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Extraction and characterization of proteins present in concentrates from quality protein maize (QPM)

Extracción y caracterización de proteínas presentes en concentrados de maíz de alta calidad proteica (QPM)

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Abstract Quality protein maize (QPM) represents an alternative protein source for food. This work aims to characterize fractions, molecular weights, and thermal stability of the proteins present in QPM protein concentrates obtained by isoelectric precipitation. Flours from two treatments, non-nixtamalized and nixtamalized, as well as three types of maize varieties: Sac Beh (QPM white), Chichén Itzá (QPM yellow), and Blanco Uxmal (control), were obtained. The experiment was conducted using a bifactorial 2x3 design. Four isoelectric precipitation pHs were evaluated, having the highest yield and maximum protein precipitation at pH 2,5 at 4 °C. The relative protein fractions in the maize varieties in both treatments showed more elevated amounts of prolamins (PR) and glutelins (GT) compared to the control. All QPM showed higher GT and lower PR. The denaturing electrophoretic profile (SDS-PAGE) showed molecular weights for the concentrates ranging from 17,6 to 225 kDa (non-nixtamalized), from 12,9 to 132,2 kDa (nixtamalized), and from the fractions with weights from 10,2 to 220,7 kDa. The thermograms showed a change in thermal stability in the concentrates from non-nixtamalized flour; there were no thermal transitions in the nixtamalized ones due to the denaturation of the proteins when obtaining the samples during nixtamalization of all the varieties studied.

Keywords: Quality protein maize (QPM), fractionation, prolamins, glutelins, electrophoresis.

Resumen El maíz de alta calidad proteica (QPM) representa una fuente alternativa de proteína para la alimentación. Este trabajo tiene como objetivo caracterizar las fracciones, pesos moleculares y estabilidad térmica de las proteínas presentes en concentrados proteicos de maíces QPM, obtenidos por precipitación isoeléctrica. Se obtuvieron harinas de dos tratamientos, sin nixtamalizar y nixtamalizado, así como tres tipos de variedades de maíz: Sac Beh (QPM blanco), Chichén Itzá (QPM amarillo) y Blanco Uxmal (BU testigo). El experimento se realizó utilizando un diseño bifactorial. Se evaluaron cuatro pHs de precipitación isoeléctrica, teniendo el mayor rendimiento y máxima precipitación de proteína a pH 2,5 a 4 °C. La cantidad relativa de fracciones en las variedades de maíz en ambos tratamientos mostró cantidades más elevadas de prolaminas (PR) y glutelinas (GT) en comparación con el control. Todos los maíces QPM mostraron mayor cantidad de GT y menor PR. El perfil electroforético desnaturalizante (SDS-PAGE) indicó pesos moleculares para los concentrados que oscilaron entre 17,6 y 225 kDa (no nixtamalizados), entre 12,9 y 132,2 kDa (nixtamalizados), y para las fracciones con pesos desde 10,2 a 220,7 kDa. Los termogramas mostraron un cambio en la estabilidad térmica en los concentrados de harina no nixtamalizados; no hubo transiciones térmicas en los nixtamalizados debido a la desnaturalización de las proteínas durante la nixtamalización de todas las variedades estudiadas.

Palabras clave: Maíz de alta calidad de proteína, fraccionamiento, prolaminas, glutelinas, electroforesis.

Introduction

The existence of quality protein maize (QPM) offers an opportunity to reduce malnutrition and diseases associated with the deficiency of essential amino acids. Two maize with high protein quality had been developed, their Mayan names being Sac Beh (white grain) and Chichén Itzá (yellow grain). The QPMs in this study come from two white and one yellow maize, to which the *opaque*-2 gene was added through the incorporation of endosperm phenotype modifier genes to the maize, which gives it the QPM or quality protein characteristic; this material retains 75 % of native germplasm and 25 % corresponds to the QPM characteristics (Mansilla, 2018).

QPM (a biofortified *opaque*-2 mutant maize variety) improves the nutritional status of the population that depends on maize as a staple crop and contains a notable amount of tryptophan, lysine, and protein content, which are significantly different from the contents of normal maize varieties (Alamu *et al.*, 2022). Among the protein fractions in normal maize endosperm, zein or prolamin usually have a proportion of 60 %, glutelins of 34 %, albumin of 3 %, and 3 % of globulin (Caballero-Rothar *et al.*, 2019). There is a need to standardize protein extraction processes as to benefit from these, obtaining protein concentrates through specific isoelectric points.

The recording of molecular weights and obtaining of fractions by the solubility of the proteins of the maize varieties will give the guideline to identify the proteins and fractions present in the studied varieties, such as the identification of albumins (soluble in water), globulins (soluble in saline solutions), prolamins (soluble in alcohol) and glutelins (soluble in alkali) so the similarities and differences of the QPM varieties can be identified.

