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Characterization of piñon seed (*Araucaria araucana* (Mol) K. Koch) and the isolated starch from the seed

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Abstract

Araucaria (*Araucaria araucana*) is a conifer tree, endemic of Chile. The seed of this tree, named piñon are composed principally of starch. In this work, piñon seeds and the starch isolated from them were evaluated. The piñon seeds are composed of starch (64%), dietary fibre (25%), total sugar (7%) and very low concentrations of phenolic compounds, lipids, proteins and crude fibre. The process performed to isolate the starch from piñon was simple and easy to realize at laboratory scale, with a yield of 36%. Starch represents a 77% of the isolated starch. The amylose content was 42%. Lipids, protein and crude fibre were very low. The starch hydration properties increased with the temperature. In the 6% suspension of starch, viscosity increases during the cooling period. The starch granules of piñon were small and round shaped. The aspects evaluated in this research, suggests that piñon seeds can be considered an interesting new starch source for the food industry.
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Keywords: *Araucaria araucana*; Piñon seeds; Starch; Technological properties; Functional properties; SEM

1. Introduction

Araucaria (*Araucaria araucana* (Mol) K. Koch) (common name Araucaria or Pehuen in the mapuche language) is a conifer tree, endemic of Chile and Argentina (Hoffmann, 1991). The natural distribution is relatively limited ranging from latitude 37°20′ to 40°20′S (Herrmann, 2005). There are two centres of distribution: the Andes Cordillera, which constitutes the main growing area (37°30′ to 40°02′S) and the coastal Cordillera of Nahuelbuta (between 37°20′ and 38°40′S) (Herrmann, 2005; Hoffmann, 1991).

The seed of this tree, named "piñon" has constituted an important source of carbohydrates for the native people that live in the south of Chile (Pehuenches). Piñon is eaten raw, boiled or toasted. It is also provide the material for the "mudai", a typical alcoholic beverage, used in indigenous ceremonies, and often ground into flour to be used as an ingredient in soups or to make bread (Aagesen, 2004; Cardemil & Reinero, 1982; Herrmann, 2005).

Piñon has oblong and cuneiform shape; and the color of their outer coat is reddish. The size is between 3.5 and 4.5 cm in length; 1.2 and 2.0 cm of diameter and the weight is between 3.5 and 3.8 g. Piñon kernel has a very resistant coat named "testa" and a thin internal membrane named "endopleura". Inside is localized the white endosperm and in the middle of it, the polycotyledon embryo (Estévez, 1993).

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Piñon has low contents of lipids (2.3–2.6%) and proteins (9.6–10.6%) (Schmidt-Hebbel, Pennacchiotti, Masson, & Mella, 1992). However, the starch is the most important compound (60–61% of the dry weight) (Cardemil & Reinero, 1982). It can be easily isolated by treatments with water under mild conditions, without addition of any additive which can be partially explained by the low content of phenolics compounds in the endosperm (Bello-Pérez et al., 2006). These are mainly localized in the internal coat (Cordenunsi et al., 2004). The polyphenols are secondary plant metabolites and major antioxidants of the diet. As antioxidants, the polyphenols may protect cell constituents against oxidative damage, and, therefore different studies have suggested that they play a preventive role in the development of various degenerative diseases, such as cardiovascular disease, various types of cancer and neurological diseases (Arts & Hollman, 2005; Kaur & Kapoor, 2001; Scalbert, Manach, Morand, & Rémésy, 2005). In foods, phenolics compounds are closely associated with the sensory and nutritional quality, contributing directly or indirectly to desirable or undesirable aroma and taste (Imeh & Khokhar, 2002).

Starch is the most important reserve polysaccharide and the most abundant constituent in many plants (Van Hung, Maeda, & Morita, 2006). Besides, it is the only carbohydrate reserve synthesized and stored in the amyloplasts of the storage organs, as well as in other non-photosynthetic tissues of higher plants (Waghorn, del Pozo, Acevedo, & Cardemil, 2003). It is also the most important carbohydrate source for human nutrition and is used extensively as an ingredient of many foods, due to its important thickening and binding properties. Furthermore, starch and it derivatives are utilized in different industrial applications (paper, textile, building materials, pharmaceutical products and chemicals) (Belitz & Grosch, 1999; Jansen, Flamme, Schüller, & Vandrey, 2001). The major components of starch are glucose polymers. Amylose is fundamentally a linear molecule of α-1,4-linked glucans, while amylopectin is a larger molecule with highly α-1,6 branched chains (Srichuwong, Sunarti, Mishima, Isono, & Hisamatsu, 2005; Vandeputte & Delcour, 2004). In addition, to amylose and amylopectin, the starch granules usually contain small amounts of proteins and lipids (Belitz & Grosch, 1999). Commercial starches are obtained from seeds (corn, waxy corn, high amylose corn, wheat and various rices) and from tubers and roots, particularly potato, sweet potato and cassava (Freitas, Paula, Feitosa, Rocha, & Sierakowski, 2004; Takizawa, Silva, Konkel, & Demiate, 2004). However, there is a constant search for new starches presenting different properties due to the continuous need for improving quality of products and processes, as well as, the development of new products (Takizawa et al., 2004). Starch granules are also complex. Their size and shape depends on the botanical source, gene-line variation, stage of development, starch hydrolysis and environment conditions (Fennema, 1985; Waghorn et al., 2003). In this context, the distribution of starch granules in seed of *A. araucana* is different from cereal grain. In this species, starch is stored in amyloplast in the embryos and megagamethophytes. However in cereals, starch is stored in the endosperm (Waghorn et al., 2003).

