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A review of the analytical methods coupled with chemometric tools for the determination of the quality and identity of dairy products

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Abstract

There is an increasing demand of the consumers and actors of the food industry sector to have means of measurement allowing the characterisation of raw materials or food. Dairy products (milk, ice cream, yogurt, butter, cheese, etc.) are in considerable demand, command premium prices and are, therefore, vulnerable to economic adulteration. Authenticity of these products is an important issue for food processors, retailers, regulatory authorities and consumers. It is also valuable for ensuring fair competition and as a mean of protecting consumers against fraud due to mislabelling. Conventional chemical methods are not able to determine the regional provenance of dairy products unambiguously. Therefore, alternative techniques such as spectroscopic techniques i.e., near infrared (NIR), mid infrared (MIR), front face fluorescence spectroscopy (FFFS), stable isotope and nuclear magnetic resonance (NMR)-coupled with chemometric tools have many potential advantages as tools for the evaluation of the identity of such products. This review article discusses the potential of destructive and non-destructive techniques for the determination of the quality and the authenticity of dairy products.

Keywords: Dairy products; Identity; Quality; Spectroscopic techniques; Chemometrics

1. Introduction

Product authenticity and authentication are emerging topics within the food sector (Karoui et al., 2004b). It is a major concern not only for consumers, but also for producers and distributors (Fernandez, Astier, Rock, Coulon, & Berdagué, 2003). Indeed, regulatory authorities, food processors, retailers and consumer groups are all interested in ensuring that foods are correctly labelled. Food adulteration has been practiced since biblical times but has become more sophisticated in the recent past. Foods or ingredients most likely to be targets for adulteration include those which are of high-value and which undergo a number of processing steps before they appear on the market. With the European harmonisation of the agricultural policy and the emergence of the international markets, authentication of such foodstuffs focuses more attention. This trend is the result of efforts made by regional authorities, as well as producers to protect and support local productions (Karoui, Mazerolles, & Dufour, 2003).

The quality of milk plays a very important role in the production of all types of cheeses, affecting both cheese yield and characteristics of the cheese (Summer et al., 2003). In regions with high production costs, agriculture needs to produce food of superior quality. The products can be labelled according to the specific conditions, which characterise their origin and the processing technology (Bosset et al., 1997). These regions can be designed for products with protected designation of origin (PDO) or protected geographical indication (PGI).

Animal feeding is one of the elements that often considered as important by cheese-makers (Buchin, Martin, Dupont, Bornard, & Achilleos, 1999; Bugaud, Buchin, Coulon, Hauwuy, & Dupont, 2001; Bugaud, Buchin, Noël, et al., 2001). Grass of natural highland pastures presents a

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highly diversified botanical composition as well as abundant secondary metabolites, which may influence milk and therefore cheese quality (Bugaud, Buchin, Coulon, et al., 2001; Bugaud, Buchin, Noël, et al., 2001). While the gramineae and legumineae families dominate artificial pastures of lowlands, permanent pastures of highlands contain significant proportions of plants belonging to many other botanical families such as rosaceae and plantaginaceae (Bugaud, Buchin, Coulon, et al., 2001; Bugaud, Buchin, Noël, et al., 2001; Jeangros, Scehovic, Troxler, Bachmann, & Bosset, 1999). The relationships between the origin of cheeses and the type of pasture was intensively highlighted by using popular and well-known analytical methods such as gas/liquid chromatography, isotope ratio mass spectrometry, olfactometry or chemical analysis (Bosset et al., 1999; Carpino, Acree, Barbano, Licitra, & Siebert, 2002; Dumont & Adda, 1978; Manca et al., 2001; Mariaca et al., 1997; Pillonel, Ampuero, Tabacchi, & Bosset, 2003; Pillonel, Badertscher, et al., 2003; Pillonel, Luginbühl, et al., 2003; Pillonel, Bütikofer, Schlichtherle-Cerny, Tabacchi, & Bosset, 2005). Other papers have been interested in studying the relationship between grass and milk (Bugaud, Buchin, Coulon, et al., 2001; Collomb, Bütikofer, Sieber, Jeangros, & Bosset, 2002; Fernandez et al., 2003; Kornexl, Werner, Rossmann, & Schmidt, 1997; Martin et al., 2002; Prache et al., 2002). Indeed, using dynamic head space-gas chromatography coupled to mass spectrometry, Fernandez et al. (2003) have found that milk collected in highland regions were richer in sesquiterpenes than those collected in lowland regions. In addition, Bugaud, Buchin, Coulon, et al. (2001) have found that the proportion of C18:1 and the total of C18:2 + C18:3 determined by gas chromatography were higher in mountain milks than in valley milks. Recently, Ritz et al. (2005) have used isotope ratio mass spectrometry to distinguish milks for both the geographic origin and the diet.

Traditional analytical strategies to uncover adulteration and guarantee quality have relied on wet chemistry to determine the amount of a marker compound or compounds in a suspect material and a subsequent comparison of the value(s) obtained with those established for equivalent material of known provenance (Downey, 1996). This approach suffers from a number of disadvantages, namely, the ever-increasing range of analytes which must be included in any test procedure and the limited knowledge of the range of each constituent in normal lots of the substance. By this, these ranges may be expected to vary with the geographic source, dairy products making procedure, etc. It may be readily appreciated that it is often not possible to make a definitive statement on the authenticity or otherwise of a material, even after its examination for a large suite of marker compounds. In addition to this uncertainly, the above mentioned methods tend to require sophisticated analytical equipments and skilled operators; they are also time consuming and require both the purchase and disposal of chemical reagents. For all these reasons, there is a continuing demand for new, rapid and relatively cheaper methods for direct quality measurement in food and food ingredients. Spectroscopic techniques, including the near infrared (NIR), mid infrared (MIR), front face fluorescence spectroscopy (FFFS), stable isotope and nuclear magnetic resonance (NMR) have been examined to assess their suitability for the determination of the quality and/or geographical origins of dairy products. This review paper examines some of the reported approaches adopted for the determination of the identity and quality of dairy products by using a particular chemometric strategy.

2. Traditional techniques used for the determination of the quality and/or authenticity of dairy products

2.1. Physico-chemical analysis

Indicators of origin for manufactured products may be subdivided into primary and secondary indicators. Primary indicators are not influenced by the technology applied for manufacture or ripening conditions but depend only on the feed of the cows, which undergoes natural variation over the year. However, secondary indicators do not depend directly on geographic origin but mainly on the technology used for the transformation of a product. Cheese making is related to local, regional or national traditions leading to differences between cheeses of the same variety but of different origin. Starters, heating temperature of the curd, brining and ripening time are some of the manufacturing parameters that are typical for a defined region and lead to chemical, physical or microbial secondary indicators.

Guinot-Thomas, Al Ammoury, and Laurent (1995) have monitored the changes that occurred in milk during storage at 4 °C for 24 and 48 h. The results obtained from their study showed that no significant difference for nitrogenous compounds (caseins, whey proteins and non-protein nitrogen) was found between the investigated milks. The main changes resulting from this treatment were apparent in mineral composition. For milk stored during 24 h at 4 °C, there was a decrease of 50% for calcium, 36% for phosphorus, 30% for magnesium and 40% for sodium, whereas milk kept during 48 h at 4 °C showed a decrease of 75% for calcium, 22% for phosphorus, 53% for magnesium and 50% for sodium.

In a different approach, Bugaud, Buchin, Coulon, et al., 2001, and Bugaud, Buchin, Hauwuy, & Coulon (2002) have studied the influence of pastures on the physico-chemical parameters of milk and cheese quality. In their researchers, it was pointed out that protein content of milk produced in mountain pastures ($n = 5$, 1500–1850 m) was similar to what was found with milk produced in valley pastures ($n = 5$, 850–1100 m). However, the level of fat content was found to be the lowest one in mountain pastures. Considering the Abondance semi-hard French cheese manufactured according to the traditional scheme, cheeses made with milk produced in mountain presented the highest values of most indicators of proteolysis (i.e., soluble

nitrogen (SN)/total nitrogen (TN), $\alpha_{S1} - I/\alpha_{S1} + \alpha_{S2}$ casein, etc.), while those manufactured with milk produced in valley showed the lowest ones (Bugaud, Buchin, Noël, et al., 2001).

Millán, Saavedra, Sanjuán, and Castelo (1996) have used some chemical parameters: ammonia nitrogen, non-protein nitrogen, moisture, salt and pH to discriminate 80 Spanish cheeses which were representative of 10 varieties of cheeses. By using discriminant analysis, all cheeses were classified in the same class as they were in the beginning (100%). The authors concluded that the physico-chemical parameters were suitable for discriminating cheeses according to their varieties.

In a similar approach, Pillonel, Badertscher, et al. (2005), and Pillonel, Bütikofer, et al. (2005) have found that some chemical parameters such as TN, WSN, 12% TCA soluble nitrogen (TCA-SN) and pH were promising parameters among others to discriminate Emmental cheeses manufactured during winter (110 samples) and summer (73 samples) and originating from different European countries.

Although the physico-chemical analyses are promising techniques, they are time consuming and need a lot of pollutant reagents. In addition, even though, a clear effects of production factors on gross chemical composition of the cheese are known, their exclusive attribution to the geographic origin seems to be difficult. This does not exclude that these traits are employed as valuable auxiliary criteria which can be obtained rapidly with little extra effort.

2.2. Liquid chromatographic techniques

For dairy compounds that cannot be volatilised readily, the liquid chromatograph can be used. The stationary phase consists of a finely powdered solid adsorbant packed into a thin metal column and the mobile phase consists of an eluting solvent forced through the column by a high-pressure pump. The mixture to be analysed is injected into the column and monitored by a detector. O'Shea, Uniacke-Lowe, and Fox (1996) have used reverse phase high performance liquid chromatography (RP-HPLC) to analyse retentate and permeate of the water soluble fraction (WSF) of 60 Cheddar cheeses, varying in age (mild, mature and extra-mature) and flavour quality (defective, non-defective). Poor classifications were obtained since only 33.3% and 48.3% of samples were correctly classified. Using the total concentration of free amino acids, 70% of correct classification was obtained for all the cheeses, while only 20% of mature cheeses were correctly classified. Better classifications were obtained for mild and extra-mature cheeses since 73.2% and 78.6% of them were correctly classified.

