Sensory and Chemical Analysis of Fractions Obtained by Gel Permeation of Water-Soluble Comté Cheese Extracts

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The pure water extraction of Comté cheese solubles and their chromatographic separation facilitate sensory analysis experiments with the fractions directly collected from the column. Two extracts, one obtained after ultrafiltration, the other after chromatography on Sephadex G25, have been separated on Toyopearl HW-40S. The sensory evaluation of the obtained fractions shows in the two cases that some fractions have interesting flavors. However, physicochemical analysis shows that organoleptic characteristics are linked more to the presence of amino acids and salts than to the presence of peptides. Apart from monosodium glutamate, most of the amino acids are present in the fractions at concentrations lower than their threshold values measured in water. Therefore, the hypothesis of synergistic effects among amino acids or between amino acids and other kinds of compounds, such as salts, can be raised. Moreover, the fractions containing free amino acids are also generally the most aromatic. The nature and the origin of these volatile compounds responsible for these aromas have to be determined.

INTRODUCTION

Proteolysis is one of the most important phenomena in cheese ripening which contributes to its flavor and texture. Degradation of caseins by proteases leads to the formation of peptides and amino acids (Desmazeaud and Gripon, 1977) which can be then enzymatically converted into various volatile compounds important for the aroma (Hemme et al., 1982; Seitz, 1990). Moreover, the amino acids can be present in their carbonyl complex form which can generate cheese flavor components (Kowalewska et al., 1985; Griffith and Hammond, 1989).

The quantities of water-soluble compounds increase during the ripening of cheese. A parallel increase of bitter taste is attributed to the accumulation of bitter-tasting peptides (Petritschek et al., 1972; Lowrie and Lawrence, 1972; Schalinatus and Behnke, 1975). What is more interesting is that the amino acids and low molecular weight peptides present in the water-soluble fraction contribute greatly to the intensity of the cheese flavor (MacGugan et al., 1979; Adda et al., 1982; Furtado, 1984; Aston and Creamer, 1986). In this water-soluble fraction, acidity, bitterness, burned, and brothy−nutty flavor were correlated with the occurrence of small peptides and amino acids, while sweetness was attributed to the interaction of calcium and magnesium ions with some of these compounds (Biede and Hammond, 1979).

Some fundamental studies have shown the tasting qualities of the amino acids (Kato et al., 1989) and low molecular weight peptides (Otagiri et al., 1985; Ishibashi et al., 1987a,b, 1988; Tamura et al., 1989; Seki et al., 1990). These compounds have tentatively been extracted and identified in different types of cheeses: Cheddar (Creamer and Richardson, 1974), Swiss-Mountain cheese (Guigoz and Solms, 1974), Roquefort (Okhrimenko and Chebotarev, 1975, 1976), Gouda (Vis-

ser et al., 1983; Kaminogawa et al., 1986), and, more recently, Blue cheese (De Llano et al., 1991), Vacherin Mont d'Or (Mojarro de Guerra et al., 1991), Comté (Roudot-Algaron et al., 1993, 1994a,b), Camembert (Tsuda et al., 1993) and in other foods such as meat (Fujimaki et al., 1973; Noguchi et al., 1975; Yamasaki and Maekawa, 1978) and beer (Dale and Young, 1989; Dale et al., 1989). Generally speaking, few peptides were identified, but most of them were found to be bitter.

In fact, compounds in water-soluble extracts are very complex and their purification and identification are difficult; moreover, most analytical techniques require non-food-grade solvents or buffers that are difficult to handle, particularly if sensory evaluations of these extracts are planned.

The purpose of this study is to achieve chromatographic separations of hydrosoluble extracts obtained from Comté cheese. Water used as eluent allows the tasting of the fractions directly obtained from chromatography to evaluate their relative sensory impact and to try linking it to their chemical composition.

MATERIALS AND METHODS

Extracts **E1** and **E2** and their corresponding purifications were achieved respectively in two different laboratories using two different extraction methods.

Preparation of Extracts. A water-soluble extract **E1** was obtained by homogenizing 100 g of frozen 12-month-old Comté cheese with 300 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 extraction was identically repeated with the pellet. Both supernatants were pooled, adjusted to pH 4.6 by the addition of 2 N HCl, centrifuged at 2000*g* at 4 °C for 30 min, freeze-dried, and frozen at −20 °C until use.

Freeze-dried material (approximately 12 g) was extracted by 40 mL of pure water. After centrifugation at 40000*g* at 4 °C for 30 min, the soluble material was ultrafiltered in a cell ($V = 50$ mL, $d = 43$ mm) (Amicon, Danvers, MA) while stirred

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at 4 °C, using a cellulose ester membrane with a molecular weight cutoff of 3000 (Filtron Technology Corp., Northborough, MA). A pressure of 3 bars was applied with nitrogen during the filtration (approximatively 12 h). The extract was then frozen and stored at −20 °C until use.

Extract **E2** was obtained by pooling fractions of a water-soluble extract of Comté cheese obtained after gel permeation on a Sephadex G-25 column showing interesting organoleptic characteristics. The water-soluble extract was prepared as follows: 160 g of grated cheese was ground with 1100 mL of pure water. After centrifugation, the supernatant was filtered through a 0.45 μm membrane; 150 mL of this fraction was injected on the gel permeation column, and the flavor fractions (11−16) were pooled, freeze-dried, and stored at −20 °C until use. The obtained powder (1.2 g) was dissolved in 150 mL of pure water before gel filtration chromatography.

Gel Filtration Chromatography. **E1** and **E2** fractions were submitted to a gel filtration chromatography on a copolymer of ethylene glycol and methacrylate resin: Toyo-pearl HW-40S (Tosoh Corp., Tokyo, Japan). The molecular weight separation range of this hydrophilic gel is 100−10 000.

