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Techno-functional properties of quality protein maize (QPM) (*Zea mays* L.) protein concentrates

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Abstract

Nowadays, new strategies and alternatives are being implemented to improve the protein quality of foods containing essential amino acids, like quality protein maize (QPM). The techno-functional properties of protein concentrate from QPM maize: Sac Beh (SB), Chichen Itza (ChI), and Blanco Uxmal (BU); non-nixtamalized (NN) and nixtamalized (N), were evaluated in the present work. The non-nixtamalized varieties showed higher amounts of protein in the QPM ChI (86.81%) and nitrogen solubility of 64.94% as the pH increased to 9. The emulsifying capacity was higher at pH 5 (60.74%). The non-nixtamalized SB samples showed higher foaming stability (1.82%) at pH 7, and the non-nixtamalized BU samples had higher foaming capacity at pH 5 (60.74%). Water holding capacities ranging from 1.2 to 3.13 g/g were achieved. The non-nixtamalized QPM concentrates had higher water and oil holding capacities. There was a predominance of elastic character over viscous character (G' > G") in all treatments behaving as weak gels, which were affected in alkaline conditions by decreasing the modulus value. The techno-functional properties were affected by the processing method. These results could be beneficial for providing a better understanding of the properties of QPM proteins, allowing innovative ways of utilising these proteins as an ingredient in food systems.

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Introduction

The food industry has undergone many changes in consumption patterns, tastes, preferences, and quality and sanitation requirements, amongst other aspects. The different technological advances provide a rapid response to consumer preferences and requirements, as well as to the frequent changes in consumption patterns, in order to remain and grow in the market (Sigaard and Laitala, 2023). Recent research showed a clear increase in consumer preference for plant-based proteins as an alternative to animal proteins (Onwezen *et al.,* 2021). The techno-functional properties of cereals primarily express the physicochemical properties of dietary proteins that determine their behaviour in foods during processing, storage, and consumption; these properties and how proteins interact with other components influence directly and indirectly in the processing applications, quality, and acceptability of foods (Fernández-Tomé *et al.,* 2023).

The techno-functional properties of a protein are determined by its physical and chemical properties that are involved in the food process, such as storage, preparation, and consumption, due to the multiple changes in protein behaviour. There are intrinsic and extrinsic factors that affect the techno-functional properties of a protein (Fuentes-Lemus and Davies, 2023; Yu *et al*., 2023). Zhang *et al.* (2023) indicated the importance and need to understand protein properties and their changes in relation to different food systems, and their relationship with the quality of food products. The main properties are water absorption capacity (WAC), oil absorption capacity (OAC), foaming capacity (FC), foaming stability (FS), gelation, and emulsification, which are the main functional properties of proteins and viscoelasticity.

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Maize is one of the cereals of greatest ancestral importance for human nutrition. The maize program of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) in the Yucatan Peninsula has implemented several strategies to generate biofortified, bioactive, and functionally improved maize, which have an impact on the health and nutrition of the consumers. The Sac Beh (white maize) and Chichen Itza (yellow maize) varieties were developed through the genetic introduction of 75% of a Mayan creole germplasm and 25% of a quality protein donor called Hybrid-519 C. They have innate characteristics of native maize adapted to stony lands where farmers practice shifting cultivation (slash and burn) as an alternative to improve their family's standard of living. For maize to be considered QPM, it must have lysine and tryptophan levels above 0.35 and 0.072 g per 100 g of grain, respectively (Ramírez-Silva *et al*., 2022). However, no studies on protein concentrate from these novel QPM have been reported; so, their techno-functional properties and potential for food production are unknown. That would be the novelty of the present work.

The research reported on the techno-functional properties of maize proteins is focused on the characteristics of normal corn meals, and not of QPM. Premkumar *et al*. (2022) extracted proteins from industrial by-product, and formulated them into a supplement that could potentially solve malnourishment. The protein was extracted using the isoelectric precipitation method. Another focus of the studies with QPM have been directed to the evaluation of physical properties, proximate composition, mineral contents, fatty acids, and amino acids of grains and meals using standard methods (Abiose and Ikujenlola, 2014). Therefore, the objective of the present work was to obtain and characterise techno-functional protein concentrates from QPM maize (Sac Beh and Chichen Itza) and a control variety (Uxmal), to evaluate their potential as food ingredients.

