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Chemometric Analysis of Ragusano Cheese Flavor

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Ragusano cheeses were produced in duplicate from milk collected from pasture-fed and total mixed ration (TMR)-fed cattle at four time intervals. The cheeses were subjected to chemical analysis, conventional sensory testing, and gas chromatography−olfactometry (GCO). Data from each type of analysis were examined by principal component and factor analysis and by pattern recognition (SIMCA) to see if sufficient information for classification into pasture-fed and TMR-fed groups was contained therein. The results clearly indicate that there are significant differences in sensory panel and chemical analysis results between the two cheeses. The data were also examined to see if models of sensory responses as a function of analytical or GCO results or both could be constructed with the modeling technique partial least-squares regression (PLS). Strong PLS models of some sensory responses (green and toasted odor; salt, pungent, bitter, and butyric sensations; and smooth consistency) were obtained.

KEYWORDS: Modeling; composition to property modeling; sensory analysis; chemometrics; GCO; multivariate modeling

INTRODUCTION

Historically, specific types of cheeses are produced in certain geographic regions. Often these cheeses have flavor characteristics that are unique for the region. Studies have suggested that local pastures play a role in determining the aroma of dairy products (*1*). There is also a large body of anecdotal evidence about the desirable effect of spring and summer grasslands on the distinctive flavor of dairy products (*2*), and some work has been done to define the difference in the flavor in terms of specific flavor compounds (*3*). French workers (*4*) have shown that Gruyere de Comtè cheese typically contains sesquiterpene hydrocarbons in summer but not in winter. Dumont et al. compared samples of Gruyere de Comtè made from milk originating from mountains, plateaus, and plains and showed that mountain cheeses were the richest in volatile compounds and typically contained terpenes and sesquiterpenes. Studies were carried out in which hays of different composition were fed. This was shown to impact flavor compounds in the milk (*5*) and also in cheese (*6*). Flavor substances may be transferred to the milk directly through inhaled air into the blood and from there to the milk, through the fodder and digestive tract, or via rumen gases to blood and milk (*7*). The unique character of some cheeses may come from the environmental conditions of milk production (*8*). Some compounds may contribute to the formation of desirable flavors in the cheese. Secondary compounds in plants, or derivatives of those compounds created by the fermentation process in the rumen, may be transmitted through the cow to the milk. It is important to define the relationship between these compounds and the special flavor of a cheese.

Ragusano cheese is produced in the Hyblean area of Sicily, Italy, utilizing local forage, often pastures, for feeding. Ragusano cheese has unique flavor characteristics. A preliminary study using Charm analysis (*9*) was conducted on plant and cheese samples produced in the Hyblean area (*10*). That study found a correlation between plant and cheese odors that provided justification for further examination. The relationships between human and instrumental perceptions are also of considerable interest. It was of interest to determine if differences between cheeses produced from pasture-fed and total mixed ration (TMR)-fed cattle could be discerned by ANOVA or pattern recognition. Because flavor perceptions are multivariate in nature, where multiple constituents produce multiple sensory sensations, it was highly appropriate to use multivariate modeling (partial least squares, PLS) to seek relationships between composition and perception.

MATERIALS AND METHODS

Experimental Design. An experiment was designed to determine the effects of feeding cattle on native pastures or a total mixed ration on the flavor and aroma of cheese.

Season. The experiment was carried out in the province of Ragusa in Sicily beginning in spring when the native pasture was available.

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Table 1. Descriptors Developed by the Panel for Intensity of Aroma, Taste, Chemesthetic, Consistency, and Mouth Structure Attributes of Ragusano Cheese Evaluated after 4 Months of Aging

aroma	taste and chemesthetic	consistency	mouth structure
pungent	pungent	smooth	soft/hard
butter	sweet	oil	doughy
floral	salt	plasticity	dispersion
green	acid	soft/hard	teeth stickness
almond	bitter	fracture	
mushroom	astringent		
toasted	butyric		
butyric	aftertaste		
odor intensity			

Choice of the Farm and Animals. The experiment was carried out on a single farm. This farm had all of the typical characteristics of a farmstead cheese producer, available native pasture, a total mixed ration facility, and a sufficient number of cows to select two similar lactation stage groups. Twenty-four Holstein cows in a similar stage of lactation were selected. Twelve cows grazed on the native pasture and 12, used as the control, were fed TMR. Information on the TMR was collected so that the possibility of confounding factors, such as the presence of native species in hay, haylage, or silage, would be known.

