Sensory Characteristics and Related Volatile Flavor Compound Profiles of Different Types of Whey

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ABSTRACT

To characterize the flavor of liquid whey, 11 samples of whey representing a wide range of types were sourced from cheese and casein-making procedures, either industrial or from pilot-plant facilities. Whey samples were assessed for flavor by descriptive sensory evaluation and analyzed for headspace volatile composition by proton transfer reaction-mass spectrometry (PTR-MS). The sensory data clearly distinguished between the samples in relation to the processes of manufacture; that is, significant differences were apparent between cheese, rennet, and acid wheys. For Mozzarella and Quarg wheys, in which fermentation progressed to low pH values, the starter cultures used for cheese making had a significant influence on flavor. In comparison, Cheddar and Gouda wheys were described by milk-like flavors, and rennet casein wheys were described by “sweet” (oat-like and “sweet”) and thermally induced flavors. The volatile compound data obtained by PTR-MS differentiated the samples as distinctive and reproducible “chemical fingerprints”. On applying partial least squares regression to determine relationships between sensory and volatile composition data, sensory characteristics such as “rancid” and cheese-like odors and “caramelized milk,” yogurt-like, “sweet,” and oat-like flavors were found to be related to the presence and absence of specific volatile compounds.

(Key words: whey, flavor, sensory analysis, headspace volatile analysis)

Abbreviation key: PC = principal components, PCA = principal component analysis, PLSR = partial least squares regression, PTR-MS = proton transfer reaction-mass spectrometry.

INTRODUCTION

The dairy industry produces several types of whey as by-products of cheese making or casein production:

(a) “sweet” whey (pH $\geq$ 5.8), which can be obtained either from the manufacture of natural enzyme-produced cheeses (e.g., Cheddar, Edam), i.e., cheese whey, or from the production of (rennet) caseinates, i.e., rennet casein whey; (b) medium acid whey (pH 5.0 to 5.8), from the manufacture of some fresh acid cheeses (e.g., Danbo, queso blanco); (c) acid whey (pH $<$ 5.0) obtained from the manufacture of fresh acid cheeses (e.g., Quarg, cottage, cream-Neufchâtel); and (d) acid casein whey, obtained from the production of acid casein by acidification of skimmed milk (Kosikowski and Mistry, 1997; Zadow, 2003).

Many whey utilization strategies involve fractionation and recovery of valuable constituents, such as proteins and lactose, for further processing or production of dry ingredients such as whey protein concentrates. However, a problem with such approaches is the low total solids content (approximately 6%, wt/wt) of whey, which reduces the efficiency of processing (Ryder, 1980; Mehrens, 2004).

Another option for whey use takes advantage of its high water content to produce beverages. Fresh liquid whey, however, is associated with high transportation costs and susceptibility to deterioration during storage (Jensen and Kroger, 2000) and also with unappealing sensory characteristics (Jelen, 1992).

It is known that different cheese and casein-making procedures result in different chemical compositions of whey (Durham et al., 1997; Ji and Haque, 2003). Further differences in composition arise from activities of coagulants (Reineccius, 1994) and starter cultures (Romero, 1992) during the initial steps of cheese making, but little is known about source-related variations in the flavor and volatile composition of liquid whey.

Although most research on whey has been devoted to the improvement of processing methods, few studies have analyzed the impact of source and processing of whey on perceived flavor. In such studies, sensory evaluation, when performed, has been applied to determine which components are responsible for undesirable flavors, particularly when whey is used as a dry ingredient added to a product (e.g., Livney and Bradley, 1994) rather than to describe flavor. The majority of reports

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Table 1. Origin of whey samples.

Origin	Source	Type of whey	Casein coagulation
Cheese making			
Cheddar cheese	Company A	"Sweet", Cheese	Unknown starter
Cheddar cheese	Company B	"Sweet", Cheese	Unknown starter
Cheddar cheese	Pilot plant	"Sweet", Cheese	Commercial starter R-604 ¹
Gouda cheese	Pilot plant	"Sweet", Cheese	Commercial starter B-11 ¹
Mozzarella cheese	Pilot plant	"Sweet", Cheese	Commercial starter TCC-3 ¹
Paneer cheese	Pilot plant	Medium acid	Citric acid solution, 1% wt/wt
Quarg (fresh cheese)	Pilot plant	Acid	Commercial starter XT-202 ¹
Casein production			
Rennet casein	Company A	"Sweet", Rennet casein	Unknown rennet
Rennet casein	Pilot plant	"Sweet", Rennet casein	Commercial rennet Std. Plus 190 ¹
Acid casein	Company A	Acid casein	Unknown acid
Lactic acid casein	Pilot plant	Acid casein	Lactic acid solution, ¹ 10% vol/vol

¹Starter cultures: R-604 = *Lactococcus lactis* spp. *cremoris*, *Lactococcus lactis* spp. *lactis*; B-11 = *Lactococcus lactis* spp. *cremoris*, *Lactococcus lactis* spp. *lactis*, *Leuconostoc mesenteroides* spp. *cremoris*, *Lactococcus lactis* spp. *diacetylactis*; TCC-3 = *Streptococcus thermophilus*, *Lactobacillus delbrueckii* spp. *bulgaricus*; XT-202 = *Lactococcus lactis* spp. *cremoris*, *Leuconostoc pseudomesenteroides*, *Lactococcus lactis* spp. *lactis* biovar. *diacetylactis*. All starters, rennet (Std. Plus 190), and lactic acid (food-grade) were provided by Chr. Hansen Ltd., Ireland.

on flavor of whey have focused on the flavor chemistry of whey protein concentrates (e.g., Morr and Ha, 1991; Mills, 1993; Laye et al., 1995).

