Carbohydrate Polymers 101 (2014) 920–927

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Isolation and characterization of starch obtained from *Brosimum alicastrum* Swarts Seeds

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ARTICLE INFO

Article history:
Received 5 August 2013
Received in revised form 3 October 2013
Accepted 4 October 2013
Available online 12 October 2013

Keywords:
Ramon
Brosimum alicastrum swartz
Starch
Carbohydrate

ABSTRACT

In this paper, the *Ramon* starch was isolated and its chemical composition and physical and microscopic characteristics were determined. Corn starch was used as reference. In general, the proximal composition was similar between starches studied. *Ramon* starch granules were oval–spherical and rounded with sizes between 6.5 and 15 μm. Starch purity was high (92.57%) with amylose content of 25.36%. The gelatinization temperature was 83.05 °C and transition enthalpy was 21.423 J/g. At 90 °C, solubility was 20.42%, swelling power 17.64 g water/g starch and water absorption capacity was 13 g water/g starch. The pH, clarity and color (Hue angle) of *Ramon* starch were higher to those reported for corn starch. The results achieved suggest that *Ramon* starch has potential for application in food systems requiring high processing temperatures and it is also a promising option for use in the manufacture of biodegradable materials.

1. Introduction

Starch is a renewable and biodegradable carbohydrate polymer from a great variety of crops. It is a raw material with a wide variety of applications ranging from its use in the food industry in order to enhance texture and consistency, to the manufacture of paper, adhesives and biodegradable packaging (Ming et al., 2011). Starch has been widely studied due to its availability and a combination of other factors such as price, abundance, easy degradability and extensive use in food products and non-food products (Flieger, Kantorová, Prell, Razanka, & Votruba, 2003; Siracusaa, Rocculib, Romanib, & Dalla Rosa, 2008).

In the food industry, starch is the principal component of many food formulations and it is responsible for important functional properties and nutritional characteristics while, in non-food industries, the development of biodegradable polymers has received much attention from the scientific community, as a consequence, it has resulted in the development of new biodegradable products (Mosab, Kotiba, & Fawaz, 2012). Biodegradable polymers are an alternative to overcome the problems related to fossil resources, recycling limitations, and the global environment.

Recently, a number of biodegradable polymers have been developed which originate from renewable natural resources (Flieger et al., 2003; Mosab et al., 2012). Among these polymers, starch obtained from renewable vegetable resources such as potato, corn, mango, banana etc. has been considered one of the most promising materials (Bello-Pérez, Aparicio-Saguilán, Méndez-Montealvo, Solorza-Feria, & Flores-Huicochea, 2005; Lopez, Lecot, Zaritzky, & Garcia, 2011). However, the increasing demand for starch in basic food products and in the manufacture of biodegradable materials is having a strong impact on the supply of these natural resources for public consumption. In this sense, current starch research is focused on searching for non-conventional starch sources which do not compete with human consumption and which can be used as raw material for industrial processes.

In this regard, one alternative recognized for its availability in the tropics, as a possible source of starch is *Ramon* seed (*Brosimum alicastrum* sw.). A number of reports have commented that corn and *Ramon* seeds were the most important food sources for the Mayan people in the Classic Period (Burns, Mosquera, & Withmre, 1998; Gillespie, Bocanegra-Ferguson, & Jimenes-Osornio, 2004; Puleston, 1968). Traditionally, the fruit was collected to be eaten raw; juices and jams were also elaborated and the seeds were ground to form dough which was mixed with corn to make tortillas (Bailey & Whelan, 1961).

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http://dx.doi.org/10.1016/j.carbpol.2013.10.012

