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Comparison of SDS–PAGE profiles of four Belgian cheeses by multivariate statistics

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Summary — Most of the classification methods so far published for cheese varieties which are based either on rheological or compositional properties appear to be inadequate. Still, a promising approach in this area of cheese research is the application to proteolytic patterns of multivariate statistics such as principal component analysis (PCA) and discriminant analysis (DA). Both techniques were therefore performed on electrophoretic profiles obtained by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) of the pH 4.6-insoluble protein fraction of cheese. Cheese samples of different Belgian varieties (Passendale, Wijnendale, Nazareth and Oud Brugge) with different grades of maturity were investigated and compared to other international brands. The SDS–PAGE method was performed by means of the PhastSystem™. The method produces volumograms which allow not only qualitative but also quantitative interpretation. Generally it can be concluded that PCA resulted in a clear separation of Nazareth and Oud Brugge and to a lesser extent of Passendale and Wijnendale; DA allowed correct classification of the four Belgian cheeses, and comparison with international brands determined known similarities between the cheeses. This tool may therefore be of use in the field of authenticity research and classification. To a lesser extent, the method can also help in determining the maturity of a ripening cheese.

cheese / proteolysis / classification / SDS–PAGE / multivariate analysis

Résumé — Comparaison des profils de SDS–PAGE de quatre fromages belges par l'analyse statistique multivariée. La plupart des méthodes de classification de variétés de fromage publiées jusqu'à présent, fondées sur les propriétés rhéologiques ou de composition, apparaissent inadéquates. Ainsi, une approche prometteuse dans ce domaine de recherche sur les fromages est l'application de l'analyse multivariée telle que l'analyse en composantes principales (ACP) ou l'analyse discriminante

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(AD) sur le profil protéolytique. Par conséquent, les deux techniques ont été réalisées sur le profil protéolytique obtenu par électrophorèse sur gel (SDS–PAGE) sur la fraction protéique insoluble à pH 4,6. Les échantillons des fromages de différentes variétés belges (Passendale, Wijnendale, Nazareth et Oud Brugge) et de différents degrés de maturité ont été étudiés et comparés avec des marques internationales. La méthode SDS–PAGE était réalisée à l'aide du PhastSytem™. La méthode résulte en volumogrammes, qui permettent non seulement l'interprétation qualitative, mais aussi quantitative. On peut généralement conclure que l'ACP donne une séparation claire des fromages Nazareth et Oud Brugge, et dans une moindre mesure, des fromages Passendale et Wijnendale. L'AD a permis une classification correcte de quatre fromages belges et la comparaison avec des marques internationales a révélé des similarités connues entre les fromages. En conséquence, cet instrument peut être utile dans le domaine de la recherche sur l'authenticité et la classification. Dans une moindre mesure, la méthode peut aussi aider à suivre l'affinage d'un fromage.

fromage / protéolyse / classification / SDS–PAGE / analyse multidimensionnelle

INTRODUCTION

While it has been claimed that there exist as many as 900 individual types of cheese (Campbell-Platt, 1987), most authors would agree that there are certainly over 400 varieties (Scott, 1986; Fox, 1993). Still, this is a large variety of different cheeses, and to assist international trade or for other reasons such as ripening and authenticity research, a number of attempts have been made to develop a classification method. However, most of the classification methods published so far, which are based either on rheological or compositional properties, appear to be inadequate (Fox, 1993).

A promising approach in this area of cheese research is the application of multivariate statistics to proteolytic patterns obtained by electrophoresis or chromatographic methods. Such pattern recognition techniques have already been used in chemistry, microbiology and medicine for identification of chemical compounds, classification of bacterial species and diagnosis of diseases, respectively (Pham and Nakai, 1984). By applying principal component analysis (PCA), linear discriminant analysis (DA), and multiple regression analysis to gas chromatographic data, Aishima (1979) reported that eight brands of soy sauce samples were classified correctly into eight groups. In addition, Pham and Nakai (1984) showed that stepwise discriminant analysis of HPLC data could be used to classify Cheddar cheese into mild, medium, old and extra-old categories.

