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# A Comparative Study of Traditional and Industrial Saint-Nectaire Cheese-Making Processes by Mid Infrared Spectroscopy and Chemometrics

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**Abstract:** The objectives of the present project were to evaluate the physicochemical characteristics of eighteen French Saint-Nectaire cheeses (ripened for 30 days) elaborated via traditional and industrial cheese-making process and to verify the ability of MIR spectroscopy (3000-900 $cm^{-1}$) to monitor the differences between the two cheese-making processes (traditional, industrial). 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 color (L* and b*). Principal component analysis (PCA) was applied to MIR spectral data. It was possible to classify different Saint-Nectaire cheese samples using MIR technology based on their spectral data. Using the spectral range of 3000-900 $cm^{-1}$, cheese samples from different manufacturers was grouped in well-separated by the PCA. We concluded that the combination of MIR spectroscopic technique with multivariate analysis could be successfully used, a rapid and simple method to discriminate cheese samples in terms of cheese-making processes.

**Key words:** Spectroscopy, chemometrics, texture, color, cheese making, Saint-Nectaire cheese.

## INTRODUCTION

Cheese production around the world occurs mainly in cheese factories, but in some areas, small-scale production of cheese using traditional methods is predominant. 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 development of microbial populations before processing. 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 (Law and Tamine, 2010).

Saint-Nectaire cheese is one of the most popular traditional semi-hard cheeses manufacture in the Auvergne region of 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; 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 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.

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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, which are laborious and time consuming (Subramanian *et al.*, 2009). In recent years, spectroscopic techniques have been used to assess food quality where characterized by relatively low cost, fast and accuracy methods (Subramanian and 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 and Dufour, 2006b). 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; Upreti and Metzger, 2006), sensory and instrumental texture parameters (Blazquez *et al.*, 2006; Karoui *et al.* 2006) and to determine the geographic origin of the cheese (Karoui *et al,.* 2006) and to use IR spectroscopy to follow compositional and molecular changes during cheese ripening (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 cheeses and to discriminate the difference among two cheese-making processes by PCA.

## MATERIALS AND METHODS

### *Cheese Samples:*

Eighteen Saint-Nectaire cheese samples ripened for 30 days were supplied by six different cheese manufacturers (three farms and three companies) location in the Auvergne region in France. Slices were cut off in the middle of the cheese height 2 cm from the rind, for physico-chemical, rheological and MIR analysis.

### *Physicochemical Analysis:*

For these determinations, the outer part of the cheese samples (2 mm under the crust) was removed and a part of about 900 g was grated and thoroughly homogenized and then used.

Grated cheese samples (900 g) were analyses for moisture, fat, ash, salt (Marshall 1993), total nitrogen and water-soluble nitrogen according to France standard methods (AFNOR, 2004). The pH was measured by put an electrode (Schott, CG840, Paris, France) in 10 g of grated cheese in 50 ml of ionized water. Total Ca in cheeses was measured by using an atomic absorption spectroscopy procedure as adapted from (Brooks IB *et al.*, 1970). Total P in cheeses was determined colorimetrically by using spectrophotometer (model SECOMAM, Jonior, Secoman, Paris, France) according to (AOAC, 1995). All analyses were done in triplicate and the results presented as mean ± standard deviation.

### *Color Measurements:*

Cheese color was determined using a colorimeter CR-400 (Konica Minolta, Tokyo, Japan). The *L*, a*, and b** color measurements were determined according to the CIELAB color space (CIE, 1976) with reference to $D_{65}$ (natural daylight, the color warmth of 6500K) and observation angle 10°. The following parameters were determined; L* (lightness or whiteness; L*=0 for black and L*=100 for white color), a*(red-green components, - a*=greenness and + a*= redness) and *b** (yellow-blue components, - b*= blueness and +b*=yellowness). Color 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 and the results represented as mean ± standard deviation.

### *Texture Measurements:*

The evaluation of the cheeses' textural properties was carried out using 3 samples (2 mm thick and 20 mm diameter) by using a rheometer (CP 20, TA Instrument, Guyancourt, France) equipped with plate geometry of 20 mm diameter. The elastic component G' (storage modulus), the viscous component G" (loss modulus), Tan δ (G"/G'), and complex viscosity (η*) were determined in the linear viscoelastic region by applying a constant force of 0.5 N and a constant frequency of 1 Hz and the temperature was fixed at 20°C. All analyses were made in triplicate.

### *Mid-Infrared Spectroscopy:*

Infrared spectra were recorded in the 3000-900 $cm^{-1}$ region with a Fourier transform spectrometer Varian 3100 FT-IR (Varian Inc., Palo Alto, USA) mounted with an ATR (horizontal ZnSe crystal. six reflections) accessory equipped with a grip (Spectra-Tech ARK Flat Plate). Slices of cheese 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. Baseline and ATR corrections and water subtraction were applied to the spectra using OMNIC 4.1a Software (Thermo Electron). The regions of the mid-infrared spectra located between 3000-2800 $cm^{-1}$ (fat region). 1700-1500 $cm^{-1}$ (protein region) and 1500-900 $cm^{-1}$ (fingerprint region) have been considered in this study (Boubellouta *et al.*, 2010).

