Thermal and Rheological behavior of native and modified starch *Araucaria angustifolia* (*pinhão*)

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Abstract

Araucaria angustifolia is known in Brazil as Paraná Pine is endangered due to reckless extraction. The preservation and maintenance of the culture could be stimulated by use derivative products like the starch from its seeds (*pinhão*). In this work were studied samples of unmodified and modified *pinhão* starch. The hydrogen peroxide (H_2O_2) were used in the concentrations 0.1, 0.2 and 0.3 mol.L^{-1} (a, b, c) and simultaneously exposed in ultraviolet rays (λ = 256 nm) for one hour. One sample (N) was maintained as received for comparisons of the analysis results. The samples were analyzed by the thermoanalytical techniques: thermogravimetry and differential thermal analysis (TG-DTA) and differential scanning calorimetry (DSC); and by the rheological analysis: Rapid Viscoamylographic Analysis (RVA). The TGA showed an increase on the stability values form the (N) sample to the modified ones. The DSC showed a strong decrease on the gelatinization enthalpy with increasing of the H_2O_2 concentrations solutions valued for the modified samples. The RVA results presented that the Setback reduced with the treatment that used hydrogen peroxide in a higher concentration. The obtained results showed that the modification method applied altered the thermal behavior of the *pinhão* starch.

Keywords
Modified Starch; Hydrogen Peroxide, Ultraviolet rays, *Araucaria angustifolia*, TG/DTA, DSC

Introduction

Araucaria angustifolia is a conifer, grows naturally in mixed forests in Brazil (south), Argentina, Paraguay and Chile, these trees can reach from 35 to 60 m in height and 0.8 to 2 m in diameter at breast height (dbh) [1]. Known locally as the Paraná pine, due to its imprudent extraction for commercial purposes or through deforestation, Araucaria is endangered. Research on the sustainable use of Araucaria derivatives could stimulate the preservation of this species. The seed of this pine tree, known as *pinhão*, is produced early in winter and constitutes a traditional high-calorie food that is usually consumed, after being cooked or roasted [2].

According to Bello-Pérez et al. [3] and Bicudo, et al. [4], the wet basis of raw seeds contains approximately: starch (36 %), proteins (3 %), lipids (1 %), soluble sugars (2.4 %), as well as fibres, minerals and phenolic compounds (< 0.2 %). Both the amount of starch and its type are critical for the texture of a given product [4].

Starch granules are made up of glucose polymers, amylase and amylopectin, and they are found inside vegetable cells.The glucose polymers that make up the starch come in two molecular forms, linear and branched. The first is referred to as amylose and the latter as amylopectin. Amylose is a linear polymer of α 1,4-linked glucose, whereas amylopectin is a highly branched polysaccharide consisting of α 1,4 -linked glucose with α 1,6- linkages at the branch points [5].

The use of starch in the food industry is to impart functional properties and to modify food texture and consistency. Efforts have been made to find native starches with the necessary properties (syneresis, transparency, freeze/thaw stability). In this way, the knowledge about the characteristics of different starches is important to select the most suitable for a specific application [3]. The modifications seek to improve the native starch so it becomes more efficient, a much studied technique is the oxidative modification [6].

The thermogravimetry analysis (TGA) can be helpful to show the behavior of starch granules when heating leads to depolymerization. The differential scanning calorimetry (DSC) is particularly appropriate to investigate the phase transitions of starch/water systems, because it allows the study of the starch gelatinization over a wide range of starch/water ratio, and the estimation of transition enthalpies [4].

The aim of this work was study the modification of *pinhão* starch by hydrogen peroxide (H_2O_2) in concentrations 0.1, 0.2 and 0.3 mol.L^{-1} with simultaneously ultraviolet rays (λ = 256 nm) exposition for one hour. In this way were investigate the changes using the following thermoanalytical techniques: thermogravimetry (TG), differential thermal analysis (DTA) and

differential scanning calorimetry (DSC), and the rheological parameters were analized by Rapid Viscoamylographic Analysis (RVA).

Material and Methods

Origin and modifications of the starch

The native *pinhão* starch (200 g) was bought in the commercial area of Colombo, PR, Brazil. The starch was divided into four parts of 50 g (dry basis). One of these was maintained as received and was designated the (N) untreated sample; the other three samples were treated following the literature methodology [7, 8] with modifications. The starches were treated with standard hydrogen peroxide (H_2O_2) solutions for one hour of simultaneously exposure in ultraviolet rays (λ = 256 nm). For samples (a), (b) and (c), 0.1, 0.2 and 0.3 mol.L^{-1} of H_2O_2 was used, respectively. The modified samples were then washed and filtered until the complete elimination of the reagent. The samples were dried in an oven with forced air circulation for 24 hours at 35 ºC and then analysis was performed.

