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Detection of formaldehyde in cheese using FTIR spectroscopy

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Abstract

A new analytical method was developed for determining formaldehyde (CH_2O) in cheese by FTIR spectroscopy. Formaldehyde (CH_2O) was also spiked at 0 to 100 mg/100g in freshly prepared cheese. Two sets, each of twenty-one (21) samples, were prepared using the same type of soft white cheese. FTIR spectra were recorded using Attenuated Total Reflectance accessory at room temperature, and the Partial Least Squares (PLS) regression statistical method was used to derive calibration models for the set of samples in triplicates. The spectral region used for correlation and cross validation were set include the data from 1650 – 800 cm^{-1}. As suggested by the correlation and variance spectra. The coefficient of determination (R^2) of correlation was found to be 0.986 with average standard error of calibration (SEC) of 2.24 mg/100g, with. The calibration model was validated by using the "leave-one-out" cross-validation method, and the R^2 of validation, the standard errors of prediction (Yang and Irudayaraj), and standard deviation (Angulo *et al*.) of the differences for repeatability and accuracy were computed and found to be 0.9662, 4.07 mg/100g and 4.61, respectively. The results support the premise that FTIR spectroscopy is an efficient, precise and rapid analytical technique for the determination of minor components such as formaldehyde / formalin in cheese samples.

Introduction

Formaldehyde is added to food because of its antiseptic and preservation properties, improving the appearance of the product, and keeping it moist and odorless (Xu *et al*., 2011). However, formaldehyde exerts toxic effects on humans, including irritation of the eyes and respiratory tract, headaches, nausea, drowsiness and allergic skin reactions (Wang *et al*., 2012). It is also considered a carcinogen by the International Agency for Research on Cancer (Cogliano, 2006) (Monakhova *et al*., 2012). As it reduces the bacteria count and increases the shelf life of milk (Souza *et al*., 2011; Abernethy and Higgs, 2013), several cases of milk adulteration by formaldehyde were recently reported (Nascimento *et al*., 2015). Threshold limits for formaldehyde in milk have not been yet established by regulatory agencies, but the World Health Organization (WHO) states that the concentration in food can vary from 3 to 27 mg/kg (Nascimento *et al*., 2015). Therefore, continuous monitoring of products that could potentially contain formaldehyde is necessary, which requires fast and simple procedures.

Formaldehyde is a highly reactive chemical which readily combines with DNA, RNA, proteins and amino acids (Chaw *et al*., 1980; Hemminki, 1982; Siomin *et al*., 1973). It has been shown that formaldehyde added to milk during the production of grana cheese reacts very rapidly with caseins (Resmi *et al*., 1980), in particular with the v-caseins and it has been suggested that formaldehyde reacts with the NHE-terminal histidine of the rE-fraction (Resmi *et al*., 1980). On the basis of this, concern has arisen with regards to the potential hazard caused by the reaction products of formaldehyde and milk proteins. In order to assess the possible toxicological risk deriving from the ingestion of these products, animal experiments were carried out using grana cheese to which radioactive formaldehyde had been added in the course of production. Following a single administration of the [~4°C] cheese to rats and mice, the evaluation of the metabolism and the toxic kinetic profile did not support the hypothesis of a toxicological risk (Galli *et al*., 1983).

Although human exposure to formaldehyde occurs most commonly by the respiratory and dermal routes, it may also occur by ingestion, for

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example by consuming the Italian Grana Padano cheese, since during its production formaldehyde is added to the milk (15-25 ppm) as an antimicrobial agent. It is generally accepted that the amount of free formaldehyde (less than 0.5 ppm) that remains in this cheese after ripening, does not give cause for concern. This is confirmed by the results of animal experiments in which formaldehyde was chronically administered at much higher concentrations in the drinking-water or in the diet (40 mg/kg body weight for 2 years in mice) and no pathological findings related to treatment was observed.

A few procedures have been described for the determination of formaldehyde in milk, which are based on high performance liquid chromatography (Kaminski *et al*., 1993) or flow injection spectrophotometry (Cerdán *et al.*, 1992). HPLC requires time-consuming sample preparation involving several steps in which analyte loss may occur, and the response range of the flow-based procedure did not meet the limits set by WHO.