The present study followed this path by applying PCA and DA to protein profiles resulting from primary proteolysis. These profiles were obtained by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) of the pH 4.6-insoluble fraction. This fraction contains polypeptides of molecular weight (MW) higher than approximately 10 000 and thus the caseins and primary degradation products (Christensen et al, 1991). Cheese samples of different varieties (Passendale, Wijnendale, Nazareth and Oud Brugge) with varying grades of maturity were analyzed and compared to international brands.

MATERIALS AND METHODS

Samples

Four Belgian cheeses were investigated in detail: two semi-hard cheeses (Passendale and Wijnendale), a high-scald hard cheese (Nazareth) and a low-scald hard cheese (Oud Brugge).

Passendale and Wijnendale were manufactured by NV Kaasmakerij Passendale (Passendale, Belgium). Nazareth and Oud Brugge were produced by CV Belgomilk (Moorslede, Belgium).

– Passendale is a mould-ripened semi-hard cheese. Spores of *Penicillium candidum* are added to the cheese milk and develop on the cheese surface after a few days of ripening. The influence of surface mould on interior cheese ripening is quite limited.

– Wijnendale is a semi-soft bacterial surface ripened cheese made from raw milk. The process of bactofugation is applied to remove undesired microorganisms. During curing the rind is washed, firstly with a suspension of *Brevibacterium linens*, then afterwards with warm water.

– Nazareth is a Gouda-type cheese with Emmentaler characteristics due to propionic acid fermentation.

– Oud Brugge is a hard Gouda-type cheese. According to the manufacturer the ripening period takes at least one year. In table I, the dry matter content, fat on dry matter and average age at consumption of the four previously described cheeses are shown.

The four Belgian cheeses were sampled according to ripening period at the production site. Table II shows the age of the different samples from the day the curd was formed. The four cheeses were also purchased at local stores together with international brands for comparison. To check the reliability of the whole procedure, some control samples were analyzed.

Fractionation at pH 4.6

A quantity of 5 g cheese dry matter was aimed at for fractionation at pH 4.6. Therefore, the dry matter content of the cheese was determined according to the IDF standard E4A (IDF, 1982) which is based on water evaporation in the presence of sand at a temperature of 102 °C in a drying oven. The fractionation procedure itself goes back to that described by Assenat (1967). The method is based on the precipitation of caseins at their isoelectric point (pH 4.6) after defatting, while the smaller (poly)peptides and whey proteins stay solubilized at this pH.

SDS–PAGE

Sodium dodecyl sulphate binds strongly to proteins, mainly through hydrophobic interactions, and carries a negative charge. The amount, which is fixed, is approximately proportional to the weight of protein: about 1.4 g SDS/g protein (Reynolds and Tanford, 1970). The indigenous net charge of the protein at any pH is thus negligible. Any protein should therefore migrate at the same velocity towards the anode in free flow electrophoresis in the presence of SDS. How-

Table I. Main characteristics of the four Belgian cheeses.
Attributs principaux des quatre fromages belges.

Cheese	*g/kg DM*	*g/kg fat on DM*	*Average age*
Passendale	500	500	4–5 weeks
Wijnendale	500	500	5 weeks
Nazareth	600	450	8 weeks (min)
Oud Brugge	700	480	1 year

DM: dry matter.
DM : matière sèche.

Table II. Sampling of the four Belgian cheeses at the production site.
Échantillonnage des quatre fromages belges à la manufacture.

