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Functional properties of fenugreek (*Trigonella foenum graecum*) protein concentrate

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Abstract

Proximate composition and physicochemical properties of a protein concentrate prepared from fenugreek seed were determined. The effects of pH and/or NaCl concentration on these properties were investigated. The protein content of fenugreek was found to be 28.4%. The crude fibre content was 9.3% and crude fat was 7.1%. The minimum protein solubility was observed at pH 4.5, which was 18.5%, while maximum protein solubility was observed at pH 11, which was 91.3%.

Measurement of emulsion and foaming properties of fenugreek protein concentrate showed that they were greatly affected by pH levels and salt (NaCl) concentration. The minimum values of both emulsion and foam properties were attained at pH 4.5 which was the isoelectric point of the protein; maximum values were obtained at pH 2 and pH 12. Results showed that fenugreek protein concentrate had high oil absorption capacity (1.56 ml oil/g protein), water absorption capacity (1.68 ml H_2O/g protein) and bulk density (0.66 g/ml).

Keywords: Fenugreek; Chemical composition; Physicochemical properties; Protein solubility; Foaming and emulsion properties

1. Introduction

In recent years, legumes are becoming an important protein source, for use as both functional food ingredients and nutritional supplements (Onweluzo, Obanu, & Onuha, 1994) together with green vegetables, which have been recognized as the cheapest and most abundant potential source of protein.

Plant proteins, to be effectively and successfully utilized in different food applications, should ideally possess several desirable characteristics, referred to as functional properties. There are many factors affecting the functional properties of proteins, such as size, shape and conformation. The method and condition of isolation of fat was also reported to affect the functional properties of protein (Finley, 1989; Luseisano, Pompeci, & Rossi, 1984).

Plant proteins are used in foods as functional ingredients to improve stability and texture as well as the nutritional quality of the product (Makri, Papalamprou, & Doxastakis, 2005).

Solubility of protein under varying conditions is one of its important functional properties, because this greatly influences other properties, such as emulsification, foaming and gelation; thus the protein may possess satisfactory properties, e.g. nutritional value, acceptable flavour, odour and texture (Kinsella, 1982).

The use of legume proteins is almost limited to the protein of soybean seed; studies should now focus on a search for proteins from other sources, such as fenugreek (*Trigonella foencem graecum*). It has historically been utilized mainly as whole seed; it is a potential protein source with high nutritive value. Fenugreek is an annual herb belonging to the legume family; it is widely grown in India, Egypt, and Middle Eastern countries (Flammang, Cifone, Ereson, & Stankowskci, 2004). The seeds are used as a cheap source of good quality protein. Salini and Sudesh (2004) reported that the addition of 10% of fenugreek flour to wheat flour increased protein content, fibre, total calcium and total iron; this indicates that fenugreek can be incorporated to

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prepare acceptable biscuits, and may also be mixed with cereals as a supplement for some limiting amino acids and hence for improving their protein quality through amino acid balance.

The objectives of this study were to determine the proximate composition of fenugreek, and to investigate the effect of pH and/or salt concentration on protein solubility and some of the functional properties of a protein concentrate from fenugreek seeds.

2. Materials and methods

2.1. Materials

Fenugreek (*T. foencem graecum*) seeds were obtained from the local market, Khartoum North, Sudan. The seeds were cleaned, then ground to a powder using an electric grinder to pass a 0.4 mm screen.

All chemical reagents used in this study were of reagent grade. Corn oil was obtained from Bittar Co. Ltd., Khartoum, Sudan.

2.2. Methods

2.2.1. Proximate composition

Official methods of the AOAC (1990) were used to determine proximate composition of fenugreek seed: drying at 105 °C for 24 h for moisture, incineration at 550 °C for ash, defatting in a Soxhlet apparatus with *n*-hexane for lipid, and micro-Kjeldahl for protein (NX6.25).

