Changes in Free Monosaccharides during Storage of Dried Milk

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Changes in the monosaccharide fraction of dried skim milk stored under different conditions of temperature and humidity have been studied. The monosaccharide fraction of freshly spray-dried milk contained only galactose and glucose. Both monosaccharides increased during storage, and the formation of tagatose and 3-deoxypentulose was also detected. These changes suggest that lactose transformations during storage take place *via* condensation with proteins (Maillard reaction), isomerization (Lobry de Bruyn–Alberda van Ekenstein reaction), and hydrolysis.

INTRODUCTION

Most of the chemical changes produced during the manufacture of milk powder are caused by the heating process to which liquid milk is submitted prior to spray-drying. The intensity of the process is in general lower than or similar to that of pasteurization, so small changes involving lactose are produced. However, milk powder is frequently stored for long periods before being consumed, and therefore some changes can take place. The Maillard reaction is relatively slow at ambient temperatures in liquid systems, but it is a predominant reaction in dried food, being the most important cause of deterioration of skim milk powder (Baltes, 1980; Renner, 1988). It is also well-known that both high values of water activity (aw) (especially within the range 0.3–0.7) and high temperatures increase browning of desiccated foods (O'Brien and Morrisey, 1989).

Most of the previous research on chemical changes during storage of skim milk powder are based on the formation of color and loss of lysine (Finot *et al.*, 1987; Renner, 1988; Lindeman-Schneider and Fennema, 1989; van Mil and Jans, 1991); a few studies have been conducted on changes in the carbohydrate fraction. Richards (1963) determined by TLC and colorimetry the changes in a lyophilized milk sample stored at 45 °C and 0.75 aw and concluded that isomerization of lactose and galactose catalyzed by the saline system of milk gave rise to the formation of lactulose and tagatose, respectively, whereas galactose was formed mainly by the breakdown of 1-amino-1-deoxy-2-ketoses. Since then, some work has been carried out on the analysis of minor carbohydrates in milk which have allowed an accurate determination of lactulose, tagatose, and galactose as well as other components such as epilactose, glucose, and 3-deoxypentulose (Olano *et al.*, 1989; Troyano *et al.*, 1992).

We have now studied the evolution of monosaccharides in dried milk stored under different conditions of humidity and temperature to obtain more information about the chemical pathways of lactose transformations and the possible utility of this determination to evaluate the deterioration of milk powder during storage.

EXPERIMENTAL PROCEDURES

Preparation of Samples. Skim milk samples were atomized with a Buchi 190 mini-spray dryer at an inlet temperature of 130 °C; the feed rate was adjusted to attain an outlet temperature of 90 °C. Freshly atomized samples were equilibrated to the desired aw using the method of Saltmarch and Labuza (1980): samples in open containers were placed in desiccators over the appropriate saturated salt solution ($MgCl_2$, 0.33 aw; K_2CO_3 0.44 aw; and $NaNO_2$, 0.65 aw). They were equilibrated under vacuum for 2 weeks at room temperature and then stored at the desired temperature (30 or 50 °C). Lactulose–lysine was synthesized as previously described (Olano *et al.*, 1992)

Analysis. One gram of milk powder was reconstituted to 10 mL with Milli-Q water for further analysis. One milliliter of a methanolic solution of *O*-methyl α-D-galactopyranoside (0.005%) was added as internal standard to 1 mL of the recombined milk and diluted to 10 mL with methanol. The mixture was filtered after 1 h, and 2 mL of the filtrate was evaporated under vacuum. The residue was dissolved in 100 μL of anhydrous pyridine and silylated with trimethylsilylimidazole/trimethylchlorosilane (2:1) as previously described (Troyano *et al.*, 1991). GC analysis of single samples was carried out in duplicate. A Hewlett-Packard 5890 chromatograph coupled to a Spectra-Physics DataJet integrator was used. TMS ethers were injected in a fused silica column (10 m × 0.2 mm) coated with OV-1. Temperatures were as follows: injector, 275 °C; detector, 250 °C; oven held at 175 °C for 17 min to elute monosaccharides and then heated at 39 °C/min to 250 °C to elute disaccharides. Mass spectra (EI, 70 eV) were obtained using a HP 5890A gas chromatograph with a HP-5170 quadrupole mass detector.

