Taste Active Compounds in a Goat Cheese Water-Soluble Extract. 1. Development and Sensory Validation of a Model Water-Soluble Extract

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The aim of this study was to evaluate the impact of the components of a goat cheese water-soluble extract (WSE) on its flavor by both physicochemical and sensory techniques with special emphasis on taste. After characterization of the organoleptic properties of the cheese, the WSE was extracted with pure water and submitted to successive tangential ultrafiltrations and nanofiltration. The physicochemical assessment of these fractions led to the constitution of a model mixture (MWSE) compared by sensory evaluation to the crude WSE, using a panel of 16 trained members. The results of both sensory profile and triangular tests indicate no significant difference, therefore proving that the reconstitution of the WSE was correct, thereby showing the sensory neutrality of lipids and peptides smaller than 500 Da, which had not been included in the MWSE. Moreover, the cheese gustatory characteristics are comparable to those of the WSE despite weaker levels of sharpness and astringency in the extract, the respective origins of which are discussed.

Keywords: *Goat cheese; water-soluble extract; peptide; composition; model mixture; taste*

INTRODUCTION

Because of its fundamental role in flavor, the cheese water-soluble extract (WSE) has been studied extensively during the past two decades. It is now well established that the nonvolatile water-soluble fraction contains the majority of the taste compounds (McSweeney, 1997). The nonvolatile WSE is a complex mixture of numerous potentially taste active compounds such as mineral salts, organic acids, sugars, amino acids, nucleotides, biogenic amines, and peptides, but if the individual taste of most of these components is relatively well-known, their respective contribution within the mixture context of the WSE has not been clearly elucidated until now (Fujimura et al., 1995). Moreover, the role of some compounds such as small peptides is not clear, probably because of the difficulty in identifying, quantifying, and purifying them (Sommerer et al., 1998).

The classical method used to study the taste active components of the WSE is to compare the composition of various cheeses or WSE fractions with their taste properties by means of tentative statistical correlation (Biede and Hammond, 1979; Aston and Creamer, 1986). In this approach nonpertinent correlation often reduced the conclusions to hypotheses because of the high number of parameters varying between the samples of cheese or WSE fractions studied. Some authors have tried to understand the global taste of the WSE or to validate their pool of taste active compounds. To reach this goal, they selected molecules on the basis of their presence at a higher concentration than their taste threshold in water or those which seemed to be correlated with taste properties. In all cases, some differences appeared between the real WSE and the synthetic model (Biede and Hammond, 1979; Aston and Creamer, 1986; Warmke et al., 1996). All of these approaches show some recurrent limitations. Trying to compare the concentration of one or several given molecules in the WSE context to their respective individual taste threshold in water implies that the other compounds present in the WSE mixture have little or no effect on the perceived taste of the molecules studied. On this subject, Stevens (1997) clearly showed that increasing the number of compounds in a model mixture permits the detection of natural substances, many components of which could be far below threshold. This concept implies that for both the interpretation of statistical correlations and the constitution of a model mixture, it is necessary to identify and quantify the maximum number of components present in the product studied.

An alternative two-step approach is proposed in this series of two papers. The first step described in this paper consists of the elaboration of a model mixture called a model water-soluble extract (MWSE) which must have the same physicochemical and gustatory properties as the goat cheese WSE. The second step involves performing omission tests (Fujimura et al., 1995; Schlichterle-Cerny and Grosch, 1998) to allow for the evaluation of the relative impact of goat cheese water-soluble compounds on the WSE taste.

To build such a model mixture, it is necessary to purchase all of the components identified in the crude WSE. In contrast with all of the other components of the WSE, peptides, when it is possible to identify them, are not commercially available. Thus, it is necessary to isolate them from the WSE. Until recently, the purifica-

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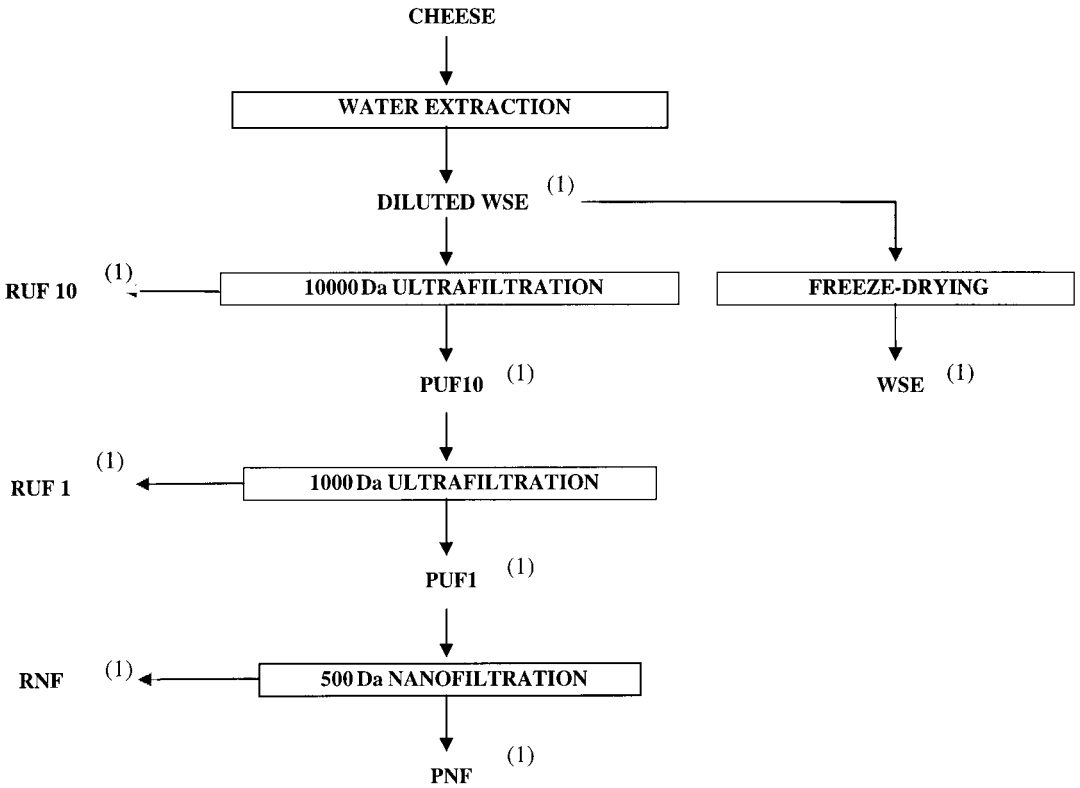

