Functional properties of a protein product from *Caryodendron orinocense* (Barinas nut)

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SUMMARY. The functional properties of *Caryodendron orinocense* protein product were investigated and compared with those of soybean (*Glycina maxima).* The product protein content was 24.47 g/100g (Nx6.25). Solubility increased at both sides of the isoelectric point (pH 4.0) and with increased NaCl concentration up to 0.5M. Compared with soybean flour (50% protein), the protein product exhibited higher water and oil absorption, but lower emulsifying activity, emulsion stability, foaming capacity, and foam stability, the last one increase at higher pH. Emulsifying activity, foaming capacity, and foam stability were ionic strength dependent. *C. orinocense* protein product increased its emulsifying activity steadily from 0.05M to 0.75M NaCl, while it remained almost constant for soybean flour. Foaming capacity increased drastically at ph 10. The minimum time and concentration to form a gel was 20% in 4 min and 10% in 8 min for the *Caryodendron* protein product and soybean flour, respectively. The bulk density was 0,5056±0,0041 g/mL.

Key words: Protein product, *Caryodendron orinocense*, functional properties.

RESUMEN. Propiedades funcionales de un producto proteico obtenido del *Caryodendron orinocense* (nuez de Barinas). Las propiedades funcionales de un producto proteico del *Caryodendron orinocense* se evaluaron y se compararon con las de soya (*Glycina maxima).* El contenido proteico del producto evaluado fue de 24.47 g/100g (Nx6.25). La solubilidad se incrementó a ambos lados del punto isoeléctrico (pH 4.0), aumentando además en presencia de NaCl, en concentraciones por encima de 0.25M. Comparado con la harina de soya (50% proteína), el producto proteico presentó una capacidad de absorción de agua y aceite mayor, pero menor actividad emulsificante, estabilidad de la emulsión, capacidad espumante y estabilidad de la espuma. La actividad emulsificante, capacidad de formación de espuma y estabilidad de la espuma, fueron dependientes de la fuerza iónica. El producto proteico del *C. orinocense* aumenta en forma continua su actividad emulsificante con concentraciones de NaCl, manteniéndose constante de 0.05M a 0.75M, mientras que permanece constante para la soya. La formación de espuma aumentó drásticamente a pH 10. El tiempo y la concentración mínima para la formación del gel fue de 20% en 4 min y 10% en 8 min para el producto proteico del *Caryodendron* y la harina de soya, respectivamente. El producto proteico presentó un densidad de 0,5056±0,0041 g/mL.

Palabras clave: Producto proteico, *Caryodendron orinocense*, propiedades funcionales.

INTRODUCTION

Proteins are important in food processing and food product development not only because of their nutritional properties but also because they are responsible for many functional characteristics that influence consumer acceptance of food. On the basis of their functionality, the proteins find use in diverse systems such as fabricated and texturized food products. Although *Caryodendron orinocense* Karst. is a tree, which grows wild at the base of The Andes in countries like Peru, Ecuador, Colombia and Venezuela, its seeds or nuts represents a good source of oil and proteins (1,2), and a potential raw material useful for the food industry. For efficient utilization and consumer acceptance of a protein product, studies on the functional properties are important. The functional properties of the flour were previously studied. However, a new protein product with a higher protein content was prepared (3) and the evaluation of its functional properties is the objective of this work.

MATERIALS AND METHODS

Preparation of protein product

Was prepared by extracting ground *Caryodendron* flour with 95° ethanol at a flour to solvent ratio of 1:30 for 15 min with constant stirring at 35°C. The slurry was centrifuged at 3000 rpm, for 30 min. The supernatant was discarded and the residue extracted twice more, then vacuum dried and stored in vacuumed plastic bags at 4°C (3). Soya (*Glycine max*) flour was obtained from Nestlé of Venezuela.

Proximate analysis

Proximate analysis of the protein product was determined according to the standard methods of the AOAC (4), with the exception of fiber, which was assessed in a Fibertec model M, (Tecator, Hoganas, Sweden). Carbohydrates were calculated by difference. Results were expressed on a dry matter basis, and all analyses were run in triplicate.

Protein solubility

Protein solubility of a 1% (w/v) water suspension was determined over a pH range from 2 to 12, and over a NaCl concentration from 0.0 to 0.75M at pH 6.4, according to the method previously described, Padilla, et al., (1). The percentage of soluble nitrogen was calculated.

