Preparation of corn (*Zea mays*) and sorghum (*Sorghum bicolor*) proteins and protein hydrolysates and their antihypertensive activities

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Abstract. The most important group of protein in cereals is prolamine, storage protein in cereal grains that contain biopeptides, which can prevent non-transmissible diseases such as hypertension. The objective of the present study was to determine the composition of sorghum, white and yellow corn proteins, as well as to prepare protein hydrolysates in order to evaluate their antihypertensive activities. Protein extraction using the alkaline method in combination with combined ultrasound reached 65.7, 63.5 and 48.1% for white corn, yellow corn and white sorghum, respectively. A comparison was made between corn and sorghum proteins; it was found that there are no significant differences between treatments ($P > 0.05$), because with the concentration of 1% of protein incubated for 3 hours reached approximately 24% of degree of hydrolysis. However, the hydrolysis time obtained a significant difference ($P < 0.05$) until reaching a saturation point at 60 minutes and 90 minutes for sorghum and corn, respectively. Corn and sorghum proteins, and their hydrolysates at the point of saturation, were subjected to the angiotensin-converting enzyme inhibition test. The best treatments obtained results of 45.4 and 43.8%, for yellow corn and white corn, respectively. The least effective treatment was white sorghum with 30.5%. It is recommended to use purification and separation methods to differentiate the biopeptides released during hydrolysis.

Key words: Alcalase, angiotensin converting enzyme, inhibitors, peptides.

Resumen. El grupo de proteínas de mayor importancia en cereales es la prolamina, proteína de almacenamiento en los cereales, en ella se encuentran los biopeptidos que pueden prevenir enfermedades no transmisibles como la hipertensión. El objetivo del presente estudio fue determinar la composición de proteínas de sorgo, maíz blanco y amarillo, así como preparar hidrolizados de proteínas con el fin de evaluar sus actividades antihipertensivas. La extracción de proteína mediante el método alcalino en combinación con ultrasonido alcanzó el 65.7, 63.5 y 48.1% para maíz blanco, maíz amarrillo y sorgo blanco, respectivamente. Se realizó una comparación entre proteínas de maíz y sorgo; se encontró que no hay diferencia significativa entre los tratamientos ($P > 0.05$), debido a que con la concentración del 1% de proteína incubada por 3 horas alcanzó aproximadamente 24% de grado de hidrolisis. Sin embargo, el tiempo de hidrolisis obtuvo una diferencia significativa ($P < 0.05$) hasta alcanzar un punto de saturación a 60 minutos y 90 minutos para el sorgo y maíz, respectivamente. Las proteínas de maíz y sorgo, y sus hidrolizados en el punto de saturación fueron sometidos a la prueba de inhibición de la enzima convertidora de angiotensina. Los mejores tratamientos obtuvieron resultados de 45.4 y 43.8%, para el maíz amarrillo y maíz blanco, respectivamente. El tratamiento menos efectivo fue el sorgo blanco con 30.5%. Se recomienda usar métodos de purificación y separación para diferenciar los biopeptidos liberados durante la hidrólisis.

Palabras clave: Alcalasa, enzima convertidora de angiotensina, inhibidores, péptidos.

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1. INTRODUCTION

Cereals are the dominant crops in world agriculture, with a total of 2,702 million tons being harvested globally in 2017, comprising 1,031 million tons of maize, 759 million tons of rice, 744 million tons of wheat and 60 million tons of sorghum (FAO 2017). They are the major source of calories and protein to the diets of humans and livestock. The reasons for the success of cereals include their adaptability, high yields, and ease of harvest and storage (Lafiandra *et al.* 2014).

The protein of corn has some specific characteristics, such as most abundant amino acids are Glutamic acid, Leucine, Proline, Alanine, Phenylalanine, and Aspartic acid. However, its properties of low water solubility and deficiency in essential amino acids such as Lysine, Histidine, and Tryptophan considerably limit its nutritional quality and the direct application in food ingredient (Zhu *et al.* 2018). On the other hand, the digestibility of sorghum proteins, especially after cooking, is lower than cereals like wheat and maize. The kafirins of sorghum are resistant to peptidase due to the formation of intramolecular disulfide bonds; this is why it has low digestibility. In varieties rich in tannins, the complexation of the kafirins with phenolic compounds can reduce the protein digestibility up to 50% (De Morais *et al.* 2015).

