Identification of physical properties and volatile and non-volatile compounds for discrimination between different Emmental-type cheeses: a preliminary study

K. I. Hartmann¹ · A. Dunkel² · H. Hillmann² · D. Hansen³ ·
P. Schieberle³ · T. Hofmann² · J. Hinrichs¹

Received: 2 February 2015 / Revised: 12 August 2015 / Accepted: 20 August 2015 /
Published online: 7 September 2015
© INRA and Springer-Verlag France 2015

Abstract Differentiation between Emmental cheese produced in diverse regions is of major interest to verify product authenticity and avoid product imitation. The objective of this study was to identify parameters that allow a differentiation between Southern German Emmental cheese and Emmental cheese produced in other regions based on physical properties and volatile and non-volatile compounds. Emmental cheese samples were collected from seven German dairies located in Bavaria and Baden-Wuerttemberg (regional Emmental cheese). Additionally, generic Emmental cheeses produced in Northern Germany, the Netherlands and Poland (European Emmental cheese) were added to the data set as well as Swiss Emmental and Allgäu Emmental cheeses. A large variety of characteristics was previously analysed by application of thermo-rheological methods and targeted quantification methods (e.g. GC-MS and dynamic oscillatory shear measurement). Stepwise discriminant analysis was used to identify parameters with high discriminant power. These were brightness L^* , loss modulus G'' at 20 °C, loss factor $\tan \delta_{\max}$, 3-methylbutyric acid, acetic acid, propionic acid, γ -Glu-Leu, γ -Glu-Val and the peptide Ile-Val-Pro-Asn. The data set resulting from the

This paper is part of the Special issue dedicated to the 9th International Cheese Symposium held in Cork, Ireland and organized by Teagasc in collaboration with University College Cork and INRA, 12th & 13th November 2014.

Electronic supplementary material The online version of this article (doi:10.1007/s13594-015-0255-0) contains supplementary material, which is available to authorized users.

✉ K. I. Hartmann
katja.hartmann@uni-hohenheim.de

¹ Department of Soft Matter Science and Dairy Technology, University of Hohenheim, Garbenstr. 21, 70599 Stuttgart, Germany

² Chair of Food Chemistry and Molecular Sensory Science, Technische Universität München, Lise-Meitner-Str. 34, 85354 Freising, Germany

³ German Research Centre for Food Chemistry, Lise-Meitner-Str. 34, 85354 Freising, Germany

nine parameters was subjected to linear discriminant analysis followed by a cross validation step. Regional Emmental cheese was classified correctly at 95%, whereas 75% of European Emmental cheese and 100% of Swiss and Allgäu Emmental cheeses were correctly classified. The results are preliminary but may provide, upon further verification, a useful tool for cheese makers to identify Emmental cheese produced in different regions based on a small number of characteristic parameters and prevent product imitation.

Keywords Emmental cheese · Physical properties · Volatile compounds · Non-volatile compounds · Classification · Multivariate statistical analysis

1 Introduction

Emmental cheese is a highly valued cheese product by many consumers because of its characteristic nutty and sweet flavour as well as its functional properties, e.g. melt behaviour.

It originates from Swiss Emmental cheese, which has been produced in certain regions in Switzerland since the 12th century according to a traditional manufacturing process regarding raw material, applied parameters during processing and quality of the final product. The requirements are summarised in a Swiss specification (FOAG 2014), and since 2002, Swiss Emmental cheese bears a label of protected designation of origin (PDO). A PDO label designates a product with certain properties that are attributed to the region of manufacturing (Kireeva 2011). From Switzerland, the knowledge of Emmental cheese production was brought to Germany. Since 1997, Allgäu Emmental cheese (PDO label) is produced in the Allgäu region in Southern Germany. Requirements regarding the manufacturing process resemble those mandatory for Swiss Emmental cheese, e.g. the use of raw milk, and are detailed in the German regulation for cheese (Deutsche Käseverordnung 2013).

In many countries (e.g. Germany, Finland, the Netherlands and Poland), generic Emmental cheese is produced. It resembles Swiss and Allgäu Emmental cheeses in the characteristic eye formation as well as in taste, aroma and texture and is often referred to as “Swiss cheese” (Bisig et al. 2010). Generic Emmental cheese is produced and evaluated according to specifications given in the Codex Alimentarius Standard 269-1967 (Codex 2013). However, technological variations in the manufacturing process of generic Emmental cheese, e.g. the use of thermised milk, are allowed as long as the typical qualitative characteristics for Emmental cheese are achieved. These include a “light yellow colour”, an “elastic but not sticky texture” and cherry-sized gas openings. Furthermore, a dry matter of $\geq 60\%$, a calcium content of $>800 \text{ mg} \cdot 100 \text{ g}^{-1}$ and a propionic acid content of $>150 \text{ mg} \cdot 100 \text{ g}^{-1}$ are mandatory (Codex 2013).

Several studies investigated Emmental cheese to identify sensory, functional and biochemical properties for discrimination between Emmental cheeses produced in different regions. Suhaj and Koreňovská (2008) analysed Emmental cheese from different European regions in terms of mineral composition. They applied multivariate statistical analyses (principal component analysis, stepwise discriminant analysis) and selected nine minerals to have large discriminant power for classification according to the producing region. Pillonel and colleagues evaluated rheological, chemical, biochemical, volatile and sensory data of Emmental cheese from different European countries (Pillonel et al. 2002, 2003a, b). In a following work (Pillonel et al. 2005),

the combined data were subjected to multivariate statistical analyses (linear discriminant analysis, artificial neural network), which resulted in the selection of 11 parameters allowing 95% of the samples to be correctly classified after cross validation according to the producing area.

