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Typicality and Geographical Origin Markers of Protected Origin Cheese from The Netherlands Revealed by PTR-MS

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ABSTRACT: Volatile fingerprints of 30 cumin cheese samples of artisanal farmers' cheese of Leiden with EU Protected Designation of Origin (PDO) and 29 cumin cheese samples of varying commercial Dutch brands without PDO protection were used to develop authentication models. The headspace concentrations of the volatiles, as measured with high sensitivity proton-transfer mass spectrometry, were subsequently subjected to partial least-squares discriminant analysis (PLS-DA). Farmers' cheese of Leiden showed a distinct volatile profile with 27 and 9 out of the 60 predominant ions showing respectively significantly higher and lower concentrations in the headspace of the cheese in comparison to the other cumin cheeses. The PLS-DA prediction models developed classified in cross-validation 96% of the samples of PDO protected, artisanal farmers' cheese of Leiden correctly, against 100% of commercial cumin cheese samples. The characteristic volatile compounds were tentatively identified by PTR-time-of-flight-MS. A consumer test indicated differences in appreciation, overall flavor intensity, creaminess, and firmness between the two cheese groups. The consumers' appreciation of the cumin cheese tested was not influenced by the presence of a name label or PDO trademark.

KEYWORDS: cumin cheese, origin classification, HS PTR-MS, PLS-DA

INTRODUCTION

Although the focus in the agriculture in Western Europe in the 20th century was on standardization of the production process and increasing production, the farmers, food producers and processors throughout Europe continue to make regional specialties. In the past decade artisanal and regional products have become increasingly popular. To protect the integrity of the regional and artisanal products, several official EU regulations were introduced which allow certain products to be named with the names of their geographical area of production such as Protected Designation of Origin (PDO), Protected Geographical Indication (PGI), and Traditional Specialty Guaranteed (TSG) certifications.[1] When a product is given a Protected Designation of Origin, it means that only items produced in a specific area, using recognized know-how, may bear that label in the European market.

The authentication of the identity and integrity of food products is an important process in monitoring fair trade and food safety and for reassurance of consumers. The verification of the integrity of food products in agriculture can aim at the authentication of ingredients, processing, geographical origin and production system (e.g., organic) or at the verification of the typicality of a product. Traditionally, monitoring quality standards of food products has been done by human sensory analysts, who rate the odor, taste and appearance of the products on prespecified standards. This method does not always offer reproducible results and is time-consuming and expensive, since it requires a highly trained and qualified panel of assessors.

In The Netherlands one of the first products that received a Protected Designation of Origin was traditional farmers' cheese from the surroundings of the city of Leiden ("*Boeren-Leidse kaas met sleutels*"). PDO protected farmers' cheese of Leiden is a semihard Dutch cumin cheese artisanally produced by farmers in the region of the city of Leiden and is appreciated for its rich cumin flavor and its firmness due to a low fat content of 30−40%. It can be recognized by its red-brown coating with an embossed trademark that is placed by all members of the Union of cheese farmers of Leiden (*Vereniging van Boeren-Leidse kaasmakers*). The trademark guarantees artisanal production according to the traditional recipe. In order to avoid misuse of the registered name, all members of the union are obliged to participate in regular inspections by expert judges who check and judge a selection of cheeses. The acceptability and judgment of the cheese is mainly dependent on the odor, flavor, and the texture of the cheese. The time required to check the cheeses, and thereby the costs of production, could be greatly reduced with the use of an instrumental technique that can reproduce the sensitivity of the human nose.[2] Because the geographical origin of food plays an important role in its quality, cheese with Protected Designation of Origin has a high commercial value, even more so given the increasing interest of consumers in regional products. An instrumental technique that can link the cheese composition to its typical features and original environment would substantially improve the detection of potential fraud on the market of this high value product.

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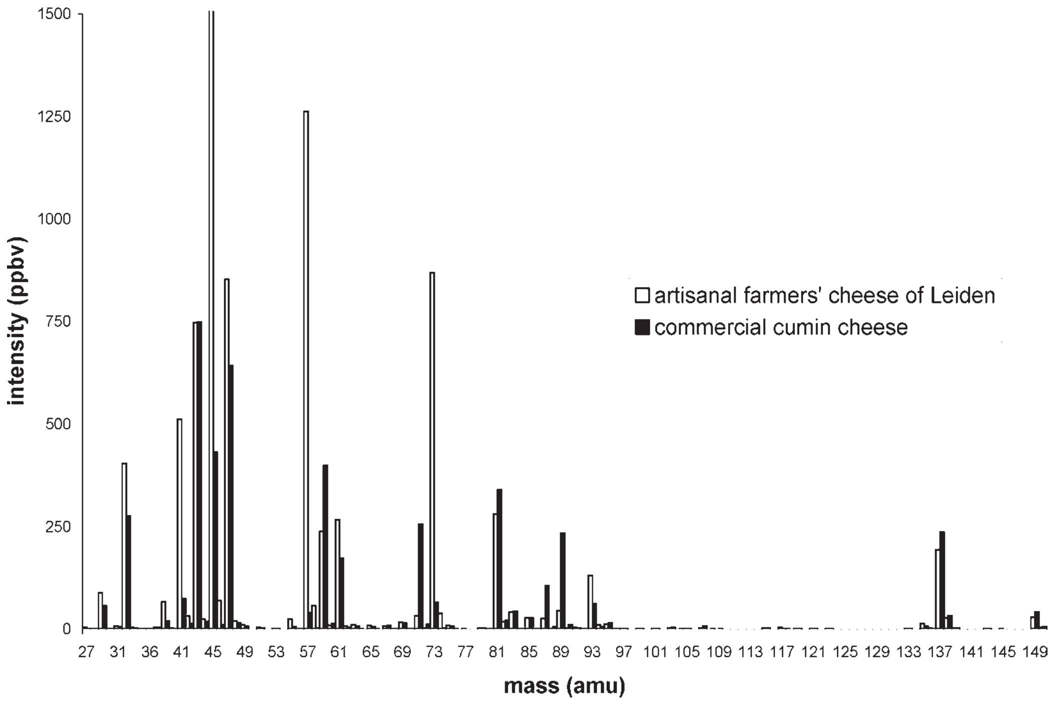

Figure 1. Mean HS PTR-MS mass spectra for PDO protected, artisanal farmers' cheese of Leiden and commercial Dutch cumin cheese without PDO protection.

