Emulsifying Capacity of Coconut Proteins as a Function of Salt, Phosphate, and Temperature

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ABSTRACT: Nitrogen solubilities of proteins in defatted coconut flour (CF) and coconut protein concentrate (CPC), prepared by ultrafiltration, were determined in water and 2% NaCl. The effect of temperature, disodium phosphate, and salt on emulsifying capacities (EC) of these products also were investigated by a model system. Between pH 4.0 and 5.0, nitrogen solubilities of CF and CPC in water were lower than those in salt solutions. In salt solutions, the nitrogen solubility was lowest at pH 1, and increased steadily as pH increased from 3.0 to 6.0. CF had higher EC values than CPC at all salt and phosphate levels. Additionally, increased phosphate level generally increased the EC at both salt levels, but these increases were not significant at 0.9% phosphate level as compared to the 0.7% phosphate level ($P < 0.05$). Although the emulsifying temperature of 40°C showed higher EC values than both 60 and 80°C at all salt and phosphate levels, the overall temperature effect was not significant ($P > 0.05$). However, EC decreased significantly with the addition of salt at all phosphate levels.

JAOCS 73, 1669–1673 (1996).

KEY WORDS: Coconut protein, coconut protein concentrate, emulsifying capacity, nitrogen solubility.

Emulsifying capacity (EC) is an important functional property of a protein in various emulsion-based food systems, such as cheese analogs, ice cream, salad dressing, and, in some cases, processed meats. EC of proteins is influenced by their structural characteristics, which are themselves affected by many environmental factors, such as protein concentration and solubility, pH of the medium, temperature, and ionic strength (1,2). The emulsifying properties of protein generally correlate with solubility (i.e., a minimum emulsifying capacity at the isoelectric point of the protein) (3). Studies of salt effects on functional properties of soy flour revealed that nitrogen solubility and emulsion capacity generally increased in high salt suspensions (1). Generally, the effects of ions on protein conformation may be divided in two categories: first, electrostatic interaction with charged groups and polar groups leads to charge opposition, thereby preventing precipitation, and second, their effect on hydrophobic interactions *via* influence on the structure of water (4).

A few studies have been reported where coconut protein could be used for production of cheese products as a partial source of protein (5). Over the years, some coconuts have been processed to prepare desiccated coconut flour, copra and coconut protein concentrate (CPC). These coconut products may be utilized extensively in cheese analogs. However, information is limited about functional properties, particularly emulsifying properties. Furthermore, processing conditions, such as heat and pressure, during oil extraction and drying could alter properties due to changes in association–dissociation and denaturation of proteins as well as surface properties (6). The majority of processed cheese operations involve formation of caseinates (combinations are generally used) with buffer salts, emulsifiers, acidulants, and fat. Heating also is involved during blending and working the mixture. Therefore, it is important to obtain reliable technological information on coconut proteins, particularly the factors that influence emulsion formation, for successfully formulating various types of emulsion-based food products with coconut proteins and oils.

The objective of this study was to investigate the effects of phosphate, salt, and temperature on the EC of CF and CPC by using a model system. Effects of these factors were studied by analysis of variance on a $3 \times 3 \times 2 \times 2$ factorial experimental design. Solubilities of CF and CPC as a function of pH and salt also were studied.

EXPERIMEMTAL PROCEDURES

Materials. All fresh coconuts used in this work were purchased locally on an as-needed basis, and commercial desiccated coconut meal was purchased from Coco Gold Manufacturing Industries Inc. (Republic of the Philippines). Desiccated coconut meal was defatted at low temperature by the conventional semipilot-scale hexane extraction method and then desolventized at room temperature. The defatted meal was ground in a coffee grinder to obtain a homogeneous flour. Coconut oil was purchased from Premier Edible Oils Corp. (Portland, OR).