The aim of this work was to differentiate between German Emmental cheese from South Germany and Emmental cheese produced in other regions based on quantitative physical properties and volatile and non-volatile compounds. Thus far, no quantitative evaluation for Emmental cheese from the South of Germany is available that allows a differentiation from other Emmental cheeses. These results may be a helpful tool to promote identification of Southern German Emmental cheese products and protect it against product imitation, which is of major interest because the name Emmental is not legally protected.

2 Materials and methods

2.1 Cheese samples and composition

Regional generic Emmental cheese samples were obtained from seven dairies located in Southern Germany (Bavaria, Baden-Wuerttemberg). Over a period of 2 years, the samples were collected directly from the dairies after ripening within a plastic film for 8–10 weeks. The samples collected were evaluated by the dairies as standard Emmental cheese in terms of appearance (eye development, colour) and sensory attributes (taste, odour).

Swiss and Allgäu Emmental cheese samples (PDO labelled) were obtained from local stores. European generic Emmental cheese samples were received from one of the German dairies. Information regarding origin, age and type of ripening of the samples is listed in Table 1.

Dry matter, propionic acid and calcium content were evaluated by an accredited external laboratory (muva Kempten, Kempten, Germany). Dry matter and calcium content were determined according to EN ISO 5534 and EN ISO 11885, respectively. The content of propionic acid was analysed according to a standard operating procedure of the laboratory with inductively coupled plasma optical emission spectrometry (ICP-OES). For Emmental cheese, a dry matter of at least 60% is required according to the Codex Stan 269-1967 (Codex 2013). For Swiss and Allgäu Emmental cheeses, a minimum dry matter of 62% is required (Deutsche Käseverordnung 2013; FOAG 2014). For further analysis, the samples that were consistent with the German and Swiss regulation as well as the Codex Alimentarius Standard in terms of dry matter, propionic acid and calcium content were chosen. Thus, a total of 28 Emmental cheese samples were analysed.

2.2 Physical properties

In a preliminary work, 25 physical properties were determined according to Schenkel (2014). The parameters and the respective methods are given in Online Resource 1. Three parameters (L^* , $G''_{80^\circ\text{C}}$, $\tan \delta_{\text{max}}$) showed high discriminant power according to stepwise discriminant analysis and were subjected to further calculations.

Table 1 Sample name, origin of cheese sample, type of milk processing, ripening time, type of ripening and season of sample production of Emmental cheese samples investigated

Emmental cheese group	Country/region of origin	Milk processing	Ripening time	Type of ripening	Season of production/number of samples	Dairy
Regional Emmental cheese	South Germany	Pasteurised	8–10 weeks	Film	Summer (5)	A
Regional Emmental cheese	South Germany	Pasteurised	8–10 weeks	Film	Summer (1)/winter (1)	B
Regional Emmental cheese	South Germany	Pasteurised	8–10 weeks	Film	Summer (2)/winter (1)	C
Regional Emmental cheese	South Germany	Pasteurised	8–10 weeks	Film	Summer (1)/winter (1)	D
Regional Emmental cheese	South Germany	Pasteurised	8–10 weeks	Film	Winter (1)	E
Regional Emmental cheese	South Germany	Pasteurised	8–10 weeks	Film	Summer (1)	F
Regional Emmental cheese	South Germany	Pasteurised	8–10 weeks	Film	Summer (3)	G
Allgäu Emmental cheese	South Germany	Raw	12 weeks	Rind	Summer (4)/winter (1)	Allgäu EC
European Emmental cheese	The Netherlands, Northern Germany, Poland ^a	Pasteurised	NA (min. 2 months)	Film	Summer (4)	European EC
Swiss Emmental cheese	Switzerland	Raw	NA (min. 4 months)	Rind	Summer (2)	Swiss EC

Summer sample: production between April and September. Winter sample: production between October and February

NA no information available, EC Emmental cheese

^a No assignment to the origin available

Brightness L^* (0=black, 100=white) of unheated cheese samples was obtained from a chromameter CR-400 (Konica Minolta Optics, Tokyo, Japan) with a D65 illuminant and 2° observer angle according to EN ISO 11664-4. Each cheese sample was freshly cut, and brightness was measured at different locations on the freshly cut inner surface. At least five measurements were performed for each sample.

Dynamic oscillatory shear measurements were conducted on a rheometer (Physica MCR 301, Anton Paar, Ostfildern, Germany) via temperature sweep experiments according to Schenkel et al. (2014). Temperature increased during the measurement from 20 to 80 °C at 3 °C.min⁻¹ at a constant deformation strain of 0.002 and a constant frequency of 1 Hz. The storage modulus, G' , and the loss modulus, G'' , were recorded as well as the loss factor $\tan \delta_{\max}$.

2.3 Volatile compounds

Preliminary, a total of 11 volatile compounds were evaluated, which are summarised in Online Resource 1. Acetic acid, propionic acid and 3-methylbutyric acid were identified to have high discriminant power according to stepwise discriminant analysis for differentiation between Emmental cheeses.

Acetic acid was determined enzymatically with a commercially available UV test (R-Biopharm AG, Darmstadt, Germany). The cheese samples were cut and subsequently ground using a cryogenic mill (Freezer/Mill 6870, Spex, Metuchen, USA). Approximately 7 g of ground cheese was measured into a volumetric flask, and deionised water was added to 100 mL. The sample was frozen for 10 min and then filtrated to remove fat. The clear filtrate was measured at 340 nm in a double-beam spectrophotometer (UV-2401 PC, Shimadzu, Duisburg, Germany). Each sample was measured threefold.

