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Full Length Research Paper

Obtainment by enzymatic and evaluation of hydrolysate of defatted corn germ (Zea mays L.)

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ABSTRACT

Defatted corn germ (DCG), contains proteins, starch, fiber and minerals and could be used for human consumption, but its palatability and low solubility limit this use. The objective of the present work was to obtain a hydrolysate of DCG by enzymatic via and evaluate its physical, chemical, microbiological and sensorial, feature in order to consider their possible use in human food. Optimum operating conditions for hydrolysis were determined using the surface response methodology. For a stirring speed of 400 rpm and a 15 % germ/water ratio, the following results were obtained for the enzyme concentration: 0.025 % and 0.08 % W/V in starches; 0.32 % W/V for fiber and 0.51 % W/V for proteins. Sensorially the hydrolysate was better qualified the DCG ($p \leq 0.05$), the dietary fiber decreased (21.4%) and the availability of nutrients increased in relation to protein digestibility (98%), energy (348.8 Kcal) and minerals. The functionality improved, as the solubility index increased and the viscosity decreased; in addition, the thermal conditions used ensured a suitable microbiological quality. The hydrolysate showed better functional properties, palatability and digestibility than the DCG. All these characteristics would allow its use as functional ingredient in the preparation of foodstuffs for human consumption.

Key words: defatted corn germ, surface response, enzymatic hydrolysis, functional properties, nutritional value.

INTRODUCTION

Corn is the most consumed cereal in Venezuela, mainly used since 1954 in the production of precooked corn flour and cooking oil (Fernández, 1991). This industry generates large volumes of germ (245,746 tons/year) used to obtain the oil, which generates a byproduct called defatted corn germ (DCG). Its availability has increased over time, being 63,756 tons/year for 2013 (Pernalete, 2014). DCG consists of a mixture of heterogeneous material, mainly seed pericarp, parts of the germ and may contain part of the aleurone layer and traces of endosperm starch, depending on the yield of the separation of the components of the grain (Granito *et al.,* 2000). DCG has been considered an important ingredient as a source of complex carbohydrates, fiber, protein and minerals, and therefore, it could be used to supplement precooked corn flour of lower protein content than the germ or as an extender for wheat, which is imported (Guerra *et al.,* 1998). The primary use of this sub-product is in animal feed formulation. The main difficulty for the direct use of DCG in the development of human food is the bran; it affects the functional properties of the germ, the appearance and taste. Attempts to reduce this difficulty have been made by using fractionation to remove the fragments of fiber from the shell (Hernández

et al., 1999). Some authors have tried to apply technological processes such as fermentation (Granito *et al.,* 2003), hydrolysis (Vioque *et al.,* 2001) and the production of protein concentrates (Guo *et al.,* 2009; Li-jun *et al.,* 2008; Pacheco, 1986; Anderson *et al.,* 1979) in different foodstuffs to improve the organoleptic and functional properties. The last two authors obtained protein concentrates from oilseeds and DCG, in which functional properties were improved, but it was not possible to remove the bitter taste and therefore, they could be incorporated only in low concentrations, to avoid the rejection of consumer (Spellman *et al.,* 2009). The use of hydrolysis to improve the sensory quality has been used in foods with high starch content, which gives dextrins, sugars or syrups as products. In the case of proteins, when fermented or hydrolyzed, low molecular weight components are produced and others that may have different flavors (salty, sweet, bitter) are removed (Guerra, 2003). The unpleasant taste of DCG has been attributed to the high content of phytate and fiber, which are associated with unpleasant tastes (Bohn *et al.,* 2008). Given that both hydrolysis and high temperature can help improve the unpleasant taste of the germ, the present work was carried out, aimed at applying an enzymatic treatment with amylases, proteases and cellulases to obtain a hydrolysate with improved the functional and sensory properties of DCG.

