Monosaccharides and *myo*-Inositol in Commercial Milks

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Monosaccharides (galactose, glucose, tagatose, 3-deoxypentulose, *N*-acetylglucosamine, and *N*-acetylgalactosamine) and *myo*-inositol were determined by gas chromatography in different types of market milk (pasteurized, dried, UHT, and in-container sterilized). Glucose, *myo*-inositol, and *N*-acetylhexosamine concentrations were similar to those previously found in raw milk and showed no variations due to sample type. Sterilized milk samples were characterized by the presence of tagatose and 3-deoxypentulose and, thus, could be clearly distinguished from UHT samples. The galactose level, which was found to be higher in the samples submitted to stronger thermal treatment, seems to be also a useful indicator for milk classification.

Keywords: *Monosaccharides; milk classification*

INTRODUCTION

The chemical changes that occur during heating and storage of milk have been widely studied since it was established that some compounds which are formed or modified under different processing conditions could have a number of uses, including the classification or differentiation of differently heat-treated milks, standardization of industrial process conditions, and identification of overprocessing or inadequate storage.

Changes in the carbohydrate composition of milk during heat processes and storage have been the subject of many papers. Lactulose, formed by the isomerization of lactose, has been shown to occur in different concentrations in ultrahigh temperature (UHT) and in-container sterilized milks (Andrews, 1986). The amount of galactose present in raw milk increases during heating as a result of lactose degradation (Olano et al., 1989). Processed milk also contains other monosaccharides, formed as a consequence of thermal treatment: tagatose, first detected in strongly heated milks (Adachi, 1958), and 3-deoxypentulose, recently characterized in sterilized milks (Troyano et al., 1992b). The monosaccharide composition of milk can be also affected by inadequate storage (Troyano et al., 1994). Glucose and *N*-acetylglucosamine levels can decrease by the action of microorganisms on raw milk (Troyano, 1993).

Although single parameters have been proposed as indicators of milk quality, better assessment can be achieved by having several parameters that show different sensitivities to heat processing and storage of milk. In spite of the potential usefulness of monosaccharides as indicators of milk quality, no data are available on the detailed monosaccharide composition of processed milks.

In this paper the monosaccharide composition of different types of market milks (pasteurized, UHT, sterilized, and spray-dried) is presented, and their possible use to distinguish different types of milk and to obtain information about milk quality is investigated.

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MATERIALS AND METHODS

Samples. Powdered, pasteurized, UHT, and in-container sterilized milks (all including both whole and skim types) from different brands were purchased at local markets. Powdered milks were reconstituted with Milli-Q water to 10% total solids.

Analysis. One milliliter of a methanolic solution of methyl α-D-galactopyranoside (0.005%) was added as internal standard to 1 mL of 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 Perkin-Elmer Model 8410 chromatograph was used. Trimethylsilyl (TMS) ethers were injected in a fused silica column (10 m × 0.2 mm) coated with methyl silicone. Temperatures were as follows: injector, 275 °C; detector, 250 °C; oven, held at 175 °C for 17 min to elute pentoses and hexoses, programmed at 5 °C/min to 195 °C, then held for 7 min to elute hexosamines, and finally programmed at 20 °C/min to 270 °C to elute disaccharides. Calibration details have been published previously (Troyano et al., 1994, 1995). Mass spectra (EI, 70 eV) were obtained using a HP 5890A gas chromatograph coupled to a HP 5970A quadrupole mass detector and a HP-1 fused silica column (12.5 m × 0.25 mm) with the same temperature program as described above.

Multiple regression and discriminant analyses (BMDP/DYNAMIC package, BMDP Statistical Software) were run in a PC microcomputer.

RESULTS AND DISCUSSION

A typical GC chromatogram of free monosaccharides from a sterilized milk is presented in Figure 1. Peaks corresponding to the different tautomeric forms of glucose, galactose, *myo*-inositol, tagatose, 3-deoxypentulose, *N*-acetylglucosamine, and *N*-acetylgalactosamine were found. Peak identities were assigned by retention times and chromatographic patterns and confirmed by GC−MS.

Table 1 shows mean values and ranges of free monosaccharides and *myo*-inositol in different types of milk. Their pattern varied broadly according to the thermal treatment type undergone by sample. Galactose, glucose, *myo*-inositol, *N*-acetylglucosamine, and *N*-acetylgalactosamine were found in all examined samples. Besides these components, tagatose and 3-deoxypentulose were present in sterilized milks.

