EFFECT OF TECHNOLOGICAL TREATMENTS ON THE QUALITY OF TRADITIONAL CHEESES

By

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14 December 2011

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ABSTRACT

The overall objective of this thesis was to study effect of some technological treatments (heat treatment, ripening, and cheese making process) on the cheese quality by using physicochemical, rheological (texture) and spectroscopic (molecular structure) methods.

The primary objective of this research was to evaluate the compositional, textural, colour and structural characteristics of the three major categories of France Cantal cheese produced from pasteurized, thermized and raw milk. Fluorescence spectroscopy had been used to evaluate the molecular structural changes in Cantal cheese caused by heat-treatment. Significant differences were observed among the investigated cheeses in their compositional characteristics. Heat treatment resulted in significant increases in the levels of pH, reduction in the contents of fat, protein, calcium and phosphorus in the cheese. Proteolysis was significantly higher in the raw Cantal cheeses milk. Heat treatment significantly influenced colour and texture attributes of cheeses. 100% of correct classification was obtained for cheeses by applying FDA to fluorescence spectral data (tryptophan and/or vitamin A).

The second objective of this research was to evaluate the compositional, physical (colour and texture) and structural changes of Cantal cheeses repined for 30-, 120- and 200-days-old by using chemical, rheology and synchronous fluorescence spectroscopy (SFS) methods. ANOVA results showed that, all the compositional characteristics of Cantal cheese increased significantly ($P<0.05$) over ripening, except for calcium and moisture contents decreased. The G', G'', Tan δ and η* values of cheese increased significantly as the ripening proceeds, but exhibited an opposite trend over 120 days as compared to 200 days. Ripening led to a decrease of L* and b* values and a slight increase in - a* value. The change in the fluorescence intensity at 295, 322 and 355 nm reflects the

physicochemical changes of cheese matrix and, in particular, structural changes in the protein network throughout ripening. PCA and FDA show that SF spectra of Cantal cheeses are clearly separated and the correct classification of 100% was observed.

The third objective of this research was to evaluate the chemical, colour (L*, a*, b* values), and texture (G', G'', Tan δ and η* values) characteristics of traditional and industrial Saint-Nectaire cheeses (ripened for 30 days) and to verify the ability of MIR spectroscopy (3000-900 cm^{-1}) to monitor the differences between the two cheese-making processes (traditional, industrial). AONVA results indicate that no significant differences occurred in the chemical parameters between the two cheese-making technologies; however, these differences were small in magnitude but gave rise to some extent of texture attributes. No differences were observed among the different samples for cheese colour (L* and b*). Using the MIR spectral range of 3000-900 cm^{-1}, cheese samples from different manufacturers was grouped in well-separated by the PCA. The best discriminatory approach was achieved in the MIR spectral region 3000-900 cm^{-1}, 100% of the original grouped cases were correctly classified by FDA.

We concluded that the combination of fluorescence or MIR spectroscopic technique with multivariate analysis could be successfully used, a rapid and simple method to discriminate cheese samples in terms of heat treatment, ripening and cheese-making processes.

Keywords: French Cheese, spectroscopy, texture, rheology, colour, structure, multivariate analysis, Cantal cheese, Saint-Nectaire cheese.

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INTRODUCTION

INTRODUCTION

Cheese is one of the oldest fermented foods. More than a thousand varieties of cheese are listed in the world. This diversity is the result of multiple factors including the type of milk used (cow, buffalo, goat or sheep), the manufacturing process and local preferences. It is produced by the coagulation of the milk protein (casein) by enzymatic action (rennet) or microbial fermentation. The solid separated and pressed into final form.

In recent years, consumers have shown interest in traditional products. Traditional cheeses represent a cultural heritage and the result of accumulated empirical knowledge passed from generation to generation. In many south European countries, several food products owe their reputation to traditional (and sometimes artisanal) production techniques used in defined geographical areas such as ‘‘Protected Denomination of Origin’’ (PDO) that make such foods very specific and well differentiated from other ones.

Today, the European market is saturated with food products. The new challenge is not to produce a standard product, which is only differentiated by price, but to produce products, which have unique characteristics and meet consumers’ expectations, which in turn requires appropriate analytical tools to investigate cheese quality.

Cheese quality is traditionally evaluated either by instrumental analysis (physical, chemical) or by sensory analysis. The physicochemical methods (such as the analysis of pH, dry matter, fat, protein and protein fractions, free amino acids and free fatty acids and triglycerides of cheese) are unsuitable to be used or adapted by the cheese industry for rapid analysis of cheese quality and it is expensive. In addition, sensory analysis is hardly possible to implement for practical use when many samples need to be analyse on-line or at time in the food industry. It is important to

have a quantitative means for assessing sensory properties in a reasonable way (faster, simple, and cheaper) to enable the food industry to rapidly respond to the changing demands of both consumers and the market. Thus, it is necessary to develop new, rapid and simple methodologies to help the cheese maker to know the cheese quality.

Cheese structure is a complex array of components such as fat globules, pools of entrapped free fat, bacterial and yeast cells, and minerals contained within a casein network interspersed with serum channels. Texture is the primary quality attribute of cheeses: it is a reflection of cheese structure at the microscopic and molecular levels **(Dufour *et al.*, 2001)**. In addition, the textural properties of cheese are widely recognized as a determinant of overall quality and consumer acceptance of cheeses and texture is an important characteristic used to differentiate many cheese varieties.

Rheological characterization of cheese in general is important as a means of determining body and texture for quality and identity as well as a means of studying its structure as a function of composition, processing techniques and storage conditions **(Konstance & Holsinger, 1992)**. Small strain dynamic has been used to define both the elastic (G') and viscous (G'') nature of cheese. The changes in dynamic rheological properties (G', G'' and Tan δ) in cheese can provide information about the nature of protein interactions in the cheese matrix. Such information is useful to characterize and differentiate cheese varieties **(Tunick *et al.*, 1990)**.

Results published in the last 10 years show that spectroscopic methods in combination with multivariate statistical analyses have broad applications in our understanding of food structure and properties. A variety of the instruments that are commonly used to analyse food characteristics are based on spectroscopy, e.g., ultraviolet and visual absorption, fluorescence,

infrared absorption and nuclear magnetic resonance spectroscopy **(Birlouez-Aragon *et al.*, 2002** and **Karoui & De Baerdemaeker, 2007)**. These instruments utilize interactions between electromagnetic radiation and matter to provide information about food properties, e.g., molecular composition, structure, dynamics and interactions. The fluorescence properties of aromatic amino acids also have been used to study protein structure and protein interactions in cheese **(Herbert *et al.*, 2000)**. The emission of tryptophan residues in protein is highly sensitive to its local environment, and is thus often used as an indicator group for protein conformational changes. On another note, the shape of vitamin A excitation spectrum (located in fat membrane globules in milk), as an intrinsic fluorescent probe, is correlated with physical state (viscosity) of triglycerides in the fat globule and protein-lipid interaction **(Dufour *et al.*, 2000)**. In addition, Wold and co-workers **(Wold *et al.*, 2002)** have demonstrated that the fluorescence properties of riboflavin can be used to measure the degree of degradation of riboflavin in dairy products. The fluorescence spectra of these intrinsic probes recorded on the cheese samples are essentially fingerprints allowing the characterization and identification of the products. MIR spectroscopy is a method used for milk and dairy product analysis. The MIR wavelength range of 3000 to 900 cm^{-1} has particular attraction, since measurements in this range provide direct information concerning the specific constituents in the sample, as well as their characteristic molecular structure. In addition, it has been reported that the MIR spectrum recorded on a cheese is a fingerprint of the sample that contains information about its physicochemical characteristics.

Therefore, the overall objective of this thesis was to investigate the use of new methods (rapid non-destructive) based on spectroscopic techniques (such as infrared spectroscopy and fluorescence spectroscopy) for evaluation cheese quality and understanding of the determinants of the structure and its relations

with the texture.

The objective of the first part of this thesis was to evaluate the influences of heat treatments on the physicochemical, rheological, textural characteristics of Cantal cheese by using fluorescence spectroscopy coupled with chemometrics.

The second objective was to evaluate changes in compositional, physical (colour, texture) and structural characteristics of Cantal cheese throughout ripening by using synchronous fluorescence spectroscopy and rheological methods.

In a third part had been studied the effect of the manufacturing process on quality characterization of Saint-Nectaire cheese by MIR spectroscopy and rheological methods.

REVIEWS OF LITERATURES

REVIEWS OF LITERATURES

I. Cheese

Cheese is a dairy product prepared by enzymatic, acid, and/or acid/heat-induced gelation of milk and concentration of the resultant gel to the desired dry matter content by dehydration techniques, such as cutting, stirring, scalding, whey drainage and/or pressing. A total of 500 to 800 **(Hermann, 1993)** different cheese varieties have been listed, but undoubtedly there are many more considering regional variations of these varieties and the anonymity of some local varieties. They differ to varying degrees in nutritive value, appearance, flavour, texture and cooking properties. Consequently, cheese is capable of satisfying a diverse range of sensory and nutritional demands and, therefore, has very wide appeal. It is an extremely versatile product, which may be consumed directly or indirectly as an ingredient in other foods.

World production of cheese was estimated at ~ 17.2×10^6 tonnes in 2008 (**IDF, 2008**), and accounted for ~25% of total milk used. While cheese-like products are produced in most parts of the world, the principal cheese-producing regions are Europe, and North America (Table 1). Within these regions, the production and consumption of cheese varies widely with country, as does the proportion of milk used for cheese, which ranges from approximately ~20% in New Zealand, Greece or Rumania to approximately ~90% in Italy. Approximately, 10% of total cheese production is traded on the global market, the major suppliers being the European Union (EU) (~38%), New Zealand (~21%) and Australia (~14%), and the major importers being Russia (~21%), Japan (~20%) and the United States (~19%) (**IDF, 2008**).

Table 1: Annual cheese production and consumption in various regions in 2007.

Region	Cheese production (× '000 tonnes)	Milk to cheese (% of total milk)	Consumption (kg person^{-1})
Europe	8904	40	—
Germany	2109	74	22.2
France	1726	71	24.3
Italy	1045	96	20.7
Netherlands	732	66	21.5
Poland	568	47	10.7
United Kingdom	375	27	12.2
Denmark	351	76	23.8[a]
Ukraine	340	28	6.0
Spain	244	40	7.3
Switzerland	176	43	22.2
Austria	149	47	18.8
Ireland	127	24	6.1
Czech Republic	116	42	17.0
Sweden	109	36	18.4
Finland	102	44	19.1
Lithuania	91	46	—
Norway	84	54	15.4
Bulgaria	73	64	—
Hungary	72	41	10.6
Belgium	66	21	19.0[a]
Rumania	62	11	—
Portugal	57	28	10.2
Slovakia	40	37	9.8
Estonia	31	45	18.7
Latvia	29	34	—
Slovenia	19	28	10.1
Greece	12	15	29.0
North America	5341	51	—
United States of America	4745	56	16.0
Canada	403	50	12.6
Mexico	193	19	2.6[b]
Oceania	642	25	—
Australia	352	39	11.9
New Zealand	290	19	6.1
Others	2187	—	—
Brazil	580	23	—
Argentina	487	50	11.2
Russia	434	13	5.5[b]
Kenya	243	69	—
Iran	230	25	4.6
Japan	125	16	2.0
Chile	70	28	—
China	18	0.5	—

a Based on data for 2003. b Estimates.

Overall, cheese consumption has increased continuously worldwide since 2000 (14.75×10^6 tonnes) at a rate of ~1.5% per annul between 1990 and 2000, and 2.5% between 2000 and 2007 **(IDF, 2008)**. The accelerated demand is being driven by a number of factors including (a) increases in global population and per capita income, (b) changes in consumer lifestyle (e.g. eating out) and (c) the expansion of food service and snack food sectors allied with the versatile functionality of cheese, which enables it to be used as an ingredient in, and enhance the quality of, prepared foods/meals and snack foods. Simultaneously, there has been an increase in demand for more consistent quality, with respect to sensory properties (e.g. taste, tactile texture, colour), usage characteristics (e.g. convenience, shreddability, melt, flowability), and nutrient profiling (e.g. ratio of saturated-to-unsaturated fatty acids, levels of calcium).

I.1. Principles of cheese manufacture

Cheese of which there are at least 500 varieties **(IDF, 2008)** is a complex and variable biochemical system. The manufacture of cheese involves coagulation of the protein of milk by the added rennet (chymosin, pepsin), acidification using starter culture or food-grade acids at a temperature of 20-40°C, to a pH value close to the isoelectric pH of casein, i.e. ~4.6; or heat-acid precipitation (e.g. heating milk to ~pH 5.6 at ~90°C). The acid and acid-heat varieties are consumed when fresh and their quality depends primarily on the composition of the curd, which depends on the chemical, microbial and organoleptic quality of the milk. However, rennet-coagulated cheeses, which represent about 75% of all cheese, are ripened for a period ranging from ~3 weeks to > 2 years, during which the characteristic flavour, body, texture and functionality of the cheese develop. The production of rennet-coagulated cheese can be divided into two phases: (a) conversion of milk to curd and (b) conversion of curd to cheese. However, the key operations are summarized in Figure (1) **(Barry & Tamine, 2010)**.

The ripening process varies depending upon the type of cheese. During ripening, chemical and enzymatic reactions occur that result in the development of flavor and changes to the body, texture and physical properties (melt, stretch) of the cheese. Ripening involves a complicated cascade of biological and biochemical events which are affected by many compositional, processing and environmental factors. The ripening agents in cheese are: (i) the coagulant (rennet), some of which is retained in the cheese curd, (ii) the starter culture (or adventitious milk microflora) used to acidify the curd, and, after the cells have died, their intracellular enzymes, (iii) the non-starter microflora, mainly mesophilic lactobacilli, which grow during ripening, and their intracellular enzymes, (iv) indigenous milk enzymes, and (v) secondary cultures (moulds and bacteria) used for some varieties. The key to good cheese making is to control and co-ordinate the activity of these agents, which is affected by (i) the composition of the curd, which is affected by the composition of the cheese milk and the manufacturing protocol, especially cooking temperature, pH and agitation, (ii) quality of the rennet, (iii) starter culture, (iv) the microflora of the milk and whether it is pasteurized or not, (v) treatment of the curd, especially cooking and salting, (vi) duration and temperature of ripening. The principal biochemical changes in cheese during ripening are: (i) glycolysis of lactose and catabolism of lactic and citrate acids, (ii) proteolysis and amino acid transformations and (iii) lipolysis and catabolism of fatty acids.

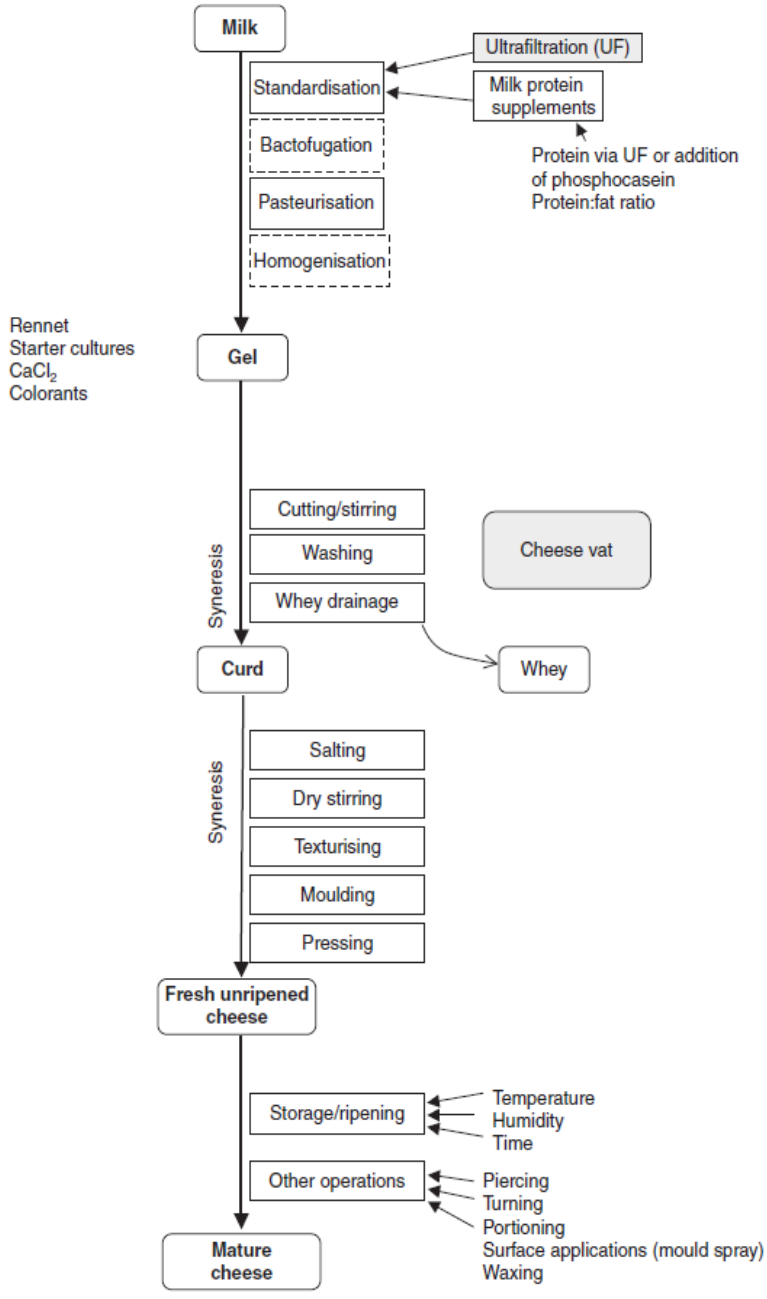


Figure 1: Overview of cheese manufacturing operations.

I.2. Classification of Cheeses

Approximately 500 varieties of cheese are recognized by the International Dairy Federation **(IDF, 2008).** For various reasons, a number of attempts have been made to classify cheeses into meaningful groups. Traditional classification schemes have been based principally on moisture content, i.e. extra-hard, hard, semi-hard/semi-soft or soft. Although used widely, this scheme suffers from serious limitations since it groups cheeses with widely different characteristics, e.g. Cheddar and Emmental are classified as hard cheeses although they have quite different textures and flavours, are manufactured by very different technologies and the microbiology and biochemistry of their ripening process are very different. In addition, cheeses traditionally developed a rind through which moisture evaporated; hence, the composition of cheese changes as it ages and there is a moisture gradient from the surface to the centre; the moisture content of long ripened cheese may decrease by 5±10% during ripening. The composition-based scheme is made more discriminating by including information on the source of the milk, coagulant, principal ripening microorganisms and cook temperature. Based on the method of milk coagulation, cheeses may be divided into four super families **(Fox *et al.*, 2000)**:

- Rennet-coagulated cheeses: most major cheese varieties
- Acid-coagulated cheeses: e.g. cottage, quark, cream
- Heat/acid coagulated: e.g. Ricotta
- Concentration: e.g. Feta.

Owing to the great diversity of rennet-coagulated cheeses, these can be classified further based on the characteristic ripening agent(s), e.g. internal bacteria, internal mould, surface mould or surface smear (bacteria), or manufacturing technology; such a scheme is shown in Figure (2).

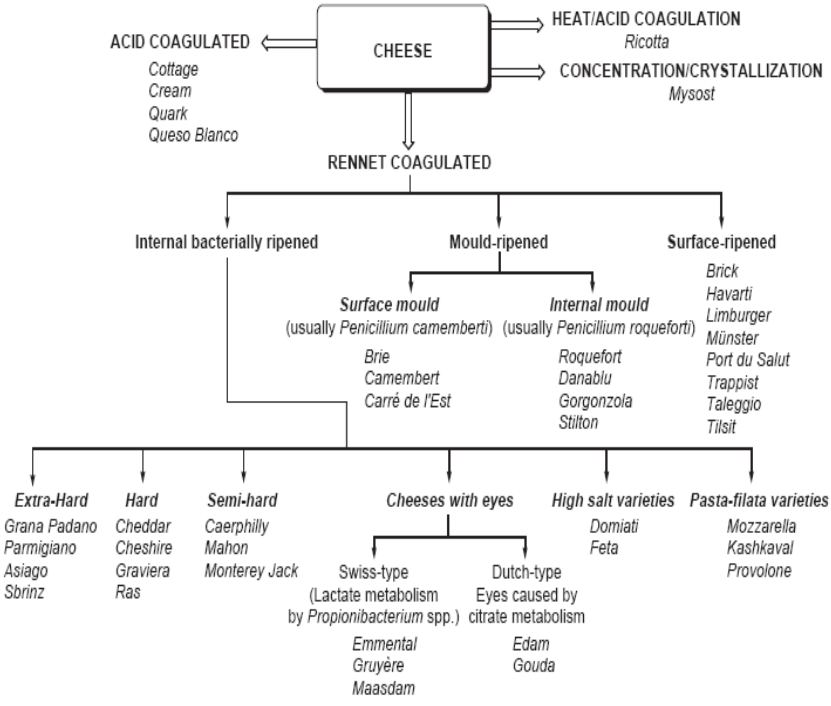


Figure 2: A Scheme for the classification of cheese (Fox et al., 2000).

II. French Cheeses

France is one of the only countries to have a wide variety of cheeses, production annually 1.850 million tons (in 2008) of cheese with a great diversity more than 400 different cheeses (93.5% of cow, 3% sheep, 3.5% goat), including 42 with a designation of origin (A.O.C). France is known as the "land of cheese, both level of production and consumption (1.420 million tons). This volume of cheese variety reflects both the rich technological opportunities available for processing the milk of several species milk (cow, sheep, goat) and an innovation of farmers and artisans of industrial.

French are, with the Greeks, the first consumers of cheese with 24.5 kilograms per person per year (66g/capita/day), 95% of them eating cheese once day at least. The cow cheeses are the most numerous and most widely consumed (93%). The cheese production is mainly located in major basins of large dairy west and east.

Traditional cheeses represent a cultural heritage and are the result of accumulated empirical knowledge passed from generation to generation. Every traditional cheese is connected to the territory of its origin and to the prevailing pedoclimatic conditions. A variety cheese, cheese local (10%) and regional farmers (90%), usually made cheese from raw milk or pasteurized milk, Some farmers cheese and/or milk receive official recognition or "sign identification of quality or origin "Appellation d'Origine Contrôlée (AOC 42 in 2007, about 11% of French cheese production). Currently, the French cheese production is an element of maintaining the socio-economic dimension environmental and many rural areas.

In France, cheese is traditionally grouped into Eight categories (Table 2), known as the eight families of cheese. In each of these families: we have cheese made from cow’s, goat’s and

sheep’s milk. It is said that there are so many different types of cheese in France that even if you ate a different one each day, you still wouldn't have tasted all of them after a year.

French cheese production is classified under four categories, and PDO/AOC rules dictate which category each protected cheese may be assigned to:

- **Fermier**: A farmhouse cheese, which is produced on the farm where the milk is produced.
- **Artisanal**: A producer producing cheese in relatively small quantities using milk from their own farm, but may also purchase milk from local farms.
- **Coopérative**: A dairy with local milk producers in an area that have joined to produce cheese.
- **Industriel**: A factory-made cheese from milk sourced locally or regionally, perhaps all over France (depending on the AOC/PDO regulations for specific cheeses).

Table 2: Classification of cheeses in French.

Family	Characterization	Examples
1. Fresh Cheeses *(Fromages Frais)*	These cheeses are white and contain a lot of water, and are not aged. Rather than adding rennet, which is used to create some cheeses, the curd is formed by adding lactic starter to the milk.	Carré Gervais Double ou Triple-crème
2. Soft Cheeses with Natural Rind *(Les Fromages à Pâte Molle et à Croûte Fleurie)*	These are soft cow's milk cheeses, aged about a month, which you will recognize by their white, almost floury surface.	Brie , Camembert
3. Soft Cheeses with Washed Rind *(Les Fromages à Pâte Molle et à Croute Lavée)*	These are cheeses made from cow's milk, but this time the rind is washed during the aging process, which prevents the formation of surface molds	Munster, Reblochon
4. Pressed Cheeses uncooked (Fromages à Pâte Pressée non-cuite)	These types of cheese are submitted to pressure during the processing, which drains the cheese of some of its moisture. After applying pressure, the cheeses are than placed in carefully controlled conditions and aged for several months.	Cantal, *Saint-Nectaire,* *Salers,* *Gouda*
5. Pressed and Cooked Cheeses *(Fromages à Pate Pressée et Cuite)*	Before being pressed, the curd is heated for an hour to make these types of cheese. They are formed in large cylinders and are ripened for a long time.	Emmental and Gruyère, Comté, beaufort

6. Goat Cheese *(Fromages de Chèvre)*	There are officially over a hundred varieties of goat cheese in France. Sometimes the goat's milk is mixed with cow's milk to create a mi-chevre. Pur chèvre contains only goat's milk.	Crottin de Chavignol, Pouligny-Saint-Pierre.
7. Blue Cheeses *(Fromages à Pâte Persillées)*	These types of cheese are easily recognized by the channels of blue or greenish-blue that run throughout them. They are mostly made from cow's milk with the notable exception of roquefort, which is made from sheep's milk. Blue cheeses are ripened a long time and have a strong flavor and smell.	Bleu d'auvergne, fourme d'ambert and Roquefort Gorgonzola, Stilton
8. Processed cheeses *(Fromages à Pâte Fondue)*	These types of cheese are made from other cheeses blended together. They are usually sold in small portions and can be flavored with various things, such as garlic, pepper, and herbs.	Fromages à tartiner, Crème de gruyère, (Vache qui rit)

The category of pressed cheeses cooked and uncooked are certainly one where there are as many varieties in France. It represents 33 % of the production national. Pressed cheeses uncooked not undergo heating or cooking during the mixing tank. Their dry matter is comprised mostly between 44% and 55%, only the Cheddar has a higher solid (63%). These cheeses, some are dry crust such, Raclette, Gouda, Edam, Cantal and Cheddar cheeses. On the other are refined with fungal flora such as Saint-Nectaire cheese.

Denomination and Designation of Origin

The idea of protecting and preserving the traditional diversity of foods, including cheese, commenced at the Paris Convention of 1883 where the term `Appelation d'Origine Controlee' (AOC) was introduced to recognize the specific heritage of food products from particular regions, while guaranteeing product authenticity. This concept became widespread in Europe and was replaced by the European Union scheme (1992), `Protected Designation of Origin' (PDO), which applies to foodstuffs that are produced, processed and prepared in a given geographical area using recognised technology.

A large number of cheeses have PDO status in different EU countries; a selection of the main productions is as follows: Belgium (1), Germany (4), Greece (19), Spain (17), France (42), Ireland (1), Italy (30), The Netherlands (4), Austria (6), Portugal (11) and United Kingdom (8). Some of the most important PDO varieties are Roquefort, Stilton, Manchego, Grana Padano, Parmigiano Reggiano and Gruyere de Comté cheeses (Table 3). Unlike commercial trademarks, PDO denomination reflects a collective heritage and may be used by all producers of a particular variety in a defined geographical area.

Table 3: The production of 42 AOC cheeses in France (in 2004).

Dénomination	Tonnage (t.)	Dénomination	Tonnage (t.)
Comté	43 555	Neufchâtel	1 114
Roquefort	18 831	Sainte-Maure de Touraine	1 103
Cantal	18 828	Rocamadour	984
Reblochon	16 637	Epoisses	877
Saint-Nectaire	13 369	Bleu des Causses	866
Camembert de Normandie	12 747	Laguiole	781
Munster	7 625	Selles sur Cher	747
Brie de Meaux	6 965	Tome des Bauges	594
Bleu d'Auvergne	6 541	Picodon	577
Morbier	6 458	Chabichou du Poitou	553
Fourme d'Ambert	6 068	Bleu de Gex	516
Beaufort	4 410	Fourme de Montbrison	512
Mont d'Or	3 724	Brocciu	494
Ossau Iraty	3 352	Langres	363
Pont-l'Evêque	3 231	Valençay	348
Maroilles	2 538	Pouligny Saint-Pierre	296
Chaource	2 194	Brie de Melun	219
Abondance	1 509	Pélardon	213
Salers	1 393	Bleu du Vercors Sassenage	169
Livarot	1 343	Chevrotin	91
Crottin de Chavignol	1 138	Banon	56

Cheeses AOC d'Auvergne

The Auvergne has produced nearly 89,000 tons of cheese in 2009, representing 5.2% of national tonnage. Only 42 (Table 3) of the 400 French cheeses have the Appellation d'Origine Contrôlée (AOC). The Auvergne is a region that counts most: Cantal, Salers, Bleu d'Auvergne, Saint-Nectaire, Fourme d'Ambert cheeses (Figure 3).