MATERIALS AND METHODS

The defatted corn germ (DCG) was obtained from a of corn oil manufacturer; the sampling was made from a total of 336,000 kg, following the general principles established by the Codex Alimentarius (CAG/GL 50-2004) and Venezuelan commission of industry standards (COVENIN 612-82). 2,030 kg of residues divided into five sub-lots were sampled. Samples were collected in plastic containers with lids, properly identified. The Sigma brand enzymes for the preparation of hydrolyzed were as follows: α-amylase from *Bacillus amylolicufaciens* and *Aspergillus oryzae*, cellulases from *Aspergillus niger*, and proteases from *Bacillus globigii* (Celtek Technologies, C.A).

Determination of the optimal conditions for enzymatic hydrolysis to obtain the hydrolysate

Initially, the working conditions (temperature, pH, water/substrate ratio and agitation rate) set by Hernández (2001) to obtain a fiberprotein cake were used as reference. Subsequently, to determine the optimal conditions for enzymatic hydrolysis, three stages were carried out using the response surface methodology, composite central design. This method enables determining the optimum operating conditions by performing a minimum number of experimental tests. To this end, a factorial design 2^2 with central points according to Montgomery (2002) was developed, considering as operating variables the factors affecting the system and the responses that reflect its behavior: germ-water ratio and enzyme concentration [E]. In addition to the central points and the factorial, axial points for the adjustment of the model were added, conforming the composite central design.

Stage 1. Enzymatic hydrolysis of starches: a α-amilasa enzyme from *Bacillus amylolicuefaciens* was used to produce the liquefaction of starches to assess the variables to be optimized, the viscosity was used as reference parameter, which was determined using the Method No. 22-10, recommended by the AACC (2004). Once the viscosity measurements were obtained, the efficiency of enzyme action was calculated, relating the viscosity of the mixture (flour-water), with the temperature after the enzymatic treatment (liquefaction) was applied, until 90 ºC was reached. O complete the hydrolysis, an α-amilasa enzyme from *Aspergillus oryzae was* used, and to evaluate the variables to be optimized, the amount of reducing sugars was used as reference parameter, which was determined by the method No. 939.03 of the AOAC (2005). **Stage 2**. Enzymatic hydrolysis of fibers: a cellulose from *Aspergillus niger* was used, and to assess the variables to be optimized, the determination of the indigestible residue (dietary fiber) was used as reference parameter, using the method No. 985.29 of the AOAC (2005), then the percentage of reduction in this component with respect to the original content of dietary fiber was determined. **Stage 3.** Enzymatic hydrolysis of proteins: a protease from *Bacillus globigii* was used, and to evaluate the variables to be optimized, the determination of the protein digestibility as a reference parameter was used, employing the method described by Hsu *et al.* (1977).

Obtainment of the hydrolysate

As a first step to obtain the hydrolyzed, the byproduct was conditioned by reducing the particle size using an Alpine brand mill (model 160 Z, Augsburg, Germany) in order to homogenize the sample of DCG used. Subsequently, applying previously established optimal conditions, it was hydrolyzed with α-amylase, cellulose, and protease using three fermentors of 14 L capacity, New Brunswick Scientific CO., model MF-114, applying previously established optimal conditions. Upon completion of this process, the hydrolysate was spray dried (Niro atomizer 11BA06 F) under the following conditions: chamber temperature: 180 - 200 °C; dryer outlet temperature: 90 - 100 ºC; evaporation rate: 9 kg/h. The hydrolysate obtained was evaluated in comparison to the defatted corn germ, raw material used for its obtention.