Two milliliters of **E1** was applied on a Superformance column (1.6 × 60 cm) (Merck, Darmstadt, Germany) fitted with a manual injection valve. The elution was realized at room temperature (20 °C) with pure water. A constant flow rate of 140 mL h^{-1} was delivered by an IP31 pump (Duramat, Heidelberg, Germany). Eighty fractions of 2.3 mL each were collected using a TDC 80 fraction collector (Gilson Medical Electronics Inc., Middleton, WI). The absorbance of each fraction was measured at 214 nm with a Lambda 15 spectrophotometer (Perkin-Elmer Corp., Norwalk, CT) and analyzed (see below).

Ten milliliters of **E2** was chromatographed on a column (2.6 × 100 cm) at 4 °C with pure water. A constant flow rate of 90 mL h^{-1} was set by a P 500 pump (Pharmacia, Uppsala, Sweden). The absorbance of the column effluent was measured at 206 nm on a 2138 Uvicord detector (LKB, Uppsala, Sweden). Ten fractions of 30 mL were collected with a Gilson Model 201 collector 90 min after injection. The separation was repeated three times, and the corresponding fractions were pooled, freeze-dried, and diluted in 20 mL of pure water for further analysis.

Sensory Evaluation. Two milliliters of **E1** was chromatographed as described above, except that the eluent was lightly mineralized water (S.A. des Eaux Minérales d'Evian, Evian, France). Its mineral composition was (in mg L^{-1}) as follows: calcium, 78.8; magnesium, 24.8; potassium, 1; sodium, 5.7; bicarbonate, 360; sulfate, 11; chloride, 3.3; nitrate, 4.0; and silicium, 14.2; its pH was 7. The separation was repeated three times, and six fractions were collected at each run, according to the absorbance profile (Figure 1). The equivalent fractions were pooled. Two milliliters of each fraction previously mentioned (1−6) and two fractions of 2 mL of lightly mineralized water percolated through the column before injection (7−8) were presented to the panelists in a well-balanced order (MacFie and Bratchell, 1989). Sensory analyses were conducted with a trained panel of 19 members in an air-conditioned room (20 °C). The panel members were selected according to their gustative and olfactive abilities. They were trained to recognize basic tastes (bitterness, saltiness, sourness, sweetness, and umami), astringency, and aromas such as vanilla, cooked milk, mushroom, and metallic in pure solutions and then in mixtures for two different sessions.

First, the panelists pinched their nostrils and put the sample on their tongue to evaluate the total taste intensity between the marks 0 and 5. Mark 5 corresponds to the highest intensity, equivalent to the intensity of a reference solution made with 0.8% sodium chloride dissolved in lightly mineralized water. Second, with open nostrils, the panelists evaluated the total aroma intensity between the marks 0 and 5.

The 10 fractions obtained from **E2** were evaluated by eight trained assessors for taste and aroma (intensity and quality). As previously, the assessors were trained to recognize basic tastes and aromas such as mushroom, butter, nut, ammonia, flower, and cabbage in pure solutions and then in mixtures
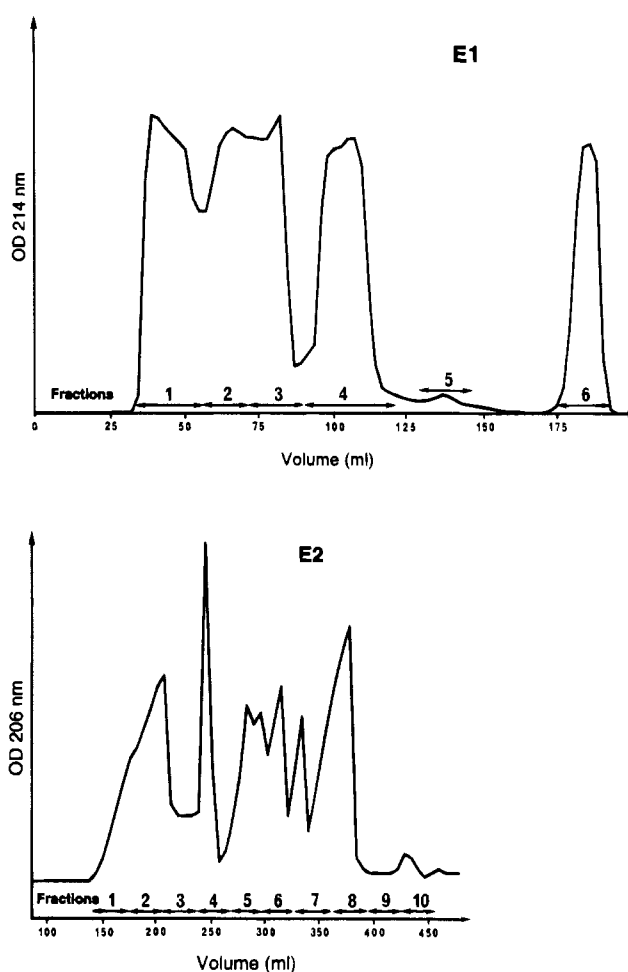
Figure 1. Toyopearl HW-40S chromatography of Comté cheese extracts (a, top) **E1** and (b, bottom) **E2**.

Table 1. Composition (Grams per 100 g of Cheese) of Extracts E1 and E2

|	E1	E2
free amino acids	2.610	2.180
peptides	0.227	0.440
lactic acid	0.180	0.029
inorganic phosphorus	0.082	0.032
chlorides	0.527	0.436
calcium	0.221	0.770
undefined	1.653	0.228
total	5.500	4.115

for two different sessions. One milliliter of each fraction was given to each panelist for its evaluation. The intensity of each basic taste (sour, bitter, sweet, salty, umami) was noted from 0 to 5 as for total flavor.

Flavor and taste were described with free vocabulary by each panel after evaluation of total intensity, but only the most frequently cited terms were selected as descriptors.