Days after curd preparation	*P*	*W*	*N*	*OB*	*Days after curd preparation*	*P*	*W*	*N*	*OB*
0	x	x	x	x	39				x
0.5	x	x		x	42			x *	
1	x	x	x	x	46				x
2	x	x	x		50	x	x		
3	x	x			53				x
4			x	x	56			x *	
5				x	57	x	x		
6	x	x		x	60				x
7	x	x	x	x	67				x
9			x	x	74				x
11				x	81				x
14			x	x	102				x
15	x	x			116				x
17	x	x	x		130				x
18				x	144				x
21				x	165				x
22	x	x			179				x
28			x		193				x
29	x	x			224				x
32				x	271				x *
36	x	x			285				x *

P: Passendale; W: Wijnendale; N: Nazareth; OB: Oud Brugge; * sample taken from a different batch.
* *Échantillon pris d'un autre lot de fromage.*

ever, in gel electrophoresis and in particular in acrylamide gels, the bigger a protein, the lower its electrophoretic mobility due to the sieving action of the gel. Therefore, the MW is easily obtained by running an electrophoresis of the proteins of interest and a mixture of proteins of known molecular weights, and by plotting log MW versus distance of migration (Ribadeau-Dumas and Grappin, 1989).

SDS–PAGE was applied for the separation of the polypeptide fraction non-soluble at pH 4.6. The method was performed by means of PhastSystem™ (Pharmacia, Uppsala, Sweden). Quantitative interpretation of the electrophoretic patterns was achieved by means of PhastImage™ (Pharmacia, Uppsala, Sweden). PhastGel® media are precast ultrathin gels (Van Hekken and Thompson, 1992) able to resolve eight lanes simultaneously; it is possible to run two gels simultaneously. In the present study, a PhastGel® homogeneous 20 was used. Homogeneous 20 consists of a discontinuous buffer system set up by a 13-mm stacking gel zone and a 32-mm separating gel zone. The stacking gel zone consists of 7.5% AA (acrylamide) and 3% crosslinker (bisacrylamide). The separating gel zone consists of 20% AA and 2% crosslinker. The buffer system in the gels consists of 0.112 mol/L acetate, 0.112 mol/L Tris, pH 6.5. The gels are run with Phastgel® SDS Buffer Strips which contain 0.2 mol/L tricine, 0.2 mol/L Tris, and 5.5 g/L SDS, pH 8.1. The eluting buffer solution consists of 10 mmol/L Tris, 1 mmol/L

EDTA, 25 g/L SDS, and 50 g/L β-mercaptoethanol, pH 8.0. For the detection of the protein bands in the gel, Coomassie blue staining was applied. The gels were scanned by means of the PhastImage™ Gel Analyzer. Lane evaluation was carried out by means of PhastImage™ software. It consisted of a number of steps: 1) defining the 16 individual slices (electrophoretograms) of the two gels; 2) defining the contours of the bands; 3) representation of the electrophoretograms as three-dimensional volumograms; 4) integration of the volumograms. The integrated values correspond to the color intensity of a band and therefore the amount of polypeptide present at that location.

For determination of the MW of the peaks appearing in the volumograms, the Low Molecular Weight Electrophoresis Calibration Kit (Pharmacia, Uppsala, Sweden) was used. To achieve greatest accuracy in the determination of the MWs, on every single gel that was run at least one lane had to be held free for the application of the calibration kit, and for every single gel a new calibration curve had to be created.

For further information on the equipment and its functioning, reading of the Pharmacia manuals (Pharmacia, 1989a, b) is recommended.

Statistical analysis

Both statistical methods applied in this study belong to the category of multivariate statistics. Whereas the basic problem of DA is to assign an observation, x, to one of two or more groups on basis of its value, PCA is simply a way of re-expressing a set of variables (Kotz and Johnson, 1982). The methods have been known for some years, but have been applied widely only since electronic computers have been available for general use. Both methods will be briefly discussed hereafter.

PCA is a method for transforming a set of variables $x_1, x_2, ..., x_p$ observed on individuals to a new set $y_1, y_2, ..., y_p$, the so-called principal components, with the following properties:

– Each principal component, y, is a linear combination of the x's, ie:

$$y_i = a_{i1}x_1 + a_{i2}x_2 + ... + a_{ip}x_p$$

– The sum of squares of the coefficients a_{ij}, where $j = 1, 2, ..., p$, is unity.

The coefficients a_{ij} are called the 'principal component loadings' and express the contribution of the original variables $x_1, x_2, ..., x_p$ to the principal components.

