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STUDY OF MODIFIED AVOCADO SEEDS STARCH  
WITH LACTIC ACID**

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## MORPHOLOGICAL AND THERMOANALYTICAL STUDY OF MODIFIED AVOCADO SEEDS STARCH WITH LACTIC ACID

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**Abstract.** Avocado seeds starch was investigated after the modification with lactic acid using thermogravimetry and differential thermal analysis (TG-DTA), differential scanning calorimetry (DSC), X-ray powder diffraction (XRD) and scanning electron microscopy (SEM). After the modification, there was a decrease in the thermal stability of the starch, also the parameters measured by differential scanning calorimetry showed lower values. These results can be correlated with the reduction in the relative crystallinity observed by XRD. There was no difference in the morphology of the granules, which presented an oval and rounded shape. These results are important for the food industry, since lactic acid is used to optimise the properties of starches.

**Keywords:** avocado starch, lactic acid, modification, thermal analysis.

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### Introduction

Avocado is a very popular fruit all over the world. The avocado plant (*Persea americana*, Miller) originates from Central/North America (Mexico), from where it has spread to several regions. Its ripe fruits are consumed on a large scale in the world and have healthy properties. Some studies have shown that a diet enriched with avocado fruit reduces total cholesterol and low-density lipoprotein levels without changing high-density lipoprotein levels [1-5]. Avocado seeds constitute a high percent in these fruits (13-25%) depending on the varieties, which are generally discarded as industrial waste [6]. These seeds contain a significant amount of starch (27-30%), as well as smaller amounts of pigments and oils. Therefore, new studies on the physicochemical properties of this biopolymer are required [4, 7-9].

Unprocessed or native starch presents some limiting characteristics restricting its application in the food industry, a problem that can be overcome by modifying the starch. Organic acids (lactic, acetic, malic, ascorbic and citric ones) are mainly known for their application in the food industry to lower pH values [10]. This would be an alternative process for the substitution of other commonly used acids associated with greater environmental impact, such as hydrochloric and sulphuric acids, to alter the pasting properties of the starches [11]. Some organic acids may be used to hydrolyse

parts of starch prior to thermal modifications, such as heat-moisture treatment, to achieve an improvement in slowly digestible and resistant starches [12]. In addition, previous studies have shown that modification by lactic acid combined with UV irradiation can improve the properties of cassava and corn starches [13]. Although the interaction between starch and lactic acid has not been fully elucidated, the authors reported that the addition of this acid in maize and cassava starches resulted in a decrease in peak viscosity [14].

The aim of this work was the extraction and modification (with lactic acid) of the starch from avocado seeds and evaluation of physicochemical characteristics using thermal analysis (TG-DTA), differential scanning calorimetry (DSC); X-ray powder diffraction (XRD) and scanning electron microscopy (SEM).

### Experimental

#### *Samples preparation*

Samples of avocado fruits (*Manteiga* variety) were purchased from local commerce in Curitiba, Paraná, Brazil. The starch was extracted from avocado seeds in aqueous solution as previously described in the literature and its yield was 28.7% [15].

For the modification, it was used the adapted methodology of Cordoba *et al.* [16]. A portion of 10 g of the native starch was suspended in 50 mL of each concentration

(0.5 and 1.0 mol L<sup>-1</sup>) of standard solution of lactic acid and kept under stirring for 120 minutes at 25°C. After this, the suspension was washed in distilled water, filtered and dried in an oven at 40°C for 24 hours.

Following the goal of this work, three samples were further used for the study: (A) native starch; (B) starch modified with 0.5 mol L<sup>-1</sup> lactic acid; (C) starch modified with 1.0 mol L<sup>-1</sup> lactic acid.

