

## Thermoanalytical study and characterization of native starches of Paraná pine seeds (*Araucaria angustifolia*, Bert O. Ktze) and European chestnut seeds (*Castanea sativa*, Mill)

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**Abstract:** Starch is the most important carbohydrate storage in plants. It is a raw material with diverse botanical origins, and is used by the food, paper, chemical, pharmaceutical, textile and other industries. In this work, native starches of Paraná pine seeds (pinhão) (*Araucaria angustifolia*, Bert O. Ktze) and european chestnut seeds (*Castanea sativa*, Mill) were studied by thermoanalytical techniques: thermogravimetry (TG), differential thermal analysis (DTA) and differential scanning calorimetry (DSC), as well as X-ray powder patterns diffractometry. Apparent and total amylose content was also determined.

**Keywords:** Starch; gelatinization; thermal analysis; enthalpy.

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### Introduction

The conifer, “Paraná pine tree” (*Araucaria angustifolia*, Bert O. Ktze), grows naturally in forest of Brazil (south), Paraguay, Chile and Argentina. Its seeds, named “pinhão”, are eaten by mammals and birds; cooked or roasted, are consumed as traditional high-caloric food during winter by humans [1, 2]. “Pinhão” (Paraná pine seeds), is a source of complex carbohydrates: starch (~ 36%), proteins (~ 3%), lipids (~ 1%), soluble sugars (~2.4%), beyond fiber, minerals and phenolic compounds (< 0.2 mg %) [3].

“Chestnut” seeds (*Castanea sativa*, Mill.), is multipurpose specie that is cultivated for timber, nut, tannin and contributes positively to the forestry landscape [4]. Its seeds chestnuts are char-

acterized by a limited shelf-life because of their high water activity and sugar contents [5].

European chestnut trees spread all over the world produce wood and chestnuts that have considerable economical value. In Brazil, however, they are not products of economic interest; trees are grown for their landscape qualities in many farms and also in some public squares, mainly in Southern Region [6].

Starch, a semi crystalline polymer, is composed of two polysaccharides: amylose and amylopectin. Amylose, a mostly linear chain, typically consists up to 3000 glucose molecules interconnected primarily by a  $\alpha$ -1,4 glycosidic linkages and is reported to contain a few branched networks [7]. Amylopectin is a large branched polymer with linkages of  $\alpha$ -1,4 that serve as the

backbone and  $\alpha$ -1,6 bridges that serve as branching points [8].

Thermogravimetry (TG) can be helpful to show the behavior of starch granules when heating leads to depolymerization [11; 17].

Starch, when heated in the presence of excess water, undergoes the order to disorder phase transition known as gelatinization over a temperature range characteristic of the starch source. This phase transition is associated with diffusion of water into the granule, water uptake by the amorphous background region, hydration and radial swelling of the starch granules, loss of birefringence, loss of crystalline order, uptake of heat, uncoiling and dissociation of double helices in the crystalline regions and amylase leaching [9; 10; 15]. The gelatinization process, which is a function of the starch : water ratio, can be studied by differential scanning calorimetry (DSC).

In recent years, substantial efforts have been made to obtain starches from non-conventional sources and to study their functional, rheological and physicochemical properties [13 - 15].

Starch is used in the food industry to impart functional properties, and to modify food texture and consistency. Both the amount of starch and its type are critical for the texture of a given product [14 - 16].

Efforts have been made to find native starches with the necessary properties for the food industry (syneresis, transparency, freeze/thaw stability), with no chemical or genetic modification. In this way, the knowledge about the characteristics of different native starches is of great importance to select the most suitable starch for a specific application [2].

Since pinhão and european chestnut were good sources of starch and they have a large potential for commercial use, the objective of this work was to evaluate some thermoanalytical and chemical, characteristics of these isolated starches.

## Experimental

Both seeds were acquired in the local market at Ponta Grossa – Pr – Brazil. All chemicals used were analytical reagent grade. Extraction and purification of each starch were performed as

described in the literature [1]. After this procedure, the obtained starches were kept in a desiccator over anhydrous calcium chloride until constant mass.

