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Carbohydrate Polymers 42 (2000) 149–158

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Relationship between baking behavior of modified cassava starches and starch chemical structure determined by FTIR spectroscopy

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Received 12 January 1999; received in revised form 26 February 1999; accepted 13 April 1999

Abstract

Cassava sour starch is a typical food ingredient from some South American countries, produced mainly in Brazil and Colombia and it shows high expansion when baked. It is known that sun-drying fermented starch is essential. Recently degradative oxidation has been considered as possibly related to chemical changes on cassava starch and was evoked as a probable cause for baking property. We produced some chemically oxidized samples presenting baking property to be compared with lactic acidified and sun- or oven-dried ones, as well as with commercial cassava sour starch and native cassava starch. All the samples were analyzed by using FTIR spectroscopy associated with chemometric data processing. Successful prediction of starch expansion value was achieved by partial least square regression of FTIR spectral data. The results of both qualitative and quantitative spectral analyses showed that presence of carboxylate groups (1600 cm^{-1}) on cassava starch as well as some other changes in the region around 1060 cm^{-1} of mean normalized spectral data are essential for baking property. The degradative oxidation is assumed to take place on the C–O bond relative to carbon 1 and oxygen 5 of the cyclic part of glucose at 1060 cm^{-1}.

Keywords: Cassava; Sour starch; Baking; Fourier transform infrared spectroscopy; Chemometrics

1. Introduction

Cassava fermented and sun-dried starch or cassava sour starch, known as *polvilho azedo* in Brazil and *almidon agrio* in Colombia (Mestres & Rouau, 1997), is used for production of special kinds of gluten-free breads and biscuits that are very popular in some countries of South America. Cassava starch is extracted from roots and naturally fermented in tanks for a period of around 30 days always in the presence of excess water which forms a layer of ca. 5 cm above the bed of starch. After this period of predominant lactic fermentation (Cereda, 1983b; Wetsby & Cereda, 1994) the moist acid starch is sun-dried for 1 or 2 days depending on the season.

The resultant fermented and sun-dried cassava starch gives doughs that when baked produce highly expanded bread-like products. Extrusion processing done to obtain high specific volume biscuits and bread-like foods is not necessary when cassava sour starch is used.

Although lactic acid as well as other organic acids such as acetic, propionic and butyric are present (Cereda, 1981, 1983a; Cereda & Lima, 1985) at a concentration that may reach 1% of the final product (Mestres & Rouau, 1997), simply acidifying cassava starch is not enough to give these baking properties. It was found that sun-drying (particularly at certain UV wavelengths) acidified cassava starch is essential (Nunes, 1994). Recently it was suggested that for cassava starch degradative oxidation could occur on exposure, to solar radiation (Mestres & Rouau, 1997; Mestres, Zakhia & Dufour,1997), but this was not experimentally tested. The authors only stressed that cassava starch seems to be sensitive to oxidation as concluded by Mat Hashim, Moorthy, Mitchell, Hill, Linfoot and Blanshard (1992), Gholap, Marondeze and Tomasik (1993) and Paterson, Mat Hashim, Hill, Mitchell and Blanshard (1994).

Many research groups have tried to understand what kind of modification is responsible for the baking properties of cassava sour starch, but until now there is no conclusive answer. The partial acid and enzymatic hydrolysis of cassava starch (Camargo, Colonna & Buleon, 1988; Cárdenas & de Buckle, 1980; Franco & Tavares, 1998) as well as the presence of some bacterial exopolysaccharides

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0144-8617/00/$ - see front matter
PII: S0144-8617(99)00152-6

Table 1
Source of modified cassava starch samples, their carboxyl content pH and expansion on baking (SV, ml/g) (n.d.: not determined)

Sample	Description[a]	COOH%	pH	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 on 1% (w/w) lactic acid solution for 4 h, 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 h instead of oven drying	0.563	3.0	11.5
LACSUNW	Washed LACSUN	0.135	4.7	10.6
LAC	NAT sample suspended on 0.1 N $KMnO_4$ solution for 15 min, drained and immersed on 1% (w/w) lactic acid solution for 30 min	0.405	4.1	18.0
LACW*	Washed LAC	0.203/**0.360**[b]	4.5/**4.2**[b]	17.3
LAC2*	NAT immersed on 0.06N $KMnO_4$ solution for 15 min, drained and immersed on 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 on 1% (w/w) citric acid solution	0.495	3.5	14.6
CITW*	Washed CIT	0.225/**0.315**[b]	3.9/**4.0**[b]	12.9
OXLAC	NAT sample suspended on 0.05% $Fe_2SO_4{\cdot}7H_2O$ solution for 15 min, drained and immersed on 0.86% lactic acid solution to which 2 ml 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 on 2.4% NaClO solution for 15 min, drained and immersed on 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

[a] Reactions were always carried out at room temperature (ca. 20°C) and oven drying was always made at 40°C.
[b] The values correspond to de-ashed samples.

produced during fermentation (Brabet & Dufour, 1993; Brabet, 1994) have been considered but it has not been shown that these are responsible for the baking behavior. Microscopic studies did not show differences between sour and natural cassava starches (Franco & Tavares, 1998).