Propionic acid was determined according to the method of the internal standard and measured via high-resolution gas chromatography-flame ionization detector (HRGC-FID). Ground cheese (50 g) was mixed with anhydrous sodium sulphate and internal standard solution (iso-butyric acid diluted in diethyl ether) and extracted three times with diethyl ether (total volume 300 mL) by stirring for 60 min. The volatiles were isolated from the filtered and combined extracts via solvent-assisted flavour evaporation (SAFE). The distillate was extracted three times with aqueous sodium carbonate solution (0.5 mol.L⁻¹, total volume 300 mL). The combined aqueous layers were adjusted to pH 2 with hydrochloric acid (32%) and subsequently extracted three times with diethyl ether (total volume 300 mL) to obtain the acidic fraction (AF). The AF was dried over anhydrous sodium sulphate, filtered and concentrated to a volume of 30 mL using a Vigreux column (50 cm×1 cm internal diameter (i.d.)). Two microliters of the AF was injected on the DB-FFAP capillary column (30 m×0.32 mm i.d.; 0.25- μ m film thickness, J&W Scientific, New Orleans, USA) and detected via FID. Mixtures of the propionic and iso-butyric acids were prepared in seven different mass ratios (1:9, 1:6, 1:3, 1:1, 3:1, 6:1, 9:1) and analysed by HRGC-FID to obtain the response factor (RF).

For the determination of 3-methylbutyric acid, the sum of the two isomers was determined via stable isotope dilution assay (SIDA) using ninefold-deuterated 2-methylbutyric acid as labelled internal standard. The samples were prepared as stated above to obtain the acidic fraction (AF). Quantitation was performed using a Varian CP

3800 gas chromatograph (Darmstadt, Germany), containing a DB-FFAP capillary column (30 m×0.32 mm i.d.; 0.25- μ m film thickness, J&W Scientific), connected to a Varian Saturn 2000 ion trap mass spectrometer (Darmstadt, Germany) operated in MS-CI mode with methanol as reaction gas. Mixtures of the labelled and unlabelled compound were prepared in five different mass ratios (1:5, 1:3, 1:1, 3:1, 5:1) and analysed under the same conditions as the sample solutions.

The ratio of 2- and 3-methylbutyric acid in the samples was determined via HRGC-MS operated in MS-EI mode. The intensity ratio of the fragments m/z 60 (3-methylbutyric acid) and m/z 74 (2-methylbutyric acid) corresponds to the mass ratio of both isomers. The system was calibrated by using mixtures with different mass ratios of 2- and 3-methylbutyric acid. The intensity ratio of m/z 60 over the sum of m/z 60 + m/z 74 was plotted against the percentage of 3-methylbutyric acid to obtain a calibration line.

2.4 Non-volatile compounds

In a preliminary study, 69 non-volatile compounds were evaluated, which are summarised in Online Resource 1. The glutamyl peptides γ -Glu-Val and γ -Glu-Leu as well as the peptide Ile-Val-Pro-Asn (IVPN) showed high discriminant power according to stepwise discriminant analysis.

Preparation of a water-soluble extract (WSE) was performed according to Toelstede and Hofmann (2008a) and stored at -20 °C until further analysis.

For the quantification of peptides, an aliquot (5 μ L) of the WSE was analysed by means of HPLC-MS/MS on a Fusion-RP80, 150×2.0 mm i.d., 5- μ m column (Phenomenex, Aschaffenburg, Germany) equipped with a guard column of the same type. Using a flow rate of 0.2 mL.min⁻¹, chromatography was performed starting with a 1% aqueous solution of formic acid for 5 min and then increasing the content of acetonitrile (containing 1% formic acid) to 50% within 45 min and to 100% within 5 min. The peptide IVPN was analysed using the mass transition given in parentheses: IVPN (m/z 442.3→70.2). Quantitative analysis was performed in triplicate by comparing the peak areas obtained for the corresponding mass traces with those of a defined standard solution of the reference compound (IVPN, EZBiolab, Westfield, USA) (Toelstede and Hofmann 2008a).

For the quantitative analysis of glutamyl peptides, lyophilised WSE extracts were dissolved in deionised water (approximately 25 mg.mL⁻¹) and membrane filtered, and aliquots (5 μ L) were injected into the HPLC-MS/MS system equipped with a 250×2.0 mm, 5- μ m MonoChrom MS column (Varian, Darmstadt, Germany). The solvent system consisted of acetonitrile (solvent A) and water (solvent B), both containing 0.1% formic acid. Operating at a flow rate of 0.2 mL.min⁻¹, chromatography was started with 100% solvent B for 10 min. The content of solvent A was increased within 10 min from 0 to 10% and then to 100% within 25 min. The following peptides were analysed using the mass transitions given in parentheses: γ -Glu-Leu (m/z 261.4f86.1) and γ -Glu-Val (m/z 247.4f72.0). Quantitative analysis was performed in triplicates by comparing the peak areas obtained for the corresponding mass traces with those of defined standard solutions of each reference peptide (γ -Glu-Leu and γ -Glu-Val, Bachem, Weil am Rhein) according to Toelstede et al. (2009).

2.5 Statistical analysis

The data set (results of 106 analysed physical properties and volatile and non-volatile compounds) was analysed by stepwise discriminant analysis to identify parameters with high discriminant power. During stepwise discriminant analysis, those parameters are selected that show high discriminant power. Every parameter is added successively during analysis until no significant amount to the canonical R is reached. The resulting data set (L^* , $G''_{20\text{ °C}}$, $\tan \delta_{\max}$, 3-methylbutyric acid, acetic acid, propionic acid, γ -Glu-Leu, γ -Glu-Val, IVPN) was subjected to canonical linear discriminant analysis (LDA). The data set was standardised to equal variance to compare the impact of variables differing in their origin and units. Canonical variables were calculated in a way that the ratio of between-group variation to within-group variation is maximised. Cross validation (leave-one-out) was performed to ascertain the quality of the prediction model. All statistical analyses were performed using SAS (SAS Institute, v. 9.2, Cary, USA).