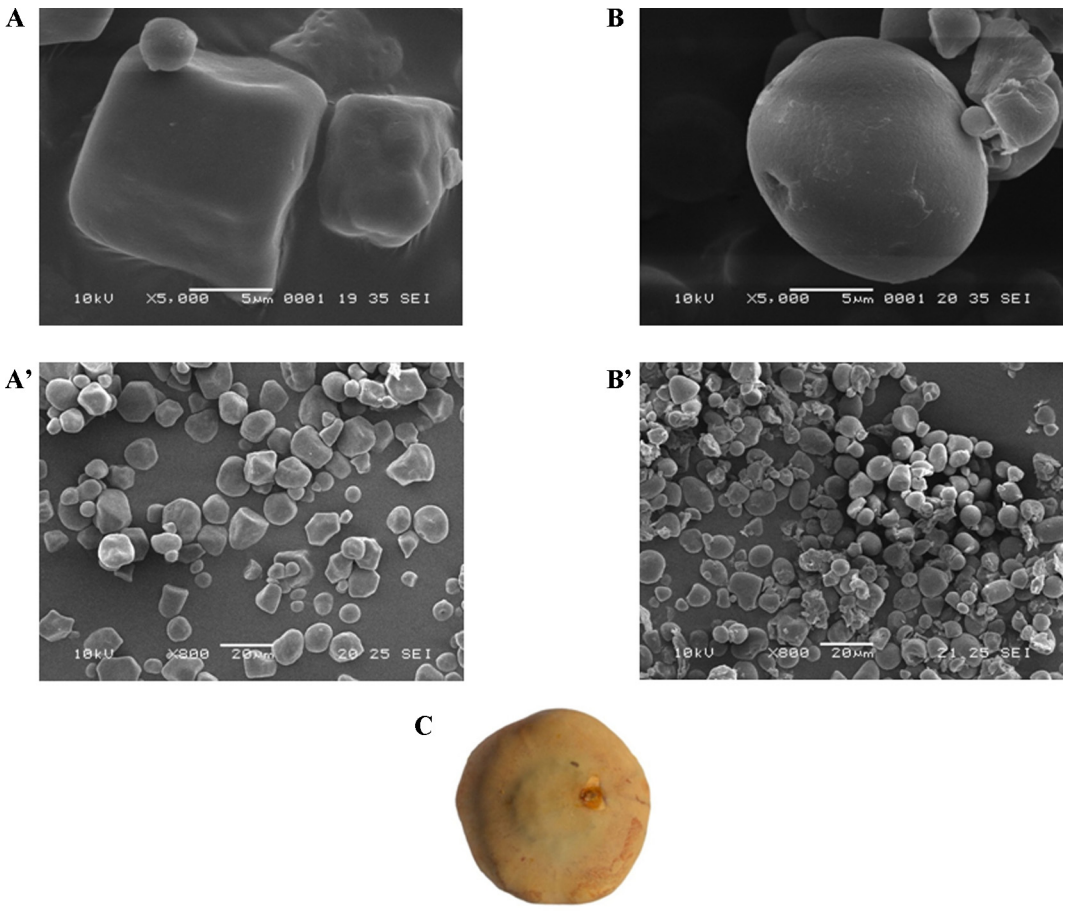

Fig. 1. Scanning electron microscopy (SEM) images. A and A′ corn granules, B and B′ *Ramon* granules, C *Ramon* seed.

Brosimum alicastrum sw., goes by more than 50 commonly recognized names, of which the most important in Latin America are: *Ramon* (from the Spanish verb *ramonear* (to browse) which refers to the consumption of its leaves and seeds by cattle and other domestic animals), Ojite, Ojoche and Capomo. It is an important component of the perennial and semiperennial tropical forest of southern Mexico and is very adaptable, growing in humid areas and in arid regions; it usually flowers in November and February and the fruit falls in March and June. The fruit of the *Ramon*, is round in shape with an average diameter of 2–2.5 cm; the color is green to greenish-orange with a pleasant sweet taste. The seed measures approximately 1.5–2 cm in diameter (Riley, Wheatley, & Asemota, 2006).

Since in Mexico, there are not reports describing the use of the seeds of *Ramon*, as a non-conventional starch source, the aim of this work is to isolate and characterize *Ramon* seed starch as an alternative for application in the industrial sectors. Nowadays, this seed is rarely used for human consumption among the population of the Yucatan Peninsula.

2. Materials and methods

2.1. Materials

Fruits of the *Ramon* tree were collected in the northern region of the State of Campeche, located in the Peninsula of Yucatan, in the southeast of Mexico. At the moment of harvesting, the fruits presented an average weight of 6.56 ± 0.09 g and an average diameter of 2.44 ± 0.67 cm. Reagent grade corn starch (Sigma–Aldrich) was used as reference.

2.2. Obtention of flour

Ramon fruits were selected based on physiological maturity, characterized by a change in color; from green to yellow-orange.

The testa was removed manually to obtain the seeds (see Fig. 1C) which were then dried in a convection oven (SHELL LAB 1350FX-10) at 70 °C for 72 h, after which they were stored in a desiccator until the milling process. Once sufficient material was obtained, the seeds were milled in a commercial blender (Osterizer®), ground in an IKA MF-10 grinder (0.5 mm sieve) and sifted through 100-mesh screen to produce flour. Finally, the resulting flour was stored in hermetically sealed glass containers until use, in order to avoid moisture absorption.

2.3. Starch isolation

Starch extraction was carried out following the technique described by Betancur, Chel Ancona, Guerrero, Camelo Matos, and Ortiz (2001), with a few modifications. Five hundred grams of *Ramon* seed flour was mixed with 5 L of sodium bisulfite (0.1%, w/v) and left to sit for 12 h; pH was adjusted to 10 with NaOH 1 N and the mixture was left to sit for a further 30 min at room temperature. The suspension was then filtered through plastic cloth (100-mesh) strainers to separate the residue (fiber) from the liquid substance (proteins and starch); the residue was subjected to the extraction process once again in order to obtain the maximum amount of starch.

The fiber residue was dried in an oven at 60 °C for 24 h. Once the material had reached room temperature, it was weighed and ground in an IKA MF 10 grinder with a sieve size of 0.5 mm. The

resulting powder was stored in hermetically sealed glass containers until further use.

The suspension (protein and starch) was sifted once again through 200-mesh screen and the liquid fraction was left to precipitate for 30 min and the supernatant removed with a siphon. The remaining liquid was washed three times by re-suspension in distilled water and the starch recovered after the final wash by centrifuging at 2500 rpm for 10 min in an Eppendorf centrifuge (model 5702-R). After isolation the starch was dried in a convection oven at 60 °C for 24 h, ground in an IKA MF-10 grinder with a sieve size of 0.5 mm and sifted through 100-mesh screen. Finally, the starch was stored in hermetically sealed glass containers until subsequent analysis.

