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Use of different thermal indices to assess the quality of pasteurized milks

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Abstract Lactoperoxidase activity and lactulose, furosine and undenatured whey protein contents were determined in Spanish commercial milks labelled as pasteurized (group A) and high-temperature pasteurized (group B), in order to assess their quality. Three samples of group A and all of the samples of group B were lactoperoxidase negative. Most samples of group A had measurable amounts of lactulose, even though their concentrations of undenatured β-lactoglobulin were higher than 2600 mg/l. In general, samples of group B showed higher lactulose and furosine and lower undenatured whey protein contents. High levels of furosine and lactulose accompanied by high levels of undenatured β-lactoglobulin could indicate the addition of milk heated at high temperatures, whereas high levels of furosine and relatively low levels of lactulose may have been due to the presence of reconstituted milk powder.

Key words Thermal indices · Pasteurized milks

Introduction

The quality of commercial milk can be related to the heat load applied during processing, therefore indicators of the heat treatments used are necessary in order to identify the heat treatment employed and as a means of process control. Under minimal conditions of pasteurization, the good organoleptic and nutritive properties of raw milk are scarcely affected. As more severe conditions are applied, the characteristics of processed milk approach those of UHT milk.

According to the heat treatment's intensity, pasteurized milks are marketed as pasteurized and high-temperature pasteurized. For pasteurized milk, the upper limit of the heat treatment is fixed by the requirement that the milk remains peroxidase positive, while high-temperature pasteurized milks are peroxidase negative. Undenatured β-lactoglobulin (β-lg) contents of at least 2600 mg/l for pasteurized and 2000 mg/l for high-temperature pasteurized milk have been proposed [1, 2]. The furosine content has also been used as an index of the heat treatment used to produce pasteurized milks, and the amount of furosine coupled with that of undenatured β-lg has been proposed as a means of detecting the addition of reconstituted milk powder [3].

The amount of lactulose is another index used to differentiate between sterilized milks [4–6], but it is not used for pasteurized milks yet since its concentration in these types of milk is very low [7, 3] and is near the detection limit of the reference HPLC method [8]. Recently, a method for the gas chromatographic determination of minute amounts of lactulose in milk based on the selective, partial removal of lactose has been reported [9].

In the present study, the concentrations of lactulose, undenatured whey proteins and furosine were determined to assess the quality of Spanish commercial pasteurized milks.

Materials and methods

Milk samples

A total of 16 samples of commercially available pasteurized milks were obtained from local stores. Nine samples were marketed as pasteurized (group A) and seven as high-temperature pasteurized (group B).

Analytical determinations

All analytical determinations were carried out in duplicate.

Protein content determination. The protein content was determined using the Kjeldahl method [10].

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Lactulose. Lactulose levels were determined by means of gas chromatography of the trimethylsilyl derivatives of the free carbohydrate fraction using a Sigma 3B gas chromatograph equipped with a 3 m × 1.0 mm (inside diameter) stainless steel column (Chrompack, Middelburg, The Netherlands) packed with 2% OV-17 on nonsilanized 120/140 Volaspher A2 (Merck, Darmstadt, Germany), following the method described by De Rafael et al. [9].

Milk samples (2.5 ml) were gently mixed with approximately 10 ml ethanol in a 25-ml volumetric flask, so that denatured protein particles would not get stuck above the volume mark; the flasks were filled to volume by adding additional ethanol. After mixing again, the mixture was held for 48 h at room temperature to allow precipitation of lactose. Four millilitres of supernatant was mixed with 1 ml 0.5% phenyl-β-glucoside in 60% methanol, evaporated under vacuum at room temperature and converted to trimethylsilyl derivatives using *N*–trimethylsilylimidazole.

Furosine. To determine the level of furosine, 2 ml milk were hydrolyzed with 6 ml of 10.6 M HCl and analyzed by reversed-phase HPLC, according to the method of Resmini et al. [11] using a C8 Alltech furosine-dedicated column [250 mm × 4.6 mm (inside diameter); Alltech, Laarne, Belgium] with a linear, binary gradient. Calibration was performed by the external standard method using a commercial standard of pure furosine (Neosystem, Strasbourg, France).

Undenatured whey protein. Undenatured whey proteins were determined on the fraction soluble at pH 4.6 by reversed-phase HPLC using a PLRP-S 8-μm column [300 Å, 150 mm × 4.6 mm (inside diameter); (Polymer, Church Stretton, UK] with a linear, binary gradient [12]. Calibrations were performed by the external standard method and curves were constructed with commercial standards of whey proteins (Sigma St. Louis, Minn.).

Lactoperoxidase activity. A qualitative analysis was performed to determine the lactoperoxidase activity [13].

Statistical analysis

The BMDP package [14] was used to determine variances (BMDP7D program).