Besides the starch, piñon of *A. araucana* is a good source of total dietary fibre (Cordenunsi et al., 2004). Dietary fibre consists of a heterogeneous mixture of non starch polysaccharides which include cellulose, hemicellulose, pectins, hydrocolloids and lignin that cannot be degraded by enzymes in the human gastrointestinal tract (Figuerola, Hurtado, Estévez, Chiffelle, & Asenjo, 2005; McKee & Latner, 2000). Dietary fibre plays an important role in the human health due to the prevention, reduction and treatment of some disease such as diverticular disease, colorectal cancer, diabetes, obesity or cardiovascular disease (Escudero & González, 2006).

The literature about the composition of the Chilean piñon is very scarce. According to previous work, and because of their high starch content, which could use for a commercial use, the objectives of this study were to characterize piñon seeds on the basis of the physical and chemical aspects and to evaluate some physical, chemical, morphological and functional characteristics of starch isolated from them, in order to use the piñon as a potential starch source for the food industry.

2. Materials and methods

2.1. Materials

Seeds of piñon (*A. araucana* (Mol) K. Koch) were collected in the Chilean forest of Malalcahuello, latitude 37°5′S (IX Region, Chile), in April 2005. After the collection, the seeds were sorted to eliminate the damaged and of poor qualities ones. Then, the samples were immediately stored at 4 °C until used. At this temperature the piñon remain viable for at least 3 months.

Before, to perform the experiment, the piñon seeds were manually peeled to remove the external coat.

2.2. Analysis of piñon

2.2.1. Proximate composition

The moisture content of the sample was calculated based on weight loss after the sample was heated in oven at 105 °C for 16 h (AOAC, 1990). The ash content was determined by incineration in a muffle furnace at 550 °C for 16 h. Lipids were determined by extraction with petroleum ether in a Soxhlet apparatus (AOAC, 1990). The total protein content was determined by the micro-Kjeldhal method, using 6.25 as conversion factor (AOAC, 1990). The crude fibre was determined gravimetrically after acid hydrolysis with 0.1275 M sulphuric acid and basic hydrolysis with 0.313 M sodium hydroxide (AOAC, 1990). The nitrogen free extract was calculated by the difference between total dry matter and the other components, determined analytically.

2.2.2. Total and reducing sugar contents

These were determined by the method of Munson and Walker (AOAC, 1990).

2.2.3. Dietary fibre

Dietary fibre was determined by the enzymatic–gravimetric methods according to Lee, Proxy, and De Vries (1992).

2.2.4. Total starch

The total starch was determined using the method of Sachse proposed by Winton and Winton (1958). This method is based on the hydrolysis from starch to glucose and the reduction from copper to cupric oxide. The total starch was calculated as glucose × 0.9.

2.2.5. Total phenolics

The total phenol content was determined using the Folin–Ciocalteau reagent and gallic acid as the standard (Sigma Chemical Co.) by the method of Singleton and Rossi (1965). Dried samples of piñon, raw and cooked coat were mixed with 150 ml of extraction solvent (70:30, acetone:water) for 5 min. The extracts were shaken in a water-bath at 20 °C for 90 min. Three 1.5 ml samples were centrifuged at 2500*g* for 15 min at 4 °C and the supernatants were used for the analysis. A 0.50 ml of the extract was mixed with 3 ml of distilled water and 0.25 ml of Folin–Ciocalteu reagent. Immediately after this, 0.75 ml of saturated sodium carbonated and 0.95 ml of distilled water were added with mixing. Then, the mixture was incubated for 30 min at 37 °C in a water-bath, and the absorbance was read at 765 nm using a spectrophotometer (Unicam Heλio α). The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

The cooked coats were obtained by the cooking of piñon seeds with boiling water (1 kg of piñon/2 l), in a pressure cooker (1.57 atm) during 30 min. Then, piñon seeds were peeled and the coats were dried in an oven at 60 °C during 16 h.

2.3. Starch isolation

The starch isolation was performed following the flow sheet proposed by Díaz (1997). The peeled piñon seeds were ground with water (1 kg/2 l) in a blender for 5 min. The homogenate was filtered through a 60 mesh nytal cloth (equivalent to a particle size of 250 μm) with 3 l of tap water. After this, the result of the first grinding was homogenized with the same condition as described previously. This homogenate was filtered through a 270 mesh nytal cloth (equivalent to a particle size of 53 μm). The result of the second grinding was left at ambient temperature for 15 h to decant. The supernatant was discarded and the precipitate was suspended in a solution of sodium hydroxide (0.2%, w/v of water) during 1 h, to solubilize the proteins. The precipitate was centrifuged for 4 min at 5000 rpm to separate the starch from the proteins. The pH of the precipitated starch was adjusted at pH 6.0 with a solution of concentrated hydrochloric acid. The precipitated starch was filtered under vacuum conditions through Whatman No. 4 filter paper, and thoroughly washed with distilled water. The samples of filtered starch were dried in an oven at 60 °C, for 1 h.