2.4. Proximal composition

Proximal chemical composition of extracted *Ramon* starch, corn starch, fiber residue and raw flour of *Ramon* seeds was determined in triplicate in accordance with AOAC methods for moisture (925.10), ash (923.03), proteins (920.87) and lipids (920.39) (AOAC, 1997). Crude fiber content was determined by acid-alkali digestion (Tejeda, 1992) and total carbohydrate as nitrogen-free extract (NFE) was calculated by difference.

2.5. Dietary fiber (DF)

An enzymatic-gravimetric procedure, AOAC Method 991.43, was used for the determination of DF contents (AOAC, 1997). In brief, starch samples were first gelatinized with heat stable α-amylase (Sigma–Aldrich, Germany). After gelatinization, the samples were digested with protease and amyloglucosidase (Sigma–Aldrich, Germany) to remove protein and starch in the samples. Subsequently, insoluble dietary fiber (IDF) was filtered and washed with warm distilled water. The filtrate and washed water were combined and added with four volumes of 95% ethanol to precipitate the soluble dietary fiber (SDF). The residues were weighed after drying at 105 °C in a hot air oven. Determination of residual protein (as Kjeldahl N × 6.25) and ash contents was carried out for corresponding corrections. Total dietary fiber (TDF) was calculated as the sum of IDF and SDF.

2.6. pH determination

A starch–water dispersion at 1% (w/v) at room temperature was prepared, pH was determined using a Metrohm 827 potentiometer.

2.7. Color evaluation

Starch sample was measured with a Minolta portable colorimeter CR-200 (Minolta Co. Ltd., Osaka, Japan). Three starch samples were selected and placed in a Petri dish before testing. The parameters '*L*', '*a**' and '*b**' were measured and the final results were expressed as hue angle (Eq. (1)) (McGuire, 1992).

$$h = \mathrm{Tan}^{-1}\frac{b*}{a*} \quad (1)$$

2.8. Amylose content

Apparent amylose content was determined using the method described by Ratnayake, Hoover, Shahidi, Perera, and Jane (2001) and by Hoover and Ratnayake (2002). Quantification of amylopectin was calculated by difference at 100% of amylose content using the colorimetric method (Morrison & Laignelet, 1983).

2.9. Granule morphology

The size and shape of starches were examined by a scanning electron microscopy (SEM). Starch samples were mounted on a metallic slide and the examination was performed with a scanning electron microscopy JEOL JSM 6360 LV electron probe microanalyzer at 15 kV in low vacuum.

2.10. Functional properties of starch

2.10.1. Solubility, swelling power and water absorption capacity

Solubility, water absorption and swelling power patterns at 60, 70, 80 and 90 °C were determined using a modified version of Sathe and Salunkhe (1981) method. Briefly, 40 ml of a 1% starch suspension (w/v) was prepared in a previously tared, 50 ml centrifuge tube. A magnetic agitator was placed into the tube, and it was kept at a constant temperature (60, 70, 80 or 90 °C) in a water bath for 30 min. The suspension was then centrifuged at 2500 rpm for 15 min, the supernatant was decanted and the swollen granules weighed. From the supernatant, 10 ml were dried in an air convection oven (SHELL LAB 1350FX-10) at 120 °C for 4 h in a crucible to constant weight. Solubility and swelling power were calculated with Eqs. (2) and (3), respectively.

$$\%\,\mathrm{Solubility} = \frac{\mathrm{Weight\ of\ solid\ solubles\,(g)}}{\mathrm{Weight\ of\ sample\,(g)}} \times 100 \quad (2)$$

$$\mathrm{Swelling\ power} = \frac{\mathrm{Weight\ of\ gel\,(g)}}{\mathrm{Weight\ of\ sample\,(g)} - \mathrm{Weight\ of\ solid\ soluble\,(g)}} \times 100 \quad (3)$$

Water absorption capacity was measured using the same conditions as above, but was expressed as weight of the gel formed per sample, divided by treated sample weight.

2.10.2. Starch gel clarity

Starch gel clarity was measured using the method of Bello-Perez, Agama-Acevedo, Sánchez-Hernández, and Paredes-López (1999). Starch suspensions (1%) in tubes with threaded caps were placed in a water bath at 100 °C for 30 min, agitated by vortexing every 5 min and left to cool to room temperature. Percentage of transmittance (*%T*) was determined from these suspensions at 650 nm using a spectrophotometer UV-Vis Perkin Elmer Lambda 11.

2.10.3. Refrigeration and freezing stability

In order to evaluate stability under refrigeration and freezing, pastes were prepared in a Brabender viscoamylograph. Briefly, 400 ml of 6% starch suspension was heated to 95 °C at a rate of 1.5 °C/min, held at this temperature for 15 min, then cooled to 50 °C at the same rate and held at this second temperature for another 15 min. Portions of 50 ml were placed in centrifuge tubes, cooling down to room temperature and stored at 4 °C and −20 °C. These were centrifuged at 4000 rpm for 10 min in a centrifuge (Eppendorf centrifuge) and measurements taken of water separation from the starch gels at 24, 48, 72, 96 and 120 h. The syneresis rate was calculated with Eq. (4) (Eliasson & Ryang, 1992).

$$\%\,\mathrm{Syneresis} = \frac{\mathrm{Weight\ of\ syneresis\ water\,(g)}}{\mathrm{Weight\ of\ gel\,(g)}} \times 100 \quad (4)$$

2.11. Total starch

Total starch was determined using the starch assay kit (Sigma–Aldrich), which is based on the hydrolysis of starch to glucose catalyzed by α-amylase and amyloglucosidase. Glucose is oxidized to gluconic acid and hydrogen peroxide by glucose oxidase. Hydrogen peroxide reacts with *o*-dianisidine in the presence of peroxidase to form a colored product.

