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Physicochemical characteristics, ATR-FTIR molecular interactions and *in vitro* starch and protein digestion of thermally-treated whole pulse flours

Chávez-Murillo Carolina Estefanía[b], Veyna-Torres Jorge Ivan[b], Cavazos-Tamez Luisa María[a], de la Rosa-Millán Julián[a,*], Serna-Saldívar Sergio Othon[a]

[a] Tecnologico de Monterrey, Escuela de Ingeniería y Ciencias, Av. Eugenio Garza Sada 2501 Sur, CP 64849 Monterrey, NL, Mexico
[b] Instituto Politécnico Nacional, Unidad Profesional Interdisciplinaria de Ingeniería campus Zacatecas (UPIIZ-IPN), Blvd. del Bote S/N Cerro del Gato Ejido La Escondida, Col. Ciudad Administrativa, C.P. 98160, Zacatecas, Zac, Mexico

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ABSTRACT

Hydrothermal treatments, annealing (ANN) and heat moisture treatment (HMT) were applied to four whole pulse flours (black bean, broad bean, chickpea and lentil) with the aim to increase their slow digestible (SDS) and resistant starch (RS) fractions. In order to assess differences in their molecular interactions, they were analyzed and compared by ATR-FTIR before and after *in vitro* digestion. Both hydrothermal treatments promoted changes on starch granular architecture, being reflected on their thermal and pasting properties, that where positively correlated with their amylose and protein contents ($R = 0.96$, $P < 0.01$). Overall, the proposed hydrothermal treatments increased their SDS and RS fractions, but they had different effect on their *in vitro* protein digestion. The ATR-FTIR analysis of cooked flours before and after digestion showed that thermal treatments promoted new physical interactions at molecular scale between starch and proteins, that were correlated with the amount of RS fraction. The outcomes of this study could help to understand the slow digestion properties and possible interactions of the flour components in these four pulses.

1. Introduction

In recent years, pulses have received great attention mainly because they present a large array of nutraceuticals (Azarpazhooh & Boye, 2012; Du, Jiang, Yu, & Jane, 2014; Ma, Boye, Azarnia, & Simpson, 2016). From the nutritional point of view, they contain a high proportion of complex carbohydrates, being starch the main fraction. The starches associated to pulses have been related with higher thermal stability during pasting, as well as significant amounts of soluble and insoluble fiber that delay its starch hydrolysis, resulting in low *in vivo* and *in vitro* digestion rate by amylolytic enzymes (Brummer, Kaviani, & Tosh, 2015; Chung et al., 2008; Jenkins et al., 1982; Maaran, Hoover, Vamadevan, Waduge, & Liu, 2016). The other nutritionally relevant macromolecule of pulses is their proteins, which presents good essential amino acids balance (Divekar et al., 2016; Ghumman, Kaur, Singh, & Singh, 2016). In Mexico there is a great diversity of pulses cultivars, that is cultivated for farmers' self-consumption and represents an important food staple with different energy value, depending on their protein and starch bioavailability. In addition, the natural occurrence of phenolic compounds has been a recurrent subject in their study as functional ingredients (Megías, Cortés-Giraldo, Alaiz, Vioque, & Girón-Calle, 2016; Moussou et al., 2016). From the technological perspective, the functional and digestion properties of starches and proteins can be modified with the use of chemical or enzymatic modifications, resulting in ingredients with improved characteristics, that are often related with their functional and digestion properties (Ahn, Kim, & Ng, 2005; Romano Giosafatto, Masi, & Mariniello, 2015). Nevertheless, one of the current trends in food processing is to avoid the use of chemical reagents that may negatively affect the consumer health and cause environmental issues due to inadequate residue disposal. For this reason, the use of thermal treatments like annealing (ANN) and heat moisture treatment (HMT) are some of the alternatives to modify some of the physicochemical as well as the starch and protein digestion characteristics in whole flours (Tester & Morrison, 1990; Tosh & Yada, 2010). Previous studies have shown that pulses seed matrix composition and their molecular arrangement delay starch hydrolysis; thus, potentially decreasing the predicted glycemic index (pGI) which may have an impact on the protein availability of the cooked flours (Torres, Rutherford, Muñoz, Peters, & Montoya, 2016). However, little attention has been paid to the possible molecular interactions that may occur among the different seed components, and their effect on viscosity and *in vitro* digestion properties. Such molecular interactions could be

* Corresponding author.
E-mail address: juliandlrm@itesm.mx (J. de la Rosa-Millán).

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qualitatively analyzed by the non-invasive ATR-FTIR, which has proven to be useful in quantify the changes on secondary structures of proteins and crystalline starch conformations (Cai & Singh, 2004; Carbonaro, Maselli, Dore, & Nucara, 2008; Manning, 2005). The aims of this investigation were to characterize the physicochemical and digestion properties of native and thermally treated whole pulse flours and to analyze their molecular interactions as during its typical consumption as food, before and after *in vitro* digestion on both the protein and carbohydrate fractions by ATR-FITR.

2. Materials and methods

2.1. Whole flour preparation

Food-grade pulse seeds from black bean (*Phaseolus vulgaris*), broad bean (*Vicia faba*), chickpea (*Cicer arietinum*) and lentil (*Lens culinaris*) were acquired with a local seed distributor in Zacatecas, México. The seeds were ground to produce whole flours in a Wiley mill (Arthur Thomas, Philadelphia, PA) equipped with a 2 mm diameter screen. All flours were sieved to pass the US 80 mesh. The overs or coarse particles were further milled to pass this sieve. Afterwards, whole flours were stored in sealed plastic bags at − 20 °C until further processing.

