Microstructural characterisation and glycemic index evaluation of pita bread enriched with chia mucilage

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Abstract

Chia seeds can be considered afunctional food due to its soluble fibre (mucilage) that could be used to reduce the glycemic index in bakery products. Hence, he aim of this work was to evaluate the effect of chi mucilage on the structure of aflat bread (crumb and crust) and its relation to in vitro glycemic index. Scanning electron (SEM), and confocal laser scanning micros-copies evaluated bread structure. X-ray diffraction analysis and differential scanning calorimetry were also carried out. Results showed that the addition offib re promoted changes in the starch structure, the crumb of all samples presenting the smallest X-ray diffraction peaks, higher gelatinization degrees(100%), and both, amylose-lipid and B-type structures. Regarding the crust, it presented an A-type diffraction pattern and the least gelatinization degree (48.75%). With respect to the glycemic index (eGI), different values were obtained depending on the section of the sample. In the crust, control bread presented higher values (73.2± 1.7, 69.4± 1.2%) than mucilage added bread (69.5± 1.9, 66.3± 0.7), while in the crumb section and for the whole bread, the eGI was higher for bread sadded withfibre (71.8 ± 1.1%, 70.0 ± 2.0%, 73.1± 0.42%). Gelatinization enthalpy values from DSC, and SEM and confocal micrographs reinforced the eGI results. From this study it can be concluded that the presence of fibre on flat bread affects the degree of gelatinization and the eGI, with the crumb of the bread having larger values; while at the crust, a portion of starch granules remained in their native state.

Introduction

Consumption of fibre has been related to the prevention of cardiovascular diseases and specifically to the reduction of glycemic index (GI). However, the effectiveness offibrerich products, flours or pure dietary fibre to reduce the glycemic index is controversial, as well as the ideal type of fibre, and the amount that should be consumed (Scazzina, Siebenhandl-Ehm,&Pellegrini, 2013).

Dietary fibre has been classified as water soluble and water insoluble (Fendri et al., 2016), where the first one includes different types of substances such as b-glucans, gums , pectin , mucilage and a rabinoxylans, and the second is mainly formed by lignin, cellulose and hemicellulose.

Both types of fibres have been tested in many starchy products such as bread, not only to reduce the glycemic response, but also to prolong freshness and to improve the bread quality (Fendri et al., 2016), but the effect on the glycemic index has be enrelated to the presence of the soluble fraction where components such asarabinoxylans (Lu, Walker, Muir, Mascara&O0Dea, 2000), orb-D glucans (Scazzina et al., 2013) seems to be responsible.

Soluble fibre can be obtained from almost all fruits, cereals, and seeds, varying the type and quantity of each compound.

Fruit sarerich in pectin, grains have b-glucans and arabinoxylans . (Abuajah , Ogbonna,&Osuji, 2015), while mucilaginous seeds, such as flax-seed or chia , have simple sugars such as xylose, arabinose, rham-nose, galactose, and glucose forming polysaccharides.

Global production of chia seed has increased mainly due to its healthy properties and popularity throughout the world (Munoz, Cobos, Diaz,& Aguilera, 2012). In 2009, chia seeds and grounded chia were approved as a novel food by the European Parliament and the European Council (Commission, E.U., 2009) for its inclusion in bread at maximum level of five percent (5%)(European Commission, 2009), althoughthispercentagewasmodifiedin2013(ECNo258/97, 2013). The use of whole or ground chia seeds in bread making has been studied, evaluating the quality of the bread (Coelho & Salas-Mellado, 2015), the gelling (Coorey , Tjoe , & Jayasena , 2014), pasting and rheological properties (Inglett, Chen , Liu , & Lee , 2014 a), the development of a gluten free bread (Costantini et al., 2014) or sugar cookies (Inglett, Chen ,& Liu , 2014b), among others. Also, but in a smaller proportion, some studies about the glycemic index, postprandial glycaemia or cardiovascular risk factors related to starchy products such as bread (Vuksan et al., 2010) or tortilla (Rendon -Villabobos, Ortíz-S´anchez, Solorza-Feria,´&Trujillo- Hernandez, 2012´) added with chia seed or flour have been carried out , but their results were inconclusive (Souza -Ferreira , Souza - Fomes , Santo da Silva , & Rosa , 2015). However, the number of published studies regarding the use of chia mucilage in bread making is null.