– Of all possible linear combinations uncorrelated with y_1, y_2 has the greatest variance. Similarly, y_3 has the greatest variance of all linear combinations of the x_i uncorrelated with y_1 and y_2, etc.

The new set of variables represents a transformation of the original variables in such a way that the new variables are uncorrelated and are arranged in order of decreasing variance. The method is perfectly general. Nevertheless, by plotting the component values on their orthogonal axes an allocation of individuals to groups, or the recognition of the existence of a new group can be achieved (Kotz and Johnson, 1982).

With DA two main canonical variables are calculated. The first canonical variable, which is a linear combination of the original variables observed on an individual and best discriminates among the groups, is plotted on the x-axis. The second canonical variable that is the next best linear combination and orthogonal to the first one, is plotted on the y-axis.

Therefore, each individual can be characterized by a pair of canonical variables and visualized in a two-dimensional map. In addition to this, DA also enables classification of unknown individuals by discriminant functions which characterize each group of individuals. The discriminant function is expressed as follows:

$$Z = a_1h_1 + a_1h_2 + ... + a_mh_m$$

where $a_1, ..., a_m$ are the coefficients of the discriminant function of a group and $h_1, ..., h_m$ are the variables 1 to m of the unknown individual. By substituting $h_1, h_2, ..., h_m$ by their respective variables, the discriminant score for each group

can be calculated. The unknown individual is assigned to the group with the largest score (Pham and Nakai, 1984).

In the present study, the individuals consisted of the different cheese samples and the original variables observed on each of the samples were the relative peak surfaces appearing in the volumograms. Groups in the case of DA were the different brands.

The analyses were performed using the SPSS-program for Windows on an IBM compatible ESCOM 486SX-33 personal computer.

RESULTS

SDS–PAGE volumograms

A schematic representation of the major peaks appearing in the volumograms is given in figure 1. By means of the appropriate MW calibration curves, the average MW of the peaks was estimated (table III). Considering these estimated MWs and the results obtained from standardized enzymic reactions (McSweeney et al, 1993; Dierckx, 1995) with different casein substrates, certain peaks can be related to certain proteins composing the fraction which is nonsoluble at pH 4.6. The origin of the remaining peaks, the MW of which is given in table IV, is uncertain. However, some relevant suggestions can still be made.

PCA of the four Belgian cheeses

Correlation PCA was performed on the four ripened Belgian cheeses. The objective was to detect possible grouping of the samples on the basis of SDS–PAGE volumograms. Figure 2 shows the scores of the first two principal components and the percentage of variability these components explain. The loadings of the first two principal components are shown in figure 3.

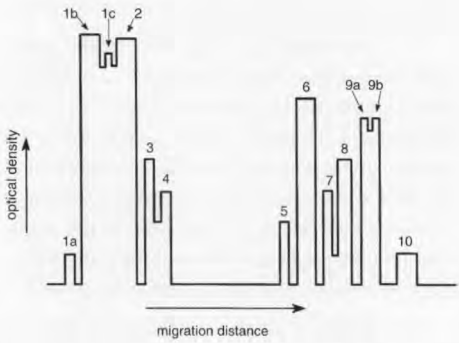

Fig 1. Schematic representation of a SDS–PAGE volumogram of the pH 4.6-insoluble protein fraction of cheese.
Représentation schématique d'un volumogramme obtenu par SDS–PAGE de la fraction protéique insoluble à pH 4,6 du fromage.

Table III. Estimated molecular weight of proteins identified by SDS–PAGE of the pH 4.6-insoluble protein fraction of cheese.
Poids moléculaire estimé des protéines identifiées par SDS–PAGE de la fraction protéique insoluble à pH 4,6 du fromage.

