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Characteristic Aroma Components of Rennet Casein

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Rennet casein, produced by enzymatic (rennet) precipitation of casein from pasteurized skim milk, is used in both industrial (technical) and food applications. The flavor of rennet casein powder is an important quality parameter; however, the product often contains an odor described as like that of animal/wet dog. Two commercial rennet casein powders were evaluated to determine the compounds responsible for the typical odor. Aroma extracts were prepared by high-vacuum distillation of direct solvent (ether) extracts and analyzed by gas chromatography−olfactometry (GCO), aroma extract dilution analysis (AEDA), and GC−mass spectrometry (MS). Odorants detected by GCO were typical of those previously reported in skim milk powders and consisted mainly of short-chain volatile acids, phenolic compounds, lactones, and furanones. Results of AEDA indicated *o*-aminoacetophenone to be a potent odorant; however, sensory descriptive sensory analysis of model aroma systems revealed that the typical odor of rennet casein was principally caused by hexanoic acid, indole, guaiacol, and *p*-cresol.

KEYWORDS: Rennet casein; odor; aroma; volatile; gas chromatography-olfactometry

INTRODUCTION

Rennet casein is widely used in both food and nonfood applications. In food applications, caseins are used as ingredients because of their flavor stability and functional properties. Usage is wide in various types of foods, especially cheese analogues, bakery, meat, and confectionery products, and desserts (*1*). Casein has a characteristic and unpleasant stale flavor. Ramshaw and Dunstone (*2*) described this flavor as "stale", "glue-like", or "burnt feathers". A similar flavor has been found in other dairy products such as stored skim milk powder (*3*, *4*) and sterilized concentrated milk (*5*). *o*-Aminoacetophenone was indicated as an important aroma component of stored skim milk powder (*6*, *7*).

The method of producing rennet casein curd is identical with that of the production of cheese curd and depends on the unique sensitivity of the $Phe_{105}-Met_{106}$ bond of κ-casein to hydrolysis by acid proteinases of rennet. Rennet is added to skim milk at about 29 °C and held for 1 h, and steam is then injected to raise the temperature to 55 °C to cook the curd before separation of whey (*1*). Other types of casein are produced by isoelectric precipitation by addition of acid or fermentation (*1*). In the production of mineral acid casein, pasteurized skim milk is mixed with dilute (0.5 N) hydrochloric or sulfuric acid to lower the pH to about 4.6 to precipitate casein. The mixture is heated to 50 °C by steam injection before whey separation. Lactic casein is produced by addition of a starter culture, usually *Lactococcus lactis*, and incubation at 22−26 °C for about 14 h. The lactic acid produced by the starter culture lowers the pH and precipitates the casein. Casein curd is drained and washed several times with water after whey separation. With any type of casein, as much water as possible is first removed by pressing or by centrifugation, and then the curd is dried using a fluidized bed drier, roller drier, or spray drier. After drying, the casein granules are cooled and packaged. The resulting range of acid and rennet casein products is used for nutritional and medical foods and imitation cheese. Casein also has a long history of use in technical applications such as paper and cardboard coating, adhesives, cosmetics, synthetic fibers, paints, and emulsions (*1*). The flavor of rennet casein is an important quality parameter. The exact chemical nature of the animal/wet dog odor of rennet casein has not been previously reported. The aim of this study was to provide information about predominant odorants responsible for the typical odor of rennet casein powder.

MATERIALS AND METHODS

Caseins. Two commercial rennet caseins (I and II) were provided by two different suppliers. Samples contained 82−90% protein.