The use of FTIR spectroscopy is increasing in all fields (Ware and Matthay, 2000) including in food analysis (Mirghani *et al.*, 2003). Infrared studies of edible oils generally use the absorption of specific bands to evaluate traditional indices and other parameters of interest in relation to the composition of edible oils (Man *et al*., 2005; Lai *et al*., 1994) had used the FTIR spectroscopy and the principal component analysis (PCA) for determining the authenticity of vegetable oils. Fourier transform infrared spectroscopy combined with chemometric analytical methods had been described as rapid and reliable techniques for the detection of adulteration in food and related materials (Rodriguez-Saona and Allendorf, 2011). For example the determination of adulteration in virgin olive oil as reported by Yang and Irudayaraj (2001) and Marikkar *et al.* (2005) used FTIR and other analytical techniques for detecting the presence of lard/randomized lard as adulterants in refined-bleached-deodorized (RBD) palm oil. Adulterated cheeses were detected successfully using Attenuated Total Reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy (Cuibus *et al*., 2014).

The objectives of this study is to establish new rapid accurate and convenient analytical techniques for the detection of minor components in food using FTIR spectroscopy. For this research the traditional analytical protocols shall be followed as well as the new technique for the same sets of samples. The results obtained shall be correlated followed by validation. The newly introduced shall be simple rapid and environmentally friendly avoiding the use of chemicals

Materials and methods

Materials and samples

White cheese samples were purchased from a local retailer and other cheese sample was prepared in the laboratory using simple technique for chemical preparation of cheese by adding acetic acid (vinegar) to fresh milk. Formaldehyde was purchased from Sigma Chemical Company (St. Louis, MO). Formaldehyde (CH_2O) was also spiked in the range from 0 to 100 mg/100g in freshly prepared cheese. Two sets, each of twenty-one (21) samples, were prepared using the same type of soft white cheese.

FTIR spectra

The IR spectra were recorded using Fourier Transform Infrared (FTIR) spectrometer (Nexus 670 Fourier Transform Infrared spectrometer, Thermo Nicolet, USA). The FTIR spectra were analyzed using "Omnic 5.2a" software. The instrument was purged with dry nitrogen, and automatic dehumidifiers were used to protect from interference by CO_2 and water vapor, respectively. The prepared samples were placed on top of the ATR element which was rinsed three times with acetone and dried with soft tissue before going for the next sample. After each five (5) measurements, the cleaned ATR element was checked spectrally to ensure that no residue of the previous sample remained on it (Vásquez-Caicedo *et al*., 2007).

Calibration spectra were obtained by co-addition of 32 scans at 1 cm^{-1} resolution and a gain of 2.0 with strong apodization over the frequency region 4000–600 cm^{-1}. The spectra were ratio against a background air spectrum. Two spectra were collected from each of the prepared samples and stored in Joint Committee on Atomic and Molecular Physical Data–Data Exchange (JCAMP) files for subsequent analysis.

Calibration models for the prediction of formaldehyde content in the cheese samples from FTIR spectral data were obtained by partial least squares (PLS) regression using Omnic 5.2a software. In the PLS analyses, spectral data predicted by the software using the FTIR spectra and actual data on formaldehyde content were correlated, and the correlation coefficients (r) were taken as estimates of the factor scores, which were then used as regressors to model both spectral and actual data.

The "leave-one-out" cross-validation technique was used to validate calibration models, and the accuracy of each model was assessed according to

the standard error of prediction and coefficient of determination (R^2). The FTIR method was further evaluated by computing the mean difference and SD of the differences for repeatability (SDDr) and accuracy (SDDa) between the predicted FTIR data and the actual formaldehyde content values in the cheese samples (Mirghani, 2010) .