Peak	*MW*	*Corresponding protein*	*Theoretical MW*	*Ref*
1a	~37 000	α_{s2}-Casein	25 230	(Eigel et al, 1984)
1b	~31 000	α_{s1}-Casein	23 614	(Eigel et al, 1984)
2	~28 000	β-Casein	23 993	(Eigel et al, 1984)
3	~24 000	γ_1-Casein	20 520	(Wilson et al, 1989)
6	~15 500	*para*-κ-Casein	12 269	(Walstra and Jennes, 1984)
9a/9b	~14 000	γ_2/γ_3-Casein	11 822/11 557	(Wilson et al, 1989)

Table IV. Estimated molecular weight of proteins unidentified by SDS–PAGE of the pH 4.6-insoluble protein fraction of cheese and some relevant suggestions.
Poids moléculaire estimé des protéines non identifiées par SDS–PAGE de la fraction protéique insoluble à pH 4,6 de fromage et quelques suggestions pertinantes.

Peak	*MW*	*Possibly corresponding protein*	*Theoretical MW (ref)*
1c	~30 000	α_{s1}-I(f24–199)-Casein	21 000 (Mulvihill and Fox, 1979)
4	~21 500	Unidentified	
5	~16 000	Proteose peptone 5	12 176 (Wilson et al, 1989)
7	~15 000	Unidentified	
8	~14 500	Unidentified	
10	~13 500	β-Casein degradation product?	

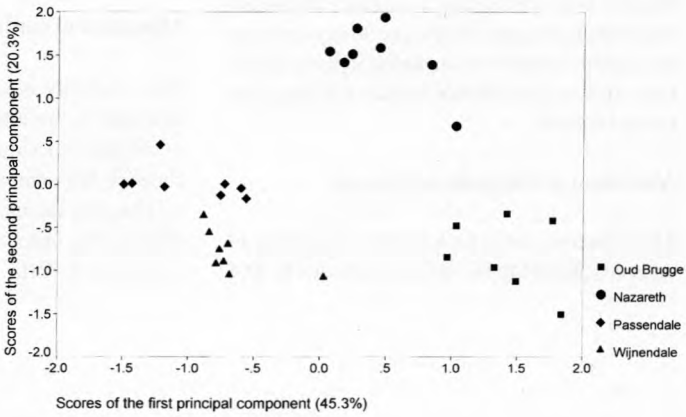

Fig 2. Scores of the principal components of the four Belgian cheeses.
Scores des composantes principales des quatre fromages belges.

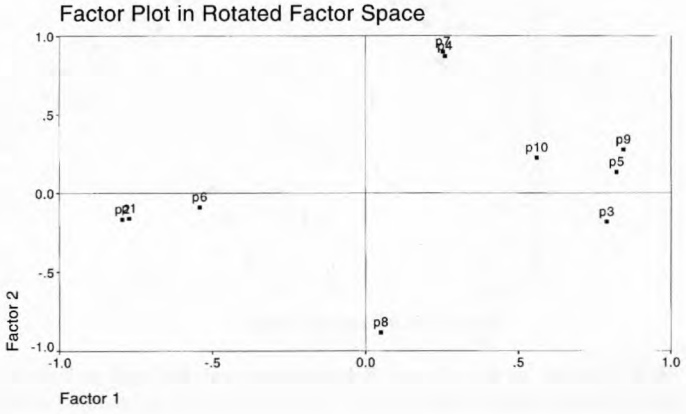

Fig 3. Loadings of the first two principal components obtained after principal component analysis of SDS–PAGE volumograms of the pH 4.6-insoluble protein fraction of cheese.
Chargements des deux premières composantes principales obtenues par l'analyse de composantes principales des volumogrammes de SDS–PAGE de la fraction protéique insoluble à pH 4,6 du fromage.