3 Results

Four groups of Emmental cheese (regional, European, Swiss and Allgäu) were analysed in terms of physical properties and volatile and non-volatile compounds. Stepwise discriminant analysis allowed the selection of nine parameters with high discriminant power. These parameters were brightness L^* , loss modulus G'' at 20 °C, loss factor $\tan \delta_{\max}$, acetic acid, propionic acid, 3-methylbutyric acid, the glutamyl peptides γ -Glu-Leu and γ -Glu-Val and the bitter peptide IVPN.

3.1 Physical properties

The brightness L^* was measured in the CIE $L^*a^*b^*$ colour space with $L^*=0$ indicating a black and $L^*=100$ indicating a white colour impression. The results are shown in Fig. 1. Brightness measured for regional generic Emmental cheese varied between 69.2 and 77.4 with a mean value of 73.3 ± 2.7 . It becomes clear from Fig. 1 that Emmental cheese samples from the other groups were in the same range as regional Emmental cheese and no discrimination according to brightness was possible. European generic Emmental cheese showed the highest values and, thus, brightest samples. Values obtained for Allgäu Emmental cheese were in the lower range of regional generic Emmental cheese whereas brightness values for Swiss Emmental cheese were found to be in the upper range.

The rheological parameters loss modulus G'' at 20 °C and maximum loss factor $\tan \delta$ are presented in Table 2. The loss modulus G'' significantly differed between regional and European generic Emmental cheeses (29.2 ± 7.6 and 20 ± 2.8 kPa, respectively). However, these results did not differ significantly from Allgäu and Swiss Emmental cheeses (28.8 ± 6.4 and 30.3 ± 10.6 kPa, respectively).

European generic Emmental cheese showed the lowest value for $\tan \delta_{\max}$ (mean value = 2.10 ± 0.39) and Allgäu Emmental cheese the highest value with 3.47 ± 0.24 (mean value $\pm$ SD). The results obtained of $\tan \delta_{\max}$ for regional and Swiss Emmental cheese were in between and did not differ significantly from each other.

Fig. 1 Results for brightness L^* for regional, European, Allgäu and Swiss Emmental cheeses; *dashed line*, mean value of regional Emmental cheese; *grey area*, SD for regional Emmental cheese; *closed circles*, regional Emmental cheese samples; *closed squares*, European Emmental cheese samples; *closed circles*, Allgäu Emmental cheese samples; *closed triangles*, Swiss Emmental cheese samples; *open squares*, mean value and SD of European Emmental cheese samples; *open circles*, mean value and SD of Allgäu Emmental cheese samples; *open triangles*, mean value and SD of Swiss Emmental cheese samples

3.2 Volatile compounds

The results for acetic, propionic and 3-methylbutyric acid are presented in Fig. 2a, b for regional and European generic Emmental cheeses as well as Allgäu and Swiss Emmental cheeses. No significant differences were detected for acetic acid between the cheese groups, except that Swiss Emmental cheese showed significantly higher values. European Emmental cheese exhibited significantly lower values for propionic acid compared to the other cheese groups.

Allgäu and Swiss Emmental cheeses differed significantly from each other in the amounts of 3-methylbutyric acid. The amounts of 3-methylbutyric acid detected for regional and European Emmental cheeses were within the same range and significantly lower compared to the results obtained for Swiss and Allgäu Emmental cheeses.

Table 2 Results of the rheological measurement for German generic Emmental cheese ($n=17$), European Emmental cheese ($n=4$), Allgäu Emmental cheese ($n=5$) and Swiss Emmental cheese ($n=2$)

	Loss modulus G'' at 20 °C in kPa	Maximum loss factor $\tan \delta$
Regional generic Emmental cheese	29.2 b $\pm$ 7.6	2.70 b $\pm$ 0.23
European generic Emmental cheese	20.0 a $\pm$ 2.8	2.10 a $\pm$ 0.39
Allgäu Emmental cheese	28.8 ab $\pm$ 6.4	3.47 c $\pm$ 0.24
Swiss Emmental cheese	30.3 ab $\pm$ 10.6	2.45 ab $\pm$ 0.21

Different letters indicate significant differences ($p \leq 0.05$)

Fig. 2 Results (mean values $\pm$ SD) for the volatile compounds acetic and propionic acid (**a**) and 3-methylbutyric acid (**b**) in regional, European, Allgäu and Swiss Emmental cheeses; different letters indicate significant differences ($p \leq 0.05$); black bars, acetic acid; light grey bars, propionic acid

3.3 Non-volatile compounds

Figure 3 provides the experimental data for glutamyl-peptides γ -Glu-Leu and γ -Glu-Val and the peptide IVPN. No significant differences were observed in the values obtained for γ -Glu-Leu and γ -Glu-Val for regional and European Emmental cheeses. The values obtained for Allgäu Emmental cheese were significantly higher compared to European and German Emmental cheese and significantly lower compared to Swiss

Fig. 3 Results (mean values $\pm$ SD) for the non-volatile compounds (γ -Glu-Leu, γ -Glu-Val, IVPN) in regional, European, Allgäu and Swiss Emmental; different letters indicate significant differences ($p \leq 0.05$); black bars, Glu-Leu; light grey bars, Glu-Val; dark grey bars, IVPN

Emmental cheese. Regional Emmental cheese showed the lowest values obtained for IVPN, whereas the other cheese groups did not differ significantly from each other in that compound.