Some confusion about flavor of whey exists in the literature, because it is not always clear if it refers to whey in dry form, as an ingredient in a formula, reconstituted, or liquid (raw). Information on the flavor of liquid whey is scarce, with a single report on flavor of acid whey (McGugan et al., 1979), and a small number on a single type of cheese (Cheddar) whey (Carunchia Whetstine et al., 2003; Karagul-Yuceer et al., 2003; Tomaino et al., 2004).

Knowledge and understanding of how the flavor of liquid whey is affected by technological variables is important, because it may help (a) to develop products such as carbonated beverages, whey-based fruit drinks, dairy type (fermented and unfermented) products, and alcoholic beverages, (b) to understand the nature of chemical reactions detrimental to the flavor of whey, and (c) to allow control of the shelf life of such products. Research on sensory evaluation of liquid whey is also necessary to improve the flavor of dried whey.

The objectives of this study were: 1) to characterize the flavor of liquid whey representing a range of cheese making and casein production procedures, 2) to assess the potential of different types of whey for specific applications due to their sensory profiles, 3) to examine, using a rapid method of headspace analysis, the flavor significance of the volatile composition for that range of samples, and 4) to establish relationships between sensory characteristics and volatile composition to enable more efficient control of whey flavor.

MATERIALS AND METHODS

Samples

Eleven samples of whey from a wide range of sources were analyzed. These represented each of the main types of whey, namely: cheese whey (from Cheddar, Gouda, and Mozzarella cheese-making), rennet casein whey (made by addition of rennet to skim milk), medium acid whey (from Paneer, a soft heat- and acid-coagulated Indian cheese), and acid whey (derived from Quarg cheese making, or acid casein making). Details about the samples, provided by industrial cheese manufacturers (companies A and B) or produced in pilot-plant cheese-making facilities, are given in Table 1.

Whey samples (4.5 L of each type) collected from pilot-scale cheese-making trials were filtered through a cheese sieve to remove curd (casein) fines, centrifuged at $3500 \times g$ for 25 min at 6°C and filtered through Whatman No. 113 filter paper to remove fat (final fat content < 0.5%). Samples from industrial manufacturers did not require filtration for curd fines but were skimmed by centrifugation and filtered under the same conditions.

Refrigerated samples from industrial cheese manufacturers were transported to the laboratory at ~6°C and subsequently frozen at -20°C until required. Pilot-plant samples, after being cooled from temperatures ranging from 43 to 32°C depending on specific protocols, were also frozen at -20°C until required.

Before analysis, samples were thawed at 6°C overnight; once thawed, samples were kept refrigerated (maximum 48 h) at 4 to 6°C until 1.5 h before sensory

evaluation or volatile headspace analysis, when they were allowed to reach room temperature (~20°C).

Compositional Analysis

Fat and protein content and total solids of samples were determined using a Milkoscan FT120 (FOSS Ireland, Dublin, Ireland). Whey pH was determined using a digital pH meter MP120 Mettler-Toledo (Schwerzenbach, Switzerland).

Sensory Evaluation

A panel of 10 assessors, with at least 2 yr of previous experience in descriptive sensory analysis of dairy products, was trained for sensory evaluation of the samples (Standard 8586; ISO, 1993). After discussion of terms in three 2-h sessions, a list of descriptors was defined (see list in Results and Discussion section). Panel homogeneous understanding of terms was verified by ANOVA and Kendall's coefficient of concordance of trial data (McDonnell et al., 2001). For evaluation, a 30-mL sample of each type of whey, in a 3-digit-coded glass, was presented to assessors in individual booths under controlled conditions of lighting and environment (Standard 8589; ISO, 1988) in a balanced order design (MacFie et al., 1989). Samples were assessed in duplicate on 2 separate days. On each day, 3 sessions, with a maximum of 4 samples per session were carried out. Samples were rated using 100-mm unstructured line scales. Unsalted crackers and water were given to assessors for palate cleansing between each sample, and 15-min breaks between sessions were allowed to avoid sensory fatigue. Data was collected using Compusense Five, version 4.0 (Compusense Inc., Guelph, Ontario, Canada).

Volatile Headspace Analysis by Proton Transfer Reaction-Mass Spectrometry

A nonexhaustive but reliable, sensitive, and rapid analytical procedure, proton transfer reaction-mass spectrometry (**PTR-MS**), was used to characterize the volatile compounds in whey samples.