Milk Sampling. The whole raw milk from the 12 cows fed with native pasture and from the 12 cows fed a TMR was collected separately, on the same day, four times with an interval of 15 days between collections. The milk was promptly transported to the Consorzio's pilot plant.

Cheese Manufacture. Ragusano cheeses were manufactured from the milk obtained from the two treatments. Two different vats of cheese were produced according to the standard technology (*11*) to make four (two from each vat) different blocks of cheese once every 15 days. The approximate weight of a block of cheese was 14 kg. Sixteen blocks of cheese in total were manufactured in the Consorzio's experimental cheese plant. Eight blocks were from raw milk of cows fed on the native pasture, and eight were from cows fed the TMR.

Cheese Aging. The 16 blocks of cheese were brined and aged in an aging center. Generally, a block of cheese remained in the brine for an average of 2 days for every kilogram of weight. Once the brine salting stage was completed, the cheese was aged in ventilated rooms at a temperature of 14−16 °C. The 16 cheeses were sampled after 4 months of aging.

Chemical Analysis. The cheeses were analyzed for total solids (*12*), total nitrogen [Method 33.2.11, 991.20 (*13*)], soluble protein by 12% TCA (*14*), soluble protein by pH 4.6 (*14*), fat (*15*), salt (*12*), and pH.

Gas Chromatography Olfactometry (GCO). The cheeses were examined by GCO (*16*) to determine the differences in the spectra of aroma compounds between the two treatments. One trained person carried out all of the sniffing runs.

Sensory Evaluation. Descriptive analysis was used to develop terms for sensory evaluation of the cheese (*17*). Twelve trained panelists were given cheese samples and asked to agree on terms in group discussion. The terms were refined, and a descriptive ballot was generated over several training sessions. A score scale (1−15) with increasing intensity from left to right was used. Panelists were asked to rate the relative intensities of four different classes of attributes (see **Table 1**).

In each case the cheeses from treatment P (pasture fed) and the cheeses from treatment U (TMR fed) were evaluated when the cheeses had been aged for 4 months. Sensory testing was conducted every 2 weeks (15 day increments) with two tests per week using 12 trained panelists. All of the panelists evaluated all of the products. Tests were carried out with panelists wearing special glasses (Post-Mydriatic sunglasses, 100% UV protection to 400 nm, Solarettes) to mask the color differences among the samples.

Data Analysis. *Designations.* Of the 16 cheese samples, 8 were from pasture-fed cattle (P) and 8 from TMR-fed cattle (U). Each was produced at four different times in duplicate, leading to sample designations P11 (i.e., pasture, time 1, block 1) and P12 (i.e., pasture, time 1, block 2) for the two time 1 pasture-fed cheeses through P41 and P42 for the time 4 cheeses. The equivalent TMR cheeses were U11, U12, ..., U41, and U42. Chemical and sensory analyses were carried out on all 16 samples (2 feeds × 4 time points × duplicate cheeses).

Statistical analysis with repeated measures ANOVA was used to determine whether treatment had a significant ($p < 0.05$) impact on panel scores for each descriptive term. When variances were not comparable, the Mann−Whitney U test was used to test for significance of mean differences.

Principal components analysis (PCA) was carried out with SCAN (software for chemometric analysis) release 1 for Windows (MINITAB Inc., State College, PA) using the nonlinear iterative partial least-squares (PLS) method. Two criteria for determining how many principal components to use were employed: examination of a Scree plot for a sharp break and/or eigenvalues ≥ 1.0. PCA was performed separately on the chemical analysis, sensory, and GCO subsets of the data. Factor analysis was performed with SCAN using Varimax rotation of the number of PCs judged to be significant. This was done separately with each subset of the data.

Soft independent modeling of class analogy (SIMCA) pattern recognition (*18*) was carried out with SCAN on the chemical analysis, sensory, and GCO subsets of the data and on the combined data set. The procedure was applied using autoscaling, proportional class prior probabilities, and cross-validation of all components. Measurements that were constant-valued for a class or zero-valued for nearly all of the samples were excluded.

PLS modeling (*18*) of the sensory descriptors as a function of the chemical analysis, GCO, and combined chemical plus GCO measurements was carried out with SCAN. The best prediction equations (lowest predictive error sum of squares) were selected, and the quality of the fits was judged by examining predicted versus observed plots and residuals versus observed plots. In cases when a single sample was a major outlier, the model was recomputed with that sample omitted. In a few cases a much improved fit could be obtained by an increase of one or two components and a small increase in predictive residual error sum of squares (PRESS); in those cases the better fitting model was used.