Pripp, Kieronczyk, Stepaniak, and Sørhaug (1999) have applied chemometric tools such as principal component analysis (PCA) and Hierarchical cluster analysis (HCA) to the RP-HPLC chromatograms of ethanol (70%)-soluble and ethanol-insoluble fractions and free amino acids to evaluate proteolysis in miniature Cheddar-type model cheeses made by the use of different single-strain starters. The PCA applied to the RP-HPLC chromatograms of ethanol (70%) soluble fraction from 2-month old showed two different clusters: the first one contained *Lactococcus Lactis* subsp. *Cremoris* SK11 and AM1, while the other large cluster contained all the other strains. Similar results were obtained from the insoluble fraction at the same ripening time. The same technique (PCA) was applied to the cheeses of 4 months ripening time: the results showed two groups for the 70% soluble and insoluble fractions: the first group grouped as for 2-month-old cheeses, except for strain *L. lactis* subsp. *Cremoris* Wg2. The researchers concluded that group strains can be classified according to their effects on chromatographic profiles and free amino acids. Ferreira and Caçote (2003) have used the same technique (RP-HPLC) to detect and quantify bovine, ovine and caprine milk percentages in milks and in Portuguese protected denomination cheeses. The chromatographic profiles of β-lactoglobulin and α-lactalbumin extracted from the investigated milks were very different. Additionally, different cheeses were manufactured using different proportions of bovine, caprine or ovine milk: (i) mixtures of 20% of bovine milk and 80% of ovine milk; (ii) 50% of bovine milk and 50% of caprine milk; and (iii) 50% of ovine milk and 50% of caprine milk. All these mixture milks were firstly analysed by RP-HPLC and then used to produce cheeses. As expected, different chromatographic profiles were obtained for each type of milk binary mixtures. In addition, similar chromatographic profiles were obtained for each milk mixture and the respective fresh and ripened cheeses. The authors concluded that the RP-HPLC is a very sensitive and accurate method for studying milk percentage as well as fresh and ripened cheeses made from binary mixtures of bovine, ovine or caprine raw milks.

In a similar approach, Di Cagno et al. (2003) have used RP-HPLC profiles of the ethanol-soluble and ethanol-insoluble fractions of the pH 4.6-soluble nitrogen to analyse three Italian PDO ewes' milk cheeses named Pecorino Romano, Canestrato Pugliese and Fiore Sardo. For both fractions, Fiore Sardo and Canestrato Pugliese showed a more complex peptide profile than the other cheeses.

Pripp, Rehman, McSweeney, and Fox (1999) compared three different Norwegian cheeses varieties: Jarlsberg, Norvegia and Sveitser by applying PCA to the proteolytic profile of RP-HPLC and capillary electrophoresis (CP). In their researchers, CP analysis gave better results for discriminating cheeses than RP-HPLC. The researchers concluded that the biochemical differences appeared to reflect the differences in starter and adjunct starter cultures used for the three cheese types. In a similar approach, Bara-Herczegh, Horváth-Almássy, and Örsi (2002) have applied multivariate statistical analysis to identify the indices of secondary proteolysis by using HPLC of 40 Hungarian Trappist cheeses throughout ripening as well as during storage for 28, 42, 56 and 70 days. The authors concluded that it could be possible to monitor using HPLC the signif-

icant changes of fractions of different molecular weight during the ripening time and shelf life. Others have used an amino analyzer by ion exchange chromatography on a sulphonated polystyrene column with post-column ninhydrin derivation to quantify free amino acids in Mahon cheese made with raw milk (three batches) or pasteurised milk (two batches) (Frau, Massanet, Rosselló, Simal, & Cañellas, 1997). The cheeses were analysed at 10, 60, 150 and 300 days of ripening. The authors applied PCA to the 27 amino acids and derived compounds and the obtained results allowed the discrimination of cheeses according to their ripening times and the quality of milk used for producing the cheeses.

The HPLC methodology used to analyse compounds in dairy products generally includes extractions with solvents. Sample extraction procedures are often regarded as bottlenecks in analytical methods. Moreover, classical sample preparation techniques are both time and solvent consuming, and sample handling can decrease the quality of the analytical results.

2.3. Gas chromatographic techniques

Flavour compounds in dairy products, especially in cheeses, are characteristics; consequently, the quantity and quality of flavour constituents analysed by gas chromatography can be used as an efficient tool for authenticity testing. Besides mineral salts, lactic acid, lactose, amino acid and peptides, the water soluble extract of cheeses contains volatile and non-volatile aroma compounds (Salles et al., 2000).

Terpene content and profile in milk and dairy products are influenced by feed and especially by grazed herbage. This relationship could be used to discriminate milk or cheese originating from grazing or not grazing system and to trace the geographical origin of these products, or production site. Indeed, Viallon et al. (2000) have used firstly dynamic head space to extract monoterpenes and sesquiterpenes in milk fat, and then gas chromatography to separate these compounds collected from different cows fed successively with forage containing high and low amounts of terpenes. Their researchers have shown, on the one hand, that a modification of the plant species composition of forages strongly influences the proportions and quantities of monoterpenes and sesquiterpenes in milk fat and, on the other hand, that a rapid transfer of these compounds takes place.

In a similar approach, Fernandez et al. (2003) have assessed the potential of dynamic head space–gas chromatography–mass spectrometry (DHS–GC–MS) to discriminate 35 representative milk samples produced either in highland region (Auvergne region, altitude 850–1050 m) or in lowland region of France (Brittany region, altitude 200 m). The milk samples were collected during both the grazing period (spring and summer) and the stabling period (winter). The authors have used multivariate statistical analysis, including analysis of variance and discriminant analysis to discriminate milk samples. The obtained results showed 100% correct classification of milk samples according to their geographical origin irrespective of season herd management pattern. The authors concluded that terpene compounds can provide useful fingerprints for the characterisation of dairy products according to their geographical origin and their production conditions. In a similar approach, Mounchili et al. (2005) have analysed milks from nine Holstein Friesian lactating cows that were selected from three commercial dairy farms using gas chromatography coupled with mass spectrometry/flame ionisation. The selected cows receiving their regular portion of barley-base concentrate were forage-starved from 07:00 a.m. to 07:00 p.m. Then, 500 mL of milk were collected from each of them before they were fed freshly opened round-bale grass silage. After 30 min and 3 h of feeding silage, the second (30 min samples) and the third (3 h samples) were also collected. The obtained chromatograms showed that 30 min post-feeding samples had significantly ($P < 0.05$) higher concentration of ethanol, propane-2-one, dimethyl sulphide, butane-2-one, hexanal, heptanal and octane-2,3-dione, while 3 h post-feeding samples had higher concentrations of four of the following compounds: propane-2-one, dimethyl sulphide, butane-2-one and hexanal. From the obtained results the authors concluded that feeding lactating cows with freshly opened paled silage up to 3 h before milking may give rise to objectionable flavours in milk produced by these cows. However, the above study has so far been done on extreme samples and it would be interesting to validate the relevance of this technique on representative milk samples.

The same technique has been used by Collomb et al. (2002) to quantify the fatty acid composition of 44 summer milk samples collected from three geographical sites located at different altitudes: lowlands (600–650 m), mountains (900–1210 m) and highlands (1275–2120 m). Their research pointed out that milk produced in the highlands had a smaller amount of saturated short- and medium-chain fatty acids, and more polyunsaturated fatty acids, than milk produced in the lowlands. The obtained results are in agreement with previous findings reporting that milk produced in the highlands had a lower content of saturated fatty acids with 4–16 carbon atoms and a higher content of stearic, oleic and polyunsaturated fatty acids, especially C18:2 and C18:3, compared to milks produced in the lowlands (Bosset et al., 1997).

Head space gas chromatography has also been proved to be a very accurate technique for a rapid examination of dried milk products to monitor flavour defects caused by lipid autoxidation (Ulberth & Roubicek, 1995). Indeed, the above researchers have found that fresh whole milk powder analysed with 24 h contained less than 10 $\mu g\ kg^{-1}$ hexanal, while those stored at room temperature (20–22 °C) had up to 28 $\mu g\ kg^{-1}$ of hexanal.

Regarding studies on cheeses, Cornu et al. (2005) have used DHS–GC–MS to study both the cheese making procedure of two French cheeses: Saint-Nectaire and Cantal

cheeses. The investigated cheeses were manufactured from raw and pasteurised milk from the same herd of dairy cows that had been grazed on the same days. The obtained results showed that milk pasteurisation did not induce significant changes in the terpenes profile of cheese, while significant difference was found between Cantal and Saint-Nectaire cheeses. Indeed, components such as α-pinene, β-myrcene and β-phellandrene were found to be 3, 5 and 5 times more abundant in Cantal cheeses, while tricyclene, α-phellandrene and geraniol were observed exclusively in Cantal cheeses. The authors concluded that the cheese making procedure was the most important factor inducing changes in terpene profiles, possibly due to the physico-chemical conditions applied, microbial population and the duration of cheese maturation period. Recently, the same research group have analysed 20 Emmental cheese samples using dynamic headspace gas chromatography followed by flame ionisation and mass spectrometry. The authors would like to test the potential of this technique to discriminate Swiss Emmental cheeses from French, Finnish, German and Austrian cheeses (Pillonel, Ampuero, et al., 2003). Their results could be considered as powerful since the percentage of correct classification was 90% and 91% for Swiss cheeses and the other samples grouped as one region.