In this study cassava starch samples including native, fermented and sun-dried (commercial sour starch), chemically treated with lactic acid and oven- or sun-dried and also native starch oxidized in the presence of potassium permanganate solution and then immersed in lactic or citric acid solutions were analyzed by FTIR spectroscopy and the data studied with aid of chemometrics. Recently this methodology was successfully employed for corn starch classification according to different chemical modifications (Dolmatova, Ruckebusch, Dupuy, Huvenne & Legrand, 1998; Dupuy, Wojciechowski, Huvenne & Legrand, 1997) and also for quantitative analysis of sugars as well as other food ingredients (De Lene Mirouze, Boulou, Dupuy, Meurens, Huvenne & Legrand, 1993; Dupuy, Meurens, Sombret, Legrand & Huvenne, 1992; Dupuy, Meurens, Sombret, Legrand & Huvenne, 1993). As the fermentation and sunlight exposure treatment modified the chemical structure of starch, probably by an oxidative process, FTIR spectra should contain significant information to provide a successful qualitative classification and prediction of the baking properties on the basis of the carboxyl content.

2. Material and methods

Modified samples were produced from a native cassava starch of commercial grade that was a gift from a Brazilian cassava starch producer, and another (NAT2) that was bought from the market. A cassava sour starch sample bought from the market was also studied.

2.1. Preparation of samples

The treatment used to produce the different samples are summarized in Table 1. This table also shows their pH, carboxyl content and expansion on baking expressed as the specific volume (SV, ml/g). Among these samples there are chemically oxidized ones, prepared by suspension in potassium permanganate solution (Mostafa, 1995) and, after draining and washing out excess oxidant, they were immersed in lactic or citric acid solutions. Sodium hypochlorite (Forssell, Hamunen, Autio, Suortti & Poutanen, 1995) and the redox pair $Fe_2SO_4 \cdot 7H_2O$/hydrogen peroxide (Parovuori, Hamunen, Forssell, Autio & Poutanen, 1995) were also tested as oxidants, associated with lactic acid treatment. These chemically oxidized samples were produced because they were expected to have a higher degree of modification than the others studied and for that reason it would be easier to detect any structural change. As these samples also showed expansion on baking they were evaluated in order to generate some structural information related to this functional property. Samples were oven dried at 40°C and analyzed. Other samples were only immersed in lactic acid and oven or sun-dried. There are also two samples of native cassava starch for comparison. Some samples were washed with de-ionized water to eliminate soluble compounds. The washed samples included the chemically oxidized ones that gave high expansions (LAC and CIT) as well as those that are chemically treated with lactic acid and sun-dried (LACSUN) or oven-dried (LACOV). It is already known that LACSUN gives baking properties similarly to cassava sour starch (SOUR and SOUR2) although LACOV does not (Nunes, 1994). Native cassava starch (NAT) was also washed to check if there was any change in its expansion property or infrared spectral profile.

Washing was carried out with de-ionized water with the aid of a Büchner funnel and the pH of the washings was determined in order to ensure complete removal of soluble acids.

De-ashing was carried out as described by Smith (1967) and employed by Parovuori et al. (1995) in order to convert all carboxyl groups to their acid form. Briefly 1 g of dry sample was immersed in 30 ml of a 0.1 M HCl solution and occasionally shaken over a 30 min period at room temperature. After this period the acidified samples were washed with de-ionized water with the aid of a medium porosity fritted glass funnel. Washings were tested with $AgNO_3$ solution for the absence of chloride. Chemical analysis was carried out to show the presence of carboxylate groups on some of the modified starch samples included in this study.