RESULTS AND DISCUSSION

Prior to formal sensory analysis of the cheeses, it was clearly demonstrated that there was a significant difference in appearance between the cheeses of the two treatments (unpublished paper). The cheese from pasture-fed cows was more yellow, whereas the cheese from the TMR-fed cows appeared to be more white. Prior research has demonstrated (unpublished paper) that large differences in appearance among samples of different treatments will produce a "halo" effect, resulting in panelists reporting larger differences in other nonappearance attributes than they can actually detect. To avoid this problem in the sensory evaluation of differences in odor, taste and chemesthetics, consistency, and mouth structure, the panelists were required to wear special glasses during training and during sensory evaluation of unknown cheeses.

Univariate Statistics. The mean values for each of the two treatment groups for each property determined by analytical measurement, sensory evaluation, and GCO are shown in **Table 2**. The means were compared by ANOVA (one-way) or, when appropriate due to significantly different variance ratios, by nonparametric tests. In most cases the means were similar and not significantly different at the $p < 0.05$ confidence level. Of the analytical measurements, only percent fat was significantly different between the two treatments; it was lower in the cheese made with milk from TMR-fed cows. None of the taste and chemesthetics or mouth structure characters were significantly different between the two feeds. Two of the odor variables, "floral" and "green", were significantly different among feeding treatments, but their overall intensity for both treatments should

Table 2. Mean Values for Analytical, Sensory, and GCO Results for Cheeses from Pasture-Fed (P) and Ration-Fed (U) Cows and ANOVA Significances (*, $p < 0.05$; **, $p < 0.01$)

Chemical Analysis

	TS (%)	protein (%)	SP 4.6 (%)	SP 12% (%)	NaCl (%)	fat (%)*	pH
P mean	61.71	26.57	3.78	3.43	3.48	28.20	5.42
U mean	60.43	26.98	4.24	3.87	3.23	26.94	5.52

Sensory Analysis[a]

	odor								
	pungent	butter	floral*	green**	almond	mushroom	toasted	butyric	odor intensity
P mean	5.76	5.66	2.85	2.94	1.65	1.52	1.55	5.43	6.22
U mean	5.68	5.60	2.48	2.40	1.56	1.80	1.48	5.52	5.82

	taste and chemesthetic							
	pungent	sweet	salt	acid	bitter	astringent	butyric	aftertaste
P mean	6.17	6.27	5.58	3.77	3.25	3.83	5.36	6.58
U mean	5.94	5.99	5.39	3.30	3.16	3.65	5.56	6.33

	consistency					mouth structure			
	smooth	oil*	plasticity	soft/hard	fracture	soft/hard	doughy	dispersion	teeth stickiness
P mean	4.77	3.31	7.44	6.07	7.39	5.40	8.38	7.40	5.21
U mean	5.14	3.83	7.60	6.01	6.60	5.17	8.66	7.63	5.30

GCO Analysis[b]

	fruity	green	potato	mushroom	fatty	plastic	wood	rancid	floral	medicinal	dairy/soapy
P mean	328.3	9.4	58.4	16.1	294.4	2.0	11.3	2.2	9.9	3.9	3.8
U mean	213.8	0.0	99.6	9.1	217.4	2.0	0.0	3.1	15.1	0.0	18.3

[a] Values for sensory descriptors are on a 1–15 scale. [b] GCO values represent relative odor intensity.

be considered low because their intensity scores were <3 on a 15 point scale; both received higher ratings in the pasture-fed cheese. Of these, the difference in the means for "green" was significant at the $p < 0.01$ level. Of the consistency characters only "oil" was significantly different between the two treatments. This received a higher average rating for the TMR-fed treatment. None of the GCO characters were significantly different between the treatment groups. The GCO rating for "green" was by far the nearest to significance, but because the variances of the two groups were quite dissimilar, nonparametric statistics had to be used and this failed to show a significant difference. Therefore, when visual cues were eliminated, there were few detectable differences in other sensory attributes of the cheese.

PCA/Factor Analysis. *Chemical Analysis.* PCA/factor analysis was performed on the duplicate results obtained from the chemical data. Three PCs were adequate to represent 86% of the variance in the seven chemical measurements. The plot of the scores on the first two PCs before rotation (**Figure 1**) showed a tendency for samples to group by feed, with some intermingling. Two of the samples were possible outliers: U41 (at the top), which had the highest TN and pH values, and P42 (to the right), which had the highest TS, fat, and NaCl. Factor analysis revealed that SP 4.6, SP 12%, and NaCl loaded in the same direction on the rotated first PC with pH opposite. The rotated PC2 mainly explained TS and fat. PC3 was mostly influenced by TN.

