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Original article

Composition and food properties of *Pachyrhizus ahipa* roots and starch

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Summary The aims of this work were to evaluate productivity and postharvest handling conditions for ahipa roots; to establish a starch extraction procedure and to characterise the morphological, physicochemical and pasting properties of ahipa starch. Plants were grown in Misiones (Argentina), evaluating three planting densities and the application of a flower pruning (FP) practice. FP increased roots yield without modifying the functional properties of the starch. Storage assays allowed to rule out refrigeration and select 25 °C as an appropriate temperature. Starch extraction yield was 56.54 g 100 g^{-1} roots (dry basis). Amylose content was 10.75%. The starch diffractograms were assigned to a type C pattern. Ahipa starch pastes gelatinised at relatively low temperature and showed low retrogradation tendency under refrigeration conditions. Rheological properties indicated that ahipa starch might be adequate as a food thickener. Thus, native ahipa starch seems to be an alternative to other traditional starch sources.

Keywords Ahipa starch, leguminous tuberous roots, long-term storage, postharvest handling, starch physicochemical properties.

Introduction

The extraction of starch from agricultural commodities and its subsequent processing is one of the most important agroindustries worldwide. Soetaert & Vandamme (2009) have reported that the estimated world production of starch as feedstock was fifty-five million tons per year and its indicative world market price reached 250 euro per ton. From the total annual starch production, approximately 67% corresponds to cereals and 33% to roots and tubers (Burrell, 2003). Roots and tubers have the advantage of supplying large quantities of dietary energy and stable yields under conditions in which other crops may fail (Scott *et al.*, 2000). Although underground plant organs have generally low respiration rates and are considered relatively non-perishable, relevant postharvest losses may arise from physical damage during harvesting and inappropriate storage conditions that favour water loss and decay. Postharvest losses also occur due to specific infection by biotic agents such as fungi, bacteria, insects and nematodes (Opara, 2003).

Another relevant issue is that, according to Piyachomkwan *et al.* (2002), the portfolio of commercial starch sources does not cover the entire spectrum of potential starch functionality. The diversity of both composition and physical parameters gives rise to different processing properties and therefore many end-uses for starch (Burrell, 2003). Amylose content is one of the characteristics that determine starch functional properties, especially when low retrogradation tendency is required. Low-amylose native starches are scarce, being cassava one of the most widely spread sources. Other physicochemical properties of starch that give rise to different end-uses are: the ability to swell, which is required in bread making; the emulsifying power, which is appreciated in salad dressing making; and the moisture binding capacity required in baking powder production, amongst several uses. Likewise,

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gelatinisation, pasting, solubility, clarity or opacity and viscosity of starch usually affect its application in food industry.

Leguminous plants are usually grown for their protein-rich seeds, forming the second most important group of crop plants after cereals (Forsyth *et al.*, 2002). Some leguminous plants also produce tuberous roots, including species of the genus *Pachyrhizus*, which is native to Southern and Central America. *P. ahipa* (ahipa) is still being grown in a very small scale in the Andes of Bolivia and Northern Argentina (Ørting *et al.*, 1996; Sørensen, 1996; Sørensen *et al.*, 1997; Forsyth *et al.*, 2002; Leidi *et al.*, 2003).

In spite of its potential in different industrial areas, ahipa starch has received little attention. Results referred to physicochemical characteristics of this starch are diverse (Forsyth *et al.*, 2002) and those relative to its pasting properties are very scarce. Likewise, few published studies on *P. ahipa* have adopted an integrated approach, including agronomic studies, root characteristics analysis, storage conditions assessment and extraction and characterisation of the starch.

Accordingly, this study has three aims: (1) to evaluate productivity and postharvest management for ahipa roots; (2) to establish a starch extraction procedure; and (3) to characterise ahipa starch from a morphological and physicochemical point of view, as well as its pasting properties.

Materials and methods

Plant material

Pachyrhizus ahipa (Wedd.) Parodi plants were grown at the INTA Montecarlo farm (Misiones, Argentina; 26° 33′ 40.15″ Latitude South and 54° 40′ 20.06″ Longitude West). Ahipa seeds (Pach-01 Dr Grau) from Perico (Jujuy, Argentina) were used. The crop was sown on 27th September 2006. Three different plant densities were assayed. In the experimental plots, planting distances were 0.5, 0.25 and 0.125 m, leading to plant densities of 40 000, 80 000 or 120 000 plants ha^{-1}, respectively. The effect of flowers removal to reduce sink competition and to increase root yield was also evaluated. At blooming, manual pruning of all flowers was applied to half the plant population of each treatment. Flower pruning (FP) was carried out at four opportunities: 105 days after sowing (DAS); 117 DAS; 141 DAS and 154 DAS. Roots were harvested on March 2007 when senescence started, (~193 DAS).

Chemical composition of ahipa roots

Harvested ahipa roots were investigated for dry matter content at 60 °C (air oven method), crude fat (Soxhlet), crude fibre and ash content, following the AOAC standard methods (1990). Total nitrogen (N) was analysed applying the technique of Kjeldahl (AOAC, 1990) and then crude protein content was calculated (N × 6.25). Reported values (% on a dry basis) corresponded to the mean ± standard deviation.

Storage assays

Ahipa roots were received at the laboratory and processed in bulk immediately. Roots were washed in tap water until removal of any soil rest and then they were dried at room temperature. Wounded or unhealthy roots were discarded.

In order to evaluate the incidence of physical treatments on quality aspects of ahipa roots, samples of twenty-five roots each were subjected to a heat shock (HS) treatment or they were UV-C irradiated. Physical treatments conditions were selected according to results of previous screening tests. For HS application, the product was heated in an oven, at 60 °C for 35 min. Eventually samples were allowed to cool at room temperature before packaging. For UV-C treatment, both sides of roots were exposed to radiation (254 nm) from four UV-C lamps (TUV G30T8, 30W, Philips), at a dose of 15 kJ m^{-2}. The flux intensity of lamps was measured with a digital radiometer (Cole-Parmer Instrument Company, Vernon Hills, IL, USA).