Bulk density

The method described by Okaka & Potter (5) was used with 25g of the prepared product.

Water and oil holding capacity

To 2 g of sample, 20 mL of water, or 12 mL of corn oil (Mazeite, Remavenca, Turmero, Venezuela), was added, stirred in a vortex and held at room temperature (~25^0 C) for 1 h. The procedure of Okaka & Potter (5) was followed.

Gel formation

Minimum gelling concentration and time needed to form a gel at this concentration was performed following the method described previously (1).

Emulsifying activity (EA) and emulsion stability (ES)

Emulsifying activity and emulsion stability were determined by the turbidimetric method of Pierce & Kinsella (6) with slight modification described by Padilla *et al.*, (1).

Foam capacity (FC) and stability (FS)

These properties were assessed by the Srinivas & Rao Narasinga (7) method, using a 3% (w/v) suspension. Both FC and FS were determined as a function of pH and NaCl concentration as described by Padilla et al., (1).

Statistical analysis

All data used were means of triplicate (n=3) determinations, and analysed by (Kruskal Wallis) no parametric ANOVA to determine statistical differences at $p<0.05$ (8).

RESULTS AND DISCUSSION

Proximate analysis

Results are shown in Table 1. The total crude protein content of the product protein evaluated was 24.47 g/100g using a conversion factor of 6.25.

TABLE 1
Proximate composition (g/100g) of protein product and flour from *C. orinocense* (Barinas nuts) [1]

Component	Flour	Protein Product
Moisture	9.85 ± 0.062	3.78 ± 0.161
Crude fat	28.29 ± 0.898	3.42 ± 0.799
Protein (N x 6.25)	17.78 ± 0.116	24.47 ± 0.415
Ash	2.80 ± 0.004	4.47 ± 0.340
Fiber (crude)	6.60 ± 0.272	4.32 ± 0.917
Carbohydrates by difference	34.60	59.54

[1] Mean ± SD of n = 3 and expressed on dry weight basis

Protein solubility

In Figure 1 the minimum nitrogen solubility for both samples was at pH 4 the isoelectric point (pI). Both samples also showed an increased solubility at both sides of the pI. Padilla et al., (1) reported similar behavior for the C. orinocense flour (protein content 17.78 g/100g), the difference is that the solubility of *C. orinocense* flours reaches a plateau at pH 8; while the protein product increase with the pH. These results suggest how the product will perform when incorporated in a food product (9), and indicate that the solubility is influenced by the origin, processing conditions and pH, besides others parameters are corroborated these results.

FIGURE 1
pH effect on the solubility profile of *Caryodendron* protein product

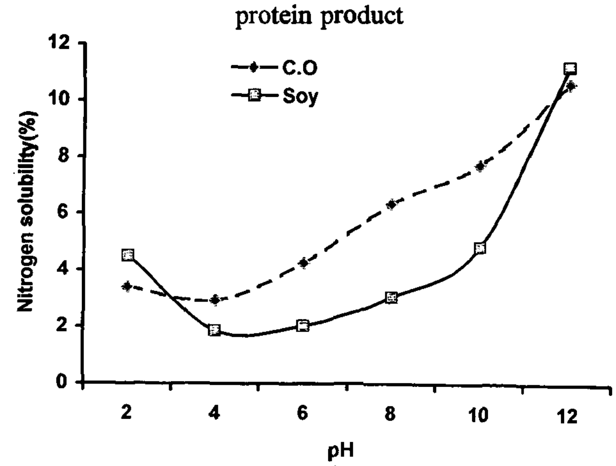
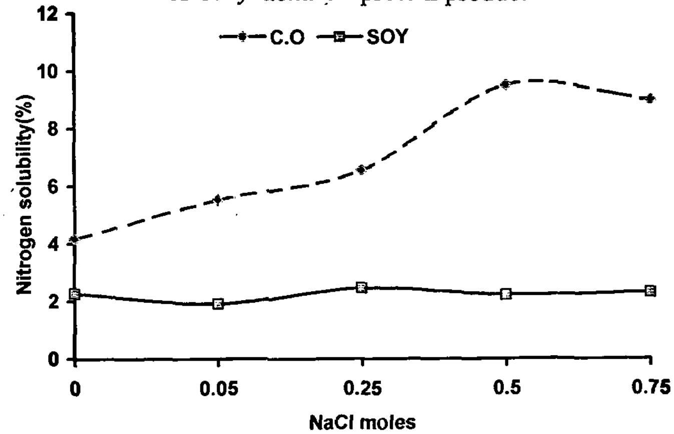


