AISME

Accademia Italiana di Scienze Merceologiche

UNIVERSITÀ DEGLI STUDI DELLA Tuscia

Via Santa Maria in Gradi, 4

XXVII CONGRESSO NAZIONALE DI SCIENZE MERCEOLOGICHE

VITERBO

Università degli Studi della Tuscia
Rettorato - Via Santa Maria in Gradi, 4

2-4 Marzo 2016

Presidente del Congresso

Prof. Luigi Ciraolo

Comitato Scientifico – Consiglio Direttivo AISME

Prof. Luigi Ciraolo
Università degli Studi di Messina (Presidente)
Prof. Riccardo Beltramo
Università degli Studi di Torino
Prof. Maurizio Boccacci Mariani
Sapienza Università di Roma
Prof.ssa Rita Jirillo
Università degli Studi della Tuscia
Prof. Giovanni Lagioia
Università degli Studi di Bari Aldo Moro
Prof. Enrico Maria Mosconi
Università degli Studi della Tuscia
Prof. Bruno Notarnicola
Università degli Studi di Bari Aldo Moro
Prof.ssa Maria Francesca Renzi
Università degli Studi Roma Tre
Prof. Alessandro Ruggieri
Università degli Studi della Tuscia
Prof.ssa Roberta Salomone
Università degli Studi di Messina

Comitato Organizzativo

Prof. Alessandro Ruggieri
Università degli Studi della Tuscia (Rettore)
Prof.ssa Rita Jirillo
Prof. Enrico Maria Mosconi
Dott.ssa Cecilia Silvestri
Dott. Stefano Poponi
Dott.ssa Ombretta Lattanzi
Dott.ssa Natalia Natali
Dott. Giuseppe Rapiti
Sig. Paolo Iantorno
Dott.ssa Rosella Bruzzichini
Dott.ssa Giuseppina Guerrini
Dott. Massimiliano Fabbri
Dott.ssa Laura Gori
Sig. Giancarlo Ludovisi

In collaborazione con l'Ufficio d'Informazione del Parlamento europeo in Italia

Con il contributo di

Con il patrocinio di

Partners

UNIVERSITA' DEGLI STUDI DELLA TUSCIA

Dipartimento di Economia e Impresa

XXVII CONGRESSO NAZIONALE DI SCIENZE MERCEOLOGICHE

QUALITÀ & INNOVAZIONE PER UNA ECONOMIA CIRCOLARE ED UN FUTURO SOSTENIBILE

Atti del Congresso

Viterbo, 2-4 marzo 2016

Copyright

Titolo del libro: Atti del Congresso AISME 2016

Autore: D.E.Im (Dipartimento di Economia e Impresa)

deim@unitus.it – cintest@unitus.it

ISBN: 978-88-941647-0-1

ISBN 978-88-941647-0-1
9 788894 164701

QUALITY AND TRADITION IN PDO CHEESES A COMPARATIVE STUDY OF CHEESE-MAKING PROCESSES, TRADITIONAL AND INDUSTRIAL, USING FT-IR/ATR

Spognardi S., Bravo I., Carelli A., Cappelli L., Papetti P.

Dipartimento di Economia e Giurisprudenza
Università degli Studi di Cassino e del Lazio Meridionale .

ABSTRACT

Twenty samples of Italian cheeses, some of which were provided by local producers and carefully selected according to the date of production and the seasoning while others were purchased at specialty stores and supermarkets, were evaluated using FTIR ATR accessory. The samples were analyzed either by destructive methods both with FTIR / ATR, which allows the measurement of quantitative characteristics directly on cheese portions (~ 0.5 g). The content of fatty substances, the level of moisture, total nitrogen and protein content were determined by official destructive methods; this quantification of the nutritional components was necessary to develop calibration models for infrared spectroscopy with Fourier transform FT / IR.
Data was valuated though multivariate analysis.
The objective of this research is to develop a simple and rapid screening tool for definition of the qualitative characteristics of Italian cheese by using Fourier transform infrared spectroscopy (FTIR)

Keywords: Italian cheese, composition, flavor, attenuated total reflectance infrared spectroscopy, multivariate analysis.

INTRODUCTION

Many factors such as the composition of the milk (eg. protein and fat levels), moisture, starter cultures, the manufacturing process, and biochemical events that occur during maturation affect the quality of cheese. There are sensible differences between traditional and industrial buffalo cheese-making processes, as well for the prices of the two final products.