Recently, the practicability and potential of comprehensive two-dimensional gas chromatography coupled to both conventional flame ionisation and time-of-flight mass spectrometric detection were compared with those of conventional one-dimensional gas chromatography for the determination of flavour compounds in butter (Adahchour, Wiewel, Ramon, Vreuls, & Brinkman, 2005). The authors reported that the two-dimensional gas chromatography was a promising and versatile technique for a rapid and wide-ranging screening of flavours and fragrances in butter.

Turkish yayik butter manufactured from goats', ewes' or cow's milk were recently analysed by gas liquid chromatography (Sağdıç, Dönmez, & Demirci, 2004). The total saturated fatty acids (%) of the yayik butter samples was found to be the highest in butter made from goats' milk (73.88 ± 0.62), while those produced with cows' milk had the lowest one (67.06 ± 0.30). However, the total monounsaturated fatty acid contents of the butters produced from cows' milk presented the highest one (24.01 ± 0.84), while butter made from the ewes' milk had the lowest content (20.22 ± 0.76).

For authentication of dairy products, headspace analysis would be interesting when distinct volatile compounds either produced by bacteria or, probably only in exceptional cases, incorporated from feed could be related to certain regions. Distinct proportions of such compounds could also be indicative. However, the bacterial population is continuously changing over time, and this might affect these key volatile compounds and their proportion. Therefore, a permanent adaptation of the target values might be necessary. Particularly promising is the analysis of volatile compounds for the determination of the geographic origin. It has to be emphasised that, in this case, these compounds code for the site where the processing is done and not for the origin of the raw product, as these sites are not necessarily identical. Processing would add flavours, e.g., from bacteria, smoke or air, which do not only characterise the specific product but also may be specific for the determination of the geographic origin of such products.

2.4. Rheological techniques

The rheological characterisation of dairy products is important as a means of determining body and texture for quality and identity as a function of composition, processing techniques and storage conditions. Commonly, fluid milk and cream are considered examples of liquids, and hard cheeses as an example of a solid. But concentrated milk, yogurt, butter, ice cream and several types of cheese can shown an intermediate behaviour as viscoelastic (Shoemaker, Nantz, Bonnans, & Noble, 1992). Texture properties of dairy products play a key role in consumer acceptance of cheese (Ak & Gunasekaran, 1992; Bugaud, Buchin, Coulon, et al., 2001; Bugaud, Buchin, Noël, et al., 2001; Jaros, Ginzinger, Tschager, Mayer, & Rhom, 1997).

Most of the published research papers about milk discuss the influence of three important variables of food processing on its rheological behaviour which are thermal treatments, milk composition and temperature storage. Gryzowska and Tuszynsky (1973) reported that temperatures less than 50 °C had no effect on the viscosity of skim milk, while temperatures above 60 °C caused an increase in the viscosity, even when applied for a short time. The obtained results were partially in agreement with those of Jeurnink and DeKruif (1993) who found that the viscosity of skim milk increased after heat treatments at temperature above 70 °C. Other researchers have monitored the changes that occurred during storage of different UHT milk samples (Swartzel, Hamann, & Hansen, 1980). The above authors related the gelation process to fat level, storage temperature and storage time.

Quality attributes such as texture, consistency, firmness, curd tension and flow properties of yogurt have been satisfactory measured and have allowed improvement of yogurt quality for consumer satisfaction. Because the network structure of yogurt plays an important role in the viscoelasticity of this dairy product, dynamic testing rheology are finding an excellent field of application in analysing the viscoelastic nature of yogurt affected by process variables and measuring conditions (Biliaderis, Khan, & Blank, 1992; Rhom & Kovac, 1994; Rönnegård & Dejmek, 1993).

Vlahopoulou and Bell (1999) have used dynamic tests to identify the viscoelastic differences between ropy and non-ropy yogurts. The authors reported that the storage and loss moduli of the ropy gels were lower than those corresponding to non-ropy yogurts. Recently the effects of different levels of water and salt on the rheological

properties and serum separation during storage of traditionally manufactured yogurt have been investigated by Köksoy and Kiliç (2003). In their studies, yoghurt samples were prepared by the addition of water at levels of 30 or 50 g 100 g^{-1} and salt at levels of 0, 0.5 or 1 g 100 g^{-1} and stored at 4 °C. Their findings showed that all samples exhibited non-Newtonian behaviour. In addition, the consistency coefficient decreased and the flow behaviour index increased dramatically with increasing levels of water and salt.

The texture of butter has also been evaluated by five methods: penetration, cutting, extrusion, compression and spreadability. Of these five instrumental methods, compression and extrusion have been the most used methods because of their high reproducibility, precision and simplicity (Dixon & Parekh, 1979).

Recently, Raphaelides, Antoniou, Vasilliadou, Georgaki, and Gravanis (2006) have used dynamic testing rheology to monitor changes occurring in Halloumi cheeses throughout ripening, manufactured either from cows' milk or ewes' milk. The results obtained from their study showed that before the starting of ripening stage, bovine Halloumi was more rigid than the ovine Halloumi. In addition, the elasticity modulus of ovine Halloumi remains practically unchanged after an aging period of 15 days, while those produced from bovine Halloumi requires an aging period of 30 days to reach a constant elastic modulus value. After 30 days the two Halloumi types presented the same values of storage modulus.

2.5. Sensory analysis

Sensory analysis of food involves the measurement, interpretation and understanding of human responses to the properties of food perceived by the senses such as sight, smell, taste, touch and hearing (Martens, 1999; Martens & Martens, 2001). It is important to have a quantitative means for assessing sensory properties in a reasonable way to enable the food industry to rapidly respond to the changing demands of both consumers and the market (Martens, 1999). Aroma intensity and flavour are among the most important properties for the consumer and numerous studies have been performed in attempts to find correlations between sensory qualities and objective instrumental measurements.

The quality of dairy products can be measured directly by sensory methods. Indeed, Wormbs et al. (2004) have compared different processing conditions on the sensory characteristics of milk. The authors reported that the process did not influence any sensory attributes significantly. Horimoto and Nakai (1998) have also used sensory analysis to detect off-flavours of milk subjected to light-induced, cooked and heated flavour milk. Light induced milk was produced by exposing the milk to 40-W cool white fluorescent lamps for 12 h at 7 °C, while cooked flavour was produced by heating milk at 75 °C for 1 min. Heated flavour milk was produced by heating milk for 15 min at 95 °C. Sensory analysis for aroma and taste indicated a significant difference ($P \leqslant 0.01$). In order to extract information from the sensory data sets, the above mentioned authors have used PCA. The authors reported that although, the heated milk samples were different from the other milk samples, PCA was not considered very successful in differentiating all the investigated milk samples. Indeed, most samples were misclassified by smelling.

Other authors have substituted the addition of non-fat dry milk with a protein standardisation by ultrafiltration (Quinones, Barbano, & Phillips, 1997, 1998). By mixing retentate and permeate from ultrafiltration, they produced milks with a true protein content in the range of 0.9–4.8%. In the experiments, the milks varied in fat content from 0.13% to 3.3%. Their results showed that the sensory texture and appearance descriptors were affected by both the protein standardisation and the fat content. The increase in protein content gave a whiter appearance and texture properties like thickness, mouthcoating and residual mouthcoating increased with higher protein content. Larger differences in sensory properties between low and high protein content were observed in samples with low fat content.

Lebecque, Laguet, Devaux, and Dufour (2001) have studied 25 Salers PDO cheeses of 3.5 months ripening time using sensory analysis. Eight attributes for texture profiling that have been chosen by the panel have been considered in their study. A 0–9 scale was used to evaluate the intensity of the attributes. The authors applied ANOVA to the sensory data. Significant differences were observed between cheeses allowing to class them in five groups. The authors have then applied PCA to the sensory data sets. Comparing the rheological and sensory data sets, the authors observed that cheeses which had high scores for humidity, adhesivity and elasticity, had the lowest stress values at 20% compression.

Recently, Ritvanen et al. (2005) have related sensory attributes to consumer liking in cheeses with different fat contents. In their study, 44 reduced and full fat cheese types (Edam, Emmental and Havarti) have been studied. The authors reported that differences in flavour and texture of full fat and reduced fat cheeses were observed. Also there were differences in attributes affecting acceptance of cheeses with different fat content. Some sensory differences between reduced and full fat cheeses were acceptable.

Although the importance of the above mentioned techniques is unquestionable, these methods are hardly possible to implement for practical use when many samples need to be analysed on-line or at-line in the food industry. For practical reasons, the quality criteria of such products should be easily measurable. Simple and rapid methods are needed for quality control and for screening many samples in a research or development situation.

Spectroscopy in the ultraviolet (UV), visible (VIS) and infrared (IR) regions of the electromagnetic spectrum is becoming a more and more attractive analytical technique for measuring quality parameters in food with decreasing

instrument prices and improved equipment and chemometric tools. The main advantages of using spectroscopic techniques are rapid sample data acquisition, the possibility of simultaneous determination of several quality parameters and the ability to replace expensive and time consuming reference techniques.

3. Spectroscopic techniques used for the determination of the quality and/or authenticity of dairy products

Discrimination between different dairy products and confirmation of the authenticity of another are basically the same analytical problem. The basic assumption behind the application of spectroscopic techniques to this problem relies on the generation of the "fingerprint" of foods. An individual dairy product with a given chemical composition exposed to a light source will have a characteristic spectrum, which is a result of the absorption by various chemical constituents. Because the exact composition of any natural material varies some what, depending on variety, season, location, etc., there exists a range of typical spectra for this material. Therefore, what is needed is a library of representative spectra, to which the spectrum of a test material may be compared in order to establish its quality or authenticity. Given the nature of the data sets involved, multivariate chemometric techniques are required and a number of commercial software packages can be used.

