Discriminant Analysis of Olomouc Curd Cheese by Fourier Transform Near Infrared Spectroscopy

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Abstract

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We used the discriminant analysis of curd cheese during storage by Fourier transform near infrared spectroscopy method (FT-NIRs). Olomouc curd cheese samples were stored at 5 and at 20°C during seven weeks. The spectra of samples were measured at the integration sphere in reflectance mode with the use of a compressive cell in the spectral range of 10 000–4000 cm^{-1} with 100 scans. Ten principal components were used for all the calibration models. Great similarity between the samples stored at 5 and 20°C was found. Twelve samples stored at 20°C for 1 week and 2 samples stored at 20°C for 2 weeks were classified as samples stored at 5°C. Different results were found out by comparing the storage time. 100% variability was described between the spectra scanned in different weeks of storage at 5°C and 99.9% variability was obtained for the samples stored at 20°C. Thus, the discriminant analysis of Olomouc curd cheese by FT-NIRs is a suitable method for the determination of ripening time.

Keywords: ripening; Mahalanobis distance

Non-destructive food testing is becoming increasingly important due to expanding automation and the incorporation of next and more efficient processes in the food industry. The safety and quality of food are the main points of interest. It is important to have a technology which will allow for a high throughput and a short response time to increase the process efficiency and to reduce waste (Woodcock *et al.* 2008a). The discriminant analysis is a classification technique which can be used to determine the class or classes of known materials which are most similar to an unknown material by computing the unknown's distance from each class centre in Mahalanobis distance units (Thermo Electron Corporation 1992). The most frequently used method with multivariate data is principal component analysis (PCA). The purpose of this technique is to obtain an overview of all the information in the data set. In PCA, orthogonal directions in a variable space describing the variation are found. In this way a new set of fewer coordinate axes called principal components (PCs) is generated. PCA allows the use of the entire spectrum for the quantitative analysis and it provides synthetic information (Karoui *et al.* 2003). When you use the method to analyse an unknown sample, the software performs a principal component analysis on the unknown sample spectrum and on the variance spectrum (or spectra) to determine score values. The score plots are used to produce Mahalanobis distance values, which in turn are used to rank the classes (Thermo Electron Corporation 1992). The aim of this study was to use the discriminant analysis by Fourier transform near infrared spectroscopy method (FT-NIRs) for the detection of storage time of curd cheese.

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MATERIAL AND METHODS

Samples and storage conditions. A total number of 180 samples of Olomouc curd cheese called Tvaruzky was examined. The samples came from the same batch and were purchased directly from the manufacturer in 2005. The samples were transported and stored in accordance with applicable legislation (Act No. 77/2003) at a temperature of 4–8°C. In the laboratory, the samples were divided into two groups. The first group (n = 84) was stored at 5°C for 7 weeks. The second group of samples (n = 84) was stored at 20°C for 7 weeks. Sampling was carried out once a week [including week 0 (n = 12) before the own storing and ripening].

FT-NIR spectroscopy analysis. Samples of the curd cheese were homogenised by blending and measured by the FT-NIR Nicolet Antaris (Thermo Electron Corporation, Madison, USA) in the spectrum range from 10 000 to 4000 cm^{-1} with 100 scans, resolution, 8 cm^{-1} (Růžičková & Šustová 2006). Spectra were measured at an integration sphere in the reflectance mode using a compression cell. Duration of one spectrum scanning took about 1.5 minutes. Measured data were processed by the TQ Analyst Version 6.2.1.509 (Thermo Electron Corporation, Medison, USA) using discriminant analysis. Ten principal components and spectrum in the same range were used for all generated models.

RESULTS AND DISCUSSION

The method of discriminant analysis was used to monitor the duration of curd cheese ripening, when stored under different conditions. Comparison of the curd cheese spectra stored at 5 and 20°C for seven weeks is shown in Figure 1. A discriminant analysis model was created under the conditions specified in the Material and Methods section using the one-point baseline type and the minimum in the region range from 4258.05 cm^{-1} to 7247.18 cm^{-1}. Though 100% variability was found, 14 spectra of samples stored at 20°C were misclassified in the group of samples stored at 5°C (Table 1).