RESULTS

The GC method of Troyano *et al.* (1991) was modified by the use of an OV-1 column instead of a FFAP column. The elution order of monosaccharides changed, but all studied components could be resolved and quantified (Figure 1a). Precision of measurement was better with the new column, as shown by the relative standard deviation which averaged 5.1% for galactose, 4.7% for glucose, 7.8% for 3-deoxypentulose, and 14.7% for tagatose in eight duplicate determinations.

GC analysis of the monosaccharide fraction of a freshly atomized milk sample showed the presence of galactose and glucose and the absence of other hexoses (see Figure 1b). In stored samples, peaks corresponding to both tagatose and 3-deoxypentulose appeared in the chromatogram (Figure 1c). Their identities were assessed by comparing their relative retention times with those of pure standards and confirmed by GC–MS.

Table 1 shows the changes of monosaccharides during storage at 30 °C.

The galactose content remained almost unaltered after the first 40 days, but a considerable increase was observed during prolonged storage; this increase was more noticeable at 0.44 aw than at 0.33 or 0.65 aw.

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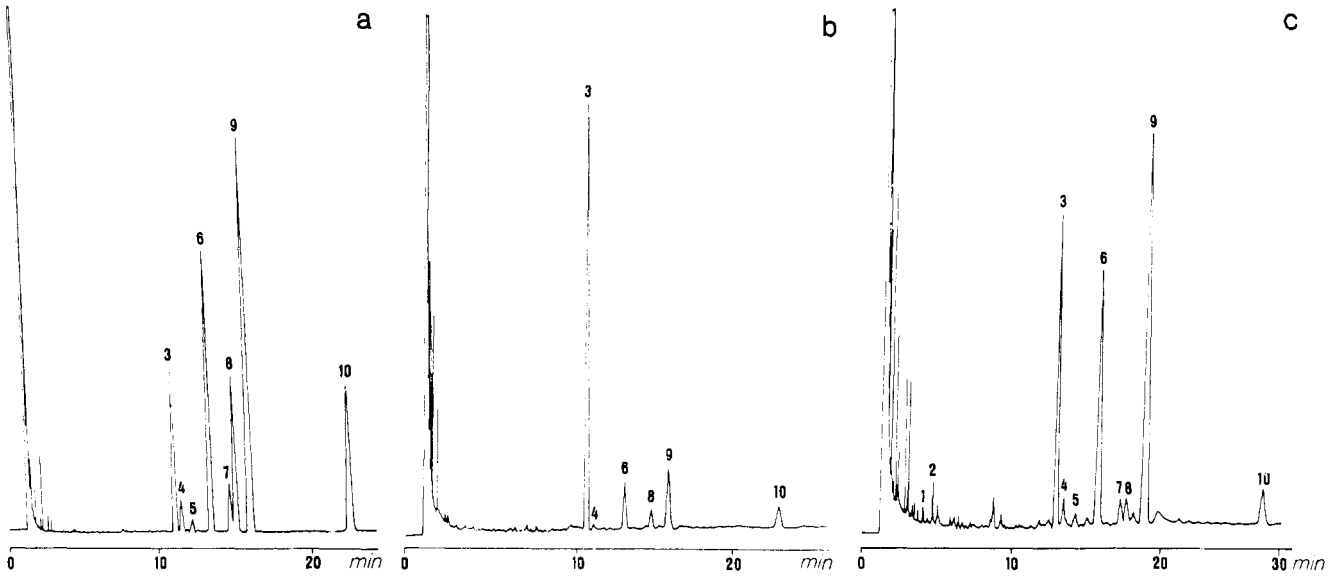

Figure 1. Chromatograms of trimethylsilyl derivatives of monosaccharides in (a, left) standard mixture, (b, middle) control sample, and (c, right) powdered milk stored at 50 °C and 0.33 aw for 100 days. Column and chromatographic conditions were as in the text. Peak numbering: 1 and 2, 3-deoxypentulose; 3, internal standard; 4, 6, and 9, galactose; 5 and 7, tagatose; 8 and 10, glucose.