0021-8561/96/1444-0815$12.00/0

Table 1. Free Monosaccharides and *myo*-Inositol in Different Kinds of Market Milks[a]

	pasteurized, $n = 12$	reconstituted, $n = 14$	UHT, $n = 11$	sterilized, $n = 12$
galactose	7.5 ± 1.29 (3.9−8.9)	7.0 ± 2.93 (3.8−13.3)	9.5 ± 1.61 (8.5−12.0)	17.3 ± 4.44 (11.2−27.1)
glucose	5.0 ± 1.54 (2.2−7.6)	5.2 ± 1.91 (0.4−7.2)	5.5 ± 1.17 (3.7−7.3)	5.1 ± 1.32 (1.2−6.1)
inositol	3.3 ± 0.70 (2.2−3.7)	3.6 ± 0.76 (2.6−5.4)	3.4 ± 0.72 (3.0−4.1)	2.9 ± 0.82 (2.5−3.1)
N-AcGlcNH$_2$	4.4 ± 1.42 (2.8−8.2)	7.0 ± 2.36 (1.1−10.3)	6.7 ± 1.61 (3.7−9.8)	5.8 ± 1.48 (3.2−7.4)
N-AcGalNH$_2$	3.5 ± 1.20 (2.8−8.2)	4.0 ± 0.93 (2.9−6.5)	2.7 ± 1.00 (2.2−3.2)	2.5 ± 1.56 (1.8−4.0)
tagatose			(none−trace)	0.4 ± 0.12 (0.2−0.6)
3-deoxypentulose				2.7 ± 1.85 (0.7−6.5)

[a] Milligrams per 100 mL. Mean values ± SD; ranges in parentheses.

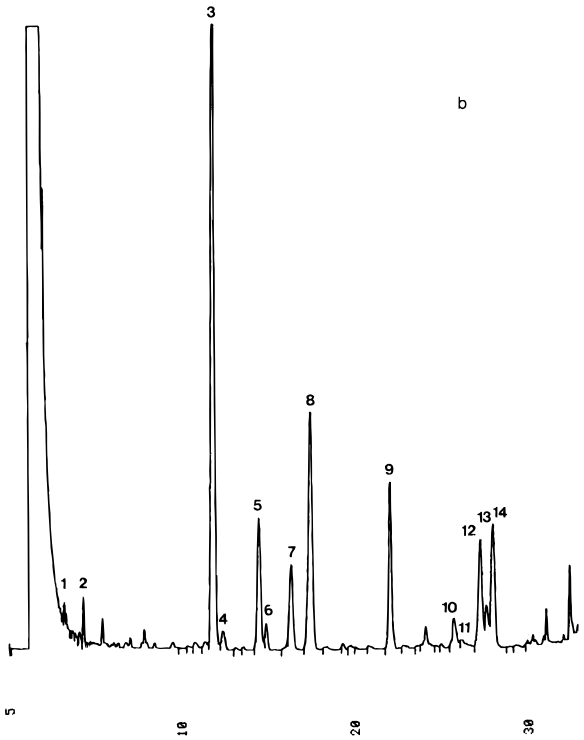

Figure 1. Chromatogram of free monosaccharides (as their trimethylsilyl ethers) from a sterilized milk sample. Peaks: 1 and 2, 3-deoxypentulose; 3, internal standard; 4, 5, and 8, galactose; 6, tagatose; 7 and 9, glucose; 10 and 11, *N*-acetylgalactosamine; 12 and 13, *N*-acetylglucosamine; 14, *myo*-inositol.