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Table 1. Predominant Neutral/Basic Odorants of Rennet Caseins

no.	compd	odor descripn[a]	retention index[b] DB-FFAP	retention index[b] DB-5MS	av $\log_3$ FD factor[c] I	av $\log_3$ FD factor[c] II
1	hexanal	green, cut-grass	1077	799		<1
2	(*Z*)-4-heptenal	rancid, crabby	1231	898		<1
3	1-octen-3-one	mushroom	1298	977		<1
4	2-acetyl-1-pyrroline[d]	popcorn	1327	921		<1
5	dimethyl trisulfide	sulfurous, cabbage	1367	969	<1	1
6	3-(methylthio)propanal	potato	1452	906	1.5	1
7	(*E*,*Z*)-2,6-nonadienal[d]	cucumber	1578	1154	1	<1
8	unknown	dried hay	1673		1	1.5
9	β-damascenone[d]	applesauce	1821	1387		<1
10	2-methoxyphenol	smoky	1840	1086	3	<1
11	benzothiazole	rubbery	1958	1267	<1	<1
12	(*E*)-4,5-epoxy-(*E*)-2-decenal[d]	fatty, unripe	1997	1379	<1	<1
13	4-methylphenol	barnyard, medicine	2077	1084	<1	<1
14	γ-decalactone	peachy	2136	1768	<1	1.5
15	δ-decalactone	peachy	2184	1494	2	<1
16	*o*-aminoacetophenone	corn tortilla, grape	2202	1307	4.5	4
17	γ-dodecalactone	cheesy, soapy	2376	1680	1.5	1.5
18	(*Z*)-6-dodecenyl-γ-lactone	cheesy, soapy	2390	1662	2	3
19	indole	mothball-like	2446	1290	<1	<1
20	3-methylindole	mothball-like, fecal	2489	1396	2	2

[a] Odor quality perceived during GCO. [b] Retention indices were calculated from GCO data. [c] Average $\log_3$ flavor dilution (FD) factor ($n = 2$). [d] Compound tentatively identified on the basis of comparison of odor property and retention indices with reference compound.

Table 2. Predominant Acidic Odorants of Rennet Casein[a]

no.	compd	odor descripn	retention index DB-FFAP	retention index DB-5MS	av $\log_3$ FD factor I	av $\log_3$ FD factor II
21	butanoic acid	cheesy	1616	804	1.5	<1
22	2-/3-methylbutanoic acid	sweaty, dried fruit	1660	876	<1	<1
23	pentanoic acid	sweaty	1718	908	1	<1
24	hexanoic acid	sweaty, vinegar	1840	1020	2.5	<1
25	maltol[b]	burnt sugar	1963	1088	2.5	2.5
26	Furaneol[c]	burnt sugar	2026	1096	2.5	2.5
27	heptanoic acid	musty	1947			2
28	octanoic acid	waxy	2054	1281	<1	1
29	sotolon[d,e]	curry, spicy	2191	1108	2.5	2.5
30	decanoic acid	soapy, waxy	2271	1387	2.5	2
31	9-decenoic acid	waxy	2357		1	<1
32	dodecanoic acid	waxy	2482		2	1
33	phenylacetic acid	waxy/rosy	2548	1265	1	1
34	vanillin[f]	vanilla	2564	1399	2	<1

[a] Refer to footnotes a–c of Table 1. [b] 3-Hydroxy-2-methyl-4-pyrone. [c] 2,5-Dimethyl-4-hydroxy-3(*2H*)-furanone. [d] Compound tentatively identified on the basis of comparison of odor property and retention indices with reference compound. [e] 3-Hydroxy-4,5-dimethyl-2(*5H*)-furanone. [f] 3-Methoxy-4-hydroxybenzaldehyde.

Chemicals. Aroma compounds listed in **Tables 1**−**3** were obtained from Aldrich Chemical Co. (St. Louis, MO) or Lancaster (Windham, NH). Compound **4** was obtained from Dr. R. Buttery (USDA, ARS, WRRC, Albany, CA). Compound **9** was provided by Firmenich Inc. (Plainsboro, NJ). Compound **12** was synthesized according to the literature (*8*).

