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Evaluation of taste compounds in water-soluble extract of goat cheeses

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Abstract

The water-soluble fractions of two goat cheeses — one denomination of origin commercial trade mark (crottin de Chavignol[®]) and one bought in a local cheese making establishment (Bouton de culotte[®]) — containing many taste and flavour molecules were studied. Ultrafiltration with a 1000 Da threshold membrane, followed by gel filtration on Toyopearl HW-40S gel using water as eluent, led to the production of edible fractions. Physicochemical and sensory analysis of these fractions showed that the most tasty fractions contained, essentially, the free amino acids and mineral salts. Some of these tasty fractions also imparted some flavours. The quantity of small peptides (MW < 1000 Da) in the ultrafiltered water-soluble fractions was rather high but the gel filtration fractions containing, essentially, the peptides were not tasty. That result minimises the direct impact of these compounds on the taste of cheeses. The concentrations of each free amino acid in the fractions were found to be lower than their respective reported threshold values. Among the quantified compounds, it seems that only mineral salts and lactic acid are direct taste-active components in cheeses. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

Proteolysis (Fox, 1989; Fox, Singh & McSweeney, 1994; Hemme, Bouillanne, Metro & Desmazeaud, 1982; Rank, Grappin & Olson, 1985; Seitz, 1990) and lipolysis (Dumont & Adda, 1979; Molimard, Le Quéré & Spinnler, 1997) are among the most important events occurring during cheese ripening, leading to the production of flavour compounds, a final result of particular importance for the consumer. Many studies during the last 25 years have contributed to the identification and the quantitation of numerous aroma compounds such as methyl ketones, short chain fatty acids and sulfur compounds, produced from the degradation of lipids and amino acids (Molimard and Spinnler, 1996).

In the meantime, much less work has been conducted for tasty compounds and very few of them have been identified. It is well established that the water-soluble fraction of cheese contains the majority of the taste compounds (Aston & Creamer, 1986; McGugan, Emmons & Larmond, 1979; Salles, Septier, RoudotAlgaron, Guillot & Etiévant, 1995) such as salts, aminoacids and low-molecular weight peptides, produced during proteolysis and some volatile compounds (Engels, Dekker, de Jong, Neeter & Visser, 1997). However, to our knowledge, only few small peptides have been identified and related to the taste of cheese in both Vacherin Mont d'Or (Mojarro-Guerra, Amado, Arrigoni & Solms, 1991) and Comté cheese variteties (Roudot-Algaron, Kerhoas, Le Bars, Einhorn & Gripon, 1994; Roudot-Algaron, Le Bars, Einhorn, Adda & Gripon, 1993; Roudot-Algaron, Le Bars, Kerhoas, Einhorn & Gripon, 1994). The real impact of small peptides on the taste of cheese, despite some claims (Aston & Creamer; Cliffe, Mark & Mulholland, 1993; McGugan et al., Roudot-Algaron, Kerhoas et al., 1994) has not been clearly demonstrated, though a lot of work on synthetic peptides, reviewed by Roudot-Algaron (1996), tends to show that some small synthetic peptides may be tasty or bland according to their structure. Conversely, many larger and hydrophobic peptides, frequently responsible for a bitter taste or "defect", were identified in milk products and reviewed by Lemieux and Simard (1992). Salles, Septier et al. (1995) showed that free amino acids and salts are probably

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involved in the taste of Comté cheese. To our knowledge, no work has been done on goat cheese.

The aim of this study was to evaluate the taste of peptide fractions obtained by size-exclusion chromatography of the ultrafiltered water-soluble extract of two goat cheeses and to relate the chemical composition of these fractions to sensory data in order to evaluate the importance of each identified compound for the taste of each fraction.

2. Materials and methods

2.1. Materials

2.1.1. Cheese

Two goat cheeses were analysed: a traditional local semi-dry goat cheese, called "Bouton de culotte[®]" (Lycée agricole, Davayé, Macon, France), and a commercial semi-dry goat cheese called "Crottin de Chavignol[®]" (France). In the text, they are respectively abbreviated as follows: BC (Bouton de culotte), CC (Crottin de Chavignol).

2.1.2. Chemicals

The pure water used in all the experiments was obtained from a MilliQ system[®] (Millipore, Bedford, MA). All the reagents used were analytical grade.

