Proximate composition of Karkadeh (*Hibiscus sabdariffa*) seeds and some functional properties of seed protein isolate as influenced by pH and NaCl

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Abstract

Seeds of an inbred line (B-11-90) of Karkadeh (*Hibiscus sabdariffa*) were investigated for their proximate composition (AOAC methods), nitrogen solubility and protein isolate (Karkadeh seed protein isolates [KSPI]) functional properties (standard methods). The fat and protein contents of the seeds were 22.43% and 32.46%, respectively. Nitrogen solubility was good in both water and 1.0 M NaCl at alkaline pH rather than at acidic pH, with better solubility at higher pH levels in water than in 1.0 M NaCl. The functional properties of the KSPI were as follows: water absorption capacity, 181 ml/100 g; fat absorption capacity, 110 ml/100 g; bulk density, 0.77 g/ml; and apparent viscosity (at 20°C), 13.42 cps. KSPI showed a maximum foaming capacity at pH 12 and 1.6 M NaCl, a maximum emulsification capacity at pH 11 and 1.8 M NaCl, and a weaker foam stability at neutral pH than at acidic or alkaline pH, with a better foam stability at alkaline pH. The foam stability was considerably improved by treatment with 1.6 M NaCl.

Keywords: *Functional properties*, Hibiscus sabdariffa, *Karkadeh seed protein isolate, pH, proximate composition, NaCl*

Introduction

World demand for protein is increasing and so more food protein is required from both conventional and new sources of protein. New proteins should have satisfactory nutritional value and acceptable flavor, color and texture, but they must also possess additional critical functional properties (the intrinsic physico-chemical characteristics that may affect the behavior of food systems during processing, preparation and storage); for example, solubility, foamability, gelation and emusification properties (Ogungbenle et al. 2002; Khalid et al. 2003) that make them compatible with, and if possible enhance, the food to which they are added (Wang and Kinsella 1976). Functional properties are most important in determining the uses of such proteins for the development of new food products by industry (Kinsella 1976), as well as for non-food industries (Anonymous 2007). Functional properties vary with the source of protein, composition, method of preparation/extraction, thermal history, and prevailing environment (e.g. pH, ionic strength, temperature, presence of salts). A great deal

of research goes into studies of functional properties by the food industry in order to understand the basics so that new processes, foods, and so on, can be developed (Anonymous 2005).

Numerous researchers have reported the preparation and functional properties of protein concentrates and protein isolates from plant, animal and microbial sources (Fernandez-Quintela et al. 1997; Akubor & Chukwu 1999; Okpala & Mamah 2001; Chel-Guerrero et al. 2002; Sogi et al. 2002; Khalid et al. 2003; Ogungbenle 2003; Aluko & Mcintosh 2004; Bora & Ribeiro 2004; Horax et al. 2004; Bernardino-Nicanor et al. 2005; Cordero-de-los-Santos et al. 2005; Makri et al. 2005; Hojillaevangelist & Evangelista 2006).

Roselle (*Hibiscus sabdariffa*), known in Sudan as Karkadeh, is an annual herb that belongs to the family Malvaceae (Mc Clean 1973). The plant is mainly grown for the large thick calyces, which are mostly used in the preparation of cold and hot beverages. Recently it has been used by the food industry in the preparation of jams, jellies and food-coloring material. Karkadeh seed is a secondary product of the crop and it is a rich source of oil (Gangadher et al. 1966; Ahmed et al. 1979; Rukinin et al. 1982; Al-Wandawi et al. 1984) and protein (Al-Wandawi et al. 1984; Ahmed & Nour 1993).

This article reports on the proximate composition of an inbred line of Karkadeh seeds, nitrogen solubility in water and in 1.0 M NaCl, and some of the surface-related (water and fat absorption, foaming and emulsification) and hydrodynamic (bulk density and apparent viscosity) functional properties of Karkadeh seed protein isolate as influenced by pH and NaCl. The study aimed at investigating the potential for utilizing Karkadeh seed proteins to improve functional properties in food preparations and other value-added products.

Materials and methods

Karkadeh seeds and seed flour

An inbred line (B-11-90) of Karkadeh seeds was obtained from the demonstration farm of the Faculty of Agriculture, Shambat, Sudan. After discarding foreign materials, seeds were sorted and milled using an electric grinder. Karkadeh seed flour (KSF) that passes mesh number 18 was obtained.