Proton transfer reaction-mass spectrometry is a mass spectrometric technique based on a particular implementation of chemical ionization using proton transfer from hydronium ions (H_3O^+) to the volatile compounds to be detected (Lindinger et al., 1998). This technique has been described in several papers (Hansel et al., 1995; Buhr et al., 2002) and has been successfully used for food analysis, especially for the determination of flavor volatiles (Boscai et al., 2003; Mayr et al., 2003; Pollen et al., 2003).

Mass spectra for each sample were obtained using an Ionicon Analytic (Innsbruck, Austria) PTR-MS system. The analytical procedure involved placing each sample of whey (50 mL) in a 500-mL glass flask. After standing for 1 h at room temperature (~20°C) to reach headspace equilibrium, the inlet of the PTR-MS was then connected by a polyethylene tube to the flask and the headspace sample was drawn by a vacuum pump at 20.8 mL/min. Masses were analyzed in a quadrupole mass spectrometer and detected as ion counts per second (cps) by a secondary electron multiplier (Balzers QMG421 and Balzers QC422, respectively, Balzers Instruments, Austria). The spectrometric data were collected over a range of mass to charge ratio (m/z) 20 to 170 atomic mass units (amu). Headspace samples were analyzed using a constant drift voltage of 600 V; drift pressure was set at 1.998 ± 0.005 mbar. Analysis of samples was performed in duplicate on 2 consecutive days, collecting the data through a computer interface connected to the mass spectrometer using Balzers Quadstar 422 software (Balzers Instruments).

Data Analysis

Sensory and volatile headspace data were analyzed by 1-way ANOVA and subsequent (posthoc) Duncan's multiple range test using SPSS for Windows version 11.0.1 (SPSS Inc., Chicago, IL). Principal components analysis (**PCA**) on each set of data, cross validated, and standardized (1/standard deviation), was performed using Unscrambler version 9.1.2 (CAMO Process AS, Oslo, Norway). An ANOVA was also carried out on PCA scores generated from the duplicate sensory ratings to determine the significance of principal components (**PC**). Partial least squares regression (**PLSR**) Types 1 and 2 (single and multiple response, respectively) (Martens and Martens, 1986) were carried out using Unscrambler version 9.1.2 for modeling relationships between chemical compounds and sensory characteristics.

Partial least squares regression is a multivariate regression method for relating the variations in one or several response variables (Y variables, in this study the sensory attributes) to the variations of several predictors (X variables, chemical compounds), with explanatory or predictive purposes. Although PSR Type 1 models a single Y variable, PLSR Type 2 models multiple Y variables simultaneously and is useful when there is collinearity between Y variables.

RESULTS AND DISCUSSION

Compositional Analysis

Compositional data of samples analyzed (Table 2) were consistent with those reported by Kosikowski and

Table 2. Gross composition and pH of wheys.

Whey sample	Composition			pH
	Total solids, %	Protein, %	Fat, %	
Cheddar, company A	7.1	0.91	0.47	6.31
Cheddar, company B	6.4	0.68	0.39	6.10
Cheddar, pilot plant	6.5	0.78	0.38	5.81
Gouda	6.5	0.82	0.49	6.27
Mozzarella	6.1	0.85	0.29	4.61
Paneer	5.7	0.70	0.18	5.52
Quarg	6.3	0.76	0.14	4.36
Rennet casein, company A	6.1	0.82	0.09	6.58
Rennet casein, pilot plant	6.1	0.84	0.12	6.68
Acid casein, company A	5.9	0.70	0.10	4.48
Lactic acid casein	6.9	0.68	0.10	4.61

Mistry (1997), except for Mozzarella whey. A possible explanation for its low pH could be that before being frozen, this sample was cooled more slowly than the other samples (as the temperature gradient was the widest) allowing further fermentation by the fast-acidification commercial starter culture used.

Processing parameters known to affect composition of whey include pasteurization of cheese milk, type and amount of bacterial starter cultures, type and concentration of coagulant used, treatment of the cheese curd before whey drainage, and removal of residual lipids from the whey (Olling and van Luin, 1988; Johansen et al., 2002).

Sensory Characteristics of Whey Samples

Karagul-Yuceer et al. (2003) reported that the flavor of whey depends on the variety of cheese produced, and Carunchia Whetstine et al. (2003) assumed that wheys made from different types of cheese have different flavor, but this was not ascertained because their results were based on observations on only one type of cheese (Cheddar) whey. These assumptions were probably based on findings by Bodyfelt et al. (1988) with reference to the flavor of whey powder, which can be produced from whey derived from production of different types of cheese.

In this study, all the sensory characteristics used in the descriptive analysis, except “caramelized” odor, significantly discriminated between the whey samples (Table 3).

The term “cheesy,” discussed across all samples during the training sessions and assessed during evaluation, although discriminating in intensity, could not be attributed specifically to whey from a particular type of cheese. This was consistent with the fact that flavor is not developed in cheese until the ripening stage (Reineccius, 1994) and with the proposition that all fresh

cheeses have a “common basic cheese taste” (Urbach, 1995). Results suggested that differences in flavor of whey basically refer to different intensities of attributes such as “acid,” “sweet,” “milky,” and a (nonspecific) “cheesy” odor and, depending on processing and handling, notes indicating thermal, enzymatic, or microbiological effects.

