LWT - Food Science and Technology 109 (2019) 63–69

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

FTIR-ATR spectroscopy as a tool for the rapid detection of adulterations in butter cheeses

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ARTICLE INFO

Keywords:
Butter cheese
Vegetable oil
Adulteration
Authentication
Chemometrics

ABSTRACT

This study assessed the potential application of Fourier-Transformed Infrared spectroscopy using Attenuated Total Reflectance (FTIR-ATR) coupled with multivariate approaches to characterize and detect adulterations in butter cheeses (BC) with soybean oil (SO). In general, as the adulterations were performed, there is a decrease in band intensities related to proteins, while for most bands related to lipid, the substitution of butter oil (BO) by SO led to increases in their intensities up to 70% of fraud. The band near 3007 cm^{-1} was evinced only in adulterated cheeses. Principal component analysis differentiated the samples validating the spectral observations. A partial least squares model allowed the prediction of the percentage of SO added into the samples with low residuals. FTIR-ATR spectroscopy associated with chemometrics showed good performance in the detection of butter cheeses' adulterations.

1. Introduction

Cheeses are consumed worldwide and have a great commercial importance within the food industry (Dankowska, Małecka, & Kowalewski, 2015). In the Northeast region of Brazil, the type of cheese named "butter cheese" (BC) has a prominent position among dairy products because of its historic and cultural roots. Its processing consists of the coagulation of whole or skim milk, desorption of the curd obtained by acidification, washing of the mass with water and/or milk, salting, melting of the mass with butter oil (BO) and molding the cheese (Nassu, Lima, & Andrade, 2009).

Milk is the only source of the fat content in BC and the addition of different fats is considered fraud. Among several possibilities of adulterations, vegetable oil such as soybean oil (SO) is a very common one. In reality, it became a real issue for the dairy industry in Brazil (Dankowska et al., 2015). The adulteration generates an uncharacterized product with problems regarding food quality and safety (Danezis, Tsagkaris, Camin, Brusic, & Georgiou, 2016).

Analytical methods for lipid detection include the determination of physicochemical properties (i.e. melting point), water-soluble and -insoluble volatile fatty acid profiles, or triacylglycerol composition (Koca, Kocaoglu-Vurma, Harper, & Rodriguez-Saona, 2010). However, most of them are based on wet-chemical analysis requiring hazardous chemicals as well as skilled and experienced staff. Also, they are time-consuming techniques with requirements of extensive sample preparation.

Attempts using spectroscopic methodologies to investigate authenticity issues have been done using mainly the UV–Visible, FT-NIR, FT-MIR and Raman spectroscopies (Brandao, dos Anjos, & Bell, 2017; Brandao, Gouvea Neto, dos Anjos, & Bell, 2017; Mishra et al., 2016). Advances in MIR spectroscopy instrumentation and analysis allowed the extraction of information related to the composition of food components from complex spectra (Koca et al., 2010) and allowed the detection of adulteration in different foods (Jawaid, Talpur, Sherazi, Nizamani, & Khaskheli, 2013; Rohman, Sismindari, Erwanto, & Che Man, 2011). A wide variety of food has been subjected to adulteration including dairy products and others (Danezis et al., 2016; Pereira et al., 2018). However, so far, little information is available on the literature attesting the authenticity of BC using MIR spectroscopy.

Considering the importance of monitoring adulterations of genuine cheeses, this study assessed the potential application of the FTIR-ATR spectroscopy, coupled with multivariate approaches (Principal Component Analysis (PCA) and Partial Least Squares (PLS) regression for characterization and quantification of BCs adulterated by SO, respectively. Besides physical-chemical parameters (protein, moisture, fat

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https://doi.org/10.1016/j.lwt.2019.04.017
Received 24 August 2018; Received in revised form 4 March 2019; Accepted 6 April 2019
Available online 08 April 2019
0023-6438/

and fat in dry basis) were analyzed.

