EFFECT OF PRE-TREATMENT ON PHYSICOCHEMICAL AND STRUCTURAL PROPERTIES, AND THE BIOACCESIBILITY OF β-CAROTENE IN SWEET POTATO FLOUR

Nalleli Trancoso-Reyes[a], *Luz A. Ochoa-Martínez[a], Luis A. Bello-Pérez[b], Juliana Morales-Castro[a] , Rocío Estévez-Santiago[c], Begoña Olmedilla-Alonso[c]

[a]Departamento de Ingenierías Química y Bioquímica. Instituto Tecnológico de Durango, Felipe Pescador 1830 Ote, Colonia Nueva Vizcaya, C.P. 34080. Durango Dgo, México.

[b]Centro de Desarrollo de Productos Bióticos del IPN, Carretera Yautepec-Jojutla, km 6, calle Ceprobi 8. Col. San Isidro, C.P. 62731, Yautepec, Morelos, México.

[c]Instituto de Ciencia y Tecnología de Alimentos y Nutrición. Calle José Antonio Nováis 10. Ciudad Universitaria. C.P. 28040. Madrid, España.

*** Correspondence:** Dra. Luz Araceli Ochoa Martínez. Departamento de Ingenierías Química y Bioquímica. Instituto Tecnológico de Durango, Felipe Pescador 1830 Ote, Colonia Nueva Vizcaya, C.P. 34080. Durango, Dgo. México.

E-mail: aochoa@itdurango.edu.mx

Tel/Fax +52(618)8186936 ext. 114

Abstract

The aim of this research was to evaluate the effect of microwave or steam pre-treatment of raw sweet potato on physiochemical and microstructural properties, and the bioaccessibility of β-carotene in sweet potato flour. It is the first time that is used the *in vitro* digestion model suitable for food, proposed in a consensus paper to assess the bioaccessibility of β-carotene in sweet potato flour. The pre-treatments produced a rearrangement in the structure of starch, which was greater by using microwaves (M6) owning to the greater increase in the gelatinization temperatures up to 4.14 °C, while the enthalpy presented the higher reduction up to (4.49 J/g), both parameters in respect to the control. The resistant starch fraction was not modified, with about 3% in all samples. Microwave (M6) and all the steam pre-treatments, presented the higher bioaccessibility of β-carotene. This flour can be used in the development of new products with high β-carotene content.

Keywords: bioaccessibility, crystallinity, microstructure, starch, β-carotene

1. Introduction

The sweet potato (*Ipomoea batatas* L.) is a root with positive attributes such as geographical variety in terms of production, adaptability to marginal conditions, a short production cycle, a high nutritional content and sensorial versatility in terms of flesh color, taste and texture. It is the sixth most important crop on a global level, after rice, wheat, potato, corn and cassava (Faostat, 2013). While the Mexican climate is suited to its cultivation, the limited production options for the sweet potato and the lack of awareness of its nutritional properties have contributed to lags in production and industrialization. Depending on flesh color, the sweet potato is rich in β-carotene, anthocyanins, phenolic compounds, dietary fiber, ascorbic acid, folic acid and minerals (Woolfe, 1992; Grabowski, Truong & Daubert, 2008). Numerous benefits, such as antioxidant, cardioprotective and anti-diabetic effects, have been attributed to sweet potato consumption, with the orange-fleshed sweet potato recognized for its pro-vitamin A activity, which contributes to preventing deficiencies of this vitamin (van Jaarsveld, Faber, Tanumihardjo, Nestel, Lombard, & Spinnler-Benadé, 2005).

While, generally, the sweet potato is consumed cooked, the dried form of the root is also used in the production of flour, which is used in the manufacture of breadmaking and breakfast cereal products, as well as baby foods and alcoholic drinks (Grabowski, Truong & Daubert, 2007; Troung & Avula, 2010; Teramoto, Hano & Ueda, 1998; Wireko-Manu, Ellis & Oduro, 2010). There is no standardized procedure for the production of sweet potato flour, in some regions a blanching process is used before drying and then milling. Different drying methods have also been used, such as solar, rotary drum, tray and spray drying (Truong & Avula, 2010; Grabowsky *et al*., 2007). On a laboratory or commercial scale, sweet potatoes are treated with a sodium metabisulfite solution to inhibit enzymatic darkening (Sablani & Mujumdar, 2007; Aprianita, Purwandari, Watson & Vasiljevic, 2009). The extraction of

compounds using microwave radiation produces improved yield, such as with anthocyanins in the purple-fleshed sweet potato (Lu *et al*., 2010), and improved retention of vitamins, such as thiamin and riboflavin in the orange-fleshed sweet potato (Dawkin & Lu, 1991). Furthermore, the use of microwave blanching on products such as rosemary (Rosmarinus officinalis L.) and marjoram (Marjona hortensis Moench.) has led to improved results in terms of color, ascorbic acid and chlorophyll retention compared to steam and water immersion pre-treatments (Singh, Raghavan & Abraham, 1996). Although there is a large market for foodstuffs prepared with microwaves (Sumnu, 2001), the effect of electromagnetic waves on physiochemical and structural properties and bioaccessibility of β-carotene in sweet potato flour has not been widely documented. The aim of this study was to carry out a comparative study on the effect of the steam and microwave pre-treatment methods (with variable time periods of 2, 4 and 6 min) on the thermal properties, the X-ray diffraction pattern, the viscosity profile, the morphology and bioaccessibility of β-carotene in sweet potato flour using the standardized static *in vitro* digestion model suitable for food, proposed in a consensus paper by Minekus *et al.* (2014).