The method of discriminant analysis did not show a demonstrable difference between the groups of samples stored at 5 and 20°C (Figure 2). Most samples are collected in the rectangular pattern on the left below. We can see a slight indication of groups, but they are not separated from each other diagonally by a bar interface and thus there is no obvious classification into individual groups.

By comparing each week results within the group stored at 5°C, a 100% variability was found (1st region 9798.04–7238.62 cm^{-1}, one-point baseline type, a minimum in the range from 9164.07 cm^{-1} to 9488.06 cm^{-1}; 2nd region 6498.93–4103.78 cm^{-1}, one-point baseline type, a minimum in the range from 5916.54 to 6171.09 cm^{-1}). 99.9% variability was obtained for samples stored at 20°C (region 8792.25–7413.03 cm^{-1}, one-point baseline type and a minimum in the range of 7833.43–8184.41 cm^{-1} applied). As published by Pappas *et al.* (2008), TQ Analyst software uses Mahalanobis algorithm for the calculation of distances. Initially, the samples were separated into actual classes. Then the software calculated the distances and created new classes – calculated classes. Afterwards, the software inserted each sample in a calculated class.

Considering the week of storage, all the spectra of the curd cheese samples stored at 5°C were classified to the correct class. In addition, one sample spectrum of curd cheese stored at 20°C for six weeks was included in the class of the seventh week of storage (Table 1). To define the classes where the samples were wrongly classified, it is possible to

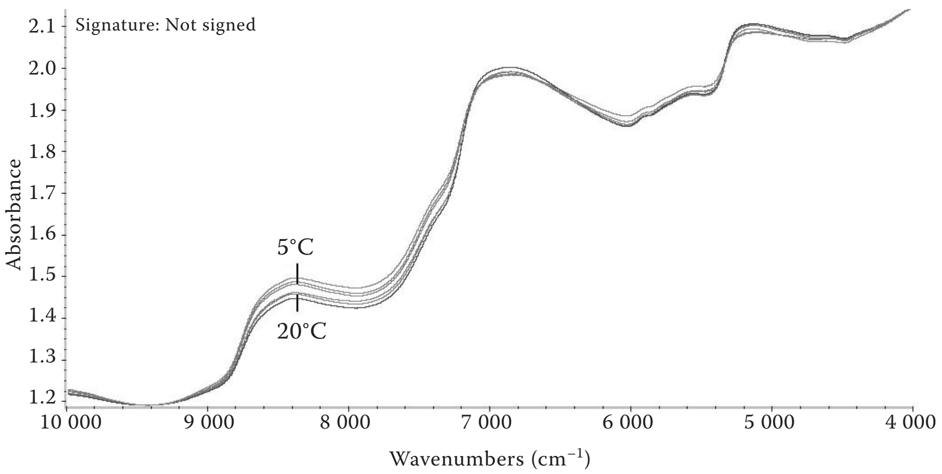

Figure 1. NIR spectra of Olomouc curd cheese stored at 5°C and 20°C for 7 weeks

Table 1. Classes and Mahalanobis distance (average values)

