

Composition and Aroma Compounds of Ragusano Cheese: Native Pasture and Total Mixed Rations*

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ABSTRACT

Raw milk from 13 cows fed TMR supplemented with native pasture and from 13 cows fed only TMR on one farm was collected separately 4 times with an interval of 15 d between collections. Two blocks (14 kg each) of cheese were made from each milk. The objective was to determine the influence of consumption of native plants in Sicilian pastures on the aroma compounds present in Ragusano cheese. Milk from cows that consumed native pasture plants produced cheeses with more odor-active compounds. In 4-mo-old cheese made from milk of pasture-fed cows, 27 odor-active compounds were identified, whereas only 13 were detected in cheese made from milk of total mixed ration-fed cows. The pasture cheeses were much more rich in odor-active aldehyde, ester, and terpenoid compounds than cheeses from cows fed only total mixed ration. A total of 8 unique aroma-active compounds (i.e., not reported in other cheeses evaluated by gas chromatography olfactory) were detected in Ragusano cheese made from milk from cows consuming native Sicilian pasture plants. These compounds were 2 aldehydes ([E,E]-2,4-octadienal and dodecanal), 2 esters (geranyl acetate and [E]-methyl jasmonate), 1 sulfur compound (methionol), and 3 terpenoid compounds (1-carvone, L(-) carvone, and citronel-

lol). Geranyl acetate and (E)-methyl jasmonate were particularly interesting because these compounds are released from fresh plants as they are being damaged and are part of a possible plant defense mechanism against damage from insects. Most of the odor-active compounds that were unique in Ragusano cheese from pasture-fed cows appeared to be compounds created by oxidation processes in the plants that may have occurred during foraging and ingestion by the cow. Some odor-active compounds were consistently present in pasture cheeses that were not detected in the total mixed ration cheeses or in the 14 species of pasture plants analyzed. Either these compounds were present in other plants not analyzed, created in the rumen or in cheese after the pasture-plant material had been consumed, or the compounds were lost in the method of sample extraction used for the plant analysis (i.e., steam distillation) versus the solid-phase microextraction method used for the cheeses. This research has demonstrated clearly that some unique odor-active compounds found in pasture plants can be transferred to the cheese.

(**Key words:** pasture, Ragusano cheese, aroma)

Abbreviation key: C/F = casein to fat ratio, FDB = fat on a dry basis, GC/MS = gas chromatography mass spectrometer, GCO = gas chromatography olfactometry, PDB = protein on a dry basis, SD = steam distillation, SN = soluble nitrogen, SORt = standard odor retention times, SPME = solid phase microextraction.

INTRODUCTION

It has been known for a long time that the diet of cows and sheep can influence the flavor of meat (Reagan et al., 1977; Field et al., 1983; Melton, 1983, 1990) and milk (Keen and Wilson, 1993). These impacts are not

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always desirable. Traditional cheeses are typically produced in small factories or on farms using a combination of preserved feeds and fresh pasture. The sensory properties of the cheeses produced in this way often reflect the characteristics of the fresh pasture plants (Dumont and Adda, 1978; Mariaca et al., 1997; Viallon et al., 2000; Bugaud, 2001b) and the contribution of the natural microflora from both the animal and cheese-making environment. Ragusano cheese is an example of a traditional aged pasta-filata Italian hard cheese produced in the Hyblean region of Sicily (Licitra et al., 1998). There are many other varieties of traditional cheeses produced throughout Europe. Often these cheeses have unique sensory characteristics that can be seasonal in nature that derive from the characteristics of the native plant species in the local pastures and the interaction of those plants with the soil and climate of the area. However, milk production practices in almost all areas are changing, and the pressure for farms to remain economically viable has increased the amount of conserved feeds and TMR feeding.

In a previous report on Ragusano cheese (Carpino et al., 2004), it was reported that there was a difference ($P < 0.05$) in color between cheeses produced from milk of cows consuming pasture versus TMR, with the pasture cheeses being more yellow. In addition, the cheeses from pasture-fed cows had significantly more floral and green odor measured by quantitative descriptive analysis of the cheeses (Carpino et al., 2004). The pastures are very rich in plants that contain a wide variety of carotenoid compounds, and during the spring, when cheese flavor quality is considered the best, many of the plant species are in flower with yellow and orange colors. Whereas carotenoids are distributed throughout the structure of plants (Britton, 1995), there is a high level of carotenoid synthesis in the flowers (Britton, 1998), for example, *Calendula*. When fresh pasture plants are consumed by cows, the damaged plants quickly activate the lipoxygenase system (Galliard and Chan, 1980; Belitz and Grosch, 1986; Wu and Robinson, 1999) that begins to break down carotenoids and lipids into a range of important volatile aroma compounds (Wachè et al., 2002) and nonvolatile compounds that can have important sensory impacts in food systems. The carotenoids, lipids, and related degradation products of these compounds are consumed by the cow, and then they are taken up at the intestinal mucosa, incorporated in chylomicra, which are transferred through the lymphatic system and then to the bloodstream (Noble, 1981; Parodi, 1996). The uptake of degradation products of lipophylic materials from plants at the mammary cell from the bloodstream will probably be in conjunction with the normal uptake of other neutral lipids. A second possible mechanism of uptake (Dou-

gherty et al., 1962) is from volatile plant odors that are inhaled by the cow during the consumption of the forage. These compounds could pass very quickly through the bloodstream into the milk.

While a wide range of volatile compounds can and have been extracted, identified, and measured with a gas chromatography mass spectrometer (GC/MS), only a small fraction of those compounds in milk and cheeses elicit a sensory response in humans. To differentiate odor-active volatile compounds from those that have no odor activity, gas chromatography olfactometry (GCO) was used in the present study (Acree et al., 1984) in combination with compound identification by mass spectrophotometry, retention index, and authentic reference compound matching to identify compounds detected in the cheeses. The impact of consumption of fresh pasture plants or different types of pasture plants on the composition of the volatile compounds in cheese (Dumont and Adda, 1978; de Frutos et al., 1991; Mariaca et al., 1997; Bugaud et al., 2001a, 2001b, 2001c) has been reported for several cheese varieties. This is particularly important in documenting the unique characteristics of European cheeses that received the Protected Denomination of Origin (PDO) designation. Ragusano cheese received this designation (Gazzetta Ufficiale della Comunità Europea, 1996). The objectives of our study were to identify plant species eaten by cows during grazing, to compare the odor-active compounds in the Ragusano cheese from cows eating TMR and native Sicilian pasture-supplemented TMR diets, and to provide the first qualitative characterization of the odor-active compounds present in Ragusano cheese.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

The experiment was conducted in spring 1999 (February to May) when native pasture was available. One farm was involved in the experiment. This farm had all the typical characteristics of a farmstead cheese producer: native pasture, TMR facility, and a sufficient number of cows to select a similar stage lactation group (cows in the late stage of lactation >150 d) for each feeding treatment. The details of diet and cow management have been described previously (Carpino et al., 2004). Raw milk from 13 cows fed TMR supplemented with native pasture and from 13 cows fed only TMR was collected separately 4 times with an interval of 15 d between collections. The 2 batches of milk (i.e., pasture and TMR) were promptly transported to the CoR-FiLaC's pilot plant, and Ragusano cheese was produced from each batch as described by Melilli et al. (2003), as modified by Carpino et al. (2004), to make 4 different blocks of cheese (2 from each vat) every 15 d. The ap-

proximate weight of a block of cheese was 14 kg. Sixteen blocks of cheese were manufactured. Eight blocks were from the raw milk of cows fed with native pasture and 8 from cows fed TMR.