Reagents and chemicals

All reagents and chemicals used in the study were of Technical Grade (BDH, Analar [VWR International, Ltd., UK]).

Defatted KSF

An exactly weighed amount of KSF was placed in a conical flask and *n*-hexane was added in the ratio of 10:1 (solvent:flour). The mixture was stirred for 16 h and then filtered. More *n*-hexane was added to wash fat traces, and the mixture was filtered again. The defatted flour (Karkadeh seed meal [KSM]) was dried in open air at room temperature (26°C).

Proximate analysis

The proximate composition of the KSF and the KSM was determined by AOAC (1990) methods.

Nitrogen solubility

The nitrogen solubility of the KSM samples in distilled water and in 1.0 M NaCl was determined over a pH range of 1–12 according to the procedure of Hagenmaier (1972) modified by Quinn and Beuchat (1975). A 2 g sample of material was added to distilled water and to 1.0 M NaCl to make a 2% suspension. The suspension was shaken in a mechanical shaker for 10 min before the desired pH was maintained by addition of 2 N HCl or 2 N NaOH over a 60-min period of constant shaking at 25–27°C. The suspension was then centrifuged at 5,000 rpm for 20 min at the same temperature. The pH of the clear supernatant was recorded and soluble nitrogen in supernatant determined by the Kjeldahl procedure. The percentage of soluble nitrogen was calculated and plotted against corresponding pH.

Extraction of Karkadeh seed protein isolates

Protein was extracted from the KSM following the method described by El-Tinay et al. (1988) using 1.0 M NaCl solution with a solvent:flour ratio of 10:1. The extraction was carried out at room temperature for 30 min. The insoluble materials were removed by centrifuging at 3,000 rpm for 15 min. The extracted liquor was dialyzed against distilled water by placing 10 ml in 25-cm-long dialysis bags. The bags with contents were placed in large containers and dialyzed against distilled water. The water was changed until it was free from the salt. The contents of the dialysis bags were centrifuged for 15 min at 3,000 rpm. The whey was discarded and the precipitate was collected and left to dry at 50°C for 10 h. A creamy powdered protein isolate (Karkadeh seed protein isolates [KSPI]) was obtained. This protein isolate was kept in a cool dry room until taken for further analysis.

Functional properties of the KSPI

Water absorption capacity. The water absorption capacity (WAC) was measured by the method of Lin et al. (1974) as modified by Quinn and Beuchat (1975). A 10% aqueous suspension of KSPI (3 g/30 ml) was stirred in a 50-ml centrifuge tube using a glass rod for 2 min at room temperature. After 30 min of equilibration, the tube was centrifuged at 4,400 rpm for 20 min. The freed water was carefully decanted into a graduated cylinder and the volume was recorded. WAC was calculated as milliliters of water retained by 100 g protein isolate.

Fat absorption capacity. The fat absorption capacity (FAC) was measured by a modified method of Lin et al. (1974). A 4-g sample was treated with 20 ml refined palm oil in a 50-ml centrifuge tube. The suspension was stirred with a glass rod for 5 min and the contents were allowed to equilibrate for a further 25 min. The suspension was centrifuged at 4,400 rpm for 20 min at room temperature. The fat was measured and the FAC was expressed as milliliters of fat bound by 100 g protein isolate.

Bulk density. The bulk density (BD) was determined according to the method of Wang and Kinsella (1976). Ten grams of material was placed in a 25-ml graduated cylinder and packed by gently tapping the cylinder on bench top (10 times from a height of 5–8 cm). The volume of sample was recorded and the BD was expressed as grams per milliliter.

Apparent viscosity. Quinn and Beuchat's (1975) method was followed for the apparent viscosity (AV) determination. Twenty grams of material was suspended in distilled water (20W/V) and left to equilibrate, and the AV was determined with a Brookfield synchroelectric viscometer (Brookfield Engineering Laboratories, MA, USA) using RVT spindle No. 4 at 100 rpm. The value was multiplied by the factor specified for the spindle in the instrument manual and the AV was expressed in counts per second (cps). Viscosity was also measured for hot slurries after heating them for 15 min at 70°C and cooling to room temperature.

