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Physicochemical, rheological, bioactive and consumer acceptance analyses of *concha*-type Mexican sweet bread containing Lima bean or cowpea hydrolysates

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ABSTRACT

Foods may be fortified to improve their nutritional value and bio-functional properties. Protein sources are used to improve physicochemical properties in foods, and hydrolyzed legume proteins are a promising alternative. A Lima bean (*Phaseolus lunatus*) protein concentrate was hydrolyzed using pepsin, and a cowpea (*Vigna unguiculata*) concentrate was hydrolyzed using a pepsin-pancreatin sequential system. The Lima bean hydrolysate had a degree of hydrolysis of 17.38%, and the cowpea one of 30.75%. When were added at 1 or 3% to dough for making *concha*-type Mexican sweet bread, dough tenacity and extensibility increased at the higher inclusion level. Physicochemical, bioactivity (ACE inhibition and antioxidant activity) and consumer acceptance analyses of the breads were performed. Protein content was higher in the bread containing hydrolysates. The control treatment had the lowest ACE inhibitory activity and TEAC value. At both inclusion levels, ACE inhibitory activity was higher in the breads containing Lima bean hydrolysates than those containing cowpea hydrolysates. This activity increased with inclusion level in both hydrolysate treatments. Bread containing the cowpea hydrolysate exhibited an acceptance level ($p > 0.05$) equal to that of the control treatment. Addition of legume hydrolysates is a promising way of improving bread nutritional and biological values without affecting sensory parameters.

1. Introduction

Protein is vital in all diets. It is one of the structural pillars in living beings, and a key factor in metabolism, nutrient synthesis and degradation (Tharantan & Mahadevamma, 2003). Because of its importance, alternative protein sources such as legumes are constantly being researched. Already an ubiquitous element in human diets, legumes are a promising source of protein due to their nutritional properties, physiological effects, low cost and techno-functional characteristics when incorporated into food products. These qualities make them a basic ingredient in human diets, particularly among low-income populations in developing countries (Suárez-Martínez, Ferriz-Martínez, Campos-Vega, Elton-Puente, De la Torre-Carbot & Teresa García-Gasca, 2016).

Legumes are rich sources of protein and carbohydrates. Both components are widely used in the food industry because they can substitute or complement much more costly animal proteins in food systems. Both Lima bean (*Phaseolus lunatus*) and cowpea (*Vigna unguiculata*) are widely cultivated in southeast Mexico. They are considered to be important crops due to their high protein content (20–35 g/100 g), high essential amino acid content (e.g. lysine and threonine) (Cai, Hettiarachchy, & Jalaluddin, 2003), and complex carbohydrates content, especially starch and dietary fiber (Campos-Vega, Loarca-Piña, & Oomah, 2010). Processing legumes with enzymatic hydrolysis can allow their use as raw materials in new food products in ways that improve biofunctional and techno-functional characteristics, thus facilitating their application in food systems (Vioque et al., 2006). Enzymatic hydrolysis of proteins has been used to reduce protein allergenicity, produce bioactive peptides, manipulate protein qualities for specific diets, and improve protein functional properties (Jamdar et al., 2010).

Recent research focused on peptides derived from food proteins

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begun the process of understanding their properties, including their biological activities and potential health benefits. Peptides extracted from partial enzymatic hydrolysates of food proteins can provide specific health benefits such as antihypertensive or antioxidant activities. Many protein hydrolysate peptides inhibit the angiotensin I-converting enzyme (ACE, EC 3.4.15.1), a dipeptidyl carboxypeptidase associated with blood pressure regulation and cardiovascular function (Brugts et al., 2014). Potent synthetic ACE inhibitors such as perindopril, captopril, enalapril, alacepril, lisinopril, and ramipril are widely used in clinical treatment of hypertension in humans. However, these synthetic drugs have several side effects including coughing, skin rashes, hypotension, hyperkalemia, headache, dizziness and renal impairment. Food-derived ACE inhibitors have safety advantages over synthetic compounds (Ahmad et al., 2014).

Antioxidant mechanisms of proteins depend on their amino acid composition. Activity is suppressed if the free radical-scavenging amino acids are located inside the protein, inaccessible to prooxidants (Elias, Kellerby, & Decker, 2008). Enzymatic hydrolysis increases exposure of antioxidant amino acids in proteins. When conducted under controlled conditions, it produces hydrolysates with potential free radical-scavenging activities. Exogenous enzymes are preferred to endogenous enzymes for antioxidant peptide production because they result in more consistent peptide composition and require shorter hydrolysis times to attain similar degrees of hydrolysis (Samaranayaka & Li-Chan, 2011). The total antioxidant capacity of foods can be attributed to the combined activity of diverse antioxidants including peptides, phenolic compounds, phytochemicals. Lee, Kim, Lee, & Lee. (2003) suggested that a 2,2'-azino-bis (3 ethylbenzthiazoline- 6-sulfonic acid) (ABTS) radical scavenging assay is more appropriate for evaluating the antioxidant activity than other methods as the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, because it can be used in both aqueous and organic solvent systems, employs a specific absorbance at a wavelength remote from the visible region, and requires a short reaction time.

