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Pachyrhizus ahipa (Wedd.) Parodi roots and flour: Biochemical and functional characteristics

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ABSTRACT

Ahipa roots' chemical composition and physiological parameters were characterised; ahipa flour preparation procedures were selected and the chemical composition and functional properties of these products were studied. Ahipa roots and flour can be considered alternative food sources of gluten-free starch, with a considerable contribution of protein, fibre and minerals, such as potassium, calcium and iron. The grating process for ahipa flour production required a pressing step (AFGP) and the recovery of the starch leached. The slicing procedure (AFS) was simpler and the resulting product showed higher contents of potassium, magnesium, calcium and protein than did AFGP, which showed lower sodium and higher acid detergent fibre contents, together with lower gelatinisation temperature. Both flours differed in terms of α-amylase activity and swelling power, characteristics that may condition their specific applications, such as the incorporation of these flours as gluten-free functional food ingredients.

1. Introduction

The commonly accepted definition for "functional foods" refers to foods or their ingredients that provide an additional physiological benefit beyond their contribution to basic nutrition (Day, Seymour, Pitts, Konczak, & Lundin, 2009). From this point of view, whole foods, such as fruits and vegetables, represent the simplest form of functional foods, since they are rich in fibre and bioactive phytochemicals (Day et al., 2009). In the development and formulation of new products, functional foods play an important role for the food industry. Some functional products are intended for people with specific health problems such as cardiovascular disease, hypertension, diabetes, morbid obesity and gluten intolerance (Villarroel, Huiriqueo, Hazbun, & Carrillo, 2009).

According to Alvarez-Jubete, Arendt, and Gallagher (2010), celiac disease (CD) is an autoimmune enteropathy triggered by the ingestion of gluten-containing grains (wheat, barley, rye and possibly oats) in genetically susceptible individuals. The reported prevalence of symptomatic CD is l in 1000 live births, with a range between l in 250 (observed in Sweden) and l in 4000 (observed in Denmark) (Branski, Fasano, & Troncone, 2006). In Latin America, some population studies in Argentina and Brazil showed an estimated prevalence of 1:167 and 1:360 individuals, respectively (Villarroel et al., 2009). The generally accepted treatment to date for CD is the strict lifelong elimination of gluten from the diet (Chand & Mihas, 2006). The dietary changes required by celiac patients to begin and maintain a strict gluten-free diet, are considerable and may have a significant impact on their daily life. Likewise, concern has been raised over the long term dietary habits and foods choices of celiac patients. Results, from a number of studies, have indicated an unbalanced intake of carbohydrates, protein, and fat, as well as limited intake of certain essential nutrients in celiac subjects compared with controls (Alvarez-Jubete et al., 2010). Results from a survey conducted in the United States (Thompson, Dennis, Higgins, Lee, & Sharrett, 2005) have also indicated that consumption of iron, calcium and fibre, in celiac patients on a gluten-free diet, may be of concern (Alvarez-Jubete et al., 2010). This situation leads to an increased interest in the study and development of gluten-free products that can provide significant quantities of fibre, starch and minerals at the same time.

Some leguminous plants from the genus *Pachyrhizus* (yam beans) produce tuberous roots (RT). This genus is native to southern and central America. Main cultivated species of this genus are: *Pachyrhizus tuberosus*, the "Amazonian yam bean", mainly grown in Bolivia, Peru, Ecuador and Brazil, *Pachyrhizus erosus*, the "jacatupe" or "Mexican yam bean", found in Central America and the

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Caribbean, and *Pachyrhizus ahipa*, the "ahipa" or "Andean yam bean", from the Andes of Bolivia and northern Argentina (Forsyth et al., 2002; Sørensen, Døygaards, Estella, Kvist, & Nielsen, 1997; Zanklan, Ahouangonou, Becker, Pawelzik, & Grüneberg, 2007). *P. ahipa* was cultivated in the past by the Incan civilisation. However, its production and use declined significantly, coinciding with the collapse of the aboriginal cultures following the Conquest of America. Currently, ahipa cultivation remains relatively low (Leidi, Sarmiento, & Rodríguez-Navarro, 2003). This species is characterised by the starch accumulation (of industrial interest) in its tuberous root, and the presence of rotenone in its seeds and leaves. The root is almost exclusively consumed raw as a fruit or even cooked; its skin lifts off quite easily from the internal portion, which is fleshy and mostly white (Milanez & Moraes-Dallaqua, 2003). References to ahipa root industrialisation are rather scarce, although this kind of plant has been studied by different research groups, showing its worldwide interest (Leidi et al., 2003; Leonel, Bortolucci Ferrari, Sarmento, & Alvares de Oliveira, 2005; Leonel, Sarmento, Cereda, & Camara, 2003; López et al., 2010).

The objectives of the present work were: (1) to characterise ahipa root chemical composition and physiological parameters, (2) to select an adequate flour-obtaining procedure, and (3) to characterise ahipa flour from a chemical and functional point of view.

2. Materials and methods

2.1. Plant material

P. ahipa (Wedd.) Parodi plants (Fig. 1a) were grown at the Instituto Nacional de Tecnología Agropecuaria (INTA) Montecarlo farm (Misiones, Argentina; 26° 33′ 40.15″, latitude south and 54° 40′ 20.06″, longitude west). Roots were harvested in April, 2009, when senescence started (~220 days after sowing).

Ahipa roots were received at the laboratory and processed immediately. Wounded or unhealthy roots were discarded. Roots were thoroughly washed with tap water for removal of any soil residue. A sanitation step was included, consisting of immersion in NaOCl solution (250 ppm of chlorine, 10 min) (Fig. 1b). Then roots were dried at room temperature.

2.2. Flour-obtaining procedures

The detailed ahipa flour (AF) preparation procedures are described in Fig. 1b. Washed and sanitised roots were hand-peeled and then they were sliced (S) or grated (G). Ahipa slices were dried at 55 °C and then milled, leading to "ahipa flour obtained by slicing" (AFS).