Since the emergence of Gluten Related Disorders (GRD) resulting from the ingestion of gluten, a mixture of prolamin proteins found mostly in wheat, gluten-free (GF) goods have become more and more popular (Hadjivassiliou *et al.* 2016; Pellegrini and Agostoni 2015; Balakireva and Zamyatnin 2016). As gluten-free crops, sorghum and corn have gained more interest to be used in gluten-free food products (Ferreira *et al.* 2016; Emendack *et al.* 2018).

The corn and sorghum proteins are also excellent source of bioactive peptides that have health effects in chronic disease prevention, particularly in regard to diabetes, cardiovascular disease, and cancer (Cavazos and Gonzalez 2013; Zhu *et al.* 2018; De Morais *et al.* 2015).

The method to extract the protein for the study was alkaline at pH 11, as described previously by De Mesa *et al.* (2010) with modifications in addition of sonication. The enzyme to hydrolyze, extrated protein for corns and sorghum was Alcalase.

The main precursor of the biopeptides is the enzymatic protein hydrolysis consisting of the cleavage of protein molecules into small peptides of various sizes, and eventually amino acids (Nielsen *et al.* 2001). Alcalase was shown to produce corn gluten meal (CGM) hydrolysates with higher degree of hydrolysis (DH) and higher ACE inhibitory activity

compared to other enzymes (Yang *et al.* 2007). Although peptide fractions with lower molecular sizes were shown to have higher antihypertensive effects (Vasudeva *et al.* 2006; Yang *et al.* 2007; Kim *et al.* 2001). Alcalase has been used for hydrolysis of Sorghum protein, 19% of DH present the higher ACE inhibitory activity with Thr-Lue-Ser (Wu *et al.* 2015).

Hypertension is the single most important risk factor for stroke, the top 2 global cause of death in 2016 (World Heart Federation 2017; WHO 2018). About 75 million American adults (29%) have high blood pressure – one in every three American adults (CDC 2016). The racial disparity in hypertension and hypertension-related outcomes has been recognized for decades with black race with greater risks than Caucasians (Lackland 2015).

Angiotensin-converting enzyme (ACE) cleaves at carboxyl terminus of His-Leu of angiotensin I resulting in the producing of angiotensin II, a potent vasopressor, and releases Phe-Arg and Ser-Pro from the carboxyl terminus of bradykinin, a vasodepressor. ACE causes hypertension by interrupting the balance between rennin-angiotensin-aldosterone system (RAS) and kallikrein kinin system (KKS). Thus, inhibition of ACE presents important pharmacological and nutraceutical values in blood pressure control and the prevention of hypertension related complications (Soffer 1976; Natesh *et al.* 2003; Wang *et al.* 2015).

To overcome the side effects of chemically synthesized drugs, a lot of efforts have been made to discover and apply the natural compounds that are capable of controlling blood pressure (De Leo *et al.* 2009). Some bioactive peptides (BP) exhibiting ACE inhibitory activities have been isolated from food protein hydrolysates, such as Proline-Serine-Glycine-Glntamine-Tyrosine-Tyrosine (Suh *et al.* 1999), Alanine-Tyrosine (Yang *et al.* 2007), and Methionine-Isoleucine/Leucine-Proline-Proline (Wang *et al.* 2015) from corn, and Threonine-Leucine-Serine (Wu *et al.* 2015) from sorghum. It was found that dipeptides and tripeptides were able to remain higher potency of bioactivity after adsorption intact through the gastrointestinal tracts than larger peptides (De Leo *et al.* 2009).

The objectives of this study were:

- To prepare corn and sorghum meals.
- To prepare protein hydrolysates using Alcalase enzyme.
- To evaluate proteins and protein hydrolysates for their antihypertensive activities.

2. MATERIALS AND METHODS

Protein content (AOAC 920.87).
Samples were digested using 500 mg of sample, ½ of catalyst tablet (Selenium reaction mixture) and 5 ml of sulfuric acid. In the digestion tubes, the samples were heated for 1.5 hours and shaked for 30 minutes. After this, the digested samples were cooled to room temperature. Kjeldahl method was used to determinate total protein using N×6.25 as conversion factor.

Moisture content (AOAC 925.10).
Three (3) grams of sample was weighed and heated for five hours 105℃ The dehydrated sample was weighed after cooling down to room temperature. The amount of moisture was determined as the difference between the three grams of sample and the dehydrated sample.

