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The Maillard reaction during the ripening of Manchego cheese

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Abstract

The Maillard reaction was studied in semi-industrial Manchego cheese during ripening, through measurement of furosine and galactose. An accumulation of galactose was observed during the initial period of ripening, probably due to the inability of *Streptococcus thermophilus* to metabolise galactose. However, a considerable decrease in galactose content and increase in furosine amount were found after 15 days of ripening. No further formation of furosine was observed after 45 days. This fact could be attributable to the exhaustion of galactose due to its participation in Maillard reaction and/or its utilisation by the microorganisms *Lactococcus* and *Lactobacillus*. Furosine seems to be a useful indicator of the Maillard reaction during ripening of Manchego cheese.

Keywords: Furosine; Galactose; Manchego cheese

1. Introduction

Manchego cheese is one of the most important Spanish cheeses and represented ~50% of the total production in 1996 (Anon, 1998). Traditional Manchego cheese is farmhouse-made from raw ewes' milk of local herds. However, an increasing consumer demand makes it necessary to produce cheese on an industrial scale from pasteurised milk. In both cases, after its manufacture, the cheese must be ripened for at least 2 months.

The manufacture of Manchego cheese produces small modifications in the constituents; the major changes occur during ripening. Proteolysis is the most important reaction that occurs during the ripening of this type of cheese, giving rise to amino acids and oligopeptides (Mora & Marcos, 1981, 1982). Hydrolysis of lactose, with the production of galactose and glucose, also occurs. The components released in these two reactions are potential reactants for Maillard browning reaction (Bley, Johnson & Olsen, 1985). The Maillard reaction plays an important role in the production of flavours and antibacterial compounds and decreases the nutritional quality (Adrian, 1973; Baltes, 1982; Van Boekel, 1998).

In dairy products, the Maillard reaction occurs between the carbonyl group of reducing sugar (lactose, glucose and galactose) and the ϵ-amino group of protein-bound lysine. Because Manchego cheese has a low amount of lactose (Fernández-García, Ramos, Polo, Juárez & Olano, 1988), the main sugars that can participate in this reaction should be galactose and glucose. On the other hand, the type of strain used in the starter culture in cheese elaboration has a big influence on the accumulation of sugar. Thus, galactose may accumulate in cheese made with *Streptococcus thermophilus* and this may cause browning of cheese (Bley et al., 1985).

Some studies on the Maillard reaction have been performed on processed cheese (Bley et al., 1985; Piergiovanni, De Noni, Fava & Schiraldi, 1989; Thomas 1969). Furosine, an amino acid formed during acid hydrolysis of the Amadori compounds (Resmini, Pellegrino & Masotti, 1993), can be used to measure the initial steps of the Maillard reaction. Furosine is a suitable indicator of the severity of heat treatment and storage conditions of dairy products (Resmini & Pellegrino, 1991; Resmini, Pellegrino & Batelli, 1990). Furosine has been used to detect illegal addition of

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reconstituted whey and milk powder to fresh cheese (Resmini et al., 1993). Furosine determination has been used to characterise the authenticity of Mozarella cheese (Pellegrino, Resmini, De Noni & Masotti, 1996), and as a ripening index of Grana Padano cheese (Pellegrino, Batelli, Resmini, Ferranti, Barone & Addeo, 1997). Also, the content of furosine in different types of Spanish commercial cheeses has been determined by Villamiel, Arias, Corzo and Olano, (in press).

In this work, a study of the progress of the Maillard reaction, during ripening of genuine semi-industrial Manchego cheese, was carried out using measurements of furosine and galactose.

2. Materials and methods

2.1. Manufacture of Manchego cheese

Three cheeses (A, B and C) were manufactured on the same day from the same batch of ewes' milk purchased from a local farm. The manufacture was carried out by traditional methods for the industrial production of Manchego cheese. Whole ewes' milk was pasteurised at 70°C for 30 min. Following pasteurisation, a starter culture of *Lactococcus lactis* spp. *lactis*, *L. lactis* spp. *cremoris*, *L. lactis*, *L. plantarum* and *S. thermophilus* was added. After heating at 30–33°C for 20 min, 0.025 g of standard rennet (strength 550 coagulant units) per l of milk was added, to give a coagulation time of 1 h at 30°C. Three cheeses were prepared from the curds, pressed and immersed (48 h) in a brine bath containing 20% NaCl at 12–14°C. The cheeses were ripened in a curing room at 13°C and 80% RH for 5 months. Portions of the cheeses, A, B and C, were analysed at 0, 15, 30, 45, 60, 90, 120 and 150 days after manufacture. All samples were analysed at least in duplicate.