The influence of the starter culture on flavor may be enhanced if it is not inactivated, as probably occurred for Mozzarella and Quarg cheese whey samples, which had significantly higher scores for yogurt odor and flavor than the rest of the samples. The starter cultures used for the manufacture of these 2 types of cheese, if active, produce ethanal (acetaldehyde) and 2,3 butadi-one (diacetyl) albeit by different biochemical pathways (Coolbear et al., 2003; Liu and Holland, 2003). Acetaldehyde and diacetyl are chemical compounds that impart characteristic yogurt aroma and flavor (Tamime and Robinson, 1999).

One of the strains of the starter culture used for manufacturing Gouda cheese (*Lactococcus lactis* spp. *diacetylactis*) also produces diacetyl and an acetaldehyde-thiamine complex (Ward et al., 2003) but the corresponding whey was not acid (pH was 6.27). On the contrary, a yogurt flavor note was reported for the lactic acid casein sample, which had no starter culture added (a score higher than the average for this characteristic). These results suggest that an acid taste-odor interaction was involved in the perception of yogurt flavor.

The lack of any characteristic flavor note in Paneer whey may be a consequence of its nonrennet/nonstarter method of manufacture.

The scores for “dirty” odor were significantly higher in industrial Cheddar (from company A) and acid casein whey than the rest of the samples; this characteristic could originate from microbial growth for industrial Cheddar (A) or, in the case of acid casein wheys due to oxidation reactions (Bodyfelt et al., 1988; Tomaino et al., 2004).

McGugan et al. (1979) reported that undesirable flavors commonly recognized in acid whey include “salty,” “bitter,” “astringent/chalky,” “nonvolatile acidity,” and “volatile acidity.” Similar sensory notes were perceived for the acid wheys in this study; however, there was a clear distinction between the whey samples from directly acidified milk and wheys (from lactic acid casein and industrial acid casein), and those resulting from fermentation (Quarg). Acid casein wheys obtained from direct acidification showed the highest sensory scores for off-odors such as “stale” and “rancid” (for industrial acid casein) and “dirty” (for lactic acid casein).

Nonvolatile compounds may have contributed significantly to explaining flavor variation, especially in differentiation of sweet and acid whey. These com-

Table 3. Sensory scores¹ (means) of samples analyzed and results of ANOVA.