2. Material and methods

2.1. Sample preparation

BC samples were prepared in duplicate, throughout two batches of skimmed milk. The milk was acquired in a dairy industry located in the city of Currais Novos, state of Rio Grande do Norte, Brazil, which processes the milk production from 10 municipalities in its surroundings. From each milk batch, cheese mass (CM) (0% fat content addition) and BCs (35% theoretical total fat content) were produced. In the cheeses, different percentages of adulterations (ranging from 0 to 100% of substitution) were accomplished by replacing the BO by SO, keeping the same industrial process as in the commercial production. The BO was obtained at the same dairy industry located in the city of Currais Novos where all the milk for BC production was acquired. The samples of SO was purchased at the local market.

The sample with 0% of fat addition was composed only of CM. During the cheese production, 400 g of CM was used and 140 g of fat content (35%) was added into it in different proportions of adulterations (0–100%) as presented in Table 1. The cheeses were then taken to a cold room with an average temperature of 10 °C, where they remained for 24 h. Then they were divided into two pieces, vacuum packed and stored at 5 °C until further analysis. One of them was submitted to physical-chemical analysis while the other was used for spectroscopic analysis. The spectroscopy was also used to evaluate the pure samples of SO and BO.

2.2. Physical-chemical analysis of the cheeses

The physical-chemical composition analysis was carried out in relation to fat, fat in dry matter (FDM), protein and moisture contents. The measurements were performed in agreement to AOAC standards. All samples were tested in triplicate.

2.3. FTIR/ATR spectroscopy

A FT-MIR spectrophotometer (Bruker, model Vertex 70) with attenuated total reflectance (ATR) accessory and OPUS 6.5 software was used. The spectra were recorded by 64 scans in absorbance mode from 4000 to 400 cm^{-1} range with a resolution of 2 cm^{-1}. The measurements were performed in triplicate in pure BO, pure SO and in the cheeses. Different cuts of the core and surface of the samples (diameter and height of 2 cm and 1.3 cm, respectively) were used as replicates for a better representation.

Table 1
Cheese composition in terms of CM, SO and BO.

Samples	Proportion of constituents		
	CM (g)	SO (g)	BO (g)
CM	400	0	0
BC - 0%	400	0	140
BC - 10%	400	14	126
BC - 20%	400	28	112
BC - 30%	400	42	98
BC - 40%	400	56	84
BC - 50%	400	70	70
BC - 60%	400	84	56
BC - 70%	400	98	42
BC - 80%	400	112	28
BC - 90%	400	126	14
BC - 100%	400	140	0

CM – cheese mass, SO – soybean oil and BO – butter oil.

2.3.1. Data processing

Prior to data analysis each spectrum was pre-processed via baseline-correction and background subtraction in order to eliminate background noises from the environment. Finally the areas of interest were selected. All the figures were performed using the software OriginPro 2015.

2.4. Statistical analysis

Differences in group's mean values of the results of physical-chemical analysis were obtained by One-way ANOVA. Tukey HSD test was carried out when significant differences among groups were obtained. As Tukey HSD may be susceptible to non-normal population, we used Shapiro-Wilk´s test to check the normality. In addition the dataset passed by homogeneity of variance through Bartlett's, Lavene's and Brown-Forsythe tests. Further checks were performed comparing Tukey HSD and Fisher LSD. The results (not shown) were almost identical. All statistical tests were done with 95% of significance and performed by softwares OriginPro 2015 and [R] (www.r-project.org).

2.5. Multivariate approach

2.5.1. Principal Component Analysis

PCA was applied to pure SO, pure BO and the cheese samples (BC – 0–100% of adulteration) to characterize the behavior of the samples as substitutions were increasing. PCA was performed using wavenumber ranges from 3500 cm^{-1} to 2800 cm^{-1}, 1800 cm^{-1} to 1050 cm^{-1} and 750 cm^{-1} to 650 cm^{-1} in a spectral resolution of 2 cm^{-1} with a total of 806 data points for each sample. The data were standardized as unit variance and subjected to the analysis using OriginPro 2015 software.

2.5.2. Partial least squares regression

To predict the percentage of SO added into the cheeses and the physical-chemical composition, PLS regression was carried out by OriginPro 2015 software. The MIR spectra were taken as the X matrix, while each proportion of adulteration with SO and the physical-chemical composition results were the Y matrix (Bassbasi, Platikanov, Tauler, & Oussama, 2014).