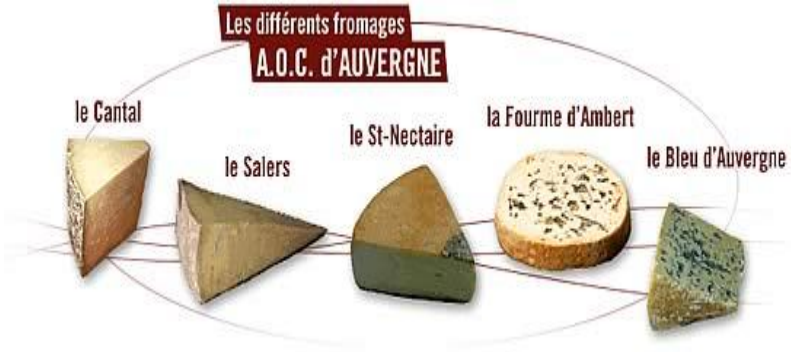


Figure 3: Cheeses AOC d'Auvergne.

The AOC is a sign of quality protected by national and European level INAO (Institut National des Appellations d'Origine), established in 1935. Since May 2009, Designation of Origin (PDO), in French, became the Protected Designation of Origin (PDO) at the European level, so that every European to recognize these high quality products.

In the Auvergne region of central France, we have five great cheeses to be awarded the status of AOC cheese. In fact, no other region of France can claim as many quality cheeses AOC.

The Auvergne cheese manufactures are divided into two specialties:

• Pressed uncooked pasta: Auvergne covers nearly a quarter of French manufacturing and is the second region behind the Loire Valley.

• Blue cheeses: Auvergne produces nearly two-thirds of this category of cheeses at the national level and ranks as the leading

French region, far ahead of Rhone-Alpes (Figure 4).

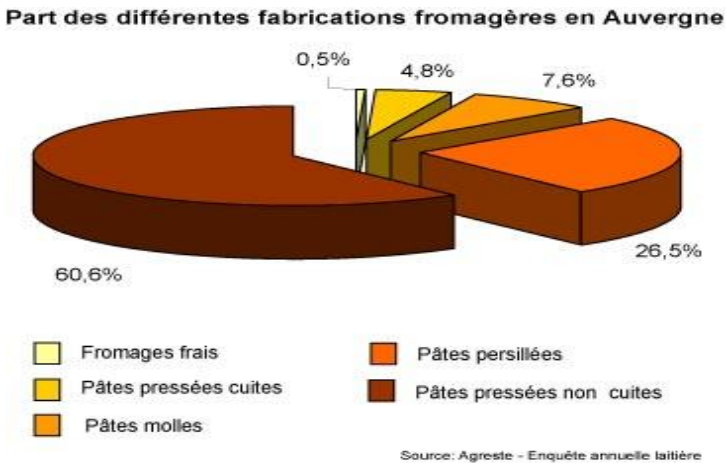


Figure 4: Cheeses made in the Auvergne region.

The weight of the various AOC:

AOC Cantal and Saint-Nectaire cheeses cover 70% of manufacturing in Auvergne (Figure 5).

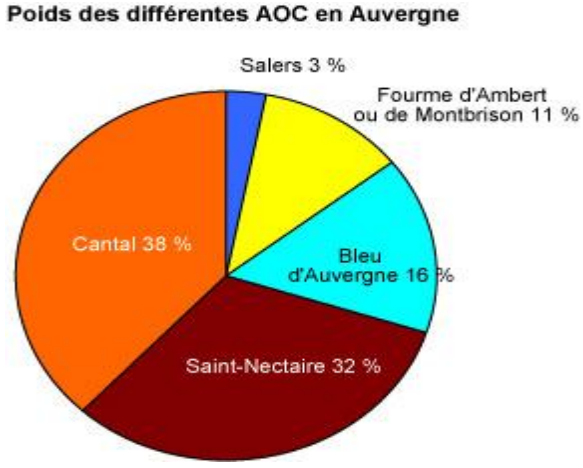


Figure 5: The weight of the various AOC of Auvergne.

Cantal cheese PDO

Cantal cheese is one of the oldest cheeses in history (There are 2000 years). Cantal cheese (dean of French cheeses) is the third most produced AOC (Appellation d'Origine Controlée) cheese variety in France with a yearly production of 15122 tonne in 2009. It is made in the Auvergne region from locally produced either raw or pasteurized cow's milk, hard cheese, pressed, uncooked, dry crust, cylindrical shape and commercialized as "yong" (ripened for at least one month) or old (ripened for over 6 months). Its making process is very similar to the Cheddar. The cheese can have a weight of between 35 and 40 kg, 40 cm height, 36-42 cm diameter round wheels with a dry crust. The total solids content and the Fat/TS ratio must be, respectively, at least 57% and 0.45 **(Isabelle *et al.*, 2005)**.

There are two types of Cantal cheese. *Cantal Fermier* is a farmhouse cheese made of raw milk. *Cantal Laitier* is the commercial, mass-produced version from pasteurized milk; both have to adhere to the same strict quality controls. Cantal cheese is shaped like a cylinder, and is one foot in diameter. Cantal is made from raw or pasteurized cow's milk of the Salers breed.

The AOC Regulatory Board has authorized two basic manufacturing processes, one artisanal and the other industrial. The former is carried out according to traditional usages, including enzymatic coagulation of raw cow's milk, cutting, pressing, salting and ripening for at least one month, although the most Cantal cheeses are aged longer. The industrial process uses pasteurized milk, and hence a starter culture is always added before coagulation; the remaining steps are similar to those for the artisanal Cantal cheese. The cheeses so prepared can thus be expected to have different organoleptic properties. It is know that the pasteurization has an effect on the biochemical modifications which occur during the cheese ripening, and ultimately on the

sensory characteristics of the cheeses (aroma, flavour and texture).

The texture of this cheese is linked to its ripening period. The crust is thin and gray white early maturing, and it thickens revealing gold buttons. There are several stages in the making of this cheese, and different types of Cantal are issued from each of these stages:

Cantal Jeune cheese (aged one month): This means young Cantal cheese. It has undergone further processing including a second pressing and aging for one month, giving the cheese a thin grayish white rind. It is appreciated for its young fruity taste.

Cantal Entre-deux cheese (aged between 2-6 months): it has a golden rind that becomes browner as it ages.

Cantal Vieux cheese (aged at least six months): This means old Cantal Cheese. It has a thick hard rind veering towards orange. This is a strong, affirmative cheese and probably should be reserved for tasting rather then cooking.

These are all available as *fermier* and *laitier*. Most (>80% of production) Cantal cheese is of the first two varieties. *Cantal vieux* cheese is already a hard cheese, if kept properly, it can last up to a year and a half without spoiling. It is not produced in large quantities. Much loved in the Cantal region, *Cantal vieux* cheese is quite rarely exported due to its strong taste, and can usually be found only in specialist stores.

Saint-Nectaire Cheese PDO

Saint-Nectaire cheese is a cow's milk (raw, pasteurized) uncooked pressed semi-hard. It is circular in shape, around 21 cm in diameter and 5 cm in height and weighing an average of 1.7 kg. It has a grey/brown rind, with white, yellow or red patches that surround a semi-hard that is creamy in appreance with occasional residual holes.

After being heated to 32°C, rennet is added to the milk and left for around an hour. The curd is milled to around the size of grains of rice and gathered into a single mass, known as the *tomme*. The *tomme* is cut into small cubes and pressed into the circular mould by hand. After pressing, the cheese is salted and pressed for around 24 hours, turning around midway through. The mould is discarded and the cheese is dried over a period of three days. The period of ripening lasts up to eight weeks, during which time they are twice washed in brine and aged on rye straw. The majority of Saint-Nectaire is transported to a professional *affineur* for the final six weeks of the ripening. The ripening is also cut short if it is decided that the flavour and scent are not developing sufficiently. Around 15 litres of milk are required to make one cheese, and the final product is at least 45% fat as a percentage of dry matter.The production of Saint-Nectaire cheeses, in 2009, five institutions have made Auvergne 7.1 thousand tonnes of Saint-Nectaire cheese which represents 20% of AOC manufactures industrial area.

III. Cheese quality

III.1. Definition of cheese quality

In an overall context, cheese quality may be defined as the degree of acceptability of the product to the end user **(Peri, 2006)**. Quality criteria involve different types of characteristics, including (Table 4):

- Sensory (taste, aroma, texture and appearance);
- Physical (e.g. sliceability, crumbliness, hardness, mouth-feel);
- Cooking (extent of flow, stringiness, browning);
- Compositional/nutritional (contents of protein, fat, Ca, sodium..etc);
- Chemical (intact casein, free fatty acids (FFA), free amino acids) and

- Safety (e.g. absence of pathogens, toxic residues, foreign bodies and conformity to approved levels of substances such as biogenic amines).

The specific combination of quality criteria depends on the application. For example, the uniform presence of meandering blue veins, the sharp flavour of methyl ketones and brittle texture are key quality attributes for the consumer of Stilton cheese. In contrast, a bland flavour, elasticity, stringiness and a surface glistening are paramount to the consumer of Mozzarella on pizza pie. The manufacturer of block processed cheese (e.g. processed American Cheddar cheese) desires high levels of calcium and intact casein to impart good sliceability and moderate meltability to the final product.

III.2. Assessment of cheese quality

Cheese quality is determined by flavor (taste and aroma), texture (hardness, stretch-ability, slice-ability,…etc.) and appearance (colour, uniformity). Cheese texture is important as a quality indicator that consumers use to accept or reject a product; proper control of the parameters that describe texture would therefore enable the cheese processor to make products with the highest quality and consumer acceptability exhibiting a wide range of textures. Also, colour is among the most important component of quality of cheese.

The assessment of quality depends on measurable criteria which provide information about the product in terms of its microstructure, composition, rheology, sensory properties and/or consumer acceptability (Table 4). Examples of measurable criteria include:

- Sensory characteristics of cheese at different maturation times using descriptive sensory analysis (DSA);

- intact casein content as an indicator of the processability of natural cheese into specific processed cheese type formulations, sauce formulations and cheese powders;

- Specific chemical components, such as propionic acid and proline, or level of short chain fatty acids (C_4–C_{10}), as indicators of quality in Swiss and Parmesan type cheeses, respectively;

- the cooking properties of heated cheese, using rheometry, or empirical assays, such as extent of flow, stretchability and viscosity under defined conditions;

- Texture-related rheological criteria, such as the G'(viscous), G"(elastic), Tan δ (viscoelastic index), and η* (as a measure of viscosity)

- viscosity under defined conditions as a measure of ease of spreadability of processed cheese spread or ripened Camembert type cheese;

- colour coordinates (*L**, *a**, *b** values; as a measure of the intensity of a particular colour, e.g. whiteness in goat cheeses, and/or

- visual assessment of eyes in Swiss-type cheeses

III.3. Sensory Analysis of cheese texture

Sensory analysis is the science of judging and evaluating the quality of a product by the use of human senses i.e. taste, smell, sight, touch and hearing **(Meilgaard *et al.*, 1991)**. The sensory characteristics of cheese that include appearance, texture and flavour are important criteria for acceptability of the cheese by the consumer. Sensory analysis is divided into two methods, analytical tests and consumer tests depending on the specific goal and sensory procedure selected. The most powerful analytical sensory test, descriptive sensory analysis that refer to a collection of techniques

that seek to discriminate between the sensory characteristics of a range of cheeses and to determine quantitative description of all the sensory differences that can be identified. Descriptive sensory analysis consists of training a group of individuals (generally 6 to 12) to identify and quantify specific sensory attributes or all of the sensory attributes of a food. Consumer tests are used when information on consumer liking and perception is desired (experts or trained panelists are NOT appropriate for determination of acceptability), and these tests require large numbers of consumers (at least 50) in order to obtain results that have any relevance to the consumer **(Lawless & Heymann, 1999 and Meilgaard *et al.*, 1999)**.

Apart from the use of sensory techniques, an alternative approach to the evaluation of texture properties involves the use of instruments specifically designed for the evaluation of the physical characteristics of foods. The instrumental evaluation of cheese texture has been studied by many researchers and several instrumental methods have been examined.

III.4. Rheology and Texture of Cheese

Rheology is the science of the deformation and flow of materials when subjected to a stress or strain **(Steffe, 1996 and Fox *et al.*, 2000)**. During mastication, food is cut by the incisors, compressed by the molars, and sheared between the palate and tongue. These mechanical processes subject food to a number of compressive and shear forces that reduce food to a size capable of being swallowed **(Fox *et al.*, 2000)**.

The rheological characterization of cheeses is important as a means of determining body and texture for quality and identity as a function of composition, processing techniques and storage conditions **(Karoui *et al.*, 2003)**. Rheological parameters of cheeses are determined to a great extend by proteolysis, lipolysis and

glycolysis during ripening. Rheological measurements are made using instruments such as rheometers, viscometers, pentratation tests and compression analyzers such as a Universal Testing Machine, these instruments introduce stresses and strains in the shear or normal direction, and also in small or large deformation modes.

Dynamic low amplitude strain testing offers very rapid results with minimal chemical and physical changes. Small strain dynamic rheological methods have been used to define both the elastic and viscous nature of cheese. Such information is useful to characterize and differentiate cheese varieties **(Tunick M.H., 1990).**Such methods are implemented within the linear viscoelastic region of the material and, therefore, are designed to be nondestructive to the basic structure of the material **(Gunasekaran & Ak, 2000)**. This method is used to determine the elastic or storage modulus (G'), viscous or loss modulus (G"), and Tan δ (=G''/G') **(Steffe, 1996)**.

Table 4: Tests for evaluation of cheese quality.

Chemical	Physical	Sensory	Compositional/ nutritional
Nitrogen solubility in different solvents Water, pH 4.6, trichloroacetic acid, tungstophosphoric acid Free amino acid analysis Petpide profiling, Reverse-phase HPLC, Gel electrophoresis, Size exclusion chromatography Organic acids Lactic, acetic, propionic Free fatty acid analysis	Rheological/texture properties *Large strain deformation (normal force)* Texture analyser/texture profile analysis (TPA), compression, bending, penetration, extensiometry, spreadability *Large strain deformation (shear force)* Torsion gelometry, shear stress, shear strain, shear rigidity, spreadability Low strain deformation *Rheometer/texture analyser* Elastic modulus, viscous modulus, loss tangent (phase angle) Viscosity Shredability/gratability Aggregation index, curd fines, image analysis, sliceability/ portionability Colour assessment (colorimeter) *Visual texture* Image texture analysis, hyperspectral imaging Microstructure Confocal laser scanning microscopy, scanning electron microscopy, transmission electron microscopy Eye features Image analysis, tomography Cooking characteristics Melt time, extent of flow, Schreiber, Arnott, Price Olson, image analysis, melt fluidity (loss tangent), stretchability	Cheese grading Triangle tests Descriptive sensory analysis Flavour and aroma, appearance, visual texture, tactile texture	Fat Protein Calcium Lactose Biogenic amines Sodium (salt) Calorific value

III.5. Colorimetry measurments

Colour is an important measure of quality in the food industry because it is considered by consumers to be related to product freshness, ripeness, desirability and food safety. Colour measurement instruments according to the International Commission on Illumination **(ICI, 1976),** transform or filter reflected spectra to produce reproducible colour space coordinates (Figure 6), namely, *L** (index of whiteness), *a** (index of redness), and *b** (index of yellowness). While colour measurements are normally carried out in a laboratory based instrument (Hunter Lab meter or Minolta Chroma meter), they can also be acquired by online instruments. Owing to ageing effects of light sources and detector systems, regular calibration of colorimetric equipment against colour standards is essential. Colorimetry is used routinely in quality control and product development to assess the colour of curd and cheese. Colour is related to diet of cow, addition of coloring and cheese variety. Recent studies also highlight the potential role of colorimetery in assessing ripening of smear-ripened cheese **(Olson *et al.*, 2006)** and for measuring defects, such as browning, during cheese maturation **(Carreira *et al.*, 2002)**.

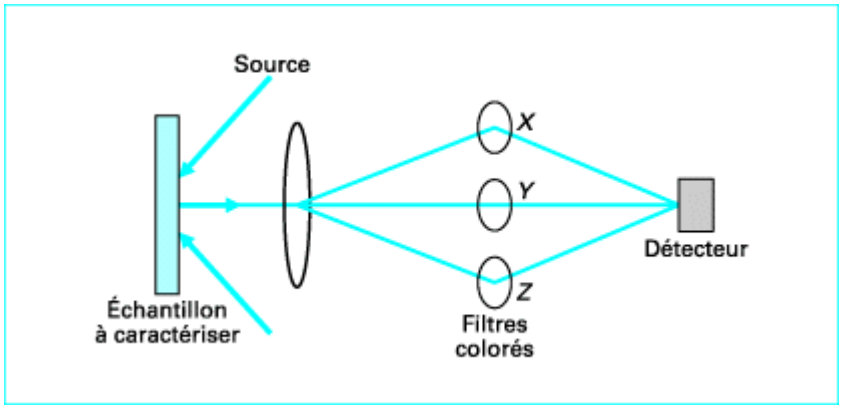


Figure 6: The operating principle of a colorimeter.

All the above-mentioned techniques are destructive, relatively expensive, time-consuming and require highly skilled operators. In recent decades, spectroscopic techniques have been used in many industries such as dairy products **(Karoui *et al.*, 2006b)**.

III.6. Spectroscopic Techniques

The development of rapid analytical methods for food products relies mainly upon two approaches: the use of physical properties of substrates as an information supply and the automation of chemical methods. The spectroscopic techniques are physical methods of characterization that can be a remarkably effective alternative to traditional analysis. They allow us to identify compounds and to observe the behavior of many complex systems. In recent years it has become increasingly clear that the application of spectroscopic methods (such as fluorescence spectroscopy frontal and infrared spectroscopy) to food analysis.

Spectroscopy can be split into two large groups **(Wilson, 1994)**: photonic spectroscopy, which is based on the study of the interaction of an electromagnetic wave with matter, and particle spectroscopy. The first group comprises spectroscopic methods exhibiting an analytical potential for rapid control. The second group is represented by mass spectrometry and derived methods. These new analytical techniques are relatively inexpensive and can be applied in basic research and online the plant to monitor milk products.

Spectroscopy is the study of the interaction of electromagnetic radiation with matter. The electromagnetic spectrum is generally divided as shown in Figure (7) in various regions depending on the wavelength of radiation: thus, we find that γ-rays are more energetic X-rays, ultraviolet, visible, Infrared (IR), microwave and radio frequency **(Lachenal, 2006)**. Each

region can associate a type of atomic or molecular transition involving different for different energies. Spectral regions, several of them being of interest for analytical purposes, can be defined as a function of wavelength (Figure 7):

● X-ray region (wavelengths between 0.5 and 10 nm) is involved in energy changes of electrons of the internal layers of atoms and molecules.

● Far-ultraviolet region (10–200 nm) is the zone corresponding to electronic emission from valence orbitals. In the near-UV region (200–350 nm), electronic transitions of the energetic levels of valence orbitals are observed. This spectral region is characterized by the absorption of peptidic bonds in proteins and of molecules presenting conjugated double bonds such as aromatic amino acids of proteins or vitamins such as vitamins A and E. In this wavelength range, luminescence (fluorescence and phosphorescence) may also be observed.

● The visible region (350–800 nm) is another zone where electronic transitions occur. Molecules exhibiting a large number of conjugated double bonds such as carotenoids, chlorophylls, and porphyrins absorb energy in this region. And their absorption properties may be used to evaluate the colour of food products.

● The near-infrared (NIR) region (800–2500 nm or 12500–4000 cm^{-1}) is the first spectral region exhibiting absorption bands related to molecule vibrations. This region is characterized by harmonics and combination bands and is widely used for composition analyses of food products.

● The mid-infrared (MIR) region (2500–25000 nm or 4000–400 cm^{-1}) is the main region of vibrational spectroscopy. This region retains information, allowing organic molecules to be identified and the structure and conformation of molecules such as proteins, polysaccharides, and lipids to be characterized. In general, the absorption of an infrared radiation corresponds to an energy change

ranging between 2 and 10 kcal mol $^{-1}$.

- In the microwave region (100 μm^{-1}), absorbed energy is related to molecule rotation. The radiofrequency region (1cm–10m) is the region investigated by nuclear magnetic resonance (NMR) and electron spin resonance.

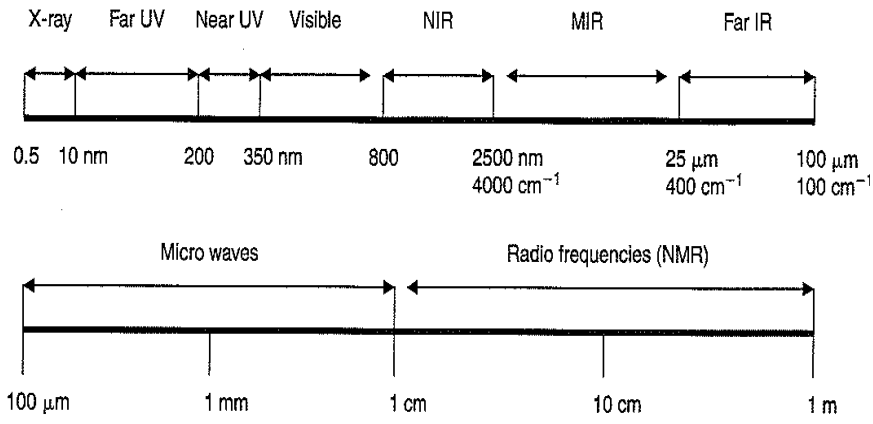


Figure 7: **Spectral regions of interest for analytical purposes (Sun, 2009).**

When matter is energized (excited) by the application of thermal, electrical, nuclear or radiant energy, electromagnetic radiation is often emitted as the matter relaxes back to its original (ground) state. The spectrum of radiation emitted by a substance that has absorbed energy is called an emission spectrum and the science is appropriately called emission spectroscopy. Another approach often used to study the interaction of electromagnetic radiation with matter is one whereby a continuous range of radiation (e.g., white light) is allowed to fall on a substance; then the frequencies absorbed by the substance are examined. The resulting spectrum from the substance contains the original range of radiation with dark spaces that correspond to missing, or absorbed, frequencies. This type of spectrum is called an absorption spectrum. In spectroscopy the emitted or absorbed radiation is usually analyzed, i.e., separated into the various frequency components and

the intensity is measured by means of an instrument called a spectroscope.

The resultant spectrum is mainly a graph of intensity of emitted or absorbed radiation versus wavelength or frequency. There are in general three types of spectra: continuous, line, and band. The sun and heated solids produce continuous spectra in which the emitted radiation contains all frequencies within a region of the electromagnetic spectrum. Line spectra are produced by excited atoms in the gas phase and contain only certain frequencies, all other frequencies being absent. Each chemical element of the periodic chart has a unique and, therefore, characteristic line spectrum. Band spectra are produced by excited molecules emitting radiation in groups of closely spaced lines that merge to form bands. These categories of emission and absorption spectra contain tremendous amounts of useful information about the structure and composition of matter. Spectroscopy is a powerful and sensitive form of chemical analysis, as well as a method of probing electronic and nuclear structure and chemical bonding. The key to interpreting this spectral information is the knowledge that certain atomic and molecular processes involve only certain energy ranges.

Infrared and fluorescent spectroscopy-based techniques have become increasingly important for a wide variety of analytical applications in biology and chemistry **(Lakowicz, 1983 and Ladokhin, 2000)**. This is due to great technical advances in both instrumentation and data analysis tools in the past two decades. Coupled with multivariate statistical tools such as principal component analysis (PCA), principal component regression (PCR), factorial discriminant analysis (FDA), and partial least squares (PLS) regression, infrared and fluorescent spectroscopies allow to extract information on composition and physicochemical properties of food systems in an accurate, high sensitive, and rapid manner **(Bertrand & Scotter, 1992 and Bro *et al.*, 2002)**.

One of the main advantages of these fast methods is their

ability to scan spectra directly on the cheese sample. In addition, the spectrum recorded on a given cheese is a fingerprint that contains information about its physico-chemical characteristics**(Herbert, 1999; Herbert *et al.*, 2000 and Lebecque *et al.*, 2001)**. Both mid-infrared and front-face fluorescence spectroscopies have been used for the characterization of cheese structure **(Dufour *et al.*, 2000 and Mazerolles *et al.*, 2001)**. In this part the infrared and fluorescence techniques are described.

III.6.1. Infrared spectroscopy

Infrared spectroscopy is defined as the study of the interaction of light with matter **(Bertrand, 2002)**. Infrared radiation (between 1-1000 µm) refers to energy in the region of the electromagnetic radiation spectrum at wavelengths longer than those of visible light, but shorter than those of radio waves. It is very commonly used in the field the Agri-Food and appears as a highly effective and often used online control production.

This band spectrum (1-1000 µm) is itself divided into three regions:

✓ Near infrared region (NIR) which is between 1 and 2.5 µm (800 and 2500 nm).

✓ Middle infrared (MIR) which is between 2.5 and 25 µm (4000-400 cm^{-1}).

✓ Far infrared region with wavelengths above 25 µm.

Mid Infrared spectroscopy (MIR)

Major food components are generally complex molecules resulting from the polymerization of monomers such as amino acids or carbohydrates. These monomers exhibit specific chemical groups such as carboxylic and amine functions in amino acids. As each chemical group may absorb in the infrared region (4000-400 cm^{-1}), it appears useful in a first step to clearly identify the characteristic absorption bands of these groups in the near- and mid-infrared regions. NIR and MIR are known as tools for

quantitative and qualitative analysis, a growing considerably in recent years **(Cadet, 2000)**.

The most informative part of the infrared spectrum is the MIR. The absorption bands observed in the MIR are mainly associated with fundamental vibrations of valence bond (ν) of functional groups in a molecule. The spectra MIR many molecules are already known. The assignments of spectral bands in the MIR are described by **(Grappin *et al.*, 2006)** (Table 5).

Water is a very strong infrared absorber with prominent bands centered at 3360 cm-1(H–O stretching band), at 2130 cm^{-1} (water association band) and at 1640 cm^{-1} (the H–O–H bending vibration) **(Safar *et al.*, 1994)**.

Carbohydrates are other important molecules found in food products. The MIR spectra of carbohydrates show four main zones of absorbance. At about 3220 cm^{-1}, an intense band resulting from the O–H bond stretching of glucose is observed. The C–H bond shows asymmetric and symmetric bending bands at 1470 cm^{-1} and 1380 cm^{-1}, respectively. Bands assigned to C–O and C–C vibrations are observed at about 1100 cm^{-1}. In the region close to 920 cm^{-1} two vibrations of C–O–C asymmetric stretching corresponding to α and β anomers are observed.

Table 5: Assignment of spectral bands in the mid-infrared to the main components of milk.

Wave number in cm^{-1}	Liaison	Fonction group	Vibration mode	Milk Component
2 955	C-H	$C-H_3$	stretching	fat
2 924	C-H	$C-H_2$	stretching zasymétrique	Fat
2 872	C-H	$C-H_3$	symmetric stretching	Fat
2 854	C-H	$C-H_2$	asymmetric stretching	Fat
1 746	C = O	ester carbonyle	stretching	Fat acides
1 651	C = O	amide I	stretching	protein
1 548	N-H	amide II	stretching	
	C-N		stretching	
1 470-1 446	-CH	CH_2	deformation	Fat
	-CH	CH_3	deformation	Fat
1 243-1 100	-C(O)-O-	Ester	stretching	Fat
	C-O		stretching	Fat
1 112-1 050	C-O	alcohol I	stretching	lactose
	O-H		deformation	

In the field of food, MIR spectroscopy has been used less for line measurement that the PIR. This is explained by the fact that water is a major constituent of these products and contributes strongly to the MIR spectrum.

Most of the spectral information useful of the analysis of the mid-infrared spectra is located in:

(1) Spectral region (3000-2800 cm^{-1}) corresponding to C-H band of methyl (CH_2) and methylene (CH_3), CH groups of fatty acids,

This area is very interesting for the identification and determination of fatty acids.