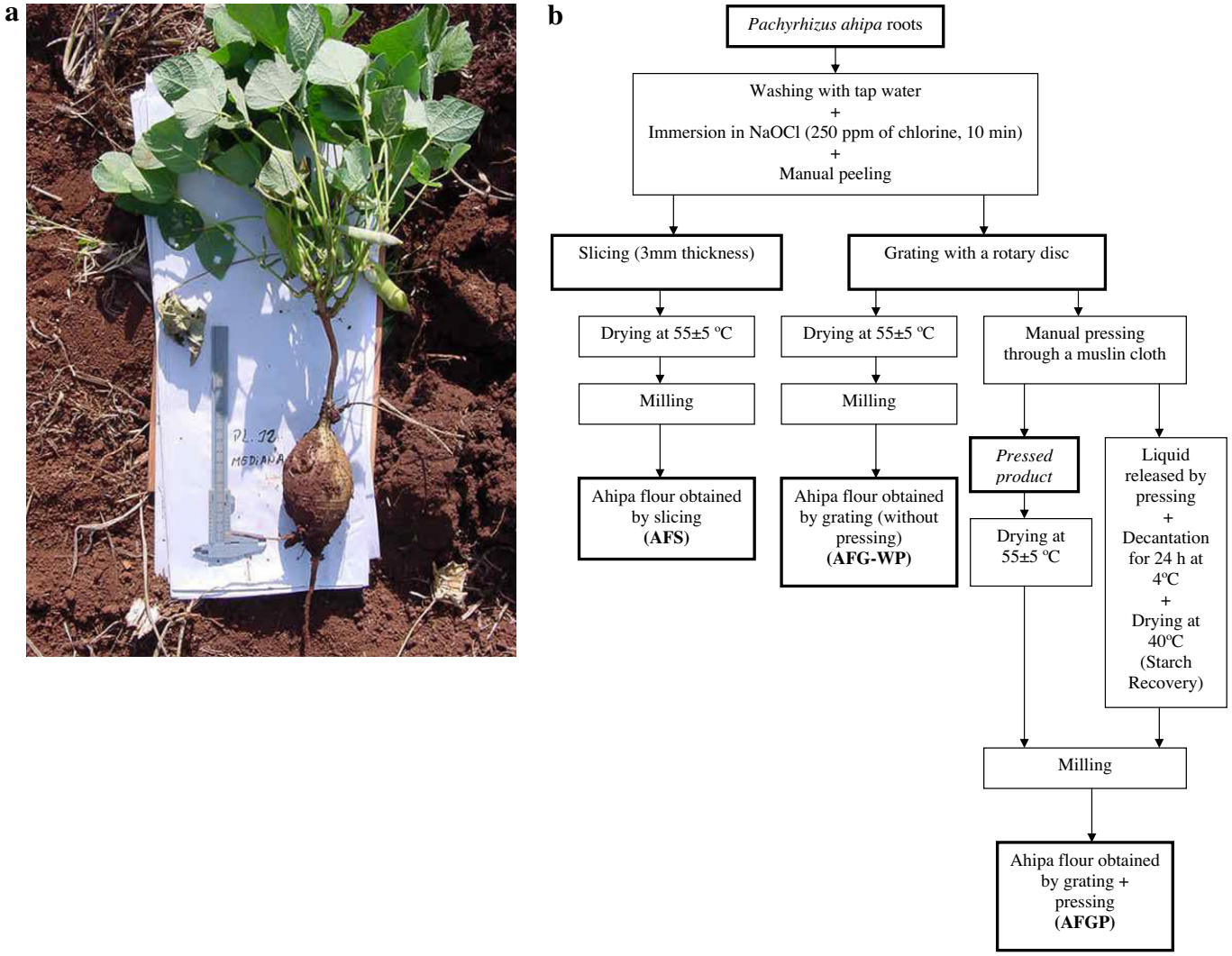

Fig. 1. (a) *Pachyrhizus ahipa* plant; (b) Fluxogram showing the processing steps for preparation of *Pachyrhizus ahipa* roots and ahipa flour obtaining.

Part of the grated roots was submitted to a pressing step and the decanted solids from the liquid released during pressing were dried at 40 °C and recovered. Solids recovered were added to the dried flour raw material, prior to milling (Fig. 1b); this flour was named AFGP. The remaining grated roots were directly dried at 55 °C and milled. This product corresponded to "ahipa flour obtained by grating, without pressing" (AFG-WP) (Fig. 1b). The processes for obtaining the different flours were performed in duplicate.

Ahipa flour yield (% w/w) was calculated through a mass balance, considering the weight of the resulting product and the initial weight of roots processed.

2.3. Chemical composition of ahipa roots and flour

2.3.1. General

For analysing their chemical composition, whole roots were cut into slices and dried at 60 °C until constant weight was reached. Dried samples were grinded in a mill and kept in hermetically closed containers until analyses were performed.

Concernig ahipa flour, chemical analysis were carried out on AFS and AFGP samples since AFG-WP developed off-flavours in a short period (one week), while AFS and AFGP kept their original quality attributes even after nine months of storage.

2.3.2. Dry matter and total ash content

Whole roots, dried at 60 °C as aforementioned, and flour samples were analysed in duplicate for residual water content and total ash content. Samples (0.5 g) were dried in an oven (San Jor H701P, Argentina) at 105 °C until constant weight was reached. Once removed from the oven, samples were allowed to cool in a desiccator and then they were weighed. Results were expressed as percentage (%) of the initial weight.

Ahipa dried samples were placed in previously burned (900 °C) and tared crucibles, weighed accurately and slowly carbonised at the flame of a Bunsen burner. Then they were incinerated in a muffle furnace (Indef 331, Córdoba, Argentina) at 550 °C until they turned into white ash and constant weight (~5 h) was reached (method 923.03 AOAC, 1990). The crucibles were weighed and the percentage (%) of total ash was calculated.

2.3.3. Quantification of sodium, potassium, calcium, magnesium, iron and phosphorus

Ash samples (0.03–0.05 g) were weighed in an analytical balance and dissolved in 50% v/v HNO_3. For calcium content determination, samples were dissolved in lanthanum oxide to a final concentration of 0.5%. The analyses of Fe (λ = 248.3 nm), Ca (λ = 422.7 nm) and Mg (λ = 285.2 nm) were carried out by atomic absorption spectroscopy and the quantification of Na (λ = 589.0 nm) and K (λ = 766.5 nm) by atomic emission spectroscopy. Measurements were carried out in a spectrophotometer Shimadzu AA-6650. The quantification of phosphorus was carried out by the photometric method 965.17 (AOAC, 1990).