After treatments application, roots were covered with PY8® film and placed in slotted plastic trays (43 cm × 33 cm × 10 cm). PY8® film is a microperforated polyolefin (~3% of the total area perforated; thickness: 15 μm; water vapour permeability: 300 g m^{-2} day^{-1}). Trays were stored at 25 °C for 5 months. Three trays were used for each treatment. The same number of trays containing ahipa roots without any treatment were stored in the same conditions and analysed as controls (C). Also, controls trays were stored at 4 or 10 °C for one month in order to assess the incidence of refrigerated storage on ahipa roots quality.

Colour was measured with a colorimeter (Konica Minolta CR 400, Osaka, Japan) calibrated with a standard white plate ($Y = 93.2$, $x = 0.3133$, $y = 0.3192$), using illuminant C. The instrument is designed in diffusion illumination 0° viewing angle geometry (specular component included). Readings were conducted by directly applying the colorimeter head on the equatorial cut section of at least fifteen different roots, for each treatment and sampling point. Coordinates L^*, a^* and b^* of the CIE scale were recorded and Chroma values [$C = (a^{*2} + b^{*2})^{1/2}$] were calculated.

Likewise, texture profile analysis (TPA) of ahipa roots was performed. Cylindrical samples (2.5-cm thick and 1.7 cm in diameter) were cut from the core of the roots and compressed 4 mm between flat plates using a TAXT2i Texture Analyzer (Stable Micro Systems Ltd,

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Godalming, Surrey, UK). A 25 kg load cell was employed. The probe, a 75 mm diameter aluminium plate, was operated in the compression mode at a constant speed of 1.0 mm s^{-1}. Hardness, springiness, cohesiveness and resilience were quantified (Andrés *et al.*, 2006).

For phytopathological analysis, roots were cut into small pieces, disinfected by immersion in 10% NaClO for 3 min and washed with sterile distilled water. Pieces were placed on Petri dishes containing 2% potato dextrose agar (PDA) as suitable medium. Five replicates of five Petri dishes each were incubated for 5–7 days at 25 °C. A total of 125 pieces per treatment were evaluated. Identification of fungi was carried out based on their morphological features using light microscope examination, the analysis of the characteristics of the colonies on PDA and the conidial structures, following the bibliography of reference (Booth, 1971; Ellis, 1971, 1976; Nelson *et al.*, 1983; Sivanesan, 1987; Barnett & Hunter, 1998). The incidence of the predominant genus was estimated as the percentage of total isolates.

Measurements of colour, texture and phytopathological analysis were carried out at the beginning and end of the storage periods.

Starch extraction and physicochemical characterisation

For these procedures, samples from trials with or without FP were analysed separately. The detailed starch extraction process is shown in Fig. 1.

Amylose content (%) was determined spectrophotometrically at 635 nm, as described in a previous work (López *et al.*, 2008). The morphology and size distribution of starch granules were studied by scanning electron microscopy (SEM) with a JEOL thirty-five CF electron microscope (Japan) (López *et al.*, 2008). Starch powder was analysed in an X-ray diffractometer X′Pert Pro PAnalytical Model PW 3040/60 (Almelo, The Netherlands) provided with a tube with a copper anode, a detector operated at 40 kV and 40 mA. Diffractograms were obtained from $2\theta = 3°$ to $2\theta = 60°$ to acquire information about the crystalline structure of the granules. The crystallinity degree (CD) was calculated as the ratio between the absorption peaks area and the diffractogram total area, and expressed as percentage (%).

Gelatinisation of starch suspensions

Swelling capacity of starch granules

Starch aqueous suspensions (5% w/w) were heated for 20 min in a beaker, immersed in a thermostatic bath (Techne 8/TE-10 D, Burlington, NJ, USA) at 90 °C, under continuous stirring. To evaluate the swelling process, two samples were taken each 30 s of heating and extended on a microscope slide to carry out microscopic observation. Samples were analysed with a Leica DMLB (Heerbrugg, Germany) microscope equipped with a Leica DC 100 digital camera. The necessary micrographs were taken in order to have a sample of at least 180 granules at each sampling point during heating. Micrographs were analysed with the GIMP 2.2 software (GNU Image Manipulation Program, under GNU licence) to obtain the average equivalent circle diameter and Sauter mean diameter ($D_{[3,2]}$) of granules as a function of heating time. Relative swelling degree (%) was calculated as follows:

$$\text{Relative swelling degree}(\%) = \frac{(D_{[3,2](t)} - D_{[3,2](t=0)})}{D_{[3,2](t=0)}} \times 100$$

where $D_{[3,2]\ (t=0)}$ and $D_{[3,2]\ (t)}$ are the corresponding values to initial and t heating times, respectively.

Thermal properties

Thermal properties were determined on 20% w/w starch aqueous suspensions as described in a previous work (López *et al.*, 2008) using a DSC model Q100 controlled by a TA 5000 module (TA Instruments, New Castle, Delaware, USA), with a quench-cooling accessory, under a N_2 atmosphere (20 mL min^{-1}). Samples were tested between 10 and 120 °C, at a heating rate of 10 °C min^{-1}.

Starch pastes properties

Paste clarity

For paste clarity evaluation, starch suspensions (1% w/v) were gelatinised in a boiling bath for 30 min. After cooling at room temperature for 60 min, the clarity of paste was measured as the percentage light transmittance (%T) at 650 nm, using water as a blank (Piyachomkwan *et al.*, 2002).