Figure 2 shows how NaCl concentration affects the solubility of proteins, which increases with NaCl concentration up to 0.5M and decreases at 0.75M. Some salts are effective in increasing protein solubility ("salting in" effect) and at the same time are also effective in destabilizing native globular and fibrous structure and increasing the rate of denaturation of native structures. During "salting out", a process that decreases solubility, there is a reverse effect on the stability and rate of denaturation of native proteins (10). The protein product presents a steady increase in solubility, more pronounce for the protein product than the flour as was reported by Padilla et al., (1). It also has a better solubility than soybean flour, as NaCl concentration increases, this is also important in the development of food products.

FIGURE 2
NaCl concentration effect on the solubility profile of *Caryodendron* protein product



Bulk density

There is no statistical difference in bulk density between both samples (Table 2). The small difference in values might be due to the smaller particle size for soybean flour.

TABLE 2
Water and oil holding capacity (WHC, OHC), emulsifying activity (EA), emulsion stability (ES), and foam capacity (FC) of protein product and soybean flour from *C. orinocense* (Barinas nuts) [1]

Sample	Density g/mL	WHC (%)	OHC (%)	EA (500nm)	ES (%)	FC (%)
Protein P.	0.5056±0.004[a]	137.56±3.28[a]	124.54±2.90[a]	0.60±0.00[a]	59.16±1.18[a]	5.76±0.00[a]
Soybean	0.5598±0.066[a]	112.43±3.31[b]	29.59±0.41[b]	0.64±0.014[a]	55.36±3.14[a]	24.38±2.02[b]

[1]Mean ± SD of n = 3. Values with different letters in the same column are statistically different ($p<0.05$)

Water and oil holding capacity

The protein product presents higher oil and water holding capacity than the soybean flour with statistical differences between them (Table 2). There is a great difference among these results and the same parameters for the *Caryodendron* flour found by Padilla, et al., (1). This might be due to the presence of other non-protein components that in flour samples could modify this characteristic (11). According to these investigators the oil and water holding capacity could be a function of the protein composition, not only due to the hydrophilic and lipophilic groups exposed but also due to the physical entrapment of oil. These results also suggest that the proteins in the protein product retain more water than oil and they are more hydrophilic and lipophilic than soybean flour. However, it was reported (12) that the high oil absorption capacity suggests the presence of an appreciable number of hydrophobic residues on the protein surface, despite high solubility in water,. Several authors (13-15) have reported higher oil binding capacity for their samples when compared to soy protein products.

Gel formation

The least concentration needed to form a gel for the protein product was 20% (w/v) with the formation of a gel in 4 min, and 10% and 8 min for soybean flour. However, Padilla et al. (1) reported 14% and 12 min. for the *Caryodendron flour* and 10% and 10 min for soybean flour. A minimum protein concentration is necessary for gelation; as the concentration is increased above this minimum, gelling time is reduced (16). Although the gelation is not only a function of protein quantify but seems also to be related to the type of protein as well as to no-protein components (17).

Emulsifying activity (EA) and emulsion stability (ES)

These properties (Table 2) are very similar in both samples studied and when compared with results for the *Caryodendron* flour reported by Padilla, et al., *(1)*. Unfolding of proteins at the water/oil interface plays an important role on the EA and ES by increasing hydrophobicity and subsequently increasing EA (18,19). The EA of proteins is related to their ability to lower the interfacial tension between water and oil in the emulsion. The surface activity is a function of the ability with which the protein can migrate to, absorb at, unfold and rearrange at an interface (20).

Figure 3 and 4 present the influence of the ionic strength on EA and ES. The EA for the product reached a minimum at 0.05M NaCl to increase thereafter, this was not so for soy flour which remained almost constant. This increase could be attributed to a greater solubility of proteins as ionic strength increases. Inyang & Iduh founds similar result, were found for sesame flour (9). The protein product ES has only a mild increase at 0.25M NaCl, while soy flour decreases slowly as the ionic strength increased. It seems that NaCl has a stabilizing effect on the protein film during emulsification (21), or might stabilize emulsions by reduction of interactions among vicinal groups (9). Good emulsifying capacity of the product suggest possible application in emulsified foods (soups and cake).

