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Identification of the Production Chain of Asiago d'Allevo Cheese by Nuclear Magnetic Resonance Spectroscopy and Principal Component Analysis

ELISABETTA SCHIEVANO,*,† GABRIELLA PASINI,§ GIULIO COZZI,# AND STEFANO MAMMI†

Dipartimento di Scienze Chimiche, Università di Padova, Via Marzolo 1, 35131 Padova, Italy; Dipartimento di Biotecnologie Agrarie and Dipartimento di Scienze Animali, Università di Padova, Viale dell'Università 16, 35020 Legnaro, Italy

In the present work, a rapid and simple NMR method to discriminate Asiago d'Allevo cheese samples from different production chains is described. A fast and reproducible extraction of the organic fraction was employed. By applying chemometric analysis to NMR data, it is possible to differentiate PDO Asiago cheese produced in alpine farms from that produced in lowland and mountain industrialized factories. PCA of both ^{1}H and ^{13}C NMR spectra showed a good separation of alpine farm products from the other ones, whereas the lowland and mountain industrialized cheeses are undistinguishable. The samples were differentiated on the basis of a higher content of unsaturated fatty acids, principally oleic, linoleic, linolenic, and conjugated linoleic acids for the alpine farm cheeses and a higher content of saturated fatty acids for the industrialized products. Conjugated linoleic acid and 1-pentene are also discriminating components.

KEYWORDS: Asiago cheese; production chain; NMR; principal component analysis

INTRODUCTION

"Asiago", a popular Italian cheese, is produced from raw dairy milk according to a regulation approved by the European Union (*1*). The cheese has a protected denomination of origin (PDO) mark, which strictly defines the geographical area of its production within the Veneto and Trentino regions. This geographical area includes both lowland and mountain zones, and in this scenario, the same cheesemaking regulation is applied in very diverse husbandry conditions. Industrialized factories localized in either lowland or mountain areas produce this cheese according to standardized practices. They process milk from dairy cattle fed high-concentrate diets, whereas alpine farms process milk from grazing cattle according to more traditional systems.

The result of these production chains is cheese with different nutritional and organoleptic properties, because the feeding plan of the dairy cows has been shown to be a predominant factor affecting milk quality. In this regard, it has been reported that volatile compounds, such as terpenes and sesquiterpenes, can be transferred, through the milk, from forage to cheese (*2*) and that they affect cheese flavor (*3*). Other authors used terpenes to differentiate mountain cheese from lowland products (*4, 5*). More recently, Favaro et al. (*6*) worked on Asiago cheese and proposed the presence of sesquiterpenes as an effective marker for the traceability of mountain cheese. Moreover, pasture grazing has been shown to modify milk components such as proteins, minerals (*7*), and specifically fatty acids (*8*). From a nutritional point of view, pasture grazing has been shown to positively affect the acidic profile of milk with an increase of unsaturated fatty acids and conjugated linoleic acid (CLA) (*9*). Recently, Zhang et al. (*10*) suggested that these fatty acids can be transferred to cheese. Therefore, the dairy cattle feeding plan and particularly the forage to concentrate ratio along with the forage quality, in terms of botanic composition and storage system, may result in a different end product within the same cheesemaking chain.

The aim of the present work is to apply nuclear magnetic resonance (NMR) to the discrimination of Asiago cheese obtained from different production chains. This spectroscopic technique is noninvasive, simple, and rapid because it does not require any complex chemical preparation of the sample. Moreover, it has the great advantage of allowing the detection of all the substances present in the sample at the same time, avoiding artifacts due to the breakdown of macromolecular structures. Recently, NMR has been proposed as a new control tool to enforce the legislation on food quality. Examples are the cases of ^{1}H NMR methods to assess the geographical origin of Italian olive oil (*11*) and wine (*12*) and to establish the age of balsamic vinegars (*13*). The same spectroscopic technique has also been used to monitor the change of the free amino

* Author to whom correspondence should be addressed (telephone 39-0498275742; fax 39-0498275239; e-mail elisabetta.schievano@unipd.it).
† Dipartimento di Scienze Chimiche.
§ Dipartimento di Biotecnologie Agrarie.
# Dipartimento di Scienze Animali.