2.2.2. Nitrogen solubility

Nitrogen solubility of the proteins at 1:5 w/v was determined by a modification of the method of Were, Hettiarachchy, and Kalapathy (1997) for the determination pH-dependent solubility profile over a pH range from 1 to 11 using 0.5 M NaOH or 0.5 M HCl. The slurries were mixed for 1 h using a magnetic stirrer before centrifugation at 12,000*g* for 20 min; the supernatants were filtered through glass wool to obtain a clear solution. The protein content in supernatants was determined by the micro-Kjeldahl method. Triplicate determinations were carried out, and the solubility profile was obtained by plotting averages of protein solubility (%) against pH; the percent soluble protein was calculated as follows:

$$\text{Solubility } (\%) = \frac{\text{amount of nitrogen in supernatant}}{\text{amount of nitrogen in sample}} \times 100.$$

2.2.3. Preparation of protein concentrate

One hundred grammes of the defatted sample was extracted by blending with 1 M NaCl using a flour to solvent ratio of 1:10. The peptized liquor was centrifuged at 12,000*g* for 30 min. The extract was isoelectrically precipitated at pH 4.5. The protein was then allowed to dry in open air at room temperature for 24 h and then ground in an electric grinder (Moulinex) to pass through a 0.4 mm screen and stored in the refrigerator (5 °C) until used. Protein content of the concentrate was found to be 73.9% with a moisture of 5.3%.

2.2.4. Water- and oil-holding capacity

The method of Lawal, Adebowale, Ogunsanwo, Sosanwo, and Bankole (2004) was used with slight modification. One gramme of the samples was mixed with 10 ml of distilled water or corn oil and the samples were then allowed to stand at room temperature (30 + 2 °C) for 30 min before centrifuging at 5000*g* for 30 min. The volume of the supernatant was measured. The water-holding capacity was expressed as the number of grammes of water held by 1 g of protein. The oil-holding capacity was expressed as the number of grammes of oil held by 1.0 g of protein. The density of the oil was found to be 0.92 g/ml.

2.2.5. Emulsion measurements

Emulsification capacity (EC) was determined according to a modification of the method used by Bera and Mukherjee (1989); 1 g of the material was blended with 25 ml of distilled water for 30 s in a Moulinex blender; after complete dispersion, refined corn oil was added cautiously (0.4 ml/s) from a burette and blending continued until there was a phase separation (visual observation/change in the shaft sound). Emulsion stability (ES) and emulsion activity (EA) were determined using the method of Neto, Narain, Silva, and Bora (2001). Emulsion properties were determined as a function of pH (2, 4.5, 6, 8, 10, and 12) and NaCl concentration (0.2–2.0 M).

2.2.6. Foam measurements

Foam capacity (FC) and foam stability (FS) of fenugreek protein concentrate were determined according to the method of Aruna and Parakash (1993) with slight modification; 3 g of the protein sample were added to 100 ml of distilled water at different pHs or NaCl concentrations. The mixture was homogenized at 300 rpm for 5 min at 25 °C and then transferred to a measuring cylinder. The volume of foam at 30 s was calculated. The foam stability was determined by measuring the reduction in volume of foam as a function of time up to a period of 105 min.

2.2.7. Bulk density

The bulk density was determined according to the method of Onuma-Okezie and Bello (1988). Ten grammes of the sample were placed in a graduated cylinder and gently packed by tapping the cylinder on the bench top (10 times), the volume of the sample was recorded. Bulk density was expressed as g/ml of the material.

2.2.8. Dispersibility

The dispersibility was measured by the method of Karuna, Kulcarni, and Ingle (1991). A 10 g sample was placed

in a stopperd measuring cylinder and distilled water was added to reach a volume of 100 ml. The mixture was stirred vigorously and allowed to settle for 3 h; the volume of settled particles was subtracted from 100, and the difference was reported as percentage dispersibility.

2.2.9. Gelation properties

Gelling properties were determined by a slight modification of the method of Alfred and Hekoronye (1986). Gel strengths were evaluated by heating 5 ml samples of aqueous dispersions (2–10% protein), adjusting the pH to 4.5, 7 and 10 with 2 N HCl or 2 N NaOH and placing them in a boiling water bath for 1 h, followed by rapid cooling under tap water. Gel formation was evaluated by inverting the tubes containing the treated aqueous dispersions. Solidified concentrations were recoded as gelled. Effect of ionic strength was investigated by preparing sample suspensions (2–10% w/v) in NaCl solutions of known ionic strength of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2 M.