2.2. Carboxyl content and pH

The carboxyl content of the samples was determined as described by Smith (1967) and employed by Parovuori et al. (1995). 500 mg of sample (DW) were suspended on 300 ml de-ionized water and boiled for 10 min with constant agitation. These solutions were titrated with NaOH 0.025 M employing phenolphthalein as indicator. Carboxyl content was calculated as shown by Eq. (1):

$$\text{COOH\%} = \text{ml NaOH} \times 0.025 \times 0.045^{*} \times 100/0.5\ \text{g} \quad (1)$$

(*COOH molecular weight/1000)

pH values were measured after suspension of 10% (w/w) starch in distilled water for 30 min under agitation at room temperature (20°C). After a further 30 min, starch was decanted and the pH of the soluble fraction was measured. Carboxyl contents and pH values are shown in Table 1.

2.3. Baking property of starches

The baking property was measured by weighing 12 g of starch sample and partially cooking by addition of 10 ml of boiling de-ionized water over this starch mass. This partially cooked starch was homogenized to produce a dough, that was molded to three small balls and baked on an electric oven at 200°C for 25 min. After baking, the doughs were weighed, and made impermeable by using paraffin and their volumes determined on graduated cylinders as the volume of water displaced. The expansion was obtained by dividing volume by weight and was expressed as specific volume (ml/g). The methodology is adequate to show differences between very high, high and low expansion values but it is not very sensitive. For the scope of this work, the measurements were not influenced by this low sensitivity and it was possible to clearly differentiate the samples.

2.4. FTIR

FTIR spectra were recorded with a IFS48 Bruker spectrometer. The absorbance spectra were computed between 4000 and 700 cm^{-1} at 4 cm^{-1} resolution with the triangular apodization function in the standard Bruker software. The FTIR spectrometer was purged to minimize spectral contributions due to atmospheric carbon dioxide and water vapor. Symmetrical interferograms on 200 scans were co-added for each spectrum. The mean of four spectra of the same sample from different pellets was then calculated.

2.5. Spectral data treatment

The reproducibility of the signal at each wavelength is defined by the relative standard deviation (RSD) according to the following formulae (Dupuy, Duponchell, Amram, Huvenne & Legrand, 1994):

$$\text{RSD} = (\sigma/x_m) \times 100 \qquad \sigma = \left(\sum_{i}^{N} (x_i - x_m)^2/N\right)^{1/2}$$

where x_i represents the absorbance of one spectrum, x_m is the average absorbance of all spectra of the same samples and N is the number of spectra.

For some applications the spectral data were first-derived

with the algorithm developed by Savitzky and Golay (1964) in order to remove unwanted spectral variations as offsets. In all cases the spectra were mean normalized.

2.6. Multivariate analysis method

Principal Component Analysis is a method for extraction of the systematic variations in one data set (Martens & Naes, 1989). The method can be used for classification as well as for description and interpretation. PCA is orientated towards modeling the variance/covariance structure of the data matrix into a model, which considers the noise as an error. The components are found one after the other. Each principal component represents the main systematic variation in the data set, which can be modeled after the extraction of the previous one. The common characteristics are modeled using one or more 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 on the basis of the scores since the characteristics of each species were established by the interpretation of specific loading (Dupuy, Duponchell, Huvenne, Sombret & Legrand, 1996).

The quantitative analysis was based on the additive nature of Beer's law. Mixtures of known concentrations are used as calibration standards and then the software calculates directly the concentrations of an unknown sample (Martens, 1979). The interference and overlapping of the absorption bands may be overcome by using powerful multicomponent quantitative analysis as partial least squares regression (PLS). PLS (Fuller & Griffiths, 1988; Haaland & Thomas, 1988) allows a sophisticated statistical approach using the full spectral region rather than unique and isolated analytical bands. The algorithm is based on the ability to correlate mathematically spectral data to concentration matrix of interest while simultaneously accounting for all other significant spectral factors that perturb the spectrum. It is a multivariate regression method based on the use of latent variables.

The evaluation of the calibration performance is estimated by computing the standard error of calibration (SEC) after comparing the real concentration with the computed one for each component:

$$\mathrm{SEC} = \left(\sum_{i=1}^{N} (C_i - C_i')^2/(N - 1 - \rho)\right)^{1/2}$$

where C_i is the known value, C_i' is the calculated value, N the number of samples and p is the number of independent variables in the regression.

The standard error of prediction (SEP) gives an estimation of the prediction performance during the step of validation of the calibration equation:

$$\mathrm{SEP} = \left(\sum_{i=1}^{M} (C_i - C_i')^2/(M - 1)\right)^{1/2}$$

where C_i is the known value, C_i' is the value calculated by the calibration equation, and M is the number of prediction samples. The chemometric applications are performed by the UNSCRAMBLER software version 6 from CAMO (Computer Aided Modeling, Trondheim, Norway).

3. Results and discussion

The aim of this study is to understand the baking properties of cassava sour starch. After the fermentation step the product is sun-dried. In order to eliminate some interference attributed to free organic acid present in the mixture the samples were washed.

