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Food Chemistry 90 (2005) 815–819

Food Chemistry

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Analysis of potential lard adulteration in chocolate and chocolate products using Fourier transform infrared spectroscopy

Y.B. Che Man [a,*], Z.A. Syahariza [a], M.E.S. Mirghani [a], S. Jinap [b], J. Bakar [a]

[a] *Department of Food Technology, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[b] *Department of Food Science, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*

Received 23 May 2003; revised 17 May 2004; accepted 17 May 2004

Abstract

Fourier transform infrared (FTIR) spectroscopy, in combination with attenuated total reflectance (ATR) and partial least square (PLS) regression, was used to detect the presence of lard in chocolate formulation. The spectral bands associated with lard, cocoa butter and their blends (ranging from 0% to 15% of lard in cocoa butter) were recorded, interpreted and identified. A semi-quantitative approach is proposed to measure the percent of lard in blends on the basis of spectral data at the frequency region 4000–650 cm^{-1}, using the equation $y=0.9225x+0.5539$. The coefficient of determination (R^2) was 0.9872 with a standard error (SE) of 1.305. In this paper, the potential of FTIR spectroscopy as a rapid analytical tool for the quantitative determination of adulterants especially lard, in chocolate, is demonstrated.

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Keywords: ATR; Chocolate; Cocoa butter; Fourier transform infrared spectroscopy; Lard; PLS

1. Introduction

The determination of food authenticity and the detection of adulteration are major issues in the food industry, and are causing concern among consumers and special attention among food manufacturers. Major authenticity issues concern the true labelling of food whereby substitution of high value raw materials with cheaper materials is common practice (Al-Jowder, Kemsley, & Wilson, 1997). This is especially so for 'value-added' products, where the potential financial rewards for substitution of a cheaper ingredients are high (Lai, Kemsley, & Wilson, 1995). Fat adulterants fall into two main categories: vegetable oils and fats, and animal body fats. Adulteration with vegetable fats and oils can be detected by several thin layer chromatographic techniques whilst animal body fat adulteration is more difficult to detect (Lambelet, 1983).

In some countries, food manufacturers choose to blend vegetable oil with lard to reduce production cost because lard is the cheapest fat currently and commonly available for the food industries. Lard or industrially modified lard could be effectively blended with other vegetable oils to produce shortenings, margarines and other speciality food oils (Marikkar, Lai, Ghazali, & Che Man, 2002). Pork and lard in food are serious matters in view of religious concerns, biological complication and health risks associated with daily intake (Rashood, Abdel-Moety, Rauf, Abou Shaaban, & Al-Khamis, 1996). From a religious point of view, Islam, Judaism and Hinduism prohibit their followers from consuming any foods containing porcine ingredients (Al-Qaradawi, 1995).

Chocolate is one of the most popular snacks and drinks worldwide. Chocolate is defined as a homogeneous product obtain from mixture of one or more of the following components: cocoa nib, cocoa mass, cocoa press cake,

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[*] Corresponding author. Tel.: +603-89468413; fax: +603-89423552.
E-mail address: yaakub@fsb.upm.edu.my (Y.B. Che Man).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.05.029

cocoa powder, with or without addition of cocoa butter and permitted optional ingredients and/or flavouring agents (Codex Alimentarius, 1981). Besides cocoa butter, limited additions (up to 5%) of other vegetable fats are also allowed. Foreign fats normally added to chocolate are cocoa butter equivalents (CBE), cocoa butter substitutes (CBS), and cocoa butter replacers (CBR) (Simoneau, Hannaert, & Anklam, 1999). For economic reasons, some small and medium industries in developing countries add lard to their chocolate and chocolate products.

In the analytical field, FTIR spectroscopy has received attention for use in the quantitative analysis of fats and oils (Guillen & Cabo, 1997b). Previous research has also shown the potential of FTIR spectroscopy as a method for detecting adulteration. Lai et al. (1995) reported that quantitative analysis of potential adulterants of extra virgin olive oil could be done using FTIR spectroscopy. Guillen and Cabo (1997a) also used FTIR spectroscopy to characterize edible oil and lard. Recently, Che Man and Mirghani (2001) developed a FTIR spectroscopic method for detecting lard in mixtures of other animal fats, such as chicken, lamb and cow. Besides, FTIR spectroscopy is also used as a tool for the rapid detection of other vegetable fats mixed in cocoa butter (Goodacre & Anklam, 2001).