#### Characterization

The simultaneous TG-DTA curves were obtained using TGA-60 thermal analysis system (Shimadzu, Japan). The samples were heated from 35°C to 600°C using opened  $\alpha$ -alumina crucibles with approximately 8.0 mg of each sample under an air flow of 100 mL min<sup>-1</sup> at a heating rate of 10°C min<sup>-1</sup>. The instrument was preliminarily calibrated with standard weight and with standard calcium oxalate monohydrate (TG) and sapphire (DTA). All percentages of mass loss were determined using TA-60 WS data analysis software. The derivative thermogravimetric curves (DTG), the first derivative of the TG curves, were calculated [15,17,18].

The DSC curves were obtained using a DSC-Q200 (TA-Instruments, USA) thermal analysis system. The DSC curves were recorded under air flow of 50 mL min<sup>-1</sup>, heating rate of 10°C min<sup>-1</sup> and samples weighing about 2.5 mg. A 4:1 (water:starch, w/w) mixture was prepared and maintained for 60 minutes in order to equilibrate the moisture content. The aluminium crucibles were sealed and then the curves were performed. The instrument was previously calibrated with indium (99.99% purity,  $T_p = 156.6^\circ\text{C}$ ,  $\Delta H = 28.56 \text{ J g}^{-1}$ ) [5,16].

X-ray diffraction powder patterns (XRD) were obtained using an X-ray diffractometer (Ultima 4, Rigaku, Japan) with CuK $\alpha$  radiation ( $\lambda = 1.541 \text{ \AA}$ ) and settings of 40 kV and 20 mA. The scattered radiation was detected in the angular range of 5-50° (2 $\theta$ ), with a scanning speed of 2° min<sup>-1</sup> and a step of 0.02°. The degree of relative crystallinity was estimated following the method described in the literature [17,18].

The morphology of the starch granules was examined using a scanning electron microscope (SEM) (Tescan, VEGA 3, Czech Republic) under an acceleration voltage of 25 kV and magnification of 1000x. All the samples were coated with gold. The area of the granules was calculated using the software Image J 1.47 for Windows [9,19].

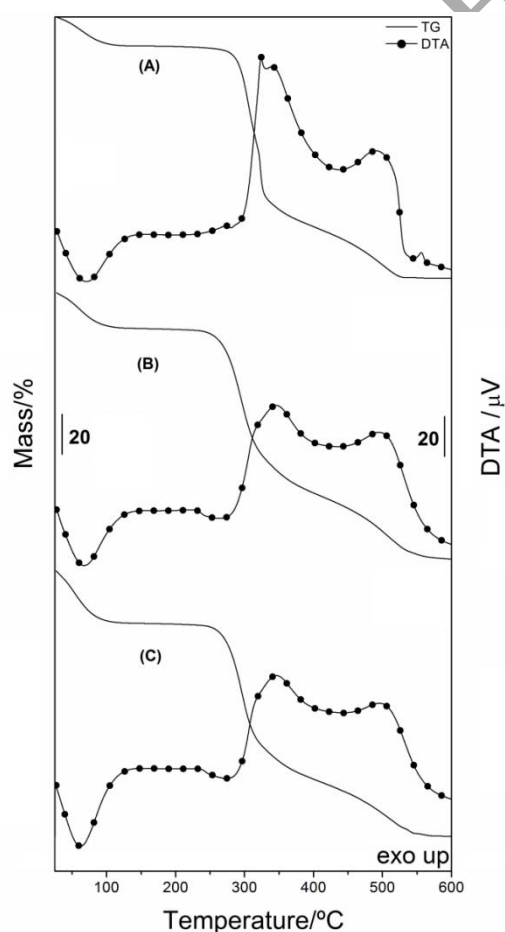
The obtained results were treated with analysis of variance (ANOVA) and compared

with Tukey's test at 95% confidence level ( $p < 0.05$ ) using SASM-Agri 8.2 software (Brazil).

#### Results and discussion

During the extraction process, the starch was washed until the aqueous solution became clear. However, avocado starch maintained the brown-yellow colour as obtained by Chel-Guerrero *et al.* [20], which may be attributed to the presence of iron [7].

TG-DTA curves (Figure 1) have been performed in conditions previously described and the results are depicted in Table 1.