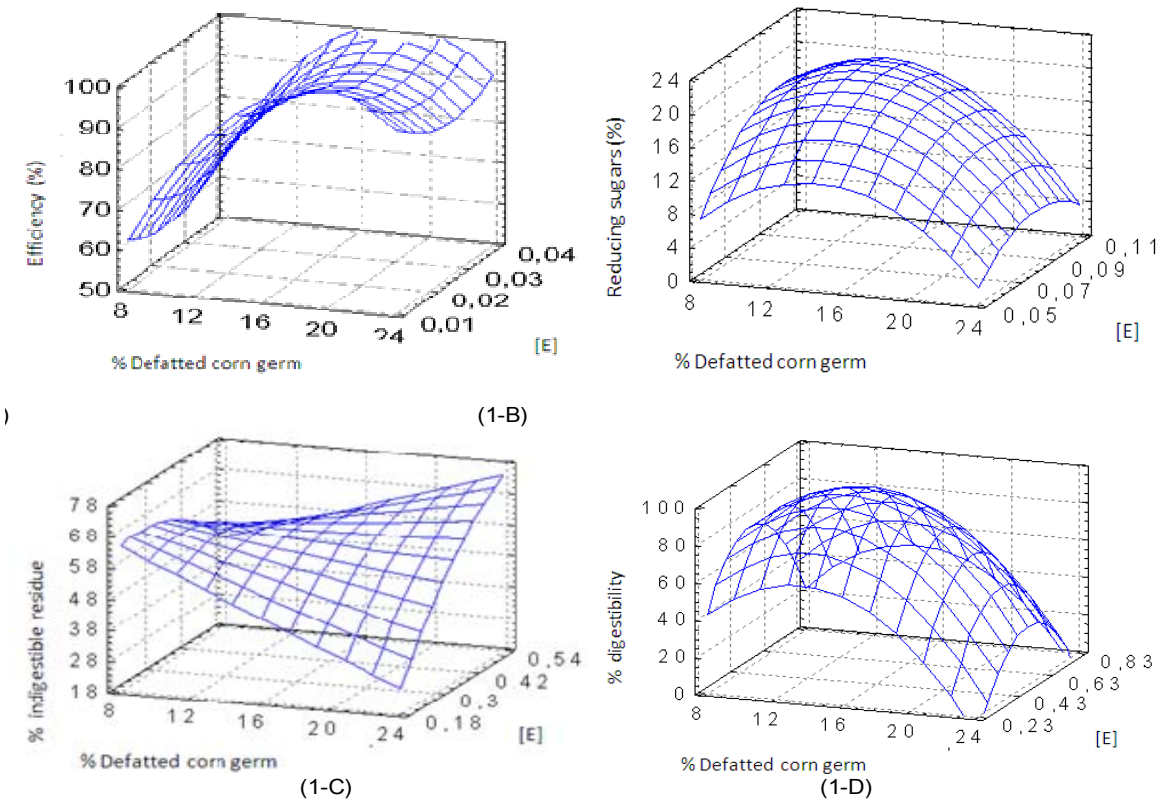

Figure 1. Graphs of surface response in each of the stages of the enzymatic hydrolysis. Expressed as viscosity (1-A), reducing sugars (1-B), indigestible material indigerible (1-C) and digestibility (1-D).

Assessment of the defatted corn germ and the hydrolysate.

The results were the mean of five determinations. Color was measured according to the of AACC method (Nº 14-22, 2004) which it is based on reflectance colorimetry, using a Hunter-Lab colorimeter; model Flex, with a white plate as standard. The granulometry was performed using the method C-136 of the ASTM (2005) and COVENIN (254-1977), a Tyler Silver Shaker modelo RX-86 and set of sieve sizes (20,30,40,50,60 mesh) were used. The proximal analysis, phytate and starch were made according to official AOAC methods (2005). Carbohydrates content was obtained by difference. The minerals (iron, magnesium, calcium, phosphorus) were determined by atomics mass absorption spectropotometry using a Spectrophotometer (Perkin Elmer 3100), according to what is established in the AOAC (2005). Amino acids (lysine, methionine and threonine) by high performance liquid chromatography (HPLC) post-columna derivatization, using a Perkin Elmer model 200 EP, method 99.13 (AOAC, 2005). Protein digestibility in vitro was performed by the multienzymatic method using trypsin, chymotrypsin and peptidase described by Hsu *et al*. (1977). Total and insoluble dietary fiber was evaluated using the methods 985.29 and 991.42 described by AOAC (2005). The energy value was calculated using the Atwater factors according to Nielsen (1994). The presence of aflatoxins in the DCG was determined by thin layer chromatography, AOAC (2005) method 975.35 based on the fluorescent characteristics these compounds show under ultraviolet light. The analysis of total coliforms, mesophilic aerobes, molds and yeasts were determined using the method recommended in APHA (2001). To determine some functional properties in the hydrolysate, farinograph and viscoamylograph analyses were carried out, which were determined according to AACC official methods Nº 54-12 and 22.10 (2004).The water absorption (WAI) and water solubility (WSI) indexes were performed according to the method of Anderson (1982). For sensory evaluation, the methodology ISO 6658 (2005) was used, with a properly trained panel of fifteen people; a test of preference was applied, followed by one of acceptability according to ISO 4121 (2003). For the statistical evaluation of the results, ISO 5725 (1994) was used.