2. Materials and methods

2.1 Sweet potato flour production

The orange-fleshed sweet potato (*Ipomoea batatas* L.) was obtained from a local supermarket in the city of Durango, in the State of Durango, Mexico. For the preparation of the flour, the sweet potato roots were washed, peeled and diced into 1 cm cubes. The sweet potato cubes were divided into 7 batches, three of which were treated with microwaves and three with steam, for 2, 4 and 6 minutes, with the last batch used as a control (a non-blanched sample). The samples were dehydrated in a tray dryer (Polinox, SEM-2 MAPISA

Internacional. Mex.) at 60 °C, for approximately 10 hours until moisture in the range of 6-7 % was reached. Finally, the samples were milled (Molino Krups F408, Cd. México, Mex.) and the flour sieved through a number 60 mesh (0.25 mm) (Mont Inox, Cd. México., Mex.). The flour was packed in polypropylene bags and stored at 4 °C until it was analyzed.

2.2 Chemical composition

The proximal chemical composition of the sweet potato flour without pre-treatment was obtained according to the official methodologies described by the AOAC (1990). Moisture (method 925.09), raw protein (method 968.06), raw fat (method 920.39), raw fiber (method 962.09), ash (method 923.03) and carbohydrates were measured by difference.

2.3 Determination of resistant and total starch

A MEGAZYME Kit was used for the resistant starch, based on the approved method from AACC 32-40.01, in which each sample was measured at a wavelength of 510 nm in a spectrophotometer (UV-Visible Thermo Scientific, Mod. Genesys 6, USA). Resistant starch was determined using Equation 1:

$$Resistant\ starch = \Delta E\ x\ F\ x \frac{10.3}{0.1}\ x \frac{1}{1000}\ x\ \frac{100}{W}\ x \frac{162}{180}$$

Ec. 1

Where:

ΔE= Absorbance (of the reaction) read against the reagent blank, F= 100 (µg of D-glucose)/absorbance of 100 µg of glucose, 10.3/0.1= Correction of the volume for the samples that contain less than 10 % of resistant starch, 1/1000= Factor of conversion of µg

to mg, 100/W= Factor that expresses the starch as a percentage of the weight of flour, W= Weight in milligrams of flour analyzed and 162/180= Conversion factor of D-free glucose to D-anhydrous glucose.

To obtain the total starch, the available starch was first measured using Equation 2:

$$Available\ starch = \Delta A\ x\ F\ x \frac{100}{0.1}\ x \frac{1}{1000}\ x\ \frac{100}{W}\ x \frac{162}{180}$$

Ec.2

Where:

ΔA= Absorbance (of the reaction) read against the reagent blank, F= 100 (µg of D-glucose)/ absorbance of 100 µg of glucose, 100/0.1= Volume correction,1/1000= Conversion factor of µg to mg, 100/W= Factor that expresses the starch as a percentage of the weight of flour, W= Weight in milligrams of flour analyzed and 162/180= Conversion factor of D-free glucose to D-anhydrous glucose.

The sum of the resistant and available starch is the total starch.

2.4 Thermal properties

The gelatinization temperature is determined using a Differential Scanning Calorimeter (DSC) (TA instruments mod. 2010, New Castle. DE), which had been previously calibrated with indium. 2 mg of flour was placed on an aluminum tray for the DSC, to which 8 µL of deionized water was added using a microsyringe. The trays were hermetically sealed and left to rest for 15 min before the thermal analysis. The samples were heated from 30 to 120 °C at a rate of 10 °C/min and the starting (T_0), peak (T_p), and concluding (T_c) gelatinization