**Figure 1. TG-DTA curves of (A) native avocado starch, (B) avocado starch modified with 0.5 mol L<sup>-1</sup> lactic acid, and (C) avocado starch modified with 1.0 mol L<sup>-1</sup> lactic acid.**

#### Production of the yoghurt samples

TG curves showed similar behaviour to the other starch samples in an oxidising atmosphere. Three main mass losses were observed: the first one due to dehydration process; in the DTA curve this phenomenon was observed as an endothermic event [21]. Once dehydrated, the samples presented some stability without mass loss and without endo- or exothermic reactions.

Table 1

TG-DTA results of (A) native avocado starch, (B) avocado starch modified with 0.5 mol L<sup>-1</sup> lactic acid, and (C) avocado starch modified with 1.0 mol L<sup>-1</sup> lactic acid.

Samples	Step	$\Delta m$ , %	$\Delta T$ , °C	$T_p$ , °C
A	1 <sup>st</sup>	10.9	30-153	65.8 (endo)
	stability	-	153-218	-
	2 <sup>nd</sup>	68.8	218-405	312.9 (exo)
	3 <sup>rd</sup>	19.1	405-584	499.0 (exo)
B	1 <sup>st</sup>	13.4	30-138	63.3 (endo)
	stability	-	138-211	-
	2 <sup>nd</sup>	61.8	211-400	269.0(endo); 295.7 (exo)
	3 <sup>rd</sup>	24.7	400-596	501.2 (exo)
C	1 <sup>st</sup>	19.7	30-140	55.8 (endo)
	stability	-	140-206	-
	2 <sup>nd</sup>	59.5	206-412	272.1 (endo); 297.2 (exo)
	3 <sup>rd</sup>	20.1	412-581	508.1 (exo)

$\Delta m$ - mass loss (%);  $\Delta T$ - temperature range (°C);  $T_p$ -peak temperature (°C).

Table 2

DSC gelatinisation, SEM and XRD results for (A) untreated avocado starch, (B) avocado starch with lactic acid 0.5 mol L<sup>-1</sup> and (C) avocado starch with lactic acid 1.0 mol L<sup>-1</sup>.

Samples	DSC gelatinisation				SEM	XRD
	$T_o$ , °C	$T_p$ , °C	$T_c$ , °C	$\Delta H_{gel}$ , J.g <sup>-1</sup>	$d_a$ , $\mu m$	Degree of relative crystallinity
A	70.2±0.4 <sup>a</sup>	76.0±0.2 <sup>a</sup>	81.5±0.1 <sup>a</sup>	11.3±0.2 <sup>a</sup>	~21.9 <sup>a</sup>	16.9±1.2 <sup>b</sup>
B	69.5±0.1 <sup>b</sup>	73.8±0.1 <sup>b</sup>	77.4±1.5 <sup>b</sup>	10.2±0.1 <sup>c</sup>	~20.9 <sup>a</sup>	14.5±0.2 <sup>b</sup>
C	69.0±0.1 <sup>c</sup>	73.2±0.1 <sup>b</sup>	78.1±0.3 <sup>b</sup>	9.7±0.1 <sup>b</sup>	~21.5 <sup>a</sup>	14.6±0.3 <sup>a</sup>

Values presented as mean values  $\pm$  standard deviation;  $T_o$  "onset" initial temperature;  $T_p$  peak temperature,  $T_c$  "endset" conclusion temperature;  $\Delta H_{gel}$  gelatinization enthalpy; " $d_a$ ": average diameter.

The degree of crystallinity was calculated as a percentage, and the peaks are determined at  $2(\theta)$ .

Values followed by the same letter in the same column are not significantly different ( $p < 0.05$ ).