RESULTS

Optimum operating conditions in the hydrolysis

Stage 1. Enzymatic hydrolysis of starches: In Figure 1-A, the behavior of the efficiency of the amyloliquefaciens enzyme as a function of enzyme concentration and germ:water ratio is presented. By appling a canonical analysis, it is predicted that the optimum operating

Table 1. Assessment of color in the defatted corn germ (DCG) and in the hydrolysate (HDCG).

Color	Sample		
	Pattern ***(white plate)***	DCG	HDCG
L	92.44[a]	84.5[b]	82.0[c]
A	-1.35[a]	1.71[b]	2.00[b]
B	0.94[a]	17.99[b]	18.00[b]

Different letters in the same row indicate significant differences (P≤0.05)

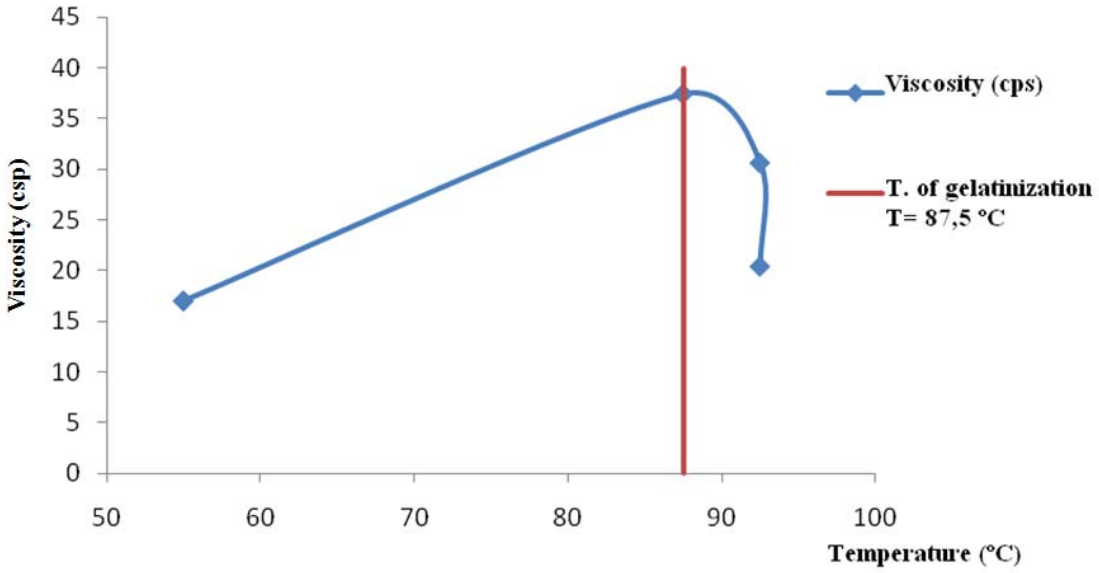

Figure 2. Effect of heating temperature on the viscosity of the hydrolysate.

