Rapid assessment of selected free amino acids during Edam cheese ripening by near infrared spectroscopy

Jiří Mlček[1], Květoslava Šustová[2], Otakar Rop[3], Tünde Juríková[4], Petr Humpolíček[5], Štefan Balla[4]

[1]Tomas Bata University, Faculty of Technology, Department of Food Technology, Zlín, Czech Republic
[2]Mendel University, Faculty of Agronomy, Department of Food Technology, Brno, Czech Republic
[3]College of Business and Hotel Management, Department of Gastronomy, Brno, Czech Republic
[4]Constantine the Philosopher University, Faculty of Central European Studies, Institute of Natural and Informatics Sciences, Nitra, Slovakia
[5]Tomas Bata University, Centre of Polymer Systems, Polymer Centre, Zlín, Czech Republic

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Abstract

The study focuses on rapid determination of free amino acids produced during the ripening of cheese, by using near infrared spectroscopy. Analyses of 96 samples of Edam cheese (30% and 45% of fat in dry matter) were performed at monthly intervals up to the ripening age of 6 months. In total, 19 amino acids were analysed with infrared spectrometer using two different methods, either in the regime of reflectance in the integrating sphere of the apparatus or using a fibre optic apparatus with the fibre optic probe. Reference data based on high-performance liquid chromatography were used for calibration of the spectrophotometer. Calibration models were developed using a partial least square algorithm and tested by means of cross-validation. When measured with the integrating sphere and with the probe, the values of correlation coefficients ranged from 0.835 to 0.993 and from 0.739 to 0.995, respectively. Paired *t*-test did not show significant differences between the reference and predicted values ($P < 0.05$). The results of this new calibration method showed the possibility of near infrared technology for fast determination of free amino acids, which occur during the ripening of Edam cheese. The content of free amino acids allow us to prepare Edam cheese quickly and efficiently for sale or to prepare the material for processed cheese.

NIR, ripeness of cheese, method of partial least squares, proteolysis

The key factor of ageing in cheese is its chemical composition. The water content is one of the most important factors in regulation of curd hydrolysis and microbial growth in manufactured cheese (Janštová et al. 2010). Nevertheless, changes in and analysis of major compounds presented in young cheese are not good indicators of the course of ripening. This is because these cheese components are stable and more or less unchanging (Hering et al. 2008). On the other hand, however, changes in contents of peptides and free amino acids are much better indicators of cheese ageing. Cheese acquires its typical taste, smell, consistency and appearance only in the course of ageing, due to fermentation processes that change all three basic milk components, i.e. lactose, proteins and fat. Proteolysis is the most complex and in the majority of cheese types also one of the most important biochemical processes that take place in the course of ageing. The concentration of amino acids, the final products of proteolysis, is dependent on the type of cheese and stage of cheese ageing. In Edam cheese, the following amino acids are the most abundant: glutamic acid, leucine, arginine, lysine, phenylalanine and serine. The type and quantity of free amino acids present in cheese influences its taste and indicates the degree of ageing and its progress. The composition and spectrum of free amino acids change depends on the type and age of cheese. During cheese ageing, the concentrations of amino acids are increasing with the exception of arginine because its concentration decreases in the final stage of ageing (McSweeney 2004). Free amino acids themselves can substantially

Address for correspondence:
Ing. Jiří Mlček, Ph.D.
Department of Food Technology
Faculty of Technology, Tomas Bata University in Zlin
nam. T.G. Masaryka 275, 762 72 Zlin, Czech Republic

Phone: +420 576 033 030
Fax: +420 576 031 111
E-mail: mlcek@ft.utb.cz
http://actavet.vfu.cz/

contribute to the taste of cheese because they are one of its sensory components (Lane et al. 1997). Good knowledge of the stage of cheese ageing is therefore an important factor of its further development and for the production of processed cheese (Bunka et al. 2004). The aim of the study was to evaluate the possibility of using near infrared spectrometry for determination of free amino acids which arise during the ripening of Edam cheese as an important indicator for the production of processed cheese. The results obtained by measurements performed in the integrating sphere and by probe were compared.