Analytical Methods. *Amino Acid Analysis.* Each fraction and crude extract was analyzed before and after hydrolysis for the determination of amino acid and peptide composition. Samples were dried under vacuum in a Speed Vac concentrator (Savant, Hicksville, NY) and, when necessary, hydrolyzed in 6 M HCl at 150 °C under vacuum for 1 h. Amino acid composition was determined on an LC5000 amino acid analyzer (Biotronik, Maintal, Germany).

Analysis of Ions. Sodium, potassium, and calcium ions were determined by flame ionization 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 with a centrifuge evaporator RC 10-

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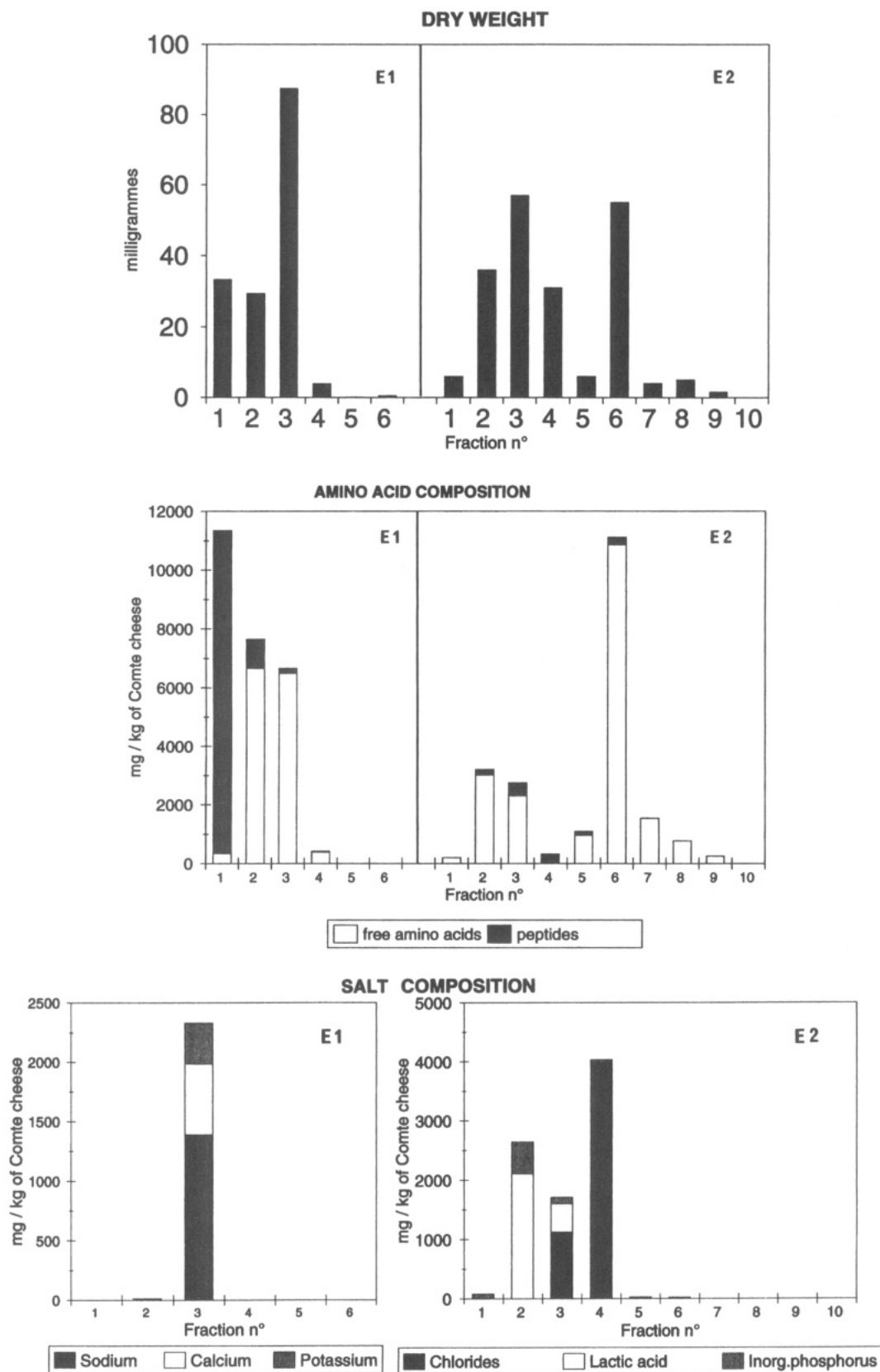
Figure 2. Dry weight, amino acid, and salt compositions of the fractions obtained after chromatography on Toyopearl HW-40S of extracts **E1** and **E2**.

10 (Jouan, St. Herblain, France) until a constant weight ($P = 0.5 \times 10^{-3}$ bar; $T = 60$ °C) was obtained.

The pure water used in all of the experiments was obtained from a Milli-Q system (Millipore, Bedford, MA).

Statistical Analysis. All of the statistical treatments of sensory evaluation data were monitored with SAS statistical software (SAS Institute, 1989) and programs developed in our

laboratory. The graphic representations (Figures 3−6) of the mean values and the confidence intervals for both total taste and total aroma of each fraction were obtained with the Charter program (P. Schlich, INRA-LRSA, Dijon, France). The analyses of variance were conducted with the GLM procedure of SAS/STAT (1989), and the LSmean test was used to compare the means obtained for the fractions of each extract.