2.4. Analysis of the isolated starch

Proximate composition, total and reducing sugar, dietary fibre and total starch were determined with the methods described previously. Yield was calculated as a percentage of starch after the procedure of the starch isolation. Yield is given as: (weight of starch/weight of piñon seeds peeled) × 100 (related to dry matter). Purity was calculated by the difference between the yield of starch and the total content of ash, lipids, protein and crude fibre. The pH was measured in a suspension of starch (10 g) with distilled water (10 ml) using a digital pH-meter (Microprocessor pH Meter 537 WTW). Acidity of starch was determined after homogenizing 10 g isolated starch with distilled water (10 ml). The extract was titrated with 0.1 M sodium hydroxide to pH 8.1, using phenolphthalein as indicator (AOAC, 1990). Acidity was expressed as anhydrous citric acid.

Ionic charge was determined using the method proposed by Schoch and Maywald (1956). The starch (50 mg) was suspended in 25 ml of 0.1% (w/v of water) of methylene blue (positive charged dye) or light green SF (negative charged dye). The suspension was agitated for 10 min, washed with distilled water until the supernatant was colored.

Colour parameters CIELab (L^*, a^* and b^*) were measured in a Minolta (Osaka, Japan) CR-200b tristimulus reflectance colorimeter. Numerical value of a^* and b^* were converted into Hue angle ($H^\circ = \tan^{-1}(b^*/a^*)$) and Chroma ($C = (a^{*2} + b^{*2})^{1/2}$) (McGuire, 1992).

Amylose content was determined by the colorimetric method according to Juliano (1971). The absorbance was read at 620 nm in a spectrophotometer (Thermo Spectronic). The amylopectin content was calculated by difference. Resistant starch content was analyzed by the enzymatic–colorimetric method proposed by Goñi, García-Diz, Mañas, and Saura-Calixto (1996). The resistant starch was calculated as glucose × 0.9.

Viscosity was measured by use of a rotational concentric cylinder viscometer (Hakke VT02). This apparatus measures the apparent viscosity of a starch suspension at certain temperature. The viscosity value (dPa s) of hot paste was determined in three starch suspensions (2, 4 and 6% (w/v) of water) and at seven temperatures (85, 70, 60, 50, 40, 30 and 25 °C). To evaluate the viscosity, the starch suspension was heated to 85 °C and then cooled until 25 °C under room temperature conditions. The temperature of the samples was controlled during the measurement with a precision of ±1 °C.

Gel firmness was evaluated as the resistance to breakdown of a gel cylinder of 25 ml prepared with a 6% (w/v of water) of starch. The gel was heated for 30 min, and then was chilled during 5 h. The gel firmness was measured with the method proposed by Kerr (1944).

2.5. Functional properties of isolated starch

Water retention capacity (WRC), Swelling capacity (SWC) and Solubility index (SI) were evaluated at different temperatures (30, 60 and 100 °C) following the method proposed by Anderson, Conway, Pheiser, and Griffin (1969). Fat adsorption capacity (FAC) was measured at ambient temperature (20 °C), according the method reported by Femenia, Lefebvre, Thebaudin, Robertson, and Bourgeois (1997).

The values reported are the means of four replications. Results were expressed as mean ± MSE (Mean Standard Error).

2.6. Scanning electron microscopy analysis

Scanning electron micrographs were obtained using a Leitz Scanning Microscope, model LEO 420 (Carl Zeiss NTS GMbh, Oberkochen, Gemany), at an accelerating voltage of 15 kV. Piñon seed slices were fixed in a mixture of formaldehyde, acetone and acetic acid in a ratio of 40:4:28:28. After fixing the tissue for at least 72 h, it was dehydrated in an alcohol and acetone battery. Solutions were: 15%, 30%, 50%, 70%, 96%, 100% ethanol; 50%/50% ethanol/acetone and pure acetone. Tissue was treated twice in each solution. After dehydration, tissue was taken to critical point with liquid CO_2. Piñon slices and piñon starch were glued on a holder using a carbon-conducting tape and sputter-coated with a gold film of 5 nm average thickness. Cell size of outer and inner cells of the seed endosperm was measured; also, shape and size of starch granules were determined. The size of cells was expressed as mean ± MSE.

3. Results and discussion

3.1. General

The process performed to isolate the starch from piñon was simple and easy to realize at laboratory scale. The yield of the starch isolation process developed was 35.9%. This value is similar to the reported by Díaz (1997) in *A. araucana* piñon. However, in piñon of *Araucaria angustifolia*, an endemic conifer of southern of Brazil, Wosiacki and Cereda (1985a) and Bello-Pérez et al. (2006) determined that the yield of the starch isolation process was around 21% and 70%, respectively. This difference can be explained because in this research a larger amount of water was used during the homogenization and filtration, than by Bello-Pérez et al. (2006) and by the use of two types of sieves with different opening.