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Table 1. Description of the Production Chains of Asiago d'Allevo Cheese

	Asiago d'Allevo cheese		
	industrialized		
	lowland	mountain	alpine farms
no. of production sites	11	6	15
av no. of wheels/site × year	20000	5300	530
dairy cows feeding plan			
forage/concentrate ratio (% total dry matter)	60:40	65:35	80:20
type of forage in the diet			
maize silage (no. of sites)	10	2	
grass and lucerne hay (no. of sites)	11	6	2
grazing pasture (no. of sites)		2	15

acid profile during the ripening of Grana Padano cheese (*14*) or to differentiate coffee or oil on the basis of their processing methods (*15, 16*).

NMR spectroscopy has already been applied in combination with multivariate statistical analysis in two exploratory studies to characterize the ripening of Parmigiano Reggiano cheese (*17*) and to determine the geographical origin of Emmental cheese (*18*) with good results. In those cases, a high-resolution MAS (magic angle spinning) approach was followed to analyze cheese samples directly in the solid state, which is very convenient in terms of sample treatment. The problems with that method reside in its limited experimental reproducibility and in sample heterogeneity, which is a more severe problem in the analysis of solid-state materials. Moreover, high-resolution solid-state NMR probes are less common than probes for liquids.

In the present study, organic extracts of cheeses of different origin were examined by ^{1}H and ^{13}C high-resolution NMR in solution with the aim to discriminate the production chain. This method entails a faster procedure relative to gas chromatographic methods (*6*) and overcomes the problems encountered in solid-state NMR (*17, 18*).

MATERIALS AND METHODS

Description of the Production Chains. The study considered Asiago d'Allevo cheese, which can be produced only with dairy milk collected within the officially recognized production zone according to its PDO regulation. All of the production sites of this cheese were considered, and they were assigned to three different types of production according to their location and production system. Eleven sites were industrial cheese factories located in the lowland and 6 in the mountains, whereas 15 were alpine farms. The main features of these three different types of production are summarized in **Table 1**. The cheese factories located in the lowland are characterized by a greater production in terms of wheels per year. They process milk collected from intensive dairy farms in which high-producing dairy cattle mainly belonging to Holstein Friesian and Italian Brown breeds are raised. These animals are fed high-concentrate diets in which the forage portion is composed of maize silage and grass and lucerne hay. Despite the location in the highlands, also the mountain industrial factories collect and process milk from high-producing cows that are fed intensive diets with an average forage to concentrate ratio very similar to those adopted in the lowland (**Table 1**). Because in many mountain areas it is difficult and inconvenient to grow maize for silage making, this forage is almost absent from the highland diets, and it is replaced by hay or by grazing pasture during the summer. The alpine farms operate only during the summer season according to an extensive system of production. Cows graze on pasture and produce less milk because of the high forage feeding regimen.

Cheese Sampling. Forty-seven wheels of cheese (weight = 8−12 kg, diameter = 30−36 cm, height = 9−12 cm) were collected during the period March 2004−January 2005 as shown in **Table 2**. All of the samples had a ripening time of 12 months, and they were collected at the site of production. The sampling scheme was designed according to the availability of the cheese. In this regard, the limited number of wheels collected from the cheesemaking factories during the summer was due to the limited production of this type of cheese at this time of year. The samples were sliced from the center to the edge and stored at −18 °C until analysis.

Table 2. Sampling Scheme Adopted in the Study Expressed as Number of Wheels

	2004				2005	
production chain	March	June	July	Sept	Jan	total
lowland factory	6	2	1	1	5	15
mountain factory	2		2	1	5	10
alpine farm		8	7	7		22

Sample Preparation. A very simple and fast method to extract the apolar components from cheeses was used. A slice of cheese (at least 150 g) was ground with a blender under liquid nitrogen after removal of 2 cm of crust all around. A weighted amount (0.2 g) of ground sample was dissolved in 1 mL of ^{2}H-$CHCl_3$, vortexed at room temperature for 10 min, filtered with a 0.2 μm filter (Millipore) to remove insoluble material, and directly placed in the NMR sample tube.

NMR Spectroscopic Analysis. All proton NMR spectra were recorded on a Bruker Avance 600 DMX instrument (Bruker Analytik GmbH, Rheinstetten, Germany) operating at 600.09 MHz for ^{1}H. A typical ^{1}H spectrum, recorded at 300 K, consisted of 1024 transients using 16K data points over a 6000 Hz spectral window with a 7 μs 90° pulse. A recycle time of 2 s was used. Each spectrum was Fourier transformed using an exponential window function with a line broadening of 0.2 Hz, phased and baseline corrected. The ^{13}C spectra were collected using inverse-gated decoupling to avoid the development of nuclear Overhauser effect to the signals, with a 200 ppm spectral width, 64K data points, and a 12.3 μs 90° pulse. ^{1}H and ^{13}C NMR chemical shifts were referenced to the chloroform signals at 7.26 and 77 ppm, respectively.