(2) the region (1700-1500 cm^{-1}) corresponding to amide I (around 1650 cm^{-1}) and amide II (around 1550 cm^{-1}) bands related to peptides bonds of protein.

The amide I band is mainly due to elongation of C=O group. The amide II band characterized mainly a combination of swing out of phase in terms of N-H group and C-N stretching. The

swing in the plane of the group C=O and the stretching CC and CN contribute little to the amide II band. The amide III band located between 1200-1400 cm^{-1} is complex and side chains of amino acids of proteins present contributions in this area.

(3) the region (1500-900 cm^{-1}) is called the fingerprint region, in this region, many chemical bonds absorb.

Generally in dairy products, the absorption of OH bonds are used for the determination of water and lactose, CH for the determination of fat and NH for protein determination. In mid infrared, a spectrum is representative of the absorption of all the chemical bonds having an infrared activity between 3000 and 900 cm^{-1}. Some parts of the spectrum are known to be mainly representative of one kind of chemical bond. For instance, the acyl chain (C-H) of fatty acids is mainly responsible for the absorptions observed between 3000 and 2800 cm^{-1}, whereas the peptidic bond C–NH is mainly responsible of the absorptions occurring between 1700 and 1500 cm^{-1}. Most of the absorption bands in the MIR region, but not in the NIR region, have been identified and attributed to chemical groups. These regions of the mid infrared spectrum, provide information on the characteristics of fats and proteins and on the environment of the chemical groups in biological samples.

The infrared bands appearing in the 3000–2800 cm^{-1} region are particularly useful because they are sensitive to the conformation and the packing of the phospholipid acyl chains**(Unemura *et al.*, 1980; Casal & Mantsch, 1984 and Mendelsohn & Mantsch, 1986)**. For example, the phase transition of phospholipids (sol to gel state transition) can be followed by MIR spectroscopy: increasing temperature results in a shift of the bands associated with C–H (2850, 2880, 2935, and 2960 cm^{-1}) and carbonyl stretching mode of the phospholipids.

The development of Fourier transform infrared (FTIR)

spectroscopy in recent years have given the possibility of obtaining unique information about protein structure and protein–protein and protein–lipid interactions without introducing perturbing probe molecules **(Casal & Mantsch, 1984)**. Also, the development of the technique of attenuated total reflection (ATR) has proven very useful for the acquisition of MIR spectra of solid food products, opaque and viscous samples, simply spread on a slide crystalline zinc selenide (ZnSe), silicon (Si) or germanium (Ge) (Figure 8).

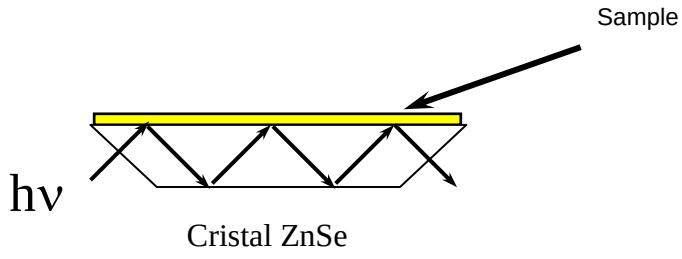


Figure 8: The principle of the attenuated total reflection (ATR) technique showing the penetration of the radiation beam into the sample material pressed closely to the crystal.

Several studies have demonstrated the potential of MIR for the characterization of cheeses. **(McQueen *et al.*, 1995)** have used mid-infrared for the prediction of protein, fat and dry matter content of 24 samples of cheese. **(Mazerolles *et al.*, 2001)** followed, through the mid-infrared modifications of the protein structure of the cheese during its ripening. Moreover, **(Karoui *et al.*, 2006a)** used for the determination of chemical parameters of Emmental cheese production in winter. Moreover, **(Picque *et al.*, 2002)** showed by using infrared spectroscopy coupled with chemometric techniques it was possible to discriminate between Emmental cheeses from different geographical origins. Finally, **(Dufour *et al.*, 2000)** have used the MIR spectra for extracting information about the physical state of triglycerides and structure of fat cheeses.

III.6.2. Fluorescence spectroscopy

Principle of fluorescence

Absorption of light by molecule causes the excitation of an electron moving from a round state to an excited state. After the electron has been excited, it rapidly relaxes from the higher vibrational states to the lowest vibrational state of the excited electronic state. After reaching the lowest vibrational state of the excited electronic state, the excited state may decay to the ground state by the emission of a photon (fluorescence). Due to energy losses, the emitted fluorescence photon always carries less energy than the absorbed photon **(Genot *et al.*, 1992a)**.

Fluorescence is the result of a three-stage process that occurs in certain molecules called fluorophores. A fluorescent probe is a fluorophore designed to localize within a specific region of a biological specimen or to respond to a specific stimulus. The process responsible for the fluorescence of fluorescent probes and other fluorophores is illustrated by the simple electronic-state diagram (Jablonski diagram) shown in Figure (9).

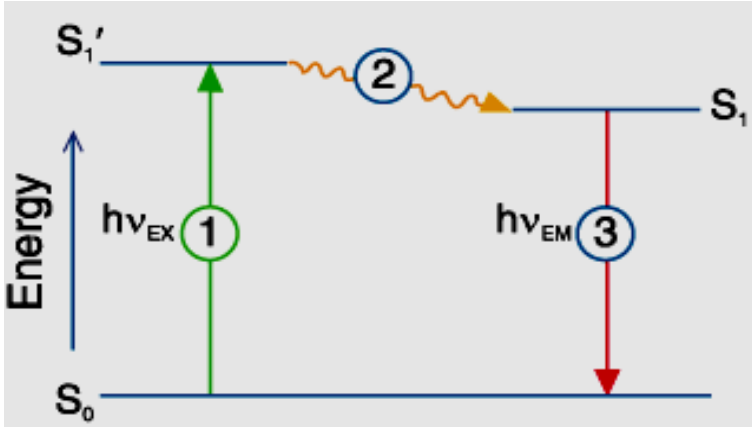


Figure 9: Excitation of molecules and emission of fluorescence or phosphorescence.

Stage 1: Excitation. A photon of energy hνEX is supplied by an external source such as an incandescent lamp or a laser and absorbed by the fluorophore, creating an excited electronic singlet state (S_1').

Stage 2: Excited-State Lifetime. The excited state exists for a finite time (typically 1–10 nanoseconds). During this time, the fluorophore undergoes conformational changes and is also subject to a multitude of possible interactions with its molecular environment. These processes have two important consequences. First, the energy of S_1' is partially dissipated, yielding a relaxed singlet excited state (S_1) from which fluorescence emission originates. Second, not all the molecules initially excited by absorption (Stage 1) return to the ground state (S_0) by fluorescence emission. Other processes such as collisional quenching, Fluorescence Resonance Energy Transfer (FRET) and intersystem crossing may also depopulate S_1. The fluorescence quantum yield, which is the ratio of the number of fluorescence photons emitted (Stage 3) to the number of photons absorbed (Stage 1), is a measure of the relative extent to which these processes occur.

Stage 3: Fluorescence Emission. A photon of energy hνEM is emitted, returning the fluorophore to its ground state S_0. Due to energy dissipation during the excited-state lifetime, the energy of this photon is lower, and therefore of longer wavelength, than the excitation photon hνEX. The difference in energy or wavelength represented by (hνEX – hνEM) is called the Stokes shift. The Stokes shift is fundamental to the sensitivity of fluorescence techniques because it allows emission photons to be detected against a low background, isolated from excitation photons. In contrast, absorption spectrophotometry requires measurement of transmitted light relative to high incident light levels at the same wavelength.

Fluorescence Spectra

Fluorescence is unique among spectroscopy techniques, because it is multidimensional. Two spectra (i.e. excitation and emission spectra) are available for identification of a certain compound instead of one (e.g. absorption spectrum). In conventional fluorescence spectroscopy, two basic types of spectra are usually measured. When a sample is excited at a fixed wavelength λex, an emission spectrum is produced by recording the emission intensity as a function of the emission wavelength λem. An excitation spectrum may be obtained when λex is scanned while the observation is conducted at a fixed λem. In food analysis, the emission spectra at a particular λex are typically studied. When a set of emission spectra at different λex is recorded, a three-dimensional landscape is obtained, the so-called fluorescence excitation-emission matrix (EEM). Recording EEMs (total excitation-emission matrix luminescence spectroscopy) enables to obtain more information about the fluorescent species present in the sample, because the bands arising in the wider axes are considered. The broad nature of conventional fluorescence spectrum and spectral overlap can be overcome and enhanced selectivity can be obtained using synchronous fluorescence scan (SFS). In SFS, the λex and λem are scanned simultaneously (synchronously), usually maintaining a constant wavelength interval, Δλ, between λex and λem. Besides the spectral overlap, the inner-filter effect, scattered light, and reflected light can also limit the applicability of conventional right-angle fluorescence spectroscopy (Figure10) under certain conditions, e.g. high concentrations of the fluorescent species. To avoid these problems, the method of front-face fluorescence spectroscopy can be used for bulk liquid samples and solid samples **(Genot *et al.*, 1992a; Genot *et al.*, 1992b; Sóti *et al.*, 1993; Herbert *et al.*, 2000 and Patra & Mishra, 2002)**. The incidence angle of the excitation radiation is usually set at 56°. Often, several spectra of the same sample are recorded to verify

reproducibility, and the average of those spectra is computed and used afterward. No special smoothing algorithms are needed when the multivariate data approach is used **(Norgaard, 1995)**.

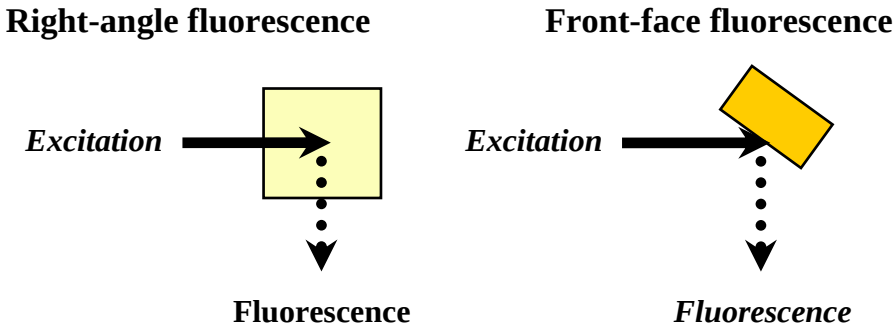


Figure 10: Right-angle and front-face fluorescence spectroscopy.

Fluorescence probes or fluorophores represent the most important area of fluorescence spectroscopy. Fluorophores can be broadly divided into two main classes: intrinsic and extrinsic. Dairy products contain a lot of 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 low or very low concentration in food. The fluorescent properties of aromatic amino acids of proteins **(Longworth, 1971; Lakowicz, 1983 and Dufour *et al.*, 1994)** can be used to study protein structure or protein–hydrophobic molecule interactions **(Dufour *et al.*, 1994)**. The six major proteins of milk, αs_1-αs_2-, β- and k-CN, β-lactoglobulin (b-LG) and α-lactalbumin (a-LA), contain at least one tryptophan residue **(Fox, 1989)**, the fluorescence of which allows the monitoring of the structural modifications of proteins. Extrinsic fluorophores are added to the sample to provide fluorescence when none exists or to change the spectral properties of the sample. Indeed, there are many instances when the molecule of interest is

non-fluorescent or when the intrinsic fluorescence is not adequate for the desired experiment.

In several studies of dairy products fluorescence emission spectra of tryptophan have been investigated as an indicator of the protein structure. Tryptophan is an essential amino acid with a well-characterized fluorescence response. Indeed, the emission spectra of tryptophan residues were recorded at 305-400 nm, with the excitation wavelength at 290nm **(Herbert *et al.*, 2000)**. The fluorescence properties of tryptophan, along with its chromophore moiety-indol ring, have been studied extensively due to its use as a standard optical probe for protein structure and dynamics **(Ladokhin, 2000)**.

Solid fat content is an important quality control parameter in the edible fats and vitamin A located in the core of fat globules can be a good fluorescent probe for the determination of this parameter. The excitation spectra of vitamin A recorded between 250 and 350 nm with the emission wavelength set at 410 nm **(Dufour & Riaublanc, 1997)** provide information on development of protein-fat globule interactions during milk coagulation (Herbert, 1999). In addition, it has been shown that the shape of the vitamin A excitation spectrum is correlated with the physical state of the triglycerides in the fat globule **(Dufour *et al.*, 1998)**. A combination of retinol fluorescence (Vit A) and indole fluorescence (Try) has been applied in several studies of cheese. the common fluorescence signal was found to correlate with the cheese type, as well as with the structure of soft cheese **(Herbert *et al.*, 2000)**.

Riboflavin, also known as vitamin B_2, is a water soluble vitamin that occurs naturally in foods containing other members of the B_2 complex. It has a strong and broad fluorescence emission peak in the region 525-53 nm **(Karoui *et al.*, 2007c)**. Riboflavin is stable to heat, oxidation, and acid, but unstable in the presence of alkali or light, especially ultraviolet light. The vitamin is photo-chemically degraded into different forms, i.e., lumichrome and

lumiflavin. These two compounds are also fluorescent with emission maxima in the regions 444 to 479 nm and 516 to 522 nm, respectively. The emission spectra of riboflavin are recorded in the zone ranging between 400 and 640 nm with the excitation wavelength set at 380 nm. The reduction of the fluorescence at about 525 nm might reflect the reduction of riboflavin content. Finally, NADH can be mentioned. The fluorescence emission spectra of NADH show two maxima observed at about 414 and 438 nm following excitation at 336 nm **(Dufour *et al.*, 2003)**.

Fluorescence spectroscopy has also been applied with some success to measurements of cheese quality **(Karoui & Dufour, 2003)**. Near-infrared (NIR) spectroscopy has been applied to cheese analysis **(Rodriguez-Otero *et al.*, 1997)**, with considerable success. NIR spectroscopy enjoys considerable advantages over these other techniques in terms of ease of sample handling and preparation prior to spectral acquisition. Specifically, it has been applied to the determination of moisture **(Wehling & Pierce, 1988)**, total solids **(Rodriguez-Otero *et al.*, 1997)**, fat **(Pierce & Wehling, 1994)**, protein and lactose **(Molt *et al.*, 1993)** in cheese. More recently, **(Sørensen & Jepsen, 1998)** reported its application to the assessment of selected sensory properties in semi-hard (Danbo) cheese.

IV. Factors Affecting the Cheese Quality

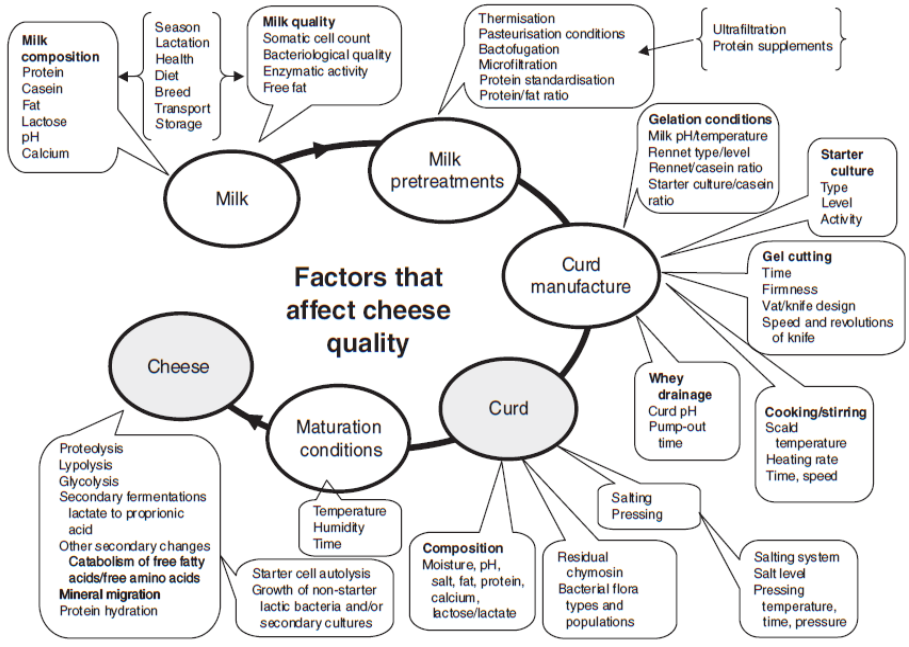
In order to be able to fully understand what determines cheese quality and texture, it is necessary to have an understanding of the physical and chemical mechanisms that occur during cheese processing. Generally, the components and processing techniques are basically the same for all cheeses, but the proportions of these components are varied. Figure (11) summarize the factors affecting cheese quality.

IV.1. Chemical Composition of Milk

The quality of cheese is influenced by many aspects of milk quality: milk composition, microbiology, somatic cell count (SCC), enzymatic activity, and chemical residues (Figure 11). Perhaps the single most important factor affecting cheese quality and yield is the composition of the milk, particularly the concentrations of fat and casein. These factors together with calcium and pH, have a major influence on several aspects of cheese manufacture, especially rennet coagulability, gel strength, curd syneresis and, hence, cheese composition and cheese yield.

The composition of milk supplied to the cheese factory is influenced many factors including species, breed, individuality, nutritional status, health and stage of lactation. Milk standardization gives the producer the ability to manipulate the composition of the final cheese by controlling the composition of the starting milk in order to meet the legal definition of the specific variety and to improve yields. There are several ways in which milk is standardized **(Lucey & Kelly, 1994)**. However, cheese composition can still vary due to variations in milk, which cannot be corrected readily by the manufacturer, for example differences in casein micelle size, in levels of individual caseins, genetic polymorphs of individual caseins, degrees of phosphorylation of αs-casein and glycosylation of k-casein, colloidal-calcium-to-casein ratio; levels of enzymatic activity and levels of hydrolysed serum- soluble casein degradation products. Hence, cheese quality is not only affected by the concentration of major cheese making constituents in milk, but also by the intactness and composition of individual caseins, the integrity of casein structural unit (casein micelle) and its equilibrium with the milk salts.

Figure 11: Overview factors affecting cheese quality.



IV.2. Cheese-making process

The manufacturing of cheese follows five basic steps: acidification, coagulation, dehydration (cutting, cooking, stirring, pressing, shaping, molding, pressing), salting, and ripening. These are discussed below.

IV.2.1. Thermization and milk Pasteruization

Thermization refers to the heat treatment of milk at sub-pasteurization temperatures (typically 50–70°C for 5–30s) on reception at the dairy to reduce the viable bacterial load in the milk and minimize changes in quality and processability prior to conversion into product. This greatly reduces the development/occurrence of bacterial-associated enzymatic activities in the milk during subsequent cold storage, as reflected by lower levels of peptides and FFAs in the stored milk. Consequently, thermization generally improves the yield and quality of cheeses

prepared from milks that have been cold stored.

Pasteurization involves heating at temperatures sufficient to inactivate the most heat-resistant pathogenic bacteria that may be potentially present in the raw milk (i.e. *Mycobacterium tuberculosis* and *Coxiella burnetii*), and to thereby make it and its products safe for human consumption **(Kelly *et al.*, 2005)**. It typically involves heating at 72–75°C for 15–30s in a continuous flow plate heat exchanger. Other pathogens that may occur in milk (e.g. *Listeria monocytogenes*, enterotoxigenic of *Escherichia coli*, e.g. *E. coli* O157 H7, *Shigella*, *Erwinia*, *Campylobacter*, *Staphlyococcus*, and *Salmonella* spp.) are also inactivated by pasteurisation. In addition, pasteurisation also eliminates non-pathogenic indigenous microflora (e.g. lactic acid bacteria), and causes partial/complete inactivation of indigenous/microbial enzymes, which otherwise contribute to the development of more diverse and regionally desired flavour and aroma profiles in raw milk cheese compared to their pasteurized milk equivalents **(Hickey *et al.*, 2007)**. Hence, substantial quantities of cheese (guesstimated at 5–10% of total cheese) continue to be manufactured from raw milk (especially in France, Germany, and Southern European countries). This is acceptable provided that the cheese is aged for a minimum of 60 days, and is in compliance with Public Health Authority Regulations and Standards, for example EU (1992). Many of these cheeses are hard, low moisture (38g 100 g^{-1}) cheese varieties (Swiss Emmental, Gruyere, Comte) the manufacture of which conforms to modern hygiene practices, involves heating the curd and whey to relatively high temperatures (50–55°C), transferring the curds while hot into the cheese moulds/forms, and slow cooling **(Fox & Cogan, 2004)**. Such conditions and the composition of the cheeses (e.g. relatively low pH, 5.3; low moisture, ~ 38 g 100 g^{-1}; pH, 5.4, and/or salt content, 2–10g 100 g^{-1} in moisture phase) are unfavourable to the growth of pathogenic bacteria.

Apart from its effects on pathogenic bacteria,

pasteurization affects cheese composition, texture, and yield to an extent dependent on the temperature used. Pasteurization of milk (72°C for 15s) results in a low level (≤ 5% of total) denaturation of whey proteins, which complex with the k-casein, and are retained in the cheese curd where they contribute to a Cheddar cheese yield increase of ~ 0.1–0.4 g 100 g^{-1} milk**.**

IV.2.2. Standardization of protein-to-fat ratio

Bovine milk varies considerably in its composition and in the relative proportions of fat and protein throughout the cheese making season, owing to factors, such as breed, stage of lactation, diet, environment and season. Consequently, milk for cheese making is standardized by adjusting the protein-to-fat ratio (PFR) and/or by increasing the protein level (milk protein standardization) so as to offset the effects of the naturally occurring variation in milk composition on product composition and quality, and to conform to end product specifications.

Increasing the PFR increases the levels of cheese moisture, protein, Ca, and P, but significantly reduces the levels of moisture in non-fat substances (MNFS), FDM, and salt in moisture (S/M). The opposite effects of PFR on MNFS and moisture in cheese reflect the depressive effect of milk fat (globules) on the permeability and syneresis of the rennet milk gels on one hand, and the dilution effect of fat on the volume fraction of moisture and protein in the cheese on the other. Owing to the impact of cheese composition on the texture, sensory properties, and quality, it is obvious that standardization of PFR of the cheese milk is essential to optimize quality and consistency. In addition, the recovery of fat decreases as the PFR is reduced, an effect attributed to a dilution effect of the protein matrix of the gel (curd) at the higher fat levels, which attenuates the ability of the protein matrix to retain occluded fat globules during gel cutting and stirring and curd handling. Conversely, the recovery of water from milk to cheese increases, as

do the actual and moisture-adjusted cheese yields, both effects due to the concomitant increase in the fat content (and, hence, cheese making solids) as the PFR is reduced.

IV.2.3. Structure formation via coagulation

In the manufacture of rennet-curd cheeses (Figure 12), the milk is typically set with the quantities of starter cultures and rennet being added to the milk. However, such a practice may lead to variations in the gel firmness at cutting, acidification rate during manufacture, composition of the curd and quality of the resultant cheese, especially when using milk displaying seasonal variations in milk composition (pH calcium, and especially protein). To minimize such variations and ensure more consistent composition and quality, rennet and starter cultures should be added at levels that kept *pro-rata* with the level of milk protein.

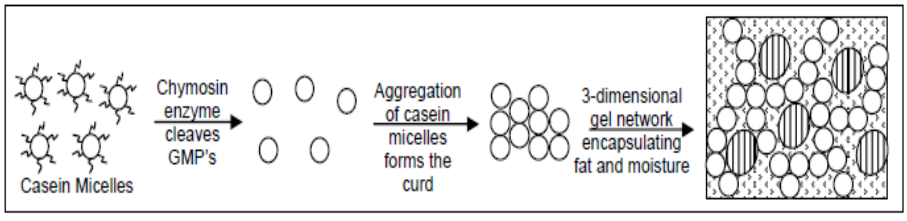


Fig.1.2: Renneting process of casein micelles (◯) for coagulation and gel network formation, encapsulating fat (▯) and moisture (⁘).

Figure 12 : Renneting process of casein micelles for coagulation and gel network formation, encapsulating fat and moisture.

Reduction in the rennet-to-casein ratio (i.e. mg rennet g^{-1} casein) have been found to reduce the extent of increase of salt (4 g NaCl 100 g^{-1}) soluble protein and the extent of degradation of αs1-casein in Gouda cheese during storage. Consistent with these trends are the decreases in the degree of proteolysis in Cheddar cheese made from milks where the protein content has been increased

without increasing the level of added rennet **(Guinee *et al.*, 1994)**.

IV.2.4. Post-coagulation

After the curd has coagulated, it is cut into smaller, cube-shaped pieces in order to expel excess moisture and whey from the protein network. The curd particles are usually stirred in the increasing volume of expelled whey for a predetermined length of time during which the majority of syneresis takes place, even though the rate of syneresis decreases with time. Hence, in commercial manufacture the whey is drained off (pumped out) after a given time, e.g. typically at 90 min after cutting in the case of Cheddar cheese. The drained whey accounts for ~80 mL 100 mL^{-1} of the milk volume, with further whey being removed during moulding, pressing and/or dry-stirring operations. The extent of cutting determines the size of curd particles, which is inversely related to the velocity of whey exudation, and directly related to moisture content of the final curd. In addition, smaller curd particles provide more surface area for syneresis, which together with the increased velocity of whey release, increases the rate of syneresis. Consequentially, smaller curd particles shrink more rapidly than larger ones.

After cutting, the curds are left alone for a short period of time known as a healing period. During the healing period, a "skin" forms on the outside of the curd, which prevents further losses of fat and moisture. The curds are then stirred and cooked in order to expel moisture and to promote shrinkage of the protein network. After cooking, whey is drained from the curds. The pH of the whey determines the proportions of chymosin and plasmin retained in the cheese **(Fox, 1989)**. Both of these have the capability to cause breakdown of the protein network during storage, which greatly impacts the final texture of the cheese.

A number of steps may now occur depending upon the variety of cheese being made. For example, curds are salted and

matted together in a process known as cheddaring when making Cheddar cheese. This process makes the protein network denser, resulting in a characteristic texture. In Mozzarella cheese, the curds are immersed into hot water, which acts as a plasticizer. The curds are stretched and kneaded, causing the protein network to have a specific orientation; this also, results in the characteristic fibrous texture. When Mozzarella is torn, as is typical in the consumption of "string cheese", the orientated protein matrix breaks, and the water and fat in the matrix is expelled **(Taneya *et al.*, 1992)**. It is at this point that the altered curds are typically pressed into molds. If the curds are not salted, the molds may be soaked in brine. Salt influences textural changes as the cheeses age. Such changes are most apparent in brined cheeses, where immediately following molding, cheeses are immersed in a sodium chloride solution. Osmotic pressure differences exist between the cheese and the brine, causing the sodium chloride to migrate into the block; simultaneously, in order to maintain equilibrium osmotic pressure, moisture from the cheese block exits into the brine **(Guinee & Fox., 1987)**.

IV.3. Cheese ripening and maturation

The cheese is now ready to be stored. Typically, as they age, a decrease in firmness (or softening) of the cheese body occurs. The degradation of the protein matrix is thought to cause this change since the lipid phase is discontinuous and would, therefore, not contribute as much to the overall consistency of the body. Two phases of texture development during storage have been identified. Phase one occurs within the first seven to fourteen days after production. During this time, the rubbery texture of the young cheese is converted into the more smooth characteristic texture of the specific variety. It is believed that during this phase, proteolysis of the casein network is taking place. Hydrolysis by residual coagulant of about 20% of the αs_1-casein, which produces the αs_1-I peptide, causes a weakening of the casein network **(Lawrence *et***

***al.*, 1987)**; specifically the Phe_{23}-Phe_{24} or Phe_{24}-Val_{25} bonds are most susceptible to hydrolysis by residual enzyme **(Fox, 1989)**. The αs_1-I peptide is present in all cheeses during the early stages of ripening. A more gradual change in cheese texture occurs during phase two of ripening. It is during this time period that the rest of the αs_1-casein and the other caseins are hydrolyzed. Unlike phase one, which takes only days, phase two occurs over a period of months **(Lawrence *et al.*, 1987)**. However, it has been shown that the β-casein does not change as much during ripening as αs1-casein **(Creamer & Olson, 1982)**.