Figure 1. Scheme of the extraction/purification/concentration pathway: (1) Diluted WSE is the diluted water-soluble extract, which was ultrafiltered on a 10 kDa cutoff membrane leading to a retentate (RUF10) and a permeate (PUF10); PUF10 was ultrafiltered through a 1 kDa cutoff membrane to obtain a retentate (RUF1) and permeate (PUF1); finally, PUF1 was nanofiltered to obtain a retentate (RNF) and a permeate (PNF). WSE is the 2.5-fold concentrated diluted WSE.

tion method most commonly used for subsequent sensory analysis of peptides is ultrafiltration followed by chromatography of the extract by gel filtration (Sommerer et al., 1998; Salles et al., 1995). The two main limitations of gel filtration are the poor resolution and the tedious repetitive steps necessary to obtain sufficient material for sensory evaluation. Nanofiltration of the ultrafiltered WSE on a Nanomax membrane allows these limitations to be overcome and has numerous advantages such as rapidity, the larger quantity of the sample treated, and the recovery of a relatively pure peptidic fraction (Sommerer et al., 1998).

The aim of this study was to build a model mixture that imparts the gustatory properties of a goat cheese WSE by means of a complete physicochemical assessment and a sensory validation. Additionally, a comparison between goat cheese and crude WSE is discussed on the basis of their gustatory attributes.

MATERIALS AND METHODS

Cheese. The semihard goat cheese called "Bouton de culotte" was bought at the Lycée Agricole of Davayé (Macon, France). It was ripened for two and a half weeks. All of the cheeses used for sensory evaluation and physicochemical analysis came from the same milk vat. Dry extract and pH value of cheeses were, respectively, 56.9% and 4.6. After purchase, the cheeses were stored at 4 °C for 3 days. Cheese blocks (1.2 kg) were then evaluated for gustatory characteristics, and 16 kg samples were frozen by plunging them into a liquid nitrogen bath at −196 °C after elimination of the rind. The frozen cheeses were stored at −80 °C until further extraction.

Chemicals. The following food grade chemical substances were purchased from commercial suppliers: L-alanine, L-arginine, L-asparagine monohydrate, L-aspartic acid, L-citrulline, L-cysteine, l-γ-aminobutyric acid, L-glutamic acid, L-glutamine, L-glycine, L-histidine, L-isoleucine, L-leucine, l-lysine, L-methionine, L-ornithine monochlorhydrate, L-phenylalanine, L-proline, L-serine, l-threonine, L-tryptophan, L-tyrosine, and L-valine (Rexim, Courbevoie, France); D-lactose monohydrate, lactic acid, sodium chloride, potassium chloride, calcium chloride dihydrate, magnesium chloride hexahydrate, disodium hydrogen phosphate dodecahydrate, monosodium dihydrogen phosphate dihydrate, and sodium hydroxyde (Merck, Darmstadt, Germany); potassium alum (Sigma, St. Quentin Fallavier, France); L-monosodium glutamate and capsaicin (Fluka, St. Quentin Fallavier, France). Pure water was obtained from a Milli-Q system (Millipore, Bedford, MA).

Extraction, Purification, and Concentration Procedure. The main steps of the extraction/purification and concentration pathways are depicted in Figure 1.

Extraction Procedure. Sixteen kilograms of frozen cheese was grated. Cheese was dispersed in pure water (w/w 1:2) and homogenized for 4 min in a 1094 homogenizer (Tecator, Höganäs, Sweden). The suspension was centrifuged at 4000*g* for 30 min at 4 °C. After filtration on gauze, the supernatant was submitted to a 100000*g* ultracentrifugation for 30 min at 4 °C. This procedure yielded 24 kg of diluted crude water-soluble cheese extract (diluted WSE) stored at −80 °C until further use.