The objective of this study is to investigate the use of FTIR spectroscopy to detect the potential presence of lard in chocolate and chocolate products available in the market, focussing on addition of lard to cocoa butter, as the main ingredient in chocolate formulation.

2. Materials and methods

2.1. Sample preparation

Lard sample was extracted by rendering the adipose tissues from various parts of slaughtered pigs, which was obtained from Seri Kembangan, Selangor, Malaysia. The rendering was done at 90–100 °C for 2 h in the oven. The melted fat was strained through triple-folded muslin cloth, dried by addition of anhydrous Na_2SO_4 and then centrifuged at 3000 rpm (Kubota Model 2010, Tokyo, Japan) for 20 min. The fat layer was decanted, shaken well and centrifuged again before being filtered through Whatman filter paper. The filtered samples were stored in tightly closed containers under a nitrogen blanket in the refrigerator. Ten brands of milk chocolate and chocolate products were purchased from a local supermarket. Extraction of fat was done using the Soxhlet method (PORIM, 1995).

2.2. Calibration standard

A cocoa butter sample was obtained from Premium Vegetable Oils Sdn. Bhd. as a reference. The calibration set was prepared by spiking lard to cocoa butter in certain ratios, covering the range 0–15%. All blends were prepared on a weight basis to calculate the exact actual percentage of the added fat. The pure fats and the blends were analyzed by means of FTIR spectroscopy.

2.3. Spectra acquisition

The FTIR spectra were obtained with a Perkin–Elmer 1725 series FTIR spectrophotometer (Perkin–Elmer Corporation, Norwalk, Connecticut, USA) equipped with a deuterated triglycine sulphate (DTGS) detector and connected to a Perkin–Elmer model 7300 PC operating under Infrared data System (IRDM) software. The samples were placed in contact with the ATR element (ZnSe crystal, 45° ends) at room temperature. FTIR data were collected over the region 4000–650 cm^{-1} by co-adding 32 scans at a resolution of 4 cm^{-1} with strong apodization. All spectra were ratioed against a background air spectrum and stored as absorbance values at each data point. All samples were scanned in duplicate.

2.4. Statistical analysis

The software programme Spectrum QUANT + version 4.1 (Perkin–Elmer) was used to construct a model of entire samples for calibration development. The adequacy of the calibration was first assessed for the goodness of fit between actual spiking value and FTIR predicted value on 25 samples used during the calibration process. A partial least squares (PLS) approach was developed for mathematical treatment of FTIR data. For the PLS equation, the assessment of accuracy was based on the smallest standard error (SE) and the coefficient of determination (R^2) (Miller & Miller, 2000).

2.5. Validation

The 'leave-one-out' cross-validation procedure was used to verify the calibration model. The standard error of prediction (SEP) and R^2 were used as the validity criteria for the calibration. The validation step was further investigated using the mean difference (MD) and standard deviation of difference (SDD) for reproducibility and accuracy.

3. Results and discussion

3.1. Spectra

Twenty-five samples were used for calibration and validation for PLS quantitative methods. Fig. 1 shows the spectra for pure cocoa butter and lard in the frequency range 4000–650 cm^{-1}. The cocoa butter spec-