3.1. Carboxyl content

In Table 1 it is possible to observe that all washed samples presented lower carboxyl contents which was due to elimination of soluble organic acids, lactic or citric, that were added to cassava starch samples. Another observation is that de-ashed (Smith, 1967) samples had higher carboxyl contents than non de-ashed ones. Both LACW and CITW samples showed higher carboxyl levels when they were acidified by de-ashing procedure, which indicates the presence of insoluble carboxylate groups in samples that were exhaustively washed. They are probably part of the starch structure and were generated by the oxidative treatments.

3.2. Baking property

Samples were evaluated as described above and their expansion values following baking (SV, ml/g) are shown in Table 1. All the samples that were produced by chemical oxidative treatment combined with organic acid treatment gave high expansion values, as did the lactic-acid-treated sun-dried sample. Both lactic and citric acids gave high expansion on baking when associated with oxidative treatment. Even after washing all samples still showed high values for expansion on baking which suggests that there are structural changes to the starch molecules.

Cassava sour starch also gave high expansion on baking. On the other hand, neither native nor oven-dried acidified samples showed this characteristic. It is established clearly that acidifying cassava starch with lactic acid alone is not sufficient to give the desired baking characteristics.

3.3. Data acquisition

It can be observed in the raw spectra (Fig. 1) that the different treatments are not easily visually detected, because the positions of absorbance peaks are similar.