Materials and methods

Raw material

Quality protein maize (QPM) of Sac Beh (SB) and Chichen Itza (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. Maize samples were harvested at the Uxmal experimental site of the Centro de Investigación Regional Sureste of INIFAP in Yucatan, Mexico.

Sample collection and preparation

Maize grains

Six kilograms of maize grains (in three samples of 2 kg each) were obtained from each variety. The grains were cleansed by removing damaged grains, as well as impurities such as stones and small remnants of corncob. The cleansed grains were stored in plastic jars, and refrigerated (4.5°C) until further use.

Non-nixtamalized maize flours

The grains of each variety were crushed in an Oakland model ME-1501 disk mill, and subsequently milled with the CT 293 Cyclotec™ mill until the flour was able to pass through a 60 mesh (0.250 mm) sieve. The flours were labelled and stored in plastic jars at room temperature.

Nixtamalized maize flours

A suspension with a 1:2 ratio maize/calcium hydroxide at 1% (w/v) was prepared and cooked at 100°C for 25 - 30 min, then left to rest for 19 h. The cooking water was removed, and the cooked maize (nixtamal) was washed three times with purified water in a 1:1 (v/v) ratio to remove excess calcium hydroxide and detached pericarp. The nixtamal samples were placed on stainless steel trays, and dried in a Thermo Scientific Savant Model SC210A 81 oven (Wyman Street Waltham, MA, USA) at 55°C for 24 h. The nixtamalized and dried material was ground in an Oakland brand disc mill, model ME-1501, and subsequently with a CT 293 Cyclotec™ mill until the flour was able to pass through a 60 mm mesh sieve. The flours were labelled and stored at room temperature (Chávez-Santoscoy *et al.,* 2016).

Protein concentrates

For the protein extraction, the modified method of Premkumar *et al*. (2022) was used. Suspensions were carried out using a 0.1% flour-NaOH ratio of 1:10 (w/v) for non-nixtamalized flours, and a 1:5 (w/v) ratio for nixtamalized flours. The suspensions were left to rest for 18 h at 18°C. Following this, they were mixed with a KN-Lab Ika T18 digital ultra-turrax homogeniser and sieved through a 100 mesh (0.150 mm). The pH of the flour suspensions was adjusted to 9 and centrifuged at 3,500 *g* for 20 min at

4°C using a Thermo Scientific Heraeus Megafuge 16R centrifuge to separate the starch. The liquid part with the solubilised protein was separated using a siphon, obtaining a supernatant with soluble protein and a solid residue of starch. The residues were washed with distilled water until the liquid obtained was a transparent, to recover the highest amount of soluble protein. The pH was adjusted to the isoelectric point with 1 N HCl to precipitate the proteins. The precipitate was centrifuged at 6,000 *g* for 20 min at 4°C in an Ortoalresa Digicen 21 R universal centrifuge. Neutralisation was then performed in a suspension of distilled water, and the pH was adjusted to 7 using NaOH 1 N, subsequently, the concentrates were freeze-dried at -47°C and 13×10^{-3} mbar in a Labconco equipment for 5 d (Laing and Christefeller, 2004). The dried protein concentrate was stored in airtight containers until further analyses.

Sample conditioning

Complete and selective removal of the non-protein compounds present in the concentrates was carried out by deffating with hexane by Soxhlet method; then they were submerged in a cold buffered solution of deionised water at pH 7.3, and dialysed for salt removal in Spectra/Por dialysis tubes of 14.6 mm diameter and MWCO of 6,000 to 8,000 Da cut-off diameter. Three 6 h dialyses were performed for each sample, and freeze-dried for 2 d. Subsequently, chemical analysis was performed to determine the amount of protein in each concentrate of the different varieties.

Protein content

Protein determination of the protein concentrates obtained from the QPM varieties (ChI and SB) and the control (BU) was performed following the official procedures described by the AOAC (2019). Nitrogen (method 954.01) was evaluated with a Kjeltec system (Tecator, Sweden), and protein content was calculated as nitrogen × 5.83.