Table 1
Proximal composition of the samples analyzed.

Starch	Moisture (%)	Ash (%)	Lipids (%)	Crude fiber (%)	Proteins (%)	Nitrogen-free extract (%)
Ramon	7.49 ± 2.26	0.47 ± 0.05	0.47 ± 0.04	1.27 ± 0.91	0.12 ± 0.03	90.16 ± 1.57
Corn	8.14 ± 1.94	0.02 ± 0.01	0.49 ± 0.04	1.24 ± 0.72	0.03 ± 0.03	90.07 ± 1.20
Raw flour	3.72 ± 0.69	3.41 ± 0.03	10.49 ± 1.67	5.21 ± 0.65	2.02 ± 0.06	75.13 ± 2.21
Fiber residue	5.70 ± 0.89	3.47 ± 0.08	3.58 ± 0.65	8.14 ± 0.42	10.81 ± 0.04	68.27 ± 0.51

Values are expressed as mean ± standard deviation ($n = 3$).

Oxidized *o*-dianisidine reacts with sulfuric acid to form a more stable colored product. The intensity of the pink color measured at 540 nm is proportional to the original glucose concentration. The analyses were performed according to the instructions supplied with the kits.

2.12. Differential scanning calorimetry (DSC)

Starch gelatinization was determined with a DSC-6 (Perkin–Elmer Corp., Norwalk, CT). Approximately 1 mg of starch was accurately weighed into an aluminum sample pan. Water (3 μl) was added with a microsyringe to obtain a starch:water ratio of 1:3 (w/w) in the DSC pans, which were sealed before analysis. The pans were heated from 25 to 110 °C, with the temperature increased at a rate of 10 °C/min, while the sample chamber was flushed with dry nitrogen to avoid moisture condensation. An empty aluminum pan was used as the reference. The onset (T_o), peak (T_p) and conclusion (T_c) temperatures were recorded. The enthalpy change of the thermal transition (ΔH_{gel}) was estimated by integrating the area between the thermogram and a base line under the peak and was expressed as Joules per gram dry weight of starch.

3. Results and discussion

3.1. Morphology of starch granules

Microphotographs of granules of corn starch (a) and *Ramon* starch (b) are shown in Fig. 1. The starches under study presented different shapes; corn starch granules were polygonal while those of the *Ramon* starch were oval–spherical; these characteristics of the starch granules are similar to the starches of the Sago and potato, respectively (Hernández-Medina, Torruco-Uco, Chel-Guerrero, & Betancur-Ancona, 2008).

Granule size was heterogeneous, with an average diameter value of 15 μm, ranging from 3 to 26 μm for corn starch and an average value of 10.8 μm, ranging from 6.5 to 15 μm for *Ramon* starch. These values were similar to those reported for a variety of starches such as, corn, cassava, makal, sweet potato and potato, with an average diameters ranging from 10.6 to 33 μm (Hernández-Medina et al., 2008; Medina & Salas, 2008).

3.2. Proximal composition

The proximal composition of *Ramon* starch, corn starch, raw flour obtained from *Ramon* seeds and the fiber residue resulting from starch extraction is shown in Table 1. *Ramon* starch and corn starch showed a similar proximal composition with respect to moisture content (7.49% and 8.14%, respectively), lipids (0.47% and 0.49%, respectively), crude fiber (1.27% and 1.24%, respectively) and nitrogen-free extract (90.16% and 90.07%, respectively), while *Ramon* starch obtained higher values of ash (0.47%) and proteins (0.12%) in comparison with corn starch (0.023% for ash and 0.03% for proteins). The moisture content of the starches under study was <10%, which is acceptable (Wolfgang, Detmold, & Hans-Peter, 1999), because commercially up to 20% of moisture is allowed in starch as raw material.

In addition, ash and protein values for the starches under study were found in lower proportions. The low protein and ash content of the starches indicates high purity. Because of its low (0.12%) protein content, *Ramon* starch can be used in the manufacture of high-glucose syrups as this protein level is lower than FDA protein content limits for corn starch (0.4%) used for this purpose.

The proximal composition of the starches evaluated in this paper was similar to those reported for maca root starch (Rondán-Sanabria & Finardi-Filho, 2009), makal starch (Torruco-Uco & Betancur-Ancona, 2007), chayote tuber starch, corn starch, potato starch (Jimenez-Hernández, Salazar-Montoya, & Rámos-Ramírez, 2007) and Parota seed starch (Jimenez-Hernández et al., 2011). It is important to note that the chemical composition of these starches may be dependent on the botanical source and on the process of procurement and purification.

In addition, the raw flour, from which the starch was obtained, showed high lipid values (10.49%), while the residue obtained from the extraction of *Ramon* starch presented higher contents of crude fiber (8.14%) and proteins (10.81%). These results indicate that the raw flour and fiber residue could be used in the elaboration of food supplements for both human and animal consumption.

