

Characterisation and classification of waxes used in dairy technology by near infrared spectroscopy

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Introduction

Some particular cheeses, like Cheddar, Edam, Gouda and Provolone cheese, are wrapped in a coating of paraffin wax: (i) to prevent development of undesirable micro-organisms;¹⁻⁶ (ii) to prevent drying-out during storage; and (iii) to obtain good stability during handling and transportation to customers. Generally speaking, waxes, which are chemically inert and non-biodegradable under ambient conditions, are obtained from the petrochemical industry.^{7,8} Previous studies suggested that waxes be replaced with plastic films.^{3,5} The main disadvantage in their use may be a migration, caused by direct surface contact,⁶ of plasticisers such as adipates and phthalates into cheese. Nowadays biodegradable waxes,⁹ which may be produced from microorganisms,¹⁰ may also be used.

The aim of this study was to evaluate the feasibility of near infrared spectroscopy (NIR) to characterise and classify different waxes applied on some Italian cheese types.

Materials and methods

Eight waxes, commonly used at Italian dairies, and four experimental biodegradable waxes, produced from coconut oil by *Pseudomonas putida*,¹⁰ were analysed by an InfraAlyzer 500 (Bran+Luebbe, Norderstedt, Germany) at room temperature.

Preliminary studies were carried out to optimise analysis conditions. Samples of suitable size were obtained by carrying out a melting process at 90°C and cutting pieces of 1.5 cm (height) × 1.5 cm (width) × 0.15 cm (thickness). NIR spectra were collected twice in the range 1100–2500 nm, at 4 nm intervals (351 data points), and data were processed by Sesame Software (Bran+Luebbe) and GRAMS/32AI (Galactic Industries, Salem, NH, USA).

Exploratory analysis was performed using principal component analysis (PCA) on spectra corrected by a standard normal variate algorithm, i.e. each spectrum was normalised by computing and removing the mean value, then scaling by the standard deviation. Discriminant analysis was used to test the qualitative classification power of NIR.

The same waxes were also analysed by FT-IR instrumentation (FT-IR/420-Jasco Europe, Cremella, LC, Italy) coupled with attenuated total reflectance (ATR) in the range 4000–600 cm⁻¹ wavenumber.

A two-dimensional correlation between NIR and FT-IR data was generated using the 2D-CORR program (Galactic Industries) to enhance NIR results.

Results and discussion

Preliminary studies were carried out to optimise sample presentation in NIR analysis.

Figure 1 shows an example of exploratory analysis (PCA) of a petrochemical wax, melted at different temperatures (85°C, 90°C, 95°C and 98°C) and cut into pieces of different thicknesses (0.5 mm, 1 mm, 1.5 mm and 2 mm). No significant differences caused by the melting temperature were found. Groups were discriminated according to the sample thickness as a result of absorbance increase. Sample preparation conditions were chosen on the basis of their easiness. In particular, a temperature of 90°C was chosen because (i) this melting temperature is commonly used at factories, (ii) this sample preparation is fast and (iii) it allows possible changes in wax composition due to extreme heat (for example, reaching the point of smoke, although in this case it did not happen) to be avoided.

Variations in the shape of absorbance spectra were related to the nature of samples. Petrochemical waxes showed an opposite trend, as compared with biodegradable waxes in the 2050–2150 nm wavelength regions. Examples of raw spectra are reported in Figure 2.

Exploratory analysis carried out on these spectra (Figure 3) showed a clear separation between the two waxes along the first principal component (PC1). The trend of the first factor loading gave prominence to three principal peaks at 1684 nm, 2136 nm and 2248 nm. These absorbances corresponded to carboxylic acid COOH vibrations, in agreement with literature data.¹¹ The second principal component (PC2) was able to further discriminate samples within each group and 1220 nm, 1748 nm, 1776 nm and 2252 nm, related to C–H vibrations,^{12,13} were found to be the most important wavelengths.