2.2. Methods

2.2.1. Extraction and purification of the water-soluble fraction

The extraction of the water-soluble fraction was done as described by Salles, Septier et al. (1995) with some modifications. Rind-less cheeses (100 g) were homogenised with 600 ml of pure water. The mixture was stirred at 40°C for 1 h and then centrifuged at 2000 g at 4°C for 30 min. The pellet obtained was reextracted and both supernatants were pooled, centrifuged at 14,000 gat 4°C for 30 min, freeze-dried, and frozen until use. Freeze-dried material was extracted by 50 ml of pure water. After centrifugation at 105,000 g at 4°C for 30 min, the soluble material was ultrafiltered by frontal filtration in a cylindrical cell made of glass and stainless steel (V = 400 ml, d = 76 mm; Millipore) while stirred at 4°C, using a cellulose ester membrane with a molecular weight cut-off of 1000 Da (Millipore). A pressure of 5 bar was applied with nitrogen during filtration. The ultrafiltered water-soluble extract (UWSE) was then stored at -20° C until use.

UWSE (2 ml) was submitted to gel filtration chromatography on Toyopearl HW-40S (Tosoh Corp., Tokyo, Japan). The sample was applied to a Superformance column (2.6×60 cm; Merck, Darmstadt, Germany) fitted with a manual injection valve (loop: V=2 ml). Elution was realised at room temperature (20° C) with pure water at a flow rate of 2 ml min⁻¹. Eluted compounds were detected at 214 nm using a L-4000 UV detector (Merck), 3.5 ml fractions being collected. All the fractions corresponding to one UV-detected peak were combined and analysed.

2.2.2. Analytical methods

The amino acids in the crude extract and in each fraction were analysed before and after hydrolysis to determine amino acid and peptide composition. Samples were dried under vacuum in a Speed Vac concentrator (Savant, Hiksville, NY) and, when necessary, hydrolysed in 6 M HCl at 150°C under vacuum for 1h. Amino acid composition was determined on a LC5000 amino acid analyser (Biotronik, Maintal, Germany). The results reported in this paper represent only one analysis but some samples were analysed in duplicate, from which the coefficient of variation was estimated to be lower than 5%.

Sodium, potassium and calcium ions were determined by flame ionisation spectrophotometry (Eppendorf, Hamburg, Germany). Lactate, inorganic phosphorus and chloride were estimated by diagnostic kits (Sigma, St Quentin Fallavier, France).

The dry extracts were obtained by evaporating the eluent contained in each fraction using a centrifugal evaporator RC 10-10 (Jouan, St Herblain, France) until constant weight ($P=0.5 \ 10^{-3}$ bar; $T=60^{\circ}$ C). Free fatty acids were extracted and analysed as described by Le Quéré, Septier, Demaiziéres and Salles (1996). The recoveries were determined by comparison of the concentration of components before and after each purification step.

2.2.3. Sensory evaluation

2.2.3.1. Preparation of the samples for sensory evaluation. UWSE (5 ml) was chromatographed with pure water, as described above. The separation was repeated twice and 10 fractions were collected on each run, according to the absorbance profiles (Fig. 1). The equivalent fractions were pooled.

2.2.3.2. Panel training. Sensory analyses were conducted with an internal panel of 10 members. The panellists were trained to recognise basic tastes (bitterness, saltiness, acidity, sweetness and umami), astringency, mixtures of tastes and different aromas during two sessions of 45 min.

2.2.3.3. Sensory evaluation session. The experiment, made in duplicate with two different extractions, was conducted in an air-conditioned room $(20 \pm 1^{\circ}C)$ under red light in separate booths. Portions (2 ml) of each fraction (1-10) were presented to the panellists. Fractions were presented individually and the presentation

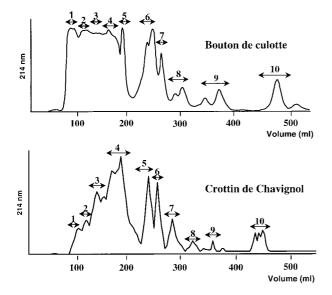


Fig. 1. Gel filtration chromatogram of the ultrafiltered water-soluble extract of goat cheeses (Toyopearl HW-40S, eluent: H₂O).

order followed Williams latin squares (MacFie, Bratchell, Greenhoff & Vallis, 1989) balanced for order and first-order carry-over effects. Firstly, the panellists closed their nostrils to suppress the olfactive sensations and put the sample on their tongue to evaluate the total taste intensity on a 6-point scale (from 0 to 5). Mark 5 corresponds to the highest intensity, equivalent to the intensity of a reference solution containing 0.8% NaCl dissolved in lightly mineralised water (Evian, France). Secondly, with open nostrils, the panellists evaluated the total flavour intensity. Flavour and taste were described with free vocabulary by each panellist after evaluation of total intensity rating, but only the most frequently quoted terms (≥ 4) were reported. For the fractions characterised by very low total taste and aroma intensities, each panellist sometimes gave several different descriptors; so they were grouped under the term "undetermined".