3.4 Physical properties and volatile and non-volatile compounds

Table 3 presents the results for the nine selected parameters for regional generic Emmental cheese (mean value $\pm$ SD), indicating a large variety in between the German Emmental

Table 3 Results of the selected parameters with high discriminant power and results obtained for the analysed regional Emmental cheese

	Parameter	Mean value $\pm$ standard deviation
Physical properties	Brightness L^* _{unheated}	73.3 $\pm$ 2.70
	Loss modulus G'' 20 °C in kilopascal	29.2 $\pm$ 7.61
	Max. loss factor $\tan \delta$	2.70 $\pm$ 0.23
Volatile compounds	3-Methylbutyric acid in mg.100 g ⁻¹	0.27 $\pm$ 0.3
	Acetic acid in mg.100 g ⁻¹	141.24 $\pm$ 61.33
	Propionic acid in mg.100 g ⁻¹	169.8 $\pm$ 78.37
Non-volatile compounds	γ -Glu-Leu in μ mol.100 g ⁻¹	1.30 $\pm$ 1.01
	γ -Glu-Val in μ mol.100 g ⁻¹	1.97 $\pm$ 1.59
	IVPN in μ mol.100 g ⁻¹	1.09 $\pm$ 1

Mean value $\pm$ standard deviation ($n=17$)

cheese group for each parameter. Figure 4 shows the spatial distribution of regional, European, Swiss and Allgäu Emmental cheeses on the first and second (Fig. 4a), and second and third (Fig. 4b) discriminant functions. A clear discrimination of Swiss Emmental cheese and Allgäu Emmental cheese from the regional and European Emmental cheese samples was achieved. Regional and European Emmental cheeses were grouped more closely.

4 Discussion

4.1 Physical properties

Figure 1 provides the experimental data for brightness L^* for regional, European, Allgäu and Swiss Emmental cheeses. The L^* values obtained for regional generic Emmental cheese in this work (Fig. 1, Table 3) are consistent with the results reported by Rohm and Jaros (1996) as well as Deegan et al. (2014), who obtained an L^* value of 73.5 for generic Finnish Emmental cheese after a ripening time of 90 days. Rohm and Jaros (1996), who analysed Austrian Emmental cheese, observed a significant decrease in brightness with increasing ripening time from 1 week ($L^*=78.6$) to 10 weeks ($L^*=74.5$). The samples analysed in this work ripened for different time periods according to the corresponding regulation. Regional and European generic Emmental cheeses ripen generally for 2 months, according to the Codex Alimentarius (Codex 2013). Allgäu Emmental cheese matures for at least 3 months (Deutsche Käseverordnung 2013) whereas, for Swiss Emmental cheese, at least 4 months are required (FOAG 2014). The L^* values for European Emmental cheese were among the highest, indicating a short ripening time compared to the results obtained for German Emmental cheese. Lower L^* values were expected for Swiss and Allgäu Emmental cheeses due to an extended ripening time and increased proteolysis, which influences light scattering resulting in lower L^* values. The values were obtained for Allgäu Emmental cheese in

Fig. 4 Spatial distribution according to linear discriminant analysis of regional, Allgäu, Swiss and Generic Emmental according to textural properties and sensometabolite concentrations on **a** the first (Can1) and second (Can2) and **b** second (Can2) and third (Can3) canonical functions

this study (71.0 ± 2.7) are consistent with results found by Pillonel et al. (2002), who obtained an L^* value of 68.9 for Allgäu Emmental cheese. However, values obtained for Swiss Emmental cheese were unexpectedly high, which may be due to differences in the manufacturing process, season of production and differences in forage. For Swiss Emmental cheese, silage feeding is not allowed in contrast to European and German generic Emmental cheese resulting in colour differences (Pillonel et al. 2002; Rohm and Jaros 1997).

Dynamic oscillatory shear measurements are widely applied to obtain information about melt and textural properties of cheese samples. The loss modulus G'' , representing viscous behaviour, decreases with increasing temperature, indicating changes in the protein structure (Guggisberg et al. 2007). The values obtained for regional Emmental cheese did not differ significantly from the results for Allgäu and Swiss Emmental cheeses indicating a similar viscous portion of the samples. However, a significant difference was observed between regional ($G'' = 29.2 \pm 7.6$ kPa) and European Emmental cheeses ($G'' = 20.0 \pm 2.8$ kPa). Differences obtained for the loss modulus G'' at 20 °C indicate differences in firmness of the cheese samples, which is most likely due to varying ripening times and, thus, differing water contents. European Emmental cheese showed the lowest G'' values, and thus, these samples were the softest caused by the short ripening time of 2 months compared to at least 3 and 4 months for Allgäu and Swiss Emmental cheeses, respectively. Regional Emmental cheese also ripens for at least 2 months; hence, it becomes obvious why the loss modulus G'' at 20 °C may be a suitable parameter for discrimination.

The loss factor $\tan \delta$ is the ratio of storage and loss modulus (G''/G') and a measure of overall meltability of a cheese sample. A value >2.0 indicates sufficient meltability (Guggisberg et al. 2007), which was obtained for all samples in this study. However, the values for European Emmental cheese were fairly low, indicating a firmer structure and reduced meltability. Schenkel et al. (2014) analysed 24 different commercial cheese types (one Emmental cheese sample and four Swiss-type cheese samples) and obtained $\tan \delta_{\max}$ values from 2.46 to 3.46 for Swiss-type cheeses (Emmental cheese = 3.12). Differences can be explained by variations in the manufacturing process such as scalding temperature, curd washing and brining duration. Variations of the processing steps influence the composition; e.g. increased scalding temperatures lead to increased dry matter (Kammerlehner 2009), which in turn influences the melt behaviour. During heating, the cheese fat melts, which causes the protein network to break down due to lubrication of the protein strands and increases meltability (Guggisberg et al. 2007; Schenkel et al. 2014).