Chia mucilage is expelled from the seed epidermis coat as it comes in contact with water, forming 18e45 nm width fibres (Salgado-Cruz et al., 2013). Its solubility (10gL—1w/vwater) is higher than that of guar and x anthic gums (Capitani, Ixtaina, Nolasco, &Tomas, 2012´), which makes it an interesting in gradient to be applied in bread making to improve the quality of bread and to reduce the glycemic response (Fardet, Leenhardt , Lioger, Scalbert, &Remesy, 2006´).

Glycemic response (GR) can be evaluated by two methodologies: in-vitro and in-vivo analysis.

In vitro methods have been developed for estimating the GR of foods. These methods are based on the rate of starch digestion, which provides the predicted or estimated glycemic index (eGI), but do not consider the metabolic responses of the human body (Capriles & Areas, 2016). Taking these limitations of in vitro studies, this kind of analysis provides insight for future in vivo trials.

However, as the GR is based on the starch digestion, and in bread making this carbohydrate is the main component, all the changes that the granular structure presents during the thermal process could affect the rate of digestion of starch and thus also affect the absorption of glucose in the small intestine (Svihus &Hervik 2016). In this regard, Schuchardt et al.(2016) published that the presence of soluble fibre, like glucan and inulin at high concentration and glucans and pentosanes(NSPS) linked to starch in bread baking products, impedes starch gelatinization and thus enzymatic vulnerability , lowering the glycemic index . They followed the changes in the starch granule structure by microscopy techniques.

Structural changes of starch during thermal process in bread-making have been studied by different techniques (Karim, Norziah,& Seow , 2000), which include light microscopy (Fleming & Sosulski , 1978), epi flourescence microscopy (Peighambardoust , Dadpour ,& Dokouhaki , 2010), visible and near-infrared reflectance spectroscopy (Xie, Dowell ,& Sun, 2004), X-ray diffraction analysis (Dragsdorf & Varriano -Marston , 1980), differential scanning calorimetry (Srikaeo , Furst , Ashton , Hosken ,& Sopade , 2005), and others. Some of these techniques, such as X-ray diffraction analysis (DRX) or differential scanning calorimetry (DSC), provide quantitative information, while others like scanning electron microscopy

(SEM) or confocal laser scanning microscopy (CLSM) result in micrographs with easily observed qualitative information about structural changes. Regarding CLSM, this technique also al- lows simultaneous observation of main bread components (starch and protein), as well as the following of structural changes on wheat dough or bread during processing (Blonk &Aalst, 1993).

Most of structural bread making studies have been applied to bread , which can be prepared with different ingredients (wheat , maize , rice, barley , rye, sorghum and millet) and process conditions that give rise to different products (flats, integral, leavened, etc.). However, most of the research regarding glycemic index has been carried out in white yeasted bread in which a low temperature (180e 210○C) and longtime baking process (20e40min) are required.

Pita bread is a flat bread highly consumed in the Middle East, North Africa and Central Asia (Al-Dmoor, 2012; Liljeberg, Granfeldt & Bjorck, 1994 €), with a very low crumb proportion, and produced under a high temperature short time baking process (HTST), having the possibility of presenting a low glycemic index as a result of its processing conditions (Indrani, Swetha, Soumya, Rajiv & Venkateswara , 2011 ; Izydorczyk et al., 2008; Smitha et al., 2008). Hence, the aim of this work was to study the changes in the microstructure of pita bread enriched with chia mucilage, and their relation to starch gelatinization and glycemic index.