DA of the four Belgian cheeses

Discriminant analysis was performed on the four ripened Belgian cheeses, resulting in the calculation of two standardized canonical discriminant functions:

$$F_1 = 1.01P_1 + 0.42P_2 - 0.08P_3 - 0.48P_4 - 0.19P_5 + 0.63P_6 - 0.21P_7 + 0.97P_8 + 0.00P_9 + 0.20P_{10}\ (75.99\%);$$

$$F_2 = 0.46P_1 + 0.75P_2 - 0.15P_3 + 0.35P_4 - 0.24P_5 + 0.25P_6 + 0.14P_7 - 0.46P_8 + 0.00P_9 - 0.20P_{10}\ (20.33\%);$$

with P_i ($i = 1,...,10$) the relative peak surface of peak i (fig 1). The value indicated between brackets is the percentage of variance of the corresponding function. Both functions were used to classify samples not included in the calculation, such as international brands, ripening, and control samples.

Allocation of international brands

The objective was to look into the possibility of correctly grouping the Belgian cheeses by DA and to determine to which of the four groups the international brands belong. By this approach, existing similarities between the cheeses may be confirmed. Figure 4 shows the canonical score plot of the DA. The international brands are plotted after calculating their canonical scores. The classification results are summarized in table V.

Allocation of ripening samples

The samples taken at the production site during ripening are plotted after calculating their canonical scores. The canonical plot is shown in figure 5. The classification results are summarized in table VI.

Allocation of control samples

The reliability of the classification procedure described in this article was checked by analysis of additional samples of the four Belgian cheeses. The samples are plotted in the canonical plot after calculation of their canonical scores (fig 6). The classification results are summarized in table VII.

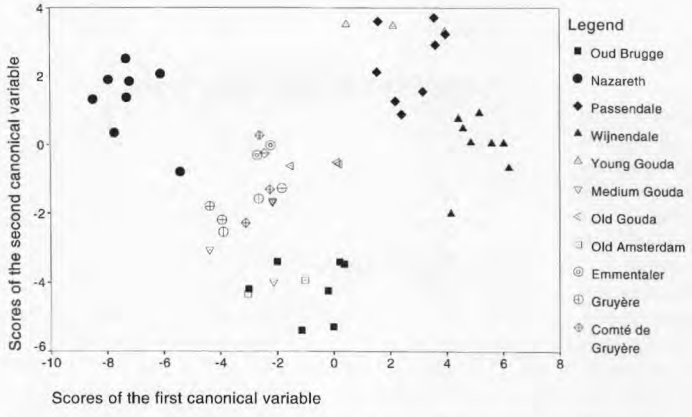

Fig 4. Canonical scores of the four Belgian cheeses and plotting of the international brands.
Scores canoniques des quatre fromages belges et allocation des marques internationales.

DISCUSSION

SDS–PAGE volumograms

From a qualitative point of view, the SDS–PAGE volumograms of the four Belgian cheeses are very similar (results not shown). From a quantitative point of view, Oud Brugge and Nazareth have slightly more individual volumograms due to the longer ripening time and higher scalding temperature which are thought to increase plasmin activity (Visser, 1993). In the Oud Brugge volumogram, pronounced peaks 3 and 9a/9b (γ_1- and γ_2/γ_3-caseins) can be distin-

Table V. Discriminant analysis classification results of the Belgian and international cheeses.
Résultats de classification des fromages belges et internationaux par l'analyse discriminante.

Actual group	*No of samples*	*Predicted group membership*			
		1	*2*	*3*	*4*
Group 1: Passendale	8	8	0	0	0
Group 2: Wijnendale	8	0	8	0	0
Group 3: Nazareth	8	0	0	8	0
Group 4: Oud Brugge	7	0	0	0	7
Allocated samples					
Young Gouda	3	3	0	0	0
Medium Gouda	4	0	0	0	4
Old Gouda	3	0	0	0	3
Old Amsterdam	2	0	0	0	2
Emmentaler	3	0	0	0	3
Gruyère	5	0	0	0	5
Comté de Gruyère	4	0	0	0	4