4.2 Volatile compounds

The Codex Alimentarius (Codex 2013) demands a minimum amount of propionic acid for Swiss-type cheese to be marketed as “Emmental cheese”. This is not required for Swiss and Allgäu Emmental cheeses. Pillonel et al. (2002) analysed the amount of propionic acid of Emmental cheese from different European regions. Allgäu and Swiss Emmental cheeses contained 177.1 and 440.8 mg.100 g⁻¹ of propionic acid, respectively. These results are consistent with the values found in this study (mean value for Allgäu and Swiss Emmental cheeses 181 ± 19 and 396 ± 36 mg.100 g⁻¹, respectively). Kocaoglu-Vurma et al. (2009) analysed 14 generic Swiss-type cheeses

produced in the USA and one imported from Europe. They obtained propionic acid contents in the range of 4.5–599 mg.100 g⁻¹. In this study, values between 57 and 359 mg.100 g⁻¹ were obtained for regional Emmental cheese. The amount of propionic acid is dependent on various factors, e.g. amount and type of added *Propionibacterium freudenreichii*, a suitable pH for propionibacteria growth during manufacturing and duration of the warm-ripening phase. However, the manufacturing process was not considered in this study.

Kocaoglu-Vurma et al. (2009) additionally analysed acetic acid and obtained values between 94 and 271 mg.100 g⁻¹ cheese, which is consistent with the results found in this study for German and regional Emmental cheese (German Emmental cheese=59.1–252 mg.100 g⁻¹, European Emmental cheese=110–316 mg.100 g⁻¹). Hintz et al. (1956) evaluated a range from 180 to 390 mg.100 g⁻¹ for raw milk Swiss-type cheese. The values obtained in this study for Swiss and Allgäu Emmental cheeses were in this range (Allgäu Emmental cheese=249–288 mg.100 g⁻¹, Swiss Emmental cheese=285–354 mg.100 g⁻¹). Acetic acid is produced during propionic acid fermentation, and thus, Emmental cheeses with a longer warm-ripening phase exhibit higher values. (Thierry et al. 2004a, b). Moreover, Swiss and Allgäu Emmental cheeses were produced from raw milk and the indigenous milk flora influences the fermentation processes.

3-Methylbutyric acid is a cheese flavour compound, produced mainly by propionibacteria, known to occur in Swiss-type cheese. The volatile acid originates from leucine catabolism and contributes to the “nutty” flavour of Emmental cheese (Patton 1966). The amount produced strongly varies depending on the strain of *P. freudenreichii* used as shown by Thierry et al. (2004a, b, 2005). Thierry et al. (2004a) compared the amount of isovaleric acid (2-methylbutyric acid and 3-methylbutyric acid) in experimental mini Swiss-type cheeses produced with added *P. freudenreichii* as well as in its absence. They obtained values for isovaleric acid of 0.67 mg.100 g⁻¹ (control cheese, absence of *P. freudenreichii*) and 4.53 mg.100 g⁻¹ (experimental cheese, fermented by *P. freudenreichii*). In this work, the amount detected for Allgäu and Swiss Emmental cheeses showed the highest values of 3-methylbutyric acid which coincides with the results found by Thierry et al. (2004b) who detected 3.3 and 0.3 mg.100 g⁻¹ isovaleric acid for Swiss-type cheese produced with and without *P. freudenreichii*, respectively. Furthermore, the results are consistent with the values by Beuvier et al. (1997), who measured 1.4 and 0.24 mg.100 g⁻¹ isovaleric acid for Swiss-type cheese made from raw, pasteurised or microfiltrated milk, respectively, and found the highest concentrations of volatile fatty acids in cheeses with an indigenous microflora (i.e. raw milk cheeses). Langler and Day (1966) analysed five different Emmental cheeses and detected 3-methylbutyric acid only in two samples. They concluded that this is possibly due to differences in specificities of the enzyme involved or alternative metabolic pathways.

4.3 Non-volatile compounds

In this study, kokumi-active γ -glutamyl peptides were quantified in Emmental cheese for the first time. The kokumi sensation enhances mouthfulness and increases the complex taste continuity of mature cheese (Toelstede et al. 2009). The formation of γ -glutamyl peptides occurs via the catalytic activity of the enzyme γ -glutamyl transferase. The enzyme transfers a glutamyl moiety to an amino acid or a peptide while

forming a γ -peptide bond. The enzyme originates from raw milk, different bacteria of the starter cultures or mould used for ripening (Tate and Meister 1981; Suzuki et al. 1986; Tomita et al. 1990), and therefore, the amount of γ -glutamyl peptides formed differs depending on the aforementioned parameters. Toelstede et al. (2009) report that γ -glutamyl dipeptides enhance the kokumi flavour in matured Gouda cheese. They obtained 0.71 and 0.26 $\mu\text{mol} \cdot 100 \text{ g}^{-1}$ of γ -Glu-Leu and γ -Glu-Val in Gouda cheese (matured for 44 weeks). The mean values in this work for regional generic Emmental cheese were 1.30 and 1.97 $\mu\text{mol} \cdot 100 \text{ g}^{-1}$ for γ -Glu-Leu and γ -Glu-Val, respectively. The amount of both γ -glutamyl dipeptides detected seemed to correlate strongly with the ripening time. The content increased with increasing ripening time in the following order: European Emmental cheese, regional Emmental cheese, Allgäu and Swiss Emmental cheeses. The low values of European Emmental cheese indicate a shorter ripening time. During ripening, a multitude of chemical, bioenzymatic and physical alterations occur which lead to the degradation of the cheese constituents. Peptides, which mostly derive from α_{s1} - and β -casein (Toelstede and Hofmann 2008b), were detected for the first time in Emmental cheese in this work. Differences in peptide concentrations may be due to varied microfloras of the cheeses resulting in a variety of proteolytic enzymes. However, due to a lack of data in literature, this subject needs to be further investigated.

