Seasonal Evolution of Hydrophilic and Hydrophobic Peptide Contents in Cheeses Made from Ewe's Goat's or Cow's Milk

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Abstract: Proteolysis is the principal and most complex biochemical event occurring during the maturation of the majority of ripened cheese varieties. In addition to softening the cheese body, proteolysis influences the development of cheese flavour via the formation of amino acid and peptides which make a direct contribution to flavour. Goat, cow and sheep cheeses have been elaborated with raw milk and calf rennet. The extent of proteolysis was monitored over six months of ripening and means of HPLC peptide profile analysis. The influence of season on the changes in hydrophobic and hydrophilic peptides and the HO/HI ratio during the ripening of the cheeses were studied.

Keywords: proteolysis; goats; ewes; cows; cheese

INTRODUCTION

In many cheese varieties the initial hydrolysis of casein is caused by the coagulant and to a lesser extent by plasmin, which results in the formation of large (water-insoluble) and intermediate-size (water soluble) peptides which are subsequently degraded by the coagulant and enzymes from the starter and non-starter microflora of the cheese. It is well accepted that the amount of total, hydrophobic and hydrophilic peptides and the hydrophobic:hydrophilic ratio is a suitable way to study the proteolysis process and the changes in these parameters were significantly influenced by cheese age (Mallatou *et al*. 2004; Picon *et al*. 2007; Tejada *et al*. 2008). Seasonal changes in milk composition affect cheese yield, quality and sensory characteristics (Izco & Torre 2000; Mendía *et al*. 2000; Fernández-García *et al*. 2004a, b, 2006; Muñoz *et al*. 2003;). Changes in cheese yield (due to increases in the concentrations of milk fat and protein) and the lipolytic activity and microbiological quality of bulk raw ewe's milk are the main aspects affected seasonaly during the cheese-making period (Barron *et al*. 2001).

EXPERIMENTAL

To perform the present study a total of 224 cheese of known composition were elaborated and controlled. Goat, cow and sheep cheese have been elaborated with raw milk and calf rennet. It was carried out during two periods, corresponding to two seasons: winter and summer. The peptides content of the water soluble cheese extracts (WSE) was determined by reversed-phased high performance liquid chromatography (RP-HPLC) and detection at 214 nm. Following the same criteria as other authors (Mallatou *et al*. 2004; Tejada *et al*. 2008), the integration area of peptides was determined and divided into two regions with the criterion being the elution times of peaks. The first group, or hydrophilic peptide portion, consists of the peaks with retention times from 3 min to 55 minutes. The second group of peptides with retention times from 61 to 100 min, was the more hydrophobic peptide portion. The ratio of hydrophobic to hydrophilic peptides was obtained by dividing the total area of the peaks in the hydrophobic peptide portion by the total area of the peaks in the hydrophilic peptide por-

tion of the HPLC run. Cheese manufacture, water soluble cheese extracts (WSE) preparation and reverse-phase HPLC were described in detail by González-Martín *et al.* (2009).

RESULTS AND DISCUSSION

As cheese ages, more caseins and high molecular weight peptides are being broken down into smaller peptides that may be water soluble. The total water soluble peptides content (total area under the peaks on the HPLC chart) in cheese made from summer's milk is higher than in cheese made from winter's milk, without differences between the races. Therefore, it was expected and observed that as the cheese aged, the total water-soluble peptides content increased. Cheeses made from cows' milk showed a higher increase than cheeses made from ewes' or goats' milk.

Total water soluble peptides content since the month 0 of ripening (one-day cheese) and the increase throughout ripening were higher in cheeses made from summer's milk than in those made from winter's milk.

