Studies on the functional properties of protein concentrate of *Kappaphycus alvarezii* (Doty) Doty – An edible seaweed

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ABSTRACT

Protein concentrate (PC) of *Kappaphycus alvarezii* (cultivated on the West coast of India), was extracted and its functional properties were evaluated. The *K. alvarezii* PC contained $62.3 \pm 1.62\%$ proteins. At pH 12, the nitrogen solubility of this PC was $58.72 \pm 1.68\%$ in the presence of 0.5 M NaCl. The emulsifying and foaming properties of this PC varied with time and pH. However, it formed remarkably stable emulsions with Jatropha oil after 720 min (i.e. $E_{720} = 53.67 \pm 1.59$). On the other hand, maximum foaming ability ($53.33 \pm 2.31\%$) of the PC was recorded at pH 4.0. This PC had high oil (1.29 ± 0.20 ml oil/g PC) and water absorption capacity (2.22 0.04 ml H_2O/g PC). DSC analysis revealed thermal transitions at about 109.25 °C at neutral pH. The results obtained in this investigation suggest the suitability of *K. alvarezii* PC as an inexpensive source of protein; thus this PC could be incorporated into several value-added food products.

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1. Introduction

Proteins are responsible for many of the functional properties that influence consumer acceptance of food products; therefore, they play a key role in food processing as well as in the development of food products (Ogunwolu, Henshaw, Mock, Santros, & Awonorin, 2009). Based on the source, food proteins could be roughly grouped into animal proteins (e.g. gelatin and milk protein) and vegetable proteins (e.g. soya protein, peanut protein and wheat protein). The availability, cost and the risk factors associated with diseases from animal protein sources make nutritionists consider alternative plant protein sources for human and feedstock preparation. Vegetable proteins have been prevalently used in various food applications, owing to their acceptable functional properties, such as emulsification, fat and water absorption, texture modifications, color control and whipping properties. In recent years, many plants have attracted a great deal of interest as a source of low-cost protein to supplement human diets. But although plant protein sources are generally cheaper as compared with the animal protein sources, these plant proteins supplements are lower in some essential amino acids, energy and minerals such as phosphorus when compared with animal protein supplements; at the same time, they constitute certain anti-nutritional factors (Yun et al., 2005). The production of plant protein concentrates

(PCs) is of growing interest to the food industry because of the increasing application of plant proteins in foods, especially in the developing countries. Plant PCs are quite extensively used in food to improve the nutritional quality of the product for economic reasons, or as a functional ingredient (e.g. the use of soya bean PCs and whey PCs (Wong, Cheung, & Ang, 2004). The demand for relatively inexpensive sources of proteins that are incorporated into value-added food products is increasing globally. It is essential to consider that plant protein sources are susceptible to climate change and require agricultural land; therefore, it is desirable to find economically viable alternatives like biomass from the marine environment.

Seaweeds are used extensively as food in coastal cuisines all around the world and have been traditionally used as food in Asia, however, their use as animal fodder has been popular in Norway (Slaski & Franklin, 2011). Approximately 250 species of seaweed have been commercially utilized worldwide, amongst which 150 species are favourably consumed as human food; however, in western countries they form a source of polysaccharides (such as agar, alginates, or carrageenans) for the food and pharmaceutical industry. From a nutritional point of view, edible seaweeds are a low calorie food, having great nutritional value owing to their vitamin, protein and mineral contents; apart from containing vitamins A, B_1, B_{12}, and C, they are also natural sources of hydrosoluble and liposoluble vitamins, such as thiamine and riboflavin, β-carotene and tocopherols (Kumar & Kaladharan, 2007). Furthermore, they possess long-chain polyunsaturated essential fatty acids from the

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omega-3 family (e.g. eicosapentaenoic acid), thereby having potential use in the development of low-cost, highly nutritive foods for human and animal consumption (Kumar & Kaladharan, 2007). In fact, due to their high essential amino acids content and relatively high level of unsaturated fatty acids, the quality of proteins and lipids in seaweeds are better suited for consumption as compared to other vegetables. Seaweeds are mostly used in human or animal foods for their mineral contents or for the functional properties of their polysaccharides, and are rarely promoted for the nutritional value of their proteins (Fleurence, 1999).