142 temperatures were calculated, as well as the enthalpy (ΔH) (Bello-Pérez, Osorio-Díaz,
143 Agama-Acevedo, Núñez-Santiago & Paredes-López, 2002).
144
145 *2.5 X-ray diffraction pattern*
146 The samples with a particle size 0.250 mm were placed in a glass sample holder with a
147 depth of 0.5 mm and, in turn, placed in an X-ray diffractometer (RIGAKU, DMAX2100,
148 Japan). The following operating conditions were used: voltage of 40 KV, 15 mA, with an
149 incident radiation λ=1.15406 Å of CuKα with scanning carried out up to 60° on a 2θ scale.
150 The data were processed using the Xpower software (Martín, 2004).
151
152 *2.6 Viscosity profile*
153 This determination was carried out on the sweet potato flour in a Rapid Visco Analyzer
154 (RVA super 4 Newport scientific PTY LTD, Sidney, Australia). Four grams of samples and
155 24 ml of distilled water was added to keep the total weight of water and sample constant at
156 28 g. The curves were obtained by applying a heating and cooling cycle which began at 50
157 °C for 1 min, after which a temperature ramp-rate of 7.5 °C/min was applied up to 90 °C,
158 with the sample remaining at this temperature for 5 min and then cooled at the same rate to
159 50 °C (Pineda-Gómez *et al*., 2012).
160
161 *2.7 Morphological characterization (SEM)*
162 The morphological analysis was carried out using Scanning Electron Microscopy
163 (Scanning Electron Microscopy, Philips, Mod. XL30 ESEM, Holland). Prior to the analysis,
164 the samples were placed in a sample carrier with carbon tape mounted on an aluminum

sample holder. The samples were coated with a fine layer of gold for sputtering. The analysis was carried out using the tension conditions for the acceleration of electrons of 10 kV.

2.8 Determination of β-carotene

2 g of the flour samples obtained using different pre-treatments were weighed to carry out the extraction of β-carotene with acetone, with the organic phase brought to dryness in a rotary evaporator. Subsequently, the sample was reconstituted with methanol and methyl tert-butyl ether (70:30 v/v) and brought to a volume of 25 mL. The β-carotene was determined by high-performance liquid chromatography (HPLC) using a system that consists of a model 600 pump, rheodyne injector and a 2998 Photodiode Array (PDA) Detector (Waters, Miliford, MA, USA). A C_{30} YMC column was used (250 mm x 4.6 mm, 5 μm particle size, Europ GmbH, Dinslaken, Germany). The mobile phase was methanol and methyl tert-butyl ether at a linear gradient from 0 min (95:5, v/v) to 30 min (70:30, v/v), maintaining this ratio up to 50 minute. The methanol was stabilized with triethylamine (TEA) to 0.1%, the flow was 0.9 mL/ min. The detection was carried out at a wavelength of 450 nm. All the chromatograms were processed using the Empower 2 software (Waters, Milford MA, USA). The quantification was carried out using a β-carotene (Sigma Chemical Co. St Louis, MO. USA) calibration curve in an concentration range of 0.6-20 ng injected (r^2= 0.998) for the digested samples and of 40-160 ng (r^2= 0.967) for the undigested samples.

2.8.1 In vitro digestion of β-carotene

Bioaccessibility was determined through the standarsized static *in vitro* digestion model proposed in a consensus paper by Minekus *et al.* (2014), adapted to assess bioaccessibility of carotenoids by Estévez-Santiago, Olmedilla-Alonso & Férnandez-Jalao (2015), Equation

3 was used for the calculations based on the protocol described by Minekus *et al.* (2014), where approximately 1 g of the sample was taken and tested for *in vitro* digestion using the simulator solutions for the salival, gastric and intestinal phases (SSF, SGF and SIF respectively) in the following way: to the sample were added 3.5 mL of the stock SSF solution at a pH of 7 (adjusted with NaOH 1 M), 0.5 mL of 1500 α-amylase solution U/mL (dissolved in SSF), 25 µL of $CaCl_2$ 0.3 M and 975 µL of water, after which the sample was gently shaken for 2 min at 37 °C. In the gastric phase, 7.5 mL of stock SGF solution at pH 3 (adjusted with 1 M HCl), 1.6 mL pepsin solution (25000 U/mL, dissolved in the SGF solution), 5 µL of $CaCl_2$ (0.3 M), which was adjusted to a pH of 3 with HCl 1 M, and water (to complete a final volume of 20 mL) were added. The sample was shaken for 2 hours at 37°C. During the duodenal phase, 10 mL of SIF at pH 7 and at 37 °C, 5 mL of pancreatin solution (800 U/mL in trypsin activity), 2.5 mL of the bile solution (160 mM of fresh bile), and 40 µL de $CaCl_2$ (0.3 M) were added, and the pH adjusted to 7 with NaOH 1 M. Finally, water was added up to make a final volume of 40 mL, after which the sample was shaken for two hours at 37 ºC. The enzymes activities assays and the in vitro protocol are described in detail in Estévez-Santiago *et al*. (2015); in the present study the cholesterol esterase was not used.

For the extraction of carotenoids, 20 mL of diethyl ether was added to the total remaining micellar fraction, and it was shaken in a vortex for 1 min, with 10 mL of NaCl (10% w/v) then added. Subsequently, it was centrifuged at 10000 *g* for 10 min at 4 °C. For each sample, the supernatant matter was collected to verify that the extraction was complete. The supernatant matter was moved to a separation flask to which anhydrous sodium sulfate was added to verify the elimination of water, after which it was dried in a rotary evaporator. The

212 methodology for the quantification of the carotenoids was carried out in the same way as that
213 described in Section 2.8.