In food consumption, prior expectations about the product are important as they may improve or degrade the perception of a product, even before it is tasted.[3] Besides advertising, packaging and brand familiarity, food labels influence the expectations of the consumer and thereby the outcome of the quality evaluation. In 1964 Allison and Uhl concluded that labels and their associations have a larger influence on the appreciation of various beer brands than the actual taste differences.[4] Identity information, in the form of word labels, is known to modulate the pleasantness of basic tastes by exerting top-down control on the neural mechanisms that regulate the appetitive value of taste and flavor.[5] Additional information in the form of a PDO label might exert a positive influence on consumers' expectations about the taste of the cheese and thereby influence their subjective appreciation.

With recent technological advances the efficiency and reliability of food authentication methods has greatly improved. Extracting complex profiles of components in a single analysis has become relatively simple with molecular spectrometry techniques. Relatively new in the field is the use of proton-transfer mass spectrometry (PTR-MS). PTR-MS, originally proposed by Lindinger,[6] is a fast and sensitive technique for volatile organic compound (VOC) detection. The volatile organic compounds in the headspace of the sample are measured and combined into a VOC profile per sample that can serve as a "fingerprint". Subsequently, products can be classified into meaningful categories based on the similarity of their "fingerprints". PTR-MS has been applied in various areas, from environmental science[7,8] to healthcare[9,10] and has been proven very useful for the investigation of the properties of agroindustrial products and processes.[11−15]

Flavor profiling based on PTR-MS spectra appears to be closely related to the traditional sensory characterization of cheese.[16] PTR-MS spectra have been used to distinguish different mozzarella cheeses[16] and Grana cheeses[11] and to authenticate the origin and the production process of varying food samples like traditional organic hams.[17] Recently, the coupling of PTR-MS with time-of-flight spectrometers has been achieved[18] confirming the high time resolution and sensitivity of the quadrupole based version and offering, at the same time, a better mass resolution and accuracy. Preliminary food applications of PTR-TOF-MS indicate the possibility to exploit these latter characteristics for the identification of the sum formula associated with the observed peaks,[19] thus increasing the analytical information provided by PTR-MS.

The aim of the present study was to investigate whether samples of PDO protected farmers' cheese of Leiden can be discriminated from other Dutch cumin cheese samples without PDO protection on the basis of their volatile profiles as measured by high sensitivity PTR-MS (HS PTR-MS). PTR-TOF-MS analysis was used for identification of the volatile compounds that characterize PDO protected farmers' cheese of Leiden. In addition to the chemical analysis of the Dutch cumin cheese, a consumer test was carried out to determine the consumer appreciation for the two types of cheeses and related sensory attributes and to study the influence of information about the origin of the cheese, in the form of word labels on the consumers' appreciation ratings.

■ MATERIALS AND METHODS

Materials. Fifty-nine low-fat cumin cheese samples were examined, 30 samples of which concerned PDO protected, artisanal farmers' cheese of Leiden, and 29 samples concerned commercial Dutch cumin cheese of varying brands without PDO protection. The PDO protected samples of farmers' cheese of Leiden were manufactured under conditions specified in the production rules of the union of cheese farmers of Leiden.[20] Four of the PDO protected samples of farmers' cheese of Leiden were obtained at local dairies and supermarkets, the other samples were collected directly at eleven registered cheese farms in the surroundings

Table 1. The 60 Most Predominant Ions Determined in HS PTR-MS Analyses of PDO Protected, Artisanal Farmers' Cheese of Leiden and Commercial Dutch Cumin Cheese without PDO Protection: Mean Concentrations, Standard Deviations and Significance of Differences (ANOVA)

	volatile compounds headspace concn (ppbv)[a]		
ions measd	farmers' cheese of Leiden (ppbv)	commercial cumin cheese (ppbv)	*P*
27	4.3 ± 2.6	1.4 ± 0.7	<0.0001
29	88.2 ± 51.4	56.1 ± 80.0	0.073
30	1.1 ± 3.2	0.5 ± 1.3	0.316
31	7.6 ± 3.3	5.1 ± 3.9	0.009
33	404.4 ± 159.6	277.6 ± 162.8	0.004
34	3.2 ± 2.0	1.8 ± 2.6	0.030
35	1.0 ± 0.4	0.6 ± 0.3	<0.0001
38	4.1 ± 0.6	4.1 ± 0.6	0.732
39	66.7 ± 37.5	18.9 ± 3.0	<0.0001
41	511.7 ± 346.6	71.7 ± 19.9	<0.0001
42	32.0 ± 13.7	13.3 ± 8.1	<0.0001
43	746.4 ± 324.3	729.4 ± 319.7	0.841
44	23.6 ± 11.5	18.2 ± 8.1	0.045
45	2946.9 ± 2965.4	439.4 ± 345.4	<0.0001
46	69.7 ± 71.6	10.5 ± 7.7	<0.0001
47	852.7 ± 498.7	632.5 ± 895.6	0.248
48	19.9 ± 11.9	14.5 ± 19.1	0.196
49	9.9 ± 3.0	6.6 ± 5.1	0.004
51	3.3 ± 1.2	2.3 ± 1.1	0.002
53	1.1 ± 0.9	1.1 ± 0.4	0.899
55	23.6 ± 26.1	6.2 ± 1.5	0.001
58	56.8 ± 49.4	2.0 ± 1.3	<0.0001
59	237.7 ± 166.9	387.2 ± 168.2	0.001
60	9.1 ± 5.8	13.0 ± 5.7	0.011
61	267.5 ± 149.2	168.5 ± 71.7	0.002
62	6.5 ± 6.9	4.3 ± 4.1	0.004
63	10.7 ± 0.2	7.2 ± 0.1	0.020
65	8.3 ± 4.8	6.2 ± 7.1	0.187
67	7.0 ± 0.2	7.7 ± 0.2	0.442
69	16.0 ± 15.4	13.9 ± 4.7	0.480
70	1.2 ± 1.1	1.0 ± 0.3	0.352
71	32.5 ± 23.0	248.7 ± 181.6	<0.0001
72	2.0 ± 1.2	11.2 ± 8.2	<0.0001
73	868.0 ± 1442.0	64.1 ± 56.1	0.005
74	38.0 ± 60.9	2.9 ± 2.5	0.004
75	8.5 ± 4.9	6.4 ± 6.8	0.175
79	2.5 ± 0.9	2.6 ± 1.1	0.702
81	280.7 ± 108.5	309.2 ± 185.5	0.474
82	18.3 ± 7.3	19.9 ± 11.1	0.530
83	41.9 ± 41.4	42.4 ± 27.0	0.954
85	26.9 ± 26.4	26.4 ± 17.0	0.936
87	25.4 ± 22.2	95.4 ± 76.4	<0.0001
88	1.2 ± 1.1	4.4 ± 3.5	<0.0001
89	44.4 ± 17.9	226.4 ± 166.6	<0.0001
90	2.0 ± 0.8	10.4 ± 7.8	<0.0001
91	4.5 ± 2.4	2.7 ± 1.5	0.001
93	131.4 ± 80.0	52.0 ± 32.1	<0.0001
94	10.1 ± 6.2	4.1 ± 2.6	<0.0001
95	12.3 ± 5.0	13.2 ± 6.9	0.581
96	0.9 ± 0.4	1.0 ± 0.5	0.887