2.2.10. Statistical analysis

Triplicate samples were analyzed for each property and the figures were then averaged. Data were assessed by analysis of variance (ANOVA) (Snedecor & Cochran, 1987) and by the Duncan multiple range test with a probability $p \leqslant 0.05$ (Duncan, 1955).

3. Results and discussion

3.1. Proximate chemical composition

The proximate chemical composition of fenugreek seed is shown in Table 1. Crude protein was found to be 28.4%. This value is higher than that reported by Sayed, Tolba, and Habashy (1986, 25%), and those reported by Patil, Niphadkar, and Bapat (1997, 26.0%) and Mir et al. (1996, 21.7%) for fenugreek forage, but similar to that reported by Niknam, Sharifizdeh, Ebrahimzadeh, and Zarre (2003, 29.0%). Compared with other legumes, the amount of protein in the fenugreek seed was found to be higher. Oil content for fenugreek was found to be 7.1% which is similar to that reported by Hemavathy and Prabhakar (1988, 7.5%) and Brummer et al. (2003, 7.2%) but higher than that reported by Patil et al. (1997, 5.8%). The seed contains 3.3% ash and 47.4% carbohydrates.

Table 1
Chemical composition of fenugreek seeds (dry basis)[a]

Moisture (%)	6.87 (±0.08)
Crude lipid (%)	7.14 (±0.28)
Crude protein (%)	28.4 (±0.64)
Crude fibre (%)	9.30 (±0.09)
Ash content (%)	3.28 (±0.09)
Total carbohydrate (%)	47.4[b]

[a] An average of triplicate samples.
[b] Calculate by difference.

3.2. Nitrogen solubility

The nitrogen solubility as a function of pH is shown in Fig. 1. The data show three regions of nitrogen solubility, at acidic pH, near to the isoelectric point and at alkaline pH. The minimum nitrogen solubility was observed at pH 4.5, which was 18.5%, indicating the isoelectric point of the protein; in the acid media pH 1, 86.3% of protein was soluble. At pH 10, 84.7% of protein was soluble and 91.3% was soluble at pH 11. Similar observations were reported by several workers for legumes e.g. Sathe and Salunkhe (1981) for Great Northern bean, Lawal et al. (2004) for African locust bean and Mwanjala, Muhammad, Jamilah, and Yaakob (1999) for cowpea and pigeon pea. High protein solubility, in both the acid and alkaline pH regions, is an important characteristic in food formulation, as reported by Idouraine, Yensen, and Weber (1991). Seena and Sridhar (2005) reported that, at highly acidic and alkaline pHs, the protein acquires net positive and negative charges, respectively, which favour the repulsion of molecules and thereby increase the solubility of the protein.

3.3. Functional properties of fenugreek protein concentrate

3.3.1. Water- and oil-holding capacity

Fenugreek protein concentrate had a water-holding capacity of 1.68 ml H_2O/g of protein, as shown in Table 2. This value is lower than the value of 3.52 ml H_2O/g of protein reported by Abdelaal, Yousif, Shehata, and ELMahdy (1986) for Egyptian fenugreek which contained about 35.8% crude protein, but was similar to that reported by Seena and Sridhar (2005) for *Canavalia maritima* seeds which was 1.53 ml H_2O/g of protein and 1.68 ml H_2O/g of protein for cowpea protein isolate reported by Mwanjala et al. (1999). The water-holding capacity is a critical prop-