For this reason, considerable interest exists in the development of instrumental techniques to enable more objective, faster and less expensive assessment for defining and controlling the qualitative characteristic of typical cheeses in order to secure the consumer's choice, to protect traditional products against cheaper industrial imitations, to limit phenomenon of Italian sounding.

Infrared spectroscopy is an attractive technology for rapid, inexpensive, sensitive, and high-throughput analysis of food components and does not require special skills by the users.

Recently Infrared spectroscopy has been applied for authentication of products by commodity, variety and geographic origin. Has been shown the potential of IR spectroscopy for monitoring the geographic origin of cheeses (Karoui *et al.*, 2004, 2005) and for discriminating them according to the nature of the milk used in cheese-making process (Picque *et al.*, 2002, Subramanian et al 2009). Specifically, mid-infrared spectroscopy (4000- 700 cm^{-1}) has been used not only for monitoring the geographic origin but also for determining the major components in cheese such as protein, fat, moisture, lactic acid, sugars, casein, cholesterol, etc. (Rodriguez-Saona *et al.*, 2006; Subramanian et al 2008), every of them with distinct and reproducible biochemical fingerprint.

The objective of this research is to develop a simple and rapid screening tool for definition of the qualitative characteristics of Italian buffalo-cheese by using Fourier transform infrared spectroscopy (FTIR), also in order to discriminate the difference among traditional and industrial cheese-making processes.

MATERIALS AND METHODS

Sampling

Twenty samples of Italian buffalo cheese (mozzarella) were analysed in this work: ten samples elaborated via traditional cheese-making process were supplied by different manufacturers, while another ten elaborated via industrial cheese-making process were purchased at local stores. Samples have been taken from different production dates and stored at refrigerated (4°C) conditions until analysis, carried in the next two days.

Physico-chemical analysis

For these determination, two sizes of Italian buffalo cheese have been analysed: 125 gr and 250 gr. Part of each sample was used for pH determination and FTIR/ATR Spectroscopy Measurements, while the other one was homogenized to define the content of fatty substances, the level of moisture, lactic acid, total nitrogen and protein.

The percentage compositions of moisture, fat, and protein were determined using the reference methods. The fat content was determined with Gerber reference method using Soxtec system, the moisture content by vacuum-oven method, and the protein content by Kjeldahl method. All samples were tested in duplicate.

FTIR/ATR Spectroscopy Measurements

Infrared spectra were recorded in the 3000-900 cm-1 region with a Fourier transform spectrometer Spectrum1000 PerkinElmer mounted with an ATR accessory equipped with a grip. Cheese slices were taken from the center of the cheese and pressed with the high-pressure clamp to ensure good contact between the sample and the crystal. In order to improve the signal to noise ratio, for each spectrum 64 scans were coadded (at 4cm-1 resolution). In this study, have been considered especially 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) (Boubellouta *et al.*, 2010).

Multivariate Data 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 $P < 0.05$. Principal component analysis (PCA) was applied to IR spectra in order to investigate differences between the samples (Bertrand et al., 2006).

RESULTS AND DISCUSSION

The results of physico-chemical analysis illustrated in (Table 1) have no showed significant differences between the two cheese-making processes.

Table 1. *Mean (±SD) of the physicochemical characteristics of traditional and industrial buffalo-cheese making processes.*

Parameters	*Traditional buffalo cheese*	*Industrial buffalo cheese*
pH	5,30 (±0,09)	5,22 (±0,08)
Lactic Acid %	0,47 (±0,02)	0,48 (±0,03)
Fat%	23,2 (±0,82)	22,6 (±0,57)
Protein%	16,2 (±0,38)	12,5 (±0,32)

FTIR Spectra of traditional and industrial buffalo cheese.

FTIR technique is applied to identify the main types of structure present in the analyzed samples, through the correct association between absorption bands and fundamental vibrations of functional groups of the molecule. Clearly, intensities of the different bands are correlated with the concentration of the chemical functional groups.

Figure (1) shows the typical mid Infrared spectrum of traditional buffalo cheese.