FIGURE 3
NaCl concentration effect on the emulsifying activity of soybean flour and *Caryodendron* product at pH 6.4

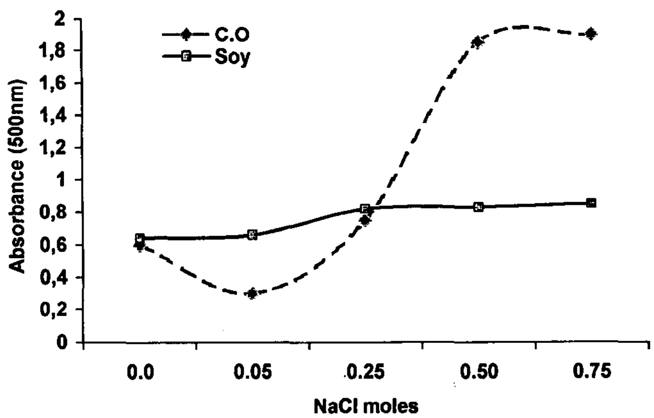
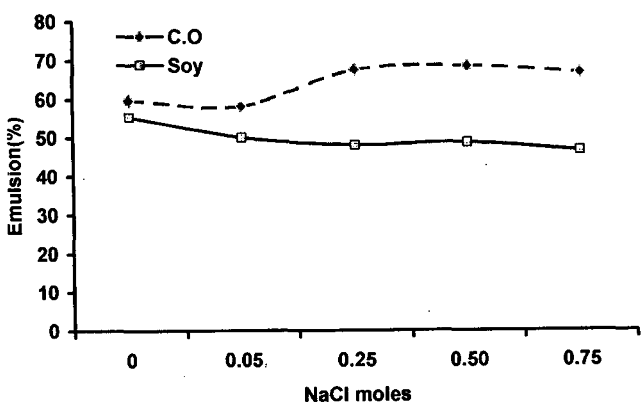


FIGURE 4
NaCl concentration effect on emulsion stability of soybean flour and *Caryodendron* product at pH 6.4



Foam capacity (FC) and stability (FS)

Foam capacity shows significant differences between both samples (Table 2). Figure 5 shows how pH influences foaming capacity. The higher FC at alkaline pH might be attributed to higher protein solubility at this pH, and the increased flexibility of the protein, which diffused more rapidly to the air-water interface to encapsulate air particles and this enhanced the foaming (22). The difference in foaming capacity of soybean flour might be due to its higher protein level. Several authors have suggested a direct relationship between FC and nitrogen solubility of seed flours (1,23,24). FC of the product increased very rapidly as the NaCl increased, but not so for soybean flour, which had a small increase with 0.05 NaCl moles decreasing thereafter (Figure 6). Some authors (1,19,24) also reported increased FC, for a bovine plasma concentrate, *Caryodendron* flour and pigeon pea, with the addition of salts. This effect has been explained differently depending on the investigator. It seems that salts usually reduce surface viscosity and rigidity of protein films but increase spreading rate, thereby weakening inter-peptide attractions and increasing foam volume for certain proteins. On the other hand the effect appears to be concentration dependent, since salt at an appropriate concentration aid foaming, presumably by aiding diffusion and spreading at the interface, but a high level of salt will depress foaming. This increased foam capacity in the presence of NaCl might make the protein product useful in the production of cakes and whipped toppings where foaming is an important property.