Materials and methods

Bread making process

Pita bread was prepared by adapting the methods reported by Maleki and Daghir (1967), Qarooni, Orth, and Wootton (1987) and Farvilli, Walker, and Quarooni (1995). Commercial wheat flour (100 g, Osasuna, Elizondo S.A. de CV, Miguel Hidalgo, DF, Mexico, ´ Alveographicdeformationenergy:300×10—4J;11.56 g protein/ 100 g sample (db) was mixed with 3g of sugar(Great Value, Walmart, Bentonville, AR, USA), 1.5gofsalt(La Fina, Sales del Istmo, S.A. de C.V, S.A. deC.V, Coatzacoalcos, Veracruz, Mexico), 1.0g of ‘instant yeast (Nevada oro; Safmex , S.A. DE C.V./Fermex , S.A. DE C.V, Toluca, Estado de Mexico) and the quantity of water (Bonafont,´LiquimexS.A. de C.V., Toluca, Estado de Mexico, Mexico) requiredto´ reach the recommended farino graphic consistency (850 e900 BU) necessary to obtain a good quality Pita bread (Qarooni et al., 1987). Dough was mixed (12 min) in the Simon extensometer mixer (Henry Simon Limited, Stockport, Cheshire, UK). Then dough was fermented(30min/30 ○C), cutin10gportions, and laminated (Atlas Wellness 150 Pasta Maker, Stainless Steel, USA) to obtain as ample of pita bread dough (4cm diameter and 6e7mmthickness). Finally, the sampleswerebakedat530 ○C for30 s in a muffle furnace (Thermo line FB1415M, Thermo-Fisher Scientific Inc., Waltham, MA, USA).
Chia mucilage was obtained as described by Salgado-Cruz (2013) and incorporated as afunctional ingredient (2gper 98 g of wheat flour) in pita bread formulation , which was elaborated as described for control bread , only varying the quantity of water , being 53 mL for control bread, and 56 mL for bread with the chia mucilage.

After baking, bread samples were cooled up to reach room temperature (20e22 ○C, 2 h approximately) and then immediately prepared to be analysed or stored for subsequent analysis. In all cases, samples were separated in two sections: top and bottom, and these sections were also divided in two: crumb and crust, resulting in 4 subsamples (Fig. 1).

Based on the test to be performed, and mainly to have the same moisture content(5e6%), some sub samples were

immediately freeze-dried after baking, packed in sealed low-density poly- ethylene bag (Ziploc, Johnson & Son, Inc., Racine, Wisconsin 53403 - 5011 USA), and kept in a desiccator until analysis , while the other subsamples were immediately analysed (Confocal laser scanning microscopy). Based on literature reports (Alhusain, Toth, Rakusz, Almasi´& Farkas, 2004; Rubenthaler & Faridi, 1981), baker's experience and preliminary tests, control bread was fixed to assure uniform samples when baking 10 g of dough based on: Diameter/height ratio 1.01 ± 0.09, color (L 70.7 ± 3.2, b* 29.6 ± 2.9), weight loss due to baking process 17.2 ± 0.32%, moisture (21.61 ± 0.09%), hardness of the crumb (bottom 2.8 ± 0.2 N and top 2.7 ± 0.3 N) and hardness of the crust (bottom 1.3 ± 0.1 N and top 1.8 ± 0.3 N). Bread added with mucilage was not restricted on physical characteristics as the addition of fibre should change these values.

X-ray diffraction analysis

X-ray diffractions patterns of lyophilized bread samples (Labconco 75034 Bench Top Freeze Dryer from Labconco, Kansas City, MO, USA) were obtained from a D8 ADVANCE diffractometer (Brucker, Santa Barbara, CA, USA) with a CuKa at 1.5406 irradiation source, 30 kV - 20 mA and scanned (2q) from 5 to 60○ (step size 0.05○, 0.5 s per step).

Differential scanning calorimetry (DSC)

Differential scanning calorimetry measurements were per- formed with a Diamond DSC (Perkin Elmer precisely, Waltham, MA, USA) calibrated with indium. The analysis was carried out following the methodology proposed by Almeida and Chang (2013). Lyophi- lized baked bread samples were added with deionized water (10 mg of sample in 30 mL of water), hermetically sealed in stainless steel DSC pans, and allowed to rest for 24 h at room temperature prior to experiments. The pans were heated from 30 to 120 ○C at a programmed speed of 10 ○C min—1. Initial temperature (T0), peak temperature (Tp) and enthalpy of phase transition (DH) expressed in Jg—1 were calculated using the software Pyris Thermal Analysis version 7.000110 (Perkin Elmer, Waltham, MA, USA). Gelatinisation percent (Equation (1)) was also calculated in relation with that of native wheat starch gelatinization enthalpy (Parada & Aguilera, 2012).