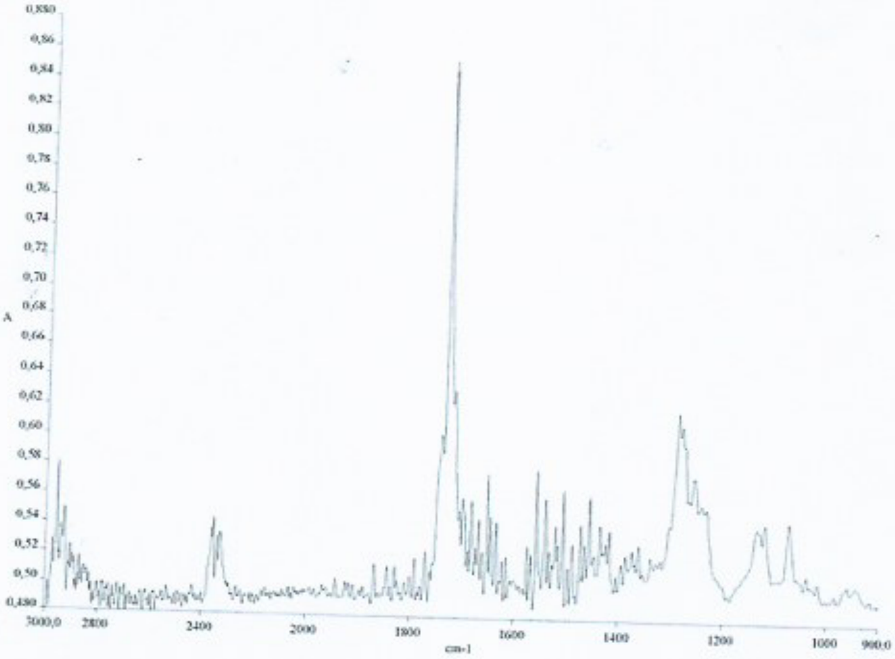
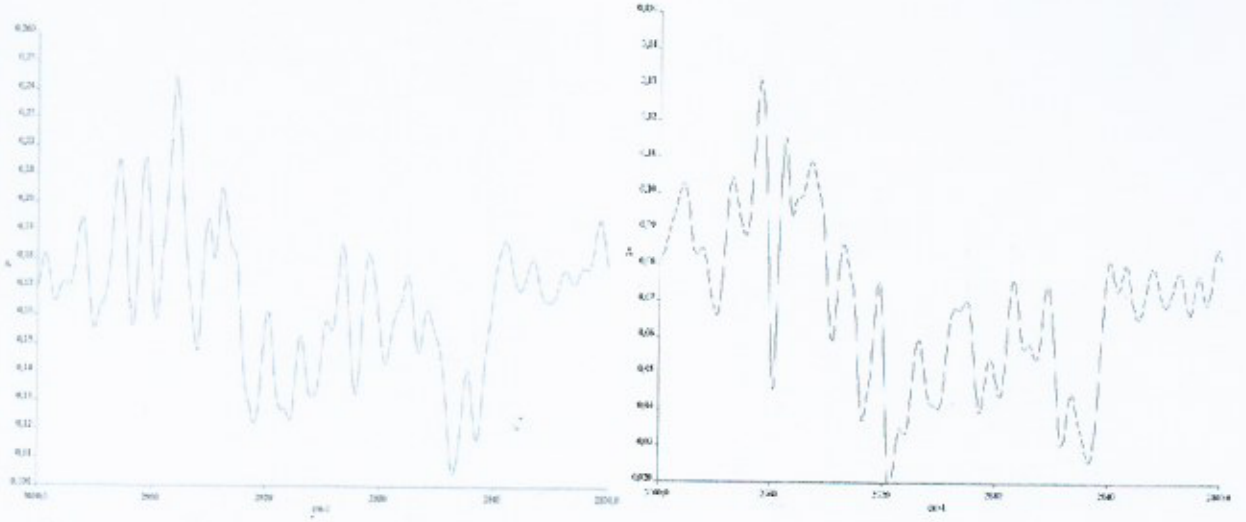


Fig. 1: Typical Mid Infrared spectrum of traditional buffalo cheese (3000-900 cm^{-1}).

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 Amide I and II bands, and the 1500-900 cm^{-1} region.