The most difficult step of the process to isolate the starch was the removal of the two coats (testa and endopleura). The first corresponds to approximately 18% of the seed weight and the second is a thin skin adhered to the seed that was eliminated during the wet milling. In addition, results showed that the color and stability of the resultant starch largely depends on the effectiveness to remove the two coats of the piñon. According to Bello-Pérez et al. (2006), the incomplete removal of them at laboratory scale resulted in brownish starch that developed a rancid smell in a short period of time, due to the high content of lipids and phenolics compounds.

The piñon starch isolated was white and odourless. Besides, it was stable for almost one year at room temperature, and did not develope dark color or off-flavour during this time.

3.2. Characterization of piñon seed and isolated starch

3.2.1. Proximate composition

Moisture represented about 43% and 11% of the total fresh weight of peeled piñon seeds and isolated starch, respectively (Table 1). The level of moisture of the piñon seed is in agreement with the amount obtained by Wosiacki and Cereda (1985a), Cordenunsi et al. (2004) and Bello-Pérez et al. (2006) in seeds of *A. angustifolia*. The level of moisture of isolated starch was similar to the values reported by Wosiacki and Cereda (1985a) and Bello-Pérez et al. (2006) in starch from seeds of *A. angustifolia*. The piñon seed had a low content of ash (2.2%), lipids (1.1%), protein (7.8%) and crude fibre (4.5%) (Table 1). The level of lipids and ash is similar to the obtained by Cardemil and Reinero (1982) and Díaz (1997), respectively. However, the content of protein is lower than values reported by Schmidt-Hebbel et al. (1992) and Díaz (1997). But, the level of crude fibre is higher than the values reported by Díaz (1997). The differences could be explained by different ecotypes, growing condition, stage of maturity or postharvest condition. On the other hand, this proximate composition is in agreement with the levels reported by Cordenunsi et al. (2004) in piñon seed of *A. angustifolia*. Besides, these values are comparable to other starchy foods such rice and beans (Cordenunsi et al., 2004).

As the piñon, the isolated starch had a low content of ash (0.05%), lipids (0.3%), protein (0.9%) and crude fibre

Table 1
Moisture (%) and proximate composition (g/100 g dry starch) of peeled piñon seeds and isolated starch

	Piñon seed	Starch
Moisture	42.72 ± 0.28[a]	10.84 ± 0.03
Ash	2.15 ± 0.01	0.05 ± 0.02
Lipids	1.11 ± 0.08	0.34 ± 0.06
Protein	7.81 ± 0.35	0.94 ± 0.10
Crude Fibre	4.51 ± 0.16	1.19 ± 0.09
Nitrogen free extract	84.30 ± 0.61	97.49 ± 0.01

[a] Mean of four replications ± MSE.

(1.2%) (Table 1). The level of protein and lipids are different to those reported in the starch isolated from piñon of *A. angustifolia* by Wosiacki and Cereda (1985a). These authors found the low level of protein in the starch fraction (0.06%), but the fraction of lipids (0.88%) was higher than the values obtained in this experiment. On the other hand, Bello-Pérez et al. (2006) reported that the content of protein and lipids were not detected by the usual methodologies used to analyze approximate composition of foods. However, they confirmed the low content of protein with other techniques that revealed only a 43 kDa major protein. These differences could be attributed to different species studied.

The nitrogen free extract was 84% and 97% in piñon and the isolated starch, respectively (Table 1). These values confirmed the high content of carbohydrates, mainly starch that this seed has. The nitrogen free extract of piñon is higher than the values reported by Schmidt-Hebbel et al. (1992), but it is similar to the value obtained by Díaz (1997).

The low content of the ash, lipids, protein and crude fibre accounts for the efficiency of the extraction and purification obtained with the treatment realized. The purity of the starch isolated was 90%.

3.2.2. Sugar

In the piñon seed, the content of total and reducing sugar was 7.1% and 0.6%, respectively. These values were lower in the isolated starch (traces and 0.03%, respectively) (Table 2). Gas chromatographic analysis of the sugars by Cardemil and Reinero (1982) indicated that there are no free monosaccharides presenting in the seed of *A. araucana*. However, when the samples are hydrolyzed, glucose, fructose and mannose are the only monosaccharides detected.

3.2.3. Starch

Piñon is a good source of complex carbohydrates (Cordenunsi et al., 2004). The starch is the main compound, with 64% of the dry weight (Table 2); this value is similar to the level reported by Cardemil and Reinero (1982); but is lower than the values obtained by Díaz (1997) in *A. araucana* piñon; and by Wosiacki and Cereda (1985a) and Cordenunsi et al. (2004) in *A. angustifolia* piñon (ranged from 87% to 72% of the dry weight). This difference could be attributed to the study of different ecotypes, growing condition, stage of maturity or postharvest condition in *A. araucana* or to the use of different species by Wosiacki and Cereda (1985a) and Cordenunsi et al. (2004). The content of starch in piñon is in the same range as that obtained by Herrera-Saldana, Huber, and Poore (1990), for five commercial cereal grains used in USA (between 58.1% and 75.7% of the dry weight).

Starch represents a 77% of the isolated starch (Table 2); this value is lower to the level reported by Bello-Pérez et al. (2006) for starch from *A. angustifolia* piñon. In addition, the level of starch obtained is lower than the values reported by McCleary et al. (2006) in regular maize starch (95.4%), high amylose maize starch (93.4%) and potato starch (92.2%). This can be explained as due to the different species studied and different methodologies used to determine the content of total starch.