Preparation of CPC. The nuts were cracked open, and the coconut water was discarded. The meats were grated from the

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shell with a grater. The grated coconut meats were mixed with 0.5 M NaCl solution (solvent-to-meats ratio of 2:1, vol/wt) at 40°C and ground in a Waring Blender for 4 min. The coconut milk obtained from pressing the pulp was passed through a cheese cloth to remove the residue. The mixture was then separated in a cream separator (Westfalia centrifuge, Westfalia Separators, Ltd.) into coconut fat and defatted milk. The defatted milk was prefiltered and then concentrated in a pilot-scale hollow-fiber ultrafiltration unit (Romicon Inc., Woburn, MA), with a molecular weight cut-off of 1 5,000 Da. After ultrafiltration, the amount of salt and sugar in the coconut milk was reduced to approximately 30% by a diafiltration technique. The diafiltered retentate was spray-dried in a pilot-scale spray dryer.

Proximate analyses. Protein, fat, moisture, and ash contents of CF and CPC (Table 1) were determined by the standard Association of Official Analytical Chemists (AOAC) methods (7). Total carbohydrates were calculated by difference.

Nitrogen solubility. Nitrogen solubility was determined according to the method of Chobert *et al.* (8). Protein samples were dispersed in distilled water or 2% NaCl solution (0.1% protein, wt/vol) by mixing with a Vortex. The pH was adjusted from 1.0 to 12.0 with HCl or NaOH of high normality to limit dilution, and the dispersion 2 was stirred with a magnetic stirrer for 1 h. The samples were centrifuged at 20,000 $\times g$ for 30 min, and the protein content of the supernatant was determined by the Bio-Rad protein assay method (9). The amount of soluble protein was expressed as percentage of total protein.

EC. EC of CF and CPC samples was determined according to the method of Webb *et al.* (10). Samples were dispersed (0.1%, wt/vol) in distilled water or 2% NaCl solution, and then the precalculated amount of Na_2HPO_4 was added to the solution while stirring. To measure EC, 40 mL of the protein solution was placed into a blender jar (Ultra-Turrax, Model SD-45), which was equipped with two electrodes connected to a volt-ohm multimeter (Simpson Electric Company, Elgin, IL), for detecting the sudden increase in electrical resistance of the dispersion that occurs upon emulsion collapse. About 10 mL of refined coconut oil was added to the solution, and the mixture was blended at low speed (3,000 rpm) for 30 s. Then, additional oil was delivered to the mixture at an average rate of 0.4 mL/s up to the inversion point of the emulsion. The blender speed was 10,000 rpm during the final emulsification step. EC was expressed as mL oil added for emulsification/100 mg protein.

TABLE 1
Proximate Analysis of Coconut Flour (CF) and Coconut Protein Concentrate (CPC)

Protein samples	Protein[a] (%)	Fat (%)	Moisture (%)	Ash (%)	Carbohydrates (%)
CF	19.70	2.19	6.31	7.21	64.59
CPC	51.79	2.20	5.00	4.35	36.66

[a]Used as the nitrogen conversion factor was 6.25.

Statistical analysis. Data were statistically analyzed by analysis of variance with a random factorial design (11). Significant treatment and interaction means were analyzed with Duncan's Multiple Range Tests (12). Differences were considered statistically significant at $P < 0.05$

RESULTS AND DISCUSSION

Nitrogen solubility. The nitrogen solubility profiles of the proteins of CF and CPC in water and NaCl solutions at pH values from 1 to 12 are shown in Figure 1. In water (Fig. 1A), the minimum solubility for both samples occurred between