Table 1. Continued

ions measd	volatile compounds headspace concn (ppbv)[a]		
	farmers' cheese of Leiden (ppbv)	commercial cumin cheese (ppbv)	*P*
103	2.6 ± 3.5	4.0 ± 2.6	0.092
107	2.7 ± 1.1	6.6 ± 3.5	<0.0001
115	2.2 ± 2.3	2.7 ± 2.1	0.471
117	3.8 ± 1.8	1.4 ± 0.5	<0.0001
119	1.1 ± 0.6	0.6 ± 0.5	0.001
121	1.3 ± 0.5	1.4 ± 0.7	0.417
133	1.2 ± 0.7	1.0 ± 0.6	0.157
135	12.7 ± 0.1	5.7 ± 0.1	<0.0001
136	2.0 ± 1.1	1.1 ± 0.7	0.001
137	193.6 ± 83.6	213.1 ± 121.3	0.477

[a] Concentration is expressed as volume mixing ratio in air.

of the city of Leiden. All PDO protected samples of farmers' cheese of Leiden have similar ripening times (2 to 4 months) and had a fat percentage of 30−40%. These samples were compared to 29 commercial cumin cheese samples of varying brands without PDO registration with similar ripening times and fat contents according to their labels, obtained at local dairies and supermarkets. Most of the samples for the HS PTR-MS measurements were collected in the last week of February 2010 and the first two weeks of March 2010 and analyzed in the first half of March 2010. A selection of six samples was collected separately. They concerned three samples of PDO protected farmers' cheese of Leiden produced at three different farms and three samples of different brands of cumin cheese which were all collected in the last week of May 2010. These three PDO farms and the three additional brands were present in the February−March HS PTR-MS sample set and were representative material for PDO protected farmers' cheese of Leiden and cumin cheese without PDO protection in the study. The newly collected samples were analyzed by HS PTR-MS in the first week of June 2010. Furthermore they were shipped to Italy and analyzed by PTR-TOF-MS in the second week of June 2010. The same sample material was also subjected to consumer testing in The Netherlands in the last week of May 2010. All cheeses had a fat percentage of 30% and similar ripening times (3−4 months).

Methods. *HS PTR-MS: Instrumental Analysis.* Following collection of the cumin cheese samples, they were stored in the absence of light at 5 °C. Cubes of approximately 3 g were equilibrated at 30 °C for 30 min in a 250 mL screw cap glass bottle, and the headspace of the samples was delivered directly to the inlet of the HS PTR-MS system (Ionicon GmbH, Innsbruck, Austria). The airflow rate was 75 mL per minute. The temperature of the inlet and drift chamber was 60 °C. The HS PTR-MS was operated at a standard E/N (ratio of electric field strength across the reaction chamber, E, to buffer gas number density, N, within the chamber) of 138 Td (1 Td = 10^{-17} cm^2 V molecule^{-1}), and measurements were carried out in the "mass scan" mode, whereby a complete mass spectrum in the range of 20−150 atomic mass units (amu), at a mass detection rate of 0.2 s mass^{-1}, was gathered in 26 s. Analyses were carried out in independent triplicates with each replicate measured for 7 mass scan cycles. Background measurements were obtained by alternating 7 sample scan cycles with 7 scan cycles of a blank air sample.

HS PTR-MS: Data Processing. The data were background corrected. The first two and the last two cycles were discarded, and the remaining three were used to provide a mean mass spectrum per replicate sample. Subsequently, a mean mass spectrum per sample was calculated from the replicate sample data. After outlier removal the data were used for multivariate analyses. A few masses associated with the HS PTR-MS ion source were removed from the mass spectra, i.e. 32 (O_2^+); 37 and 57 (major water cluster ions). The averages of triplicate measurements were subjected to partial least-squares discriminant analysis (PLS-DA; Pirouette 4.01, Infometrix, Seattle, WA) in order to estimate a classification model of PDO protected, artisanal farmers' cheese of Leiden and commercial Dutch cumin cheese without PDO protection. The results of several preprocessing techniques were analyzed. Optimal results were obtained after autoscaling and optimization of the number of factors. The performance of the model was evaluated using a leave-one-out cross validation method. A one-way analysis of variance was performed on the concentration data of the 60 most predominant ions in order to determine significant differences between the farmers'cheese of Leiden and the other cumin cheeses. The p-value for testing statistical significance was 0.05 throughout the study.