The resistant starch is defined as the sum of starch and products of starch degradation which are not absorbed in the small intestine of healthy individuals (Goñi et al., 1996). It represented 76% of the native isolated starch (Table 2). This value is similar to the reported by McCleary et al. (2006) in potato starch (78.8%), but it is higher than the results reported by these authors in amylomaize starch (native) (50.8%) and amylomaize starch (retrograded) (30.1%).

Typical levels of amylose and amylopectin in starch are 15–30% and 70–85%, respectively (Belitz & Grosch, 1999; Srichuwong et al., 2005). In this experiment, the amylose and amylopectin content of the starch isolated from *A. araucana* piñon was 42% and 58%, respectively (Table 2). The amylose content is higher than the values founded by Wosiacki and Cereda (1985a) and by Bello-Pérez et al. (2006) in starch from *A. angustifolia* piñon (22.25% and 25%, respectively). The differences could be explained by the use of different species of *Araucaria*. Besides, this value is higher than the amylose contents reported by Srichuwong et al. (2005) and Takizawa et al. (2004) in other botanical sources of starches, such as corn (23.4–24.5%), potato (18.0–25.4%), cassava (17.9–18.6%) and rice (13.2%). The variation in amylose contents among the starches from different and similar plant sources may also be attributed to the different starch isolation procedures and analytical methods used to determine amylose content (Singh, Singh, Kaur, Singh Sodhi, & Singh Gill, 2003). The different amylose/amylopectin ratios led to differences in granular structure, physicochemical properties and quality of end-use products (Van Hung et al., 2006).

3.2.4. Total dietary fibre (TDF)

TDF represented about 25.4% of the dry weight of peeled piñon seeds (Table 2). This value is higher than

Table 2
Content of sugar, dietary fibre and starch (g/100 g dry starch) of peeled piñon seeds and isolated starch

	Piñon seed	Starch
Sugar		
Total	7.10 ± 0.002[a]	Traces
Reducing	0.59 ± 0.01	0.03 ± 0.01
Dietary fibre		
Total	25.43 ± 1.99	5.30 ± 0.25
Insoluble	22.58 ± 2.61	3.91 ± 0.23
Soluble	2.85 ± 0.62	1.39 ± 0.48
Starch		
Total	63.67 ± 2.55	77.17 ± 1.01
Resistant	ND	76.29 ± 1.39
Amylose	ND	42.1 ± 2.58
Amylopectin	ND	57.9 ± 2.58

[a] Mean of four replications ± MSE. ND: not determined.

the value found by Cordenunsi et al. (2004) (approximate 10% of the dry weight) in *A. angustifolia*. This difference could be attributed to different species studied. Most of the TDF content of piñon corresponds to insoluble dietary fibre (IDF) (22.6%) in comparison with the content of soluble dietary fibre (SDF) (2.9%). The IDF/SDF ratio was 7.9:1 that is similar to the proportion obtained by Cordenunsi et al. (2004) (6.8:1).

Piñon seed had 89% and 11% of insoluble and soluble dietary fibre, respectively. These values are in agreement with the percentage obtained by Cordenunsi et al. (2004) (87% of insoluble and 13% of soluble dietary fibre). However, this ratio is not considered a well balanced proportion for physiological purposes, because a 70–50% of insoluble and 30–50% of soluble dietary fibre, is required to complement each other (Gorinstein et al., 2001).

The content of TDF in the isolated starch was 5.3% (Table 2). The content of insoluble and soluble dietary fibre was 3.9% and 1.4%, respectively; and the IDF/SDF was 1:2.8. The proportion of insoluble and soluble dietary fibre in the piñon starch was 74% and 26%, respectively. These values were lower, than the values obtained for piñon seed. However, according to Gorinstein et al. (2001), this ratio was considered a well balanced proportion.

3.2.5. Phenolic compounds

These are commonly found in both edible and non edible plants, and they have been reported to have multiple biological effects, including antioxidant activity (Kähkönen et al., 1999). Phenolics contents vary among different species, cultivar and within different tissues (Imeh & Khokhar, 2002). In this context, the total phenol in the peeled piñon seeds was 0.71 ± 0.02 mg GAE/g DW, and in the raw coat was 66.34 ± 1.89 mg GAE/g DW. The piñon seeds presented a very low content of phenolics in comparison to the seed coats. This is an agreement with the reported by Cordenunsi et al. (2004). This can be explained for the removal of the peel, because generally this fraction is one of the major sources of natural antioxidants in seeds and fruits, and may potentially contain more antioxidant quantitatively or qualitatively than the other fractions; for example, the peel of apple, pear and peaches contain about two times higher polyphenolic content than their peeled fruit (Gorinstein et al., 2002). Or depending on cultivar, the peel of apples contains about two to six times more phenolic contents than their pulp (Wolfe, Wu, & Liu, 2003). In low concentration, phenolics may protect food from oxidative deterioration (Imeh & Khokhar, 2002).