Isolation of Volatiles. Caseins were subjected to direct solvent extraction followed by high-vacuum distillation (*7*). Fifty grams of rennet casein was mixed with 25 g of solid NaCl and then hydrated with 100 mL of odor-free distilled−deionized water. The mixture was thoroughly blended with a hand-held mixer (Bio Homogenizer M133/1281-0, Biospec Products, Inc., Bartlesville, OK). The mixture was evenly divided between two 250 mL Teflon bottles, and 50 mL of ether was added to each bottle. Bottles were sealed with Teflon caps and shaken by hand for 5 min and then for 30 min on a Roto Mix (Mistral Multi-Mixer Lab-Line Instruments Inc., Melrose Park, IL). Ether layers were pooled, and the extraction was repeated two more times. Extractions were conducted in duplicate for each casein sample.

Compound Class Fractionation. Each ether extract was subjected to high-vacuum distillation as previously described (*7*). The volatile extract (distillate) was concentrated to 20 mL under a gentle stream of nitrogen gas (N_2). It was then washed with sodium carbonate (Na_2CO_3) (0.5 M; 2 × 7.5 mL) and then with a saturated solution of sodium chloride in water (3 × 2.5 mL). The upper ether phase, containing the neutral/basic volatiles, was collected and concentrated to 10 mL under N_2. It was then dried over anhydrous Na_2SO_4 (2 g) and concentrated to 0.5 mL under N_2. The pooled aqueous phase (bottom layer) was acidified with HCl (10% v/v) to pH 1.5−2, and the acidic volatiles were extracted with ether (3 × 10 mL). The pooled ether extract was then concentrated to 10 mL under N_2, dried over anhydrous Na_2SO_4, and further concentrated to 0.5 mL under N_2.

Gas Chromatography−**Olfactometry**. The GCO system consisted of a HP6890 GC (Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector (FID), a sniffing port (DATU, Geneva, NY), and cool on-column injector. Each extract (2 μL) was injected into a capillary column (DB-FFAP 30 m length × 0.25 mm i.d. × 0.25 μm film thickness (d_f) or DB-5ms 30 m length × 0.32 mm i.d. × 0.25 μm d_f; J & W Scientific, Folson, CA). The GC oven temperature was programmed from 35 to 200 °C at a rate of 10 °C/min with initial and final hold times of 5 and 30 min, respectively. Carrier gas was helium at a constant flow of 2.2 mL/min. Two experienced panelists conducted GCO. The extracts containing the neutral/basic and acidic volatiles were diluted stepwise with diethyl ether at a ratio of 1:3 (v/v). The aroma extract dilution procedure was performed until no odorants were detected by GCO. The highest dilution was defined as flavor dilution (FD) factor (*9*).

Table 3. Concentrations and Odor Activity Values of Selected Volatile Components of Rennet Caseins

		concn (ng/g)[a]			odor activity value[c]	
no.	compd	I	II	odor threshold (μg/L in water)[b]	I	II
			Neutral/Basic Compounds			
10	2-methoxyphenol	27 ± 1	75 ± 72	10.9	2.5	6.9
13	4-methylphenol	3.0 ± 0.1	132 ± 120	2.7	1.12	49.4
15	δ-decalactone	5.8 ± 1	44 ± 4	30	0.19	1.5
16	*o*-aminoacetophenone	5.8 ± 1	13 ± 6	0.28	20.7	46.4
17	δ-dodecalactone	23 ± 1	36 ± 43	4.6	5	7.8
18	(*Z*)-6-dodecenyl-γ-lactone	0.32 ± 0.06	4.5 ± 4.6	0.7	0.45	6.4
19	indole	2.9 ± 0.1	22 ± 27	21	0.14	1.1
20	3-methylindole	2.2 ± 0.3	11 ± 7	3	0.73	3.7
			Acidic Compounds			
35	acetic acid	1910 ± 1550	1350 ± 611	22000	0.09	0.06
36	propanoic acid	191 ± 108	225 ± 84	2190	0.09	0.1
21	butanoic acid	722 ± 172	2240 ± 56	1274	0.6	1.76
22	2-/3-methylbutanoic acid	17 ± 1	87 ± 3	250	0.07	0.3
23	pentanoic acid	17.7 ± 0.6	89.3 ± 0.4	1207	0.01	0.07
24	hexanoic acid	720 ± 46	2380 ± 180	35.6	20.2	66.8
27	heptanoic acid	30 ± 5	153 ± 37			
25	maltol[d]	110 ± 26	663 ± 210	210	0.5	3.2
28	octanoic acid	531 ± 105	2160 ± 1180	1405	0.4	1.54
37	nonanoic acid	82 ± 6	243 ± 214			
30	decanoic acid	727 ± 330	1890 ± 2170	10000	0.07	0.2
38	undecanoic acid	33 ± 32	14 ± 3			
31	9-undecenoic acid	273 ± 213	490 ± 636			
32	dodecanoic acid	238 ± 225	691 ± 692			
34	vanillin[e]	9.7 ± 1.1	30 ± 27	64	0.15	0.5