Purification Procedure. Fifteen kilograms of the diluted WSE was pooled and successively submitted to two tangential ultrafiltrations (respectively, 10 and 1 kDa cutoff) with polysulfone membrane (Millipore) and tangential nanofiltration (0.5 kDa cutoff) using a Nanomax 50 membrane (Millipore). Ultra- and nanofiltrations were performed in a pilot apparatus (MSP 006239 Prolab, Millipore) as described by Garem et al. (1996). The filtration temperature and the transmembrane pressure were respectively maintained at ∼15 °C and ∼4 Pa. At each step, an aliquot of retentate and permeate was kept for physicochemical measurements. The permeates of 10, 1, and 0.5 kDa nanofiltration were respectively called PUF10, PUF1, and PNF. In the same way the corresponding retentates were called RUF 10, RUF1, and RNF.

Concentration Procedures. Three hundred and sixty milliliters of diluted WSE was concentrated by cryoconcentration according to the method of Langlois et al. (1997) except that the temperature was maintained at −7 °C. The cryoconcentration was performed until a concentration factor of 2.7 was reached. Nine kilograms of the diluted WSE was freeze-dried to concentrate it ∼4 times. Afterward, the concentration factor was adjusted to 2.5 with pure water to obtain the WSE for further gustatory comparison to the MWSE.

Physicochemical Analysis. All measurements were done in triplicate.

pH was measured using an HI 9017 pH meter (Hanna Instruments, Tannerie, France) equipped with a glass electrode.

Dry Extract. A drying oven (106 °C) was used to determine the dry matter content.

Minerals. Sodium, potassium, calcium, magnesium, and iron ions were determined with a Spectra 100 atomic absorption spectrophotometer (Varian, les Ulys, France). Inorganic phosphorus and chloride ions were estimated by diagnostic kits (Sigma).

Lactose and Organic Acids. Lactose, lactate, and acetate were quantified using Boehringer-Mannheim kits (Meylan, France).

Nucleotides were quantified by HPLC with a Synchropack AX anion exchange column (250 × 4.6 mm, Synchrom Inc., Lafayette, IN) and a UV detection at 254 nm as described by Valentin et al. (1997).

Biogenic amines were determined by RP-HPLC of the dabsyl derivatives as described by Bockhardt et al. (1996).

Lipid Content. The total lipid content was determined using the Iatroscan TLC-FID system (Sebedio and Juaneda, 1991) after extraction carried out according to the method of Folch et al. (1957).

Amino Acids. The free and total amino acids were determined on an LC5000 amino acid analyzer (Biotronik, Maintal, Germany), respectively, before and after acid hydrolysis (6 N HCl, 110 °C, 24 h).

Volatile Composition. Volatiles were extracted, identified, and quantified as described by Le Quéré et al. (1996).

Elaboration of the MWSE. MWSE was elaborated in physicochemical accordance with the WSE, using, on the one hand, synthetic chemical compounds and, on the other hand, ultra- and nanofiltration retentates as the source of peptides.

Taking into account the physicochemical composition of each purified water-soluble fraction, the loss at each purification step was determined for each compound by subtraction of the total amount of the compound in question in permeate and retentate to its initial quantity in the fraction to filtrate. These calculated loss rates were used to evaluate concentration factors to apply to each retentate to compensate for losses.

Sensory Analysis. *General Conditions.* The evaluations were conducted under red light in an air-conditioned room (21 ± 1 °C). The room was fitted with 16 separated booths according to ISO 45486. To suppress olfactive sensations, panelists' nostrils were pinched. At each measuring session, products were presented in a monadic way, according to a Williams Latin square design (MacFie et al., 1989) to balance report and position effects. Data were recorded thanks with a FIZZ computerized system version 1.20 (Biosystemes, Couternon, France).

Analysis of the Crude Goat Cheese. Eight experienced assessors were selected for their ability to recognize and quantify flavor characteristics of cheese. During five 1 h sessions, panelists were trained to the evaluation procedure and elaborated by consensus a list of adequate terms to describe the goat cheese gustatory properties. During the three measuring sessions, the panelists were instructed to taste the product after elimination of the rind and to mark the intensity of each attribute relative to the total gustatory intensity of the cheese. Thirteen-centimeter unstructured line scales were used to assess each of the seven attributes, and the scores were converted from 0 to 100, the 100 mark referring to the total gustatory intensity. Three samples of the same goat cheese from different vats were tested to introduce variability. The cheese sample further studied was assessed in triplicate. Between samples, panelists were instructed to rinse their mouths with a piece of apple, some bread crumbs, and mineral water in that order.

Table 1. Nature and Concentration of Reference Solutions for Each Attribute

attribute	reference compd	concn in pure water
sour	lactic acid	1.38 g/L
bitter	L-leucine	8 g/L
salty	sodium chloride	4.5 g/L
umami	L-monosodium glutamate	0.6 g/L
sweet	D-lactose	23.75 g/L
astringent	potassium alum	0.33 g/L
sharp	capsaicin	0.15 mg/L

