Carbohydrate and whey protein composition of dried milk as a possible criterion of heat treatment intensity

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1. Introduction

The suitability of dried milk for particular usages is largely dependent on the effect of heat treatment applied during processing. It is therefore necessary to distinguish between dried milks subjected to different heat treatments. Most of the methods which have been developed for heat classification are based on direct measurement of the undenatured whey proteins. The most widely used heat classification method is based on the turbidimetric determination of undenatured milk serum protein nitrogen (UMSPN) and the dried milk is classified into one of the following classes: low heat ($\geq$ 6.0 mg UMSPN/g), medium heat (5.99-1.51 mg UMSPN/g) and high heat ($\leq$ 1.5 mg UMSPN/g) (2). Other methods include measurement of the cysteine number (9, 11) and high performance liquid chromatography (HPLC) determination of whey proteins (8, 15).

Although extensive work on quantitative PAGE of whey proteins has been carried out (6, 14) this method has not been applied to heat classification of dried milk.

Due to seasonal variations in the concentration and relative proportions of the proteins in raw bulk milk, a test based on the changes of these constituents requires for its interpretation an accurate knowledge of the protein content of the unheated milk. Since in practice, except for the manufacturer, it is most unlikely that a sample of the raw milk is available, it is necessary to assume a constant value for the protein composition in unheated milk. This imposes some limitation of the accuracy of all protein-based heat classification methods.

The determination of a compound formed during heat treatment, which is absent in unheated milk or present in insignificant quantities would be suitable for a classification test. Lactulose is absent in unheated milk, is formed by isomerization of lactose during the thermal process (1) and the extent of heat treatment applied to liquid milk can be followed by the amount of lactulose formed (3, 5, 10). Recently, it has been proved that the concentration of galactose present in liquid milk increases with the intensity of the heating process (13).

In spite of the work carried out in studying the changes in the lactose content during heat treatment of milk, no data are available on the carbohydrate composition of dried milk and its application to heat classification.

The main objective of this work has been the study of the free carbohydrate and individual whey protein composition of dried milk products as well as the heat classification of commercial spray-dried milk samples according to this composition.

2. Materials and methods

Samples

Dried milk samples submitted to different heat treatments were purchased in various parts of Spain. The samples were reconstituted with deionized water to 10 % total solids.

Gas chromatography

The derivatives were prepared by mixing 1 ml of milk with 1 ml of 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 trimethylsilylimidazol as reported earlier (10).

Gas chromatographic analysis was performed on a Sigma 3B gas chromatograph (Perkin Elmer) equipped with a 3 m x 1.0 mm i.d. stainless steel column (Chrompack) packed with 2 % OV-17 on non-silanized 120/140 Volaspher A-2 (Merck). Nitrogen was used as carrier gas. The temperature of the injector and detector was 300 °C. Analysis was 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 (12).

Polyacrylamide gel electrophoresis (PAGE)

The milk serum proteins were obtained by acidification to pH 4.6 by adding 1 volume of acetic acid and 1 volume of sodium acetate 1M to 5 volumes of skimmilk. The supernatant containing the whey proteins was analyzed by electrophoresis.

Quantitative PAGE was performed by the method of HILLIER (6) in cylindrical gel rods using β-lactoglobulin A (β-lg A) as internal standard. This was achieved by loading 3.31 μg of β-lg A on the gel and applying 5 mA/gel for 15 min. In every run 2 gels containing known amounts of β-lg (3.34 μg) α-lactalbumin (α-la) (1.20 μg) and bovine serum albumin (SA) (0.14 μg) were included.

Gels were stained with Coomassie blue without further decoloration (4). Quantification was based on the measurement of the height of peaks of densitograms obtained with a Chromoscan MKII (Joyce Lobel).

Determination of undenatured whey protein

The ADMI (2) method was used. 20 ml of milk were mixed with 8 g NaCl, kept for 30 min at 37 °C and filtered. 5 ml of the filtrate were mixed with 50 ml of a saturated solution of NaCl. One drop of HCl was

mixed with 5.5 ml of the diluted sample. After 5-10 min the level of undenatured whey protein was measured using turbidimetric determination at 420 nm. The results were expressed as UMSPN mg/g of dried milk.

3. Results and discussion

Table 1 shows the UMSPN and β-lactoglobulin (β-lg), serum albumin (SA), α-lactalbumin (α-la), galactose and lactulose content of 17 dried milk samples. Although some differences were found between UMSPN determined by turbidimetry or PAGE the same heat classification of milk was obtained using both methods. The SA content of high heat samples was lower than 0.31 mg/g and only one medium heat sample gave a SA content lower than 0.31 mg/g. The β-lg content was lower than 4.9 mg/g in high heat samples and higher than 5.6 mg/g in medium heat samples. The α-la content in high heat samples was in most cases lower than in medium heat samples, however a clear distinction between both types of samples according to their α-la content could not be achieved.