Recently, people have been looking into using seaweed as an economic alternative source for protein concentrates; additionally, reports suggest that the nutritional potential of seaweed as a source of protein has been known to vary with species (Fleurence, 1999). However owing to the complications of extraction and preparation of protein concentrates, very few studies are reported on the quality of seaweed protein (Wong, Cheung, & Ang, 2004); in fact, barely any research has been performed on the protein of seaweeds for human consumption. However, the ultimate success of utilizing any plant protein as food ingredients depends largely on its function l and nutritional properties. To increase its utilization, there is a need to process the whole seaweed biomass into high protein products such as protein concentrate and isolates, and then examine the suitability of these products for use as functional ingredients and food supplements. Studies on the functional properties of proteins, such as solubility, water/oil holding capacity, emulsifying activity, foaming ability and stability, viscosity, and gelation, which are in turn highly dependent on many factors such as pH and type and amount of salt present etc., have been reported by several authors (Ganesan, Kumar, & Subba Rao, 2012; Gbadamosi, Abiose, & Aluko, 2012). Rhodophtyic seaweeds possess good amount of protein (Wong, Cheung, & Ang, 2004); particularly *Kappaphycus alvarezii* (Doty) Doty constitutes significant amount of protein (Rajasulochana, Krishnamoorthy, & Dhamotharan, 2012). Moreover, this edible seaweed having multifarious food applications, is also used in pet food and as an aquaculture feed. However, research on the functional properties of the protein concentrate of this seaweed has not been undertaken and brought to the public domain. The present study therefore aimed to investigate the functional properties of protein concentrates from widely cultivated seaweed *K. alvarezii* (Doty) Doty, considering its use as an ingredient various in food formulations.

2. Materials and method

2.1. Sample preparation

Fresh *K. alvarezii* collected from cultivation farm, Port Okha (22°28.65′N and 69°04.01′E), Gujarat, Northwest coast of India, was sun-dried, and thoroughly washed with distiled water to remove epiphytes. This cleansed seaweed was then oven dried at 60 °C for 16 h to a constant weight. The dried moisture-free sample was then pulverized to obtain uniformly sized (0.5 mm) particles. The milled seaweed sample was then stored in airtight plastic bags in a desiccator at room temperature (25 °C) prior to extraction of the protein concentrate (PC).

2.2. Extraction of protein concentrate

K. alvarezii PC was extracted according to a slightly modified methodology of Fleurence, Le Coeur, Mabeau, Maurice, and Landrein (1995). In brief, seaweed powder was suspended in deionized water (1:20 w/v); this suspension was gently stirred overnight at 35 °C. After incubation, the suspension was centrifuged at 10,000*g* at 4 °C for 20 min. The supernatant was collected, and the pellet was re-suspended in de-ionized water in the presence of 0.5% (v/v) 2-mercaptoethanol. The pH of the mixture was then adjusted to 12 with 1 M NaOH. The mixture was gently stirred at room temperature for 2 h before centrifugation under the same conditions as stated above. The second supernatant was collected and combined with the previous supernatant. The combined supernatant was stirred at 0 ± 4 °C, and its pH was adjusted to 7 before precipitation with solid ammonium sulphate. This extraction procedure (mentioned above) was repeated five times on the residue. The seaweed PCs were precipitated from the supernatant by gently adding solid ammonium sulphate along with stirring until 85% saturation (60 g/100 ml) was reached. Then this mixture was allowed to stand for 30 min before centrifugation under conditions mentioned above. The pellet (PC) obtained was dialyzed against distiled water until the total dissolved solutes (TDS) (mg/l) of dialysate, were similar to those of the distiled water. Finally, the retentate containing the seaweed PCs were freeze-dried, powdered and stored in air-tight bags in desiccators before evaluation of its functional properties.

2.3. Determination of total protein content

The nitrogen content of the PC was determined by the Kjeldahl method (Wathelet, 1999) using a KEL PLUS-KES 20L Digestion unit attached to a KEL PLUS-CLASSIC DX Distillation unit (M/s PELICAN Equipment, Chennai, India); thereafter, the crude protein content of PCs was calculated by multiplying its nitrogen content by a factor of 6.25.