3.3. Physicochemical characteristics

Table 2 shows the physicochemical characteristics of *Ramon* and corn starches. From these results, it is possible to notice that the apparent amylose content of *Ramon* starch was similar, without significant difference, to that of corn starch and in consequence, the same can be said of the amylopectin content; both starches also contain amylose and amylopectin values similar to those reported for pigmented maize (Agama-Acevedo, Ottenhof, Farhat, Paredes-López, Ortíz-Cereceres, & Bello-Pérez, 2005) and to those reported for *Trichosanthes kirilowii* starch (Maa, Changb, Zhengc, Yud, & Mad, 2010). The ratio of these two components is important given the functional properties they provide; amylose is responsible for the formation and stability of the gels while amylopectin provides

Table 2
Physicochemical characteristics of the starch samples analyzed.

Parameter	*Ramon* starch	Corn starch
Amylose (%)	25.36 ± 2.37	27.33 ± 0.56
Amylopectin (%)	74.64 ± 2.37	72.67 ± 0.56
Amylose/amylopectin ratio	1:2.94	1:2.65
Total starch (%)	92.57 ± 2.89	98.86 ± 0.47
Starch yield (g kg^{-1} dry weight)[a]	300 ± 2.03	–
pH	9.1 ± 0.14	5.92 ± 0.02
Clarity (% transmitance at 650 nm)	12.13 ± 1.53	7.53 ± 1.17
Total dietary fiber (%)	1.30 ± 0.01	1.55 ± 0.09
Soluble fiber (%)	1.15 ± 0.01	1.40 ± 0.15
Insoluble fiber (%)	0.15 ± 0.02	0.15 ± 0.05
L^*	86.0 ± 0.09	95.8 ± 0.08
a^*	0.36 ± 0.015	−0.57 ± 0.005
b^*	12.6 ± 0.15	5.56 ± 0.03
Hue angle	88.34 ± 0.04	95.89 ± 0.05

Values are expressed as mean ± standard deviation ($n = 3$).
[a] Values based on seeds weight without testa.

viscosity (Billiaderis, 1991). Amylose/amylopectin ratio of the starches evaluated was 1:2.94 and 1:2.65 for *Ramon* starch and corn starch, respectively, which was similar to that found in starch obtained from 10 potato cultivars (Alvani, Qui, Tester, & Snape, 2011), indicating the same predominance of amylopectin. Starches with an amylopectin high content could form gels with a low trend to retrogradation (BeMiller, 1993).

The *Ramon* starch yield, based on seeds weight without testa, was 300 g kg^{-1} dry weight. *Ramon* starch also showed an average purity of 92.57%, which was slightly lower in comparison with that of corn starch (98.86%). These purity values for both of the starches evaluated herein were higher than those found for Chayote tuber starch (Jimenez-Hernández et al., 2007), indicating that the *Ramon* seed can be considered as a novel non-conventional source of high purity starch.

Furthermore, *Ramon* starch presented an alkaline pH value of 9.1, whereas the corn starch was acid (5.9). In relation to this point, it has been reported that a high pH favors the degree of ionization in the amylose and amylopectin chains (Pérez, Lares, & González, 1997), thus it is achievable to infer that *Ramon* starch presents a high degree of ionization. The degree of ionization affects hydration behavior in starches by facilitating the interaction between water molecules and amylose and amylopectin chains (Jimenez-Hernández et al., 2011).

Ramon starch was isolated using the alkaline steeping (NaOH 1 N), which influenced the *Ramon* starch showing a high value of pH. The pH value obtained from the starch *Ramon* is comparable to those reported by Jimenez-Hernández et al. (2007) for a new starch from Chayote tuber (pH = 8.12). It is also important to mention that, it might be insufficient the amount of water washing in order to obtain a *Ramon* starch with values close to neutrality.

With respect to the clarity of starch pastes evaluated, the transmittance values (%*T*) obtained for *Ramon* starch (12.13%) demonstrated that it is more transparent than corn starch (7.53%) (Table 2). A similar value was reported for the sago starch (Bello-Perez et al., 1999; Hernández-Medina et al., 2008) and for banana (Hoover, Swamidas, Kok, & Vasanthan, 1996).

Ramon starch showed greater swelling power and in consequence higher paste clarity in comparison with corn starch. It is important to note that clarity is a key parameter in starch paste quality as it provides luminosity and opacity to the color of the product (Torruco-Uco & Betancur-Ancona, 2007).

Dietary fiber is defined as the endogenous material of the cell wall which is not digested by the gastrointestinal secretions of humans (Chau & Cheung, 1998). In this regard, corn starch presented higher dietary fiber content (1.55%) in comparison with *Ramon* starch (1.30%); similarly, corn starch also obtained a higher amount of soluble fiber (1.40%). These results are an indication that both of the starches evaluated herein are characterized by a greater content of soluble dietary fiber, compared to insoluble, due to their high degree of purity. Dietary fiber values found in this study are comparable to those reported for *Canavalia ensiformis* starch (Novelo-Cen & Betancur-Ancona, 2003).