conditions for the stage of liquefaction of starch are (G/W) 18.8 % and [E] 0.026 %. In Figure 1-B, the formation of sugars during hydrolysis of starches using the enzyme Fungamyl is shown. By using a canonical analysis, it is predicted that the optimum operating conditions for the stage of the hydrolysis of starches is (G/W) 13.7 % and [E] 0.086%. **Stage 2.** Enzymatic hydrolysis of the fiber: In Figure 1-C, the behavior of the indigestible residue, which decreases during the hydrolysis of the fiber using the enzyme celluclast, is shown as a function of the concentration of enzyme and the germ:water ratio. Finally, through the canonical analysis, it was found that for [E] equal to 0.33%, a ratio (G/W) of 13.1 % is required. **Stage 3.** Enzymatic hydrolysis of proteins: In Figura 1-D it is evidenced that with the highest [E] (0.51%) and the ratio (G/W) of 15 %. Using canonical analysis, the optimum operating conditions for the stage of the hydrolysis of proteins of (G/W) 17.16 % and [E] 0.55 % are predicted. Once the optimum operating conditions were generated, a pilot scale hydrolysis was carried out; in this stage, the yield of the process was determined using a mass balance, obtaining a yield of 85 %; this indicates that the process is efficient, and therefore it has a potential application on a larger scale.

Assessment of the germ and the hydrolisate.

In Table 1 it can be observed that the defatted corn germ (DCG) had a yellow color with reddish tones. The defatted corn germ (DCG) consists of 68.54 % of large particles and 31.46 % of fine particles. The hydrolysate had a lower granulometry than the DCG (80.1 % of fine particles) of less than 0.84 mm.

The hydrolysate presented a water soluby index (WSI) of 44.83%; and a water absorption index (WAI) of 2.54 g/g. In Figure 2, the effect of heating temperature on the

Table 2. Proximal composition, starch and energy content in the defatted corn germ (DCG) and in the hydrolysate (HDCG).

Parameters	DCG	HDCG
Moisture (%)	9.3± 0.72	4.6± 0.10
Ash (%)	4.1± 0.26	8.9± 0.00
Proteins (%) N x 6.25	11.6± 0.36	12.0± 0.00
Raw fiber (%)	6.1± 0.20	4.0± 0.00
Fat (%)	0.6± 0.20	0. 53± 0.02
Carbohydrates (%) *	68.3± 0.44	69.97± 0.02
Starch (%)	62.0± 1.39	56.0± 0.00
Energy Value (Kcal/100g)	320.59	348.80

*From difference

Table 3. Mineral content of the defatted corn germ (DCG) and the hydrolysate (HDCG).

Parameters	DCG	HDCG
Calcium (mg/100g)	40.4± 1.73	49.61±0.00
Magnesium (mg/100g)	384.4± 1.00	397.34± 0.00
Iron (mg/100g)	7.14±0.53	7.25± 0.00
Phosphorus (mg/100g)	900.00±0.10	1,300±0.00

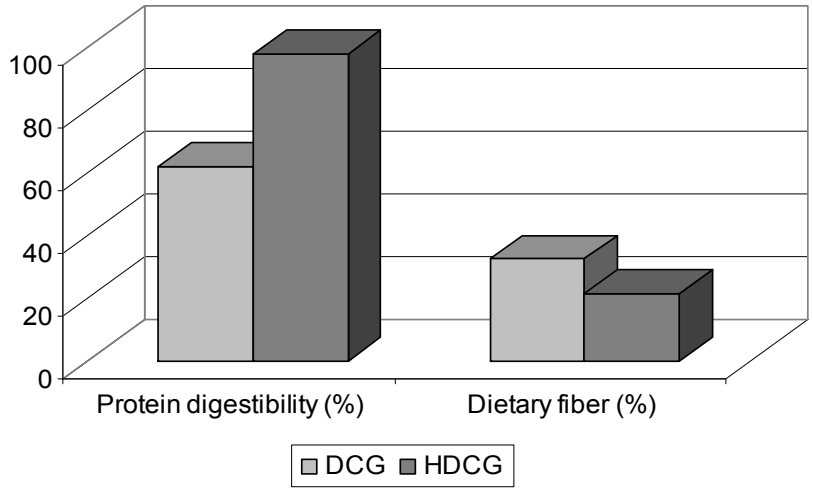

Figure 3. Protein digestibility in vitro and dietary fiber of the defatted corn germ and the hydrolysate.