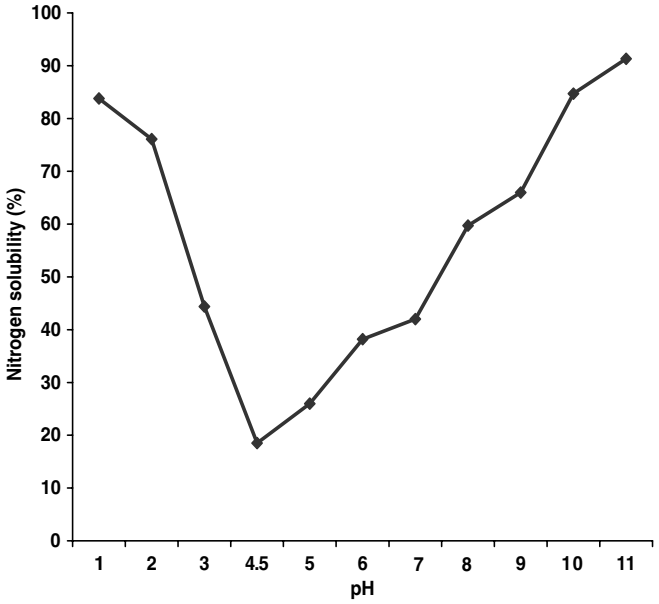

Fig. 1. Effect of pH on the nitrogen solubility of fenugreek protein.

Table 2
Functional properties of fenugreek protein concentrate

Functional property	Value[a] (±SD)		
Water-holding capacity (ml/g)	1.68 (±0.01)		
Oil-holding capacity (ml/g)	1.56 (±0.32)		
Bulk density (g/ml)	0.66 (±0.05)		
Dispersibility (%):			
pH	4.5	7	10
Value[a] (±SD)	41.30 (±1.79)	65.35 (±0.346)	69.2 (±0.96)

erty of proteins in viscous foods, e.g. soups, dough, custards and baked products, because these are supposed to imbibe water without dissolution of protein, thereby providing body, thickening and viscosity (Adeyeye, Oshodi, & Lpinmoroti, 1994; Seena & Sridhar, 2005).

The oil-holding capacity of fenugreek protein concentrate was found to be 1.56 ml oil/g of protein which is higher than that reported by Milan-Carrillo, Reyes-Moreno, Armirenta-Rodelo, Carabez-Trejo, and Mora-Escobedo (2000) for chickpea flour and that of *Mucuna pruriens* flour, as reported by Adeboeale, Adeyemi, and Oshodi (2005), but similar to that (1.66 ml oil/g of protein) reported by Mwanjala et al. (1999) for cowpea protein isolate.

The mechanism of fat/oil absorption capacity was explained by Kinsella (1979) as a physical entrapment of oil. Fat/oil absorption capacity is a critical determinant of flavour retention, while fat emulsion capacity and stability are important attributes of additives for the stabilization of fat emulsions. Chau and Cheung (1997) reported that surface area and hydrophobicity improve oil absorption capacity. Results obtained in this study indicate that fenugreek had good oil absorption capacity.

3.3.2. Dispersibility

The dispersibility of fenugreek protein concentrate is shown in Table 2. Highest dispersity was obtained at pH 10 (69.2%) while 65.35% at pH 7. The lowest dispersibility was 41.3% obtained at pH 4.5. Fenugreek proteins concentrate had good dispersibility in both acidic and basic media. Kinsella (1979) reported that high dispersibility enhances other functional properties like emulsifying and foaming properties during processing for the manufacture of cookies.

3.3.3. Gelation capacity

The effect of pH on the least gelation concentration of fenugreek protein concentrate is shown in Table 3a. The data show that a very weak gel was formed at 6% (w/v) at pH 4.5 and 7, while a weak gel was formed at pH 10. A strong gel was formed at 8%; the appearance of gel at 10% was very strong. No gel was formed at 2% and 4% (Table 3). This property is dependent on pH (especially at neutral and alkaline pHs) and protein concentration; this observation agrees with the finding of Lawal et al. (2004), who found that gelation was pH-dependent.

Table 3a
Effect of pH on the least gelation of fenugreek protein concentrate

pH	Concentration				
	2%	4%	6%	8%	10%
4.5	–	–	±	+	++
7	–	–	±	++	+++
10	–	–	±	++	+++

(–) No gel, (±) very weak gel, (+) weak gel, (++) strong gel, (+++) very strong gel.