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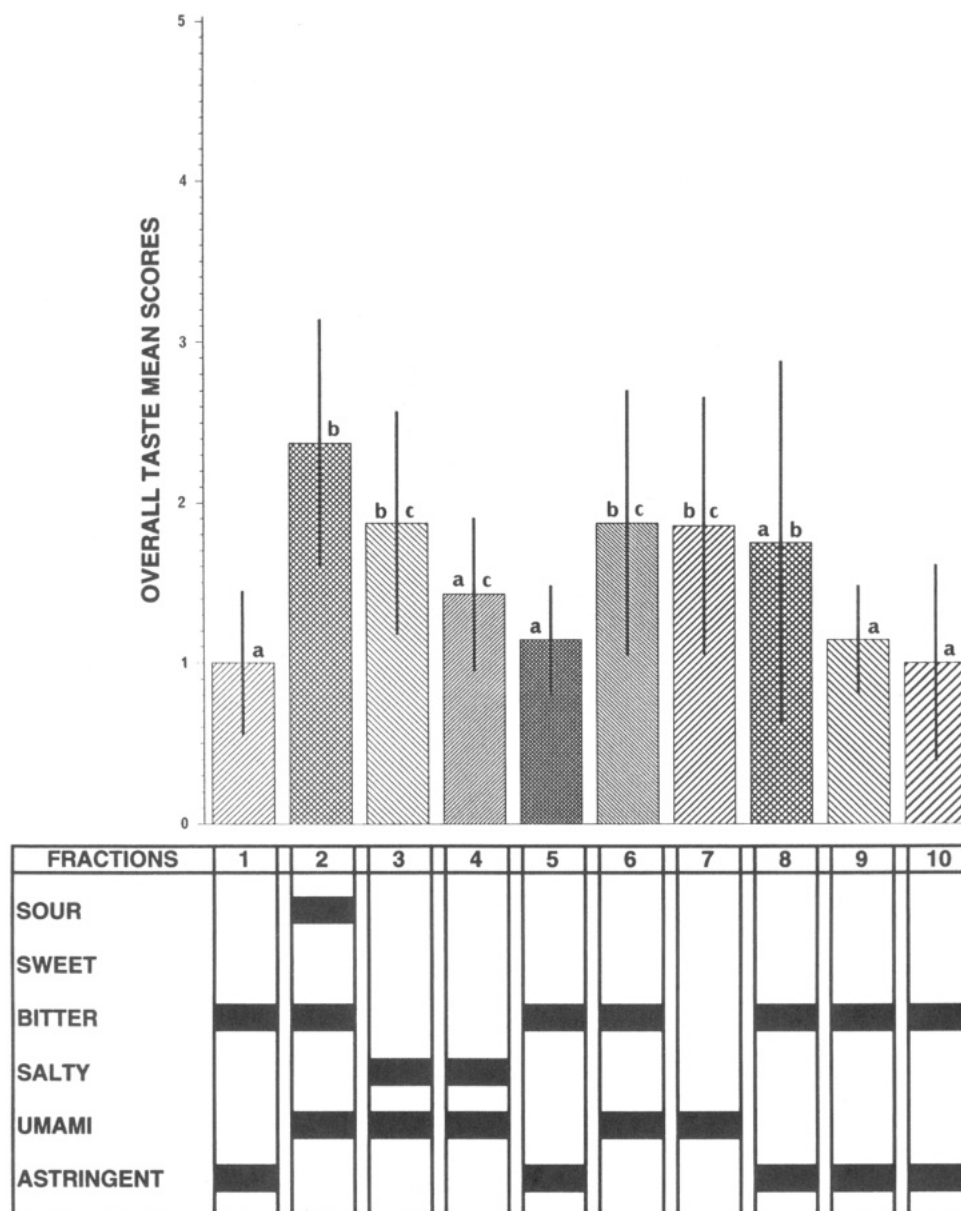
Figure 3. Overall taste mean scores and descriptors for each fraction obtained by chromatography on Toyopearl HW-40S of extract **E1**. The bars assigned the same letter (a–c) are not significantly different at the level of 5% (test LSmeans, SAS). The confidence interval is drawn at the top of each bar.

RESULTS AND DISCUSSION

Cheese Extracts. Many solvents have been used for the extraction of low molecular weight nitrogenous components and precipitation of the largest molecules from food: phosphotungstic acid solutions (Reiter et al., 1969), trichloroacetic acid and ethanol (Reville and Fox, 1978), and methanolic solvent (Lemieux et al., 1989). Kuchroo and Fox (1982) compared various homogenization techniques and operational parameters such as temperature, duration of homogenization, cheese/water ratio, and the use of various reagents for selective precipitation to optimize the extraction. Bican and Spahni (1991) showed that an acidic extraction procedure with a solvent containing 1% NaCl, 1% TFA, 5% formic acid, and 1 N HCl is the most promising approach for low molecular mass nitrogen component isolation.

However, none of the methods described above, except mixing with water, allow one to taste the hydrosoluble fraction. So, the extraction of cheese with water as described by Kuchroo and Fox is the most appropriate method for this study.

For **E1**, most of the caseins and high molecular weight

peptides are precipitated at pH 4.6. The elimination of those molecules is to prevent the clogging of the ultra-filtration membrane. The interest of the ultrafiltration is to discard the large peptides which are tasteless or bitter (Lemieux and Simard, 1992). Bitterness is generally a cause of rejection of food, so we are more interested in the other tastes which are more attractive to consumers.

Respectively, 4.1 and 5.5 g of dry extracts **E1** and **E2** were obtained from 100 g of cheese. The composition of these two extracts is given in Table 1.