- DG % 1 DHsample 100 (1)
- DHnative
- DGð%Þ ¼ Gelatinization percent
- DHsample ¼ Gelatinization enthalpy of the sample
- DHnative ¼ Gelatinization enthalpy of the wheat flour

Scanning electron microscopy

Bread samples were freeze-dried (5 h, 50 ○C, Labconco 75034 Bench Top Freeze Dryer from Labconco, Kansas City, MO, USA), mounted on metal stubs with double-sided conductive tape and coated with a gold layer (4 min, 10 mA, 50 to 100 milli Torr, Denton Vacuum Desk II, Denton Vacuum, Moorestown, NJ, USA). Samples were observed (1000) in a scanning electron microscope (Jeol JSM 5800 Mod LV; Jeol Inc., Peabody, MA, USA) using an accelerating voltage of 10 kV or 15 kV, depending on the susceptibility of the sample to bleaching.

Confocal laser scanning microscopy (CLSM)

Interactions among protein-starch-xylans were evaluated using confocal laser scanning microscopy as reported by Schober, Bean, Boyle, and Park (2008) for proteins, Kolmakov et al. (2010) for starches and Autio and Salmenkallio-Marttila (2001) for xylans, with some modifications. Bread samples taken from different sections, were placed on heparin (1 mL, 5000 units, In hepar® PiSA) for10 min, rinsing the sample by adding 5 mL of water and repeating this procedure four times; the excess of water was removed with an absorbent material (100% polyester), and three dyes were subsequently added: fluorescein 5-isothiocyanate (FITC, 0.85% in water) for protein, rhodamine B (0.15% in water) for starch and calco fluor (1% in water) for xylans. Samples were viewed under the CLSM microscope (LSM 710, Carl Zeiss, Oberkochen, Baden-Württemberg, Germany). The excitation laser wavelength was at 488, 568 and 350 nm for FITC, Rhodamine B, and calcofluor respectively, and the maximum emissions were at 518, 625 and 405 nm.

In vitro starch digestion (glycemic index)

The glycemic index was measured following the Gon~i, Garcia- Alonso, & Saura-Calixto, (1997) and Frei, Siddhuraju, and Becker (2003) methods with some modifications. Pita bread sections were obtained by separating baked bread in two layers (Top and bottom, Fig. 1). Samples from these sections were separated ac- cording to characteristics of both color and texture with a single edge razor. The first sample was obtained from the top crust, this being identified by a brown color, the residues from this same zone correspond to the top crumb; the second samples were obtained in a similar fashion out of the bottom crust and crumb, identified by a lighter color. Each sample section (10 mg) was mixed with 2 mL of HCl-KCl buffer (pH 1.5); this suspension was added with 0.04 mL of pepsin solution (1 g pepsin (Merk 107185)/ 10 mL of HCl-KCl buffer) and incubated at 40 ○C for 1 h in a shaking water bath. After incubation, approximately 5 mL of Trise Maleate buffer (pH 6.9) were poured to adjust pH (6.75e7.10), and immediately 1 mL of Trise Maleate buffer containing 2.6 U of a-amylase from porcine pancreas (A-3176, SigmaeAldrich Inc.) was added; this solution was incubated at 37 ○C in a shaking water bath. Ali- quots of 0.5 mL were taken every 30 min from 0 to 180 min. To inactivate the enzyme (a-amylase), these aliquots were heated up to 100 ○C and kept at this temperature for 5 min while shaking. Then, 0.6 mL of 0.4 M sodium-acetate buffer (pH 4.75) and 12 mL of amyloglucosidase from Aspergillus Niger (A7420, Sigmae Aldrich Inc.) were added; these samples were incubated at 60 ○C for 45 min. Lastly, glucose concentration was measured using the glucose oxidaseeperoxidase kit (GAGO20, Sigma e Aldrich Inc.). The kinetic of the in vitro starch digestion was followed by a nonlinear model and the results were multiplied by 0.9 (stoichiometric glucose/starch conversion factor) to convert glucose into starch as reported by Gon~i et al. (1997). The glycemic index was calculated using the area under the hydrolysis curve (AUC), which was obtained by fitting experimental data to an exponential model of two parameters. The equation applied was: C ¼ Cf.1 — e—kt Σ (2) where C is the percentage of starch hydrolyzed at time t.Cf is the percentage at equilibrium of hydrolyzed starch, normally after 180 min, k is the kinetic constant and t is the time (min). The parameters Cf and k were estimated using the software SIGMAPLOT version 12 for MS Office and a lineal model was used to calculate AUC (Equation (3)): AUC ¼ C .tf — t0Σ — .CfΣh1 — exph — k.tf — t0Σii (3) Where, tf is the final time (180 min), t0 is the initial time (0 min). The hydrolysis index (HI) was expressed as the ratio of the area under the hydrolysis curve (AUC) of the sample to the AUC of the white fresh bread. Then, the estimated Glycemic Index (eGI) was calculated by using the equation reported by Granfeldt, Bjo€rck, Drews, and Tovar (1992). eGI ¼ 8:189 þ ð0:862 × HIÞ (4)