Sensory Data. The data for all 26 sensory attributes for all 16 samples were subjected to PCA. More PCs were required to represent the data set of 26 sensory parameters than were needed for the analytical or GCO results (see later). The first nine PCs described 92% of the variance in the sensory data. The plot of the first two PC scores (**Figure 2**) tended to group the pasture-fed samples in the center, but some of these were close to some of the U samples. The first nine PCs were subjected to Varimax

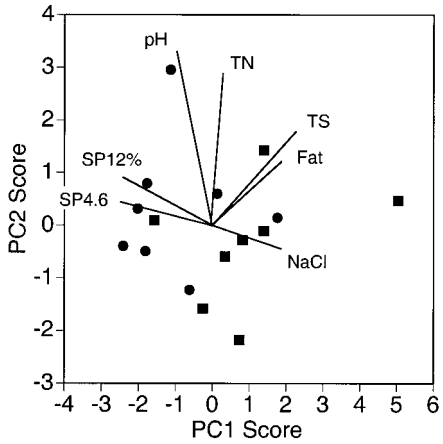

Figure 1. First two principal component scores of the chemical analysis data of cheeses made with milk from pasture-fed (■) and TMR-fed (●) cattle.

rotation to bring them into better alignment with the original measurements. Rotated factors 1−9 explained from 15.5 to 6.4% of the variance. PC1 was strongly influenced by "softness" and "dispersion" in one direction and by "sweetness" and "plasticity" in the other. PC2 showed "almond" odor and "astringent" sensation in opposition to "fracture". PC3 was associated with "floral" odor and "acid" taste and PC4 with "pungent" and "butyric" odors and high odor intensity. Consistency and mouth structure attributes were strongly associated with the PCs explaining most of the variance (always the first few extracted), with odor characters secondarily important and taste and chemesthetic attributes ranking third. These results are somewhat different from the significant mean differences of the sensory properties of the cheeses (**Table 2**), where the texture attribute "oil" and the "floral" and "green" odors were significant ($p < 0.05$).

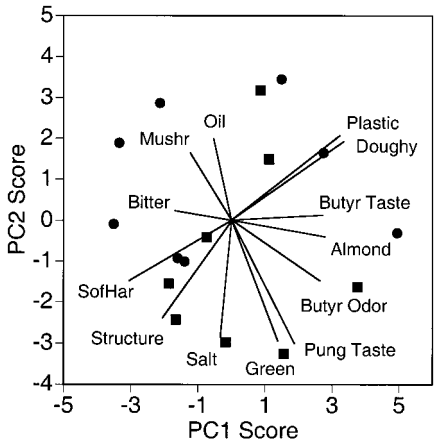

Figure 2. First two principal component scores of the sensory analysis data of cheeses made with milk from pasture-fed (■) and TMR-fed (●) cattle.

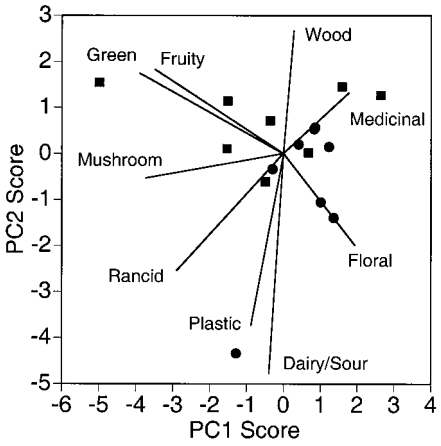

Figure 3. First two principal component scores of the GCO data of cheeses made with milk from pasture-fed (■) and TMR-fed (●) cattle.

GCO Results. PCA was applied to the 11 GCO measurements made on each sample. The results indicated that five (based on eigenvalue > 1) or six PCs contain the meaningful variance in the data set. These correspond to 86 or 90.9% of the total variance. The plot of the first two PCs (**Figure 3**) indicates that samples P12 and U22 are possible outliers. P12, in the upper left corner, received the highest scores for "green", "fruity", and "mushroom" and was also high in "wood" and "rancid". U22, at the bottom, was the only TMR-fed sample with nonzero scores for "plastic" or "rancid". The remaining U samples form a fairly tight grouping that is surrounded by the P samples. The first few PCs did not reveal an obvious tendency for samples to group by treatment. The first five PCs were Varimax rotated, and these loadings indicated that "green" was opposite to "floral" on PC1, "fruity" and "fatty" were aligned on PC2, "plastic", "rancid", and "dairy/sour" mapped to PC3, "wood" and "medicinal" on PC4, and "potato" on PC5.