2.3.4. Extraction and quantification of crude fat

For the determination of crude fat content, whole roots dried at 60 °C and ahipa flour samples (12 g) were extracted with hexane in a Soxhlet apparatus until completing eight cycles of extraction. The residue obtained in the flask, after solvent recovery and evaporation (crude fat), was weighed. Results were expressed as percentage (%) on a dry basis.

2.3.5. Quantification of crude protein

Whole roots, dried at 60 °C, and ahipa flour samples (0.8 g) were analysed for total nitrogen content by the Kjeldahl method. A Büchi K-435 digestion unit (Flawil, Suiza) and a Büchi K-350 distillation unit (Flawil, Suiza) were used. The crude protein content was calculated, using the conversion factor 6.25. Results were expressed as percentages (%) on a dry basis.

2.3.6. Quantification of acid detergent fibre (ADF)

Dried whole roots and flours samples were weighed (1.0 g) in duplicate, and the respective quantifications of ADF were performed by the Van Soest detergent system (Robertson & Van Soest, 1981), following the 973.18c method (AOAC, 1990). Results were expressed as percentages (%) on a dry basis.

2.3.7. Total starch content

Dried ahipa whole root and flour samples (0.10–0.15 g) were analysed for total starch content. The method used started with a conversion of the starch to glucose during two stages of an enzymatic treatment that was followed by a colorimetric determination of the glucose produced, using a glucose-specific coupled enzyme reaction and a chromogen system (K-TSTA 05/06 Megazyme©, Ireland). The method involved the initial enzymatic treatment of the finely powdered plant material with thermostable α-amylase. During this step, the initial breakdown of the starch to dextrins and oligosaccharides occurred, ensuring a more effective quantitative conversion to glucose during a second incubation with amyloglucosidase. The colorimetric determination of glucose was done using a single-solution reagent method, which involved the coupled enzymatic glucose oxidase/peroxidase reaction, in combination with the 4-amino antipyrine chromogen system. A D-glucose standard solution (1.0 mg ml^{-1}) in 0.2% (w/v) benzoic acid was used. Absorbance readings were obtained at 510 nm in a spectrophotometer (Shimadzu UV mini 1240 UV–VIS, Japan). Results were expressed as percentages (%) on a dry basis.

2.4. Physiological parameters

2.4.1. Respiratory activity

Ahipa roots, of known weight and volume, were placed in hermetically sealed glass flasks (2 L). CO_2 accumulated was quantified using a CO_2 Sensor Compuflow 8650 Indoor Air Quality Meter incorporated into the flask. CO_2 concentration was plotted against time. From the slope of the line, the CO_2 production was calculated and expressed as ml kg^{-1} h^{-1}.

2.4.2. α-Amylase activity

α-Amylase activity, in ahipa roots and flour samples, was measured by means of the Ceralpha (K-CERA 08/05 Megazyme©, Ireland) procedure, which employs, as substrate, the non-reducing end-blocked *p*-nitrophenyl maltoheptaoside reagent in the presence of excess levels of a thermostable α-glucosidase. The excess quantities of α-glucosidase present in the mixture give instantaneous and quantitative hydrolysis of the *p*-nitrophenyl maltosaccharide fragment to glucose and free *p*-nitrophenol. The reaction was terminated (and colour developed) by the addition of a weak alkaline solution. The absorbance at 400 nm was measured in a spectrophotometer Shimadzu UV mini 1240 UV–VIS (Japan). Results were expressed as Ceralpha Units g^{-1} dry sample.

2.5. Ahipa flour physicochemical characteristics

2.5.1. Particle size distribution

Particle size distribution was measured using a laboratory sifter Buhler MLU-300 with the following set of meshes: 32D (666 μm), 45D (472 μm), 60D (341 μm), 6xx (230 μm) and pan. The sieving procedure was done for 100 g of flour during 12 min. The fractions retained in each mesh were separated and weighed (Palacios-Fonseca, Vázquez-Ramos, & Rodríguez-García, 2009). The fractionation

process was performed in duplicate. Values reported correspond to the means ± standard deviations.

2.5.2. Scanning electron microscopy

Morphology and size distribution of ahipa flour particles smaller than 230 μm were studied by scanning electron microscopy (SEM), with a JEOL 35 CF electron microscope (Japan), (López et al., 2010). The images were obtained with a software programme designed to acquire images digitally (IDX), with a resolution of 1024 × 800 pixels. The AnalySis Pro 3.0 software programme was used for the processing and analysis of the images.

2.5.3. Colour measurements

Colour was measured using a Chroma Meter CR-400 (Konica Minolta Sensing Inc., Japan) and expressed in terms of lightness ($L*$), red–green coordinate ($a*$ value), blue–yellow coordinate ($b*$), hue angle ($h°$) and Chroma ($C*$); $h° = \tan^{-1}(b*/a*)$ and $C* = (a*^2 + b*^2)^{1/2}$.

2.6. Ahipa flour functional properties

2.6.1. Gelatinisation of ahipa flour suspensions

Thermal properties of ahipa flours were determined by DSC according to López, García, and Zaritzky (2008), using a Q100 differential scanning calorimeter 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}). Approximately 7 mg of ahipa flour suspensions (20% w/w) were weighed into aluminium pans and closed hermetically; an empty pan was used as reference. The scanning rate was 10 °C/minute. Heating ranges varied from 10 to 120 °C for all samples.

Swelling power (SP) of ahipa flours was measured according to a modification of the method described by Tsai, Li, and Lii (1997). Flour suspensions (1% w/w) were placed in centrifuge tubes with caps; the tubes were heated at 55, 65, 75, 85, 90 and 95 °C for 1 h. Samples were cooled to room temperature and centrifuged at 850*g* for 15 min. SP was determined as the ratio of the weight of the hydrated flour (sediment) to the initial weight of dry flour. The supernatant was removed for amylose and amylopectin leached concentration measurement according to the spectrophotometrical method proposed by García, Martino, and Zaritzky (1995).