The concentration of total phenolics in piñon is higher than the values reported by Cordenunsi et al. (2004) in raw and cooked piñon of *A. angustifolia* (0.23 and 0.11 mg catechin equivalent/g fresh sample, respectively) and by Kähkönen et al. (1999) in cereals grain, ranging from 0.2 mg GAE/g dry weight (wheat) to 0.4 mg GAE/g of dry weight (barley). On the other hand, this content is lower than the values obtained by Wu et al. (2004) in common nuts in the United State, ranging from 0.68 mg GAE/g (pine nuts) to 20.16 mg GAE/g (pecans). This difference could be attributed to different species studied and the type of solvent extraction used, because aqueous acetone extraction have shown to be slightly superior to aqueous methanol in extracting phenolics in some fruits such as berries and apples (Kähkönen, Hopia, & Heinonen, 2001).

It is well known that many food antioxidants can be significantly lost as a consequence of food processing, due to the fact that most of the compounds are relatively unstable. However, it was observed that the cooked coat presented more content of total phenols (73.91 ± 1.58 mg GAE/g DW) than the raw coat (66.34 ± 1.89 mg GAE/g DW). Unlike this study, Cordenunsi et al. (2004) observed that the total phenolics of cooked peeled piñon are lower than the raw peeled piñon; however, in normal condition of cooking (piñon with the coats) there was a migration of phenolics from the seed coat into the seed, and as result the white seed become brownish in their surface. In contrast, the results showed an increase of 12% in the total phenolics of cooked coats and this could be explained by the liberation of phenolics from the matrix or other cell components; for example, during processing, complex phenolic compounds, such as tannins and lignins, are subjected to hydrolysis, which lead to the release of lower molecular weight phenolics (Al-Farsi, Alasalvar, Morris, Baron, & Shahidi, 2005). Besides of the disruption of cell walls as a result of the thermal processing, there is also a release of oxidative and hydrolytic enzymes that can destroy the antioxidants in fruits and vegetables, but the use of high temperatures inactivates these enzymes to avoid the loss of phenolics acids (Dewanto, Wu, Adom, & Liu, 2002).

3.3. Other chemical and physical characteristics

In general, most foods have pH values in the range between 4 and 7 (Fennema, 1985). The pH of the piñon starch was 5.9 (Table 3). This value is similar to that the pH reported by Takizawa et al. (2004) in tropical starches such as cassava (5.9), potato (5.8) and sweet potato (5.7); and by Perez-Sira (1997) in corn starch (5.6). The acidity of the piñon starch was 0.03% (Table 3). This value is similar to obtained by Perez-Sira (1997) in corn starch.

Table 3
Chemical and physical characteristics of isolated starch

pH	5.87 ± 0.05[a]
Acidity (%)	0.03 ± 0.002
Ionic charge	Anionic
Colour	
L^* value	92.03 ± 0.37
a^* value	0.77 ± 0.07
b^* value	4.68 ± 0.72
Hue angle	80.05 ± 0.43
Chroma	4.03 ± 0.13
Gel firmness (g/cm^2)	8.20

[a] Mean of four replications ± MSE.

The granules of piñon starch were stained only with a positively charged dye (methylene blue) and it was observed the absence of any staining with a negatively charged dye (light green SF). This reaction indicated that the starch was anionic (Table 3). This characteristic would be associated to the presence of phosphate groups, proteins or anionic lipids (Gray & BeMiller, 2004; Takizawa et al., 2004; Wosiacki & Cereda, 1985a). This result is similar to the reported by Wosiacki and Cereda (1985a) in starch isolated from *A. angustifolia* and in corn starch. The ionic charge of the isolated starch could indicate its potential use in the formulation of foods (Schoch & Maywald, 1956). According to Bergthaller (2004), there is a relation of phosphate group concentration in potato starch and its quality characteristics to be used in food application, such as gelatinisation, paste formation and viscosity.

The color of the isolated starch from piñon was almost white (L about 92%); a^* parameter was near to cero, and the coordinate b^* indicate a slight yellow color (Table 3). This could be explained for the use the sodium hydroxide to isolate the starch, because it could solubilize some tannins of the testa, which give the yellow color into the starches. The Hue angle and the Chroma values obtained were 80 and 4.0 (Table 3), respectively, which indicated that the isolated starch was of light yellow color and dull.

The starch isolated from piñon formed translucent and white color gels. The gel firmness is mainly caused by retrogradation of starch gels, which is associated with the syneresis of water and crystallization of amylopectin, leading to harder gels. Starches that exhibit harder gels tend to have higher amylose content and longer amylopectin chains (Sandhu & Singh, 2007). In the piñon starch the gel firmness was 8.2 g/cm^2 (Table 3). This value is similar than the obtained by Díaz (1997) in starch isolated from piñon of *A. araucana*.

3.4. Functional properties

The hydration properties of starch refer to its ability to retain water within the matrix. The starch granules are insoluble in cold water, but they can reversibly imbibe water and swell slightly due to the presence of a larger numbers of hydroxyl groups. However, as the temperature is increased, the starch molecules vibrate more vigorously, breaking intermolecular bonds and allowing their hydrogen-bonding sites to absorb more water molecules (Fennema, 1985).

As shown in Table 4, water retention capacity (WRC) of the starch ranged between 2.4 g water/g of dry starch at 30 °C and 6.1 g water/g of dry starch at 100 °C. The WRC increased 250% as the temperature increased. Wosiacki and Cereda (1989) determined that the piñon starch of *A. angustifolia* showed higher capacity of cold water absorption compared with that of cassava and corn starches.