Nitrogen solubility

Nitrogen solubility tests were carried out according to Were *et al.* (1997). Briefly, 25 mL of a 0.5% (dry base) sample suspension was prepared. The pH was adjusted (5, 7, or 9) with HCl or NaOH 0.1 N, and shaken for 30 min. Subsequently, it was centrifuged at 4,320 *g* for 30 min. The volume of the supernatant was measured, and an aliquot was taken to determine its nitrogen content by the Kjeldahl method, following the AOAC (2019). Nitrogen solubility was expressed as the percentage of solubilised nitrogen in relation to the nitrogen content in the sample.

Foaming capacity and stability

This was carried out according to Chau *et al.* (1997) by preparing 10 mL of a suspension of each sample at 1.5%. The pH was adjusted (5, 7, and 9), and blended at low speed for 5 min in an Osterizer model 04652-13 blender. Then, it was transferred to a 250 mL graduated cylinder, and the foam's volume after 30 s was recorded. The result was expressed as percentage increase in foam volume after the 30 s. It was left to rest, and the foam volume was measured after 5, 30, and 120 min. Foam stability was determined as a percentage of the remaining foam volume after the indicated periods.

Emulsifying capacity and emulsion stability

The Chau *et al.* (1997) methodology was used. This test was performed at pH values of 5, 7, and 9, for which 10 mL of a 1.5% (w/v) sample suspension was prepared. Then the pH was adjusted to the desired level, and shaken in a Caframo model RZR1 homogenising equipment at a speed of 1,000 rpm for 2 min. Next, 10 mL of maize oil was added and homogenised for an additional 1 min. The resulting solution was centrifuged in 15 mL graduated tubes at 1,200 *g* for 5 min, and the volume of the emulsion was measured. The emulsifying activity was expressed as the ratio between the volume of emulsified layer and volume of the whole layer in the tube × 100. For emulsion stability (ES), the sample was heated at 80°C for 30 min, and cooled to room temperature. Subsequently, they were centrifuged in 15 mL graduated tubes at 1,200 *g* for 5 min, and the volume of the emulsion was measured. The ES was calculated as the percentage of the remaining volume of the emulsified layer in relation to the volume of the original emulsion.

Water and oil absorption capacity

The methodology reported by Chau *et al.* (1997) was used by weighing 0.5 g sample (dry base) of the protein concentrate, and adding 10 mL of distilled water or maize oil, depending on the test. It was placed in constant agitation (speed 6) in a Corning PC 320 magnetic plate for 1 min, then centrifuged at 2,200 *g* for 30 min. At the end of the centrifugation, the volume of the supernatant was

measured in 10 mL test tubes, and with the density of the oil (0.91 g/mL), the mass of water or separated oil was determined in g. The water or oil absorption capacity was expressed as g of water or oil absorbed by g of sample.

Rheological properties

Dispersions of protein concentrate with 5% (w/v) total solids were used, and the pH of each sample was adjusted to 5, 7, and 9 to determine the viscoelastic properties with an AR 2000 rheometer (TA Instruments, New Castle, DE). Oscillatory tests were performed in triplicate. A parallel plate geometry with a diameter of 60 mm was used. Before measurement, the samples were homogenised by agitation at 30/s for 2 min at room temperature, and left to stabilise for 50 s. The sample temperature was adjusted to 25°C. The linear viscoelastic region (LVR) was identified by running sweep amplitudes (at a constant frequency of 1 Hz) from 0.01 to 1% at 25°C. Frequency sweeps (0.1 - 10 Hz) were run, with the previously determined amplitude value (LVR). The elastic or storage modulus (G') and the viscous or loss modulus (G'') were evaluated for each test (Chel-Guerrero *et al.*, 2021).

Experimental design and statistical analysis

The effect of the treatments on the techno-functional properties was performed based on a 2 × 3 bifactorial design, with the factors being the process of obtaining the flours (non-nixtamalized and nixtamalized) and the type of maize varieties: Sac Beh (SB), Chichen Itza (ChI), and Blanco Uxmal (BU). Three replicates were performed for each sample, and the results were expressed as mean ± standard deviation. The statistical analysis was performed with a Two-way analysis of variance (ANOVA), using LSD mean comparison to establish differences between the results obtained for the two QPM varieties and the control variety. The STATGRAPHICS Centurion XVIII statistical software was used for data analysis.