Chromatography of the Extracts. Gel permeation has been frequently used to isolate peptidic fractions from different foods: Aston and Creamer (1986) fractionated the water-soluble fraction of Cheddar cheese with Sephadex G-15; Lemieux et al. (1989) isolated astringent and bitter fractions also from Cheddar cheese with TSK-G 2000 SW gel; Dale and Young (1989) separated low molecular weight beer compounds on Sephadex LH-20 and small peptides fractions on Sephadex G-10, while De Llano et al. (1991) separated nitrogenous compounds on Sephadex G-10. The main

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Table 2. Comparison between Free Amino Acids Contained in the Fractions Obtained after Chromatography on Toyopearl HW-40S and Their Threshold Values

free amino acid	E1 (mg L⁻¹)				E2 (mg L⁻¹)									threshold in water[a] (mg L⁻¹)	taste[a]
	1	2	3	4	1	2	3	4	5	6	7	8	9		
Asp/AspNa	3.1	**45.9**[b]			2.6	**41.6**	**32.9**							30/1000	sour/umami
Thr	0.8	6.9	11.3						69.5	100.1				2600	sweet
Ser	2.6	33.1	4.8						20.8	128.5				1500	sweet
Asn									111.8	112.9				1000	sour
Glu/GluNa	26.9	**527.8**			40.8	**614.0**	**475.1**							50/300	sour/umami
Gln	1.7								2.0	126.5				nd[c]	flat
Pro		50.5	55.3						1.4	515.9				3000	sweet, bitter
Gly	1.4	37.5	12.7						0.7	117.4				1300	sweet
Ala	1.1	45.9	17.9						1.8	165.0				600	sweet
Val	3.3	96.8	117.8							**438.5**				400	bitter
Cys														nd	
Met	1.1		125.6							90.0	37.8			300	bitter
Ile	12.0	8.2	231.5							259.6	22.5			900	bitter
Leu	3.8	12.3	504.2							388.3	211.9			1900	bitter
Tyr				4.7										nd	bitter
Phe	2.4			66.5								143.8	53.1	900	bitter
Lys	2.7	25.9	33.4						3.5	16.5				500	sweet, bitter
His		26.1	41.2								73.9	26.4		200	bitter
Arg		0.5												500	bitter
Cit		81.4	23.7							45.6				nd	
Orn		30.5	4.1											nd	
Gaba		8.9	5.1											nd	
total	62.9	1038.2	1188.6	71.2	43.4	655.6	507.9		211.4	2504.6	346.0	170.1	53.1		

[a] Kato et al. (1989). [b] The bold values are above threshold. [c] nd, not determined.

interest of this chromatographic technique is the possibility to use pure water as eluent.

Typical separation patterns obtained after chromatography of the extracts **E1** and **E2** on HW-40S gel are presented in Figure 1. Dry extracts, amino acid composition of the fractions obtained from **E1** and **E2**, and their ion composition (Na⁺, K⁺ and Ca²⁺ for **E1** fractions; lactate, inorganic phosphorus, and Cl⁻ for **E2** fractions) are presented in Figure 2.

The pattern obtained by UV detection from extract **E1** shows six peaks. Peaks 1–3 are not well resolved but they correspond to the fractions containing the largest quantity of solids; cations are essentially present in the fraction corresponding to peak 3.

The six fractions collected from the chromatographic pattern are analyzed for amino acids before (free amino acids) and after acid hydrolysis (total amino acids). The difference indicates the peptide amino acids. Peptides are essentially localizated in fraction 1. The important ratio of glutamic acid in their composition indicates that they are polar peptides.

Fraction 2 contains only some peptides and the largest quantity of free glutamic and aspartic acids, serine, glycine, alanine, citrulline, and ornithine.

Fraction 3 is made up of the other free amino acids, essentially, methionine, leucine, and isoleucine. Free phenylalanine and tyrosine are present in fraction 4, and no nitrogenous component was identified in fraction 5 or 6.

It is noteworthy that the chromatography of synthetic mixtures of amino acids and peptides in the same conditions leads to the same elution volume as those obtained with the cheese extracts. Particularly, tryptophan has the same elution volume as fraction 6. Moreover, synthetic polar dipeptides containing glutamic acid and generally other small polar peptides show the same elution volume as fraction 1.

The chromatography of **E2** allows one to observe a good separation between polar and nonpolar components, and a better resolution than that obtained with **E1** due to the greater height of the column. Fractions 1–3 are made up of free or bound acidic amino acids. The amino acid analysis reveals exclusively the presence

of Asp and Glu in these fractions. The other free amino acids are eluted in fractions 5–9 in a decreasing polarity order, the majority of them in fraction 6. Fraction 7 has about the same composition as fraction 3 of extract **E1** and fractions 8 and 9 correspond to fraction 4 of **E1**. For the peptides, the separation is less evident but we can observe acidic peptides in fractions 2 and 3 and the others in fractions 4–6.

Moreover, the chromatograms obtained with **E1** and **E2** indicate that several amino acids have a higher elution volume than aspartic and glutamic acid, which are the more polar amino acids, whereas their molecular weights are in the same range of mass (75–204). This retention on the column is probably caused by hydrophobic interactions between the gel matrix and these compounds; this phenomenon has been already mentioned with other components (Salles et al., 1990).

The ions Cl⁻ and PO_4^{3-} and lactic acid were observed in the first eluting fractions (2–4) with the more polar compounds. However, Ca²⁺ ions were detected only in extract **E2** but not in its fractions. We suspected that the concentrations are probably too low to be detected in the fractions or that interactions with the stationary phase immobilize all of the calcium ions, which are present in lower concentration than in the equivalent fraction (3) from **E1**.

Sensory Analysis of the Fractions. *Taste.* The total taste and individual scores of each fraction of extract **E1** are shown on Figure 3.

Fractions 7 and 8 obtained with percolated mineral water have a very low global taste intensity (near 0.5) and are noted as bitter and astringent. It is surprising that if pure water (Milli-Q) is used for chromatography, the intensity of all the equivalent fractions is increased by 1 unit.