According to Takizawa et al. (2004), the swelling capacity (SWC) and solubility of starch granules showed a great evidence of interaction of the starch chains between the amorphous and crystalline regions. SWC of starch was between 2.5 ml water/g of dry starch in 30 °C and 6.3 ml water/g of dry starch in 100 °C (Table 4). The swelling values increased when the temperature increased due to the increase of water mobility and the diffusion of water, which allows bulk water absorption (Bello-Pérez et al., 2006). In this context, the SWC increased in 255% with the increase of temperature. The values of SWC were similar to those reported by Wosiacki and Cereda (1989), in their study of hydration of the granules of starch isolated from piñon of *A. angustifolia*. But they are lower than those obtained in the same species by Bello-Pérez et al. (2006). And, if it is considered one common source of starch, the SWC of various corn starches was higher, ranging from 20.7 to 13.7 (g water/g dry starch) (Sandhu & Singh, 2007). This could be related to different species, different amylose: amylopectin ratio, starch isolation method and methodology used for determining swelling values.

Table 4
Hydration properties and oil absorption capacity of isolated starch

	Temperature (°C)		
	30	60	100
WRC (g water/g dry starch)	2.44 ± 0.11[a]	2.55 ± 0.10	6.10 ± 0.38
SWC (ml water/g dry starch)	2.45 ± 0.11	2.57 ± 0.10	6.25 ± 0.40
SI (g water/100 g dry starch)	0.27 ± 0.01	0.59 ± 0.05	2.38 ± 0.23
FAC (g oil/g dry starch)	1.89 ± 0.01		

WRC: water retention capacity; SWC: swelling capacity; SI: solubility index; FAC: fat absorption capacity.
[a] Mean of four replications ± MSE.

The solubility index (SI) was between 0.3% in 30 °C and 2.4% in 100 °C (Table 4). As expected, the SI increased by 883% with the increase of temperature. When starch is submitted to heating in excess of water, there is a relaxation of the crystalline structure and the groups of amylose and some amylopectin chains associate with water molecules through hydrogen bonds, being responsible for the increasing value during starch gelatinization (Bello-Pérez et al., 2006; Takizawa et al., 2004). Wosiacki and Cereda (1989) and Bello-Pérez et al. (2006) reported a similar pattern to that determined in this study for piñon seed starch, but the solubility indices were higher than those obtained in this experiment. In addition, if it is considered one common source of starch, the solubility of various corn starches was higher, ranging from 9.7% to 15% (Sandhu & Singh, 2007).

According to Figuerola et al. (2005), fat absorption capacity (FAC) depends on the surface properties, overall charge density, thickness and hydrophobic nature of the particle. The value determined was 1.9 g of oil/of dry starch at room temperature (20 °C) (Table 4).

3.5. Viscosity

When starch is heated in excess water, its granules are swollen and at the same time, granule components are solubilized, giving rise to a swollen and dispersed particle

suspension in a continuous phase (Bello-Pérez et al., 2006). The volume and morphology of starch dispersion play an important role in the rheological behaviour (Sandhu & Singh, 2007). One way to monitor gelatinization, as a function of temperature, is to measure the viscosity of a starch suspension (Belitz & Grosch, 1999). The viscosity results from the flow resistance of the enlarged granules that occupy the entire sample volume (Fennema, 1985). The viscosity of the starch paste increased during the cooling period. This indicates a tendency of various constituents present in the hot paste (swollen granules, fragments of swollen granules, colloidally- and molecularly-dispersed starch molecules) to associate or retrograde as the temperature of the paste decreases (Belitz & Grosch, 1999; Singh et al., 2003). As shown in Fig. 1, the viscosity increased upon cooling with the decrease of the temperature, probably due to the reorganization of linear chains (mainly amylose) of the starch that were solubilized during heating. That process produces a higher number of cross-links between the molecule strands, forming a network which retains a high amount of water molecules (Bello-Pérez et al., 2006). Changes in the physical properties vary considerably, depending on the composition of the starches in terms of amylose and amylopectin, the granular structure, molecular weight of each component, intensity of internal stabilizing bonding forces, extension of crystalline and amorphous zones, the temperature used, and the water content of the suspension (Belitz & Grosch, 1999; Wosiacki & Cereda, 1985b). As shown in Fig. 1, 2% (w/v) starch suspension, did not undergo changes, whereas the 6% suspension of starch (w/v) presented the highest viscosity, which increased significantly upon cooling at 40 °C.

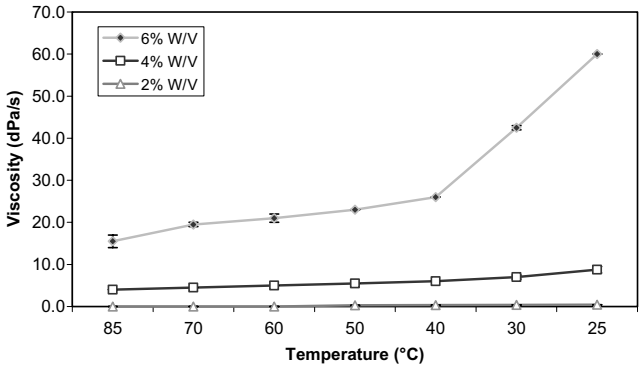

Fig. 1. Viscosity (dPa s) of three dispersions of starch paste under cooling.