Rheological characterisation

The assays were performed in a Rheo Stress 600 ThermoHaake (Haake, Germany) rheometer using a plate-plate system PP35 (gap size 1 mm) at controlled temperature (25 °C). Starch suspensions (5% w/w) were prepared and heated at 90 °C for 20 min. Rotational mode was used to investigate time-dependent behaviour of starch suspensions. Rheological curves were obtained after a stabilisation time of 3 min at 25 °C. The shear stress was determined as a function of shear rate between 0 and 500 s^{-1}, with the following program: 3 min to reach the maximum shear rate, then it was maintained during 1 min and finally it was allowed to reach 0 shear rate in 3 min. This program allows to evaluate the rheological behavior of the solutions and its time-dependence. Flux behaviour was mathematically modelled as described in a previous work (López *et al.*, 2008).

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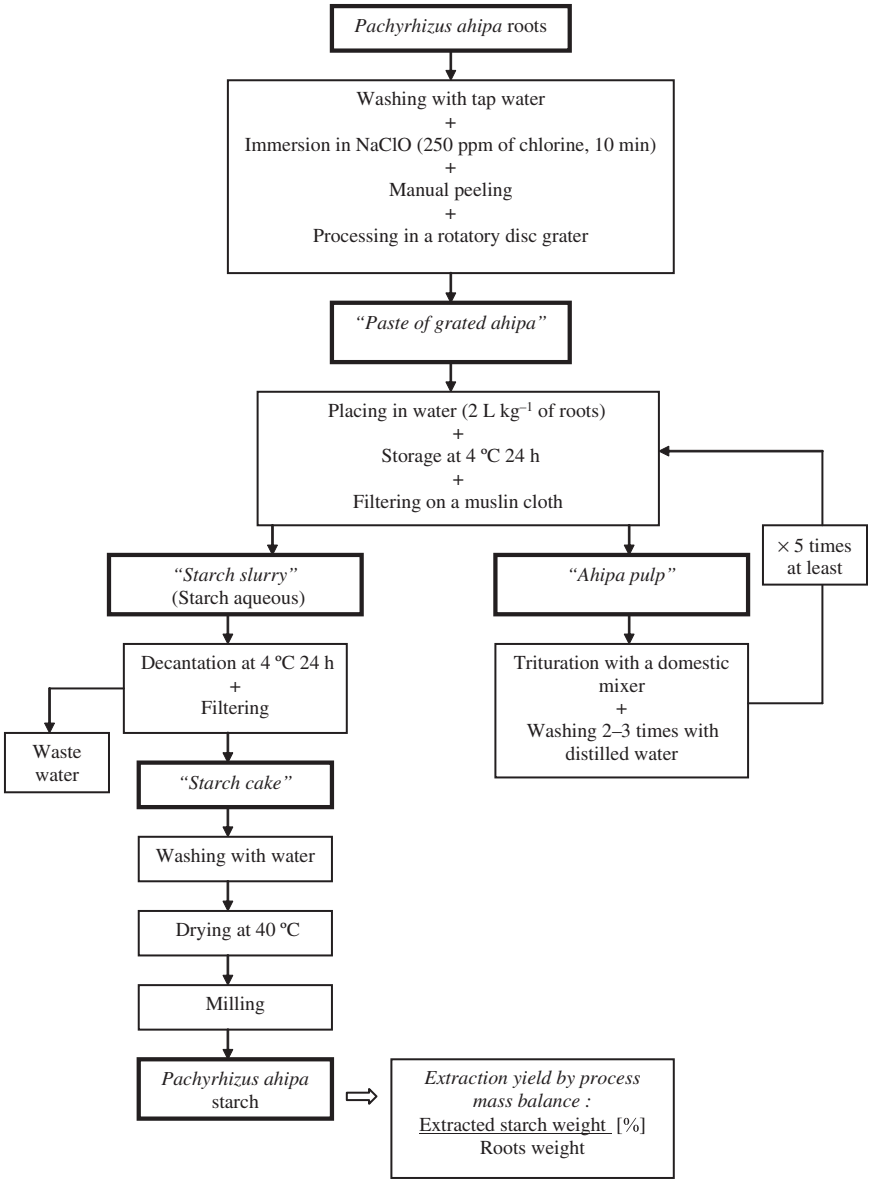

Figure 1 Fluxogram showing the *Pachyrhizus ahipa* starch extraction procedure.

Retrogradation

Starch retrogradation during refrigerated storage of gelatinised starch pastes was analysed by DSC evaluating the amylopectin retrogradation and the syneresis degree for amylose retrogradation. Ahipa starch suspensions (7% w/w) were gelatinised by heating 20 min at 90 °C. For DSC evaluation, approximately 5 g of gelatinised starch suspension were placed into plastic containers (diameter: 2.5 cm; height: 1.5 cm) and covered with a PVC film during storage at 4 °C. Samples were taken at different storage times and kept for 1 h at room temperature before the analysis. Starch pastes samples were weighed in aluminium pans, closed hermetically and analysed as described above.

Likewise, TPA of pastes samples conditioned as mentioned was performed. Besides hardness, springiness and cohesiveness, gumminess and adhesiveness were quantified (Andrés *et al.*, 2006).

To determine the syneresis degree of pastes, approximately 10 g of gelatinised ahipa starch suspension were placed into weighted centrifuge tubes, which were stored under refrigerated conditions as described previously. At each storage time, two stored tubes were centrifuged (5000 × ***g***) for 15 min. The obtained liquid was carefully discarded and the remained pastes were weighted in an analytical balance. The syneresis degree (%) was determined by evaluating the weight of the tubes before and after liquid separation.

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Experimental design and statistical analysis

In field trials, a split plot design with completed randomised blocks was used with two treatments and three replications per treatment. The treatments were plant density (40 000; 80 000 and 120 000 plants ha^{-1}) and flower pruning (*with* FP and *without* FP).