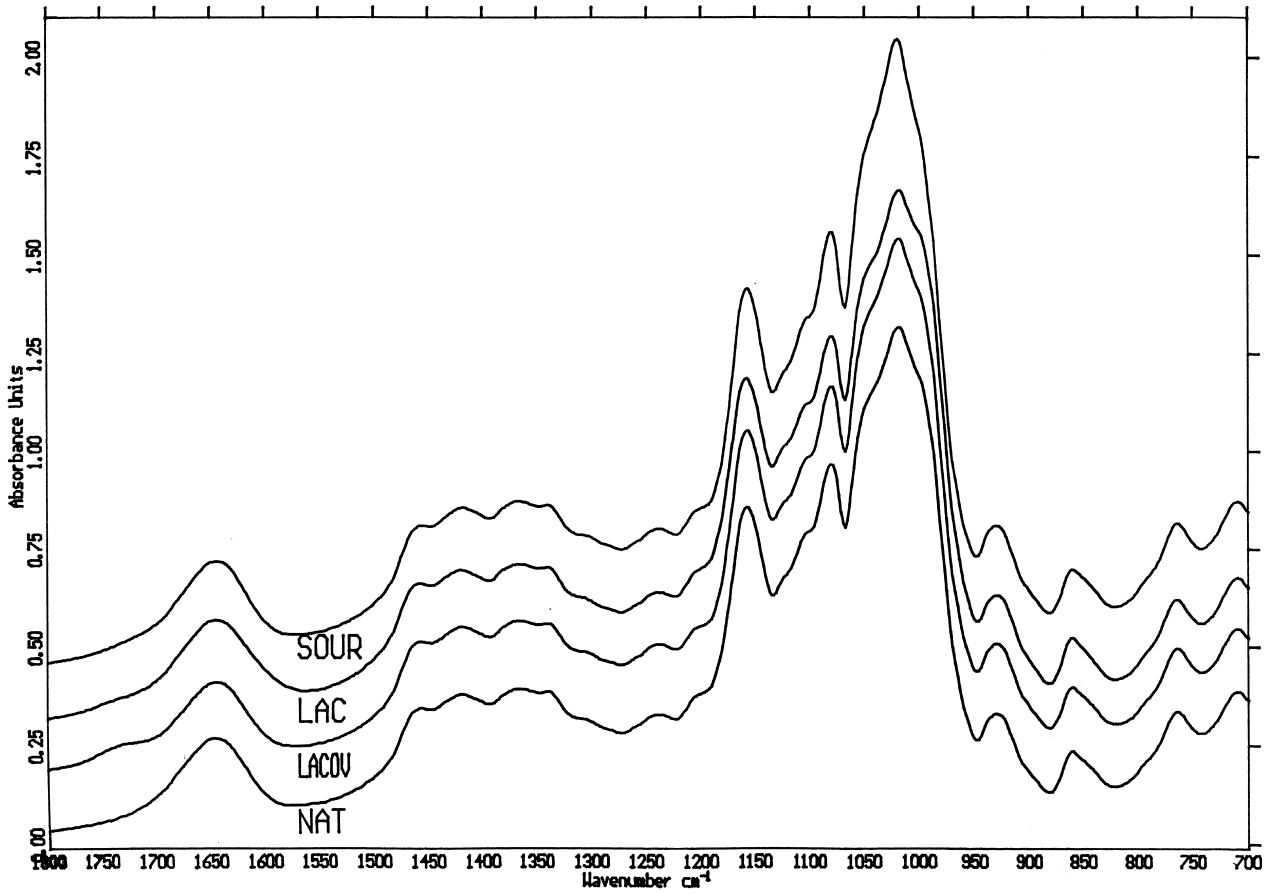

Fig. 1. Infrared spectra of some samples in the [1800–700] cm^{-1} spectral region.

All samples were produced from cassava starch and contain the same major components (amylose and amylopectin). The chemical treatments induce changes of less than 1% in weight in the native starch. It is therefore necessary to use a sample presentation technique that allows high light penetration with adequate reproducibility.

Since the most commonly used method for powder sample preparation is the KBr pellet technique (Harrick, 1979), the method was used. The spectra of 10 samples of the same compound at levels of 1.5% in KBr were recorded. The relative standard deviation calculated at different wavenumbers was about 10%. These results can be explained by the inhomogeneity of the starch powder in the pellets. Since the performance is not very good with respect to reproducibility, we decided to investigate each kind of starch four times with different pellets, and average the spectra. In this case the relative standard deviation was 4.5% and justifies the choice of average spectra.

3.4. Principal component analysis

In order to eliminate variations due to the starch's inhomogeneity, we worked on the first derivative of the spectra in the region [1800–1540] cm^{-1}, searching for differences relative to the organic acid function. This spectral region was also selected because the native starches do not absorb in it and because when a broader region was considered there was poor differentiation between certain samples, probably because of differences in the moisture content.

In Fig. 2 it can be seen that the three first extracted loadings explained 83% of the data matrix variance. The fourth component and the further ones contribute less than 5% of the residual variance and we may assume that these components are due to noise.

The first principal component explains 50% of the data set variance and represents the most dominant information since the scores associated with this component reveal that the extracted spectral features are highly correlated with the degree of sample acidity as acid carboxyl content. The samples that presented carboxyl contents higher than 0.4% are negatively projected while the others are positives. The first loading intensity is maximum around 1730 cm^{-1} that corresponds to the first derivative of the C=O vibration band.

Table 1 shows that all the washed samples had lower carboxyl contents when compared to the respective non-washed samples, which is due to elimination of soluble organic acids (lactic or citric). Samples coded LACOV