PTR-TOF-MS: Instrumental Analysis. The volatile compounds of six representative samples of both types of cheese, three of each, were tentatively identified using a PTR-TOF-MS 8000 instrument from Ionicon GmbH (Innsbruck, Austria) in its V mode configuration. The sampling procedure was similar to the HS PTR-MS procedure described in HS PTR-MS: Instrumental Analysis. Samples were presented in a 120 mL screw cap glass bottle (Supelco, Belefonte, US), and the temperature of the inlet and drift chamber of the PTR-TOF-MS system was 110 °C. The sampling time per channel of TOF acquisition was 0.1 ns, amounting to 350000 channels for a mass spectrum ranging from m/z 10 to 400. The ionization conditions in the reaction chamber were maintained at a drift voltage of 600 V and a drift pressure of 2.25 mbar. The instrument was operated at an E/N value of 140 Td (1 Td = 10^{-17} cm^2 V^{-1} s^{-1}). We refer to Fabris et al.[19] for a more detailed description of the PTR-TOF-MS analysis.

PTR-TOF-MS: Data Processing. Base line removal and spectra alignment by internal calibration of the TOF data were performed according to the procedure described by Cappellin et al.[18] After outlier removal the average of triplicate measurements of the entire spectrum data set (m/z 33−200, m/z 37 excluded) was subjected to a principal component analysis (PCA; STATISTICA release 8, Statsoft Inc., Tulsa, OK, USA). One-way ANOVA was performed on the concentration data of the individual ions in order to determine significant differences between this selection of farmers' cheese of Leiden and the other cumin cheeses.

Consumer Preference Testing: Procedure. In addition to the instrumental analysis of Dutch cumin cheese using HS PTR-MS, a consumers' preference test was carried out. Two hundred seventeen Dutch visitors of an open day of the RIKILT research institute were invited to taste samples of PDO protected farmers' cheese of Leiden, originating from three different farms, and samples of three brands of cumin cheese without PDO protection and rate their preferences. In total 217 people participated in the cheese tasting, 97 men and 120 women, in the age range of 8−88 years (m = 43 ± 17).

The samples were presented at room temperature in cubes of approximately 10 g. Participants were instructed to taste a prespecified

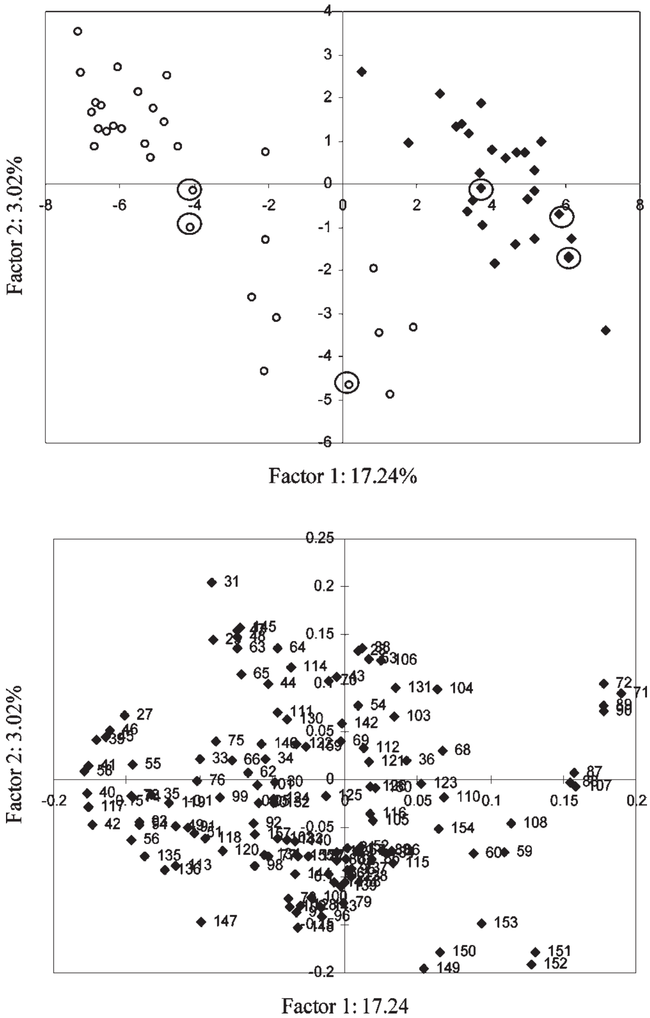

Figure 2. Scores (upper) and loadings (lower) plots of the first two dimensions of PLS-DA on the mass spectral data of 30 samples of PDO protected, artisanal cumin cheese (open circles) and 29 samples of varying brands of Dutch cumin cheese without PDO protection (solid diamonds) determined by HS PTR-MS. Circled samples were used in PTR-TOF-MS analysis and consumer testing.

sample and to rate the overall appreciation of the sample on a 7-point Likert scale ranging from 1 = "very bad" through 4 = "not good/not bad" to 7 = "very good". Subsequently they were asked to rate the quality of the cheese on the following characteristics: overall flavor intensity, cumin flavor intensity, creaminess intensity, and firmness intensity on a 5-point scale ranging from 1 = "too low" through 3 = "exactly right" to 5 = "too high".

The attributes were generated and agreed on by participants of an internal expert panel session prior to the consumer test. The panelists were experienced in dairy product tasting.