2.6.2. Water holding capacity (WHC)

WHC was measured according to Szymonska, Krok, Komorowska-Czepirska, and Rebilas (2003). Ahipa flour samples (1 g) were placed in centrifuge tubes and 10 ml portions of distilled water were added. After homogenisation and stabilisation for 15 min (with intermittent agitation each 5 min), suspensions were centrifuged at 850*g* for 15 min. Supernatants were discarded and the gels were weighed. WHC was calculated as follows:

$$\mathrm{WHC}(\%) = [((F - G) - C)/C] \times 100 \quad (1)$$

where F is the weight of the tube containing the wet sample after it was decanted (g); G is the weight of the centrifuge tube (g) and C is the weight of the dried sample (g).

2.7. Statistical analysis

All determinations were carried out at least in duplicate. For the statistical analysis of the results, the programme Systat® Software (Version 10.0) was used. Analysis of variance (ANOVA) and comparison of means with the Fisher's least significant difference (LSD) test were conducted, at a significance level $p = 0.05$.

3. Results and discussion

3.1. Chemical composition of ahipa roots and flour

Ahipa flour yield, calculated considering non-peeled root weight, was 14 ± 1.3 g flour per 100 g of fresh roots for AFGP and 18 ± 1.5 g flour per 100 g fresh roots for AFS. Although yield values of non-traditional flours reported in the literature are rather scarce, Bou Rached, de Vizcarrondo, Rincón, and Padilla (2006) have pointed out that white and purple varieties of mapuey (*Dioscorea trifida*) yielded 18.48 and 19.90 g of flour/100 g of pulp (dry basis), respectively. These values are close to those found for ahipa samples.

Dry matter content (quantified at 60 °C) of whole ahipa roots was 23.6 ± 0.3%. The residual water content (quantified at 105 °C) was 12.9 ± 0.2%. AFGP and AFS flours had 90.7 ± 0.7% and 88.8 ± 0.2% dry matter contents, respectively, AFGP dry matter content being significantly ($p < 0.05$) higher than that of AFS.

Results from the proximate chemical analyses carried out in ahipa samples are shown in Table 1. As expected, the total ash content of the entire root was significantly higher than that of flour, since the processing for obtaining flour began with the peeling of the roots. This indicates that root skins could provide some mineral elements.

Total ash contents found for both ahipa flour samples were within the values reported by Lebot, Champagne, Malapa, and Shiley (2009) for other tropical root and tuber crops, such as cassava (*Manihot esculenta*), sweet potato (*Ipomoea batatas*), taro (*Colocasia esculenta*) and yam (*Dioscorea spp*). Total ash content for AFS was significantly higher than that for AFGP, and this observation is also related to the processing method. During AFGP preparation, part of the soluble components are lost into the supernatant released after pressing. Total ash contents of both ahipa flour types were higher than those reported in traditional flours, such as bread wheat (0.50%), durum wheat (0.80%), rice (0.48%) and corn (1.2%) flours (Sabanis & Tzia, 2009). However, ash content of ahipa flours were below those found in soy flour (4.1%).

Table 2 shows the contents of sodium, potassium, calcium, magnesium, iron and phosphorus for ahipa samples. Except for sodium, the contents of the mineral elements analysed were higher for ahipa whole roots than for flours. Sodium content for AFS was 1.25 times higher ($p < 0.05$) than that for AFGP. Phosphorus and potassium were the predominant elements in all samples. For AFS, potassium content was significantly higher than that for AFGP (1.35 times). Ogbuagu (2008) also found that potassium

Table 1
Proximate chemical composition (% w/w) and α-amylase activity (Ceralpha units, CU g^{-1} dry sample) of *Pachyrhizus ahipa* roots and flour. AFGP: ahipa flour obtained by grating plus pressing; AFS: ahipa flour obtained by slicing.

Sample	Total ash	Crude fat	Crude protein	Acid detergent fibre (ADF)	Total carbohydrates	Total starch content	α-amylase activity
Whole root	3.50 ± 0.02[a]	0.65 ± 0.04[a]	8.61 ± 0.29[a]	12.8 ± 0.6[a]	87.2 ± 0.3[a]	37.0 ± 2.7[a]	2.7 ± 0.3[a]
AFGP	1.98 ± 0.05[b]	0.25 ± 0.03[b]	5.74 ± 0.08[b]	7.4 ± 0.1[b]	92.03 ± 0.05[b]	60.5 ± 8.4[b]	1.4 ± 0.1[b]
AFS	2.51 ± 0.01[c]	0.39 ± 0.01[c]	9.0 ± 0.4[a]	5.9 ± 0.5[c]	88.1 ± 0.4[c]	53.0 ± 0.9[c]	1.9 ± 0.1[c]

Note: Reported values correspond to the means ± standard deviation. Results are expressed on dry basis. Different letters in the same column indicate significant differences ($p < 0.05$).

Table 2
Mineral content (mg/100 g) of *Pachyrhizus ahipa* roots and flour. AFGP: ahipa flour obtained by grating plus pressing; AFS: ahipa flour obtained by slicing.

Sample	Sodium	Potassium	Calcium	Magnesium	Iron	Phosphorus
Whole root	12.2 ± 2.5[a]	773 ± 15[a]	210 ± 15[a]	64.3 ± 0.5[a]	37 ± 2[a]	849 ± 92[a]
AFGP	16.4 ± 0.3[b]	459 ± 6[b]	97 ± 3[b]	31.1 ± 0.6[b]	4 ± 1[b]	329 ± 21[b]
AFS	20.6 ± 1.4[c]	619 ± 25[c]	109.8 ± 0.4[c]	42 ± 1.4[c]	4.0 ± 0.7[b]	435 ± 48[c]

Note: Reported values correspond to the means ± standard deviation. Results are expressed on dry basis. Different letters in the same column indicate significant differences ($p < 0.05$).

was one of the main elements in *Dioscorea bulbifera* (potato yam) and *Dioscorea dumentorum* (bitter yam).

Magnesium and calcium contents were higher for AFS than for AFGP. Calcium content of ahipa flour was in the range of that found by Aboubakar, Njintang, Scher, and Mbofung (2008) for taro flours from the varieties KW1 and CE.

Among the essential elements analysed, iron content was found to be the lowest, since iron is classified as a plant micronutrient, unlike the other elements quantified. No significant differences in iron levels were observed between AFGP and AFS.