In the case of storage assays and starch extraction and characterisation, results were subjected to analysis of variance (ANOVA) using a Systat® Software (Version 10.0). Comparison of means was conducted with the Fisher's least significant difference (LSD) test at a significance level $P = 0.05$.

Results and discussion

Concerning plant material production, the effect of plant density was not significant, however higher yields (20 839 ± 9894 kg ha^{-1} with FP; 10 716 ± 4667 without FP) were obtained with a stock of 120 000 plant ha^{-1}. Leidi *et al.* (2004) reported a positive correlation between root yield and planting density and a negative correlation with root and pod mass per plant. This trend was also observed in this trial. Flower pruning practice significantly ($P < 0.05$) increased root yields, which were 1.9, 1.5 and 1.4 times higher with FP than without FP, for 120 000, 80 000 and 40 000 plants per ha, respectively (data not shown). Similar results were obtained by Vaz *et al.* (1998), Nielsen & Sørensen (1998), Leidi *et al.* (2004) and Zanklan *et al.* (2007). The average maximum production obtained was 20 839 kg ha^{-1} with a plant density of 120 000 plants ha^{-1} and applying FP. This yield was close to those reported by Leidi *et al.* (2004) and Zanklan *et al.* (2007), in the late case without FP but with irrigation. These authors have reported *P. ahipa* root yields ranging from 5.5 to 19.3 ton ha^{-1}.

Concerning the chemical composition of ahipa roots, water content quantified at 60 °C (81.9 ± 5.0%) was the same that those reported by Ørting *et al.* (1996) and Leonel *et al.* (2003). Crude protein content on a dry basis of ahipa roots was 6.5 ± 0.7%. This value was higher than the ones mentioned for *Canna* rhizomes (Piyachomkwan *et al.*, 2002), cassava storage roots (Stupak *et al.*, 2006) and *Colocassia esculenta* flour (Aboubakar *et al.*, 2008). Results found in this work were in agreement with those reported by Forsyth & Shewry (2002), although they were lower than those mentioned by Zanklan *et al.* (2007) for *Pachyrhizus tuberosus* (10.7%), *P. erosus* (12.4%) and *P. ahipa* (9.0%). Differences could be probably attributed to deficient soil fertility levels and low root nodulation. Results concerning ash (3.7 ± 0.6%), total fat (1.5 ± 0.5%) and crude fibre (13.3 ± 6.1%), all of them expressed on a dry basis, were in agreement with the reports of Leonel *et al.* (2005), particularly those corresponding to *Pachyrhizus ahipa* roots planted in October and harvested after 7 months.

Storage assays

Colour evaluation is a relevant issue since the acceptability of fresh vegetables is usually based on consumers' colour perception. Considering other roots and tuber crops, it was visually observed that soon after peeling and slicing, trifoliate yam (*Dioscorea dumetorum*) quickly underwent browning and its colour changed from pale yellow to deep brown (Njie & Rumsey, 1998). Conversely, cassava (*Manihot esculenta*) had a very white flesh, which underwent very little observable browning after peeling and slicing (Njie & Rumsey, 1998).

The chromaticity coordinate a^* that characterised the internal colour of ahipa roots ranged from –1.49 to 1.51 and the coordinate b^*, from 2.96 to 11.56. Thus, a^* and b^* values were close to 0. The degree of saturation (Chroma) was rather low, meanwhile lightness was high (80/100) (Table 1). Flesh colour of ahipa roots might be described as pale, white but dull. The high standard deviation of the chromaticity values (data not shown) could be explained considering that cross sections of ahipa roots exhibited purplish dots, being highly heterogeneous.

Colour measurements results for treated and stored ahipa roots are shown in Table 1. No significant differences were observed during storage at 4, 10 or 25 °C, both for L^* and Chroma values. Thus, the internal colour of samples was retained at least for 5 months at 25 °C or 1 month at 4 and 10 °C. The assayed physical treatments did not cause significant differences on colour measurements of ahipa roots.

Firmness of vegetables is generally quantified as the maximum force produced upon compression of the sample, because this parameter best relates to the texture customer perception. Table 1 shows the results of the TPA carried out on ahipa roots samples.

Referring to other root crops, maximum compression force (up to a 15% deformation of the sample) for raw cassava was 231.5 N (Menoli & Beleia, 2007). Authors have stated that turgid cells had high compression force, since fresh cassava tissue had approximately 65 g of water/100 g of material. Ahipa roots samples showed similar characteristics. Hardness can be related to the force necessary to break the product with the incisors during mastication. No significant differences in hardness of ahipa roots were observed during storage of control and treated samples (Table 1).

With respect to the mycoflora isolated from ahipa roots, the genus *Fusarium* (*F. solani*, *F. oxysporum*, *F. equiseti*) was predominant (77–100%) in control, heated and UV-treated samples (Table 2) at the beginning of the storage period. Being *Fusarium* a soil fungus, direct contact between roots and soil could be the primary source of contamination by this genus (Gnonlonfin *et al.*, 2008).