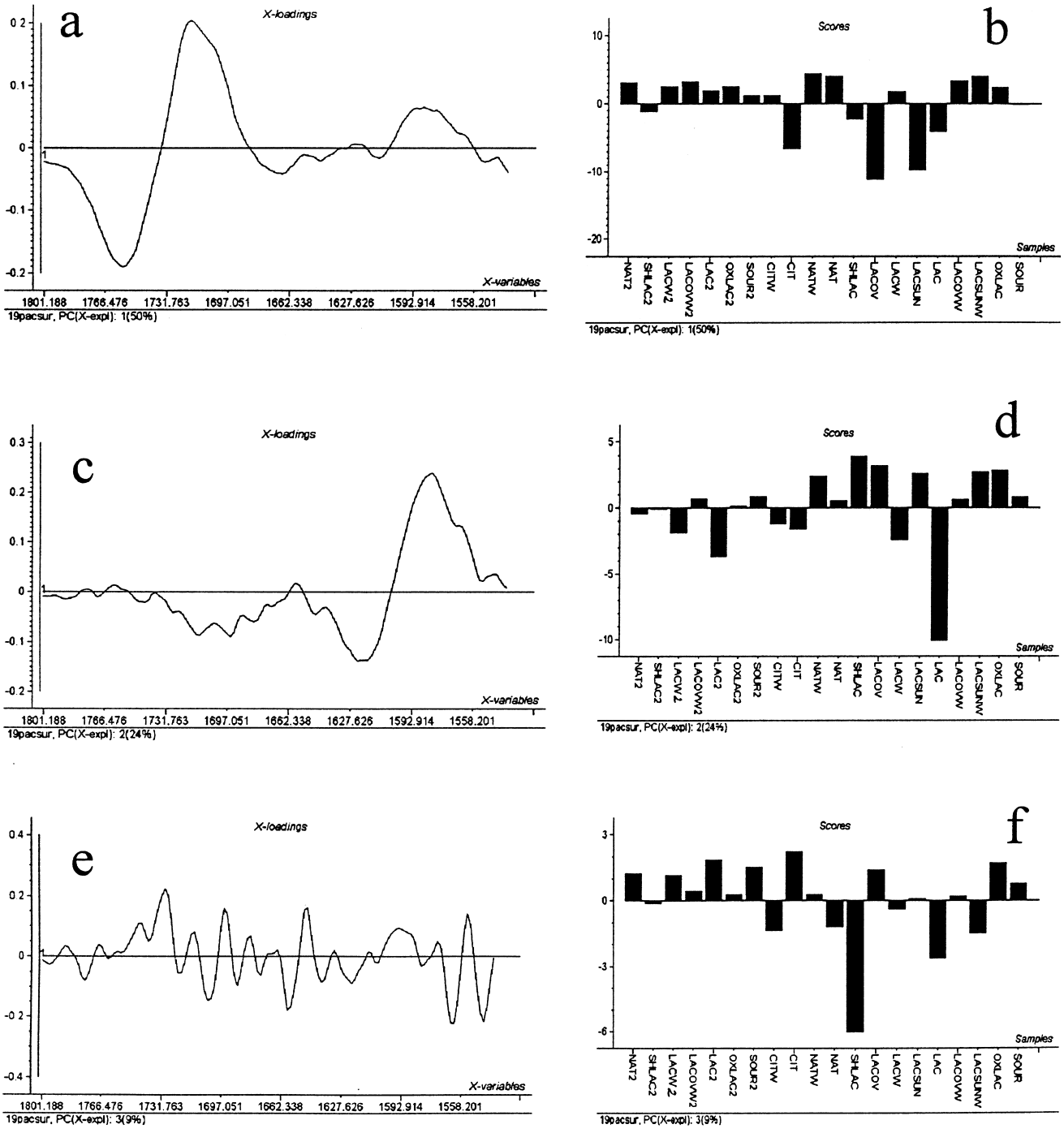

Fig. 2. The three principal components (a, c, e) and the associated scores (b, d, f).

and LACSUN were acidified with addition of the same amount of lactic acid and their difference in the projection on the first component should be attributed to sun-drying degradation (Dufour, Larsonneur, Alarcon, Brabet & Chuzel, 1996; Mestres & Rouau, 1997). The action of $KMnO_4$ oxidizing agent also resulted in a lower projection in the carboxyl direction. Differences between carboxyl contents of samples LAC and CIT should be related to different molar concentrations of acid solutions employed to modify native cassava starch: as the concentration was always 1% (w/w), this represents about 0.11 mol/l for lactic acid (LAC); and about 0.048 mol/l for citric acid (CIT). The same explanation could cause the difference between LAC and LAC2 (ca. 0.089 ml/l).

The second principal component explains 24% of the data set variance and its intensity is maximum at 1600 cm^{-1}. This information cannot be related to water even if water presents a major contribution in