Pattern Recognition (SIMCA). The SIMCA pattern recognition procedure was used to attempt to classify the samples into the P or U feeding classes from their analytical, sensory, and GCO measurements. This was done separately with the chemical data, the GCO data, and the sensory data and also with all of the measurement data combined. The results are shown in **Table 3**. SIMCA computes envelopes completely bounding each class (in this case P and U). If the envelopes do not overlap, a completely successful classification is obtained. If they do overlap, then classification is difficult.

Table 3. Summary of SIMCA Classification Success Rates for Three Subsets and the Entire Data Set

data set	overall classification success[a] (%)	overall cv classification success[b] (%)
chemical analysis	100	62.5
sensory evaluation	100	31.25
GCO	87.5	75.0
combined[c]	100	87.5

[a] Percentage of cheese samples correctly classified according to cattle feed. [b] Percentage of cheese samples correctly classified according to cattle feed after cross-validation. [c] Omitting chemical analysis variable TS and GCO variables green, fatty, fruity, medicinal, plastic, potato, rancid, and wood.

A more conservative estimate of the predictive ability of a classification model is obtained by recomputing it multiple times, with each sample omitted once. In each computation the class identity of the omitted sample is estimated using the model produced without it. This procedure is called cross-validation and tests the class models for their sensitivity to particular samples. It produces a more realistic estimate of the performance of a classification model with new samples.

The GCO data were problematic for constructing class models. When data are invariant (constant valued) within a class, a classification model cannot be computed. All of the U samples received scores of 0 for the green, medicinal, and wood characters. Also troublesome were situations when only one sample in the class received a nonzero score for a character; this occurred for the dairy/sour, medicinal, and plastic characters for the P samples and for the plastic and rancid characters for the U samples (actually both of these were in the U22 sample, which appeared to be an outlier in **Figure 3**). As a result these measurements were not used in the procedure.

Except for the GCO data (see **Table 3**), SIMCA was able to successfully classify the samples into the two feed classes. With cross-validation, however, the success rate declined markedly but was still reasonably good for the GCO data (75% success) and combined data (87.5% success).

Modeling (PLS). For purposes of constructing models of behavior, the system was highly overdetermined; that is, there were many more measurements (7 chemical + 26 sensory + 11 GCO = 44) than there were samples (16 samples). As a result, one of the main assumptions of multiple linear regression was violated, and this technique could not be used. PLS regression, on the other hand, can produce useful results with overdetermined systems (*18*) and was used in this study.

The multiple correlation coefficient squared, R^2, gives an indication of the fit of a model to the samples. When several variations in models under consideration, such as calculations using different numbers of PLS latent variables, are compared, the one with the highest R^2 is called the "best-fit" model. A more conservative estimate of the predictive ability of a model is obtained by fitting a model to the data set with one sample omitted. The measurement values for the omitted sample are substituted in the model to estimate its response value, and the difference between this and the actual result is the cross-validated residual. The omitted sample is returned to the data set, another is taken out, and the operation is repeated until each sample has been omitted once. The cross-validated residuals for each sample are squared and summed to arrive at the PRESS. The PRESS is used to compute the cross-validated R^2, which is considered to give a more realistic estimate of the ability to fit a new sample to the model. When several variants are used, the one with the lowest PRESS is considered to be the "best prediction" model.

It is presumed that some aspects of sample composition can be perceived by chemical measurement and that the sensory properties are determined by the product composition. Although GCO measurements are perceived sensorially, the responses are, for the most part, to single chemical entities and, as a result, mainly represent the levels of compounds of olfactory significance in a sample. For each sensory character there are two possibilities: that enough of the chemical properties associated with it are represented in the data set to explain its perception or they are not. In the latter case a poor model (poor predictive ability and/or fit) will result. When a good model is obtained, there are two possibilities: that the measurement variable treated as independent actually causes (in whole or in part) the sensory perception or some variable that was not measured but which is correlated with the measured variable participates in the perception.

Some of the GCO characters were not well suited for use in modeling because they had nonzero values for only one, two, or three samples (plastic, rancid, wood, and medicinal). These characters were, as a result, problematic in computations and of questionable use in modeling. These characters were not used in calculating the results.