Comparing calcium content of ahipa and wheat flours, levels found in this work were, on average, 4.77 times higher than those of wheat flour (Peterson, Johnson, & Mattern, 1986). At the same time, ahipa flour samples had iron contents 3.05 times higher than those of wheat flour. This finding indicates that ahipa flour could provide higher calcium and iron quantities than traditional flours, such as wheat flour.

Ahipa whole roots had a higher ($p < 0.05$) phosphorus content than had ahipa flours, being 49% and 61% higher than those of AFS and AFGP, respectively (Table 2). Besides, AFS phosphorus content was 24% higher ($p < 0.05$) than that of AFGP.

Mean crude fat content of ahipa whole roots (Table 1) was significantly higher than those of other roots and tuber (R&T) crops. Afoakwa and Sefa-Dedeh (2001) found that mean fat contents for *Dioscorea dumetorum* were 0.35–0.38%. Authors have pointed out that these levels were quite low and comparable to the values found for other R&T crops, such as potato (0.4/100 g) and edible aroids (0.2/100 g). Nevertheless Padonou, Mestres, and Coffi Nago (2005) reported a mean value of 0.56% (dry basis) for lipid content of cassava dried roots.

Likewise, crude fat content of ahipa whole roots was significantly higher than that of flours. Although most *P. ahipa* root tissues are formed by parenchyma cells, root external tissues (periderm) probably contain suberised cells and a certain proportion of waxes and other hydrophobic substances that act as a protection and waterproofing barrier.

Concerning whole root crude protein content, Zanklan et al. (2007) have mentioned that *Pachyrhizus spp* (yam bean) produces relatively high levels of protein, up to 3–5 times higher than that of commonly grown tropical R&T crops, such as cassava, sweet potato and yam. Authors reported a 9.0% crude protein content for *P. ahipa* roots, close to those found in the present study (Table 1). In a previous work (López et al., 2010), a 6.5 ± 0.7% crude protein content of ahipa whole roots was reported. Since determinations correspond to two different crops, grown successively in the same plot, differences in crude protein content could be probably attributed to better soil fertility status and root nodulation in the second cycle, among other factors.

Results of the present study showed that in the case of the flours, AFS had a 36% higher ($p < 0.05$) level of crude protein than had AFGP. One possible explanation is the loss of soluble proteins during the pressing step implemented in getting AFGP. According to Forsyth and Shewry (2002), salt-soluble proteins comprised about 60% of the total nitrogen of *P. ahipa* roots, with low-molecular-mass nitrogenous components comprising a further 30%. Electrophoretic analysis of the salt-soluble proteins showed that none of the components in six different *P. ahipa* accessions was present in amounts sufficiently high to suggest a role as storage proteins. Forsyth and Shewry (2002) have concluded that the primary roles of those proteins were probably related to aspects of the tuberisation process and root metabolism/development, in some cases conferring protection to pests and pathogens. These authors have pointed out that true storage proteins were not present and that the absence of storage proteins was consistent with the biological role of *P. ahipa* roots as carbohydrate storage organs rather than as propagules.

Compared with traditional flours, protein content of AFS flour (Table 1) was slightly below the values reported by Sabanis and Tzia (2009) for bread wheat (11.8%) and durum wheat flour (13.8%) and similar to the values found in corn (7.5%) and rice (7.0%) flour.

With respect to the fibre content (Table 1), acid detergent fibre (ADF) for ahipa whole roots was 53.5% and 42.2% higher than the ADFs for AFS and AFGP, respectively. This difference could be indicative of the root skin fibre content. When comparing the ADF content of the flours, AFGP had 19.6% more fibre than had AFS.

Total carbohydrate content (TCC) was estimated by difference, after quantifying all other components of the proximate analysis. TCC would correspond to the contents of soluble sugars, starch and structural carbohydrates (cellulose, hemicelluloses) taken together. TCC of ahipa flours (Table 1) was slightly below the values reported by Aboubakar et al. (2008) for taro flours (90.5–95.5%).

Concerning total starch content (Table 1), whole ahipa roots had between 18–27% less starch than had ahipa flours. There were no significant differences between total starch levels of AFGP and AFS. Thus, the solids (mainly leached starch) recovery step, implemented during the AFGP preparation procedure, was quite effective. In a previous work (López et al., 2010), it was reported that the starch yields obtained by a technique, designed to extract ahipa roots starch on a laboratory scale, were 56.54% w/w on a dry basis. These starch yields were close to the values of total starch content found in AFGP and AFS samples.

Total starch content of AFGP was lower than that reported by Lebot et al. (2009) for cassava, sweet potato, taro and yam. AFS had a total starch content similar to that of sweet potato (Krishnan, Padmaja, Moorthy, Suja, & Sajeev, 2010; Lebot et al., 2009).

3.2. Physiological parameters

Three major physiological disorders occur in tropical R&T (tuberous root) crops after harvest and subsequent storage. According to Ravi and Aked (1996), these are the increase in respiratory activity in association with loss of water and carbohydrates, breakage of dormancy and low-temperature-induced damage. Ahipa roots respiratory activity, measured at room temperature (~20 °C), was 25.4 ± 5.34 ml CO_2 kg^{-1} h^{-1}.

In the case of sweet potato, respiration contributed to loss in weight and alteration of internal and external appearance (Ravi & Aked, 1996). Picha (1986) reported that the respiration rate of sweet potato on the day of harvest was 27 ml of CO_2 kg^{-1} h^{-1},

similar to the rate found in *P. ahipa*. Measurements of respiration in both healthy and decaying *Dioscorea rotundata* tubers, by Coursey and Rusell (1969), showed respiratory activity varying between 5 and 20 ml of CO_2 per kilogramme of fresh weight per hour in healthy tubers (dependent on age and dormancy stage), whereas decay was associated with rates above 35 ml CO_2 kg^{-1} h^{-1}.

Referring to respiratory activity of the corms of Aroids during harvest and storage, Passam (1982) reported that, in taro and tannia corms, respiratory rates were 22 ± 5 and 41 ± 11 ml CO_2 kg^{-1} h^{-1} at 27 and 32 °C, respectively.