A total of 11 samples were used for PLS, 8 for the calibration curve and 3 for the external validation. The samples for the calibration and the external validation were chosen randomly amongst the range of 0–100% of adulteration level. Full cross-validation (leave-one-out) was used to obtain the optimum number of latent variables. The root mean square error (RMSE), the relative prediction errors (RE%) and the coefficient of determination (R^2) were used to describe and compare the model performance, for both calibration and validation data sets. In general, as low as the RMSE and RE% value can be, and R^2 as close as possible to 1, the better will be the model predictions. RMSE and RE% parameters were calculated according to Andrade et al. (2019).

3. Results and discussion

3.1. Physical-chemical analysis

The analyses of the CM used to produce the BCs revealed levels of 24.60 ± 1.64% of protein, 54.71 ± 2.41% of moisture, 6.00 ± 0.89% of total fat and 13.21 ± 1.24% of FDM. As expected, this sample showed non-standard results for FDM, since no fat was added to it. The results of the cheeses are shown in Fig. 1.

The highest protein and moisture level was obtained with the CM. High protein levels lead to higher water binding capacity, which increases the final moisture of the cheese (Arslan, Topcu, Saldamli, & Koksal, 2014). During the cheese production, 35% (w/w) of the CM was substituted by fat (as shown in Table 1). Therefore, the BCs (0–100% of adulteration) presented a lower protein and moisture content. Nassu et al. (2009) studied six brands of BC. Those with lower milk fat levels

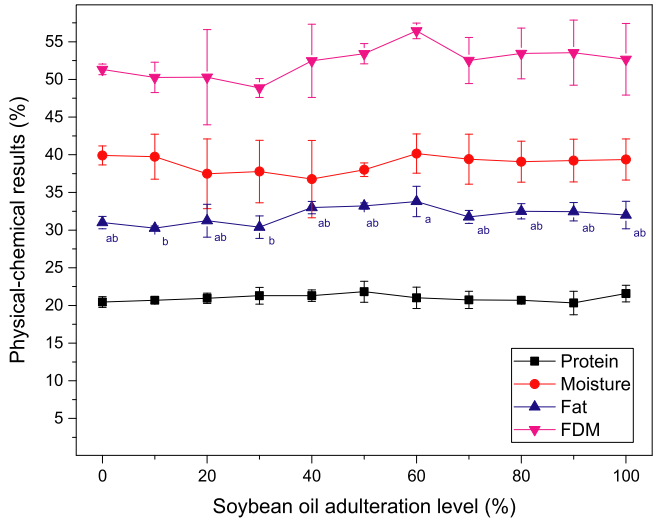

Fig. 1. Variation in the physical-chemical parameters (protein, moisture, fat and fat in dry matter - FDM) obtained for the butter cheeses (0–100% of adulteration). Superscript letters mean statistically significant differences ($p < 0.05$) obtained by TukeyHSD test with 95% of significance.

had higher protein content. Moreover, a reduced fat content in cheeses results in a greater interaction between casein molecules, obtaining a denser protein matrix (Guinee & Kilcawley, 2004).

Statistical analysis did not reveal significant changes in the physical-chemical analyses of the BC, except for fat content as shown in Fig. 1. In this figure, one may observe that the presence of SO did not influence the overall results. In addition, all samples could still be classified as BC as the variables evaluated were within the levels established by the Brazilian regulatory agency (Brasil, 2001). This fact demonstrates that the physical-chemical analyses carried out in our work were not capable of detecting this type of fraud.

3.2. Spectroscopic analysis

The main absorbance bands obtained were from 3600 to 2750 cm^{-1} and 1800 to 625 cm^{-1} in agreement with the literature regarding other cheeses (Andrade et al., 2018). The spectra of the CM and adulterated samples are presented in Fig. 2.