TG and DTA curves were recorded using a simultaneous TG 60 system (Shimadzu) under a  $100 \text{ mL min}^{-1}$  air flow and a  $10 \text{ }^\circ\text{C min}^{-1}$  heating rate of. The initial sample mass was about 9 mg. Alumina crucibles were used for the TG and DTA experiments. DSC curves were recorded using a DSC 60 (Shimadzu) under an air flow of  $100 \text{ mL min}^{-1}$ , heating rate of  $5 \text{ }^\circ\text{C min}^{-1}$ . Sealed aluminum crucibles were used and the studies were carried out in order to study the gelatinization process, according the following conditions: 1<sup>st</sup> condition - a 4:1 (water:starch w/w) mixture was prepared and left for two hours stirring in order to equilibrate the moisture content. With a micropipette  $10 \text{ }\mu\text{L}$  of this emulsion was transferred for an aluminum crucible, which was sealed and carried out in order to study the gelatinization process. The 2<sup>nd</sup> condition was: a 10:1 (water:starch w/w) was prepared directly by weighting  $1,0 \text{ mg}$  of each starch and up to this mass  $10 \text{ }\mu\text{L}$  of water was added; the aluminum crucible was sealed and after one hour a new DSC curve was realized. The 3<sup>rd</sup> condition was: a 5:1 (water:starch w/w) was prepared directly by weighting  $2,0 \text{ mg}$  of each starch and up to this mass  $10 \text{ }\mu\text{L}$  of water was added; the aluminum crucible was sealed and after one hour a new DSC curve was realized.

X-ray diffraction powder patterns were obtained by using a D-5000 X Ray diffractometer (Siemens), with  $\text{CuK}\alpha$  radiation ( $\lambda = 1.544 \text{ \AA}$ ) and a setting of  $40\text{kV}$  and  $20 \text{ mA}$ .

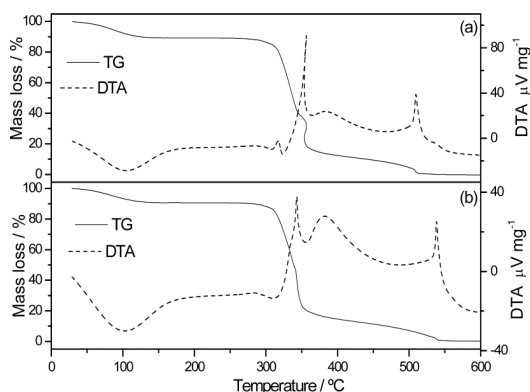
Total and apparent amylose were determined according the technique described by Hoover and Ratnayake [12], in a spectrophotometer (Shimadzu UV mini 1240) at  $\lambda = 600 \text{ nm}$ .

The procedure for apparent amylose content was: starch sample ( $20 \text{ mg}$ ) was dissolved in 90% of dimethylsulfoxide ( $8 \text{ ml}$ ) in  $10 \text{ ml}$  at screw-cap reaction vials. The content of the vial was vigorously mixed for  $20 \text{ min}$  and then heated in a water bath (with intermittent shaking) at  $85 \text{ }^\circ\text{C}$  for  $15 \text{ min}$ . The vial was then cooled to ambient temperature, and the content diluted with water to  $25 \text{ mL}$  in a volumetric flask. An aliquot ( $1.0 \text{ mL}$ ) of the diluted solution was mixed with water ( $40$

ml) and 5mL of I<sub>2</sub>/KI solution (I<sub>2</sub> = 2.5 × 10<sup>-3</sup> mol L<sup>-1</sup> and KI = 6.5 × 10<sup>-3</sup> mol L<sup>-1</sup>) and then adjusted to a final volume of 50 ml. The contents were allowed to stand for 15 min at ambient temperature, before absorbance measurements at 600 nm.

## Results and Discussion

The simultaneous TG and DTA curves of the European chestnut and Paraná pine seeds (pinhão) starches are shown in Figure 1.



**Figure 1.** Simultaneous TG and DTA curves of: (a) European chestnut seeds starch, *m* = 9.46 mg, and (b) Paraná pine seeds starch, *m* = 8.62 mg.