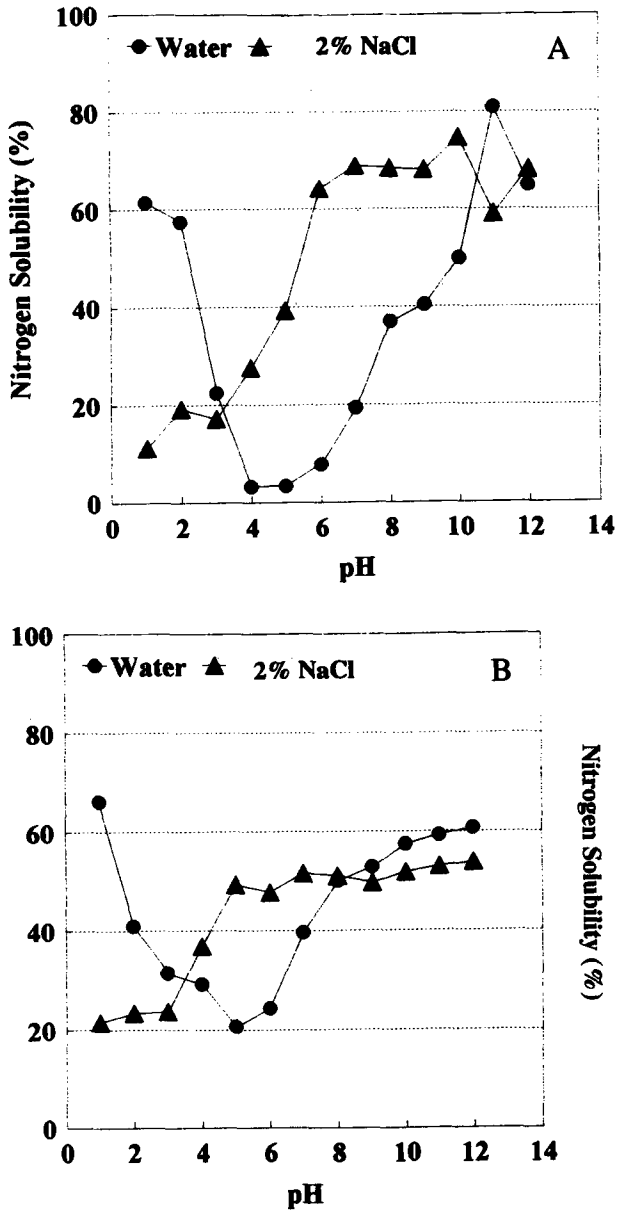

FIG. 1. Nitrogen solubility of defatted coconut flour (A) and coconut protein concentrate (B) in water and 2% NaCl solution as a function pH.

pH 4 and 5, which is the isoelectric point (pI) region of coconut proteins (13). As expected, nitrogen solubility increased at both sides of the pI region. The pH affects charge and electrostatic balance within and between proteins. Below and above the pI, proteins have a positive or negative net charge, which enhances solubility. At the pI (net charge is zero), attractive forces predominate, and molecules tend to associate (14). About 80% of the CF protein in water was soluble at pH 11. CPC samples in water (Fig. 1B) were more soluble than CF at pH between 3 and 10, indicating probably partial denaturation of water-soluble proteins of CF during processing. Heat denaturation of protein during processing can decrease the solubility of the macromolecule in aqueous solvent (15). These protein solubility curves are similar to those of other plant proteins (1,16).

The protein solubility profiles of CF and CPC (Fig. 1A and 1B) in the presence of 2% NaCl were quite different to those of CF and CPC in water. Nitrogen solubility in NaCl decreased at the extreme acidic region (pH 1–3), and increased at pH above 4. In the acidic region, near the pKa of glutamic and aspartic acids, the number of charges is reduced, and salts compete with proteins for water. Thus, protein-protein hydrophobic interactions could have increased, followed by hydrophobic aggregation and loss of solubility (2). CF and CPC proteins in NaCl (Fig. 1A and 1B) showed similar nitrogen solubility in the acidic pH range. However, CF protein in NaCl (Fig. 1A) was more soluble than CPC protein (Fig. 1B) at pH greater than 6.

Proximate composition Protein contents were about two and half times higher in CPC than in CF, while CF was higher in carbohydrate content than CPC (Table 1). These differences in protein and carbohydrate (comprised of fiber, sugar, some starch, and other materials) could be the factors that influence the EC values of these protein ingredients in a model system.

pH of sample solutions. When phosphate was added, the pH values of both CF and CPC solutions increased (Table 2). However, increasing phosphate level from 0.7 to 0.9% or addition of salt did not appreciably alter the solution pH, presumably due to a buffering effect of phosphate (17)

EC. In emulsions, the role of protein is to form an interfacial film and stabilize the system against flocculation, coalescence, creaming, or oiling off. Thus, in processed cheese manufacturing, in which structure formation presumably relates to the emulsification, gelation and hydration properties of the protein used, film-forming properties are needed, but the thermal stability of the film during heating and the ability to set to a gel on cooling are important properties of the protein. EC measurements can be considered as an index of the ability of the protein to absorb onto the newly created surface and retard coalescence during emulsification (18).