Materials and Methods

Analyses were performed on Edam cheese samples originating from two dairy factories (marked as A and B) in the Czech Republic. Each factory supplied four different products. Two of them contained 45% and 30% of fat in dry matter (FDM), respectively, and two were so-called starting cultures (*Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis, Leuconostoc mesenteroides* subsp. *cremoris, Lactococcus lactis* subsp. *diacetylactis, Streptococcus thermophilus, Lactobacillus helveticus*). Each of the samplings involved three samples. Analyses of these samples were performed at monthly intervals up to the ripening age of 6 months. The central part (core) and rind of cheese were evaluated separately and therefore the total number of cheese samples was 96.

Every month the cheese samples were analysed for the presence of following 19 aminoacids: soluble tyrosine, tryptophan, arginine, ornithine, lysine, histidine, glutamine, serine, asparagine, glycine, threonine, alanine, proline, aspartic acid, valine, glutamic acid, leucine, isoleucine and phenylalanine with the aim to calibrate the Fourier transform infrared (FTIR) apparatus for individual components which change in dependence on the degree of cheese ripeness. Concentrations of free amino acids were estimated in the liquid chromatograph (HPLC) Agilent 1100 Series with the mass detector Agilent MSD 1456 VL (Agilent Technologies, USA) based on the method of Ardo and Polychronidau (1999). All reagents including standards were provided in the EZ:faast™ amino acid analysis sample testing kit by Phenomenex Inc. (Torrance, CA, USA). Data obtained from HPLC were used for calibration of the near infrared spectrophotometer. Edam cheeses were analysed with the Nicolet Antaris near infrared spectrometer following the procedure according to Mlček et al. (2011). Two different methods were used: integrating sphere (IS) and fibre optic apparatus with a fibre optic probe. Calibration models were created with the TQ Analyst version 6.2.1.509 (Thermo Electron Corporation, Madison, USA); statistical methods used were similar as described by Mlček et al. (2006).

Results

Altogether 96 samples of Edam cheese were used to evaluate the amino acids. The results obtained by measurements performed in the integrating sphere and using the probe were compared. All developed calibration models showed high correlation coefficients (Table 1). The highest correlation coefficients were found in models for phenylalanine (0.993) and tryptophan (0.992) in samples of Edam cheese measured in the integrating sphere and for phenylalanine (0.995) and ornithine (0.993) measured by the probe. When using the integrating sphere and probe, the lowest values of correlation coefficients were found in the calibration models for glycine (0.835) and proline (0.739), respectively. In general, it can be concluded that there were no marked differences in values of correlation coefficients when using both aforementioned methods.

Correlation coefficients of validation ranged from 0.733 to 0.983 and from 0.558 to 0.960 when using the integrating sphere and probe, respectively. Similarly as with calibration results also in case of the correlation coefficient of validation the best results were obtained for phenylalanine (R = 0.983 and 0.960 when measuring in the integrating sphere and using the probe, respectively). The worst results were recorded for glycine (R = 0.733) and proline (R = 0.558) when measuring with the integrating sphere and using the probe, respectively.

Table 2 indicates that in case of the probe, better standard deviations of calibration values were obtained for ten amino acids (ornithine, lysine, histidine, glutamine, glycine, threonine, alanine, aspartic acid, leucine, phenylalanine) whereas better standard deviations of validation values were obtained only for five amino acids (histidine, tyrosine, glutamine, glycine and alanine). Except for the amino acids mentioned above, other amino acids

Table 1. Values of free amino acids in Edam cheese, established by reference methods (n = 96), calibration and validation results of free amino acids in Edam cheese established by integrating sphere

