JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Discrimination of Cheese Products for Authenticity Control by Infrared Spectroscopy

Jana Hruzikova,[†] David Milde,[†] Pavla Krajancova,[†] and Vaclav Ranc*[,†,§]

[†]Department of Analytical Chemistry, Faculty of Science, Palacky University Olomouc, 17 listopadu 12, Olomouc CZ77146, Czech Republic

[§]Department of Physiology, Faculty of Medicine, University of Fribourg, Chemin du Musee 5, 1700 Fribourg, Switzerland

ABSTRACT: Quality and authenticity control serve as the customers' and manufacturers' insurance, and thus the development of analytical tools providing these tasks represents an important step of each product development. The control of authenticity in food manufacturing is even more important due to the direct influence of its products on the health of the population. This study sought to develop an easy to use and robust method for the authenticity control of cheese products. The method is based on the measurement of infrared spectra of the gas phase obtained by heating of selected cheese under controlled conditions. Two different procedures, that is, treatment of samples in a desiccator and their freeze-drying, were compared, and also various temperatures and heating times were studied. It was found that suitable fingerprint infrared spectra can be obtained by both techniques; however, freeze-drying offered faster analysis times. The sample heating temperature and time were evaluated using advanced statistical approaches, and it was found that suitable results could be obtained using 120 °C heating for 90 min. This method was tested for the authenticity control of two cheese families, Tvaruzky and Romadur, for which four cheese products were evaluated and successfully discriminated for each family. This method can be potentially used as a cheap and easy to use alternative to other commercially available options.

KEYWORDS: *infrared spectrometry, gas phase, cheese, Olomouc curd cheese*

INTRODUCTION

The control of quality and authenticity presents an important task in all product development with an essential influence on the final consumer's satisfaction.[1−5] Both tasks serve as monitoring indicators of levels of product quality and could serve as customers' and manufacturers' insurance.[6]

Authenticity and quality control in a food production process could be seen as a task with a high importance, mainly due to its effects on the quality of life of customers as well as its effect on the manufacturer's prosperity.[7−9]

This study covers the development of pilot protocols for an evaluation of the authenticity of cheese products, that is, Olomoucke Tvaruzky[10] and Romadur.[11] Both products have a strong aroma flavor, which was used as a discrimination marker in this study. Tvaruzek presents a model example of a regional product (Region Hana, Czech Republic) with a registered trademark, and thus the authenticity control of such a product is highly demanding. On the other hand, Romadur presents an ideal model of cheese, for which a simple quality control could meaningfully increase consumers' satisfaction.[12]

From an analytical point of view, the analysis of milk products is usually performed using chromatographic approaches, with well-developed protocols and routine platforms, summarized, for example, in refs 13−17 and with more in-detail insights in the works by Chung et al.[18] or others.[19,20] On the other hand, infrared spectroscopy is a main technique in the quality control of milk products at the manufacturer's levels, especially regarding the control of intermediate products throughout all production steps.[21] Nonetheless, infrared spectroscopy was also used for a testing of cheese quality, for example, for fats,[22] control of the ripening process,[23] total cheese constitution control,[24] control of milk quality,[25] or testing for free amino acids contaminations.[23]

Control of authenticity of cheese products represents an analytical question, and a fast and robust method for an initial screening in this field is still in great demand. Some pilot works have been conducted, mostly on Chedar cheese,[26,27] Swiss cheese,[28] and general cheese products[29] with considerable advantages and disadvantages of demonstrated protocols.

In contrast to previously used techniques, our discrimination method is based on an evaluation of the gas phase of respective products, in which the main differences are expected.[30−34] According to our best knowledge, the total composition of cheese products and their nonoriginal, mostly illegal replications does not considerably differ on the general level, on one hand, and, on the other, the usually employed chromatographic approaches could detect even nonimportant variability between measured samples, which dramatically complicates the decision among relevant markers and random composition changes without any importance. Here the presented study sought to discriminate milk products (cheese) on a more fundamental level defined by a presence of volatile compounds as results of differences in the production and postproduction processes (e.g., different ingredients, diversity in ripening). This evaluation was accomplished by en evaluation of gas phase (flavor).