EC of CF as a function of temperature and phosphate level in the absence and in the presence of 2% NaCl is shown in Figure 2A and 2B. Analysis of variance for EC values (Table 3) showed significant ($P < 0.05$) interactions of protein, temperature, and salt level ($C \times T \times N$). Strong statistical interactions on EC values existed between any two of the four factors, but no interaction effect of temperature was observed for phosphate level (Table 3). In water, the EC of CF tended to decrease with increasing temperature from 40 to 60°C at all phosphate levels. At 80°C, EC remained essentially the same at 0.7 and 0.9% phosphate. The lowest EC was found at 80°C and zero phosphate. In NaCl, an increase in temperature (40–60°C) slightly increased the EC in all samples, but at higher temperature (80°C), the EC decreased (Fig. 2B). This maximum may be explained by unfolding the protein at the oil–water interface, thus forming a more stable interfacial film. The emulsifying properties of soy protein generally tend to decrease if the protein solution is heated (3). Venktesh and Prakash (6) have reported that heat does not affect the EC values of sunflower proteins profoundly, although a marginal decrease did occur in samples that had been exposed to high pressure and temperature. Overall, the addition of 2% NaCl (Fig. 2B) decreased the EC values of all CF samples as compared to the EC values in water (Fig. 2A). It is evident from the data that salt, by ionic interaction, affected the EC values of the samples. This phenomenon has been previously reported for other vegetable protein systems (1,6).

The EC value of CF at 0.7% phosphate level in water (Fig. 2A) was higher compared to the control (no phosphate) across the temperature range, but it was not significantly af-

TABLE 2
pH of Protein Solution in NaCl–Na_2HPO_4 for Emulsifying Capacity Measurement

Na_2HPO_4 level (%)	Coconut flour		Coconut protein concentrate	
	0% NaCl	2.0% NaCl	0% NaCl	2.0% NaCl
0.00	6.70	6.72	4.00	4.40
0.70	8.45	8.42	7.95	7.90
0.90	8.55	8.47	8.06	8.05

TABLE 3
Analysis of Variance (*P*-values) for Emulsifying Capacity Values as Influenced by the Type of Coconut Protein, Temperature and Na_2HPO_4, and NaCl Levels

Source of variation	Degrees of freedom	*P* value
Coconut Protein (*C*)	1	0.0001[a]
Temperature (*T*)	2	0.0001
Phosphate level (*P*)	2	0.0001
NaCl (*N*)	1	0.0001
$C \times T$	2	0.0012
$C \times N$	1	0.0001
$C \times P$	2	0.0001
$T \times N$	2	0.0001
$N \times P$	2	0.0044
$T \times P$	4	0.1393
$C \times N \times P$	2	0.0031
$C \times T \times N$	2	0.0001
$T \times N \times P$	4	0.3437
$C \times T \times P$	4	0.4915

[a]Significant at 5% confidence level ($P < 0.05$).