[a] Average concentration ± standard deviation ($n = 2$). [b] Orthonasal detection threshold. [c] Odor activity value = average concentration divided by odor detection threshold. [d] 3-Hydroxy-2-methyl-4-pyrone. [e] 3-Methoxy-4-hydroxybenzaldehyde.

Gas Chromatography−Mass Spectrometry. The system consisted of an HP6890 GC/5973 mass selective detector (MSD, Agilent Technologies). Separations were performed on a fused silica capillary column (DB-FFAP, 30 m length × 0.25 mm i.d. × 0.25 μm d_f, J&W Scientific). Carrier gas was helium at a constant flow of 1 mL/min. The oven temperature was programmed from 35 to 200 °C at a rate of 3 °C/min with initial and final hold times of 5 and 45 min, respectively. MSD conditions were as follows: capillary direct interface temperature, 280 °C; ionization energy, 70 eV; mass range, 33−350 amu; EM voltage (Atune+200 V); scan rate, 2.2 scans/s. Each extract (2 μL) was injected in the cool on-column mode.

Identification of Odorants. Positive identifications were made by comparing retention indices (RI), mass spectra, and odor properties of unknowns with those of authentic standard compounds analyzed under identical conditions. Tentative identifications were based on matching the RI values and odor properties of unknowns against those of authentic standards. Retention indices were calculated by using a series of *n*-alkanes (*10*).

Quantification of Odorants. Casein samples were subjected to direct solvent extraction/high-vacuum distillation and compound class fractionation as described above, except that prior to extraction each sample was spiked with 5 μL of a multiple internal standard (IS) solution (containing 20.8 mg of ethyl maltol, 50.6 mg of 2-ethylbutanoic acid, 12.5 mg of 6-amyl-α-pyrone, 0.50 mg of *o*-toluidine, and 0.51 mg of *tert*-amylphenol in 5 mL of methanol). Extractions were performed in duplicate for each sample. GC-MS analysis was conducted as described above. For generation of calibration curves, solutions of selected compounds found in **Table 3** were prepared in deodorized water, at three levels bracketing the concentration found in the casein samples, and then subjected to the same extraction procedure as casein samples. GC−MS response factors were determined for the following IS/analyte combinations: ethyl maltol for **25** and **34**; 2-ethylbutanoic acid for **21**−**24**, **27**, **28**, **30**−**32**, and **35**−**38**; 6-amyl-α-pyrone for **15**, **17**, and **18**; *o*-toluidine for **16**, **19**, and **20**; and *tert*-amylphenol for **10** and **13**. For each analyte (i) a response factor (R_i) was determined by plotting mass ratio ($\text{mass}_i/\text{mass}_{IS}$) versus total ion peak area ratio ($\text{area}_i/\text{area}_{IS}$), where R_i equals 1/slope. The analyte concentration was then calculated as

$$\text{Concn}_i\ (\text{ng/g}) = (\text{area}_i/\text{area}_{IS})R_i[\text{mass}_{IS}\ (\text{ng})/\text{mass}_{\text{sample}}\ (\text{g})]$$

Table 4. References for Descriptive Sensory Evaluation of Rennet Casein[a]

descriptor	definition	reference
cooked	aromatics associated with cooked milk	skim milk heated to 85 °C for 30 min
sweet aromatic	aromatics associated with white cake mix	white cake mix
animal/wet dog	aromatics associated with gelatin solution	one bag of gelatin (28 g) dissolved in 500 mL of odor-free water
potato/brothy	aromatics associated with vegetable soup stock	canned potatoes
cardboard	aromatics associated with wet cardboard	cardboard paper soaked in water
overall aroma	overall aroma intensity	

[a] Adapted from Drake et al. (*12*).