The galactose content was independent of the heat classification. This is probably due to the fact that the amount of galactose increases during storage of dried milk samples (16). The lactulose content was higher in high heat samples (0.30-0.44 mg/g) than in medium heat samples (0.09-0.20 mg/g).

Only one sample which was not included in the study shown in Table 1 was classified as medium heat when UMSPN was determined by turbidimetry (1.98 mg/g) whereas the content of UMSPN by PAGE (1.13 mg/g), SA (0.21 mg/g), β-lg (4.46 mg/g) and lactulose (0.34 mg/g) correspond to high heat samples.

According to these results it can be concluded that the quantitative PAGE determination of UMSPN, SA and β-lg can be used as a method for heat classification of dried milk. These results are in agreement with those of KNEIFEL and ULBERT (8) using the HPLC determination of the β-lg.

Since it has been recognized that no single heat classification method is likely to be suitable for all purposes (7) it could be useful to take also the denaturation of serum proteins and the formation of lactulose in dried milk into account.

Table 1: Undenatured milk serum protein nitrogen (UMSPN) determined by turbidimetry (*) and by PAGE (**), β-lactoglobulin (β-lg), bovine serum albumin (SA), α-lactalbumin (α-la), galactose and lactulose content of dried milk samples

	High heat			Medium heat		
	x̄ n = 6	Maximum (mg/g)	Minimum	x̄ n = 6	Maximum (mg/g)	Minimum
UMSPN*	1.32	1.50	1.20	2.34	3.10	1.60
UMSPN**	1.05	1.50	0.77	2.34	3.21	1.53
SA	0.19	0.31	0.11	0.57	0.88	0.22
α-la	3.05	4.59	2.09	4.51	5.72	3.18
β-lg	3.47	4.83	2.65	9.83	13.93	5.68
Galactose	1.17	1.42	0.89	1.10	1.43	0.99
Lactulose	0.38	0.44	0.30	0.15	0.20	0.09

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5. Summary

OLANO, A., CALVO, M.M., RAMOS, M., MORAIS, F.: **Carbohydrate and whey protein composition of dried milk as a possible criterion of heat treatment intensity.** Milchwissenschaft **44** (2) 80-82 (1989).

74 **Milk powder** (heat classification)

17 dried milk samples were heat-classified as medium heat and high heat according to their undenatured milk serum protein nitrogen (UMSPN) content; bovine serum albumin (SA), β-lactoglobulin (β-lg) and lactulose mean content were 0.19, 3.47 and 0.38 mg/g, respectively, for high heat samples and 0.57, 4.15 and 0.15 mg/g, respectively, for medium heat samples. It is concluded that the determination of SA, β-lg or lactulose can be used for heat classification of dried milk.

OLANO, A., CALVO, M.M., RAMOS, M., MORAIS, F.: **Kohlenhydrat- und Molkenproteinzusammensetzung von Milchpulver als mögliches Merkmal der Intensität der Wärmebehandlung.** Milchwissenschaft **44** (2) 80-82 (1989).

74 **Milchpulver** (Hitzebehandlungsklassen)

Es wurden 17 Milchpulverproben durch die Bestimmung des Gehaltes an undenaturiertem Molkenproteinstickstoff in die Hitzebehandlungsklassen „medium heat" und „high heat" klassifiziert.

Die Mittelwerte des Blutserumalbumin(SA)-, β-Laktoglobulin(β-Lg)- und Laktulose-Gehalts betrugen 0,19 bzw. 3,47 bzw. 0,38 mg/g für „high heat" und 0,57 bzw. 4,15 bzw. 0,15 mg/g für „medium heat"-Proben.

Es wird die Schlußfolgerung gezogen, daß die Bestimmung des Gehaltes an SA, β-Laktoglobulin oder Laktulose als Methode zur Ermittlung von Hitzebehandlungsklassen von Trockenmilch eingesetzt werden kann.

OLANO, A., CALVO, M.M., RAMOS, M., MORAIS, F.: **La composition du lait en poudre en glucide et séroprotéine comme critère possible de l'intensité du traitement thermique.** Milchwissenschaft **44** (2) 80–82 (1989).

74 **Lait en poudre** (classification thermique)

OLANO, A., CALVO, M.M., RAMOS, M., MORAIS, F.: **Composición de leche en polvo en carbohidratos y seroproteina como posible criterio de la intensidad del tratamiento térmico.** Milchwissenschaft **44** (2) 80–82 (1989).

74 **Leche en polvo** (clasificación térmica)