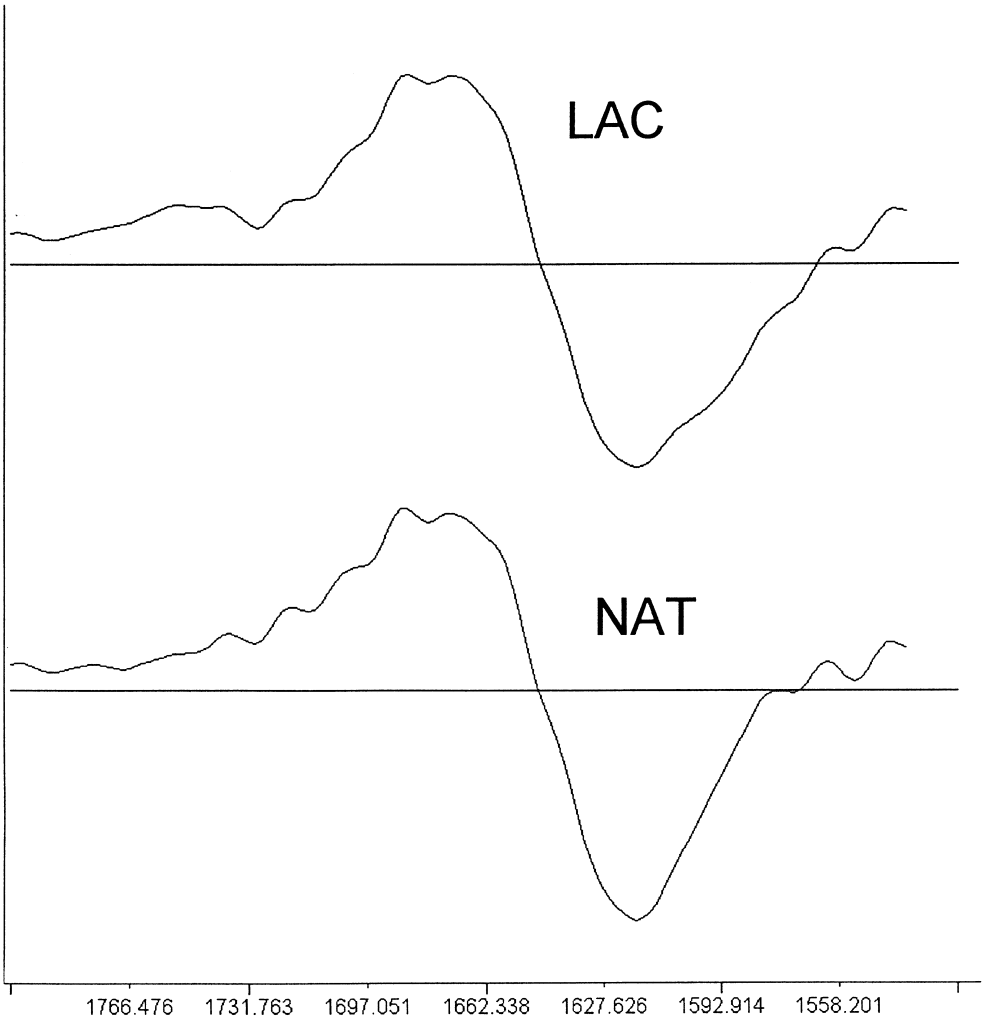

Fig. 3. First derivative spectra of NAT and LAC sample in the [1800–1540] cm^{-1} infrared region.

this area at 1630 cm^{-1}. As a matter of fact we can see in Fig. 3 the first derivative spectra of LAC and NAT sample, and the shoulder on the LAC spectrum at 1600 cm^{-1} that corresponds to the anti-symmetrical vibration band of COO^- structure of the carboxylate anion (Bellamy, 1975). Fortunately this band is much more characteristic of ionized carboxyl than the other around 1400 cm^{-1} (symmetrical COO^-) that is at a region where many other skeletal vibrations may occur. The samples that presented a high COO^- content are negatively projected on the second principal component. The difference between LAC and CIT must be explained by the different values of the dissociation coefficients (lactic acid: $pK_a = 3.86$ and citric acid: $pK_{a1} = 3.08$, $pK_{a2} = 4.20$ and $pK_{a3} = 5.40$). LAC gave a pH higher than the lactic acid pK_a which means that this sample contains significant concentrations of COO^- groups, whereas CIT had a pH of 3.5 and only one carboxyl group would be in the COO^- form.

A high pH associated with the presence of carboxyl groups was a determinant for the separation on this principal component. High pH from NAT and NAT2 may explain their tendency to be projected on the direction of carboxylate presence, even if they are expected to contain a low concentration of this group; NATW did not present the same projection because washings eliminated some lactic acid that may be present at low concentration on native cassava starch (de Carvalho, Canhos, Vilela & de Carvalho, 1996). On the other hand, low pH values of SOUR and SOUR2, that contain higher concentration of carboxyl groups, did not allow their projection in this direction. The third principal component explains only 9% of data set variance and shows no important sample separation, being influenced probably by some interference from the purge.

3.5. Prediction of baking behavior

An attempt was made to predict baking behavior from the mid infrared spectra of the samples. If the baking behavior is related to structural changes in cassava starch, infrared

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] and [1360–1030] cm^{-1} regions
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

spectra must contain some information relative to these modifications. The samples marked with an asterisk in Table 1 were used to build the calibration set and the others were tested on the prediction step. The calibration was done using PLS software in two spectral regions: (1) [1800–1500] cm^{-1}; and (2) [1800–1525] *and* [1360–1030] cm^{-1}, on the absorbance spectra obtained after mean normalization of data. The results are reported in Table 2. The baking volume is correctly predicted when the second spectral region was considered and in this case the error in specific volume was about 2.9 ml/g. Fig. 4 shows the regression loading obtained on this spectral region and it must be observed that there is a great contribution of the carboxylate group associated with some other difference in starch structure at around 1060 cm^{-1}. This spectral region was necessary for prediction of baking property; the analysis performed on the shorter spectral region gave bad results, especially on samples, which did not have an important contribution of carboxylate group. It is possible to observe that the region at 1600 cm^{-1} is positively correlated to