Received: October 27, 2011
Revised: January 24, 2012
Accepted: January 26, 2012
Published: January 26, 2012

■ MATERIALS AND METHODS

Cheese Sample Preparation. Eight kinds of ripening cheeses were purchased from different local supermarkets and are described in Table 1. Cut up cheese pieces (size approximately 0.5 cm) were dried in a desiccator, under laboratory temperature, for 6 days. Freeze-drying was used as an alternative drying method and was carried out for 24 h by two steps with different conditions in the freeze-dryer (Christ, ALFA 1-2 LD plus, SciQuip Ltd.). The first step was main drying under conditions of −42 °C and 0.097 mbar, and the second step was final drying under conditions of −55 °C and 0.0049 mbar. These two procedures were tested as methods of choice to eliminate water from the sample, which could dramatically influence the resulting infrared spectra and thus interfere in the discrimination process.

Table 1. Samples of Ripening Cheeses

original name	country of origin	class name
Olomoucké tvarůžky	Czech Republic	Olomouc curd cheese (A1)
Jemné tvarůžky Loose	Germany	soft curd cheese 1 (A2)
Jemné tvarůžky	Germany	soft curd cheese 2 (A3)
Quargel natur	Austria	Austrian curd cheese (A4)
Romadur	Czech Republic	Romadur 1 (B1)
Original Rahm-Romadur (St. Mang)	Germany	Romadur 2 (B2)
Jarošovký dezertní pivní sýr	Czech Republic	dessert cheese (B3)
Sedlečanský Romadůžek	Czech Republic	Romadůžek (B4)

A short path EC gas cell (PIKE Technologies) was used for the measurement.

FT-IR Analysis. The measurement of each sample was carried out on an FT-IR spectrometer (Nicolet 6700, Thermo) with an ETC EverGlo radiation source, a deuterated triglycine sulfate detector, and a potassium bromide beam splitter. The infrared spectra were obtained in the range of 4000−650 cm^{-1} with a spectral resolution of 4 cm^{-1}. Each evaluated spectrum is a mean of 64 scans. Dried cheese (2.00 g) was heated in an infrared gas cell made of glass with an effective length of 10 cm and a diameter of 2.5 cm equipped with ZnSe windows (2.5 cm × 3 mm). The two most intense spectral bands interpreted as carboxylic groups vibrations (966 and 930 cm^{-1}) were selected as monitoring indicators of the heating process. Infrared spectra of corresponding gaseous phases were measured at temperatures from 70 to 150 °C; 120 °C was selected for all subsequent measurements. The time of the heating process presents another essential parameter and was studied from 30 to 300 min for each temperature; an interval of 90 min was selected. The histogram showing mean intensities of these two bands at different experimental conditions is shown in Figure 1 (see also Table 2). The background was measured using an empty infrared gas cell with ZnSe windows before measurement of samples. From each ripening cheese three samples were taken and were measured six times if not stated otherwise.

Multivariate Analysis. Discriminant analysis was applied for an evaluation of spectral data of gaseous phases of samples using spectroscopic software TQ Analyst 8 (Thermo Scientific). Discriminant analysis was used to statistically evaluate the differences among objects from different groups (various cheese samples) and to find similarities among objects inside one group (several measurements of one cheese sample). The indicator of such difference/similarity was a Mahalanobis distance.

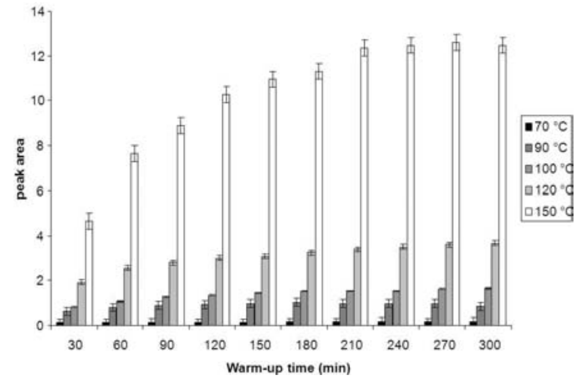

Figure 1. Dependence of peak area on warmup time. Error bars indicate standard deviations.