Therefore, the objective of this work was to determine some characteristics of the proteins present in protein concentrates obtained by isoelectric precipitation and fractionation by the solubility of QPM Sac Beh and Chichen Itzá maize varieties and a non-nixtamalized and nixtamalized control variety.

Materials and methods

Raw Materials

Quality protein maize (*Zea mays*, QPM) of Sac Beh (SB) and Chichén Itzá (ChI) varieties, developed by the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) for the state of Yucatán, and a control maize V-539 Blanco Uxmal (BU) were obtained from the Uxmal experimental site in Yucatan, Mexico.

Sample collection and preparation

The kernels were cleaned by removing damaged kernels, as well as impurities (stones and remnants of the husk) and were stored in refrigeration at 4 °C until use.

Non-nixtamalized maize flours

The kernels were crushed in an Oakland disk mill model ME-1501 and subsequently milled with the Cyclotec™ mill until a flour capable of passing through a 60 mesh (0,250 mm) was obtained.

Nixtamalized maize flours

A suspension was prepared with a ratio of 1:2 maize/calcium hydroxide at 1 % w/v, cooked at 100 °C for 25 - 30 min, and then allowed to rest for 19 h. The cooked maize (nixtamal) was washed 3 times with purified water in a 1:1 (v/v) ratio. The nixtamal was placed on stainless steel trays and dried in a Thermo scientific oven (Wyman Street Waltham, MA, USA) at 55 °C for 24 h, grounded in an Oakland disc mill, and subsequently with the Cyclotec™ mill until a flour capable of passing through a 60 mm mesh was obtained.

Isoelectric precipitation of proteins

The procedure reported by Wang and Wang (2004) with some modifications to adjust the isoelectric pH conditions, was used to obtain the concentrate. A pre-treatment was carried out for the separation of starch and protein through the use of the solvent NaOH at 0,1 % on the flours, with a solid-liquid ratio of 1:5 and 1:10 (w/v); two ratios were tested to evaluate in which ratio the protein of each treatment was better solubilized, leaving them at rest for 18 h at 18 °C, then adjusted to pH 9, homogenized, centrifuged, and separated. The supernatant was then recovered and the protein precipitation was performed. To determine the isoelectric point for protein precipitation, different pH values were used: 2,0; 2,5; 3,0; 3,5, and 4,0, all of them adjusted with 0,1% NaOH or 1 N HCl at times of 5, 10, 20 and 30 min at a temperature of 4 °C in the centrifugation. The isoelectric point was established at the pH value where the precipitate presented the highest yield of protein extractable.

Protein concentrates

For protein extraction, a suspension of the flour to NaOH 0,1% and 1:10 w/v ratio was used for non-nixtamalized maize and a 1:5 w/v ratio for the nixtamalized one. The differences in the ratios used were due to the dispersion capacity of the flours. The mixtures were left to rest for 18 h at 18 °C. After this time, were mixed with a KN - Lab IKA T18 digital Ultra - turrax homogenizer and sieved through 100 mesh (0,150 mm). The pH was adjusted to 9 and centrifuged at 3500 x g for 20 min at 4 °C using a Thermo Scientific Heraeus Megafuge 16R centrifuge to separate the starch. Using a siphon, the solubilized protein was separated.

The residues were washed with distilled water and then adjusted to the isoelectric point with 1 N hydrochloric acid to precipitate the proteins. The precipitate was centrifuged at 6000 x g for 20 min at 4 °C in an Ortoalresa Digicen 21 R universal centrifuge, the pH was adjusted to 7 with 1N NaOH, and were freeze-dried at -47 °C and 13 x 10^{-3} mbar in a Labconco freeze-dryer for 5 days (Laing & Christefeller, 2004).

Protein Composition

Determination of this component for QPM (ChI and SB) and the control (BU) protein concentrates was performed according to the Nitrogen method 954,01 of the Association of Official Agricultural Chemists (AOAC, 2005).

Sample conditioning process

The complete and selective elimination of the soluble non-protein compounds present in the concentrates was carried out through defatting with hexane by Soxhlet method and dialysis for the removal of salts in Spectra/Por dialysis tubes of 14,6 mm diameter and MWCO 6000 to 8000 of cut off, subsequently they were immersed in a cold buffer solution of deionized water with pH 7,3. Three 6-h dialysates were performed for each sample and freeze-dried for 2 days. Protein quantification by the Bradford method was performed on the samples, using bovine serum albumin as a reference standard.

Fractionation by solubility of non-nixtamalized and nixtamalized protein concentrates

The extraction of the albumin (AB) and globulin (GB) fractions was performed using a modification of the method reported by Barba de la Rosa *et al.* (1992). The supernatant containing the AB and GB fractions was dialyzed with distilled water at 4 °C. It was then centrifuged to separate the AB (soluble) fraction from the GB (insoluble) fraction. The residue from AB and GB extraction was dispersed with 70 % isopropanol for prolamins (PR) extraction. Glutelin (GT) extraction was carried out by dispersing the residue of PR extraction with a 1 M NaOH solution at a 1:15 w/v ratio, stirring for 1 h. The percentage of protein extracted in each fraction

was calculated as: (g of protein extracted in the fraction / g of protein in the concentrate) x 100. The protein content of the maize concentrates was calculated by the method of Bradford, using bovine serum albumin as a reference standard.