Selective 1D-TOCSY experiments were obtained with a Gaussian-shaped pulse and a 70 ms mixing time. The TOCSY spectra were acquired as 512 experiments of 2K data points and 64 scans each; the spectral width was 10 ppm and the delay time, 1 s. The HMQC spectra were acquired as 120 experiments of 2K data points and 64 scans each; the spectral width was 10 ppm in the ^{1}H dimension and 150 ppm in the ^{13}C dimension; the delay time was 1 s.

The 1D spectra were processed using the ACD software (ACD/Specmanager 7.00 software, Advanced Chemistry Development Inc., Toronto, ON, Canada). The spectra were phased and baseline corrected using the ACD manual routine. Each ^{1}H spectrum was segmented into intervals ("buckets") of identical, 0.02 ppm, width over the region 0.00−10.00 ppm, and the signal intensity in each interval was integrated. Prior to principal component analysis (PCA), each integral interval was normalized to the integral of the entire spectrum (*19*).

The exact resonance position of the ^{13}C peaks is very sensitive to parameters related to the sample (such as relative concentration or pH) or to the instrument (field homogeneity variations, shimming effects) (*20*). For this reason, the integration intervals were chosen manually. The integrals were normalized with respect to glycerol.

In each case, the interval corresponding to the residual solvent peak was removed.

^{1}H and ^{13}C spectral assignment to link the content of each bucket to a specific compound was based on published data (*21, 22*) and was confirmed through the addition of pure standard compounds (triglycerol standards: tributyrin, tricaprin, tricaproin, tricaprylin, trilaurin, trilinolein, trimyristin, triolein, tripalmitin, tripalmitolein, tripentadecanoin, tristearin, and 1-pentene) purchased from Sigma Chemicals.

Statistical Analysis. The NMR data matrix was imported into the software SIMCA-P11 (Umetrics, Umea, Sweden) for statistical analysis in terms of PCA.

To validate the robustness of the discrimination, the samples were divided into training set and test set. The latter was constructed by randomly selecting two samples from each production class. The statistical analysis was performed on the training set. Normalized prediction distances (DmodX) provided distances to the models; critical values (D_{crit}) were computed with 0.95 confidence intervals. The distance to each of the PC models was computed and plotted in a Coomans' plot along with the critical distances. This approach was used to assess the classification performance of samples by predicting class membership in terms of distance to the model and to evaluate the specificity of the models (*23*).

A PLS-DA model was also applied splitting the samples into classes according to their origin. The validity of the PLS-DA model was assessed by statistical parameters: the correlation coefficient R^2 and the cross-validation correlation coefficient Q^2. Q^2 was derived using the default option of SIMCA-P. The same training set and test set were used as in the PCA.

RESULTS

Sample Extraction. The method of organic phase extraction used in this work is very simple and rapid and proved to be quite appropriate for our purposes. Prior to its application to the sample set, we compared our procedure with the modified-Folch method commonly used to extract triacylglycerols from solid matrices (*21, 24*). The latter method allows quantitative measurements but requires a considerably longer experimental time (*25*). The NMR spectra obtained with both methods showed the same fatty acid profile (data not shown). One advantage of our procedure is that volatile components that are lost with the modified-Folch method are retained and can, in principle, be used to discriminate the various samples (**Figure S1**; Supporting Information).

To demonstrate the reproducibility of this method, three repetitions of the same sample were performed. The relative standard deviation of selected integrals, reported in Table S1, is low. In all cases, the error on a specific peak is lower than the variation within a group of samples of the same production origin. These data indicate that the procedure can be used to compare different cheese samples on a relative basis without the ambition of being quantitative. When dealing with a high number of samples to be analyzed on a statistical basis, the method appears to be extremely effective.

Spectral Analysis. Figure 1A shows portions of the ^{1}H NMR spectra of a representative sample of Asiago d'Allevo cheese from an alpine farm. The signals of the triglycerides dominate the ^{1}H spectra, but signals from minor components, such as alcohols and CLA, are also present (**Figure 1B**). Industrial and farm samples display differences in the intensity of resonances relative to both the bisallylic and olefinic protons, appreciable by simple visual inspection (insets in **Figure 1A**, where portions of a spectrum from an industrial factory are compared to the alpine farm one). These findings are consistent with a higher content of unsaturated fatty acid in the cheese from alpine farms.