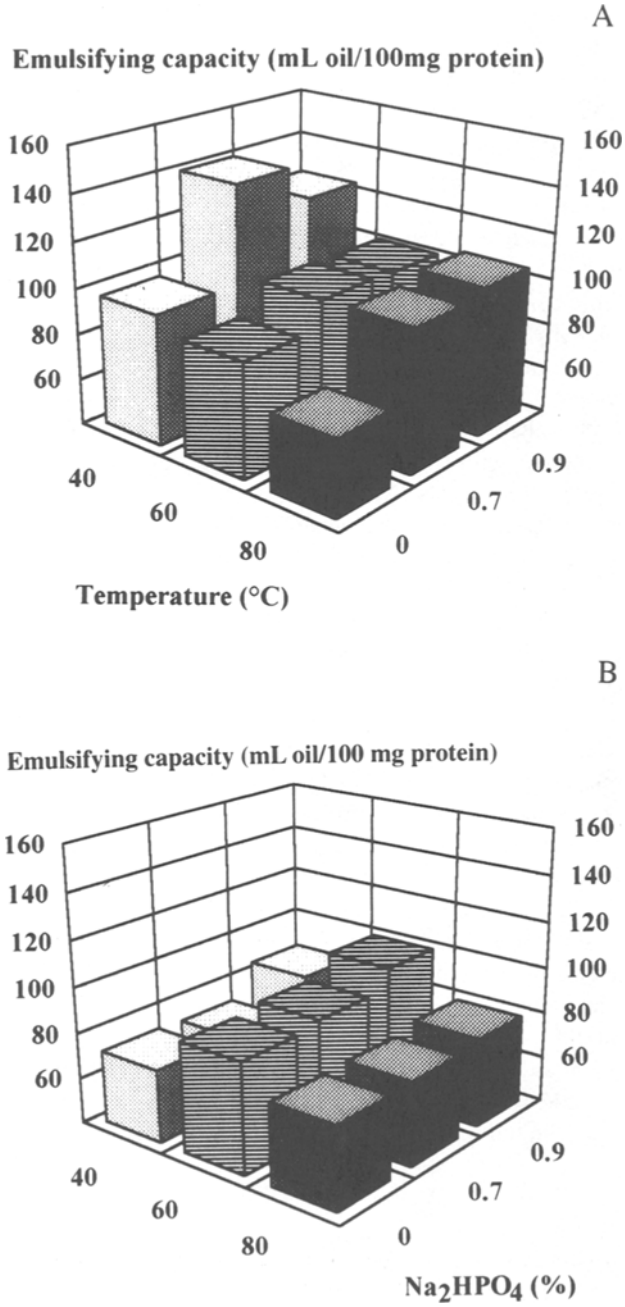

FIG. 2. Effect of temperature and phosphate levels on emulsifying capacity of coconut flour in the absence (A) and in the presence (B) of 2% NaCl.

fected at the higher phosphate level (0.9%). In the presence of NaCl, EC slightly increased with increasing phosphate levels. It has been known that inorganic, alkaline sodium phosphates enhance water-holding capacity in meat products (19) and they are reported to have a considerable effect on processed cheese made from dairy ingredients (20). Although the emulsifying mechanism remains somewhat less than fully defined, anions from the salt added to processed cheese participate in ionic bridges between protein molecules, thereby providing a stabilized matrix that entraps the fat in processed cheese (21). The EC of CF was maximum (149.0) at 40°C with 0.7% phosphate in water (Fig. 2A).

Figure 3 shows the EC of CPC as a function of temperature, phosphate and NaCl. No significant ($P > 0.05$) effects of temperature, phosphate, and salt were found (Table 3 and Fig. 3). Compared to CF, CPC had lower EC values at almost all phosphate levels and temperatures in water (Fig. 2A and 3A). These differences in EC values between CF and CPC may be accounted for by the more than 60% carbohydrate contained in CF, which could play an important role in protein–carbohydrate interactions. Lin *et al.* (22) found that flours were far superior in EC, compared to corresponding sunflower concentrates and isolates. EC values of CPC (pH of solutions is about 4.0) at 40°C in water without phosphate were lower than those of any other samples. It appears that EC is influenced by the pH (near pI) to which the sample is exposed. McWatters and Cherry (23) reported that emulsion properties of soybean, pecan, and peanut flours were poorest at pH 4.0, a level that encompasses the apparent pI of many seed proteins. In general, the addition of NaCl slightly decreased the EC of all CPC samples as compared to the EC values in water (Fig. 3B). Increasing the phosphate level tended to slightly increase the EC values in water but did not affect EC values in the presence of NaCl. Also, the temperature of the protein dispersion did not significantly ($P > 0.05$) affect the EC of CPC at all phosphate levels. EC of CPC slightly increased in water at all phosphate levels (Fig. 3).

According to mean EC values, showing the effect of proteins, temperatures, and levels of phosphate and NaCl (Table 4), CF had higher EC than CPC, and the optimal EC was at 40°C . A lower EC from the addition of NaCl may be related to protein solubility. EC significantly ($P < 0.05$) increased with addition of phosphate probably due to probably elevated pH values and the emulsifying effect described above.