Hydrophobic and hydrophilic peptides concentrations in the WSE of cheese do not present the same evolution, so hydrophilic peptides increase significatively throughout ripening, being more extensive in the first month of ripening. Similar results were reported by Tejada *et al.* (2008) which show that the ripening time significantly affected HI content, with a significant increase being shown throughout the ripening period elapsed over the first 30 days of ripening. On the other hand, the hydrophobic peptides showed a high variability in all kind of samples without significantly differences in their contents throughout ripening, for all types of milk and seasonality. In Feta cheese was observed that as the age of cheese increased, the HO peptides in the WSF of cheese decreased, whereas the HI peptides increased (Katsiari *et al.* 2000).

When the amount of hydrophobic peptides present in the WSE of cheese was expressed relative to the amount of hydrophilic peptides, the ratio decrease with increasing cheese age (Table 1). These results are lower than those reported by other authors, perhaps because of the different manufacturing processes of cheeses. It should be noted that there are differences in the first portion of chromatograms called HI by different authors, which could explain the greater values found for the ratio HO/HI, in these studies. It is evident that the ratio of HO/HI peptides of cheese decreased sharply in the first month of ageing.

This evolution is showed for all kind of milk and season. This decrease could be attributed mainly to the degradation of HO peptides and the formation of HI peptides as well as highly HO peptides that are no longer water soluble. Similar results were reported in Teleme cheese made from ewes' goats' and a mixture of ewes' and goats' milk (Mallatou *et al.* 2004) in manchego cheese from ewes' milk (Picon *et al.* 2007), in Feta cheese (Katsiari *et al.* 2000), in white and red Afuega'l pitu cheese

Table 1. Means and standard deviations for the hydrophobic:hydrophilic ratio (HO/HI) after 0, 1, 2, 3, 4, 5, and 6 month of ripening of each one of the two replicates of cheese

Ripening month	Cows'		Ewes'		Goats'	
	winter	summer	winter	summer	winter	summer
0	0.36 ± 0.03^{c}	0.37 ± 0.07^{a}	0.67 ± 0.01^{f}	0.28 ± 0.00^{a}	0.50 ± 0.05^{c}	0.85 ± 0.01^{a}
1	0.20 ± 0.00^{b}	0.17 ± 0.01^{a}	0.21 ± 0.0^{e}	0.16 ± 0.02^{a}	0.24 ± 0.04^{ab}	0.23 ± 0.04^{a}
2	0.20 ± 0.01^{b}	0.13 ± 0.00^{a}	0.19 ± 0.01^{de}	0.11 ± 0.04^{a}	0.21 ± 0.03^{ab}	0.09 ± 0.00^{a}
3	0.17 ± 0.01^{ab}	0.09 ± 0.03^{a}	0.16 ± 0.01^{cd}	0.17 ± 0.09^{a}	0.26 ± 0.00^{b}	0.12 ± 0.01^{a}
4	0.19 ± 0.00^{b}	0.11 ± 0.00^{a}	0.15 ± 0.00^{bc}	0.06 ± 0.02^{a}	0.18 ± 0.01^{ab}	0.09 ± 0.03^{a}
5	0.16 ± 0.00^{a}	0.11 ± 0.02^{a}	0.12 ± 0.01^{ab}	0.08 ± 0.00^{a}	0.14 ± 0.01^{a}	0.08 ± 0.02^{a}
6	0.21 ± 0.00^{b}	0.12 ± 0.01^{a}	0.11 ± 0.02^{a}	0.08 ± 0.05^{a}	0.20 ± 0.04^{ab}	0.10 ± 0.02^{a}

^{a,b,c}results of the Fisher's least significant differences (LSD) test; means of the same parameter in the same column without a common letter (a–f) differ significantly ($P < 0.05$)

(Gonzalez de Llano *et al*. 1995) and in goats' milk cheese (Tejada *et al*. 2008).

The ratio HO/HI evolution shows the sharper decrease throughout ripening process in cheeses made from cow´s and goat's milk in summer's cheeses than those made in winter. However, cheeses made from ewe's milk does not show differences between the summer's and the winter's milk. No significatively differences were found in the values of the ratio HO/HI because of the type of milk neither the season.

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