Table 2. Value of Peak Area and RSD for Each Temperature

temperature (°C)	average peak area	RSD (%)
70	0.16	14.42
90	0.91	17.93
100	1.39	2.85
120	3.08	10.87
150	10.37	35.89

■ RESULTS

Protocol Development. The gaseous phase of each sample was measured by FT-IR spectrometry under defined heating conditions in a glass cuvette (for details see Materials and Methods and Figure 1). Representative mid-infrared spectra of the gaseous phase of Olomouc curd cheese and Romadur are shown in Figure 2, top and bottom panels, respectively. The regions from 4000 to 3500 cm^{-1} and from 2000 to 1300 cm^{-1} consist of vibrational bands of O−H groups, which could belong to the water vapor present. The spectral range from 1300 to 650 cm^{-1} contains two major bands at 966 and 930 cm^{-1} that have been ascribed as C−H bending vibrations of fatty acids and O−H out-of-plane bending vibration of carboxylic acids. Both low molecular weight fatty acids and carboxylic acids are key factors of the resulting flavor of curd cheese. In contrast to Tvaruzky, spectra of ripening cheese type Romadur contain C−H stretching vibrations of $-CH_3$ and $>CH_2$ groups of fatty acids in the region from 3000 to 2800 cm^{-1} (for details see Figure 3).

Two methods of sample drying were tested and compared, and the influence of further statistical evaluation was evaluated. Sample drying was found as an essential step in the sample preparation process due to the considerable influence of the water vapor present on the resulting infrared spectra. First, drying in a desiccator was tested. On the basis of observed infrared spectra (data not shown), it was found that the minimum time for a suitable sample drying is several days; an interval of 6 days was selected as a compromise between time consumption and obtained results. Second, freeze-drying was evaluated. This type of sample processing allowed shortening the sample preprocessing times to several hours, and this process also offers easy to use automation. Spectra obtained by both drying techniques showed moderate bands of water vapor (for details see Figure 2); however, they did not interfere with further statistical evaluation.

Discriminant Analysis (DA). DA was applied to classify two groups of ripening cheeses, Tvaruzky and Romadur, in the

dx.doi.org/10.1021/jf204267d | J. Agric. Food Chem. 2012, 60, 1845−1849

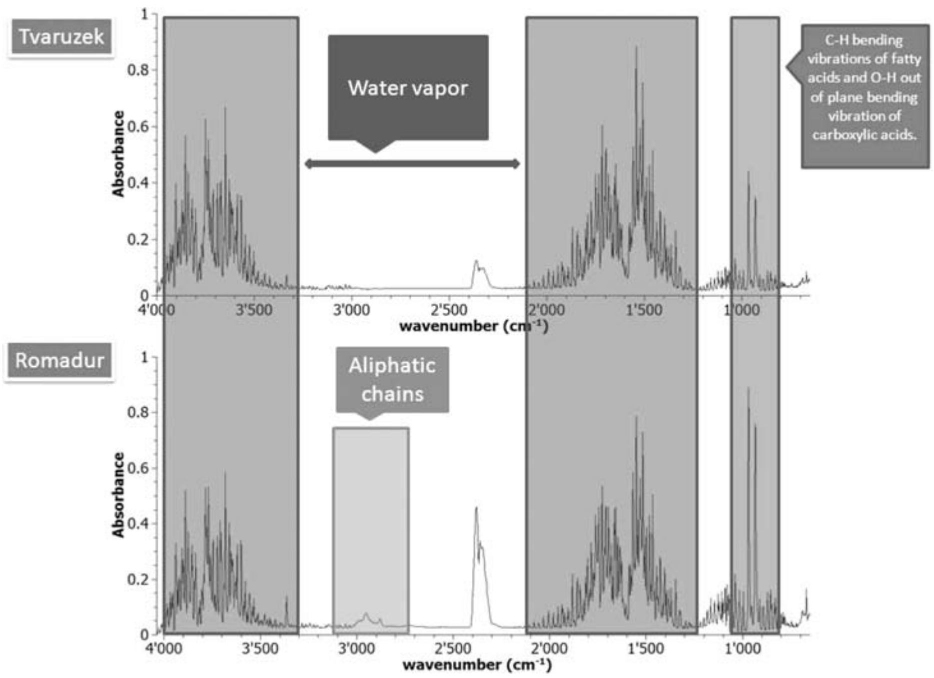

Figure 2. FT-IR spectrum of gaseous phase of Olomouc curd cheese and Romadur 1 measured after 90 min of heating at 120 °C.