A 2D correlation with FT-IR spectra was performed to carefully recognise the wavelengths involved, to point out other functional groups, possibly covered in the NIR region and to correctly allo-

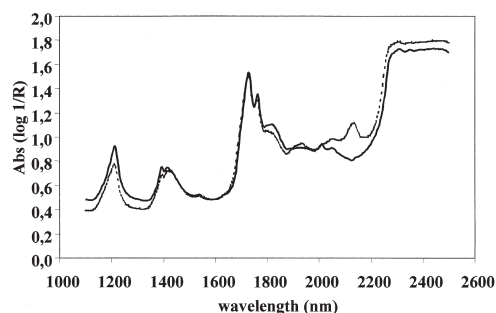


Figure 2. Examples of raw NIR spectra of (—) paraffin and (---) biodegradable wax.

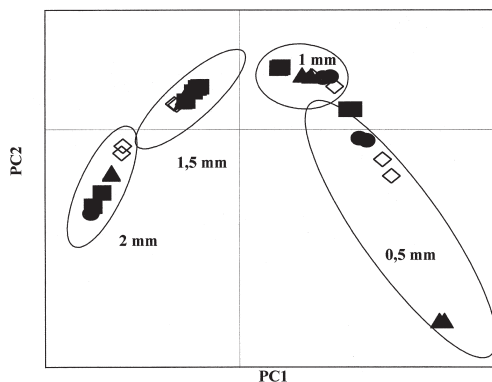


Figure 1. Example of an exploratory analysis (PCA) of a petrochemical wax, melted at different temperatures: (●) 85°C, (■) 90°C, (▲) 95°C and (◇) 98°C.

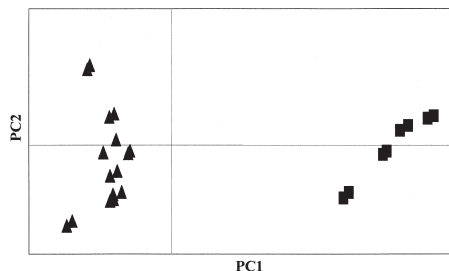


Figure 3. Exploratory analysis (PCA) of (▲) paraffin and (■) biodegradable samples on NIR spectra corrected by an Standard Normal Variate algorithm.

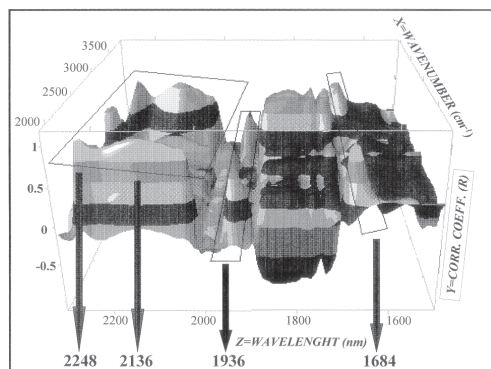


Figure 4. 2D correlation between NIR and FT-IR spectra, displayed as three dimensional "correlation map".

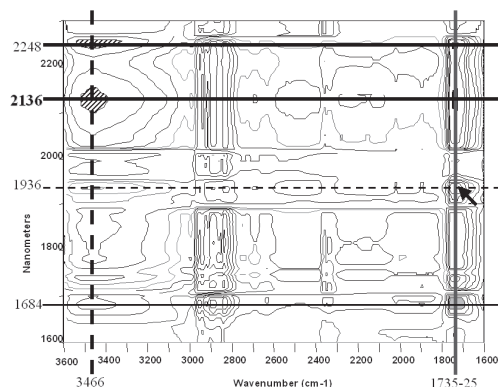


Figure 5. Contour map of bidimensional correlation FT-IR/NIR.

cate some functional groups to a precise chemical class. Figure 4 shows results as a three-dimensional "correlation map": regions of high positive correlation appear as "mountains", where individual bands for the two types of spectra were most similar and regions of negative correlation appear as "valleys" where spectra were most different.