IV.4. Effect of cheese composition

The composition of cheese has a marked influence on all aspects of quality, including sensory properties, texture, and cooking properties. This trend is consistent with the effects of composition on the extent of calcium solubilization, protein hydration, enzyme activity, glycolysis, proteolysis, lipolysis, and microbiology. Cheeses having a higher fat content are less firm and more elastic; the recently popular low fat cheeses are firmer and less smooth due to the increase in the amount of protein matrix (and lack of lipid filler). Likewise, the level of protein directly affects the firmness of the cheese; the more protein the cheese has initially, the firmer the cheese is. It has also been shown that small variations in water content greatly affect firmness; water content is affected by cheese making conditions and by surface evaporation during ripening **(Addeo, 1992)**.

MATERIALS AND METHODS

MATERIALS AND METHODS

I. Materials

I.1. Cheese Samples

Two different French cheeses, Cantal (hard) and Saint-Nectaire (semi-hard) cheeses were considered in this study. They were supplied directly from three different manufacturers located in the Massif Central area of France, weighing between 2-3 kg. They were taken on different manufacturing process and at different aging times.

Twenty four different samples of Cantal cheeses were collected according to (1) their age times; 30, 120 and 200 days-old cheeses, (2) heat treatment of milk (raw, thermized, and pasteurized).

Twenty four different samples of Saint-Nectaire cheeses ripened for 30 days were obtained according to their manufacturing methods; 12 samples industrial and 12 samples traditional cheese making.

1.2. Cheese Sample Preparation

I.2.1. Chemical analysis

In order to make them uniform, we take 2 quarts in the center of the cheese (2 mm under the crust) and about 900 g was grated to yield particles of 1 mm as described by **(Guinee, 2002)**. Mix the crushed and stored in an airtight container. Freeze the sample until analysis.

I.2.2. Rheological measurements

A slice of cheese (2 cm thick) was taken at the center of the cheese and slices were cut into thin discs (2 mm thick and 20 mm in diameter) using of a cheese slicer. The sliced samples were placed into plastic bags to prevent dehydration and stored at 5ºC

until analysis.

I.2.3. Spectral measurements

A slice of 2 cm thick was taken at the center of the cheese and then cut into samples for (2 cm x 1 cm x 0.3 cm) and placed in a quartz cell which is then placed in an accessory with of a spectrofluorimeter.

II. Methods of analysis

II.1. Physicochemical Analysis

Grated cheese samples (900 g) were analyses for pH, dry matter, fat, protein, water-soluble nitrogen (WSN), Total nitrogen (TN,) ash, total Ca, total P and NaCl contents were determined according to French standard **(AFNOR, 2004)** as follows and all the analyses were done in triplicate and the results reported as mean ± standard deviation:

II.1.1. pH value

The pH was measured using a pH meter CG840 (Schott, Paris, France) calibrated at 20°C. Three measurements were made by inserting the electrode in 10 g of grated cheese dispersed in 50 ml of distilled water.

II.1.2. Moisture content

Moisture content in cheese was estimated by using the thermogravimetric method by drying 3 g of cheese in an oven at 103ºC for 24 h. After cooling in a desiccators containing a desiccant for a period of 45 minutes, the dry samples were weighed and moisture subtraction calculated according to French standard (**AFNOR**, **2004**) (AFNOR: NF EN ISO 5534).

II.1.3. Fat content

Fat content was estimated using a method of acid-butyrometric Gerber according to French standard **(AFNOR, 2004)**

(AFNOR: NF V04-287) by weighing 3 g of sample prepared in a butyrometer cheese. Add concentrated sulfuric acid (p20 = 1.522g/ml) to a height equal to 2/3 of the chamber of butyrometer. Place butyrometer in a water bath at 65°C for 1 h. Shake vigorously for 10 min until completely dissolved proteins. Remove butyrometer from the water bath and add 1 ml of amyl alcohol. Stir at least 3 sec and add concentrated sulfuric acid until the solution reaches the graduation mark located 35% of the scale. Replace butyrometer for 5 min in a water bath (65°C) and centrifuge for 10 min butyrometer. Replace butyrometer still for 5 min in a water bath prior to reading.

II.1.4. Total nitrogen content

The concentration of total nitrogenous matter was obtained in two steps: a first step, the cheese was mineralized with concentrated sulfuric acid at 400°C, and total nitrogen content in the digest was determined according to Kjeldahl method **(AFNOR, 2004)** (French standard NF En ISO 8968-1) with a mineralizing model K370 and automatic distillation unit model K370 (Buchi, Flawil, Switzerland). The nitrogen content was then converted into protein using the conversion factor 6.38.

II.1.5. Water soluble nitrogen content

Water Soluble nitrogen (WSN) was obtained in several steps. First, the extraction of soluble nitrogen in water by weighing 3 g of cheese sample, put in a stomacher bag, add 50 ml of distilled water and stirring for 5 minutes. The resulting homogenate was immersed in a water bath at 40°C for 60 min and shake again to stomacher for 5 min. Centrifuge the mixture at 1200 at 4°C for 30 min. The mixture was then filtered through filter paper holding 30 microns. The filtrate (20 ml) was mineralized as described in the total nitrogen. Nitrogen in the digest was determined according to the Kjeldahl method as for total nitrogen **(AFNOR, 2004)** (AFNOR: NF EN ISO 8998-1).

II.1.6. Ash content

The ash content was determined using the method of incineration of a sample (5 g) in a muffle furnace at 550°C for 6 h. After cooling in a desiccator for a period of 45 minutes, the% of total ash content is calculated on a dry basis according to French standards **(AFNOR, 2004)** (AFNOR: NF ISO 8070).**II.1.7. Mineral contents**

The content of minerals was determined from the ashes. The content of minerals was obtained in different stages, first dissolve the ash obtained from 1 ml of 25% nitric acid and then follow the protocol used for determining the content of Na, total Ca and total phosphorus as fellows **(AFNOR, 2004)** (AFNOR: NF ISO 8070).

II.1.7.1. Total Calcium content

The calcium content was determined by atomic absorption spectrometry after dissolving the ash using the spectrometer Varian Spectr AA 220 FS (Varian SA, Les Ulis, France) as described by IDF **(IDF, 2003)**.

II.1.7.2. Total Phosphorus content

Phosphorus content was determined from the ash by a calorimetric method using a spectrometer UV/visible (Model Secoman, Jonior, Secoman, Paris, France) according to AOAC **(AOAC, 1995)**. Organic matter in cheese is destroyed at temperature high and phosphorus is converted to inorganic orthophosphate with ammonium molybdate ammonium formed phoshopomolybdate with colour blue and then the optical density is measured by spectrometer à 436 nm.

II.1.7.3. Chloride content

Chloride content was determined according to French standard (AFNOR: NF ISO 5843) using an automatic titrator

(TitroLine easy, Model III, Schott, France) which is based on the Volhard titrimetric test according to Marchall method **(Marshall, 1993)**. The content of chloride is expressed in grams of NaCl per kg of cheese.

II.2. Colour Measurements

Cheese colour was determined using a colorimeter CR-400 (Konica Minolta, Tokyo, Japan). The *L*, a*, and b** colour measurements were determined according to the CIELAB colour space **(CIE, 1976)** with reference to D_{65} (natural daylight, the colour warmth of 6500 K) and observation angle 10°. The following parameters were determined; L* (lightness or whiteness; L*=0 for black and L*=100 for white colour), a*(red-green components, - a*=greenness and + a*= redness) and *b** (yellow-blue components, - b*= blueness and +b*=yellowness). The colorimeter was calibrated with a white standard plate 3.5 cm thick layer (X = 0.3155, Y =0.3319, Z=94.0) before the measurements. Colour measurements were made 5 times, 1 on the middle and 4 on different parts of cheese surface after removing a 0.5 cm layer of upper surface.

II.3. Texture analysis

II.3.1. Rheological analysis

The evaluation of the cheeses' textural properties were determined by dynamic small-amplitude oscillatory rheometer (CP 20, TA Instrument, Guyancourt, France) equipped with a Peltier plate geometry of 20 mm diameter (upper plate) and a Peltier plate (lower plate) that provided very accurate and rapid temperature control of the sample. The cheese discs (2 mm thick and 20 mm diameter) were attached to the upper plate. The upper plate was lowered to contact with the cheese discs such that the normal force did not exceed 1N. The tests were started after the normal force readings were relatively stable at ~1N. Oscillation analyses were

performed in the linear viscoelastic region by applying a constant force of 0.1 N and a constant frequency of 1 Hz and the temperature was fixed at 20°C according to **(Karoui *et al.*, 2003c)**. The parameters measured were the elastic component G' (storage modulus), the viscous component G" (loss modulus), Tan δ (=G"/G'), and complex viscosity (η*).

All analyses were made in triplicate for the same cheese to ensure repeatability. Data were collected and rheological parameters were calculated using TA instrument software programme.

II.3.2. Fluorescence Spectroscopy

Fluorescence spectra were recorded using a Fluorolog-2 spectrofluorimeter (Spex-Jobin Yves, Longjumeau, France). The emission spectra of the tryptophan residues (305-400 nm) and riboflavin (400-640 nm) were recorded with the excitation wavelength set at 290 and 380 nm. The excitation spectra of vitamin A (250-350 nm) was recorded with the emission wavelength set at 410 nm **(Karoui *et al.*, 2003c)**. All spectra were corrected for instrumental distortions in excitation using a rhodamine cell as a reference channel. For each cheese sample (2 cm long, 1 cm wide and 0.2 cm thick) mounted between two quartz slides, three spectra were recorded at 20°C for different samples.

II.3.3. Synchronous Fluorescence Spectroscopy

Synchronous fluorescence spectra were recorded using a FlyotoMax-2 spectrofluorimeter (Spex-Jobin Yvon, Longjumeau, France) mounted with a front-surface accessory. The incidence angle of the excitation radiation was set at 56° to ensure that reflected light, scattered radiation and depolarization phenomena were minimized. Spectra of cheese slices (2 cm long,1 cm wide, 0.2 cm think) mounted between two quartz slides were recorded at 20°C with emission and excitation slits set at 4 nm. SF spectra were

collected in the 250-500 nm excitation wavelength range using offsets of 80 nm **(Boubellouta & Dufour, 2010)** between excitation and emission monochromators. For each cheese sample, three spectra were recorded on 3 different slices.

II.3.4. Mid-infrared Spectroscopy

Infrared spectra were recorded in the 3000 and 900 cm-1 region with a Fourier transform spectrometer Varian 3100 FT-IR (Varian Inc., Palo Alto, USA) mounted with an ATR accessory equipped with a grip (Spectra-Tech ARK Flat Plate). The ATR cell is six reflections and was made of a horizontal ZnSe crystal that presented an incidence angle of 45°.

Slices of cheese samples were set on the crystal and a pressure on the grip ensured a good contact between the two elements. For each cheese, the spectra were recorded at 20°C in triplicate using different samples. To improve the signal-to-noise ratio, 64 scans (at 4 cm^{-1} resolution) were accumulated for each spectrum. The reference spectrum was first recorded using a blank m-ATR cell. Before each measurement, the spectrum of the ZnSe crystal was recorded in the conditions described above and used as background. Baseline and ATR corrections, smoothing and water subtraction were applied to the spectra using OMNIC 4.1 a software (Thermo electron).The regions of the mid-infrared spectra located between 3.000 and 2.800 cm^{-1} (fat region), 1.700 and 1.500 cm^{-1} (protein region) and 1.500 and 900 cm^{-1} (fingerprint region) have been considered in this study.

III. Statistical Analysis

III.1. Univariate analysis

One-way ANOVA was carried out to determine the significance of the chemical, physical and rheological results among the samples. Least Significant Difference test ($P < 0.05$)

was applied for comparison of the means using XLSTAT software version 2009 (Addisnoft, France).

III.2. Multivariate analysis

Before multivariate analysis of the spectral data, the fluorescence spectra were normalized by reducing the area under each spectrum to a value of 1 according to Betrand and Scotter **(Bertrand & Scotter, 1992)** in order to reduced scattering effects. Subsequently, all the spectral scans from each sample at each condition were averaged and the average was used for further analysis. The averaged spectra were then analyzed by PCA in order to get the best possible view of the spectral structure and distribution of samples. Scores, loadings, and explained variance were studied for the first two principal components (PC). Loadings plots were used to interpret the spectral variation contained in each PC, while score plots were used to visualize the relation between samples in the corresponding PC. Factorial discriminant analysis (FDA) was applied to the first 10 principal components of the PCA performed on the MIR spectral and the fluorescence spectral data (tryptophan and vitamin A) in order to investigate the presence of certain relationship between a qualitative explanatory characters of the 2 kinds of cheeses **(Bertrand & Dufour, 2006)**. From the first 10 principal components selected, FDA assessed new synthetic variables called discriminant factors, which were not correlated and allowed the best separation of the qualitative groups.

RESULTS AND DISCUSSION

PART I

Chemical, rheological and Structural Characteristics of Cantal cheese made from Raw, Thermized, and Pasteurized milk

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PART I: Chemical, rheological and Structural Characteristics of Cantal cheese made from Raw, Thermized, and Pasteurized milk

1. INTRODUCTION

Cheese characteristics strongly depend on technological processes which is in turn affecting on the composition, dynamics, and interactions between components of the cheese. Of all these characteristics, texture and colour are the most important criteria used to evaluate cheese quality by consumers. Several steps in the cheese making process are known to affect on these characteristics. The heat treatments are usually the first step in cheese making. The effects of heat treatment on the components of milk (proteins, lipids, carbohydrates and minerals) are very important for the final product character, since they undergo modifications that affect the quality attributes of cheese **(Burton, 1984)**.

In dairy processes, heat treatment of cheese milk is intended to reduce microbial loads and eliminate pathogens and most of the spoilage microorganisms that may be present in milk. However, milk pasteurization is also known to adversely affect the development of many quality attributes of cheese. Milk pasteurization affects cheese texture, giving rise to an open structure with numerous and irregular cavities that is less firm and more fracturable compared with that of raw milk cheeses **(Creamer & Olson, 1982 and Buffa *et al.*, 2001)**. Milk for cheese manufacture is generally pasteurized at 72°C typically for 15s. There are a number of alternatives to pasteurization for the decontamination of cheese milk: thermization- heat treatment at a sub-pasteurization temperature (typically 50-65°C/5-30s); thermization is intended to reduce the microflora of raw milk, minimize changes in milk quality and processability prior to conversion into product. Although thermization does not meet the

requirements for pasteurization from the public health viewpoint, it is widely used for cheese milk and in combination with other hurdles, e.g., cooking of the cheese curd, low pH, high S/M, is probably adequate to render good-quality milk free of pathogens and food poisoning bacteria.

About 700.000 tonnes of raw milk cheeses are produced annually in Europe, particularly in France, Italy and Switzerland **(Barry & Tamine, 2010)**. Raw-milk cheeses constitute a major portion of French cheeses with an appellation of origin. These cheeses are highly praised for their expressive sensory characteristics, their ties to unique areas of production and production techniques that have been handed down for generations. About 15% (210,000 tons/year) of ripened French cheese is made from raw milk **(Odet, 1999)**. Cantal cheese is the one of the 42 cheeses Protected Denomination of Origin (PDO) produced in France. It is uncooked, hard cheese produced in the Massif Central area of France, made from raw, thermized and pasteurized cow's milk. Its making process is very similar to Cheddar cheese.

Several analytical chemical methods have been described to characterize heat-induced changes in cheese quality. Tedious and time-consuming chemical methods are often replaced by more rapid and noninvasive spectroscopic methods **(Karoui & De Baerdemaeker, 2007)**. Caseins in cheese contain the amino acid tryptophan and vitamin A, which are naturally occurring fluorescent substances. When milk is subjected to thermal treatment, many changes occur in milk like denaturation of protein, degradation of some fluorophores (tryptophan residues in proteins, vitamins), and development of some new fluorophores (Maillard-reaction products), which consequently changes the fluorescence signals of cheese. Measure the spectra of tryptophan in cheese by using fluorescence spectroscopy have been used to predict the microstructure of cheese **(Karoui *et al.*, 2003 and Garimella *et al.*, 2005)**. On another note, fluorescence spectroscopy has also been

used to evaluate changes in protein-lipids interaction by measuring the fluorescence properties of vitamin A spectra **(Dufour *et al.*, 2001)**.

Interpretation of spectroscopic data commonly requires the use of chemometrics to draw conclusions. There have been several reports on the use of multivariate statistical analysis for evaluation of fluorescence spectral data obtained by the use of fluorescence spectroscopy analysis to monitor changes in protein-lipids interaction in cheese making and ripening **(Karoui & De Baerdemaeker, 2007)**. In that sense, Dufour and Riaublanc **(Dufour & Riaublanc, 1997)** used a front-face fluorescence method to distinguish between raw, heated, homogenized and homogenized + heated milks by multivariate statistical analysis (Principal Component Analysis and Discriminant Analysis) of fluorescence spectral data and obtained results equal to or better than physicochemical analysis.

The objective of this research were (1) to evaluate the influence of heat treatments on the main quality characteristics of Cantal cheeses ripened for 90 days that may influence the consumers' acceptance: compositional, textural, colour characteristics of Cantal cheeses made from raw, thermized, and pasteurized milk, (2) to investigate the potential of fluorescence spectroscopy coupled with chemometric tools as a rapid and low-cost technique for monitoring cheese molecular structural characteristic.

2. RESULTS AND DISCUSSION

2.1. Compositional characteristics of Cantal cheese

The effect of heat treatments on the compositional characteristics of Cantal cheeses samples ripened for 90 days are reported in Table (6). Heat treatments had the largest impact on cheese composition, significantly ($P < 0.05$) affecting in all compositional characteristics.

pH value and proteolysis

As shown in Table (6) Cantal cheeses made from thermized and pasteurized milk had pH values higher than raw Cantal cheese milk, although no significance difference between thermized and pasteurized cheese milk. This may be attributed to the high microbial content of raw milk cheese and the greater utilization of lactic acid leading to low pH value, while pasteurized milk cheese contained the lowest bacterial content owing to the effect of pasteurization **(Barry & Tamine, 2010)**.

Rates of proteolysis in Cantal cheese were found to be significantly ($P < 0.05$) affected by heat treatment. Levels of WSN/TN%, as an index of proteolysis, were significantly ($P < 0.05$) higher in raw-milk cheese than in thermized and pasteurized milk cheese. The highest values of W.S.N./T.N. % were recorded with the raw Cantal cheese milk followed by pasteurized and thermized cheese milk, respectively. The lower rate of ripening in heat treated milk cheese may be due to the destructive effect of heat treatment on the natural flora and milk enzymes which in turn affect fat and protein degradation **(Lucey & Fox, 1993)**. Similar observations have been reported previously **(Benfeldt *et al.*, 1997 and Barry & Tamine, 2010)**.

Moisture content

Moisture is the major component of cheese which acts as a lubricant or plasticizer in the protein matrix thereby making it less elastic and more susceptible to fracture upon compression **(Fox *et al.*, 2000)**. Heat treated milk and pasteurized milk cheese revealed higher moisture content than raw milk cheese and were significantly differences among the cheese samples (Table 6). This may be attributed to the effect of pasteurization on kappa casein forming complex with β-lactoglobulin which increase water-holding capacity **(Lucey & Kelly, 1994 and Fox *et al.*, 2000)**.

Protein and fat contents

The protein and fat in cheese are the most important constituent for the texture of the cheese. The fat content of the heat-treated cheese milk was significantly lower ($P<0.05$) than the raw cheese milk, while the protein content in the heat-treated cheese milk was significantly higher with compare with raw Cantal cheese (Table 6). Contrary to moisture contents, the protein contents increased with decreasing fat contents of cheese. The decrease in levels of protein and fat with heat treatments are due to concomitant increase in moisture, and hence, the reduction in level of cheese dry matter **(Guinee *et al.*, 1996; Bulca *et al.*, 2004 and Guinee *et al.*, 2006)**.

Ash and salt contents

The ash content in the food stuff represents the inorganic matters remaining after the organic matters have been burnt. No major differences in the ash contents were noticeable between raw and thermized cheese milk, despite the significant differences found between the raw and pasteurized cheese milk **(Fox & McSweeney, 2004 and Barry & Tamine, 2010)**.

Concerning the salt %, the higher salt content was detected in raw cheese milk than the other types of cheese. Higher levels of salt induce a swelling of the casein phase with subsequent adsorption and absorption of moisture by the casein matrix. Salt content significantly affects cheese structure by increases the level of protein hydration and results in a swelling of the protein matrix (solubilization of caseins). Increased protein hydration results in decreased protein-protein interactions and increased protein-water interactions **(Bulca *et al.*, 2004; Celik *et al.*, 2005; Considine *et al.*, 2007 and Donato & Guyomarch, 2009)**.

Calcium and phosphorus contents

Cheese matrix is essentially Ca-P paracasein matrix linked together by various interactions between paracaseins and fat

globules, moisture and dissolved substances, and enzymes exist in the pores of this matrix. A dynamic equilibrium exists for the concentration of calcium and phosphate between the paracasein matrix and cheese serum could be affect the structure and texture development of cheese. This equilibrium is influenced by pH and other factors **(Fox *et al.*, 2000)**. The pH values significantly affect the texture and structure of cheese because of its influence on calcium and phosphate solubilization, which results in changes in the protein network.

Calcium and phosphorus contents significantly differed in cheese samples (Table 6). A non significant difference was noted in Ca and P contents between thermized and pasteurized cheeses. Heat treatment resulted in significant increases in the levels of pH, reduction in the contents of fat, protein, calcium and phosphorus in the cheese **(Lucey & Fox, 1993; Lucey & Kelly, 1994 and Lucey *et al.*, 2001)**.

Table 6: Mean (±SD) of the compositional characteristics and colour values of Cantal cheese made from raw, thermized and pasteurized cow's milk.

Compositional parameters	Cantal cheese made from		
	Raw milk	Thermized milk	Pasteurized milk
pH	5.23 (±0.01)^{b}	5.37 (±0.01)^{a}	5.38 (±0.01)^{a}
Moisture (%)	39.62 (±0.25)^{c}	41.30 (±0.19)^{a}	39.96 (±0.11)^{b}
Fat (%)	32.85 (±0.14)^{a}	31.58 (±0.14)^{b}	30.50 (±0.00)^{c}
Protein (%)	24.48 (±0.08)^{c}	25.18 (±0.19)^{b}	28.38 (±0.46)^{a}
WSN/TN %	26.09 (±0.25)^{a}	22.04 (±0.43)^{c}	23.89 (±0.58)^{b}
Salt (%)	1.46 (±0.00)^{a}	1.32 (±0.01)^{b}	1.01 (±0.00)^{c}
Ash (%)	4.25 (±0.01)^{a}	4.24 (±0.02)^{a}	3.87 (±0.01)^{b}
Total Ca (%)	0.612 (±0.10)^{b}	0.824 (±0.01)^{a}	0.790 (±0.05)^{a}
Total P (%)	0.478(±0.15)^{b}	0.529(±0.13)^{a}	0.527(±0.23)^{a}
Colour values			
*L** value	80.91 (±0.53)^{a}	79.47 (±0.70)^{b}	79.92 (±0.98)^{b}
*a** value	-2.77 (±0.11)^{a}	-1.78 (±2.76)^{a}	-2.34 (±0.11)^{a}
*b** value	23.63 (±0.80)^{a}	22.73 (±0.39)^{b}	20.93 (±2.45)^{c}
Δ E	--	2.58 (±0.72)^{a}	2.87 (±0.50)^{a}

SD: standard deviation
One-Way ANOVA was applied to data and values with different superscript letter are significantly different ($P<0.05$, LSD test).

2.2. Colour values of Cantal cheese

The colour of cheese is an important factor in the consumer appeal of the product. The cheese colour is influenced by several factors including light scattering of fat, protein particles and water drops **(Rudan *et al.*, 1998)**. Table (6) presents the mean values obtained for L*, a*, and b* parameters of the Cantal cheese made from raw, thermized and pasteurized milk. The results illustrated that the colour parameters (L*, a* and b* values) were higher in raw Cantal cheese milk than the other cheeses made from heated milk, although, there was no significant difference between the thermized and pasteurized milk cheese concerning the L* values. With regard to the red component (- a*) was not significant ($P < 0.05$) between the cheeses, indicating that the pasteurization of milk had not an effect on a* value. The b* values were significantly differed between all cheeses. However, the changes in b* value for thermized Cantal cheese milk were much smaller than those found for pasteurized Cantal cheese milk.

In order to determine the effect of heat treatments applied to cheese milk on the total colour of the investigated cheeses, total colour difference (ΔE) was calculated according to the formula given by **(Buffa *et al.*, 2001)**. Although the lowest value of this parameter was observed for thermized cheeses with a value of 2.58 and the highest one was observed for pasteurized cheese ($\Delta E=2.87$), no significant difference was found among them ($P \leq 0.05$). The almost identical colour values found in thermized and pasteurized cheeses could be attributed to their similar structure. The obtained results were in agreement with those of **(Buffa *et al.*, 2001)**, who reported that no significant difference in the colour values was observed between cheeses made from raw, pasteurized or high pressure treated goats' milk.

2.3. Textural attributes of Cantal cheese

Texture is a group of physical properties that derives from the structure of food and the way its constituent ingredients interact **(Creamer & Olson, 1982)**. The textural properties of a food product can be achieved by examination of its rheological behavior.

Effects of heat treatments on elastic modulus (*G′*, elastic rigidity), viscous modulus (G'', viscous rigidity), Tan δ (indication of relative viscous and elastic components) and η* (complex viscosity) of the three Cantal cheese are shown in Figure (13). Briefly, the heat treatment gave rise to an increase of G' and G'' values. Since, the raw Cantal cheese had significantly lower G', G'', Tan δ and η* than the other cheeses (Figure 13).

The adverse effects of heat treatments (HT) on texture attributes are probably due, in part, to the presence of denatured whey protein-k-casein complexes on the surface of the rennet-treated micelles and in other part, adverse effect of HT on cheese constituents (reduction in fat, WSN/TN%; increase in protein, pH, Ca, P contents) **(Visser, 1991 and Joshi *et al.*, 2003)**. Increase in contribution of whey proteins in gel network cause significantly increase in storage modulus (*G′*) because denatured whey proteins act as bridging material between casein particles, which would increase the number and strength of bonds.**(Kelly & O'kennedy, 2001 and Pastorino *et al.*, 2003)**.

For all cheese samples values of Tan (δ) were lower than 1, an indication that the cheeses exhibited elastic, solid-like behavior. These results are in agreement with the work of other investigators **(Vliet & Keeteles, 1995; Lucey & Singh, 1997; Lucey *et al.*, 1997; Kelly & O'kennedy, 2001; Lucey *et al.*, 2001 and Vaziri *et al.*, 2001)**. **(Lucey *et al.*, 1997)** and **(Vasbinder *et al.*, 2003)** concluded that formation of a disulphide-linked protein structure during heat treatments resulted in gels with an increased elastic modulus and hardness. However, the amount of whey proteins that

denatured at 65°C was lower than 72°C. Thus, cross-linking of the denatured whey proteins was low, and *G′* was lower than in the high-heat treatment. In addition, the amount of whey protein denatured present at the micellar surface is dependent on the heating and intensity. This results in a smoother surface with decreased hydrophobicity and an increased water-holding capacity of the protein matrix **(Tamine & Robinson, 1999)**.

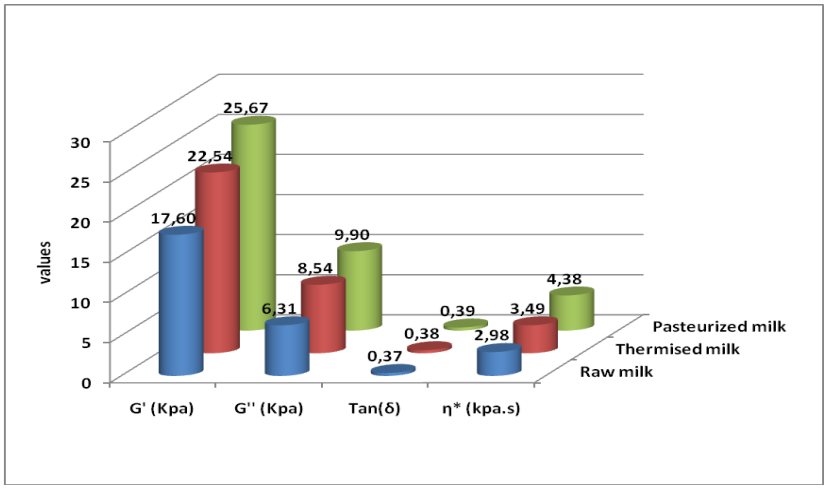

Figure 13: The effect of heat treatment on textural attributes (G', G'', Tan δ and η*) of Cantal cheeses made from raw, thermized and pasteurized milk.