2.2.3.4. Statistical treatment. The data were processed with the SAS (1989) statistical package. An analysis of variance was conducted on each sensory variable using the following model: total taste (flavour) intensity= fraction + panellist + replicate + fraction \times panellist + fraction \times replicate + panellist \times replicate, with panellist and replicate as random variables as suggested by Schlich (1997). Comparison of means were then performed using least significant different (LSD) test.

3. Results and discussion

Extraction of the water-soluble fraction of cheese with pure water as solvent is currently used (Engels & Visser, 1994; Mojarro-Guerra, Amado, Arrigoni & Solms, 1991; Warmke, Belitz & Grosch, 1996), leading to an

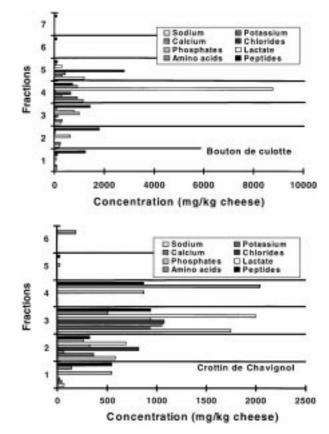


Fig. 2. Concentration of amino acids, peptides, mineral salt and lactic acid in the fractions obtained after chromatography on Toyopearl HW-40S of ultrafiltered water-soluble extract of goat cheeses.

edible extract with good recovery of nitrogenous compounds (Kuchroo & Fox, 1982). An alternative for the extraction of the water-soluble fraction is the use of a hydraulic press. However, although it gave satisfactory results in the case of hard cheese, such as Emmental (Salvat-Brunaud et al., 1995), its use failed for the goat cheese we studied (BC): the application of a pressure of 40 bar for 3 h to 1 kg grated goat cheese mixed with sand did not permit the extraction of any aqueous layer; only oil cheese was obtained.

The choice of techniques for fractionating the watersoluble extract of cheese is limited when the fractions

Table 1

Composition of the ultrafiltered water-soluble extracts of goat cheeses: Bouton de culotte (BC) and Crottin de Chavignol (CC)

Cheese (mg/g dry matter)	BC	CC	
Dry matter (%)	55.3	53.0	
Sodium	3.9	3.8	
Potassium	2.7	2.5	
Calcium	2.1	1.8	
Chloride	8.3	9.3	
Phosphorus	0.9	1.1	
Lactate	0.15	0.03	
Amino acids	2.6	7.4	
Peptides	10.7	5.6	

10

Table 2

	BC cheese		CC cheese		
	Overall taste mean scores ^a	Taste descriptors ^b	Overall taste mean scores ^a	Taste descriptors ^b	
1	1.1c	Bitter	1.0b	Bitter	
2	1.2c	Bitter	3.7a	Acid, salty, umam	
3	2.5b	Acid	3.1a	Salty, umami	
4	3.8a	Salty, acid, umami	1.2b	Bitter	
5	3.5a	Salty, umami	0.7b	Bitter	
6	1.5c	Bitter	0.3b	(Undetermined)	
7	1.4c	Bitter	1.2b	Bitter	
8	1.1c	Bitter	0.6b	Bitter	
9	1.1c	Bitter	0.6b	(Undetermined)	

0.3b

Overall mean taste scores and descr	riptors for each fraction obtained h	ov chromatography on To	yopearl HW-40S (cheeses BC and CC)

^a The means within a column assigned with the same letter are not significantly different (P < 0.05, LSD).

^b Only the descriptors quoted more than four times are reported (the most frequently quoted descriptor is in bold).

Bitter

obtained are to be tasted by a panel. So, we used only ultrafiltation using membranes with a molecular weight cut-off of 1000 Da to isolate low molecular weight material and gel filtration with pure water as eluent without any modifier, which generally may have a taste and be difficult to remove completely.

1.6c

On ultrafiltration, we did not observe any substantial loss of mineral or organic compounds apart for phosphate (14%) and peptides (9%), probably due to adsorption on the membrane (measured with BC cheese).

The composition of the ultrafiltered water-soluble extract of the cheeses is given in Table 1. It should be noticed, in particular, that the peptides/free amino acids ratio was around 1 for CC cheese and 5 for BC cheese. Small peptides are relatively abundant in these kinds of cheese, in contrast to Comté cheese which contains a high level of free amino acids and very few low molecular weight peptides (Salles, Septier et al., 1995).