viscosity of the hydrolysate is shown. The results of the proximate composition of the samples are presented in Table 2. The protein quality of the DCG, expressed as g aa / 100 g of protein were: lysine (3.68), methionine (1.84) and threonine (4.74).The content of amino acids in the hydrolysate were as follows: 3.85; 1.72 and 5.41 g aa / 100 g of proteins, for lysine, methionine and threonine, respectively. Both samples showed significant levels of phosphorus, iron, calcium and magnesium (Table 3). In Figure 3 data on protein digestibility and dietary fiber of DCG and HDCG are presented. In the germ, aflatoxins were not detected in the types B and G and of phytate were detected high levels, it could demonstrate that the process of enzymatic hydrolysis reduced by 55% (Figure 4). The microbiological evaluation indicates that there was a reduction in mesophilic aerobes, molds and yeasts

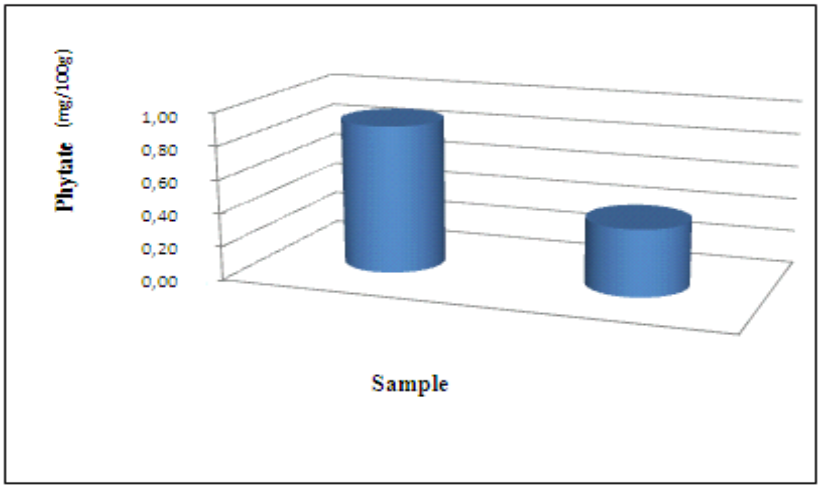

Figure 4. Levels of phytate in the defatted corn germ (DCG) and the hydrolysate (HDCG).

in the hydrolysate, due to the temperatures (50 - 90 °C) used during the hydrolysis. Total means by attribute of defatted corn germ (DCG) and of the hydrolysate (HDCG) in the acceptability test are presented in Table4.