Data analysis

All experiments were carried out at least in triplicates, reporting the average and standard deviation values. Data were statistically analysed (SigmaPlot V. 12.0. Systat Software Inc., San Jose, CA, USA). $P < 0.05$ values were considered significantly different.

3. Results and discussion

3.1. X-ray diffraction

Fig. 2 shows the X-ray diffraction patterns of control (CB) and chia mucilage enriched (Mu) pita breads, evaluated at the crust (2A) and at the crumb (2B).

The presence of A-type diffraction pattern given by a 15○, 23○, and unresolved 17○ and 18○ peaks (2q angle) was observed in all crust samples (Fig. 2A), while at the crumb (Fig. 2B), a V-type diffraction pattern (7○ 13○ and 20○, 2q angle) was obtained. The presence of this A-type pattern in the crust confirmed a low gelatinization degree during pita bread making that could be the result of a rapid dehydration process that normally occurs when applying high temperatures (Ratnayake, Otani, & Jackson, 2012). This kind of pattern is also related to a minimal loss of crystallinity (Osella, Sa´nchez, Carrara, De la Torre, & Buera, 2005), and the permanence of the starch granules in their native form (Zobel, Young, & Rocca, 1988).

In the case of the crumb section, the V-type pattern is related to amylose-lipid complex, as has been stated by Primo-Martín, Van Nieuwenhuijzen, Hamer, and Van Vliet (2007), which means that some part of the starch was gelatinised, other remained native and other reacted with flour lipids.

These differences in the starch diffraction patterns between the crust and the crumb sections, such as the disappearance of peaks at 18○ and 23○ (2q angle) from the crust, and the emerging of the peaks at 13, 22○ and 24○ (2q angle) at the crumb, and those at 17○ and 20○ that keep their intensities almost invariable, can be related to changes in the starch structure. As mentioned before, the peaks at 13○ and 20○ have been connected to the presence of the amylose-lipid complex (Ribotta, Cuffini, Leo´n, & An~on. 2004), and although the addition of shortening to pita bread formulation was minimal, the reaction of flour native lipids with the amylose could be taking place (Morrison, Tester, Snape, Law, & Gidley, 1993). Also, this change in the crumb structure, as compared to crust could be the result of the gelatinization process, as has been pointed out by several authors (Wang, Ya, Bogracheva & Hedley, 1998), which could be leading to the formation of a B-type starch structure, supposition that is reinforced by the presence of the peaks at 5.6○, 15○, 17○, 20○, 22○ and 24○ (2q angle) at low intensity as observed in Fig. 2B for chia mucilage samples. In this regard, Dragdsford &Varriano-Marston, (1980) reported the presence of both V and B patterns in bread, while Ribotta et al. (2004) expressed the change from an A type starch to a B type during baking, giving rise to the starting of retro gradation process.