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Table 1 Colour attributes (L^*, lightness and Chroma, saturation) and parameters of the texture profile analysis (TPA) of *Pachyrhizus ahipa* roots

Treatments and conditions	Color attributes		TPA parameters			
	L^*	Chroma	Hardness (*N*)	Springiness	Cohesiveness	Resilience
Control samples at the beginning of the storage	80.7 ± 4.5[a]	7.7 ± 3.7[a]	135.4 ± 22.4[a]	0.79 ± 0.06[a]	0.45 ± 0.24[a]	0.53 ± 0.28[a]
Control samples stored for 5 months at 25 °C	82.8 ± 2.6[a]	11.6 ± 1.1[a]	147.0 ± 22.6[a]	0.72 ± 0.08[a]	0.58 ± 0.18[a]	0.42 ± 0.19[a]
Heat treated samples stored for 5 months at 25 °C	82.7 ± 4.0[a]	12.4 ± 1.0[a]	152.3 ± 35.7[a]	0.75 ± 0.21[a]	0.72 ± 0.05[a]	0.42 ± 0.07[a]
UV-C treated samples stored for 5 months at 25 °C	81.0 ± 2.4[a]	11.9 ± 1.2[a]	150.5 ± 18.0[a]	0.74 ± 0.03[a]	0.71 ± 0.02[a]	0.41 ± 0.02[a]
Control samples stored for 1 month at 4 °C	83.0 ± 2.7[a]	11.3 ± 1.7[a]	121.8 ± 30.5[a]	0.69 ± 0.11[a]	0.61 ± 0.16[a]	0.48 ± 0.13[a]
Control samples stored for 1 month at 10 °C	76.5 ± 7.3[a]	12.6 ± 6.2[a]	149.8 ± 26.7[a]	0.77 ± 0.24[a]	0.40 ± 0.23[a]	0.46 ± 0.22[a]

Reported values correspond to the mean ± standard deviation. Values within each column followed by different letters indicate significant differences ($P < 0.05$).

Table 2 Percentage of incidence of *Fusarium* in control and treated *Pachyrhizus ahipa* roots

Treatments and conditions	Incidence of *Fusarium* (%)	
	Initial counts (%)	Final counts (%)
Control samples	100.0 ± 0.0[a]	47.2 ± 18.4[b]
Heat treated samples	96.0 ± 6.9[a]	84.8 ± 18.0[a]
UV-C treated samples	76.8 ± 13.1[a]	60.8 ± 10.4[a]
Samples stored at 4 °C	100.0 ± 0.0[a]	72.0 ± 16.5[b]
Samples stored at 10 °C	100.0 ± 0.0[a]	75.2 ± 12.1[b]

Reported values correspond to the mean ± standard deviation. Values within each row followed by different letters indicate significant differences ($P < 0.05$).

In control and treated ahipa roots, the incidence of *Fusarium* decreased after the storage period, being accompanied by other genera representatives such as *Alternaria*, *Bipolaris*, *Cladosporium*, *Curvularia*, *Penicillium* and *Trichoderma* (Table 2).

Previous research about ahipa mycoflora is rather scarce. However, there is some available information about fungal population affecting similar underground plant organs. Studying the root-surface mycoflora of cassava, Ikediugwu & Ejale (1980) have isolated a small group of fungi, which included *Aspergillus niger*, *Botryodiplodia theobromae*, *Fusarium solani*, *Penicillium* sp. and *Trichoderma* sp. Concerning the predominance of *Fusarium*, the results of the present work were in agreement with those found for cassava roots by Lozano (1992), Msikita *et al.* (1997) and Bandyopadhyay *et al.* (2006).

Ahipa samples stored at 4 and 10 °C showed a rapid deterioration and they evidenced symptoms and signs of fungi attack, after 1 month of refrigerated storage. Conversely, control, heated and UV-treated roots could be kept for at least 5 months at 25 °C. These results indicated that *Pachyrhizus ahipa* roots were strongly affected by chilling injury that made tissues more susceptible to pathogens attack.

Roots and tubers can be stored underground (by delaying harvest), at room temperature or under refrigeration. The last alternative would not be viable for ahipa roots since they suffered chilling injury. Leaving roots in the field until needed (a common practice for, e.g. cassava roots) shows the disadvantage of withholding land from production. Thus, postharvest clean technologies such as heat shock and UV-treatments could be considered as useful tools for prolonging storage life of ahipa roots.

Starch extraction and characterisation

During the starch extraction process (Fig. 1), the water volume used was optimised as well as the number of times that the operation of starch slurry obtaining and its later sedimentation had to be repeated. With four repetitions the optimum results were achieved. Washing the roots with chlorinated water and the election of a low storage temperature (4 °C) of the pulp and the starch slurry were determining to avoid fungi proliferation. The starch suspensions centrifugation was unnecessary, since the starch slurry pouring off was sufficient to recover the extracted starch.

The technique designed for starch extraction from ahipa roots allowed to the following yields: 11.31% w/w on wet basis and 56.54% w/w on dry basis. These starch yields were in agreement with those reported by Forsyth *et al.* (2002) and they were similar to those reported for other Andean root and tuber crops, such as *Mirabilis expansa* and *Oxalis tuberosa* (Espín *et al.*, 2004). Flower-pruning practice increased 1.6 times starch extraction yield. In order to evaluate the efficiency of the starch extraction procedure, crude protein by Kjeldahl method and total ash content were quantified on ahipa starch samples. Total nitrogen was not detected in the extracted starch powders and total ash content ranged from 0.28 to 0.34%, evidencing a good performance of the starch isolation method used.

Physicochemical starch characterisation

Amylose content was not affected by the performed cultural practice since values obtained for starch