2.4. Principal component analysis of compositional data

In order to determine the relationship between physicochemical parameters, Principal component analysis (PCA) was applied to the data sets. PCA of the physicochemical results indicated that 87.29% of the total variation among the samples could be explained by two principal components (PC), PC_I and PC_2 (Figure 14 a-b). Principal component one explained 68.08 % of this variation and was positively driven by pH, protein, Ca, P contents and rheological parameters. PCA of physicochemical results distinguished the samples into three different groups. The first

group included samples that showed positive scores in PC_I (due to high values of Ca, P and G') and were located in the down closely to zero. Cheese in Group 2 had positive scores in PC_I because of their high protein, G'', low fat, and ash contents. Cheese in Group 3 had negative scores in PC_I because of their high fat, ash, WSN and salt contents. Cantal cheeses made from thermized milk had characteristics similar to this Group 1 while those from pasteurized milk belong to Group 2 and from raw milk were belongs to Group 3.

It was shown that the results from PCA analysis are in agreement with ANOVA results which demonstrate that the heat treatments of cheese milk had an effect on the physicochemical parameters of cheese. In addition, it appeared that cheese samples produced from raw milk was characterized by the highest amount of fat, salt, ash contents, while those thermized and pasteurized presented the highest level of protein and textural attributes (Figure 14 b).

Pearson correlation coefficients between the physicochemical parameters of cheese produced from raw, thermized and pasteurized milks were determined (Table 7). Significant negative correlations ($P < 0.05$) were observed between fat and protein. Similar negative correlations were observed between protein and salt, protein and ash, and protein and b*. However, a strong positive correlation was found between the protein and the following physicochemical parameters: G', G'' and η^*.

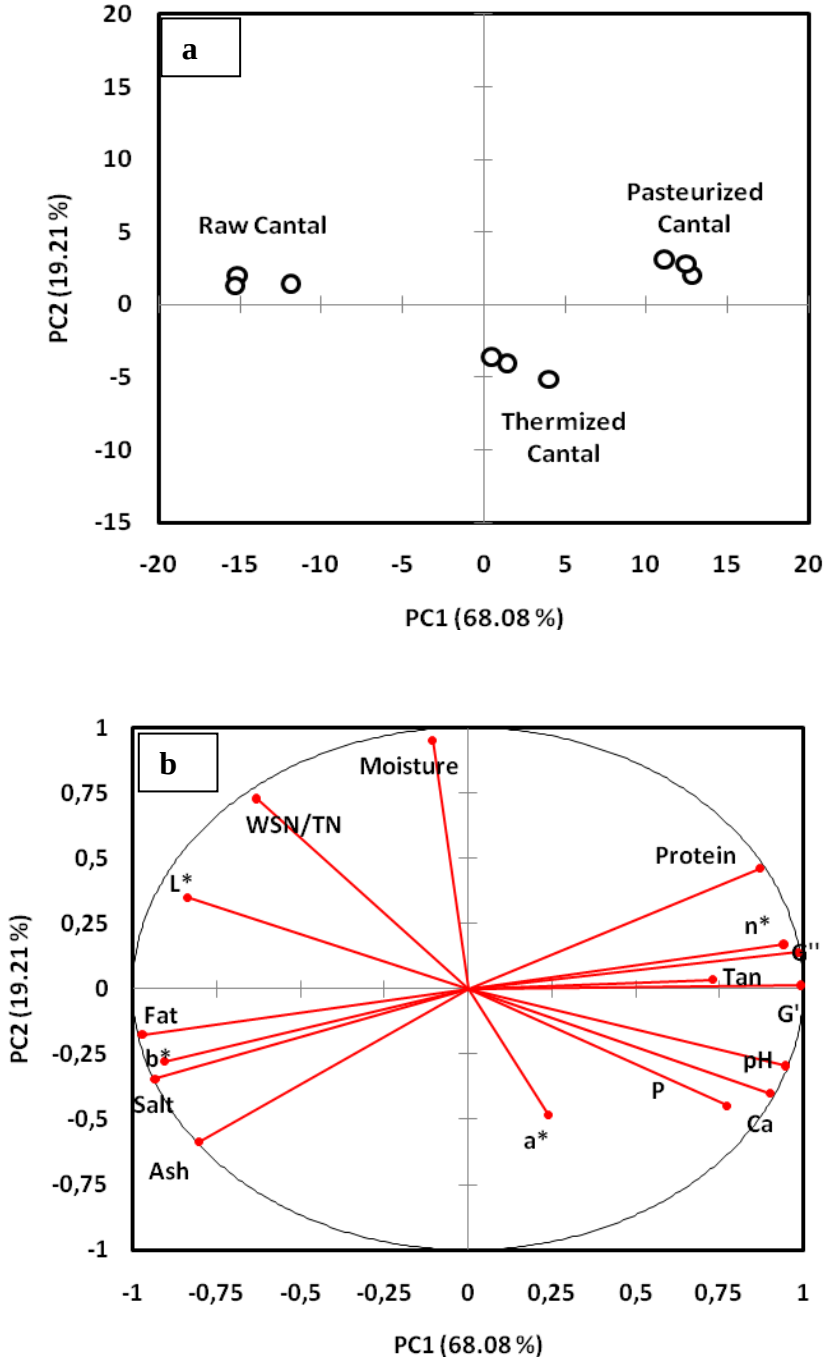

Figure 14: (a) PCA similarity map (b) loading plot of the PC_1 vs. PC_2 for the physicochemical parameters measured in Cantal cheese made from raw, thermized and pasteurized milk.

Table 7: Pearson correlation coefficients (P<0.05) between the physicochemical parameters of Cantal cheese produced from raw, thermized and pasteurized milk.

Variables	pH	Moisture	Fat	Protein	Salt	Ash	Ca	P	WSN/TN	L*	a*	b*	G'	G''	Tan (δ)	η*
pH	1															
Moisture	-0,401	1														
Fat	**-0,875**	-0,028	1													
Protein	**0,701**	0,335	**-0,928**	1												
Salt	**-0,788**	-0,210	**0,975**	**-0,982**	1											
Ash	-0,589	-0,480	**0,878**	**-0,982**	**0,956**	1										
Ca	**0,835**	-0,466	**-0,680**	0,424	-0,539	-0,344	1									
P	**0,983**	-0,520	**-0,829**	0,604	**-0,714**	-0,485	**0,853**	1								
WSN/TN	**-0,810**	**0,810**	0,521	-0,207	0,346	0,072	**-0,865**	**-0,886**	1							
L*	**-0,887**	0,356	**0,706**	-0,585	0,663	0,484	**-0,724**	**-0,852**	**0,702**	1						
a*	0,357	-0,298	-0,037	0,016	-0,031	0,051	0,283	0,316	-0,288	-0,622	1					
b*	**-0,771**	-0,197	**0,922**	**-0,881**	**0,926**	**0,870**	-0,632	**-0,689**	0,358	**0,687**	-0,078	1				
G'	**0,945**	-0,124	**-0,982**	**0,877**	**-0,941**	**-0,805**	**0,734**	**0,909**	-0,633	**-0,804**	0,173	**-0,884**	1			
G''	**0,898**	0,002	**-0,993**	**0,928**	**-0,976**	**-0,874**	**0,671**	**0,847**	-0,533	**-0,760**	0,123	**-0,912**	**0,992**	1		
Tan (δ)	0,650	0,026	-0,646	0,628	**-0,683**	-0,640	0,497	0,599	-0,358	-0,662	0,402	-0,609	**0,703**	**0,711**	1	
η*	**0,837**	0,074	**-0,964**	**0,886**	**-0,927**	**-0,842**	**0,725**	**0,786**	-0,491	**-0,682**	0,105	**-0,926**	**0,933**	**0,943**	0,558	1

P-values in bold are NOT significant

2.5. Molecular structural characteristics of Cantal cheese

Cheese structure characteristics depending on the cheese technology process and the operation temperature because several of its constituents present as a solid matrix (para-casein), some as a liquid phase (serum), and others (e.g. fat) as either solid or liquid **(Pierre *et al.*, 1999)**.

As mentioned previously, fluorescence spectroscopy method have be used to evaluate the structural characteristics of cheeses via evaluation the fluoresce properties of tryptophan and vitamin A in cheese **(Herbert *et al.*, 2000; Karoui & Dufour, 2003; Karoui & De Baerdemaeker, 2007 and Dufour, 2010)**.

Since the investigated cheeses differed in their physiochemical parameters due to differed in manufacturing processes, it was assumed that their molecular structural characteristics and, as a consequence, the environment of the intrinsic fluorophores, such as tryptophan residues in the proteins and vitamin A in the fat globules, were different.

2.5.1. Fluorescence properties of tryptophan and vitamin A

Figure (15) shows the tryptophan emission and vitamin A excitation fluorescence spectra of the Cantal cheeses manufacture from raw, thermized and pasteurized milk.

Concerning tryptophan emission spectra of the three cheeses types exhibited a maximum at about 339 nm for all investigated cheeses, while the shape of tryptophan spectra varied slightly among the three cheeses (Figure 15 a).

This difference in the shape of the spectra among the three cheeses types of Cantal cheeses could be due to the difference in environments of the tryptophan residues resulting from the differences in the structure of cheese matrices **(Herbert, 1999 and Herbert *et al.*, 2000)**. Indeed, the changes of the environment and

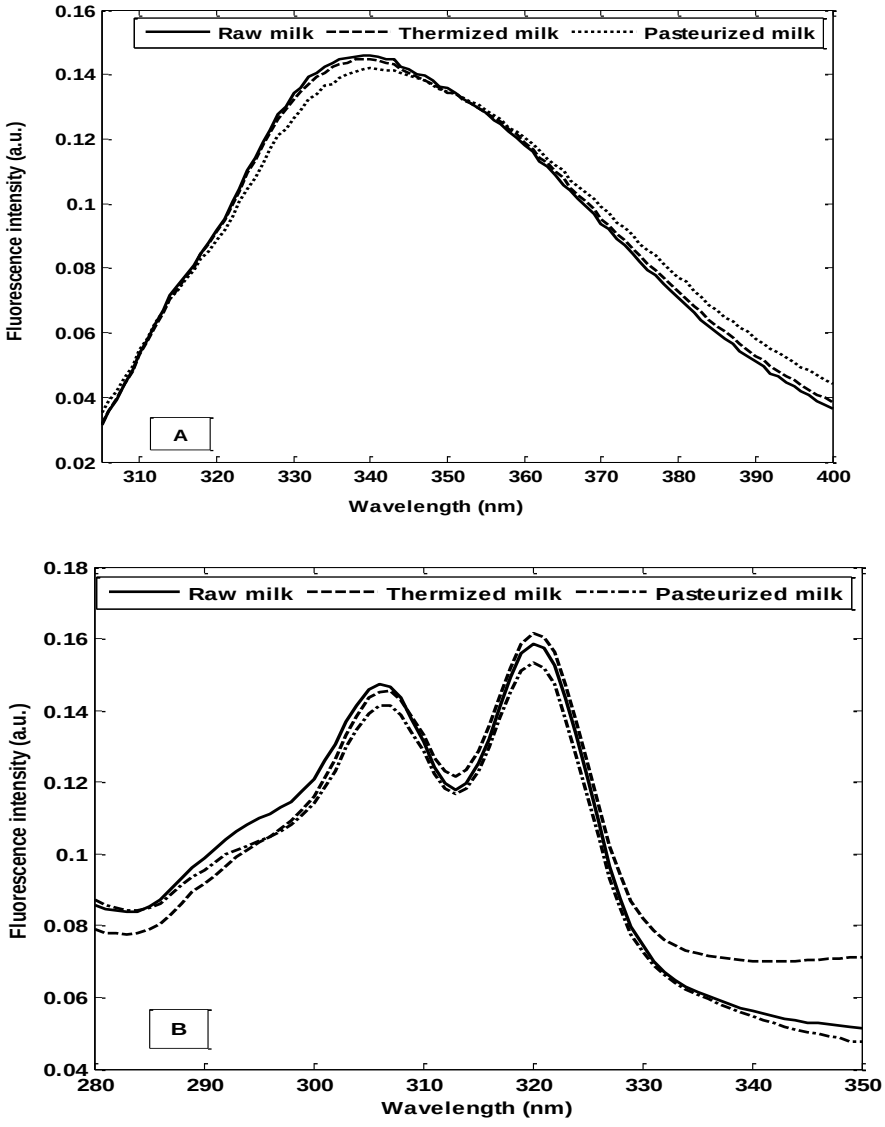

Figure 15: Normalized fluorescence spectra of Cantal cheese made from raw (--), thermized (----) and pasteurized (……) milk: (A) Tryptophan emission and (B) Vitamin A excitation.

solvent viscosity could be change the fluorescence properties of tryptophan as has been reported previously by **(Dufour & Riaublanc, 1997 and Dufour *et al.*, 2001)**.

With regard to the vitamin A, the shape of the spectra showed two maxima at 320 and 305 nm. The shapes of the spectra scanned on cheese samples were overall similar, varying mainly in the maximum/shoulder intensity ratios. The changes in the shapes of the vitamin A spectra could be related to the modification in the physical state of triglycerides in the fat globules as well as to different protein–lipid interactions **(Herbert, 1999)**. Indeed, the shape of the vitamin A spectrum depends on the physical state of the triglycerides and the heat treatment applied to the milk during cheese manufacture **(Dufour & Riaublanc, 1997 and Herbert *et al.*, 2000)**.

2.5.2. Multivariate analysis of spectral data

Due to the large number of variables and the slight difference among fluorescence spectra and difficult to distinguish by visual observation of the spectrum, univariate analysis of fluorescence spectral data is not appropriate. Multivariate statistical analysis such as PCA and FDA make it possible to extract information from spectral data. FDA was applied in order to monitor the ability of fluorescence spectroscopy to discriminate cheeses as a function of their molecular structural changes resulting of heat treatment of cheese milk.

To compare the ability of tryptophan emission and/or vitamin A excitation fluorescence spectra to emphasize the similarities and differences among the cheese samples, PCA, in a first step, was carried out separately on each spectral data set (tryptophan and vitamin A) and in a second step FDA was applied on the first10 PCs of PCA performed on each spectral data set to cheese discrimination.

2.5.3. Cheese discrimination from their tryptophan spectra

Firstly, the PCA performed on tryptophan emission spectra (30 observations and 100 variables), the similarity map of the two first PCs allowed a good discrimination of pasteurized Cantal cheese from raw and thermized cheese milk (Figure 16). Indeed, according to PC_1 which took into account 84.2% of the total variance, pasteurized Cantal cheeses presented negative scores, whereas those produced from raw and thermized had positive scores. Less good discrimination was obtained from the similarity map of the tryptophan fluorescence spectra for Cantal manufactured from raw or thermized cheese milk.

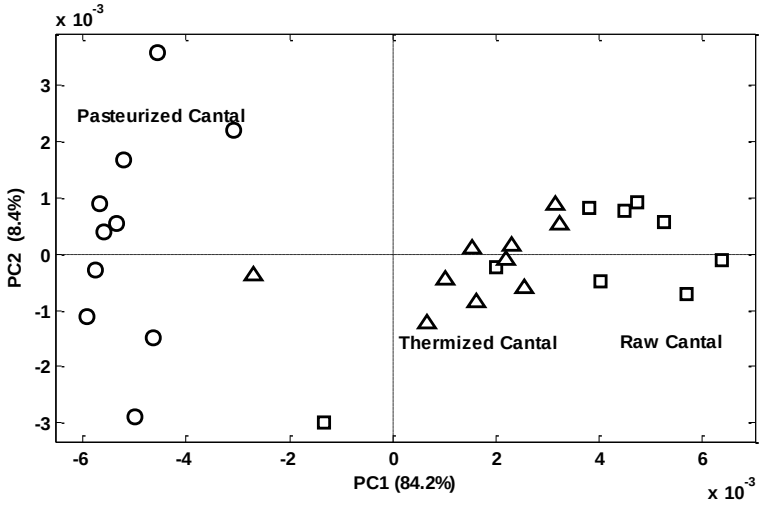

Figure 16: Principal component analysis similarity map determined by principal components 1 and 2 for the tryptophan fluorescence spectra of Cantal cheeses made from raw (□), thermized (Δ) and pasteurized milk (o).

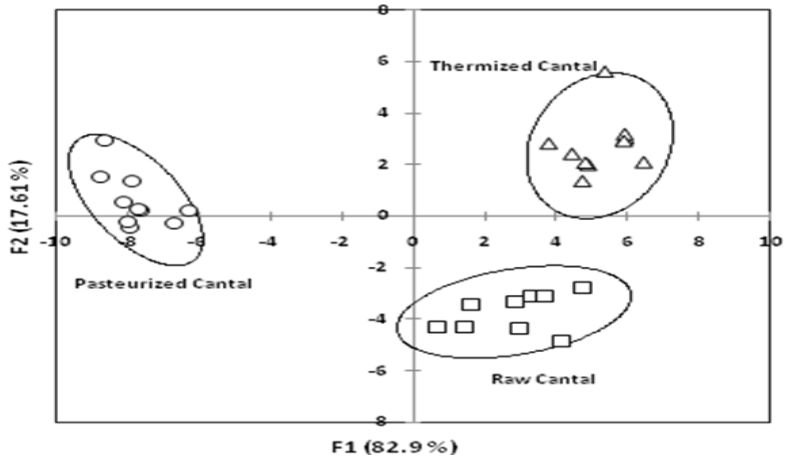

Figure 17: Discriminant analysis similarity map determined by discriminant factors 1 (F_1) and 2 (F_2) of the FDA performed on the first10 PCs of PCA of tryptophan fluorescence spectra of Cantal cheeses made from raw (□), thermized (Δ) and pasteurized milk (o).

Secondly, FDA was performed on the first10 PCs of PCA performed on the tryptophan spectra. The data matrix for FDA included 30 objects and 10 variables (the first 10 principal components). The similarity map defined by the discriminant factors 1 and 2 took into account 100% of the total variance with discriminant factor 1 accounting for 100 % (Figure 17). Considering discriminant factor 1 accounting for 82.9% of the total variance, pasteurized Cantal cheeses were observed on the left, whereas cheese from the raw and thermized were observed on the right. Raw Cantal cheeses were discriminated than thermized Cantal cheeses according to discriminant factor 2. 100% correct classification was obtained for the three investigated cheeses (Table 8).

2.5.4. Cheese discrimination from vitamin A spectra

Firstly, the PCA performed on vitamin A excitation spectra (30 observations and 80 variables) and the similarity map of the PCA performed on vitamin A fluorescence data set is shown in Figure (18). Again, a quite good separation of thermized Cantal cheese (TM) was observed according to the PC_1 accounting for 62.70% of the total variance. However, less good discrimination was obtained between the raw and pasteurized Cantal cheese.

Secondly, FDA was performed on the first10 PCs of PCA performed on the vitamin A spectra. The data matrix for FDA included 30 objects and 10 variables (the first 10 principal components). The similarity map defined by the discriminant factors 1 and 2 took into account 100% of the total variance with discriminant factor 1 accounting for 98.87% (Figure 19). A discrimination of raw Cantal cheese from the others was essentially observed according to discriminant factor 1, where pasteurized Cantal cheese were observed on the right, whereas thermized and raw Cantal cheese were observed on the left. The discriminant factor 2 essentially discriminated raw from thermized Cantal cheese

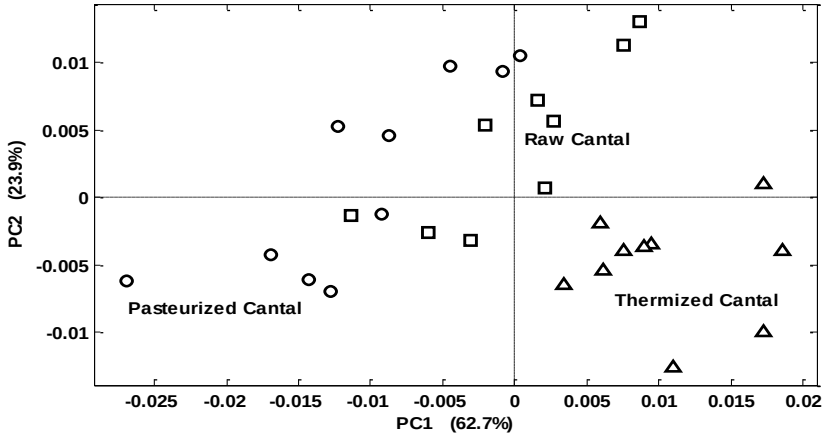

Figure 18: Principal component analysis similarity map determined by principal components 1 and 2 for the vitamin A fluorescence spectra of Cantal cheeses made from raw (□), thermized (Δ) and pasteurized milk (o).

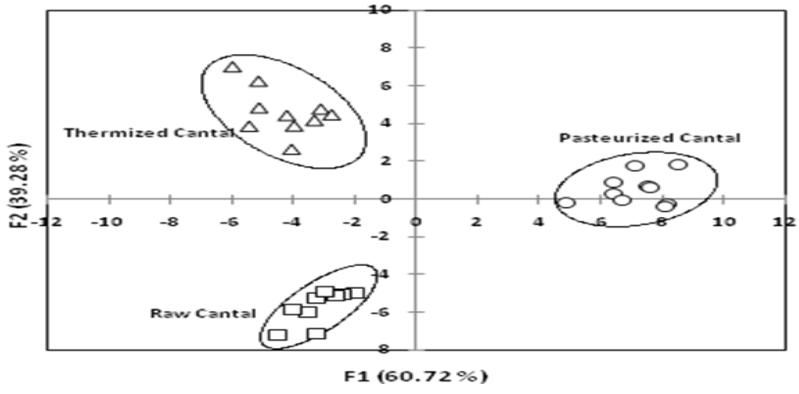

Figure 19: Discriminant analysis similarity map determined by discriminant factors 1 (F_1) and 2 (F_2) of the FDA performed on the first10 PCs of PCA of vitamin A fluorescence spectra of Cantal cheeses made raw (□), thermized (Δ) and pasteurized milk (o).

(Figure 19). 100% correct classification was obtained for the three investigated cheeses (Table 8).

2.5.5. Concatenation of the fluorescence spectral data

Finally, the first 10 PCs of the PCA performed on each of the 2 data sets (i.e., tryptophan and vitamin A spectra together) were pooled into one matrix and this new table (matrix of 30 individuals and 20 variables) was analyzed by FDA. This concatenation approach helped to improve the discrimination between cheeses by using different fluorescence spectra, as well as to assess the ability of fluorescence spectroscopy to discriminate cheeses according to their heat treatment of cheese milk.

The similarity map defined by the discriminant factors 1 and 2 represented 100% of the total variance with discriminant factor 1 accounting for 86.11% of the total variance (Figure 20). A good discrimination of cheeses independently of their heat treatments was observed according to discriminant factor 1: where pasteurized Cantal cheese were observed on the lift side, whereas thermized Cantal cheese were located on the high far right side and raw Cantal cheese were located on the low far right side according to F_1. Raw Cantal cheeses were discriminated than thermized Cantal cheese according to discriminant factor 2. 100% correct classification was obtained for the three investigated cheese (Table 8).

It appeared that the approach using concatenation of the two data sets allowed us to manage in a very efficient way all of the spectroscopic information collected on the cheeses. The current study is in agreement with previously published data, indicating that fluorescence spectra can be used for the study molecular structural characteristics of cheeses **(Karoui & Dufour, 2003; Karoui *et al.*, 2003c and Boubellouta & Dufour, 2008)**.

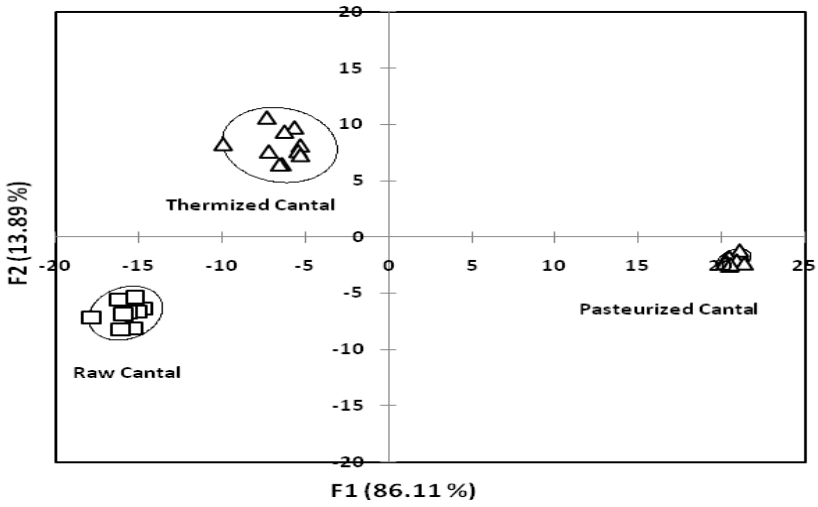

Figure 20: Discriminant analysis similarity map determined by discriminant factors 1 (F_1) and 2 (F_2). FDA was performed on the 20 concatenated PCs corresponding to the PCA performed on the tryptophan and vitamin A fluorescence spectra of Cantal cheeses made raw (□), thermized (Δ) and pasteurized milk (o).

Table (8) gives the classifications of the spectra data for the three groups of Cantal cheeses. This table shows 100% correct classification was observed for the tryptophan fluorescence spectra recorded on the three investigated cheeses. All of the Cantal cheeses were well discriminated in a function of their heat treatment of cheese milk. Similar results were observed with the spectra of vitamin A and joint tryptophan and vitamin A spectra. It could be concluded that fluorescence spectra is fingerprints that allow accurate discrimination of Cantal cheeses according to their heat treatments of cheese milk. The discrimination of cheese samples from the others could be explained by the changes in the environment of the intrinsic fluorophores (tryptophan and vitamin A) during cheese making. Similar results were obtained from the FDA evaluation of fluorescence spectral data **(Herbert, 1999; Dufour *et al.*, 2001; Karoui *et al.*, 2003c; Karoui *et al.*, 2005c; Karoui *et al.*, 2007c and Boubellouta & Dufour, 2008)**.

Table 8: Correct classification % by FDA of Cantal cheeses made from Raw (RM), Thermized (TM) and Pasteurized milk (PM) according to their fluorescence spectral data sets.

Predicted\Real	RM cheese	TM cheese	PM cheese	Total	% Correct classification
Tryptophan fluorescence spectra					
RM cheese	10	0	0	10	100%
TM cheese	0	9	0	9	100%
PM cheese	0	0	10	10	100%
Total	10	9	10	29	100%
Vitamin A fluorescence spectra					
RM cheese	10	0	0	10	100%
TM cheese	0	9	0	9	100%
PM cheese	0	0	10	10	100%
Total	10	9	10	29	100%
Concatenation: Tryptophan and vitamin A fluorescence spectra					
RM cheese	10	0	0	10	100%
TM cheese	0	9	0	9	100%
PM cheese	0	0	10	10	100%
Total	10	9	10	29	100%

3. CONCLUSION

There were considerable variations in the chemical, colour and textural properties of Cantal cheese made from raw, thermized and pasteurized milk. These differences probably reflect processing condition used in the manufacture these cheeses. Raw Cantal cheeses milk significantly differed in colour and texture from the others cheeses made with thermized or pasteurized milk.

The structural changes as determined by rheological and fluorescence methods modified the level of rheological parameters (elastic and viscous moduli) and the shape of spectra of tryptophan and vitamin A curves.

It can be concluded that, emission spectra of tryptophan and excitation spectra of vitamin A could be considered as fingerprints allowing a good identification of cheese samples according to their heat treatments.