The 16 blocks of cheese were aged in an aging center. Once the brine-salting stage was completed at the aging center, the cheeses were aged in ventilated rooms at 14 to 16°C. At 4 mo, each 14-kg block was cut in half. One half was used for the evaluation at 4 mo, whereas the cut surface of the other half was sealed with Rindol A (Paramelt B. V. Heerhugowaard, The Netherlands) and returned to the aging center for chemical analysis at 7 mo. The half block was cut in cubes (1 cm³), the cubes were mixed, and then a randomly selected portion of the cubes was removed for chemical and sensory analysis. The sensory analysis has been reported (Carpino et al., 2004).

Data for cheese composition and for total FFA and individual FFA were analyzed using the GLM procedure of SAS (Version 8, 1999, SAS Institute, Cary, NC). For analysis of the cheese composition data, the ANOVA model included terms for the effect of feed type (pasture vs. TMR), day of cheese making (0, 15, 30, and 45), both as a linear and quadratic term, and the interaction of feed with the linear term for day of cheese making. No significant interactions of feed type by the quadratic term for day of cheese making were found, and this term was dropped from the model. When the day of cheese making was treated as a continuous variable in the ANOVA model, the linear and quadratic terms for day of cheese making would be correlated. Distortion of the ANOVA model by multicollinearity of these terms was minimized by transforming the day of cheese making (Glantz and Slinker, 2001). The day was transformed as follows: time = day of cheese making - [(last day of cheese making - first day of cheese making)/2]. This transformation made the data set orthogonal with respect to day of cheese making. For statistical analysis of the mean total and mean individual FFA concentration data, a *t*-test (LSD, *P* < 0.05) was used to determine if there was a significant effect of feed type on FFA content of the cheeses.

Sampling of Pasture Plants

On each sampling day, 4 of the cows were selected randomly for observation of their pasture grazing. Each cow was followed as closely as possible for a period of about 20 min. During that time, the types of plants selected and the plant parts selected were recorded. Fourteen individual plant species consumed by the cows were selected for analysis to represent the major plants in the diet and the diversity of plant species consumed by the cows in this study. These 14 plant

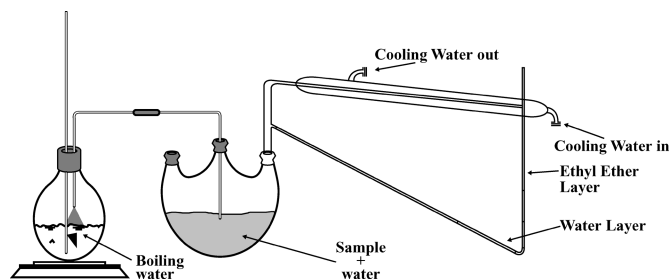


Figure 1. Steam distillation apparatus.

species were sampled once during the study for determination of the odor-active compounds present in each species during the cheese-making trial. Plant samples were collected, brought directly to the lab, and frozen at -40°C.

Composition Analysis of Cheeses

All analyses were done in duplicate. The cheese analyses were the following: moisture content was determined by drying a 3-g sample in a forced-air oven at 100°C for 24 h (AOAC, 2000, method number 33.2.44; 990.20), the salt content by the Volhard method (AOAC, 2000, method number 33.7.1; 935.43), and total nitrogen by Kjeldahl using a 1-g sample size (AOAC, 2000; method number 33.2.11, 991.20) with conversion to protein content using a factor of 6.38. The fat content of cheese was determined by the Gerber method using a butyrometer with a calibrated range from 0 to 40% fat (Licitra et al., 2000). During cheese aging, pH 4.6 acetate buffer and 12% TCA soluble nitrogen (SN) were determined (Bynum and Barbano, 1985) and expressed as a percentage of the total nitrogen content of the cheese at each stage of aging. Proteolysis during aging may contribute to both texture and flavor development in Ragusano cheese. The quantity of total FFA and individual FFA was determined using a resin binding technique to remove FFA from an acidic ether extract of cheese, followed by quantitative GLC analysis (IDF, 1991). Changes in FFA content of Ragusano cheese may be related to flavor development.

Isolation and Separation of Aroma Compounds from Plants and Cheese

Volatile compounds were extracted from 100 g of frozen plant sample by SD (Figure 1) for 1 h, and the extract was collected in 20 mL of ethyl ether. The plant extracts were kept in 3 mL-glass Teflon capped vials at 4°C until analysis. Frozen cheese samples were warmed to 35°C and were extracted using a solid-phase microextraction (SPME) apparatus; SPME is an ex-

traction method utilizing a 1 to 2 cm of fiber coated with a stationary phase that extracts volatiles from liquids and headspace gases. In preliminary work, fibers of varying polarities and phase thickness were evaluated for use with cheese at 35°C (data not shown).

Carbowax/nonpolar polydimethylsiloxilane, polydimethylsiloxilane, and carbowax/divinylbenzene-coated fibers were used to test extraction of several Ragusano cheese samples. Carbowax/divinylbenzene was selected because of its ability to extract a wide range of low- to mid-molecular weight molecules commonly found and reported in the literature (Kataota et al., 2000) to be important to cheese quality.

The carbowax/divinylbenzene SPME system recovered 18 compounds detectable by GCO, whereas the other 2 only recovered 11 (data not shown). Therefore, an SPME fiber with 65 μm thickness of carbowax/divinylbenzene coating (Supelco Co. Bellefonte, PA) was used. Before the initial use, the fiber was conditioned for 1 h at 250°C. Before each extraction, the fiber was held at 225°C for 5 min and allowed to come to room temperature for 10 min. The fiber was exposed to headspace gases 1 cm above 5 g of grated cheese, in a sealed 20-mL vial for 1 h at 35°C. A temperature of 35°C was selected to provide a temperature similar to that expected in an oral nasal cavity. After extraction of volatiles from the headspace, the fiber was placed in the injection port of the GLC to release the volatile compounds. The plunger depth was set at 3 cm to allow for maximum desorption by injecting into the hottest part of the injection port.

Gas Chromatography Olfactometry Analysis of Plants and Cheese

Gas chromatography olfactometry (GCO) was conducted on the cheese samples and plant materials using charm analysis (Acree et al., 1984). The SD extracts from the 14 plant species were analyzed in triplicate, and the SPME extracts of each of the 16 cheeses were run in duplicate using an OV101 HP-fused silica capillary column coated with cross-linked methyl silicone (12 m $\times$ 0.32 μm , film thickness 0.52 μm) installed in a high-resolution GCO made from a modified Hewlett Packard 5890 gas chromatograph (Datu Inc., Geneva, NY).

Chromatographic conditions were as follows: the GLC oven was temperature programmed from 35 to 190°C at 4°C/min (first held for 3 min), from 190 to 225°C at 30°C/min, and held for 3 min with helium as the carrier gas. The injection temperature was at 250°C. A single sniffer was trained for GCO analysis with a procedure and standard compound mixture specifically designed for GCO subject selection (Marin et

al., 1988). The mixture contained 8 compounds designed to evaluate a person's olfactory acuity and to determine if the sniffer has anosmia for some specific common odors. The sniffer had no anosmia for these compounds. The sniffer was exposed for 30 to 35 min to a continuous stream of humidified air (10 L/min) mixed with the GLC effluent in a high resolution GCO (Acree et al., 1976). The times after injection and duration of the responses to odor were recorded, along with a word chosen to describe the odor character of each response. The odor information was converted to retention index information (Acree et al., 1984). Data were collected and stored using the Charmware 1.08 software (Datu Inc., Geneva, NY). Normal hydrocarbons from heptane to octadecane were chromatographed daily with the GLC in the flame-ionization detection mode, and their retention times used to convert GCO retention values to retention indices (Kovats et al., 1965). Selected common odor reference compounds were also used as identification standards. These were used as chemical standard odor retention times (**SORt**). Each odor detected in the analysis was defined by a retention index value and an odor description.