4.4 Physical properties and volatile and non-volatile compounds

The data obtained for physical properties and volatile and non-volatile compounds were combined and subjected to linear discriminant analysis (LDA). During LDA, latent variables are calculated so that the ratio of between-group variation to within-group variation is maximised. The aim was to discriminate between regional, European, Allgäu and Swiss Emmental cheeses based on the data obtained for nine characteristics parameters. The spatial distributions on the first and second, and second and third are shown in Fig. 4a, b, respectively. The clear discrimination between Swiss and Allgäu Emmental cheeses from European and German Emmental cheese is mainly due to the different values obtained for γ -Glu-Leu.

The results after cross validation are shown in Table 4. The selection of nine parameters resulted in a good differentiation between regional, Swiss, Allgäu and European Emmental cheeses. For regional Emmental cheese, 16 out of 17 samples were classified correctly after cross validation (94.1%). One sample was classified to be

Table 4 Results for classification of different Emmental cheeses after cross validation

	Regional Emmental cheese	Allgäu Emmental cheese	Swiss Emmental cheese	European Emmental cheese	Total number of samples
Regional Emmental cheese	16	0	0	1	$n=17$
Allgäu Emmental cheese	0	5	0	0	$n=5$
Swiss Emmental cheese	0	0	2	0	$n=2$
European Emmental cheese	1	0	0	3	$n=4$

Rows, classified cheeses; columns, group that each cheese was classified into

a European generic Emmental cheese. European Emmental cheese was classified correctly at 75%; one sample was classified as regional Emmental cheese. Swiss and Allgäu Emmental cheeses were classified correctly at 100%. This leads to an overall total of 92.9%. Pillonel et al. (2005) analysed 183 different Swiss-type cheeses and applied statistical methods for classification according to the region of production. The concentration of five free amino acids (asparagine, glycine, lysine, phenylalanine and proline) allowed accurate classification of the cheeses produced in Switzerland, and including certain casein fractions enabled to further discrimination between cheeses from “Finland”, “Bretagne” and “Savoie”.

The clear discrimination of Swiss and Allgäu Emmental cheeses is likely due to the use of raw milk and the extended ripening time as well as the different types of ripening (rind/film). Raw milk cheeses typically exhibit a more intense flavour due to higher concentrations of free amino acids, free fatty acids and volatile compounds developed because of the indigenous milk microflora and enzymes (Beuvier et al. 1997).

A differentiation between regional and European Emmental cheeses is more difficult because of the use of pasteurised milk and similar maturation times. However, the selected parameters seem to demonstrate differences between regional Emmental cheese and European Emmental cheese samples, which may result from variations in ripening time and/or manufacturing process, e.g. cultures, curd washing, scalding, size and weight of the cheeses.

5 Conclusion

In this work, physical properties and volatile and non-volatile compounds, which enable a discrimination between regional Emmental cheese and other Emmental cheese, were identified. Stepwise discriminant analysis allowed the reduction from 106 to nine parameters with high discriminant power. The application of LDA analysis leads to a clear differentiation of Swiss and Allgäu Emmental cheeses and a correct classification of 94% of regional Emmental cheese. Furthermore, γ -glutamyl peptides were quantified for the first time in Emmental which possibly contribute to the kokumi impression of Emmental cheese.

The results provide first indications for analytical parameters that are highly valuable for the identification and characterisation of German regional Emmental cheese (Bavaria and Baden-Wuerttemberg) as well as for discrimination between different Emmental cheese types. Thus, these preliminary results may be useful, after further verification, for cheese makers to promote a label for protection of their Emmental cheese. However, a large variety of parameters influence the results obtained, e.g. the season of production, type of ripening and raw material. Hence, an increased number of Emmental cheese samples will be analysed for more accuracy of the statistical results as well as to verify the discriminant suitability of the selected parameters for discrimination.

Acknowledgments The authors would like to thank the dairies for the generous supply of Emmental cheese samples with special thanks to Hubert Dennenmoser. Furthermore, we thank Thomas Westermair for the analysis of the composition of all Emmental cheese samples.

This research project was supported by the German Ministry of Economics and Technology (via AiF) and the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn). Project 17068N.

Compliance with ethics requirement statements

Conflict of interest All authors declare that they have no conflict of interest.