In order to enhance results, a contour map of the bidimensional correlation FT-IR/NIR was plotted (Figure 5). The X dimension (horizontal) is the X matrix spectral axis of FT-IR and the Z dimension (vertical) is the Z matrix spectral axis of NIR.

The maximum value of the correlation coefficient, R , was close to 1 (see black contours under the thick lines). These contours related the interval 1750–1722 cm^{-1} in the mid-IR to two wavelength intervals in the NIR: 2100–2156 and 2234–2252 nm. At 1725 cm^{-1} wavenumber absorption of C=O stretching of carboxylic acids is reported. At 2136 nm the absorption of C–O stretching of long chain fatty acids is attributed. At 2248 nm the absorption is associated with the 2nd overtone stretching of coupled C–O / OH. At 1684 nm a correlation to the 3rd overtone absorption of this group was present with lower values ($R = 0.75$) of correlation coefficient (see gray contour under the thin line). Wavelengths of 2136 and 2248 nm exhibited further correlations of lower values, i.e. $R = 0.5$ (see striped contours under thick-dotted lines), with 3466 cm^{-1} where absorption of O–H stretching of carboxylic acids is reported.¹⁴ The interval corresponding to the absorption of C=O stretching of saturated acyclic esters correlated quite well ($R = 0.6$) to 1936 nm (see black arrow), ester 2nd overtone C=O stretching.¹⁵

From these relationships it can be concluded that the NIR wavelengths able to differentiate the chemical compounds giving rise to biowaxes and paraffins were well established. Biowaxes are polymeric chains of fatty acids (3-hydroxy fatty acids) containing the ester group, which permits them to be distinguished from paraffins, i.e. simple hydrocarbon chains with few branches, both in the mid-IR and in the NIR. In addition, they contain, though in lower amounts, natural waxes, other fats and primary alcohols such as stearic alcohol (saturated C 18). The wavelengths that discriminate waxes (1684 nm, 2136 nm and 2248 nm) were related to COOH group vibrations and the higher correlation coefficient values (R near 1) found, indicated that waxes had a similar behaviour in the near and mid IR regions. Surprisingly, ester C=O stretching did not seem to influence the classification of waxes as expected because biowaxes were made from fatty acid esterification processes.

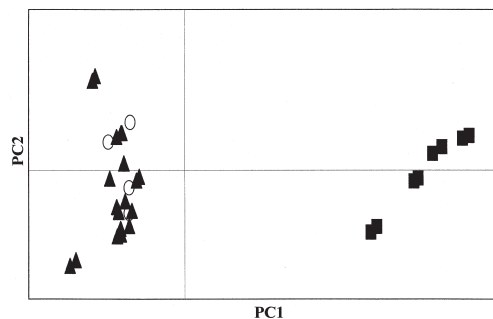


Figure 6. Qualitative classification of unknown samples using discriminant analysis applied on NIR spectra (▲) paraffin, (■) biodegradable samples, and (○) unknown samples.

In fact wavelength 1936 nm had a positive correlation with $1740\text{--}1735\text{ cm}^{-1}$ ($\text{C}=\text{O}$ stretching) and a negative correlation with 3466 cm^{-1} (carboxylic acid $\text{O}\text{--}\text{H}$ stretching).

On the basis of its classification power, NIR spectroscopy appeared to be a promising tool when used in routine analysis for a qualitative control of raw materials also on account of a further discrimination within each individual wax group according to the chain length.

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The discriminant analysis performed on unknown samples (Figure 6) showed that NIR was able to correctly classify waxes using simple criteria (i.e. a qualitative classification), based on the information collected from raw spectra. These data suggested that NIR may be used in routine analysis for the control of raw materials, although further investigations are required to confirm preliminary results.

Conclusions

A two-dimensional correlation between NIR and FT-IR spectra allowed us to explain why the two types of wax were grouped so clearly by NIR on the basis of their chemical compounds.

Interestingly, the correlation NIR/FT-IR permitted us to know the wavelength of absorption exactly, due to ester 2nd overtone $\text{C}=\text{O}$ stretching.