After tasting the first sample the participants in the consumer test were requested to repeat the procedure with the second, prespecified sample. Participants took part in one of three different conditions. In the first condition ($n = 83$) the samples were labeled with the veridical name of the cheese (i.e., PDO protected farmers' cheese of Leiden or supermarket cheese). In the second condition ($n = 84$) the labels of the cheese samples were switched without the participants being aware of the switch. In this condition the participants were told that they were tasting the PDO protected farmers' cheese of Leiden when in fact they were tasting the cumin cheese without PDO protection and vice versa. In the third condition ($n = 50$) none of the cheese samples were labeled and the

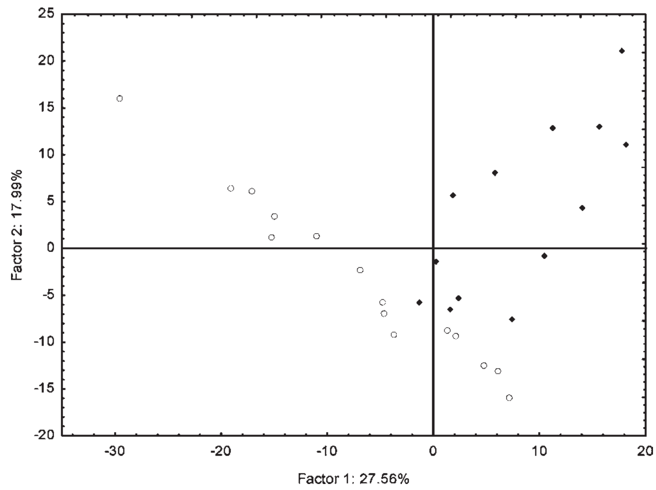

Figure 3. Scores plot of the first two dimensions of PCA on the mass spectral data (m/z 30−200) of 15 samples of PDO protected, artisanal cumin cheeses (open circles, 3 samples, 5 replicates) and 15 samples of varying brands of commercial Dutch cumin cheese without PDO protection (solid squares, 3 samples, 5 replicates) determined by PTR-TOF-MS.

participants were not informed about the type of cheese they were tasting. Conditions were alternated each hour, from 10 a.m. to 4 p.m. In addition participants were asked to indicate how often they consumed cumin cheese in daily life on a 5-point scale ranging from "almost never" to "often" and whether or not they were familiar with PDO protected farmers' cheese of Leiden (yes/no).

Consumer Preference Testing: Data Processing. Consumers' preferences were investigated using descriptive statistics. Overall appreciation of the cumin cheese samples of varying brands without PDO protection were compared to the appreciation ratings of PDO protected farmers' cheese of Leiden, using a repeated measures ANOVA. A one-way ANOVA was applied to test the effect of the presence or absence of veridical labels, familiarity with PDO protected farmers' cheese of Leiden, and frequency of cumin cheese consumption on consumers' appreciation of PDO protected farmers' cheese of Leiden and cumin cheese without PDO protection. Consumers' ratings of the characteristics of the cheese samples that were measured on a 5-point scale were compared using a nonparametric Friedman two-way analysis of variance using PASW Statistics 17.0.3 (SPSS, Inc. Chicago, IL).

RESULTS AND DISCUSSION

HS PTR-MS: Fingerprint Analyses. The mass spectral data were used as "fingerprints". The masses present in each sample and their corresponding signal intensities (ppbv) served as a pattern for sample comparison. Mean mass spectra for both types of cheese are displayed in Figure 1. A comparison of the average intensities of the 60 most predominant masses of PDO protected farmers' cheese of Leiden and commercial cumin cheese is displayed in Table 1. In comparison, the mean coefficient of variance for the masses listed in Table 1 within each of the Boeren-Leidse cheese samples (between replicate samples) was 15.1% on average. Out of the 60 predominant masses, 27 showed significantly higher concentrations in the PDO group and 9 showed significantly lower concentrations in the PDO group (ANOVA, $P < 0.05$). The remaining 24 masses showed no significant differences between de PDO group and the commercial cumin cheese samples. The considerable differences between the groups indicated the typicality of the PDO protected Farmers' cheese

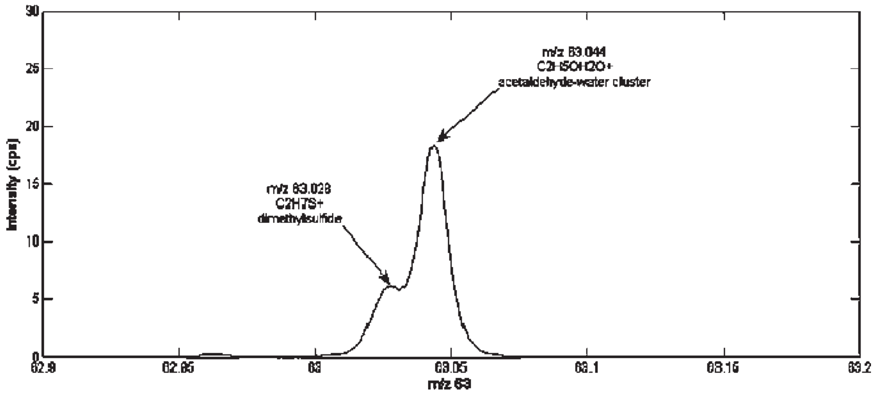

Figure 4. Mass spectral region at nominal mass 63 demonstrating the existence of a double peak corresponding to dimethyl sulfide (observed at *m*/*z* 63.028) and acetaldehyde−water cluster (*m*/*z* 63.044). The higher headspace concentration for *m*/*z* 63 in the case of HS PTR-MS originates from the high intensity of signal detected for the acetaldehyde−water cluster, and thus the higher mass resolution of PTR-TOF-MS provides additional information.

of Leiden, and this result was promising in view of development of an authentication test.

A PLS-DA classification model was estimated and optimized to classify the samples into two categories: PDO protected farmers' cheese of Leiden and cumin cheese of varying brands without PDO protection, using their HS PTR-MS mass spectra. Cross validation showed correct prediction of 28 out of 28 (100%) of non-PDO protected cumin cheese samples and 29 out of 30 (96%) of the PDO protected samples of farmers' cheese of Leiden using an autoscaled, 3 factor model. The scores and loadings plots of the first two dimensions of the PLS-DA model for all samples are presented in Figure 2. The PDO protected farmers' cheese of Leiden and the cumin cheeses of varying brands without PDO protection are clearly separated.