The results of the model fitting are shown in **Table 4**. Equations in which each sensory character was used as the dependent variable and each of three sets of data (the 7 chemical analysis results, seven of the 11 GCO results, and the combined 7 chemical analysis plus seven GCO results) was used as the independent variables were calculated:

$$\text{sensory character} = b_1\,\text{chem}_1 + b_2\,\text{chem}_2 + ... + b_7\,\text{chem}_7$$

$$\text{sensory character} = b_1\,\text{GCO}_1 + b_2\,\text{GCO}_2 + ... + b_7\,\text{GCO}_7$$

$$\text{sensory character} = b_1\,\text{chem}_1 + b_2\,\text{chem}_2 + ... + b_7\,\text{chem}_7 + b_8\,\text{GCO}_1 + b_9\,\text{GCO}_2 + ... + b_{14}\,\text{GCO}_7$$

None of the models of the mouth structure characters (**Table 4**) appeared to be strong. It is likely that the mouth structure sensations result from particular classes of macromolecules, and presumably these are not assessed at all by GCO and perhaps only to a small extent by the chemical analyses used. As a result the lack of a good relationship is not surprising.

The consistency relationships (**Table 4**) were somewhat stronger than those for mouth structure. The chemical analysis models predicted "oil" and "soft/hard", whereas the GCO results predicted "smooth" and "fracture". All of these but "smooth" appeared weaker with the combined data. The agreement between the model produced with the combined data and the observed result for smooth ($R^2 = 0.709$) is shown in **Figure 4**. This, like all of the other models, is a linear combination of all the independent variables used. The factors have different degrees of influence, which can be expressed as the predictor importances. The factors with the greatest importance for this model are the GCO variables potato (+), fatty (−), and floral (−); the arithmetic sign in parentheses shows the direction of the influence. It is not readily apparent how any volatile compound would influence "fracture". "Smooth", on the other hand, might be influenced by simultaneous odor perceptions that are not actually perceived in the mouth.

The taste and chemesthetic descriptors had models that were stronger than for the other attributes (**Table 4**). Some of these (pungent, salt, bitter, and aftertaste) appeared stronger with chemical measurements than with GCO results; this is not surprising because most of these are thought to be perceived in the mouth rather than by olfaction. "Butyric" and possibly "acid" had stronger fits to the GCO than the chemical analysis results. In most cases the results were strongest with the combined GCO and chemical data. "Pungent", "salt", "bitter", "butyric", and "aftertaste" all gave quite respectable models. The "pungent taste" model with the combined data had an R^2 of 0.842 (see **Figure 5**), and the highest importances were for TN (−), fat (+), and fruity (+).

The model for salt using the chemical analysis data alone was quite strong, with an $R^2 = 0.880$ and high predictor importances for TS (+), SP 12% (+), and fat (−). When the GCO data were added, the model strengthened to $R^2 = 0.931$

Table 4. Multiple Correlation Coefficients Squared (R^2) for Best Prediction Models of Individual Sensory Results as a Function of Chemical Analysis, GCO, and Combined Chemical Analysis plus GCO Data

	chem[a]	GCO[b]	GCO + chem
odor			
pungent	0.323	0.148	0.409
butter	0.273	0.381	0.585
floral	0.114	0.554	0.563
green	0.520	0.273	0.997
almond	0.335	0.580	0.518
mushroom	0.474	0.107	0.388
toasted	0.191	0.758	0.926
butyric	0.304	0.410	0.451
odor intensity	0.307	0.204	0.545
taste and chemesthetic			
pungent	0.485	0.239	0.842
sweet	0.359	0.353	0.496
salt	0.880	0.445	0.931
acid	0.275	0.474	0.572
bitter	0.598	0.435	0.815
astringent	0.216	0.241	0.315
butyric	0.446	0.687	0.981
aftertaste	0.679	0.080	0.751
consistency			
smooth	0.112	0.663	0.709
oil	0.430	0.189	0.386
plasticity	0.361	0.242	0.430
soft/hard	0.475	0.233	0.405
fracture	0.128	0.518	0.453
structure			
soft/hard	0.135	0.089	0.303
doughy	0.329	0.405	0.316
dispersion	0.244	0.308	0.353
teeth stickiness	0.271	0.142	0.375

[a] Fit using the seven chemical analysis results as independent variables. [b] Fit using the seven GCO results as independent variables (omitting plastic, wood, rancid, and medicinal).

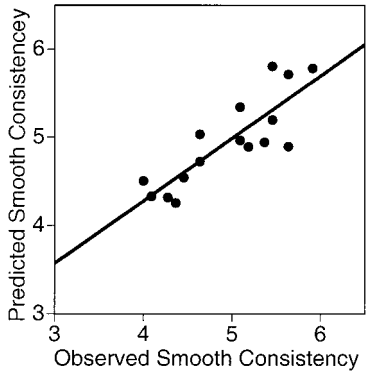

Figure 4. Predicted versus observed plot for the model of "smooth" consistency based on combined chemical analysis and GCO data ($R^2 = 0.709$).