Actual class	Calculated class	Distance	Next class	Next distance
5°C × 20°C				
5°C (*n* = 84)	5°C (*n* = 84)	0.93	20°C (*n* = 84)	1.27
20°C (*n* = 84)	20°C (*n* = 70)	0.93	5°C (*n* = 70)	1.34
	5°C (*n* = 14)	0.64	20°C (*n* = 14)	0.90
Samples stored at 5°C				
0^{th} week (*n* = 12)	0^{th} week (*n* = 12)	1.28	1^{st} week (*n* = 12)	3.22
1^{st} week (*n* = 12)	1^{st} week (*n* = 12)	1.11	0^{th} week (*n* = 2)	2.68
			2^{nd} week (*n* = 10)	2.63
2^{nd} week (*n* = 12)	2^{nd} week (*n* = 12)	0.80	3^{rd} week (*n* = 6)	1.63
			7^{th} week (*n* = 6)	1.73
3^{rd} week (*n* = 12)	3^{rd} week (*n* = 12)	0.84	2^{nd} week (*n* = 3)	1.58
			4^{th} week (*n* = 9)	0.98
4^{th} week (*n* = 12)	4^{th} week (*n* = 12)	0.69	3^{rd} week (*n* = 9)	1.02
			6^{th} week (*n* = 3)	0.98
5^{th} week (*n* = 12)	5^{th} week (*n* = 12)	0.77	6^{th} week (*n* = 12)	1.33
6^{th} week (*n* = 12)	6. week (*n* = 12)	0.71	4^{th} week (*n* = 8)	1.15
			5^{th} week (*n* = 4)	1.04
7^{th} week (*n* = 12)	7^{th} week (*n* = 12)	0.92	2^{nd} week (*n* = 2)	2.18
			3^{rd} week (*n* = 10)	1.36
Samples stored at 20°C				
0^{th} week (*n* = 12)	0^{th} week (*n* = 12)	1.11	1^{st} week (*n* = 12)	2.22
1^{st} week (*n* = 12)	1^{st} week (*n* = 12)	0.96	0^{th} week (*n* = 11)	2.15
			2^{nd} week (*n* = 1)	1.71
2^{nd} week (*n* = 12)	2^{nd} week (*n* = 12)	0.84	3^{rd} week (*n* = 12)	1.72
3^{rd} week (*n* = 12)	3^{rd} week (*n* = 12)	0.77	2^{nd} week (*n* = 2)	1.45
			4^{th} week (*n* = 10)	1.27
4^{th} week (*n* = 12)	4^{th} week (*n* = 12)	0.76	3^{rd} week (*n* = 12)	1.35
5^{th} week (*n* = 12)	5^{th} week (*n* = 12)	0.93	4^{th} week (*n* = 5)	1.90
			6^{th} week (*n* = 7)	1.70
6^{th} week (*n* = 12)	6^{th} week (*n* = 11)	0.89	5^{th} week (*n* = 1)	1.39
	7^{th} week (*n* = 1)	0.55	6^{th} week (*n* = 1)	0.80
			7^{th} week (*n* = 10)	1.28
7^{th} week (*n* = 12)	7^{th} week (*n* = 12)	1.11	6^{th} week (*n* = 12)	1.41

5°C (20°C) – samples stored at 5°C (20°C); *n* – number of samples

develop another calibration where only the wrongly classified samples will be used.

Differences between curd cheese samples stored for 0 and 2 weeks at 5°C are shown in Figure 3. Each rectangle defined by the diagonal arrangement includes a single class.

The differentiation between the samples of individual storage weeks was confirmed using discriminant analysis. The same method was used by Pappas *et al.* (2008). Numbers of samples and the next closest class, identification based on the Mahalanobis distance, are shown in Table 1. For the purposes of accuracy verification, external calibration was performed, which confirmed the classification of unknown samples always in the correct class in weeks of storage.

Storing the curd cheese at 5°C does not cause any radical changes during ripening and thus does not influence the spectra as much as the ripening at 20°C. Table 3 shows an increase in the average