Ramon starch also exhibited a slightly yellow-white color, while corn starch was white. Isolated *Ramon* starch, therefore, showed a low lightness L^* value (86.0) compared to the reference, corn starch (95.8). Based on these results for lightness L^*, in the starch samples analyzed, the value of L^* for corn starch corresponds to a lighter shade of white in comparison with *Ramon* starch. The values of a^* and b^* obtained for corn starch were different from those found in *Ramon* starch. Results show that *Ramon* starch obtained greater values of b^* (12.6) than corn starch (5.56), indicating that *Ramon* starch presents yellow tones, with a hue angle of 88.34 corresponding to a slightly yellow-white color. The results found in the present study are similar to color parameters reported for chestnut starch (Cruz, Abraao, Lemos, & Nunes, 2013).

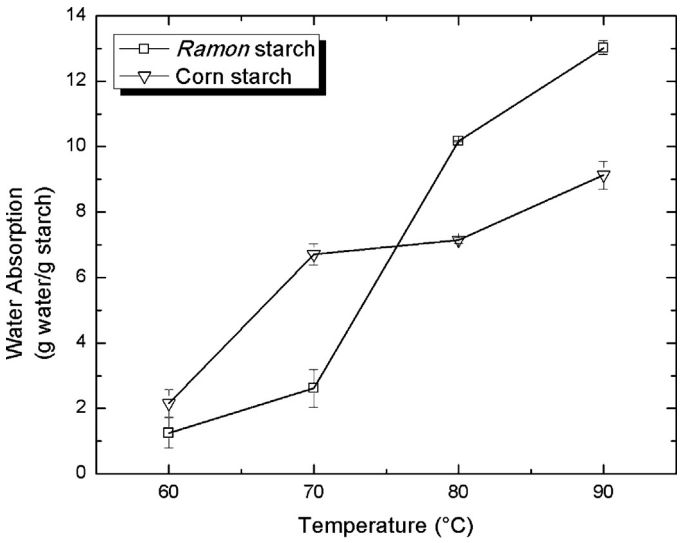

Fig. 2. Water absorption of *Ramon* and corn starches.

3.4. Functional characterization

When an aqueous suspension of starch granules is heated, these structures are hydrated and swelling takes place. Water absorption capacity corresponds to the amount of water that the starch granule is capable for absorbing and the swelling faculty is related to the ability for retaining such water. Solubility index indicates the level of degradation of the polymers contained in the starch granule (Ruales, Valencia, & Nair, 1993). Swelling power, water absorption capacity and solubility of studied starches are directly correlated to the increment in temperature. From these results we can appreciate that, during the interval from 60 °C to 70 °C, *Ramon* starch granules absorbed less water in comparison with corn starch granules (Fig. 2), due to their high gelatinization temperature (Hernández-Medina et al., 2008); the effect was reversed as the temperature continued to rise from 70 °C to 90 °C. Moreover, *Ramon* starch absorbed water at a greater rate of change compared to corn starch, probably due to the rupture of intermolecular hydrogen bond in the amorphous areas, thereby facilitating irreversible and progressive water absorption (Lii, Shao, & Tseng, 1995).

Swelling power and solubility can be used to assess the extent of interaction between starch chains, within the amorphous and crystalline domains of the starch granule (Ratnayake, Hoover, & Warkentin, 2002). Fig. 3 shows the results of swelling power in the starches evaluated. As can be seen, *Ramon* starch presented greater swelling power (17.64 g water/g starch), while corn starch showed a swelling power of 11.04 g water/g starch. These results are similar to those reported by Hoover and Ratnayake (2002) for starches of chick pea (18.2 g water/g starch) and smooth pea (18.5 g water/g starch). The starches evaluated in this study showed similar values at 60 °C and both starches reached their maximum swelling power values at 90 °C. This hydrothermal behavior is related to a number of factors such as, stability, starch granule at a certain temperature value, size distribution, lipid content, as well as content and length of amylose and amylopectin chains (Beynum & Roels, 1985; Crosbie, 1991). *Ramon* starch also presented a smaller granule size, which facilitated a more effective interaction between granules and water molecules, in comparison with the corn starch granules. This behavior provides a partial explanation as to why the *Ramon* starch granules swell more rapidly.

Corn starch presented lower values of solubility (Fig. 4). It is also appreciated that the solubility increases in response to a corresponding increase in temperature. This increase is produced at

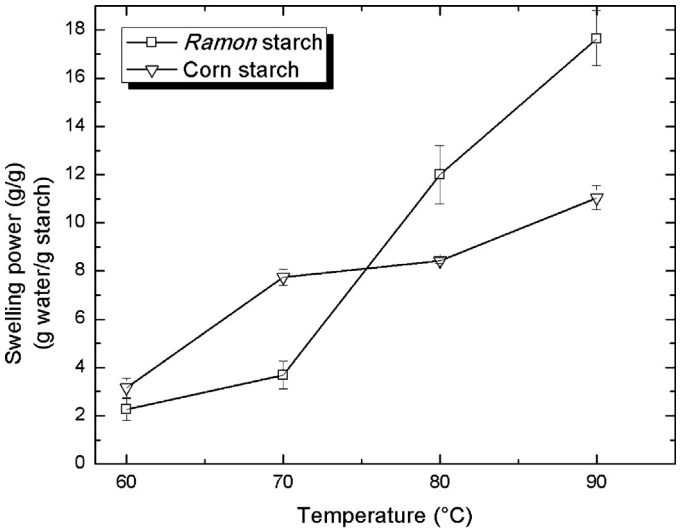

Fig. 3. Swelling power pattern of *Ramon* and corn starches.