Lipid content (AACC 30-25).
Five (5) grams of sample was weighed. The sample was transferred to the extractor and extracted with petroleum ether for 5 hours at a condensation rate of 5 to 6 drops/seconds. The petroleum ether was removed from collection flask. The deffated samples were weighed. The amount of fat is the different between the sample weight and the weight from the deffated sample.

Preparation of corn and sorghum protein (CP and SP).
The corn and sorghum flours were passed through 60 MESH to obtain uniform particle sizes, and defatted using flour: hexane (1:4 ratio) at ambient temperature (25 ℃). The flours were dispersed in deionized (DI) water (1:9 ratio), the pH was adjusted to 11.0 with sodium hydroxide (NaOH 1N) and sonicated for 30 min to disrupt the cell walls. To prevent the overheating of the sample, the beaker containing the sample was placed in an ice and cold-water bath during sonication. Dispersions were stirred for 3 hours followed by a centrifugation at 10,000 *g* for 30 min at 20 ℃ using a centrifuge (model J2-21, Beckman, Fullerton, Calif., U.S.A.) to extract the protein. The residue was re-extracted two more times to obtain the residual protein. The supernatants were combined and the pH adjusted to 4.5 (isoelectric pH) with hydrochloridic acid (HCl 1N), to isoelectrically precipitate the protein, and then centrifuged at 10,000 *g* for 20 min at 4 ℃ to obtain the protein isolate. After this, the precipitate was reconstituted in water adjusted to pH 7.0, freeze-dried, and stored at 5 ℃.

Preparation of corns (CPH) and sorghum protein hydrolysates (SPH) by Alcalase. Corn protein (CP) or sorghum protein (SP) were prepared in a dispersion (1% in DI water) adjusted to pH 7.0. Based on previous studies, Alcalase (0.5 AU/g protein) was used to obtain CPHs and SPHs. After adding the Alcalase, the samples were incubated at 60 ℃ at varying times (0, 30, 60, 90, 120 and 180 min) in a shaking water bath at 120 rpm (Grant Model OLS200, Cambridge, England). Alcalase was inactivated at 85 ℃ for 5 min and cooled at ambient temperature, then stored at 5 ℃.

Degree of hydrolysis of CPH and SPH.
o-phthaldialdehyde (OPA) method as described by Nielsen *et al.* (2001) was used to determine the degree of hydrolysis (DH) with slight modifications. The OPA reagent solution was prepared with 250 mg of sodium-dodecyl sulfate (SDS) and 9.525 g disodium tetraborate decahydrate in 155 ml water; 200 mg OPA in 5 ml ethanol; both solutions were mixed, added with 220 mg dithiothreitol and stirred to a clear solution. A volumetric flask was used to complete 250 ml with DI water. Samples were diluted 5 times to do the spectrophotometric readings. 400 µl of the diluted hydrolysate solutions were added into the OPA reagent (3 ml) and mixed. Absorbance at 340 nm (UV-1601 spectrophotometer, Shimadzu, Kyoto, Japan) was taken exactly 2 min after the addition of the OPA reagent. Blank and standard solutions were prepared using 400 µl of DI water and a serine solution (0.01%), respectively. DH was calculated using the following equations:

$$(\text{Serine-NH}_2) = \frac{\text{Sample}_{OD} - \text{Blank}_{OD}}{\text{Standard}_{OD} - \text{Blank}_{OD}} \times \frac{(0.9516 \times V \times 100)}{(X \times P)} \qquad [1]$$

Where, V is sample volume (in liter); X is sample weight (in gram); P is protein content (in %) of the sample, and Serine–NH_2 is in meq Serine-NH_2/g protein.

$$h = \frac{(\text{Serine-NH}_2) - \beta}{\alpha} \qquad [2]$$

Where, α and β values are estimated to be 1.00 and 0.40, respectively, and *h* is the number of hydrolyzed bonds. The h total values are estimated to be 9.2 and 7.9 for corn and sorghum, respectively.