Electrophoretic analysis of maize protein concentrates.

The fractions obtained were subjected to the following evaluations:

- ***Denaturing (SDS-PAGE):*** following Laemmli (1970), 12 % polyacrylamide gels and proteins of 225, 150, 100, 75, 50, 35, 25, 15, and 10 kDa Bio-Rad catalog number V849A as standards were used. The protein loaded was 20 µg per lane. The electrophoretic analysis was performed with a constant current of 8 - 10 V/gel for 5 h. At the end of the electrophoretic analysis then they were stained with a 0,1% solution of Coomassie blue G-250 (Fluka 27815) in water:methanol:acetic acid in a 4:1:5 ratio (v/v/v) for 1 h. Subsequently, they were decolorized using a mixture of water:acetic acid:methanol in 5:1:2 ratio (v/v/v) for 12 h. Molecular weights were determined by linear regression analysis based on the standard RFs.

The electrophoretic analysis was performed with a constant current of 8 - 10 V/gel for 5 h. At the end of the electrophoretic analysis then they were stained with a 0,1 % solution of Coomassie blue G - 250 (Fluka 27815) in water:methanol:acetic acid in a 4:1:5 ratio (v/v/v) for 1 h. Subsequently, they were decolorized using a mixture of water:acetic acid:methanol in 5:1:2 ratio (v/v/v) for 12 h. Molecular weights were determined by linear regression analysis based on the standard RFs.

-***Native-PAGE:*** modification of Laemmli's technique (1970), in which SDS was excluded and replaced by distilled water. Proteins of 225, 150, 100, 75, 50, 50, 35, 25, 15, and 10 kDa (Bio-Rad) and 7 % concentration gels containing 30 % acrylamide (Sigma A - 9099) and 0,8 % bisacrylamide (Sigma M-2022) were used. The sample volume loaded was 5 to 10 µL. The electrophoretic analysis was equal to the denaturation technique.

- ***Reducing denaturants (SDS-PAGE Me):*** the method of Schägger and Jagow (1987) was used. The distilled water was replaced by 0.5 ml of mercaptoethanol. Proteins of 225, 150, 100, 75, 50, 35, 25, 15, and 10 kDa (Bio-Rad) and 13% polyacrylamide gels were used. The gels were stained in the same way as denaturing SDS-PAGE and Native-PAGE, staining with the same dye, for the same time and decolorizing for 12 h with the same mixture.

- ***Denaturing electrophoretic analysis of maize protein concentrates fractions (SDS-PAGE):*** The technique described by Laemmli (1970) as explained before was used with the same standards, sample volume, constant current, and time as previously established with the protein concentrates. The gels were stained and decolorized as indicated by the established technique.

Differential scanning calorimetry (DSC) of maize concentrates

The modified methodology of Michnik and Drzazga (2010) was used. 2,5 mg (d.b.) of sample were weighed and suspended in 12,5 µL of deionized water (30 %). Sample pans (Perkin-Elmer No. 0219 - 0062) were used, allowing the samples to stabilize for 30 min at room temperature (25 °C) and performing a heating flow rate of 10 °C /min at a range of 30 to 120° with a Perkin Elmer DSC-6 Pyris.

Experimental design and statistical analysis.

A 2x3 bifactorial design was carried out, the factors were flour process (Non-nixtamalized and nixtamalized) and the maize varieties Sac

Beh (SB), Chichén Itzá (ChI) and Blanco Uxmal (BU, control). This was performed in triplicate. The statistical analysis was carried out with a two-way analysis of variance (ANOVA). The statistical software STATGRAPHICS Centurion XVIII was used.

Results and discussion

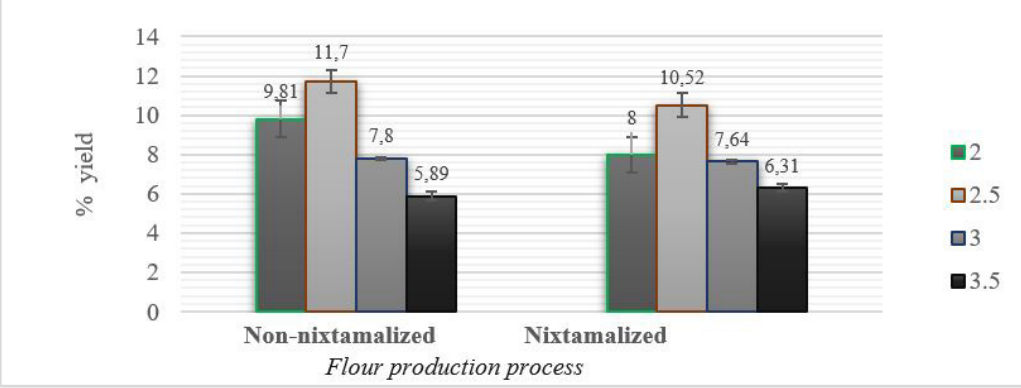
The results obtained indicated significant differences ($p < 0,05$) in the protein content of the flours of the varieties under study. The non-nixtamalized and nixtamalized Sac-Beh varieties, with 10,79 and 10,31 %, presented the highest protein percentages among the varieties studied; the results were lower compared to the hybrid Sac Beh (11,37 %) and higher than the predecessor Sac Beh (9,77 %) according to Chan-Chan *et al.* (2021). Palacios *et al.* (2020) conducted studies with QPM hybrids that reported between 8,2 and 9,4 %, lower than those found in this study.