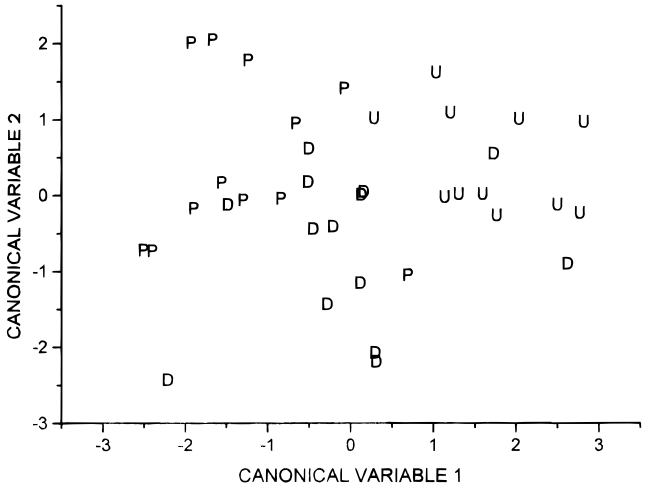

Figure 2. Classification of 37 market milks as obtained by discriminant analysis using five variables (galactose, glucose, *myo*-inositol, *N*-acetylgalactosamine, and *N*-acetylglucosamine). D, recombined dried milks; U, UHT milks; P, pasteurized milks.

The values found for glucose agree with those previously reported (Faulkner et al., 1981; Marschke and Kitchen, 1984; Kuncewicz and Jarkowski, 1987). Although glucose had a considerable variation range, the means showed no differences among the different types of milk. The values found for *myo*-inositol agree with those previously reported in raw milk (Byun and Jenness, 1982; Indyk and Woollard, 1994; Troyano et al., 1995), and they were broadly similar in the four milk types. Although we have not found references on the thermal behavior of *myo*-inositol in milk, it can be considered as the most stable of the studied compounds since inositols have no reducing group; it seems likely that its level is not affected by usual thermal treatments. *N*-Acetylglucosamine (Hoff, 1963) and *N*-acetylgalactosamine (Troyano et al., 1995) detected as free components in raw milk were also present in all examined samples. Mean values of *N*-acetylglucosamine content were similar for all types of milk; however, UHT and sterilized samples showed lower mean values of *N*-acetylgalactosamine content than did pasteurized and reconstituted milks. The present results seem to indicate that thermal treatments have little influence on the free hexosamines level in milk.

Galactose averaged 7.5 mg/100 mL in pasteurized samples, 7.0 mg/100 mL in reconstituted samples, 9.5 mg/100 mL in UHT samples, and 17 mg/100 mL in sterilized ones. The concentration of galactose increased with the severity of thermal treatment; it has been proved that it follows a first-order kinetics (Calvo and Olano, 1989; Troyano et al., 1992a).

Tagatose and 3-deoxypentulose appeared only in sterilized milks. Tagatose was present at very low concentration (0.2−0.6 mg/100 mL), whereas the level of 3-deoxypentulose ranged from 0.7 to 6.5 mg/100 mL and correlated with that of galactose ($r = 0.91$).

Two samples displayed very low levels of glucose and *N*-acetylglucosamine: a dried reconstituted milk gave 0.38 mg/100 mL glucose and 1.09 mg/100 mL *N*-acetylglucosamine; a sterilized sample gave 1.21 mg/100 mL glucose and 3.22 mg/100 mL *N*-acetylglucosamine, respectively. Similar low values have been found in some raw milks stored at room temperature and attributed to the development of microorganisms (Troyano, 1993).

An attempt was made to classify the samples using the monosaccharides concentration. Since 3-deoxypen-

tulose and tagatose were present only in the in-container sterilized samples, these parameters are clearly enough to distinguish sterilized milks from the rest of the commercial samples. UHT, pasteurized, and reconstituted dried milks were submitted to discriminant analysis (BMDP package, 7M program) to differentiate among the three types of milk. UHT samples were correctly classified using the five remaining variables (Figure 2), whereas reconstituted and pasteurized samples were only partially distinguished. The galactose level was the variable of highest discriminant power, followed by *N*-acetylgalactosamine. Two reconstituted dried milk samples were classified as UHT because of their high galactose content (11 and 13 mg/100 mL). The presence of high galactose content in dried milk could be due to inadequate storage conditions (Troyano et al., 1994). Since the use of lactulose as a thermal treatment indicator may not be applicable for all purposes (Andrews, 1989; Olano et al., 1989), the determination of the monosaccharides composition could afford additional information to distinguish among different types of heat-processed milks.

ACKNOWLEDGMENT

We thank M. J. Rodríguez for technical assistance.

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Received for review May 1, 1995. Revised manuscript received December 5, 1995. Accepted December 28, 1995.[⊗] This work was supported by Comunidad Autonoma de Madrid (Project COR0025/94).

JF950260D

[⊗] Abstract published in *Advance ACS Abstracts,* February 1, 1996.