Since the principle of the above mentioned spectroscopic techniques and chemometric tools have been reviewed in detail by Karoui et al. (2003), this study will only focus on the application of these techniques for the determination of the quality and/or identity of dairy products. The advantages and disadvantages of each technique are reported in Table 1.

3.1. Near infrared spectroscopy

The NIR spectroscopy is widely used in the food industry as a quality control tool. It was utilised for monitoring rennet coagulation in cow's milk (Laporte, Martel, & Paquin, 1998; O'Callaghana, O'Donnellb, & Paynec, 2000), reconstituted skim milk powder (Giardina, Sinelli, Cattaneo, & Giangiacomo, 2004) and the modifications that occurred in delactosated milk during shelf-life (Giardina, Cattaneo, & Barzaghi, 2003). It has also been extensively used to determine the physico-chemical parameters of cheeses (Adamopoulos, Goula, & Petropakis, 2001; Mazerolles, Duboz, & Hugot, 2000; Purnomoadi, Batajoo, Ueda, & Terada, 1999) and butter (Hermida, Gonzalez, Sanchez, & Rodriguez-Oterob, 2001).

Burns and Ciurczak (1992) have used the NIR to study the ripeness age of different varieties of Dutch cheeses (Edam and Gouda), with the purpose to classify them into different aging groups as young (minimum 28 days), young-matured (minimum 2 months), matured (minimum 4 months) and extra matured (minimum 7 months). The results obtained from their study were promising showing a correlation coefficient of 0.92 and a standard error of calibration values (SEC) of 28 days for samples of ripeness interval between 25 and 412 days. The statistical data were more valuable when testing specific calibrations models of subgroups of reduced samples with ripeness intervals of 160 days, reporting a correlation coefficient and SEC of 0.96 and 11, respectively. Recently, the NIR-Fourier transform infrared (FTIR) has been applied on the Italian fresh cheese Crescenza (Cattaneo, Giardina, Sinelli, Riva, & Giangiacomo, 2005). The authors found a successful discrimination between cheeses according to their storage time using the PCA. The authors reported that the map defined by the first two PCs was sufficient to provide three well-separated groups corresponding to the fresh (0–6 days), aged (8–10 days) and old (storage time >10 days). The authors concluded that NIR could be a suitable technique for the evaluation of the shelf-life in which Crescenza freshness is maintained. However, no interpretation at the molecular level was provided by the above mentioned study.

The potential of the NIR diffuse reflection in combination with multivariate chemometrics for discriminating Emmental cheeses of various geographic origins has been investigated by Pillonel, Luginbühl, et al. (2003). The linear discriminant analysis (LDA) applied on the PCA scores allowed a classification of the investigated cheeses since 100% correct classification was obtained for the Emmental cheeses produced in the six European regions. However with only 20 samples, the models suffer from over-fitting and for consequent were not very robust against the inclusion or exclusion of samples. Further analyses with more samples should be necessary to substantiate these models. This would allow including more variability of the chemical properties and thus developing general mathematical models for better accuracy of the NIR technique. To request with this comply, Karoui, Bosset, Mazerolles, Kulmyrzaev,

Table 1
Advantages and disadvantages of the near infrared (NIR), Fourier transform infrared (FT-IR) fluorescence and nuclear magnetic resonance (NMR) for measuring undiluted milk

Spectroscopic techniques	Sensitivity	Information content	Absence of interferences	Repeatability	Absence of light scatter
NIR	**	**	*	**	**
FT-IR	***	***	*	***	**
Fluorescence	***	*	***	**	*
NMR	**	***	*	**	***

Symbols. Sensitivity and information content: *, low; ***, high; absence of interferences: *, many interferences; ***, few interferences; repeatability: *, poor; ***, good; absence of light scatter: *, severe light scatter; ***, no light scatter.

and Dufour (2005), Karoui, Dufour, et al. (2005), and Karoui, Martin, and Dufour (2005) attempted to discriminate 91 Emmental cheeses produced during winter period and originating from different European countries. The NIR spectra were recorded in the wavelength range of 1000–2500 nm. The authors have used FDA to discriminate cheeses according to their geographical origin. Correct classification of 89% and 86.8% was observed for the calibration and validation sets, respectively. The authors concluded that NIR delivered fingerprints allowed a fairly good recognition of the geographic origin of the Emmental cheeses. However, the above researchers have applied the FDA on the first 20 PCs of the PCA performed on NIR spectra which could increase the rate of correct classification. Further investigation that should consider a few number of PCs (5) are needed before claiming the potential of this technique to determine the geographic origin of cheeses.

Recently, Filho and Volery (2005) have used NIR to quantify total solid contents of fresh cheeses which present low, medium and high solid contents. The plot of solid contents determined by the NIR allowed a good discrimination of cheeses according to their solid contents. In fact, all samples found in the top cluster along the regression line belong to those which had the highest solid contents, while those in the bottom cluster contain cheeses which exhibited the lowest solid contents. In a similar approach, Blaquez, Downey, O'Donnell, O'Callaghan, and Howard (2004) have used NIR reflectance spectroscopy to predict moisture, fat and inorganic salts in processed cheeses. The above mentioned authors claimed that the results obtained are sufficiently accurate to recommend this technique for off-line quality assessment of processed cheese.

Recently, the potential of NIR to predict maturity and sensory attributes of 24 Cheddar cheeses produced using five renneting enzymes and stored at 4 °C for up to 9 months has been investigated (Downey et al., 2005). The authors showed that NIR spectroscopy has demonstrated the ability to predict cheese maturity and several sensory attributes namely crumbly, rubbery, chewy, mouthcoating and massforming with sufficient accuracy to be industrially useful. The authors noticed that the obtained models merit evaluation on commercially-produced cheeses. However, due to the low number of cheeses investigated in their study, the obtained results should be regarded with precaution until validation on a large number of cheeses.

In a similar approach, Blaquez et al. (2006) have used NIR reflectance spectroscopy in the range of 750–2498 nm to record spectra on cheeses which were stored for 2 and 4 weeks at 4 °C. Nine sensory properties, five instrumental parameters and cheese meltability were determined on cheese samples. The authors reported that sensory attributes and instrumental texture measurements were predicted with sufficient accuracy. The authors recommended the use of NIR reflectance spectroscopy for routine quality assessment of processed cheese.

The most interesting advantage of the NIR is its ability to use longer path lengths than MIR, and that the optical equipment used is much simpler. Indeed, optical fibers made from quartz glass can be used. The main disadvantage is the low sensitivity of the signal compared to what is compared from for example MIR. Thus, low concentration components cannot be expected to be determined by the use of NIR. Another disadvantage is the superposition of many different overtone and combination bands in the NIR region which causes a very low structural selectivity for NIR spectra compared to the MIR where many fundamentals can usually be observed in isolated positions. The many overlapping signals give rise to a spectrum with broad peaks, making it difficult to interpret compared to the conventional MIR spectrum. Starting from these explanations, it appears very difficult to study the secondary structure of proteins in dairy product using NIR.

3.2. Mid-infrared spectroscopy

At present, the MIR spectroscopy is the most preferred method for milk and dairy product analysis. This is due to the fact that almost every chemical substance (apart from some salts and very simple chemical compounds) has its own distinctive spectrum. Only substances occurring in very low concentrations can be difficult to determine, as the noise level of the method might be encountered in such a case.

The ability of MIR to determine the geographic origin of dairy products has been investigated by several authors. The analyses focused on the measurement in the 4000–900 cm^{-1} spectral region. Within this region, three spectral regions were used: 3000–2800, 1700–1500 and 1500–900 cm^{-1}. These spectral regions were selected because they are rich in information while the inclusion of the other spectral data (i.e., 4000–3000 and 2800–1700 cm^{-1}) might interfere with the extraction of useful information.The potential of the MIR to monitor the ripening time of 16 experimental semi hard cheeses at four different times of ripening of 1, 21, 51 and 81 days has been investigated by several authors (Mazerolles et al., 2001; Mazerolles, Devaux, Dufour, Qannari, & Courcoux, 2002). Following the PCA performed in the 1700–1500 cm^{-1} spectral region recorded on cheeses at different ripening time, the pattern of each component was examined. The authors clearly demonstrated the potential of the PCA to facilitate discrimination between cheeses. In addition, the authors have provided a rational molecular basis for the observed discrimination by a thorough discussion of the spectral patterns and their relation to known absorptions due to amide I and II. The researchers reported that one or several continuous phenomena that occurred during ripening were detected at the level of amide I and II absorption bands.

In a similar approach, Karoui et al. (2005) reported that the MIR provided relevant information on the geographical origin of both experimental Jura hard cheeses and Swiss Gruyère and l'Etivaz PDO cheeses. In addition, a good dis-

crimination of 12 experimental French hard cheeses was achieved using identical and controlled cheese making conditions with milks originating from three different regions in Jura (France) by using the 1700–1500 cm^{-1} spectral region (Fig. 1). Indeed, cheeses made with milk produced from Oussières region were located of the far right, whereas the other cheeses were observed on the far left according to discriminant factor 1 accounting for 76% of the total variance. The discriminant factor 2 differentiates between cheeses made with milk produced in Tourmont region from those manufactured with milk produced in Chilly region.

Using a similar approach, the same researchers satisfied to discriminate 25 Swiss Gruyère PDO cheeses manufactured at three different altitudes-l'Etivaz, highland and lowland regions. The authors concluded that MIR was an accurate technique for the discrimination between different brands of French Emmental cheeses, which was in agreement with the findings of Picque, Cattenoz, and Corrieu (2002).

Recently, the potential of the MIR for determining the geographic origin of Emmental cheeses manufactured during winter and summer times has also been investigated (Karoui et al., 2004b; Karoui, Bosset, et al., 2005; Karoui, Dufour, et al., 2005; Karoui, Martin, & Dufour, 2005). The authors reported a successful discrimination between different kinds of commercially-produced Emmental cheeses by using the 1500–900 cm^{-1} since 96.7% and 96.7% of the calibration and validation samples was obtained. Although, the FDA has been performed on the first 20 PCs, such rates are practically useful and merit evaluation on commercially-produced cheeses.