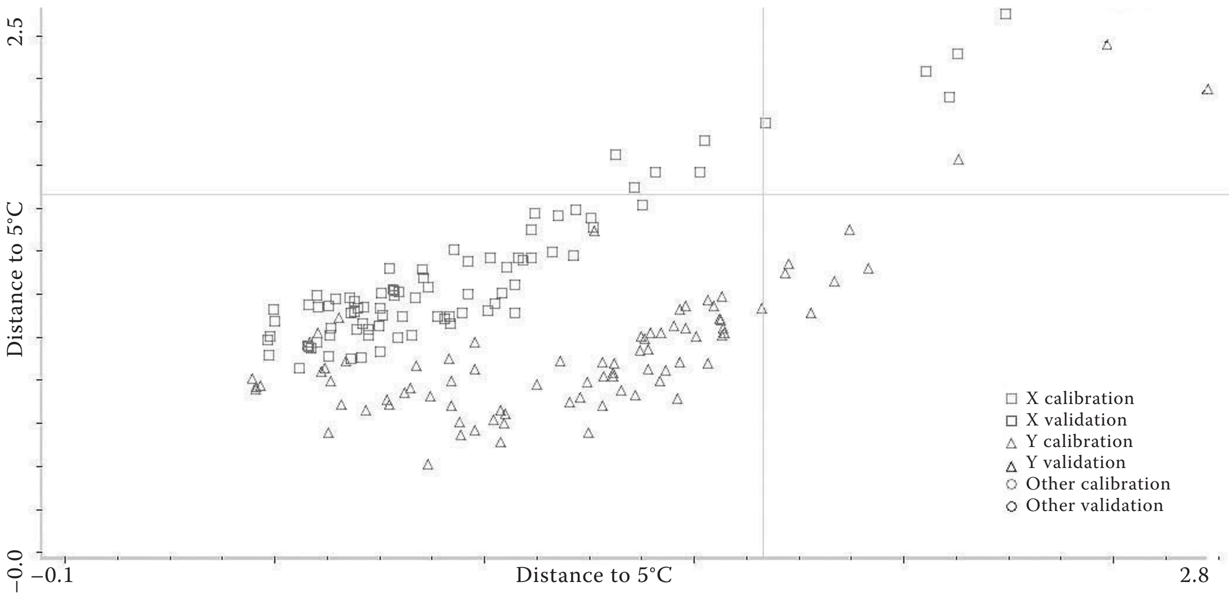

Figure 2. Discriminant analysis of samples stored at 5°C (□), 20°C (Δ), and other temperature (◇)

distance between the individual classes with the time of storage.

Infrared spectroscopy has been identified as an ideal process analytical technology tool, and recent publications have demonstrated the potential of both NIR and MIR spectroscopy, coupled with chemometric techniques, for monitoring coagulation, synaeresis, and ripening as well as determination of authenticity, composition, sensory and rheological parameters. Spectroscopic and chemometric methods are employed to assess the potential of infrared spectroscopy as a technology for improving process control and quality in cheese manufacture (Woodcock *et al.* 2008b).

During the maturation and ripening phases, cheese undergoes a complex series of chemical, bacterial, and enzymatic reactions which are responsible for the breakdown of the protein matrix and ultimately for the development of the texture and sensory characteristics that are typical of mature cheese (Woodcock *et al.* 2008b). Cattaneo *et al.* (2005) applied PCA to discriminate the storage time of Crescenza cheese. The authors obtained three well-separated groups corresponding to fresh (0–6 days), aged (8–10 days),