Gas Chromatography Mass Spectrometry

Volatile compounds from both types of extraction were analyzed using an HP 6890 GLC and 5973 mass selective detector fitted with a nonpolar capillary column HP-1 (25 m $\times$ 0.20 μm i.d., film thickness 0.11 μm , HP 19091 A-002-methyl siloxane). The gas chromatograph oven was temperature-programmed (first held for 3 min) from 35 to 190°C at 4°C/min, from 190 to 225°C at 30°C/min, and held for 3 min, with helium as the carrier gas. The injection temperature was at 250°C. The mass selective detector scanned ions 33 to 400 atomic mass units. To correlate gas chromatography mass spectrometer (**GC/MS**) results to the GCO retention index information for odor-active compounds, a paraffin series (C7 to C18) was run on the GC/MS under the above conditions and peak times for the alkanes integrated and reported. Kovats retention indices were calculated from the intervals between the carbons for all peaks eluting in subsequent cheese sample runs under the same chromatographic conditions.

RESULTS AND DISCUSSION

Cheese Composition and Proteolysis

Ragusano cheese is an aged pasta-filata cheese made from raw milk (Licitra et al., 1998, 2000). Time-dependent changes in composition are the largest during brine salting (Melilli et al., 2003) and continue during aging (Licitra et al., 2000). The average moisture of

Table 1. Influence of feed type on composition of Ragusano cheeses (n = 4) at 4 and 7 mo of age made from milk produced by pasture and TMR fed cows.

Component	Pasture	TMR	Feed	Day	Day × day	Day × feed	R ²
4 mo							
Moisture, %	38.29	39.57	NS ¹	***	NS	NS	0.76
Fat, %	28.20	26.94	NS	**	NS	NS	0.70
Protein, %	26.57	26.98	NS	NS	NS	*	0.55
Salt, %	3.41	3.23	NS	NS	NS	NS	0.33
pH 4.6/TN, % ²	14.18	15.48	NS	NS	NS	NS	0.49
12% TCA/TN, % ³	12.72	14.15	NS	NS	NS	NS	0.41
NaCl/M, % ⁴	8.95	8.18	NS	NS	NS	NS	0.46
FDB, % ⁵	45.68	44.58	NS	NS	NS	NS	0.46
PDB, % ⁶	43.07	44.64	NS	***	NS	***	0.90
pH	5.42	5.51	NS	NS	NS	NS	0.34
7 mo							
Moisture, %	34.49	35.48	NS	***	**	NS	0.87
Fat, %	30.03	28.28	NS	***	NS	*	0.88
Protein, %	28.14	28.82	NS	***	*	NS	0.87
Salt, %	4.10	4.30	NS	NS	*	NS	0.52
pH 4.6/TN, %	MD ⁷	MD	...	...	...	...	...
12% TCA/TN, %	MD	MD	...	...	...	...	...
NaCl/M, %	12.08	12.23	NS	**	**	NS	0.74
FDB, %	45.78	43.82	NS	***	NS	*	0.79
PDB, %	43.00	44.66	NS	*	*	*	0.85
pH	5.36	5.42	NS	NS	NS	NS	0.52

P* < 0.05; *P* < 0.01; ****P* < 0.001.¹NS = not significant, *P* > 0.05.²pH 4.6/TN = pH 4.6 acetate buffer soluble nitrogen as a percentage of total nitrogen.³12% TCA/TN = 12% trichloroacetic acid soluble nitrogen as a percentage of total nitrogen.⁴NaCl/M = ((sodium chloride)/moisture) × 100.⁵FDB = Fat on dry basis.⁶PDB = Protein on dry basis.⁷MD = missing data.

Ragusano cheese decreases from about 44 to 35%, whereas fat (26 to 28%) and protein (26 to 27.5%) content increase during 1 yr of aging (Licitra et al., 2000).

Significant impacts of day of cheese making and pasture feeding on cheese composition were detected (Table 1). The cheese making spanned a period of 45 d when pasture quality was excellent. However, even during this period, the presence of various plant species and the mix of maturity levels among plant species were changing and could have influenced milk and cheese composition. In addition, the cows were at >150 d in lactation, so stage of lactation impacts on milk composition during the 45 d-period of cheese making was also expected to influence milk composition (and indirectly cheese composition) for both groups of cows. Mean milk casein and fat from pasture and TMR-fed cows were 2.55 and 2.62% casein and 3.44 and 3.30% fat, respectively. The mean milk casein content was relatively constant during the cheese-making period for both pasture- and TMR-fed cows, but the fat content of the milk increased during the period of the study. The milk casein to fat ratio (C/F) was 0.74 for pasture and 0.79 for TMR (data not shown), with the ratio staying relatively constant during the cheese-making period for TMR,

while the C/F ratio for the pasture milk decreased from about 0.80 to 0.68 over the 45 d cheese-making period. These differences in milk composition were expected to influence cheese composition. Previous work on another pasta filata cheese (Rudan et al., 1999) demonstrated that as C/F ratio decreased, cheese moisture and protein concentration decreased, whereas fat content and fat on dry basis (FDB) increased and protein on dry basis (PDB) decreased. In general, the differences (Table 1) in moisture (Figure 2), fat, FDB, and PDB (Figure 3) for pasture and TMR cheeses were due to the differences in milk C/F ratio. The lower milk C/F ratio and the larger decrease in C/F ratio during the cheese-making period for the milk of pasture-fed cows was related to the detection of significantly lower PDB at 4 and 7 mo and a higher FDB at 7 mo (Table 1).

High- plus low-molecular weight products of proteolysis (i.e., extent of proteolysis) are reflected by the amount of pH 4.6 SN, whereas the amount of low molecular weight proteolysis products (i.e., depth of proteolysis) is reflected by the amount of 12% TCA SN developed during cheese aging. The extent of proteolysis (i.e., pH 4.6 SN) at 4 mo (Table 1) was similar to previously reported values, but the depth of proteolysis (i.e., 12%

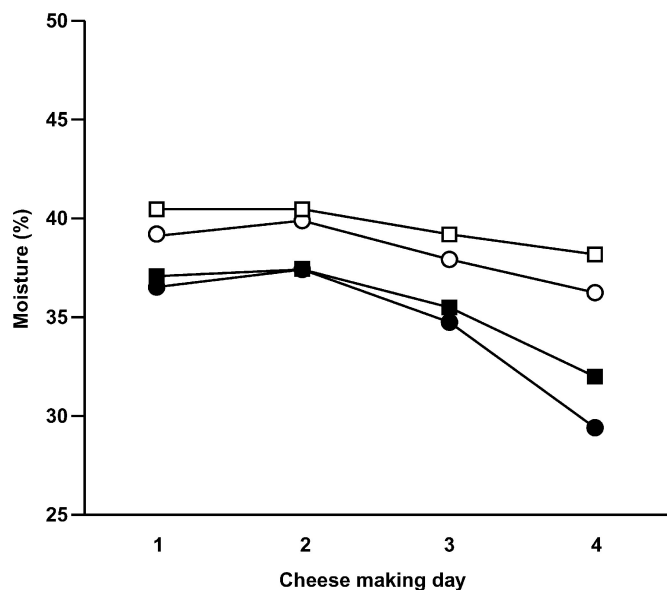


Figure 2. Average moisture content of cheese for the 4 different days of cheese making 1 = d 0, 2 = d 15, 3 = d 30, and 4 = d 45 for pasture fed cows at 4 mo (○), and 7 mo (●) and TMR fed cows at 4 mo (□) and 7 mo (■).

