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FTIR SPECTROSCOPY OF MODIFIED CASSAVA STARCHES PRESENTING EXPANSION PROPERTY#

Abstract

The property of expansion of cassava sour starch is very valued allowing the production of expanded gluten-free biscuits without need of extrusion, baking powder or yeast addition. Lactic fermentation and sun-drying are involved in the modification of cassava starch and are linked with the baking ability. Photo-oxidation was suggested as possibly responsible for chemical alterations of the starch macromolecules and in the present work cassava starch oxidation was considered. Potassium permanganate was employed for starch oxidation followed by lactic (LAC) or citric (CIT) acid treatment. Native cassava starch (NAT) as well as lactic acid treated and oven (LACOV) or sun-dried (LACSUN) samples were considered for comparison. One sample of commercial cassava sour starch (SOUR) was also analyzed. The results showed that both chemically (LAC and CIT) and photo-chemically (LACSUN and SOUR) modified samples presented baking property, but not NAT and LACOV. The carboxyl content was higher for the chemically oxidized samples indicating that they were more extensively modified. The FTIR spectroscopy data of these and some other samples resulted in a separation by their spectra, after being studied by principal component analysis (PCA). The presence of carboxylate groups (1600 cm^{-1}) was essential for differentiating the samples. By using partial least squares regression (PLS) on mean normalized data, it was possible to predict the expansion of the samples, that was positively related with carboxylate band (1600 cm^{-1}) and negatively related with another band at around 1060 cm^{-1}, that was assumed to be due to a degradative oxidation taking place at C-O bond (carbon 1 and oxygen 5) of the cyclic part of glucose.

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Introduction

Cassava starch presents physico-chemical characteristics of interest both for native starch application as well as for using as raw-material for modifications. Cassava sour starch is an example of a different product obtained by fermentation followed by sun-drying in some South American countries. Mainly in Brazil and Colombia, known as *polvilho azedo* and *almidón agrio*, respectively, is very valued and unique for production of gluten-free biscuits and bread-like foods [1]. Studies indicated that only after sun-drying the fermented cassava starch presented expansion property. UV-irradiation of lactic acid treated cassava starch also promoted structural modifications on cassava starch that became expansible when baked [2]. There is no need of extrusion or addition of baking powder or yeast to the dough.

As cassava starch seems to be very susceptible to oxidation, and there were some indications that cassava sour starch could be partially degraded by photo-oxidation [3, 4], oxidizing chemical treatments were tested for starch modification [5]. Potassium permanganate associated with lactic acid was employed for treating cassava native starch and some properties of the modified samples were evaluated. FTIR spectroscopy was used for detecting structural changes on the samples in comparison with native and other modified ones [5, 6]. Sodium hypochlorite as well as hydrogen peroxide/Fe_2SO_4 associated with lactic acid were also considered as modifying agents and the samples tested for some characteristics [5].

The FTIR spectral study was selected due to previous results described in the literature [7-13]. Considering mid-infrared techniques for food analysis, Wilson & Tapp [13] cite qualitative and quantitative applications as advances that caused impact on the food sector; they predict that MIR spectroscopy is likely to continue to increase and develop in the near future.

The spectral data, when exploited by multivariate analysis (chemometrics), allow best interpretation of the information, that in many cases is not evident on the raw spectra. It is possible to relate latent variables with structural differences that appear in the spectra when they are conveniently explored. Both qualitative and quantitative methods can be used for data analysis and will make possible to classify the samples and to associate structural information with some physico-chemical or functional properties. Principal component analysis (PCA) is a qualitative method that when employed on FTIR spectral data allows classification of foods to be undertaken without any other chemical analysis [14].

Partial least squares regression (PLS) allows multicomponent quantitative analysis in mixtures, being able to choose the best spectral components for the regression with regard to the variation of the concentration [15]. PLS regression uses all spectral data to determine analyte concentration by factoring all wavelengths into the resulting equation on any selected area of a spectrum. In the PLS approach, the concentration matrix is used at the same time that the spectral data matrix and, by balancing the spectral information and the related concentrations the method reduces the impact of large but irrelevant variation in the spectra [16].

Material and methods

Cassava native starch (NAT) was a gift of a Brazilian factory and was employed for producing the modified samples and also analyzed for comparison. Cassava sour starch samples (SOUR and SOUR2) were bought in Brazil on supermarkets and analyzed directly. For modifying the starch, reagent grade chemicals were used. Detailed description is found on Demiate [5] and Demiate et al. [6].