Thermogravimetric study (TG/DTA)

The TG/DTA curves were obtained using a thermal analysis system (Shimadzu, DTG-60H model); the samples were heated from 30 ºC to 600 ºC using open alumina crucibles with approximately 5.0 mg of the sample under a nitrogen flow of 150 mL.min^{-1} at a heating rate of 10 ºC min^{-1}. The instrument was preliminarily calibrated with standard weight and with standard calcium oxalate monohydrate. All mass loss percentages were determined using TA-60 WS data analysis software [8, 9].

Differential scanning calorimetry (DSC)

The DSC curves were obtained using a thermal analysis system (TA-Instruments, DSC-Q200 model, USA). The DSC curves were recorded under an air flow of 50 mL.min^{-1}, heating rate of 10 ºC min^{-1} and the samples weighed about 2.5 mg. The 4:1 (water:starch w/w) mixture was prepared and maintained for 60 minutes in order to equilibrate the moisture content. The aluminum crucibles were sealed and then the curves were performed. The instrument was previously calibrated with 99.99 % purity Indium, melting point of T_p. = 156.6 ºC, ΔH = 28.56 J.g^{-1} [8, 9].

Rapid viscoamylographic analysis (RVA)

The pasting properties of the samples were obtained by using a viscometer (Newport Sci., RVA-4 model, Australia). A suspension of 3.0 g of starch in 25.0 g of distilled water underwent a controlled heating and cooling cycle under constant shear where it was held at 50 ºC for two min, heated from 50 to 95 ºC at 6 ºC min^{-1}, held at 95 ºC for 5 min, cooled to 50 ºC at 6 ºC min^{-1} and held at 50 ºC for 2 min [9, 10].

Statistical analysis

All the results were analysed for variance (ANOVA) with the Tukey test to compare sample means at 95% confidence level ($p<0.05$) using STATISTICA 7.0 software (StatSoft, Inc., Tulsa, OK, USA).

Results and Discussion

Simultaneous thermogravimetry and differential thermal analysis (TG–DTA)

The thermogravimetric curves (TG/DTA), Fig. 1, were performed and it was possible to verify that the profile of each curve were very similar showing three main events of mass loss. The first event, well marked with a endothermic peak in the DTA curve, is attributed to the evaporation of the water and volatile compounds, followed by stability. Once dehydrated, the second and third main regions in the TG curves were due to the degradation of the organic matter (amylose) and the formation of final residues (ash). Similar behavior were found in earlier works [1, 4]. The calculated results (TG/DTA) are shown in Table 1.

On Table 1, the first step correspond to the evaporation, which can be related to the moisture content of the samples. Thermal analysis permit the determination of moisture content in samples on a temperature range of 30 to 150 ºC [11]. This fact was verified by Costa et al [1], that studied the characterization of native starches of seeds of *Araucaria angustifolia* from four germplasm collections, which were found similar values by both official method and thermogravimetric ones. The thermogravimetric analysis presents advantages in relation of the official method by requiring a much less amount of sample and it is faster could show the results in less than a half hour [1]. It can also be observed in Table 1, that the stability values increased significantly from the sample (N) to the modified ones, in a range of 209 - 243 ºC, demonstrating that the modification by H_2O_2+UV caused alterations on the thermal properties of the *pinhão* starch.