Results and discussion

Table 1 shows the concentrations of furosine, lactulose and undenatured whey protein and lactoperoxidase activity in group A and group B milks. Lactulose was detected in all commercial samples except in sample 2 of group A. Samples of group B had been treated at higher temperatures since they showed higher lactulose and furosine contents and a lower undenatured β-lg level than group A samples.

In group A, the lactulose concentration ranged from 0 mg/l to 5.8 mg/l, and a concentration of undenatured β-lg higher than 3000 mg/l was present in all the milk samples. The undenatured β-lg contents were in the range of those obtained by Andreini et al. [15] for this type of pasteurized milk. Five samples (1–5) were lactoperoxidase positive, showing furosine levels lower than the limit of 8.5 mg/100 g protein proposed for genuine pasteurized milks [16]. The rest of the samples showed a higher furosine content, and only one of these, number 6, was lactoperoxidase positive.

All the samples of group B were peroxidase negative, as is usually found in this type of milk [17], and the contents of undenatured whey protein were lower than in group A. The lactulose concentration ranged from 6.7 mg/l to 20.3 mg/l, and that of furosine from 10.1 mg/100 g protein to 31.4 mg/100 g protein. In general, these results are in agreement with those De Rafael et al. [9] for lactulose and those of Pellegrino et al. [18] for furosine. Samples 15 and 16 showed similar, considerably high values of furosine, but they differed in their lactulose and undenatured β-lg contents. In sample 15, the high content of lactulose and the low content of undenatured β-lg could have been an indication of excessive heating or addition of milk heated at a high tempera-

Table 1 Values of different thermal indicesof commercial milks marketed as pasteurized (*Group A*) and high-temperature pasteurized (*Group B*). *TP* Total protein, *β-lg* β-lactoglobulin, *α-lac* α-lactalbumin, *BSA* bovine serum albumin, + present, − absent

Type of milk	Samples	TP (g/100 ml)	Lactoperoxidase	Furosine (mg/100 g protein)	Lactulose (mg/l)	Undenatured whey protein (mg/l)			β-lg/TP (%)
						α-lac	BSA	β-lg	
	1	3.18	+	6.9	2.7	1021.5	70.5	3069.7	9.6
	2	3.39	++	7.2	−	1113.5	84.7	3539.2	10.4
	3	3.39	+	7.4	4.1	1075.8	72.0	3485.8	10.4
	4	3.17	+	7.4	5.7	980.9	143.8	3775.8	11.9
Group A	5	3.15	+	8.3	5.1	979.7	75.5	3140.4	10.0
	6	3.19	+	8.7	5.8	1064.0	93.8	3295.3	10.3
	7	3.03	−	8.7	4.9	1045.1	65.3	3199.6	10.6
	8	3.00	−	9.0	5.2	921.2	116.8	3576.4	11.9
	9	2.96	−	10.0	3.3	974.6	103.6	3143.3	10.6
	10	3.06	−	10.1	6.7	899.8	63.6	2049.6	6.7
	11	2.97	−	12.2	10.3	905.3	85.0	2600.5	8.7
	12	3.02	−	13.0	14.1	785.6	44.0	1340.5	4.4
	13	3.05	−	13.9	7.3	788.6	112.7	2503.4	8.2
Group B	14	3.20	−	15.0	12.3	741.2	46.5	1445.6	4.5
	15	2.97	−	27.7	20.3	754.0	26.4	792.2	2.7
	16	2.69	−	31.4	12.0	753.4	52.1	1884.0	7.0

ture, whereas in sample 16 the furosine content exceeded the level expected with regard to the lactulose and undenatured β-lg contents, suggesting the presence of reconstituted milk powder.

Table 1 also shows the content of acid-soluble β-lg expressed as a percentage of total protein (%β-lg/TP). This variable seemed to be a good indicator of the severity of high-temperature pasteurization, and a threshold concentration of 10% would be an appropriate limit to assess the heat damage of pasteurized milk [18]. Group A showed a range of %β-lg/TP between 9.6% and 11.9%. Three of these samples (7, 8 and 9) were peroxidase negative, Pellegrino et al. [18] also found %β-lg/TP concentrations of 10% in few pasteurized, peroxidase-negative milk samples.

In group B the %β-lg/TP concentrations were lower than 10% and all the samples were peroxidase negative. Three samples, 12, 14 and 15 had very low %β-lg/TP concentrations, which could indicate that they had been subjected to a severe heat treatment. This was supported by the high levels of lactulose measured.

The ANOVA included all the variables shown in Table 1, as well as the ratios between them, and it revealed highly significant differences ($P \leq 0.001$) between group A and group B milks for all the variables studied.

The present study shows the usefulness of different thermal indices to differentiate between pasteurized milks according to the heat treatment applied. The advantage of using combined indices to assess the quality of pasteurized milks is also shown.

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