Foaming capacity. Two grams of material was blended with 100 ml water in a Moulinex blender. The suspension was whipped at 11,600 rpm for 3 min. The mixture was poured into a 250-ml measuring cylinder and the foam volume was recorded after 30 sec. The foaming capacity (FC) was determined as a function of pH (1–12) and NaCl molar concentrations (0.2–3.4 M), and was expressed as the percentage increase in volume after whipping according to Lawhon et al. (1972).

Foam stability. The foam stability (FS) was determined by recording the foam volume at 30-min intervals for 150 min after pouring the material into a cylinder, and was calculated using the formula: FS = (foam volume after a specific time × 100)/(initial foam volume). The FS was determined as a function of selected pH values (pH 1, 7 and 12) and a selected NaCl molarity (1.6 M).

Emulsification capacity. The emulsification capacity (EC) was determined by the method of Beuchat et al. (1975). Two grams of material was blended with 23 ml distilled water for 30 sec in a Moulinex blender at 11,600 rpm. After complete dispersion, refined palm oil was added cautiously (0.4 ml/sec) from a burette and blending was continued until phase separation was visually observed. The EC was expressed as milliliters of oil emulsified by 1 g isolate. The EC was determined as a function of pH (1–12) and NaCl molarities (0.2–3.6 M).

Statistical analysis

The SPSS software (SPSS Inc., Illinois, USA) was used in the statistical analysis of results.

Results and discussion

Proximate composition

The proximate compositions of the KSF and the KSM are presented in Table I. The moisture and fat contents of KSF were 4.95% and 22.43%, respectively, which were not significantly different ($P < 0.01$) from the values of 4.73% and 21.70% reported by El-Nour (1991) for the Elrahad variety of Karkadeh. The crude fiber content was

Table I. Proximate composition[a] of Karkadeh seed flour and defatted flour.

Component (%)	Seed (KSF)	Defatted flour (KSM)
Moisture	4.95 (0.6)	7.05 (0.8)
Fat	22.43 (2.1)	4.34 (0.6)
Crude protein	32.46 (3.4)	46.46 (4.3)
Crude fiber	13.90 (1.6)	9.65 (1.3)
Ash	9.40 (1.2)	12.45 (1.4)
Nitrogen-free extract	16.86 (1.7)	20.05 (2.2)

Data presented as mean (standard deviation). [a]Average of six replicates.

13.90%, which was not significantly different ($P<0.01$) from the value of 13.68% reported by El-Nour (1991); whereas the ash content was 9.40%, which was significantly higher than the value of 7.10% reported by Bakheet (1989). The crude protein was 32.46%, which was lower than the value of 33.40% reported by Bakheet (1989) for the Elrahad variety of Karkadeh and was significantly higher than the value of 25.20% reported by Al-Wandawi et al. (1984) for the same variety. The crude protein content of the cold defatted flour was 46.46%, which was significantly higher than the value of 35.0% reported by El-Nour (1991).

Nitrogen solubility of the KSM

Nitrogen solubility in distilled water at different pH values. Figure 1 shows the percentage of soluble nitrogen at various pH values (pH 1–12). The lowest nitrogen solubilities were 12% and 16% at pH 3.0 and 4.0, respectively. The highest nitrogen solubility (87%) was obtained at pH 9.0. El-Nour (1991) reported that highest nitrogen solubility (73%) for Karkadeh seed meal was obtained at pH 10.0. Nitrogen solubility was higher at alkaline pH values; this agrees with the results obtained by Shehata and Thannoun (1981).

Nitrogen solubility in 1.0 M NaCl at different pH values. Figure 1 also shows the percentage of soluble nitrogen in 1.0 M NaCl at various pH values (pH 1–12). The percentage of soluble nitrogen increased at both acidic and neutral pH. Nitrogen