214 $$\%\text{Bioaccessibility} = (\beta\text{carotene digested } (\mu g/100\ g)/\beta\text{carotene in flour } (\mu g/100\ g))\ x100$$

215 Ec. 3

216 *2.9 Statistical analysis*

217 All analysis was carried out in duplicate except for the carotenoid and starch content,
218 which were carried out in triplicate. The data was analyzed using the *Statistica 7.0* statistics
219 program (Statsoft, 2 Tulsa, Oklahoma, EUA). A variance analysis (ANDEVA) and Tukey's
220 multiple comparison test (α=0.05) were carried out.

221

222 **3. Results and discussion**

223 *3.1 Proximal analysis*

224 The protein composition of sweet potato flour without pre-treatment was 5.28 g/100 g,
225 with a lipid concentration of 1.13 g/100 g, a carbohydrate content of 87.5 g/100 g, a raw fiber
226 content of 2.10 g/100 g, and 4.00 g/100 g of ash, all in dry basis. While the protein content
227 was greater than that previously reported for sweet potato flour (3.48 g/100 g), there was a
228 similar lipid content (1.27 g/100 g) (Ahmed, Akter, & Eun, 2010). Moorthy, Naskar,
229 Shanavas, Radhika & Mukherjee (2010) reported ash content between 1.28 and 6.45 g/100 g
230 in different sweet potato varieties. The nutritional composition of the sweet potato depends
231 on the variety, the conditions in which the crop was cultivated, maturity and storage (Truong,
232 Avula, Pecota & Yencho, 2011). Table 1 shows the total starch content of the sweet potato
233 flours obtained from different pre-treatments. Significant differences in the concentration of
234 total starch ($p<0.05$) were not found after the different treatments. Amhed *et al*. (2010) did

not find differences ($p<0.05$) in the quantity of starch on subjecting the sweet potato to different pre-treatments (with sodium sulfite) and drying temperatures for flour production, reporting a range of 64.81- 65.37 g/100 g. This was as expected, owing to the fact that the determination of total starch quantifies the glucose released and that this does not change when the treatments modify the structure of the starch, which would be seen in the physiochemical and functional properties. The resistant starch (RS) and available starch (AS) content in the orange-fleshed sweet potato flour were around 3 g/100 g and 48 g/100 g, respectively, and were not affected by the pre-treatments. Moongngarm (2013) determined a 3.19 g/100 g concentration of resistant starch in white-fleshed sweet potato flour, while Ramesh-Yadav, Guha, Tharanathan & Ramteke (2006) reported only 1.4 g/100 g in sweet potato treated using traditional cooking methods (100 °C/30 min) and 1.3 g/100 g from pressure cooking (121 °C/10 min), indicating that there was no significant effect on the starch content on applying one treatment or another. Similar results were found in this study, where the pre-treatment did not present any effect on the quantity of RS, AS and TS (Table 1)

3.2 Thermal properties

Table 2 presents the effect on the gelatinization temperature and gelatinization enthalpy in sweet potato flour when different pre-treatments were applied on raw sweet potato. It was observed that all pre-treatments increased the gelatinization temperature and decreased the gelatinization enthalpy in respect to control. The behavior of the above parameters is associated to the material properties and composition of the sample mainly to the starch concentration. The factors such as granule type and size, degree of heterogeneity, the interaction of starch with lipids, protein and fiber would alter the thermal properties. Steam pre-treatment for 2, 4 and 6 min changed the gelatinization temperatures and, produced a

decrease in the enthalpy value; however, this decrease was lower compared to microwave pre-treatment (Table 2). Microwave and steam pre-treatments produced a rearrangement in the structure of starch, which was greater by using microwaves owning to the greater increase in the gelatinization temperatures and a greater decrease in enthalpy value. While the production of greater interactions between the components of starch during the treatments require a higher temperature to become disorganized, these interactions are not strong (a decrease in the content of double helixes of the amylopectin chains), owing to the fact that they require less energy for their disorganization during the experiment in the calorimeter (De la Rosa-Millán, Agama-Acevedo, Osorio-Díaz, & Bello-Pérez, 2014).