PTR-TOF-MS: Tentative Identification. In order to identify the characteristic volatile compounds of both types of cheese, more detailed analysis of the volatile profile of the cumin cheese samples was carried out by PTR-TOF-MS. Three representative samples of PDO protected farmers' cheese of Leiden and three representative commercial cumin cheese samples without PDO protection were analyzed. The identity of the selected samples is indicated in the PLS-DA plot in Figure 2.

Principal component analysis on the entire mass data set (*m*/*z* 33−200) as measured with PTR-TOF-MS clearly separated the PDO protected farmers' cheese of Leiden (farm 1−3) samples from the non-PDO protected cumin cheese samples (brand 1−3) (Figure 3). This separation confirms the results of PLS-DA analysis on HS PTR-MS data described in HS PTR-MS: Fingerprint Analyses.

The fingerprint analyses revealed 39 peaks in both types of cumin cheese (similar to Figure 1) that could be tentatively attributed to aldehydes, ketones, diketones, fatty acids, esters, and sulfur compounds, as well as three volatile compounds that are related to the presence of cumin seeds (*p*-cymene, cuminaldehyde and cumin alcohol) and their corresponding fragments.

The concentrations observed with HS PTR-MS and PTR-TOF-MS were different as different sample sets were used. However both techniques revealed generally the same trends at the individual nominal masses, with the exception of ions *m*/*z* 63 and 93. By checking the peaks found at nominal masses 63 and 93 we observe that the reversed order in the volatile organic compound concentration in the case of HS PTR-MS is related to the presence of isobaric peaks with different intensities. In the case of *m*/*z* 63, for example, the total signal detected by HS PTR-MS consists of the signal of dimethyl sulfide (that has a lower intensity) and of the acetaldehyde−water cluster signal (that has a higher intensity). The PTR-TOF-MS spectrum reveals that the acetaldehyde−water cluster signal is dominant (Figure 4). Table 2 lists examples of compounds that could be tentatively identified by comparing the measured with the theoretically expected masses, and by considering the available fragmentation pattern data.[12,21,22] So in general HS PTR-MS gives a good picture of the samples but in a few cases the high resolution of PTR-TOF-MS appears to be necessary to separate relevant isobaric peaks. A strong dependence on the type of cheese was observed for many compounds including acetaldehyde, ethanol, acetone, acetic acid/acetates, 2-butanone/butanal, diacetyl and acetoin/butyrates/butyric acid.

The four predominant compounds for PDO protected farmers' cheese of Leiden as identified by HS PTR-MS were, in decreasing order, acetaldehyde (*m*/*z* 45), 2-butanone/butanal (*m*/*z* 73), ethanol (*m*/*z* 47), and alkyl fragment (*m*/*z* 43). Three out of these four ions were also found to be the ions in highest concentrations in the selection of samples analyzed by PTR-TOF-MS. They were, in decreasing order, ethanol (*m*/*z* 47.048), 2-butanone-butanal (*m*/*z* 73.063), *p*-cymene (*m*/*z* 137.129), and acetaldehyde (*m*/*z* 45.033). The slight differences are due to the limited set of samples used in the PTR-TOF-MS analyses, as well as some slight instrumental differences.

In order to further explore which masses were specific for the different types of cumin cheese, a one-way ANOVA was carried out on the masses measured in the six samples. Out of the 39 masses (Table 2), 19 of them showed significantly higher concentrations in the PDO group, and 7 of them showed significantly lower concentrations in the PDO group (ANOVA, $P < 0.05$). For 13 masses no significant differences between the PDO group and the commercial samples were observed. This pattern is in line with the results obtained with HS PTR-MS. Strongly discriminating compounds included e.g. diacetyl, 2-propanone, 2-butanone/butanal, 2,3-pentadiene, hexanoic acid, *p*-cymene, formic acid, and 2-propenal (low *p*-values).

Regarding the compounds in the volatile profiles of the cumin cheeses, some information on their origin/formation is available. For instance aldehydes, responsible for a fruity note, are the major secondary products of autoxidation of unsaturated fatty acids.[23] Besides the fermentation production by microorganisms

Table 2. Ions Determined in PTR-TOF-MS of PDO Protected, Artisanal Farmers' Cheese of Leiden and Commercial Dutch Cumin Cheese without PDO Protection: Tentative Identification, Mean Concentrations, Standard Deviations and Significance of Differences (ANOVA)