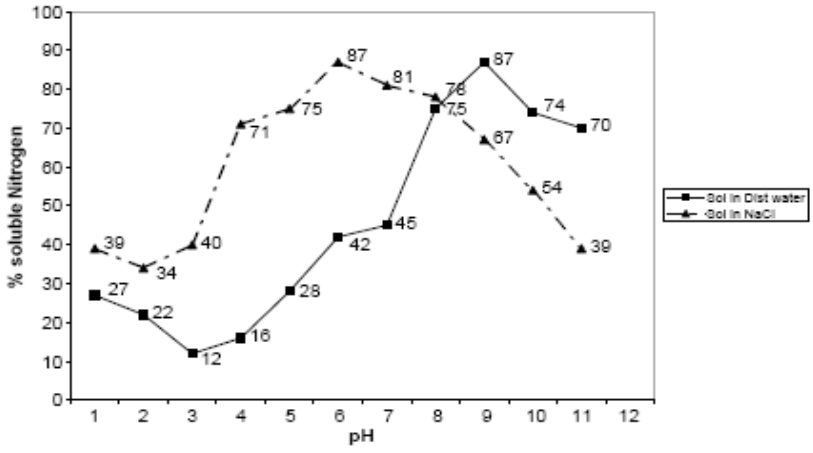

Figure 1. Nitrogen solubility of KSM in distilled water and in 1.0 M NaCl at different pH values.

solubility increased steadily as pH was increased from 3.0 to 6.0. Addition of 1.0 M solubilized the greatest percentage of nitrogen (87%) at pH 6.0, followed by a rapid decrease in solubility at extreme alkaline pH. McWatters and Holmes (1979) reported that maximum nitrogen solubility for soy flour was obtained at pH 6.0, with minimum solubility at pH 2.0 and a slight decrease between pH 6.0 and 8.0. The present investigation indicated that Karkadeh seed protein has good solubility in both water and 1.0 M NaCl at alkaline medium rather than in acidic medium with better solubility at higher pH levels in water than in 1.0 M NaCl.

Solubility characteristics, under various conditions, are very useful in selecting optimum conditions for extracting proteins from natural sources. Solubility behavior provides a good index of the potential applications of proteins. The nitrogen solubility profile, over a range of pH values, is being used increasingly as a guide to protein functionality, since this relates directly to many important properties (e.g. use in beverages, emulsification, foaming capacity and gelation). Good solubility can markedly expand potential applications of proteins.

Functional properties of KSPI

Many of the important functional properties of food proteins relate to water protein interactions (i.e. solubility, viscosity, gelation, foaming and emulsification).

Water absorption capacity. The WAC of KSPI was found to be 181 ml/100 g protein isolate (Table II). This is not significantly ($P<0.01$) different from the WAC of safflower protein isolate (180 ml/100 g) reported by Lopez and Falomir (1986), but is significantly ($P<0.01$) higher than that of sunflower protein (70 ml/100 g) reported by Canella et al. (1985). The storage quality and stability of foods are related to their moisture content. Bread can be fortified with proteins possessing good water binding qualities to improve its freshness. KSPI possesses reasonable WAC for incorporation in food products such as bread.

Fat absorption capacity. The KSPI was found to absorb 110 ml/100 g isolate (Table II). This value is close to, but significantly ($P<0.01$) different from, that of sunflower protein (130 ml/100 g) reported by Canella et al. (1985). Many properties of foods involve interactions of proteins and lipids (e.g. as in meat products). KSPI can be incorporated into meat products to enhance their fat absorption characteristics. The ability of proteins to bind fat is very important for such applications as meat replacers

Table II. Water absorption capacity, fat absorption capacity, bulk density and apparent viscosity of Karkadeh seed protein isolate.

Functional property	Value[a]
WAC (ml/100 g)	181 (2.6)
FAC (ml/100 g)	110 (1.8)
BD of KSM (g/ml)	0.50 (0.0)
BD of KSPI (g/ml)	0.77 (0.1)
AV at 20°C (cps)	13.42 (0.7)
AV at 70°C (cps)	44.74 (1.2)

Data presented as mean (standard deviation). [a]Average of six replicates.

and extenders, particularly because it enhances flavor retention and improves mouthfeel.

Bulk density. The BD of KSPI was found to be 0.77 g/ml, while the BD of KSM was 0.50 g/ml (Table II). Dench (1982) reported the BD of soy meal as 0.5 g/ml and that of soy protein isolate as 0.33 g/ml, while Wang and Kinsella (1976) stated that the BD of soy protein concentrate was 0.52 g/ml. High BD is a desirable characteristic when powdered food materials are to be packed in a limited space or area. High BD, as in KSPI, also finds use where products can be incorporated into light snacks or baby foods.