In general, the samples showed very similar spectra with differences mainly in relation to the intensity of the bands. The main bands

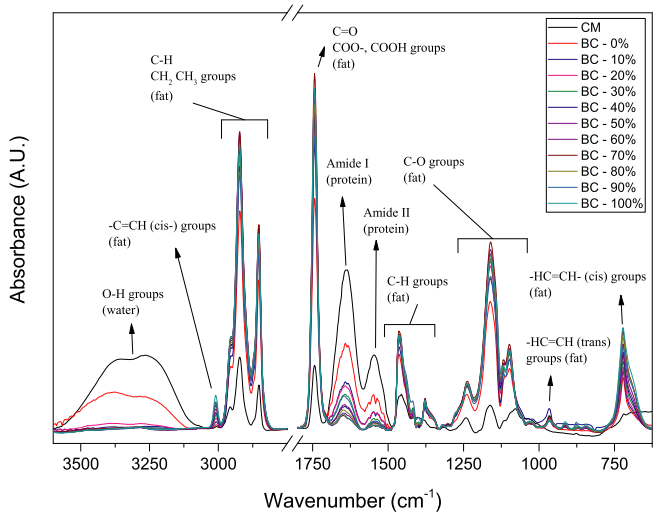

Fig. 2. FTIR/ATR spectra of the CM (0% of fat) and those with 35% of fat content (BC - 0–100% of adulteration by SO).

obtained are related to the presence of water, lipids and proteins, which belong to the region called "functional group" (4000–1500 cm^{-1}) (Lohumi, Lee, Lee, & Cho, 2015). According to Cuibus et al. (2014), such a variety of contributions (hydroxyl group, acids, esters, amides I and II, aliphatic chain fatty acids and amino acids) make the cheese spectrum difficult to understand. This fact also causes difficulties in the discovery of small variations owing to adulteration procedures. However, a clear differentiation was noticed when compared the sample without fat to those with BO and SO, especially in the wavelength range from 3600 to 3050 cm^{-1} and from 1000 to 400 cm^{-1}.

3.2.1. Lipid regions

In cheeses, the range between 3100 and 2800 cm^{-1} is characterized by absorption bands with symmetrical and asymmetric stretching vibrations of C-H molecules associated with methyl and methylene groups characterizing fatty acids (Cuibus et al., 2014). The results presented in Fig. 2 show that in this region there were 2 main bands, at 2922 cm^{-1} and 2852 cm^{-1}. Karoui et al. (2006) found two intense bands occurring at 2915 cm^{-1} and 2846 cm^{-1} associated with methylene anti-symmetric and symmetric stretching, respectively. Two other bands occurred at 2954 cm^{-1} and 2860 cm^{-1}, which are results of asymmetric and symmetrical stretches of methyl groups, respectively.

Other relevant bands are the one at 1743 cm^{-1} (stretches of carbonyl groups C = O) (Sauer-Leal, Okada, & Peralta-Zamora, 2008), and the bands around 1464 cm^{-1} (C-H groups in CH_2) and 1160 cm^{-1} (C-O group in esters) (Rodriguez-Saona, Koca, Harper, & Alvarez, 2006). Regarding fatty acids, the band at 966 cm^{-1} corresponds to trans-CH out-of-plane deformation vibrations, being reported as a marker for the determination of trans fats (Koca et al., 2010). In Fig. 2 this band does not appear in the CM, while in the others samples, it doesn't have a regular behavior due to band overlap in the so called "fingerprint region" (1500–500 cm^{-1}) (Lohumi et al., 2015).

The band near 3007 cm^{-1} represents stretches of groups -C = CH (cis-) of double bonds in unsaturated fatty acids (Kadamne, Jain, Saleh, & Proctor, 2009; Koca et al., 2010). The band around 721 cm^{-1} is produced by rocking vibrations of groups -HC = CH- (cis), characteristic of long chain unsaturated fatty acids (Kadamne et al., 2009). Also in this region occurs wagging vibration, common in compounds containing more than four bound methylene groups linked to the formation of polyunsaturated compounds (such as ω-3 and ω-6). About 8% of the fatty acid content of SO is of α-linolenic acid, a ω-3 compound (Fonseca & Gutierrez, 1974).

In Fig. 2 the CM sample presented the lipid related bands with the lowest intensities. This behavior agrees with the physical-chemical results once the CM was made with skim milk (0.5% fat) with no lipid addition. When comparing the CM with the BCs, the former had a fat content of 6.0% and FDM of 13.21%, while the latter (manufactured with addition of 35% of fat) showed average values of 32% and 52.5%, respectively (Fig. 1).

Even though all the BC samples had the same fat content addition (35%), the absorbance intensities of the lipid related bands were higher in the adulterated BCs. This can be due to the presence of different types of fat (SO and BO) in different proportions. Such fact is corroborated analyzing the spectra of pure BO and pure SO as presented in Fig. S1 (supplementary material).