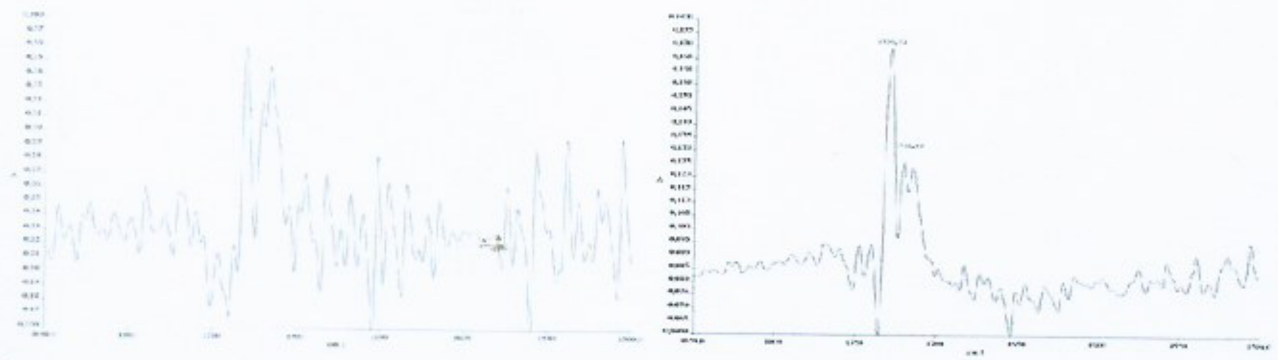
The spectral circa values of 1800-700 cm-1 are considered to be "fingerprint" values, specific fingerprint of each compound (Cevoli et al. 2013; Nurrulhidayah et al. 2013; Reid et al., 2006). In literature this area has a coverage between 800-1200 and 1000-1500 cm^{-1} (Van de Voort *et al.*, 1994) but in this study, authors have extended it to 1850 cm^{-1}.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, 2006). Specifically, the interval between 2900-2827 cm^{-1} correspond to fat content in cheese (as well the range from 1782 to 1705 cm^{-1}). Figure 2 shows two major bands corresponding to methylene/methyl groups, that were observed at ~ 2963 and at ~2955 cm^{-1}. The observed changes in methyl and methylene bands were attributed to difference in nature concentration and physical state o fatty acids (Boubelouta *et al.*, 2010).



14. b.

Fig. 2: Spectra of traditional buffalo cheese (a), and industrial buffalo cheese (b) in the region between 3000 and 2800 cm^{-1}.

The spectral region between 1701-1507 cm^{-1} corresponded to protein content. Figure 3 shows spectra of traditional buffalo cheese (a), and industrial buffalo cheese (b) in the region between 1850 and 1500 cm^{-1}. Two well-defined peaks were observed at 1715 and 1730 cm^{-1} associated with esters and organic acids. Besides, in the figure 3 a) were noted many other picks, two of which associated to the Amide I at ~1651 cm^{-1} and to Amide II at~ 1556 cm^{-1}. (Koca et al 2007, Mendenhall & Brown 1991) Probably, these two peaks are associated with hydrolysed proteins according to Bertrand and Dufour, 2006. 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 (Othman *et al.*, 2012; Karoui et al 2006, Mazerolles *et al.*, 2001; Bertrand and Dufour, 2006).

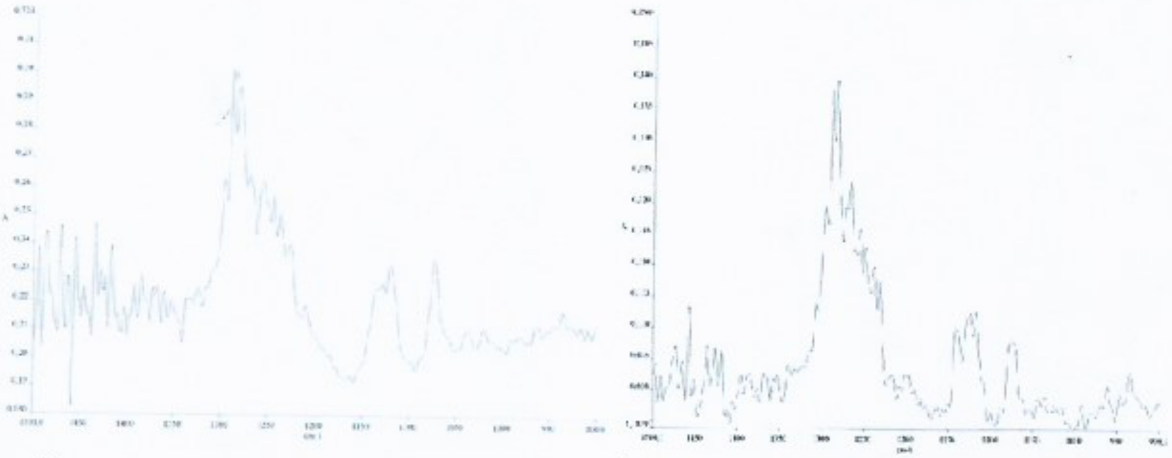


a. b.