The FTIR has been used to determine the quality of a typical "Pasta Filata" cheese (Giardina, Cattaneo, Giangiacomo, 2003). These latter have analysed 112 cheese samples coated with biodegradable wax or paraffin at 90 and 120 days of shelf life. The authors found that the 1100–1035 and 1720–1690 cm^{-1} spectral regions can be used to classify cheese samples according to the days of shelf life and the type of coating.

The main advantage of this technique as reported in Table 1 is its high repeatability and very easy sampling. The main disadvantage on aqueous samples is the strong absorptions of water. The O–H bending band (at $\approx$1650 cm^{-1}) effectively obscures potentially useful absorptions from protein. Another disadvantage of the MIR is that infrared radiation cannot be transmitted through quartz glass and that the weak infrared radiation only measures the surface of a sample or can only be transmitted through very small amounts of sample, which complicates sample presentation.

3.3. Front face fluorescence spectroscopy

Dairy products contain a lot of important intrinsic fluorophores, which represent the most important area of fluorescence spectroscopy. They include the aromatic amino-acids – tryptophan, tyrosine and phenylalanine in proteins, vitamin A and B_2, NADH derivatives of pyridoxal and chlorophyll, some nucleotides and numerous other compounds that can be found at a low or very low concentration in food.

Dufour and Riaublanc (1997) have investigated the potential of FFFS to discriminate between raw, heated (70 °C during 20 min), homogenised and homogenised + heated milks. Following the PCA applied on the tryptophan and vitamin A fluorescence spectra, a good discrimination between samples as a function of homogenisation has been found according to the PC1, whereas the PC2 discriminated samples as a function of heat treatment. From the results obtained, the authors concluded that the treatment applied to the milks induced specific modifications in the shape of the fluorescence spectra.

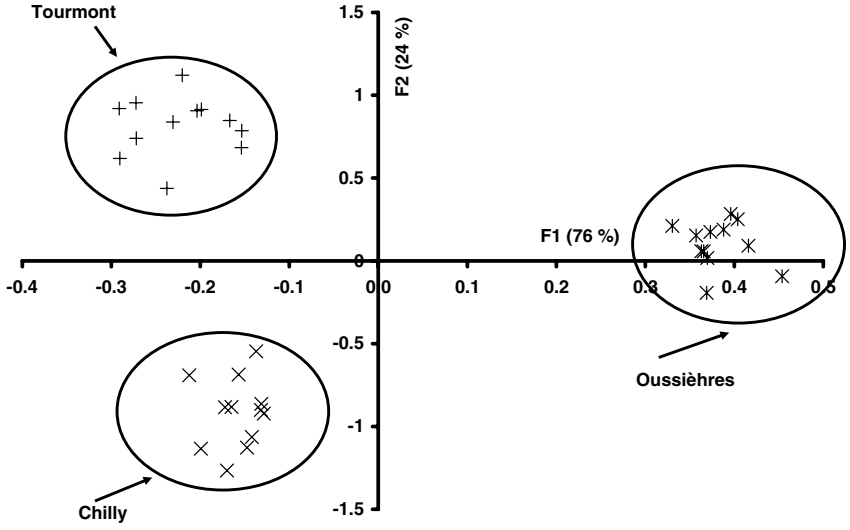

Fig. 1. Discriminant analysis similarity map determined by discriminant factors 1 and 2 for mid-infrared spectra (1700–1500 cm^{-1}) for hard cheeses produced in three different regions in Jura (France).

These earlier findings were confirmed by Kulmyrzaev, Levieux, and Dufour (2005). In their research, emission and excitation spectra of different intrinsic probes (i.e., aromatic amino acids, NADH and FADH) were used to evaluate changes in milk following thermal treatments in the range of 57–72 °C for 0.5 up to 30 min. The PCA applied on the normalised spectra allowed a good discrimination of milk samples subjected to different temperatures and times of thermal treatment. Recently, the potential of FFFS to monitor the development of Maillard browning in milk during thermal processing has been assessed (Schamberger & Labuza, 2006). The authors reported that fluorescence levels were found to increase with higher time temperature combinations. The fluorescence spectra of milks which were processed for 5, 15, 20, 25 and 30 s in 5 °C increments from 110 to 140 °C were correlated with hydroxylmethylfurfural (HMF). The R^2 values of 0.95 and more were found continuously throughout the emission wavelength range of 394–447 nm. The authors concluded that FFFS is a very promising method for measuring Maillard browning in milk and can also be used to as an online instrument for monitoring and control of thermal processing of milk.

Most of the previous studies regarding the potential of FFFS to discriminate between milks have been done so far on extreme and controlled samples. Milk products from mountain areas are reputed to have specific organoleptic and nutritional qualities (Bosset et al., 1999; Coulon & Priolo, 2002; Renou et al., 2004). The tracing of milk production sites is essential to avoid fraud. Thus, it would be interesting to validate the accuracy of FFFS to discriminate between different representative milk samples collected from highland and lowland regions. Recently, a total of 40 milk samples; 8 milks produced in lowland (430–480 m), 16 milks produced in mid-mountain (720–860 m) and 16 others produced in mountain (1070–1150 m) areas of Haute-Loire department (France) at key periods of animals feeding were analysed by FFFS (Karoui, Martin, & Dufour, 2005). Tryptophan fluorescence spectra, aromatic amino acids + nucleic acid (AAA + NA) spectra and riboflavin spectra were recorded directly on these milks with excitation wavelengths set at 290, 250 and 380 nm, respectively. The excitation spectra of vitamin A were also recorded with the emission wavelength set at 410 nm. The authors applied the FDA to the first 5 PCs of the PCA performed on each of the investigated probe. In this case, a trend to a good separation between milks as a function of their altitude was observed. The best results were obtained with AAA + NA fluorescence spectra since 81.5% and 76.9% of the calibration and validation spectra, respectively, were correctly classified. However, the authors observed some misclassification of milks produced in mid mountain with the other milks. This discrepancy has been attributed to the heterogeneity of the location of the farms in the mid-mountain regions which varies in altitude between 720 and 860 m. Whereas, the altitude of the farms of the lowlands and mountains were more homogeneous and were located at about 460 and 1100 m, respectively.

Milk coagulation is the primary step in the development of texture of most dairy products. Herbert, Riaublanc, Bouchet, Gallant, and Dufour (1999) have used FFFS to monitor milk coagulation at the molecular level. Three different coagulation processes have been studied: the glucono-δ-lactone (GDL), rennet-induced coagulation system and a mix system of GDL + rennet-induced coagulation system. Emission fluorescence spectra of the protein tryptophanyl residues were recorded for each system during the milk coagulation kinetics. The PCA applied to the collection of normalised fluorescence spectral data of the three systems showed that FFFS allowed detection of structural changes in casein micelles during coagulation and discrimination of different dynamics of the three coagulation systems. The authors concluded that FFFS allowed the investigation of network structure and molecular interactions during milk coagulation.

Miquel Becker, Christensen, Frederiksen, and Haugaard (2003) have monitored the effect of both packaging (polylactate and polystyrene) and light on the oxidation of yogurt during storage (0, 7, 14, 21, 28 and 35 days) using FFFS. Regarding yogurt samples stored during 35 days at light, tryptophan seems to be present, while the riboflavin signal seems considerably decreased. The authors observed a degradation of riboflavin when the samples were exposed to the light. This degradation was found to be higher in yogurt samples packaged in polystyrene than those packaged in polylactate. In a similar approach, Wold, Jørgensen, and Lundby (2002) have demonstrated the potential of FFFS to assess the oxidation of different dairy products such as Swiss cheese, cream cheese and sour cream. To detect changes in fluorescence spectra, all products were stored under different conditions at 4 °C: light with exposure to air, light with no exposure to air, dark with exposure to air and dark without exposure to air. The obtained results showed a significant decrease in the fluorescence intensity at approximately 525 nm and a corresponding increase in the 415–490 nm spectral region. Variation in two smaller peaks located around 520 and 630 nm was related to the interaction effect between exposure to light and air. The authors ascribed these changes in the fluorescence spectra to the photodegradation of riboflavin and lipid oxidation.

The texture of cheese at both macroscopic and molecular levels are influenced by many factors including milk origin, milk treatment, type and amount of starter added (Baer, Ryba, & Casey, 1997), manufacture conditions and ripening time (Pillonel et al., 2002) and temperature. The climate, geology, forage and breed itself influence the milk quality, while local regional or national traditions influence the cheese making (Pillonel, Luginbühl, et al., 2003).

The potential of FFFS to discriminate between eight groups of soft cheeses has been evaluated by Herbert et al. (2000). In their research, the authors have used tryp-

tophan (excitation 290 nm) and vitamin A (emission 410 nm) spectra to discriminate the soft cheeses as a function of their ripening time and/or cheese-making procedure. The above mentioned paper provided a rational molecular basis for the discrimination of cheeses. The authors reported that the environment of tryptophan residues was relatively more hydrophilic for the old cheeses than for those at the young stage. This phenomenon was attributed to the partial proteolysis of caseins resulting in an increase of tryptophan exposure to the solvent.

In order to test the accuracy of FFFS for differentiating between the eight soft cheeses, the authors applied FDA to the most relevant PCs. The results obtained from their study showed that a better classification was obtained with vitamin A spectra (96% and 93% for the calibration and validation samples, respectively) than with tryptophan spectra (95% and 92% for the calibration and validation samples, respectively). However, in their investigations, the authors have used only parts taken from the centre of cheese, which constituted limited interpretation in the case of soft cheese. Indeed, protein breakdown, lipolysis, pH, etc. are significantly different between the surface and the centre of soft ripened cheeses.