TCA SN) was greater for the cheeses in the present study than in a previous survey (Licitra et al., 2000). The reason for this difference from previously reported data is not known. No differences ($P > 0.05$) in SN content of cheeses due to pasture feeding were detected.

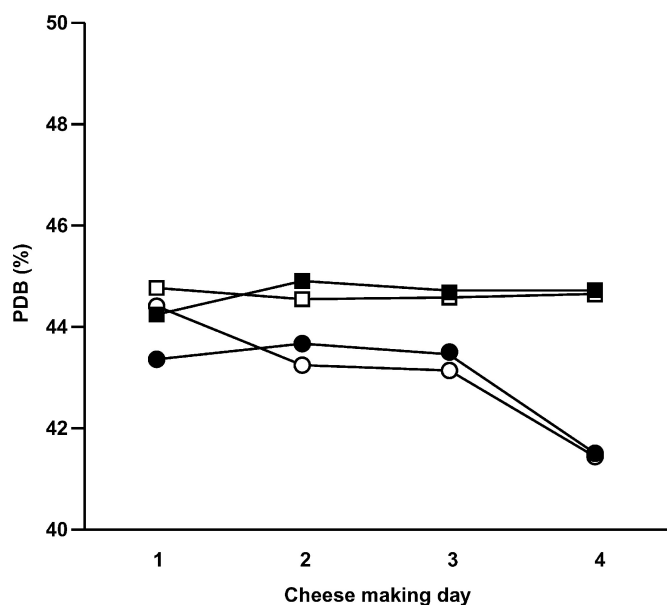


Figure 3. Average protein on a dry basis (PDB) content of cheese for the 4 different days of cheese making 1 = d 0, 2 = d 15, 3 = d 30, and 4 = d 45 for pasture fed cows at 4 mo (○) and 7 mo (●) and TMR fed cows at 4 mo (□) and 7 mo (■).

Free Fatty Acid Content

High levels of FFA are characteristic of Italian hard cheeses and contribute to typical flavors and aromas (Battistotti and Corradini, 1993). Aged Ragusano cheese contains levels of FFA (Licitra et al., 2000) similar to Provolone (De Felice et al., 1999). No differences ($P > 0.05$) were detected in the mean total FFA content (Table 2) within both 4- and 7-mo old cheeses due to feed type, and the levels were similar to a previous report (Licitra et al., 2000). Butyric (C4) as a percentage of the total FFA content was in range of 44 to 51% of the total FFA for the cheeses in the present study. Previously reported values for Ragusano cheese (Licitra et al., 2000) for butyric acid were about 17 to 22% of the total FFA content in cheese. The cheeses in the present study were made from milk produced on one farm with the cheese produced in the pilot plant at CoRFiLaC, whereas the previous report (Licitra et al., 2000) reflected an average of cheeses made on 10 different farms. The medium- and long-chain FFA (C12 to C18:2) at 4 and (C10 to C18) 7 mo were consistently present in higher concentration ($P < 0.05$) in pasture cheese, but the reason for this is not clear. The differences in FFA content due to feed type were relatively small compared with the increase in level of all FFA with cheese age (Table 2). Short and intermediate chain length FFA in Ragusano cheese can contribute directly to both flavor and aroma and can act as precursors to the production of other flavor and aroma compounds. Short chain FFA may interact with alcohols (produced in low concentration by bacteria) present in cheese to produce esters (McSweeney and Sousa, 2000) such as, ethyl butyrate and ethyl hexanoate.

Unique Odor-Active Compounds in Ragusano Cheese

Odor-active compounds detected by GCO in the cheese after 4 (Table 3) and 7 mo (Table 4) of aging are shown. In the headspace, 31 individual odor-active compounds were detected in the 4-mo-old cheeses, whereas 23 were detected in the 7-mo-old cheeses. Different classes of odor compounds were identified, including 3 acids, 8 aldehydes, 1 alkane, 5 esters, 4 ketones, 1 lactone, 1 pyrazine, 4 sulfur compounds, 3 terpenoids, and 2 unknowns.

Many of the same odor-active compounds have been reported as constituents of other cheeses (Curioni and Bosset, 2002), but the present study shows, for the first time, the specific compounds identified in Ragusano cheese. Acetic, butyric, and hexanoic acids are common odor-active FFA contained in cheeses. These have been detected in many cheese varieties by GCO (Curioni and Bosset, 2002) and were expected in Ragusano cheese.

Table 2. Free fatty acid content (mg/100 g of cheese) of the Ragusano cheeses (n = 4) at 4 and 7 mo of age made from milks produced by pasture and TMR fed cows.

FFA	4 mo			7 mo		
	Pasture	TMR	LSD ¹	Pasture	TMR	LSD
C4	149.68	168.34	NS ²	245.91	233.41	NS
C6	33.23	33.36	NS	57.35	54.79	NS
C8	7.85	7.26	NS	14.69	3.86	NS
C10	14.78	13.13	NS	25.02 ^a	22.84 ^b	1.54
C12	13.16 ^a	11.38 ^b	1.34	20.55 ^a	18.65 ^b	0.91
C14	20.33 ^a	15.80 ^b	2.25	32.43 ^a	28.36 ^b	2.63
C14:1	3.46 ^a	2.79 ^b	0.38	5.23 ^a	4.76 ^b	0.36
C16	40.20 ^a	30.92 ^b	5.45	63.08 ^a	56.31 ^b	5.71
C16:1	3.46 ^a	2.75 ^b	0.47	5.96 ^a	5.12 ^b	0.52
C18	8.70 ^a	6.52 ^b	1.38	13.53 ^a	12.04 ^b	1.32
C18:1	34.98	29.70	NS	52.42	49.95	NS
C18:2	8.75 ^a	7.03 ^b	1.42	13.71	12.70	NS
Total	338.67	329.02	NS	549.88	512.81	NS

^{a,b}Means within the same age not having a common superscript differ ($P < 0.05$).

¹LSD = least significance difference ($P < 0.05$).

²NS = not significant.

Phenylacetaldehyde, nonanal, (E)-2-nonenal, and (Z)-2-nonenal are odor-active compounds that have been detected by GCO in a variety of cheeses (Curioni and Bosset, 2002), whereas 2,4-decadienal and vanillin have only been reported in Gruyere and Emmental, respectively. Two aldehydes that have not been previously reported in cheese (E,E-2,4-octadienal and dodecanal) were detected by GCO in the Ragusano cheese in the present study. An alkane, hexadecane, was also found in Ragusano cheese and has not been previously identified by GCO in other cheeses. Ethyl 2-methyl butyrate has been detected and reported for Gruyere cheese (Rychlik and Bosset, 2001) and was also found in Ragusano, whereas ethyl hexanoate has been reported in many varieties of cheese (Curioni and Bosset, 2002). Two unique odor-active esters were found in Ragusano—geranyl acetate and (E)-methyl jasmonate—that have not been reported in other cheeses. All of the odor-active ketones and delta decalactone found in Ragusano (Tables 3 and 4) have also been reported for numerous other cheeses (Curioni and Bosset, 2002). Three sulfur-containing, odor-active compounds (dimethyl disulfide, methional, and dimethyl tetrasulfide) that were detected in Ragusano cheese by GCO have also been reported for numerous other cheeses (Curioni and Bosset, 2002); however, methionol was detected in Ragusano cheese and has not been reported previously. The odor-active terpenoid compounds (1-carvone, L(-)-carvone, and citronellol) were found in Ragusano but have not been detected previously by GCO in other cheeses. Thus, a total of 9 unique odor-active compounds were detected in the Ragusano cheese in this study, and, of these, 8 were only found in the cheese produced by cows consuming fresh native pasture (Ta-

bles 3 and 4). This clearly indicates that consumption of fresh native-pasture plants in the region of cheese production contributes to the presence of specific odor-active compounds in the cheese.

