Extraction and characterization of polisaccharides of *Lepidium meyenii Walpers* root. comparison of a processed product and fresh root after drying and grinding

Larry Ladislao Ramos Paredes* y Carmen Lucia de Oliveira Petkowicz**

*Universidad Católica San Pablo, Facultad de Ingenierias - Arequipa – Perú; E-mail: larryramos1@hotmail.com.
**Universidade Federal do Paraná, Departamento de Bioquímica - Curitiba - Paraná – Brasil.

Abstract

Maca (*Lepidium meyenii Walpers*) is a Peruvian plant. The roots are used as food and popular medicine. In this work, we have isolated and characterized the cell-wall polysaccharides. The material was defatted, the enzyme inactivated and treated with α-amylase and then subjected to aqueous (25 and 93°C) and alkaline extractions sequences (1M, 2M and 4M NaOH at 25 °C). For extractions two materials were used: a commercial product known as maca powder and fresh roots dryed and ground. The composition of the neutral monosaccharides was determined by GLC and acidic iones colorimetrically. Although the monosacaride composition of the fractions was similar, in general, there was a higher content of xylose and less glucose in fractions obtained from maca powder when compared to the roots, suggesting the occurrence of changes during post-harvest processing that affect the solubility of polymers.

Keywords: *Lepidium meyenii,* maca, polysaccharides.

Introduction

Maca (Lepidium meyenii) is a plant native of Peru, the roots have economic interest due to their medicinal and nutritional properties. Since the sixteenth century the native people of the Central Andes of Peru believed that the use of maca improves human reproduction and invigorates those who consume it (CHACON, 1961; GONZALES, 2001). This plant is grown from centuries ago and is used by the natives as food and folk medicine, in treating anemia, tuberculosis and sterility (CICERO *et al.*, 2001; ZHENG *et al.*, 2000).

Several investigations attempt to prove the properties attributed to this root, as well as some components of low molar mass were studied (CUI *et al.*, 2003; RONCERO *et al.*, 2005, ZHENG *et al.*, 2000).

The maca root contains a large amount of starch (PAULET; CARRASCO, SÁNCHEZ, 2006), but after an extensive literature review, no data have been found on other polysaccharides. In this study we isolated and characterized the polysaccharides extracted from the cell wall of maca root (*Lepidium meyenii*).

Polysaccharides are important chemical constituents of plants because they are the main components of the cell wall (structural support for plant cells) (REID, 1997). The cell wall is a dynamic mechanical and biological structure, that suffer changes along with life (McCANN *et al.*, 2001) and is involved in the morphology, growth and development of cells and interactions between host plants and their pathogens, and works as a physical barrier against microorganisms and other harmful agents (CARPITA and McCANN, 2000, TALMADGE *et al.*, 1973).

An important part of the cell wall is formed by hemicellulose, which are polysaccharides intimately associated with cellulose, defining structural properties of the cell wall such as regulating the growth and development of plants. The hemicellulose compounds include xyloglucans, glucurono arabinoxylans, mannans, galactomannans, glucomannans and galactoglucomannans. In dicotyledons such as maca, the principal hemicellulose component are xyloglucans.

Materials and methods

Samples

A commercial processed powder of maca root (*Lepidium meyenii*) was purchased in the market (Maca powder of Santa Natura by Agroindustrias Floris SAC; Lima, Peru). This sample was identified as "M".

A second sample correspondED to a fresh maca root bought in the municipal market (San Camilo) in Arequipa city, Peru. The sample was washed with sodium hypochlorite, sun dried, ground to powder and identified as "R".

Extraction of polysaccharides from maca powder

The maca powder was submitted to enzyme inactivation with methanol–H_2O (4:1, v/v) under reflux for 20 min, immediately cooled to room temperature, and centrifuged. Insoluble material was sedimented at 15,400g for 20 min, washed with ethanol, dried under vacuum, milled, and defatted with p-toluene– ethanol (2:1, v/v) in a Soxhlet extractor. The solid was dried and used to remove the starch. The maca powder was treated with alpha amylose enzyme TDF-100A A3306 from Sigma Aldrich, at 50 ± 5 ° C. The degradation of starch was monitored with the iodine test. The material was centrifuged and the residue washed with water and ethanol and dried, resulting in maca powder free of starch, which was used for polysaccharide extraction. Successive extractions were performed in a mechanical blender and after each one, centrifugation was carried out and the residue was submitted to the next extraction. Each extract was concentrated and treated with ethanol (2:1, v/v) in order to obtain precipitated polysaccharides, which were then washed three times with ethanol and dried under vacuum.