After the modification with lactic acid, a decrease in the stability plateau was observed in comparison to the native starch. This behaviour was also observed for the pinhão starch modified with lactic acid (0.1 and 0.2 mol L<sup>-1</sup>) [16]. Other researchers have observed that the heat treatment of starches under oxidative atmosphere, normally leads to depolymerisation around 300°C [22,23]. The second mass loss was attributed to degradation of the organic matter (amylose and amylopectin) with the endothermic reaction observed on DTA curves (except for the untreated starch) followed by two exothermic reactions. The third mass loss occurred consecutively to the second, due to oxidation of the organic matter with the formation of ashes, which was for sample (A) 1.2%, (B) 0.6% and (C) 0.7%, respectively. The ash content of the native starch was close to that reported by Saavedra *et al.* [24].

DSC curves (Figure 2) were performed with 1:4 ratio of starch:water in sealed crucibles after 60 minutes. The aim of this procedure was to obtain the gelatinisation values of the samples before and after treating with lactic acid. The values obtained for the onset, peak and conclusion temperatures of the untreated sample (Table 2)

were close to those reported in the literature, but the gelatinisation enthalpy was lower [5]. In comparison to pinhão starch and wheat starch modified with lactic acid, avocado starch showed a slight displacement of the gelatinisation peak to lower temperatures in the same way as the gelatinisation enthalpies ( $\Delta H_{gel}$ ) [10,16].

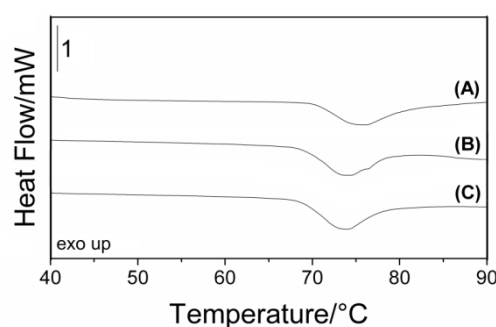


Figure 2. DSC gelatinisation curves for (A) native avocado starch, (B) avocado starch modified with 0.5 mol L<sup>-1</sup> lactic acid, (C) avocado starch modified with 1.0 mol L<sup>-1</sup> lactic acid.

It has been suggested that lactic acid may have promoted partial hydrolysis of amylopectin chains, decreasing the crystallinity of granules, as observed by X-ray diffraction, and therefore the

modified avocado starch requires less energy during the occurrence of gelatinisation. Hydrochloric or sulphuric acids are commonly used to modify starch properties, but in most studies this chemical modification causes an increase in gelatinisation temperatures and a decrease in the pasting viscosity, depending on the acid concentration that is used, besides involving strong acids should be totally eliminated from food industry [15,25,26].

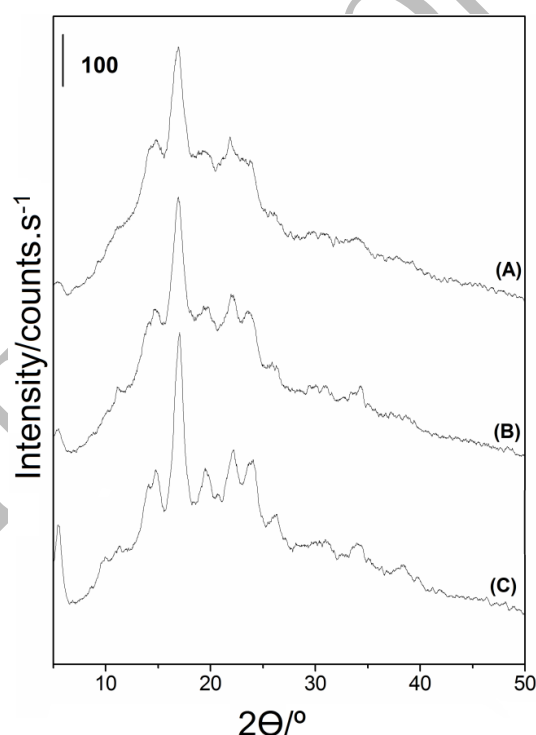
The diffraction pattern of each starch sample can be classified by X-ray powder diffractometry (XRD). The main peaks observed at  $2(\theta)$  were: 15; 17.3 and 23.2° and the diffraction pattern can be classified as C-type. After modification with acid, two peaks were intensified: 5.6 and 24° at  $2(\theta)$ , but without changing on the diffraction pattern. The peak identified at 20° (diffraction angle) may be related to the complexes between amylose and lipids as reported in the literature [27], which were more intense after modification with lactic acid.