Among the six fractions isolated from cheese, only fractions 2 and 3 are characterized by a noteworthy global taste intensity. The umami taste of fraction 2 can be easily explained by an important concentration of glutamate monosodium, which is approximately 10 times the threshold value of this compound (Kato et al., 1989), while the concentrations of the other amino acids are under their threshold values in this fraction (Table

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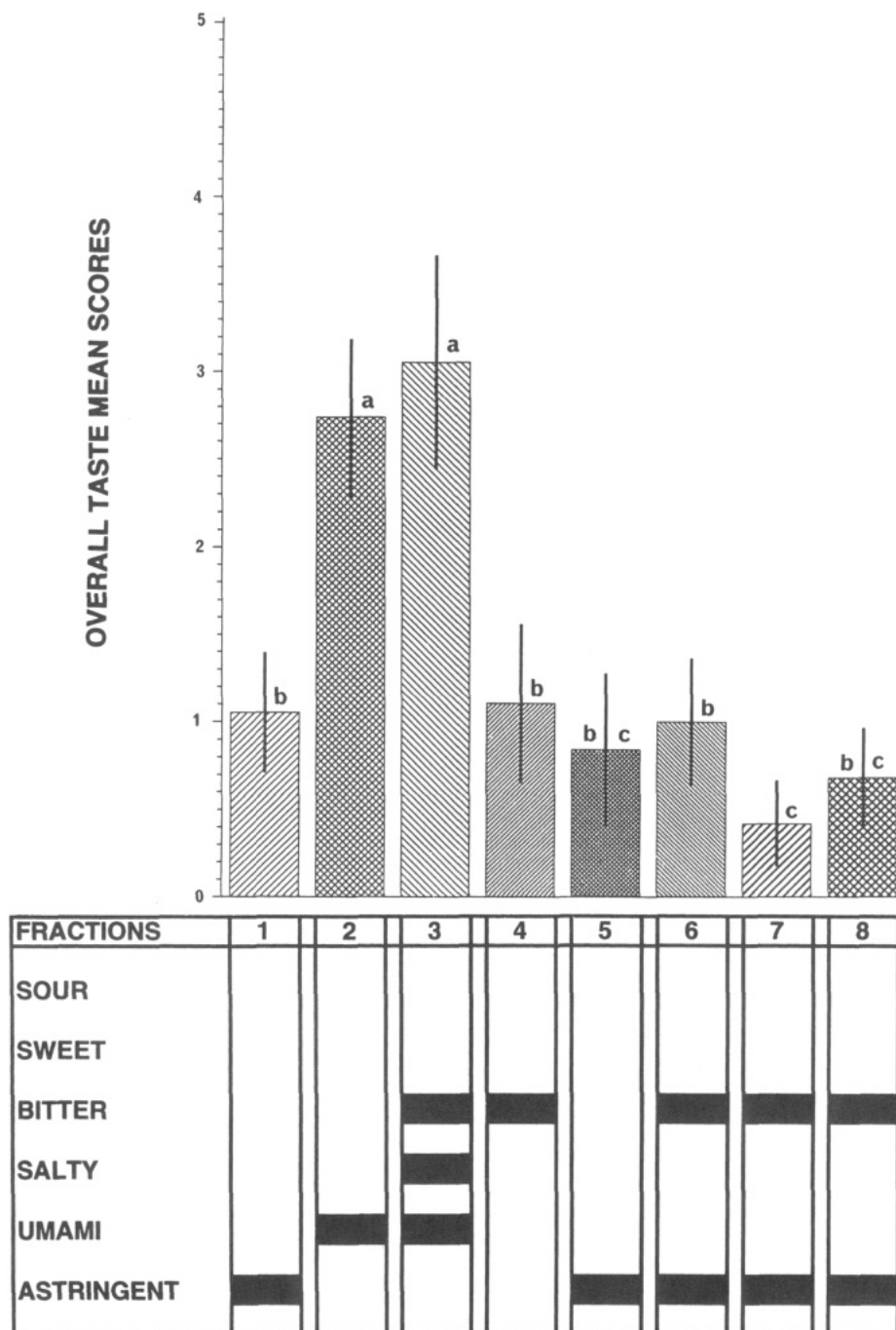
Figure 4. Overall taste mean scores and descriptors for each fraction obtained by chromatography on Toyopearl HW-40S of extract **E2**. The bars assigned the same letter (a–c) are not significantly different at the level of 5% (test LSmeans, SAS). The confidence interval is drawn at the top of each bar.

2). For fraction **3**, the taste seems to be more complex. If the salty taste can be explained by the presence of salts in this fraction, the bitter and umami tastes cannot be explained by a specific amino acid because their respective concentrations are lower than their threshold values (Table 2). The hypothesis of synergistic effects of several molecules can be advanced. As far as we know, only Nishimura and Kato (1988) reported a synergistic effect in umami taste in the mixture monosodium glutamate–5'-ribonucleotide and the following free amino acids: Gly, Ala, Cys, His, Met, Pro, and Val. Noone else has ever reported the eventual synergistic effects of many amino acids, but preliminary data clearly show that the presence of salts and particularly NaCl enhances the taste of amino acids (Konosu et al., 1987; Petro-Turza and Teleky-Vamossy, 1989; Ugawa

et al., 1992). However, both hypotheses can be put forward.

Fractions 1 and 4–6 have a low global taste intensity which is not significantly different from that observed for fractions 7 and 8. For fractions 4–6, the very low quantity of material they contained may be an explanation. For fraction 1, which contains more peptidic material, the explanation is different. Le Bars (personal communication) has shown that for this kind of cheese small tasty peptides are minor compared to larger tasteless peptides. Thus, we suggest that this fraction contains mainly tasteless peptides.