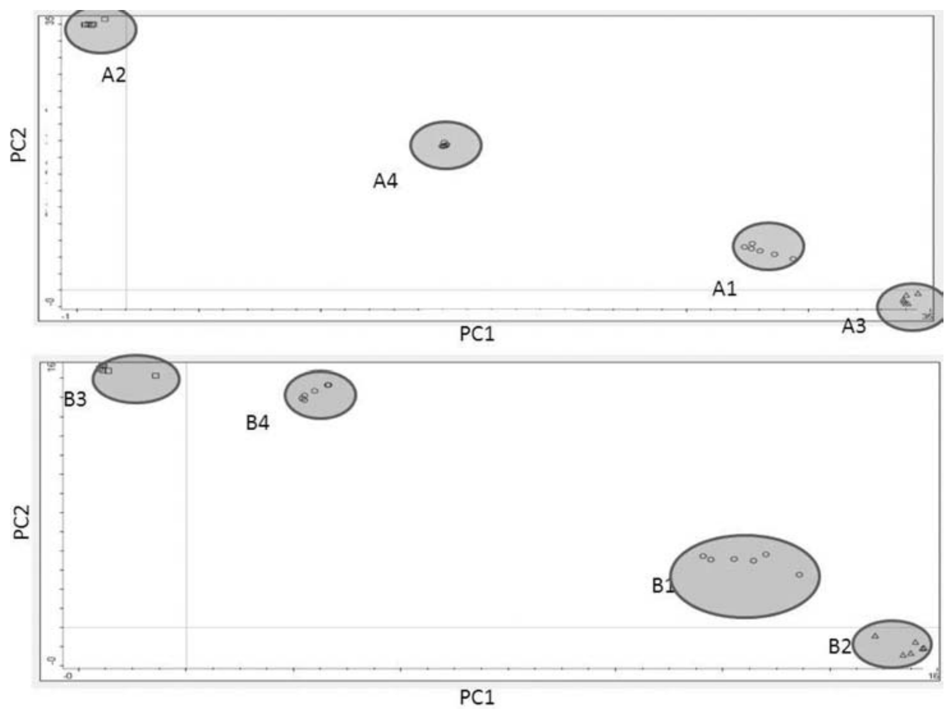

Figure 3. Plot of classification of ripening curd type cheese obtained by a statistical analysis. A1, Olomouc curd cheese; A2, soft curd cheese 1; A3, soft curd cheese 2; A4, Austrian curd cheese; B1, Romadur 1; B2, Romadur 2; B3, dessert cheese; B4, Romadůžek.

infrared region from 4000 to 700 cm^{-1}. Both groups were represented by four samples of cheeses (with six measurements of each sample). DA is the differentiation of individual classes of samples, and it can be graphically expressed in a two-dimensional space by the Mahalanobis distance describing the space between centroids of two classes. Figure 3 shows the classification of Tvaruzky curd cheese using six principal components for the classification. The *x*-axis shows the Mahalonobis distance to the soft curd cheese 1, whereas the *y*-axis shows the distance to the soft curd cheese 2. There, it can be seen that individual samples of curd cheeses make separate clusters, which have different interclass Mahalanobis distances (Table 3). Resulting interclass distances are presented in Table 3. Significant differences in distances were found for classes Olomouc curd cheese (A1)−soft curd cheese 1 (A2) and soft curd cheese 2 (A3)−soft curd cheese 1 (A2).