2.4. Nitrogen solubility

Nitrogen solubility was determined by the method of Bera and Mukherjee (1989). Here, PC samples (100 mg each) were dispersed in varying concentrations (0.1 and 0.5 M) of NaCl solutions as well as in 5 ml of distiled water. The pH of the mixture was adjusted to 2–12 using 0.1 N HCl or NaOH. Samples were shaken at 145 rpm for 30 min at room temperature and then centrifuged at 4000*g* for 30 min. Nitrogen content of the supernatants was determined by the Kjeldahl method, and percent nitrogen solubility was calculated as follows:

$$\text{Solubility } (\%) = \frac{\text{Amount of nitrogen in the supernatant}}{\text{Amount of nitrogen in protein concentrate}} \times 100 \tag{1}$$

2.5. Water-holding capacity (WHC)

Water-holding capacity (g of H_2O/g of PC) was determined using the method of Bencini (1986). Protein concentrate (0.5 g) was transferred into a pre-weighed 15 ml centrifuge tube and 10 ml of distiled water was added to it; this was then mixed at a high speed using a vortex mixer (Tarson, India) for 2 min. After the mixture was uniformly wet and consistent, it was allowed to stand at room temperature for 30 min, and then centrifuged at 2000*g* for 30 min. The supernatant obtained thereby was decanted and the centrifuge tube containing sediment was weighed. Water-holding capacity was calculated by the following formula.

$$\text{WHC } (g\ H_2O) = \frac{W_2 - W_1}{W_0} \times 100 \tag{2}$$

where W_0 is the weight of the dry sample (g), W_1 the weight of the tube plus the dry sample (g) and W_2 weight of the tube plus the sediment (g).

2.6. Fat absorption capacities (FAC)

In order to determine the fat absorption capacities (g of oil/g of PC), 1.0 g of PC sample was taken in a pre-weighed centrifuge tube and thoroughly mixed with 5 ml of sunflower oil. This protein–oil mixture was then centrifuged (3000g for 30 min); immediately after centrifugation, the supernatant was carefully removed and the tubes were weighed. FAC (grams of oil per gram of protein) was calculated as

$$FAC(g\ Oil) = \frac{F_2 - F_1}{F_0} \times 100 \qquad (3)$$

where F_0 is the weight of the dry sample (g), F_1 is the weight of the tube plus the dry sample (g) and F_2 is the weight of the tube plus the sediment (g).

2.7. Emulsifying and surface-active properties

Emulsifying activity was measured using a modified method of Cooper and Goldenberg (1987). Oil was added to the aqueous phase containing the protein concentrate (10 mg/ml); here the hydrocarbon:PC ratio was 3:2 (v/v). This mixture was stirred vigorously for 2 min on a cyclo-mixer and thereafter left undisturbed. The oil, emulsion and aqueous layers were measured at different time intervals and an emulsification index (E) was calculated as follows

$$Emulsifying\ index\ (E) = \frac{Volume\ of\ the\ emulsion\ layer}{Total\ volume\ of\ the\ mixture} \times 100 \qquad (4)$$

The emulsification index was noted with respect to time (15, 30, 90, 210, 390, 720 min) and was represented accordingly, i.e. the emulsification index after 15, 30, 90, 210, 390, and 720 min was represented as E_{15}, E_{30}, E_{90}, E_{210}, E_{390}, and E_{720}, respectively.

In order to understand the surface-active properties of the PC, the surface tension of 0.1 and 0.5% (w/v) PCs were determined using a Dataphysics Dynamic Contact Angle Meter and Tensiometer (DCAT 21) (Dataphysics Instruments GmbH, Germany) using a Wilhelmy plate (PT 11) made of platinum-iridium.

2.8. Foaming capacity and stability

A modified method of Nath and Rao (1981) was used to determine the foaming capacity and stability of the protein concentrate. A 100 ml solution of the protein concentrate (20 µg/ml) was vortexed for 5 min at room temperature and transferred to a measuring cylinder. The volume increase is expressed as percent foaming capacity.

$$Foaming\ capacity\ (\%) = \frac{V_2 - V_1}{V_1} \times 100 \qquad (5)$$

where V_1 is the volume of protein solution before whipping and V_2 is the volume of protein solution after whipping.