Determination of the isoelectric point

Higher yields were recorded based on the weight and protein quantification for isoelectric precipitation at pH 2,5 (**Figure 1**). The extractable concentrate is smaller in quantity than the initial raw material. The behavior of pH does significantly affect the yield; this coincides with Aquino-Méndez *et al.* (2015), who states that factors such as the type of solvent, raw material, ionic strength, temperature, and mainly pH, significantly affect the protein extraction process.

Figure 1

Yield of protein concentrates obtained from maize flours subjected to different treatments: non-nixtamalized (A) and nixtamalized (B) at pH 2, 2.5, 3 and 3.5.



The protein concentrates from non-nixtamalized and nixtamalized flours showed a relative balance between the amount of fat and the amount of protein, which indicate that there is a ratio of up to 30 % fat and 70 % protein existent in the non-nixtamalized concentrates in the varieties, However, the amount of protein in the concentrates of the nixtamalized varieties was reduced, a consequence of the loss of pericarp and protein during nixtamalization (**Table 1**).

Therefore, it is congruent that the non-nixtamalized ChI defatted varieties registered a higher percentage of protein in all the treatments and varieties present in the research, while the nixtamalized ChI non-defatted variety presented a lower percentage of protein. There were significant differences ($p < 0,05$) between the QPM concentrates and the control when non-nixtamalized and nixtamalized. Molina-Paredes *et al.* (2018) pointed out that there isn't just one pH value as an isoelectric point, since

it depends on the primary composition of the protein chains that make up the protein, the amino acids, and peptides that constitute it, as well as the interactions that exist between these molecules and the medium. The results for fiber and nitrogen-free extract did not show significant differences ($p < 0,05$) between varieties.

Table 1

Protein content of non-nixtamalized (A) and nixtamalized (B) QPM Chichen Itzá (ChI) and Sac Beh (SB) maize concentrates and a control maize Blanco Uxmal (BU) (% d.b. 1).

Variety	Protein from fat concentrates	Protein from defatted concentrates
ChI(A)	42,09±2,3^{c}	86,81±1,15^{f}
ChI(B)	21,99±4,54^{b}	59,62±1,67^{c}
SB (A)	43,35±2,3^{cd}	75,137±2,66^{e}
SB (B)	19,12±5,23^{a}	57,083±1,75^{b}
BU (A)	44,08±1,29^{d}	64,393±1,55^{d}
BU (B)	22,56±4,43^{b}	51,347±1,94^{a}

Note. $^{a-f}$ Different letters in the same column indicate that the means are statistically different ($p < 0,05$), [1]Dry basis, data are the average of three replicates.

Solubility fractionation of proteins

The protein fraction with the highest relative percentage in the QPM varieties were prolamins (PR), followed by globulins (GB), albumins (AB) and glutelins (GT) (**Table 2**).

A higher percentage of total protein was obtained in the BU (non-nixtamalized) variety with86,81%,andtherewasasignificantdifference ($p < 0,05$) in the protein content obtained between varieties from non-nixtamalized and nixtamalized flours. The majority fraction for all varieties was PR, followed by GL; the variety with the highest amount of PR and GL was BU (non-nixtamalized) with 38,68 % and 25,41 % respectively with significant differences ($p < 0,05$) when compared with all varieties from non-nixtamalized and nixtamalized flour of the present study. In reference to the fractions obtained from PR in the QPM varieties, there was no significant difference ($p > 0,05$), the variety ChI (non-nixtamalized) presented a significant difference ($p < 0,05$), presenting the lowest percentage of 22,66 %. Aguirre-Mancilla *et al.* (2020) reported in a study with QPM accession (non-nixtamalized), a highest protein quality because its endosperm content includes high levels of albumins and globulins with a decreased zein content. These proteins constituted the main fraction of the storage proteins, representing 50 to 70 %.