Table 3b
Effect of NaCl concentrations on the least gelation of fenugreek protein concentrate

NaCl (M)	Concentration				
	2%	4%	6%	8%	10%
0.2	–	–	±	++	+++
0.4	–	–	±	+++	+++
0.6	–	–	±	±++	+++
0.8	–	–	++	+++	+++
1.0	–	–	+	++	+++
1.2	–	–	+	++	+++
1.4	–	–	+	++	+++
1.6	–	–	+	++	++
1.8	–	–	±	+	++
2.0	–	–	±	+	++

(–) No gel, (±) very weak gel, (+) weak gel, (++) strong gel, (+++) very strong gel.

Addition of NaCl (0.2–2 M) to the fenugreek protein concentrate at concentrations of 8% and 10% had a profound effect on the gel-forming ability of the protein, which was found to be strong (Table 3b). No gel was formed at 2% and 4% at any NaCl concentration (0.2–2.0 M). Similar results were reported by Ragab, Babiker, and El Tinay (2003) for cowpea protein isolate.

Gelation is not only an indication of protein quality but is related to type of protein. Sathe and Salunkhe (1981) reported that various factors affect the formation of gels, mainly non-protein components and protein solubility. Gelation is an important function in food formulation.

3.3.4. Emulsifying properties

Fig. 2 shows the effect of pH on emulsion capacity of the protein concentrate. This property was markedly affected by pH; minimum capacity (14.6 ml/g) was observed at pH 4.5, where proteins tend to precipitate, leading to reduction in emulsion formation. Maximum emulsion capacity of fenugreek protein concentrate was 135 ml of oil/g at pH 12; alkaline pHs improved emulsion capacity more than did acidic pHs. Halling (1981) has suggested that pH exerts its effect on emulsification properties primarily by altering the charge on protein molecules. Kiosseoglou, Doxastakis, and Alevisopoulos (1999) reported that the emulsifying properties of seed proteins are related to the processing procedure and to the protein composition. Several authors have reported that emulsion capacity depends on the hydrophobic-lipophilic balance, which is affected by pH.

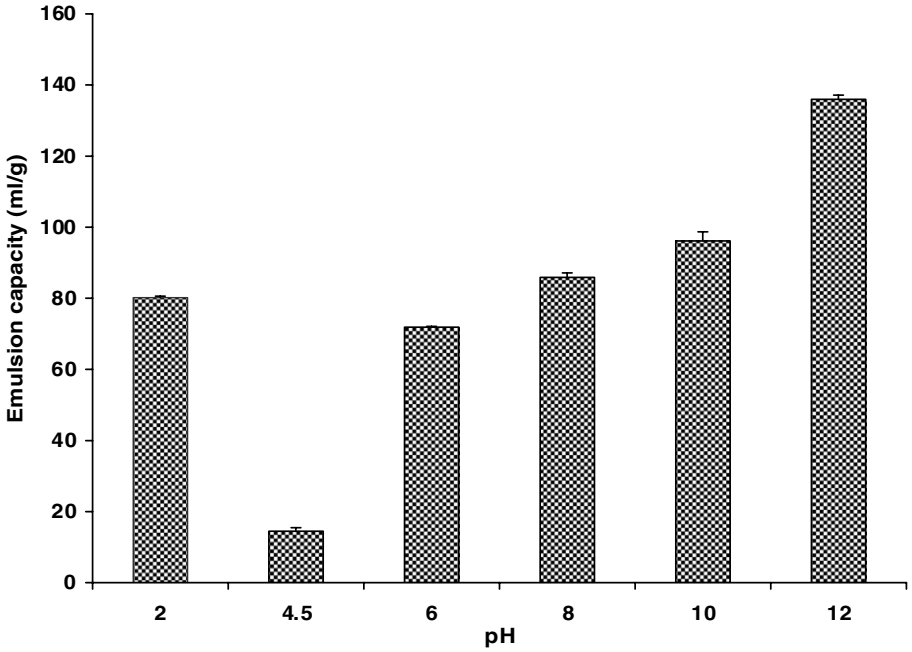

Fig. 2. Effect of pH on the emulsion capacity of fenugreek protein concentrate.