The foam stability was determined by measuring the decrease in volume of foam as a function of time up to a period of 90 min with an interval of 30 min at different pH level ranged from 2 to 10.

$$Foam\ stability\ (\%) = \frac{Volume\ after\ standing - Volume\ before\ whipping}{Volume\ before\ whipping} \times 100 \qquad (6)$$

2.9. Differential scanning calorimetric (DSC) analysis and thermal gravimetric analysis (TGA)

Differential scanning calorimetry (DSC) was carried out using a Mettler Toledo Star SW 7.01, according to the procedure of Meng and Ma (2001), with slight modifications. The protein sample (5 mg) was dissolved in 1 ml of 0.06 M phosphate buffer (pH 7.0) containing 0.1 M NaCl. A 45 µl sample of the protein solution was hermetically sealed in a stainless steel pan and was heated from 0 to 300 °C at a rate of 10 °C/min. The thermal properties were referenced against another pan containing 45 ml of buffer without protein. The denaturation peak temperature (dT) and enthalpy (DH) were calculated by a thermal analysis software programme. The temperature at which denaturation started, known as the onset denaturation temperature (T onset), was calculated by taking the intercept of the baseline and the extrapolated maximum slope of the peak. The peak denaturation temperature (T peak) was considered to be the temperature at maximum heat flow. The enthalpy of thermal denaturation was calculated from the area of the endothermic peak. Thermal gravimetric analysis (TGA) was carried with a Mettler Tole-doTGA/SDTA System (Greifensee, Switzerland) and the thermogram was obtained in the range of 30–480 °C at a rate of 10 °C/min.

2.10. FT-IR spectroscopy

The lyophilized protein concentrate was ground with potassium bromide at a 1/100 ratio (w/w). This protein concentrate was pressed at high pressure into a KBr pellet. The spectral analysis was carried out using NXR FT-IR module (Thermo electron corporation, USA). The FT-IR spectrum of sample was recorded in the 4000–400 cm^{-1} region at room temperature.

2.11. Statistical analysis

Analysis of variance (ANOVA) was conducted to determine the differences amongst all treatments and multiple comparison tests with the least significance difference (LSD) were performed to determine significant differences.

3. Result and discussion

3.1. Protein content, recovery and yield of K. alvarezii

K. alvarezii biomass constituted 18.16 ± 0.03% total protein determined on dry weight which is analogous to the report of Rajasulochana, Krishnamoorthy, and Dhamotharan (2012) who reported 18.78% protein content for the same species. The protein content of *K. alvarezii* obtained herein was comparable or in fact higher than that reported for seaweeds such as *Caulerpa veravelensis* (7.77 ± 0.59%), *Caulerpa scalpelliformis* (10.50 ± 0.91%), and *Caulerpa racemosa* (12.88 ± 1.17%), *Plocamium brasiliense* (15.72%), *Ochtodes secundiramea* (10.10%,), *Laminaria japonica* (9.1%), *Palmaria palmate* (13.5%) and *Ulva rigida* (17.8%) (Gressler et al., 2011; Kumar, Gupta, Kumari, Reddy, & Jha, 2011).

Using the ammonium sulphate precipitation method, 7.81 ± 2.42% of protein concentrate could be recovered; this PC was comprised of 62.3 ± 1.62% protein. The protein content of the PC was much higher than that reported by Ganesan et al. (2012); they studied total protein content and protein contents in PC of *Enteromorpha tubulosa* (19.09 ± 0.91; 53.83 ± 0.70%), *Enteromorpha compressa* (17.48 ± 0.41; 60.35 ± 2.01%), and *Enteromorpha linza* (12.5 ± 1.26; 33.36 ± 1.04%) and could recover up to 6.16, 6.48, and 5.71% of PC, respectively. The values were also higher than

those obtained by Wong and Cheung (2001) who studied the total protein content and protein content of the PC from *Hypnea charoides* (18.13 ± 0.29; 55.4 ± 0.63%, respectively), *Hypnea japonica* (19.4 ± 0.33; 56.67 ± 0.25%) and *Ulva lactuca* (7.13 ± 0.21; 50.87 ± 0.5%).