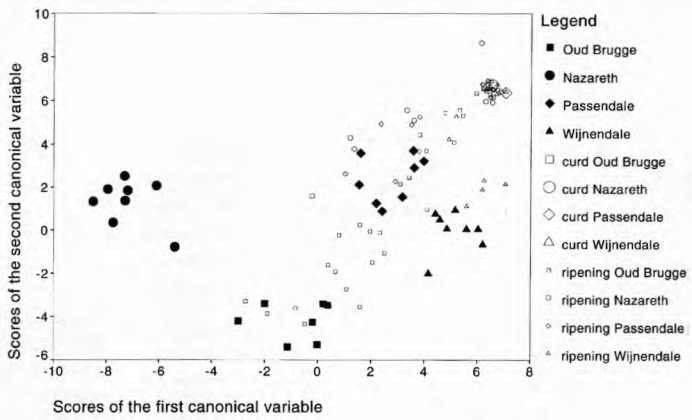

Fig 5. Canonical scores of the four Belgian cheeses and plotting of the ripening samples.
Scores canoniques des quatre fromages belges et allocation des échantillons de différents degrés de maturité.

Table VI. Discriminant analysis classification results of ripening and ripened Belgian cheeses.
Résultats de classification des fromages belges de différents degrés de maturité par l'analyse dicriminante.

Actual group	*No of samples*	*Predicted group membership*			
		1	*2*	*3*	*4*
Group 1: Passendale	8	8	0	0	0
Group 2: Wijnendale	8	0	8	0	0
Group 3: Nazareth	8	0	0	8	0
Group 4: Oud Brugge	7	0	0	0	7
Allocated samples					
Passendale					
Curd	1	1	0	0	0
From day 0.5 to 50	12	12	0	0	0
Wijnendale					
Curd	1	1	0	0	0
From day 0.5 to 17	8	8	0	0	0
From day 22 to 50	4	0	4	0	0
Nazareth					
Curd	1	1	0	0	0
From day 1 to 42	9	9	0	0	0
Oud Brugge					
Curd	1	1	0	0	0
From day 0.5 to 102	20	20	0	0	0
From day 116 to 271	8	0	0	0	8

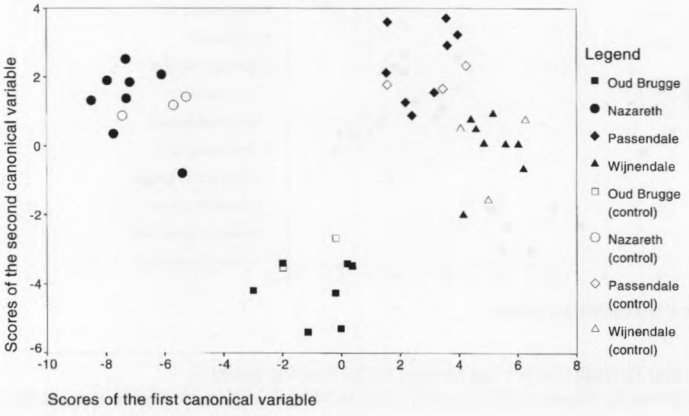

Fig 6. Canonical scores of the four Belgian cheeses and plotting of the control samples.
Scores canoniques des quatre fromages belges et allocation des échantillons de contrôle.

Table VII. Discriminant analysis classification results of the four Belgian cheeses and control samples.
Résultats de classification des quatre fromages belges et des échantillons de contrôle.

Actual group	*No of samples*	*Predicted group membership*			
		1	*2*	*3*	*4*
Group 1: Passendale	8	8	0	0	0
Group 2: Wijnendale	8	0	8	0	0
Group 3: Nazareth	8	0	0	8	0
Group 4: Oud Brugge	7	0	0	0	7
Ungrouped cases					
Passendale	3	2	1	0	0
Wijnendale	3	0	3	0	0
Nazareth	3	0	0	3	0
Oud Brugge	2	0	0	0	2

guished, probably caused by a more intense plasmin activity due to the longer ripening period. The Nazareth volumogram possesses a special feature regarding the absence of peak 8, which can be differentiated in the volumograms of the three other cheeses. No relevant explanation can be formulated, since peak 8 is unidentified. The pronounced peaks 5 and 10 further underline the assumption that these peaks represent degradation products of β-casein. Summarizing, the similarity between the four volumograms is explained by the fact that the SDS–PAGE technique used only visualizes proteins and peptides with a MW above 10 000. Still, quantitative differences can be detected and this is further investigated by the multivariate statistical techniques.