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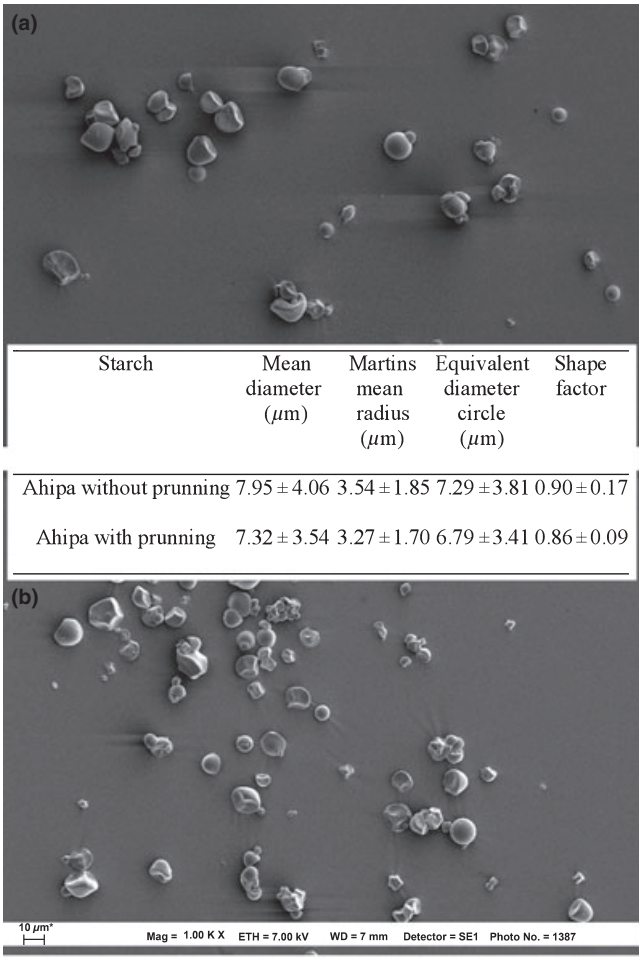
extracted from ahipa with and without FP were similar. The corresponding amylose content was 10.75 ± 0.71%. It might be argued that the high digestibility and stability of starch pastes is a consequence of this low value (Skrabanja *et al.*, 1999). Ahipa starch would be suitable for food processing since low retrogradation tendency is associated to low amylose starches (Forsyth *et al.*, 2002).

Figure 2 corresponds to the SEM micrographs of extracted starch, with and without FP. Ahipa starch granules were not associated as clusters; they exhibited round and polygonal shapes with irregular borders with a mean diameter of 7.95 ± 4.06 μm for ahipa starch granules without FP and 7.32 ± 3.54 μm for those roots where the deflowering practice was performed. Starch granules exhibited smooth surfaces without evidence of rupture or damage due to the extraction procedure. With regard to the granule size, ahipa starch exhibited a monomodal straight distribution ranged from 2 to 18 μm, in both starch samples. Granular sizes were lower than those reported for potato starch, with an average size ranging between 30 and 70 μm, and those reported for cassava starch which exhibited average sizes of 12–15 μm (Piyachomkwan *et al.*, 2002). Besides, Mélo *et al.* (1994) reported that yam bean (*P. erosus*) starch exhibited granules with an average size lower than 10 μm with different geometric forms, such as round, cupuliform and convex-biconcave.

The diffraction pattern type assignation of ahipa starches was difficult since they did not correspond to type A, which is characteristic of cereal starches, neither to type B, the one corresponding to tubers and roots starches. The diffractograms (Fig. 3) could be assigned to a type C diffraction pattern, which reflects at 5.5, 15.4, 17.0, 18.0, 20.0 and 23.5° 2θ, (Whistler, 1984; Geng *et al.*, 2007). The C-type pattern is an intermediate between A- and B-type and is typical of legume seeds (Zobel, 1988), tropical tubers and fruits (Luengwilai & Beckles, 2009). Huang (2009) working on different root and tuber starches assigned an A-type profile to taro and yam bean starch while yam and potato starches exhibited four main reflection intensities typical of B-type. However, a C-type close to an A-type (Ca-type) was assigned to sweet potato starch. Geng *et al.* (2007) also assigned a C-type pattern to kudzu root starch (*Pueraria hirsute*) and stressed that type C starches are highly susceptible to environmental temperature, as indicated in the starches of soybean and sweet potato (Suzuki *et al.*, 1992).

Likewise, resistant starches are associated to a B-type crystal structure (Hung & Morita, 2005; Luengwilai & Beckles, 2009); therefore, appropriate digestibility properties could be expected for ahipa starch based products.



Starch	Mean diameter (μm)	Martins mean radius (μm)	Equivalent diameter circle (μm)	Shape factor
Ahipa without prunning	7.95 ± 4.06	3.54 ± 1.85	7.29 ± 3.81	0.90 ± 0.17
Ahipa with prunning	7.32 ± 3.54	3.27 ± 1.70	6.79 ± 3.41	0.86 ± 0.09

Figure 2 Morphological parameters and scanning electron micrographs of *Pachyrhizus ahipa* starch granules. (a) without FP, (b) with FP.

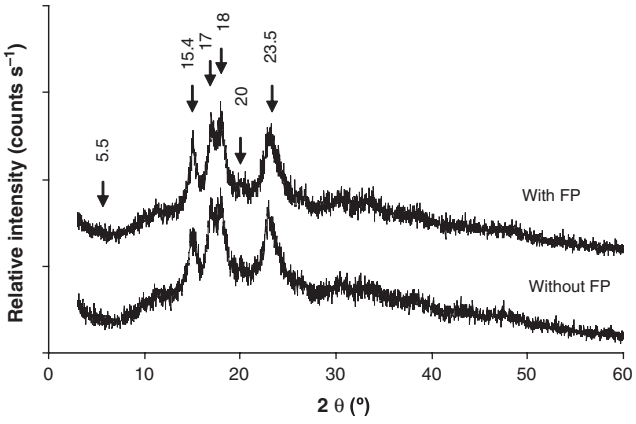


Figure 3 X-ray diffractogram of *Pachyrhizus ahipa* starch.

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Flower pruning practice did not modify the diffraction pattern of ahipa starches neither their crystallinity degree (Fig. 3). Ahipa starch extracted from roots corresponding to flower-pruned plants exhibited a crystallinity degree of 44.51 ± 1.33% and 41.88 ± 2.33% for those obtained from non flower-pruned ones.