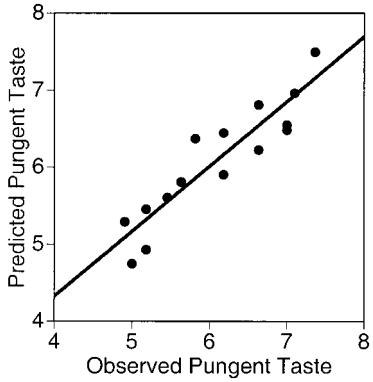

Figure 5. Predicted versus observed plot for the model of "pungent" sensation based on combined chemical analysis and GCO data (R^2 = 0.842).

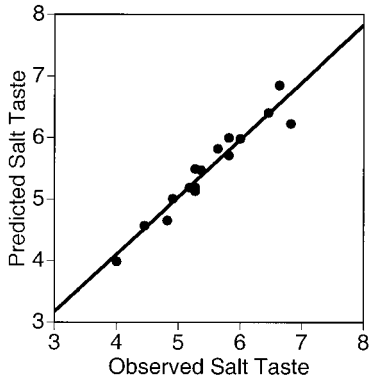

Figure 6. Predicted versus observed plot for the model of "salt" taste based on combined chemical analysis and GCO data (R^2 = 0.931).

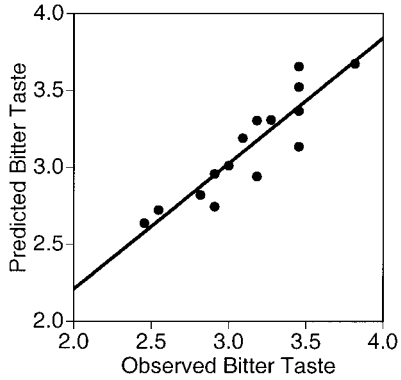

Figure 7. Predicted versus observed plot for the model of "bitter" taste based on combined chemical analysis and GCO data (R^2 = 0.815).

(see **Figure 6**) and the high predictor importances were for TN (−), fat (−), and 'fatty' (+). It is interesting to note that the NaCl concentration was not strongly associated with the salt taste perception.

Bitter taste was modelable to some extent with both the chemical analysis and GCO data separately, but was much stronger (R^2 = 0.815) when these were combined (see **Figure 7**). The largest predictor importances were for TS (+), fat (+), and the GCO results "potato" (+), "fatty" (+), and "floral" (−).

The butyric sensation, like bitter taste, was modelable to some extent with both the chemical analysis and GCO data separately, but was very strong (R^2 = 0.981) when these were combined (see **Figure 8**). This model had high predictor importances for NaCl (−), pH (−), and the GCO characters "fruity" (+), "green" (−), "fatty" (−), and "dairy/sour" (−). This suggests that the

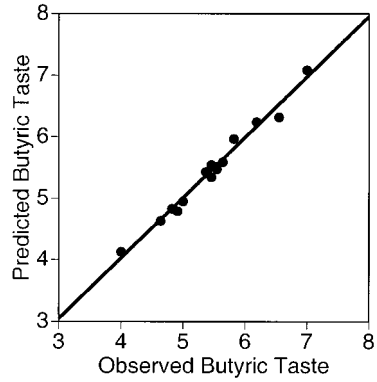

Figure 8. Predicted versus observed plot for the model of "butyric" sensation based on combined chemical analysis and GCO data (R^2 = 0.981).

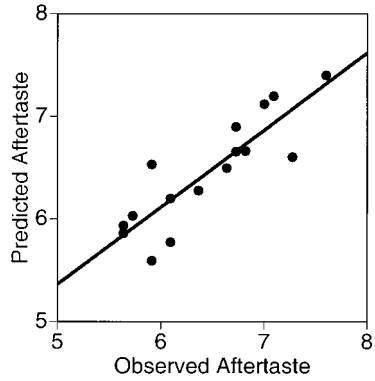

Figure 9. Predicted versus observed plot for the model of "aftertaste" based on combined chemical analysis and GCO data (R^2 = 0.751).

character is more detectable with low levels of most other characteristics. Perhaps it is easily masked.