Starch modification

Cassava starch was suspended on 0.1 N potassium permanganate solution for 15 min under mild agitation at room temperature (*ca.* 20°C). After this period it was recovered by paper filtration with aid of a Büchner funnel and a vacuum pump. The moist starch was washed with de-ionized water and re-suspended in 1% (w/w) lactic (LAC) or citric (CIT) acid solution. This suspension was kept at room temperature for 30 min and then oven dried at 40°C. A part of the sample was washed with de-ionized water for complete elimination of excess acid (LACW and CITW).

Some other samples were considered, including another cassava native starch (NAT2) and modified ones (LAC2, LACW2, SHLAC, SHLAC2, OXLAC, OXLAC2, LACSUN, LACSUNW, LACOV, LACOVW, LACOVW2). The description of how these samples were produced is presented in Table 1, that also shows their carboxyl content, pH and expansion on baking.

pH and carboxyl content

The pH was measured in starch suspension after a 30 min stabilization period by direct electrode immersion. Carboxyl content was determined as described by Smith [17], by NaOH titration of the hot starch paste.

Expansion property

The expansion of the samples was determined as described on Demiate [5] and Demiate et al. [6]. The expanded biscuits were weighed and, after been made impermeable with paraffin, their volumes were determined by water displacement on graduated cylinders. Expansion values were expressed as specific volumes (SV) in mL/g.

Table 1

Source of modified cassava starch samples, their carboxyl content, pH and expansion on baking (SV, mL/g)[#]

Sample	Description[1]	COOH %	*p*H	SV (ml/g)
NAT	Native commercial cassava starch	0.113	6.0	3.2
NATW*	Washed NAT	0.090	5.8	3.2
NAT2*	Another sample of native commercial cassava starch	n.d.	6.0	3.2
SOUR*	Commercial cassava sour starch	0.349	3.5	10.0
SOUR2	Another sample of commercial cassava sour starch	n.d.	3.7	10.0
LACOV*	NAT immersed in 1% (w/w) lactic acid solution for 4 hours, drained and oven dried	0.698	3.0	3.2
LACOVW*	Washed LACOV	0.135	4.7	3.2
LACOVW2	Same as LACOVW but produced with NAT2	n.d.	4.9	3.2
LACSUN*	Produced with the same treatment as LACOV, but sun-dried for 8 hours instead of oven drying	0.563	3.0	11.5
LACSUNW	Washed LACSUN	0.135	4.7	10.6
LAC	NAT sample suspended in 0.1 N $KMnO_4$ solution for 15 min, drained and immersed in 1% (w/w) lactic acid solution for 30 min	0.405	4.1	18.0
LACW*	Washed LAC	0.203/***0.360***[2]	4.5/***4.2***[2]	17.3
LAC2*	NAT immersed in 0.06 N $KMnO_4$ solution for 15 minutes, drained and immersed in 0.79% lactic acid solution for 30 min	n.d.	6.0	17.4
LACW2	Washed LAC2	n.d.	5.8	16.0
CIT	Produced.by the same procedure as LAC sample but immersed in 1% (w/w) citric acid solution	0.495	3.5	14.6
CITW*	Washed CIT	0.225/***0.315***[2]	3..9/***4.0***[2]	12.9
OXLAC	NAT sample suspended on 0.05% $Fe_2SO_4.7H_2O$ solution for 15 min, drained and immersed on 0.86% lactic acid solution to which 2ml of 30% H_2O_2 were added. After 30 min starch was recovered and dried	n.d.	4.1	10.0
SHLAC*	NAT sample suspended in 2.4% NaClO solution for 15 min, drained and immersed in 0.86% lactic acid solution for 30 min	n.d.	3.7	8.0
OXLAC2*	Sample produced by the same procedure as OXLAC but with NAT2	n.d.	n.d.	10.8
SHLAC2	Sample produced by the same procedure as SHLAC but with NAT2	n.d.	n.d.	8.3

[1] Reactions were always carried out at room temperature (ca. 20°C) and oven drying was always made at 40°C.

[2] The values correspond to de-ashed samples

n.d.: not determined

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Infrared spectra

The mid-infrared spectra were collected employing an IFS48 Bruker spectrophotometer. The absorbance spectra were computed between 4000 and 700 cm^{-1} at 4 cm^{-1} resolution with the apodization function of the standard Bruker software. The 2000–700 cm^{-1} region was preferred for multivariate analysis due to the presence of well described absorption peaks. Starch samples were diluted into KBr pellets (1.5% w/w) and the transmission was the chosen technique for getting the spectra. Detailed description of the methodology is discussed on Demiate et al. [6].