Results and discussion

The results of the protein analysis of the concentrates indicated higher protein levels when the varieties did not undergo nixtamalization. The data for the SB, ChI, and BU nixtamalized varieties were 57.08, 59.62, and 51.34%, respectively. While for the same non-nixtamalized varieties they were 75.13, 68.81, and 64.39%, respectively.

Nitrogen solubility

The solubility of proteins is an important factor for their functional application in the food industry (Sun *et al.*, 2023). The nitrogen solubility index (NSI) of the proteins from non-nixtamalized and nixtamalized protein concentrates is shown in Table 1.

Table 1. Nitrogen solubility index (NSI) of defatted protein concentrates from QPM (SB and ChI) and control (BU), between non-nixtamalized and nixtamalized maize flour.

		Nitrogen solubility index	
Sample	**pH**	**Non-Nixtamalized (%)**	**Nixtamalized (%)**
ChI	5	4.05 ± 1.54^{cA}	0.6 ± 0.27^{aB}
ChI	7	3.36 ± 0.4^{cA}	0.99 ± 0.67^{aA}
ChI	9	64.94 ± 0.43^{cB}	1.34 ± 0.02^{aA}
SB	5	5.4 ± 0.09^{bA}	7.09 ± 3.12^{bB}
SB	7	2.57 ± 0.09^{bA}	1.66 ± 0.88^{bA}
SB	9	39.03 ± 1.14^{bB}	$2.05 \pm .058^{bA}$
BU	5	2.11 ± 0.06^{aA}	6.29 ± 0.74^{bB}
BU	7	2.25 ± 0.57^{aA}	1.25 ± 0.19^{bA}
BU	9	15.07 ± 0.59^{aB}	1.25 ± 0.34^{bA}

Values are mean ± standard deviation of triplicate (n = 3). Means followed by different lowercase superscripts between columns, and different uppercase superscripts between rows are significantly different ($p < 0.05$).

Based on the results, the minimum NSI percentage was recorded in the Uxmal maize concentrate at a pH of 5 (2.11%), and progressively increased with the increase in pH, up to a maximum value of pH 9 (64.94%) in the ChI variety; as for the percentage of NSI in the nixtamalized maize, the minimum solubility was at pH 9 (0.021%) in the ChI variety, and progressively increased with the increase in pH, up to a maximum value of pH 5 (7.09%) in the SB variety. The QPM concentrates from the non-nixtamalized treatment had higher solubility index compared to the nixtamalized treatment. These results were similar to those found for protein fractions of cowpea, soybean, winged bean, kidney bean, and wheat (Fernández-Sosa *et al.*, 2021).

The non-nixtamalized protein concentrates yielded higher percentage of solubility than the nixtamalized ones, implying that by subjecting them to high temperatures and the incorporation of salts during nixtamalization, the proteins of QPM concentrates were affected, reducing their solubility ($p < 0.05$). It was also observed that solubility increased as the pH became more basic. The results obtained in the present work were similar to those described by other authors in amaranth, quinoa, and chia, who found that at a pH near the isoelectric point, the percentages of solubility in flours and protein concentrates were lower, while at basic pH, solubility increased (Manzoori *et al.*, 2023).

López *et al.* (2019) stated that of all the functional activities, solubility is of vital importance because of its definite influence on the other functional properties. High solubility in aqueous media ensures good whipping, foaming, gelling, and emulsifying characteristics. In this sense, the proteins present in the QPM varieties could be used for the production of food systems as beverages, dehydrated soups, and sauces, among others.

Foaming capacity and stability

The values of foaming capacity (FC) and foam stability (FS) presented in Table 2 indicate that there were significant differences ($p < 0.05$) in the parameters evaluated due to the effect of the nixtamalization treatment. The highest FC and FS values were recorded at pH 9 with the SB (NN) variety, followed by the same variety nixtamalized. The presence of lysine and tryptophan in the QPM varieties along with the nixtamalization process represented an important factor that could be influencing the protein conformation and development of stable foams (Yang and Sagis, 2021) compared to the BU control. The difference in results between the treatments used to obtain the concentrates might have been due to the reduced capacity to form surface membranes around the air bubbles (Daliri *et al.*, 2021). These effects on the foaming activity of the proteins could be attributed to the addition of calcium hydroxide or hydrated lime, and the subjection to high temperatures that produced changes in the structure of the proteins and their consequent denaturation, modifying their ability to interact superficially with the air, as has been reported by Noman *et al.* (2018).