DISCUSSION

Optimum operating conditions in the hydrolysis

Stage 1. Enzymatic hydrolysis of starches: at the start, the liquefaction of starches occurs, due to the action of an α-amylase, and enzyme that reduces the viscosity of the mixture defatted germ:water (G/W). This enzyme is an endoamylase that randomly hydrolyzes the 1, 4-α-glucosidic linkages of amylose and amylopectin, yielding products such as oligosaccharides and dextrins of various sizes (Sigma, 2008-2009). It was observed that at concentrations of the enzyme [E] between 0.025 and 0.035, and the ratio (G/W) between 16 % and 20 % the highest efficiency of the enzyme α-amylase is obtained, related to the maximum reduction in viscosity (99.3 %) forming a liquid mixture. Subsequently an α-amylase enzyme was used according to the [E] and the ratio (G/W); this is a exoamylase, which hydrolyzes amylose chains causing the successive separation of the maltose units; in the case of the amylopectin, the rupture originates glucose units from branch points α (1-6) (Sigma, 2008-2009). It was evidenced that when the enzyme concentration [E] is 0.08 and the ratio (G/W) between 10 % and 15 %, the highest levels of reducing sugars (22.1 %) are obtained. **Stage 2.** Enzymatic hydrolysis of the fiber: for this stage, a cellulase enzyme was used to hydrolyze the fiber present in the initial residue. This hydrolyzes the cellulose molecules into glucose, cellobiose and larger glucose polymers (Sigma, 2008-2009). It was observed that with the ratio (G/W) 15% and [E] 0.32 %, the greatest reduction in indigestible residue (62.1 %) is obtained. **Stage 3.** Enzymatic hydrolysis of proteins: in this stage an endopeptidase that acts on the peptide bonds, breaking them and releasing the amino group and carboxyl group (Sigma, 2008-2009) was used. it is evidenced that with the highest [E] (0.51%) and the ratio (G/W) of 15 %, the highest digestibility (97.6 %) was obtained, comparable to the digestibility of high quality proteins, determined both in vivo and in vitro (Suárez *et al.,* 2006), which shows the hydrolytic action of the enzyme, which improves digestibility by 36 %. Once the optimum operating conditions were generated, a pilot scale hydrolysis was carried out; in this stage, the yield of the process was determined using a mass balance, obtaining a yield of 85 %; this indicates that the process is efficient, and therefore it has a potential application on a larger scale.

Assessment of the germ and the hydrolisate.

The defatted corn germ (DCG) had a yellow color with reddish tones a trend similar to the values obtained in the coarse fraction of the germ by Guerra *et al*., (1998) and consists of 68.54 % of large particles and 31.46 % of fine particles. The hydrolysate obtained by enzymatic treatment is colored darker than the germ ($P \leq 0.05$), with shades of yellow similar to those of the germ and very marked compared to the pattern ($P \leq 0.05$). The hydrolysate had a lower granulometry than the DCG (80.1 % of fine particles) of less than 0.84 mm. The HGDM had lower moisture content than the DCG, because of the drying treatment, which favors its conservation.

Regarding the functional properties, the hydrolysate presented a water soluby index (WSI) of 44.83%; this value is considered high when compared to the value for DCG (2.92 %), a result similar to that obtained by Hernández *et al.* (1999) for DCG prepared in the

TablE 4. Total means by attribute of defatted corn germ (DCG) and of the hydrolysate (HDCG) in the acceptability test.

Attribute	Total means	
	DCG	HDCG
Smell	5.95 [a]	6.73 [b]
Taste	5.82 [b]	7.41 [c]
Color	6.77 [d]	7.82[e]

Different letters in the same row indicate significant differences (P≤0.05)