These peaks are characteristics of two types of structures: one that corresponds to the amylose lipid complex, (V-type pattern), while the other corresponds to the B-Type pattern. The path regarding retro gradation (17○ at 2q angle) has been reported by Kadan, Robinson, Thibodeaux, and Pepperman (2001).

Differential scanning calorimetric (DSC)

DSC results for control bread and chia mucilage bread presented two peaks corresponding to gelatinization process and to

the lipid- amylose complex, as has been reported by Szczodrak and Pomeranz (1992), who cited that the endotherm between 95 and 130 ○C corresponds to the amylose-lipid complex. The presence of this second peak confirms the information obtained in the diffraction study about the formation of the lipid-amylose complex. Peak gelatinization temperatures and enthalpy changes values for these samples are presented in Table 1.

From these data it is possible to appreciate that there were no significant differences between gelatinization temperatures among crust sections and the top crumb in the control bread, or crust sections (top and bottom) in the bread added with mucilage. At the same time, no gelatinization temperatures were detected at the bottom of the crumb, neither for control bread nor in the crumb sections of mucilage bread. This lack of data could be related to a complete starch gelatinization in this section of the breads during the baking process before the DSC analysis and also might indicate, for the other samples, that some native starch remained after baking.

Based on these enthalpy results, the percentage of gelatinization (DG) was calculated, observing that the top crust of both, the control (CB) and the mucilage breads (Mu), presented the largest proportion of native starch (DG 48.8 ± 0.7%, DG 76.6 ± 0.7% respectively), followed by the bottom crusts of CB (DG 82.6 ± 0.6%) and Mu breads (DG 86.1 ± 0.6%) and finally the top crumb of control bread (DG 90.6 ± 0.6%). This information confirmed that the high temperature-short time bread making process used in this work results in a larger proportion of native starch in the crust and gelatinised starch in the crumb. In this regard, Fukuoka, Ohta & Watanabe (2002) and Schirmer, Hussein, Jekle, Hussein & Becker (2011) mention that starch gelatinization is a function of temperature and moisture content, which is expected to result in higher gelatinization degrees at larger water and water vapour contents, like those found in the crumb.

Regarding the effect of the mucilage, its presence seemed to increase the gelatinization degree in all bread sections. This result could be a consequence of the larger proportion of water in the mucilage bread dough, as compared to control bread. Referring to the amylose-lipid complex peak, all the bread sections (control and mucilage added) showed its presence. These broader peaks, located at higher temperature ranges (approximately80e110 ○C), were attributable to the presence of the lipid-amylose complex as has been reported by Almeida and Chang (2013).

Other important fact observed from these results is that the addition of soluble fibre did not produce a reduction in the gelatinization degree, as no enthalpy data for the crumb zone in the bread added with mucilage were found, which means that all starch was already gelatinised before it was evaluated at the DSC system. This could also be related to a higher glycemic index.

CB: Control bread; Mu: Chia mucilage added bread. Tp: peak temperature (○C); DH: Enthalpy of gelatinization or enthalpy vinculated to amylose-lipid complex (J g-1); ND: Non detected; Data presented are the mean values ± standard deviation of at least three independent experiments. Values with the same letter in the same col- umn are not significantly different ($P < 0.05$).

Scanning electron microscopy

From diffraction and DSC data it is expected that some starch granules remain in their native state in the crust and also in the top section of the crumb forth control bread and only in the crust for the bread added with the chia mucilage. This

information was corroborated by the SEM analysis. Fig. 3A(1e4) and3B(1e4) showed both, small and large starch (St) granules , from which, their granular form appeared to be intact, suggesting that they were non-gelatinised . Prabhasankar, Indrani, Jyotsna, and Venkateswara (2004) have reported similar results. In another study, Almeida and Chang (2013) described that starch in the bread shows a differential degree of gelatinisation, decreasing the presence of intact starch from the crust to the center of the crumb.

Regarding gelatinised starch , micrographs of crumb sections in both types of breads, show that starch granules are swollen, fused to then eighbouring granules and partially is integrated, changing their original shape and size, and presenting a more complex structure due to the presence of the soluble fibre (mucilage), which generates junctions with starch and protein, and a more porous structure as compared to control bread.