Houde *et al.* (2018) studied the foaming capacity and stability of barley protein concentrates at different pH values of 3, 5, and 8, finding that the foaming capacity of barley concentrates did not vary significantly within the same pH range, decreasing only slightly from 88.7 to 75.3% with increasing pH from 3 to 8. These results contrasted with the values obtained in the present work for protein concentrates from SB and ChI, most likely because they are QPM varieties rich in lysine and tryptophan, and particularly because of the overtreatment that might have been caused by nixtamalization. Although the QPM protein concentrates presented a good foaming capacity that would suggest its use as a foaming agent in food systems as whipped cream, soufflés, mousses,

Table 2. Foaming capacity (FC) and foaming stability (FS) of protein concentrates from QPM (SB and ChI) and control (BU), between non-nixtamalized and nixtamalized maize flour.

Sample	pH	Non-Nixtamalized	Nixtamalized	Non-Nixtamalized	Nixtamalized
		FC (%)	FC (%)	FS (%)	FS (%)
ChI	5	62.4 ± 19.84^{Aa}	39.2 ± 4.17^{Aa}	0.02 ± 0.00^{Aa}	0.01 ± 0.002^{Ba}
ChI	7	54.0 ± 11.61^{Aa}	116.2 ± 12.11^{Ac}	0.05 ± 0.01^{Ac}	0.75 ± 0.10^{Bb}
ChI	9	100.2 ± 7.98^{Ab}	111.5 ± 15.6^{Ab}	0.34 ± 0.16^{Ab}	0.88 ± 0.05^{Bb}
SB	5	55.2 ± 21.42^{Aa}	79.0 ± 18.13^{Ca}	0.11 ± 0.04^{Ca}	0.05 ± 0.01^{Aa}
SB	7	61.2 ± 8.8^{Ab}	118.3 ± 4.01^{Ca}	1.66 ± 0.06^{Cb}	0.97 ± 0.18^{Ab}
SB	9	101.2 ± 0.00^{Ac}	163.8 ± 12.95^{Cb}	1.82 ± 0.28^{Cc}	1.23 ± 0.65^{Ab}
BU	5	74.2 ± 10.43^{Aa}	74.6 ± 10.43^{Ba}	0.05 ± 0.01^{Ba}	0.05 ± 0.01^{Ba}
BU	7	89.1 ± 4.13^{Ac}	89.1 ± 4.13^{Ba}	0.65 ± 0.09^{Bc}	0.04 ± 0.009^{Bb}
BU	9	87.9 ± 10.43^{Ab}	101.2 ± 8.07^{Bb}	0.61 ± 0.02^{Bb}	0.61 ± 0.02^{Cb}

Values are mean ± standard deviation of triplicate ($n = 3$). Means followed by different lowercase superscripts between columns, and different uppercase superscripts between rows are significantly different ($p < 0.05$).

and marshmallows, the poor stability of the foams formed limited their use in foods based on these colloidal dispersion systems.

Emulsifying capacity and emulsion stability

Emulsifying capacity (EC) refers to the property of proteins to interact with fats and water in the same colloidal dispersion system. The proteins form an interfacial monolayer between the two phases that make up the emulsion, the apolar and polar phases, thus changing their conformation by orienting their groups based on the exposed phase. Table 3 shows the differences in the emulsifying capacities and stabilities of the protein concentrates. It was observed that the emulsifying capacity showed no significant differences ($p > 0.05$) due to the type of variety for the non-nixtamalized and nixtamalized treatments, but it was affected ($p < 0.05$) by the pH of the test. The highest emulsifying activity was found at pH 5 in the concentrates of non-nixtamalized maize, and at pH 7 for nixtamalized maize.

Table 3. Emulsifying capacity (EC) and emulsifying stability (ESs) of protein concentrates from QPM (SB and ChI) and control (BU), between non-nixtamalized and nixtamalized maize flour.

