DOI 10.1002/star.200600606 *Starch/Stärke* **59** (2007) 284–289

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Starch Characterisation of *Araucaria angustifolia* and *Araucaria araucana* Seeds

Araucaria angustifolia and *Araucaria araucana* are conifers that cover different areas of South America. Their seeds have been consumed from prehistoric times until today in Brazil, Argentina and Chile. In this work, the starch of *Araucaria angustifolia* and *Araucaria araucana* seeds were analysed by light and environmental scanning electron microscopy, X-ray diffraction, and differential scanning calorimetry. The starch granules of *A. angustifolia* and *A. araucana* were round or slightly oval, with a central hilum. Both starches gave X-ray diffraction patterns compatible with the A-type, with strong peaks at 15°, 17°, and 23°. The gelatinisation temperature of *A. angustifolia* starch (68.5°C) was higher than that of *A. araucana* (66.6°C), probably due to the higher amylose content of the former (22.4 % and 17.3 %, respectively). The thermograms of *A. araucana* starch presented a minor peak at about 71°C, which was attributed to the fact that the starch granules population of *A. araucana* was heterogeneous, with large and small granules, whereas *A. angustifolia* starch contained mainly large granules.

Keywords: Araucaria seeds; X-ray diffraction; Environmental scanning electron microscopy; Differential scanning calorimetry

1 Introduction

Araucaria is a genus of Southern hemisphere coniferous trees with large and nutritious seeds. Starch is an important energy source in the human diet and the principal reserve of araucaria seeds. *Araucaria angustifolia* and *Araucaria araucana* seeds have been consumed from prehistoric times until today, cooked in water, baked, or as raw flour in regional dishes, especially by natives of Brazil, Argentina and Chile [1]. *A. angustifolia* covers areas of the South and South East of Brazil and North East of Argentina [1], whereas *A. araucana* is a South American conifer restricted to high mountain areas in the South of Argentina, mainly in the province of Neuquén, and Chile, between Ñuble and the Villarica volcano [2]. In the province of Nuequén it is possible to eat "alfajores" made with araucaria seed flour, a regional food which is characteristic in this region.

Some studies have been performed to characterise *A. angustifolia* starch: light microscopy, hydration of the granules, susceptibility to enzymatic hydrolysis, and rheological properties of the paste [3-5]. Also, the size of starch granules and the digestion of them during germination were studied on *A. araucana* starch [6].

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However, the literature about the starch characteristics of *A. araucana* and *A. angustifolia* seeds is scarce. In order to contribute to the knowledge of the starch of these regional seeds and compare the properties of them, the starch was characterised by environmental scanning microscopy, X-ray diffraction, and differential scanning calorimetry (DSC). DSC is particularly appropriate to investigate the phase transitions of starch/water systems, because it allows the study of the starch gelatinisation over a wide range of starch/water ratio, and the estimation of transition enthalpies [7].

2 Materials and Methods

2.1 Materials

The seeds of *Araucaria angustifolia* were obtained from the field "Manuel Belgrano" (EEA Montecarlo, INTA), San Antonio, province of Misiones, Argentina, whereas the seeds of *Araucaria araucana* were obtained from local shops in the province of Neuquén, Argentina.

2.2 Light and environmental scanning electron microscopy (ESEM)

Starch granules were observed with a light microscope with polarised light (Leica Microsystems GMBH, Wetzlar, Germany) with a magnification of 400 ×, and with an en-

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vironmental scanning electron microscope ElectroScan 2010 (Philips, Cambridge, UK), with a magnification of 1000 ×. The diameters of the starch granules were determined from the environmental scanning electron micrographs using a calibre.