The relative percentages of the QPMs in GT were higher compared to the control maize, although the relative % of PR in the QPMs was lower in comparison. Approximately 80 % of the grain proteins were storage proteins. PR or zeins represent the major part of grain protein (52 %), AB and GB represent 5 - 7 % of grain nitrogen, and GT represents 25 % of grain nitrogen according to Mansilla (2018). The fractionation of QPM (ChI and SB) and control (BU) maize concentrates showed differences. This phenomenon occurs because in common maize the zein content is 47 % and GT 35 % while in maize modified by the *o2* genes the zein content drops to 22 % and GT increases to 50 % of its initial value, Ortiz-Martínez *et al.* (2017). Therefore, the increase of GT fractions, which have a good amino acid balance of lysine and tryptophan, is preferred, Chavez *et al.* (2022).

Table 2

Proportion of fractions of protein concentrates of QPM varieties (ChI and SB) and a control variety (BU) non-nixtamalized (A) and nixtamalized (B).

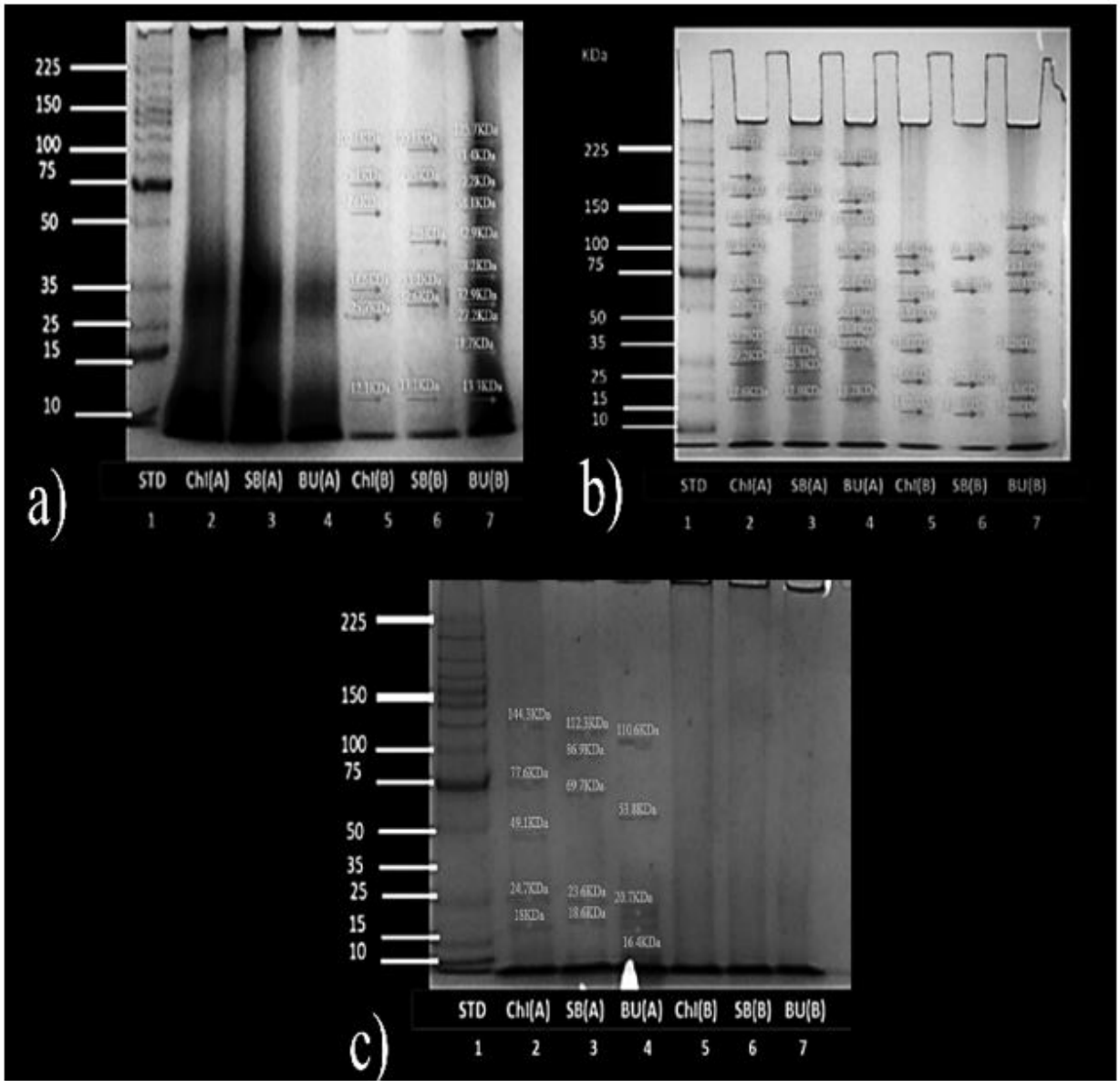
Variety	Fractions	Extracted protein (%)	% Relative
ChI(A)	AB	1,83±0,25[a]	3,59
ChI(A)	GB	4,84±1,13[b]	9,43
ChI(A)	PR	22,66±0,46[d]	44,13
ChI(A)	GT	22,01±0,09[c]	42,85
	TOTAL	**51,35**	**100**
ChI(B)	AB	0,143±0042[a]	0,25
ChI(B)	GB	10,32±452[b]	17,31
ChI(B)	PR	25,96±0,042[d]	43,54
ChI(B)	GT	23,19±0,268[c]	38,9
	TOTAL	**59,62**	**100**
SB(A)	AB	16,64 ± 1,35[b]	22,17
SB(A)	GB	8,54 ± 0,18[a]	11,37
SB(A)	PR	25,96 ± 0[d]	34,55
SB(A)	GT	23,98 ± 0,01[c]	31,91
	TOTAL	**75,14**	**100**
SB(B)	AB	5,63 ± 0,5[a]	9,71
SB(B)	GB	8,549 ± 0,56[b]	14,72
SB(B)	PR	25,94 ± 0,08[d]	44,67
SB(B)	GT	17,94 ± 0,11[c]	30,9
	TOTAL	**58,08**	**100**
BU(A)	AB	6,07 ± 0,04[a]	7,02
BU(A)	GB	16,63 ± 0,14[b]	19,15
BU(A)	PR	38,68 ± 0,03[d]	44,56
BU(A)	GT	25,41 ± 0,15[c]	29,27
	TOTAL	**86,81**	**100**
BU(B)	AB	5,68 ± 0,7[a]	8,84
BU(B)	GB	7,66 ± 0,46[b]	11,9
BU(B)	PR	31,60 ± 0,56[d]	49,07
BU(B)	GT	19,44 ± 0,45[c]	30,19
	TOTAL	**64,39**	**100**