3.2. Nitrogen solubility

The effect of pH and salt concentration on nitrogen solubility of *K. alvarezii* PC is elaborated in Fig. 1. The minimum nitrogen solubility (33.72 ± 1.23%) was observed at pH 4; this might be due to the fact that the isoelectric point (pI) of the protein might be corresponding here. Sorgentini and Wagner (2002) suggest that the majority of the food proteins are acidic and exhibit minimum solubility at pH 4–5 and maximum solubility at alkaline pH. Not much difference in nitrogen solubility values were noticed at pH 8 and 10. The PC showed only a gradual increase in solubility from pH 8 to 12 in water as well as NaCl concentrations. However, at pH 12, *K. alvarezii* PC reached 58.72 ± 1.68% solubility in the presence of 0.5 M NaCl, which was comparatively higher than PC of *E. tubulosa* (13.60 ± 0.85–25.41 ± 1.94%), *E. compressa* (14.96 ± 0.35–26.38 ± 0.88%) and *E. linza* (10.87 ± 1.12–20.31 ± 1.66%) (Ganesan et al., 2012); it was also lower than that reported for fenugreek PC (86.3% at pH 10) (El Nasri & El Tinay, 2007). According to Seena and Sridhar (2005), at highly acidic and alkaline pHs, the protein acquires a net positive and negative charge, respectively, which favors the repulsion of molecules and thereby increases the solubility of the protein. The solubility is a physico-chemical property of a protein that critically affects its functional properties as manifested in foods, mainly emulsifying, foaming and gel forming abilities. On this accord, *K. alvarezii* PC demonstrated remarkable solubility values in the presence of salt at varying pH. It could be stated that this PC would definitely hold a promising market in various food products.

3.3. Water-holding and fat absorption capacity (WHC and FAC)

The water-holding capacity of *K. alvarezii* PC was 2.22 ± 0.04 g water/g of PC; this was higher than that reported for *E. compressa* (1.53 ± 0.07 g water/g PC), *E. tubulosa* (1.32 ± 0.11 g water/g PC) and *E. linza* (1.22 ± 0.06 g water/g PC) (Ganesan et al., 2012); however, this value was lower than that reported for the protein concentrate of Egyptian fenugreek (3.52 ml water/g of protein)
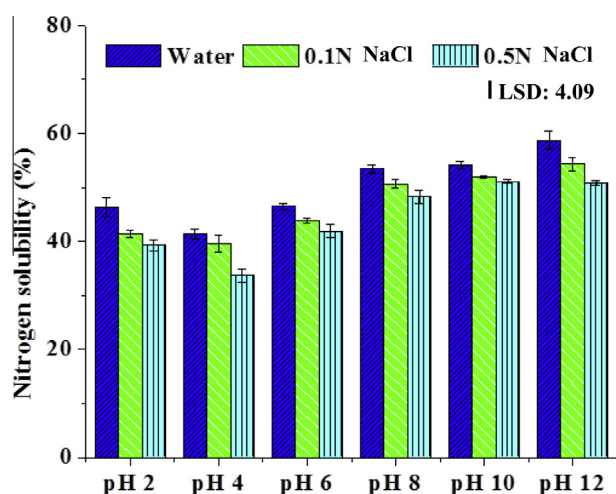
(Abdel-Aal, Yousif, Adel-Shehata, & El-Mahdy, 1986). High water absorption of proteins helps to reduce moisture loss in packaged bakery goods; moreover water-holding is indispensably required to maintain freshness and a moist mouth feel of baked foods. The water-holding capacity is a critical property 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 (Cooper & Goldenberg, 1987; Ganesan et al., 2012). However, the intrinsic factors affecting the water-binding capacity of food proteins include amino acid composition, protein conformation, and surface polarity/ hydrophobicity. The very fact that *K. alvarezii* PC has tremendous water-holding capacity, suggests its appropriateness in being used in various delicacies requiring moist foods.

The fat absorption capacity of *K. alvarezii* PC (1.29 ± 0.20 g oil/g of protein) was slightly lower than that of *E. compressa* (1.34 ± 0.10 g oil/g PC) and fenugreek (*Trigonella foenum graecum*) protein concentrate (1.56 g oil/g PC); but it was slightly higher than that reported for *E. linza* (1.05 ± 0.07 g oil/g PC) and *E. tubulosa* (1.08 ± 0.04 ml oil/g PC) (El Nasri & El Tinay, 2007; Ganesan et al., 2012). In food systems, good interactions of water and oil with proteins are imperative as this would indirectly affect the flavour and texture of foods. However, food processing methods have important impacts on the protein conformation and hydrophobicity. The fat/oil holding/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. High oil absorption/holding is requisite for the formulation foods such as sausages, cake batters, mayonnaise and salad dressings (Chandi & Sogi, 2007).