Fig. 3 shows the behavior of the maximum absorbance intensity of the main lipid bands. In general, there was an increase in the absorbance values up to 70% of adulteration. However, in the samples with higher levels of SO (BC - 80%, BC - 90% and BC - 100%) the intensities of the bands at 2922 cm^{-1}, 2852 cm^{-1}, 1743 cm^{-1}, 1464 cm^{-1}, 1160 cm^{-1} and 721 cm^{-1} have shown a decrease in absorbance with an increase of the SO content. This behavior may be explained by an excess of SO in higher adulterated BCs. Visually the brightness and oiliness could be homogeneously observed in the whole cheese samples. The ones with high SO levels showed a substantial release of the oil from the cheese protein network. The higher the proportion of SO in the cheese,

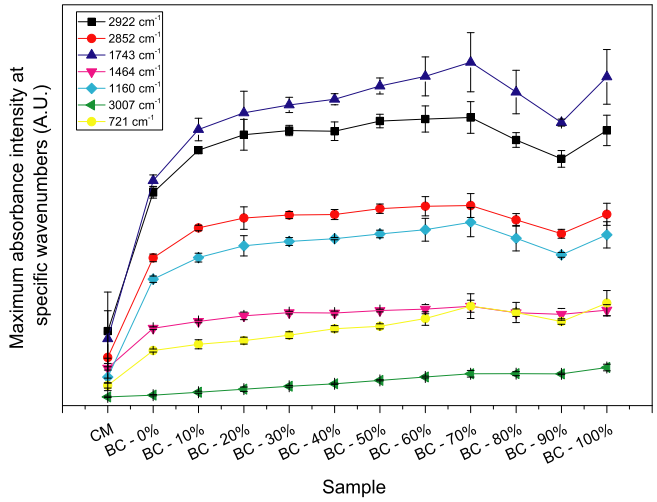

Fig. 3. Evolution of the points of maximum absorbance regarding the bands related to lipid compounds.

the greater the release. This lead to the formation of a thin film of SO on their surface, which interferes in the MIR measurement. Therefore, the measurement might be more related to the SO film than the cheese itself, once the laser beam penetrates maximum of 5 μm in the sample. As the bands of the spectrum of SO are less pronounced than that of pure BO (Fig. S1), this behavior of decreasing spectra intensity in cheeses with higher levels of fraud can be justified.

Fig. S2 presents the variation in the oil present on the surface of the cheeses with 35% of fat addition (BC - 0%, BC - 50% and BC – 100%).

Fig. 3 shows that the band at 3007 cm^{-1} was the only one in which there was a regular increase in the absorbance values because of the increase of SO content in the cheeses, even in those with adulterations of 80%, 90% and 100%. Such an increase may be explained by observing the peak intensity at 3007 cm^{-1} at Fig. S1, where the absorbance level is substantially higher in pure SO than in pure BO. This band is characteristic of stretches of –C = C–H bonds present in double bonds of unsaturated fatty acids, such as linoleic acid (C18:2) and α-linolenic acid (Kadamne et al., 2009; Koca et al., 2010), which are highly present in vegetable oils, such as soybean (Fonseca & Gutierrez, 1974), but in low concentrations in butter as shown in Table 2. Similar behavior was observed in adulteration of butter with margarine (Koca et al., 2010).

Another aspect used to analyze spectra is the band shift. This happened at 3007 cm^{-1} (Fig. 2). In the BC - 0% and BC - 10% cheeses, the band showed maximum absorbance at 3007 cm^{-1}. From 20% to 100% of adulteration, the maxima were shifted to 3009 cm^{-1}. It was found that the proportion and composition of unsaturated fatty acids affect the position and intensity of this band (Koca et al., 2010). In our case, we attribute a similar origin to the band shift and band increase, the higher presence of unsaturated fatty acids in SO.

3.2.2. Protein and water regions

In Fig. 2, the region from 3600 to 3050 cm^{-1} is characterized by the O-H stretching of hydroxyl groups (Cuibus et al., 2014). The broad band in this region has a specific bell shape, which is closely linked to presence of hydrogen bonds.