The PCA conducted on these spectra revealed that some minor components affect the separation (see below). To obtain the assignment of these components, selective 1D and 2D NMR experiments were performed. The selective ^{1}H TOCSY spectra are very clean, and the absence of the TGA signals results in a dramatic increase of the dynamic range of the experiment (**Figure 2**). This was therefore the experiment of choice to identify the spin system of the compounds present in small amounts. 2D TOCSY was performed to confirm proton assignment, whereas HMQC was performed to obtain ^{13}C assignment. By inspection of these spectra, we assigned the resonances of two compounds to CLA and 1-pentene (**Table 3**). The correct assignment of 1-pentene was confirmed by addition of the standard compound.

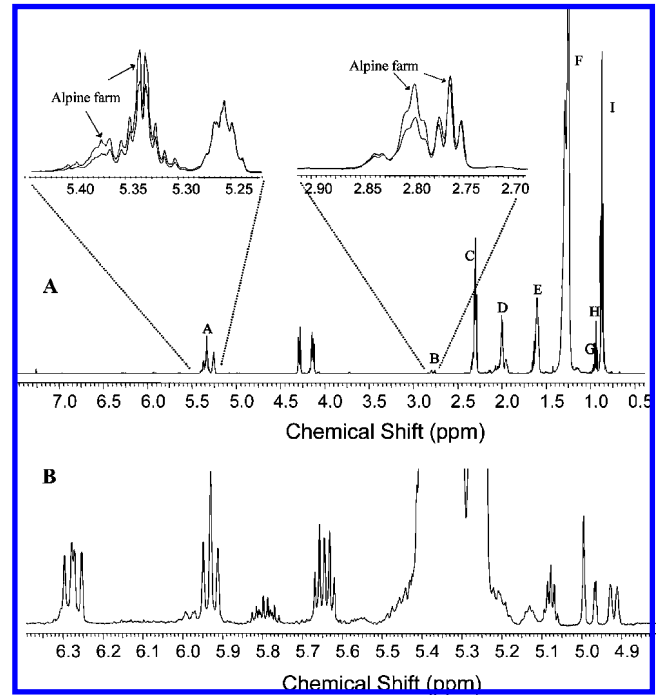

Figure 1. ^{1}H NMR spectra recorded at 600 MHz. (**A**) Spectrum of the organic extract of an Asiago cheese from an alpine farm with the assignment of the peaks originating from the fatty acids (major components). The letters indicate the following protons: A, olefinic protons of all unsaturated chains; B, bisallylic protons; C, methylenic protons bonded to C2 of all fatty acid chains; D, allylic protons; E, methylenic protons bonded to C3 of all fatty acid chains; F, methylenic protons; G, methyl protons of linolenic acid; H, methyl protons of butyric acid; I, all other methyl protons. In the top insets, the bisallylic and olefinic regions of two representative spectra from an alpine farm and an industrial factory are expanded and overlapped. Arrows indicate the spectrum from the alpine farm, which contains higher amounts of both bisallylic (at 2.80 ppm) and olefinic (at 5.34 and 5.38 ppm) protons. (**B**) Expansion of a region in which some important resonances from CLA and 1-pentene (minor components) fall. For the assignment, see **Table 3**.

Table 3. Assignment of Secondary Resonances in the 600 MHz Spectrum of the Organic Extract

	proton δ	carbon δ
CLA[a]		
11	6.28 (d,d J = 15.3 Hz, 10.3 Hz)	125.49
10	5.94 (t, 10.3 Hz)	128.43
12	5.65 (d,t J = 15.3, 7.3 Hz)	135.14
9	5.29 (d,t J = 10.3, 7.3 Hz)	
2	2.30	
3	1.61	
8	2.14	
13	2.09	
17−14, 7−4	1.30−1.36	
18	0.88	
1-pentene[a]		
1a	4.92 (d,d J = 10 Hz, 1.6 Hz)	114.06
1b	4.98 (d,d J = 17 Hz, 1.6 Hz)	114.06
2	5.79 (m)	139.07
3	2.03	
4	1.41	
5	0.89	

[a] The numbers correspond to IUPAC nomenclature.