TABLE 4
Mean Emulsifying Capacity (EC) Values as Affected by the Type of Proteins, Temperature and NaCl and Na_2HPO_4 Levels

Variables[a]	Mean values of EC (mL oil/100 mg protein)[b]
Coconut protein (n = 54)	
Coconut flour	92.63[a]
Coconut protein concentrate	76.84[b]
Temperature (n = 36)	
40°C	87.00[a]
60°C	86.84[a]
80°C	80.36[b]
NaCl levels (n = 54)	
0%	92.82[a]
2%	76.66[b]
Na_2HPO_4 levels (n = 54)	
0.0%	77.70[a]
0.7%	87.67[b]
0.9%	88.85[b]

[a]n: Number of observations.
[b]Values with the same superscripts within a group for a given variable are not significantly different from each other at $P < 0.05$.

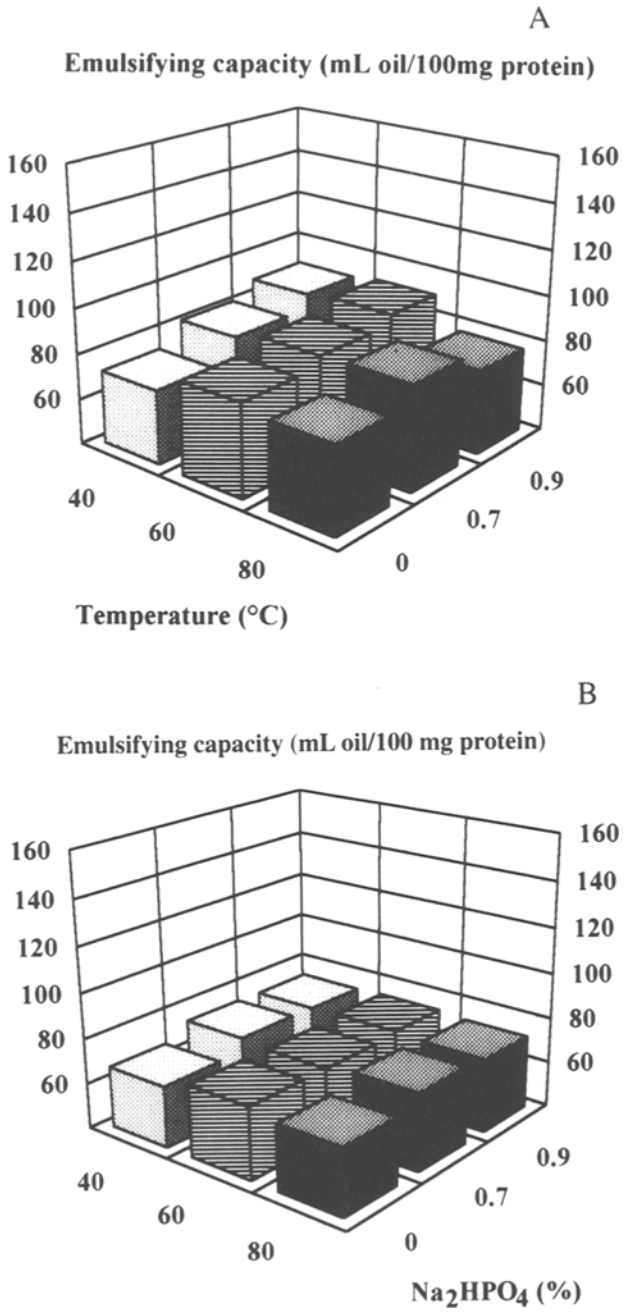

FIG. 3. Effect of temperature and phosphate levels on emulsifying capacity of coconut protein concentrate in the absence (A) and in the presence (B) of 2% NaCl.

ACKNOWLEDGMENTS

This research was supported in part by the United Nations Industrial Development Organization and Texas Engineering Experiment Station. Part of this paper was presented at the 54th Annual Meeting of the Institute of Food Technologists, Atlanta, Georgia, June 25–29, 1994.

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[Received January 23, 1996; accepted September 8, 1996]