$$DH = \frac{h}{h_{tot}} \times 100 \qquad [3]$$

Determination of ACE-I inhibitory activity.
Inhibitory antihypertensive activity was a modified method of Horax *et al.* (2017). The mixture of 50 µl CPH or SPH [10 mg hydrolysate/ml in 0.1 M phosphate buffer (PB) (pH 8.3)], 50 µl ACE-I solution [125 mU/ml in 0.1 M PB (pH 8.3)], and 150 µl hippuryl-L-histidyl-L-leucine (HHL) solution [12.5 mM of HHL in PB (pH 8.3) containing 0.5 M

NaCl] were incubated at 37 ℃ for 1 hour. 250 µl of 1 N HCL were used to stop the reaction. Negative control was used with the following conditions: 1 N of phosphate buffer was added before the addition of the enzyme. The hippuric acid (HA) liberated from HHL by ACE-I activity was extracted with 1.0 ml of ethyl acetate and centrifuged. The solution of ethyl acetate extract (0.7 ml) was evaporated to drynesss at 90 ℃ in a water bath, then to dissolve the residue, 1 ml of DI water was added. The amount of HA liberated was measured spectrophotometrically at 228 nm. The percentage (%) of inhibition was calculated as follows:

$$\left[1-\left(\frac{A-B}{C-B}\right)\right]\times 100 \quad [4]$$

Where A is absorbance in the presence of hydrolysate; B is absorbance of negative control; C is absorbance in the absence of hydrolysate.

Statistical analysis.

A statistical analysis was performed using the program SAS, Version 9.4® to find if there were significant differences between corns and sorghum proteins, as well as ACE inhibitory activities.

Experimental design.

A completely randomized design (CRD) was used, with a factorial arrangement 3 × 7, as shown in Table 1 and 3 × 2, as shown in Table 2 for degree of hydrolysis and ACE-I inhibitory activity, respectively. The separation of means was performed using Duncan and LSMEANS.

Table 1. Degree of hydrolysis depending the treatment and time.

Time (min)	Yellow Corn	White Corn	White Sorghum
	Degree of hydrolysis (%)		
0	TRT1	TRT8	TRT15
30	TRT2	TRT9	TRT16
60	TRT3	TRT10	TRT17
90	TRT4	TRT11	TRT18
120	TRT5	TRT12	TRT19
150	TRT6	TRT13	TRT20
180	TRT7	TRT14	TRT21

Table 2. Angiotensin-converting enzyme (ACE) inhibitory activities of the treatments in the higher degree of hydrolysis.

Treatments	Time (min)	DH	ACE inhibitory rate
Yellow Corn	0	TRT1	TRT1
Yellow Corn	90	TRT4	TRT4
White Corn	0	TRT8	TRT8
White Corn	90	TRT11	TRT11
White Sorghum	0	TRT15	TRT15
White Sorghum	60	TRT17	TRT17

Location of the study.

The analyzes of antihypertensive activities of corns and sorghum were done in a laboratory of protein functionality in the University of Arkansas, USA.

3. RESULTS AND DISCUSSION

Proximal composition of flours.
Table 3 shows that white sorghum had the highest amount of protein with 9.8% in comparison to corns that had 5.7 - 6.0%. As previously shown in others researched grains, protein content range is between 7 - 18% and 6 - 12% for sorghum and corn, respectively, depending on many factors such as the variety of the crop, variation in cultivation practices (e.g. fertilization), as well as environmental conditions (Bean *et al.* 2011; Williams 1981).

For moisture, the highest was obtained for is the white sorghum with 12.3%, but still lower than 15% of moisture as established by the FAO (Codex Alimentarius 2019). In the other hand, lipids in corns were higher with 6.4 and 6.6% and sorghum 5.5%; similar results were reported in previous study (Gwirtz & Garcia 2013; Stefoska 2015).

Table 3. Moisture, lipid and protein contents of corns and sorghum flours.[1]

Flour	Moisture Content (%)	Lipid content (%)	Protein content (%)
Yellow Corn	11.7 ± 0.4^{b}	6.4 ± 0.1^{a}	5.7 ± 0.8^{b}
White Corn	11.0 ± 0.1^{b}	6.6 ± 0.4^{a}	6.0 ± 0.9^{b}
White Sorghum	12.3 ± 0.3^{a}	5.5 ± 0.3^{b}	9.8 ± 0.3^{a}
CV (%)	1.26	5.12	8.45

[1]Values are presented as means ± standard deviation of three replication analysis. Protein content was determined on dry basis.
[a-b]: Different letters for each column represent statistical differences among treatments ($P < 0.05$).
CV: Coefficient of variation.