m/z				volatile compounds headspace concn (ppbv)		
measd	theor *m/z*	chem formula	tentative identification of volatile compds	Farmers' cheese of Leiden	commercial cumin cheese	*p*
29.039	29.0386	$C_2H_5^+$	alkyl fragment (ethanol)	1573 ± 182	1070 ± 51	0.012
33.033	33.0334	CH_5O^+	methanol	1094 ± 109	794 ± 32	0.014
34.995	34.9949	H_2SH^+	hydrogen sulfide	0.177 ± 0.031	0.117 ± 0.014	0.039
41.038	41.0385	$C_3H_5^+$	alkyl fragment	2299 ± 522	705 ± 53	0.005
42.033	42.0338	$C_2H_4N^+$	acetonitrile	58.8 ± 5.5	43.8 ± 3.8	0.035
43.054	43.0542	$C_3H_7^+$	alkyl fragment	969 ± 171	587 ± 35	0.038
44.997	44.9971	CO_2H^+	carbon dioxide	11.9 ± 0.3	11.7 ± 0.4	0.691
45.033	43.0334	$C_2H_5O^+$	acetaldehyde	4199 ± 804	2001 ± 245	0.015
47.013	47.0127	$CH_3O_2^+$	formic acid	20.6 ± 0.3	17.6 ± 0.9	0.003
47.048	47.0491	$C_2H_7O^+$	ethanol	7113 ± 502	6889 ± 341	0.715
49.010	49.0106	CH_5S^+	methanethiol	17.4 ± 4.3	4.07 ± 0.6	0.005
57.034	57.0334	$C_3H_5O^+$	2-propenal	176 ± 37	66.9 ± 2.8	0.007
57.068	57.0698	$C_4H_9^+$	alkyl fragment	5613 ± 1471	120 ± 8	0.001
59.048	59.0491	$C_3H_7O^+$	2-propanone	1252 ± 88	4388 ± 400	<0.001
61.028	61.0284	$C_2H_5O_2^+$	acetic acid/acetates	536 ± 109	501 ± 66	0.784
63.027	63.0262	$C_2H_7S^+$	dimethyl sulfide	12.8 ± 2.0	20.3 ± 3.1	0.039
69.067	69.0698	$C_5H_9^+$	isoprene, 3-hexen-2-ol	76.0 ± 9.6	55.1 ± 3.9	0.034
73.063	73.0647	$C_4H_9O^+$	2-butanone/butanal	6710 ± 1561	791 ± 73	0.001
75.044	75.0441	$C_3H_7O_2^+$	propionic acid/methyl acetate	43.8 ± 6.6	61.3 ± 14.7	0.273
79.054	79.0542	$C_6H_7^+$	benzene	87.1 ± 10.6	87.9 ± 7.4	0.952
81.068	81.0698	$C_6H_9^+$	alkyl fragment (*p*-cymene)	3851 ± 587	3947 ± 669	0.915
83.049	83.0491	$C_5H_7O^+$	1,4-pentadien-3-one	10.1 ± 0.6	8.71 ± 0.23	0.032
87.042	87.0441	$C_4H_7O_2^+$	diacetyl	96.0 ± 10.6	968 ± 96.6	<0.001
87.078	87.0804	$C_5H_{11}O^+$	2-pentanone/pentanal	52.3 ± 7.5	70.6 ± 8.4	0.082
89.056	89.0597	$C_4H_9O_2^+$	acetoin/butyric acid/butyrates	410 ± 52	4953 ± 640	<0.001
93.068	93.0698	$C_7H_9^+$	methyl benzene	841 ± 116	1057 ± 136	0.240
101.060	101.0597	$C_5H_9O_2^+$	2,3-pentanediene	14.5 ± 0.6	11.2 ± 0.7	0.001
101.095	101.0961	$C_6H_{13}O^+$	2-hexanone/hexanal	4.93 ± 0.65	3.37 ± 0.35	0.045
103.076	103.0754	$C_5H_{11}O_2^+$	isovaleric acid	15.6 ± 2.7	10.8 ± 1.4	0.124
107.085	107.0855	$C_8H_{11}^+$	dimethylbenzene	111 ± 15	143 ± 13	0.041
115.113	115.1117	$C_7H_{15}O^+$	2-heptanone/heptanal	16.0 ± 0.9	33.5 ± 7.9	0.038
117.091	117.0910	$C_6H_{13}O_2^+$	hexanoic acid	20.3 ± 2.7	10.4 ± 0.6	0.001
119.053	119.0525	$C_5H_{11}OS^+$	1-methylthio-2-butanone	0.123 ± 0.005	3.01 ± 0.80	0.002
133.102	133.1012	$C_{10}H_{13}^+$	alkyl fragment (cumin alcohol)	88.2 ± 13.7	46.2 ± 3.9	0.007
137.129	137.1325	$C_{10}H_{17}^+$	*p*-cymene	4717 ± 742	3771 ± 467	0.291
143.144	143.1430	$C_9H_{19}O^+$	2-nonanone/nonanal	5.87 ± 0.21	9.98 ± 3.09	0.061
145.123	145.1223	$C_8H_{17}O_2^+$	octanoic acid	15.9 ± 4.2	6.44 ± 0.68	0.034
149.096	149.0961	$C_{10}H_{13}O^+$	cuminaldehyde	1518 ± 203	1509 ± 167	0.973
151.110	151.1117	$C_{10}H_{15}O^+$	cumin alcohol	370 ± 49	458 ± 52	0.229

(lactose metabolism) acetaldehyde can derive from threonine degradation, a process that could be of importance during cheese ripening.[24] Acetaldehyde is the most common aldehyde found in cheese and contributes to the flavor of various varieties of cheese.[25] Diacetyl, which gives the odor a buttery caramel note, may be a product of microbial action involving lactobacteria[26,27] and citrate metabolism. Diacetyl is one of the most characteristic odors of cultured milk and fresh cheeses[28] and has been found to increase during ripening in Swiss Emmental[29] and Pecorino Sardo[2] and was found in other two hard cheeses, Gruyere[30] and Parmigiano Reggiano.[27] Methanethiol has been associated with the aroma of cooked cabbage and has been recognized as a contributor to the characteristic aroma of cheese being particularly influential in the aroma and flavor of many surface ripened cheeses.[31]

Consumers' Preferences. Exploration of consumers' appreciation of PDO protected and non-PDO cumin cheese revealed that both PDO protected, artisanal farmers' cheese of Leiden and commcercial Dutch cumin cheese without PDO protection were liked by this consumer group. The overall appreciation ratings of both types of cheese were above average. The average consumers' appreciation score for PDO protected farmers' cheese of Leiden was 4.4. This score lies between 4 (not good/not bad) and 5

Table 3. Consumer Test Results of PDO Protected, Artisanal Farmers' Cheese of Leiden and Commercial Dutch Cumin Cheese: Mean Consumer Scores, Standard Deviations, and Significance of Differences (Appreciation: ANOVA; Overall Flavor/Cumin Flavor/Creaminess/Firmness: Friedman's Two-Way Analysis of Variance)