FIGURE 5
pH effect on foam capacity of *Caryodendron* product and soybean flour

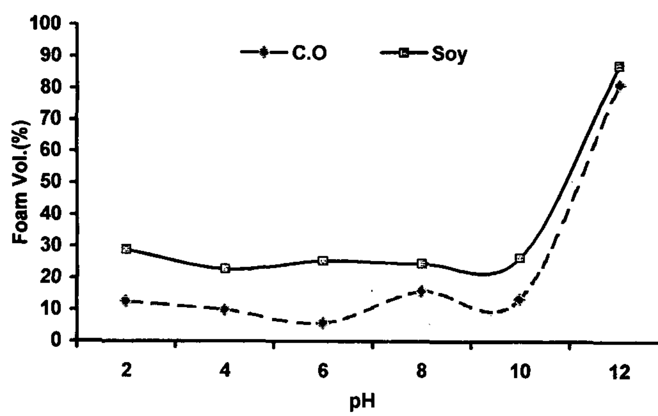
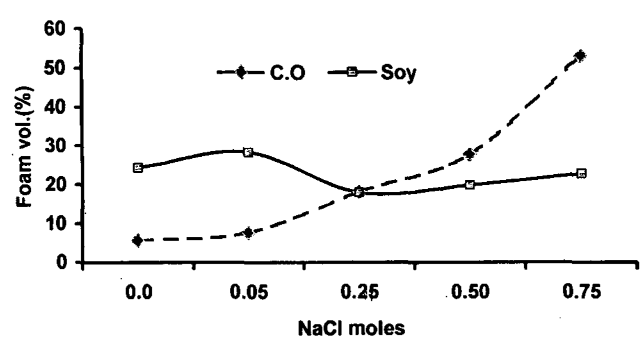


FIGURE 6
NaCl effect on foam capacity of soybean flour and *Caryodendron* product at pH 6.4



The influence of the pH and presence of NaCl at pH 6.4 on FS after standing for 5, 30, 60 and 120 min are presented in Figure 7 and 8. In both samples foam was stable at all pH values below 8.0, even though soybean flour had a higher FC at all pH values. Nevertheless, soybean flour FS decreased at pH above 8 while the *Caryodendron* product presented a smaller decrease, but increase drastically at pH 12 being stable during the time. The stability to the protein product is highest at 0.75M NaCl. This property is related to the protein solubility and concentration of the sample. According to Deland, Hojilla-Evangelista, & Jonson, (25) the protein film at the interface provides foam stability but this requires some denaturation of the protein during whipping and also rearrangement of the hydrophilic and hydrophobic groups; suggesting that the aminoacid composition and conformational structure might develop foam with an adequate volume. However, they might not be enough to stabilize the foam.

FIGURE 7
pH effect on foam stability of *Caryodendron* product compared with soybean flour

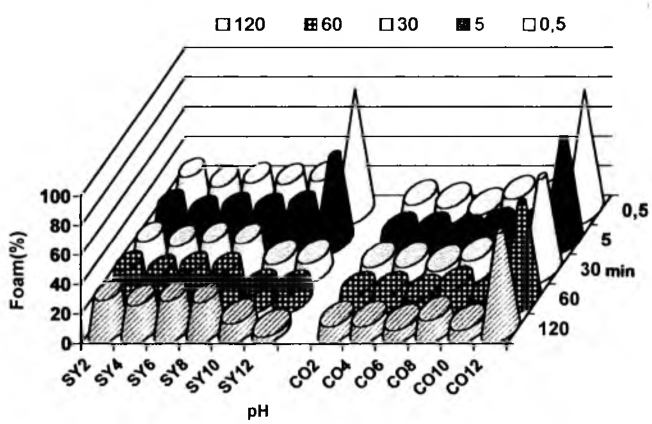
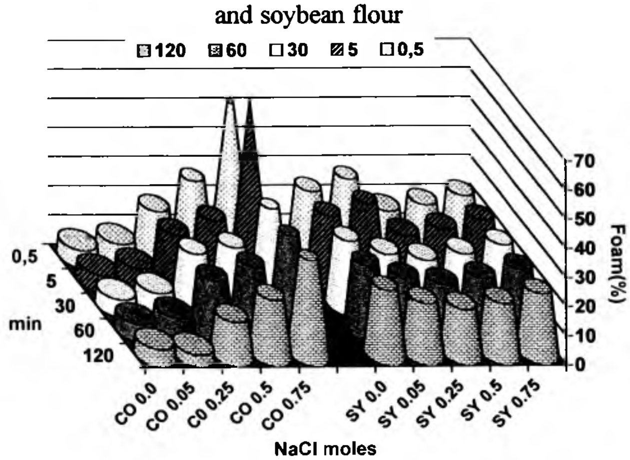


FIGURE 8
NaCl effect on the foam stability of *Caryodendron* product and soybean flour



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