It was observed that only the CM and BC – 0% showed the band indicative of the presence of water, at approximately 3265 cm^{-1} (Fig. 2). In other cheeses (BC - 10% to 100%) this band almost disappear. In relation to the CM, the high intensity of the band is due to the highest moisture content among the analyzed samples (approximately 55%) and the lowest fat content (6.00%). Among the BCs, even though all of them had similar fat content (35%), only in the BC – 0% the OH band was detected. This fact is related to the previous issue of the SO release in adulterated samples. As the SO is a liquid lipid at room

Table 2
Fatty acid composition of soybean oil (SO) and butter oil (BO) (Fonseca & Gutierrez, 1974).

Fatty acid	% by weight of total fatty acids	
	SO	BO
4:0	–	2.00
6:0	–	1.00
8:0	–	1.00
10:0	–	1.50
12:0	–	1.50
13:0	–	0.20
14:0	0.20	9.00
15:0	–	1.90
16:0	11.35	24.00
17:0	–	1.00
18:0	4.15	13.00
19:0	–	0.40
20:0	0.15	0.50
Total saturated	15.85	57.00
6:1	–	0.01
8:1	–	0.02
10:1	–	0.20
10:3	–	0.09
12:1	–	0.20
12:2	–	0.20
12:3	–	0.40
14:1	–	0.80
14:2	–	0.45
14:3	–	0.50
16:1	0.05	2.50
16:2	–	0.5
18:1	25.30	32.00
18:2	50.60	3.00
18:3	8.20	1.00
Total unsaturated	84.15	41.87

SO – soybean oil and BO – butter oil.

temperature, less viscous than BO, and unfamiliar to the cheese, it is easy to leak from the cheese lattice migrating to its surface (Fig. S2). Therefore, by having more SO on the surface, it is natural that the adulterated BCs present spectra more similar to the pure SO (Fig. S1). The same does not happen with BC - 0%, because its fat content comes exclusively from the BO, which comes from the milk itself and might interact better with the cheese network during the processing, preventing its release. Therefore, a possible indicative of fraud is the visualization of the OH band.

The region between 1700 cm^{-1} to 1500 cm^{-1} is associated with proteins, being characterized by two important bands: Amide I at 1641 cm^{-1} (ν C = O, ν C – N) and Amide II at 1549 cm^{-1} (δ N – H e ν C – N) (Andrade et al., 2018).

Among the samples studied, the CM presented the highest intensities in the two bands (Amide I and II), which is in agreement with its protein content found in the physical-chemical analysis. Also, the CM has lack of fat (especially at the sample surface, compared to the adulterated samples), therefore better exposing the proteins to the IR radiation.

According to Arslan et al. (2014), protein levels correlate with water binding capacity. The CM sample showed high moisture content. Thus, there is a direct correlation between the band intensity of Amide I, II and OH groups, but they are inversely correlated to the oil content present in the cheeses (Fig. 2). The same authors noted that the replacement of milk fat by oil had a significant effect on fat and protein levels in the dry matter of white Turkish cheese. As the fat content decreased, the moisture and protein content increased. According to Foda, Bahgaat, Kassem, and Aly (2013), differences in moisture content may be related to differences in protein content, the type of vegetable