Apparent viscosity. The viscosity of cold and hot slurries of KSPI is presented in Table II. The AV was 13.42 cps at 25°C and was 44.74 cps at 70°C. This agrees with the findings of Circle et al. (1964), who stated that heated dispersions exhibited greater viscosity than unheated dispersions at a given concentration. Knowledge of the flow properties and viscosity of protein dispersions are of practical significance in food processing and process design, new product development, designing of quality control tests, and mouth-feel and physical appearance.

Foaming capacity. Effect of pH on foaming capacity. The FC (percentage of foam) of KSPI as a function of pH (1–12) is shown in Figure 2. The minimum FC (16%) was observed at pH 3.0 near the iso-electric region, and high foams were formed in the extreme alkali region. The maximum FC (98.6%) for KSPI was noted at pH 12.0, which was similar to the value obtained by Holm and Breedon (1983) for sunflower protein.

Effect of NaCl on foaming capacity. The FC (percentage of foam) as a function of NaCl molarity in the range of 0.2–3.4 M NaCl is shown in Figure 3. There was a noticeable increase in FC (0.2–1.6 M NaCl) from 42% to 96%, followed by a considerable

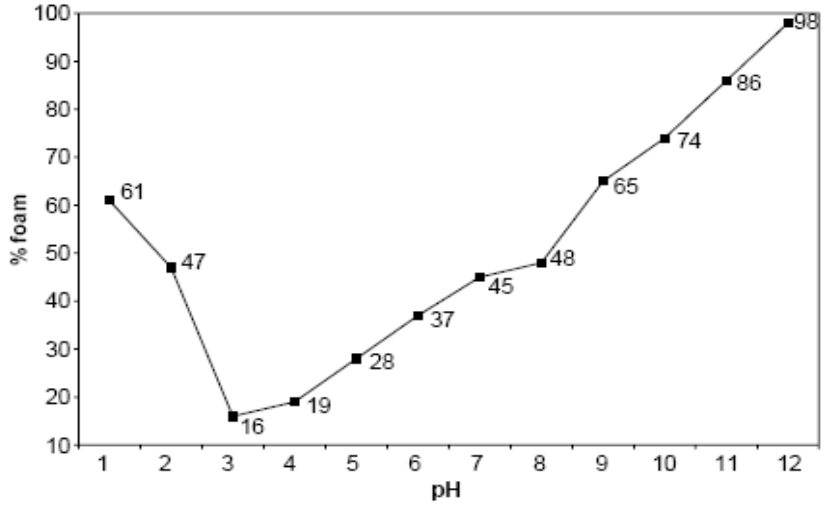

Figure 2. Foaming capacity of KSPI at different pH values.

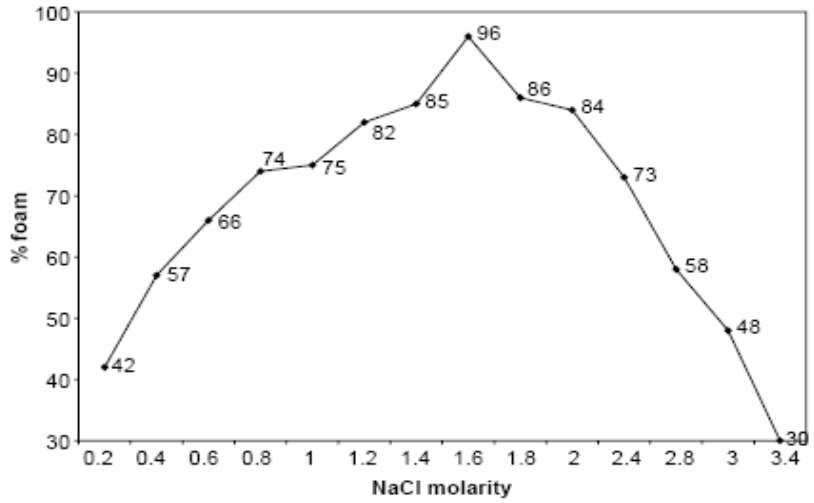

Figure 3. Foaming capacity of KSPI at different NaCl molarities.

decrease afterwards. Sathe and Salunkhe (1981) mentioned that addition of NaCl improved the FC of winged bean protein concentrate. They reported that maximum improvement in foaming was observed at a salt concentration of 0.8% (w/v) in the slurry. Such improvement in foaming due to addition of salt was attributed to increased protein solubility through partial denaturation (Sosulski 1977).