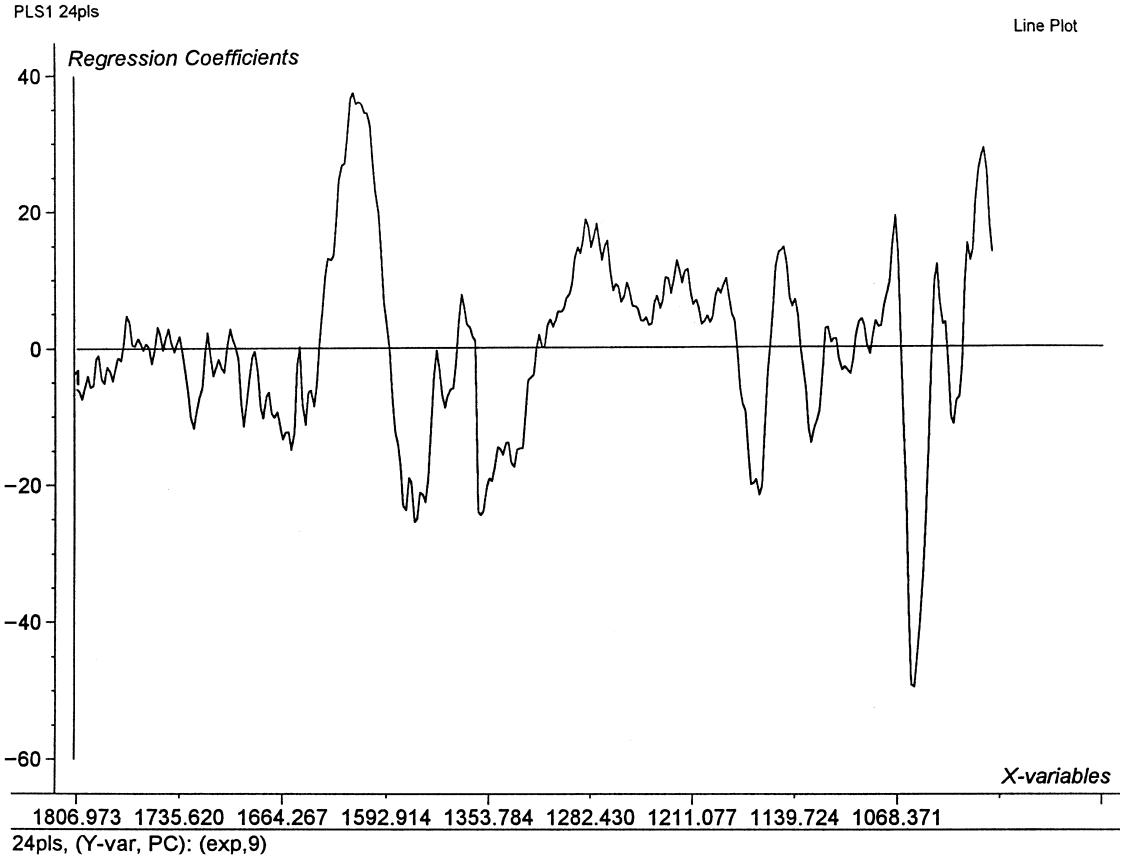

Fig. 4. The regression coefficients obtained in the second spectral region.

baking property while the other spectral region around 1060 cm^{-1} is linked to some non-expansion related structural information (negative part of the regression coefficient). The infrared information at 1060 cm^{-1} may be attributed to C–O vibration on carbon 1 and oxygen 5 of the cyclic part of glucose (Sekkal, 1990). Because this region is negatively correlated to expansion values the degradative oxidation causes a change on this part of molecule which is associated with the appearance of a carboxylate group at 1600 cm^{-1}.

4. Conclusions

- Principal component analysis showed a clear separation between samples containing different levels of added organic acids and, of particular interest, different contents of carboxylate groups, even after elimination of excess reagents by washing. This suggested that some carboxylate groups are part of starch structure and are not just mixed with native starch.
- Carboxylate groups are present in samples giving high expansion on baking and are important for this characteristic as demonstrated by a quantitative prediction of baking behavior.
- It was possible to predict expansion on baking for the samples using a PLS regression of the mean normalized absorbance in the spectral regions [1800–1525] and [1360–1030] cm^{-1}. There is a particular contribution from the bands at ca. 1600 cm^{-1} (COO^-) and at ca. 1060 cm^{-1}.

Acknowledgements

I.M.D., M.P.C. and G.W. are grateful to CAPES, CNPq and RHAE/Agroindustria for financial support (scholarships) and also to CNRS that allowed utilization of its laboratories (LASIR) for development of this research.

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