The relative impact of each of these unique compounds (detected in the present study) on the overall aroma of the cheese was not determined because the data in the present study were qualitative. No relative odor-intensity data or quantitative measures of the concentration of these components are currently available. The cheeses analyzed in this study were from one farm, with one, very well-characterized “typical” native pasture (Carpino et al., 2004). Future work to define typical Ragusano cheese aroma needs to systematically analyze cheeses from a large number of different farms, at different points in the forage season, to obtain a more representative qualitative picture of the diversity of odor-active compounds present in the population of Ragusano cheeses. At the same time, the intensity of the various odor-active compounds needs to be measured to define which compounds make the largest contribution to the aroma profile of Ragusano cheese.

Effect of Pasture on Aroma Profile

There is a large body of anecdotal evidence about the desirable effect of spring and summer grasslands on the flavor of dairy products (Wigan, 1951). Urbach (1990) reported regional differences in butter color and flavor. The GC-MS analysis performed on Beaufort cheese volatiles has led to the identification of 140 components, including 9 sesquiterpenes. Sesquiterpenes were only found in cheeses made from summer milk when cows were grazing on high-altitude pastures (Dumont and Adda, 1978). The transfer of mono and sesquiterpenes

Table 3. Comparison of odor compounds isolated with solid phase microextraction (SPME) and detected by gas chromatography olfactometry (GCO) in pasture (n = 4) and TMR cheeses (n = 4) at 4 mo of aging.

Odor description	RI ¹	Odorant	Compound class	Identification method	Cheese	
					Pasture	TMR
Acetic	747	Acetic acid	Acid	MS ² /PI ³	+ ⁴	+
Dirty-sock	823	Butyric acid	Acid	MS/PI	+	+
Sweaty	890	Hexanoic acid	Acid	PI	— ⁵	+
Daisy	1007	Phenylacetaldehyde	Aldehyde	MS/PI	+	—
Soapy	1079	Nonanal	Aldehyde	MS/PI	+	+
Green flower	1086	(E,E)-2,4-octadienal	Aldehyde	SORt ⁶ /PI	+	—
Green	1128	(E)-2-nonenal	Aldehyde	MS/PI	+	—
Hay	1133	(Z)-2-nonenal	Aldehyde	MS/PI	+	—
Fried	1290	2,4-decadienal	Aldehyde	MS/PI	+	—
Vanilla	1348	Vanillin	Aldehyde	MS/SORt/PI	+	—
Lilly	1391	Dodecanal	Aldehyde	PI	+	—
Woody	1100	Hexadecane	Alkane	PI	—	+
Fruity	787	Ethyl butyrate	Ester	MS/SORt/PI	+	+
Sweet	835	Ethyl 2-methyl butyrate	Ester	SORt/PI	+	—
Orange	981	Ethyl hexanoate	Ester	MS/PI	+	+
Fresh flower	1560	Geranyl acetate	Ester	MS/PI	+	—
Jasmine	1612	(E)-methyl jasmonate	Ester	MS/PI	+	—
Buttery	701	3-Hydroxy-2-butanone	Ketone	PI	+	—
Mushroom	956	1-octen-3-one	Ketone	MS/PI	+	+
Body odor	1055	8-nonen-2-one	Ketone	PI	—	+
Hot milk	1080	2-nonanone	Ketone	MS/PI	+	—
Peach	1452	δ -decalactone	Lactone	SORt/PI	+	—
Nutty	883	2,6 dimethyl pyrazine	Pyrazine	PI	+	—
Onion	725	Dimethyl disulfide	Sulfur	MS/SORt/PI	+	—
Potato	864	Methional	Sulfur	MS/SORt/PI	+	+
Orange	941	Methionol	Sulfur	PI	+	—
Sulfur	1173	Dimethyl tetrasulfide	Sulfur	MS/PI	+	+
Herbaceous	1210	1-carvone	Terpenoide	SORt/PI	+	—
Rose	1228	Citronellol	Terpenoide	SORt/PI	+	—
Metallic	912	Unknown	...	NI ⁷	—	+
Sulfuric	936	Unknown	...	NI	+	+

¹RI = retention index.²MS = GC/mass spectrophotometer.³PI = published index (Flavornet – Acree T. E. and Arn H. 1997–1999 – ©Cornell University GCO of natural products (<http://www.nysaes.cornell.edu/flavornet>)).⁴+ Indicates the compound was detected in each of the four cheeses.⁵— Indicates the compound was not detected in any of the four cheeses.⁶SORt = chemical standard odor retention time.⁷NI = not identified.

from diet to milk and cheese has also been reported by others (Bugaud et al., 2001a, 2001b, 2001c; deFrutos et al., 1991; Mariaca et al., 1997). Pastures rich in dicotyledons, in particular *Apiaceae*, contained a greater quantity and a wider variety of terpenes than milks from pastures rich in *Gramineae* (Bugaud et al., 2001a). It has been suggested that carotenoids and carotenoid-derived compounds in cheeses could be used as biomarkers of origin of production (Viallon et al., 1999; Prache et al., 2002), of botanical composition, and of even stage of maturity of the forage. Viallon et al. (1999, 2000) did a controlled study to determine the relationship between volatile terpenes in both forage and cheese. They found 15 volatile terpenoid compounds in the Saint-Nectaire-type cheese. Of the variation in the volatile terpenes in the cheese, 71% was explained by

the type of forage and the amount of mono and sesquiterpenes in milk. This percentage increased and decreased quickly when certain plants were added or removed from the diet (Viallon et al., 2000). Viallon et al. (1999) indicated that the aroma activity of these compounds remains to be demonstrated. Carotenoid and related terpene compounds are not the only volatile compounds transferred from diet to cheese. Our results are novel because they relate odor-active compounds present in fresh pasture plants to their presence in cheese and demonstrate a difference in odor-active compounds in cheese produced from cows eating only TMR versus those consuming fresh pasture as part of their diet (Carpino et al., 2004).

In the 4-mo-old cheese, 27 individual odor-active compounds were detected in the pasture cheeses, whereas

Table 4. Comparison of odor compounds isolated with solid phase micro extraction (SPME) and detected by gas chromatography olfactometry (GCO) in pasture (n = 4) and TMR (n = 4) cheeses at 7 mo of aging.