The coupling constants extracted from the 1D spectra of CLA indicated a cis-9, trans-11 configuration and the absence of any

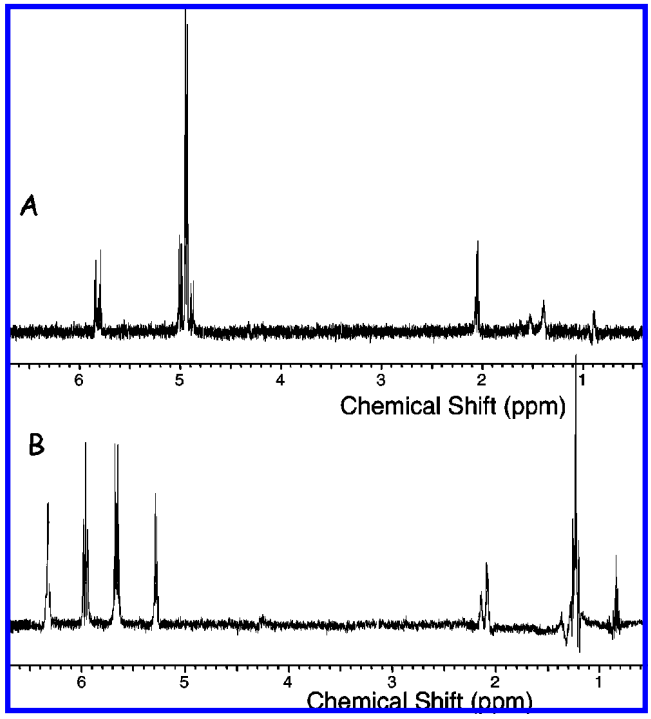

Figure 2. Expanded region of 1D TOCSY experiments obtained with selective excitation at (**A**) 4.92 ppm (1a proton of 1-pentene) and at (**B**) 6.28 ppm (11 proton of CLA).

other geometric isomer of this conjugated acid. The resonance assignment presented here coincides with that of CLA produced by *Lactobacillus plantarum* from linoleic acid (*26*), whereas it disagrees with the hypothesis of possible hydroperoxides (*27*).

Statistical Analysis. Data were visualized either through score plots, in which each point represents an individual sample, or through loading plots, which permit the identification of the most important spectral regions to separate and cluster the samples and, therefore, reveal which compounds are responsible for such clustering (markers).

For the purpose of this work, a covariance matrix was used; that is, the data were mean centered with no scaling (*28*). The loadings produced in this way retain the scale of the original data, and this allows a more useful interpretation of the loadings of the NMR resonances. In this approach, though, the contribution of the triglycerides may mask that of the minor components in the proton spectra. Although a good sample separation was obtained already in this way, a more effective evaluation of the variability of minor metabolites may be carried out by applying PCA separately to the triglycerides and to the remaining components.

At first, the triglyceride analysis was performed integrating both the ^{1}H and the ^{13}C spectra. The scores scatter plot of the PCA performed on the proton resonances is shown in **Figure 3**. Cheeses from alpine farms are clearly separated from all of the remaining ones and are clustered in the left-hand side of the score plot, whereas samples from industrial factories, regardless of their altimetric location, are located on the right-hand side. The first component (PC1) describes 66% of the variance, whereas the second component (PC2) describes an additional 15%. The two PCs together are an accurate representation of the clustering of the products according to the different production chains. **Figure 3** also shows how the test set observations fit the model.

The examination of the loadings (**Figure 3**) is useful to understand the main markers responsible for the observed separation. PC1 shows negative values for resonances from the olefinic and allylic protons and the methyl groups of linolenic acid as well as the bisallylic protons, where the higher contribution is from linolenic acid. These results are in line with the fact that alpine farm samples contain higher amounts of unsaturated fatty acids. On the other hand, PC1 loadings show positive values at positions corresponding to saturated fatty acids known to be present in higher amounts in cheeses from the industrial factories.

To evaluate the possibility to discriminate lowland from mountain industrial factories, each class of observation was modeled separately by disjoint PCA models and the specificity of the three models was computed according to the DmodX criterion on the critical values (D_{crit}) with 95% confidence interval (*23*). The comparison between lowland industrial factories and alpine farm models shown in the Coomans' plot of **Figure 4** is the most informative. The four quadrants correspond to the following memberships of the samples: (1) lowland factories model; (2) alpine farm model; (3) common area (the samples fit both models); (4) samples that do not belong to either model. As shown in the plot, the common area is free, demonstrating the specificity of the two compared models.

The mountain industrial factories are also plotted in **Figure 4**. Almost all of them are clustered with the lowland factories, indicating that the samples from these two types of factories are very similar. Interestingly, two samples from mountain factories are erroneously classified as alpine farms, and one falls in quadrant 4. Noting that these cheeses were produced during the summer, these data can be explained in two ways. Often, mountain factories process milk obtained in part from alpine farms, which do not process it themselves. Alternatively, the milk used comes from intensive farms where the cows are allowed to graze during the daylight hours in the summer.