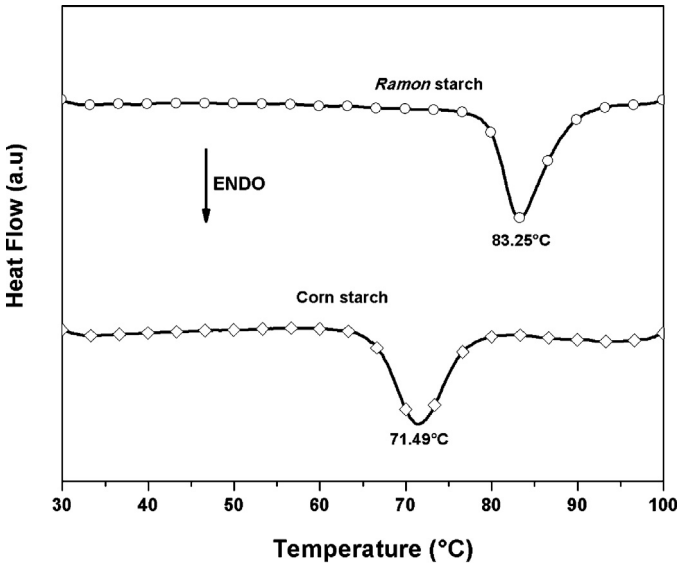

Fig. 5. DSC thermogram of corn and *Ramon* starches.

a greater rate of change for *Ramon* starch than for corn starch and is due to the fact that the swollen starch granules allow exudation of amylose (Gujska, Reinhard, & Khan, 1994). The differences in solubility of the studied starches could largely be due to structural differences. Differences in chain length distributions in the starches cause differences in solubility (Bello-Pérez, Contreras-Ramos, Jiménez-Aparicio, & Paredes-Lopez, 2000). Granular size also affects solubility of the starches. The smaller the granule size, the higher the solubility (Tian, Rickard, & Blanshard, 1991). This could also probably explain the higher solubility of *Ramon* starch, as they had smaller sized granules compared to corn starch.

3.5. Freeze–thaw stability and syneresis

Results of stability in the starches studied under conditions of refrigeration and freezing are shown in Table 3. The evaluation of refrigeration and freeze–thaw stability consists in verifying the expulsion of water (syneresis) contained in gels as a consequence of the reorganization of starch molecules (Ovando-Martínez, Bello-Pérez, Whitney, Osorio-Díaz, & Simsek, 2011). In general, as the storage period advanced, both starches showed high syneresis

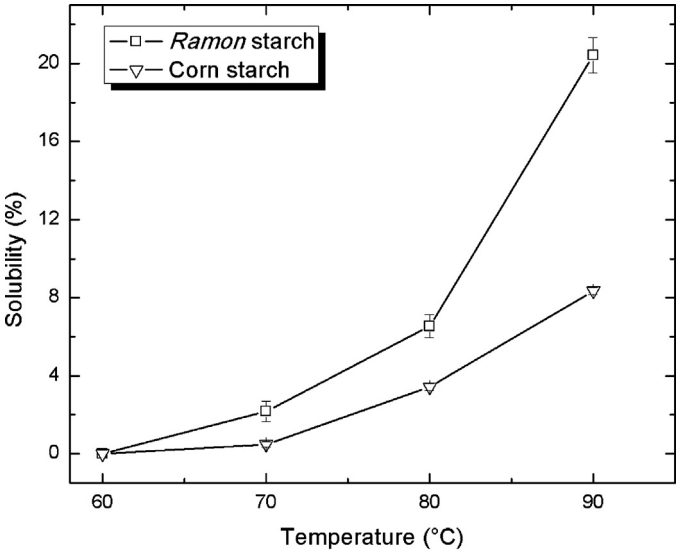

Fig. 4. Solubility pattern of *Ramon* and corn starches.

under refrigeration (4 °C) and freezing (−20 °C); however, *Ramon* starch did present greater syneresis in comparison with corn starch. These results, therefore, indicate the low refrigeration and freeze–thaw stability of both starches studied.

In relation to this, Thoufeek Ahamed, Singhal, Kulkarni, and Mohinder (1996), mention that, when starches are subjected to successive freezing cycles, their structure is affected by the redistribution and dilution of starch paste caused by ice crystal growth and dissolution. According to the literature, starches with high amylose content such as potato (20.1–31.0%), maize (22.4–32.5%), taro (28.7–29.9%) and cassava (18.6–23.6%) present high syneresis, due to the large amount of water expelled during the retrograding process (Gunaratne & Hoover, 2002; Sing, Sing, Kaur, Sodhi, & Gill, 2003).

3.6. Differential scanning calorimetry (DSC)

The gelatinization temperatures (onset, T_o; peak, T_p; and conclusion, T_c), enthalpy of gelatinization (ΔH_{gel}), peak height index (PHI) and gelatinization temperature range (Gel_{TR}) for corn and *Ramon* starches, measured using DSC are presented in Table 4.