2.2. Model system

ε-*N*-Deoxytagatosyl-lysine was prepared following the method of Finot and Mauron (1969). Galactose (3.25 g) and *N*-α-acetyl-L-lysine (0.5 g) in 47.5 ml of methanol were heated under reflux for 4 h and then evaporated to dryness under vacuum.

2.3. Chemical analyses

NaCl and dry matter (DM) content were determined following the official methods of the IDF (1972, 1982). Protein content was determined by the Kjeldahl method of the IDF (1993). Water activity (a_w) was determined by using a Hygrometer Decagon Device CX-2 (USA) operating at 20°C. The pH of the samples was measured in a Crison 2002 pH meter (Barcelona, Spain).

2.4. Gas liquid chromatography (GLC) analysis of carbohydrates

2.4.1. Preparation of the samples

Samples for GLC analysis of carbohydrates were prepared by the method of Fernández-García, Olano, Cabezudo, Martin-Alvarez and Ramos (1993); 4 g of cheese were homogenised in 20 ml of distilled water and 5 ml of internal standard (1 mg/ml). The mixture was filtered through Whatman No. 1 paper and 5 ml of the filtrate were diluted to 25 ml with methanol, held for about 1 h at room temperature and filtered through Whatman No. 42 paper. The filtrate was dried on a rotatory evaporator at 38–40°C.

2.4.2. GLC analysis of lactose

Lactose was analysed by GLC by the method of Olano, Calvo and Reglero (1986); 100 μl of trimethylsilyl imidazole were added to the dry sample and the mixture was held for several seconds in an ultrasonic bath to dissolve the whole sugar fraction. Derivatisation was carried out at 65–70°C for 30 min. Hexane (100 μl) and 200 μl water were added, and 2 μl of the organic fraction were injected into a stainless steel column (Chrompack, Middelburg, The Netherlands) packed with 2% OV-17 on nonsilanized 120/140 Volaspher A-2 (3 m×1.0 mm) (Merck, Darmstadt, Germany). Phenyl-β-glucoside was used as internal standard.

2.4.3. GLC analysis of monosaccharides

Free monosaccharides (galactose and glucose) were determined by gas chromatography as their trimethylsilyl ethers following the method of Troyano, Olano, Fernández Diaz, Sanz and Martínez-Castro (1991). Pyridine (100 μl), 100 μl of trimethylsilyl imidazole and 100 μl of trimethyl chlorosilane were added to the dry sample. Derivatisation was carried out at room temperature; 100 μl hexane and 200 μl water were added, and 2 μl of the organic fraction were injected into a fused silica column (10 m×0.2 mm) coated with methyl silicone. Methyl-α-D-galactopyranoside was the internal standard.

2.5. High performance liquid chromatography (HPLC) analysis of furosine

2.5.1. Sample preparation

For furosine determination in the model system, the reaction mixture containing ε-*N*-deoxytagatosyl-lysine was hydrolysed with 500 ml of 6 N HCl (Finot, Bricout, Viani & Mauron, 1968). For analysis of furosine in cheese, 200–300 mg of sample were hydrolysed with 8 ml of 7.95 N HCl at 110°C for 23 h.

2.5.2. HPLC analysis

Furosine analysis was performed by RP-HPLC by the method of Resmini et al. (1990), using a C8 column

Table 1
Changes in composition during Manchego cheese ripening[a]

Storage (days)	a_w	pH	Dry matter[b]	NaCl[c]	Protein[c]
0	0.970	5.82	52.98	1.59	36.2
15	0.961	5.03	55.76	2.44	35.5
30	0.968	5.11	57.03	2.10	36.5
45	0.956	5.10	59.27	3.18	35.7
60	0.950	5.16	62.03	3.03	37.0
90	0.934	5.13	66.23	3.35	36.9
150	0.924	5.14	68.47	3.10	35.6

[a] Values are the mean of A, B and C cheeses analysed in duplicate.
[b] g/100 g of cheese.
[c] g/100 g of dry matter.

(250×4.6 mm inside diameter) (Alltech Furosine-dedicated; Alltech Associates, Laarne, Belgium) with a linear binary mobile phase gradient. Calibration was performed by the external standard method using a commercial standard of pure furosine (Neosystem Laboratories, Strasbourg, France).

3. Results and discussion

The overall composition of the cheese and the changes observed during the ripening period are presented in Table 1. In general terms, all the values were close to those reported in the literature for stored Manchego cheese (Fernández-García et al. 1988, 1993; Marcos, Esteban & Fernández-Salgueiro, 1979; Pardo, Pérez, Gómez, Tardáguila, Martínez & Serrano, 1996; Serrano, Garcia, Medina & Serrano, 1997).