As the PC of *K. alvarezii* demonstrated acceptable water-holding and oil-holding capacity, it could be definitely be used for multiple food applications such as water-holding, or as a texture enhancer. It could also be used suitable for improving the viscous nature of food formulations.

3.4. Emulsifying and surface active properties

Emulsifying properties of food proteins can be determined by studying by the emulsifying activity (EAI) and emulsifying stability (ESI) indices. The emulsifying activity (EAI) is a measure of the capacity of the protein to help formation and stabilization of the emulsion created, while ESI provides a measure of the ability of the protein to impart strength to the emulsion to resist changes to its structure (e.g., coalescence, creaming, flocculation or sedimentation) over a defined time period (Boye et al., 2010). *K. alvarezii* PC efficiently emulsified oils such as silicone oil, paraffin oil, groundnut oil, cotton seed oil, jatropha oil, cedar wood oil, jojoba oil, sunflower oil and olive oil. The emulsification indices of *K. alvarezii* PC with various oils are shown in Fig. 2; here, maximum emulsification indices were observed with cedar wood oil (99.67 ± 0.58), jatropha oil (99.33 ± 1.15) and olive oil (99 ± 1.73) after 15 min. Moreover, this PC showed good emulsifying activity with groundnut oil (77 ± 1.00) and cotton seed oil (75.68 ± 0.58) after 15 min. Formation of stable emulsions was observed using cedar wood oil (E_{720} = 75.33 ± 2.08), olive oil (E_{720} = 54.33 ± 1.16) and jatropha oil (E_{720} = 53.67 ± 1.59) at 10 mg PC/ml concentration, the emulsion stability of *K. alvarezii* PC would probably depend on the strength of the hydrophilic and hydrophobic properties of proteins, rather than the balance between hydrophilic and lipophilic phases. Reports suggest that the hydrophobic lipid portions in an emulsion are generally responsible for emulsifying action (Gbadamosi et al., 2012). In this study, the *K. alvarezii* PC not only formed emulsions with superior emulsifying indices, but also demonstrated stable emulsions with various oils including edible oils. This observation is essential for its application as an emulsifier. Dickinson, Galazka,
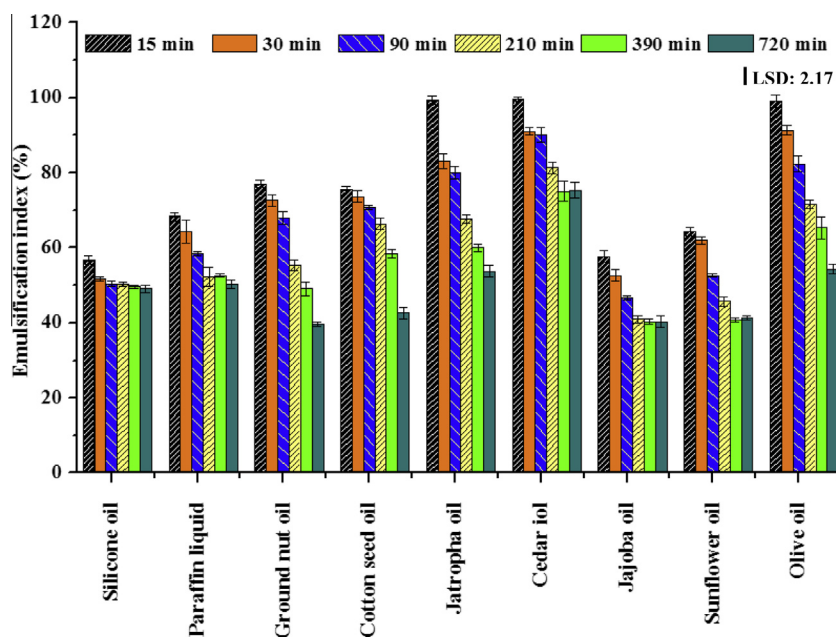

Fig. 1. Influence of pH and ionic strength contributed by NaCl on nitrogen solubility of *K. alvarezii* protein concentrate.

Fig. 2. Emulsifying index of *K. alvarezii* protein concentrate using different oils and emulsion stability.

and Anderson (1991) suggest that the emulsifying activity of products often depend to a great extent on the nature and concentration of the protein present in it (e.g. acacia gums). Moreover, a high percentage of hydrophobic amino acids in the protein moiety favour emulsification (Dickinson et al., 1991). Thus, in the present study, the amount of protein present in the concentrate might be responsible for the formation of stable emulsions.