3.3 X-ray diffraction

The x-ray diffraction pattern of sweet potato flour showed itself to be of a Type A polymorphism, due to the appearance of the characteristic peaks, the first around 15°, with another two occurring close to 17° and 18°, and finally at 23° (Figure 1). Type A polymorphism is related to a greater quantity of short chains (A chains) in the structure of amylopectin (Hizukuri, Kaneko, & Takeda, 1983). Sweet potato flour subjected to the treatments (microwave and steam) showed a decrease in the intensity of the crystallinity peaks (Figure 1). This reduction increases with longer the treatment time and was greater with microwave treatment. The decrease of the crystallinity peaks is due to disorganization in the structure of starch caused by the temperature. Microwave heating generates greater movement of the water molecules within the flour, producing greater disorganization in the starch granules, on the other hand, steam heating occurs first on the surface and, then moves towards the interior. Flour subjected to microwaves from 4 minutes onward showed an amorphous pattern (without the presence of crystallinity peaks), which indicates a complete

disorganization (gelatinization) of the starch structure. The results of the X-ray diffraction coincide with the gelatinization enthalpy values, where the microwave treatment showed lower values than steam heating (Table 2), indicating a greater disorganization of the crystalline starch structure. Ahn *et al.* (2013) used X-ray diffraction to evaluate the effect of the hydrothermal treatment on sweet potato flour, observing a decrease in crystallinity caused by the hydrothermal treatment, and reporting a crystallinity loss of up to 34%.

3.4 Viscosity profile

The rheological properties of the flours obtained from different pre-treatments are shown in Table 3. The peak viscosity of sweet potato flour decreased on increasing the treatment time, decrease that was greater with microwaves than with steam. This behavior coincides with the calorimetry and X-ray diffraction results, which shows disorganization of the structure caused by the pre-treatments, provoking the starch granules to swell less. In addition, the complex composition of the sweet potato flour, containing protein, fiber and some polysaccharides like pectin, hemicellulose and cellulose may affect their viscosity characteristics. When peak viscosity was reached, and the sample continues to be shaken, it was found that the flour treated with both processes were more susceptible to rupture, for the above reason, the flours presented higher viscosity values in hot paste as the pre-treatment time was increased. These values were greater with microwaves than with steam. This corroborates the level of disorganization of the structure increasing along with the increase in treatment time. It is known that during the pre-treatments, the viscosity profile is modified due to the solubilisation of cell wall causing disorganization and rupture of cellulose, fiber and pectin. In the same sense, the rheological properties of sweet potato flour are affected by several factors, such as the type of starch, amylose/amylopectin ratio, temperature, pH, and

the presence and concentration of other macromolecules (proteins and gums) and low-molecular-weight solutes (salts, acids and sugars) (Choi & Yoo, 2008). The greater disorganization of the structure, and, therefore, the greater level of rupture during the formation of the paste, ensured that the viscosity would increase in higher proportion in the flour treated with microwaves, thus forming a firmer gel due to the higher quantity of linear chains that are responsible of gelatinization (Morris, 1990).

3.5 Morphological characterization

Scanning electron microscopy was used to analyze the effect of pre-treatment on the morphology of sweet potato flour, thus finding that the microstructure of the starch granules in the sample without pre-treatment showed different sized granules, of an oval and polygonal shape. Chen, Schols & Voragen (2003), found the same type of morphology in three varieties of sweet potato. Noda, Isono, Krivandin, Shatalova, Błaszczak, & Yuryev (2009), indicated that the granules of sweet potato starch are characterized by rather smooth and non-cracked surface, which is observed in Figure 2 in the granules which remained intact. On the other hand, the effect of the pre-treatments on the microstructure was dependent on the pre-treatment time with either steam or microwaves. It was observed that increasing the pre-treatment time the morphology of the starch granules was clearly affected (Figure 2). In the same sense it has been proven that hydrothermal treatments influence the structure and starch granules porosity, affecting the integrity of them and altering their original shape (Lee *et al.*, 2011) because of the gelatinization. In the pre-treatment with steam and microwave for 6 minutes (V6 and M6) it was observed a marked loss in the form of starch granules, and this change in form also had a significant effect on the thermal properties and the results of X-ray diffraction obtained in this research. In the micrograph of pretreatment with 4 minutes

of steam is observed swollen starch granules and a higher agglomeration that pre-treatment with steam 2 minutes, due to longer hydrothermal pretreatment and generation of gelatinized starch.

3.6 Bioaccessibility of β-carotene through in vitro digestion

Table 4 shows the concentration of β-carotene in the flour samples obtained from sweet potato without pre-treatment and with both pre-treatment, steam and microwave, all of them before and after *in vitro* digestion. Microwave pre-treatment for 2 and 4 minutes enabled the extraction of β-carotene, but not significantly ($p<0.05$) in comparison to the control flour, while with 6 minutes of pre-treatment, the concentration decreased. The steam pre-treatments provoked a decrease in the concentration of β-carotene, in such a way that pre-treatment V4 caused an average decrease of 5928 μg/100 g, while pre-treatment V6 caused an average decrease of 4186 μg/100 g. However, after *in vitro* digestion, the steam pre-treatments (for 2 and 4 min) favored a higher β-carotene concentration and, therefore, the percentage of micellization as shown in Table 4. Bioaccessibility (average 25%) is slightly higher in the flours pre-treated with steam (4 and 6 min) and lower with the application of microwaves. The average bioaccessibility (considering the three pre-treatment times) of β-carotene in the flour obtained with steam was 32%, while in the flour obtained with microwaves the level obtained was 20%, compared to the 22% bioaccessibility of the control flour. The microwave pre-treatment for 6 minutes increased the bioaccessibility of β-carotene by 5.1%, while the steam pre-treatment for 4 minutes raised this by 20.8%, as compared to the control in both cases. The aforementioned is most likely due to the fact that during processing, the cellular structures of the food matrix were damaged, thus giving access to digestive enzymes and improving bioaccessibility (Thakkar, Huo, Maziya-Dixon & Failla, 2009). It has been shown