Similarly, the classification of ripening Romadur type cheese was performed, and the resulting Mahalanobis distances are summarized in Table 3. Figure 3 also shows a graphical expression of classification of ripening Romadur type cheeses

Table 3. Mahalanobis Distances between Classes of Ripening Curd Type Cheese (A1, Olomouc Curd Cheese; A2, Soft Curd Cheese 1; A3, Soft Curd Cheese 2; A4, Austrian Curd Cheese) and Romadur Type Cheese (B1, Romadur 1; B2, Romadur 2; B3, Dessert Cheese; B4, Romadůžek) from Discriminant Analysis

type of curd cheese	A1	A2	A3	A4
A1		1.98[b]	7.03[b]	17.73[b]
A2	28.13[a]		5.27[b]	16.75[b]
A3	6.77[a]	34.07[a]		13.65[b]
A4	13.70[a]	15.06[a]	19.46[a]	
type of Romadur cheese	B1	B2	B3	B4
B1		5.49[b]	12.40[b]	11.93[b]
B2	10.32[a]		15.44[b]	14.26[b]
B3	15.00[a]	16.26[a]		4.38[b]
B4	60.78[a]	68.65[a]	54.73[a]	

[a]Samples dried in the desiccator. [b]Samples dried in the freeze-dryer.

using six principal components. Significant differences in Mahalanobis distances were found for classes Romadur 1 (B1)−Romadůžek (B4), Romadur 2 (B2)−Romadůžek (B4), and Romadůžek (B4)−dessert cheese (B3) when sample pretreatments were compared.

DISCUSSION

On the basis of the conducted comprehensive characterization of infrared spectra of Tvaruzky and Romadur cheeses, it could be stated that the general compositions of these two milk products from our point of view are similar, but with considerable differences especially in the region of 2500−3500 cm^{-1}, where the aliphatic groups of fatty acids could be observed and are distinctive especially for products with high content of fats (for details, see Figure 2). Romadur cheese can contain up to 40% fat, and thus the bands at these regions are observed with higher intensities; on the other hand, Tvaruzky contains no more than 2% fat, and these bands are missing in the spectra.

With regard to the results of a comparison between freeze-drying and drying in a desiccator, it could be stated that both techniques provide suitable results of DA expressed by respective space distances. Nonetheless, the freeze-drying process could be performed overnight in contrast to desiccator drying, which takes up to 6 days (depending on cheese amount, type, and initial conditions), and also additional ripening and chemical decomposition processes could be expected. These processes could be then a reason for the different Mahalanobis distances observed for both drying processes. In summary, both groups of ripening cheeses (Tvaruzky and Romadur) can be distinguished using a desiccator or freeze-dryer; however, the Mahalanobis distances are higher when a desiccator is used, at the price of longer sample pretreatment times.

DA of Tvaruzky cheese uncovered significant differences (expressed by space distances) among original Tvaruzky cheese and its nonoriginal variants labeled as A2−A4 samples. These changes could be a result of the different ingredients used, variations in athe ripening process, or changes in product postproduction. Similar results were obtained also for Romadur cheese, for which statistically relevant differences were observed among four types of selected Romadur cheese.

The development of suitable tools that provide a control of authenticity of food products presents an important task usually performed by analytical or food chemists. The study presented here sought to develop a protocol for an authenticity control of selected cheeses, that is, Tvaruzky and Romadur, based on the utilization of infrared spectroscopy. The developed protocol uses an evaluation of cheese flavor measured as a gas phase after a sample heating process performed in a glass cuvette. The performed DA allowed us to statistically distinguish among original Tvaruzky cheese and its nonoriginal copies. The differences among samples are expressed using Mahalanobis distances in corresponding graphical expressions (Figure 3). The protocol development was based on the comparison of two drying procedures: desiccator drying and freeze-drying. Both approaches afford suitable results; however, freeze-drying represents a faster approach for the prize of slightly lower space distances among sample clusters in a DA. Similar results were obtained for the Romadur cheese analysis, where the protocol allows four different types of this particular type of cheese to be distinguished and thus the control of the authenticity of such products.

This protocol could be potentially used in food manufacturing processes or by authenticity control institutions, where it could serve as a cheap and relatively easy to automate process of screening. Nonetheless, more detailed cheese characteristics could be provided by chromatographic approaches when needed.

AUTHOR INFORMATION

Corresponding Author

*E-mail: vaclav.ranc@unifr.ch.