For the most abundant free fatty acids (hexanoic, octanoic and decanoic acids), which were also identified and quantified in the water-soluble extract (Le Quéré et al., 1996), we observed a recovery of only ca. 1% before ultrafiltration, due to losses during the freeze-drying step. However, in goat cheese, they do not seem to be

directly responsible for any taste (Salles, Dalmas et al., 1995).

(Undetermined)

The elution profile obtained on gel filtration of UWSE of cheese BC and CC on Toyopearl HW-40S is given in Fig. 1, showing large peaks in the first part of the chromatogram with smaller peaks in the second part.

Ten fractions were constituted according to the absorbance profile. Almost all the organic material was recovered, but the recovery of minerals, particularly calcium, was poor due to an interaction with the gel matrix.

Sensory evaluation and physicochemical analysis were performed on each gel filtration fraction in order to detect the tasty fractions. The results obtained for the mean overall taste scores of gel filtration fractions for BC and CC cheese are reported in Table 2. Free amino acids, peptides, mineral salts and lactic acids, prominent in the UWSE, were quantified in each fraction (Fig. 2). Fractions 1, 2 and 6-10 of BC cheese and fractions 1 and 4-10 for CC cheese had a low total intensity which can be considered to be the base level.

For BC cheese, acidity was observed in fractions 3 and 4 (Table 2) and may be directly related to the presence of lactic acid (Fig. 2) which was present in these

Table 3

Concentration of the non-nitrogenous compounds identified in each tasted fraction obtained by gel filtration (Cheese BC)^a

Parameters (mg l ⁻¹)	Fracti	ions			Taste threshold value in water(mg l^{-1}) ^b				
	1	2	3	4	5	6	7		
Na ⁺ (NaCl)	40	97	212	487	725	5	5	$0.45 - 1.4 \times 10^{3}$	(NaCl)
K ⁺ (KCl)	42	112	262	400	200	_	_	1.3×10^{3}	(KCl)
Ca^{2+} (CaCl ₂)	5	10	45	125	275	_	_	$0.85 - 1.1 \times 10^{3}$	(CaCl ₂)
Cl-	_	_	50	114	683	_	_		
$P(H_3PO_4)$	14	120	338	8	-	-	_		
Lactic acid	_	_	277	1505	70	_	_	38-144	

^a Means of three measures: the coefficients of variation are lower than 5%.

Table 4	
Concentration of free amino acids identified in each fraction obtained by gel filtration and comparison with their threshold value (Cheese B	C)

Amino acids (mg/l)	Fractions							Threshold value (mg/l) and taste free amino acids in water ^b		
(Vt) ^a	1 (35 ml)	2 (32 ml)	3 (18 ml)	4 (35 ml)	5 (25 ml)	6 (49 ml)	7 (25 ml)	nee annio acids in water		
Asp(Na)	3	27	74	3	_	_	_	1000	umami	
Thr	12	2	_	56	-	-	-	2600	sweet	
Ser	15	4	_	86	_	_	_	1500	sweet	
Glu(Na)	21	9	146	21	-	-	_	300	umami	
Gln	6	-	_	69	-	-	_	nd	flat	
Pro	_	_	_	91	_	_	_	3000	sweet, bitter	
Gly	5	-	_	12	-	-	_	1300	sweet	
Ala	10	_	_	111	_	_	_	600	sweet	
Cit	-	-	_	18	-	-	_	nd		
Val	16	-	_	89	-	-	_	400	bitter	
Cys	_	4	_	_	-	-	_	nd		
Met	7	-	_	16	28	-	-	300	bitter	
Ile	9	_	_	60	_	_	_	900	bitter	
Leu	11	-	_	144	14	6	10	1900	bitter	
Tyr	-	-	_	-	-	20	70	nd	bitter	
Phe	_	_	_	_	90	90	_	900	bitter	
Gaba	_	_	_	73	_	_	_	40	acid ^c	
Orn	_	-	—	3	_	_	_	nd		
Lys	12	_	_	70	_	_	_	500	sweet, bitter	
his	_	_	_	26	_	_	_	200	bitter	
Arg	3	-	-	54	-	-	-	500	bitter	
Total	130	46	220	1002	132	116	80			

^a Vt = Total volume of the fractions obtained after chromatography of 2 ml UWSE fraction.

^b Kato, Rhue and Nishimura (1989).