An analysis of the ^{13}C spectra was performed with the aim to better cluster the samples from the industrial factories and to have more specific information about the fatty acids responsible for the separation. The score plot of the first two components derived by ^{13}C data is shown in **Figure 5**. Together, they express 76% of the total variability (PC1 = 66%, PC2 = 10%). Also in this case, only two groups of samples are observed, with clear overlap of lowland and mountain factories. All of the test samples were correctly classified.

The loading values indicate which fatty acids are responsible for this separation. As shown in **Figure 5**, the industrial cheeses are characterized by a higher content of saturated fatty acids with <14 carbon atoms, principally capric and caprylic acids, whereas the alpine farms contain higher amounts of butyric and stearic acids. Moreover, more unsaturated acids, principally oleic and linoleic acids, are present in the farm cheeses.

In an attempt to improve discriminations of the samples, PLS-DA was performed to both ^{1}H and ^{13}C data sets, but no further information was obtained with respect to PCA.

To account for the contribution of the minor components to the variability within the cheeses analyzed, a PCA was also performed on data matrices created by eliminating the regions of the ^{1}H spectra in which triglyceride signals fall. Similar separations were obtained. Examination of scores and loading plots, **Figure 6**, showed that the first principal component is mainly positively correlated with the CLA peaks and with an unknown compound. PC1 is also negatively correlated with a set of resonances assigned to 1-pentene, a substance that characterizes the industrial samples. This compound was demonstrated to derive from the decomposition of 13-hydrop-

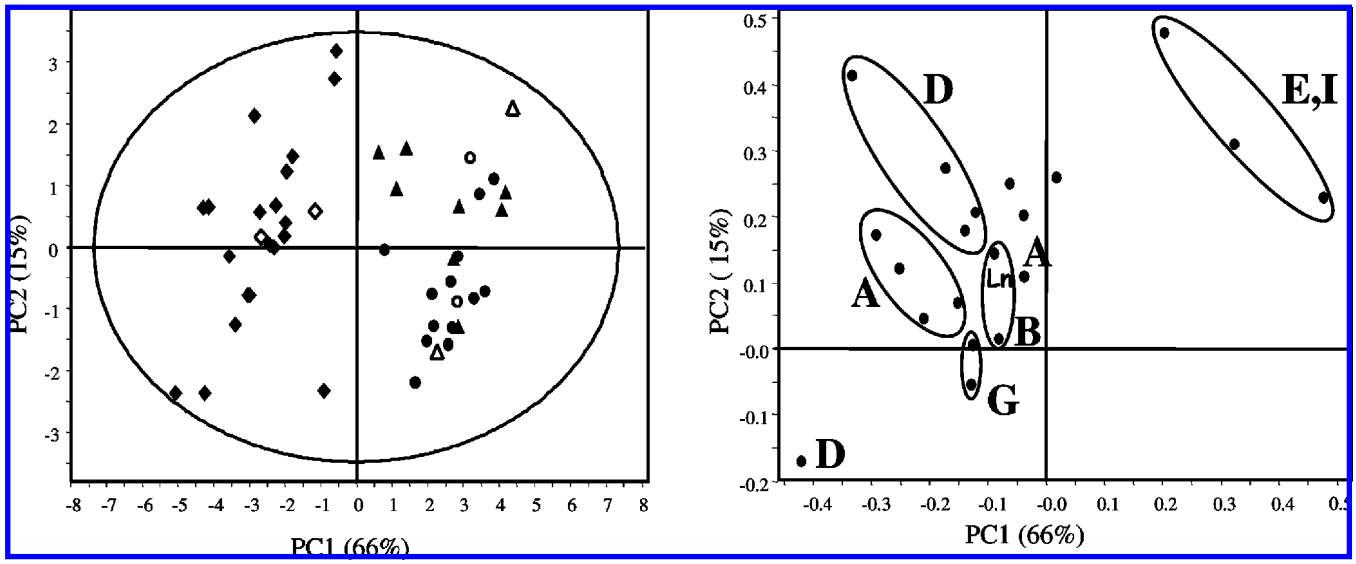

Figure 3. PCA of the fatty acid ^{1}H NMR resonances: (**A**) score plot of PC1 vs PC2 [training set (◆) alpine farms, (●) lowland industrialized factories, (▲) mountain industrialized factories; test set (◇) alpine farms; (○) lowland industrialized factories, (△) mountain industrialized factories]; (**B**) loadings profile (letters according to **Figure 1A**).

