Formation of galactose during heat treatment of milk and model systems

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SUMMARY. The formation of galactose, lactulose and epilactose during heating of milk was studied. The concentration of these carbohydrates increased with pH and the severity of heat treatment. The presence of proteins reduced the amount of lactulose formed whereas it increased that of epilactose and galactose. The galactose in heated milks was derived from lactose degradation. Under the conditions studied, degradation of the Amadori rearrangement product did not result in the formation of galactose. The interaction between proteins and galactose increased the thermal stability of the monosaccharide.

Although changes in the free carbohydrate fraction in heated milk have been studied extensively during the last 10 years, most publications have been concerned with the possibility of using the lactulose content of a milk sample as a measure of the severity of the heat treatment to which the milk has been subjected (Martinez-Castro & Olano, 1978; Geier & Klostermeyer, 1983; Andrews, 1984; Fink & Kessler, 1988). However, during heating processes, other carbohydrates such as epilactose (4-*O*- β -D-galactopyranosyl-D-mannose) and galactose are also formed (Martinez-Castro & Olano, 1980). Epilactose is formed by the epimerization of lactose and it is present only in milk submitted to severe heat treatment (Corzo *et al.* 1986). Galactose is present in raw milk in variable amounts (Walstra & Jenness, 1984) and is formed during thermal degradation of lactose. It has been shown to occur in different concentrations in ultra heat treated (UHT) and in-container sterilized milks (Olano *et al.* 1989).

Since the mechanisms of epilactose and galactose formation in heated milk have not been studied, the effects of variation in milk composition cannot be predicted. In this paper we report an investigation on the effect of the saline system, protein, pH and initial galactose on the content of galactose after thermal processing of milk.

MATERIALS AND METHODS

Samples

Fresh raw milk was obtained from herds in the Central region of Spain. Whey proteins were obtained from milk by precipitation at pH 4.6 with 10% (v/v) acetic acid and 1 M-Na acetate to remove caseins, followed by centrifugation. The supernatant containing the whey proteins was dialysed before analysis. Caseins were obtained by ultracentrifugation of milk at 47000 *g* for 60 min.

Milk, twice concentrated, and the corresponding ultrafiltrate were obtained by

Table 1. *Carbohydrate composition of milks submitted to pasteurization (63 °C for 30 min) or sterilization (120 °C for 20 min)*

Carbohydrate, mg/100 ml	Heat treatment	Milk samples		
		1	2	3
Galactose	None	7.08	6.34	8.22
	Pasteurization	12.51	7.79	9.45
	Sterilization	21.17	22.95	24.75
Lactulose	None	—	—	—
	Pasteurization	0.53	0.76	2.07
	Sterilization	101.22	136.01	140.01
Epilactose	None	—	—	—
	Pasteurization	—	—	—
	Sterilization	15.70	20.81	19.52

passing raw milk through a Pellicon (Millipore, Madrid, Spain) stirred ultrafiltration cell, fitted with a 10000 M_r exclusion membrane. A simulated milk ultrafiltrate solution, simulating milk serum and containing no N, was prepared according to the method of Jenness & Koops (1962).

Heat treatment

Portions (10 ml) of samples were heated at 120 °C for 20 or 30 min, and at 63 °C for 30 min, in a silicone oil bath, in tightly stoppered Pyrex glass tubes (16 × 162 mm).

Carbohydrate determination by gas chromatography

Heated milk (1 ml) was mixed with 0.5% phenyl- β -glucoside (1 ml) in 60% methanol. The mixture was diluted to 10 ml with methanol, kept for 1 h at room temperature and filtered. A portion (1 ml) of the filtrate was evaporated under vacuum at room temperature and converted to trimethylsilyl derivatives using trimethylsilylimidazole as previously described (Martinez-Castro & Olano, 1978).

The gas chromatographic analyses were performed on a Sigma 3B gas chromatograph (Perkin Elmer, Madrid, Spain) equipped with a 3 m × 1.0 mm i.d. stainless steel column (Chrompack, Madrid, Spain) packed with 2% OV-17 on non-silanized 120/140 Volaspher A-2 (Merck, Barcelona, Spain). The temperature of the injector and detector was 300 °C and the analyses were performed using temperature programming from 200 to 270 °C at a heating rate of 15 °C/min with an initial holding time at 200 °C for 2 min.