that thermal pre-treatments such as blanching in boiling water and then drying, substantially improve the bioaccessibility of β-carotene in various foods, such as dehydrated carrot (Hiranvarachat, Devahastin & Chiewchan, 2011). Also, treatments such as pressure cooking and frying have enabled an increase in bioaccessibility in pumpkin and spinach (Veda, Platel & Srinivasan, 2010).

4. Conclusion

The application of a blanching pre-treatment, either with steam or microwaves to fresh sweet potato in the production of flour, modifies its physiochemical and morphological properties, thus presenting alternative uses of this product in the food industry. Soluble and resistant starch fraction did no change with pre-treatments to obtain sweet potato flour, however microstructure of starch granules was affected. Microwave and steam treatments produced a rearrangement in the structure of starch, which was greater by using microwaves owning to the greater increase in the gelatinization temperatures and a greater decrease in enthalpy value. In the same sense a decrease in enthalpy as well as on the intensity of the X-ray diffraction pattern indicated damage to crystalline region of starch. Final viscosity was greater in the flours produced using microwave pre-treatments for 4 and 6 minutes, with these flours being able to be used in the production of puree type foods. The pre-treatments did not affect the bioaccessibility of β-carotene, although there is a tendency to increase with the application of steam (4 and 6 min). Flours pre-treated with steam represent an option for the preparation of foods enriched with vitamin A, such as pasta and biscuits.

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Table 1. Fractions of starch in orange sweet potato flour obtained with different pre-treatments (g / 100 g dry sample)

Treatment	RS	AS	TS
C	3.38 ± 0.29a	48.36 ± 0.61a	51.74 ± 0.57a
M2	3.13 ± 0.23a	48.17 ± 1.13a	51.30 ± 1.25a
M4	3.01 ± 0.07a	49.90 ± 0.56a	52.91 ± 0.52a
M6	3.01 ± 0.11a	49.98 ± 0.39a	52.99 ± 0.32a
V2	3.38 ± 0.28a	48.12 ± 2.74a	51.50 ± 2.85a
V4	3.19 ± 0.23a	48.29 ± 2.64a	51.49 ± 2.70a
V6	3.25 ± 0.18a	48.25 ± 1.68a	51.51 ± 1.80a

C: control flour; M: microwave; V: steam; 2,4,6: time of pre-treatment (min). RS: Resistant starch; AS: Available starch; AT: Total starch. Letter a, means without significative difference ($p \geq 0.05$).

514 Table 2. Thermal properties of the flours obtained by different pre-treatments

Treatment	T_0 (°C)	Tp (°C)	Tf (°C)	ΔH (J/g)
C	65.46 ± 0.35b	72.12 ± 0.38c	77.43 ± 0.70b	5.00 ± 0.05a
M2	66.92 ± 0.68b	73.16 ± 0.38b	78.29 ± 0.48b	4.02 ± 0.02c
M4	69.79 ± 0.13a	75.35 ± 0.14a	81.46 ± 0.47a	0.82 ± 0.19f
M6	71.32 ± 0.93a	76.26 ± 0.21a	81.62 ± 0.50a	0.51 ± 0.00g
V2	66.19 ± 0.94b	73.14 ± 0.21b	78.49 ± 0.89b	4.13 ± 0.02b
V4	66.65 ± 0.24b	73.53 ± 0.14b	78.11 ± 0.32b	3.51 ± 0.01d
V6	69.46 ± 0.21a	75.65 ± 0.17a	80.66 ± 0.31a	1.82 ± 0.01e

515 C: control flour; M: microwave; V: steam; 2,4,6: time of pre-treatment (min). T_0: onset
516 temperature; T_p: peak temperature; T_f: conclusion temperature. Means with different letters
517 (a, b, c,..) in the same column are significantly different ($p \leq 0.05$)
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Table 3. RVA pasting parameters of sweet potato flour obtained by different pre-treatments

Treatment	Peak viscosity (cP)	Hot paste viscosity (cP)	Final viscosity (cP)
C	426.50 ± 13.43a	79.50 ± 0.70cd	105.50 ± 0.70de
M2	205.50 ± 10.60cd	95.00 ± 4.24c	121.00 ± 4.24d
M4	196.00 ± 7.07d	161.00 ± 4.24a	211.00 ± 2.82b
M6	182.00 ± 11.31d	173.00 ± 4.24a	252.00 ± 2.82a
V2	296.50 ± 16.26b	69.50 ± 2.12d	82.50 ± 2.12e
V4	337.00 ± 2.82b	101.00 ± 1.41c	132.00 ± 2.82d
V6	248 .00 ± 16.97c	126.00 ± 12.72b	173.00 ± 16.97c