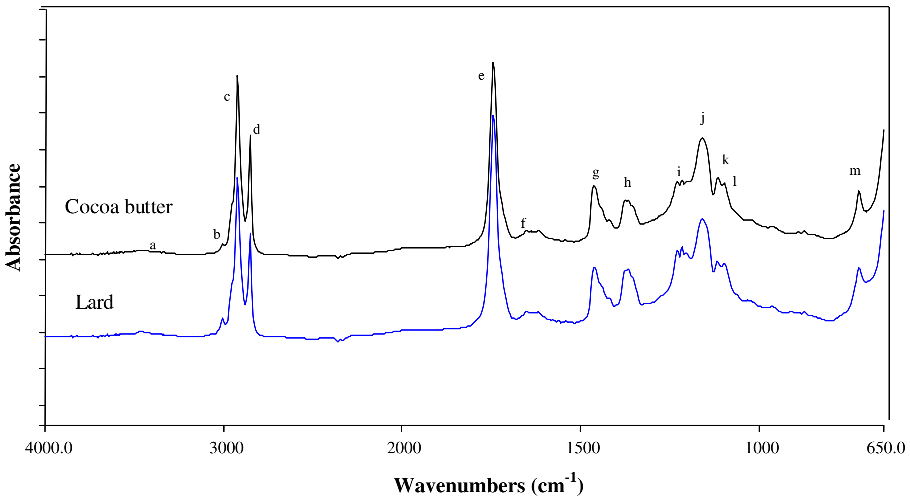

Fig. 1. FTIR spectra of pure lard and cocoa butter.

trum showed the characteristic absorption bands for common vegetable oils (Safar, Bertrand, Robert, Devaux, & Genot, 1994).

The greatest differences between spectra of the two samples, due to the intensity of bonds, observable with the naked eye, are at absorptions of approximately 3006 cm^{-1} (band b), 1238 cm^{-1} (band i), 1163 cm^{-1} (band j), 1118 cm^{-1} (band k), 1097 cm^{-1} (band l), and 723 cm^{-1} (band m) (Guillen & Cabo, 1997a).

The two spectra show a small band, a, near to 3464 cm^{-1}, which is associated with the overtone of the glyceride ester carbonyl absorption. A shoulder, b, approximately 3006 cm^{-1}, is observed in both spectra. These are due to stretching vibration of the cis olefinic double bond. Both the methylene asymmetrical stretching band, c, at approximately 2924 cm^{-1} and at the methylene symmetrical band, d, at 2853 cm^{-1}, are obviously present in both samples.

The C=O group of triglycerides shows a stretching vibration band, e, at approximately 1746 cm^{-1}. The C=C stretching mode of unconjugated olefins usually shows moderate to weak absorption at 1667–1640 cm^{-1}. In samples, one small band, f, at 1648 cm^{-1} can be detected.

The bands between 1400 cm^{-1} and 1000 cm^{-1} are the most difficult to assign. However, in this region, the differences to the eye in Fig. 1 are also the most significant. At approximately 1465 cm^{-1}, g, both spectra show the scissoring band, of the bending vibration of the methylene group.

A band, h, at 1377 cm^{-1} could be due to symmetrical bending vibration of methyl groups. The following group of bands, i, j, k, and l, at approximately 1238, 1163, 1118 and 1097 cm^{-1}, respectively, and a shoulder, m, at approximately 723 cm^{-1} results from the overlapping of the methylene rocking vibration and the out-of-plane bending vibration of *cis*-disubstituted olefins.

The 4000–650 cm^{-1} region was used for prediction using the PLS statistical techniques to develop calibrations from the FTIR spectra and actual data. Fig. 2 shows FTIR spectra for cocoa butter, lard and some blends of cocoa butter–lard with certain ratios.

3.2. PLS calibration and cross-validation

The power of PLS is based on its ability to use spectral information from broad spectral regions and to correlate spectral changes in the concentration of a component of interest while simultaneously accounting for other spectral contributions that may perturb the spectrum. A PLS calibration model was developed based on the calibration standard that included the different weighted amounts of blended lard and cocoa butter.

Fig. 3 plots the data from actual data against the PLS FTIR predicted data for lard content in cocoa butter using the None baseline type. A good linear regression of the actual value against FTIR predicted value was obtained ($y = 0.9225x + 0.5539$) with the standard deviation (SD) 3.7875.

Table 1 compares the predicted FTIR values and the actual values of lard content in terms of MD and SDD for overall accuracy (*a*) and repeatability (*r*). The term accuracy is defined as the closeness of agreement between actual data and the predicted FTIR result. Low MDa and SDDa (0.2325, 0.3923), respectively, show that the FTIR is superior in determining lard adulteration. Meanwhile, low MDr and SDDr (0.2072, 0.4064), respectively, indicate that the FTIR method has appreciably high repeatability. The SD relative to the mean of the analytical method is expressed as the coefficient of variation (CV). It has been argued that a CV of <20% suffices for most analytical purposes. The CV obtained from the PLS FTIR predicted data and actual data was 5.209%.