The effect of NaCl on the emulsion capacity of fenugreek protein concentrate is shown in Table 4. Addition of NaCl (0.6 M) to the fenugreek protein concentrate gave the maximum emulsion capacity (63.6%) while the minimum emulsion capacity was obtained at 2.0 M. Addition of NaCl enhances protein solubility by weakening the hydrophobic interactions of the protein. The fenugreek protein concentrate had a better EC, in both acidic and alkaline media, than in salt media.

The effects of pH on the emulsion activity (EA) and emulsion stability (ES) of fenugreek protein concentrate are shown in Tables 5 and 6. Emulsion activity and emulsion stability were affected by pH; at pH 2, EA and ES were 67% and 63%, respectively; these values were less than those (amounting to 85% and 74% for EA and ES, respectively) obtained by Bera and Mukherjee (1989) for rice bran protein concentrate. Poor emulsification properties of fenugreek may be due to the presence of carbohydrates.

Table 4
Effect of NaCl concentration on the emulsion capacity of fenugreek protein concentrate

NaCl (M)	Emulsion capacity (%)
0.2	61.0b (±0.912)
0.4	59.9b (±0.424)
0.6	63.6a (±1.83)
0.8	60.1b (±1.27)
1.0	60.2b (±0.412)
1.2	60.0b (±0.49)
1.4	58.8c (±0.458)
1.6	59.8b (±0.282)
1.8	51.5d (±0.495)
2.0	50.5d (±0.420)

Values are means of triplicate samples (±SD).
Means with the same letter are not significantly different at $p \leqslant 0.05$.

Table 5
Effect of pH on the emulsifying activity of fenugreek protein concentrates

pH	Emulsion activity (%)
2	67.0a (±0.00)
4.5	13.0e (±0.06)
6	57.5d (±1.34)
8	61.0c (±0.13)
10	61.5c (±1.426)
12	62.7b (±1.112)

Values are means of triplicate samples (±SD).
Means with the same letter are not significantly different at $p \leqslant 0.05$.

Table 6
Effect of pH on the emulsion stability of fenugreek protein concentrate

pH	Emulsion stability (%)
2	63.2a (±1.33)
4.5	15.3e (±1.092)
6	47.0d (±1.10)
8	61.0b (±0.13)
10	58.6c (±0.365)
12	57.1c (±1.65)

Values are means of triplicate samples (±SD).
Means with the same letter are not significantly different at $p \leqslant 0.05$.

McWatters and Cherry (1997), Nakai and Voutainas (1983) reported that emulsifying properties are not only dependent on the protein solubility but also on hydrophilic balance of the particular protein.

The effect of NaCl on the emulsion activity of fenugreek protein concentrate is shown in Table 7. Fenugreek protein concentrate had a maximum emulsion activity of 58% at 0.6 M NaCl, as it is known that, at low salt concentrations, most proteins are soluble (salting-in) and thus have maximum emulsion activity. Aluko and Yada (1995) reported

Table 7
Effect of NaCl concentration on emulsion activity of fenugreek protein concentrate

NaCl (M)	Emulsion activity (%)
0.2	51.1c (±1.642)
0.4	52.2c (±0.063)
0.6	58.8a (±0.774)
0.8	55.4b (±0.435)
1.0	56.1a (±2.771)
1.2	51.3c (±1.443)
1.4	50.6c (±0.268)
1.6	41.1e (±1.480)
1.8	48.5d (±0.790)
2.0	44.6d (±1.484)

Values are means of triplicate samples (±SD).
Means with the same letter are not significantly different at $p \leqslant 0.05$.