2.2. Hydrothermal treatments

For ANN, 100 g of each pulse flour (d.s.b.) were weighed into hermetic glass containers, blended with 400 mL of distilled water and homogenized during 30 min. The containers then were tight sealed and kept for 24 h at 10 °C. Afterwards the containers were incubated at 65 °C for 24 h in a convection oven. After the incubation time, the containers were opened and the flour samples vacuum dried at 40 °C for 24 h, followed by milling to pass through a US 80 mesh screen and kept into hermetic containers until analysis. For HMT treatment, the flours (100 g) were weighted into a plastic bag, where the appropriate amount of distilled water was added until reach a 30% moisture content. The bags were tight closed and their contents were homogenized until evenly wetted without the presence of dry lumps. Afterwards, the bags were stored at 4 °C for 4 h to stabilize the moisture content within the flour; then, their contents were poured into hermetic glass containers which were immediately incubated at 120 °C for 24 h in a forced air convection oven. After the time, the containers were cooled down to 25 °C and opened, its content spread on an aluminum tray followed by 24 h of a vacuum dry process at 40 °C, thereafter, the dried flours were grounded until pass the US 80 mesh screen and stored in hermetic containers in a tempered room (≈ 25 °C) until further use. The processing conditions (both for ANN and HMT) were based on previous pilot plant trials and were selected due to pilot plant process efficiency.

2.3. Physicochemical characteristics

Native and heat-treated pulse flours were analyzed for moisture, protein, fat and ash according to approved AACCI methods 44-01.01, 46-13.01, 30-20.01 and 08-01.01, respectively (AACCI, 2000) and their carbohydrates calculated by difference. The total and damaged starch (TS and DS, respectively), as well as the total amylose and total dietary fiber contents (TDF) were determined with commercial available kits K-TSTA, K-SDAM, K-AMYL and K-TDFR respectively following the procedures described by Megazyme (Wicklow, Ireland).

2.4. Starch granule morphology

The starch morphology and birefringence patterns were observed with a Motic BA-210 digital microscope (Hong Kong, China). The images were acquired at 40 × magnification (× 40) under normal and polarized light.

2.5. Color characteristics

Color characteristics of native and thermally treated flours were measured on the CIE 1976 color scale using a Minolta CM-600, Chroma Meter (Konica Minolta Co., Osaka, Japan). For each sample, 5 readings were taken from powder samples and recorded as lightness (L∗), redness-greenness (a∗) and yellowness-blueness (b∗) values. To establish color differences of the different whole pulse flour treatments and to observe the effect of thermal treatments, the color index value (ΔE) was calculated with the following equation.

$$\Delta E = ((\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)^{1/2} \quad (1)$$

2.6. Colorimetric total phenolic determination

Determination of total phenolic content was performed using the Folin–Ciocalteu method as described by Singleton, Orthofer, Lamuela-Raventós, and Lester (1999). The samples and gallic acid standard curve (0.016–0.1 mg gallic acid mL^{-1}; $y = 3.0161x - 0.0216$; $R^2 = 0.9915$) were read at 725 nm. Results were expressed as mg of gallic acid equivalents per gram of flour sample (d.s.b.). Absorbance values of colorimetric assays were read in a microplate reader Synergy HT, Biotek (Winooski, USA); with Gen5™2.0 data analysis software spectrophotometer.

2.7. Rapid viscosity analysis

The pasting profiles of flours were measured with a Rapid Visco Analyzer (RVA Model 1170, Newport Scientific, Warriewood, NSW, Australia) using a constant 25.5 g suspension with 12% solids. The heating profile was: hold at 50 °C for 2 min, heat to 90 °C at 15 °C/min (heating cycle), hold at 90 °C for 4 min (high temperature hold), cool to 50 °C at − 15 °C/min (cooling cycle) and hold at 30 °C for 4 min (low temperature hold).

2.8. Differential scanning calorimetry features of whole pulse flours

For this analysis, 2 mg (based on total starch content) of whole pulse flours were placed in semi-hermetic anodized aluminum capsules (Perkin Elmer, B02190062, US), hydrated with the appropriate amount of distilled water (3 volumes, based on total sample weight) and containers carefully sealed. Once hydrated, samples were kept for 30 min at room temperature (≈ 25 °C) and subsequently heated from 30 to 120 °C at a temperature rate of 10 °C/min in a Diamond DSC (Perkin Elmer, NorTcolk, VA, USA) apparatus which was calibrated with an Indium reference cell before experimental measurements were performed. An empty capsule was used as reference for each determination. The transition parameters, onset (To), peak (Tp), and conclusion (Tc) temperatures of gelatinization as well as the endothermic enthalpy (ΔH) were calculated using the Pyris manager software (Perkin Elmer, Nortfolk, VA, USA).

2.9. Starch digestion fractions

The *in vitro* starch digestibility of whole pulse flours was determined according the Englyst, Kingman, and Cummings (1992) protocol with slight modifications. Pulse flours (400 mg) were hydrated with 10 mL of deionized water and heated in a boiling water bath for 20 min with vortexing every 5 min. The tubes were cooled at 37 °C and 8 mL of pepsin dispersion (5.21 mg/mL) added and incubated in a shaking water bath at 37 °C and 200 strokes/min for 30 min. Then, 8 mL of 0.5 M sodium acetate buffer (pH 5.2) were added and homogenized. To each reaction tube, 4 mL of an enzyme solution (pancreatin, amyloglucosidase and invertase) and 7 glass beads (7 mm diameter) were added. After 20 and 120 min of reaction, 1 mL aliquots were taken and immediately mixed with 2 mL of 80% ethanol, its glucose content was