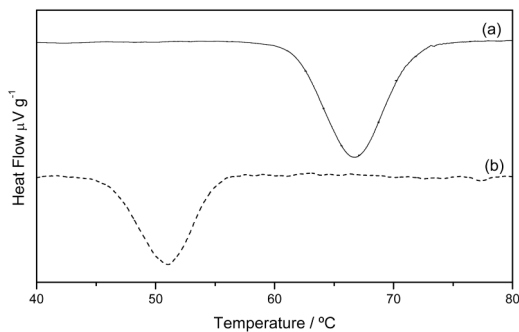
The TG-DTA curves of European chestnut starch seeds, Fig 1(a), show mass losses in four steps and thermal events corresponding to these losses. The first mass loss between 30 – 126 °C (10.5 %) corresponding to the endothermic peak at 104 °C which is attributed to the dehydration, that occurs in a single step.

Once dehydrated, the compound is stable up to 270 °C and above this temperature the thermal decomposition occurs in three consecutive and/or overlapping steps between 270 and 520 °C. The first mass loss of the anhydrous compound observed between 270 – 345 °C with loss of 50 %, corresponding to the endothermic peaks at 308 and 323 °C, which is attributed to the thermal decomposition that occurs without oxidative process. The second mass loss, between 345 – 365 °C with loss of 15.9 %, corresponding to the

sharp exothermic peak is ascribed to the oxidation of the organic matter. The profiles of the TG and DTA curves in this step, suggest that the oxidation of the organic matter is accompanied by combustion. The last step, between 365 – 520 °C, with loss of 23.6 %, the mass loss occurs through a slow process corresponding to the small and broad exotherm between 370 and 410 °C and a sharp exothermic peak at 509 °C is attributed to the total oxidation of the organic matter.

The TG-DTA curves of Paraná pine seeds starch, Fig 1(b) show mass losses in three consecutive steps, in spite of the second mass loss to suggest two overlapping steps, and thermal events corresponding to these losses. The first mass loss observed between 30 – 128 °C, with loss of 9.4 %, corresponding to the endothermic peak at 104 °C is attributed to the dehydration that occurs in a single step.

The anhydrous compound is stable up to 276 °C and above this temperature the thermal decomposition occurs in two consecutive steps between 276 and 550 °C. The first mass loss observed between 276 and 386 °C with loss of 70.5 %, corresponding to the endothermic peak at 310 °C, followed by a sharp exothermic one at 342 °C is attributed to the thermal decomposition that initiate without oxidative process, followed by oxidative one. The second mass loss that occurs between 386 and 550 °C, through a slow process with loss of 20.1 %, corresponding to the exothermic peaks at 381 and 538 °C, respectively is attributed to total oxidation of the organic matter.



**Figure 2.** DSC curves of European chestnut seeds starch (a) and Paraná pine seeds (pinhão) starch (b), obtained at the 1<sup>st</sup> conditions.

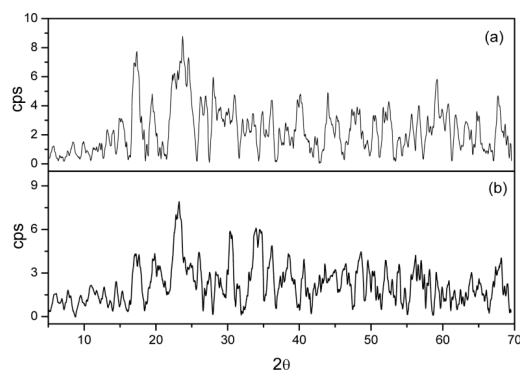
DSC curves of the European chestnut seeds starch and Paraná pine seeds starch are shown at Fig. 2. The instrument was calibrated with indium, and an empty aluminum crucible was used as reference. The characteristics of the transitions, including onset temperature ( $T_o$ ), peak temperature ( $T_p$ ) and gelatinization enthalpy ( $\Delta H_{gel}$ ) were calculated and show in Table 1.