Table 8
Effect of NaCl concentration on foam capacity of fenugreek protein concentrate

NaCl (M)	Foam capacity (%)
0.2	50.6a (±0.424)
0.4	51.6a (±1.35)
0.6	50.0a (±1.04)
0.8	46.2b (±0.141)
1.0	45.0b (±1.37)
1.2	45.5b (±1.272)
1.4	43.6b (±2.532)
1.6	47.6b (±0.420)
1.8	42.2b (±1.680)
2.0	42.3b (±0.707)

Values are means of triplicate samples (±SD).
Means with the same letter are not significantly different at $p \leqslant 0.05$.

that the emulsifying activity of cowpea globulin isolate was higher at low NaCl concentrations at various pH values while, at high salt concentration, the protein precipitates (salting-out), showing a minimum emulsion activity of 41%, obtained at 1.6 M, which is lower than the 47% and 49% obtained by Mwanjala et al. (1999) for pigeonpea and cowpea, respectively. They also observed that no clear-cut relationship exists between varying NaCl concentrations and emulsifying properties of cowpea and pigeon pea protein isolates, but the effect of NaCl may still be due to differences in the subunit molecular weight distribution and amino acid composition of the seeds. Several other authors have reported that different factors may affect emulsifying properties, such as net charge, pH, interfacial tension, protein conformation and the effect of salting-in of NaCl (Hung & Zayas, 1991; Lawal et al., 2004).

3.3.5. Foaming properties

The foam capacity (FC) of fenugreek protein concentrate is shown in Fig. 3. This property is pH-dependent. The lowest FC (27.5%) was obtained at pH 4.5 (isoelectric point of the protein); at this point the molecules are in more compact form than at other pH values.

FC significantly ($p \leqslant 0.05$) increased, especially at pH 10 and 12, reaching 89.5% and 94.6%, respectively 111% for *Phaseolus angularis* reported by Chau and Cheung (1997). The basic requirements for a protein to be a good foaming agent are the ability to adsorb rapidly at the air–water

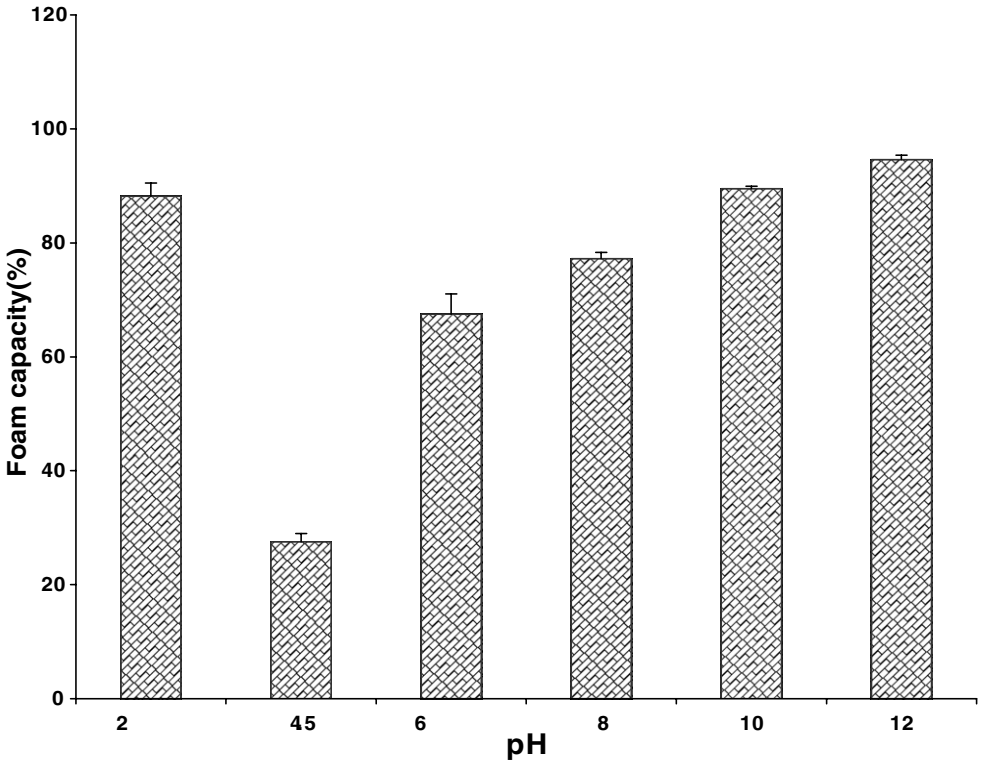

Fig. 3. Effect of pH on the foam capacity of fenugreek protein concentrate.

