

THERMAL PROPERTIES OF SELECTED FRACTIONS OF PROTEINS AND LIPIDS OBTAINED FROM CORN FLOUR.**PROPIEDADES TÉRMICAS DE LA HARINA DE MAÍZ Y DE DIFERENTES FRACCIONES DE PROTEÍNAS Y LÍPIDOS**

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Abstract

Whole (COM=commercial and WL=experimental) and degermed (DEG) corn flours were subjected to protein and lipid extraction to obtain nixtamalized starch base to evaluate the effect of proteins fractions albumins and globulins (A+G) and oil reconstitution (50%, and 100% of the original values). Differential scanning calorimetry (DSC) was used to obtain the thermal characteristics of corn flours treatments. Oil extraction and reconstitution did not modify the typical endotherm of whole corn masa. Protein reconstitution of DEG flour showed three transition states in which the T_o , and T_{max} of all endothermic peaks increased ($p<0.05$) as protein content increased. The third endothermic peak of these flours showed very small enthalpies (0.67 and 0.62 J/g, $p<0.05$) as the T_{max} increased (119°C), as compared with the value for the whole flour (73.7°C, and 2.6 J/g); which resulted from protein aggregation. Higher temperature of gelatinization (T_{max}) was required when water was partitioned between starch and protein in the reconstituted DEG flour. DSC was sensitive in monitoring declination on protein and oil composition and can be used to establish moisture requirements for appropriate enrichment and processing conditions of corn masa flours.

Keywords: nixtamalized corn, DSC, thermodynamic properties, corn starch, alkaline cooking.

Resumen

Harina de maíz nixtamalizada (comercial (COM), experimental (WL)) y degerminada (DEG) fueron sujetas a extracción de lípidos y proteínas para obtener una base de almidón nixtamalizado. El objetivo fue evaluar el efecto de las fracciones proteicas albúminas+globulinas (A+G) y aceite (50% y 100% de reconstitución) en las propiedades calorimétricas de las harinas usando un calorímetro diferencial de barrido (DSC). La extracción y reconstitución del aceite no modificó las características típicas de las endotermas de la masa integral. Sin embargo, las masas DEG reconstituídas con proteína mostraron tres estados de transición; donde, la temperatura requerida para las endotermas aumentó significativa y proporcionalmente al contenido de proteína. El tercer pico endotérmico aquí observado mostró menor valor de entalpía (0.67 y 0.62 J/g, $p<0.05$) con un incremento en T_{max} (119°C) resultante de agregación proteica, comparada con los valores de la masa integral (73.7°C y 2.6J/g). Las masas DEG requirieron mayor temperatura de gelatinización (T_{max}) cuando el agua se fracciona entre almidón y proteína. El uso del DSC mostró sensibilidad para monitorear la cantidad de proteína y aceite presente, permitiendo exitosamente establecer las condiciones apropiadas de procesamiento y enriquecimiento durante la elaboración de masas de maíz nixtamalizado.

Palabras clave: maíz nixtamalizado, DSC, propiedades termodinámicas, almidón de maíz, nixtamalización.

1. Introduction

The corn is transformed in corn masa by an alkali cooking process called nixtamalization (Arámbula-Villa *et al.*, 1998). The masa is used to make corn tortillas, tortilla shells and a number of other mexican dishes. It is known that during cooking, a number of physical and chemical interactions occur among proteins, carbohydrates, lipids, and lime ($\text{Ca}(\text{OH})_2$) (Salinas *et al.*, 2003;

Gómez *et al.*, 1989). A total of 12.8% solids of corn grain – called “nejayote” – are lost in the cooking water. Gómez *et al.* (1989), reported that a considerable amount of corn lipids and proteins (12.2-17.5% w/w) are eliminated during the alkali cooking process, which modifies the quality of corn tortillas. Arámbula-Villa *et al.* (2001), studied the role of nonpolar lipids in masa prepared by extrusion. They found that an addition of 0.5% (w/w) of nonpolar lipids to the extruded whole corn flour

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improved the textural properties of masa and tortillas.