R_i values (given in parentheses) for the selected analytes were as follows: **10** (0.177), **13** (0.421), **15** (8.01), **16** (8.72), **17** (7.10), **18** (14.9), **19** (0.667), **20** (0.821), **21** (0.566), **22** (0.877), **23** (0.648), **24** (0.858), **25** (0.878), **27** (0.993), **28** (1.02), **30** (1.03), **31** (1.16), **32** (1.24), **34** (0.885), **35** (0.314), **36** (0.408), **37** (1.14), and **38** (1.15).

Sensory Evaluation. *Caseins.* For odor evaluation, 10 g of casein was suspended in 100 mL of odor-free water. A descriptive sensory analysis of odor was conducted on casein samples by seven trained and experienced panelists using a sensory language developed for dried dairy ingredients, including caseins and caseinates (*11*, *12*). Terms identified and selected by the panelists are listed in **Table 4**. Panelists marked their responses on a 10-point numerical intensity scale anchored on the left with "none" and on the right with "extreme". Panelists each had previously received more than 50 h of training in descriptive sensory analysis of dried dairy ingredients. Three 1 h sessions were conducted to focus on sensory properties of caseins and casein aroma, prior to sensory analysis of caseins. Caseins were evaluated in duplicate. Differences among samples were evaluated by analysis of variance with means separation (ANOVA) using SAS version 7.0 (*13*).

Determination of Threshold Values. A modification of ASTM procedure E679-91, an ascending forced-choice method of limits, was used to determine threshold values (*14*). Three samples (two water and one containing an aroma compound) of about 20 mL each were

Table 5. Sensory Aroma Comparison of Odorant Combinations and Casein I

model	combination[a]	respective concn (ng/g)[b]	similarity score[c]	comments
A	**10**, **16**, **19**, and **24**	27, 5.8, 2.9, and 720	6.0 ± 0.4	sweet/perfumey/grapelike
B	**10**, **16**, **20**, and **24**	27, 5.8, 2.2, and 720	4.0 ± 1.0	overwhelmingly mothball-like, jasmine/floral
C	**19** and **24**	5.8 and 1478	7.0 ± 0.5	sweaty, not animal-like or brothy enough
D	**20** and **24**	4.4 and 1478	3.0 ± 1.2	overwhelmingly mothball-like
E	**13**, **19**, and **24**	3, 2.9, and 1478	7.5 ± 0.3	not brothy enough
F	**10**, **13**, **19**, and **24**	27, 3, 2.9, and 1478	8.5 ± 0.5	most similar to sample

[a] Numbers correspond to compounds listed in **Tables 1**–**3**. [b] Concentration of odorant on solids basis. [c] Average similarity score ± standard deviation.

presented at five ascending concentration levels at room temperature. Solutions were presented in 2 oz souffle cups with lids. Panelists (n = 30) were instructed to partially remove the lid and sniff the headspace. Panelists were then asked to choose the odd sample from the three and give a certainty judgment (sure/not sure). The individual best estimate threshold was taken as the geometric mean of the last concentration with an incorrect response and the first concentration with a correct response, except for the following sequence: if the subject indicated a "not sure" response for the correct choice, that concentration was increased by a factor of 1.41 to bring it up to the geometric mean between the two concentration steps, to adjust for the possibility of chance correct response (*15*). Duplicate analyses were conducted for each compound. Group thresholds were taken as the geometric mean of the individual best estimate thresholds.