The level of endogenous α-amylases in starchy products significantly affects the industrial use of these commodities. Depending on the end use, it may be useful to have a level of α-amylase sufficient to produce saccharides available to yeast, but not so high as to cause excessive dextrinisation. α-Amylase activity (Table 1) was significantly higher (30% and 50%) in ahipa whole roots than in flours. Likewise, AFS showed 1.4 times higher α-amylase activity than did AFGP. Possibly, the supernatant released during the pressing step implemented for AFGP contained some soluble enzymes, including part of the α-amylase.

3.3. Ahipa flour physicochemical characteristics

The particle size distributions of ahipa flours were studied at the macroscopic level, using a laboratory sifter, and at the microscopic level by SEM. With regard to the sieving process, the percentages of particle size distribution for AFGP and AFS flours are shown in Fig. 2; approximately 36% of AFGP was retained in the 32D, 45D, 60D and 6xx sieves and 64% of AFGP passed through the sieves and was retained in the pan.

With AFS, although 41% of the flour passed through the sieves and reached the pan, a higher proportion of coarser fractions (59%) was retained, especially by the 32D sieve.

Fig. 3a–c show the scanning electron micrographs of ahipa flour pan samples; images correspond to starch granules as well as other flour components, such as fibre and protein complexes. In all samples, deformed, truncated particles, with little sphericity, were observed. AFG-WP exhibited a particle size distribution with a mean value around 10 μm and a high contribution of particles larger than 18 μm that could be analysed as the aforementioned complexes (Fig. 3d). They probably could not be disaggregated by the milling process. Samples corresponding to AFGP presented a high contribution of small particles (lower than 6 μm) that might be assigned to the added recovered solid (mainly starch) (Fig. 3d). Likewise, particles larger than 18 μm were also observed for this sample, although in a smaller proportion than that for AFG-WP. Possibly the pressing process allowed the solubilisation of part of the proteins. AFS showed a significant contribution of particles with size lower than 6 μm (Fig. 3d); the mean particle size was similar to that corresponding to the other samples (11 μm). Again, a high contribution of particles larger than 18 μm was observed.

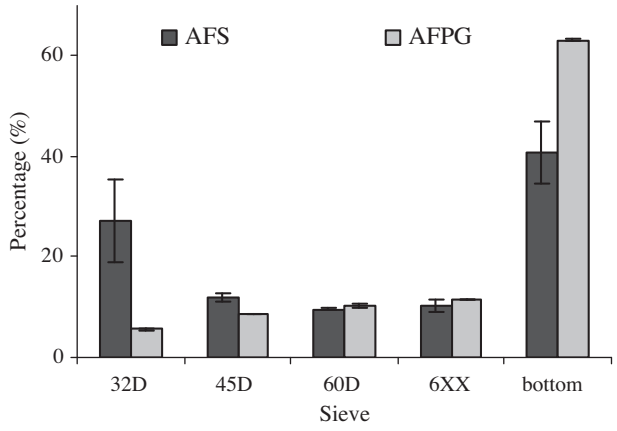

Fig. 2. Particle size distribution (%, w/w) of *Pachyrhizus ahipa* flour, determined by sieving.

As can be seen in Fig. 3, and as previously reported, ahipa starch granules were not associated as clusters; they exhibited round and polygonal shapes with irregular borders (Leonel, 2007). In granule size, ahipa starch exhibited a monomodal straight distribution, ranging from 2 to 18 μm, with a median value of 7.6 μm (López et al., 2010).

Concerning colour of ahipa flours, lightness ($L*$) value was 85.4 ± 2.0 and 84.1 ± 1.1 for AFGP and AFS, respectively. The $a*$ coordinate showed similar values for both products (1.04 ± 0.08 and 1.22 ± 0.16 for AFGP and AFS, respectively). Major differences were observed in $b*$ coordinates (11.2 ± 0.5 for AFGP and 15.0 ± 0.9 for AFS). Hue values corresponded to 84.7° ± 0.2 and 85.4° ± 0.4 for AFGP and AFS, respectively. $L*$ values of ahipa flours were slightly below than those of marama (*Tylosema esculentum*) bean flour (92.2–96.5) and higher than those found for heated defatted soya flour (72.2). Ahipa flour $L*$ values were close to those of unheated defatted soya flour (89.1) (Maruatona, Duodu, & Minnaar, 2010).

Analysing the chromaticity coordinates, ahipa flour samples had $a*$ values close to those of taro RIE variety flour (1.4 ± 0.1); ahipa $b*$ values were close to those of taro KW1 and KW2 flour varieties (Aboubakar et al., 2008).

As previously mentioned (Section 2.3), AFGP and AFS maintained their original quality attributes, even after nine months of storage, while AFG-WP samples developed off-flavours in a short period (one week). This fact could be attributed to fermentation processes that could have been occurred during the drying of the grated pulp. The high water and carbohydrate contents of the roots and the relatively low drying temperature could have favoured this process.

3.4. Ahipa flour functional properties: gelatinisation of ahipa flour suspensions

Thermograms showed that the main peak was associated with starch gelatinisation (Fig. 4). Thermal parameters of the ahipa flour samples are shown in Table 3. Non-significant differences ($p > 0.05$) were obtained between thermal parameters of raw and defatted ahipa flour, which is in agreement with the low lipid content of ahipa roots (Table 1). Onset peak temperature was the thermal parameter most affected by the preparation procedure, being lower for the flour obtained by grating and pressing (Table 3). AFGP exhibited significantly ($p < 0.05$) lower peak temperature values than AFS and AFG-WP. This could be attributed to the loss in the supernatant of high melting temperature soluble components. Besides, AFS and AFG-WP maintained all the structural components that constitute the roots. The peak temperature of AFGP was similar to those reported for the gelatinisation of ahipa starch (López et al., 2010); this statement reinforces the hypothesis of soluble components loss during ahipa flour preparation. Differences between thermal parameters of starch and flours have also been reported for taro (Jane, Shen, Lim, Kasemsuwantt, & Nip, 1992), although Aboubakar et al. (2008), working on taro flour and starches, stressed that thermal behaviour tendencies depended on the variety.