Looking at the taste of fractions from extract **E2** (Figure 4), bitterness is detected in almost all of the fractions, but with a low intensity which could be partially attributed to the water used. In fact, fraction

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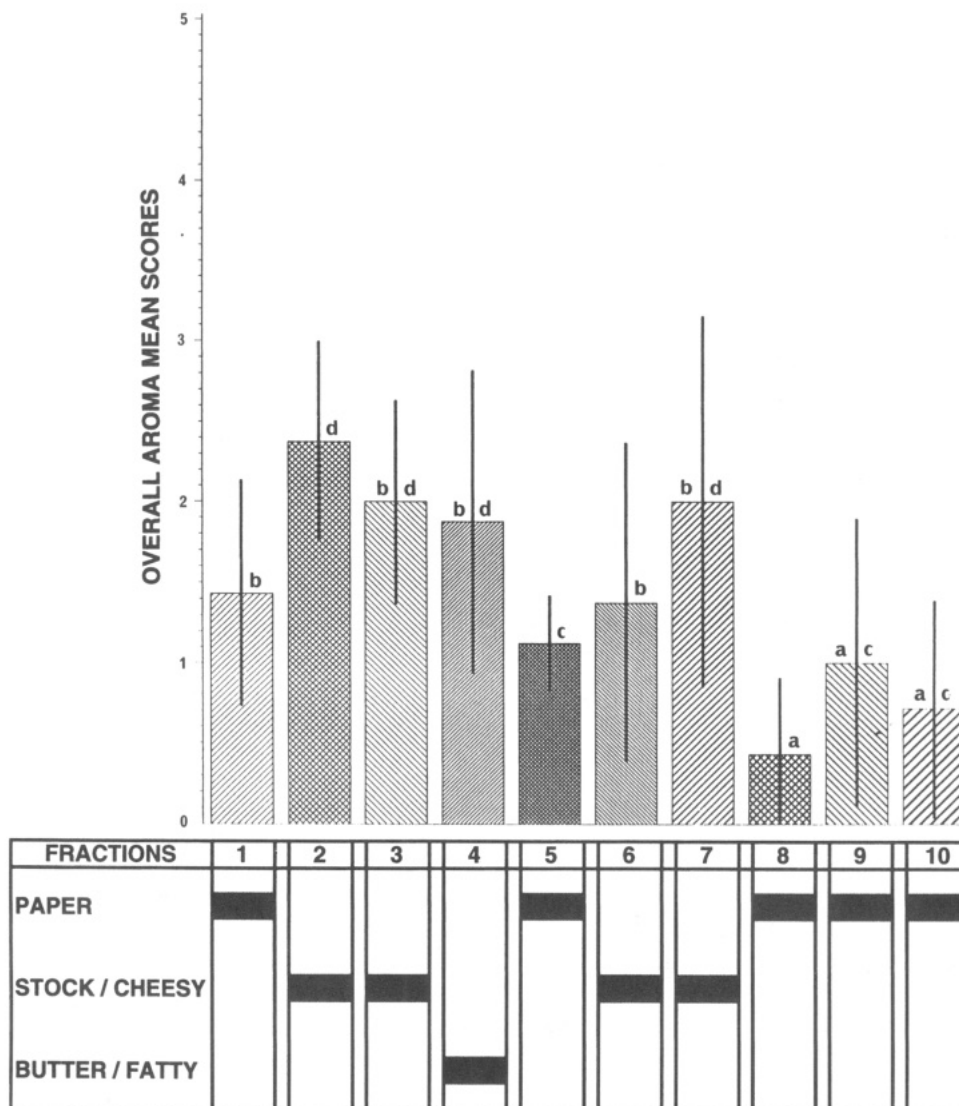*J. Agric. Food Chem.,* Vol. 43, No. 6, 1995 **1665**
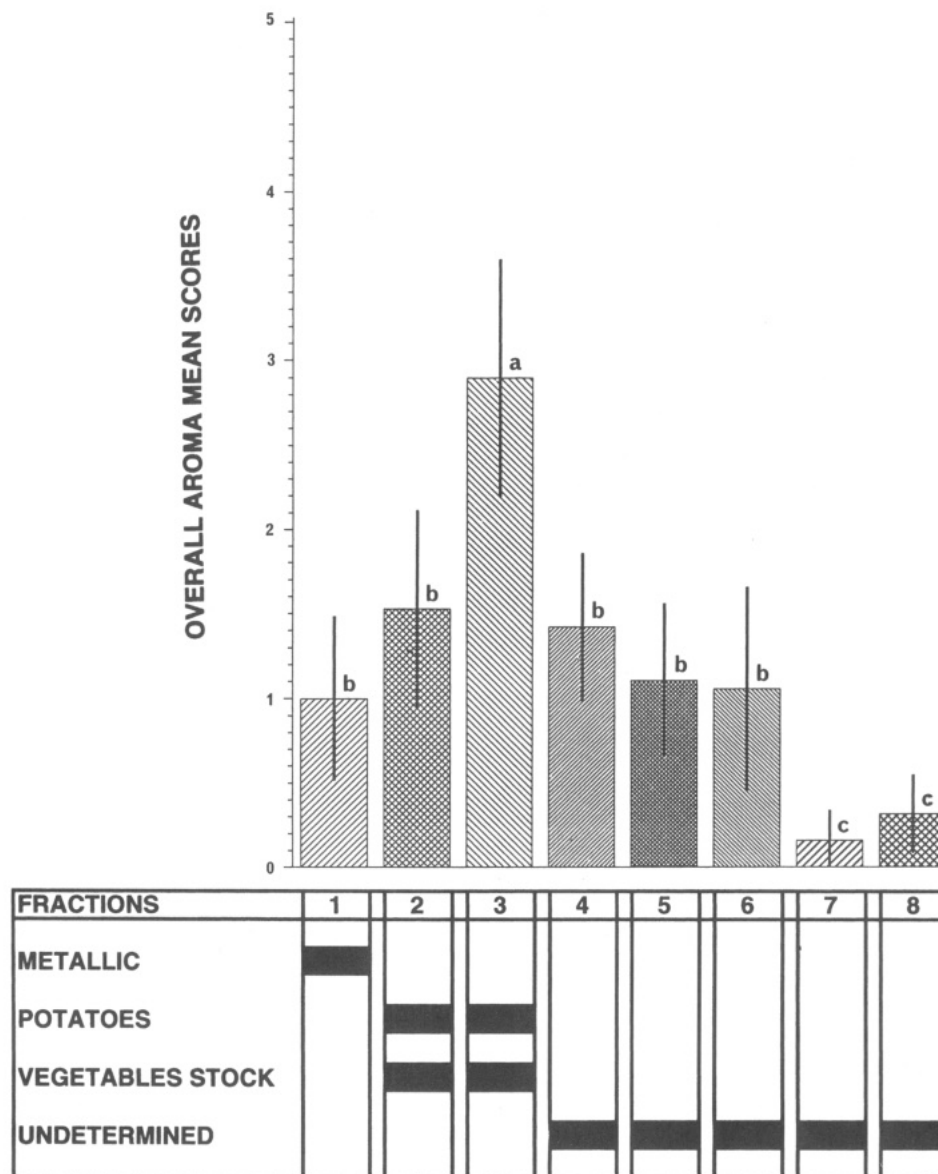
Figure 5. Overall aroma mean scores and descriptors for each fraction obtained by chromatography on Toyopearl HW-40S of extract **E1**. The bars assigned the same letter (a–c) are not significantly different at the level of 5% (test LSmeans, SAS). The confidence interval is drawn at the top of each bar.