Maca powder defatted, inactivated and free of starch was subjected to sequential extractions with water at 93 °C and NaOH solutions at concentrations: 1, 2 and 4 mol L^{-1}. Alkaline extractions were performed in the presence of sodium borohydride; acidified with 50% acetic acid until pH 5. The polysaccharides were separated by centrifugation, dried under vacuum and labeled as hemicellulose A. The supernatant obtained after precipitation was dialysated, concentrated and precipitated with ethanol 3:1 v/v, yield in formation of the hemicellulose B fraction, which was separated by centrifugation and dried under vacuum.

Determination of total sugar, protein and uronic acid content

Total carbohydrate was measured by the phenol–sulfuric acid method (DUBOIS *et al.,* 1956), using glucose as standard. Uronic acid was estimated by the sulfamate/ 3-phenylphenol colorimetric method (FILISETTI-COZZI and CARPITA, 1991), using galacturonic acid as standard. Protein was determined according to Peterson (1977), using BSA as standard.

Monosaccharide composition

Polysaccharides were hydrolyzed with 2 M trifluoroacetic acid (5 h, 100 °C), and after concentration to dryness, the residues were reduced with $NaBH_4$ (WOLFROM and THOMPSON, 1963b) and acetylated with pyridine–acetic anhydride (1:1, v/v, 16 h, at 25 °C) (WOLFROM and THOMPSON, 1963a).

The final residue was solubilized and partially hydrolyzed with 72% (w/w) H_2SO_4 for 1 h at 0–4 °C, diluted to 8% and kept at 100 °C for 15 h (BIERMANN, 1989). The hydrolysate was neutralized with $BaCO_3$ and the insoluble material removed by filtration. Monosaccharides were reduced and acetylated as described above.

The resulting alditol acetates were analyzed by gas–liquid chromatography (GLC) using a model 5890 S II Hewlett–Packard gas chromatograph at 220 °C (flame ionization detector and injector temperature, 250 °C) with a DB-210 capillary column (0.25 mm internal diameter x 30 m), film thickness 0.25 µm, with nitrogen as carrier gas (2.0 ml/min).

HPSEC–MALLS analysis

High pressure size exclusion chromatography (HPSEC) was carried out using a multidetection equipment: a Waters 2410 differential refractometer (RI) and a Wyatt Technology Dawn F multi-angle laser light scattering (MALLS) detector. Four Waters Ultrahydrogel 2000/500/250/120 columns were connected in series and coupled to the multidetection equipment. A 0.1 mol L^{-1} $NaNO_2$ solution, containing NaN_3 (0.5 g L^{-1}), was used as eluent.

Previously filtered samples (0.22 μm; Millipore) were analyzed at 1.5 mg mL^{-1} and the data were collected and processed by a Wyatt Technology ASTRA program.

Results and discussion

After the deffating, the material was subjected to enzyme inactivation with methanol-water (4:1, v/v). Normally this treatment is also effective in removing low-mass carbohydrates, as saw in this case, where the extract was viscous, indicating the presence of compounds with higher molar mass. The extract was treated with ethanol (3v), promoting the precipitation of a fraction that was called MMMe.

According to Paulet, Carrasco and Sanchez (2006) dried maca roots contain 20% of starch. This was characterized (RONDAN-SANABRIA and FINARDI-FILHO, 2008), the maca powder inactivated, and defatted was subjected to hydrolysis with α-amylase to remove starch and thus facilitate the extraction and study of other present polysaccharides. After the enzymatic digestion, the powder was centrifuged and the residue was washed with water and ethanol and subsequently dried, resulting in maca powder free of starch, which was used for the extractions of the cell wall polysaccharides. The supernatant was boiled for 30 minutes and the denatured enzyme was removed by centrifugation. After removing the enzyme, the supernatant dialysate was concentrated and precipitated with ethanol, resulting in the fraction labeled as MFW.

The maca powder defatted, inactivated and free of starch was subjected to sequential extractions with water at 93 °C and NaOH solutions at concentrations of 1, 2 and 4 mol L^{-1} at 25 °C.