Multivariate analysis

Principal component analysis (PCA) was employed for extracting systematic variation of the spectra. It models the variance/covariance structure of the data matrix into a model that considers noise as an error. The objective of applying PCA was to search for a separation among the samples based only on their FTIR spectral differences. Due to the characteristics of the mid-infrared absorption bands, it would be possible to find out the structural changes responsible for that separation.

The quantitative analysis was made by using the partial least squares regression (PLS). By employing this method it is possible to correlate mathematically the spectra matrix and other experimental data. In the present paper the expansion values were considered in order to find out structural changes that might be associated with this functional property. Representative samples were selected and a calibration model developed (*samples of Table 1). The expansion ability of the remaining samples was predicted.

Both qualitative and quantitative chemometric applications were performed by "The Unscrambler" software, version 6, from CAMO – Computer Aided Modeling (Trondheim, Norway).

Results and discussion

Cassava modified starches were produced and presented expansion property. It was possible to evaluate the samples in relation to their pH values as well as carboxyl contents. The FTIR spectral study was also done and, when evaluated by chemometric techniques, made evident some structural differences among the samples.

All samples that were exposed to oxidizing treatments (sun-drying or chemicals) presented expansion property. Native cassava starch samples and that only exposed to lactic acid and oven-dried did not have expansion when baked (Table 1). Even after elimination of soluble compounds by washings, the oxidized samples presented expansion, what suggests structural modification.

FTIR spectra

When the raw FTIR spectra were evaluated it was very difficult to discover any difference among the samples. As all samples were chemically very similar, their spectra should not evidence important changes (Figure 1).

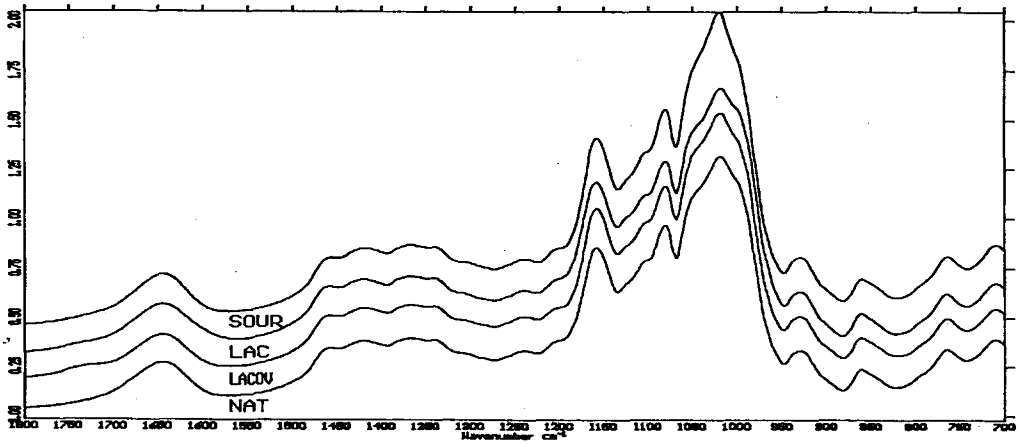

Fig. 1. Infrared spectra of some samples in the 1800-700 cm^{-1} spectral region
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Multivariate analysis

The spectral data were initially considered as a broad region (4000–700 cm^{-1}) for PCA analysis. In order to eliminate undesirable variations, the first derivative of the spectra was considered and the region 1800–1540 cm^{-1} was selected as the most important for sample separation. This selection was done after a long period of data exploitation without any important result. Also, the previous knowledge about chemical characteristics of the samples was important in the definition of the spectral region. Acid carboxyl groups have a high absorption at around 1730 cm^{-1} whereas the carboxylate form absorb at around 1600 cm^{-1}.

In Figure 2 it is possible to observe the sample separation and the structural information related to that. The principal components called "loadings" are presented as a distribution of the variance with respect to the wavenumbers. For each sample the original data may be reconstructed by the sum of the loadings multiplied by coefficients called "scores". The other figure presents, for each principal component, the scores with respect to the sample numbers. The common characteristics are modeled in one or several principal components for which the scores are not significantly different according to the species. On the other hand, the information which differentiates the species contributes to principal components whose scores were significant. The classification of the samples was done by the scores since the characteristics of each species was established by the interpretation of the specific loading [18].