Electrophoretic analysis of maize concentrates

In the electrophoretic profile of maize concentrates by Native - PAGE electrophoresis (**Figure 2**), bands between 12,1 to 125,7 kDa were observed in lanes 5, 6, and 7 in the tree varieties of nixtamalized maize. The corresponding varieties of non-nixtamalized maize in lanes 2, 3, and 4 didn't show any defined bands (**Figure 2**). The nixtamalization process of the maize reduced the solubility of albumins and globulins and the same occurred with the solubility of PRs. The appearance of high-molecular-weight GTs was also observed.

Figure 2

Native-PAGE (a), denaturing SDS-PAGE (b), and reducing denaturing SDS-PAGE-Me (c) electrophoresis of protein concentrates from QPM (SB and ChI) and control (BU) maize flour subjected to treatment A (non-nixtamalized) and B (nixtamalized).



In commercial zein, a molecular weight of approximately 21,5 kDa were reported and in studies performed with zein fractions obtained through polyacrylamide gel electrophoresis (SDS - PAGE), molecular sizes between 18 to 10 kDa were observed (Nuñez-Terrones, 2018). This author observed that QPM presented a lower prolamin content than normal maize ranging from 35,39 to 61,28 kDa. The denaturing electrophoresis (SDS-PAGE) was chosen to determine the molecular weights of the protein fractions, obtaining the following electrophoretic profiles (**Figure 3**).

Figure 3

Denaturing electrophoresis (SDS-PAGE) of the fractions (Albumins (a), Globulins (b), Prolamines (c) and Glutelins (d) of the QPM protein concentrates (SB and ChI) and control (BU) subjected to treatment A (not nixtamalized) and B (nixtamalized) obtained by solubility.

