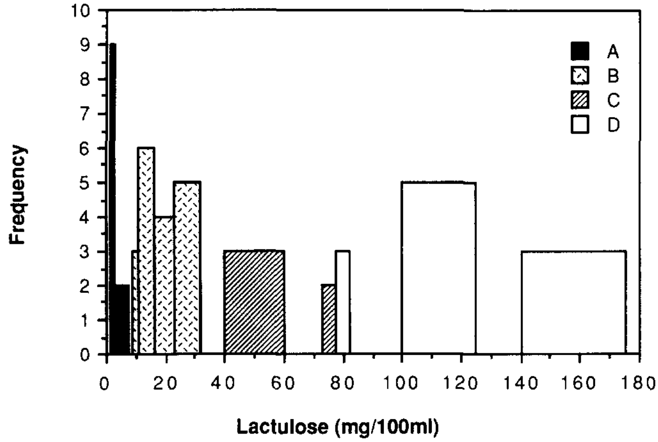

Fig. 1. Lactulose concentrations found in commercial milk samples. A: pasteurized and reconstituted dried milks; B: directly heated UHT; C: indirectly heated UHT; D: sterilized.

These findings support the results of previous studies on the differentiation of commercial milks by their lactulose content (Martinez-Castro & Olano, 1978; Geier & Klostermeyer, 1983; Andrews, 1984; Corzo *et al.*, 1986).

The distribution of galactose levels in commercial milk samples, given in Fig. 2 shows that the lowest galactose content was found in four samples of reconstituted dried milks (6·5–7·0 mg/100 ml) whereas pasteurized and UHT milks, as well as two samples of reconstituted dried milks, showed galactose contents in the range of 9·5–16·5 mg/100 ml. Although most samples from

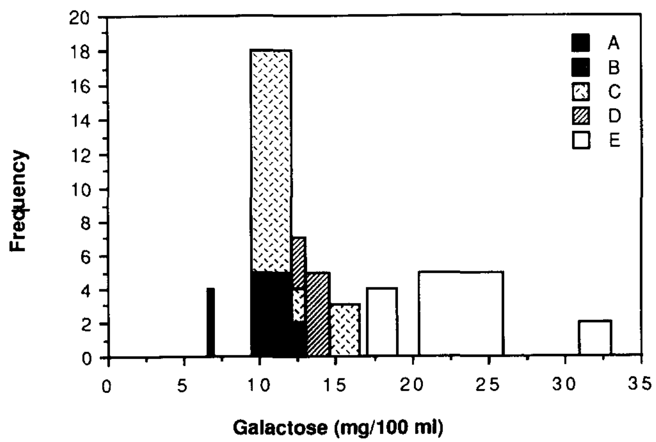

Fig. 2. Galactose concentrations found in commercial milk samples. A: reconstituted dried milk, B: pasteurized; C: directly heated UHT; D: indirectly heated UHT; E: sterilized.

directly heated UHT plant showed lower galactose levels than samples from indirectly heated UHT plant, they could not be differentiated on the basis of their galactose content. However, the lower limit for in-container sterilized milks was 17 mg galactose/100 ml and no UHT milk analyzed contained more than 16·5 mg/100 ml. Epilactose was present in all samples of in-container sterilized milks in a concentration range of 5·3–18·6 mg/100 ml. No epilactose was detected in dried, pasteurized or UHT milk.

The International Dairy Federation (IDF) are establishing definitions of UHT and sterilized milks but it is necessary to provide methods to distinguish between different types of milk sterilization processes. The present results seem to indicate that galactose and epilactose determinations could be suitable procedures to distinguish UHT and sterilized milks.

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