Foam stability. Effect of pH on foam stability. Figure 4 shows the FS of KSPI at pH 1.0, pH 7.0 and pH 12.0. After 150 min standing, the FS was 40%, 36% and 48%, respectively. The KSPI exhibited the highest FS value at pH 12.0, where it was 75% after 30 min standing, compared with 55% and 40% at pH 1.0 and pH 7.0, respectively. This indicates a weaker FS at neutral pH compared with alkaline and

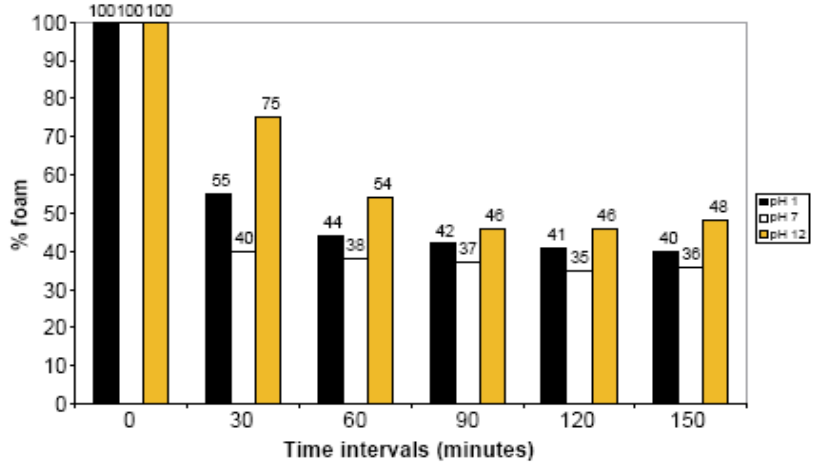

Figure 4. Foam stability of KSPI at selected pH values.

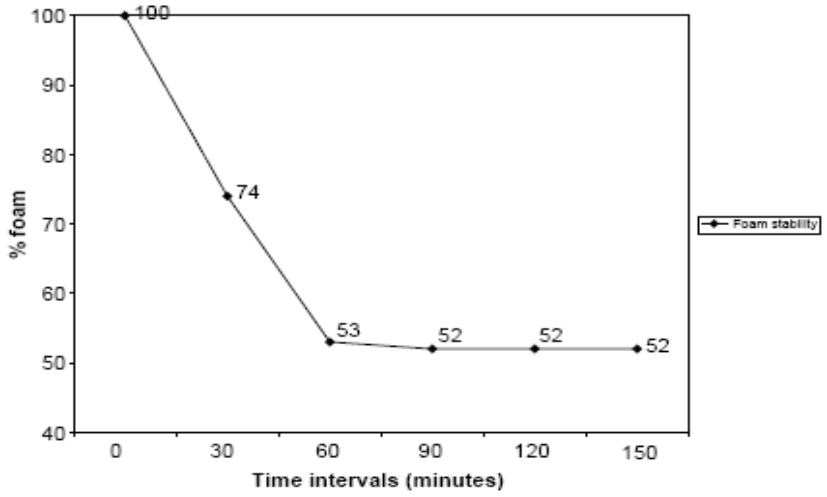

Figure 5. Foam stability of KSPI at 1.6 M NaCl.

acidic pH values. The maximum FS for sunflower seed protein was obtained at pH 9.0 (Huffman et al. 1975). The present investigation indicates that the KSPI exhibits stable foams especially at alkaline pH, and hence represents a useful foaming agent to be used in food systems.

Effect of NaCl on foam stability. The FS of KSPI in the presence of 1.6 M NaCl at different time intervals is shown in Figure 5. The FS of KSPI was considerably improved by treatment with NaCl. The FS was 74% after 30 min standing, almost similar to the stability produced earlier at pH 12, an extreme alkalinity practically not common in food systems.