Preparation of corn and sorghum protein (CP and SP). In Table 4, it is shown that about 63 - 65% of protein was obtained from both corns and these results are consistent with previous research of corn protein extraction, which reported extractions ranging 60 - 71%. On the other hand, the lower protein recovery was obtained for white sorghum with 48%. Tapia-Hernández *et al.* (2019) reported similar results 35.33 - 46.51% of protein recovery in sorghum. Higher amounts of protein, in the range of 66%.

The proteins in corn and sorghum are soluble in selected solvents due to their types of proteins, such as: albumins (water), globulins (salt) and glutelin (alkali), zein and kafirin (alcohol). Zein and kafirins are hydrophobic storage proteins in grains that form 45 - 50%

and 50 - 60% of the total protein, respectively. Also, prolamins are soluble in pH 11 or above (Shukla and Cheryan 2001; Kamath *et al.* 2007). Kafirin presents the most hydrophobic trend of the prolamins (Sun *et al.* 2016). That is why the protein extraction is lower than corns.

Table 4. Comparison between protein content in flour and protein extraction.[1]

Flour	Protein content (%)	Extraction	Protein content (%)
White Sorghum	9.8 ± 0.3^{aX}	WSP[2]	48.1 ± 3.4^{bY}
White Corn	6.0 ± 0.9^{bX}	WCP[3]	65.7 ± 5.3^{aY}
Yellow Corn	5.7 ± 0.8^{bX}	YCP[4]	63.5 ± 4.7^{aY}
P value	<0.0001		
C.V. (%)	10.14		
R^2	0.991		

[1]Values are presented as means ± standard deviation of three replication analysis. Protein content was determined on dry basis.
[2]White sorghum protein (WSP), [3]white corn protein (WCP) and [4]yellow corn protein (WCP).
[a-c]: Different letters in the same column represent statistical differences among treatments ($P < 0.05$). [X-Y]: Different letters in the same row represent statistical differences within the same treatment ($P < 0.05$).
CV: Coefficient of variation.

In plants, the cell wall consists mainly of cellulose microfibrils embedded in a matrix of hemicelluloses, pectins, proteins and phenolics (Santiago *et al*. 2013). Proteins represent 10% of the extracellular matrix mass (Komatsu and Yanagawa 2012). The main purpose of ultrasound is to extract the protein in the cell wall. The mechanism of sonication involves high power of ultrasound to cause disruption of cell walls (Tang *et al*. 2002) and the breaking of covalent bonds (Singh *et al*. 1990). However, disruption of cell walls has a limited effect in protein extraction.

Degree of hydrolysis of CPH and SPH.

The proteins previous extracted were subjected to hydrolysys, the process was conducted using a Alcalase enzyme concentration of 0.5 AU/g of protein. Degree of hydrolysis (DH) is the percentage of the number of peptide bonds broken among the total number of peptide bonds in the substrate during protein hydrolysis (Wu *et al*. 2015). Previous studies reported that ultrasonic bubble cavitation causes crushing, which results in large mechanical shearing forces, which degrade protein structure and open up hydrophilic groups. This opening of structure increases the protein solubility and allows the protease to bind more easily with the protein substrate, which increases the efficiency of hydrolysis (Kadam *et al.* 2015).

As observed in Table 5, the DH increased as the time of incubation was increased, reaching a plateau at 90 min for both white (WCPH) and yellow corn protein hydrolysates (YCPH),

and 60 min for white sorghum protein hydrolysate (WSPH). All the treatments presented similar degree of hydrolysis, around 24%. There was significant difference between times ($P < 0.05$).

Table 5. Degree of Hydrolysis (%) of different corns and sorghum.[1]

Time (min)	Yellow Corn	White Corn	White Sorghum
	Degree of hydrolysis (%)		
0	5.68 ± 0.2^{dY}	5.51 ± 0.4^{dY}	6.60 ± 0.4^{dX}
30	21.55 ± 0.5^{bX}	21.10 ± 0.9^{bX}	22.05 ± 0.9^{bX}
60	22.41 ± 0.7^{bcY}	22.54 ± 1.4^{bcY}	24.51 ± 1.1^{aX}
90	24.07 ± 0.5^{aX}	24.85 ± 0.4^{aX}	24.01 ± 1.1^{aX}
120	23.28 ± 0.7^{abX}	24.17 ± 0.7^{abX}	23.77 ± 0.7^{aX}
150	23.92 ± 1.1^{aX}	23.85 ± 0.8^{abX}	23.80 ± 0.8^{aX}
180	23.60 ± 0.6^{aX}	24.21 ± 0.7^{abX}	24.50 ± 0.6^{aX}
P value	<0.0001		
C.V. (%)	4.31		
R^2	0.997		