Odor description	RI ¹	Odorant	Compound class	Identification method	Cheese	
					Pasture	TMR
Acetic	747	Acetic acid	Acid	MS ² /PI ³	+ ⁴	+
Dirty-sock	823	Butyric acid	Acid	MS/PI	+	+
Sweaty	890	Hexanoic acid	Acid	PI	- ⁵	+
Daisy	1007	Phenylacetaldehyde	Aldehyde	MS/PI	+	-
Soapy	1079	Nonanal	Aldehyde	MS/PI	+	+
Green flower	1086	(E,E)-2,4-octadienal	Aldehyde	SORT ⁶ /PI	+	-
Green	1128	(E)-2-nonenal	Aldehyde	MS/PI	+	-
Hay	1133	(Z)-2-nonenal	Aldehyde	MS/PI	+	-
Fried	1290	2,4-decadienal	Aldehyde	MS/PI	+	-
Vanilla	1348	Vanillin	Aldehyde	MS/SORT/PI	+	-
Fruity	787	Ethyl butyrate	Ester	MS/SORT/PI	+	+
Orange	981	Ethyl hexanoate	Ester	MS/PI	+	+
Fresh flower	1560	Geranyl acetate	Ester	MS/PI	+	-
Jasmine	1612	(E)-methyl jasmonate	Ester	MS/PI	+	-
Buttery	701	3-Hydroxy-2-butanone	Ketone	PI	+	-
Mushroom	956	1-octen-3-one	Ketone	MS/PI	+	+
Hot milk	1080	2-nonanone	Ketone	MS/PI	+	-
Onion	725	Dimethyl disulfide	Sulfur	MS/SORT/PI	+	+
Potato	864	Methional	Sulfur	MS/SORT/PI	+	+
Orange	941	Methionol	Sulfur	PI	+	-
Sulfur	1173	Dimethyl tetrasulfide	Sulfur	MS/PI	+	+
Green	1275	L (-) carvone	Terpenoid	SORT/PI	+	-
Sulfuric	936	Unknown	. . .	NI ⁷	+	+

¹RI = retention index.²MS = GC/mass spectrophotometer.³PI = published index (Flavornet – Acree T. E. and Arn H. 1997–1999 – ©Cornell University GCO of natural products (<http://www.nysaes.cornell.edu/flavornet>)).⁴+ Indicates the compound was detected in each of the four cheeses.⁵- Indicates the compound was not detected in any of the four cheeses.⁶SORT = chemical standard odor retention time.⁷NI = not identified.

13 were detected in the TMR cheeses (Table 3). Odor-active compounds detected and identified in the pasture cheeses but not in TMR cheeses at 4 mo included aldehydes (phenylacetaldehyde, [E,E]-2,4 octadienal, [E]-2-nonenal, [Z]-2-nonenal, 2,4 decadienal, vanillin, and dodecanal); esters (ethyl 2-methyl butyrate, geranyl acetate, [E]-methyl jasmonate); ketones (3-hydroxy-2-butanone, 2-nonanone); lactones (δ -decalactone); pyrazine (2,6 dimethyl pyrazine); sulfur compounds (dimethyl disulfide, methionol); and terpenoid compounds (1-carvone, citronellol). Odor-active compounds detected and identified in the TMR cheese samples but not in pasture cheeses at 4 mo were an acid (hexanoic), an alkane (hexadecane), and a ketone (8-nonen-2-one). There were 18 out of the total of 27 odor-active compounds consistently present in the pasture cheeses that were absent in the cheeses produced from milk of cows fed exclusively a TMR diet. The absence of these odor-active compounds may indicate that some cheese aromas and flavors may derive specifically from certain compounds originating from fresh pasture plants.

The GCO data for the 7-mo-old pasture and TMR cheeses are summarized in Table 4. The total number

of odor-active compounds detected in both pasture and TMR cheeses was lower at 7 mo than at 4 mo. A decrease in the number of odor-active compounds in Grana Padano cheese with increasing age was reported by Moio and Addeo (1998). They reported the key neutral odorant constituents with an olfactometric index >20 during Grana Padano ripening and found that fruity, green, and apple odors generated by ethyl butanoate, ethyl hexanoate, 2-heptanol, and 2-heptanone tended to decrease with age. Twenty-two different odor-active compounds were consistently detected in 7-mo-old pasture cheeses, whereas 11 odor-active compounds were consistently detected and identified in the TMR cheeses. At 7 mo there was only one odor-active compound that was detected (hexanoic acid) in the TMR cheeses that was not present in the pasture cheeses. There was only one odor-active compound (L (-) carvone) detected in the pasture cheeses at 7 mo that was not detected in the pasture cheeses at 4 mo. The 1-carvone found in the 4-mo cheeses and L (-) carvone found in the 7-mo cheeses are closely related because they are optical. Odor-active compounds identified in the pasture cheeses but not in TMR cheeses at 7 mo were aldehydes (phenylacetalde-

hyde, E,E-2,4-octadienal, [E]-2-nonenal, and [Z]-2-nonenal), 2,4-decadienal, vanillin (3-methoxy-4-hydroxybenzaldehyde); esters (geranyl acetate, [E]-methyl jasmonate); ketones (3-hydroxy-2-butanone and 2-nonanone); sulfur (methionol), and terpenoid (L (-) carvone). Clearly, cheeses made from the milk produced by cows that had their diets supplemented with fresh pasture contained more odor-active compounds.

Aroma Compounds in Forage and Cheese

The compounds found in the cheeses made from pasture-fed and TMR-fed cows and their occurrence in 14 selected plant species out of more than 40 from the pasture sampled in this study are shown in Table 5. Compounds found in pasture plants and cheeses at 4 and 7 mo, but not in cheeses made from milk produced by cows eating only TMR include the following seven odor-active compounds: aldehydes (phenylacetaldehyde, [E,E]-2,4 octadienal, [E]-2-nonenal, [Z]-2-nonenal, 2,4 decadienal, and vanillin); and esters (geranyl acetate). Five additional odor-active compounds found only in cheese from pasture-fed animals but at only one of the times of aging include: an ester (ethyl 2-methyl butyrate), lactone (δ -decalactone), and terpenoid compounds (1-carvone, L (-) carvone, and citronellol). Several other compounds were found only in cheeses from pasture-fed cows but were not detected in the 14 plant species analyzed. These compounds are aldehyde (2, 4-decadienal, dodecanal), esters ([E]-methyl jasmonate); ketone (3-hydroxy-2-butanone, 2-nonanone); and sulfur compound (methionol). These compounds may have been present in other plants in the pasture that were not analyzed, or they may have been produced from other plant compounds during the process of consumption and digestion of the plant materials. The cheeses were extracted with SPME, and the plant materials were extracted with SD. It is possible that some of the compounds found in the cheeses of pasture-fed cows, but not in the 14 pasture plants, may be difficult to recover with SD.

Possible Origins of Odor-Active Compounds in Ragusano Cheese

The odor-active compounds identified in Ragusano cheeses in this study are listed in Table 6, with literature references and an indication of the possible origin of each compound. Low levels of acetic acid can be found in most cheeses and is usually produced by fermentation of carbohydrates or amino acids in cheese during aging (Aston and Dulley, 1982; Colchin et al., 2001; Dimos et al., 1996). Acetic acid was detected in all pasture and TMR cheeses. Little, if any, acetic acid is trans-

ferred from the diet to milk. Butyric and hexanoic acids are typically released from triglycerides by lipases during cheese aging (McSweeney and Sousa, 2000). Ragusano cheese is made with a rennet source that has high lipase activity, and FFA are part of the typical flavor and aroma of Ragusano cheese.

More odor-active aldehydes (8) were detected in Ragusano cheese than any other class of compounds. Of these 8 compounds, 7 were unique to the cheeses produced by cows consuming fresh pasture. Phenylacetaldehyde is involved in plant metabolism related to both lignin and amino-acid synthesis and is a vitamin-like substance for some rumen bacteria (Van Soest, 1994). Phenylacetaldehyde was detected in 6 out of 14 plant species (Table 5). Phenylacetaldehyde also may be produced in cheese via Strecker degradation of phenylalanine (Dunn and Lindsey, 1985). Some other aromatic aldehydes, such as vanillin, are also related to lignin synthesis in plants (Belitz and Grosch, 1986) and were detected in 9 out of 14 of the plant species (Table 5).