Funding

This research was supported by a grant from the Ministry of Education (MSM6198959216), Czech Republic, and by the student project PrF_2011_025 of Palacky University.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Romano, J. Advanced technologies for food and beverage quality control. *Agro Food Ind. Hi-Tech* **2011**, *22* (3), 2−2.

(2) News related to foreign food quality control events. *Elelmiszervizsgalati Kozl.* **2010**, *56* (3), 194−200.

(3) Hron, J.; Macak, T. Control of food products' quality. *Agric. Econ. (Zemedelska Ekonomika)* **2009**, *55* (5), 221−228.

(4) Mannino, S.; Scampicchio, M. Nanotechnology and food quality control. *Vet. Res. Commun.* **2007**, *31*, 149−151.

(5) Robinson, R. K. *Modern Dairy Technology*; Elsevier Applied Science Publishers: London, U.K., 1986.

(6) Ma, X. Study on quality and safety supervision control system for food supply chain. *2009 IEEE 16th International Conference on Industrial Engineering and Engineering Management, Vol. 1 and 2, Proceedings*; Piscataway, NJ, 2009; pp 1141−1144

(7) Hardin, R. S.; Kundinger, M. M.; Woodward, C. L.; Donalson, M.; Golbach, J. L.; Ricke, S. C. Development of a quality control laboratory design project for poultry science undergraduate students enrolled in an advanced food microbiology course. *J. Dairy Sci.* **2004**, *87*, 76−76.

(8) Duran, L. Measurement of sensory attributes in food quality control. *Acta Aliment.* **2004**, 33 (2), 97−100.

(9) Alli, I. Food quality assurance − process-control. *Can. Inst. Food Sci. Technol. J.* **1990**, *23* (4−5), 156−159.

(10) Standarova, E.; Vorlova, L.; Kordiovska, P.; Janstova, B.; Drackova, M.; Borkovcova, I. Biogenic amine production in Olomouc curd cheese (Olomoucke tvaruzky) at various storage conditions. *Acta Vet. Brno* **2010**, *79* (1), 147−156.

(11) Foster, R. D. *Cheese: Types, Nutrition, and Consumption*; Nova Science Publishers: Hauppauge, NY, 2011.

(12) Behnke, U.; Lieske, B. [Studies on the incidence of volatile sulfur compounds during the ripening of Camenbert, Romadur, Tollen and Edam cheeses. 2. Chemical and organoleptic studies]. *Nahrung* **1973**, *17* (4), 479−498.

(13) Styrelsen för internationell utveckling. Manual of food quality control. In *FAO Food and Nutrition Paper 14*; Food and Agriculture Organization of the United Nations: Rome, Italy, 1979; p v.

(14) *Validation of Analytical Methods for Food Control*; report of a Joint FAO/IAEA Expert Consultation, Vienna, Austria, Dec 2−4, 1997; Food and Agriculture Organization of the United Nations: Rome, Italy, 1998; p 19

(15) TACIS Technical Dissemination Project. European Commission. DG IA−External Political Relations. *Setting up a Food Control Laboratory: The Example of Pushchino*; European Commission; UNIPUB Distributor: Brussels, Belgium, 1996; p 60.

(16) Wilson, S.; Weir, G. *Food and Drink Laboratory Accreditation: A Practical Approach*; Chapman and Hall: London, U.K., 1995; p xxiv, 262 pp.

(17) Chiaradia-Bousquet, J.-P. *Legislation Governing Food Control and Quality Certification: Authorities and Producers*; Food and Agriculture Organization of the United Nations: Rome, Italy, 1995; p x, 125 pp.

(18) Chung, S. W. C.; Chan, B. T. P. Validation and use of a fast sample preparation method and liquid chromatography-tandem mass spectrometry in analysis of ultra-trace levels of 98 organophosphorus pesticide and carbamate residues in a total diet study involving diversified food types. *J. Chromatogr., A* **2010**, *1217* (29), 4815−4824.

(19) Di Corcia, A.; Bogialli, S.; Coradazzi, C.; Lagana, A.; Sergi, M. A rapid method based on hot water extraction and liquid chromatography-tandem mass spectrometry for analyzing tetracycline antibiotic residues in cheese. *J. AOAC Int.* **2007**, *90* (3), 864−871.