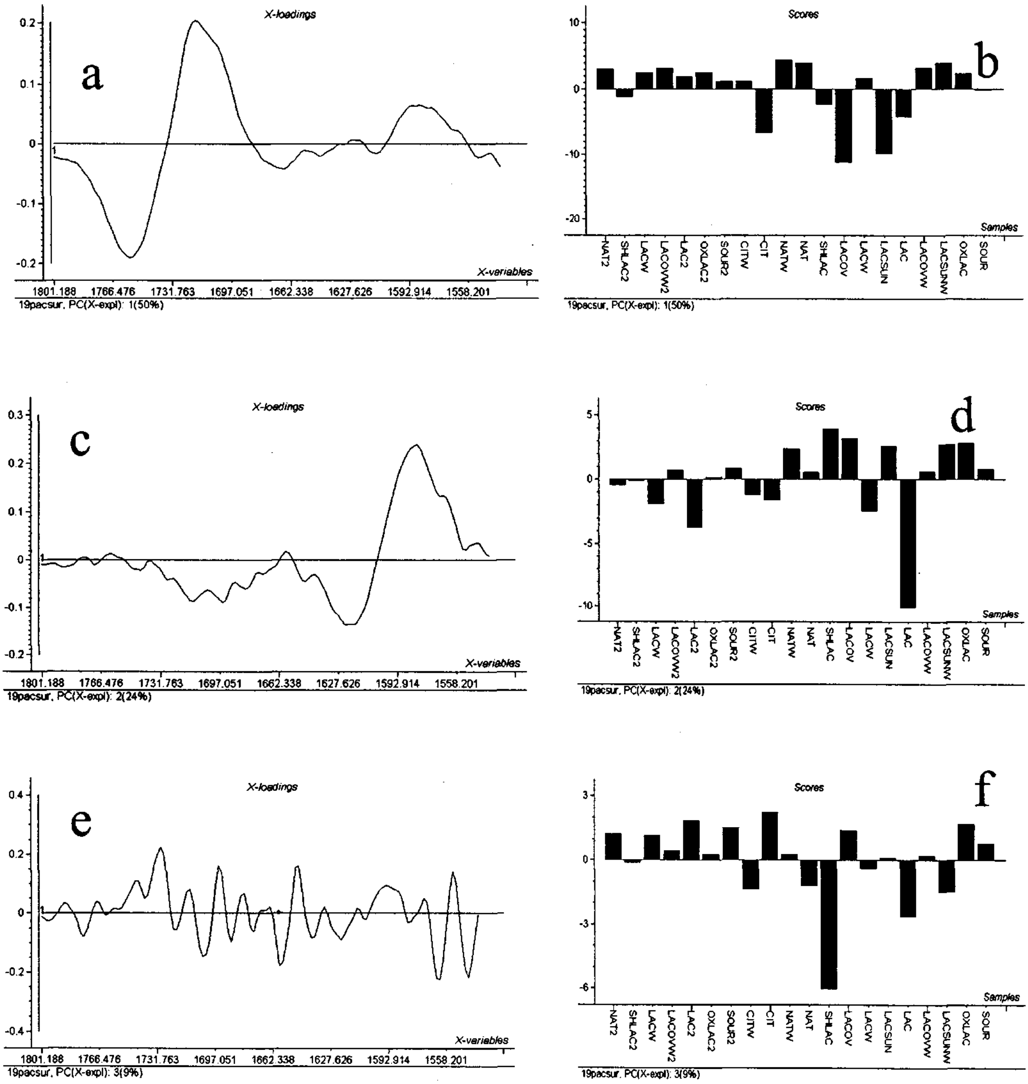

Fig. 2[#]. The three principal components (a, c, e) and the associated scores (b, d, f).

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The first and the second principal components showed the sample classification based on their acid and ionized carboxyl contents, respectively, and explained 74% of total variance. The third principal component did not present important sample separation.

The use of PLS regression allowed the prediction of sample baking behavior based on their FTIR spectra indicating a structural relationship. The calibration was done by using the PLS software in two spectral regions: [1800-1540] cm^{-1} and [1800-1525] and [1360-1030] cm^{-1} on the absorbance spectra obtained after mean normalization of data. The results are shown in Table 2.

Table 2

Predicted values of expansion by PLS regression, compared with observed values[#].