Enthalpy values of ahipa flours obtained by grating (AFGP and AFG-WP) were similar and differed significantly ($p < 0.05$) from those obtained by AFS (Table 3), although the values were closer and similar to those reported in previous work on ahipa starch (López et al., 2010). Cooke and Gidley (1992) have postulated that

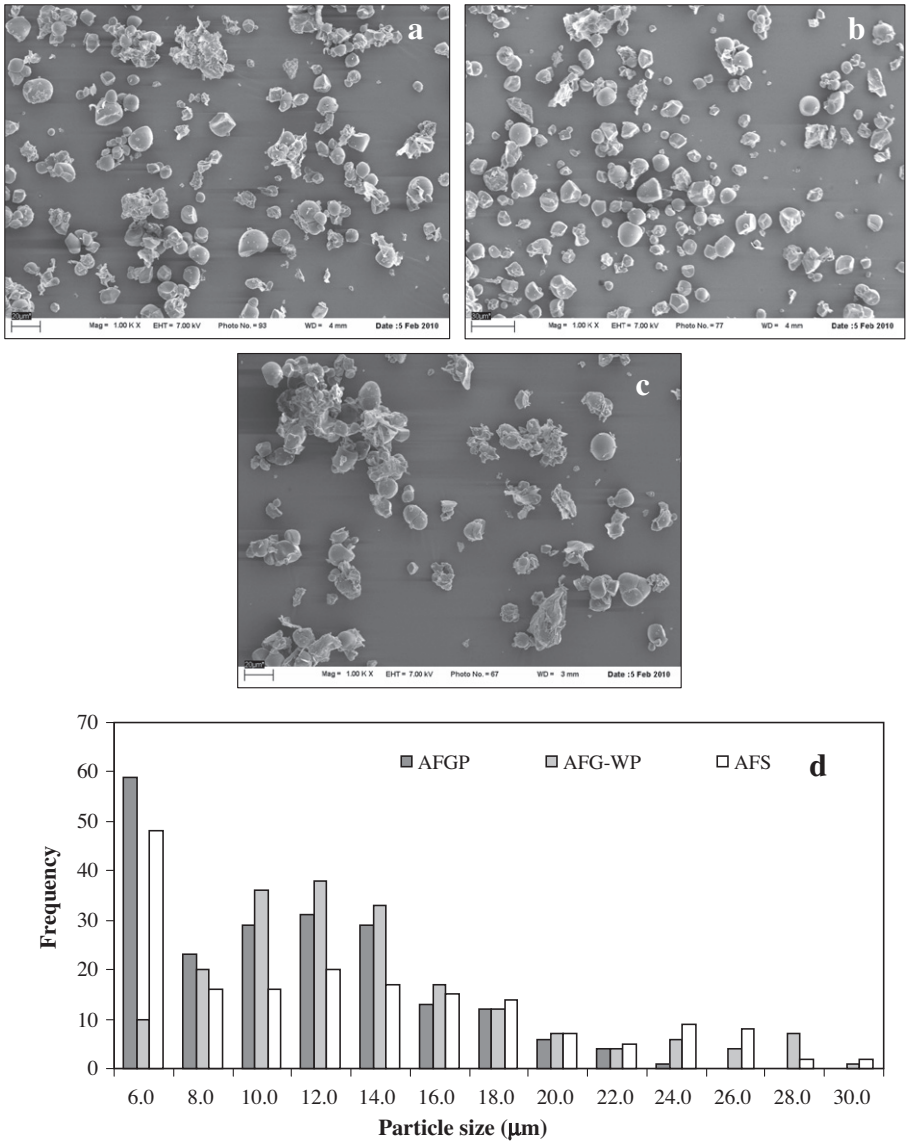

Fig. 3. Scanning electron micrographs of *Pachyrhizus ahipa* flour samples retained in the pan after the sieving process. (a) AFG-WP: ahipa flour obtained by grating, without pressing; (b) AFGP: ahipa flour obtained by grating plus pressing; (c) AFS: ahipa flour obtained by slicing; (d) Histogram corresponding to particle size distribution of ahipa flour samples. Magnification is indicated in the micrograph.

gelatinisation enthalpy reflects the loss of double helical order, while Tester and Morrison (1990a,b) have postulated that it reflects the overall crystallinity (quality and amount of starch crystallites) of amylopectin. Furthermore, due to limited information on amylose chain length and amylopectin branch chain length distribution of ahipa starches, it is very difficult to discuss the influence of molecular structure on the DSC parameters of ahipa flours.

Gelatinisation profile of ahipa flours may be influenced, not only by the starch composition (amylose to amylopectin ratio), but also by other characteristics, such as the granular architecture (crystalline to amorphous ratio), and the molecular structure of amylopectin (extent of branching, unit chain length) (Aboubakar et al., 2008). Furthermore, high amylose starches, with longer average chains, have been reported to exhibit higher transition temperatures (Jane et al., 1992).

Among thermal parameters of flours from different sources, ahipa flours exhibited peak temperatures higher than those of taro, which varied between 55.56 and 68.67 °C (Aboubakar et al., 2008) and lower than those of green banana flour, with a peak temperature of 68.13 °C (Tribess et al., 2009). Comparing the associ-

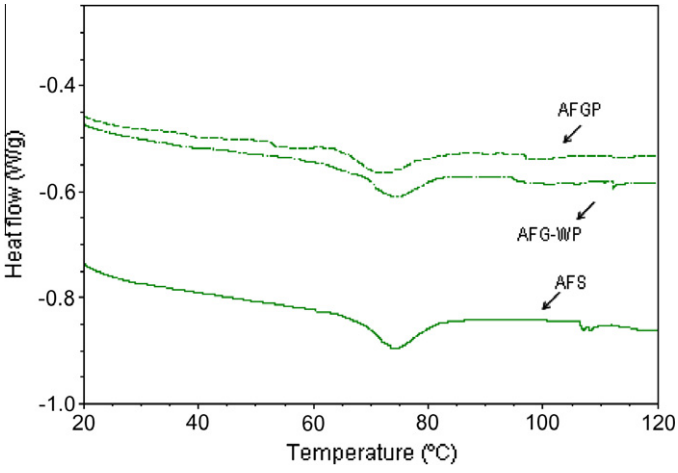

Fig. 4. Thermograms obtained by DSC for *Pachyrhizus ahipa* flour samples. AFG-WP: ahipa flour obtained by grating, without pressing; AFGP: ahipa flour obtained by grating plus pressing; AFS: ahipa flour obtained by slicing.

ated enthalpies, ahipa flours, in general, exhibited similar values to those reported for taro but lower than those of green banana (Aboubakar et al., 2008; Tribess et al., 2009).

