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Changes in the Functional Properties of Three Starches by Interaction with Lima Bean Proteins

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Summary

The functional properties of starches determine their potential applications in food systems. These properties depend largely on granular and molecular structure and can be physically, chemically or enzymatically modified. One way of modifying starch functional properties is by interaction with other food components, such as proteins. Starch-protein interactions are frequent in plant foods, particularly cereals and legumes, which are formed mainly of starches and proteins. An evaluation has been done of changes in the functional properties of three native starches (corn, *Zea mays* L.; cassava, *Manihot esculenta*; and lima bean, *Phaseolus lunatus* L.) when blended with lima bean protein concentrate. The gelatinization temperature of each blend increased compared to its corresponding native starch. The cassava starch/lima bean protein blend had the highest overall swelling power and water absorption capacity values at all temperatures. Maximum viscosity for each blend was higher than for the corresponding native starches. The blends of lima bean protein with cassava and corn starches did not exhibit syneresis. The lima bean starch/lima bean protein blend had the highest gel firmness values, followed by the blends with corn and cassava starches. The protein-starch mixtures are an alternative in the improvement of the starch functional properties which are useful in the development of nutritional products.

Key words: cassava starch, corn starch, lima bean, starch-protein blends, functional properties

Introduction

Native starches generally have limited functional properties, which complicates their incorporation into food systems. These properties can be improved by physical, chemical or enzymatic modification of starch granule integrity, and consequently of starch physical or chemical properties (*1*). Polysaccharide-containing protein mixtures can stabilize these functional properties. The polysaccharide-protein interaction influences the structure and stability of many processed foods, so the control and manipulation of these macromolecular interactions is a key factor in developing new foods and processed products. Among the protein-polysaccharide interactions, the protein-starch interaction deserves special attention since it is a functional component in many nutritional systems. Protein-starch mixtures constitute a polymeric system lacking covalent interaction. However, this system can be important because if one or both polymers have gelling capacity, it has the potential to form nutritious products with different textural properties (*2*). Starches have ionizable molecules and, therefore, the bonds in starch-protein interactions can be made by hydrogen bonds, ionic bonds and in some cases hydrophobic bonds (*3*). The presence of both amylose and amylopectin in the starch means that the ramified polymer chains form a larger matrix with proteins than do linear polymers. At temperatures high enough for starch gela-

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tinization (and higher), the probability of interaction with proteins is much greater in the presence of amylopectin than of amylose because amylopectin's long ramifications increase potential interaction with the hydrophilic portions of the protein molecule (*4*). Using differential scanning calorimetry, Eliasson and Tjerneld (*5*) demonstrated that in a system with starch and limited water, gluten competes with starch for the water available for gelatinization, a process which is influenced by protein quantity and composition (*6*). Eliasson and Tjerneld's (*5*) results support the hypothesis that in a protein/wheat-starch interaction the protein is adsorbed onto the granule surface. This was valid only at low protein concentrations, because the results were very disperse at high concentrations, probably due to protein precipitation. In addition, the high molecular mass protein fraction was absorbed to a greater degree (approx. 10 mg protein per m^2 starch) than the low molecular mass protein fraction. The highest association occurred at neutral pH, meaning that the interactions were probably hydrophobic. Friedman (*7*), in contrast, reported that amylose aggregates with gliadins, while amylopectin does not.

Guerrieri *et al.* (*6*) used amyloglucosidase action as a parameter for measurement of starch-protein interactions. After thermal treatment, they concluded that the amylose systems were less accessible to enzyme action, probably because some amyloglucosidase reaction locations were occupied by the protein interaction (particularly gliadins), while amylopectin hydrolysis was unaffected. In a study of starches with different amylose and amylopectin contents, Chedid and Kokini (*4*) observed that at higher amylose levels, amylose-amylose interactions were favoured over protein-amylose interactions due to the helical shape of the latter. In another study, it was observed that interaction can occur between both protein and starch molecules in gels of whey protein (concentrate or isolate; protein concentration >60 %) and wheat starch. Depending on the concentration of whey protein isolate, the molecules were found to form an emulsion (single phase), to lack interaction (double phase) or sometimes both phases can be present. Protein denaturalization increased at higher viscosities (*8*). These controversial results do not clearly demonstrate with which starch components the protein is interacting. This interaction depends on protein composition as well as conformation, and what kind of interaction occurs will in turn determine the intensity with which the functional properties of both components are affected.