Aftertaste was reasonably well modeled by chemical analysis variables, but not at all by GCO data alone. However, the two combined produced a slightly stronger model (R^2 = 0. 751; see **Figure 9**) than the chemical analysis data alone. Variables with high predictor importances were TS (−), TN (−), and pH (−). This indicates that lower levels of total solids and total nitrogen were associated with greater aftertaste, and so was lower pH, which of course corresponds to greater acidity.

One would think that the models likely to fit well would be those between odor characters and GCO results. This appeared to be the case for "floral", "almond", and "butyric", where adding the chemical data to the GCO data produced little change in modeling ability.

The model for "toasted" was the strongest for GCO data alone (R^2 = 0.758), but was strengthened quite a bit (to R^2 = 0.926) by adding the chemical data (see **Figure 10**). The high predictor importances were for TN (+), fat (+), "fruity" (+), "green" (−), "floral" (+), and "dairy/sour" (−). In the GCO only model three of the four GCO variables had high predictor importances in the same directions. A fourth, "mushroom" (+), was substituted for "floral".

Surprisingly, the model for "green" was stronger for the chemical data than for the GCO alone, although the two combined were very much stronger (R^2 = 0.997; see **Figure 11**). The predictor importances here were greatest for TN (−), pH (−), "potato" (+), and "dairy/sour" (−). To a lesser extent the same type of pattern also occurred with odor intensity.

Many of the models relating sensory responses to analytical and GCO observations (**Table 4**) were weak. Of the mouthfeel

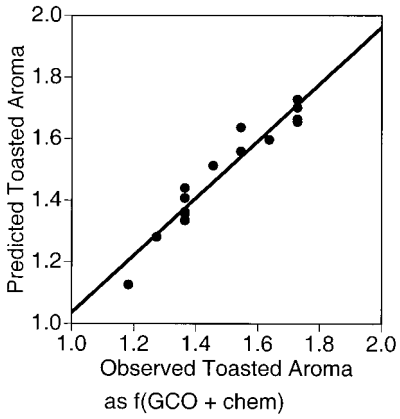

Figure 10. Predicted versus observed plot for the model of "toasted" aroma based on combined chemical analysis and GCO data ($R^2 = 0.926$).

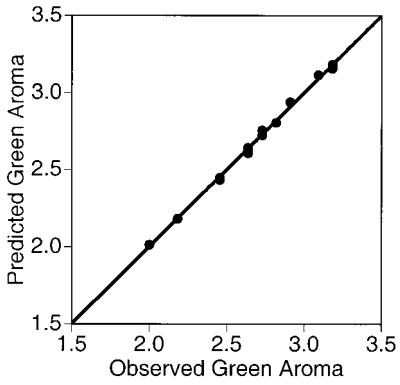

Figure 11. Predicted vs observed plot for the model of "green" aroma based on combined chemical analysis and GCO data ($R^2 = 0.997$).

Table 5. Ranges and R^2 Values for the Stronger Models of Sensory Attributes

character	sensory score range	R^2
smooth	1.9	0.709
pungent	2.5	0.842
salt	2.8	0.931
bitter	1.8	0.815
butyric	3.0	0.981
aftertaste	2.0	0.751
toasted	0.6	0.926
green	1.3	0.997

relationships only "smooth" consistency was well explained. The odor descriptions "green" and "toasted" were well explained by combined chemical analysis and GCO results. The characters "salt" and "aftertaste" were well explained by chemical analysis measurements alone, whereas "pungent", "bitter", and "butyric" sensations could be explained by combined chemical analysis and GCO results.

Of the models produced, in addition to the strength of the regression coefficient, one should consider the range of scores used by the sensory panel (see **Table 5**). The larger the range used, the more convincing the significance of the model. A larger range indicates a better signal to noise ratio in the sensory result and better explaining power of the model. Viewed in this way the "toasted" aroma model, although it has a good R^2 (0.926), is not so impressive because the range of sensory scores spanned (0.6) is small. The models for "salt" taste and "butyric" sensation, on the other hand, are impressive because they span larger ranges (2.8 and 3.0, respectively) and have high R^2 values (0.931 and 0.981). The "green" aroma model has the highest R^2 (0.997) and spans a modest range of responses (1.3).

ABBREVIATIONS USED

GCO, gas chromatography−olfactometry; PCA, principal component analysis; PLS, partial least-squares regression; PRESS, predictive residual error sum of squares; SIMCA, soft independent modeling of class analogies; SP 12%, soluble protein by 12% TCA; SP 4.6, soluble protein by pH 4.6; TMR, total mixed ration; TN, total nitrogen; TS, total solids.

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