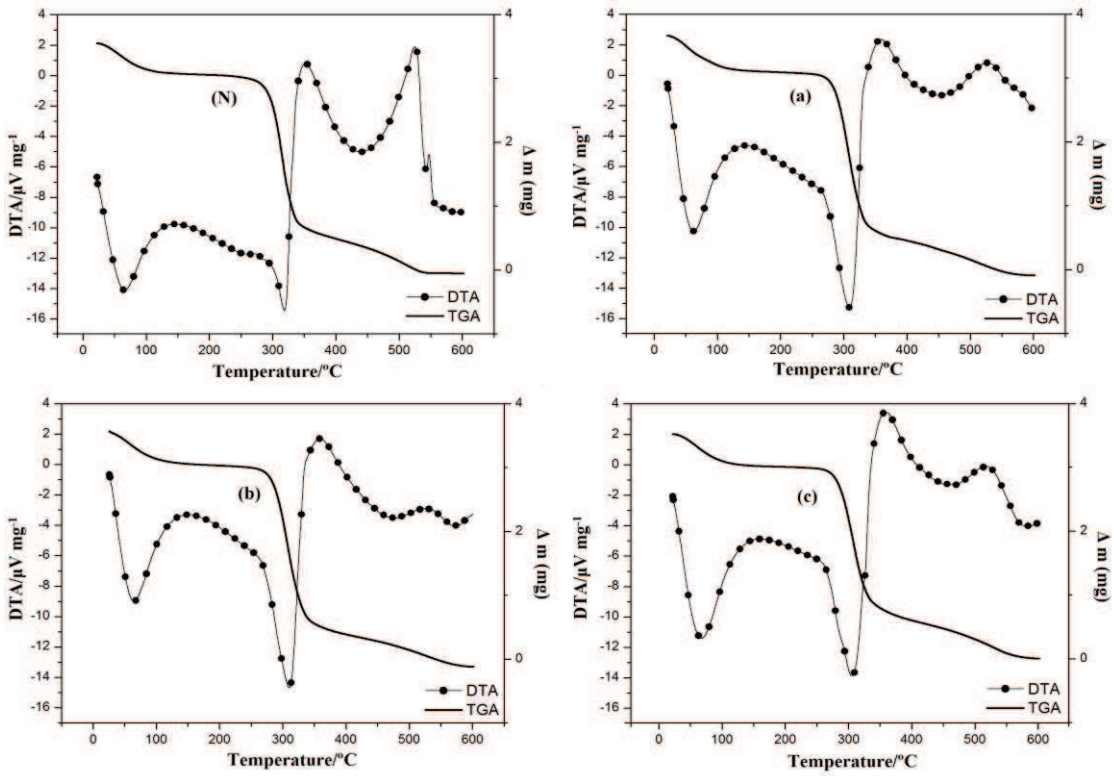

Fig.1 - TG/DTA curves: (N) native *pinhão* starch; (a; b; c) native *pinhão* starch treated with UV-rays (λ = 256 nm) at one hour exposure and H_2O_2 standard solutions 0.1; 0.2 and 0.5 mol L^{-1}, respectively.

Table 1 TG and DTA results of: (N) native *pinhão* starch; (a; b; c) native *pinhão* starch treated with UV-rays (λ = 256 nm) at one hour exposure and H_2O_2 standard solutions 0.1; 0.2 and 0.5 mol L^{-1}, respectively.

Samples	TG Results		DTA Results	
	Step	Δm/%	ΔT/°C	Tp/°C
(N)	1st	13.02	30 - 133	59.26
	stability	-	133 - 209	-
	2nd	72.99	209 - 424	315.60
	3rd	13.25	424 - 576	510.64
(a)	1st	11.90	30 - 140	62.11
	stability	-	140 - 235	-
	2nd	74.06	235 - 419	316.77
	3rd	12.85	419 - 573	514.44
(b)	1st	11.86	30 - 145	62.70
	stability	-	145 - 243	-
	2nd	72.01	243 - 428	316.58
	3rd	14.93	428 - 569	507.74
(c)	1st	14.24	30 - 149	56.41
	stability	-	149 - 238	-
	2nd	72.49	238 - 414	315.19
	3rd	12.05	414 - 567	494.23

Δm mass loss (%), ΔT temperature range, Tp peak temperature.

Differential scanning calorimetry (DSC)

DSC was used to determine de gelatinization properties of native and oxidized *pinhão* starch samples. The results are showed in Fig. 2 and Table 2.

As can be seen in Table 2, the oxidized samples (a, b and c) had a higher onset temperature (To) than sample (N), however samples (a) and (b) did not differ significantly from sample (N) with respect to the peak temperature (Tp), and only a higher H_2O_2 concentration (sample c) increased the same. Final temperature (Tc) decreased significantly when low concentrations of H_2O_2 were used (samples a and b) and sample (c) (highest concentration of H_2O_2) not differ significantly from sample (N). Sangseethong et al. [12] showed an increase in all transition temperatures (To, Tp and Tc) after oxidize cassava starch with

H_2O_2 (3 %). Liu et al. [13] showed similar results after oxidize corn starch with H_2O_2 (12 %). In this study, very low concentrations of H_2O_2 were used, then some results are different from those found in the literature. Oxidation promoted a reduction in gelatinization enthalpy (ΔH_{gel}) and this reduction was more pronounced in higher concentrations of H_2O_2 (samples a and b). Pinto et al. [2] and Klein et al. [14] found that gelatinization enthalpy of *pinhão* starch reduced after heat-moisture treatment. The reduction in ΔH_{gel} can be attributed to degradation of the starch chains caused by modification, consequently, less energy was needed to gelatinize starch [12].