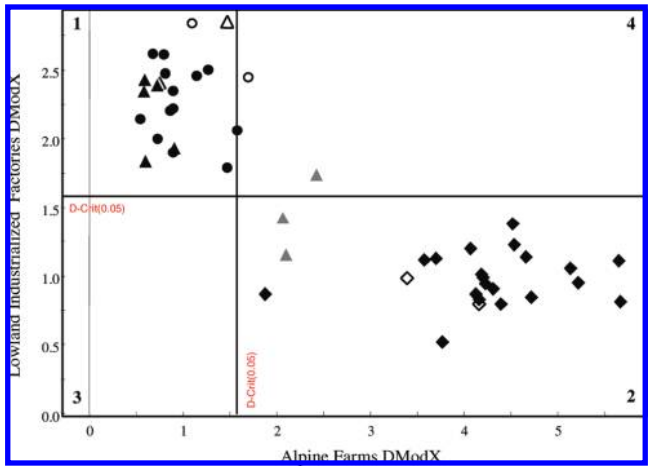

Figure 4. Coomans' plot: upper left corner (1), lowland industrialized factories area; lower right corner (2), alpine farm area; lower left corner (3), common area of the two models; upper right corner (4), area of exclusion from either model. Training set: (◆) alpine farms; (●) lowland industrialized factories; (▲) mountain industrialized factories, winter production; (gray triangles) mountain industrialized factories, summer production. Test set: (◇) alpine farms; (○) lowland industrialized factories; (△) mountain industrialized factories.

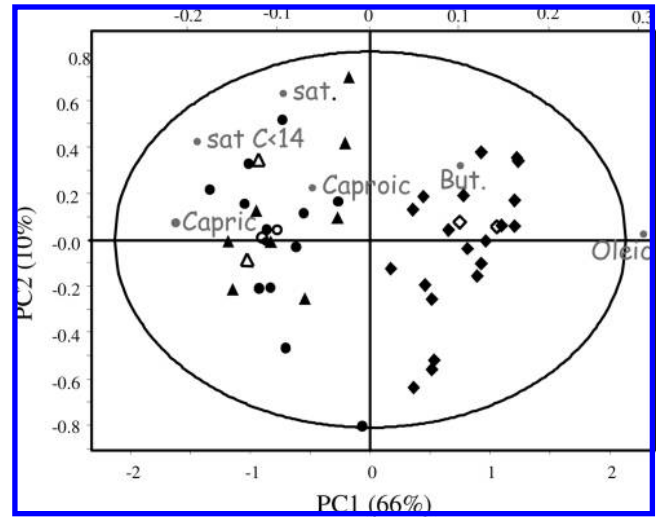

Figure 5. PCA of ^{13}C spectra: score plot of PC1 vs PC2. Training set: (◆) alpine farms; (●) lowland industrialized factories; (▲) mountain industrialized factories. Test set: (◇) alpine farms; (○) lowland industrialized factories; (△) mountain industrialized factories. Fatty acids generating the more discriminating ^{13}C variables are also reported at the corresponding loading values. The loadings *x*-axis is reported above the plot, whereas the loadings ordinate scale is the same as that of the score plot.

eroxy-9-*cis*-11-*trans*-octadecadienoic acid during lipid peroxidation of linoleic acid (*29*). Remarkably, 91% of the sample variability is explained by PC1.

Different from the lowland factories, which display very similar PC1 values, the alpine farms show great dispersion along PC1. This variability, mainly attributable to variable amounts of CLA in alpine farm cheeses, can be explained by diverse grazing conditions in different farms. The much lower dispersion for the lowland industrialized factories arises from a more standardized diet.

DISCUSSION

A rapid and simple NMR method to discriminate Asiago d'Allevo cheese samples from different production chains is described. A fast and reproducible extraction of the organic fraction was employed. Although not exhaustive, the extraction has the advantage of not requiring the extensive chemical manipulation of the samples needed by other methods, such as the one proposed by Folch, and still guarantees a homogeneous sampling.

The analysis of the NMR spectra of the extracts allowed us to distinguish between alpine farm and industrialized factory products. The samples were differentiated on the basis of a higher content of unsaturated fatty acids and CLA for the alpine farm cheeses and a higher content of saturated fatty acids for the factory products.