References

- Beuvier E, Berthaud K, Cegarra S, Dasen A, Pochet S, Buchin S, Duboz G (1997) Ripening and quality of Swiss-type cheese made from raw, pasteurized or microfiltered milk. *Int Dairy J* 7:311–323
- Bisig W, Fröhlich-Wyder MT, Jakob E, Wechsler D (2010) Comparison between Emmentaler PDO and generic emmental cheese production in Europe. *Aust J Dairy Technol* 65:206–213
- Codex (2013) Codex standard for Emmental, codex standard 269-1967. Formerly codex stan C-9-1967-standard for Emmentaler. Adopted 1967. Revision 2007. Amendments 2008, 2010, 2013. Codex Alimentarius Commission
- Deegan KC, Holopainen U, McSweeney PLH, Alatossava T, Tuorila H (2014) Characterisation of the sensory properties and market positioning of novel reduced-fat cheese. *Innov Food Sci Emerg Technol* 21:169–178
- Deutsche Käseverordnung (2013) Established 1965 revision 1986, Amendment 2010, 2013. German Federal Ministry for justice and consumer protection
- FOAG (2014) Emmentaler PDO. Swiss federal office for agriculture, Bern. <http://www.blw.admin.ch>. Accessed 11 March 2015
- Guggisberg D, Bütikofer U, Albrecht B (2007) Melting and solidification characteristics of Swiss raclette cheese measured by small amplitude oscillatory shear measurements. *J Texture Stud* 38:297–323
- Hintz PC, Slatter WL, Harper WJ (1956) A survey of various free amino and fatty acids in domestic Swiss cheese I. *J Dairy Sci* 39:235–244
- Kammerlehner J (2009) Cheese technology, 1st edn. Josef Kammerlehner Verlag, Freising, Germany
- Kireeva I (2011) How to register geographical indications in the European community. *World Patent Inf* 33: 72–77
- Kocaoglu-Vurma NA, Yurdugul S, Koca N, Harper WJ, Courtney PD (2009) Chemical, microbiological, and physical characteristics of commercial Swiss-type cheeses. *Milchwissenschaft* 64:379–383
- Langler JE, Day EA (1966) Quantitative analysis of the major free fatty acids in Swiss cheese. *J Dairy Sci* 49: 91–93
- Patton S (1966) Volatile acids of Swiss cheese. *J Dairy Sci* 47:817–818
- Pillonel L, Badertscher R, Bütikofer U, Casey M, Dalla Torre M, Lavanchy P, Meyer J, Tabacchi R, Bosset JO (2002) Analytical methods for the determination of the geographic origin of Emmentaler cheese. Main framework of the project; chemical, biochemical, microbiological, colour and sensory analyses. *Eur Food Res Technol* 215:260–267
- Pillonel L, Albrecht B, Badertscher R, Bütikofer U, Chamba JF, Tabacchi R, Bosset JO (2003a) Analytical methods for the determination of the geographic origin of emmental cheese. Parameters of proteolysis and rheology. *Ital J Food Sci* 15:49–62
- Pillonel L, Ampuero S, Tabacchi R, Bosset JO (2003b) Analytical methods for the determination of the geographic origin of emmental cheese: volatile compounds by GC/MS-FID and electronic nose. *Eur Food Res Technol* 216:179–183
- Pillonel L, Bütikofer U, Schlichtherle-Cerny H, Tabacchi R, Bosset JO (2005) Geographic origin of European Emmental. Use of discriminant analysis and artificial neural network for classification purposes. *Int Dairy J* 15:557–562
- Rohm H, Jaros D (1996) Colour of hard cheese. 1. Description of colour properties and effects of maturation. *Eur Food Res Technol* 203:241–244
- Rohm H, Jaros D (1997) Colour of hard cheese: 2. Factors of influence and relation to compositional parameters. *Eur Food Res Technol* 204:259–264
- Schenkel P (2014) Innovative technologies in cheese milk pre-treatment and its impact on thermo-physical properties of semi-hard cheese. Dissertation, Verlag Dr. Hut
- Schenkel P, Hartmann KI, Samudrala R, Hinrichs J (2014) Characterization of commercial cheese regarding thermophysical properties and application of multivariate statistical analysis to elaborate product mapping. *J Texture Stud* 45:440–451
- Suhaj M, Koreňovská M (2008) Study of some European cheeses geographical traceability by pattern recognition analysis of multielemental data. *Eur Food Res Technol* 227:1419–1427

- Suzuki H, Kumagai H, Tochikura T (1986) γ -Glutamyltranspeptidase from *Escherichia coli* K-12: purification and properties. *J Bacteriol* 168:1325–1331
- Tate SS, Meister A (1981) γ -Glutamyl transpeptidase: catalytic, structural and functional aspects. *Mol Cell Biochem* 39:357–368
- Thierry A, Maillard M-B, Hervé C, Richoux R, Lortal S (2004a) Varied volatile compounds are produced by *Propionibacterium freudenreichii* in Emmental cheese. *Food Chem* 87:439–446
- Thierry A, Richoux R, Kerjean J (2004b) Isovaleric acid is mainly produced by *Propionibacterium freudenreichii* in Swiss cheese. *Int Dairy J* 14:801–807
- Thierry A, Richoux R, Kerjean J (2005) *Propionibacterium freudenreichii* strains quantitatively affect production of volatile compounds in Swiss cheese. *Lait* 85:57–74
- Toelstede S, Hofmann T (2008a) Quantitative studies and taste re-engineering experiments toward the decoding of the nonvolatile sensometabolome of Gouda cheese. *J Agric Food Chem* 56:5299–5307
- Toelstede S, Hofmann T (2008b) Sensomics mapping and identification of the key bitter metabolites in Gouda cheese. *J Agric Food Chem* 56:2795–2804
- Toelstede S, Dunkel A, Hofmann T (2009) A series of kokumi peptides impart the long-lasting mouthfulness of matured Gouda cheese. *J Agric Food Chem* 57:1440–1448
- Tomita K, Yano T, Tsuchida T, Kumagai H, Tochikura T (1990) Purification and properties of γ -glutamyltranspeptidase from *Penicillium roqueforti* IFO 4622. *J Ferment Bioeng* 70:128–130