Table IV shows an overestimation of the MW obtained by SDS–PAGE of the caseins and peptides, probably due to presence of denaturating agents such as urea and β-mercaptoethanol, both altering the three-dimensional protein conformation.

PCA of the four Belgian cheeses

The scores of the principal components plotted in figure 2 clearly show a grouping of the Belgian cheeses. The less pronounced differentiation in the Passendale and Wijnendale groups is most probably due to the fact that these cheeses are both quite young (4 to 5 weeks), and hence differences in primary proteolysis of the caseins are quite small. Therefore, basic differences in age and the presence or absence of propionic acid fermentation are expressed by correlation PCA. From figure 3 can be concluded that all peaks have a significant contribution to one of the principal components (factors 1 and 2), since no peak is located in the centre of the factor plot.

DA of the four Belgian cheeses

Allocation of international brands

The four Belgian cheeses are correctly classified by means of DA, as illustrated in figure 3 and table V. This is in agreement with the PCA results. The samples of young Gouda (ripening period: ± 2 months) are perfectly classified in the Passendale group. Passendale is much more similar to Gouda than to Wijnendale, which is a real Saint-Paulin type of cheese. The samples of medium Gouda (ripening period: 4–7 months) and old Gouda (ripening period: 10–12 months)

are classified in the Oud Brugge group, which is a real Gouda-type cheese with a long ripening period and possibly an accelerated ripening. Nevertheless, Oud Brugge is easy to cut in slices when it is fully ripened, and this can explain the similarity with medium Gouda. The allocation of Old Amsterdam to the Oud Brugge group is as expected, since both cheeses are very similar in taste and texture. Although the Swiss-type cheeses are classified in the Oud Brugge group, most probably due to their longer ripening time, they show a tendency towards Nazareth, explained by their common secondary fermentation. Discriminant analysis therefore confirms the relationships between the examined cheeses.

Allocation of ripening samples

From figure 5 and table VI, it can be seen that the curds of the four Belgian cheeses are located very near to each other and in the area of Passendale. The clotting of cheese milk is very similar in the case of the four cheeses and based on a combination of acid and rennet coagulation. From table VI, the following conclusions can be drawn. The Passendale samples taken during the ripening period are all located in the Passendale group. Therefore, no real distinction in maturity could be made. Wijnendale is located in the area of Passendale until 17 days after curd preparation. Between days 17 and 22 of ripening, the pattern changed towards a ripened Wijnendale, most probably due to the typical ripening conditions including surface microflora of *B linens*. During the last two weeks, no Nazareth samples were taken and therefore a detailed migration in the canonical plot cannot be noticed. Still, it can be concluded that the pattern stays very close to that of Passendale and Wijnendale for quite a long time (at least 42 days). At the end of the ripening period, the pattern becomes specifically a Nazareth one, mainly due to the propionic acid fermentation. Oud Brugge is located in its own group after about one-third of its total ripening time (1 year). Proteolytic changes in a hard Gouda-type cheese are slow, mainly due to the slow action of plasmin (Lawrence et al, 1987).

Allocation of control samples

Figure 6 and table VII illustrate that all control samples are correctly classified, except for one sample of Passendale. This further proves the reliability of the classification method which enables the clear distinction between Oud Brugge, Nazareth, and the semi-hard cheeses (Passendale and Wijnendale).

CONCLUSION

Generally, it can be concluded that quantitative SDS–PAGE of the pH 4.6-insoluble protein fraction of cheese combined with multivariate statistics permits the demonstration of certain relationships between several cheeses. It follows that this tool may be of use in the field of cheese authenticity research and classification. To a lesser extent, the method can also help in determining the maturity of a ripening cheese. A combination of the previously described method and analysis of the pH 4.6-soluble fraction for example by size exclusion chromatography might conceivably further reduce the scattering of the different samples plotted by both statistical methods.

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