Significant difference was observed in T_o, T_p and T_c among starches from corn and *Ramon*. The lowest T_o, T_p and T_c of 64, 71 and 80 °C, respectively, were observed for corn starch, whereas *Ramon* starch showed the highest value for the same (Fig. 5). As shown in Table 4, the peak of gelatinization temperature (T_p) of corn and *Ramon* starches were found to be 71.08 °C and 83.05 °C, respectively. The gelatinization temperature for *Ramon* starch is higher than those reported for corn (62.4, 66.3 and 72.9 °C) (Betancur et al., 2001) potato (60, 69 and 80 °C) and white sweet potato starches (66.7, 70.7 and 74.8 °C) (Osundahunsi, Fagbemi, Kesselman, & Simón, 2003) starches. These variations are influenced by various factors including the composition of the granule of starch (amylose:amylopectin), structure (relation crystalline:amorphous), shape and size, the molecular structure of amylopectin (bunch size, length chains) and the content of other components such as proteins, lipids and phosphorus (Gunaratne & Hoover, 2002; Noda, Takahata, Sato, Ikoma, & Mochinda, 1996; Singh & Singh, 2001; Singh, Kaur, & Singh, 2004; Yuan, Thompson, & Boyer, 1993).

The gelatinization enthalpy (ΔH_{gel}) is indicative of the loss of molecular order (crystalline region) which occurs in the starch granules during gelatinization. The ΔH_{gel} values obtained for the

Table 3
Refrigeration and freezing stability of *Ramon* starch compared to corn starch.

Time (h)	Syneresis to refrigeration 4 °C (%)		Syneresis to freezing −20 °C (%)	
	Corn starch	*Ramon* starch	Corn starch	*Ramon* starch
24	56 ± 1	76.33 ± 0.58	70.33 ± 0.58	72.33 ± 0.58
48	61.67 ± 0.58	75.67 ± 0.58	74.33 ± 0.58	72 ± 1.0
72	68 ± 1	77.67 ± 0.58	76.0 ± 1.0	74.33 ± 0.58
96	69.67 ± 0.58	79.67 ± 0.58	76 ± 1.0	75 ± 1.0
120	73.67 ± 0.58	80 ± 1.0	78.0 ± 1.0	76.33 ± 0.58

Values are expressed as mean ± standard deviation ($n = 3$).

Table 4
Thermal properties of corn and *Ramon* starch.

Starch	Parameters					
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{gel} (J/g)	GEL_{TR} (°C)	PHI (J/g °C)
Corn	64 ± 1	71.08 ± 0.5	80 ± 1	14.89 ± 0.5	14.16	2.10
Ramon	75 ± 1	83.05 ± 0.5	95 ± 1	21.42 ± 0.5	16.10	2.66

T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH_{gel}, enthalpy of gelatinization; GEL_{TR}, gelatinization range $2(T_p - T_o)$; PHI, peak height index $\Delta H_{gel}/(T_p - T_o)$.

corn and *Ramon* starch were 14.893 J/g and 21.423 J/g, respectively. There is a significant difference between them, indicating that the *Ramon* starch requires more energy to gelatinize. The difference in ΔH_{gel} reflects melting of amylopectin crystallites. The variations in ΔH_{gel} could represent differences in bonding forces between the double helices that form the amylopectin crystallites, which resulted in different alignment of hydrogen bonds within starch molecules (McPherson & Jane, 1999).

PHI, a measure of uniformity in gelatinization, was found to be the lowest for corn (2.10) starch, whereas it was found to be the highest for *Ramon* (2.66).

The values Gel_{TR} obtained show that there is a higher content of crystalline phases present in the *Ramon* (16.19 °C) in comparison with corn starch (14.16 °C). This implies that it is necessary a larger amount of heat to melt all the crystalline phase in *Ramon* starch. These observed changes may be influenced by intrinsic factors such as type of starch granule size and by extrinsic factors such as heating rate, moisture content, mechanical damage of the granules, the thermal history of the sample and the conditions extraction of starch, among others.

4. Conclusions

The chemical composition and functional properties of starch from *Ramon* seed, a non-conventional source, suggest it may have numerous possible uses as an ingredient in food systems and other industrial applications.

Ramon seeds without testa produced a starch yield of 300 g kg^{-1}. The extracted *Ramon* starch showed a high degree of starch purity (92.57%), a granule size ranging from 6.5 to 15 μm and an oval–spherical-shaped granule. A high degree of ionization was obtained (pH 9.1) and the amylose/amylopectin ratio was 1:2.94, indicating a predominance of amylopectin which results in a low tendency to retrograde. The thermal properties presented by *Ramon* starch allow assuming that it possesses longer chains of amylopectin molecules and a greater arrangement of double helices than corn starch, indicating a higher degree of molecular structure and thus the need for more energy to initiate fusion. Raw flour and fiber residue resulting from *Ramon* starch extraction showed high contents of crude fiber (8.14%) and proteins (10.81%). Such materials could be employed in the elaboration of food supplements.

The results of this study suggest that *Ramon* starch could be used in food systems requiring high processing temperatures and that it is a promising material for application in the manufacture of biodegradable materials.

Acknowledgement

The authors would like to express their gratitude to the Program PROMEP-SEP, for the financial support to the project and to the Mexican Council for Science and Technology (CONACYT) for the provision of Grant 60204/CM0042.

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