10 is considered to be representative of pure water: slightly bitter and astringent. A little sourness is detected in fraction 2 corresponding to acidic compounds (Asp, Glu, and lactic acid). In this fraction, the concentrations of these amino acids are above their threshold values (Table 2). Fractions 2, 3, 6, and 7 are described as umami. In fractions 2 and 3, this taste could be attributed to the presence of Glu and Asp (bound with Na^+) because their respective concentrations are higher than their threshold values. For the fraction 6, only Val concentration is near its threshold value. The umami taste of fractions 6 and 7 is interesting because these fractions do not contain glutamic acid. It could be the result of interactions between the components present in the fractions or due to other compounds not detected as nucleotides. The fractions also contain Met, which could be responsible for this flavor. Met and its decomposition compounds have low detection thresholds. The salty taste corresponds to the presence of Cl^- ions in the fractions. We can suppose that Cl^- ions are present as NaCl salt. We can also observe that the intensity is generally proportional to the total weight of each fraction (dry matter) except for fraction 7, where intensity is high with less than 5 mg of dry solids.

Aroma. The global aroma mean intensity from each **E1** fraction and the corresponding descriptors are presented in Figure 5. Fractions 1 and 4–8 have a low aroma intensity, making it difficult to describe by the panel members. Only fractions 2 and 3 showed an interesting aroma described almost unanimously as potatoes and vegetable stock.

Concerning the overall flavor for the **E2** fractions (Figure 6), we can observe as for the taste that it varied from fractions and was correlated with its dry matter content except for fraction 7. Umami taste is accompanied by a stock/cheesy flavor. The stock note of fractions 6 and 7 can be explained indirectly by the presence of methionine due to the low detection thresholds of its decomposition compounds. Fraction 4 is buttery and fatty. This fraction contains very low quantities of peptides but also probably fatty acids.

Concerning the results obtained with each extract, it is difficult to compare them rigorously because several parameters are different, such as the origin of the cheese, the preparation of the extracts, and the chromatographic and sensory evaluation conditions. However, the results obtained are not contradictory because,

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Figure 6. Overall aroma mean scores and descriptors for each fraction obtained by chromatography on Toyopearl HW-40S of extract **E2**. The bars assigned the same letter (a−d) are not significantly different at the level of 5% (test LSmeans, SAS). The confidence interval is drawn at the top of each bar.

in the two cases, the fractions containing free amino acids have the highest taste and aroma intensities.

The nature of the volatile compounds responsible for the aroma in the water-soluble extract is not yet known but is currently under study in our laboratory. It is interesting to observe that only the fractions containing mainly free amino acids are the most aromatic. This phenomenon has already been observed, specially in Swiss cheese by Biede and Hammond (1979), who described the aroma of the water-soluble extract as nutty and burned and correlated this with the presence of small peptides and free amino acids. Later on, Aston and Creamer (1986) correlated, after gel filtration chromatography of a water-soluble extract of Cheddar cheese, the aroma to the presence of free amino acids. Two hypotheses may be put forward to explain such a phenomenon:

(1) Some hydrosoluble volatile compounds are present in the extract and coeluted with free amino acids after gel filtration chromatography either because they have the same elution volume or because they interact with amino acids to form some kind of complexes.

(2) The aroma is generated by the degradation of amino acids or of some derived compounds as suggested by Kowalewska et al. (1985) and Griffith and Hammond (1989). Unfortunately, noone reports the identification

of these volatiles in Comté cheese, which is necessary to validate these hypotheses.

The water-soluble fraction composition of cheese is very complex and its purification by chromatography is necessary to evaluate the organoleptic importance of each of its components in tasting, in particular amino acids and peptides. However, the taste of a fraction may result from a synergistic combination of several compounds, as shown by Tamura et al. (1989) for peptides, and the drawback of chromatography is the loss of taste due to the separation of these kinds of compounds. Moreover, sensory analyses are solely conducted with compounds dissolved in pure water. These conditions are not representative of a cheese medium because pH, matrix, salt concentrations, and texture are different. So, now our objectives are to make sensory analyses of the purified fractions introduced in a cheese model, to study other food models containing smaller peptides, and to determine the sequence of isolated peptides by mass spectrometry and also to identify all of the volatile compounds responsible for the aroma of the water-soluble fraction.

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INRA, Dijon, France) for the sensory evaluation data and D. Le Bars (Station de Recherches Laitières, INRA, Jouy-en-Josas, France) for the amino acid analysis and for their helpful suggestions.

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