Validation of the MWSE. The gustatory properties of the MWSE were compared with those of the crude WSE. The panel consisted of 16 students in the Master of Sensory Sciences program at ENSBANA (Dijon, France). Panelists were trained during 18 1-h sessions to recognize and quantify each basic taste, astringency and sharpness in simple solutions and in complex mixtures. For each sensation studied, an appropriate reference solution was chosen. During the training sessions, the concentration of each reference chosen was adjusted in accordance with its intensity in the WSE or its purified fractions. The quantification of each attribute intensity was evaluated in comparison with the perceived intensity of the corresponding reference solution adjusted to a given concentration (Table 1). One measurement session allowed the gustatory profile of both the WSE and MWSE to be obtained to compare them. Four and a half milliliters of both solutions was presented twice to the panelists in a 90 mL coded plastic cup. MWSE was prepared 3 h before the session. Both the MWSE and WSE were kept at 21 ±1 °C for 1 h before their evaluation. Prior to every other task, panelists were asked to taste the reference solutions and to memorize their respective intensity corresponding, by mutual agreement, to 50% of their respective assessment scale. Afterward they had to taste each product to mark the intensity of each attribute on a 13-cm unstructured linear scale anchored from "no sensation" to "strong". A mark at the middle of the scale corresponded for each descriptor to the intensity of the reference solution. Between samples, the assessors rinsed their mouths with bread crumbs and mineral water. During the measurements, they could taste the reference solutions to recall each gustatory sensation and its intensity.

Triangular Test. It was done in duplicate. The samples were prepared in the same way as for the profile evaluation. Four and half milliliters of both solutions was presented to the panelists in 130-mL coded black glasses. For each replication of the test, three coded black glasses were presented to the assessors. The two replications were designed in order that, for one panelist, the repeated sample was different for each replicate. For the panel, the presentation order was designed to assess all of the possible combinations of two products.

Statistical Treatments. The data were processed with the SAS statistical package version 6.11, 4th edition (SAS Institute Inc., Cary, NC). ANOVA analyses were performed at level $\alpha = 0.05$, according to the model attribute = product + subject + product × subject, with subject considered as a random effect. Means were compared with the Newman−Keuls multiple comparison test (Student *t* test).

Triangular test replication results were assessed according to the method of Brockhoff and Schlich (1998), to take into account the panel heterogeneity effect. The Binrisks SAS macro (Schlich, 1993) was used to compute risk tables, for which the percentage of discriminators (p_c) in the population was estimated at 50% as the test was performed by a trained panel.

RESULTS AND DISCUSSION

Profile Evaluation of the Crude Cheese. The aim of this step was to characterize the gustatory properties of the goat cheese sample to compare its main sensory

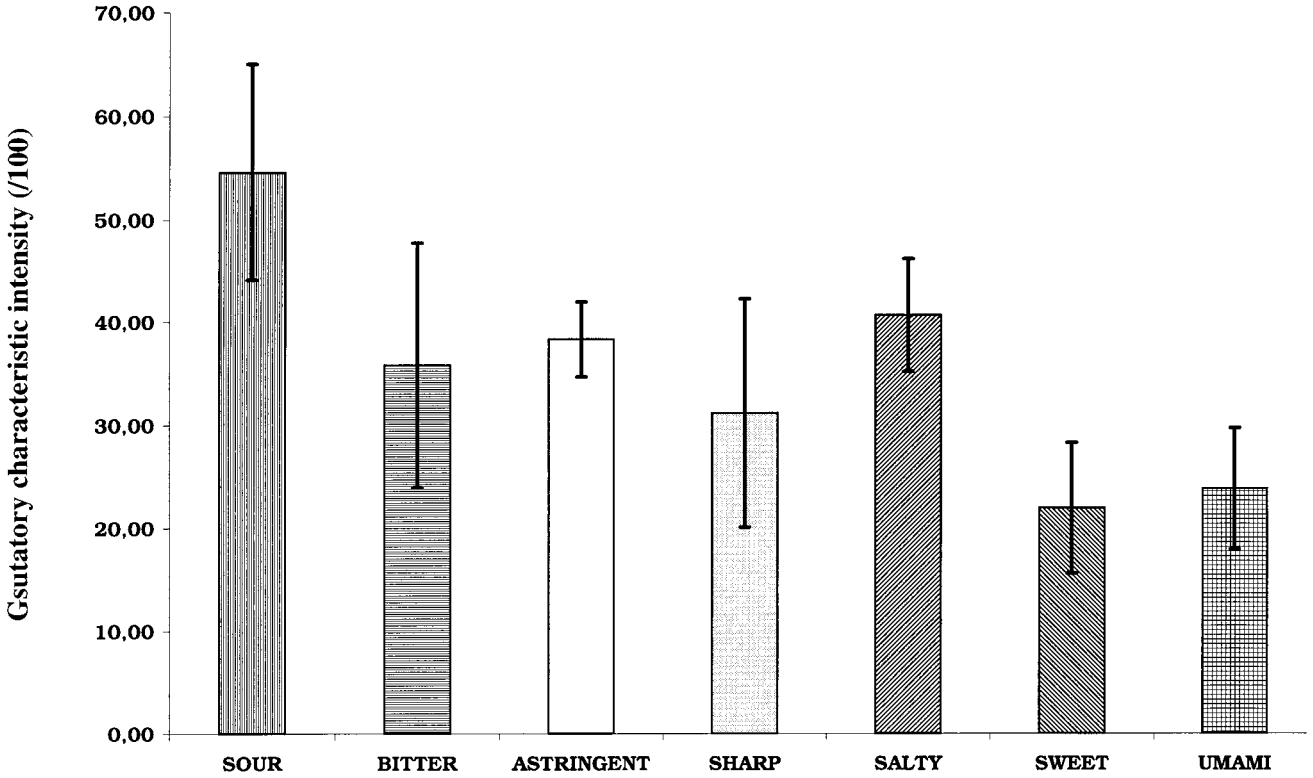

Figure 2. Taste and chemesthesic sensations profile of the goat cheese studied. The bars figure the mean gustatory intensity of the considered attribute for three replications, and standard deviation is drawn at the top of each bar.