The extracted fraction after the alkaline treatment was acidified with acetic acid 50% and polysaccharides that precipitated under these conditions were called hemicellulose A. The resulting supernatant dialysate was concentrated and then precipitated with 3 volumes of ethanol, forming the hemicellulose B fraction. For the extract obtained with the1 mol L^{-1} NaOH solution, a new precipitate was formedduring the dialysis which was called hemicellulose D (dialysis).

The identification codes followed the next criteria: all fractions initially received the letter "M" regarding to maca, followed by "M" that identifies the initial sample as maca powder. The next characters refer to the extraction medium used: HW (hot water or in the case of alkaline extractions, a number corresponding to the molar concentration of the solution was used followed by "A","B"or "D", according to the type of hemicellulose A, B or D.

The final residue obtained after the extraction was washed with water to neutrality for subsequent hydrolysis.

Table 1 shows yield of polysaccharide, total sugar and protein content in the fractions obtained. All fractions have low yields; among these, the fractions MMMe, MMW and MMHW had the highest yield. From the alkaline extraction, the fraction MM4B obtained with 4 mol L^{-1} NaOH, had the highest yield (1.9%), followed by MM2B (1.3%), obtained with 2 mol L^{-1} NaOH.

In the alkaline fraction, the yield of hemicellulose B was higher than that of hemicellulose A, suggesting the predominance of high molar mass polysaccharides.

To investigate the differences between polysaccharides present in fresh roots of maca and the commercial product

Table 1. Yield, total sugar and protein content in polysaccharide extracts from maca powder

Fractions	Yield[a] % (m/m)	Total sugar[b] (%)	Protein[c] (%)
MMMe	2.2	52.2	10.6
MMW	2.5	52.1	18.2
MMHW	2.0	43.0	2.3
MM1A	1.0	29.9	59.1
MM1B	1.1	39.8	33.4
MM1D	0.2	11.6	8.4
MM2A	0.8	9.2	26.7
MM2B	1.3	81.0	14.8
MM4A	0.5	4.3	17.3
MM4B	1.9	34.3	34.2

[a] Of maca powder defatted.
[b] Dubois method (1956).
[c] Peterson method (1977).

known as maca powder, extractions were done using the maca root. This was sun-dried, ground (50g) and subject to the same treatment applied on maca powder. The roots defatted, inactivated and free of starch were extracted in turn with water and alkaline solution using the protocol previously used for extraction of the maca powder. For the identification of fractions, the same criteria used above was followed, except for the substitution of "M" by "R" (roots).

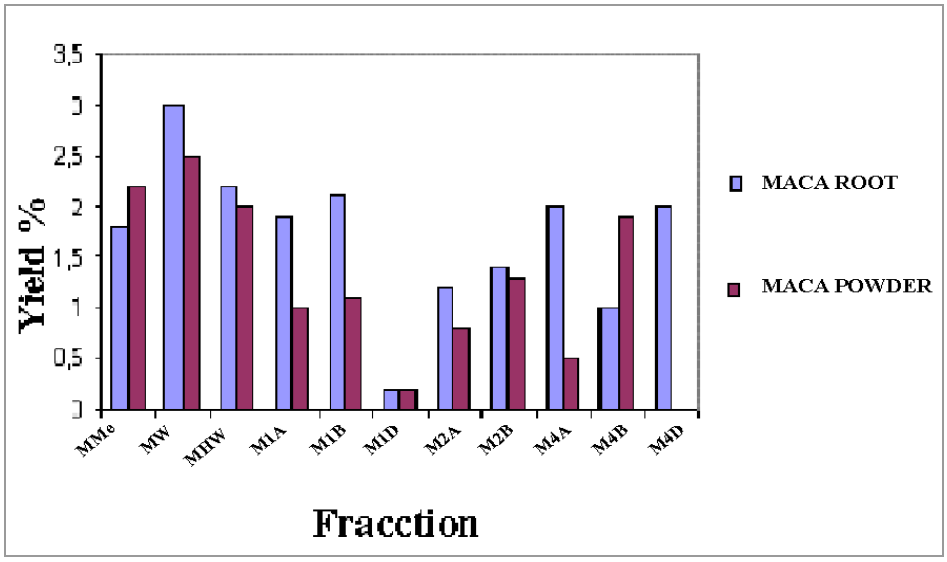

Figure1. comparison of the polysaccharides yields from roots and maca powder

The yields of fractions obtained from the roots and maca powder in water had similar values, whereas with the alkaline extractions larger differences were observed. In general, the yield of alkaline fractions from the roots of maca was higher than that obtained from the maca powder. The fractions obtained with 1 mol L^{-1} NaOH from the root (MR1A and MR1B) was the double of the fractions obtained from the maca powder (MM1A and MM1B) and the fraction obtained with 4 mol L^{-1} NaOH from the root MR4A was four times higher than the fraction from maca powder (MM4A).

