CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

EFFECT OF ACID-ALCOHOL TREATMENT ON PHYSICOCHEMICAL PROPERTIES OF *CARIOCA* BEAN (*Phaseolus vulgaris* L.) STARCH

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Article history:
Received:
4 December 2017
Accepted:
10 September 2017

Keywords:
Bean starch;
Hydrolysis;
Methanol;
Ethanol.

ABSTRACT
Starches from different sources present unknown potential for new technological applications mainly when modifications are considered. In the present study acid-alcoholic treatments of *Carioca* bean starch were evaluated considering selected physicochemical and thermal properties. Ethanol, methanol and their mixture (1:1) were employed as solvents for hydrochloric acid hydrolysis of bean starch. The results showed changes in DSC parameters, including gelatinisation temperatures that increased from around 70 to 80 °C and enthalpy changes that decreased from around 10 to 4 J g^{-1}. The viscographic behaviour analysed by RVA, L*, a* and b* colour parameters and also some changes in the granular morphology by SEM were observed. On the other hand, no changes were detected for the relative crystallinity by X ray diffraction. The acid alcoholic treatment resulted in different starch properties including the expected acid thinning due to depolymerisation.

1. Introduction

Starch is a natural biopolymer arranged as semi-crystalline microscopic granules comprising fractions of linear amylose and branched amylopectin (Zeeman et al., 2010; Dutta et al., 2011). Due to some limited properties of natural starch, chemical modification is widely used to enhance its functionality for application in the food industry(Xiao et al., 2012).

Common bean (*Phaseolus vulgaris* L.) is a very important starchy seed in human nutrition, because it presents high amounts of carbohydrates (50-60%) and proteins (20-25%). Starch is the most abundant carbohydrate (22-45%), presenting high levels of amylose which gives interesting properties for applications and uses (Hoover et al., 2010; Ovando-Martinez et al., 2011). In Brazil, this kind of common bean represents an important crop for small farmers and is a diary food for a population of almost 200 million people (Otsuboet al., 2013); the variety Carioca makes around 85 % of the Brazilian bean market (Silva et al., 2014).

Acid hydrolysis involves the suspension of granular starch in an aqueous solution of hydrochloric or sulfuric acid. The action of

the strong acid undercontrolled temperature results in partial cleavage of polysaccharides (Yiu et al., 2008).According to Shi et al. (2014), acid modification changes the physical and chemical properties of starch, but the granular structure remains the same.

Acid hydrolysis modifies the starch to be incorporated in foods in order to control viscosity, texture and to improve moisture retention, in addition to presenting low viscosity in aqueous solution (Gao et al., 2012). Alcohol-acid modifications were already described in the literature for several conditions as alcohol type, starch source and concentration, reaction time (Dutta et al., 2011; Luo et al., 2011). Those modifications were reportedforseveral starch sources such as lentils (Sodhi et al., 2009), barley (You and Izydorczyk, 2007) and sago starch (Yiu et al., 2008). Using acid hydrolysis in different alcohols, Ma and Robyt (1987) prepared and characterized soluble potato and waxcy maize starches having different molecular sizes and composition.

The aim of this study was to assess the effect of alcohol-acid hydrolysis on the main physicochemical and thermal properties of *Carioca* bean (*Phaseolus vulgaris* L.) starches, which is not found in literature.

2. Materials and methods

2.1. Materials

Starch, in its native form, was extracted from *Carioca* beans (*Phaseolus vulgaris* L.) acquired in a local retail market in Ponta Grossa, Paraná, Brazil. The reagents used for modification were anhydrous methanol, CH_3OH (J.T. Baker, Xalostoc, Mexico), ethanol, C_2H_5OH (Biotec, Porto Alegre, Brazil), hydrochloric acid, HCl 37 % (in weight), d=1.19 g mL^{-1} (Synth, São Paulo, Brazil) and sodium bicarbonate, $NaHCO_3$ (Caal, São Paulo, Brazil), all of analytical grade.