Hydrolysis of protein alters many of its functional characteristics, such as solubility, viscosity, flavor, and foaming and emulsion properties, among others (Benitez, Ibarz, & Pagan, 2008). Protein hydrolysates are important in the food industry as an ingredient in a wide variety of products, including meats, breads, nutritional drinks and soup bases (Megías et al., 2009). Protein and other functional ingredients can also be found in bread products. For example, milk protein and amaranth were added to bread to improve dough functionality, biological value and digestibility since amaranth effectively uses nitrogen (Güemes-Vera, Totosaus, Hernández, Soto, & Aquino, 2009). In another study, chia (*Salvia hispanica*) hydrolysates were added to white bread, resulting in a less sticky crumb, lower kneading energy, and an agreeable flavor. The chia hydrolysate was considered as a functional ingredient with the possible benefit of lower consumer risk of arterial hypertension (Segura-Campos, Salazar-Vega, Chel-Guerrero, & Betancur-Ancona, 2013a). Lima bean hydrolysates have been incorporated, along with cassava and corn starch, into sausages resulting in a higher water absorption capacity and a positive relationship between mechanical, sensory and textural (hardness and elasticity) properties (Huerta-Abrego, Segura-Campos, Chel-Guerrero, & Betancur-Ancona, 2010).

The present study objective was to evaluate the effects of including Lima bean (*Phaseolus lunatus*) and cowpea (*Vigna unguiculata*) hydrolysates with bioactive properties in dough for a typical Mexican sweet bread known as *concha* on dough rheological properties, bread product physicochemical and bioactive properties, and consumer acceptance.

2. Materials and methods

2.1. Materials

Lima bean (*Phaseolus lunatus*) and cowpea (*Vigna unguiculata*) seeds were purchased in bulk in the city of Merida, Yucatan, Mexico. Analytical grade reagents and enzymes were purchased from J. T. Baker (Phillipsburg, NJ, USA), Sigma Chemical Co. (St. Louis, MO, USA), Merck (Darmstadt, Germany) and Bio-Rad Laboratories, Inc. (Hercules, CA, USA). Food ingredients were wheat flour Selecta® (Gruma S.A.B. de CV, México, D.F.), sugar Great Value® (Nueva Wal-Mart de México, D.F.), salt Sol® (Industria Salinera de Yucatán, México), butter Lala® (Grupo Lala, Durango, México), skim milk Lala® (Grupo Lala, Durango, México) and egg Bachoco® (Corporativo Bachoco S.A, México, D.F.). All these ingredients were purchased in a local supermarket.

2.2. Bean meal production

The cowpea seeds were dried in a convection oven at 60 °C for 4 h, broken in a manual disc mill, the seed hulls removed with compressed air, and then ground in an impact mill (Cyclotec, Tecator, Sweden). The Lima bean seeds were ground successively in a disc mill (Cemotec 1990; Tecator, Sweden) and the impact mill. The resulting meal was screened through a 200 mesh sieve to produce a fine (74 μm particle) flour and stored in containers until use (Segura-Campos, Espinosa-García, Chel-Guerrero, & Betancur-Ancona, 2012).

2.3. Protein concentrate production

Protein concentrates were produced separately for each type of legume seed, but using the same procedure to fractionate meal components (Segura-Campos, Chel-Guerrero, & Betancur-Ancona, 2011). Ten kg of seed meal were suspended in distilled water at a 1:6 (w/v) ratio. Suspension pH was adjusted to 11 with a 1 mol/L NaOH, and agitated with a mechanical agitator (Caframo RZ-1, Caframo Lab Solutions, Ontario, Canada) at 400 rpm for 1 h. The suspension was passed through 80 and 100 mesh to separate the bagasse from the starch and protein mixture. The bagasse was washed five times with distilled water and the resulting filtrate was added to the starch and protein mixture. This was left to rest for 45 min at room temperature. Then, the protein-rich supernatant decanted, leaving the starch-rich sediment. The supernatant was adjusted to pH 4.5 with a 1 mol/L HCl and centrifuged (Mistral 3000i centrifuge, Sanyo Gallenkamp PLC, UK) at 5000 rpm for 15 min. The precipitate was recovered and dried at −47 °C and 1.3 Pa in a freeze drier (Labconco, Kansas City, MO, USA).

2.4. Enzymatic hydrolysis

Hydrolysis of the Lima bean protein concentrate was done with pepsin (Sigma-P7000) following the method described by Segura-Campos, Chel-Guerrero, and Betancur-Ancona (2010). Digestion with the enzyme was done using a 1:10 enzyme:substrate ratio, and run for 90 min at 37 °C and pH 2. Hydrolysis was done in a 2 L precipitate vessel in a water bath (Brunswick R76, New Brunswick Scientific, NJ USA) under constant agitation at 300 rpm (Caframo RZ-1, Caframo Lab Solutions, Ontario, Canada), with a thermometer to control temperature and an electrode to adjust pH. The substrate was a 4 g/100 mL protein solution, prepared by dissolving 57.9 g Lima bean protein concentrate (dry basis) in 1 L distilled water. The hydrolysis reaction was stopped by placing samples in a water bath (Lindberg Blue M1110, Thermo Fisher Scientific, Waltham, MA USA) at 80 °C for 20 min.