Integrating sphere	x ± SD (nmol/g)	Calibration			Validation		
		R	SEC (nmol/g)	CCV (%)	R	SEP (nmol/g)	PCV (%)
Arginine	10.57 ± 7.68 ^{ns}	0.970	1.87	17.69	0.837	4.22	39.92
Ornithine	230.06 ± 146.96 ^{ns}	0.977	31.6	13.74	0.966	38.1	16.56
Lysine	258.89 ± 149.34 ^{ns}	0.958	42.7	16.49	0.930	55	21.25
Histidine	66.40 ± 46.23 ^{ns}	0.929	17.1	25.75	0.874	22.6	34.03
Tryptophane	17.43 ± 9.03 ^{ns}	0.992	11.6	6.65	0.949	28.6	16.41
Tyrosine	95.85 ± 45.43 ^{ns}	0.949	80.9	9.60	0.884	213.0	22.22
Glutamine	166.61 ± 118.54 ^{ns}	0.983	22.1	13.27	0.915	47.9	28.75
Serine	162.23 ± 90.15 ^{ns}	0.912	36.9	22.75	0.881	42.7	26.32
Asparagine	282.07 ± 177.73 ^{ns}	0.890	81	28.72	0.849	94.1	33.36
Glycine	126.75 ± 85.32 ^{ns}	0.835	47	37.08	0.733	58.6	46.23
Threonine	110.59 ± 71.19 ^{ns}	0.941	24	21.70	0.918	28.2	25.50
Alanine	238.52 ± 160.57 ^{ns}	0.848	85.2	35.72	0.788	99.4	41.67
Proline	200.80 ± 179.42 ^{ns}	0.935	63.8	31.77	0.821	102	50.80
Aspartic acid	46.75 ± 29.45 ^{ns}	0.975	6.5	13.9	0.961	8.16	17.46
Valine	292.14 ± 232.48 ^{ns}	0.967	59.5	20.37	0.940	79.5	27.21
Glutamic acid	610.20 ± 453.28 ^{ns}	0.959	129	21.14	0.919	179	29.34
Leucine	497.40 ± 380.19 ^{ns}	0.973	88.2	17.73	0.953	115	23.12
Isoleucine	92.57 ± 85.88 ^{ns}	0.969	21.2	22.90	0.940	29.3	31.65
Phenylalanine	274.69 ± 150.39 ^{ns}	0.993	17.8	6.48	0.983	27.8	10.12

x - average values, SD - standard deviation, R - correlation coefficient, SEC - standard error of calibration, CCV - calibration coefficient of variation, SEP - standard error of prediction, PCV - prediction coefficient of variation, ^{ns} - non-significant difference ($P < 0.05$)

showed better results obtained by measuring in the integrating sphere. This means that the integrating sphere can be recommended for the estimation of amino acids in cheese by means of NIR spectroscopy because lower error values are obtained.

For all amino acids the values of calibration coefficients of variation (CCV) were higher than 5% and the values of prediction coefficient of variation (PCV) exceeded 10%. The increased variability resulted from complex dependences and also from a minor presence of amino acids in cheese. The lowest values of CCV were recorded for phenylalanine when measuring both in the integrating sphere and using the probe (CCV = 6.48% and CCV = 5.84%, respectively). As far as the value of PCV was detected, the lowest values were recorded also in case of phenylalanine (PCV = 10.12% and PCV = 15.81%, respectively).

Discussion

NIR spectroscopy shows good ability to set values of fats, water content and proteins in natural and processed cheese and the obtained results correlate well with other reference methods (Rodriguez-Otero et al. 1995; McQueen et al. 1995; Adamopoulos et al. 2001; Curda et al. 2001; McKenna 2001). These methods are of interest especially when monitoring the process of cheese ageing because they enable analysis of changes in their composition. The composition of young cheeses is a key factor for their further ageing and the moisture content is one of the most important factors of the regulation of curd hydrolysis and of cheese microbial growth.

Table 2. Values of free amino acids in Edam cheese, established by reference methods (n = 96), calibration and validation results of free amino acids in Edam cheese, established by probe

Probe	x ± SD (nmol/g)	Calibration			Validation		
		R	SEC (nmol/g)	CCV (%)	R	SEP (nmol/g)	PCV (%)
Arginine	10.7 ± 7.57 ^{ns}	0.925	2.87	27.15	0.821	4.35	41.15
Ornithine	242.64 ± 152.7 ^{ns}	0.993	18	7.42	0.946	49.5	20.40
Lysine	285.73 ± 186.02 ^{ns}	0.976	40.2	14.07	0.927	70.1	24.53
Histidine	71.56 ± 53.91 ^{ns}	0.970	13.1	18.31	0.911	22.2	31.02
Tryptophane	16.89 ± 7.96 ^{ns}	0.951	24.7	14.62	0.819	46	27.23
Tyrosine	93.74 ± 44.04 ^{ns}	0.957	128.0	13.66	0.929	163.0	17.39
Glutamine	171.09 ± 118.48 ^{ns}	0.991	16.1	9.41	0.931	43.4	25.37
Serine	160.39 ± 86.18 ^{ns}	0.900	37.6	23.44	0.844	46.3	28.87
Asparagine	316.46 ± 234.94 ^{ns}	0.937	82.3	26.01	0.901	102	32.23
Glycine	155.73 ± 119.56 ^{ns}	0.940	40.7	26.14	0.888	55	35.32
Threonine	117.98 ± 76.06 ^{ns}	0.986	12.8	10.85	0.889	35.2	29.84
Alanine	264.84 ± 193.46 ^{ns}	0.912	79.3	29.94	0.873	94.8	35.80
Proline	188.34 ± 144.10 ^{ns}	0.739	97	51.50	0.558	122	64.78
Aspartic acid	51.68 ± 29.00 ^{ns}	0.981	5.62	10.88	0.934	10.4	20.12
Valine	306.08 ± 244.40 ^{ns}	0.946	79.4	25.94	0.877	118	38.55
Glutamic acid	656.52 ± 471.24 ^{ns}	0.955	140	21.32	0.915	191	29.09
Leucine	577.19 ± 416.78 ^{ns}	0.979	84.5	14.64	0.935	148	25.64
Isoleucine	121.70 ± 120.81 ^{ns}	0.975	26.7	21.94	0.916	48.7	40.02
Phenylalanine	282.67 ± 160.03 ^{ns}	0.995	16.5	5.84	0.960	44.7	15.81