Swelling power (SP) of ahipa flour obtained by different processing alternatives was plotted as a function of temperature (Fig. 5a). Up to 75 °C (near gelatinisation temperature), no significant differences of SP were observed between flours obtained by different processing methods. A similar trend was reported by Aboubakar et al. (2008), working on taro flours and starches. Agunbiade and Longe (1999) also reported that starches and flours from different sources exhibited a slow swelling pattern at relatively low temperatures. When gelatinisation temperature was reached, the starch granules swelled, increasing by several times, their original size due to hydration. At higher temperatures, SP of ahipa flour, obtained by grating and pressing, was higher than that obtained by the slicing procedure (Fig. 5a).

Table 3
Thermal parameters of ahipa flours: effect of preparation procedure. AF-WP: ahipa flour obtained by grating, without pressing; AFGP: ahipa flour obtained by grating plus pressing; AFS: ahipa flour obtained by slicing.

Ahipa flour obtaining procedure		Onset peak temperature (°C)	Peak temperature (°C)	Enthalpy (J/g dry basis)
AF-WP	Raw	65.9 ± 0.9^{a}	73.6 ± 0.5^{a}	9.9 ± 0.3^{a}
	Defatted	65.7 ± 1.6^{a}	73.5 ± 0.3^{a}	9.6 ± 0.2^{a}
AFGP	Raw	$64.3 \pm 1.2^{a,b}$	71.2 ± 0.8^{b}	9.4 ± 0.5^{a}
	Defatted	63.5 ± 0.3^{b}	70.5 ± 0.2^{b}	9.4 ± 0.3^{a}
AFS	Raw	68.5 ± 0.8^{c}	73.1 ± 0.9^{a}	8.6 ± 0.2^{b}
	Defatted	65.9 ± 1.0^{a}	73.4 ± 0.2^{a}	8.5 ± 0.2^{b}

Note: Reported values correspond to the means ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$).

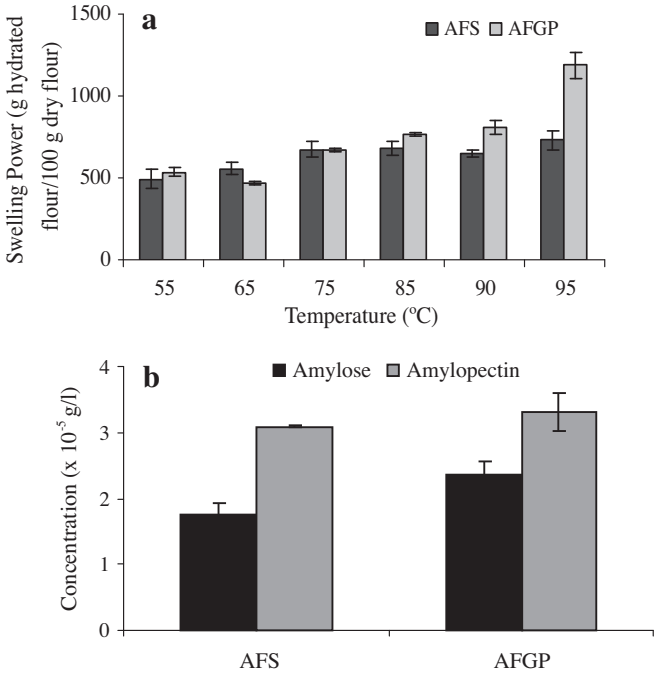

Fig. 5. (a) Swelling power (g hydrated flour/100 g dry flour) of *Pachyrhizus ahipa* flour samples as a function of temperature (°C). (b) Concentration ($\times 10^{-5}$ g/l) of amylose and amylopectin leached from ahipa flour samples during heating at 95 °C. AFGP: ahipa flour obtained by grating plus pressing; AFS: ahipa flour obtained by slicing.

Swelling is accompanied by solubilisation of starch granule constituents. Again, below the gelatinisation temperature, amylose and amylopectin amounts leached from starch granules were negligible. At higher temperatures, the lixiviation process became relevant and the concentrations of both components in the supernatant increased with temperature. This effect is observed in Fig. 5b, that shows the concentrations of amylose and amylopectin leached from the flour starch granules during heating at 95 °C. Flour preparation procedure did not significantly affect ($p > 0.05$) leached amylopectin concentration while amylose heated was significantly ($p < 0.05$) higher for AFGP than for AFS.

Ahipa flour WHC was 191 ± 0.8% and 132 ± 0.6%, for AFS and AFGP, respectively. Although WHC and SP are related determinations, WHC (which is performed at room temperature) gives information about physical properties that influence sample behaviour. Meanwhile SP, which is determined at different heating temperatures, is one of the indicative parameters used for gelatinisation process studies. The crystalline molecular structure of starch flour is broken and the water molecules are bonded to the free hydroxyl groups of amylose and amylopectin by hydrogen bonds, which could cause an increment in the water holding capacity (Singh, Singh, Kaur, Sodhi, & Gill, 2003).

4. Conclusions

The ahipa roots and flour can be considered alternative food sources of gluten-free starch, suitable for people with specific nutritional needs. Compared to other R&T, ahipa roots and flour had a more balanced chemical composition from a nutritional point of view. They might make a contribution of protein, fibre and minerals, such as potassium, calcium and iron.

Among the ahipa flour-obtaining procedures assayed, the grating process had to be accompanied by a pressing step; likewise, this alternative required the recovery of the starch leached. When the pressing step was not implemented, AFG-WP developed a marked off-flavour in a short time, limiting its use and conservation. The slicing procedure for obtaining AFS was simpler and the resulting product showed the advantages of higher contents of potassium, magnesium, calcium and protein than in the case of AFGP. Water-holding capacity of AFS was higher, this functional property being relevant to many technological applications. AFGP showed a lower sodium content, and higher ADF content, widely desirable nutritional characteristics. An additional technological advantage of this product was its lower gelatinisation temperature, which would be related to its lower protein content, with associated high melting points. Further investigations about ahipa protein characteristics and their interaction with fibre are required. The differences observed for both flours, in terms of α-amylase activity and swelling power (measured at temperatures above the gelatinisation one), must be taken into account in relation to specific applications.

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