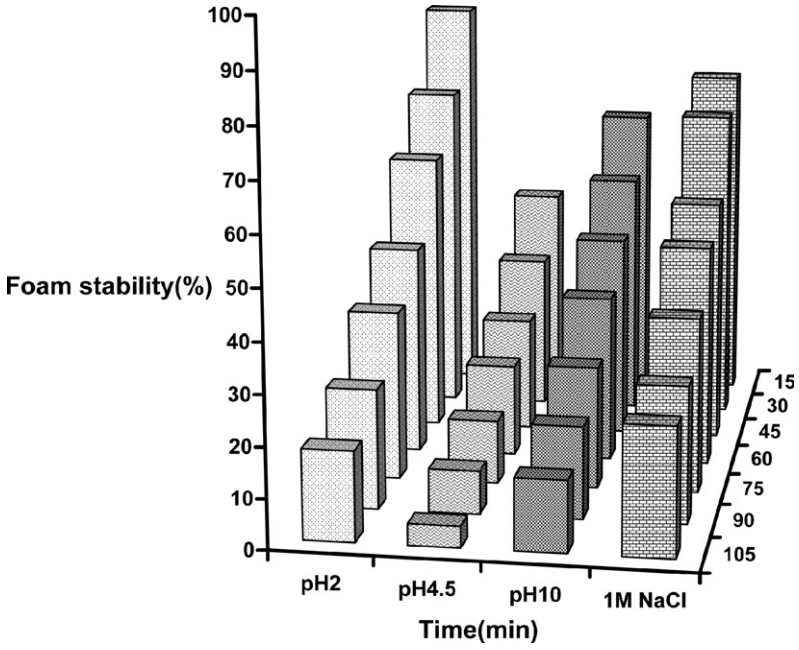

Fig. 4. Effect of pH and NaCl on foam stability of fenugreek protein concentrate.

interface during bubbling; and the ability to undergo rapid conformational changes and re-arrangement at the interface (Fidantsi & Doxastakis, 2001). The profile of FC against pH for fenugreek protein concentrate was more or less similar to the profile of nitrogen solubility of the seed due to an increase in the net charge of the protein which weakens hydrophobic interaction and increases protein solubility and flexibility, allowing the protein to spread to the air–water interface more quickly, encapsulating air particles and thus increasing foam formation, as reported by Lawal et al. (2004). Similar observations were reported by Aluko and Yada (1995).

Effects of salts depend upon their concentration; probably they affect foaming by enhancing solubility initially, whereas salting-out may occur when high concentrations are used. The maximum FC for fenugreek protein concentrate was observed at 0.4 M (51.6%), which is markedly and significantly ($p \leqslant 0.05$) reduced (Table 8). This may be attributed to the fact that addition of NaCl enhances the protein solubility, while higher salt concentrations decrease the FC due to salting-out of the protein. Seena and Sridhar (2005) reported that addition of salt improved the foam capacity to a maximum value of 30.5% at 0.4 M NaCl for *C. maritima* flour, due to the increased protein solubility.

The effect of pH and salt on foam stability, as a function of time, is shown in Fig. 4. The data show that the FS decreased with time. At pH 2 and pH 10, the foam stability significantly ($p \leqslant 0.05$) decreased and reached 17.9% and 14.2%, respectively, when the foam stood for 105 min; this property depend on pH and time.

Addition of salt (1.0 M) improved FS of the protein, due to increase in solubility and surface activity of the soluble protein.

4. Conclusion

The analytical data on crude protein content for fenugreek seeds suggests their high potential as a cheap source of alternative protein for human consumption.

The protein of fenugreek seeds was found to be more soluble at acidic and alkaline pHs than near neutral pH. Emulsifying and foaming properties for the concentrate were greater than those of other legumes, indicating an important role in food systems, such as salad dressing, comminuted meat, ice cream, cake batters and mayonnaise. The good fenugreek protein concentrate solubility can be of use for the production of beverages. It can also be used as supplement to enhance the low nitrogen content of traditional staples of cereals and tubers.

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