No research has been done to date evaluating the interaction of native starches with legume proteins. Legumes grow worldwide and are of particular economic and dietary importance in Latin America. On the Yucatan Peninsula, Mexico, a wide variety of legumes are in use, including lima bean (*Phaseolus lunatus*) (*9*). One way of exploiting legumes is to process them into a starch fraction, a protein concentrate and a fibre fraction, which decreases and/or inactivates their antinutritional factors (*10*). Betancur-Ancona *et al.* (*11*) support the finding that wet fractionation of *Phaseolus lunatus* seeds allows to obtain a starch fraction containing 98 % starch and a concentrated protein at 71 %, which increases the integral use of those seeds. Their possible uses depend on their functional properties, which can affect sensory characteristics and the physical behaviour of foods or ingredients during processing and storage (*12*). With the purpose of improving the technological application of three starches, in the present study starch-protein mixtures are made. Therefore, the objective of this study is to determine the functional properties of corn (*Zea mays* L.), cassava (*Manihot esculenta*) and lima bean (*Phaseolus lunatus* L.) starches when they are blended with lima bean protein concentrate.

Materials and Methods

Seeds, roots and chemicals

Lima bean seeds and fresh cassava roots were obtained from the 2007 harvest in the state of Yucatan, Mexico, and milled to produce flour from which the native starch and protein concentrate were extracted. Commercial corn starch was purchased from Productos de Maíz, S.A. (Guadalajara, Jalisco, Mexico). All chemicals were of reagent grade and purchased from J.T. Baker (Phillipsburg, NJ, USA).

Extraction of starch and protein concentrates from lima beans

A single extraction was done with 6 kg of lima beans. Impurities and damaged seeds were removed and healthy seeds were milled in a Thomas Wiley laboratory mill (Model 4, Thomas Scientific, Swedesboro, NJ, USA). The resulting flour was sifted through a 20-mesh screen and then processed using the wet fractionation method reported by Betancur-Ancona *et al.* (*11*). Briefly, whole flour (20-mesh) was suspended in distilled water at a 1:6 ratio (by mass per volume) to obtain an adequate separation of the flour components (protein and starch). The pH was then adjusted to 11 with 1 mol/L NaOH, and the suspension was stirred for 1 h at 400 rpm with a mechanical agitator (Caframo Rz-1, Heidolph Schwabach, Germany). It was then wet-milled with a KitchenAid mill (St. Joseph, MI, USA) and the fibre solids were separated from the starch and protein mix by straining through 80- and 150-mesh sieves. The residue was washed five times with distilled water. The starch suspension was allowed to sediment for 30 min at room temperature to recover the starch fraction, after which the solubilised protein was removed by decanting. The protein in the solution was adjusted to pH=4.5 with 1 mmol/L HCl, centrifuged at 1317×*g* for 12 min and the precipitate was dried at –47 °C and 13 bar. The starch fraction was washed three times by resuspension in distilled water (250 mL) and centrifuged at 4250×*g* for 10 min. The product was dried at 50 °C for 12 h in an air convection laboratory oven (Imperial V, Labline, Maharashtra, India), weighed and then milled in a Cyclotec mill (Tecator, Höganäs Sweden) until it passed through a 20-mesh screen.