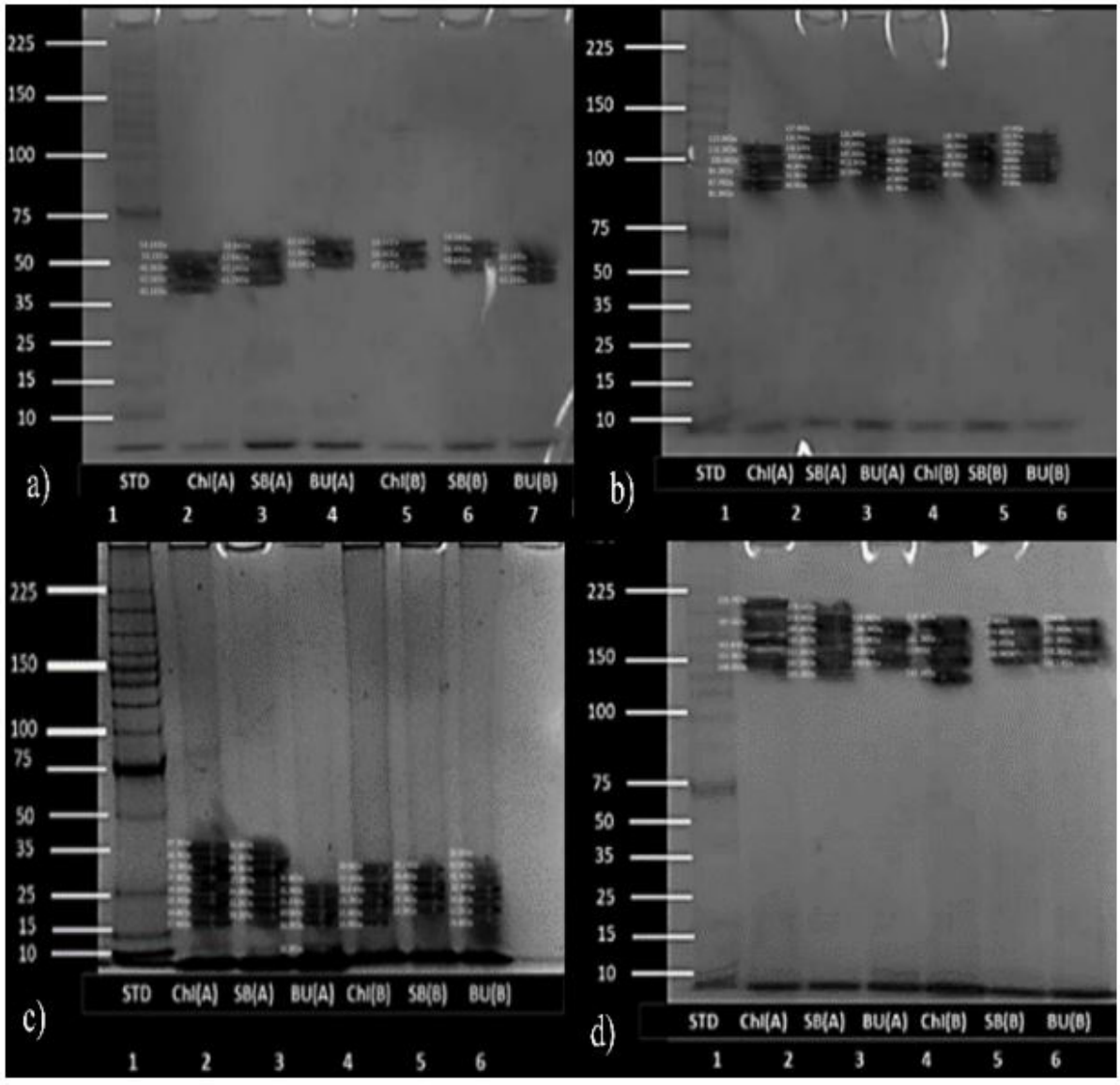


Table 3 shows the molecular weights and number of bands of the fractions obtained by denaturing electrophoresis (SDS-PAGE) of the different varieties of concentrates obtained from maize for the present study. The GTs exhibit higher molecular weights. The variety with the highest molecular weight was recorded in BU (nixtamalized) with 150,5 kDa and the band with the lowest molecular weight was observed in variety SB (nixtamalized) with 142,1 kDa. The band with the lowest molecular weight was 200 kDa in BU (nixtamalized).

The non-nixtamalized QPM showed the highest number of bands and molecular weights compared to the nixtamalized QPM. The QPM ChI (non-nixtamalized) in lane 2 showed the highest molecular band weight with 37,7 kDa, followed by SB (non-nixtamalized) with 36,2 kDa in lane 3. The nixtamalized QPMs obtained lower molecular weights compared to the BU control (19,8 to 30,3 kDa) and a lower number of bands in their respective lanes, 4 and 5 (ChI 16,9 to 29,0 kDa and 22,3 to 29,1 kDa). AB and GBs

presented a lower number of bands in relation to PRL fractions, and their molecular weights ranged from 40,1 to 50,0 kDa in ABs and from 85,3 to 124,4 kDa in GB.

Table 3

Molecular weights of fractions of protein concentrates of nixtamalized (A) and non-nixtamalized (B) QPM (ChI and SB) and BU maize obtained by denaturing electrophoresis (SDS-PAGE).

Fractions	ChI (A) (kDa)	ChI (B) (kDa)	SB (A) (kDa)	SB (B) (kDa)	BU (A) (kDa)	BU (B) (kDa)
AB	40,1 - 54,1	47,6 - 59,6	41,7 - 59,8	48,6 - 59,5	50,0 – 62,0	42,3 - 50,1
GB	85,3 - 123,9	85,7 - 123,3	86,5 - 127,4	89,1 - 126,7	92,2 - 125,3	87,8 - 127,4
PR	17,4 - 37,7	16,9 - 29,0	20,2 - 36,2	22,3 - 29,1	10,2 - 26,8	19,8 - 30,3
GT	148,6 - 220,7	142,1 - 219,1	143,2 - 219,1	150,3 - 216	149 - 218,9	150,5 - 200

PLR had generally lower molecular weights. As observed in both ChI and SB without nixtamalization, they had some bands with more weight in GT and GB, and lower in AB and PR when compared to the other treatments. According to previous research maize prolamins are grouped into α-zeins of 19 kDa and 22 kDa, β-zeins of 15 kDa, γ-zeins of 16 kDa, 27 kDa, and 5 kDa, and δ-zeins of 10 kDa and 18 kDa (Li *et al.*, 2021).