Recently, the matrix structures of three different retailed soft cheeses (M1, M2 and M3) with different manufacturing processes were studied from the surface to the centre of the cheese using FFFS (Karoui & Dufour, 2003). The PCA applied on the tryptophan fluorescence spectra recorded on each cheese variety showed a good discrimination of cheese samples as a function of their location. In addition, the authors concluded that the tryptophan fluorescence spectra were larger for external zone than those of central zone. The environment of tryptophan residues at the molecular level was found to be more heterogeneous in the surface samples than in the centre samples. The authors attributed these spectroscopic differences to the changes in the extent and type of protein–protein interactions in the protein network due to ripening. The interactions modified at the surface of cheeses, resulted from an increase in the capacity of water sorption by caseins (Ruegg & Blanc, 1976). Karoui and Dufour (2003) reported that spectra reflect changes in the environment of the fluorophores and are indicative of structural modifications of the protein network during ripening.

In a similar approach, Karoui, Bosset, et al. (2005) attempted to discriminate 25 Gruyère and PDO cheeses. The FDA was applied separately on the emission spectra following excitation at 250 and 290 nm and excitation spectra following emission at 410 nm. Hundred per cent (100%) correct classification was obtained from the emission and excitation spectra. The authors concluded that FFFS could be an accuracy technique for the determination of the geographic origin of cheeses. These findings was fully confirmed on Emmental cheeses providing from different geographic origin and manufactured during both winter and summer times (Karoui et al., 2004b; Karoui, Bosset, et al., 2005; Karoui, Dufour, et al., 2005; Karoui, Martin, & Dufour, 2005). Following the PCA performed on tryptophan normalised spectral data recorded on Emmental cheeses produced during winter time, Karoui, Bosset, et al. (2005), Karoui, Dufour, et al. (2005), and Karoui, Martin, and Dufour (2005) reported that tryptophan fluorescence spectra allowed discrimination of cheeses according to the treatment applied to the milk used for manufacturing Emmental cheeses. These results were fully confirmed by earlier findings reporting that the tryptophan fluorescence spectra of raw and pasteurised milks were different (Dufour & Riaublanc, 1997).

In order to assess the ability of FFFS to discriminate between cheeses made from raw milk and those produced with thermised milk, the same researchers performed PCA on a sub-data set containing 90 spectra recorded on 30 French Emmental cheeses made from raw milk ($n_1 = 15$) or thermised milk ($n_2 = 15$). A good discrimination of these cheeses as a function of the treatment applied to the milk was observed (Fig. 2). Indeed, according to PC3 accounting for 1.2% of the total variance, cheeses produced with raw milk exhibited mostly positive score values, whereas those made with thermised milk had negative values.

The authors reported that the obtained results supported earlier findings using destructive techniques (Lau, Barbano, & Rasmussen, 1991) reporting that the level of proteolysis in Cheddar cheeses from heated milk was lower than that in Cheddar cheeses made from raw milk.

It is worth noting that the emission spectrum is highly dependent on the excitation wavelength, both an excitation and an emission spectrum can be obtained. Thus, the advantage of fluorescence spectroscopy is the absence of interferences and that higher-order data can be obtained from it. However, the most severe disadvantage is the strong dependence of light scatter, and there are no means for making mathematical corrections, because no information on the amount of scatter is contained in the spectrum.

3.4. Stable isotope

The ratios of stable isotopes (either given as proportions or as an excess, δ, of the respective rare isotope compared with its natural occurrence) provide an interesting analytical tool to confirm quality and/or identity of dairy products as there are sometimes region-specific patterns in environmental isotopic ratios (soil, water). Similar to the trace elements, isotopes are incorporated in local feeds and in the body of the animals. Therefore, these ratios may be specific for those areas. The ratios of hydrogen (H/D) and oxygen ($^{16}O/^{18}O$) isotopes in body tissues are primarily influenced by drinking water. Isotopic ratios of H, C, N, S and Sr ($^{12}C/^{13}C$, $^{14}N/^{15}N$, $^{32}S/^{34}S$, $^{86}Sr/^{87}Sr$) are more indicative of soil and feed origin (Pillonel, Ampuero, et al., 2003; Pillonel, Badertscher, et al., 2003; Pillonel, Luginbühl, et al., 2003).

Attempts utilising the isotopic ratios of H, O, N and S have been applied to determine the identity of dairy prod-

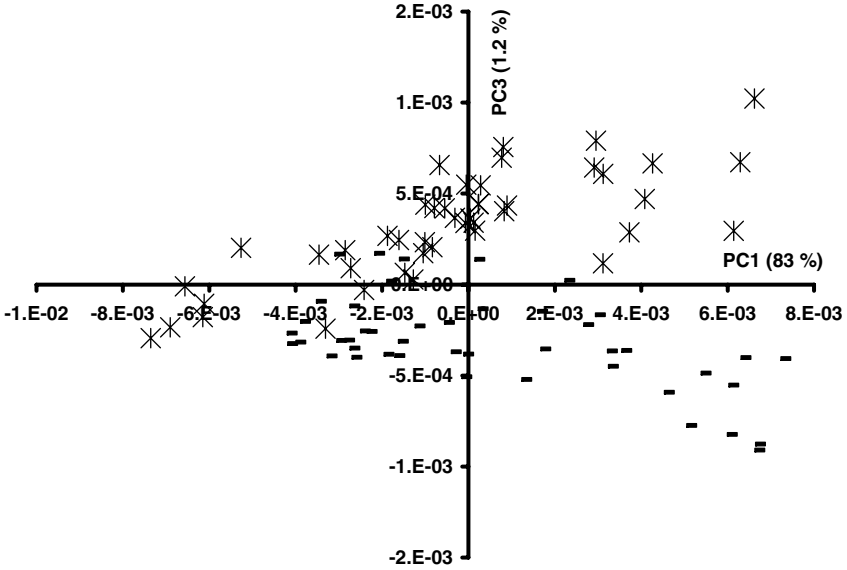

Fig. 2. Principal component analysis similarity map determined by principal components 1 (PC1) and 2 (PC2) for the tryptophan spectra of French Emmental cheeses made from raw (✳) and thermised (▬) milks.

ucts (Fortunato et al., 2004; Manca et al., 2001; Pillonel, Ampuero, et al., 2003; Pillonel, Badertscher, et al., 2003; Pillonel, Luginbühl, et al., 2003; Rossmann et al., 2000). On the basis of the O and H isotopic ratios, it was possible to differentiate between milks produced in plain (altitude 200 m) and those produced in mountain (altitude 1100 m) (Renou et al., 2004). Their research showed that milk enrichments differed significantly between sites for both ^{18}O and ^{2}H. On the plain, the ^{18}O enrichments were significantly higher for grazing cows than those fed on maize silage or hay. However, for the latter two diets, no significant differences were observed in $\delta^{18}O$ or $\delta^{2}H$. The obtained results were found to be different for milks produced in mountain, since the ^{18}O and ^{2}H enrichments both varied between cows fed on grass, grass silage or hay. Manca et al. (2001) have applied PCA to the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ of casein and a good discrimination was found according to the place of origin of cheeses. These findings were fully supported, recently, by Pillonel, Ampuero, et al. (2003), Pillonel, Badertscher, et al. (2003), and Pillonel, Luginbühl, et al. (2003) who attempted to discriminate European Emmental cheeses using different stable isotope ratios. In their study, the authors observed that cheeses from Finland, Bretagne and Savoie were well separated using $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, $\delta^{87}Sr$-values. However, cheeses from Switzerland, Allgäu and Vorarlberg were found to be similar. From the obtained results, the authors concluded that analysing stable isotope ratios appeared to be a promising method to obtain information about the geographic origin of milks and cheeses.

The isotopic ratios of H and O, depending on the amount of drinking water consumed, cannot be easily falsified or masked by feeding diet ingredients from an origin outside of the region. Additionally, a method based on the properties of drinking water is not influenced by grazing versus feeding in a barn. In turn, the isotopic ratios of C and N give some indication of the type of diet fed, particularly when the diet differs in the proportions of C_3 and C_4 plants. The isotopic ratios of C and N are often also characteristic for production systems and feeding intensity (increasing maize proportions with intensive fattening of cattle) and the isotopic signature of previous feeding seems to persist (Renou et al., 2004). Conclusions on, for example, the proportion of maize in the diet could be helpful to confirm or disprove claims of a certain regional origin, but only when a certain type of feeding is very common in a certain area.

Rossmann et al. (2000) have used stable isotope analysis to determine the geographic origin of butter originating from alpine region, western Germany, The Netherlands, northern and eastern Germany, Denmark, eastern European and Scandinavian countries, western Europe, and other parts of the world such the USA and New Zealand. The authors satisfied to discriminate butter using discriminant analysis on stable isotope analysis for C, N, O, S and Sr together. The authors concluded that this technique could be a very potent tool with which to solve the problem of butter origin assignment.

However, the stable isotope approach also has some important constraints. Conclusions made from results using the stable isotope must be based on uniform environment features (e.g. climate, altitude, distance from oceans) allowing few or no differences in isotopic ratios of the dairy products. Therefore, dairy products from animals originating from different, but climatically or geologically similar areas might have an identical isotopic signature. In addition, Ritz et al. (2005) demonstrated that the breed of cows can influence the isotopic enrichment of milk, even in circumstances where the food and water consumed are similar. Another disadvantage of analysing stable isotopes is

the time-consuming and expensive preparation of samples for some elements and the high costs of the analytical equipment.