2.3 Raw seed flour

In order to reduce the external microbial content, the seeds were previously washed with 5% NaClO, and the remaining solution was removed with an absorbent paper. The flour of raw seeds was obtained by separating the resistant coat, and then milling the seeds. The ground seeds were dried at 80°C for 2 h, milled again (flour passed through a 0.500 mm sieve), and left at 50–55°C until the moisture content was lower than 10%. The raw seed flour of *A. angustifolia* contained, on a dry basis, 7.3% protein (N × 6.25), 1.7% lipid, 88.0% carbohydrates, and 3.0% ash. The raw seed flour of *A. araucana* contained 7.5% protein, 2.2% lipid, 87.5% carbohydrates, and 2.8% ash.

2.4 Starch isolation

Seeds of *A. angustifolia* and *A. araucana* were dehulled and peeled, and the germs were removed. The isolation of starch from the resulting gametophyte was performed by the method of *Singh Sandhu* et al. [8], with few modifications, using 0.16% sodium hydrogen sulphite in order to break the disulphide bonds of proteins that may surround the starch granules [9], and 0.2% NaOH to disperse proteins with minimum losses of starch [10].

2.5 Composition of the isolated starch

The total protein content was determined by the Kjeldahl method (N × 6.25). The ash content was determined by incineration in a muffle furnace at 525°C, and the moisture content was estimated through the weight loss after heating the sample in an oven at 105°C [11]. Lipids were extracted with petroleum ether (35-60) in a Soxhlet extractor and weighed [11].

2.6 Amylose content

The amylose content was determined by the method described by *Chrastil* [12] in about 20 mg starch from both *A. angustifolia* and *A. araucana*. Measures were performed in triplicate.

2.7 X-ray diffraction

X-ray diffractograms of starch and raw seed flour were obtained with a Philips PW 1394 diffractometer (Philips, Eindhoven, The Netherlands), operating at 40 kV and 20 mA with copper radiation. The samples were scanned through the 2θ (diffraction angle) range of 5° – 35° at 1°/min.

2.8 Differential scanning calorimetry

A differential scanning calorimeter (Rheometric Scientific Ltd., Epsom, Surrey, UK) calibrated with indium was used. Samples of 2.5 – 6.0 mg of raw seed flour or starch were placed into aluminium DSC hermetic pans, and distilled water was added to achieve different sample: water ratios. Flours were passed through a 0.300 mm sieve. An empty double pan was used as reference. Sample and reference were heated between 25 and 120°C at a heating rate of 10°C/min. Onset temperature (T_o); peak temperature (T_p); conclusion temperature (T_c) and enthalpy of gelatinisation (ΔH_{gel}) were computed from the endothermic peaks. The gelatinisation temperature range (R) was calculated as ($T_c - T_o$) [13]. At least three independent replicates were made for each sample. Enthalpies were calculated on a dry basis.

2.9 Statistics

An analysis of variance (ANOVA) of the data was performed by using a Systat statistical computer program.

3 Results and Discussion

3.1 Starch characterisation

Tab. 1 shows the proximate composition of the isolated starch of *A. araucana* and *A. angustifolia*. The starch of *A. angustifolia* presented higher protein and ash and lower lipid contents than results reported by *Wosiacki* and *Cereda* [3], probably due to differences in the extraction method. The moisture content of isolated starch from both *A. angustifolia* and *A. araucana* was 10.7%.

The amylose content of *A. angustifolia* starch was higher ($P < 0.05$) than that of *A. araucana*, as observed in Tab. 1. The amylose content of *A. angustifolia* starch agrees with the value reported by *Wosiacki* and *Cereda* [3], and is similar to the amylose content of corn and potato starch [3, 7]. The amylose content of *A. araucana* starch, on the other hand, is similar to that of cassava starch [3]. Other sources of starch contain higher amylose per-

Tab. 1. Proximate composition (mean ± sd) of isolated starch of *A. angustifolia* and *A. araucana* seed (dry basis).