Attribute	Whey sample ²											<i>F</i> value	<i>P</i> value
	ACASI	CHEDA	CHEDB	CHEDP	GOUDA	LACAS	MOZZ	PAN	QUARG	RCASA	RCASP		
Odor attributes													
Pungent	21 ^{ab}	19 ^b	5 ^d	8 ^{cd}	6 ^d	25 ^a	14 ^{bc}	13 ^{bc}	21 ^{ab}	9 ^c	10 ^c	8.4	0.001
Sulfurous	7 ^b	3 ^{cd}	2 ^{cd}	5 ^c	3 ^{cd}	9 ^b	10 ^b	4 ^c	15 ^a	2 ^{cd}	3 ^{cd}	16.0	0.000
Milky	15 ^c	17 ^c	29 ^a	23 ^{ab}	26 ^a	8 ^d	21 ^b	24 ^{ab}	11 ^d	24 ^{ab}	22 ^{ab}	5.1	0.006
Rancid	25 ^{ab}	20 ^b	8 ^d	11 ^{cd}	9 ^{cd}	32 ^a	15 ^c	13 ^c	19 ^b	13 ^c	12 ^c	4.5	0.010
Caramelized milk	2	3	11	8	9	4	8	6	2	10	7	1.7	0.201
Natural yogurt	4 ^c	0 ^d	5 ^c	11 ^b	6 ^c	5 ^c	30 ^a	6 ^c	26 ^{ab}	3 ^{cd}	5 ^c	6.0	0.003
Oaty	4 ^d	19 ^{ab}	19 ^{ab}	9 ^c	16 ^b	5 ^d	3 ^d	12 ^{bc}	2 ^d	20 ^{ab}	14 ^b	5.9	0.003
Cheesy	5 ^b	5 ^b	4 ^b	6 ^b	5 ^b	6 ^b	16 ^a	6 ^b	17 ^a	1 ^c	7 ^{bc}	5.6	0.004
Heated/boiled milk	18 ^b	27 ^{ab}	29 ^a	26 ^{ab}	33 ^a	6 ^c	17 ^b	28 ^a	4 ^c	31 ^a	28 ^a	4.3	0.012
Dirty	37 ^a	32 ^{ab}	4 ^d	0 ^d	5 ^d	40 ^a	6 ^d	6 ^d	16 ^c	9 ^d	8 ^d	24.9	0.000
Chemical	15 ^{ab}	9 ^b	1 ^c	1 ^c	1 ^c	24 ^a	2 ^c	5 ^{bc}	14 ^{ab}	7 ^{bc}	10 ^b	27.2	0.000
Flavor attributes													
Heated/boiled milk	27 ^b	39 ^a	41 ^a	36 ^{ab}	39 ^a	9 ^d	20 ^c	30 ^b	13 ^d	39 ^a	35 ^{ab}	15.9	0.000
Caramelized milk	4 ^d	12 ^b	21 ^a	15 ^b	14 ^b	3 ^d	4 ^d	7 ^{cd}	3 ^d	12 ^b	6 ^{cd}	11.7	0.000
Acid	36 ^{bc}	18 ^{cd}	5 ^d	23 ^{cd}	5 ^d	44 ^a	39 ^b	18 ^{cd}	47 ^a	8 ^d	7 ^d	66.5	0.000
Sweet	12 ^{de}	37 ^{ab}	37 ^{ab}	22 ^{cd}	28 ^c	11 ^{de}	8 ^e	21 ^{cd}	7 ^e	44 ^a	26 ^c	59.7	0.000
Bitter	7 ^{bc}	5 ^{bc}	3 ^c	2 ^c	3 ^c	11 ^{ab}	14 ^{ab}	6 ^{bc}	18 ^a	2 ^c	4 ^c	11.2	0.000
Natural yogurt	5 ^d	5 ^d	4 ^d	11 ^c	4 ^d	16 ^b	29 ^a	5 ^d	26 ^a	3 ^d	3 ^d	27.5	0.000
Stale	22 ^a	22 ^a	9 ^c	10 ^c	8 ^c	18 ^{ab}	9 ^c	12 ^{bc}	15 ^b	18 ^{ab}	12 ^{bc}	3.2	0.035
Rancid	38 ^a	31 ^b	16 ^{de}	5 ^e	6 ^e	32 ^b	24 ^c	12 ^{de}	28 ^b	8 ^e	8 ^e	14.6	0.000
Salty	18 ^a	17 ^a	6 ^c	4 ^c	6 ^c	17 ^a	12 ^b	10 ^{bc}	9 ^{bc}	12 ^b	4 ^c	5.5	0.005
Oaty	7 ^c	19 ^a	8 ^c	2 ^d	8 ^c	2 ^d	1 ^d	4 ^d	2 ^d	16 ^{ab}	9 ^c	33.0	0.000
Dirty	35 ^b	24 ^c	2 ^{de}	3 ^{de}	2 ^{de}	44 ^a	7 ^d	4 ^{de}	5 ^{de}	2 ^{de}	6 ^{de}	41.1	0.000
Mouthfeel attributes													
Mouth coating	34 ^b	37 ^a	32 ^b	25 ^{bc}	26 ^{bc}	28 ^{bc}	22 ^c	26 ^{bc}	24 ^c	33 ^b	24 ^c	5.5	0.005
Chalky	8 ^{bc}	7 ^{bc}	3 ^c	9 ^{bc}	4 ^c	7 ^{bc}	9 ^{bc}	8 ^{bc}	18 ^a	3 ^c	8 ^{bc}	3.4	0.029
Residual flavor attributes													
Metallic	4 ^c	1 ^c	1 ^c	2 ^c	2 ^c	10 ^{ab}	12 ^a	4 ^c	13 ^a	1 ^c	2 ^c	9.4	0.000
Acid	23 ^{bc}	14 ^{bc}	5 ^c	16 ^{bc}	5 ^c	28 ^b	37 ^a	14 ^{bc}	40 ^a	5 ^c	6 ^c	29.3	0.000
Bitter	8 ^{bc}	5 ^c	3 ^c	2 ^c	3 ^c	11 ^{ab}	14 ^a	4 ^c	17 ^a	3 ^c	3 ^c	18.2	0.000
Astringent	14 ^{ab}	10 ^b	3 ^c	7 ^{bc}	4 ^c	15 ^{ab}	18 ^a	10 ^b	19 ^a	7 ^{bc}	4 ^c	10.0	0.000
Chemical	6 ^{bc}	9 ^b	2 ^c	4 ^c	1 ^c	22 ^a	6 ^{bc}	2 ^c	9 ^b	2 ^c	2 ^c	18.3	0.000
Salty	14 ^{ab}	13 ^{ab}	4 ^c	5 ^{bc}	3 ^c	17 ^a	12 ^{ab}	7 ^b	8 ^b	7 ^b	3 ^c	10.0	0.000
Sweet	14 ^c	28 ^a	23 ^b	13 ^{cd}	20 ^b	8 ^{cd}	3 ^d	16 ^c	2 ^d	32 ^a	13 ^c	29.1	0.000

a,b,c,d,e After Duncan multiple range test, means within a row not sharing the same superscript were significantly different ($P < 0.05$).

¹Sensory scores on 1-to-100 scale.

²Whey samples: ACASI = acid casein; CHEDA = Cheddar, company A; CHEDB = Cheddar, company B; CHEDP = Cheddar, pilot plant; GOUDA = Gouda cheese; LACAS = lactic acid casein; MOZZ = Mozzarella cheese; PAN = Paneer cheese; QUARG = Quarg cheese; RCASA = rennet casein, company A; RCASP = rennet casein, pilot plant.