attributes to those of its WSE further studied by physicochemical methods. By means of consensus, the panel established a list of seven attributes to describe the goat cheese gustatory properties: sour, bitter, astringent, sharp, salty, sweet, and umami. The crude goat cheese profile (Figure 2) shows three major tastes (sour, salty, and bitter) and two chemesthesic sensations (astringent and sharp). It is noticeable that a sharp note, already described for goat cheeses by Heil and Dumont (1993) and De la Fuente et al. (1993), is present in the goat cheese sample studied. Additionally, the astringent note that is detected had never been mentioned to characterize goat cheese but was mentioned in Cheddar (Harwalkar and Elliott, 1971) and Comte (Roudot-Algaron et al., 1993).

Physicochemical Assessment of the WSE. *Purification Procedure.* Most of the compounds responsible for taste in cheese are extracted directly by pure water and are found in the 1 kDa ultrafiltration permeate and, even, for some authors, in the 0.5 kDa one (McSweeney, 1997). Admitting without any a priori basis that peptides could be involved in the WSE taste, it was interesting to isolate peptides of different molecular weights to further evaluate their contribution to WSE gustatory properties. For that reason, the diluted WSE was successively filtered through 10, 1, and 0.5 kDa cutoff membranes. As the compositions of the WSE and PUF10 given by Table 2 attest, the 10 kDa ultrafiltration step permitted the elimination of nearly two-thirds of the lipids that had not been discarded during the previous ultracentrifugation. The nanofiltration led to a retention of ~80% of the PUF1 peptides and to a preferential retention of the phosphates compared to the other ions, a phenomenon previously observed by Sommerer et al. (1998). Moreover, nearly 60% of the lactose present in the PUF1 was recovered in the nanofiltration retentate, which is in accordance with the suggestion of Guu and Zall (1992) to use nanofiltration to concentrate lactose. As the goal of those purification steps was to obtain representative peptidic fractions of the WSE for sensory validation, it proved to be necessary to use each class of peptide fractions at its WSE concentration. As Table 2 attests, each filtration step led to some losses due to retention in the void volume of the pilot apparatus and to interactions with the membrane. This was taken into account by correcting the peptide concentration of each filtration step. The relative quantities of each class of peptides are given in Table 3.

Composition of the WSE. The results of the physicochemical measurements given in Table 2 show that the WSE contains mainly mineral salts (39.7%), lactose (30.6%), peptides and proteins (17.5%), and lactic acid and lactate (8.5%) and a weak quantity of amino acids and lipids. In constrast, nucleotides (UMP and IMP), iron, and acetic acid were found at the trace level, and no biogenic amines could be detected. In comparison with the sample of the goat cheese studied by Sommerer et al. (1998), the weaker pH value (4.6 versus 5.1) and the higher lactic acid/lactate concentration seemed to indicate a less matured cheese, which is supported by its relatively high lactose content. However, the rather high level of lactose did not bring any unbalanced sweet taste in the crude cheese as Figure 2 attests.

Assessment of the WSE and Its Purified Fractions. The percentage of the dry extract that has been quantified (identification rates) is given for each fraction in Table 2. Most compounds present in each fraction are quantified even for RUF10 for which ~90% of components were identified. Thus, we can conclude an assessment validation of each fraction.

Constitution of the MWSE. These physicochemical assessments allowed the constitution of the MWSE in which all possible components are likely to be present at the same concentration as in the crude WSE. The

Table 2. WSE and Purified Fractions Composition (Grams per Kilogram of Cheese)[a]