Isolation of starch from cassava

Starch extraction from cassava was done following Novelo-Cen and Betancur-Ancona (*10*). Briefly, the cassava roots were manually peeled, cut into cubes (approx. 3 cm^3) and soaked for 30 min in a sodium bisulphite so-

lution (concentration of SO_2 1500 ppm) at a ratio of 1:3 (by mass per volume). After soaking, the cubes were milled in a cutter (Fatosa C-3527, Barcelona, Spain) for 2 min to reduce particle size and the resulting mass was distributed in recipients containing a sodium bisulphite solution (concentration of SO_2 1500 ppm) at a ratio of 1:1 (by volume). The solution was then run twice through a colloidal mill (Koromex G-91T085-18, Monterrey, Mexico) to further reduce particle size and extract the largest possible amount of starch. The starch slurry was then filtered through 80-mesh plastic cloth to eliminate fibre, and the filtrate was allowed to settle at 4 °C for 4 h. After settling, the liquid was removed; the sediment (starch) was washed three times with water and centrifuged for 12 min at 1317×*g*. The product was dried at 50 °C for 12 h in an air convection laboratory oven (Imperial V, Labline, Maharashtra, India), weighed and then milled in a Cyclotec mill (Tecator, Höganäs, Sweden) until it passed through a 20-mesh screen.

Preparation of starch/protein blends

The three starches (lima bean, cassava and corn) were blended individually with lima bean protein concentrate (LPC) at a ratio of 1:5 (by mass) on a dry basis for 5 min in a KitchenAid mixer (St. Joseph, MI, USA). The ratio of 1:5 (by mass) was employed because this is the estimated ratio of these components in cereals such as corn. Samples were taken from these blends for the analyses.

Proximate composition of starch/LPC blends

Proximate composition of starch/LPC blends was determined using AOAC methods for moisture content (Sec. 925.09); ash (Sec. 923.03); crude fat (Sec. 920.39); crude protein, using a 6.25 nitrogen protein conversion factor (Sec. 954.01); and crude fibre (Sec. 962.09). Carbohydrate content was estimated as nitrogen-free extract (NFE) (*13*).

Differential scanning calorimetry

Starch/LPC blend gelatinization was determined with a differential scanning calorimeter (DSC-7, PerkinElmer Corp., Norwalk, CT, USA) using the technique described by Ruales and Nair (*14*). The DSC-7 was calibrated with indium and the data were analyzed using the Pyris software program (PerkinElmer Corp., Norwalk, CT, USA). A mass of 2 mg of dry sample was placed in an aluminium pan and moisture content was adjusted to 70 % by adding deionized water. The pan was hermetically sealed and left to equilibrate for 1 h at room temperature. It was then placed in the calorimeter and heated from 30 to 120 °C at a rate of 10 °C/min, with an empty pan as reference. Gelatinization temperature was determined by automatically computing the onset temperature (T_o), peak temperature (T_p), final temperature (T_f), and gelatinization enthalpy (ΔH) from the resulting thermogram.

Solubility, swelling power and water absorption capacity

Solubility, water absorption capacity (WAC) and swelling power (SP) were determined at 60, 70, 80 and 90 °C using a modified version of Sathe *et al.* (*15*). Briefly, 40 mL of a 1 % sample suspension (by mass per volume) were prepared in a previously tared, 50-mL centrifuge tube. A magnetic agitator was placed in the tube, and the suspension was kept at a constant temperature (60, 70, 80 or 90 °C) in a water bath for 30 min. The suspension was then centrifuged at 2120×*g* for 15 min, the supernatant was decanted and the swollen granules were weighed. From the supernatant, 10 mL were dried in an air convection laboratory oven (Imperial V, Labline, Maharashtra, India) at 120 °C for 4 h to constant mass. Percentage solubility and swelling power were calculated using Eqs. 1 and 2 respectively:

$$\text{Solubility}=(\text{dry mass at 120 °C}\times 400/\text{sample mass})/\% \quad /1/$$

$$\text{Swelling power}=\text{mass of swollen granules}\times \times 100/\text{sample mass}\times(100-\text{solubility}) \quad /2/$$

Water absorption capacity was measured using the same conditions as above, but expressed as gel mass per sample, divided by sample mass:

$$\text{Water absorption capacity}=\text{mass of swollen granules}/\text{sample mass} \quad /3/$$

Pasting properties of starch/LPC blends

A viscoamylograph (Brabender PT-100, Duisburg, Germany) was used to evaluate pasting properties of the blends according to Wiesenborn *et al.* (*16*). Briefly, 500 mL of 8 % (on dry basis) starch suspension were heated to 95 °C at a rate of 1.5 °C/min, held at this temperature for 15 min, cooled to 50 °C at the same rate and held at this second temperature for another 15 min. Maximum viscosity, consistency, breakdown and setback were calculated in Brabender units (BU) from the resulting amylograms.