laboratory. The high solubility can be attributed to the hydrolytic action that reduces some of the components of the germ to smaller molecules that have more interaction with water; these properties incorporating water to solubilize components or keep it in a network gives characteristics for its use in beverages or doughs similar to bakery products, cookies and pasta. The hydrolysate presented a water absorption index (WAI) of 2.54 g/g similar to that of DCG (3.02 g gel / g sample) and to the value (3.27 g gel / g sample) reported by Hernández *et al* (1999), indicating that hydrolysis hardly affected the absorption. The effect of heating temperature on the viscosity of the hydrolysate is shown, in the viscoamylograph points evaluated, which shows that as the temperature raises (87,5 ºC), the viscosity increases up to a value of 37.42 cps, after which it starts decreasing to a value of 17.01 cps, when the temperature is 55 ºC. This behavior occurs by enzymatic modification of the starches, which decrease with hydrolysis, which facilitates the solubility of lower molecular weight components thereby decreasing the viscosity. Since according to William and Keith (1982), decreasing native starch content increases the solubility. In the germ and the hydrolisate, carbohydrates are the largest component, consisting of starches, which represent 80 % of them. There is a decrease of approximately 15 % compared to the starch content of the DCG. This reduction is low compared to that reported by Hernández (2001) while obtaining a fiber-protein cake, the value of which was 29 %. There were no variations in the protein content, since with the hydrolysis a breakdown of the structure occurs. The protein quality of the DCG, expressed as g aa / 100 g of protein were: lysine (3.68), methionine (1.84) and threonine (4.74), similar to those reported by Dondero and Meneses (1981) of 3.83, 1.81 and 4.76, for the same aminoacids, respectively. Other authors such as Guerra *et al*. (1998) reported for lysine 2.50, 3.84 and 3.13 g aa /100 g of proteins, in different fractions of DCG. The content of amino acids in the hydrolysate were as follows: 3.85; 1.72 and 5.41 g aa / 100 g of proteins, for lysine, methionine and threonine, respectively, indicating that there was no change in the amino acid content during hydrolysis, and therefore, the hydrolysate maintains a reasonable content of lysine, greater than that of the endosperm of corn flour, and both the germ and the hydrolysate are a good source of sulfur amino acids (Guerra *et al.,* 1998; Bressani *et al.,* 2001; Mendoza *et al.,* 2006). Raw fiber was reduced by approximately 34 %, which improves the energy value of the hydrolysate, since reducing sugars are one of the products of the hydrolysis of the fiber. Both samples showed significant levels of phosphorus, iron, calcium and magnesium. In the hydrolysate, a slight increase in the levels of calcium, magnesium and phosphorus is observed, possibly because these minerals were bound to the fiber and/or proteins in the germ, were released during the hydrolysis, as it has been reported that these components bind minerals and reduce their use (Drago y Valencia, 2008 a, b). The protein digestibility obtained for DCG was 62 % which is within the range (63.08 % to 88.20 %) reported by Mosqueda *et al.* (1986). It is observed that with the hydrolysis, the protein digestibility increased by 36 %, improving the availability of proteins, which is indicative of the favorable effect of the enzymatic hydrolysis on them. The value of dietary fiber in the germ was 32.5 %, close to the levels reported in the coarse fraction of DCG (35.9 %) by Granito *et al.* (2000). The dietary fiber present in the DCG is basically insoluble (27%), both results justify modifying enzyme via the germ, for their major use. Due to the hydrolysis, dietary fiber decreased (21.4%), and as a result, the energy value increases (348.8 Kcal/100g).

In the germ, aflatoxins were not detected in the types B and G. He was also the determination of phytate, high levels in the diet are associated with adverse nutritional effects in man (Frontela *et al.,* 2008; Bhon *et al.,* 2008). It could demonstrate that the process of enzymatic hydrolysis reduced by 55% levels of AF of the GDM (0.89%) attributable to the action of cellulase on fiber, since the phytates coexist with it. According to Khan (1988), the phytate decreases as a result of hydrolysis, enzymatic or chemical. This can be beneficial, since using the hydrolysate in the preparation of a food, is favouring the absorption of these minerals, such as calcium, iron, zinc, etc (Drago and Valencia, 2008 a).

The microbiological evaluation indicates that there was a reduction in mesophilic aerobes, molds and yeasts in the hydrolysate, due to the temperatures (50 - 90 °C) used during the hydrolysis. There was no growth of total and fecal coliforms, indicating proper handling of the germ during the hydrolysis.

The results of the preference test conducted in the germ and in the hydrolyzate, where significant differences (P ≤ 0.05) were observed. Results that can be attributed

to decreased levels of fiber and phytate, since these compounds may influence the low palatability of the germ.

According to the statistical test of the physical, chemical, nutritional and sensory assessments made to germ and to the hydrolysate, in the parameters, there is little or no dispersion among their replicas. In the microbiological determinations, there are no significant differences (at 95% probability) among their replicas.

CONCLUSIONS

A hydrolysate whit pleasant taste, high solubility, with proteins of good digestibility, high content of dietary fiber and with a reasonable supply of calcium, phosphorus, iron and magnesium was obtained. All these characteristics would allow its use as functional ingredient in the preparation of solid and liquid foodstuffs for human consumption.

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