Sample	pH	Non-Nixtamalized	Nixtamalized	Non-Nixtamalized	Nixtamalized
		EC (%)	EC (%)	ESs (%)	ESs (%)
ChI	5	58.33 ± 2.89^{Ab}	8.33 ± 2.89^{Aab}	33.33 ± 5.77^{Aa}	5.00 ± 0.00^{Aa}
ChI	7	50.00 ± 0.00^{Aa}	8.52 ± 2.57^{Ab}	29.26 ± 5.16^{Aa}	10.17 ± 0.30^{Ab}
ChI	9	50.00 ± 0.00^{Aa}	5.18 ± 0.32^{Aa}	43.33 ± 2.89^{Ab}	3.33 ± 2.89^{Aa}
SB	5	52.03 ± 6.09^{Ab}	13.89 ± 3.47^{abAB}	50.37 ± 8.98^{Ba}	10.37 ± 0.64^{Ba}
SB	7	51.85 ± 3.2^{Aa}	10.17 ± 0.29^{abB}	32.78 ± 2.55^{Ba}	10.17 ± 0.29^{Bb}
SB	9	50.00 ± 0.00^{Aa}	11.67 ± 2.89^{abA}	50.00 ± 0.00^{Bb}	5.00 ± 0.00^{Ba}
BU	5	60.74 ± 5.59^{Ab}	29.29 ± 2.70^{Bb}	37.4 ± 3.57^{Ba}	5.00 ± 0.00^{Ba}
BU	7	50.00 ± 0.00^{Aa}	10.00 ± 0.00^{Bab}	50.00 ± 0.00^{Ba}	17.20 ± 3.29^{Bb}
BU	9	50.00 ± 0.00^{Aa}	10.00 ± 0.00^{Ba}	45.00 ± 0.00^{Bb}	6.67 ± 2.89^{Ba}

Data are the mean of $n = 3 \pm$ standard deviation. Different lowercase letters between columns and uppercase letters between rows were significantly different ($p < 0.05$).

The pH influenced the emulsifying capacity of the concentrates of all the varieties; it was observed that the concentrates of non-nixtamalized maize had greater emulsifying capacity than those of nixtamalized maize. This behaviour was similar to that found by Mir *et al*. (2023) who reported that the emulsifying activity depends on the pH, being better at acidic pH. In this regard, Islam *et al.* (2023) mentioned that close to the isoelectric point, protein molecules are found in a more compact configuration than at other pH values, and they are absorbed onto the interface in this configuration, which could provide a higher concentration of protein molecules per unit area of the interface, and consequently, a higher number of interconnections per unit area, compared to other pH values. Therefore, it can be highlighted that the isoelectric point of the protein concentrates of QPM and BU maize are found at acidic pH. The nixtamalization process, the use of high temperatures, and the presence of salts could be responsible for the reduction in the percentage of EC in all varieties since it could cause a decrease in the interfacial tension, and the formation of a cohesive film that could affect the formation of the emulsion.

In the results of emulsifying stability (ESs), greater stability was found in the proteins of the non-nixtamalized QPM and control compared to the nixtamalized ones, with the SB non-nixtamalized variety presenting greater ESs with 50.37% at pH 5, and the lowest value was observed at pH 9 with the ChI nixtamalized variety with 3.33% of ESs. A previous study indicated that changes by physicochemical treatments of proteins could have positive effects on the capacity and stability of emulsions because the hydrophobic chains linked to the amino groups could decrease the polarity of the protein, and increase its capacity to act in hydrophobic interactions at the oil-water interface. However, an overtreatment could decrease the solubility of the protein in the aqueous phase because a higher degree of substitution could lead to a lower emulsifying capacity since an optimal

hydrophilic/hydrophobic ratio is required. The latter might have happened with the QPM SB and ChI protein concentrates that were subjected to combined treatments of nixtamalization and alkaline extraction with isoelectric precipitation in the present work. This would explain the differences found between the samples evaluated. EC and ESs values obtained in the present work were moderate to high. This makes possible the inclusion of QPM SB and ChI maize protein concentrates in food systems such as sauces, mayonnaise, and meat emulsions.

Water and oil absorption capacity

Water absorption capacities (WAC) were obtained between 1.2 and 3.13 g/g, with significant differences ($p < 0.05$) among the varieties evaluated (Table 4). Similar results have been reported for soybean meals, soy concentrates, and soy isolates, with WACs of 1.3, 2.2, and 4.4 g/g, respectively (Onder *et al.*, 2023). Nixtamalized protein concentrates showed higher water and oil absorption capacity compared to non-nixtamalized protein concentrates for the QPM varieties. The potential increases in ionic strength in the nixtamalization treatments could be attributed to the effect of the salts used since they saturate the electrostatic charges present in the protein and its environment. In addition, WAC depends on water parameters such as size, shape, steric factors, and the hydrophilic-hydrophobic balance of amino acids in molecules such as lipids and carbohydrates (Chaparro-Acuña *et al.*, 2015).