Starch retrogradations, and fast dehydration rate, are two of the main factors that contribute to the hardening or staling of corn starch (Ottenhof *et al.*, 2005). A hardened tortilla shows deficient texture properties and lack of flexibility, which is unacceptable for the consumer (Arámbula-Villa *et al.*, 2001). Some additives, such as gums and colloids, offer a partial solution for the prevention or reduction of the dehydration rate of corn tortillas (Gómez *et al.*, 1989). Addition of oil improves texture but could arise health and shelf life issues. Hypothetically, we think that water retention is the key for staling, in which quantity and degree of modification of functional protein (albumins and globulins) determine water distribution between starch and proteins of nixtamalized corn. However, the staling of tortillas is still the main problem for the consumer as well as for the tortilla industry.

Calorimetric techniques have contributed to the understanding of native and annealing starch gelatinization, and its interactions with proteins and lipids (Bogacheva *et al.*, 2002; Kar *et al.*, 2005). Differential scanning calorimetry (DSC) is particularly used to study the phase transitions of the starch-water system (Cruz-Orea *et al.* 2002; Randzio *et al.* 2003; Knutson, 1990). The graphic evolution of temperature over a mixture of the sample and water shows a peak for starch gelatinization, and the area under the curve is an estimation of the transition enthalpy. The DSC has been used to study protein denaturation, it gives information about the thermal transformation during the processing of raw materials into foods (Erdogdu *et al.*, 1995b). Temperature is the mayor factor for protein unfolding and loss of protein functionality during processing (Erdogdu *et al.*, 1995a,b; Kalichevsky and Blanshard 1992; León *et al.*, 2003; Biswas and Kayastha, 2002; Micard, 2000; Choi and Ma, 2005). In fact, nixtamalization is an alkaline cooking process, and the second strongest factor which leads to modify protein water absorption and retention.

Beyond the acceptability based in hardness or rollability of corn tortilla, nutrition is a main concern for consumers. For institutional programs, tortilla can be an excellent carrier of nutrients in México and Latin-American countries. We have extracted the neutral lipids and proteins of commercial and experimental-made corn masa to obtain the starch fraction. This starch fraction of corn masa could be the carrier of oil and proteins to fortify masa flour for specific population needs. In this study DSC analysis was used to monitor thermal characteristic of starch corn masa reconstituted with neutral lipids and proteins (A + G) to prepare wet masa. Our objective was to investigate the thermal properties of the starch fraction of corn masa as a function of oil and protein added. Results of our study would provide a basis for the understanding of the interaction of selected

fractions of protein and lipids with the starch of corn masa.

2. Materials and methods

2.1 Materials and preparation of the masa

White corn samples (Asgrow 465), commercial alkali Ca(OH)_2 (Monterrey, S.A.), and instant commercial corn masa (COM) (Maseca, Cd. Obregón) were used in all of the experiments. Whole (LW) and degermed (DEG) nixtamalized corn masa were prepared as reported by Vidal-Quintanar *et al.* (2001).

2.2 Lipids extraction and reconstitution

Total neutral lipids were extracted from whole corn masas by two sequential extractions with petroleum ether and hexane (5h, bp 35-65 °C) (AACC method 30-26 (2001)). Oil was purified evaporating the solvent using a rotoevaporator (model 461, Büchi, Ltd.) at 30 rpm and 45°C for 30 min. Spare masa samples (5-10 g) were homogenised with 10 ml of 2.47%, and 4.95% w/w oil in hexane solutions (for 50 and 100% reconstitution of original corn oil content, respectively). The excess of solvent was evaporated placing them under a hood for 24 hr. All treatments were analysed for moisture (AACC method 44-40), and total lipid content (AACC method 30-26) (2001).