C: control flour; M: microwave; V: steam; 2,4,6: time of pre-treatment (min). Means with different letters (a, b, c,..) in the same column are significantly different ($p \leq 0.05$)

553 Table 4. Concentration and bioaccessibility of β-carotene in sweet potato flour obtained by
554 different pre-treatments

Treatment	All-trans-β-carotene (µg/100g)	All-trans-β-carotene after *in vitro* digestion process (µg/100g)	bioaccessibility (%)
C	13749 ± 1528abc	3023 ± 434ab	22.0
M2	16357 ± 1381a	2464 ± 144b	15.1
M4	14556 ± 2163ab	2536 ± 758ab	17.4
M6	11980 ± 1810bc	3241 ± 420a	27.1
V2	13419 ± 2125abc	3043 ± 119ab	22.7
V4	7821 ± 567d	3350 ± 332a	42.8
V6	9563 ± 1567cd	2879 ± 774ab	30.1

555 C: control flour; M: microwave; V: steam; 2,4,6: time of pre-treatment (min). Means with
556 different letters (a, b, c,..) in the same column are significantly different ($p \leq 0.05$)
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568 Leyendas
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570 Figura 1. Effect of pre-treatment on X-ray diffraction of sweet potato flour
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573 Figura 2. Effect of pre-treatment on the microstructure of starch granules in sweet potato
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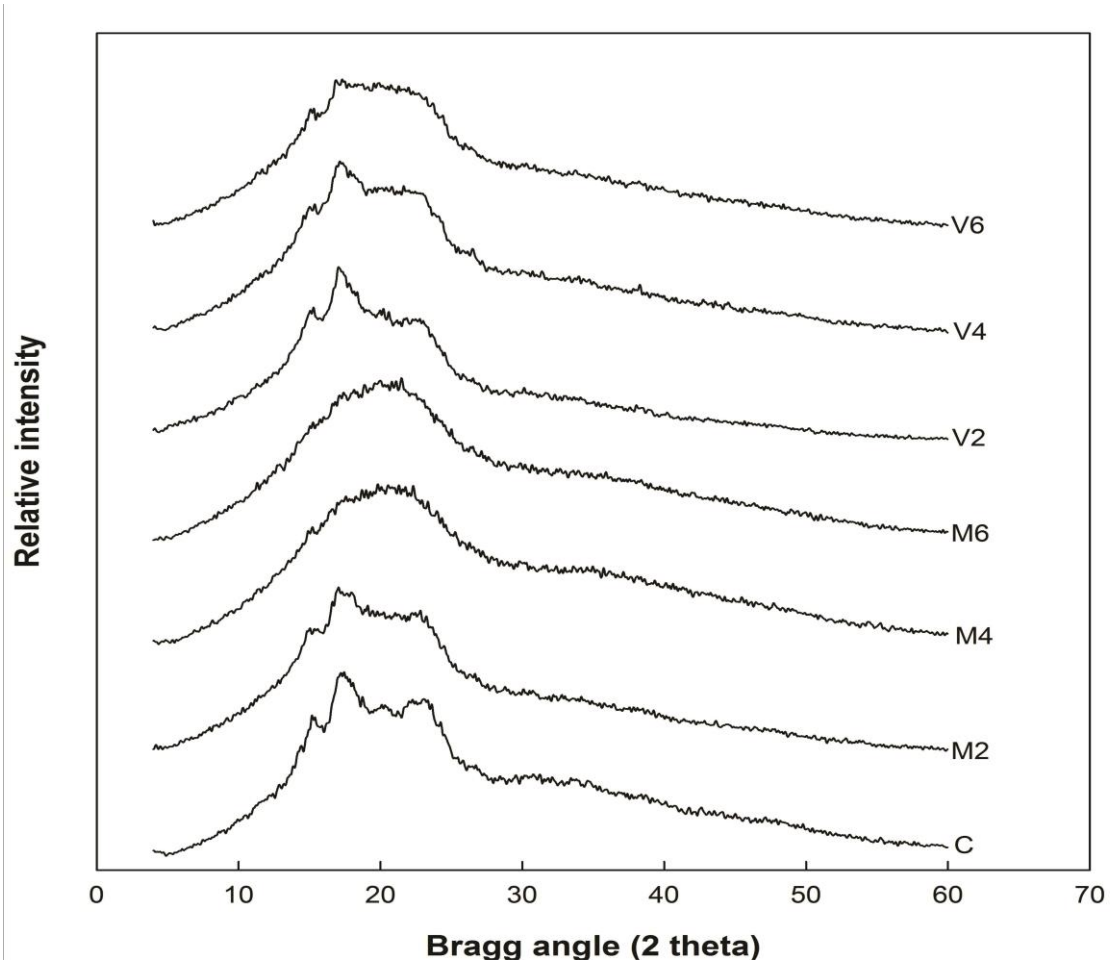