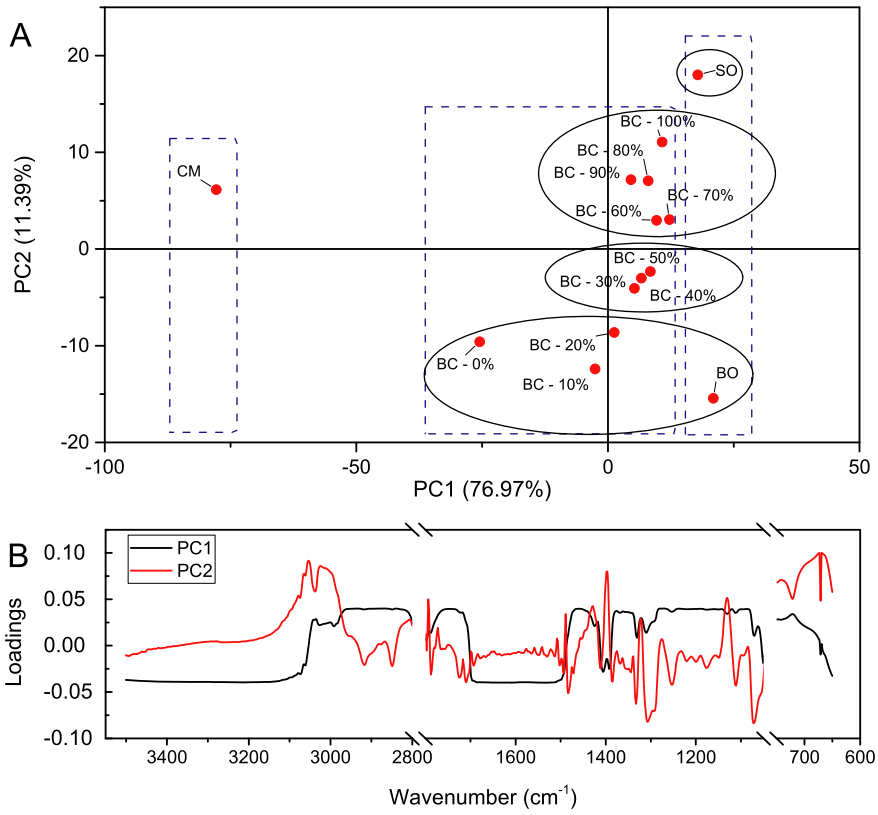

Fig. 4. Principal Component Analysis of the dataset containing the spectra of the samples: CM, pure BO, pure SO and the BC - 0–100% with SO. (A) Score plot; (B) Loading plot.

oil used in cheese making, and its ability to hold water.

3.3. Principal components analysis (PCA)

Fig. 4 presents the score plot of PCA where the first two principal components accounted for approximately 88% of the total variability of the data.

The score plot (Fig. 4A) shows a clear separation between the CM from the other samples in relation to PC1. As previously seen, this sample has high moisture and protein content, and the others have the presence of fat added. These differences may have influenced the variability of most of the dataset. In Fig. 4B, the loading plot reveals how each wavenumber influenced the samples present in the score plot. The range from 3600 to 3050 cm^{-1} (water region) and the one between 1700 and 1500 cm^{-1} (protein) weighted significantly on the negative scale of PC1. On the other hand, the fat regions (3000–2800 cm^{-1}, 1800 to 1700 cm^{-1}, 1480 to 1415 cm^{-1}, 1385 to 1060 cm^{-1} and 750 to 680 cm^{-1}) affected the positive side of PC1. Therefore it can be concluded that PC1 is related to the amount of fat present into the samples and can be divided in three groups (dashed rectangles in Fig. 4A). The first is related to CM (0% of fat addition) and is positioned on the negative side of PC1. The second, which encompasses the BCs (35% of fat content addition) is positioned from the beginning of negative scale to the beginning of positive scale of PC1. Lastly, the third group, including samples with expected 100% of fat content (BO and SO), is located in the extreme right side of PC1.

In relation to PC2, Fig. 4A shows that the pure components (BO and SO) assumed almost opposite positions, evincing the differentiation in relation to the type of lipid present. The samples of BCs – 0%, 10% and 20% are the closest to the pure BO, precisely because they have a higher BO content.

In Fig. 4A, four main groups can be highlighted in relation to PC2. BO and BC samples up to 20% of adulteration can be grouped into the first one. From BC - 30–50% in a second group, which is positioned more distant than the BC – 0%. Therefore, PCA was capable to differentiate fraud above 20% of addition of SO. This fact agrees with previous conclusions regarding bands related to lipids. In addition, the wavenumbers at approximately 3007 cm^{-1} and 721 cm^{-1} weighted significantly in the positive scale of PC2, confirming the influence of the regions of double bonds of unsaturated fatty acids.

Another group contains samples from 60% to 100% of fraud, which is located in the positive scale of PC2, showing more similarities with pure SO than with pure BO. Finally, pure SO remained isolated at the most positive value of PC2. Thus, it can be inferred that PC2 indicates correlations between the mixing qualities of different lipids, where the more negative, the more correlated with the BO, and the more positive, the more associated with SO.