2.2. Methods

Acid-alcoholic starch treatment was performedaccording to Ma and Robyt(1987) and Lin et al. (2003) with some modifications: a quantity of starch (25 g) was suspended in 100 mL of methanol, ethanol, their mixture (1:1) or alcoholic-acid solutions (1 mL of HCl) in 400 mL flasks. Seven treatments were studied with varying methanol, ethanol and hydrochloric acid proportions (Table 1). The reaction was maintained at 65 °C for 1 hour(Ma and Robyt, 1987)in a Dubnoffshaker bath (TECNAL TE-053, Brazil). The reaction was quenched by the addition of 14 mL of 1 mol L^{-1} $NaHCO_3$ and then placed in an ice bath. The starch was centrifuged (ROTINA 420R, Hettich, Germany) at 3000 ×*g* for 5 minutes and then washed four times with 50 % ethanol (v/v). The precipitate was dried in an oven with air renewal and circulation (TECNAL TE394/2, Brazil) at 40 °C for 12 hours.

Table 1. Proportions of reagents used for each acid-alcohol hydrolysis treatment

Treatment	Volume (mL)		
	Methanol	Ethanol	HCl
1	-	-	-
2	100	-	-
3	100	-	1
4	-	100	-
5	-	100	1
6	50	50	-
7	50	50	1

2.2.1 Differential Scanning Calorimetry (DSC)

The DSC curves were obtained using a thermal analysis system model DSC-Q200 (TA-Instruments, USA). The DSC curves were recorded under the following conditions: air flow of 50 mL min^{-1}, heating rate of 10 ºC min^{-1} and samples weighing about 2.0 mg. A proportion 4:1 (water:starch, w/w) was considered and the

aluminum crucibles were sealed and rested for 60 minutes in order to equilibrate the moisture; after that, the curves were performed. The instrument was previously calibrated with indium standard 99.99% purity, with melting point T_p = 156.6 ºC, ΔH = 28.56 Jg^{-1}. Gelatinisation occurs as an endothermic event and the "onset", "peak" and "conclusion" transition temperatures (T_o, T_p and T_c) as well as the gelatinisation enthalpy change (ΔH_{gel}) were calculated using the "Universal Analysis 2000" software (Vatanansuchartet al., 2005; Granzaet al., 2014).

2.2.2 Pasting Properties (RVA)

The pasting properties of the starch samples were determined using the RVA-4 (Newport Scientific Pvt. Ltd., Narabeen, Australia). Anaqueous starch suspension (8 % w/w, dry basis) with 28 g of total mass underwent a controlled heating and cooling cycle under constant stirring (160 rpm) when it was held at 50 ºC for one minute; heating from 50 to 95 ºC at 6 ºC min^{-1}, and held at 95 ºC for 5 minutes; cooling until 50 °C at 6 ºC min^{-1}, and held at 50 ºC for 2 minutes (Franco, 2002). The apparent viscosity curve during the aqueous starch suspension heating, cooking and cooling was recorded producing the viscoamlylograms.

2.2.3 X-Ray Diffraction

The X-ray diffractograms were collected in theRigakuUltima IV (Rigaku, Tokyo, Japan) equipment with CuKα radiation (λ = 1.544 Å) at 40 kV and 20 mA (Beninca et al., 2008). The analysis was performed at 20 °C in a 2θ angle range of 7-30° with a measuring period of 5 s/2θ.

2.2.4 Colour Parameters

For determining the colour parameters of the starch, the MiniScan EZ 4500L (HunterLab, USA) was used. Three colour components were measured: L*,brightness ranging from 0 (black) to 100 (white); a* ranging from positive (red) to negative (green); and b*, which varies from positive (yellow) to negative (blue) (Falade and Onyeoziri, 2012).

2.2.5 Scanning electron microscopy

The morphology of the starch granules was examined using a scanning electron microscope (Tescan, VEGA 3, Kohoutovice, Czech Republic). The starch samples were coated with gold and examined in the scanning electron microscope under an acceleration voltage of 25 kV and magnification of 1,000 times.

2.2.6 Statistical Analysis

The normality of the experimental data was evaluated considering the Shapiro-Wilk test and thehomogeneity of variance, using Brown-Forsythe ($p>0.05$ was considered parametric). Then the parametric data were evaluated by analysis of variance (ANOVA) complemented with the Fisher LSD mean comparison test. A value of $p<0.05$ was considered significant. Statistical analyses were performed by Statistica software version 7.0 (Statsoft, Tulsa OK, USA).

3.Results and discussions

3.1. Differential Scanning Calorimetry (DSC)

The DSC curves were performed to study the gelatinisation process. They were obtained in a heating rate of 10 ºC min^{-1} with a 1:4 proportion of starch:water in sealed aluminum crucibles. Figure 1 shows the profile of the DSC curves.