	A. angustifolia	*A. araucana*
Protein [%]	3.25 ± 0.17	3.40 ± 0.29
Lipid [%]	0.52 ± 0.50	0.45 ± 0.18
Ash [%]	0.28 ± 0.02	0.42 ± 0.07
Carbohydrates [%][a]	95.95	95.73
Amylose [%][b]	22.4 ± 1.9	17.3 ± 1.4

[a] Estimated by difference.
[b] Calculated on starch basis.

centages; thus, beans and smooth pea have amylose contents from 32.5 to 36.0%, and lentil starch presents 45.5% amylose [7].

The starch granules of *A. angustifolia* and *A. araucana* presented in both cases a round or slightly oval shape, with a central hilum (Fig. 1). The average size of the starch granules of *A. angustifolia* was higher than that of *A. araucana*: 12.2 ± 2.7 μm and 8.4 ± 2.9 μm, respectively, and significant differences were found in this parameter between these two seeds ($P < 0.05$). The starch granules of *A. araucana* were more heterogeneous in size, with large and small granules, whereas mainly large granules were observed in *A. angustifolia* starch. The size distribution of the starch granules of the megagametophyte of *A. angustifolia* and *A. araucana* is shown in Fig. 2. Results indicate that more than 50% of the starch granules of *A. angustifolia* presented a diameter between 10.41 and 14.40 μm, whereas *A. araucana* showed similar percentages of starch granules in the ranges from 4.41 to 12.40 μm.

The size and shape of starch granules depend on several factors, such as botanical source, gene-line variation, stage of development and starch hydrolysis [6]. *Wosiacki* and *Cereda* [3] have described the starch granules of *A. angustifolia* as round-shaped, with an irregular surface and a dark and central hilum. However, the starch granules of Fig. 1 do not show an irregular surface; this difference could be due to the extraction method. On the other hand, *Waghorn* et al. [6] have studied the amylases of *A. araucana*, and reported that all the starch granules of the megagametophyte of *A. araucana* have a similar size, with an average diameter of 13.8 ± 3.2 μm, whereas in the embryo there are different sizes of starch granules: 2.90 ± 0.79 μm (small), 6.24 ± 1.37 μm (medium), and 10.30 ± 1.35 μm (large). These results do not agree with the values obtained in the present study, in which this distribution of starch granules was observed in the megagametophyte, with an average diameter of 8.36 μm (Fig. 2). It is possible that these differences were due in part to different growing conditions.

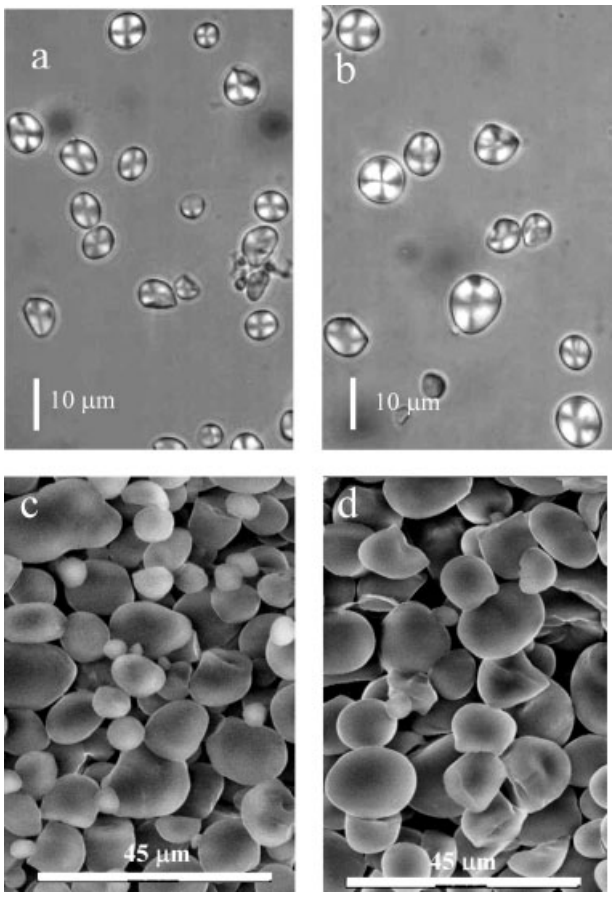

Fig. 1. (a, b) Light micrographs of starch granules of *Araucaria araucana* (a) and *Araucaria angustifolia* (b) observed with polarised light. Magnification: 400 ×.
(c, d) Environmental scanning electron micrographs of starch granules of *Araucaria araucana* (c) and *Araucaria angustifolia* (d). Magnification: 1000 ×.