compounds	WSE[b]	PUF10[b]	RUF10[b]	PUF1[b]	RUF1[b]	PNF[b]	RNF[b]
Na	3.69 (±0.06)	3.17 (±0.06)	0.26 (±0.06)	2.86 (±0.09)	0.21 (±0.01)	2.08 (±0.01)	0.36 (±0.01))
K	1.81 (±0.06)	1.47 (±0.14)	0.08 (±0.01)	1.19 (±0.10)	0.08 (±0.00)	0.85 (±0.02)	0.14 (±0.01)
Ca	1.23 (±0.01)	0.99 (±0.00)	0.13 (±0.01)	0.89 (±0.01)	0.08 (±0.00)	0.56 (±0.01)	0.17 (±0.00)
Mg	0.14 (±0.00)	0.13 (±0.00)	0.02 (±0.00)	0.11 (±0.00)	0.01 (±0.00)	0.07 (±0.00)	0.02 (±0.00)
Cl	7.89 (±0.29)	5.89 (±0.25)	0.49 (±0.01)	5.37 (±0.18)	0.39 (±0.01)	4.41 (±0.01)	0.60 (±0.00)
phosphates	3.34 (±0.51)	2.82 (±0.37)	0.30 (±0.05)	2.15 (±0.34)	0.18 (±0.03)	0.90 (±0.29)	0.75 (±0.05)
total minerals[c]	18.1 (±0.94)	14.48 (±0.82)	1.27 (±0.08)	12.57 (±0.71)	0.94 (±0.05)	8.87 (±0.45)	2.04 (±0.08)
total lipids	0.77 (±0.3)	0.29 (±0.11)	0.23 (±0.09)	0.23 (±0.09)	0.02 (±0.01)	0.16 (±0.06)	0.04 (±0.02)
lactate	3.86 (±0.23)	2.87 (±0.20)	0.26 (±0.03)	2.56 (±0.24)	0.21 (±0.01)	1.81 (±0.08)	0.44 (±0.03)
lactose	13.95 (±0.15)	11.04 (±0.05)	1.74 (±0.00)	8.81 (±0.04)	0.99 (±0.00)	0.50 (±0.07)	5.00 (±0.00)
amino acids	0.67 (±0.06)	0.22 (±0.03)	0.07 (±0.00)	0.39 (±0.00)	0.01 (±0.00)	0.10 (±0.02)	0.13 (±0.02)
peptides	8.03 (±0.74)	2.03 (±0.08)	5.46 (±0.11)	0.94 (±0.22)	0.36 (±0.02)	0.22 (±0.03)	0.76 (±0.25)
total quantified[c]	45.38 (±2.28)	30.93 (±1.30)	9.04 (±0.31)	25.88 (±1.33)	2.54 (±0.09)	11.67 (±0.63)	8.41 (±0.40)
dry extract	45.59 (±3.08)	31.68 (±2.67)	10.19 (±0.21)	27.54 (±1.04)	2.8 (±0.75)	12.15 (±0.57)	8.87 (±0.18)
identification rate[d]	95.5% (±6.75%)	97.6% (±8.64%)	88.7 (±2.68%)	94.0 (±4.31%)	90.6 (±26.96%)	96.1 (±5.28%)	94.9 (±3.6%)

[a] For each quantified compound the mean concentration of three replicates is expressed in grams per kilogram of crude cheese. Confidence intervals at 5% are indicated within parentheses. [b] WSE is the crude water-soluble extract, which was ultrafiltered on a 10 kDa cutoff membrane leading to a retentate (RUF10) and a permeate (PUF10). PUF10 was ultrafiltered through a 1 kDa cutoff membrane to obtain a retentate (RUF1) and permeate (PUF1). Finally, PUF1 was nanofiltered to obtain a retentate (RNF) and a permeate (PNF) [c] Total minerals and total quantified are, respectively, the total mineral amount and the sum of all compounds identified and quantified in WSE or purified fractions. [d] Identification rate, expressed in percent, indicates the percentage of the dry extract that had been quantified for each fraction. Confidence intervals at 5% are indicated within parentheses.

Table 3. Peptide Profile of the Goat Cheese WSE

	peptide mol wt				
	<500 Da	500−1000 Da	1000−10000 Da	>10000 Da + proteins	total WSE
quantity[a] (g/kg of cheese)	0.32	1.10	0.44	6.18	8.03
percentage	4.00	13.75	5.5	76.75	

[a] Corrected values taking into account the loss due to each purification step.

composition of the MWSE is given in Table 4. The quantity of each class of peptides brought to the mixture had to be in accordance with the concentration indicated in Table 3, which subsequently led us to adjust the quantity of each retentate added to the MWSE.

Moreover, it was necessary to concentrate 2.5-fold the diluted WSE, to compensate for the initial dilution of the cheese due to the extraction procedure and to obtain a WSE representative of the water-soluble fraction actually present in the cheese. The amount of water-soluble molecules in 1 kg of water was regarded as equivalent to the amount of the water-soluble molecules in 1 kg of cheese. Two methods of concentration were compared: cryoconcentration (Langlois et al., 1997) and freeze-drying (Salles et al., 1995). As indicated in Table 5, cryoconcentration leads to a loss of ~30% of the water-soluble compounds, due to trapping of nonvolatile and volatile compounds in the ice, which was also observed by Langlois et al. (1997). In contrast, as the freeze-drying recovery yield of water-soluble compounds was near 100%, this technique was chosen. However, it was necessary to be sure to limit the loss of volatile compounds during this concentration step because chemesthesic sensations such as sharp note might have a volatile origin (Woo et al., 1984; De la Fuente et al., 1993; Heil and Dumont, 1993). For that reason, freeze-drying was stopped when the solution was concentrated 4 times. After dichloromethane extraction of both diluted and freeze-dried WSE, the total amount of volatile compounds was measured by GC: 65% of the volatile fraction was recovered with this concentration method.

However, there are some composition differences between the crude WSE obtained by freeze-drying and the MWSE. First, the small peptides present in the nanofiltration permeate could not be included in the MWSE, hypothesizing that their sensory contribution would be negligible according to Sommerer et al. (1998). Second, no lipids were added apart from those brought by the retentates, as it is admitted that they have only a minor gustatory impact (McSweeney, 1997). Third, the volatile compound contents of the MWSE and WSE might be different: on one the hand, only part of the volatile was added with the retentate for the constitution the MWSE and, on the other hand, part of the volatile was lost during the freeze-drying of the diluted WSE. If some gustatory differences had appeared between the WSE and MWSE, it would have been necessary to adjust as exactly as possible the composition of the MWSE in terms of volatiles.