Syneresis

A paste was prepared as above, 50-mL portions were placed in centrifuge tubes, allowed to cool to room temperature and stored at 4 °C for 24 h. The tubes were then centrifuged at 8000×*g* for 10 min (J2-HS centrifuge, Beckman Instruments, Inc., CA, USA), and the amount of water separated from the starch was measured (*17*).

Gel firmness

Gel firmness was evaluated according to a modified version of Hoover and Senanayake (*18*) using an Instron Universal Testing Machine (Instron, Norwood, MA, USA). Briefly, 400 mL of 8 % (on dry basis) sample suspension were heated to 95 °C at a rate of 1.5 °C/min in the viscoamylograph, held at this temperature for 10 min and 40-mL portions of the paste were transferred into 50-mL beakers. These were allowed to cool to room temperature, covered with parafilm and stored at 4 °C for 24 h. The gels (3 cm in diameter) were then removed from the beaker, cut at a height of 3 cm and gel penetration was measured. Each gel was placed perpendicularly in the equipment and compressed at a rate of 1 mm/s using a 5-mm probe and a 5-kg cell.

Statistical analysis

A variance analysis was applied using the starch/LPC blends as a factor and levels. Chemical analyses

and functional property determinations were done in triplicate and treated as the response variables. The differences between the starch/LPC blend means were compared using a 5 % significance level. All statistical analyses were done with the Statgraphics plus v. 5.1 software and according to Montgomery's methods (*19*).

Results and Discussion

Proximate composition of starch/LPC blends

Protein contents of the starch/LPC blends (*Z. mays*, M/LPC; *M. esculenta*, C/LPC; and *P. lunatus*, L/LPC) were not significantly different ($p>0.05$) and exhibited increased protein content compared to their respective native starches due to the addition of the LPC (LPC protein content=71.13 %) (*10*) (Table 1). Ash content in the blends was higher than in the native starches, with the highest level in the M/LPC, which was in turn lower than the LPC alone (2.8 %) (*10*). The C/LPC had the highest fibre content, suggesting that the fibre residue was contaminated during the separation stage of the extraction process.

Differential scanning calorimetry

All blends exhibited a single transition, probably due to the sum of the thermal events of each blend component (*i.e.* starch gelatinization and protein denaturation), since gelatinization temperature and enthalpy were higher in the blends than in the separate components. The C/LPC had the lowest gelatinization temperature (72.3 °C), followed by the M/LPC (75 °C) and the L/LPC (81.4 °C). This behaviour was similar to that observed for the native starch gelatinization temperatures (Table 2). The temperature of legume starch granule gelatinization is higher than that of maize or cassava starches, probably because its supramolecular packing complex produces a crystal structure that requires higher temperatures for fusion (*1*). In addition, lima bean starch has higher amylose content compared to the corn and cassava starches, and this generally translates into higher gelatinization temperature (*10*).

Solubility, swelling power and water absorption capacity

The solubility, swelling power (SP) and water absorption capacity (WAC) of all the blends increased at higher temperatures. The C/LPC had the highest WAC (3.9, 10.8, 15.1 and 24.2 g water/g sample at 60, 70, 80 and 90 °C, respectively) (Fig. 1) and SP (4.1, 11.6, 17.1 and 35.7 g sample/g water at 60, 70, 80 and 90 °C, respectively) values (Fig. 2). Its SP values may be due to the high amylopectin content in the cassava starch because the amylose acts as a dilutor and swelling inhibitor, while long ramifications of amylopectin increase potential interaction with the water (*20*). Below their gelatinization temperatures, the blends had lower SP and WAC values, while at or above their gelatinization temperatures these values increased. Solubility of the C/LPC exhibited a pattern similar to M/LPC and L/LPC at temperatures below 80 °C, but it had the highest solubility at 90 °C (Fig. 3). During gelatinization, amylose spreads towards water, but if heating continues, the granules fracture and partial solubilization occurs. The cassava native starch showed extreme granule swelling as temperature increased, until its granules fractured and were solubilized. However, the C/LPC had lower solubility than the cassava native starch, probably due to the interaction between the protein and starch, particularly amylopectin, which interact with their long ramifications containing hydrophilic portions of the protein molecule, reducing the interaction with the available water (*4*).