[1]Values are presented as means ± standard deviation of three replication analysis.
$^{a-d}$: Different letters in the same column represent statistical differences among treatments ($P < 0.05$). $^{X-Y}$: Different letters in the same row represent statistical differences within treatments ($P < 0.05$).
CV: Coefficient of variation.

As shown in table 5, the DH rapidly increased in the first 30 min and then showed a slow increase from 30 to 60 min, or 90 min depending on the treatment. The reduction of the speed in the degree of hydrolysis it could be related with a reduction of substrate in the solution. The analysis of the degree of hydrolysis for yellow corn (YCPH), white corn (WCPH) and sorghum (WSPH). No significant differences were found ($P > 0.05$) for any of the treatments.

WSPH showed similar results to WCPH and YCPH corns, but the difference in the time was because the sorghum protein has less peptide bonds, as previously discussed. These results are consistent with previous studies for sorghum and corn, where it was reported that the concentration of protein was hydrolyzed and this is why the DH did not increase after 90 min (Yang *et al*. 2007; Wu *et al*. 2015).

Determination of ACE-I inhibitory activity.
The samples with the highest DH (the time point where the DH just reached the plateau) and the lowest DH were subjected to the evaluation of ACE inhibitory activities. The highest inhibitory activities were 45.4, 43.5 and 30.5% for YCPH, WCPH, and WSPH,

respectively. These results suggest that extensive hydrolysis may release many low-molecular weight peptides and results in high ACE inhibition. This seems to be consistent with previous reported results (Bao *et al*. 2017; Aluko 2018). These inhibitory activities were obtained with the highest DH for each treatment. When the DH was low, the ACE inhibition rate was also lowered to 5.68, 5.51, and 6.60% for yellow corn, white corn, and white sorghum shown as controls, respectively. As expected, lower degree of hydrolysis had less inhibitory activity, due the limited release of low- molecular weight peptides. (Table 6)

Table 6. Angiotensin-converting enzyme (ACE) inhibitory activities of protein and protein hydrolysates from corns and sorghum.[1]

	Treatments	[2]**Initial time**	[3]**Final time**
Degree of Hydrolysis (%)	YCPH[4]	5.68 ± 0.2^{aY}	24.07 ± 0.7^{aX}
	WCPH[5]	5.51 ± 0.4^{aY}	24.85 ± 0.4^{aX}
	WSPH[6]	6.60 ± 0.4^{aY}	24.51 ± 1.1^{aX}
ACE inhibitory rate (%)	YCPH	5.03 ± 0.4^{aY}	45.43 ± 1.9^{aX}
	WCPH	5.27 ± 0.3^{aY}	43.82 ± 1.7^{aX}
	WSPH	5.95 ± 0.3^{aY}	30.51 ± 2.3^{bX}
P value	<0.0001		
C.V. (%)	3.82		
R^2	0.998253		

[1]Values are presented as means ± standard deviation of three replication analysis.
[2]Inicial time is 0 min for YCPH, WCPH and WSPH. [3]Final time is 90 min for YCPH and WCPH and 60 min for WSPH.
[4]Yellow corn protein hydrolysate (YCPH), [5]white corn protein hydrolysate (WCP) and [6]White sorghum protein hydrolysate (WSPH).
[a-b]: Different letters in the same column represent statistical differences among treatments ($P < 0.05$). [X-Y]: Different letters in the same row represent statistical differences within treatments ($P < 0.05$).
These results were obtained using the same concentration of hydrolysate protein at 10 mg/ml.
CV: Coefficient of variation.

Lower reduction of ACE activity in this study could be related with the low protein extraction rate (De Mesa 2010; Shukla and Cheryan 2001). Several studies reported that bioactives fragments are located in the prolamins, in this specific case, zein and kafirin (Kamath *et al.* 2007; Zhou *et al.* 2012; Yano *et al.* 1996).