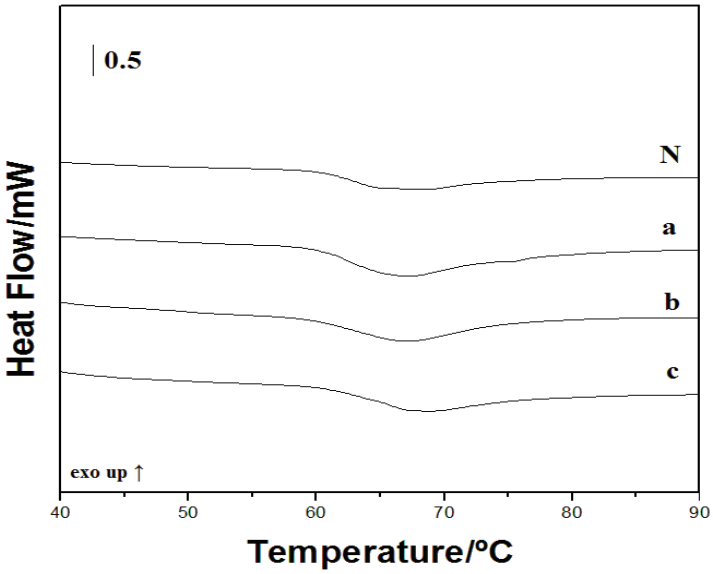

Fig. 2 DSC gelatinization curves: (N) native *pinhão* starch; (a; b; c) native *pinhão* starch treated with UV-rays (λ = 256 nm) at one hour exposure and H_2O_2 standard solutions 0.1; 0.2 and 0.5 mol L^{-1}, respectively.

Table 2 DSC gelatinization of: (N) native *pinhão* starch; (a; b; c) native *pinhão* starch treated with UV-rays (λ = 256 nm) at one hour exposure and H_2O_2 standard solutions 0.1; 0.2 and 0.5 mol L^{-1}, respectively.

Samples	DSC gelatinization			
	To/ºC	*Tp*/ºC	*Tc*/ºC	ΔH_{gel}/J.g$^{\pm 1}$
(N)	59.08±0.04^{a}	66.02±0.05^{a}	75.54±0.03^{c}	14.64±0.02^{d}
(a)	60.41±0.23^{b}	66.49±0.09^{a}	70.96±0.18^{a}	6.75±0.04^{c}
(b)	60.78±0.22^{b}	66.74±0.06^{a}	74.50±0.22^{b}	5.85±0.01^{b}
(c)	62.08±0.05^{c}	67.80±0.07^{c}	75.14±0.11^{c}	5.24±0.06^{b}

(*) *To* "onset" initial temperature, *Tp* peak temperature, *Tc* "endset" final temperature, ΔH_{gel} gelatinization enthalpy, (**) different letters in the same column represents significative difference according to Duncan test ($p<0.05$).

Rapid viscoamylographic analysis (RVA)

The RVA results are shown in Fig. 3 and Table 3. Oxidation caused a reduction in trough (hot paste viscosity) and viscosity peak, regardless of the concentration of peroxide used. Similar results were obtained by Pietrzyk et al. [15] to corn starch oxidized with 2 % and 4 % NaOCl and Gumul et al. [16] to potato starch oxidized with H_2O_2 2 %. The reduction in these viscosities can be attributed to degradation of the starch chains caused by oxidation, with consequent reduction of the molecular weight [17]. On the other hand, final viscosity of samples (a), (b) and (c) were higher than sample (N). During the heating step, amylose chains were solubilized forming a network of more compact structure in cooling step and increasing final viscosity [18].

The results of the pasting temperature, oxidation provided a reduction, except for the sample (b) that was not significantly different when compared with the sample (N). Similar results were obtained by Tethool, Jading and Santoso [19] after oxidation of sago starch using hydrogen peroxide (3 %) and catalyzed by UV irradiation.

Breakdown is an average resistance to degradation of the starch granule [20]. The oxidized samples (a), (b) e (c) had a reduction in breakdown indicating the higher tendency of starch to resist shear force. Setback reduced with the treatment that used hydrogen peroxide in a higher concentration (c), however in lower hydrogen peroxide concentrations occurred a increase of setback. Chon et al. [21] showed similar results. Higher setback value could be due higher amount of solubilized starch granules, which have a greater tendency to recouping during paste cooling [22].