3.5. Nuclear magnetic resonance

NMR is a versatile spectroscopic technique for studying opaque heterogeneous samples, which has already proved to have a number of useful applications in dairy research (Duce, Amin, Horsfield, Tyszka, & Hall, 1995). Some processes such as pressure, heating or changes in pH alter the milk protein conformation and/or the aggregation state. Both aspects can be studied by NMR. Indeed, the evolution of the unfolding processes of whey proteins, induced by heat and foaming have been followed by using ^{1}H NMR in combination with deuterium exchange reactions. The conformational changes occurring in β-lactoglobulin when heated at pH 2 and 7.4 have been studied (Belloque & Smith, 1998b). The authors reported that at pH 2, much of the structure is preserved and two-dimensional spectra can be obtained, while at 55 °C strand E and the A–B loop unfolded. Strand A became partially flexible at 55 °C and lost the protective action of the α-helix at 75 °C, which became unfolded. In another study, the same research group has investigated the conformational behavior of β-lactoglobulin during foaming (Belloque & Smith, 1998a). The authors have monitored the structural events that occurred during the foaming of β-lactoglobulin. They related the early stage of foaming to flexible structures that were present in the native protein. Indeed, the authors reported that higher stability of the foam was attributed to a higher degree of conformational flexibility.

Recently, NMR has been used to determine the effect of formulation on ice cream structure (Lucas, Le Ray, Barey, & Mariette, 2005; Lucas, Wagener, Barey, & Mariette, 2005). The researchers demonstrated the effect of nature of protein milk on the behaviour of NMR signal. Indeed, very little effect has been observed when the aqueous phase contains serum protein powder. However, when the aqueous phase contain milk protein, the addition of stabilises modified both the relaxation time and relative intensities (Lucas, Wagener, et al., 2005). In another study, the authors have interested to determine the effect of the nature fats, protein and emulsifiers on the behaviour of fats (Lucas, Le Ray, et al., 2005). The most significant factor was found to be the nature type fat. Indeed, the relaxation time of fat milk crystals were between 220 and 260 ms, while those from vegetable fat were between 420 and 555 ms.

The potential of NMR to determine the geographic origin of milk and cheese has been studied. In milks from grazing cows, the percentage of polyunsaturated fatty acids was significantly greater in mountain than in plain, while no significant difference was found between the two sites for monounsaturated and saturated fatty acids (Renou et al., 2004). The researchers attributed these differences to the nature of pasture. Indeed, mountain pastures are rich in non-leguminous, while plain pastures are mainly composed of graminae and leguminous plants.

The accuracy of ^{13}C NMR to differentiate cows' milk from buffaloes' milk has been investigated by Andreotti, Trivellone, Lamanna, Di Luccia, and Motta (2000). In their study, fifteen milk samples have been studied and the PCA applied to the 10 NMR parameters showed a good discrimination between the investigated milks. However, with only 15 milks, the results obtained were still not very robust and further research that should include more milk samples is needed.

In a similar approach, Hinrichs et al. (2004) have used low resolution NMR to characterise water holding capacity of experimental fresh cheeses which contained 6.5%, 26.5%, 3.5% of protein, fat and lactose, respectively. The investigated cheeses had a dry matter of 38% and a pH of 5.2. Cheeses were manufactured by addition of differently treated whey protein concentrate (native and modified). All fresh cheeses were sheared up to 30 min at 50 rpm at about 80 °C in a shear reaction. The authors showed that the shearing procedure during processing had an influence on the viscosity of the final product, the syneresis behaviour and the firmness of fresh cheeses.

De Angelis Curtis et al. (2000) have monitored the ripening of Italian PDO cheeses (6, 12 and 18 months) using low and high NMR. Using low NMR, the authors reported an increase in the amount of free water and a decrease in the amounts of bound water and total water for the cheese samples cut at 2, 5, 8 cm from the base of the wheel as well as for those cut at 2, 8, and 14 cm from the rind side of the wheel. The authors attributed this phenomenon to the hydrolysis of protein during ripening. The lipolysis was also reported to contribute to this phenomenon, but this was of secondary importance with respect to proteolysis. In a second step, the same authors have used high resolution NMR to quantify amino acids present in cheeses. Significant variations in the levels of the amino acids were found. Indeed, an increase in the level of serine, alanine, phenylalanine and methionine and a decrease in the amounts of glutamate, leucine and valine have been found during the ripening time. The authors related these variations to the proteolysis and to metabolic processes during the ripening time.

Kuo, Gunasekaran, Johnson, and Chen (2001) have investigated changes in molecular mobility of water of Pasta Filata and non-Pasta Filata Mozzarella cheeses after 10 days of storage using NMR. The observed modifications using NMR have been attributed to the changes in the physico-chemical environments due to the structural rearrangements of protein matrix, contributing to the change of water mobility during aging. Moreover, a change of both T_1 and T_2 have been observed during aging and has been attributed to the increase of hydration of proteins and to the change in the structure of protein matrix caused by proteolysis.

The advantage of NMR spectroscopy is that the decay of a given proton is influenced by its surroundings. Other

types of spectroscopy (e.g. MIR and fluorescence) are also sensitive to the molecular surroundings, but not at the same extent. For example, the relaxation of water is much faster when it is in bound state than when it is free. NMR is rich in information of this type compared to the above mentioned spectroscopic techniques. Therefore, NMR can provide information which cannot be gathered anywhere else. In addition, NMR might be a better choice for heterogeneous samples, as larger quantity of a sample is probed with NMR than with, for example, NIR reflectance or transmittance. The main disadvantage of this technique is that the equipment is complex to optimise compared with, for example, the NIR instrument, and that measurements are time dependent. Indeed, the pulse sequence used is crucial to obtain valuable results. The use of wrong pulse sequence or parameter settings might result in a negative outcome of the experiments. Therefore, NIR might be preferred for fast, non-invasive quantitative measurements on samples which are relatively homogeneous.

3.6. Joint analysis of the spectral data obtained from infrared and fluorescence spectra

In order to take into account the whole information given by spectroscopic techniques cited here above, coupling these techniques could be an appropriate tool for determining the quality and identity of dairy products (cheese, milk, etc.). Common Components and specific Weights Analysis (CCSWA) was applied to determine the geographic origin of Emmental cheeses manufactured during winter time (Karoui, Bosset, et al., 2005, 2005, 2005). The CCSWA method was performed on six normalised data sets containing physico-chemical data, NIR spectra, three MIR spectral regions (3000–2800, 1700–1500 and 1500–900 cm^{-1}) and tryptophan fluorescence spectra. The authors concluded that the analysis of the six data sets by the CCSWA enabled the most efficient means of analysing the spectroscopic and physicochemical data collected on the 91 cheeses. Although, the spectroscopic techniques applied in this study were independent, each of them provided information, which can be partially used for recognising the origin country of European Emmental cheeses. In addition, unpublished results assessed the use of this multivariate statistical method (CCSWA) in order to characterise French Emmental cheeses having different brand of products which were produced in different regions of France. Indeed, a discrimination of French Emmental cheeses according to their making procedures, ripening times and physico-chemical compositions was observed. The same technique has been applied recently by Karoui, Dufour, Schoonheydt, and De Baerdemaeker (2006) on FFFS spectra (tryptophan, vitamin A and riboflavin) recorded on traditional (M1 and M2) and stabilised (M3) soft cheeses at both the external and central zones. The plane defined by the common components 1 (q_1) and 3 (q_3) showed a clear discrimination between the cheese varieties and sampling zones (Fig. 3).

Regarding q_1, cheese samples cut at the central zone (M1 and M3) were located on the left, whereas the other cheese samples were located on the right. The q_1 showed also a good discrimination between the external and central zones of M1 and M3 cheeses. The results obtained from CCSWA were not given by the PCA performed separately on tryptophan, riboflavin and vitamin A fluorescence spectra.

The same approach has also been applied to 12 semi-hard (Raclette) cheeses produced during summer period

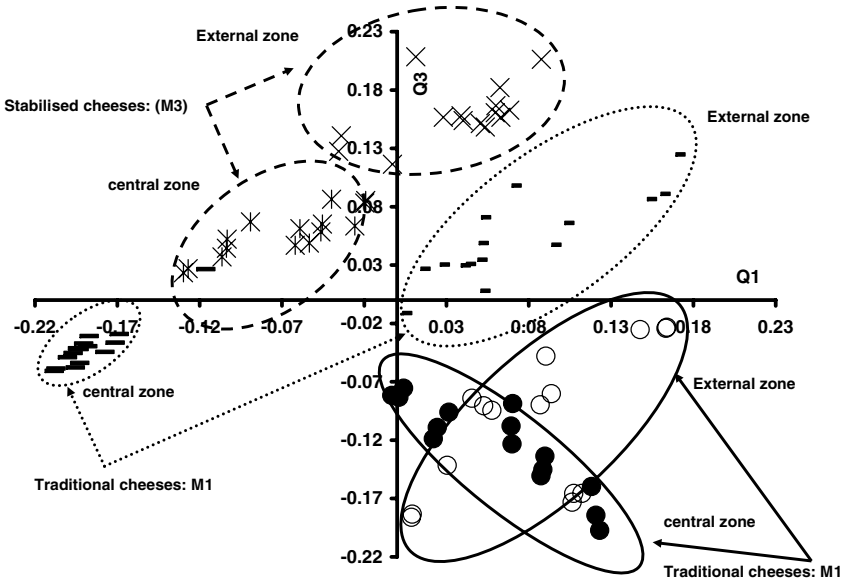

Fig. 3. Common components and specific weights analysis (CCSWA) similarity map defined by the common components 1 (q_1) and 3 (q_3) of external M1, central M1, external M2, central M2, external M3 and central M3 zones of two traditional (M1 and M2) and stabilised (M3) cheeses.

and belonging to four brand products (Karoui, Dufour, De Baerdemaeker, in press). CCSWA was applied on the physico-chemical data and fluorescence spectral data sets at the end of ripening (60 days). The first common component q_1 expressed 94.4% and 59% of the inertia of the vitamin A and tryptophan spectral data, respectively and a less part (24.2% and 13.2%) of the inertia of riboflavin and physico-chemical data sets, respectively. The authors observed a common structure highlighted by the common component q_1 which has been identified in the tryptophan and vitamin A data sets. The q_3 expressed 34.6% and 23.9% of the inertia of the physico-chemical data and tryptophan fluorescence spectra, respectively and a small amount of the riboflavin and vitamin A spectra data sets (3.2 and 0.7, respectively). The authors concluded that the spectral data recorded using, on the one hand, tryptophan and vitamin A fluorescence spectra and, on the other hand, riboflavin fluorescence spectra were independent.