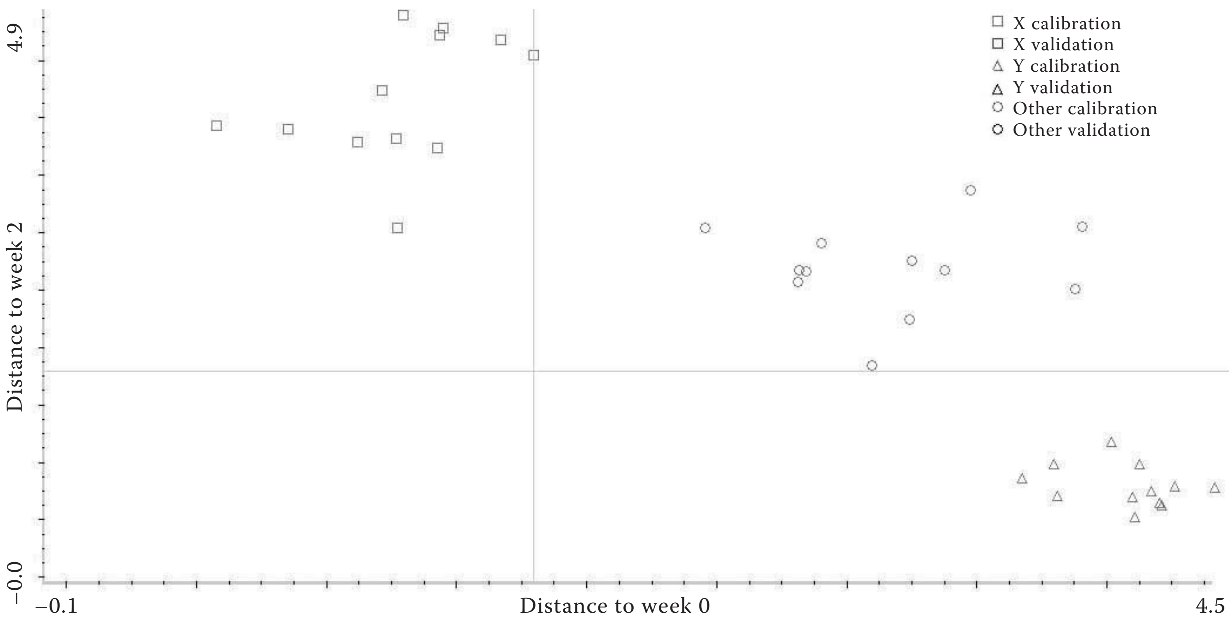

Figure 3. Discriminant analysis of samples stored at 5°C for 0 week (□), 2 weeks (Δ) and other weeks of storage (○)

Table 2. Mahalanobis distance (average values) from the actual class to individual weeks of storage at 5 and 20°C

Actual class	Distance to week							
	0	1	2	3	4	5	6	7
5°C								
0th week	**1.28**	3.22	4.18	5.48	5.85	5.99	5.93*	5.22*
1st week	3.17	**1.11**	2.69	3.70	4.29	4.59	4.44*	3.49*
2nd week	4.07	2.57	**0.80**	1.76	2.25	3.14	2.67*	1.71*
3rd week	5.41	3.63	1.81	**0,84**	1.16	2.30	1.61*	1.45*
4th week	5.77	4.19	2.24	1.07	**0.69**	1.90	1.13*	1.57*
5th week	5.92	4.52	3.15	2.29	1.95	**0.77**	1.33	2.65
6th week	5.85	4.35	2.66	1.56	1.16*	1.27	**0.71**	1.98
7th week	5.17	3.45	1.81*	1.51*	1.69*	2.70	2.07	**0.92**
20°C								
0th week	**1.11**	2.22	3.07	4.16	4.80	5.50	6.32	6.99
1st week	2.13	**0.96**	2.47	3.37	3.83	4.37	5.45	6.13
2nd week	2.97	2.42	**0.84**	1.72	2.38	3.66	4.10	4.62
3rd week	4.08	3.30	1.68	**0.72**	1.33	2.62	2.84	3.31
4th week	4.74	3.78	2.35	1.35	**0.76**	1.87	2.06	2.57
5th week	5.47	4.35	3.67	2.68	1.95	**0.93**	1.92	2.55
6th week	6.29	5.43	4.10	2.88	2.10	1.91	**0.88**	1.23
7th week	6.99	6.15	4.67	3.42	2.70	2.65	1.41	**1.11**

*Mahalanobis distance did not increase with the time of storage; in bold – average distance from the centre of the cluster to each sample within the actual class

and old (storage time > 10 days) cheese. PCs score plot showed that it was possible to obtain a good sample distribution along the PC1 axis according to the increase of storage time.

Reflectance spectroscopy has been demonstrated to be more suited to industrial applications than transmittance spectroscopy. The potential of these technologies for compositional, ripening stage, rheological and process monitoring applications at a laboratory scale is also well documented (Woodcock *et al.* 2008b). Cervera *et al.* (2006) successfully classified the Manchego cheese in 3 maturity classes. 90% of samples were correctly assigned to the right category.

CONCLUSION

The method of discriminant analysis belongs among qualitative methods which use near-infrared spectrometry. Using this method, it was possible to classify curd cheese samples into predefined classes (ripening duration in weeks) with ~100% variability. The average distance between the individual classes of the samples was increasing with the length of storage in most cases. Discriminant analysis thus represents a simple and quick test of ripening in the production of curd cheese.

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