Results and discussion

Figure 1 shows IR spectrum of pure cheese sample that have peaks at 3380 and 3277 cm^{-1} for amide I absorbance and N–H band and may be some –OH for moisture content. Other region has bands at 2923 and 2853 cm^{-1} for C–H stretching and saturated (–CH) which is similar to the result obtained by Foda *et al.* (2010). The peak at 1745 cm^{-1} is for –C=O, peaks at 1628 and 1541 cm^{-1} are for amide II and protein, respectively. The band at 1456 cm^{-1} indicated the presence of acidic amino acids such as glutamic acid and may be aliphatic chain of fatty acids (Hug and Bahnemann, 2006). Other peaks in the same spectra of white soft cheese samples at 1236 cm^{-1} indicationg the presence of ester aliphatic chain and C–C, 1161 cm^{-1} for C–O and C=O and the peak at 1081 cm^{-1} for C–H in plane bending of aromatic C–H.

Figure 2 shows the spectrum of formaldehyde that has bands at 3308, 2982 and 2914 for –CH stretch, clear peak at 1636 for –C=O for aldehyde, and bands at 1429, 1271, 1103, 1019, and 989 cm^{-1}. Some of them have the same interpretation as for the cheese sample but others different and not found in the cheese spectrum such as the peak at 1271 cm^{-1} for C–O and C–O of phenolic OH, at 1103 cm^{-1} for –CH probably in plane bending and may be symmetric stretching and at 1019 and 989 for =C–O–C symmetric stretching. However, Figure 3 shows the spectra in Figures 1 and 2 in addition to the cheese spiked with formaldehyde spectrum that allows the select the region to detect the presence of formaldehyde if added to that type of cheese. The spectral response to changes in formaldehyde content was investigated by examination of the correlation and variance spectra. The variance spectrum distinguishes between the active and inactive spectral regions. Thus, the correlation spectrum can be used to choose which peak or region is selected for the calibration (Mirghani, 2010). The correlation spectrum is calculated by multiplying the differences between each standard spectrum and the mean spectrum, at each wavelength, by the difference between the corresponding property concentration and the mean property concentration, and summing over all the standards.