consumer ratings[a]	PDO protected farmers' cheese of Leiden	commercial cumin cheese	significance
Scores of All Consumers Combined			
appreciation	4.4 ± 1.7	5.0 ± 1.5	$F(1)17.88$, $P = 0.000$
overall flavor intensity	3.2 ± 1.2	2.8 ± 1.0	$\chi^2(1)15.91$, $P < 0.000$
cumin flavor intensity	2.9 ± 1.2	2.8 ± 1.0	$\chi^2(1).95$, $P = 0.330$
creaminess intensity	2.0 ± 0.9	3.0 ± 1.0	$\chi^2(1)84.57$, $P < 0.000$
firmness intensity	3.5 ± 1.0	2.6 ± 0.8	$\chi^2(1)84.1$, $P < 0.000$
Consumers Preferring PDO Protected, Artisanal Farmers' Cheese of Leiden over Commercial Dutch Cumin Cheese			
appreciation	5.7 ± 1.2	3.7 ± 1.5	$F(1)174.47$, $P < 0.000$
overall flavor intensity	3.2 ± 0.75	2.7 ± 1.2	$\chi^2(1)7.08$, $P = 0.008$
cumin flavor intensity	3.0 ± 0.8	2.9 ± 1.3	$\chi^2(1)1.88$, $P = 0.170$
creaminess intensity	2.5 ± 0.9	3.0 ± 1.3	$\chi^2(1)4.79$, $P = 0.029$
firmness intensity	3.2 ± 0.9	2.4 ± 0.9	$\chi^2(1)24.2$, $P < 0.000$
Scores of Consumers Preferring Commercial Dutch Cumin Cheese over PDO Protected, Artisanal Farmers' Cheese of Leiden			
appreciation	3.4 ± 1.4	5.7 ± 1.1	$F(1)326.05$, $P < 0.000$
overall flavor intensity	3.2 ± 1.4	2.9 ± 0.7	$\chi^2(1)2.53$, $P = 0.112$
cumin flavor intensity	2.7 ± 1.3	2.9 ± 0.9	$\chi^2(1)1.64$, $P = 0.201$
creaminess intensity	1.7 ± 0.8	3.0 ± 0.8	$\chi^2(1)74.49$, $P < 0.000$
firmness intensity	3.7 ± 1.0	2.7 ± 0.7	$\chi^2(1)41.86$, $P < 0.000$

[a] Overall flavor, cumin flavor, creaminess, and firmness: 1 = too little, 3 = exactly right, 5 = too much.

(moderately good). The average consumers' appreciation score for the three brands of Dutch cumin cheese without PDO protection was 5.0 (5 = moderately good). The main effect of type of cheese on consumers' appreciation yielded an F ratio of $F(1)15.48$, $p < 0.001$, indicating that the mean appreciation score was higher for the three brands of cumin cheese without PDO protection (5.0 ± 1.5) than for PDO protected farmers' cheese of Leiden (4.4 ± 1.7) (Table 3).

Qualitative analyses of the preferences of the sensory panel revealed that more than half the panel, 110 out of 217 people, preferred the non-PDO cumin cheese over the PDO protected farmers' cheese of Leiden (commercial cheese likers), whereas 44 people gave similar ratings to both types of cheese and 63 people preferred the PDO protected cheese farmers' cheese of Leiden over the non-PDO protected cumin cheese (PDO likers). The majority of the PDO-likers were female (61%) and aged 20−50. Seniors (age 60−80) seemed to prefer the commercial cheese predominantly. The average appreciation score in the PDO likers group was 5.7 for the PDO protected farmers' cheese of Leiden, whereas it was 3.4 in the commercial cheese likers group (Table 3). For the commercial cheeses, the opposite was observed: the appreciation of that type of cheese was rated in the PDO likers group 3.7 on average, and in the commercial cheese likers group 5.7. Both groups rated the overall flavor intensity higher for the PDO protected farmers' cheese of Leiden, and the cumin flavor intensity fairly similar for both types of cheeses. Both groups rated the creaminess of the PDO cheese lower and its firmness higher than for the commercial cheese. However, the commercial cheese likers perceived the PDO protected farmers' cheese of Leiden to a larger extent "not creamy enough" than the PDO likers group (average scores 1.7 and 2.5, respectively). Similar differences were observed for firmness, but now the commercial cheese likers rated the intensity of this attribute more severely 'too strong' (average score 3.7) than the PDO likers group (average score 3.2).

It can be concluded that the consumers perceived strong overall flavor and firm texture of the Farmers' cheese of Leiden, whereas difference in appreciation depended mostly on the perception/preference of the texture qualities.

In both the PDO likers and commercial cheese likers groups few people were familiar with PDO protected farmers' cheese of Leiden. Familiarity with PDO protected farmers' cheese of Leiden did not influence consumers' overall appreciation scores ($F(1)0.33$, $p = 0.564$) and no significant interaction was observed between familiarity with PDO protected farmers' cheese of Leiden and type of cheese on appreciation scores ($F(1).46$, $p = 0.830$).

The main effect of cumin cheese consumption on the overall appreciation rates was significant ($F(4)15.15$, $p = <0.001$) indicating that the appreciation of cumin cheese increased linearly with an increase in frequent consumption of cumin cheese. No differential effect was observed of frequency of cumin cheese consumption on consumers' appreciation ratings of PDO protected farmers' cheese of Leiden and non PDO protected cumin cheese ($F(4)0.09$, $p = 0.99$).

Prompting expectations about the cheese samples by supplying extra information in the form of correct or incorrect labels did not exert significant influence on consumers' ratings of appreciation of both cheese types. No effect of condition on consumers overall appreciation ratings was observed ($F(2)0.74$, $p = 0.48$), and no significant interaction effect of condition and type of cheese was observed on the ratings of appreciation ($F(2)0.67$, $p = 0.51$). It is important to note that the informative value of the PDO label was limited as only a small portion of the panel (18%) had heard of the brand of PDO protected farmers' cheese of Leiden before the cheese tasting. These results contradict earlier findings of a modulatory effect of word labels on the appreciation of food products.[3−5] In the present study the potential influence of word labels on appreciation might have been overshadowed by strong consumers' preferences due to large differences in sensory qualities of both types of cheese.

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ABBREVIATIONS USED

AMU, atomic mass unit; ANOVA, analysis of variance; EU, European Union; HS PTR-MS, high sensitivity proton-transfer mass spectrometry; PDO, Protected Designation of Origin; PGI, Protected Geographical Indication; PLS-DA, partial least squares discriminant analysis; PPBV, parts per billion by volume; PTR-MS, proton-transfer mass spectrometry; PTR-TOF-MS, proton-transfer time-of-flight mass spectrometry; TSG, Traditional Specialty Guaranteed; VOC, volatile organic compound

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