Figure 1. Results of principal component analysis of sensory attributes of wheys showing the first 2 principal components (PC1 and PC2). o- = odor, f- = flavor, mf- = mouthfeel, rf- = residual flavor (after swallowing); yogurt = natural yogurt; heated milk = heated or boiled milk. Whey samples: ACASI = acid casein; CHEI(A) = Cheddar, company A; CHEI(B) = Cheddar, company B; CHEP = Cheddar, pilot plant; GOUDA = Gouda cheese; LA_CASEIN = lactic acid casein; MOZZ = Mozzarella cheese; PANEER = Paneer cheese; QUARG = Quarg cheese; RCASA = rennet casein, company A; RCASP = rennet casein, pilot plant.

pounds should therefore be considered and analyzed further as some of them may be relevant to the flavor of processed whey or whey beverages.

PCA of Sensory Data

The PCA diagram of sensory data (Figure 1) clearly illustrates differences between the samples and relationships between attributes on the first 2 PC, which accounted for 70% of explained variance. The ANOVA of PC scores, from means based on duplicate ratings (ANOVA not shown) found that the first 5 PC significantly discriminated ($P < 0.05$) between samples accounting for 93% (cumulative sum) of the total variance.

The PCA diagram showed that rennet and cheese wheys were differentiated from acid wheys. Component 1, which explained 50% of the variance in the data set, showed that discrimination was mainly based on “acid,” and “bitter” flavor and an “astringent” residual flavor (positive correlation to PC 1), and “heated or boiled milk” and “caramelized milk” flavors (negative correlation). Principal component 2 explained an additional 20% of the variance and was primarily a function of “natural yogurt” flavor (negative correlation to PC 2) and off-flavors such as “dirty” and “stale” (positive cor-

relation). Loadings for “salty” (flavor and residual flavor) discriminated the acid wheys from the rest of the samples in an additional sensory dimension (PC 3).

Headspace Volatile Compounds in Whey Samples

Twenty-three volatile compounds detected by PTR-MS significantly discriminated among samples (Table 4). Other compounds were detected in the samples but did not discriminate among them, and therefore were not included in subsequent analysis.

It was not possible to define key chemical compounds and concentrations that could be associated with a particular sample of whey. However, the PTR-MS spectra constituted a “chemical fingerprint” for each one of the samples; examples of the headspace volatile profiles of a sample with a fermented aroma, that is, a cheese (Mozzarella) whey and a less complex profile from lactic acid casein whey are shown in Figures 2 and 3.

Attribution of volatiles to the masses detected by PTR-MS in the samples was based on the molecular weight of compounds with a proton transferred (i.e., molecular weight + 1), and cross-referenced to flavor studies in dairy products (Badings, 1991; Friedrich and Acree, 1998), processed whey (Mills, 1993; Rankin,

Table 4. Average concentration (ppb) of significant volatile compounds detected by proton transfer reaction-mass spectrometry in the headspace of samples and results of ANOVA.