Gelatinisation of starch suspensions

When starch granules are heated with sufficient water, the crystalline structure of starch is disrupted, which causes increased granule swelling (Huang *et al.*, 2009). Granule swelling capacity plays an important role in the rheological behaviour of starch suspensions. Sauter mean diameter ($D_{[3,2]}$) and the typical swelling behaviour of ahipa starch granules were plotted as a function of heating time (Fig. 4). The major increase of $D_{[3,2]}$ of starch granules occurred between 1 and 2 min of heating, regardless the sample. At longer heating times starch granules of ahipa without FP maintained their integrity more than those corresponding to ahipa with FP. Thus, relative swelling degrees of starch granules of ahipa with and without FP were 213% and 199%, respectively.

Ahipa starch suspensions exhibited thermograms with a single endothermic transition, typical of the gelatinisation process of suspensions with high water content. This process is of primary importance in the food industry because of its high impact on the texture of starch-based food. Thermal properties of ahipa starch were slightly affected by the FP practice performed (Table 3). Starch granules extracted from ahipa with FP exhibited a higher peak temperature and a narrower gelatinisation range than those without FP. A low temperature range is associated to a highly cooperative

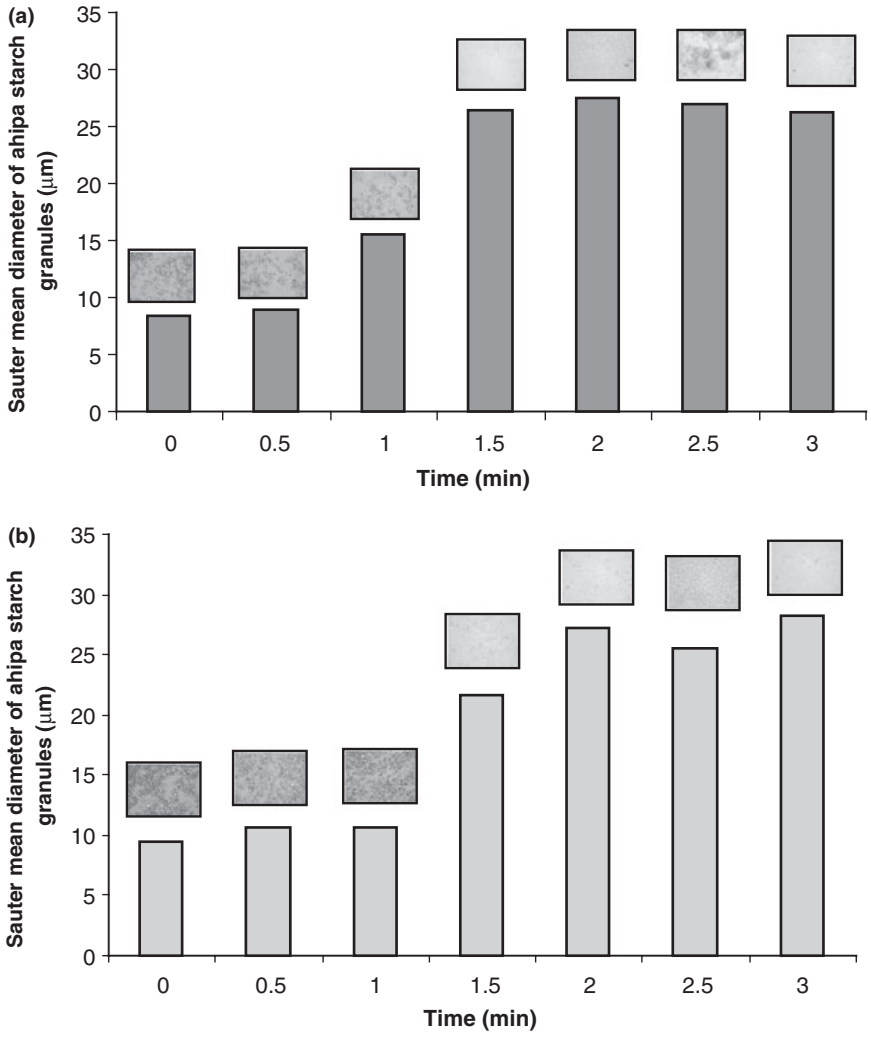

Figure 4 Flower pruning (FP) effect on Sauter mean diameter ($D_{[3,2]}$) of *Pachyrhizus ahipa* starch granules at different heating times. (a) with FP, (b) without FP.

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Table 3 Thermal and rheological properties of ahipa starch

Property		Ahipa starch with flower pruning	Ahipa starch without flower pruning
Thermal properties	Onset temperature, T_o (ºC)	65.19 ± 1.18[a]	62.33 ± 0.69[b]
	Peak temperature, T_p (ºC)	69.13 ± 0.50[a]	67.04 ± 1.01[b]
	Gelatinisation enthalpy, $\Delta H_{gelat.}$ (mJ mg^{-1})	9.10 ± 0.13[a]	9.96 ± 0.20[b]
Rheological properties	Thixotropic index (Pa s^{-1})	653.43 ± 29.82[a]	693.55 ± 133.01[a]
	Consistency coefficient*, k (Pa s^{-n})	2.09 ± 0.23[a]	2.39 ± 0.38[a]
	Flow behavior index*, n	0.48 ± 0.01[a]	0.43 ± 0.01[b]
	Apparent viscosity at 500 s^{-1} (mPa s)	82.01 ± 1.046[a]	81.87 ± 0.313[a]

*Ostwald de Waele model fitting parameters. Reported values correspond to the mean ± standard deviation. Values within each row followed by different letters indicate significant differences ($P < 0.05$).

process (Huang, 2009), which could be indicative of the narrowness size chains distribution of amylose and amylopectin. Besides, thermal results are in agreement with those of swelling capacity.

Enthalpy values obtained were similar to those reported for the gelatinisation of starches from different botanical sources, such as corn. Gelatinisation temperatures of ahipa starch were within the range of those reported by Mélo *et al.* (2003) for potato (56–66 °C) and cassava (53–63 °C) starches. Besides, they were lower than those reported for cereals such as corn (70–72 °C). These results indicate that the associative forces, which maintain granular integrity of ahipa starch, were similar to those from other tubers and roots. These starches gelatinise at relatively low temperature, which is an indicator of the easiness of cooking, and exhibit a higher paste clarity and viscosity compared to cereal starches (Mélo *et al.*, 2003).