Table 1. Proximate composition of starch/LPC blends and native starches (on dry basis)

Component	M/LPC	L/LPC	C/LPC	LPC	Corn starch	Lima bean starch	Cassava starch
Moisture	8.55[a]	10.35[b]	9.8[b]	7.87	9.90	11.93	12.72
Protein	11.75[a]	11.82[a]	11.71[a]	71.13	0.35	0.20	0.05
Fat	0.93[a]	0.77[b]	0.48[c]	0.68	0.35	0.12	0.16
Crude fibre	0.46[a]	0.75[b]	0.94[c]	0.20	0.62	1.25	1.74
Ash	1.20[a]	0.92[b]	0.99[b]	2.80	0.06	0.04	0.34
Nitrogen-free extract	85.66[a]	85.74[a]	85.89[a]	25.12	98.93	98.49	97.71

[a–c]different letters in the same row indicate statistically significant difference ($p<0.05$)

Table 2. Gelatinization temperature of starch/LPC blends and native starches

Sample	Onset temperature/°C	Peak temperature/°C	Final temperature/°C	Enthalpy/(J/g)
M/LPC	68.1[a]	75.0[a]	81.7[a]	11.6[a]
L/LPC	73.5[b]	81.4[b]	91.4[b]	15.0[b]
C/LPC	65.1[a]	72.3[c]	82.6[a]	14.7[b]
Corn starch	62.3	66.3	72.9	10.3
Lima bean starch	75.2	80.2	87.6	10.7
Cassava starch	57.6	65.2	75.3	9.6

[a–c]different letters in the same column indicate statistically significant difference ($p<0.05$)