3.2 X-ray diffractometry

Native granular starches give X-ray diffraction patterns that have been classified as A-, B- or C-types [14]. Most tuber and root starches, as potato, present a B-type X-ray diffraction pattern, which is characterised by a small peak at 5.6°, only one peak at 17° and a doublet at 22° and 24° [14]. On the other hand, most cereal starches display A-type starch crystals [15], with a peak at 15°, a doublet at 17° and 18° and a single peak at 23° [14, 16]. Finally, tapioca starch gives the C-pattern, which is similar to A-type, except for the appearance of the 5.73° line on the C-pattern [14]. The V-pattern arises from complexes formed

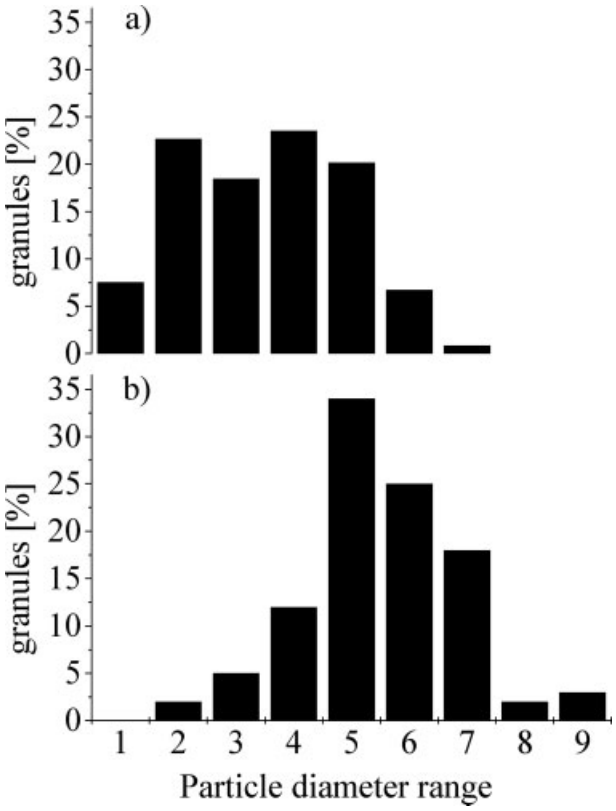

Fig. 2. Size distribution of starch granules of the megagametophyte of (a) *Araucaria araucana* and (b) *Araucaria angustifolia.* Particle diameter range: 1: 2.41-4.40 μm; 2: 4.41-6.40 μm; 3: 6.41-8.40 μm; 4: 8.41-10.40 μm; 5: 10.41-12.40 μm; 6: 12.41-14.40 μm; 7: 14.41 -16.40 μm; 8: 16.41-18.40 μm; 9: 18.41-20.40 μm.