Nonanal, (E,E)-2,4-octadienal, (E)-2-nonenal, (Z)-2-nonenal, and 2,4-decadienal are typical oxidation products of unsaturated fatty acids in plants (Ho and Chen, 1994; Hsieh, 1994) produced by the lipoxygenase system (Galliard and Chan, 1980). The (E)-2-nonenal and (Z)-2-nonenal were common in pasture plants (Table 5). Lipoxygenase activity is exhibited in damaged plant tissues and may be part of an injury response system (Galliard and Chan, 1980). This occurs when a cow is grazing, and aldehydes are produced very quickly in the damaged plants prior to entering the rumen. Lipoxygenase also is involved in cooxidation reactions of fatty acids, and carotenoids can be oxidized, decolorized, and degraded (Galliard and Chan, 1980; Belitz and Grosch, 1986; Wu and Robinson, 1999). These reactions may also relate to the diversity of carotenoid-based compounds found in some cheeses when cows consume fresh pasture plants (Dumont and Adda, 1978; Mariaca et al. 1997). Because of the negative oxidation-reduction potential both in the rumen and in most cheeses, it is unlikely that these compounds are produced either in the rumen or in cheese. Their presence only in the cheese from cows consuming pasture plants was very consistent.

Some odor-active esters were also found in cheeses from pasture-fed cows and not TMR. Ethyl butyrate and ethyl hexanoate were found in all pasture and TMR cheeses and in plants. Buchin et al. (1999) reported the influence of the botanical composition of the pasture on the chemical, rheological, and sensory characteristics of ripened Abondance cheese. They found that branched aldehydes and ethyl esters were the result of the microbial metabolism of amino acids, fatty acids, and carbohydrates of milk and not from direct transfer from the

Table 5. Odor-active compounds detected in various pasture plant species and in cheese made from milk of pasture and TMR fed cows after 4 and 7 mo of aging (+ indicates the detection of the compound).

	Compound Class	Plant code (species name given below) ¹													Cheese							
		Ant	Cal	Cer	Beta	Dipl	Sin	Eup	Med	Scor	Erod	Ger	Mal	Fum	Rum	Pasture			TMR			
																4	7	mo	4	7	mo	
Odorant																						
Acetic acid	Acid																+	+	+	+	+	+
Butyric acid	Acid																+	+	+	+	+	+
Hexanoic acid	Acid																-	-	-	-	-	-
Phenylacetaldehyde	Aldehyde			+				+	+				+				+	+	+	+	+	+
Nonanal	Aldehyde					+				+							+	+	+	+	+	+
(E,E)-2,4-octadienal	Aldehyde							+									+	+	+	+	+	+
(E)-2-nonenal	Aldehyde	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
(Z)-2-nonenal	Aldehyde	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2,4-decadienal	Aldehyde																+	+	+	+	+	+
Vanillin	Aldehyde			+	+	+		+	+	+				+	+	+	+	+	+	+	+	+
Dodecanal	Aldehyde															+	+	-	-	-	-	-
Hexadecane	Alkane																-	-	-	-	-	-
Ethyl butyrate	Ester		+			+				+		+	+	+	+	+	+	+	+	+	+	+
Ethyl 2-methyl butyrate	Ester							+					+	+	+	+	+	+	-	-	-	-
Ethyl hexanoate	Ester		+	+		+						+	+			+	+	+	+	+	+	+
Geranyl acetate	Ester	+														+	+	+	+	+	+	+
(E)-methyl jasmonate	Ester															+	+	+	+	+	+	+
3-Hydroxy-2-butanone	Ketone															+	+	+	+	+	+	+
1-octen-3-one	Ketone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8-nonen-2-one	Ketone															+	+	+	+	+	+	+
2-nonanone	Ketone															+	+	+	+	+	+	+
δ-decalactone	Lactone							+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2,6 dimethyl pyrazine	Pyrazine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dimethyl disulfide	Sulfur															+	+	+	+	+	+	+
Methional	Sulfur	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Methionol	Sulfur															+	+	+	+	+	+	+
Dimethyl tetrasulfide	Sulfur															+	+	+	+	+	+	+
1-carvone	Terpenoide							+					+			+	+	+	+	+	+	+
L (-) carvone	Terpenoide									+	+	+	+	+	+	+	+	+	+	+	+	+
Citronellol	Terpenoide	+	+	+	+	+	+		+	+	+	+	+	+	+	+	-	-	-	-	-	-

¹Plant Species: Ant = *Anthemis arvensis*; Cal = *Calendula arvensis*; Cer = *Cerinth major*; Beta = *Beta vulgaris*; Dipl = *Diplotaxis erucoides*; Sin = *Sinapis arvensis*; Eup = *Euphorbia helioscopia*; Med = *Medicago hispida*; Scor = *Scorpiurus subvillosus*; Erod = *Erodium moscatum*; Ger = *Geranium rotundifolium*; Mal = *Malva silvestris*; Fum = *Fumaria officinalis*; and Rum = *Rumex bucephalophorus*.

Table 6. Possible origins of odor-active compounds found in Ragusano cheese.

Odorant	Compound class	Possible origin	Reference
Acetic acid	Acid	Microbial fermentation of carbohydrate or amino acids in cheese	Colchin et al. (2001), Aston and Dullely 1982), and Dimos, et al. (1996)
Butyric acid	Acid	Lipolytic release from triglycerides in cheese	McSweeney and Sousa (2000)
Hexanoic acid	Acid	Lipolytic release from triglycerides in cheese	McSweeney and Sousa (2000)
Phenylacetaldehyde	Aldehyde	Strecker degradation of phenylalanine in cheese	Dunn and Lindsay (1985)
Nonanal	Aldehyde	Oxidation of unsaturated fatty acid in plants	Ho and Chen (1994), Hsieh (1994)
(E,E)-2,4-octadienal	Aldehyde	Oxidation of unsaturated fatty acid in plants	Ho and Chen (1994), Hsieh (1994)
(E)-2-nonenal	Aldehyde	Oxidation of unsaturated fatty acid in plants	Ho and Chen (1994), Hsieh (1994)
(Z)-2-nonenal	Aldehyde	Oxidation of unsaturated fatty acid in plants	Ho and Chen (1994), Hsieh (1994)
2,4-decadienal	Aldehyde	Oxidation of unsaturated fatty acid in plants	Ho and Chen (1994), Hsieh (1994)
Vanillin	Aldehyde	Compound transferred (either vanillin or ferulic acid) from plants related to synthesis of lignin	Belitz and Grosch (1986), Van Soest (1994)
Dodecanal	Aldehyde	Oxidation of unsaturated fatty acid	Ho and Chen (1994), Hsieh (1994)
Ethyl butyrate	Ester	Interaction of free butyric acid with alcohol	McSweeney and Sousa (2000)
Ethyl 2 (or 3)-methyl butyrate	Ester	Strecker degradation of branched chain amino acid with alcohol	McSweeney and Sousa (2000), Urbach (1993)
Ethyl hexanoate	Ester	Interaction of free butyric acid with alcohol	McSweeney and Sousa (2000)
Geranyl acetate	Ester	Precursor of carotenoid synthesis in plants	Belitz and Grosch (1986)
(E)-methyl jasmonate	Ester	Oxidation of unsaturated fatty acid in plants	Ho and Chen (1994), Hsieh (1994)
3-Hydroxy-2-butanone	Ketone	Strecker degradation of amino acid	McSweeney and Sousa (2000)
1-octen-3-one	Ketone	Oxidation of unsaturated fatty acid	Ho and Chen (1994), Hsieh (1994)
2-nonanone	Ketone	Oxidation of unsaturated fatty acid	Ho and Chen (1994), Hsieh (1994)
δ -decalactone	Lactone	Hydroxy fatty acid of milk fat	McSweeney and Sousa (2000), Belitz and Grosch (1986), Dimos et al. (1996)
2,6 dimethyl pyrazine	Pyrazine	Maillard browning product or microbial metabolite	Belitz and Grosch (1986), McSweeney and Sousa (2000), Meinhardt and Schreier (1986)
Dimethyl disulfide	Sulfur	Degradation of methionine	Belitz and Grosch (1986), McSweeney and Sousa (2000)
Methional	Sulfur	Degradation of methionine	Belitz and Grosch (1986), McSweeney and Sousa (2000)
Methionol	Sulfur	Degradation of methionine	Belitz and Grosch (1986), McSweeney and Sousa (2000)
Dimethyl tetrasulfide	Sulfur	Degradation of methionine	Belitz and Grosch (1986), McSweeney and Sousa (2000)
L (-)-carvone/1-carvone	Terpenoid	Precursor or degradation product of carotenoids	Belitz and Grosch (1986)
Citronellol	Terpenoid	Precursor or degradation product of carotenoids	Belitz and Grosch (1986)