The total amylose contents of starch samples were determined by the above procedure, but with prior defatting with hot n-propanol–water (3:1 v/v) for 7 h. In order to correct for over estimation of apparent and total amylose content (due to complex formation between  $I_2$  and the outer branches of amylopectin), amylose content was calculated from a standard curve prepared using mixtures of pure potato amylose and amylopectin (over the range 0–100% amylose).

Amylopectin (%) was calculated by subtraction of total amylose (%) of 100 %; amylose leaching (%) was obtained by subtraction of total

amylose (%) and apparent amylose. The results are shown at Table 2.

X-ray diffraction powder patterns of the studied starches are shown in Figure 3. Starches tend to present pertinent crystalline arrangements



**Figure 3.** X-ray diffraction powder patterns of the compounds: (a) European chestnut starch and (b) Paraná seeds pine starch.

**Table 1.** Experimental values obtained of gelatinization enthalpy ( $\Delta H_{gel}$ ), onset temperature ( $T_o$ ), peak temperature ( $T_p$ ) and initial mass ( $m_i$ ) for studied starches.

Sample	$\Delta H_{gel}$ (J g <sup>-1</sup> )	$T_p$ (°C)	$T_o$ (°C)	$m_i$ (mg)
European chestnut starch (1 <sup>st</sup> conditions)	15.0	66.7	61.5	-
European chestnut starch (2 <sup>nd</sup> conditions)	7.2	65.0	61.2	1.110
European chestnut starch (3 <sup>rd</sup> conditions)	5.6	65.0	61.5	2.000
Paraná seeds pine starch (1 <sup>st</sup> conditions)	2.4	50.9	46.5	-
Paraná seeds pine starch (2 <sup>nd</sup> conditions)	2.2	49.8	46,9	1.031
Paraná seeds pine starch (3 <sup>rd</sup> conditions)	6.5	50.8	47.0	2.040

**Table 2.** Obtained values of apparent amylose, total amylose, amylopectin and amylose leaching.

Samples	apparent amylose(%)	total amylose (%)	amylopectin (%)	amylose leaching (%)
European chestnut starch	21,3	27,8	72,2	6,5
Paraná pine seeds starch	25,6	25,3	74,4	0,0

depending on their botanical origin; the intensity of few diffraction peaks can suggest different characteristic of crystalline patterns. Some authors classify the starches in three types: A-for cereals, B-for tuberous, banana and corn waxy, and C-for seeds and legumes [17 - 22]. In this work, the studied starches shown characteristics of the C-type.

## Conclusions

TG curves of European chestnut starch and Paraná pine seeds starch show mass losses in four and three steps respectively; helped to observe the dehydration and the thermal stability of each raw starch sample, in agreement with endo or exother-

mic peaks observed at DTA curves. By using DSC technique the starch gelatinization process was observed and determined the enthalpy according the experimental conditions, which should be observed carefully. X-ray diffraction confirmed the characteristic of native pattern C-type.

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S. C. W. Bicudo, I. M. Demiate, G. Bannach, L. G. Lacerda, M. A. S. Carvalho Filho, M. Ionashiro and E. Schnitzler. Estudo termoanalítico e caracterização de amidos nativos de sementes do pinheiro do Paraná (*Araucaria angustifolia*, Bert O. Ktze) e sementes de castanha européia (*Castanea sativa*, Mill).

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**Resumo:** O amido é o mais importante carboidrato de reserva em plantas. É uma matéria-prima originária de diversas fontes botânicas, e muito usado nas indústrias de alimentos, papéis, químicas, farmacêuticas, têxteis e outras. Neste trabalho, amidos nativos das sementes de pinheiro do Paraná (pinhão) (*Araucaria angustifolia*, Bert O. Ktze) e de castanha Européia (*Castanea sativa*, Mill) foram estudados pelas técnicas termoanalíticas: termogravimetria (TG), análise térmica diferencial (DTA) e calorimetria exploratória diferencial (DSC), bem como difratometria de raios-X. Também os teores de amilose aparente e total foram determinados.

*Palavras chave:* Amidos; gelatinização; análise térmica; entalpia.

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