Sample	Reference values	Predicted values in [1800 – 1540] cm^{-1} region	Predicted values in [1800 – 1525] - [1360 – 1030] cm^{-1} region
SHLAC2	8.3	9.5	11.8
LACW2	16.0	13.0	14.1
LACOVW2	3.2	6.1	2.2
SOUR2	10.0	9.3	7.5
CIT	14.6	18.4	16.2
NAT	3.2	11.3	2.6
LAC	18.0	44.8	23.6
LACSUNW	10.6	-6.9	7.2
OXLAC	10.0	-29.6	8.5
SEC		0.5	0.26
SEP		17.8	2.9

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The second spectral region predicted the expansion values with a lower standard error (SEP) if compared with the first one. There was positive interference of the 1600 cm^{-1} band and a negative contribution of a band at *ca.* 1060 cm^{-1}, as shown in figure 3. The first band is due to carboxylate presence in the starch molecules. The second band (1060 cm^{-1}) is not very well described as the first but may be attributed to C-O vibration on carbon 1 and oxygen 5 of the cyclic part of glucose (19).

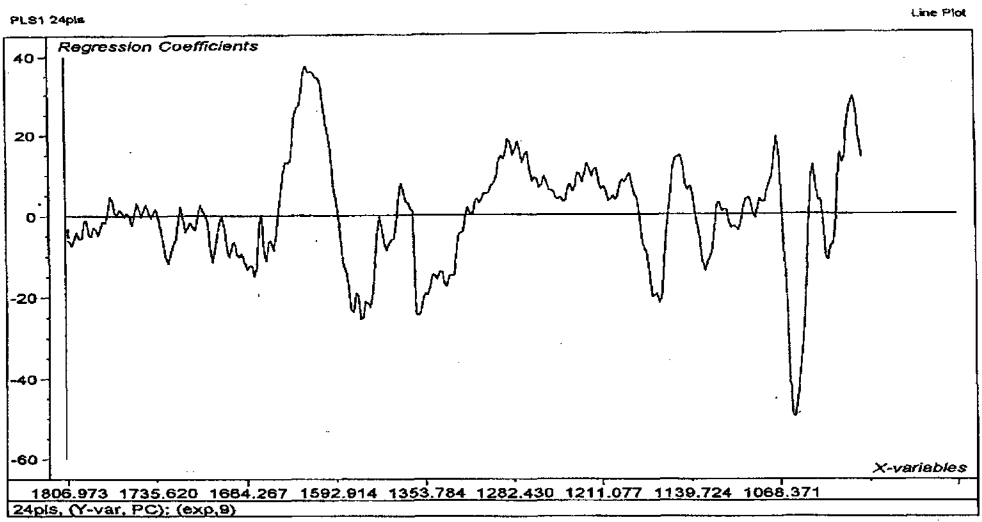

Fig. 3[#]. The regression coefficients obtained in the second spectral region.
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SPEKTROSKOPIA FTIR MODYFIKOWANYCH SKROBI TAPIOKOWYCH O WŁAŚCIWOŚCIACH EKSPANDUJĄCYCH

Streszczenie

Ekspandowanie kwaśnej skrobi tapiokowej pozwala otrzymywać bezglutenowe ciasto biszkoptowe bez potrzeby stosowania ekstruzji, proszku do pieczenia lub drożdży. Efekt ten osiąga się prowadząc fermentację mlekową skrobi i suszenie sfermentowanego produktu w słońcu.

Obserwowane zmiany właściwości chemicznych, zachodzące w trakcie tego procesu, przypisuje się fotoutlenianiu. W celu potwierdzenia tego poglądu porównano właściwości skrobi tapiokowej utlenionej $KMnO_4$, a następnie zakwaszanej kwasem mlekowym (LAC) lub cytrynowym (CIT), ze skrobią natywną (NAT), skrobią traktowaną kwasem mlekowym, a następnie suszoną w suszarce (LACOV) i w słońcu (LACSLIN) oraz handlową skrobią fermentowaną (SOUR).

Okazało się, że w przeciwieństwie do skrobi NAT i LACOV, skrobie LAC i CIT oraz LACSLTN i SOUR nadają się do wypieków.

Skrobie utleniane posiadały większą liczbę grup karboksylowych (1600 cm^{-1}). Na podstawie zmian intensywności tego pasma można było przeprowadzić interpretacje skutków utlenienia, posługując się analizą głównego składnika (PCA). Zmiany zachodzące w intensywności pasma przy 1600 i 1060 cm^{-1} zależały liniowo od stopnia eskpandowania.

Na podstawie nachylenia korelacji przypuszcza się, że degradacyjne utlenianie zachodzi pomiędzy C1 i O5 jednostek glukozowych.