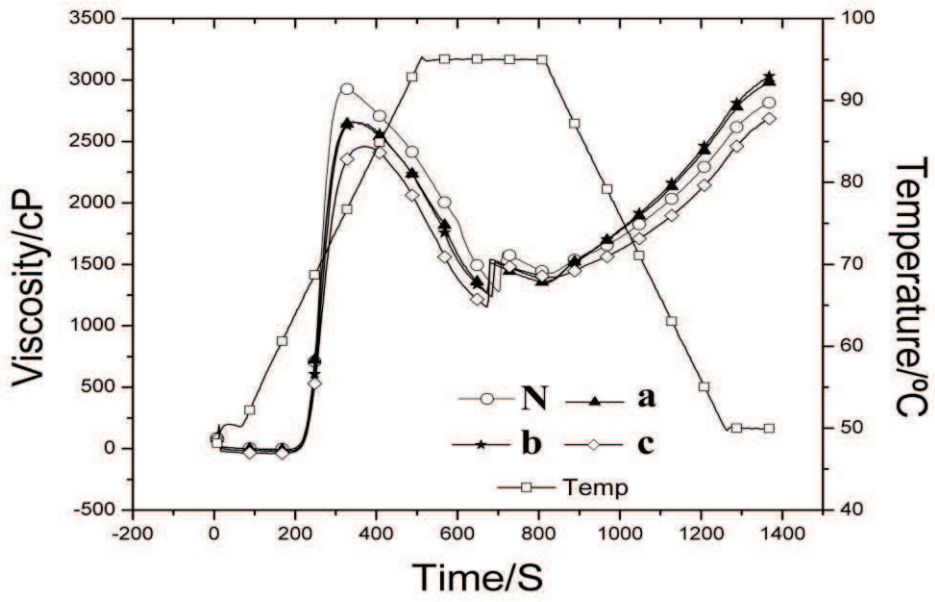

Fig. 3 RVA curves: (N) native *pinhão* starch; (a; b; c) native *pinhão* starch treated with UV-rays (λ = 256 nm) at one hour exposure and H_2O_2 standard solutions 0.1; 0.2 and 0.5 mol L^{-1}, respectively.

Table 3 RVA results of: (N) native *pinhão* starch; (a; b; c) native *pinhão* starch treated with UV-rays (λ = 256 nm) at one hour exposure and H_2O_2 standard solutions 0.1; 0.2 and 0.5 mol L^{-1}, respectively.

Samples	Pasting temperature/°C	Trough/cP	Viscosity peak/cP	Setback/cP	Break/cP	Final viscosity/cP
(N)	65.55±0.02^{a}	1426±2.64^{a}	2925±1.73^{a}	1398±2.00^{c}	1499±1.00^{a}	1426±2.00^{d}
(a)	64.66±0.01^{c}	1349±2.64^{d}	2659±2.64^{b}	1645±3.60^{b}	1310±2.00^{c}	2994±1.73^{b}
(b)	65.55±0.03^{a}	1362±2.64^{c}	2659±3.33^{b}	1680±3.00^{a}	1297±2.64^{b}	3042±2.00^{a}
(c)	65.20±0.02^{b}	1392±1.73^{b}	2459±1.00^{c}	1308±2.00^{d}	1067±3.00^{d}	2700±2.64^{c}

(*) cP "centipoises", sec "seconds", (**)(**) different letters in the same column represents significative difference according to Duncan test ($p<0.05$).

Conclusion

The TG-DTA analysis showed that the thermal properties of the *pinhão* starch was altered, highlighting the increased on the thermal stability event.
The DSC curves showed a similarity in their profiles related to the (N) sample, except for sample (c), and the calculated results showed a great decreased of the gelatinization enthalpy with the increasing of the H_2O_2 concentrations solutions.
The RVA results presented that the oxidation caused a reduction in trough (hot paste viscosity) and viscosity peak of the modified samples related to unmodified one.
By the results obtained with the thermal analysis , it possible to conclude that the modification by H_2O_2 and UV- rays exposure, altered the thermal behavior of the native *pinhão* starch.

Acknowledgements

The financial resources for this study were provided by EMBRAPA-Brazil, CAPES-Brazil and CNPq-Brazil, which the authors gratefully acknowledge.

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