Fig. 3: Spectra of traditional buffalo cheese (a), and industrial buffalo cheese (b) in the region between 1850 and 1500 cm^{-1}.

In the third region of FTIR spectra (1500-900 cm^{-1}), wavelengths for specific chemical groups were recognised: 1477-1400 and 1195-1129 cm^{-1} for ester carbonyl C-H and C-O group (Belton *et al.*,1988). In figure 4 a. were observed more peaks in the range between 1477 and 1400 cm^{-1}, but we can find in both spectra (a. and b.) peaks at ~ 1420, 1435, 1470 cm^{-1} although with different absorbance. The highest peak was observed at ~1289 cm^{-1}.

In both spectra were observed two peaks: at~ 1073 cm^{-1} and another one at ~ 1120 cm^{-1}, probably related to sum of lactose, monosaccharide (Karoui et al 2006,, Petibois *et al.*, 2000), and the ester linkage of lipids (Martin del Campo *et al.*, 2007-2009); instead no well-defined peaks were assigned to glucose and galactose (that may be located at ~ 1377 cm^{-1}, according to Othman *et al.*, 2012).



a. b.

Fig. 4: Spectra of traditional buffalo cheese (a), and industrial buffalo cheese (b) in the region between 1500 and 900 cm^{-1}.

Throughout the region MIR (3000-900 cm^{-1}), and specifically in the intervals between 2900-2827 and 1782-1705 cm^{-1} (which correspond to fat content in cheese), in the range between 1701 and 1507 cm^{-1} (corresponding to protein content) and finally in that from 1200 to 967 cm^{-1} (carbohydrates), the buffalo cheese samples produced in a traditional manner, have showed higher absorbance, demonstrating the results of chemical and physical analysis according to which these samples possessed a higher content of protein and fat respect those obtained through industrial buffalo cheese-making processes.

These results highlight the importance of different regions across the entire spectral range used in predicting the quality attributes of cheese.

FTIR could be used to check any variations in buffalo cheese samples, especially when they have to be marketed abroad, to implement the traceability of products, to identify substances (such as soda and bleaching) commonly used in phenomena like "Italian sounding", in order to protect Italian products and consumer choices.

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 PC1 and PC 2 was analyzed. The main loading peaks observed for PC1 were positive associated with lipids (2963, 2955 cm^{-1}). Negative loading peaks associated in PC2 with lipids (2920, 2855, 1742 cm). Positive loadings peaks associated with Amide I (1651 cm^{-1}). Amide II (1556 cm^{-1}), C-H bending (1462 cm) were observed for PC2. These results also suggest that MIR spectroscopy is primarily differentiating between samples on the basis of manufacturing process of cheese.

CONCLUSION

Results from this study showed that industrial technology has a large impact on Italian buffalo cheese (mozzarella) quality, significantly affecting the texture attributes of cheeses, although they had similar composition characteristics. FT-IR spectroscopy and chemometric analysis, could be provide an exceptional opportunity for confirming cheese quality and to classify according to their manufacturing process. FT-IR spectroscopy 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. Results from the study showed that industrial technology has a large impact on cheese quality.

REFERENCES

AOAC., 1995. Chapter 33, Dairy products. In:Official methods of analysis of Association of Official Analytical Chemistry. 16th ed. Virginia: AOAC.

Belton P. S., Alfred M. S., Reginald H. W., 1988 Use of Fourier transform infrared spectroscopy for quantitative analysis: A comparative study of different detection methods. Analyst 112:1117-1120.

Bertrand, D. and É. Dufour, 2006. La spectroscopie infrarouge est ses applications analytiques. paris, France: Tec and Doc, Lavoisier.

Boubellouta, T., R. Karoui, A. Lebecque, Dufour É., 2010. Utilisation of attenuated total reflectance MIR and front-face fluorescence spectroscopies for the identification of Saint-Nectaire cheeses varying by manufacturing conditions. European Food Research and Technology, 231(6): 873-882.

Cevoli, C., Gori, A., Nocetti, M., Cuibus, L., Caboni, M. F., & Fabbri, A. 2013. FT-NIR and FT-MIR spectroscopy to discriminate competitors, non compliance and compliance grated Parmigiano Reggiano cheese. Food Research International, 52(1), 214–220.

Koca N, Rodriguez-Saona L. E., Harper W. J., Alvarez V. B., 2007 Application of Fourier transform infrared spectroscopy for monitoring short-chain free fatty acids in Swiss cheese. J Dairy Sci 90:3596-3603.