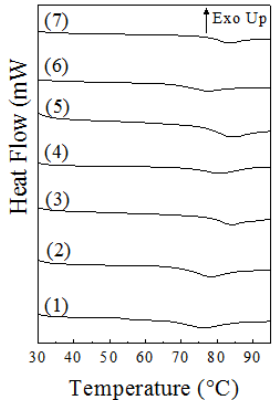

Figure 1. DSC curves of starches of *Carioca* beans after acid-alcohol treatment

Table 2 shows the DSC results and compared with the (N) native bean starch sample, the peak temperatures (T_p) of the modified samples did not show significant differences. These effects are dependent on the moisture level of the treatment, the starch source, and the amylose content(Lin *et al.*, 2012; Granza*et al.*, 2014). The calculated gelatinisation enthalpy change (ΔH_{gel}) presented an increase, contrasting with the result observed for sample from treatment 3 confirming that the modification occurred in this sample. Similar values for enthalpy changes arefound in the literature: 15.4, 14.3, 14.4 and 15.0 J g^{-1}, for annealed navy bean starch, for navy bean starch submitted to heat-moisture treatment and dual modified,i.e, annealed starch that was submitted to heat-moisture treatment (ANN–HMT) and heat-moisture treated starch that was submitted to annealing (HMT-ANN),respectively (Chung *et al.*, 2010). The differences between the results of ΔH_{gel} were due to the different treatments that were performed (reagent concentrations, time of treatment, genetic varieties and the employed physical methods).

The temperatures of gelatinisation (onset, peak and conclusion temperatures)of the starches are shown in Table 2 and Figure 1; the hydrolysis (treatments 2-7) increased the values of $T_o$andT_p, and reduced those of T_c and $\Delta H_{gel}$when compared with native starch (treatment 1). The increase and reduction of these parameters were more pronounced on the treatments with use of HCl (treatments 3, 5 and 7) than those with ethanol/methanol only.

The hydrolysis increased the gelatinisation temperature while ΔH_{gel}values were lower than that of native starch. These results are in line with other studies,as observed with potato and maize starches hydrolysedbyHCl in methanol. The modified starches presented higher gelatinisation temperature after treatment (Lin et al., 2003); the treatment with ethanol and HCl in waxy corn starch presented the same tendency for ΔH_{gel}.The reduction of ΔH_{gel}on treated starch may be attributed to its partial gelatinisation (Chang *et al.*, 2004).

3.2. Pasting properties

The RVA results are shown in Figure 2 and Table 3. When methanol alone was used to produce the starch suspension,in the absence of HCl (treatments 2 and 6), there was a significant increase in peak viscosity (V_p) and conclusion viscosity (V_c). However, when ethanol instead of methanol was used (treatment 4), a significant reduction of those viscosities occurred. That viscosity change was also reported previously (Lin et al., 2005) for potato starch suspended in methanol.

The addition of HCl (treatments 3, 5 and 7) had a marked effect on pasting properties of bean starch. Both viscosities (V_p and V_c) were significantly reduced and in some samples the peak viscosity values were undetectable. Similiar results were reported by Chang et al. (2004), Cavallini and Franco (2010) and Luo et al. (2011) when starches were treated with HCl in alcohol.

Table 2. DSC gelatinisation results of *Carioca* bean starches modified with different treatments

Treatments	DSC gelatinisation			
	T_0/ºC	Tp/ºC	Tc/ºC	ΔH_{gel}/J g^{-1}
1	67.5±0.13^{f}	76.6±0.00^{g}	92.2±0.69^{a}	10.2±0.11^{a}
2	73.1±0.00^{d}	80.1±0.00^{e}	87.7±0.69^{d}	8.3±0.03^{b}
3	78.6±0.10^{a}	83.6±0.00^{b}	91.2±0.16^{b}	5.0±0.48^{d}
4	73.4±0.15^{c}	81.1±0.00^{d}	87.8±0.24^{d}	7.7±0.28^{c}
5	77.5±0.02^{b}	84.0±0.00^{a}	89.2±0.35^{c}	3.7±0.03^{e}
6	72.7±0.18^{e}	79.6±0.00^{f}	86.4±0.30^{e}	4.8±0.07^{d}
7	78.5±0.04^{a}	83.1±0.00^{c}	87.5±0.28^{d}	3.9±0.09^{e}
p (Brown-Forsythe)*	0.73	1.00	0.72	0.41
p (Anova)**	<0.01	<0.01	<0.01	<0.01