### *Statistical Analysis:*

One-way analysis of variance was carried out to determine the effect of cheese-making technology on the dependent variables (chemical and physicochemical parameters). Least significant difference (LSD) test was applied for comparison of the means and the statistical significance was determined at $P < 0.05$ using XLSTAT software version 2007 (Addinsoft, France). Principal component analysis (PCA) was applied to MIR spectra in order to investigate differences between the samples (Bertrand *et al.*, 2006).

## RESULTS AND DISCUSSION

### *Physicochemical Characteristics of Saint-Nectaire Cheeses:*

Table (1) 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 and proteolysis, but higher levels of protein, ash, fat/dry matter than traditional cheeses.

### *Cheese Color Values:*

The results illustrated in Table (1) showed the effect of cheese-making process on Saint-Nectaire cheese color 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:*

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 (1) and illustrated in Figure (1). 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 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 (Gunasekaran and Mehmet, 2000).

Generally, the differences in composition between the investigated cheeses may be probably attributed to the differences in milk composition, treatment applied to milk and the cheese-making process which had a significant effect on the 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; Cabezas *et al.,* 2007).

**Table 1:** Mean (±SD) of the physicochemical characteristics of traditional and industrial Saint-Nectaire cheeses. One-Way ANOVA was applied to data and values with different superscript letter are significantly different (P<0.05, LSD test).

| Parameters | Traditional Saint-Nectaire | | Industrial Saint-Nectaire | |
|---|---|---|---|---|
| | 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 |
| Cheese Color 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; proteolysis index = (WSN/TN) x 100: water-soluble nitrogen/total nitrogen.

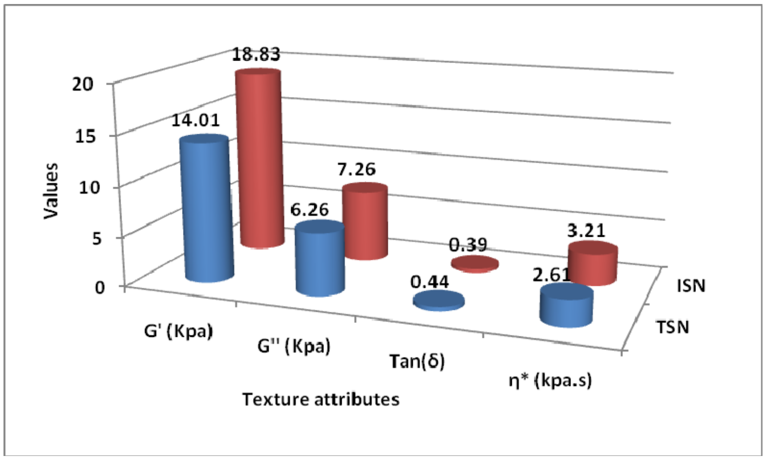


**Fig. 1:** Texture attributes of traditional (TSN) and industrial Saint-Nectaire cheeses (ISN).

***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. The band intensities vary with the overall concentration of the chemical functional groups in the sample. Figure (2) 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 and Dufour, 2006a). Two major bands corresponding to methylene groups at ~ 2920 $cm^{-1}$($\nu_{as}$ $CH_2$) and 2855$cm^{-1}$ ($\nu_s$ $CH_3$), as well as one minor band corresponding to methyl groups at 2956 $cm^{-1}$ ($\nu_{as}$ $CH_3$) 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 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 lactate consumption (Martın-del-Campo *et al.*, 2009) while the increment with higher concentration of carbonyl groups generated during lipolysis and proteolysis (Chen *et al.*, 1998).

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 and Dufour 2006b). 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.,* 2005; Bertrand and 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 and Dufour, 2006). Visual comparison of the raw spectra showed numerous differences between cheeses, especially in the spectral region 1500-900 $cm^{-1}$. The peaks located at ~1377$cm^{-1}$ ($\delta_s$ $CH_3$) 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 absorbance bands of this region is due to an increase in quantities of cheese compounds which could be vary in cheese due to complex biochemical reactions, such as glycolysis, lipolysis and proteolysis which occurred during cheese ripening.