The fraction MR4B presented half of the yield from the fraction MM4B. When the total polysaccharides extracted with 4 mol L^{-1} NaOH are considered, the yield from the roots was 2.1 times higher than for "maca powder".

Shiga, Lajolo and Filisetti (2004) studied changes in the cell wall of beans after 24 months storage at room temperature and observed that the amount of soluble material in water practically was not altered. In the meantime, the authors noted that the amount of material extracted with NaOH 4 mol L^{-1} presented a small increase after the storage of the beans.

The observed differences may be due to changes that occur during the storage or even may be due to differences during the post-harvest processing, such as time and intensity of exposure to the sun while drying. Graefe *et al.*, (2004) evaluated the effects of post-harvest processing on oligosaccharides of yacon roots (sonchifolius Smallanthus) and found that the exposure to the sun for six days decreased the levels of oligosaccharides.

Monosaccharide composition of the fractions obtained from extraction of the maca root is indicated in Table 1.

Comparing the composition of the fractions obtained from roots with those of maca powder, it is noted that the aqueous fractions are similar (Figure 2). The hot water fraction obtained from roots presented a higher content of glucose when compared with maca powder. Moreover, in the same fraction of maca powder there is a higher extraction of polymers containing xylose and arabinose compared to the extract obtained from the root. A much

higher percentage of uronic acid and xylose was observed for MMW fraction when compared to the equivalent fraction obtained from roots (MRW).

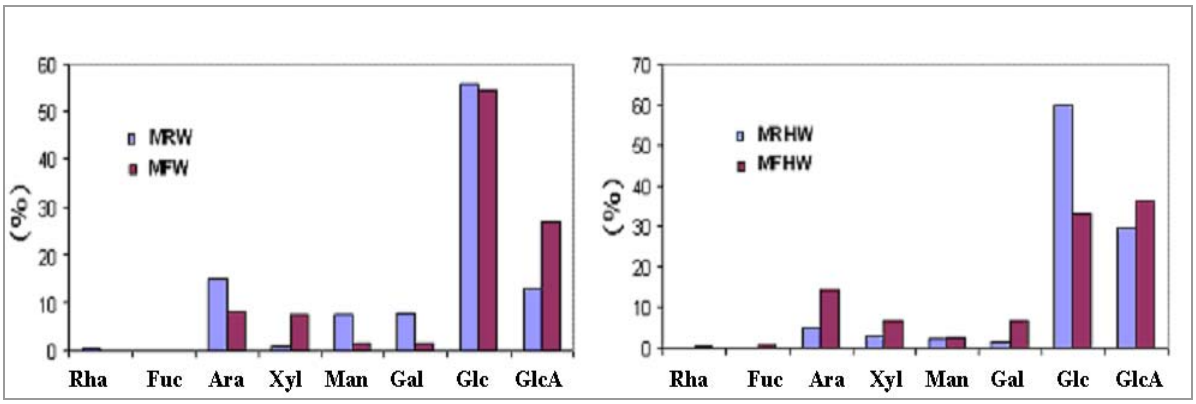

Figure 2. Composition of monosaccharide fractions extracted with water (normal and hot) obtained from root and maca powder

In Figure 3 the monosaccharide compositions of hemicellulose A and B obtained with NaOH 1, 2 and 4 mol L^{-1} are compared.

The alkaline extractions also yielded a higher amount of Xyl in hemicellulose A fractions obtained from maca powder compared to the fractions obtained from roots. Just as observed for aqueous extractions, a greater amount of glucose was detected in the fractions from roots. These results suggest that during drying and storage can occur alterations in the quantity, structure and/or molar mass of polysaccharides, which affect their solubility. Possible changes that may occur in the polysacchardes during post-harvest processing could alter not only the individual polymers, but also could affect the degree of intermolecular association and hence its solubility.

Conclusion

Based on the aqueous extraction of maca powder (*Lepidium meyenii*) inactivated, defatted and free of starch, the mixtures of polysacharides were obtained, whose composition suggests the presence of glucans and acid polysaccharides.