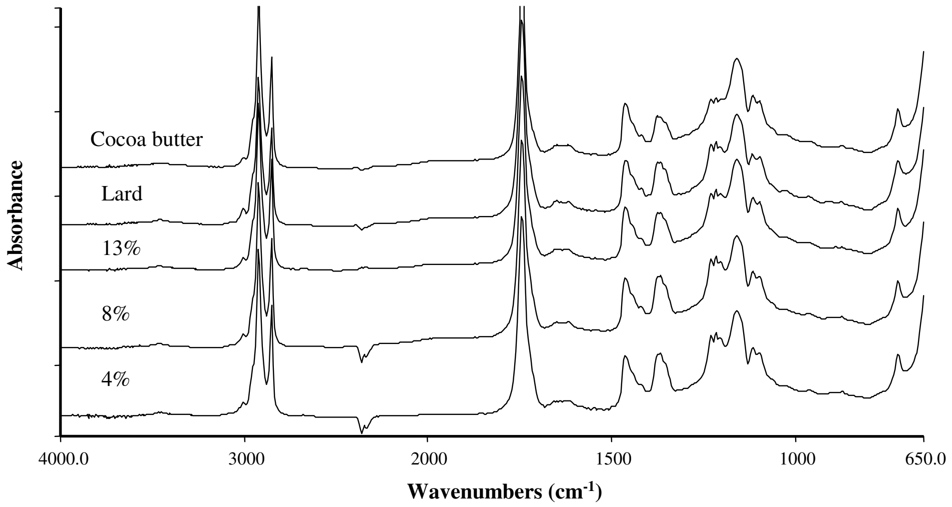

Fig. 2. FTIR spectra of lard, cocoa butter and some blends of lard–cocoa butter.

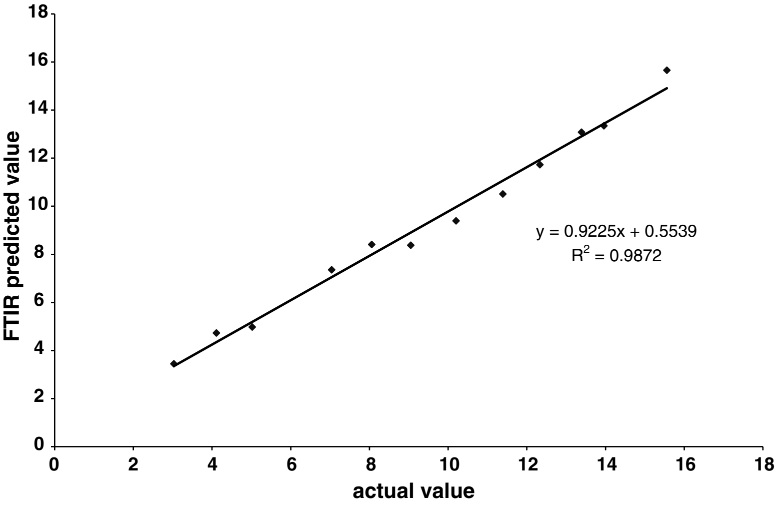

Fig. 3. Data from actual value versus FTIR-predicted values for lard content by the 'leave-one-out' cross-validation.

Table 1
Repeatability and accuracy of FTIR prediction of lard content obtained by cross-validation[a]

Statistics	Actual data	PLS FTIR
MDa	0.2325	
SDDa	0.3923	
MDr	–	0.2072
SDDr	–	0.4064

[a] MD, mean difference; SDD, standard deviation of difference; *a*, accuracy; *r*, repeatability.

4. Conclusions

From this study, we conclude that FTIR spectroscopy, combined with ATR and PLS regression, can be used to determine the lard content when blended with cocoa butter. The results from this work provide some basic information about the detection of lard in the real chocolates sample in the future. Detection of lard adulteration will be extended to various types of fats normally used in chocolate production, such as cocoa butter substitutes (CBS), cocoa butter equivalents (CBE) and cocoa butter replacers (CBR).

With a total analysis of less than 2 min and less than 2 ml of sample, FTIR spectroscopy offers a rapid, accurate, low cost and environmentally friendly tool for quantitative analysis of adulteration.

Acknowledgement

We would like to thank the Universiti Putra Malaysia (UPM) for funding (IRPA Grant No. 09-02-04-0457-EA001) this study.

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