Confocal laser scanning microscopy

Confocal laser micrographs of control and chia mucilage breads (Fig. 4AeB) at their different sections (crust and crumb, to pan bottom) showed the presence of gelatinised and ungelatinised starch granules that could be identified by their shape and size. In the same micrographs, gluten proteins and yeast were also identified (stained in green) as well as xylans and mucilage strands (stained in blue); these last ones presented similar structure to fibres of gluten as observed by SEM (Amend & Belitz, 1991).

In Fig. 4 A, regarding flour components (gluten and starch), two phases were observed: continuous phase formed by a network of gluten (green colour) and discontinuous phase clearly composed by starch granules (red color) embedded in the continuous phase. These interactions can also be seen in the micrographs of chia mucilage bread (Fig. 4B), where the protein appearance is denser than in control bread, maybe as are sult of the fibre presence that could be avoiding there lease of starch granules. Regarding the crumb samples, with or without chia mucilage (Figs. 4A-2, 4A-4, 4B- 2 and 4B- 4) they presented larger and more deformed granules which could mean a higher degree of gelatinization and an increase in a-amylase accessibility , as reported by Kim et al.(2008), while those of the crust were smaller and less deformed . These structural changes are in agreement with the results obtained from the X-ray diffraction and DSC analysis. In this respect, Sidhu, Seibel & Dietrich Meyer (1990), reported that the starch obtained from the crumb of flat breads (chapathi, parontha and poorie) was gelatinised to a greater extent than that acquired from the crusts, results that are in agreement with our data.

In vitro starch digestion (glycemic index)

The starch hydrolysis kinetic curves used to calculate AUC are presented in Fig. 5. In this figure a rapid hydrolysis rate in the first 30 min is observed for both the crust and crumb samples, followed by a decrement of the rate of hydrolysis. The values of the estimated glycemic index (eGI) of chia mucilage bread (Mu) and the control bread (CB) are shown in Table 2. The eGI values for the top and bottom crumbs in control bread(68.7± 1.9 and 66.9 ± 1.8% respectively) were significantly smaller ($P < 0.05$) than those obtained in the breads added with mucilage(71.8 ± 1.1and 70.0 ± 2.0%). This could be related to the larger degree of starch gelatinization (DG) of the chia mucilage bread. However, at the crust, the results were different, as the samples added with chia mucilage did show smaller eGI values at the crust , even when they had presented a larger gelatinization degree (DG) than the control samples . These unexpected results could have a correlation with the bread

microstructure. In this regard, as seen in Fig. 4, that gelatinization is not a homogenous process, being the crumb the most affected section of the bread. In the same figure, fibre and protein are not homogenous in the sample, and they seem to play a" barrier " role because they are covering the swollen starch granules probably protecting them from enzymatic action, giving as a result the different values in the glycemic index.

However, when analyzing the whole bread, the one added with mucilage presented larger eGI values, which could mean that under the experimental conditions of this work, the gelatinization process had more effect than the protective action of the fibres. More studies at higher levels of fibre addition are needed. It is important to mention that the eGI values obtained in this work were lower than those reported for Buckwheat(GI¼ 80), Quinoa(GI¼ 95), Sorghum(GI¼ 72) and Teff (GI¼ 74) added breads and even lower than those reported for wheat bread (GI ¼ 100) (Wolter, Hager, Zannini, & Arendt, 2014).

Conclusions

High temperature -short time baking process produces different degrees of gelatinization on Pita bread, maintaining the unaffected starch granules in the crust (top or bottom) in its native form, while those from the crumb were almost completely gelatinized at different degrees. Microscopy, X-ray diffraction and thermal studies confirmed the presence of a larger proportion of gelatinised starch in the crumb in the presence of soluble chia mucilage as compared to control bread and a larger glycemic index for the crumb (top or bottom) of mucilage added bread.

Acknowledgments

This research was financed through the projects Catedra-CONACYT1688, andSIP20160097fromInstitutoPolitecnicoNacional.´The authors also wish to thank the Centro de Nanociencias y Micro y Nanotecnologías of IPN and Centro de Investigation en Ciencia´ Aplicada y Tecnología Avanzada, Unidad Legaria.

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