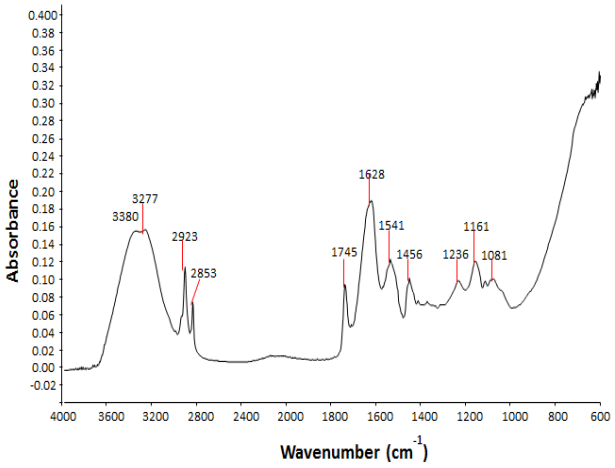

Figure 1. FTIR spectra for soft white cheese sample

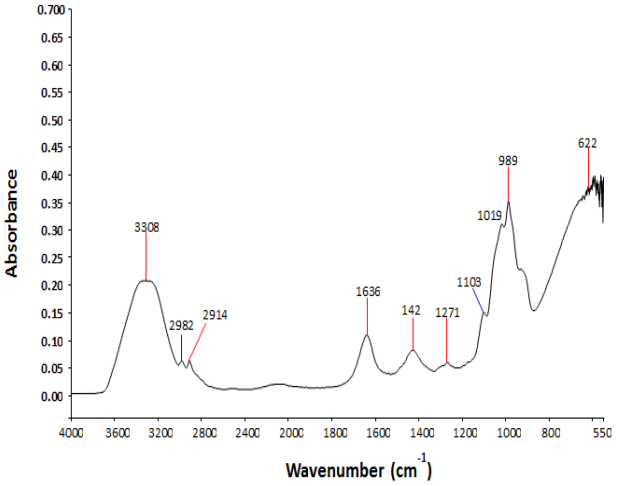

Figure 2. FTIR Spectrum of formaldehyde

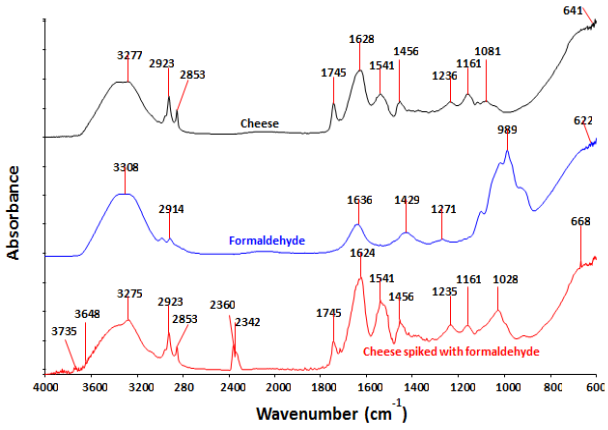

Figure 3. FTIR spectra of cheese, formaldehyde and cheese spiked by formaldehyde

The correlation spectrum was used to select regions that showed a mathematical correlation between spectral changes and the formaldehyde content in the cheese samples, and the variance spectrum was used to distinguish between active and inactive spectral regions (Deng *et al.*, 2012).

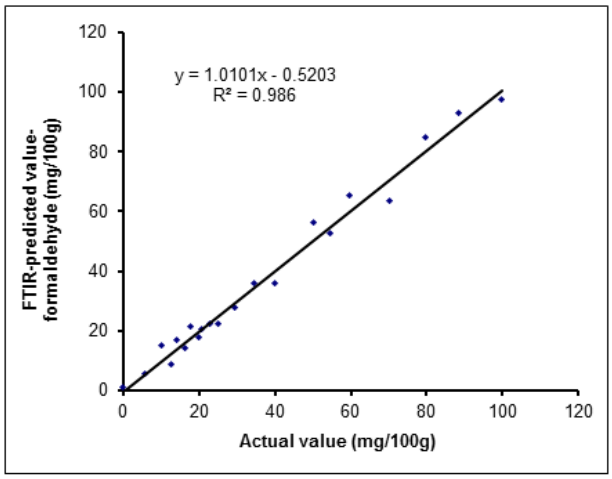

Figure 4. A plot of actual value of formaldehyde in cheese versus FTIR-spectroscopic predicted values, calculated with partial least square (PLS) calibration using the spectral data in the region 1650 – 800 cm^{-1}.

Table 1. Calibration and cross-validation statistics for formaldehyde content in soft white cheese measured by FTIR spectroscopic method at the wavelength ranges 1650 – 800 cm^{-1}[a]

	Soft white cheese				
Data set	Mean	SD	R^2	SEC	SEP
Calibration	58.55	4.86	0.9860	2.24	3.91
Validation	55.07	4.61	0.9662	3.72	4.07

[a] Abbreviations: FTIR: Fourier transform infrared; SD: standard deviation. R^2: coefficient of determination; SEC: Standard Error of Calibration; SEP: Standard Error of Prediction

The spectral regions used in the calibration were set to include the data from 1650 – 800 cm^{-1}, using the information showing the active and inactive regions in the correlation and variance spectra.

The regression equation (y = 1.0101 x – 0.5203) and correlation coefficient (R^2) of the determination is found to be 0.986 (Figure 4) revealed a good linearity response for the method developed, since the R^2 for standard curve is >0.98 indicating that the calculated line could explain more than 98% of the experimental data. Table 1 shows the data computed for calibration and cross validation, respectively, for the determination of spiked formaldehyde in soft white cheese by FTIR spectroscopy using PLS statistical analysis. The mean values, R^2, Standard deviations (Angulo *et al.*), standard errors of calibration (SEC) and of prediction (Yang and Irudayaraj) obtained using these spectral regions.

Conclusion

In this study, we report the development of an ATR-FTIR with PLS statistical regression strategy to reveal the detection of Formaldehyde/formalin in dairy products such as cheese. The analysis is rapid, requires only a minimal sample size (<2 mL), and avoids the use of chemicals. Precise monitoring of food quality using FTIR spectroscopy can help avoid risk to public health. This will widened the applications of FTIR spectroscopy to food quality assessment and control as one of the latest food industrial techniques.

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