RESULTS AND DISCUSSION

Six raw milk samples containing different amounts of galactose were submitted either to pasteurization (63 °C/30 min), or to sterilization, (120 °C/20 min) and the carbohydrate compositions determined (Table 1). Differences in the initial galactose contents did not affect the amount of galactose present in the heated milks. Galactose, lactulose and epilactose concentrations increased with the severity of the thermal treatment.

Since formation of epilactose and lactulose in heated milk is both temperature- and pH-dependent (Martinez-Castro & Olano, 1980; Geier & Klostermeyer, 1983),

Table 2. Carbohydrate composition of milk and concentrate at different pH values heated at 120 °C for 20 min

Sample	Initial pH	Carbohydrate, mg/100 ml		
		Galactose	Lactulose	Epilactose
Milk	6.80	29.77	200.19	18.49
	6.62	24.50	119.80	14.80
	6.40	19.56	49.46	7.10
	6.20	17.61	42.75	0
	6.00	16.70	26.90	0
Concentrate	6.80	40.85	164.08	23.11
	6.62	35.82	117.06	21.35
	6.40	24.86	56.17	12.82
	6.20	21.33	37.41	11.35
	6.00	18.79	23.21	8.91

Table 3. Carbohydrate composition of ultrafiltrate, milk and concentrate heated at 120 °C for 20 or 30 min

Sample	Heat treatment	Carbohydrate, mg/100 ml		
		Galactose	Lactulose	Epilactose
Ultrafiltrate	120 °C/20 min	17.53	181.97	12.56
Milk		27.53	119.80	14.81
Concentrate		34.65	118.05	19.05
Ultrafiltrate	120 °C/30 min	21.51	263.89	18.12
Milk		42.59	211.63	22.63
Concentrate		68.74	195.14	35.32

differences in composition between samples submitted to similar thermal treatments could be attributed to variations in composition of milk constituents, such as salts and proteins, producing a buffering action.

Table 2 shows the influence of pH on the carbohydrate formation during heating of milk and milk concentrate prepared by ultrafiltration. The amounts of the three carbohydrates studied increased with pH. However, the amounts of galactose and epilactose present in milk were lower than amounts present in the corresponding concentrate, whereas the lactulose content was higher in milk. Similar results were found when milk and the corresponding ultrafiltrate and concentrate were heated (Table 3).

Previous studies (Greig & Payne, 1985) have shown that the presence of casein reduced the amount of lactulose formed on heating so that the lower lactulose content found in the concentrate could be attributed to the higher protein concentration. The increase of galactose with protein concentration in milk could indicate that part of the galactose originated via reaction of lactose with the free amino groups of lysine in proteins followed by further degradation of the lactulose-lysine formed. However when *N*-acetyl-lactulosyl-lysine was added to model systems or to milk before heat treatment, the amount of galactose formed did not increase (Table 4).

When galactose solutions were heated in the presence of caseins or whey proteins degradation of galactose was reduced (Table 5). On the other hand no formation of galactose was found when model systems containing proteins in the absence of

Table 4. Galactose formation in different model systems heated at 120 °C for 20 min

Sample	Galactose, mg/100 ml
A	0.02
B	1.23
C	24.96
D	25.63

A, 40 mg α -acetyl-lactulosyl-lysine in 100 ml simulated milk ultrafiltrate; B, 80 mg α -acetyl-lactulosyl-lysine in 100 ml simulated milk ultrafiltrate; C, milk; D, 80 mg α -acetyl-lactulosyl-lysine in 100 ml milk.

Table 5. Galactose content (mg/100 ml) of different model systems heated at 120 °C for 20 or 30 min

Model system	Heat treatment	
	120 °C/20 min	120 °C/30 min
A	19.39	16.42
B	24.93	21.19
C	29.73	28.73
D	0.00	0.00

A, 35 mg galactose in 100 ml simulated milk ultrafiltrate; B, 0.6% seroproteins in system A; C, 2.6% caseins in system A; D, 2.6% caseins + 0.6% seroproteins in simulated milk ultrafiltrate.

lactose were heated, indicating that galactose was not liberated from glycoproteins during thermal treatment.

The results obtained from the model milk solutions and from milk indicate that the formation of galactose took place via degradation of lactose and was dependent on the severity of the heat treatment and the pH of the media. An interaction between proteins and galactose which increased the thermal stability of the monosaccharide also took place.

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