Taking into account the retentate composition, the quantities of other chemical species to be added in

Table 4. MWSE Composition

	Global Composition (g/L)																				
	mineral salts							lactic acid/lactate			peptides										
	NaCl	KCl	$CaCl_2$	$MgCl_2$	Na_2HPO_4	NaH_2PO_4	lactose	lactic acid	NaOH	amino acids	RUF10	RUF1	RNF								
MWSE	5.40	2.82	2.15	0.33	1.30	1.10	3.74	2.79	0.50	0.32	6.12	0.44	1.10								
	Amino Acid Composition (mg/L)																				
	Asp	Thr	Ser	Asn	Glu	Gln	Pro	Gly	Ala	Cyt	Val	Cys	Met	Ile	Leu	Tyr	Phe	GABA	His	Orn	Lys	Arg
MWSE	4.9	5.3	14.6	11.3	10.4	23.1	28.0	2.3	17.0	0.6	14.2	3.1	2.7	6.7	50.7	19.8	31.5	27.8	0.0	6.2	16.4	21.9

Table 5. Respective Yield of Water-Soluble Molecules and Volatile Recovery of both Cryoconcentration and Freeze-Drying of Goat Cheese WSE

concn method	extract	concn factor[a]	recovery yield[b] (%)
cryoconcentration	WSE dry matter	2.7	70
	WSE volatile compounds[c]		nd[d]
freeze-drying	WSE dry matter	4	100
	WSE volatile compounds[c]		65

[a] Volumic concentration factor of the WSE by each of the two methods. [b] Percentage of WSE dry extract recovered in the concentrate. [c] Total quantified volatile compounds after CH_2Cl_2 extraction and quantification according to Le Quéré et al. (1996). [d] nd, not determined.

synthetic form were calculated by difference from the WSE composition. With respect to the pH value, it is noticeable that the resulting sodium level in the MWSE was in excess by 7% compared to the WSE composition. For all other compounds, the composition of the MWSE is in agreement with the WSE physicochemical assessment.

Sensory Validation of the MWSE. For all of the attributes the appropriate *t* test showed no significant difference at $\alpha = 5\%$ between the respective mean gustatory intensities of the WSE and MWSE as reported in Figure 3. Considering the possible existence of other sensory dimensions that could differentiate the two solutions, which could be due, for instance, to differences in volatile content, and to improve the statistical power of the validation, triangular tests were conducted to fully conclude the absence of any difference between the two samples. The correction method for replication in discrimination tests (Brockhoff and Schlich, 1998) revealed that of the 27 subjects who performed the test, only 9 right answers (judges who were able to recognize the different samples) were given. According to Schlich's table (Schlich, 1993) the β risk to wrongly conclude that there is no difference between the two samples is 0.04%, thus meaning that there is no detectable difference be-