Table 4. Water absorption capacity (WAC) and oil absorption capacity (OAC) of protein concentrates of flour from QPM (SB and ChI) and control (BU), between non-nixtamalized and nixtamalized maize flour.

Variety	Non-Nixtamalized WAC g water absorbed/ g sample	Nixtamalized WAC g water absorbed/ g sample	Non-Nixtamalized OAC g oil absorbed/ g sample	Nixtamalized OAC g oil absorbed/ g sample
ChI	1.2 ± 0.06^{a}	3.13 ± 0.00^{b}	1.06 ± 0.11^{a}	5.56 ± 0.02^{c}
SB	1.56 ± 0.22^{b}	2.50 ± 0.05^{a}	2.34 ± 0.16^{b}	5.38 ± 0.01^{b}
BU	2.76 ± 0.07^{c}	2.93 ± 0.18^{b}	4.35 ± 0.15^{c}	5.22 ± 0.00^{a}

Values are mean ± standard deviation of triplicate ($n = 3$) on a dry basis. Means followed by different lowercase superscripts in the same column are significantly different ($p < 0.05$).

The oil holding capacity increased considerably in the nixtamalized protein concentrates, while the water holding capacity of the nixtamalized concentrates remained constant compared to the Uxmal maize concentrate, and increased compared to the non-nixtamalized and nixtamalized ChI and SB. As reported by Vahed *et al.* (2023), water holding capacity of proteins depends on the type and amount of polar hydrophobic groups present in the protein. However, it also depends on the pore size; the smaller the pore size, the better they are distributed in the structure of the product, and the better the water molecules are held. In the case of gels, water holding capacity is greater if the pore size is between 1 - 100 nm according to Ketnawa and Rawdkuen (2023). Therefore, nixtamalization could be modifying the pore size of the particles in the protein concentrates, which resulted in an increase in the capacity to retain moisture (Susilowati *et al.* 2023). The abilities of QPM protein concentrates could help improve the interactions in its structure, which is able to retain flavour, moisture, improve palatability, and reduce losses of water and fat in meat and bakery products, thus extending its shelf life.

Rheological properties

The linear viscoelastic zone occurred at a strain of 0.4% over a frequency range of 0.1 to 10 Hz. This strain value was used in the study of the viscoelastic behaviour of the protein concentrate dispersions by means of frequency sweeps, which are shown in Figure 1. It can be observed that all treatments had a predominantly elastic behaviour in the studied interval since the elastic modulus was higher than the viscous modulus ($G' > G''$) throughout the studied frequency interval. This behaviour indicated the formation of a three-dimensional structure between the proteins of the aqueous dispersion, allowing no rapid relaxation of the chains that make up the network, and preventing the dissipation of energy in the form of heat. On the other hand, a low-frequency dependence of the modules was observed; so, these

systems can be considered weak gels (Picout and Ross-Murphy, 2003).