In addition, the similarity map defined by the q_1 and q_3 allowed a good discrimination between the 4 cheese brand products (Fig. 4). The correlation coefficients of the physico-chemical variables according to common components q_1 and q_3 were found high for all the parameters, except for the variables fat in dry matter and soluble calcium which were poorly correlated with the common components q_1 and q_3, respectively.

The authors observed an evidence relationship between tryptophan and vitamin A fluorescence spectra and physico-chemical variables. Indeed, regarding the saliences associated with common components q_1 and q_3, the information contained in the tryptophan and vitamin A fluorescence spectra (according to common component q_1) and the tryptophan fluorescence spectra (according to common component q_3) refereed to the physico-chemical parameters (according to common components q_1 and q_3). However, no relationship was observed between the physico-chemical parameters and the riboflavin fluorescence spectra. The authors explained this phenomenon by the fact that the 400–640 nm emission riboflavin spectra recorded after excitation wavelength set at 380 nm were related to the formation of lipid oxidation products and no parameter related to this phenomenon has been measured in their study.

The results obtained from CCSWA showed that the relationships between all the data sets leads to the formation of two common components which allowed a global characterisation of the various brand cheese products.

Recently, a concatenation technique has also been applied to determine the geographic origin of Emmental cheeses independently of their manufacture periods. A total of 163 Emmental cheeses produced in winter ($n = 91$) and summer ($n = 72$) periods were investigated by the MIR and FFFS (Karoui et al., 2004a). Correct classification of 89% and 76.7% was observed for the calibration and validation samples, respectively. The authors reported that although, this statistical technique did not allow 100% correct classification for all the groups, the obtained results could be considered as promising considering the significant effect of the season on the quality of investigated cheeses. Even though, many factors influence the final quality of Emmental cheeses i.e., milk origin, milk treatment, season, type and amount of starter added, manufacture conditions and ripening time and temperature, the above study confirmed the interest of the joint analysis (concatenation) of spectroscopic data obtained

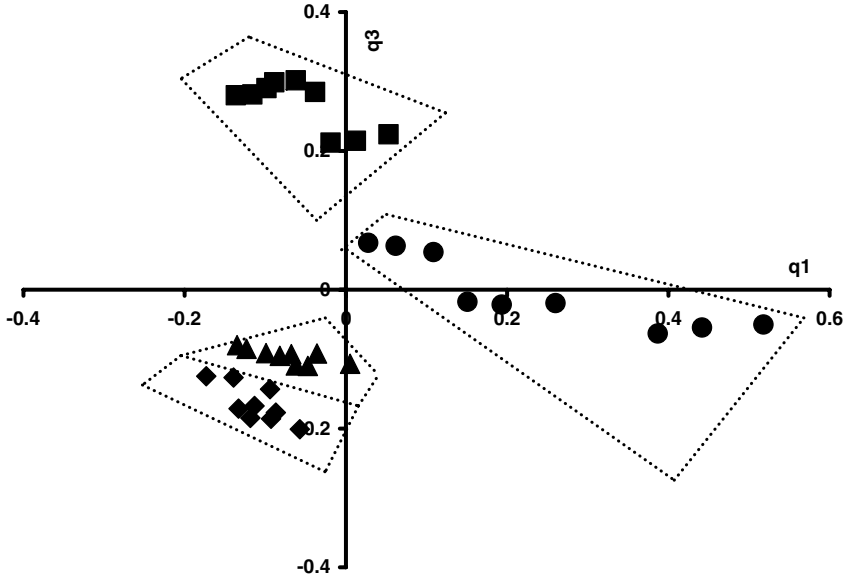

Fig. 4. Common components and specific weight analysis (CCSWA) similarity map defined by the common components 1 (q_1) and 3 (q_3) of A (♦), B (■), C (▲) and D (●) semi-hard cheeses at 60 days of ripening. CCSWA was performed on data tables obtained using riboflavin, tryptophan and vitamin A fluorescence spectra and physico-chemical data.

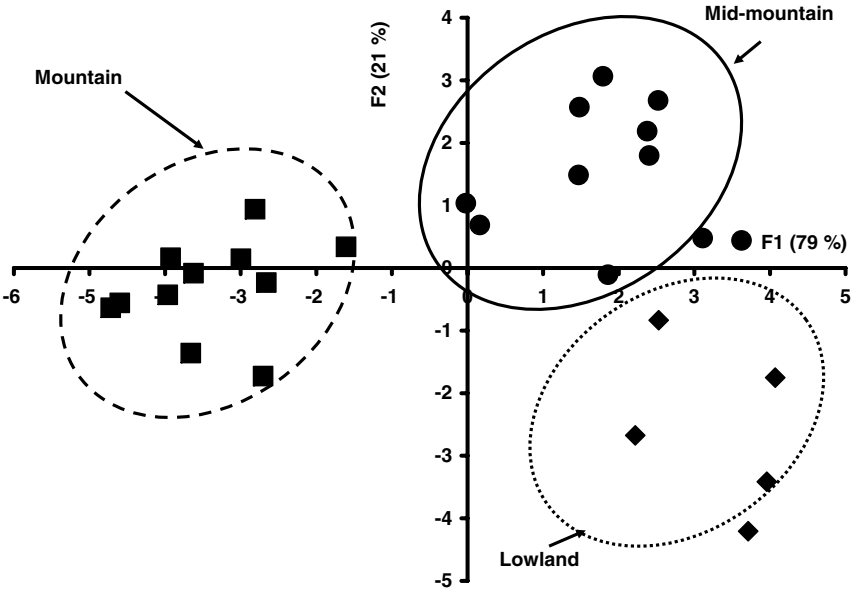

Fig. 5. Discriminant analysis similarity maps determined by discriminant factors 1 and 2. FDA performed on the first 20 concatenated PC of the PCA performed on the fluorescence spectral data of the investigated: milks produced in lowland regions (♦), milks produced in mid-mountain regions (●) and milks produced in mountain regions (■).

by different techniques to determine the geographic origin of cheeses.

The same technique (concatenation) has been applied for the determination of the geographic origin of milks produced in lowland, mid-mountain and mountain areas of Haute-Loire department (France) (Karoui, Martin, & Dufour, 2005). The obtained results showed a good discrimination of milks according to their production sites (Fig. 5). Indeed, milks were discriminated according to discriminant factor 1: considering this axis, milks from the lowlands were observed on the far right, whereas milks from the mountains were located on the far left. Milks produced in the mid-mountain had co-ordinates close to the origin. In addition, the authors observed 100% correct classification for milks from lowlands. Two milks produced in the mid-mountains were misclassified as belonging to milks produced in the lowlands and *vice versa*. The authors explained this misclassification to the close altitudes of certain tours which are nevertheless classified into two different groups: mountain or mid-mountain.

NIR spectrometry and electronic nose (EN) data have been used for on-line monitoring of yogurt and filmjölk (a Swedish yogurt-like sour milk) fermentations under industrial conditions (Navrátil, Cimander, & Mandenius, 2004). PCA was applied firstly to the NIR and electronic nose signals, and loadings vectors were then analysed. Then, the first PCs for the NIR and the EN signals were used for on-line generation of a process trajectory plot visualising the actual state of fermentation. The researchers reported that by using five or six PLS factors, the models yielded acceptable predictions that could be further improved by increasing the number of reliable and precise calibration data. The authors concluded that the fusion of the NIR and EN signals has a potential for rapid on-line monitoring and assessment of process quality of yogurt fermentation.

NMR and isotope ratios ($^{13}C/^{12}C$, $^{15}N/^{14}N$) techniques have been used recently to determine the geographic origin of buffalo milk and mozzarella cheese (Brescia, Monfreda, Buccolieri, & Carrino, 2005). A good discrimination of samples according to their geographical origin was obtained by applying PCA to the NMR spectral data sets. The authors related this finding to the different type of feeding. The authors reported that they will extend this method to the characterisation of other typical cheeses.

4. Conclusion

There can be a little doubt that the spectroscopic techniques such as the near infrared (NIR), mid infrared (MIR), front face fluorescence spectroscopy (FFFS), stable isotope, nuclear magnetic resonance (NMR) have demonstrated considerable potential for determining the quality and the geographic origin of dairy products. Ready transfer of these techniques to the dairy plants as either on- or in-line methods is already possible. Regarding the chemometric tools, a greater research into the wide array of potential models will continue and it seems likely that, with the ever-increasing power of personal computers will be more extensively studied in the future. There is no feasible ideal method for all purposes. An accurate determination of the quality and/or identity of dairy product seem feasible only when a combination of all parameters is applied. By way of such a multi-factorial approach, all data must be carefully interpreted and cross-validated with tools of multivariate statistics in order to establish links to the

origin. Indeed, the results illustrated in this review showed that the methodology of coupling different spectroscopic techniques by using appropriate chemometric tools like common components and specific weights analysis (CCSWA) and concatenation enabled a better discrimination of dairy products according to their geographical origin. These techniques provide spectroscopic fingerprints of the dairy products, which by comparison with authentic samples can be used to detect certain fraudulent practices and to authenticate the geographical origin. They can also provide an efficient means of enforcing the restricted rules associated with PDO labelled products.

The development of spectroscopic methods should increase our understanding of the determinants of food texture and may allow devising a structure engineering of cheese.

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