3.4. PLS regression

PLS regression was calculated to estimate the percentage of substitution of SO in BC and the physical-chemical results. The calculations were made based on the spectral regions directly associated with unsaturated fatty acids, highly present in SO (from 3040 to 2985 cm^{-1} and 780 to 635 cm^{-1}).

The calibration model was developed from a set of 8 samples (BCs - 0, 20, 40, 50, 70, 80, 90 and 100%) and other 3 different samples (BC - 10, 30 and 60%) were used for validation. Full cross-validation was used and it obtained a model with 3 latent variables, which described practically all the cumulative variance of the X (MIR spectra – 99.91%) and Y (percentage of SO in BC and physical-chemical results – 99.61%) effects. The results of the PLS model regarding the prediction of the physical-chemical parameters were not acceptable because R^2 values were between 0.348 and 0.854 (calibration) and 0.175 to 0.636 (validation) without superposition of the curves, therefore, non-correlated. On the other hand, the estimates of the percentage of SO present in the

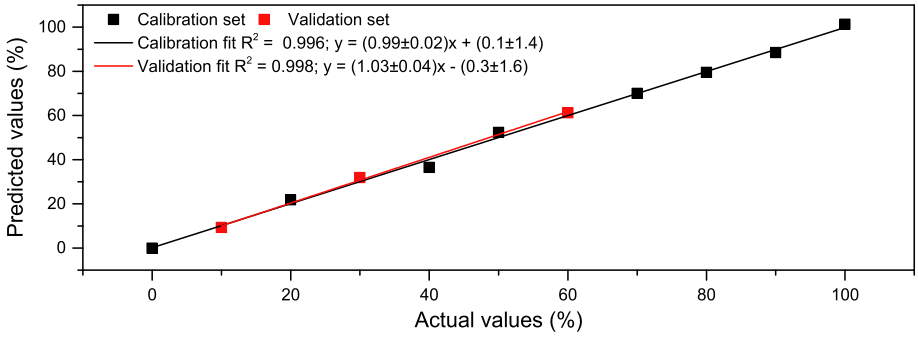

Fig. 5. Partial Least Squares regression results regarding the calibration and validation datasets and the respective predictions of percentage of addition of SO in BC.

Table 3
Residual values obtained for calibration and validation sets used on PLS regression model.

Data set	Addition of soybean oil (%)	Residuals (%)
Calibration	0	0.070
	20	−1.860
	40	3.494
	50	−2.343
	70	−0.002
	80	0.447
	90	1.506
	100	−1.312
Validation	10	0.720
	30	−1.935
	60	−1.262

BC showed overlaid curves with R^2 values of 0.997 and 0.998 for calibration and validation, respectively. Fig. 5 shows the predicted and true values as well as the linear fits. Combined with low values obtained for RMSE and RE (1.780 and 2.7% for calibration, and 1.397 and 3.6% for validation, respectively), the model indicated a good overall fit.

Table 3 shows the residual values, which corresponds to the difference between the true values and those obtained from the prediction model. There is a random distribution of data, without a tendency to increase or decrease the predicted values as a function of addition of SO, indicating a homoscedastic distribution. Therefore, it can be concluded that the model fitted well the experimental values and made good predictions for the percentage of SO present in the adulterated samples of BC.

4. Conclusions

In this study the analysis of infrared spectra allied with multivariate analysis allowed the identification and quantification of frauds in BCs. Because of the physical-chemical analyses were not decisive in the detection of adulterations, the substitution of BO by SO led to decrease in absorbance intensities of protein related bands, and to increases (up to 70% fraud) in lipid related bands. Also, it was noted a band near 3007 cm^{-1} characteristic of unsaturated fatty acids, indicating the presence of SO in the samples. Another indication of fraud could be the lack of the water band in adulterated cheeses. Finally, PCA differentiated the samples confirming the similarities found in the spectral analysis, while PLS regression resulted in good predictions regarding the substitution levels, with low residual errors.

The results obtained in this study confirms that MIR spectroscopy allied with multivariate analysis has great potential and can be a powerful analytical technique to be employed in the quality assurance and authenticity of dairy products in general.

Acknowledgements

This work was funded by CAPES, FAPEMIG and Conselho Nacional de Desenvolvimento Científico e Tecnológico.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2019.04.017.

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