The electrophoretic pattern of prolamins found in this work can be seen in the presence of bands of molecular weights located from 10,2 to 37,7 kDa, corresponding to fraction α-zeins (21,00-26 kDa), which matches that reported by Huang *et al.* (2022). Zein is a mixed polypeptide comprised of α-zein (21 - 25 kDa, 75 - 85 %), β-zein (17 -18 kDa, 10 - 15 %), γ-zein (27 kDa, 5 - 10 %) and δ-zein (10 kDa, 3 %), respectively, Molecular weights from 16,7 to 24,2 kDa correspond to the β-zein fraction (18,00 - 24 kDa), similar to those reported by Abdelsalam *et al.* (2021), (18 to 24 kDa). The bands ranging from 10,00 to 17,2 kDa and lower than 10 kDa correspond to zein peptides, as indicated by Li *et al.* (2021). According to Aguirre-Mancilla *et al.* (2020), the prolamins of INIFAP-QPM accession (non-nixtamalized) had an electrophoretic pattern that included bands 67, 45, 25, 20, 16 and 13,2 kDa. One band at 33,1 kDa that the other materials under study did not present was reported.

It can be concluded that there are differences in the bands and molecular weights when comparing non-nixtamalized and nixtamalized maize, as well as a decrease in the solubility of the different protein fractions obtained by the different techniques used. In relation to the effect observed in the QPM and the control maize, there were differences in bands and molecular weights in the non-nixtamalized and nixtamalized maize, the ones with the modified gene presented a decrease in the solubility of the different fractions in relation to the control and the most notable changes were noticed in the nixtamalized QPM where high temperatures were applied and an adjustment of pH was done for the isoelectric precipitation to obtain the concentrate.

Differential scanning calorimetry (DSC) of QPM and control maize concentrates

The concentrates from non-nixtamalized flour of all varieties presented endothermic transitions during the analysis of thermal behavior, the samples under study recorded for the QPM varieties showed a slight increase in the denaturation enthalpy (DHtotal = 7,12 ± 0,52 J/g) and transition temperatures (Tp of 82, 40 ± 1,91 °C and 89,28 ± 0,76 °C), for ChI (non-nixtamalized) and DHtotal = (6,23 ± 0,32 J/g), (Tp of 80,20 ± 1,12 °C and 81,16 ± 0, 71 °C), for SB (non-nixtamalized), respectively. For the control BU (non-nixtamalized) a slight increase

in denaturation enthalpy (DHtotal = 5,54 ± 01,02 J/g), (Tp of 67,90 ± 1,10 °C and 70,17 ± 0,41 °C) was recorded.

This result was confirmed doing an assay with a standard egg albumin sample with which a transition was recorded at 87,765 ± 1,21 °C to 76 ± 1,32 °C, with a DH of 66,4 ± 01,00 J/g. The starting raw material (non-nixtamalized concentrates) and the absence of denaturing treatments during the obtaining of the concentrate justify the absence of peaks in the thermograms of concentrates from nixtamalized flour and the presence of peaks in the non-nixtamalized ones, this due to the preservation of that part of the native structure of the proteins present in the varieties of non-nixtamalized maize concentrates.

The nixtamalized concentrates didn't register denaturation enthalpies and transition temperatures could have been affected in the process of obtaining the sample by the alkaline treatment realized before obtaining the concentrate, which attributes this effect to the decrease in solubility, and formation of disulfide bridges between zein monomers and cross-linking of disulfide bonds Khalid *et al*, (2022). Therefore, in the varieties of maize concentrates of the present study, the disulfide bonds of the non-nixtamalized varieties were preserved during sample collection, the increase in denaturation enthalpy and transition temperature was observed during the differential scanning analysis; the opposite happened with the concentrates from nixtamalized flours, the proteins were denatured in the process of obtaining the samples during nixtamalization.

Conclusions

The varieties of non-nixtamalized and nixtamalized maize flours were precipitated at pH 2,5 obtaining protein concentrates, the defatting of the concentrates had positive effects by notably increasing the percentages of protein in both treatments. Nixtamalization affected the purity of the concentrates and denaturation of the proteins present due to the high temperatures and the presence of salts.

The decrease in the fraction of prolamins (*zeins*) and increase of glutelins in the QPM varieties used in this study, compared to the BU confirmed the positive effect of the presence of *opaque* -2 gene. Denaturing electrophoresis (SDS-PAGE) allowed the determination of the molecular weight of the unknown proteins.

Differential scanning calorimetry proved that the non-nixtamalized QPM wasn't denatured during the breeding process, confirming the results of the gel electrophoresis in the native state, where it was observed that the lanes showed protein bands with a structure corresponding to QPM and control maize. With non-nixtamalized maize there was an increase in the denaturation enthalpy and the transition temperature. A different case occurred with the concentrates from nixtamalized maize where the proteins were denatured in the process.

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