Preparation of Rehydrated Milk Retentate (RMR). Rehydrated milk retentate was used as the matrix for the sensory analysis of model casein solutions (*16*). Skim milk was centrifuged at 12 000*g* for 40 min at 7 °C. Then the upper phase (supernatant) was removed. The solid bottom phase was collected and rehydrated with odor-free distilled−deionized water. Sensory analysis indicated that RMR was odorless and tasteless. The pH of RMR was 6.6 ± 0.1, and the protein content was 1.0 ± 0.05% (w/v).

Simulation of Casein Aroma. Aroma models for descriptive sensory analysis were prepared by dissolving selected odorants at the concentrations found in the rennet caseins into RMR. Model preparation was conducted on selected compounds which exhibited high odor activity values (OAVs) (**Table 3**). The compounds, which included **10**, **13**, **16**, **19**, **20**, and **24**, were evaluated at six different combinations, chosen primarily on the basis of concentration data for casein I (**Table 5**). The aromas of the six models were evaluated by the trained panel in duplicate for overall similarity to rehydrated casein I. Panelists marked similarity scores on 10-point numerical intensity scales anchored on the left with "not similar" and on the right with "very similar". The most similar model was selected on the basis of the overall similarity scores. This model was then evaluated in duplicate for specific aroma attributes by sensory descriptive analysis. All models were freshly prepared just prior to evaluation.

RESULTS AND DISCUSSION

Predominant odorants of the two casein samples were identified by means of aroma extract dilution analysis (AEDA). Twenty neutral/basic and 14 acidic odorants were identified in the $\log_3$ flavor dilution (FD) factor range of <1−4.5 (**Table 1**). A greater number of neutral/basic odorants were identified in sample II than in sample I. However, the same number of acidic odorants, consisting mainly of short-chain volatile acids, were identified in both caseins (**Table 2**). On the basis of its high average $\log_3$ FD factor the most potent odorant in rennet casein was *o*-aminoacetophenone (**16**), which contributed a corn tortilla and grapelike odor. It was previously reported to be the contributor of a flavor like that of glue/burnt feathers in stored casein (*2*), stored dry milk (*6*), and sterilized concentrated milk (*5*). *o*-Aminoacetophenone was recently identified by use of GCO techniques as an off-flavor compound in micromilled milk powder (*3*) and as a predominant odorant in stored skim milk powder (*4*). Walker and Manning (*17*) reported this compound to be the major contributor to the overall musty/stale flavor of stored dried lactic casein. Nevertheless, results of sensory studies of aroma models (described later) indicate that *o*-aminoacetophenone may not be responsible for the typical odor of casein powders.

In addition to *o*-aminoacetophenone, other neutral/basic odorants with relatively high average $\log_3$ FD factors (≥1.5) were (*Z*)-6-dodecenyl-γ-lactone (**18**), 2-methoxyphenol (guaiacol, **10**), γ-dodecalactone (**17**), and 3-methylindole (skatole, **20**) (**Table 1**). Predominant acidic odorants (with average $\log_3$ FD factors of 2.5) were hexanoic acid (**24**), maltol (**25**), Furaneol (**26**), sotolon (**29**), and decanoic acid (**30**) (**Table 2**). With the exception of guaiacol (**10**), γ-decalactone (**14**), (Z)-6-dodecenyl-γ-lactone (**18**), indole (**19**), heptanoic acid (**27**), and 9-undecenoic acid (**31**), all of the compounds listed in **Tables 1** and **2** were previously reported as odor-active components of milk powders (*3*, *4*, *7*). However, compounds **10**, **14**, **18**, **19**, **27**, and **31** are known volatile constituents of other dairy products (*18*). Because of the high protein content (∼82−90%) of rennet casein, it is possible that the typical odor develops primarily as a result of the degradation of free amino acids. For example, compounds **6** and **13** may be derived from methionine (*19*, *20*) and tyrosine (*21*), respectively, while compounds **16**, **19**, and **20** may form as a result of tryptophan degradation (*19*, *22*, *23*). The amino acid degradation products *p*-cresol, 2-phenylethanol, indole, acetophenone, and benzaldehyde have been previously reported in lactic casein (*17*).