The concentrations of lactose, glucose and galactose were also determined. Lactose (results not shown) was detected at low levels: 15.1, 9.4 and 3.6 mg/100 g DM after 15, 30 and 150 days of ripening, respectively. Fernández-García et al. (1988) found 16.1 and 6.77 mg of lactose/100 g DM in Manchego type cheese stored for 15 and 30 days, respectively. At the 15th day of storage, the amount of glucose (results not shown) was 22.6 mg/100 g DM and this decreased to 7.0 mg/100 g DM at the end of ripening. At the beginning of the storage period, galactose (Fig. 1) was the most abundant sugar, reaching 470 mg/100 g DM on the 15th day of storage. Galactose content decreased markedly after 15 days of ripening and no changes were detected during the rest of the storage period. The low lactose content, together with the high galactose level found at the beginning of storage, is due to the hydrolysis of lactose by the culture microorganisms (Fernández-García et al., 1988) and the inability of *S. thermophilus* to metabolise galactose (Tinson, Hiller & Jago, 1982). The comparatively low glucose content indicates rapid assimilation of glucose by bacteria.

Fig. 1 also shows the formation of furosine in Manchego cheese during ripening. No appreciable Maillard reaction occurred in the curd, since the initial furosine content of the cheese (8 mg/100 g of protein) was close to that found in pasteurised milk (Resmini & Pellegrino, 1991). A considerable increase in the concentration of furosine was observed during the initial period of storage, reaching a plateau on day 45 (22 mg/100 g protein approx). This increase was consistent with the above-mentioned decrease in the amount of galactose. No progress of Maillard reaction after 45 days of storage was detected. This observation could be

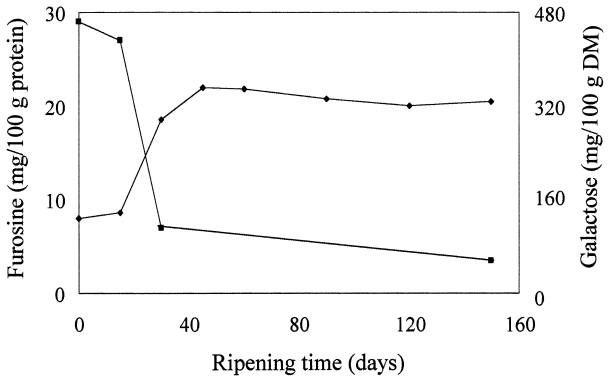

Fig. 1. Concentration of furosine (mg/100 g protein) (◆) and galactose (mg/100 g DM) (■) in Manchego cheese during ripening for up to 150 days.

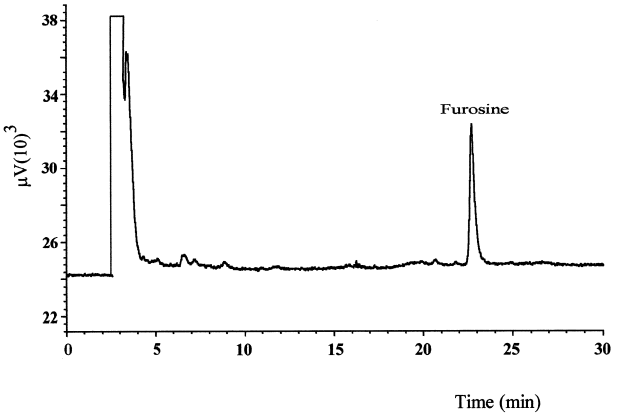

Fig. 2. HPLC chromatogram of the acid hydrolysate of the Amadori compound ε-*N*-deoxitagatosyl-lysine.

attributed to the exhaustion of galactose due to its participation in this reaction and/or its utilisation by the microorganisms *Lactococcus* and *Lactobacillus*.

Resmini and Pellegrino (1991) reported furosine values for ripened soft and hard cheeses in a range 13 to 40 mg/100 g of protein. Villamiel et al. (in press) reported concentrations of furosine from 3.5 to 43.8 mg/100 g protein in industrial hard-pressed cheese (some of them were Manchego type) from the Spanish market.

In view of these results, it is reasonable to suggest that galactose, present at a relatively high level during the initial period, is the main reducing sugar involved in the early stages of the Maillard reaction during the ripening of Manchego cheese and the corresponding protein-bound Amadori product, ε-*N*-deoxitagatosyl-lysine can be formed. In order to test whether acid hydrolysis of ε-*N*-deoxitagatosyl-lysine produces furosine, additional experiments were carried out using a model system containing galactose and *N*-α-acetyl-L-lysine. The HPLC analysis (Fig. 2) of acid hydrolysate of reaction mixture gave a single peak with the same retention time as furosine, confirming the occurrence of the Amadori compound during the ripening of cheese.

The results obtained in this study show that furosine could be used as a useful indicator of Maillard reaction during the ripening of Manchego cheese.

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