2.3 Proteins fractionation and reconstitution

Protein fractions were obtained from whole and degermed corn flour according to Laundry and Moreaux (1980). Proteins were sequentially extracted from 20 g for 30 min under constant mixing with 100 ml of each of the following solvents: deionized water (albumins); 50mM Tri-HCL, pH 7.8, with 100 mM KCl and 5 mM EDTA (globulins); 50% 1-propanol (gliadins); and 50% 1-propanol with 1% SDS (for sodium dodecyl sulfate soluble glutenins). After protein extraction, the degermed corn flour was composed mainly of nixtamalized starchy endosperms. These endosperms may be used to carry proteins and lipids to enhance the flavour and nutrition of corn masa products. Proteins fractions were freeze-dried and further used for reconstitution. Samples were reconstituted at three levels; 0% (control), 50% (56.3mg/g A + 13mg/g G), and 100% (112.6mg/g A + 26g/g G), for the DSC analysis. The textural and sensory properties of the tortillas made from these masas had been reported by Vidal-Quintanar *et al.* (2001). The protein and moisture content were determined by AACC official methods (2001).

2.4 Differential scanning calorimetry

The thermal behaviour of masas was studied by DSC using a 1020 Series DSC7 thermal analysis system (Perkin-Elmer, Norwalk, CN). The evaluation of the signals was done with a computer using the PE Nelson model 1022 processor from Perkin-Elmer. The instrument was calibrated for the temperature baseline using indium as a standard, and an empty capsule was used as a reference (Erdogdu *et al.*, 1995a). The samples (7.5 ± 0.2 mg) and 10 μ l of water were placed into DSC hermetic pans (PE no. 0319-0218) and the test was run from 30 to 130°C at a rate of 10°C/min. Peak temperature (°C) and the transition enthalpies (ΔH) expressed as J/mg of sample were recorded.

Analyses for sample were carried out in triplicate. The results were reported as mean values. One-way ANOVA procedures were employed for data analysis. The means differences were established by the least significant difference procedure (LSD, $p > 0.05$) Tukey test. The data were analysed using the JMP4 statistical data analytical software (SAS, 2001).

3. Results and discussion

Extraction yields of oil (95%) and protein (43%) of these masas obtained respect to corn grain composition were similar to those reported by Arámbula-Villa *et al.* (2001). The DEG masa had $0.68 \pm 0.1\%$ oil and $2.5 \pm 0.5\%$ residual proteins. DEG masa after oil and protein extraction serve as a model of nixtamalized corn masa starch, and is suited for study of the interaction of lipids and proteins reincorporated to corn masas.

3.1 Thermal properties of the neutral oil masa flour

The extraction and reconstitution of neutral oil did not modify the thermal properties; our results agree with those reported by Knutson (1990). The starch-transition endotherm occurred at the expected temperature ($74 \pm 2^\circ\text{C}$). The mean temperature at gelatinization peak was reached at $75.1 \pm 1.4^\circ\text{C}$ (Table 1) and the enthalpy values varied from 2.0 to 3.4 J/g. The mean value was 2.7 ± 0.4 J/g for all treatments studied (COM, WL, DEG). The DEG corn masa flour behaved as a semi-purified starch rather than as a complex mix of corn masa flour because, during germs striping oil and functional proteins were took along. Therefore, the observed thermal behaviour corresponds mainly to starch as principal component of corn endosperm. Our findings are in agreement with those obtained by Kalichevsky and Blanshard (1992), where the sample's thermal history is related to modification on miscibility of their components and reflected as small variation on T_{max} and enthalpy. In addition, Toro-Vazquez *et al.* (2001) also reported that corn