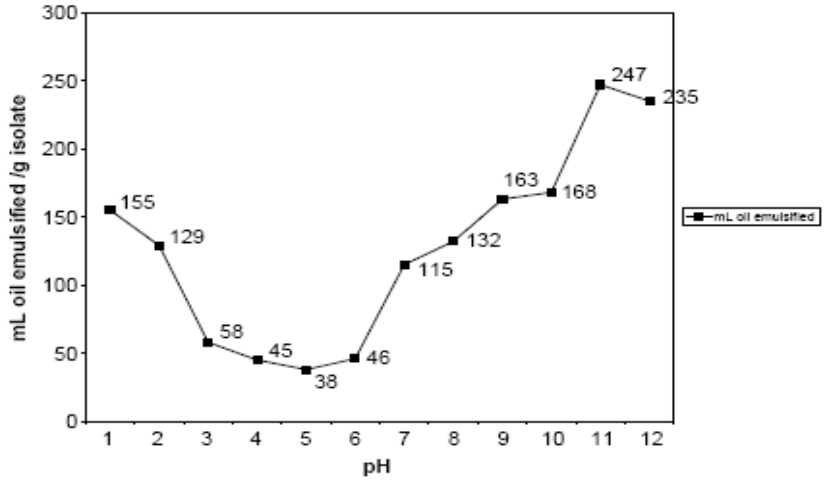

Figure 6. Emulsification capacity of KSPI at different pH values.

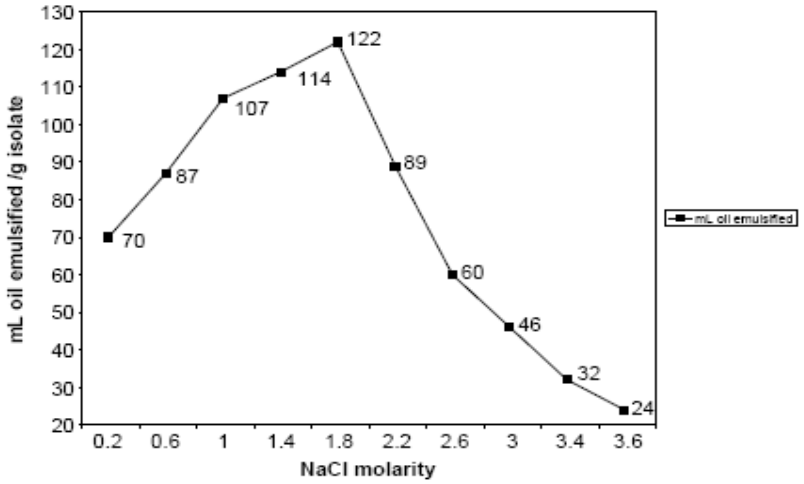

Figure 7. Emulsification capacity of KSPI at different NaCl molarities.

Emulsification capacity. Effect of pH on emulsification capacity. The EC of KSPI expressed as milliliters of oil emulsified by one gram of protein isolate as a function of pH is shown in Figure 6. The EC was found to be pH dependent. An alkaline pH improved the EC more than did acidic pH. Several investigators (Lin et al. 1974; Franzen and Kinsella 1976) have reported a similar pH dependence of EC of proteins. The EC of KSPI was found to be maximum at pH 11.0 (247 ml oil emulsified/g protein isolate), whereas a minimum EC value of 38 ml/g was obtained at pH 5.0, a level near to the isoelectric point. Similar results were obtained by Ramanatham et al. (1978) for groundnut proteins. At pH 1.0, the EC of the KSPI was found to be 155 ml/g. These results indicate that KSPI has good potential for use in the preparation of salad dressings and meat products.

Effect of NaCl on emulsification capacity. The effect of NaCl molarities in the range of 0.2–3.6 M on the EC of KSPI is shown in Figure 7. The EC of KSPI increased with increasing NaCl molarity up to 1.8 M (122 ml/g) and then decreased steadily to reach a minimum of 30 ml/g at 3.4 M NaCl.

Emulsifying, foaming and whipping properties are primary functional requirements in several food proteins. Surfactant properties are related to the capacity of proteins to lower the interfacial tensions between the hydrophobic and hydrophilic components in foods. Foaming or whipping (i.e. the capacity to form stable foams with air), is an important functionality of proteins in several products (e.g. angel food cakes, sponge cakes, confections, candy, meringue, soufflés, various whipped toppings, icings, fudges, nougats, etc.).

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