In addition, the viscosity of the starch solutions depends also on the kinds and amount of other constituents present (Fennema, 1985). For example, residual lipids in the starch fraction may substantially modify the rheological behaviour of the pastes (Wosiacki & Cereda, 1985a).

The starch isolated from piñon formed thick, cohesive, translucent, and colorless pastes. This starch could be used as an ingredient in many foods, because it has the ability to form thick pastes (Fennema, 1985).

3.6. Microscopic studies

Microscopic observation of the cells of *A. araucana* piñon allowed the determination of their size distribution. In this research, the dimensions of the outer and inner cells were 60.51 ± 5.87 and 140.74 ± 8.67 μm, respectively (Fig. 2). These values are similar to the dimensions reported by Cardemil and Reinero (1982) in the inner cell ($177.3 + 15$ μm).

The size and shape of the starch granules is diverse and species specific (Srichuwong et al., 2005). The granule size is variable and ranges from 1 to 110 μm (Singh et al., 2003). The size found here was 14.61 ± 1.05 μm (Fig. 3). The morphology of starch granules depends on the biochemistry of the chloroplast or amyloplast, as well as physiology of the plant (Singh et al., 2003). The starch granules of piñon were small, round shaped and the surface was smooth with no irregularities or erosion and most of them showed a flat surface on one side. This value is in agreement with Cardemil and Reinero (1982) and Waghorn et al. (2003), who found the dimensions of the starch granules of *A. araucana* were 13.5 ± 2.8 and 13.80 ± 3.20 μm, respectively. In addition, Bello-Pérez et al. (2006) determined that the granules size of *A. angustifolia* piñon was

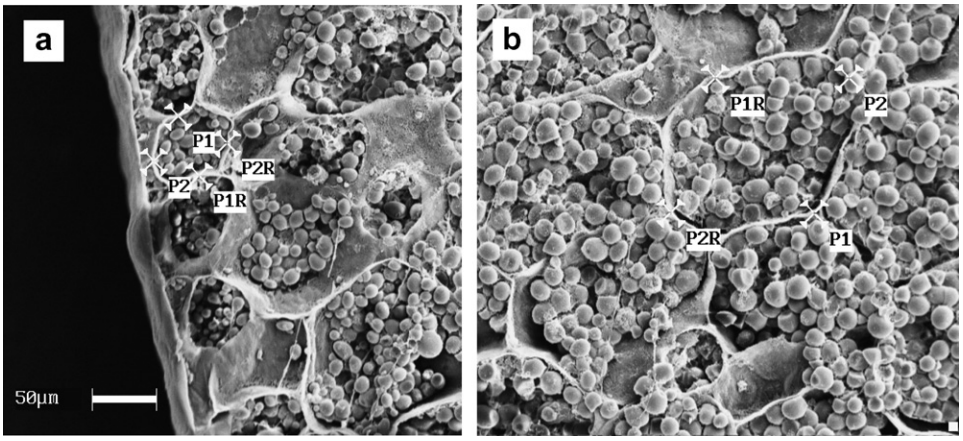

Fig. 2. Scanning electron micrographs of cells of *Araucaria araucana* piñon: (a) outer cells and (b) inner cells.

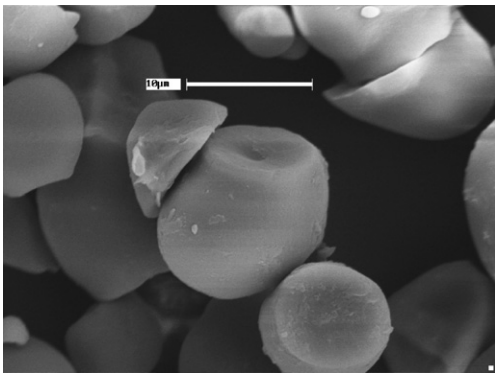

Fig. 3. Scanning electron micrographs of starch granules of *Araucaria araucana* piñon.

between 10 and 25 μm. On the other hand, the size determined in this research is similar to the values reported by Srichuwong et al. (2005) and Belitz and Grosch (1999) in different plant origins. According to Bello-Pérez et al. (2006), the absence of irregularities on the surface, could be an interesting characteristic, because channels and pores of corn starches granules are believed to affect starch reactivity when it is modified by chemical treatment, as well as its physicochemical and functional properties. Also, it is interesting to point out, that the isolation process caused some damages in some granules and it was very effective in separating other compounds different to starch.

4. Conclusions

The isolation of starch from *A. araucana* piñon is a simple and easy process to realize at laboratory scale. It could be attractive for a pilot plant and also for commercial production. The low contents of ash, lipids, protein and crude fibre account for the efficiency of the extraction and highest purification obtained with the treatment used. This aspect and the physical, chemical, nutritional, functional and microscopic properties, suggests that piñon seeds can be considered an interesting new starch source that could be useful in the food industry to produce, to develop or to replace starches that are currently used in different food products.

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