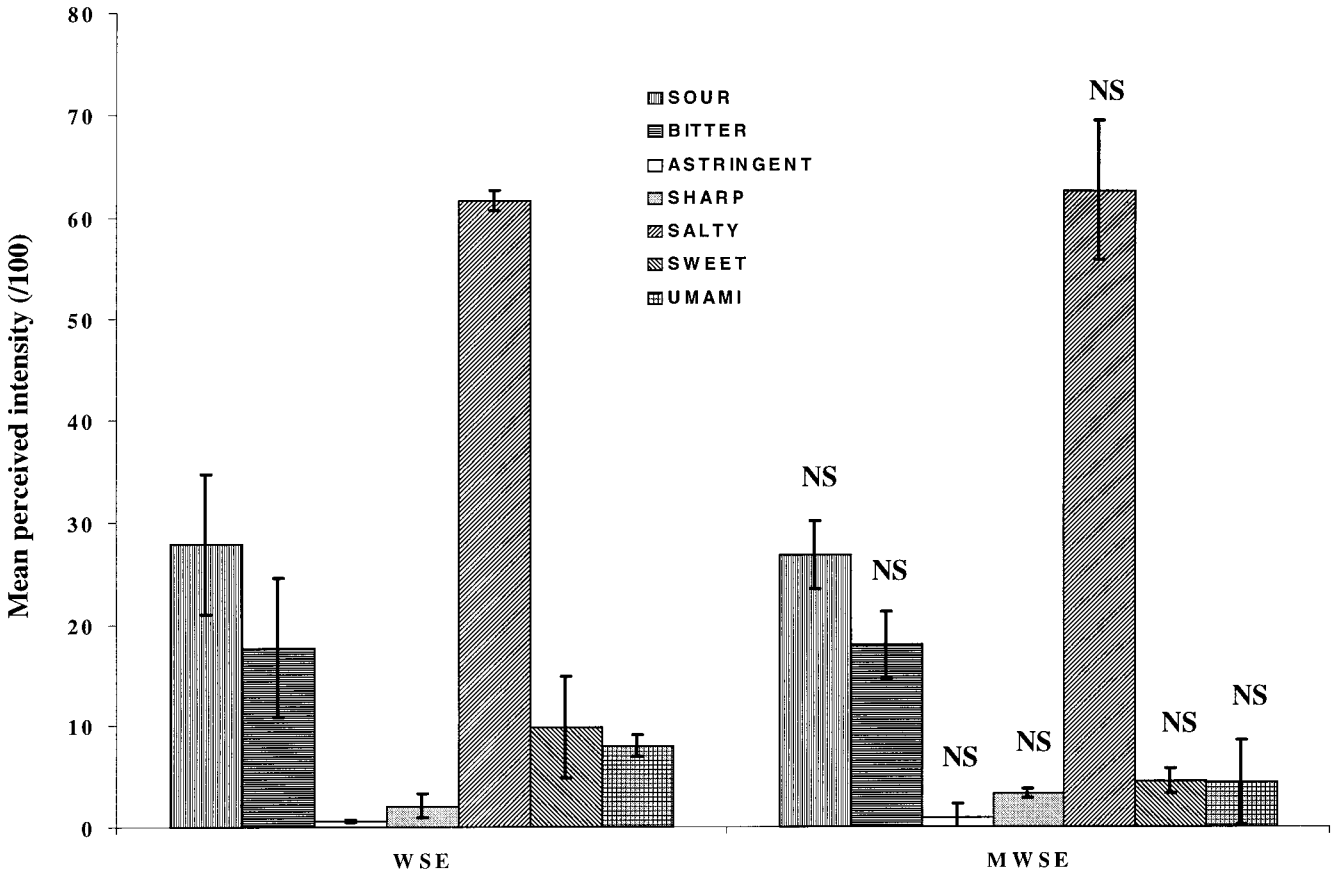

Figure 3. Gustatory profile of both WSE and a model mixture elaborated on the basis of WSE physicochemical assessment (MWSE). The bars figure the mean gustatory intensity of the considered attribute for three replications, and standard deviation is drawn at the top of each bar. NS indicates that there was no significant difference between WSE and MWSE for the considered attribute.

tween the WSE and MWSE. Therefore, from the sensory point of view the MWSE could be considered in full gustatory accordance with the WSE.

These results indicate that the absence of the peptides which were quantified in the nanofiltration permeate (molecular weight <500 Da) did not have any perceptible consequences on the sensory properties of the WSE. However, as most of those small peptides are recovered in the 500 Da nanofiltration retentate (Sommerer et al., 1998), it is necessary to perform further omission tests on this fraction to fully conclude as to the gustatory impact of the peptides of a molecular weight <500 Da (see Physicochemical Assessment of the WSE). Moreover, the fact that no lipid was added to the MWSE to adjust their quantity to their WSE level did not have any effect on the gustatory characteristics of the MWSE. The volatile content discrepancy between the two samples did not seem to imply any difference in their gustatory characteristics either. These results are in accordance with those of a previous study done on another sample of the same goat cheese (Salles and Le Quéré, 1998), which showed that both medium-size fatty acids and volatiles did not have any impact on this cheese gustatory properties.

The comparison between the crude cheese gustatory profile (Figure 2) and its WSE shown in Figure 3 leads to two main conclusions. First, the three main taste characteristics of the cheese, which are sour, bitter, and salty, are recovered in its extract. As previously mentioned by numerous authors (Biede and Hammond, 1979; MacGugan et al., 1979; Aston and Creamer, 1986; McSweeney, 1997), the fundamental role of the water-soluble fraction in cheese taste is confirmed. In contrast, the chemesthesic notes, astringent and sharp, which are well represented in the cheese profile, are only slighty perceived in the WSE. Harwalkar and Elliott (1971) isolated an astringent fraction from Cheddar cheese, but the nature of the astringent compounds was not clearly elucidated. Lemieux et al. (1989) studied Harwalkar and Elliott's fraction by size exclusion high-performance liquid chromatography, the astringent fraction eluting as three peaks. This last work suggested that both intact caseins or their large degradation products and peptides ranging from 2 to 18 kDa could be involved in the astringent note. In the case of the goat cheese sample studied, it seems that a great part of this chemesthesic sensation is due more to nonsoluble material, for instance, casein, than to water-soluble extractable compounds, as the weak astringency of the WSE attests. The slight sharp note perceived in the WSE in comparison with the crude cheese could be due either to a non-water-soluble compound contribution or to the loss of small-chain fatty acids, which are supposed to be responsible for sharpness in several cheeses previously studied (Woo et al., 1984; De la Fuente et al., 1993; Heil and Dumont, 1993). It is indeed very likely that the 35% loss of volatiles due to freeze-drying had preferentially affected the short-chain fatty acids because of their higher volatility.

Conclusion. According to the first goal of this study, a MWSE was constructed that was in physicochemical and gustatory accordance with the crude WSE. Peptides <500 Da, as well as lipids, were found to have no impact on both taste and chemesthesic properties of this extract. Nevertheless, the comparison between the crude cheese and its WSE gustatory profile confirmed the fundamental role of water-soluble molecules in the cheese taste and suggested a possible implication of short-chain fatty acids in the sharpness of the cheese as well as an involvement of nonsoluble compounds in its astringency. These last two points will be further investigated.

Relying on this representative MWSE, omission tests have been performed to determine, qualitatively and quantitatively, the relative gustatory impact of each of the water-soluble components in the WSE and consequently in the goat cheese. Those results are presented in the second paper of this study.

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