Comparing the composition of the fractions obtained by aqueous and alkaline extractions of the commercial product known as maca powder and fresh roots after drying and grinding, some compositional differences were detected, suggesting the occurrence of changes during post-harvest processing that affect the solubility of polymers.

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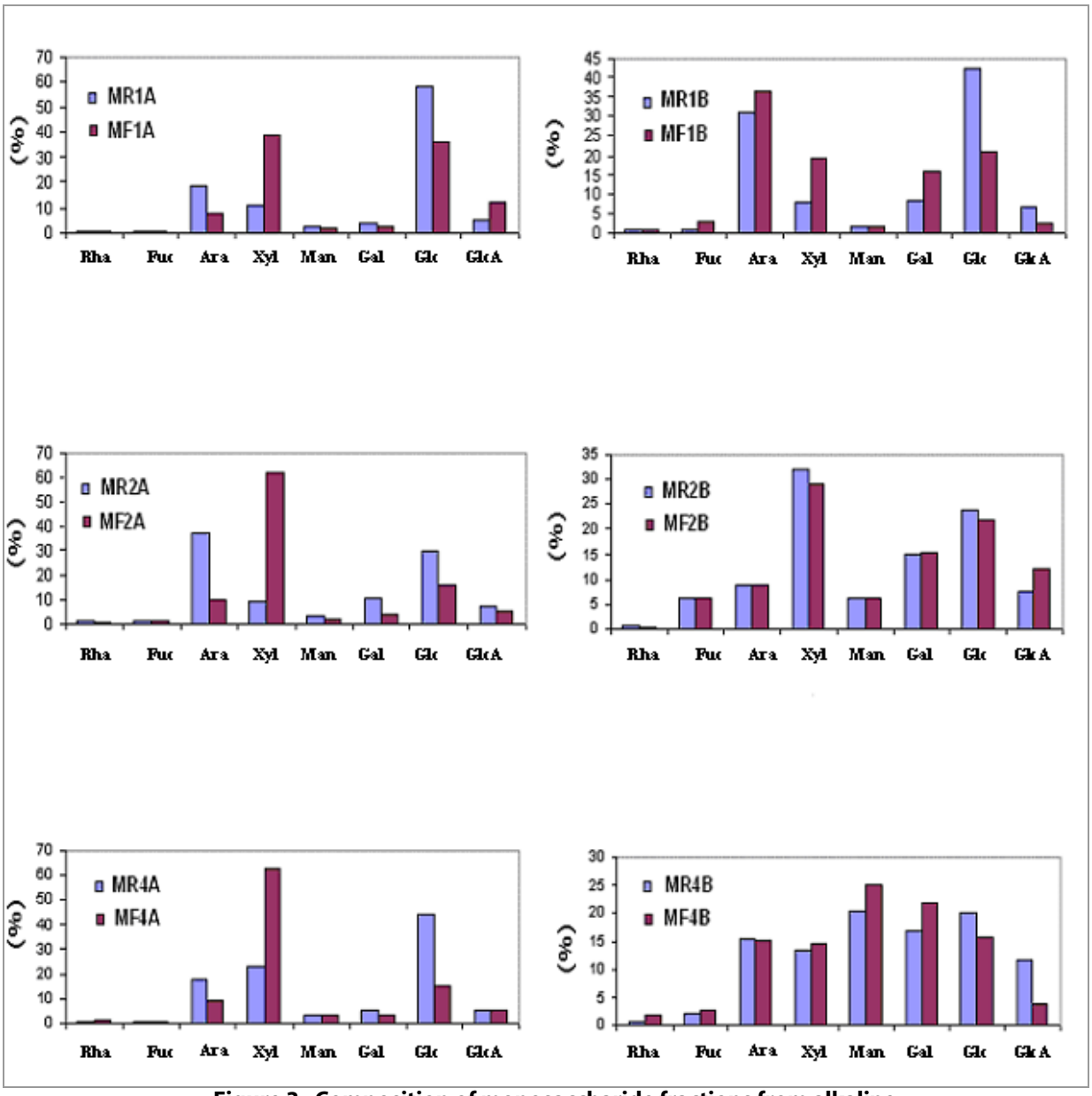

Figure 3. Composition of monosaccharide fractions from alkaline treatment inobtained roots and maca powder".

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