T_0: on set temperature; Tp: peak temperature; T_c: endset temperature; ΔH_{gel}: gelatinisation enthalpy change.
Different letters in the same column represent significant difference according to Fisher LSD test ($p<0.05$)
* Probability value obtained by Brown–Forsythe test for homogeneity of variance
** Probability value obtained by Anova one-factor

Table 3. Pasting propertiesof *Carioca* bean starches modified with different treatments

Sample	T_p(°C)	V_p(cP)	V_c (cP)	Breakdown (cP)	Setback (cP)
1	80.5±0.10^{c}	1436.7±1.53^{c}	2134±2.00^{c}	342.7±2.52^{c}	1040.3±2.08^{b}
2	84.9±0.09^{b}	1677.3±1.15^{a}	2195±1.00^{b}	469.0±1.00^{b}	987.3±2.08^{c}
3	-	11.0±0.06^{e}	5.00±0.00^{e}	15.1±0.15^{f}	9.0±0.03^{e}
4	86.1±0.10^{b}	1000.3±2.89^{d}	1489.0±2.64^{d}	113.7±1.53^{d}	603.0±2.64^{d}
5	-	0	0	19.0±0.17^{e}	3.9±0.06^{g}
6	84.7±0.01^{b}	1656.7±0.15^{b}	2224.3±3.05^{a}	628.3±1.53^{a}	1195.3 ±3.21^{a}
7	-	0	0	9.0±0.00^{g}	7.0±0.06^{f}
p (Brown-Forsythe)*	0.47	0.39	0.30	0.19	0.82
P (Anova)**	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01

T_p: pasting temperature; V_p: peak viscosity; V_c: conclusion viscosity.
* Probability value obtained by Brown-Forsythe test for homogeneity of variance.
** Probability value obtained by Anova one-factor.
*** Different letters in the same column represent significant difference according to Fisher LSD test ($p<0.05$).

Table 4. Colour parameters of *Carioca* bean starches modified with different treatments

Treatment	L*	a*	b*
1	91.39±1.09^{a}	0.67±0.1^{e}	4.51±0.11^{g}
2	84.33±1.1^{c}	1.98±0.04^{f}	7.11±0.03^{b}
3	82.08±1.96^{d}	2.55±0.14^{c}	5.43±0.26^{f}
4	85.95±0.69^{c}	2.28±0.02^{d}	7.93±0.07^{a}
5	81.03±0.84^{d}	5.27±0.13^{a}	6.50±0.13^{d}
6	88.21±0.45^{b}	1.87±0.01^{f}	6.81±0.04^{c}

7	81.08±0.69[d]	4.04±0.06[b]	5.89±0.15[e]
p (Brown-Forsythe)**	0.76	0.41	0.53
p (Anova)***	<0.01	<0.01	<0.01

** Probability value obtained by Brown-Forsythe test for homogeneity of variance.
*** Probability value obtained by Anova one-factor.
**** Different letters in the same column represent significant difference according to Fisher LSD test (p<0.05).

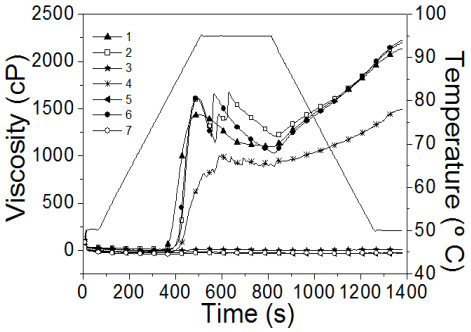

Figure 2. RVA curves of starches of *Carioca* bean modified by acid-alcohol treatment

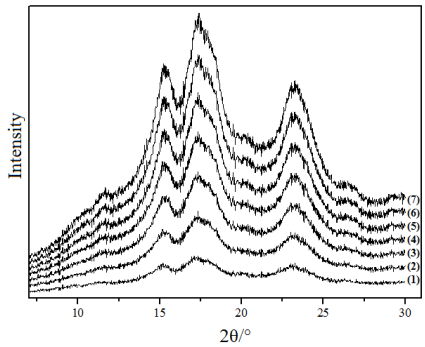

Figure 3. Diffractograms of starches of *Carioca* bean modified by acid-alcohol treatment

The reduction of the viscosities occurs due to degradation of the starch chains, caused by the presence of acid, with consequent molecular weight reduction (Cavallini and Franco, 2010). As the viscosities of the starch pastes that were submitted to acid-alcohol treatment were extremely low, the pasting temperature was not detected (Table 3).