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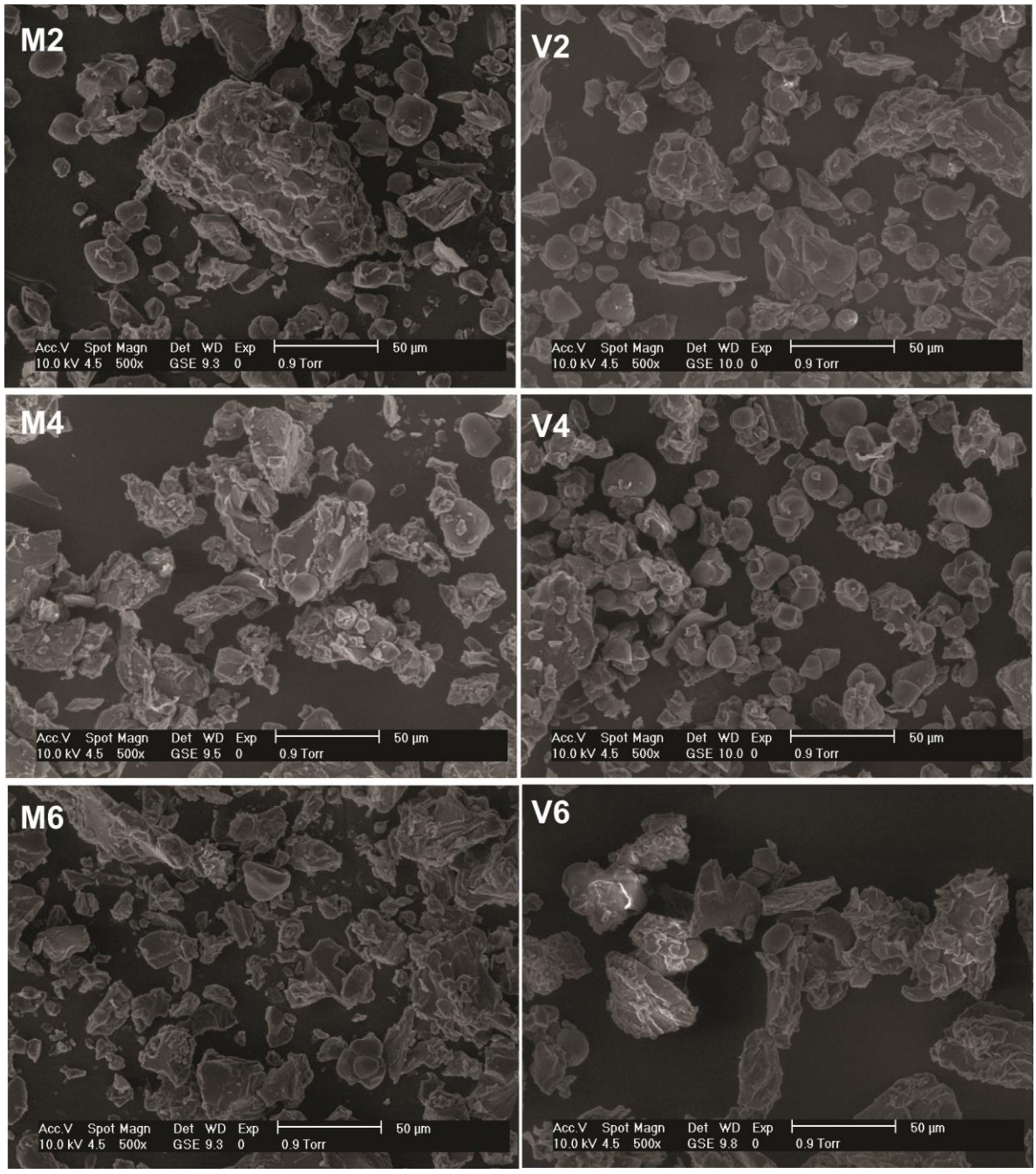
M2
Acc.V Spot Magn Det WD Exp 50 µm
10.0 kV 4.5 500x GSE 9.3 0 0.9 Torr
V2
Acc.V Spot Magn Det WD Exp 50 µm
10.0 kV 4.5 500x GSE 10.0 0 0.9 Torr
M4
Acc.V Spot Magn Det WD Exp 50 µm
10.0 kV 4.5 500x GSE 9.5 0 0.9 Torr
V4
Acc.V Spot Magn Det WD Exp 50 µm
10.0 kV 4.5 500x GSE 10.0 0 0.9 Torr
M6
Acc.V Spot Magn Det WD Exp 50 µm
10.0 kV 4.5 500x GSE 9.3 0 0.9 Torr
V6
Acc.V Spot Magn Det WD Exp 50 µm
10.0 kV 4.5 500x GSE 9.8 0 0.9 Torr

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