m/z ²	Volatile compound attributed ³	Whey samples ¹											<i>F</i> value	<i>P</i> value
		ACASI	CHEDA	CHEDB	CHEDP	GOUDA	LACAS	MOZZ	PAN	QUARG	RCASA	RCASP		
41	1,2 propadiene; alkenyl (fr)	3 ^b	12 ^b	ND	17 ^b	7 ^b	14 ^b	7 ^b	44 ^a	12 ^b	2 ^b	45 ^a	7.21	0.002
42	Allyl (r); dimethylamine	4 ^c	8 ^c	70 ^a	3 ^c	11 ^c	8 ^c	11 ^c	54 ^b	11 ^c	13 ^c	13 ^c	37.37	0.000
43	Propan-1-ol; alkyl (fr)	34 ^c	255 ^a	55 ^c	23 ^c	90 ^c	177 ^b	88 ^c	159 ^b	83 ^c	41 ^c	59 ^c	13.18	0.000
45	Acetaldehyde	11 ^d	107 ^{cd}	117 ^{cd}	3 ^d	462 ^a	18 ^d	786 ^a	209 ^{bc}	841 ^a	31 ^d	281 ^b	91.60	0.000
46	Hydrocarboxyl (r)	3 ^{cd}	4 ^{bcd}	4 ^{bcd}	4 ^{bcd}	21 ^a	12 ^{abc}	19 ^a	3 ^{cd}	14 ^{ab}	ND	5 ^{bcd}	5.74	0.004
47	Ethanol	ND	ND	16 ^b	ND	1 ^c	11 ^{bc}	ND	31 ^a	ND	2 ^c	30 ^a	11.68	0.000
55	Alkenyl (fr)	43 ^b	17 ^b	22 ^b	23 ^b	33 ^b	74 ^a	24 ^b	42 ^b	24 ^b	23 ^b	19 ^b	5.04	0.007
59	Acetone; propanal	1977 ^{bc}	870 ^{fg}	1137 ^{ef}	2815 ^a	2033 ^{bc}	1719 ^{cd}	2251 ^b	1395 ^{de}	2261 ^b	602 ^g	2131 ^{bc}	27.63	0.000
61	Acetic acid	ND	12 ^c	9 ^{cb}	1 ^c	21 ^c	215 ^a	2 ^c	74 ^b	2 ^c	18 ^c	20 ^c	43.31	0.000
63	Dimethyl sulfide	9 ^c	4 ^c	153 ^b	4 ^c	5 ^c	ND	8 ^c	7 ^c	3 ^c	19 ^c	260 ^a	13.93	0.000
71	Alkane (fr)	7 ^{cd}	106 ^a	19 ^{bcd}	2 ^d	30 ^{bcd}	3 ^d	33 ^b	33 ^{bc}	38 ^b	9 ^{cd}	2 ^d	13.10	0.000
73	2-butanone; butanal	52 ^{bcd}	34 ^{de}	39 ^{cde}	31 ^e	63 ^b	43 ^{cde}	77 ^{ab}	56 ^{bc}	71 ^{ab}	9 ^f	84 ^a	14.52	0.000
75	Propionic acid	166 ^a	44 ^e	73 ^{de}	93 ^{cd}	123 ^{bc}	82 ^{cde}	97 ^{cd}	137 ^{ab}	91 ^{cd}	72 ^{de}	140 ^{ab}	8.147	0.001
83	Butadiene; 3-methyl furan	18 ^a	ND	ND	ND	3 ^b	12 ^a	2 ^b	13 ^a	2 ^b	ND	1 ^b	5.470	0.005
87	Diacetyl; 2-/3- methyl butanal; pentanal	6 ^d	154 ^a	26 ^{cd}	ND	41 ^c	ND	46 ^c	84 ^b	46 ^c	11 ^d	12 ^d	36.76	0.000
89	Butanoic acid; isobutyric acid; acetoin	3 ^{cd}	57 ^a	5 ^{cd}	ND	28 ^b	11 ^c	28 ^b	21 ^b	23 ^b	8 ^{cd}	2 ^{cd}	41.44	0.000
97	2-furfural	185 ^a	2 ^d	ND	ND	ND	91 ^b	1 ^d	1 ^d	16 ^c	8 ^{cd}	2 ^d	422.44	0.000
104	NA	ND	ND	ND	1 ^b	1 ^b	ND	18 ^a	ND	3 ^a	1 ^b	ND	5.25	0.006
107	2 ethyl pyrazine; hexanoic acid	3 ^b	1 ^b	ND	1 ^b	21 ^a	ND	ND	1 ^b	ND	1 ^b	1 ^b	9.58	0.000
115	Heptanone; heptanal	20 ^a	3 ^b	8 ^b	1 ^b	3 ^b	ND	28 ^a	2 ^b	28 ^a	2 ^b	1 ^b	8.99	0.001
116	Hexanoic acid; caproic acid	1 ^b	ND	2 ^{ab}	ND	ND	ND	15 ^a	2 ^{ab}	17 ^a	ND	1 ^b	3.18	0.035
126	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	18 ^a	ND	9.00	0.001
143	Nonanone; nonanal	6 ^{ab}	ND	14 ^a	1 ^{bc}	ND	ND	6 ^{ab}	1 ^{bc}	6 ^{ab}	1 ^{bc}	1 ^{bc}	3.04	0.041

^{a,b,c,d,e,f,g}After Duncan multiple range test, means within a row not sharing the same superscript were significantly different ($P < 0.05$). ND = Not detected.

¹Whey samples: ACASI = acid casein; CHEDA = Cheddar, company A; CHEDB = Cheddar, company B; CHEDP = Cheddar, pilot plant; GOUDA = Gouda cheese; LACAS = lactic acid casein; MOZZ = Mozzarella cheese; PAN = Paneer cheese; QUARG = Quarg cheese; RCASA = rennet casein, company A; RCASP = rennet casein, pilot plant.

² m/z = Mass to charge ratio.

³Tentative identification; (fr) = fragment, (r) = radical, NA = Not attributed.

Figure 2. Proton transfer reaction-mass spectrometry headspace profile of lactic acid casein whey sample.

2003; Mahajan et al., 2004), and fresh whey (Carunchia Whetstine et al., 2003). Knowledge of fractionation patterns, and isomeric and isobaric compounds in using the PTR-MS technique (Buhr et al., 2002) was also considered.

PCA of Volatile Compound Data

The ANOVA of PC scores of the volatile compound data, based on duplicate measurements (as for the sensory data), indicated that the first 9 PC significantly discriminated ($P < 0.05$) between samples (ANOVA not

shown). This finding demonstrated that PTR-MS is a technique capable of analyzing a remarkable sample complexity that can be reproducibly measured.

The PCA diagram in Figure 4 showed that the first PC was a function of volatile compounds that develop during fermentation in milk-based products (Reineccius, 1994), that is, acetaldehyde, 2,3 butadione (diacetyl), and 2 butanol-3-one (acetoin). Quarg and Mozzarella were positioned close to each other on the PCA diagram of PC 1 against PC 2 consistent with their sensory evaluation results (for odor). This PCA diagram explained 43% of the total variance and was based on

Figure 3. Proton transfer reaction-mass spectrometry headspace profile of Mozzarella whey sample.