Breakdown is the difference between the viscosity of the swollen starch granules and viscosity when the granules are disrupted (Han and Hamaker, 2001); setback is the difference between the viscosity after the disruption of the swollen granules after cooling and the hot paste viscosity (Shirai et al., 2007). Therefore, as a result of the reduction of V_p and V_c, the breakdown and setback values of the starches treated with alcohol and acid (treatments 3, 5 and 7) were significantly lower than those of the native bean starch. This reduction was also observed by Ferriniet al. (2008), Dutta et *al.*(2011) and Lin *et al.* (2012) for starches from cassava, jackfruit seed and maize, respectively.

3.3. X-Ray Diffraction

The X-ray diffractograms of the native starch and starches submitted to acid-alcohol hydrolysis are shown in Figure 3. The native starch and the modified starches presented diffraction peaks at 15°, 17° and 23°,characteristic of C-type starch (Wang and Ratnayake, 2014).

The native starch showed relative crystallinity of 24%, whereas treated starches showed values around 22.5%, without a significant difference between samples. The crystallinity revealed that the acid-alcohol treatment did not affect the crystalline structure of starches. In a study by Chang et al. (2004) native and modified waxy maize starches also did not present difference between the crystallinities. Luo et al. (2011) treated maize starches with different amylose contents (0%, 23% and 55%) in anhydrous methanol, ethanol, 2-propanol, 1-butanol with 0.36% HCl and concluded that the degradation occurred preferentially in the amorphous regions and the different changes depended on the

crystal structure and amylose content of starch. Additionally the authors reported that the extent of the changes caused by acid treatment in the same alcohol depended not only on the crystal structure of starch but also on its amylose content.

3.4. Colour Parameters

The L*, a* and b* colour parameters indicate the tendency of samples to white or black, green or red and yellow or blue, respectively. In Table 4 it is possible to verify the colorimetric variation among samples of starch from *Carioca* beans modified by acid alcoholic hydrolysis.

According to Table 4 the L* value was higher for native starch and a significant reduction in brightness for the samples modified by acid alcohol hydrolysis was found. As for the parameter a* the modified samples exhibited a greater tendency to red and native starch tended to neutrality between red and green.

The samples 5 and 7 showed higher a* due to treatment with ethanol and HCl and ethanol:methanol and HCl, respectively; ethanol and HCl was the treatment that most influenced the occurrence of this effect. Similarly, evaluating the parameter b*, the modified samples showed a greater tendency to yellow when compared to native starch. Thus, the acid-alcoholic hydrolysis promoted slight changes in the colour of the bean starches.

3.5. Scanning Electron Microscopy

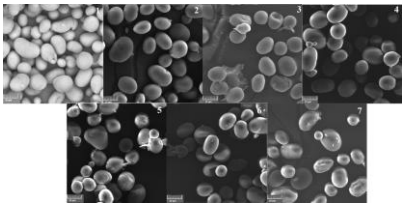

Figure 4. Scanning electron microscopy of native and modified starches of *Carioca* bean (1,000 x)

The native bean starch granules observed by SEM showed smooth surface without visible fissures, oval and spherical shapes according to Fig. 4 (treatment 1) and agreeing with data found in the literature (Ovando-Martinez et al., 2011; Granza et al., 2014; Demiate et al., 2016).Samples that were treated only with alcohol, according to Fig. 4 (treatments 2, 4, 6), showed no significant changes in shape and surface such as fragmentation or swelling, as reported for potato starch by Lin et al.(2005). On the other hand, samples 3, 5 and 7, that were modified with alcohol and acid (Fig. 4; 3, 5 and 7) showed some fissures randomly distributed as well as deformation and degradation in some granules, in line with previously published results for starches from other sources (Han et al., 2001; Lin et al., 2005).

4. Conclusions

The hydrolysis treatment resulted in decreased ΔH_{gel} as showed by DSC analysis and caused significant changes in pasting properties of bean starch due to depolymerisation of starch chains.

The modifications have not changed the X-ray diffraction pattern and relative crystallinity of the studied starches, but modified the colour of starches and the samples treated with ethanol and HCl presented a greater tendency to red, with substantial variation of the a*value.

By using SEM it was observed that the treatment with alcohol alone did not affect the morphology of the granules while the use of acid resulted in a partial deformation and apparent degradation.

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