PART II

Structural changes of Cantal cheese throughout ripening by synchronous fluorescence spectroscopy and rheology methods

PART II: Structural changes of Cantal cheese throughout ripening by synchronous fluorescence spectroscopy and rheology methods

1. INTRODUCTION

Quality attributes of food products are closely related to structure. Cheese structure can be described as protein units (mostly caseins) held together by physical forces with fat, and moisture (contains minerals, vitamins and organic acids) dispersed throughout this structure **(David & Mark, 2008)**. Much of the major changes in cheese structure, which ultimately affects final quality, occur during ripening process. Cheese ripening is complex process of physical, chemical and microbiological changes affecting the principal components (i.e., protein, fat, carbohydrate…etc) of cheese matrix that affect the structure and texture of cheese **(Fox *et al.*, 1990)**. Texture is the primary quality attribute of cheeses: it is a reflection of cheese structure at the microscopic and molecular levels **(Dufour *et al.*, 2001)**.

In cheese factories, evaluation of ripening stage is made by the cheese maker on the basis of a limited number of measurements (pH and weight) as well as on the basis of visual and tactile examination. In addition, several analytical techniques have already been developed in different domains of expertise (chemistry, biochemistry, rheology…) for follow cheese ripening at the laboratory level. All these methods are relatively expensive, time-consuming, require highly skilled operators and are not easily adapted to on-line monitoring **(Karoui & De Baerdemaeker, 2007)**. For this reason, there is a pressing need to develop new analytical techniques, which are rapid, non-destructive and relatively low-cost and can be applied in both fundamental research and in the factory as on-line sensors for monitoring the ripening

process.

Rheological properties obtained in the linear viscoelastic region are useful tools for the food industry. Elastic and viscous contributions to the internal structure of the cheese can be obtained performing oscillatory measurements **(Konstance & Holsinger, 1992 and Karoui *et al.*, 2003)**. Such studies provide an insight into the fundamental nature of the physical basis of food texture **(Gunasekaran & Ak, 2000 and Tunick, 2000)**.

Synchronous fluorescence is a type of spectroscopy used in food science, analytical chemistry, biochemistry, environmental science and others fields. Synchronous fluorescence detects so-called fluorophores, molecules with a structure that allows emission of light when relaxing to the ground state from an excited singlet state. Synchronous fluorescence spectrum recorded on a cheese sample following excitation between 250-500 nm (offset 80 nm) included information on several intrinsic fluorophores presented in cheese and may be considered as a characteristic fingerprint which allows the sample to be identified **(Boubellouta & Dufour, 2010)**. The best known fluorescent molecules in dairy products include: tryptophan residues of proteins, vitamin A and riboflavin, which all have been reported to be affected during structural changes in cheese **(Dufour *et al.*, 2001)**. Tryptophan fluorescence spectra is often used as a reference group for protein structure changes, binding of ligands and protein-protein associations **(Herbert *et al.*, 2000)**. Moreover, using vitamin A excitation, as an intrinsic fluorescent probe, can also provide information on the physical state of triglycerides and protein–lipid interactions **(Dufour *et al.*, 2000)**. Riboflavin can be used for the evaluation of oxidative changes in processed cheese during storage **(Wold *et al.*, 2002)**.

Cantal cheese is a hard uncooked, pressed cheese variety granted the status of a Protected Denomination of Origin (PDO) by European Commission and produced in the Auvergne region in France, with an annual production of 19 000 T (**CNIEL, 2009**). Its

making process is very similar to Cheddar cheese. It is made from either raw or pasteurized cow's milk and commercialized as "young" (ripened for at least one month), "between the two" (ripened for 2 to 6 months) or "old" (ripened for over 6 months). Cantal is characterized as a cylinder-shaped (round wheels) cheese with a dry crust; its weight ranges between 35 to 40 kg, 40 cm height, 36 to 42 cm diameter. The dry matter content and the Fat/dry matter ratio must be, respectively, at least 57% and 45% **(République Française, 1986)**.

The objective of this research were (1) to evaluate changes in compositional (pH, moisture, protein, fat, WSN/TN, salt, Ca and ash contents) and physical (colour and texture) characteristics of Cantal cheeses throughout ripening process. (2) to evaluate the potential of synchronous fluorescence spectroscopy to follow the ripening phenomena of Cantal cheese. In order to discriminate between these cheeses in term of ripening period, the principal component analysis (PCA) and factorial discriminant analysis (FDA) were applied to synchronous fluorescence data.

2. RESULTS AND DISCUSSION

2.1. Compositional changes of Cantal cheese throughout ripening periods

Changes in pH value, moisture, fat, protein, fat/dry matter, ash, salt, Ca, and water soluble nitrogen/total nitrogen (WSN/TN %) contents of Cantal cheeses throughout ripening period (30, 120 and 200 days) are reported in Table (9).

As ripening progressed, fat, protein, salt, WSN/TN % and ash contents of Cantal cheeses continuously increased, as a result of the significant decrease in moisture content, whereas their calcium and fat in dry matter contents decreased. This can be related to during cheese ripening, released amino acids raise pH value to a somewhat higher level **(Waagner, 1993)**. The WSN/TN % of

cheeses increased during the ripening period, indicating progressive proteolysis. It has also been reported that there is an appreciable reduction in the amount of calcium content of Cantal cheese during the ripening period because of the solubilization of colloidal Ca phosphate (CCP). In parallel, the reduction in the amount of calcium associated with casein molecules (i.e., CCP) would be expected to alter cheese texture **(Lucey *et al.*, 2003 and Lucey *et al.*, 2005)**. As shown in Figure (21), there were an increase in cheese pH value, dry matter, proteolysis indicators and a decrease in calcium content during cheese the ripening period.

Table 9: Mean (±SD) of chemical characteristics and texture attributes of Cantal cheese throughout the ripening periods.

Parameters	Young Cantal (30 days)	Mild Cantal (120 days)	Old Cantal (200 days)
pH	5.23(±0.01)^{c}	5.41(±0.02)^{b}	5.79(±0.01)^{a}
Moisture (%)	42.92 (±0.06)^{c}	38.61(±0.05)^{b}	34.90(±0.05)^{a}
Protein (%)	24.43(±0.08)^{c}	25.11(±0.19)^{b}	28.40(±0.46)^{a}
Fat (%)	29.92(±0.14)^{c}	31.83(±0.14)^{b}	32.50(±0.00)^{a}
Fat in dry matter (%)	52.41(±0.28)^{a}	51.85(±0.21)^{b}	49.92(±0.04)^{c}
WSN/TN (%)	11.27(±2.30)^{c}	25.62(±0.64)^{b}	39.95(±0.51)^{a}
Salt (%)	1.34(±0.03)^{b}	1.43(±0.08)^{b}	1.63(±0.01)^{a}
Ash (%)	4.20(±0.03)^{b}	4.33(±0.02)^{b}	4.62(±0.03)^{a}
Total Ca (%)	0.765(±1.41)^{a}	0.743(±2.30)^{ab}	0.717(±2.22)^{b}
Texture attributes			
G' (KPa)	12.69(±2.09)^{b}	58.64(±4.33)^{a}	51.15 (±1.56)^{a}
G'' (KPa)	4.36(±0.74)^{b}	19.21(±1.15)^{a}	18.02 (±0.54)^{a}
Tan δ (G''/G')	0.34(±0.00)^{ab}	0.33(±0.00)^{b}	0.35 (±0.02)^{a}
η* (KPa.s)	2.14(±0.35)^{b}	9.83(±0.71)^{a}	8.14 (±0.26)^{a}

One-Way ANOVA was applied to data and values with different superscript letter are significantly different ($P<0.05$, LSD test)

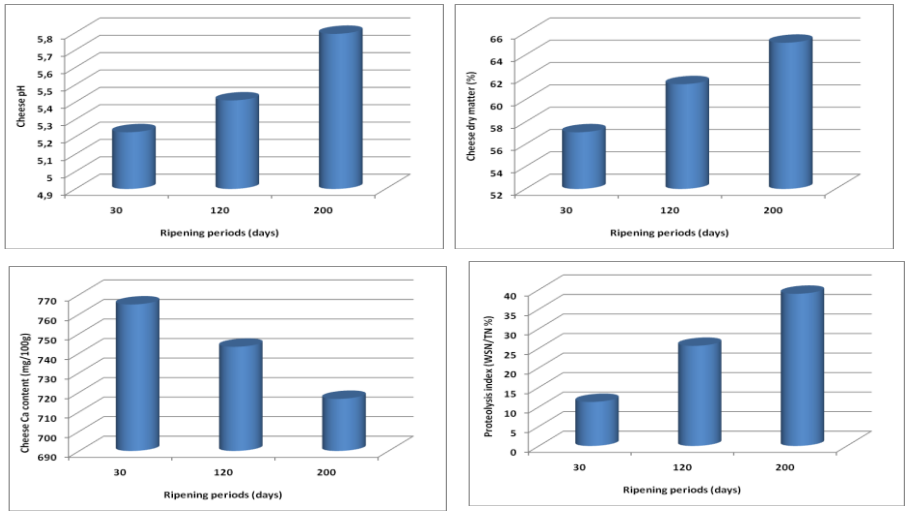

Figure 21: Change in pH values, dry matter, Ca and WSN/TN % of Cantal cheese during the ripening period.

2.1.1. Changes in colour values of Cantal Cheese throughout the ripening periods

No colour (L* and a* values) significant differences were observed from Cantal cheese samples ripened for at 30, 120, and 200 days, although a slightly lower degree of L* and a* values, as shown in Figure (22). The value of the L* and b* indicates that the cheeses studied had a colour tending towards lighter yellow which was decreased throughout ripening progress. Regarding the a* parameter the cheeses had, in general, negative values. Negative numbers for the *a** value indicate that cheeses are more green than red. The values of the b* confirms that the predominant colour of the cheeses was yellow.

The whiteness of cheese is influenced by several factors including light scattering of fat and protein particles **(Rudan *et al.*, 1998)** and whey pockets **(Paulson *et al.*, 1998)**. Since the micelles have colloidal dimensions, they are capable of scattering light and the white colour of milk is due largely to light scattering by the casein micelles, with a contribution from fat globules. As ripening

progressed, whey in serum pockets diffused from the cheese body out, as seen by moisture loss. The surface area occupied by light-scattering centers was therefore decreased. Thus, changes in Cantal colour throughout ripening is probably attributed to the loss of moisture content which in turn increase the dry matter content and in parallel to changes in the decreased light scattering, and hence, lower L* and b* values.

Our results were in agreement with other authors who described a decrease in both lightness (L*) and yellowness (b*) and a slight increase in redness (a*) during cheese ripening **(Rohm & Jaros, 1996 and Pillonel *et al.*, 2002)**.

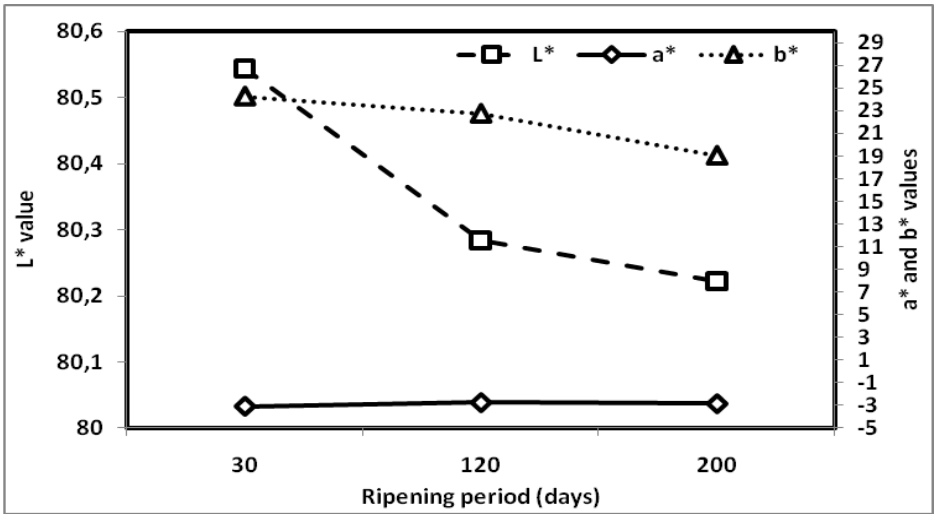

Figure 22: Changes in colour values (L*, a* and b*) of Cantal cheese ripened for 30, 120 and 200 days.

2.1.2. Rheological changes of Cantal cheese throughout the ripening periods

Changes in the rheological characteristics of Cantal cheese during the ripening based on its storage modulus (G'), loss modulus (G''), loss tangent (Tan δ) and viscosity complex (η*) are reported in Table (9) and Figure (23) that reflected the biochemical changes in Cantal cheese throughout ripening.

In general, at any ripening stage the G'(index of the firmness), was always higher than G'' which indicates the predominating solid character of cheeses **(Ustunol *et al.*, 1995)**. Moreover, the value of Tan delta (δ) for Cantal cheese was less than 1.0 indicating that the elasticity nature (G') of the samples was higher than their viscous nature (G''), an indication that the cheese exhibited solid-like behavior **(Tunick *et al.*, 1993)**. Since, Tan δ (ratio of G'' to G', viscoelasticity index) was ranged from 0.33±0.00 for the 30 days-old to 0.35±0.02 for the 200 days-old cheeses.

One-way ANOVA showed that there were significant differences ($P < 0.05$) in all textural properties among the cheeses ripened for 30 and 120 days, although at 200 days little differences were observed compared with 120-days-old cheeses. Since, there was no statistical difference in texture attributes of Cantal cheese ripened for 120 and 200-days-old cheeses, as reported in Table (9). The increase in the viscosity complex (η*) observed throughout ripening, since as proteolysis occurs, the amount of soluble casein that is in the serum phase increases, accounting for the increase in viscosity of the filler component **(Visser, 1991 and Olson *et al.*, 1996)**.

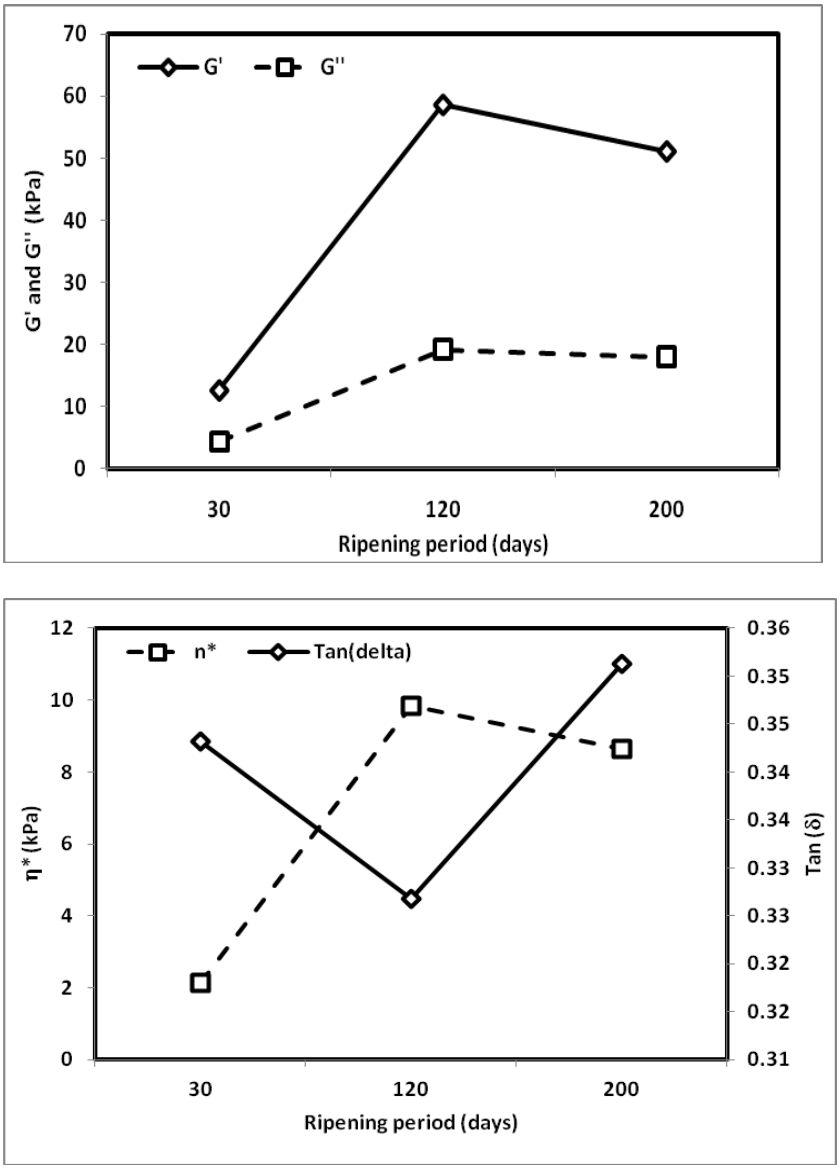

Figure 23: Changes in rheological characteristics (G', G'', Tan δ, η*) of Cantal cheeses ripened for 30, 120 and 200 days.

These differences in rheological parameters could be explained by two opposite effects (weakening of cheese matrix and firming effect) throughout the ripening periods which would be predominant depending on the extent of proteolysis, pH and water content.

Differences between the cheese ripened for 30 and 120 days was probably attributed to, in the early stages of ripening time, the degree of curd fusion and contact area between casein particles was low which we believe to be responsible for the increase in the rheological parameters. The long-ripened cheese (120 and 200 days) the rheological parameters were decreased, but this decrease did not statistically important, due to extended proteolysis **(Khosroshahi *et al.*, 2006)**, a gradual breakage of the network calcium bonds **(Ehsani *et al.*, 1999)** and the loss of water available of solvation of the protein chains and the consequent formation of a more compact cheese matrix (firmness cheese). Similar results were in agreement with those obtained for soft cheese **(Karoui & Dufour, 2003)** and Cheddar cheeses **(Wick *et al.*, 2004)**.

In conclusion, this generally can be attribute to during cheese ripening, hydrolysis of casein to low molecular weight peptides and amino acids, solubilization of insoluble Ca, decrease of water available and pH changes due to lactic acid production are responsible for the changes in the textural properties of Cantal cheese.

2.1.3. Monitoring structural changes of Cantal cheese throughout the ripening periods by SFS

Cheese structure is determined by the spatial arrangement of its components (especially casein) and their interaction with them during manufacture and ripening processes. During ripening, the main components of cheese are subject to physical and chemical changes, which determine the fluorescence properties of the final product.

SFS technique is a physical method of characterization that can be a remarkably effective alternative to traditional analysis. SFS is a method wherein simultaneous (synchronously) scanning of both the excitation and emission spectra is done at a constant offset value or differences between the emission and excitation wavelengths, $\Delta\lambda$ (λ_{em}-λ_{ex}). Unlike conventional fluorescence, SFS has the advantage of being able to characterize several fluorophores from a single spectrum.

The ripening of Cantal-type cheeses was studied in terms of various structural changes at the molecular level-protein structure and interactions associated with protein-protein and protein-lipids interaction by following the changes in the intrinsic fluorophores exist in cheese.

The synchronous scans performed on Cantal cheese throughout the ripening periods showed the presence of three major fluorophores, namely; 295, 322 and 355 nm, as shown in Figure (24). The synchronous fluorescence spectra showed slightly different shapes between the investigated cheeses and the fluorescence intensity decreased in accordance with the degree of ripening. Differences in the spectral shape might be explained by the physico-chemical changes in the proteins and fat occurring during ripening period (Table 9).

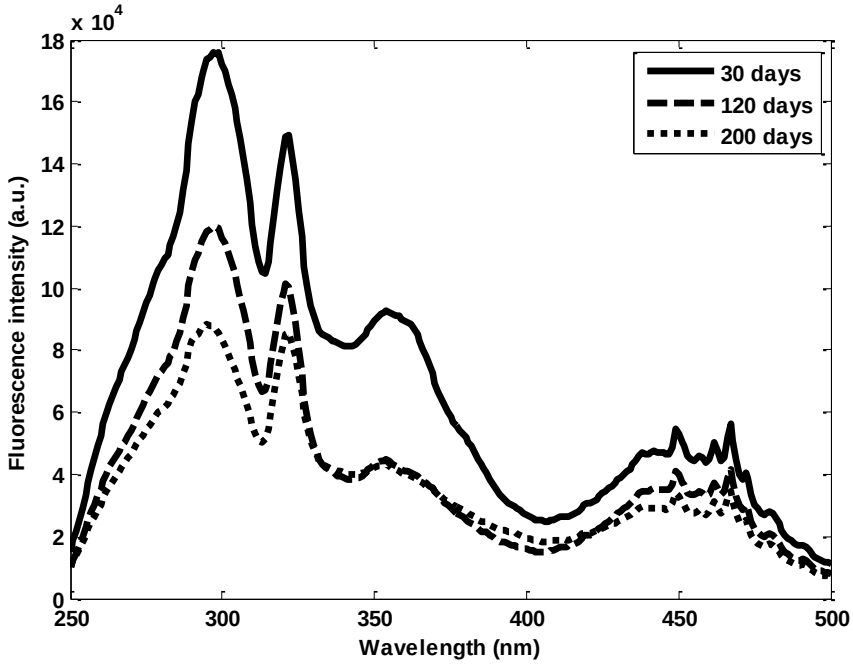

Figure 24: Changes in synchronous fluorescence spectra of young (30 days), mild (120 days) and old (200 days) Cantal cheeses collected in the 250-500 nm excitation wavelength range using offsets of Δλ 80 nm.

From Figure (24), for all cheeses, the highest synchronous fluorescence peaks are seen with excitation at 295 nm (emission at 375 nm), at 322 nm (emission at 402 nm), and at 355 nm (emission at 435 nm). Apart from this three major peaks, some smaller peaks of aromatic amino acids are observed around 449-490 nm. The band observed at 295 nm could be attributed to tryptophan residues of proteins **(Karoui, 2004 and Karoui *et al.*, 2004)**, while the band appeared at 322 nm (emission 402 nm) was probably related to vitamin A **(Karoui, 2004)** and the band appeared at 355 nm (emission at 435 nm) was probably related to riboflavin **(Karoui *et al.*, 2007c)**. Finally, the bands around 449-490 nm could be assigned to some coenzymes (*e.g.* NADH, FADH) **(Kulmyrzaev *et al.*, 2005b)**, riboflavin found in milk **(Boubellouta & Dufour, 2008)** and Maillard-reaction products **(Kristensen *et al.*, 2001 and Karoui *et al.*, 2007a)**.

Moreover, we observed a red shift of tryptophan excitation spectra to larger wavelength (from 295 to 297 nm) of the cheese ripened for 200 days. This shift can be explained by the proteolysis of casein during the ripening periods which resulting in an increase of tryptophan exposure to the aqueous phase for old cheeses **(Herbert *et al.*, 2000)**. Thus, it is suggested that native caseins and their degradation products induce changes in the molecular environment of tryptophan residues **(Herbert *et al.*, 2000 and Dufour *et al.*, 2001)** which explain the broadening of the tryptophan excitation spectra between 30 and 200 days of cheese ripening. Indeed, young cheeses (30-days-old) had the highest fluorescence intensity at 295, 322 and 355 nm, whereas cheeses of 200 days old had the lowest ones.

The differences observed for vitamin A fluorescence spectra (band at 322 nm) are consistent with changes of lipid structure and, as a consequence, changes of the physical state of triglycerides in the fat globules throughout the ripening periods **(Dufour & Riaublanc, 1997 and Dufour *et al.*, 2000)**. Concerning the changes in the band at 355 nm excitation fluorescence spectra throughout ripening period, this could be attributed to the lipid oxidation of cheeses throughout ripening which could contribute to the change observed on the riboflavin spectra.

Because of the complexity of the fluorescence spectra, univariate analysis ANOVA was not appropriate for the study of large data sets. Multivariate statistical techniques such as PCA, and FDA make it possible to extract information related to the structural changes cheese from the environment of the intrinsic probes in cheese **(Saporta, 1990)**.

2.1.4. Multivariate analysis of SF spectra

Our second objective was to test the ability of synchronous spectra data to the discrimination between differently stored cheese samples. Principal component analysis and FDA were used to examine how the synchronous fluorescence method was able to differentiate between the Cantal cheeses throughout the ripening periods.

Firstly, PCA was applied to the set (24 objects and 251 variables) of synchronous fluorescence spectra recorded on Cantal cheese throughout ripening period. This treatment has shown that three ripening groups were ordered in accordance with ripening time in the factorial map defined by the principal components 1 and 2. The first two principal components accounted for 94% of the total variance with a large predominance of the principal component 1 (explains 76.36%). Figure (25 a) shows the score plot of PC_1 (explains 76.36% of total variance) versus PC_2 (explains 17.92% of total variance) of PCA plot applied on the synchronous fluorescence spectra of young (30 days), between the two (120 days) and old (200 days) Cantal cheeses.

Three groupings of cheese are observed; the first grouping (30 days) is seen in the upper right quadrant which have high PC_1 values; the second grouping (120 days) is seen in the lower right quadrant of the low PC_1 values and the third grouping (200 days) is seen in the upper left quadrant according to PC_1. Indeed, it appeared that first and second groups exhibited positive values according to PC_1, the third group (200 days) showed negative values according to PC_1 and positive values according to PC_2 (Figure 25 a). These differences reflected changes in the structure of cheese matrix, the physical state of triglycerides and protein-lipid interactions throughout cheese ripening. It was concluded that one (or more) continuous phenomenon, taking place during the ripening, was detected when the fluorescence of the intrinsic was

considered.

In order to point out which wavelengths were involved in the discrimination of the cheese samples, the factor loadings associated with the PC_1 and PC_2 were analyzed (Figure 25 b). The factor loadings for PC_1 and PC_2 shows the importance of the bands with maxima at 295 (assigned to tryptophan) at 322 (assigned to vitamin A) and 355 nm (assigned to riboflavin), and they describes changes in these bands throughout the ripening periods.

Factor loadings 1 (Figure 25 b) characterized the samples on the right of the map (Figure 25 a) which it were characterized by a maximum at a relatively higher fluorescence intensity than those on the left side. It indicated that during ripening process, the main components of cheese (casein and fat) are subject to physical and chemical changes, which effect on the fluorescence intensity of tryptophan, vitamin A and riboflavin, resulting in changes in the structure of casein micelles. These structural changes can induce a more hydrophilic environment of the tryptophan of caseins in accordance with the blue shift of the maximum for the older cheeses and change in the shape of vitamin A spectra which was found to correlate with lipid oxidation of cheese. Moreover, ripening involves mainly an increase in pH, a change in protein-protein and the physical state of triglycerides and protein-lipid interactions. The pH values of 30, 120 and 200 days-old cheeses were 5.23, 5.41 and 5.79, respectively (Table 9).

Factor loadings 2 (Figure 25 b) indicated that the shape of fluorescence spectra was larger for cheeses located on the positive side (30 and 120 days) than for those on the negative side (200 days). It appeared that changes in fluorescence spectra observed could be due to different protein-protein/fat interactions and different network structures resulting from the ripening process.

The results showed that the factor loadings 1 and 2 allow us to derive the information on the protein structure, protein-protein

and protein-fat globule interactions, and the degree of riboflavin degradation to be derived at the molecular level during the ripening periods. Thus, our results confirm previous findings **(Herbert, 1999; Dufour *et al.*, 2001; Mazerolles *et al.*, 2001; Karoui *et al.*, 2007a and Karoui *et al.*, 2007b)** reporting that three intrinsic fluorophores presented in the cheese could be considered as fingerprints allowing a good identification of changes in the cheeses as a function of their ripening time.