It is well-known that the composition of cheese is affected by stage of lactation, diet, breed, and other factors. Specifically, the acidic profile of milk is affected by higher level of polyunsaturated fatty acids (PUFA) present in particular herbaceous species (*30*).

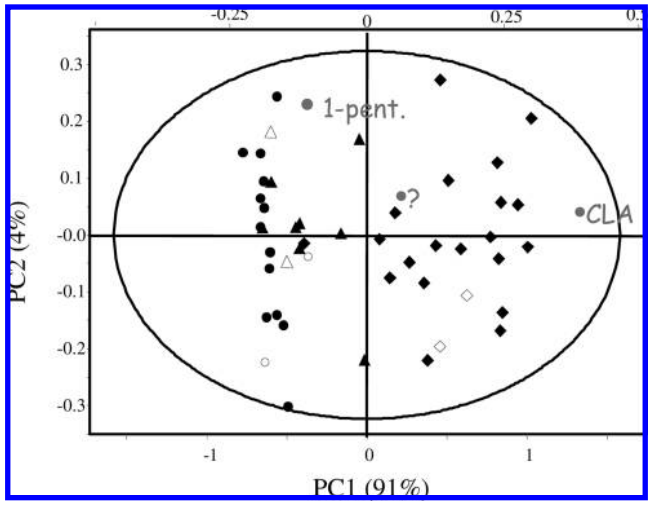

Figure 6. PCA of ^{1}H resonances of minor components: score plot of PC1 vs PC2. Training set: (◆) alpine farms; (●) lowland industrialized factories; (▲) mountain industrialized factories. Test set: (◇) alpine farms; (○) lowland industrialized factories; (△) mountain industrialized factories. The loadings of important variables are also highlighted. The loadings *x*-axis is reported above the plot, whereas the loadings ordinate scale is the same as that of the score plot.

Recent studies confirm that milk and cheese obtained from pasture-grazing dairy cows are richer in CLA (*10*). The different distribution of fatty acids in the various products is therefore not unexpected. The novel and attractive aspect of our approach is how such differences were identified through a fast NMR analysis, which could become a useful tool to identify and promote milk production chains on the basis of pasture grazing.

In line with this consideration, our approach was unable to distinguish between cheese samples produced in industrialized factories located in either lowland or mountain areas. This result must not be considered to be a failure of NMR spectroscopy, but as a logical consequence of the recent trend observed in the husbandry of dairy cattle in mountain areas. In this geographical area, the increase of the milk price along with the low cost of the energy concentrates observed in the past decade has oriented the dairy farmers toward the introduction of high-producing dairy cattle and their management according to very intensive solutions absolutely similar to those of the lowland. In the feeding plan of these animals, the local forage and the pasture have been progressively replaced with roughage sources such as lucerne hay and maize silage produced in the lowland and bought from the feed market. The consequence of this feeding strategy has been increased dairy production, but a standardized quality of milk and cheese, which resemble the lowland products. Therefore, location and altitude cannot lead to a difference in cheese quality unless the production chains operate in different ways in terms of cattle feeding and management as in the case of the alpine farms.

If the conclusions of the present study could be transferred to other typical cheeses, we can state that there is a real risk for the consumer, when buying a mountain product, to pay additional money for a food product that has lost most of its typical traits because it is the expression of a standardized production system absolutely unrelated to its environmental location. In fact, we expect that the method presented here can be expanded to the analysis of milk samples, in which the same metabolites can be found. This method could therefore be used to ascertain, a posteriori, the feeding regimen of dairy cows.

The results of this study indicate that cheeses produced in an alpine farm setting are significantly different from the industrial ones based on objective, physical−chemical parameters. The organoleptic difference in the two types of cheese is a direct consequence of the different fatty acid compositions. On the one hand, fatty acids are of fundamental importance as aroma precursors, because they are a source of volatile aroma substances (*5*). Moreover, the fatty acid composition has an influence on the structural features of the paste of the cheeses, which are less solid if they are produced from milk obtained from pasture-grazing cows (*31*).

Finally, the health benefits perceived by the consumer who prefers "genuine" alpine products can be justified by the higher contents of PUFA in such products. A higher level of PUFA in the human diet is recommended to prevent cardiovascular disease. Specifically, the CLA fraction is interesting for its antitumoral, immunomodulating, and antidiabetic activities (*32*).

Supporting Information Available: Selected expanded regions of the ^{1}H NMR spectra of cheese samples obtained by two different extraction methods and table with reproducibility and variability data. This material is available free of charge via the Internet at http://pubs.acs.org.

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