^c Petro-Turza and Teleky-Vamossy (1989).

fractions at a higher concentration than its taste threshold. For CC cheese, the relation between acidity in fraction 2 and the presence of lactic acid was more difficult to assess because it was present at much lower concentration. As the majority of volatile acids were eliminated during freeze-drying, lactic acid was the main organic acid present in the fractions. In Swiss cheese, Biede and Hammond (1979) found no correlation between lactic acid and acid taste but the concentration of lactate may be very different according to the type of cheese and its perception may be influenced by other compounds (Breslin, 1996; Stampanoni & Noble, 1991).

Fractions 4 and 5 for BC and 2 and 3 for CC were described as salty, with a relatively high intensity. These fractions contained most of the mineral salts, with a concentration of sodium chloride higher than its threshold value, as shown in Table 3 for BC cheese (not shown for CC cheese), while the concentrations of potassium and calcium chloride that may contribute to bitterness (McSweeney, 1997) are lower than their threshold values.

Some panellists reported a umami taste for fractions 4 and 5 of BC and for fractions 2 and 3 of CC. However, in these fractions, the molecules usually responsible for this taste, monosodium glutamate and aspartate, were at a much lower concentration than their threshold value for the two cheeses (Table 4, shown only for BC). As no molecule likely to have a umami taste has been identified in these fractions, the more plausible explanation is a possible confusion of panellists who were not trained enough to recognise this particular taste.

We observed that fractions 1 and 2, which contained mainly peptides for BC cheese, were almost tasteless. The peptides contained in these two first fractions, some of which have been isolated and identified (Sommerer, Salles, Promé, Promé & Le Quéré, 1998; Sommerer, Septier, Salles & Le Quéré, 1998), constituted only a half of the total peptide content. In the following fractions of BC cheese and in most of the fractions of CC cheese (Fig. 2), the peptides coeluted with other compounds (amino acids, organic acids, salts) which have some taste per se, so, under these conditions, we cannot evaluate their taste impact. For CC cheese, we observed that more peptides eluted later than for BC cheese, indicating that they are probably more hydrophobic and were retained by interactions with the gel matrix.

Concerning the aroma of the fractions, for each cheese, only two fractions exhibited an interesting cheese flavour with a relatively high intensity, described as cheesy or/and brothy (Table 5). In each case, the aroma compounds coeluted with compounds for which

Overall aroma mean scores and descriptors for each fraction obtained by chromatography on Toyopearl HW-40S (Cheeses BC and CC)

Table 5			

Fractions	BC cheese		CC cheese			
	Overall aroma mean scores ^a	Aroma descriptors ^b	Overall aroma mean scores ^a	Aroma descriptors ^b		
1	0.9c	(Undetermined)	0.4c	(Undetermined)		
2	0.6c	(Undetermined)	2.1b	Brothy		
3	1.1c	Milky	3.2a	Cheesy, brothy		
4	2.5c	Cheesy, brothy	0.4c	(Undetermined)		
5	3.3c	Cheesy, brothy	0.4c	(Undetermined)		
6	0.8c	(Undetermined)	0.6c	(Undetermined)		
7	0.7c	(Undetermined)	0.3c	(Undetermined)		
8	0.8c	(Undetermined)	0.4c	(Undetermined)		
9	0.7c	(Undetermined)	0.8c	(Undetermined)		
10	0.9c	(Undetermined)	0.4c	(Undetermined)		

^a The means within a column assigned with the same letter are not significantly different (P < 0.05, LSD).

^b Only the descriptors quoted more than four times are reported (the most frequently quoted descriptor is in bold).

the molecular weights are in the same range, such as lactate and mineral salts and amino acids. In these fractions, we have identified some volatile free fatty acids, in particular hexanoic, octanoic and decanoic acids, well known for their large contribution to the cheesy notes. These aroma compounds present in the crude water-soluble extract were recently described by Le Quéré et al. (1996) for goat cheeses and Engels et al. (1997) for cow cheeses. These volatiles, perceived by the olfactory receptor via retronasal passage during the sensory evaluation sessions of the fractions, are probably soluble enough in water to be extracted from the cheese.

These results suggest that only lactic acid and mineral salts seem to be directly involved in the taste of the water-soluble extract. Small peptides and free amino acids do not seem to have any direct taste activity. However, synergistic effects for taste may occur between peptides, amino acids and mineral salts (Wang, Maga & Bechtel, 1996) and interactions between tastes in mixtures may be important (Breslin, 1996). The taste evaluation of model mixtures of compounds identified and quantified in the water-soluble extract is now under investigation. This evaluation should make the relative sensory impact of each component or family of compounds on the overall taste of the water-soluble extract of goat cheese more precise.

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