Figure 4. Results of principal component analysis of volatile compounds (numbers refer to m/z) detected by proton transfer reaction-mass spectrometry showing the first 2 principal components (PC1 and PC2). Whey samples: ACASI = acid casein; CHEI(A) = Cheddar, company A; CHEI(B) = Cheddar, company B; CHEP = Cheddar, pilot plant; GOUDA = Gouda cheese; LA_CASEIN = lactic acid casein; MOZZ = Mozzarella cheese; PANEER = Paneer cheese; QUARG = Quarg cheese; RCASI = rennet casein, company A; RCASP = rennet casein, pilot plant.

significant masses only. Principal component 2 was mainly a function of volatile compounds such as 2-butanone (m/z 73), propionic acid (m/z 75), and butadiene (m/z 83); and PC 3 was essentially a function of propan-1-ol (m/z 43), an alkane fragment (m/z 71), diacetyl (m/z 87), and nonanone (m/z 143). The 9 PC that significantly discriminated ($P < 0.05$) between samples accounted for 98% (cumulative sum) of the total variance.

PLSR of Volatile Compound and Sensory Data

Partial least squares regression Type 2 (multiresponse) of chemical and sensory data fitted well the sensory and chemical data of this study (calibration correlation coefficient was > 0.7 for most characteristics); however, using this procedure, no PC was validated, possibly due to complex interrelationships between chemical compounds or high multiple collinearity of sensory attributes.

Partial least squares regression Type 1 (single response) was then applied as an alternative. Using this procedure, relationships between volatile compounds and sensory attributes were determined, one attribute at a time, and several successful regression models were

developed (Table 5). Attributes for which regression models with moderate to high validation coefficients ranging 0.40 to 0.94 were developed included “rancid” and “natural yogurt” odor and, to a lesser extent, “cheesy” and “dirty” odor, and “caramelized milk,” “sweet,” and “oaty” flavors.

“Rancid” odor could be determined by the presence of m/z 83 (attributed to butadiene or to 3-methyl furan) and 97 (not attributed), and the absence of m/z 42 (attributed to an allyl radical or to dimethylamine) and m/z 47 (attributed to ethanol). The regression model for “natural yogurt” odor also had a reasonable predictive ability based upon the presence of m/z 45 (attributed to acetaldehyde), 46 (attributed to a hydrocarboxyl radical), 87 (attributed to diacetyl), 104 (not attributed), 115 (attributed to heptanone), and 116 (attributed to hexanoic acid).

Most of the volatiles detected in this study have been reported to have flavor significance in dairy products but only some coincide with descriptive or associative terms (Bodyfelt et al., 1988). To our knowledge, there are no studies on flavor of acid whey obtained from mineral acid casein whey production, to which the results obtained could be compared. However, the deter-

Table 5. Results of partial least squares regression analysis, indicating positive and negative correlations between volatile compounds and specific sensory attributes. Integers refer to mass to charge ratio. Best fit calibration coefficients (measured) and validation coefficients (predictive ability) of models are included.

Attribute ¹	Positive correlation	Negative correlation	Calibration	Validation
(o)-Milky	42, 107	55, 83	0.70	0.40
(o)-Rancid	83, 97	42, 47	0.96	0.94
(o)-Cheesy	143	104	0.86	0.63
(o)-Dirty	55, 83	47, 115	0.78	0.45
(f)-Caramelized milk	42, 126	59, 73, 83	0.75	0.60
(f)-Natural yogurt	45, 46, 87, 104, 115 116		0.78	0.65
(f)-Sweet	126	45, 59, 61, 73	0.76	0.60
(f)-Oaty	87, 126	59, 73	0.75	0.60

¹(o) = Odor; (f) = flavor.

mination of moderate validation coefficients may help to understand the importance of the proportions and even absence of volatile compounds to elicit particular sensory characteristics such as “dirty” odor, which characterized acid casein wheys, and “oaty” odor and flavor, which characterized rennet casein wheys in this study.

CONCLUSIONS

Cheddar, Gouda, and rennet wheys (“sweet” cheese and rennet wheys), as raw materials, provided a bland, “sweet”/“milky” flavor base, adequate for producing liquid dairy beverages. Differentiation of flavor of whey based on the activity of the starter cultures used for cheese making at the time of whey drainage was limited to the extent in which a nonspecific “cheesy” aroma (as for unripe cheese) was perceived in cheese whey (Gouda and Cheddar) samples.

Results suggested that starter cultures might influence flavor of whey to different degrees, acquiring a more specific cheese-like character relative to the variety from which it originated, only if fermentation progresses to low pH values before drainage of whey. Precise control of the temperature of whey or inactivation of starter cultures, particularly fast-acidification or fast-fermenting commercial strains, is therefore important. For other types of products to be made from whey, a cheese-like flavor could be advantageous.

The absence of a starter culture in the process of obtaining rennet whey (both industrial and pilot-plant scale) led to a sweet-tasting product with a subtle odor suitable as a neutral base for production of dairy-type beverages.

On the other hand, off-flavors such as “bitter,” “stale,” “rancid,” and “chemical” perceived in acid casein whey samples may be caused by reactions induced by the acid added during the manufacture of the casein.

The use of PTR-MS provided a reliable volatile fingerprint of each sample, useful for quality control purposes, as it efficiently provided chemical information

related to the sensory quality of the raw material. Application of PTR-MS is also capable of guiding a more effective and efficient product development through the potential for generation of a desired sensory profile starting from adequate sensory characteristics in the raw material.

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