diet. Whereas it would be possible for these compounds to transfer from diet to cheese, the esters can also be produced in cheese by the interaction of butyric or hexanoic acid with alcohol (Urbach, 1993; McSweeney and Sousa, 2000) produced by bacterial action in the cheese (Table 6).

Geranyl acetate, a precursor in carotenoid synthesis, and (E)-methyl jasmonate, a lipid-oxidation product in plants (that is produced rapidly when a plant is damaged), are both odor-active esters that produce floral and jasmine aroma responses, respectively. It is interesting to note that (E)-methyl jasmonate is a key compound in the physiological response mechanism of plants to resist damage by insects (Dicke et al., 1999; Rodriguez-Saona et al., 2001). When plants are damaged by herbivorous insects, volatile compounds are produced that induce the production of other volatile compounds by the plant that have a negative effect on the physiology of the herbivorous insects and that attract carnivorous insects that attack the herbivorous insects. The compounds that attract carnivorous insects typically have high odor activity. For example, in lima bean plants, jasmonic acid and methyl jasmonate (that are emitted in response to plant tissue damage) are involved in the induction of production of compounds such as (E)- β -ocimene, linalool, and other related terpenoid compounds (Dicke et al., 1999). Wounding of the plant tissues activates the octadecanoid/lipoxygenase pathway, a lipid-based signaling sequence (Rodriguez-Saona et al., 2001), resulting in the production of a wide range of terpenoid and other lipid-derived volatile compounds. Therefore, it is not surprising that some of these lipophylic volatile compounds would be found in milk and cheese of cows consuming fresh pasture. The native plant species of the spring Sicilian pastures are like a wildflower garden, with a diversity of colors and aromas. Geranyl acetate, a precursor in carotenoid synthesis, was found consistently in the cheeses of pasture-fed animals but was only detected in one of the 14 plant species analyzed. (E)-methyl jasmonate was also consistently present in the cheeses from pasture-fed animals but was not detected in any of the 14 plant species analyzed. Either these compounds were being produced by degradation of other compounds consumed from the plants, they were present in other plants in the pasture (not analyzed) that were consumed by the cows, or they were easily lost from the plant sample during sample handling, preparation, and SD.

The transfer of lipophylic compounds from the pasture diet into Ragusano cheese that produced a color difference was demonstrated and reported in a previous paper (Carpino et al., 2004a). The color of the pasture cheeses was much more yellow than cheeses produced from milk of cows on the TMR diet, indicating a transfer

of carotenoid compounds from diet to milk. Carotenoids are transferred from diet to milk via a chylomicron-mediated uptake mechanism (Noble, 1981; Parodi, 1996). Most of the lipophylic odor-active compounds found in the cheese produced from milk of pasture-fed cows would transfer from diet to milk by a similar mechanism.

Two ketones were found uniquely in the cheeses from pasture-fed cows—3-hydroxy-2-butanone and 2-nonanone. Typically, 3-hydroxy-2-butanone is produced by a Strecker degradation of an amino acid (McSweeney and Sousa, 2000) and 2-nonanone is typically an oxidation product of unsaturated fatty acid (Ho and Chen, 1994). Neither of these compounds were detected in the 14 plant species analyzed, yet they were consistently present in the pasture cheeses and not in the TMR cheeses. The ketone 1-octen-3-one and the lactone δ -decalactone were both very common in the pasture plants (Table 5) and were detected in the cheeses. All of the sulfur-containing, odor-active compounds found in the cheese (Table 5) are thought to be degradation products of methionine (Belitz and Grosch, 1986; McSweeney and Sousa, 2000). These could come from the diet or be formed from methionine released from protein structures during cheese aging. The terpenoid compounds found in the cheese made from milk of pasture origin are odor-active degradation products of carotenoids (Belitz and Grosch, 1986), with both 1-carvone and citronellol being detected in several plant species. Carotenoid compounds are rapidly destroyed during harvesting of plants (Bauernfeind, 1972). Clearly the lipoxygenase pathway plays an important role in the production of many odor-active volatile compounds in plants. Much of this transformation may take place during the mastication of the plants by the cow while they are in the pasture (Bugaud et al., 2001a) before the plant material reaches the negative oxidation-reduction potential environment in the rumen. It is apparent that the odor-active compounds in pasture-derived cheeses are dependent on diets diverse in plant species. The TMR diet based on maize or corn silage (Carpino et al., 2004) had a simpler flavor spectrum.

CONCLUSIONS

Milk from cows that consumed native pasture plants produced cheeses with more odor-active compounds. In 4-mo-old cheese made from milk of pasture-fed cows, 27 odor-active compounds were identified, whereas only 13 were detected in cheese made from milk of TMR-fed cows. The pasture cheeses were much more rich in odor-active aldehyde, ester, and terpenoid compounds than cheeses from cows fed only TMR. A total of 8 unique aroma-active compounds (i.e., not reported in other

cheeses evaluated by GCO) were detected in Ragusano cheese made from cows consuming native Sicilian pasture plants. These compounds were 2 aldehydes ([E,E]-2,4-octadienal and dodecanal), 2 esters (geranyl acetate and [E]-methyl jasmonate), 1 sulfur compound (methionol), and 3 terpenoid compounds (1-carvone, L(-) carvone, and citronellol). Geranyl acetate and (E)-methyl jasmonate were particularly interesting because these compounds are released from fresh plants as they are being damaged and are part of a possible plant defense mechanism against damage from insects. Most odor-active compounds that were unique in Ragusano cheese from pasture-fed cows appeared to be compounds created by oxidation processes in the plants that may have occurred during grazing and ingestion by the cows. Some odor-active compounds were consistently present in pasture cheeses that were not detected in the TMR cheeses or in the 14 species of pasture plants analyzed. Either these compounds were present in other plants not analyzed, created in the rumen or in cheese after the pasture plant material had been consumed, or the compounds were lost in the method of sample extraction used for the plant analysis (i.e., steam distillation) vs. the SPME method used for the cheeses. This research has demonstrated clearly that some unique odor-active compounds found in pasture plants can be transferred to the cheese.

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