Selected volatile constituents were quantified in order to calculate their odor activity values (OAVs), which also can serve to indicate or rank relative odor potencies of the compounds (**Table 3**). Compounds **26** and **29** were present at levels below quantification limits and were, therefore, excluded from **Table 3**. *o*-Aminoacetophenone (**16**), hexanoic acid (**24**), guaiacol (**10**), 4-methylphenol (*p*-cresol, **13**), δ-decalactone (**15**), (*Z*)-6-dodecenyl-γ-lactone (**18**), and 3-methylindole (**20**) had the highest OAVs, which was in general agreement with the AEDA results. One notable exception was hexanoic acid, which had OAVs higher than expected in both caseins, primarily due to the relatively low odor detection threshold of this compound compared with that of the other short-chain volatile acids measured.

Sensory profiles of both caseins were nearly identical, except that casein I contained more sweet aroma than sample II (**Figure 1**). The animal/wet dog attribute was rated highest in the two samples, followed by cooked and potato/brothy attributes. Sensory analysis of model systems indicated that the aroma of model F, which included indole, guaiacol, *p*-cresol, and hexanoic acid, was the most similar to rehydrated casein I (**Table 5**). Rehydrated casein I received average intensity scores of 5.0 for musty/wet dog and 2.4 for potato/brothy aroma attributes. Meanwhile, model F received intensity scores of 4.0 for animal/wet dog like and 1.0 for potato/brothy notes. Even though *o*-aminoacetophenone (**16**), and to a lesser extent 3-methylindole

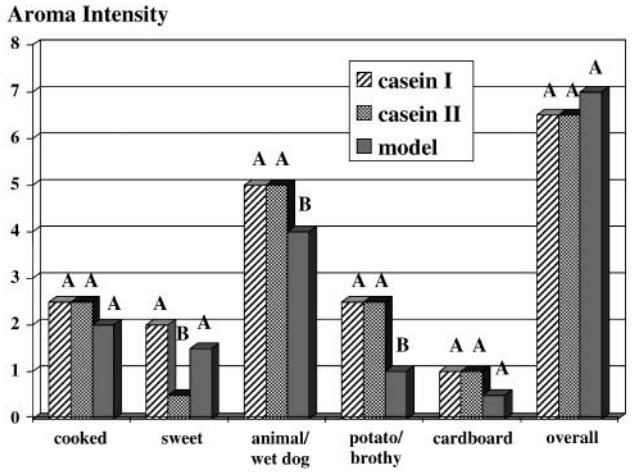

Figure 1. Aroma profiles of rehydrated rennet caseins and the most similar model F by sensory analysis. Bars within each aroma attribute group having different lettering are significantly different; $p \leq 0.05$.

(**20**), exhibited high OAVs (**Table 3**), the addition of these compounds into models resulted in low overall similarity scores (**Table 5**). This was because aromas of models containing *o*-aminoacetophenone were described by the panelists as "sweet and grape-like" and those with 3-methylindole as "floral/perfumey" as opposed to "animal/wet dog" and "potato/brothy", the key sensory descriptors for casein aroma. The above findings agree with results of our previous studies with nonfat milk powders, where no correlation was found to exist between an animal/gelatin/wet dog like note and *o*-aminoacetophenone (*4*). Meanwhile, in that same study hexanoic acid was positively correlated with a barny/animal-like sensory attribute.

In summary, application of GCO and AEDA to the study of the typical aroma components of rennet casein indicated the presence of 20 neutral/basic and 14 acidic odorants. Among these, *o*-aminoacetophonene was detected at the highest $\log_3$ FD factor. However, despite its high odor potency, sensory studies of model mixtures demonstrated *o*-aminoacetophonene may play only a minor role in the typical odor of rennet casein. Instead, hexanoic acid, indole, guaiaco,l and *p*-cresol are the major contributors to the typical animal/wet dog like odor of rennet casein.

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