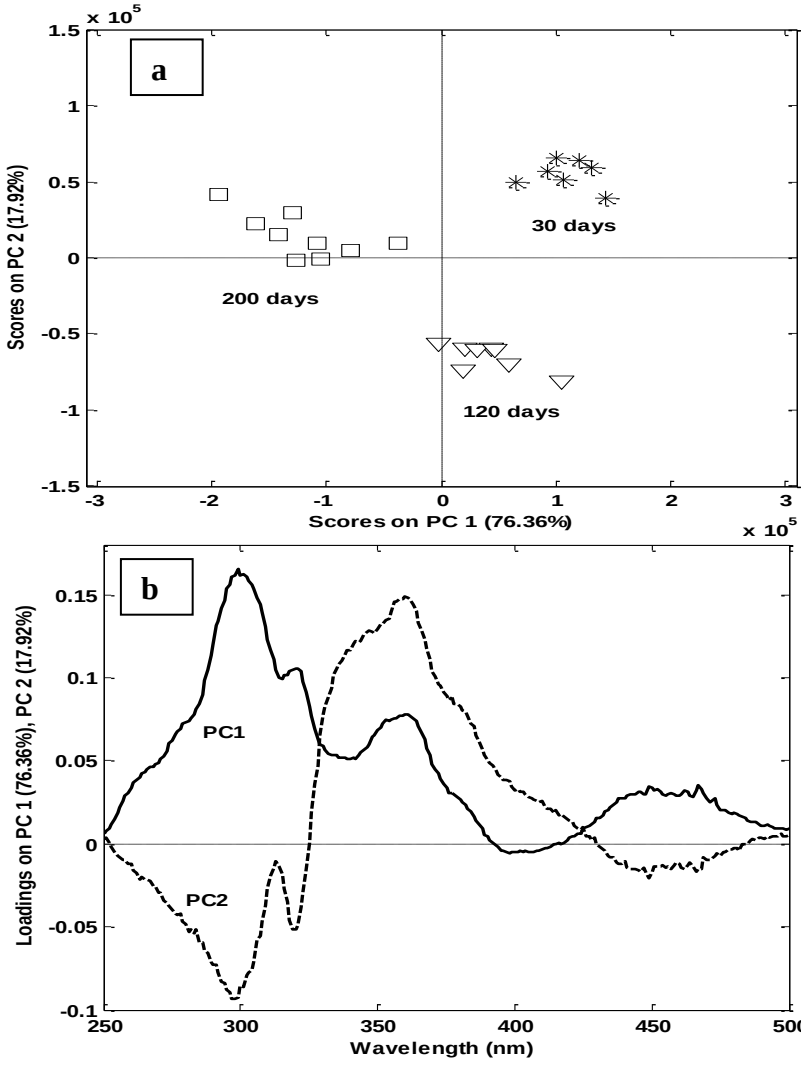

Figure 25: (a) Principal component analysis similarity map (score plot) determined by principal components 1 (PC_1) and principal component 2 (PC_2) and (b) factor loadings corresponding to PC_1 and PC_2 performed on the synchronous fluorescence spectra of the Cantal cheese ripened for 30, 120 and 200 days. The lines in (b) indicate: PC_1 (solid) and PC_2 (dotted).

Secondly, in order to find out the differences between cheeses at the molecular level-protein structure and interactions throughout ripening, the FDA was applied on the first 5 PCs of the PCA performed on the synchronous fluorescence spectra of Cantal cheese throughout ripening. The similarity map of the FDA allowed a good discrimination of the investigated cheeses. The map defined by the discriminant factors 1 and 2 represented 100% of the total variance with discriminant factor 1 accounting for 82.10% (Figure 26). Considering discriminant factor 1, Cantal cheeses ripened for 120 days and 200 days exhibited negative scores, whereas Cantal cheeses ripened for 30 days had positive score values. The discriminant factor 2 which took into account 17.90% of the total variance differentiated between 120-days-old and 200-days-old Cantal cheeses. A correct classification of 100% was obtained, as shown in Table (10).

Synchronous fluorescence spectra allowed the changes of cheese structure during ripening to be discriminated. These results suggest that SFS could be considered as a fingerprint, allowing a good identification of cheese based on their structural changes, resulting from the changes of chemical characteristics of cheese throughout ripening period.

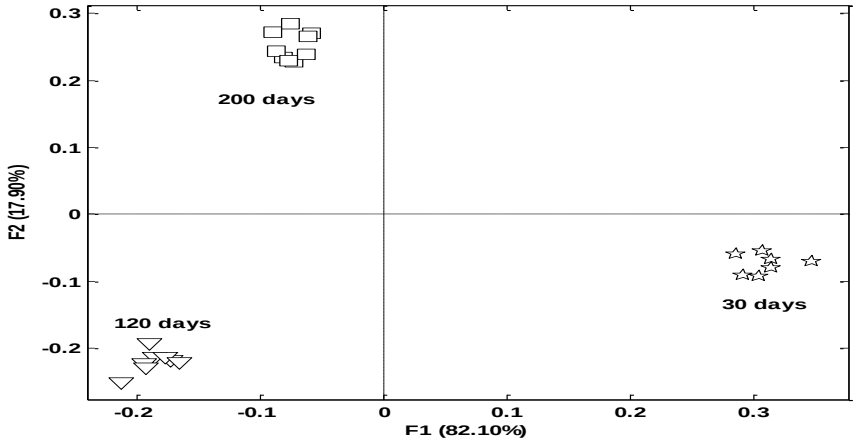

Figure 26: Discriminant analysis similarity map determined by discriminant factors 1 (F_1) and 2 (F_2) for the FDA performed on the first 5 principal components (PCs) of the PCA applied to the synchronous fluorescence spectra of Cantal cheeses ripened for 30, 120 and 200 days.

Table 10: Classification of Cantal cheese ripened for 30, 120 and 200 days based on their synchronous fluorescence spectra.

The number of predicted samples	The number of observed samples				% correct classification
	30 days	120 days	200 days	Total	
30 days	7	0	0	7	100%
120 days	0	8	0	8	100%
200 days	0	0	9	9	100%
Total	7	8	9	24	100%

3. CONCLUSION

The compositional characteristics (pH, fat, protein, salt, WSN/TN% and ash contents) increased significantly during the ripening period but calcium and the moisture contents decreased to some extent.

Ripening significantly influenced colour, resulting in a decrease of L* and b*, but it was observed a slight increase in a* value over ripening.

Rheological characteristics increased with the ripening periods, showing that ripening contributed to changes in the structure of cheese matrix, where the differences in G' and G" were observed.

The results of FDA performed on PCs, showed a good discrimination of the cheeses from their spectral data. Synchronous fluorescence spectroscopy presents a suitable alternative for monitoring changes in the chemical characteristics of Cantal cheese throughout the ripening periods compared with the routine analysis. This method allows obtaining information on several fluorescent constituents in a single scan.

PART III

A comparative study of traditional and industrial Saint-Nectaire cheese-making process by mid infrared spectroscopy and chemometrics

PART III: A comparative study of traditional and industrial Saint-Nectaire cheese-making process by mid infrared spectroscopy and chemometrics

1. INTRODUCTION

Cheese manufacture is essentially a dehydration process of milk in which the fat and casein in milk are concentrated between 6- and 12-fold, depending on the variety. Cheese production around the world occurs mainly in cheese factories, but in some areas, small-scale production of cheese using traditional methods is predominant. The quality and unique character of some local cheeses with distinctive flavor and texture attributes are closely related to the environmental conditions of milk production, to the type of milk employed, to the particular technology applied and closely guarded family secrets.

In a large scale, cheese manufacture implies changes in milk production, with consequences for the quality of the milk. In particular, milk collection has changed; milk is collected over a wider area, resulting in co-mingled milks and increased transport and the storage time before processing. This induces the development of microbial populations which are different from those present in milk at the farm. In order to destroy pathogens and standardize the milk microflora, pasteurization of milk has become widespread which reduce of quality of cheese **(Fox *et al.*, 2004)**. One of the consequences is the standardization of milk and use of a secondary microflora (starters) however; this leads to cheeses with a more constant and uniform quality. Another consequence in the modification of the cheese manufacturing practices is heat treatment of milk prior to cheese making had an effect on microbial flora, proteolysis, free amino acids, free fatty acids, volatile fractions and sensory characteristics. In contrast, in the farmhouse cheese, milk is made immediately after lactation process to cheese without any technological treatment; use of natural microflora as a

starter culture namely ‘wild’ starters and ripening under natural environment.

Saint-Nectaire cheese is one of the most popular traditional semi-hard cheeses manufacture in the Auvergne region of central France by processes recognized by Protected Denomination of Origin (PDO). Production may be artisanal or industrial, depending on whether the cheeses are manufactured with raw or pasteurized milk. The traditional process is carried out according to traditional usages, including raw milk, unprocessed milk, natural microflora as a starter culture and farmhouse rennet, cutting, salting and ripening in cave nature. The industrial process used pasteurized milk, and hence a starter culture is always added before coagulation; the remaining steps are similar to those for the traditional process but ripening process performed in ripening room with high technology.

The traditional method is still used in farms in small scale and villages. Local forage, often pastures, is utilized for feeding cows, and the raw milk is coagulated with rennet paste, using wooden cheese making equipment and without adding any starter cultures. The nature and quality of the finished cheese are determined largely by the method of manufacture which influences on the physical, chemical and sensory characteristics of the cheese. The PDO mark should represent a guarantee for the consumers that cheeses were produced according to local milk production regulations and traditional cheese-making techniques and cheese ripening processes **(Karoui *et al.*, 2005a)**. Therefore, considerable interest exists in the development of instrumental techniques to enable more objective, faster and less expensive assessments for defining and controlling the qualitative characteristics of typical cheeses in order to secure the consumer’s choices and to protect traditional products against cheaper industrial imitations.

The Manufacturers have traditionally depended on a wide range of conventional methods for assessment of cheese quality such as oven drying, Gerber or standard micro/macro-Kjeldahl

procedures for the determination of moisture, fat and protein contents, respectively. are laborious and time consuming **(Subramanian *et al.*, 2009)**.

In the recent years, spectroscopic techniques have been used quite often in the agricultural and food industries. These analytical techniques are relatively of low cost and can be utilized in both fundamental research and in the factory as on-line sensors for monitoring dairy processes **(Subramanian & Rodriguez-Saona, 2008)**.

Spectroscopic methods for measurements of food quality include ultraviolet and visible absorption, fluorescence emission, near-infrared and mid-infrared absorption spectroscopy. Fourier transform infrared (FTIR) spectroscopy is a direct, reliable and fast method that makes it possible to obtain specific information about different parameters simultaneously. mainly in the 3000-900 cm^{-1} region since bands are associated to vibrations of functional groups of the molecules **(Bertrand & Dufour, 2006)**. The associated bands of proteins, fats, lactose and lactic acid are well known and have been described in milk and cheese.

In recent years, infrared (IR) spectroscopy has been applied to measure cheese composition **(Rodriguez-Saona *et al.*, 2006 and Upreti & Metzger, 2006)**, sensory and instrumental texture parameters **(Blazquez *et al.*, 2006 and Karoui *et al.*, 2006c)** and to determine the geographic origin of the cheese **(Karoui *et al.*, 2005b)** and to use IR spectroscopy to follow compositional and molecular changes during the cheese ripening periods **(Subramanian *et al.*, 2009)**.

The objective of this study was to (1) examine the physico-chemical properties of cheese elaborated via traditional and industrial methods and (2) to evaluate the potential of FTIR spectroscopy to examine the spectral characteristics for Saint-Nectaire cheese and to discriminate the difference among two

cheese-making processes by PCA and FDA.

2. RESULTS AND DISCUSSION

2.1. Physical and chemical characteristics of Saint-Nectaire cheese

The physical properties (i.e., body/texture, colour…etc) and chemical composition of cheese are influenced by initial cheese milk used, manufacturing procedures. and maturation conditions **(Guinee, 2002)**. Table (11) presents average values and standard deviations for physical properties and chemical composition for traditional and industrial Saint-Nectaire cheeses. In the context of physiochemical characteristics, industrial cheeses in this study had similar levels of dry matter, pH, fat, Ca, P contents and proteolysis, but higher levels of protein, ash, fat/dry matter than traditional cheeses.

Cheese Colour values

Cheese colour is influenced by many factors such as the milk pigments content, like β-carotene with regard to the feed and to the botanical composition of pasture in grazing cattle.

Changes in the cheese colour could be also related to the biochemical activity of the native microflora, the technological processes and the ripening technique **(McSweeney, 2004 and Dufossé *et al*., 2005).**

The results illustrated in Table (11) showed the effect of cheese-making process on Saint-Nectaire cheese colour in terms of redness (- a*), yellowness (b*) and lightness (L*). It is clear that cheese-making process did not significant effect on the lightness (L*) as well as the yellowness (b*), whereas (a*) value showed a significant difference. This could be explained by the cheese milk composition and the treatments applied to milk. Indeed, industrial cheeses produced from pasteurized milk whereas the traditional

cheeses were made with raw milk.

Textural characteristics

Cheese has a complex structure that causes differences which depend on compositional factors, their changes during ripening and manufacturing process. The parameters of G', G", Tan δ and η* were used for comparing the texture and rheology of cheeses and its results are summarized in Table (11) and illustrated in Figure (27). However, traditional and industrial Saint-Nectaire cheeses are quite similar in composition but significantly different in texture attributes. Industrial Saint-Nectaire cheeses exhibited the highest values of G', G", and η* whereas traditional cheeses showed the lowest ones. The parameters of G', G" and η* generally increased with network firmness trends **(Gunasekaran & AK, 2000)**. The significant modification of protein matrix, fat globule and redistribution of fat during cheese making are probably responsible for the observed differences in dynamic properties of traditional and industrial Saint-Nectaire cheeses.

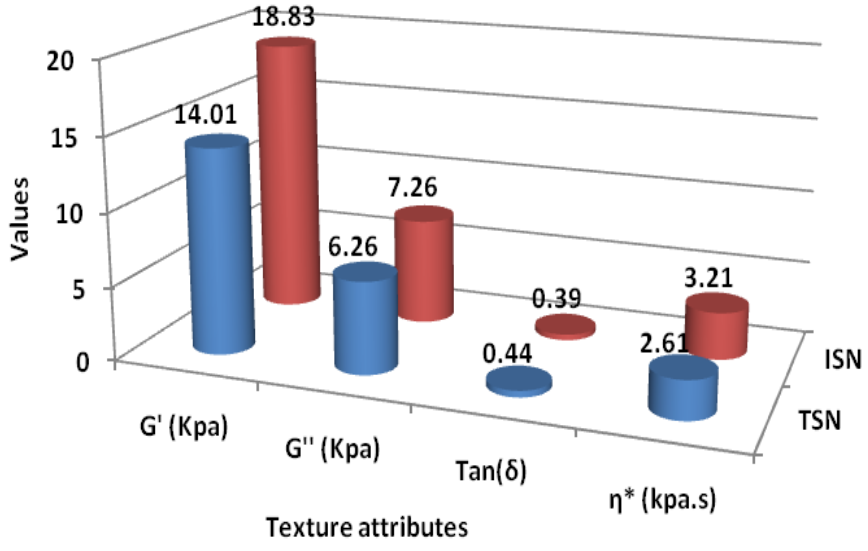

Figure 27: Texture properties attributes of traditional (TSN) and industrial Saint-Nectaire cheeses (ISN).

Table 11: Mean (±SD) of the physical and chemical characteristics of traditional and industrial Saint-Nectaire cheeses.

	Traditional Saint-Nectaire		Industrial Saint-Nectaire	
Parameters	**Mean (±SD)**	***CV**	**Mean (±SD)**	***CV (%)**
pH	5.66 (±0.12)[a]	2.12	5.74 (±0.15)[a]	2.61
Dry matter %	53.50 (±2.21)[a]	4.14	55.22 (±1.18)[a]	2.13
Fat %	27.78 (±2.05)[a]	7.43	26.78 (±1.24)[a]	4.63
Protein %	20.96 (±0.66)[b]	3.14	23.44 (±0.36)[a]	1.54
WSN/TN %	31.74 (±1.64)[a]	5.16	33.37 (±0.51)[a]	1.53
Salt %	0.97 (±0.02)[b]	2.01	1.23 (±0.03)[a]	2.44
Ash %	3.17 (±0.16)[b]	5.05	3.63 (±0.17)[a]	4.68
Total Ca %	0.55 (±0.07)[a]	3.63	0.54 (±0.02)[a]	3.70
Total P %	0.42 (±0.03)[a]	7.14	0.45 (±0.01)[a]	2.22
Colour values				
*L** value	79.65(±1.03)[a]	1.29	80.34(±0.87)[a]	1.09
*a** value	-1.79(±0.23)[a]	12.92	-2.34(±0.11)[b]	4.65
*b** value	23.20(±0.64)[a]	2.75	21.23 (±2.75)[a]	12.12
Texture attributes				
G' (KPa)	14.01(±5.91)[a]	4.21	18.83(±6.43)[b]	3.40
G" (KPa)	6.26(±2.41)[a]	3.84	7.26(±4.41)[b]	0.60
Tan δ (G'' /G')	0.44(±0.03)[a]	6.82	0.39(±0.01)[b]	2.80
η* (KPa.s)	2.61(±1.01)[a]	3.87	3.21(±0.98)[b]	3.06

*CV (%): coefficient variation; One-Way ANOVA was applied to data and values with different superscript letter are significantly different ($P<0.05$, LSD test).

2.2. Principal component analysis of physicochemical data

To support the hypothesis that cheese-making technology was responsible for the differences, the physicochemical data (significant only) were subjected to principal component analysis (PCA), in order to ascertain which physicochemical variables contributed most to the total variance and to differentiating between the different cheeses. Two principal components (PC) accounted for 91% of the total variance; PC_1 accounted for 81.86%, and correlated with protein, salt and rheological properties; PC_2 accounted for 9.46% of the total variance, and correlated with fat/dry matter, Tan δ and a* values. Figure (28) shows the distribution of the cheese samples on the map formed by the two principal components (PC_1 and PC_2). A very clear separation of the samples was observed in the score plot of PC_1 vs. PC_2; separate groups were formed for each type of the cheese. Industrial cheeses were grouped to the right of PC_1, indicating high levels of protein, ash, salt and texture attributes. While, traditional cheeses were grouped on the negative side of PC_1. These samples of cheeses contained the highest concentrations of fat/dry matter.

The obtained results in this study indicated that few chemical or physical differences between the different cheeses from the two cheese-making technologies; there were, however, differences in nitrogen fractions and texture attributes. This may be due to the action of different enzymes, mostly of microbial origin, during the ripening. These results gave rise to small but characteristic texture differences between the different cheeses from different technology, with texture attributes contributing the most to differentiation among them.

Generally, the differences in composition between the investigated cheeses may be probably attributed to the differences in milk composition, different treatments applied to cheese milk and the cheese-making process which had a significant effect on viscoelastic characterization of cheeses. These results are consistent

with those reported for other artisanal cheeses produced in Europe **(Beuvier *et al.*, 1997; Ballesteros *et al.*, 2006 and Cabezas *et al.*, 2007)**.

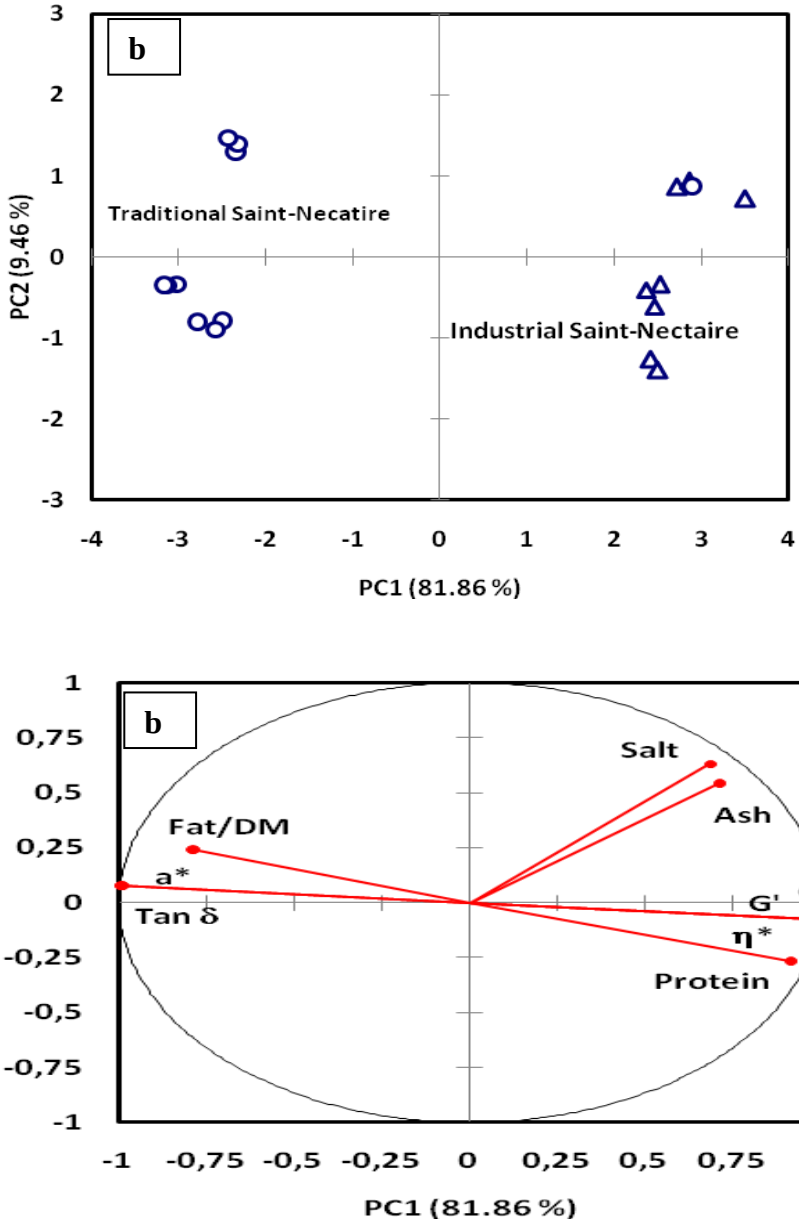

Figure 28: PCA of the physicochemical data of traditional and industrial Saint-Necatire cheeses.

(a) Score plot of PC_1 vs. PC_2, (b) Loading plot of PC_1 vs. PC_2.

2.3. Characterization of FTIR Spectra of Saint-Nectaire cheeses

In the MIR (3000-900 cm^{-1}) region, the absorption bands are associated with fundamental vibrations of functional groups of the molecule particles. The band intensities vary with the overall concentration of the chemical functional groups in the cheese samples.

Figure (29) shows a typical MIR spectrum of traditional and industrial Saint-Nectaire cheeses ripened for 30 days, indicating a number of the spectral bands arising from specific functional group vibrations for fat, protein, lactose and lactic acid.

The absorbance intensity of fat-and protein-related bands in the investigated cheese samples were different as expected due to the variation in composition, rate of proteolysis and lipolysis occurring in each cheese samples which depending on cheese-making procedure.

The spectral regions that were the most important to assess the quality attributes of cheese were found to be 3000-2800 cm^{-1} region corresponding to lipids, 1700-1500 cm^{-1} corresponding to the Amide I and II bands, and the 1500-900 cm^{-1} region called the fingerprint region. This indicates the role of fat content and protein structure in determining the quality attributes of cheese.

The region between 3000-2800 cm^{-1} consists of absorbance from C-H stretching vibrations of $–CH_3$ and $> CH_2$ functional groups of fatty acids **(Bertrand & Dufour, 2006)**.

Two major bands corresponding to methylene groups at ~ 2920 cm^{-1}(ν_{as} CH_2) and 2855cm^{-1} (ν_s CH_3), as well as one minor band corresponding to methyl groups at 2956 cm^{-1} (ν_{as} CH_3) were observed. There is a variation in absorbance intensity in the investigated cheese samples, where traditional Saint-Nectaire (TSN) cheeses presented the highest intensity at 2920 cm^{-1} and 2855 cm^{-1}, while industrial Saint-Nectaire (ISN) cheeses presented the lowest ones. Furthermore, a slight shift to higher wavenumber

of the CH_2 stretching mode was observed for industrial Saint-Nectaire (ISN) cheeses.

The observed changes in methyl and methylene bands were attributed to the difference in nature, concentration and physical state of fatty acids **(Boubellouta *et al.*, 2010)**.

The peak at ~ 1742 cm^{-1} (ν –C=O), associated with esters and organic acids. A reduction in the intensity of this band has been associated with the lactate consumption **(Martın-del-Campo *et al.*, 2009)** while the increment with higher concentration of carbonyl groups generated during the lipolysis and proteolysis **(Chen *et al.*, 1998)**.

Also obtained results showed that, two well-defined peaks were observed in the frequency range 1700 to 1500 cm^{-1} to the Amide I at ~1643 cm^{-1} (*ν* C=O, *ν* C-N) for TSN cheeses, at ~1641 cm^{-1} for ISN cheeses while the Amide II at ~1547 cm^{-1} (*δ* N-H and *ν* C-N) for TSN cheeses, and at ~1545 cm^{-1} for ISN cheeses. These two peaks are associated with hydrolysed proteins **(Bertrand & Dufour, 2006)**. The changes in intensity and position of these bands in the 1700-1500 cm^{-1} range have been associated with changes in casein secondary structure. protein aggregation and protein-water interaction **(Mazerolles *et al.*, 2001; Kulmyrzaev *et al.*, 2005a and Bertrand & Dufour, 2006)**.

The C-H bending (1462, 1417, 1377 cm^{-1}) and C-O stretching (1242, 1165 cm^{-1}) functional groups of polypeptides, amino acids, carbonyl groups of fatty acids, hydroxyl groups, carboxylic acid groups, and fatty acid esters appear in spectral range 1500-900 cm^{-1} (fingerprint region) **(Bertrand & Dufour, 2006)**. Visual comparison of the raw spectra showed numerous differences between the different cheeses, especially in the spectral region 1500-900 cm^{-1}.

The peaks located at ~1377cm^{-1} (δ_s CH_3) assigned to glucose and galactose and the ~1165 cm^{-1} peak, which related to

sum of lactose (ν C-OH). monosaccharide (ν C-O) **(Petibois *et al.*, 2000)** and the ester linkage of lipids (ν C-O) **(Martín-del-Campo *et al.*, 2007)**. The bands located between 1103 and 966 cm^{-1} have been attributed to the lactate by **(Picque *et al.*, 2002)**. The increase in the absorbance bands of this region is due to an increase in the quantities of cheese compounds which could be vary in cheese due to the complex biochemical reactions, such as glycolysis, lipolysis and proteolysis which occurred during the cheese ripening periods.

These results highlight the importance of the different regions across the entire spectral range used in predicting the quality attributes of cheese. The importance of different spectral regions in predicting quality attributes is related to the effect of manufacturing process, the composition of milk (such as protein and fat level) and the biochemical events that occur during ripening on final quality of the cheese. Changes resulting in manufacturing process of on cheese composition directly affect its molecular structure and hence its mid-infrared spectra.

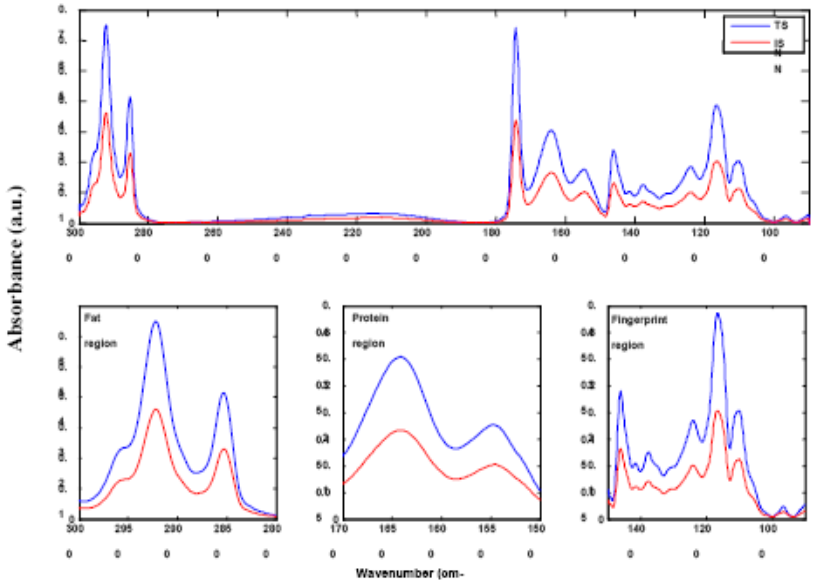

Figure 29: **Typical Mid infrared spectrum of traditional (solid line) and industrial Saint-Nectaire cheese (dashed line) of 30 days-old aged in the three regions. AU = arbitrary unit**

2.4. Cheese discrimination based on FTIR spectra

PCA was applied to the set of mid-infrared spectra in order to obtain important information that described the spectral changes during cheese-making process and the association with corresponding biochemical reactions.

The PCA factorial map and the factor loadings plot of Saint-Nectaire cheese spectra are shown in Figure (30 a and b). It is defined by the principal components 1 and 2 which account 91.29 % and 8.02% of the total variance, respectively. From the PCA results, it was found that samples could be discriminated on the basis of cheese-making process into two groups of samples according to $PC_{1,}$ first group (farm cheese) located at the positive score of $PC_1$where as second group (industrial cheese) located at the negative score of PC_1 (Figure 30 a).