starches, without the nonconstitutive lipid fractions showed more consistent thermal properties, such as gelatinization behaviour. We found that lipid extraction did not caused structural modification on masa starch, or proteins as shown by only one endothermic peak after lipid reconstitution. This can be explained considering that the masa is structured as a mixture of gelatinized and dispersed starch, hydrated and denatured protein matrix, and emulsified lipids (Gómez *et al.* 1989). In this complex mixture, lipids and proteins are only the means for starch granules to aggregate (Toro-Vazquez *et al.* 2001). Nixtamalization, grinding, and drying transform corn into instant flour, in which gelatinization and reorientation of starch polymers facilitates the formation of a unique net called masa (Cruz-Orea *et al.* 2002). Those starch changes make possible the incorporation of additional nutrients as exogenous fat or proteins. Incorporation of lipid on masa without modification on starch thermal properties suits our objective of using nixtamalized corn as carrier of nutrients to improve nutrition of specific segments of Mexican population.

3.2 Thermal properties of the corn masa plus albumins and globulins fraction

COM and LW masas showed only one peak in the DSC. The changes in transition characteristics for these two masa flours were not significantly different among the five treatments [COM, LW, and LW with 0%, 50%, and 100% A + G] (Table 2). The COM masa showed a mean peak temperature (T_{max}) of $74.7 \pm 0.01^\circ\text{C}$, whereas the LW masa showed a lower value ($73.7 \pm 0.03^\circ\text{C}$); however, both masas showed the highest temperature ($77.3 \pm 0.21^\circ\text{C}$) after reconstitution (50%, 100% A + G). A similar tendency was observed for the onset temperature (T_o) in all treatments. The LW masa showed a T_o of $67.4 \pm 0.35^\circ\text{C}$, and $67.3 \pm 0.10^\circ\text{C}$ for the 100% A + G reconstituted masa. COM masa showed higher transition enthalpy (2.8 ± 0.65 J/g) than WL flour (2.6 ± 0.56 J/g); but, the 100% A + G reconstituted masa flour showed the highest value (2.9 ± 0.08 J/g). The moisture content used was the same for all samples (10 μ L). These endothermal results are characteristic of corn starch gelatinization. Ma and Harwalkar (1988), had established a shift to lower temperature of protein denaturation endotherm working with fix moisture in wheat proteins. In recent study Choi and Ma (2005), reported that protein unfolding, reassociation, and aggregation caused progressive increases in T_{max} and pronounced decreases in enthalpies. Further studies are needed to establish if corn proteins fractions might need additional water to be detected as independent peak proteins of whole corn masa.

The first endothermic peak of protein reconstituted COM and DEG masas (Fig.1) was observed at a typical starch gelatinization

temperature (mean value of 74.18°C), and at a transition enthalpy with a mean value of 2.68 J/g. DEG corn masa showed only one endotherm. Then, when DEG masa was subjected to protein extraction two independent endotherms were formed. After protein reconstitution (50% and 100%) of masas three separated endotherms were observed (Fig. 1).

The 50%, and 100% A + G reconstituted DEG masas showed higher Tmax at each consecutive endothermal peaks. In addition, protein DEG reconstituted masas showed second peaks with higher enthalpy values (1.4 J/g) than third peaks (0.6 J/g).

Table 1. Thermal characteristics of starch for neutral lipids reconstitution of the three types of corn masa flours.