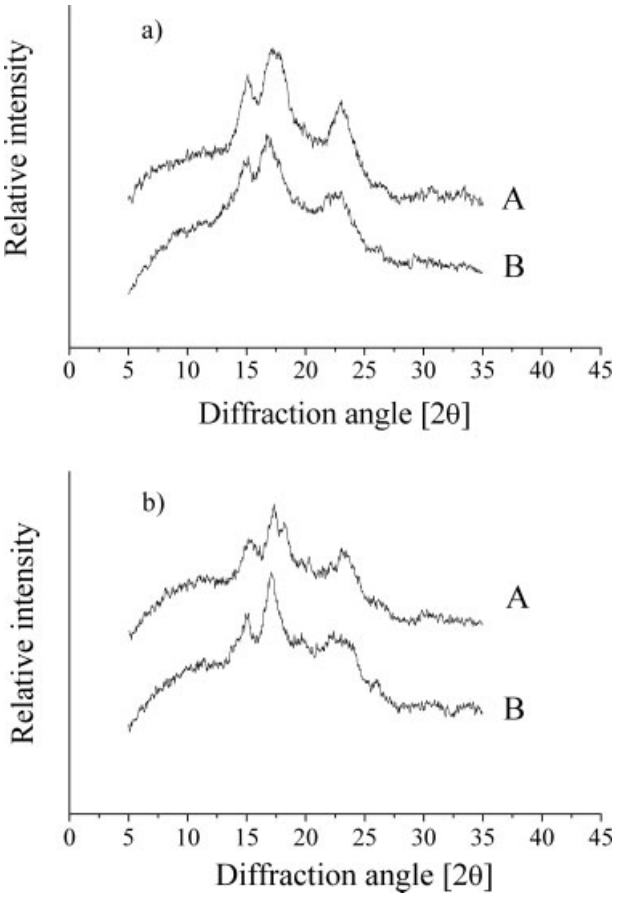

Fig. 3. X-ray diffraction patterns of (a) starch and (b) raw seed flour from (A) *Araucaria angustifolia* and (B) *Araucaria araucana*.

by amylose with a variety of polar organic molecules, as higher fatty acids [14]. Peaks at 2θ = 8, 13.5 and 20° are typical to V-type structure [14, 17].

Fig. 3 shows the X-ray diffraction patterns of isolated starch and seed flour from *Araucaria araucana* and *A. angustifolia*. Even when the doublet at 17° and 18° was not evident in all cases, especially in *A. araucana*, the patterns were compatible with the A-type, with strong peaks at 15°, 17° and 23°.

3.3 Differential scanning calorimetry

Starch heated with sufficient water presents an endothermic peak near 60°C in the thermogram due to starch gelatinisation [18]. In a first series of measurements, starch and raw seed flour from *A. araucana* and *A. angustifolia* seeds were heated in a DSC apparatus with a sample to water ratio $\leq$ 30%. These results are presented in Tab. 2. The gelatinisation enthalpies of *Araucaria araucana* and *A. angustifolia* starch were in the range reported in the literature for other starches, which is between 9 and 18 J/g for canna, mung bean, faba bean, garbanzo bean, lentil, corn, cassava, wheat and potato starches [7, 13, 16, 18, 19]. The enthalpy for starch gelatinisation has been associated with many factors as the molecular architecture of the crystalline region of the starch granules, the amylose/amylopectin ratio, and the size of the granules [13, 16]. However, measurements of gelatinisation enthalpy are not very precise, and results of different authors do not agree with respect to the effect of these factors [18]. No significant differences in the ΔH_{gel} were observed between *Araucaria araucana* and *A. angustifolia*.

The peak temperature of starch gelatinisation (T_p) of *A. angustifolia* was higher than that of *A. araucana* ($P < 0.05$), both for starch and raw seed flour (Tab. 2), whereas the onset temperature for starch gelatinisation (T_o) of *A. angustifolia* was higher than that of *A. araucana* ($P < 0.05$) for the starch samples but not for raw seed flour. Several factors contribute to the starch gelatinisation tempera-

Tab. 2. Onset temperature (T_o); peak temperature (T_p); conclusion temperature (T_c) and enthalpy of gelatinisation (ΔH_{gel}) of raw seed flour and starches from *A. angustifolia* and *A. araucana*. The gelatinisation temperature range (R) was calculated as ($T_c - T_o$). Water content of the samples $\geq$ 70%. Values are the mean $\pm$ standard deviation of at least four determinations.