In order to determine the spectral wavenumbers and the associated functional groups that were responsible for the classification of the cheeses in PCA plot, the loading plot for PC_1 and PC_2 was analyzed. Figure (30 b) provides us important information about the characteristic absorption bands and the biochemical reactions involved which explain the discrimination. These bands could be provided valuable structural information about the changes that occurred in the acyl chains during the cheese manufacturing.

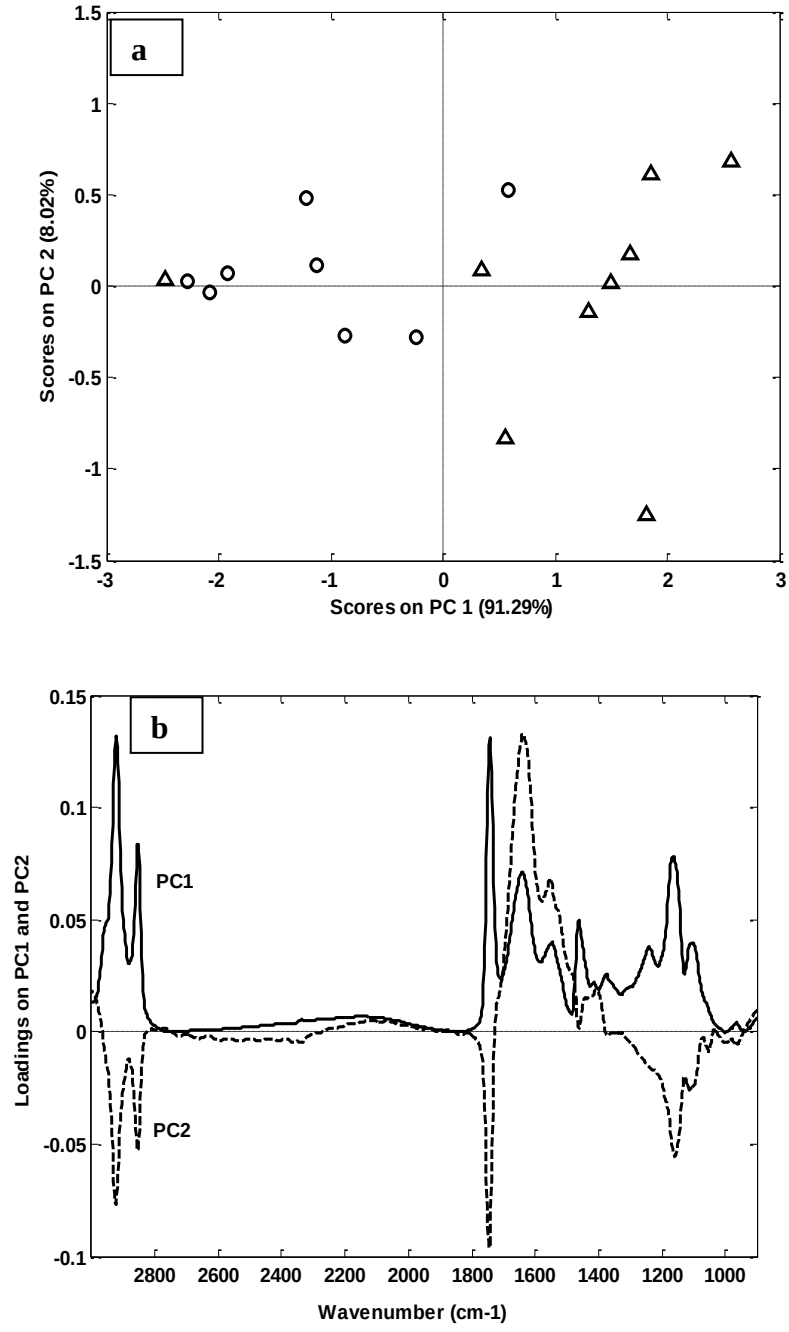

Figure 30: **(a) Principal component analysis similarity map determined by principal components 1 and 2 for MIR spectra of traditional (Δ) and industrial Saint-Nectaire cheese (o). (b) Loading plots corresponding to the principal components PC_1 (—) and PC_2 (- - -) for FTIR spectra**.

The main loading peaks observed for PC_1 were positive associated with lipids (2920, 2855, 1742 cm^{-1}). Amide I (1643 cm^{-1}), Amide II (1547 cm^{-1}), C-H bending (1462, 1417, 1377 cm^{-1}) and C-O stretching (1103, 1242, 1165 cm^{-1}). Negative loading peaks associated with lipids (2920, 2855, 1742 cm^{-1}). C-O stretching (1165. 110 $3cm^{-1}$) and the positive loadings peaks associated with Amide I (1643 cm^{-1}). Amide II (1547 cm^{-1}), C-H bending (1462 cm^{-1}) were observed for PC_2. These observations suggest that both loading peaks described the phenomena correlated with lactate consumption as well as proteolysis and lipolysis of cheese. These results also suggest that MIR spectroscopy is primarily differentiating between samples on the basis of manufacturing process of cheese.

In the second step, FDA was applied to the first 10 PCs of PCA applied to MIR spectra (3000-900 cm^{-1}) in order to differentiate between the two groups of cheeses (TSN and ISN). It was possible to classify different Saint-Nectaire cheese samples using MIR technology based on their spectral information. Using the spectral range of 3000-900 cm^{-1}, cheese samples from different manufacturers was grouped in well-separated by the FDA.

Table (12) shows the real versus the predicted values for the samples investigated. According to these observations, two groups could be distinguished as shown by the FDA, the first comprising samples was made by traditional methods from cheeses made by industrial methods. A discriminant analysis made on the basis of these two groups led to a correct classification rate of 100% for data sets. These results are consistent with the biochemical evolution of Saint-Nectaire cheeses.

Table 12: Results of factorial discriminant analysis classification of samples by cheese-making process.

Real [a]	Predicted [b]		Classification Success (%)
	Traditional SN	Industrial SN	
Traditional SN	9	0	100%
Industrial SN	0	9	100%

[a] The number of cheeses predicted from the model.
[b] The number of real cheeses

3. CONCLUSION

Results from the comparative study showed that industrial technology has a large impact on cheese quality, significantly affecting the texture attributes of cheeses, although they had similar composition characteristics.

FT-IR spectroscopy, coupled with chemometric analysis, could be provided an exceptional opportunity for confirming cheese quality and to classify according to their manufacturing process.

The information-rich infrared spectral range for Saint-Nectaire cheese samples was from 3.000 to 2.800 cm^{-1}, 1700-1500 cm^{-1} and 1500-900 cm^{-1}. This technique could contribute to the development of simple and rapid protocols for monitoring complex biochemical changes, and predicting the final quality of cheese. Variation in absorbance intensity of fat and protein-related bands changed greatly due to variation in cheese-making procedure. PCA and FDA of Saint-Nectaire cheese spectra made it possible to identify the spectral bands that exhibited the changes resulting from cheese-making process.

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SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

This work was conducted in collaboration with Food Quality Department, Academy of Agriculture Sciences and Veterinary (VetAgroSup), Clermont-Ferrand, France. This study included 3 projects research to study the effect of some treatments technology on the quality of Cantal and Saint-Nectaire cheeses by using some fast techniques which rely on the interaction of light with food material.

The quality of cheese can be evaluated by many instrumental methods and sensory analysis that are expensive, need to chemical materials, and time-consuming. Alternatively, spectroscopy methods has been successfully utilized to evaluate molecular-level interactions between protein-protein, fat and proteins in various food-based emulsions, as well as monitor structural changes in cheese and Maillard browning in milk and dairy products.

Therefore, the overall objective of this thesis was to investigate the use of new methods (rapid non-destructive) based on spectroscopic techniques (such as infrared spectroscopy and fluorescence spectroscopy) for evaluation cheese quality and understanding of the determinants of the structure and its relations with the texture.

The third chapter of this research work presents the results and discussion of experiments. This chapter is organized into three parts and the results obtained in this work are summarized briefly as follows:

Part I: Chemical, rheological, and Structural Characteristics of Cantal cheese made from Raw, Thermized, and Pasteurized milk

The objective of this part was to evaluate of Cantal cheese quality produced from raw, thermized and pasteurized milk by using physicochemical and fluorescence spectroscopy methods coupled with chemometrics.

Significant differences were observed between Cantal cheeses in their compositional characteristics. Heat treatment resulted in significant increases in the levels of pH, reduction in the contents of fat, protein, calcium and phosphorus in the cheese. Proteolysis was significantly higher in the raw Cantal cheeses milk.

Heat treatment significantly influenced colour, since colour values (L*, b* and negative a*) were high in cheeses made from raw milk than made from thermized or pasteurized milk, while the heat treatment effect was not significant for negative a* values. Texture attributes of raw and heat-treated milk cheeses were not significantly different. The PCA performed on the physicochemical parameters allowed to a good discrimination between cheese samples according to their physicochemical changes.

100% of correct classification was obtained for cheeses by applying FDA to fluorescence spectral data (tryptophan and/or vitamin A). It is shown that tryptophan emission or vitamin A excitation fluorescence spectra of cheeses may be considered as a characteristic fingerprint which allows the cheese samples to be discriminated, since it were retained information related to molecular structural changes resulting from heat treatment of cheese milk.

Part II: Structural changes of Cantal cheese throughout ripening by synchronous fluorescence spectroscopy and rheology methods

The aim of the present project was to study of changes occurring throughout ripening of Cantal cheese (at 30-, 120- and 200-days-old) by physicochemical, physical (colour, texture) and synchronous fluorescence spectroscopy methods.

Anova results showed that, all the compositional characteristics of Cantal cheese increased significantly ($P<0.05$) over ripening, except for calcium and moisture contents decreased. The most important biochemical change of Cantal cheese during aging was the extent of proteolysis (WSN/TN %). The water-soluble nitrogen to total nitrogen ratio increased significantly during ripening.

The changes in the rheological characteristics throughout ripening reflected the biochemical changes in Cantal cheese. The G', G'', Tan δ and η* values of cheese increased significantly as the ripening proceeds, but exhibited an opposite trend over 120 days as compared to 200 days. Ripening led to a decrease of L* and b* values and a slight increase in - a* value.

The change in the fluorescence intensity at 295, 322 and 355 nm reflects the physicochemical changes of cheese matrix and, in particular, structural changes in the protein network throughout ripening. The spectral pattern associated with the first two PCs shows the importance of the band with a maximum at 295, 322 and 355 nm which are the most suitable for separating the spectra. PCA and FDA show that SF spectra of Cantal cheeses are clearly separated and the correct classification of 100% was observed.

Part III: A comparative study of traditional and industrial Saint-Nectaire cheese-making process by mid infrared spectroscopy and chemometrics

In this part, was concern on effect of manufacturing process (traditional, industrial) on Saint-Nectaire cheese quality in term of chemical, colour, rheological properties by MIR spectroscopy and rheological methods.

The AONVA results indicate that no significant differences occurred in the chemical parameters between the two cheese-making technologies; however, these differences were small in magnitude but gave rise to some extent of texture attributes. Texture attributes of industrial Saint-Nectaire cheeses showed slightly greater than the other ones and the differences were significant. No differences were observed among the different samples for cheese colour (L* and b*).

Using the spectral range of 3000-900 cm^{-1}, cheese samples from different manufacturers was grouped in well-separated by the PCA. The best discriminatory approach was achieved in MIR spectral region 3000-900 cm^{-1}, 100% of the original grouped cases were correctly classified by FDA.

كلية الزراعـــــــة

تأثير المعاملات التكنولوجية علي جودة الجبن التقليدية

رسالة مقدمه من

شيماء محمد حمدي محمود محمد عثمان

للحصول على

درجة الدكتوراه فى العلوم الزراعية

(علوم وتكنولوجيا الألبان)

قسم علوم وتكنولوجيا الألبان

كليـــــة الزراعـــــــة

جامعة الفـــــيوم

2011

تأثير المعاملات التكنولوجية علي جودة الجبن التقليدية

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الملخص العربي

تأثير المعاملات التكنولوجية علي جودة الجبن التقليدية

Effect of technological treatments on the quality of traditional cheeses

هذه الدراسة تم إجرائها بالتعاون مع قسم الأغذية التابع لأكاديميـــة العلـــوم الزراعية والبيطرية بمدينة كليرموفيرا جنوب فرنسا وشملت الدراسة 3 دراسات بحثية بهدف دراسة تأثير بعض المعاملات التكنولوجية علي جودة الجين الكانتـــال والســـان نيكتير واللذان يمثلان أكثر من 70% من الجبن التقليدية المنتجة في مقاطعة الاوفـــرن بجنوب فرنسا وذلك باستخدام بعض الطرق التحليلية الحديثة والسريعة التي تعتمد علي تفاعل الضوء مع المادة الغذائية.

الجبن التقليدية Traditionel Cheese تمثل التراث الثقافي للجـــبن فـــي منطقة جغرافية معينه نتيجة لتراكم المعرفة التجريبيـــة Empirical knoweldge التي انتقلت من جيل إلي جيل عبر التاريخ , ولحماية هذه الجبن أعطت لهـــا علامـــة الجودة المتصلة بالأراضي Terroir والخبرة التي يتمتـــع بهـــا الصـــانع cheese maker experiences الذي يعيش في هذه المنطقة و للماشية أيضا و تعرف بأنهـــا جبن PDO وفي كثيرا من بلدان الجنوب الأوروبي يوجد العديد من المنتجات الغذائيـــة التقليدية (PDO) بسمعتها في استخدام تقنيات الإنتاج التقليدية. و فرنسا مـــن البلـــدان الوحيدة التي لديها عدد كبير من الجبن، وتم إحصاء أكثر من 400 نوعـــا، منهـــا 42 تخضع لتسمية المنشأ (PDO).

وتعرف فرنسا بأنها "أرض الجبن" على حد سواء في الإنتاج والاســـتهلاك. والفرنسيون من أولي العاشقين للجبن حيث يصل متوسط استهلاك الفرد السنوي قـــدره 24،5 كلجم/ للفرد في عام 2008 فقد لا تخلو المائدة الفرنسية من احد أصناف الجـــبن ومن بين الجبن التي تصنع من اللبن البقري والتي تلاقي أفضلية الفرنسيين هي ، أولا ، الأجبان الطازجة، ثم ، في ترتيب تنازلي ، الجـــبن الطـــري والجـــبن المســـموطه المكبوسه ، والغير مكبوسه والجبن الأزرق المعرق بالفطر وتمثل الجـــبن المســـموطه المكبوسه والغير مكبوسة حوالي 13% من الإنتاج القومي الفرنسي وتتـــراوح نســـبة

المادة الجافة بها ما بين 44–55% ومن بين هذه العائلة الكنتال Cantal cheese (عميد الجبن الفرنسي) وهي من الجبن الجاف والسان نكتير (جبن نصف جاف)Saint-nectaire واللذان يمثلوا أكثر من 70% من الجبن (PDO) التي يتم إنتاجهم في منطقة الاوفرن Auvergne region جنوب فرنسا,

وتعرف جودة الجبن بأنها درجة مدي قبول المنتج للمستهلك النهائي وهناك معايير مختلفة لتقييم جودة الجبن Cheese Quality منها معايير حسية (القوام والتركيب والمظهر والمذاق والنكهة) معايير فيزيقيه (اللزوجة الصلابة والمرونة) ومعايير كيميائية وغذائية (محتوي البروتين الدهن الكالسيوم الصوديوم والأحماض الأمنية والدهنية الحرة) ومعايير وظيفية (المطاطية والقابلية الأنصار والنعومة والتدفق اثناء التسخين) ومعايير الأمان (الخلو من البكتريا الضارة والمرضية وبقاية السموم والأجسام الغريبة) ومعايير المستهلك (القوام واللون).

ويمكن تصنيف الطرق التقليدية المستخدمة لتقييم جودة الجبن إلي طرق معملية (تقدير المحتوي الرطوبي والدهني والبروتيني– اللزوجة والقوام والتركيب) وطرق التقييم الحسي ، وجميع هذه الطرق مكلفة نسبيا وتستغرق وقت طويلا لتنفيذها كما أن التقييم الحسي لا يسمح بتقييم الجبن online في خطوط الإنتاج .

وحديثا ظهرت عدد كبير من التقنيات الطيفية التي تستخدم في تحديد خصائص التركيب الجزيئي للجبن مثل مطياف الأشعة تحت الحمراء و الطيفي الفلورسنتي وهذه الطرق (التحليل الطيفي) هي من الطرق الفيزيائية لتوصيف المنتجات والتي يمكن أن تكون بديلا فعالا بشكل ملحوظ لطرق التحليل التقليدية وتتميز هذه التقنيات التحليلية الجديدة بأنها غير مكلفة نسبيا وسريعة ولا تحتاج إلي مواد كيميائية ولا تؤثر علي قوام المنتج ويمكن تطبيقها في مجال الأغذية وعلى خطوط الإنتاج في مصانع الألبان لرصد جودة منتجات الألبان حيث أظهرت نتائج الأبحاث التي نشرت في إل 10 سنوات الماضية أن هذه الطرق الطيفية مع التحليل الإحصائي متعدد المتغيرات لها قدرة عالية لدراسة هيكل مصفوفة الجبن على المستوى الجزيئي. وقد أظهرت هذه التقنيات انه يتم تحديد الخصائص البنائية للجبن علي حسب

الخصائص الفيزيائية والكيميائية للجبن والمعاملات التكنولوجية التي أجريت عليها أثناء التصنيع.

لذا فكان الهدف الرئيسي لهذا البحث هو لدراسة تأثير بعض المعاملات التكنولوجية (المعاملات الحرارية ، والتسوية ، وعملية تصنع الجبن) على جودة الجبن التقليدية الفرنسية (Cantal and Saint-Nectaire cheeses) باستخدام طرق التحليل الكيميائية والريولوجية وطرق التحليل الطيفي الوميضي وطرق التحليل في مجال الأشعة تحت الحمراء.

وتنقسم هذه الدراسة إلي ثلاث أقسام رئيسيه :

الجزء الأول :

الخصائص الكيميائية والريولوجية لجبن الكانتال المصنع من اللبن الخام، المعامل حراريا والمبستر

Chemical and rheological characteristics of Cantal cheese made from Raw, Thermized, and Pasteurized milk

الخصائص البنائية لـلجبن تعتمد بشكل كبير على العمليات التكنولوجية التي تؤثر بدورها على التكوين والتركيب الكيميائي والديناميكية والتداخلات بين مكونات الجبن. ومن كل هذه الخصائص يعتبر اللون والقوام من أهم المعايير المستخدمة لتقييم جودة الجبن من قبل المستهلكين. وتخضع صناعة الجبن للعديد من العمليات التكنولوجية التي تؤثر على العديد من الصفات النوعية للجين ومنها المعاملات الحرارية التي تعتبر من أول واهم العمليات التكنولوجية في صناعة الجبن. ومعرفة آثار تلك المعاملات الحرارية على مكونات اللبن (البروتينات والدهون والمعادن) مهمة جدا للحكم علي جودة المنتج النهائي، نظرا لأنها تحدث تعديلات كثيرة تؤثر على الصفات النوعية لـلجبن.

والهدف من هذا البحث هو دراسة :

(1) تأثير المعاملات الحرارية علي بعض الصفات النوعية لجبن الكانتال المسواة لمدة 90 يوما والناتجة من لبن خام –لبن معامل حراريا – لبن مبستر وذلك باستخدام الطرق التقليدية للتقييم من طرق كيميائية (تقدير الدهن–البروتين– الملح–الرماد–معامل التسوية– الكالسيوم والفوسفور...) وفيزيقية (اللون والقوام)

(2) دراسة قدره طرق التحليل الطيفي الفلورسنتي المستحدثة (بروتين التربتوفات – فيتامين أ الموجود في حبيبات الدهن) كطريقة سريعة وغير مكلفة مع استخدم بعض طرق التحليل الإحصائي المتقدمة (PCA, FDA) في فحص التغيرات البنائية للجبن ،

وأظهرت نتائج التحليل التباين أنه يوجد اختلاف معنويا بين جبن الكانتال الناتجة من لبن خام ولبن معامل حراريا ولبن مبستر في خصائصها الكيميائية حيث كان للمعاملات الحرارية لبن الجبن تأثير معنويا علي معامل التسوية والمعادن ودرجة ال pH للجبن الناتج بينما كان تأثيرها طفيف علي لون وقوام الجبن الناتج . كما بين تطبيق طريقة المكونات الأساسية Principal components analysis علي المعلومات الفيزوكيميائية التميز الجيد لعينات الجبن وفقا للتغيرات الفيزيائية والكيميائية الناتجة من المعامل الحرارية للبن المستخدم في صناعة الجبن.

وأظهرت نتائج التحليل الإحصائي متعدد المتغيرات لل Fluorescence spectral data انه تم الحصول على 100 ٪ من التصنيف الصحيح للجبن من خلال تطبيق تكنيك التحليل التمييزي أو التصنيفي Factoriel discriminant analysis (FDA) على البيانات الطيفية (تريبتوفان أو/ و فيتامين A). وتبين أنه يمكن اعتبار التربتوفان أو فيتامين (أ) أطياف الإثارة من الجبن كبصمة مميزة والذي يسمح للتمييز بين عينات الجبن علي أساس المعلومات المتعلقة بالتغييرات الهيكلية الجزيئية الناتجة عن المعاملات الحرارية للبن الجبن.

استنتج من هذه الدراسة أن التحليل الطيفي مع الأساليب الإحصائية متعددة المتغيرات يمكن أن تعتبر كطريقة سريعة وغير مكلفة و بدون استخدام المواد الكيميائية في دراسة التركيب الجبن علي المستوي الجزيئ مقارنا بالطرق التقليدية للتحليل كما توصي هذه الدراسة باستخدام تكنيك Conactenation وذلك للحصول علي جميع المعلومات التي تحملها Fluorescence spectra في تميزي عينات الجبن .

الجزء الثاني :

تغيرات التركيب البنائي لجبن الكانتال أثناء التسوية بواسطة طـرق التحليـل الطيفي الفلورسنتي والطرق الريولوجية

Structural changes of Cantal cheese throughout ripening by synchronous fluorescence spectroscopy and rheology methods

ترتبط جودة الجبن ارتباطـا وثيقـا بالتركيـب البنـائي للجـبن Cheese structure الذي يوصف بأنه تجمع وحدات من بروتين الكـازين لتكـوين الشـبكة البروتينية (ميسيلات الكازين) التي تحتجز بداخلها حبيبات دهن اللبن بواسـطة قـوي فيزيائية وينتشر الماء بين فجوات هذا التركيب مذابا به العديد من الأملاح والفيتامينات والأحماض العضوية ويتأثر هذا التركيب بدرجة كبيرة بعملية التسوية التي ينشأ عنهـا تغيرات مختلفة (كيميائية وفيزيقية وميكروبيولوجية) في تركيب وقوام الجين.

لذا فالهدف من هذه البحث

(1) تقييم التغيرات الكيميائية والفيزيائية (اللون والقـوام) والتركيـب البنـائي للجين الكانتال (جبن جاف فرنسي تقليدي) أثنـاء التسـوية (30يومـا –120 يومـا– 200يوما) بواسطة طرق التحليل الكيميائي والريولوجية

(2) تقييم قدرة طريقة التحليل الطيفي الفلورسنتي مع تطبيق بعـض أسـاليب التحليل الإحصائي المتعدد المتغيرات (تحليل العنصـر الرئيسـي – تحليـل العـاملي التمييزي) علي تقييم هذه التغيرات الناتجة من التسوية.

وأظهرت نتائج ال ANOVA أن جميع العناصر التركيبية لجبن الكانتـال (الدهن– البروتين –الرماد– الملح...) تزداد معنويا أثناء التسـوية بنسـب متباينـة ، باستثناء الكالسيوم والرطوبة تنخفض معنويا ، وزيادة نسبة النيتروجين الذاب في الماء إلى نسبة النيتروجين الكلي بشكل كبير خلال التسوية. وتعكس التغيرات الحادثـة فـي القيم الريولوجية أثناء التسوية مدي التغيرات البيوكيميائية في جبن الكانتال حيث أن قيم G' (مقياس للمرونة) ، G''(مقياس للزوجة) ، Tan δ (معامل اللزوجة للمرونـة) ، η* (مقياس اللزوجة المركبة) زادت معنويا أثناء التسوية ، ولكن أظهـرت اتجـاه

معاكس في الجبن المسواه لمدة 120 يوما مقارنتا 200 يوما. كما أدت التسوية إلى انخفاض قيم *L (مقياس للبياض) ، *b (مقياس للاصفراء) وزيادة طفيفة في قيمة *a -.

التغير في شدة الوميض الفلورسنتي للروابط عند طول موجي 295(التربتوفان)،322(فيتامين أ) و355 (الريبوفلافين) نانومتر يعكس التغيرات الفيزيائية والكيميائية الحادثة للتركيب البنائي للجبن في جميع مراحل التسوية.

وأظهرت نتائج تحليل العنصر الرئيسي أهمية الروابط 295 ، 322 و 355 نانومتر في تتبع التغيرات في التركيب البنائي للجبن. كما تم تصنيف الجبن على أساس التغيرات الحادثة في هذه الروابط الثلاثة باستخدام التحليل العاملي التمييزي وبلغت نسبة التصنيف الصحيح 100٪. وبذلك هذه النتائج تشير إلى أنه يمكن اعتبار طريقة SFS مع تحليل بيانات متعددة المتغيرات بمثابة البصمة،حيت أنها تسمح لتوصيف وتصنيف جيد للجبن على أساس التغيرات البنائية طوال فترات التسوية.

الجزء الثالث :

دراسة مقارنة بين الطريقة التقليدية والصناعية المستخدمة في تصنيع الجبن سان نيكتير بواسطة التحليل الطيفي بالأشعة تحت الحمراء المتوسطة وطرق التحليل الإحصائي

A comparative study of traditional and industrial Saint-Nectaire cheese-making process by mid infrared spectroscopy and chemometrics

جبن سان نيكتير هي واحدة من الأكثر الجبن شعبية في فرنسا وينتج على نطاق صغير باستخدام الطرق التقليدية (بدون أي تقنيات صناعية) و باستخدام الطرق الصناعية علي نطاق أوسع واكبر(أي استخدام تقنيات صناعية متطورة من بسترة وخلافه).

والهدف من هذه الدراسة

(1) دراسة الخصائص الفيزيائية (اللون والقوام) والكيميائية لجبن سان نيكتير الناتجة عبر الطرق التقليدية والصناعية

(2) تقييم إمكانيات استخدام طرق التحليل الطيفي في مجال الأشعة الحمراء (FTIR) لدراسة الخصائص الطيفية لجبن سان نيكتير وإمكانية تميز الفرق بين طريقتين الصناعة باستخدام الطرق الإحصائية المتعددة المتغيرات (تحليل المكونات الأساسية وتحليل التمييزي).

وتشير نتائج تحليل التباين إلى أن عدم وجود اختلافات كبيرة في التركيب الكيميائي بين الجبن تحت الدراسة وعلي الرغم من ذلك هناك اختلافات ملموسة في قوام الجبن حيث أظهر سمات قوام الجبن سان نيكتير الصناعية أكبر قليلا من تلك المصنعة بالطريقة التقليدية وعلي الجانب الأخر لم يلاحظ أي اختلافات بين عينات الجبن من حيث اللون .

تطبيق تحليل العنصر الرئيسي (PCA) علي بيانات التحليل الطيفي MIR و كذلك (FDA) كان من الممكن تصنيف عينات الجبن سان نيكتير المختلفة باستخدام التكنولوجيا على أساس بياناتها الطيفيةMIR حيث بلغت نسبة التصنيف الصحيح 100 ٪ وبذلك يمكن من النتائج السابقة استنتاج أنه يمكن استخدام تقنية MIR الطيفية مع التحليل متعدد المتغيرات كطريقة سريعة وبسيطة لتميز عينات الجبن علي أساس طريقة صناعة الجبن.

الكلمات الدالة للبحث : الجبن التقليدي – البسترة – التخزين – طريقة التصنيع – جودة الجبن (جين الكانتال والسان نيكتير) – قوام وتركيب الجبن والمظهر – الخواص الريولوجية – طرق التحليل الطيفي – طرق التحليل الإحصائي.