(20) Leonil, J.; Gagnaire, V.; Molle, D.; Pezennec, S.; Bouhallab, S. Application of chromatography and mass spectrometry to the characterization of food proteins and derived peptides. *J. Chromatogr., A* **2000**, *881* (1−2), 1−21.

(21) Zheng, L. M.; Wang, T. Z.; Tian, L. J.; Wu, P.; Zhu, H.; Ren, F. Z. Analysis of milk and dairy products by near-infrared spectroscopy: a review. *Spectrosc. Spectral Anal.* **2010**, *30* (12), 3208−3212.

(22) Wehling, R. L.; Pierce, M. M. Application of near-infrared reflectance spectroscopy to determination of fat in Cheddar cheese. *J. Assoc. Off. Anal. Chem.* **1989**, *72* (1), 56−58.

(23) Skeie, S.; Feten, G.; Almoy, T.; Ostlie, H.; Isaksson, T. The use of near infrared spectroscopy to predict selected free amino acids during cheese ripening. *Int. Dairy J.* **2006**, *16* (3), 236−242.

(24) Rohm, H.; Sultaneh, A. Using near infrared spectroscopy for the determination of total solids and protein content in cheese curd. *Int. J. Dairy Technol.* **2007**, *60* (4), 241−244.

(25) Vukasinovic, Z.; Antunac, N.; Mikulec, N.; Mioc, B.; Barac, Z. Production and milk quality of Pag sheep. *Mljekarstvo* **2008**, *58* (1), 5−20.

(26) Subramanian, A.; Harper, J. W.; Rodriguez-Saona, L. E. Classification of cheddar cheese based on flavor quality using Fourier transform infrared spectroscopy. *J. Dairy Sci.* **2007**, *90*, 146−146.

(27) Rodriguez-Saona, L. E.; Subramanian, A.; Harper, W. J. Cheddar cheese classification based on flavor quality using a novel extraction method and Fourier transform infrared spectroscopy. *J. Dairy Sci.* **2009**, *92* (1), 87−94.

(28) Rodriguez-Saona, L. E.; Kocaoglu-Vurma, N. A.; Eliardi, A.; Drake, M. A.; Harper, W. J. Rapid profiling of Swiss cheese by attenuated total reflectance (ATR) infrared spectroscopy and descriptive sensory analysis. *J. Food Sci.* **2009**, *74* (6), S232−S239.

(29) Xu, H. R.; Sun, T.; Ying, Y. B. Progress in application of near infrared spectroscopy to nondestructive on-line detection of products/food quality. *Spectrosc. Spectral Anal.* **2009**, *29* (1), 122−126.

(30) Drake, M. A.; Miracle, R. E.; McMahon, D. J. Impact of fat reduction on flavor and flavor chemistry of Cheddar cheeses. *J. Dairy Sci.* **2010**, *93* (11), 5069−5081.

(31) Evans, J.; Zulewska, J.; Newbold, M.; Drake, M. A.; Barbano, D. M. Comparison of composition, sensory, and volatile components of thirty-four percent whey protein and milk serum protein concentrates. *J. Dairy Sci.* **2009**, *92* (10), 4773−4791.

(32) Januszkiewicz, J.; Sabik, H.; Azarnia, S.; Lee, B. Optimization of headspace solid-phase microextraction for the analysis of specific flavors in enzyme modified and natural Cheddar cheese using factorial design and response surface methodology. *J. Chromatogr., A* **2008**, *1195* (1−2), 16−24.

(33) Hayaloglu, A. A.; Cakmakci, S.; Brechany, E. Y.; Deegan, K. C.; McSweeney, P. L. Microbiology, biochemistry, and volatile composition of Tulum cheese ripened in goat's skin or plastic bags. *J. Dairy Sci.* **2007**, *90* (3), 1102−1121.

(34) Carpino, S.; Mallia, S.; La Terra, S.; Melilli, C.; Licitra, G.; Acree, T. E.; Barbano, D. M.; Van Soest, P. J. Composition and aroma compounds of Ragusano cheese: native pasture and total mixed rations. *J. Dairy Sci.* **2004**, *87* (4), 816−830.