Table 1. Changes of Free Monosaccharides (Milligrams per Gram) in Dried Milks Stored for 100 Days at 30 °C and Different Water Activity Levels

aw	days	galactose	glucose	3-deoxypentulose	tagatose
control	0	0.8	0.3		
0.33	40	0.9	0.4	0.07	0.02
	60	1.0	0.4	0.06	0.02
	80	2.1	0.9	0.04	0.07
	100	2.0	0.9	*a*	0.05
0.44	40	0.8	0.2	0.08	0.02
	60	2.6	0.7	0.2	0.08
	80	2.7	0.7	0.2	0.07
	100	2.9	0.8	0.3	0.07
0.65	40	0.7	0.2	0.07	0.02
	60	0.9	0.2	0.09	0.03
	80	1.9	0.4	*a*	0.09
	100	2.1	0.4	0.1	0.05

[a] These peaks could not be measured.

Table 2. Changes of Free Monosaccharides (Milligrams per Gram) in Dried Milks Stored for 100 Days at 50 °C and Different Water Activity Levels

aw	days	galactose	glucose	3-deoxypentulose	tagatose
control	0	0.8	0.3		
0.33	40	1.7	0.3	0.2	0.08
	60	2.0	0.3	0.2	0.08
	100	7.0	0.9	0.5	0.4
0.44	40	3.8	0.2	0.7	0.3
	60	10.4	0.6	1.7	1.1
	100	10.1	0.6	1.2	1.4
0.65	40	4.8	0.2	0.1	0.2
	60	10.8	0.6	0.5	0.4
	100	8.0	0.7	1.5	0.3

The evolution of glucose did not follow a regular pattern: a decrease was observed after the first 40 days, and then its concentrataion increased up to about 0.8 mg/g, when stored at 0.33 and 0.44 aw. A lower increase was observed during storage at 0.65 aw.

Tagatose and 3-deoxypentulose appeared as traces after the first 40 days of storage. At the end of the studied period, the maximum amount of tagatose formed was lower than 0.1 mg/g; therefore, no differences due to the effect of aw could be estimated. The amount of 3-deoxypentulose formed was higher than that of tagatose; the maximum concentration (about 0.2 mg/g) was found in samples stored at 0.44 aw, whereas storage at 0.33 aw gave rise to the lower formation of this compound.

Samples stored at 50 °C displayed a clear brownish color. Table 2 shows the changes of monosaccharides in samples stored at 50 °C and different aw values. They underwent a similar but more marked behavior than samples stored at 30 °C. All monosaccharides increased markedly. The galactose concentration attained 10.1 mg/g, 3-deoxypentulose 1.2 mg/g, and tagatose 1.4 mg/g after 100 days at 0.44 aw. Nevertheless, the glucose increase was less than that in the samples stored at 30 °C.

DISCUSSION

Analysis of the free monosaccharide fraction afforded data related to storage conditions of powdered milk. Both Lobry de Bruyn–Alberda van Ekenstein (L–A) and Maillard reactions are considered the main pathways to changes in the carbohydrate fraction of heated milk, but none of them give rise to the formation of glucose. The observed increase of this monosaccharide during storage could be due to lactose hydrolysis, even though this type of reaction has not been reported in powdered milk. Galactose was also formed during lactose hydrolysis, as it started to increase after 60 days of storage, but it could be also formed by hydrolysis of lactulose and lactulose–lysine.

The formation of tagatose, which increased markedly during storage, can only be due to isomerization of galactose. 3-Deoxypentulose is a product of alkaline degradation (L–A reaction) of lactose and other 1,4-disaccharides (Lindström and Samuelson, 1977; Löwendahl and Samuelson, 1976). We have hypothesized that it could also be formed as a byproduct of Maillard reaction, because it has been detected by TLC after thermal treatment of a solution containing 3 mg/mL lactulose–lysine in 0.035 M NaOH (Troyano, 1993). Formation of 3-deoxypentulose during sterilization of milk has been recently reported (Troyano *et al.*, 1992). Thus, it seems well established that 3-deoxypentulose can be formed from lactose not only in alkaline media but also in the milk buffer system; both formation pathways (L–A and Maillard reactions) could be possible in the case of dried milks.

Neither tagatose nor 3-deoxypentulose appears in freshly processed milk powders, and their presence can be considered an indicator of severe deterioration.

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