Karoui, R. J.O. Bosset, G. Mazerolles, A. Kulmyrzaev, Dufour E., 2005a. Monitoring the geographic origin of both experimental French Jura hard cheeses and Swiss Gruyère and l'Etivaz PDO cheeses using mid-infrared and fluorescence spectroscopies. International Dairy Journal, 15: 275-286.

Karoui, R. É. Dufour, L. Pillonel, E. Schaller, D. Picque, T. Cattenoz,. Bosset J.O, 2005b. The potential of combined infrared and fluorescence spectroscopies as a method of determination of the geographic origin of Emmental cheeses. International Dairy Journal, 15(3): 287-298.

Karoui, R. A.M. Mouazen, H. Ramon, R. Schoonheydt , Baerdemaeker J.D., 2006. Feasibility study of discriminating the manufacturing process and sampling zone in ripened soft cheeses using attenuated total reflectance MIR and fiber optic diffuse reflectance VIS–NIR spectroscopy. Food Research International, 39: 588-597.

Martín-del-Campo, S.T., Picque D., Cosío-Ramírez R.,. Corrieu G, 2007. Middle infrared spectroscopy characterization of ripening stages of Camembert-type cheeses. International Dairy Journal, 17(7) : 835-845.

Martın-del-Campo, S.T., N. Bonnaire, D. Picque and G. Corrieu, 2009. Initial studies into the characterisation of ripening stages of Emmental cheeses by mid-infrared spectroscopy. Dairy Science Technology, 89: 155-167.

Mazerolles, G., M.F. Devaux, G. Duboz, M.H. Duployer, Mouhous Riou N., Dufour E., 2001. Infrared and fluorescence spectroscopy for monitoring protein structure and interaction changes during cheese ripening. Le Lait., 81: 509-527.

Mendenhall I. V., Brown R. J., 1991 Fourier transform infrared determination of whey powder in nonfat dry milk. J Dairy Sci 74:2896-2900.

Nurrulhidayah, A.F., I., Che Shuhaimi, M. Man, Y.B., Rohman, A., Amin I., Khatib, A. 2013. Authentication analysis of butter from beef fat using Fourier Transform Infrared (FTIR) spectroscopy coupled with chemometrics. International Food Research Journal 20(3): 1383-1388

Othman S., Abbas K., Lebecque A., Bayoumi R., Ibrahim G., Degheidi M. 2012. A Comparative Study of Traditional and Industrial Saint-Nectaire Cheese-Making Processes by Mid Infrared Spectroscopy and Chemometrics Australian Journal of Basic and Applied Sciences, 6(10): 477-482,

Petibois, C., A.-M. Melin, A. Perromat, G. Cazorla Déléris G., 2000. Glucose and lactate concentration determination on single microsamples by Fourier-transform infrared spectroscopy. Journal of Laboratory and Clinical Medicine, 135(2): 210-215.

Picque, D., Cattenoz T., Corrieu G., 2002. Discrimination of Emmental cheeses by infrared spectroscopy. Milchwiss, 57: 202-204.

Reid, L. M., O'Donnell, C. P. O. , Downey, G. 2006. Recent technological advances for the determination of food authenticity. Trends in Food Science and Technology, 17, 344–353

Rodriguez-Saona, L.E., N. Koca, W.J. Harper , Alvarez V.B., 2006. Rapid Determination of Swiss Cheese Composition by Fourier Transform Infrared/Attenuated Total Reflectance Spectroscopy. Journal of Dairy Science., 89(5): 1407-1412.

Subramanian, A. and L.E. Rodriguez-Saona, 2008. Fourier transform midinfrares spectroscopy. in: Sun DW, editor. Infrared spectroscopy for food quality analysis and control. Dublin, Ireland: Elsevier.

Subramanian, A., W.J. Harper , Rodriguez-Saona L.E., 2009. Cheddar cheese classification based on flavor quality using a novel extraction method and Fourier transform infrared spectroscopy. Journal of Dairy Science, 92(1): 87-94.

Van de Voort F. R., Ismail A. A., Sedman J., Dubois J., Nicodemo T., 1994a Rapid quantitative determination of free fatty acids in fats and oils by Fourier transform infrared spectroscopy. J Am Oil Chem Soc 70:335-341.

Van de Voort F. R., Ismail A. A., Sedman J., Emo G., 1994b Monitoring the oxidation of edible oils by Fourier transform infrared spectroscopy. J Am Oil Chem Soc 71:242- 252.