As previously shown, in other studies it was reported that hydrolyzed protein from corn (CPH) and sorghum (SPH) with a DH between 17 - 19% exhibited higher ACE inhibitory activity (Kamath *et al.* 2007; Yang *et al.* 2007; Wu *et al.* 2015). As previous reported for Kamath *et al.* 2007 and Yang *et al* 2007 ACE rate of inhibition is 7 - 46% and 13 - 83% for sorghum and corn, respectively. These inhibition rate values could be beneficial to the state of health of hypertensive individuals. Huang *et al.* (2011) achieved a reduction of 26.57

mmHg in systolic blood pressure to hypertensive rats in four weeks. The reduction in ACE activity could be due to the biopeptides reported in previous studies. For example, hexapeptide [Pro-Ser-Gly-Gln-Tyr-Tyr] and a dipeptide [Ala-Tyr] obtained from corn protein, and from sorghum the tripeptide [Thr–Leu–Ser] (Huang *et al.* 2011; Wu *et al.* 2015).

Kamath *et al.* (2007), reported that peptides obtained by enzymatic hydrolysis with Alcalase inhibited the (ACE) enzyme activity by competitively binding with the substrate for the active sites and uncompetitive inhibitors. Also, it was observed that antihypertensive peptides from zein (proline containing) were not susceptible to proteolysis by enzymes of the digestive tract such as chymotrypsin, trypsin or pepsin. The previous author used the same methodology and enzyme so is expected that results obtained was similarities whit the results of this study.

4. CONCLUSIONS

- The highest amount of protein extract was obtained from white corn and yellow corn, while the least amount of protein extracted was obtained from white sorghum.

- The degree of hydrolysis increased during incubation time with Alcalase reaching a plateau after 60 min for sorghum and 90 min for yellow and white corn.

- Treatments with a higher degree of hydrolysis showed higher ACE inhibitory activities. Yellow and white corn exhibited higher inhibitory efficiency than white sorghum.

5. RECOMMENDATIONS

- Evaluate another method of protein extraction in order to reach higher protein concentrations.

- Use a method of purification and fractionation to differentiate the biopeptides that were released.

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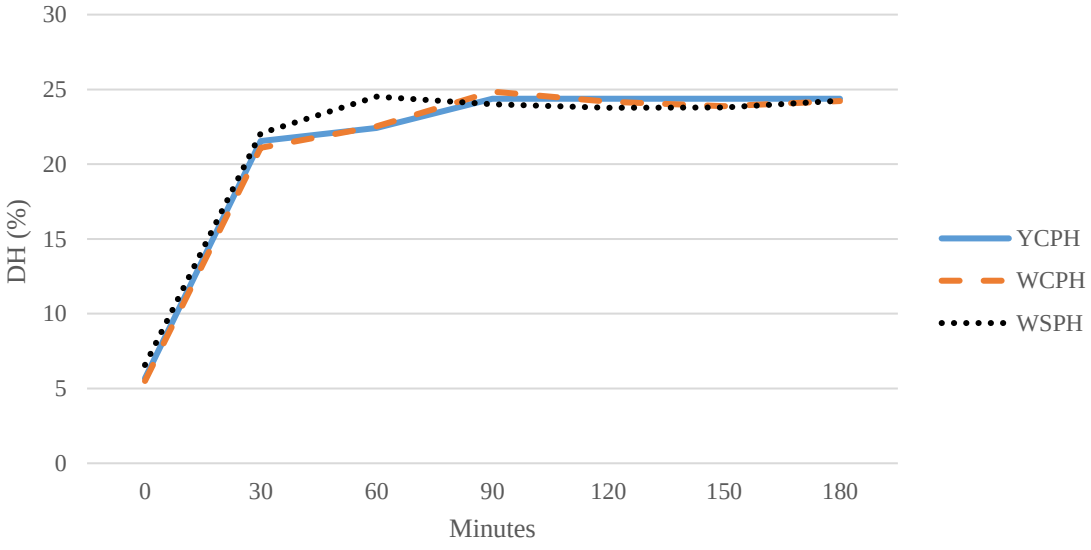
7. APPENDICES

Appendix 1. Degree of hydrolysis of yellow corn, white corn and sorghum protein hydrolysate at different times.



Appendix 2. ACE Inhibitory activities of protein and protein hydrolysates from corns and sorghum.