The diffractograms and the degree of relative crystallinity are depicted in Figure 3 and Table 2, respectively. The calculated relative crystallinity decreased as compared to native avocado starch and followed the same behaviour of avocado starch modified with sodium hypochlorite or by heat moisture treatment [4,5] and of wheat starch modified with *L*-ascorbic acid [28]. This indicates that the crystalline structure was affected by the addition of lactic acid corroborating with the decrease in enthalpy observed by DSC. Majzoobi and Beparva pointed out that modification with lactic acid can promote depolymerisation of amylose and amylopectin chains due to low pH value [10].

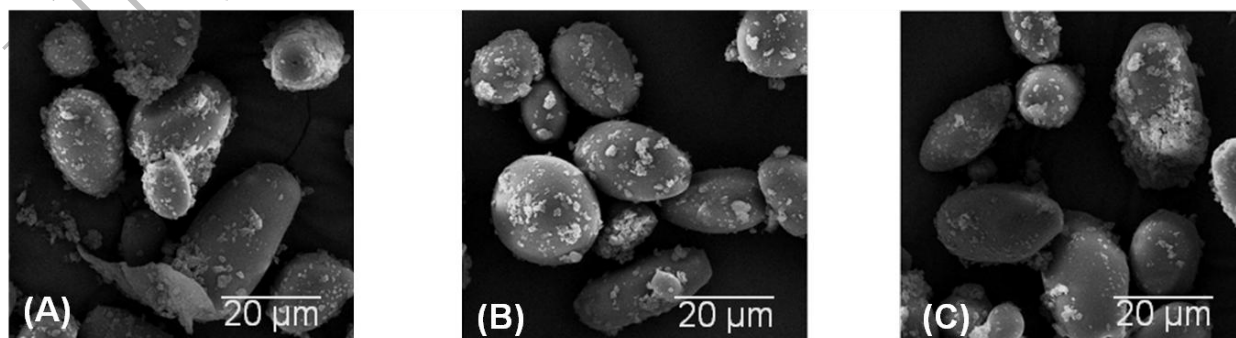
The SEM images of avocado starch granules are shown in Figure 4. As observed, avocado starch presented rounded or oval shapes, as obtained by Silva *et al.* [29].

As the starch isolation process takes place in strictly aqueous medium, other components may remain adhered to the granules, even in small amounts. Thus, using SEM, it was possible to observe materials impregnated on the surface of the starch granules.

It was possible to calculate the average diameter (width and length) of the starch granules. Native and modified avocado starch maintained the diameter around 21  $\mu\text{m}$ , with no significant difference after modification (Table 2). Kahn obtained similar values for the average diameter of avocado starch [9].



**Figure 3.** X-ray diffractograms of (A) native avocado starch, (B) avocado starch modified with 0.5 mol L<sup>-1</sup> lactic acid, and (C) avocado starch modified with 1.0 mol L<sup>-1</sup> lactic acid.



**Figure 4.** SEM images of (A) native avocado starch, (B) avocado starch modified with 0.5 mol L<sup>-1</sup> lactic acid, and (C) avocado starch modified with 1.0 mol L<sup>-1</sup> lactic acid (magnification 2000 X).

## Conclusions

Being considered as an industrial residue, avocado seeds can be used as a non-conventional starch source; and their physicochemical properties can be altered through modification with lactic acid. It was observed that thermal stability of starch decreased after the modification. Also, decreasing the temperatures and gelatinisation enthalpy after the action of lactic acid induces a decrease in the relative crystallinity of the starch. No visible morphological changes after modification were registered by SEM.

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