Treatment	To (°C)	Tmax (°C)	ΔH (J/g)
COM	63.7 ^a (0.35)	73.3 ^b (0.35)	2.7 ^a (0.64)
After lipid extraction	66.4 ^a (0.16)	75.7 ^{ab} (0.15)	2.2 ^a (0.05)
50% reconstituted neutral lipids	63.0 ^a (0.12)	73.8 ^a (0.02)	3.1 ^a (0.31)
100% reconstituted neutral lipids	65.7 ^a (0.14)	76.0 ^a (0.12)	3.4 ^a (0.30)
LSD	7.26	2.47	2.45
WL	67.0 ^a (0.11)	75.4 ^a (0.08)	2.0 ^a (0.71)
After lipid extraction	66.2 ^a (0.27)	75.3 ^a (0.99)	2.3 ^a (0.18)
50 % reconstituted neutral lipids	66.4 ^a (0.04)	76.9 ^a (0.6)	2.8 ^a (0.47)
100% reconstituted neutral lipids	66.7 ^a (0.15)	78.0 ^a (1.01)	3.2 ^a (0.28)
LSD	2.72	5.54	1.86
DEG	65.9 ^b (0.44)	73.7 ^a (0.52)	2.6 ^a (0.01)
After lipid extraction	65.6 ^b (0.54)	73.6 ^a (0.02)	2.4 ^a (0.17)
50 % reconstituted neutral lipids	66.1 ^{ab} (0.35)	74.6 ^a (1.17)	2.9 ^a (0.34)
100% reconstituted neutral lipids	66.6 ^a (0.58)	75.1 ^a (0.82)	2.8 ^a (0.21)
LSD	0.68	3.62	1.02

¹Standard deviation is in parenthesis (±).

^{a,b} significantly different ($p < 0.05$) in the same column.

LSD is the least significantly difference ($p < 0.5$).

COM is whole commercial, WL is whole experimental, and DEG is degermed.

Table 2. Thermal characteristics of COM and WL corn masa flour.
On set (To) and peak (Tmax) temperatures, gelatinization enthalpies (ΔH).

Treatments	Peak I		
	To (°C) ¹	Tmax (°C)	ΔH (J/g)
COM	66.7 ^a (0.25)	74.7 ^a (0.01)	2.8 ^a (0.65)
WL	67.4 ^a (0.35)	73.7 ^a (0.03)	2.6 ^a (0.56)
WL after protein extraction	65.9 ^a (0.16)	74.0 ^a (0.15)	2.1 ^a (0.15)
Reconstitution fractions A + G			
50%	66.8 ^a (0.56)	77.3 ^a (0.21)	2.4 ^a (0.31)
100%	67.3 ^a (0.10)	77.3 ^a (0.02)	2.9 ^a (0.08)
LSD	5.71	5.24	0.85

¹Standard deviation is in parenthesis (±).

^a Different letter for the same column indicate significant differences ($p < 0.05$).

LSD is the least significantly difference ($p < 0.5$).

COM is whole commercial and WL is whole experimental.

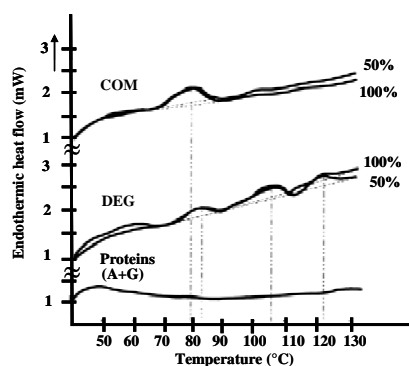


Fig. 1. DSC-thermograms of degermed and commercial corn masa flour with 50% and 100% albumins (A) and globulins (B) reconstitution.

Table 3 shows thermal characteristics for all DEG treatments; DEG masa showed one peak, samples after protein extraction had two peaks, and three peaks were observed for protein reconstitution samples. The first endothermic peak of DEG masa after protein extraction was a typical starch gelatinization temperature (76.42 ± 0.28 °C) with a transition enthalpy of 0.75 ± 0.02 J/g. The second peak, corresponding to starch-lipids, has been reported with a higher Tmax and lower enthalpy. These peaks showed Tmax of 101.2 ± 0.38 °C and a transition enthalpy of 1.25 ± 0.31 J/g, both parameters were higher values but not significantly different than those observed for the first or gelatinization peak (Table 3). These results agreed with those of Biliaderis *et al.* (1980) who had reported that the fusion of the amilose-lipid complex occurred from 96°C to 104°C.