		T_o [°C]	T_p [°C]	T_c [°C]	R [°C]	ΔH_{gel} [J/g][a]
Flour	*A. angustifolia*	65.1 ± 0.3	72.1 ± 0.4	81.0 ± 0.7	15.9 ± 0.8	10.7 ± 4.0
	A. araucana	64.1 ± 0.4	70.5 ± 0.5	81.4 ± 1.4	17.3 ± 1.1	13.4 ± 3.4
Starch	*A. angustifolia*	63.6 ± 1.1	68.5 ± 1.1	75.8 ± 2.9	12.2 ± 3.0	10.3 ± 2.6
	A. araucana	61.0 ± 1.4	66.6 ± 0.4	73.5 ± 1.2	12.5 ± 2.7	10.7 ± 3.2

[a] dry basis, based on starch weight.

ture, as differences in the granular structure and amylose content, and the presence of other components, such as proteins and sugars [19, 20]. Large granules were associated with lower gelatinisation temperatures [13]. However, as it was discussed earlier, large granules are more abundant in *A. angustifolia* starch, whereas in *A. araucana* starch there are large and small granules. The higher gelatinisation temperature of *A. angustifolia* starch could be attributed to its higher amylose content.

Tab. 2 shows that the starch gelatinisation shifted to higher temperatures in raw seed flour, when it was compared with isolated starch. This is probably due to the presence of other components in the flour, such as proteins and minerals.

In a second series of experiments, starch and raw seed flour were heated with lower amounts of water. These results are shown in Fig. 4. The thermograms of starch from *A. araucana* presented a major peak at 66.6°C, and a minor peak superimposed to the first one at about 71°C. The presence of two peaks could be explained by the heterogeneous starch granules population observed in these seeds. This behaviour was not observed in raw seed flour, in which the presence of other components would mask the two peaks.

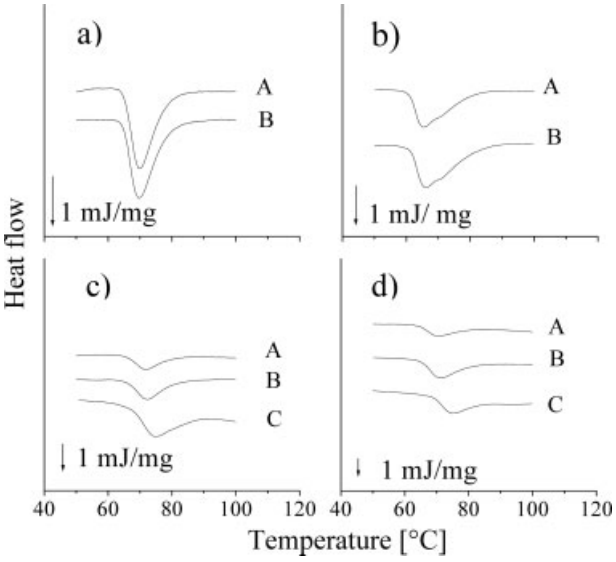

Fig. 4. Differential scanning calorimetry thermograms of (a, b) starch, and (c, d) raw seed flour from (a, c) *Araucaria angustifolia* and (b, d) *Araucaria araucana*. Percent concentrations of starch or flour: (a) A: 29.7, B: 31.3; (b) A: 28.9, B: 31.2; (c) A: 16.0, B: 20.3, C: 47.8; (d) A: 19.7, B: 24.5, C: 47.0.

4 Conclusion

The starch granules of *A. angustifolia* and *A. araucana* are round or slightly oval, with a central hilum. Both starches gave X-ray diffraction patterns compatible with the A-type, with strong peaks at 15°, 17°, and 23°.

The gelatinisation temperature of *A. angustifolia* starch is higher than that of *A. araucana*, probably due to the higher amylose content of the former. On the other hand, the thermograms of *A. araucana* starch present a second peak at a higher temperature, attributed to the fact that the starch granules population of *A. araucana* is heterogeneous, with large and small granules, whereas *A. angustifolia* starch contains mainly large granules.

Further studies will be needed, particularly in relation to starch digestibility, to complete the starch and seed characterization of *A. angustifalia* and *A. araucana.*

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