x - average values, SD - standard deviation, R - correlation coefficient, SEC - standard error of calibration, CCV - calibration coefficient of variation, SEP - standard error of prediction, PCV - prediction coefficient of variation, ^{ns} - non-significant difference ($P < 0.05$)

In the course of cheese ageing, the curd is hydrolysed by enzymes originating from rennet, by native enzymes of milk, and by microorganisms present in the cheese matrix. Casein is degraded to peptides and these are further degraded to individual amino acids that can become precursors of a bad taste, which is caused by various acids, alcohols, aldehydes, and ketones (McSweeney et al. 2000). Types and amounts of free amino acids present in cheese influence its taste and indicate or characterise the stage of ageing. The spectrum of free amino acids in cheese changes in dependence on its type and age. The authors found that there were differences in the content of free amino acids not only between individual types but also between individual batches of cheese; these differences were caused by varying contents of dry matter, salt, amounts of added lactic acid bacteria, and of so-called non-starter lactic acid bacteria (NSLAB) which occur in cheese under so far unexplained conditions (Østlie et al. 2004; Østlie et al. 2005).

The degradation of some specific free amino acids is important for the development of sensory substances contained in cheese (Yvon et al. 2001). Methionine is degraded to methanethiol, which is the cause of unpleasant sulphurous stench of cheese. The degradation of branched-chain amino acids (leucine, valine and isoleucine) and of aromatic amino acids (phenylalanine, tyrosine a tryptophan) results in formation of various aldehydes and alcohols (i.e. true aromatic cheese components) and of various carboxylic acids (i.e. precursors of aromatic compounds) (Yvon et al. 2001). Recently it was found that also degradation of serine (Ser) resulted in production of strong aromatic compounds (Liu et al. 2003).

Good knowledge of the stage of ripeness of natural cheese is important information for the subsequent manufacturing of processed cheese (Forman 1996; Fox 2004).

Based on the highest values of correlation coefficients of both calibration and validation experiments, the model developed for the estimation of phenylalanine using both aforementioned methods can be recommended as the most reliable. Estimation of proline, alanine, asparagine and glycine are the least reliable because they showed not only the highest differences between coefficients of correlation but also the highest variability.

Parametric *t*-test corroborated that the evaluated reference and instrumental methods did not give different results ($t_{stat} < t_{krit}$).

For all models used for the estimation of free amino acids, NIR spectroscopy seems to be suitable for orientation purposes because only in the model for phenylalanine the PCV value measured in the integration sphere was at the level of 10% (PCV = 10.12%); whereas using the probe the CCV value was equal to 5.84%. For the measurement of this amino acid we can evaluate this model as reliable. However, comparing both methods of measurement, measuring with the integrating sphere seems to be more suitable.

Although the estimation of free amino acids by means of NIR spectroscopy gave, as expected, a little worse results (mainly due to the fact that compared to the basic components of cheese, the accuracy of estimation of some less frequent compounds was lower), it is possible to recommend the use of NIR spectroscopy for an orientation test and estimation of these substances mainly due to its speed. It could contribute not only to the evaluation of the degree of ripeness of Edam cheese but also when selecting optimum raw material for making processed cheeses. Compared to traditional methods of determination of the chemical composition and quality of cheese, the NIRS method enables a fast, simple and simultaneous evaluation of more components at once and properties of cheese without the need of any chemicals. It is also a non-destructive method suitable for food evaluation even in inter-operational analysis.

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