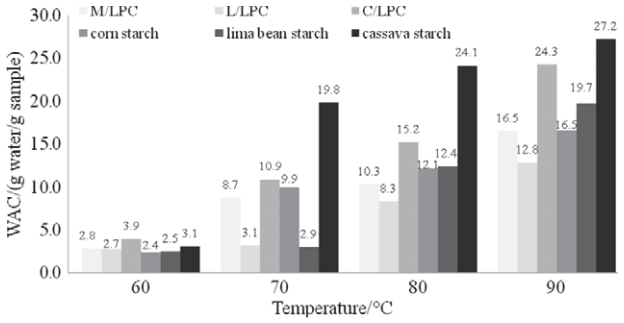

Fig. 1. Water absorption capacity (WAC) of LPC/starch blends and native starches

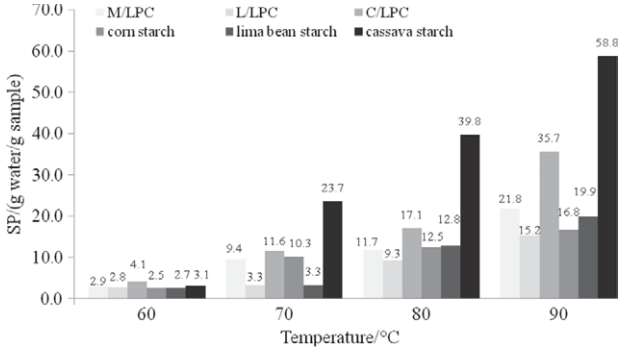

Fig. 2. Swelling power (SP) of starch/LPC blends and native starches

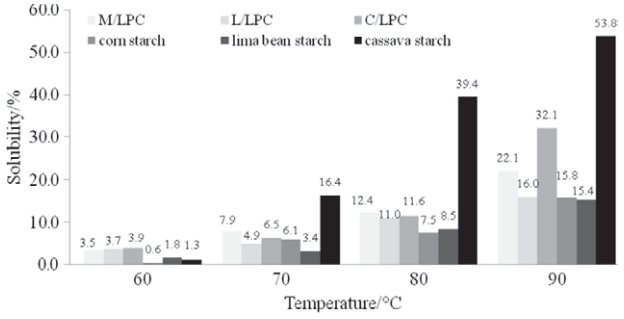

Fig. 3. Solubility of starch/LPC blends and native starches

Pasting properties of starch/LPC blends

Maximum consistency for the L/LPC (910 BU), M/LPC (416 BU) and C/LPC (960 BU) was higher than the reported values for their respective native starches (680, 252 (*1*) and 468.8 BU (*10*), respectively) (Table 3). A number of factors can significantly affect starch viscosity, including the presence of proteins, the amylose/amylopectin ratio, protein type, temperature, contact time and moisture content (*4*). Most likely because of its ramified structure, amylopectin increases viscosity at higher gelatinization temperatures, whereas gelatinization in starches containing 100 % amylose does not involve the same type of synergistic interactions (*4*). This explains why the C/LPC, which had the highest amylopectin content among the blends, also had the highest viscosity.

Syneresis

The corn and cassava starches did not present syneresis to the studied concentrations, which explains the absence of this parameter in the M/LPC and C/LPC blends. The L/LPC, in contrast, exhibited syneresis of 15 g water/50 mL sample, higher than reported for lima bean native starch (8.8 g/50 mL). High degree of syneresis of legume starches is explained based on their high amylose content. The linear structure of amylose tends to increase in size during gelatinization and to precipitate quickly at initial gelatinization, thus favouring development of rigidity when gels are cooled (*21,22*).

Gel firmness

Firmness was the highest ($p<0.05$) in the L/LPC gels (0.2182 N) (Table 4), which coincides with the highest firmness of the lima bean starch and the lowest swelling power. This was probably a function of low swelling power of this blend since blends with low swelling power (*i.e.* L/LPC) produced firmer gels than those with high swelling power (*i.e.* C/LPC) (*23*).

Conclusions

The cassava starch/LPC blend had the lowest gelatinization temperature (72.3 °C), with higher values for the corn starch/LPC (75.0 °C) and lima bean starch/LPC blends (81.4 °C). The cassava starch/LPC blend also had

Table 3. Pasting properties of starch/LPC blends and native starches

Parameters	M/LPC	L/LPC	C/LPC	Corn starch	Lima bean starch	Cassava starch
Pasting temperature/°C	76	81	68	80	87	69
Maximum viscosity/BU	416	910	960	252	680	469
Viscosity at 95 °C/BU	390	830	910	244	680	281
Viscosity at 95 °C for 15 min/BU	318	638	580	230	650	162
Viscosity at 50 °C/BU	470	798	710	534	800	231
Viscosity at 50 °C for 15 min/BU	542	960	760	520	840	237
Consistency/BU	54	–112	–250	304	150	75
Fragility/BU	98	272	380	22	30	306
Settling/BU	152	160	130	282	120	–231

Table 4. Gel firmness of starch/LPC blends and native starches

Sample	Max load/N	Deformation/mm
M/LPC	0.338[a]	4.377[a]
L/LPC	2.182[b]	4.522[a]
C/LPC	0.112[c]	14.885[b]
Corn starch	0.18	3.32
Lima bean starch	8.5	6.9
Cassava starch	0.1	10.86

[a–c]different letters in the same column indicate statistically significant difference ($p<0.05$)

the best functional properties, with the highest water absorption (24.2 g water/g sample), swelling power (35.71 g water/g sample) and solubility (32.1 %) at 90 °C, as well as a lack of syneresis and the highest viscosity (960 BU). The lima bean starch/LPC gel was the firmest (0.2182 N) and the cassava starch/LPC gel was the least firm (0.0112 N). Gelatinization temperature and maximum viscosity were higher in the starch/LPC blends than in their respective native starches. Solubility in the corn starch/LPC and lima bean starch/LPC blends was higher than in their respective native starches, whereas the cassava starch/LPC blend had lower solubility than its native starch. The elaboration of protein-starch mixtures is an alternative in the improvement of the starch functional properties, which are useful in the development of nutritional products to confer physical and chemical properties such as solubility, syneresis, viscosity, *etc*.

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