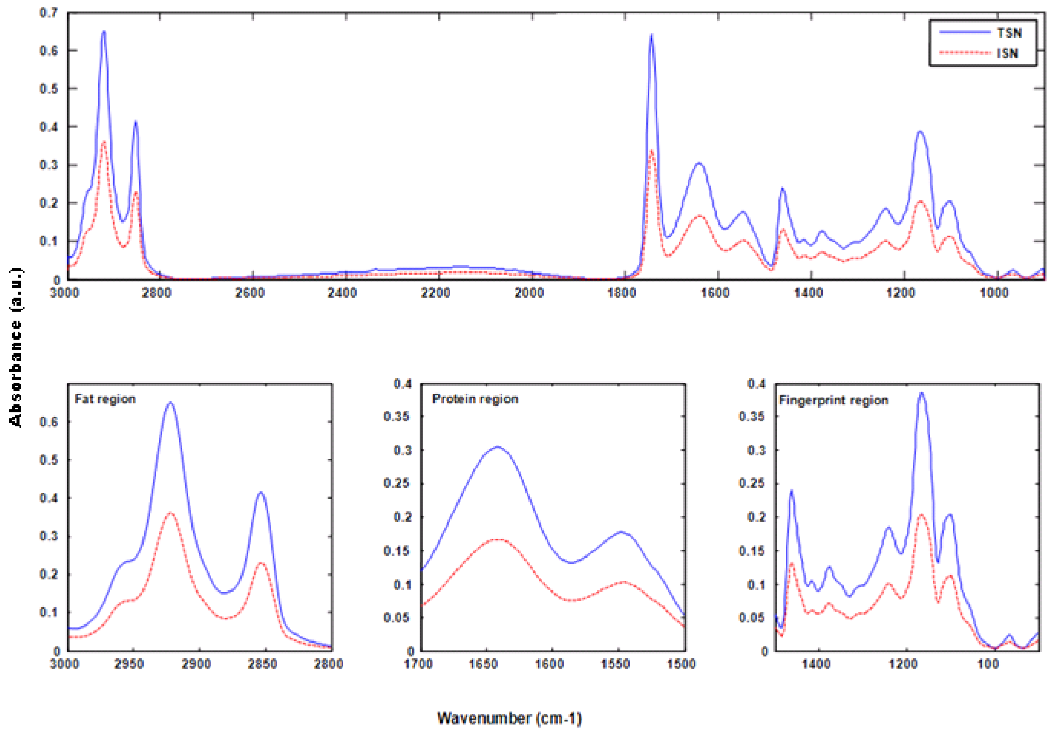


**Fig. 2:** Typical Mid infrared spectrum of traditional (solid line) and industrial Saint-Nectaire cheese (dashed line) of 30 days-old aged in the three regions. AU = arbitrary unit.

These results highlight the importance of 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.

***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 spectra are shown in Figure (3 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 3 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 (3 b) provides us important information about the characteristic absorption bands and the biochemical reactions involved which explain the discrimination. These bands could provide valuable structural information about the changes that occurred in the acyl chains during cheese manufacturing. 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 3$cm^{-1}$) and 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. These results also suggest that MIR spectroscopy is primarily differentiating between samples on the basis of manufacturing process of cheese.

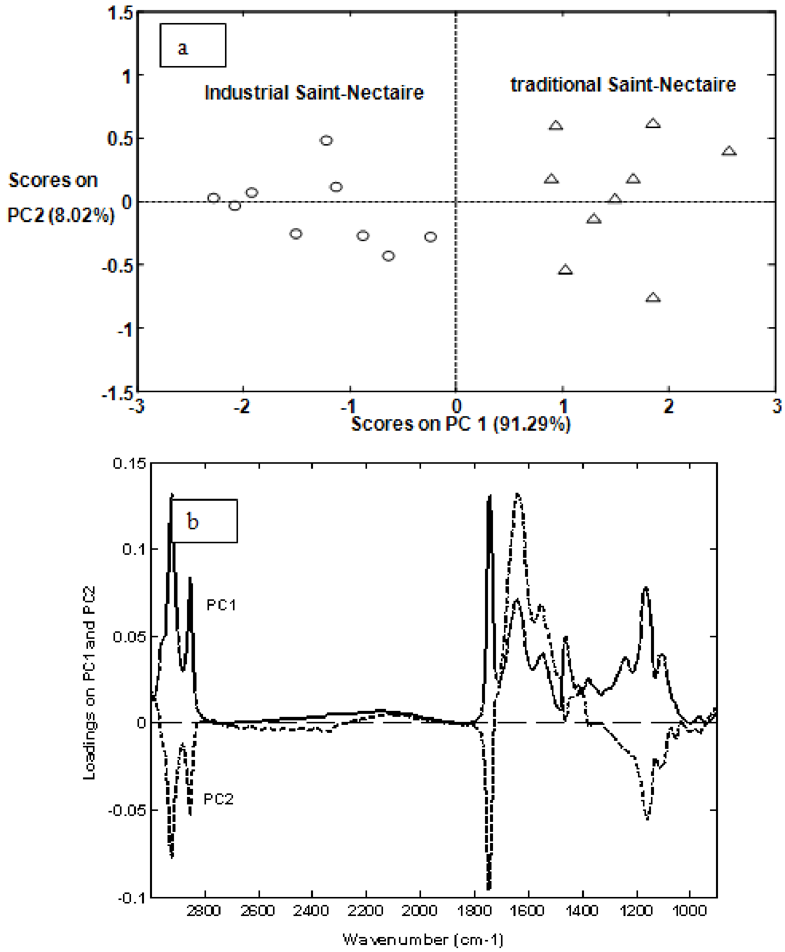


**Fig. 3 a-b:** (a) Principal component analysis similarity map determined by principal components 1 and 2 for MIR spectra of traditional (Δ) and industrial Saint-Nectaire cheese (o). (b) Loading plots corresponding to the principal components $PC_1$ (—) and $PC_2$ (- - -) for FTIR spectra.

***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 provide an exceptional opportunity for confirming cheese quality and to classify according to their manufacturing process. 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.

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