Starch pastes properties

Rheological properties of starch pastes have high technological impact on food developments. Rotational assays allow to analyse the rheological behaviour of samples under high shear stress, which are close to technological process conditions.

Ahipa starch exhibited a pseudoplastic behaviour ($n < 1$, Figure S1), which was satisfactorily fitted by Ostwald de Waele model ($r^2 > 0.9979$) and showed a thixotropic behaviour. The thixotropic indexes, the consistency coefficients and the apparent viscosities at 500 s^{-1} are presented also in Table 3. The flower pruning practice only affected the flow behaviour index of starch pastes.

Paste clarity index was 7.26 ± 0.25%, which was lower than those reported for cassava starch pastes (21.84%), but higher than those reported for corn starch (5.6%) by Mélo *et al.* (2003). These authors also informed that yam bean (*P. erosus*) starch produced pastes with a clarity index of 11.44% analysed with an equivalent technique. The lower paste clarity found in ahipa starch may be explained by the presence of molecules less susceptible to retrogradation.

Starch retrogradation is defined as a process, which occurs when the molecular chains in gelatinised starches begin to reassociate in an ordered structure. Since no significant differences on rheological behaviour of starch pastes were detected, retrogradation analysis was performed on a blended sample of ahipa starch. Starch pastes were stored under refrigeration during 11 days since longer periods led to microbial deterioration. At the end of the storage, DSC curves of ahipa starch pastes did not show any changes in the thermograms, indicating that no structural changes due to amylopectin retrogradation occurred.

Since retrogradation causes an increase in the starch pastes rigidity inducing phase separation, thus the presence of an exudate (syneresis phenomenon) is commonly used as an indicator of this detrimental process. The syneresis degree for ahipa starch pastes stored 11 days at 4 °C resulted very low (0.21 ± 0.01%), in comparison to cereal starch pastes (40.09 ± 1.04% for corn starch), which could be attributed to the low amylose content of ahipa starch.

Finally, textural changes of starch pastes are used to evaluate the performance of starch in a food system and are related mainly to amylose retrogradation (Sandhu & Singh, 2007). Table 4 shows the parameters obtained from TPA analysis of ahipa pastes stored under refrigerated conditions. The absence of fracturability peak in the mechanical pattern of ahipa starch pastes is in agreement with DSC results. Storage time significantly affected ($P < 0.05$) hardness, chewiness and springiness of ahipa starch pastes while gumminess and cohesiveness did not vary significantly ($P > 0.05$) (Table 4). Adhesiveness, which is a surface characteristic, decreased with storage time. The comparison of the mechanical properties of starch gels obtained with those reported by other authors is difficult since these properties depend upon various factors including starch suspension concentration, storage time and temperature as well as experimental conditions.

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Table 4 Ahipa starch pastes retrogradation evaluation by different analysis techniques

Analysis technique		Initial	11 days stored at 4 °C
DSC		Non-detected	Non-detected
Syneresis degree (%)		Non-detected	0.21 ± 0.01
TPA	Hardness (N)	1.69 ± 0.10[a]	1.22 ± 0.29[b]
	Adhesiveness (N mm)	0.24 ± 0.06[a]	0.17 ± 0.05[a]
	Cohesiveness (adimensional)	0.90 ± 0.24[a]	0.88 ± 0.29[a]
	Gumminess (N)	1.53 ± 0.27[a]	1.07 ± 0.40[a]
	Springiness (mm mm^{-1})	1.15 ± 0.06[a]	1.07 ± 0.04[a]
	Chewiness (N)	1.76 ± 0.18[a]	1.15 ± 0.38[b]

Reported values correspond to the mean ± standard deviation. Values within each row followed by different letters indicate significant differences ($P < 0.05$).

Conclusions

The implementation of a flower pruning practice, which is quite easy and simple, allowed to improve ahipa roots yield without modifying the functional properties of the starch extracted. This practice could be applied in both small-scale and commercial production. However, since sowing is the common propagation method of ahipa, it should be considered that a few plants of the crop must always be left to produce seeds. Considering that the crop was conducted without the input of fertilisers and pesticides, *Pachyrhizus ahipa* might be included in crop rotations in marginal agricultural regions. However, further effort must be made in genetic improvement and agronomic practices in order to improve *P. ahipa* yields.

The implementation of refrigerated storage for ahipa roots was limited by the development of chilling injury. This physiological disorder was accomplished by fungal attack. Thus, from the assayed alternatives, storing at 25 °C seemed to be the most adequate. After 5 months of storage at room temperature, control and treated roots maintained their colour and texture attributes. The assayed postharvest clean technologies (heat shock and UV-treatment) could be considered as viable tools to extend the storage life of ahipa roots at 25 °C.

The developed ahipa starch extraction procedure included relatively few stages and was similar to that used for other root and tuber crops. Because of its low amylose content, and the low temperatures required to gelatinise its suspensions, ahipa starch appears as a promising alternative to other traditional starch sources. SEM analysis indicated that ahipa starch granules presented round and polygonal shapes and a C-type diffraction pattern. Thermal properties revealed that ahipa starch could be processed at low temperatures. Rheological properties of pastes, mainly apparent viscosity, indicate that ahipa starch might be adequate as a food thickener additive. Moreover, these pastes that showed adequate clarity index had the advantage of a low retrogradation tendency under refrigeration conditions. Thus, these interesting functional properties of *Pachyrhizus ahipa* starch would be relevant to the development of food applications, among other uses.

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Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1. Rotational flow curves of ahipa (with and without flower pruning) starch suspensions.

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