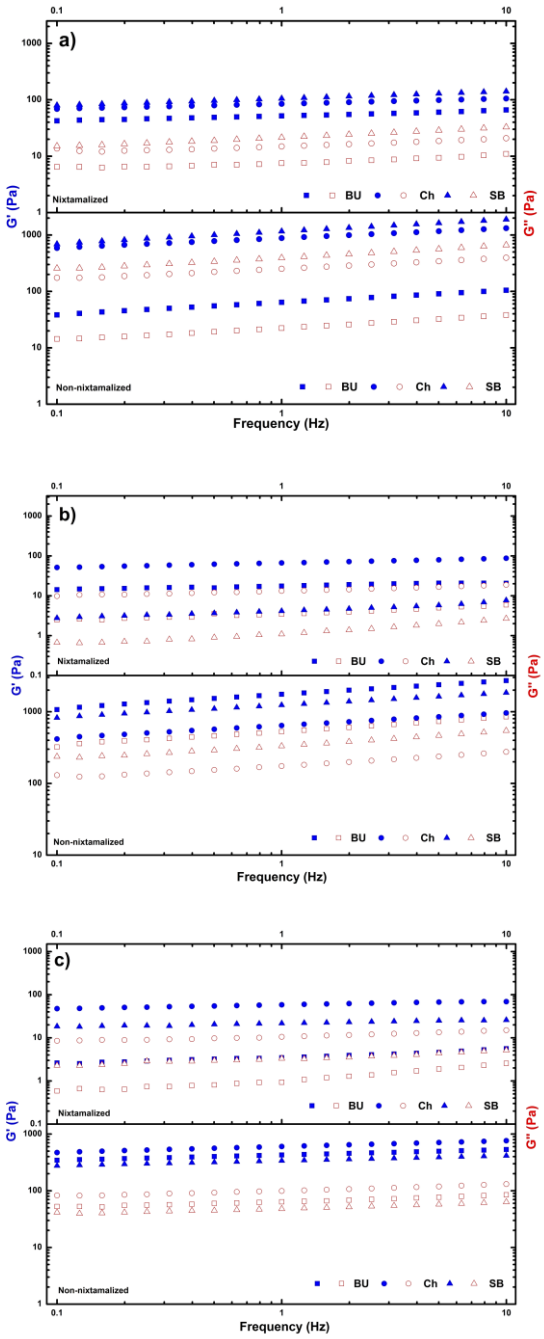

Figure 1. Frequency sweeps of protein concentrates from non-nixtamalized and nixtamalized maize of QPM (ChI, SB) and Control (BU) varieties at different pH: **(a)** pH 5, **(b)** pH 7, and **(c)** pH 9.

The nixtamalization process caused a decrease in the values of the modulus (G' and G") of the dispersions at the pH studied (Figures 1a, 1b, and 1c) without losing the weak gel behaviour. This could be explained by the fact that during the alkaline process of nixtamalization, the proteins underwent changes in hydrogen bonds, disulphide bonds, as well as the conversion of thiol-disulphide bonds (Deleu *et al*., 2019) causing the three-dimensional network to have a lower rigidity. This result was obtained in all maize varieties studied. However, the magnitude of the decrease in modulus of protein concentrates varied depending on maize variety, pH, and processing; at pH 5 (Figure 1a), non-nixtamalized ChI and SB maize had similar values in G' and G" while BU presented the lowest values. It was observed that the effect of nixtamalization was more pronounced in ChI and SB maize while it was less for BU. Figure 1c shows the value of G' and G" at pH 9, where it can be noted that the values of the modules decreased compared with the values at pH 7 and 5 (Figures 1a and 1b); as previously discussed, this decrease was also observed by the alkaline process of nixtamalization, whereby the alkaline conditions weaken the structure formed by the proteins of the system. All these results demonstrated that the QPM protein concentrates can be used in food systems like thickening agents in jellies, marmalades, and sauces, as well as in dry foods or in mixtures for soups to obtain a desired viscosity when it is reconstituted with water.

Conclusion

The techno-functional properties of the protein concentrate from QPM, and the control BU were affected by the thermal processing method to which the nixtamalized maize was subjected. Solubility was higher at basic pH, and decreased considerably in the sample of non-nixtamalized maize. The emulsifying capacity was affected by the effect of pH, and higher at basic and neutral pH. The foaming capacity and foam stability were affected by the type of processing used to obtain the maize concentrates, and higher in the non-nixtamalized Sac Beh protein concentrate. Water holding capacity increased in all the samples that were nixtamalized for the Blanco Uxmal maize and the QPM (Chichen Itza and Sac Beh). Oil holding capacity increased with nixtamalization of all QPM and control maize varieties. The dispersions of the maize protein concentrate formed a weak gel. The alkaline process of nixtamalization caused a reduction in the values of the elastic and viscous modulus, probably due to the denaturation of the protein.

The results obtained in the present work highlighted its novelty, and clearly concluded that QPM protein concentrates could be used as food additives like foaming, emulsifying, water, and oil

holding agents; also, as protein supplements to prepare many protein-enriched products such as protein-based beverages, sauces, sausages, and meat emulsions. Further instrumental analyses, including scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), pore size, and surface hydrophobicity could be performed to reveal the physical properties of proteins.

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