The third peak of the endotherm was observed only for 50% and 100% A + G reconstitution at a significantly high temperature (119.5°C) with the lowest and significantly different transition enthalpy (≈ 0.65 J/g) (Table 3). The high temperature required

for the third endothermic peak corresponds to a typical interaction of protein with the starch-lipid complex, as reported by Erdogdu *et al.* (1995a,b). This indicates that the A + G were denaturated during nixtamalization, dialysis and ultrafiltration steps. In addition of having a more open structure, it may present some low molecular weight components of any of the protein fractions, which played an important role in the interaction process. Therefore, we understand the existence of higher Tmax and lower enthalpy for the third peak might be explained as a response of the interaction among A + G fractions with the residual natured or annealing starch. This agrees with the presence of non-starch components that facilitate starch aggregation into a continuous net as showed on microphotographs by Toro-Vazquez (2001). The protein fractions added, and the natural starch-lipid complex of these masas, self-associate during the hydration to form a more complex polymer; in addition, protein acted as a starch water competitor, which is reflected in the endothermic temperature observed. In addition, Ma and Harwalker (1988) reported that the globulin fraction of cereals denatures at temperatures above 97°C and up to 108°C. The decrease in enthalpy showed a re-distribution of water in favor of the starch fraction. These results showed lower self-association of these polymers, which resulted in two Tmax peaks corresponding to the two components. The above resulted in a positive transition enthalpy, similar to those reported by Kalichevsky and Blanshard (1992). A perfect polymer of two components showed a high degree of self-association with a single Tmax between the Tmax of the two components. In contrast, a widening separation of Tmax could be observed, as it occurred in these samples as their components became more immiscible.

Table 3. Thermal characteristics presented for degermed corn masa flour (DEG). Peak temperatures (Tmax), transition enthalpies (ΔH).

Treatments	Peak I		Peak II		Peak III	
	Tmax (°C)	ΔH (J/g)	Tmax (°C)	ΔH (J/g)	Tmax (°C)	ΔH (J/g)
DEG	73.66 ^c (0.52)	2.57 ^a (0.01)	-----No peak-----			
DEG after protein extraction	76.42 ^{bc} (0.28)	0.75 ^b (0.02)	101.22 ^b (0.38)	1.25 ^a (0.31)	No peak	
Reconstitution fractions A + G						
50 %	80.23 ^{ab} (0.02)	0.85 ^b (0.01)	103.65 ^a (0.89)	1.49 ^a (0.07)	119.54 ^a (0.091)	0.67 ^a (0.17)
100%	82.04 ^a (0.03)	0.90 ^b (0.17)	104.27 ^a (0.07)	1.47 ^a (0.02)	119.88 ^a (0.05)	0.62 ^a (0.02)
LSD	4.02	0.16	1.91	0.64	3.89	0.61

¹Standard deviation is in parenthesis (\pm).

^{a,b} Different letter for the same column indicate significant differences ($p < 0.05$).

LSD is the least significantly difference ($p < 0.5$). DEG is degermed masa.

These results should be considered for the enrichment of corn masa, in which an uneven hydration and dehydration process will yield quick development and an unacceptable staling rate of tortillas. A mechanically-automated corn masa process should prevent the stripping of lipid and protein during nixtamalization, to reach optimal masa properties.

Conclusions

Corn masa behaved as a good base for carrying lipid and protein fortification of corn products. The lipid extraction and its reconstitution did not modify the thermal property values of whole corn masa flour. Miscibility and hydration of corn masa with protein fractions depended on sample history, which modified protein interaction with the starch-lipid complex. This behaviour can be the explanation for the higher temperature required for the second and third endothermic peaks of degermed and protein reconstituted corn masa flour. Since, protein and neutral oil extraction, and reconstitution of corn nixtamalized masa did not modify thermal starch properties; corn masa is a potential carrier for protein and oil components. This study enables researchers and industry to monitor changes in thermal behaviour during food processing of corn masa enriched with functional proteins and could prevent deleterious changes on final products.

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