The surface tension of distiled water (which was used as a reference or control, i.e. without any surface-active agent) was 72.05 ± 0.04 mN/m, while the surface tension of 0.1% and 0.5% of *K. alvarezii* PC was 50.10 ± 0.03 and 44.02 ± 0.03 mN/m, respectively; this was comparable with milk proteins in various products such as Prolacta 90, Promilk 852 FB, Promilk 852 B and skimmed milk powder, as well as sodium caseinate (46.1, 46.2, 49.4, 48.4, and 46.4 mN/m, respectively). Emulsifiers are most effective and stable in lowering surface tension (Rouimi, Schorsch, Valentini, & Vaslin, 2005); therefore the very fact that the *K. alvarezii* PC was surface active and could lower the surface tension of distiled water, qualifies it as a emulsifying agent. There are several reports available on the use of several biological products of animal, plant and bacterial origin for the reduction of surface tension, however, the surface tension properties of seaweed protein has hardly dealt with.

3.5. Foaming capacity (FC) and stability (FS)

The foaming capacity (FC) of PC investigated herein was pH-dependent (Fig. 3); lowest FC (38 ± 2%) was recorded at pH 6.0. On the other hand highest FC (53.33 ± 2.31%) was obtained at pH 4.0; this value was slightly lower than that of *E. compressa* (55.0 ± 2.6%; Ganesan et al., 2012), and much lower than the fenugreek protein concentrate i.e. 89.5% (El Nasri & El Tinay, 2007). It was much higher than that of *E. tubulosa* (31.9 ± 2.7%) and *E. linza* (33.3 ± 5.7%) (Ganesan et al., 2012). Here, maximum foaming stability (FS) (45.33 ± 1.15) recorded after 30 min at pH 2.0 was higher than *E. compressa* (37.5 ± 2.0%), *E. tubulosa* (16.7 ± 1.5%) and *E. linza* (4.4 ± 2.0) (Ganesan et al., 2012). The basic requirements for a protein to be a good foaming agent are the ability to rapidly adsorb at the air–water interface during bubbling and the ability to undergo rapid conformational changes at the interface. Furthermore, the
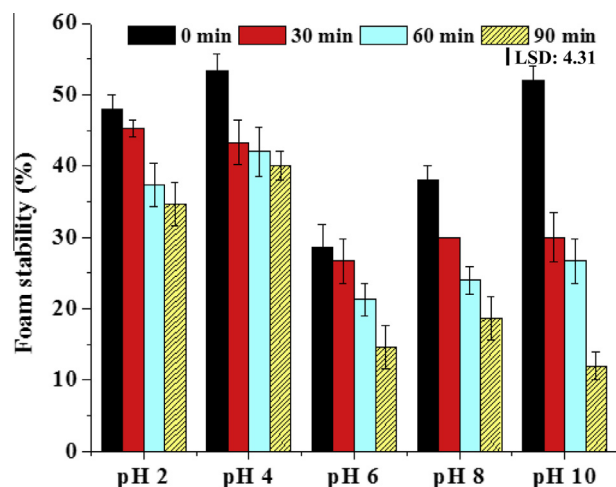

Fig. 3. Effect of pH and time on the foaming stability of *K. alvarezii* protein concentrate.

high foaming ability mainly depends on the protein dispersing ability, but stability of foaming is primarily influenced by the degree of denatured protein (Fidantsi & Doxastakis, 2001).

3.6. Differential scanning calorimetric (DSC) analysis and thermal gravimetric analysis (TGA)

It is known that the formation of unique structures of biological macromolecules, such as proteins and their specific complexes is, in principle, reversible, and the reactions are thermodynamically driven; therefore, thermodynamic investigations of these processes are of high priority (Gill, Moghadam, & Ranjbar, 2010). DSC is a rapid and easy technique for supplying both thermodynamic (heat capacity, enthalpy and entropy) and kinetic data (reaction rate and activation energy) on protein denaturation, and has been used extensively in various food systems. The information on protein thermal properties is useful for food-processing strategies and heat-processing design (Ju, Hettiarachchy, & Rath, 2001).
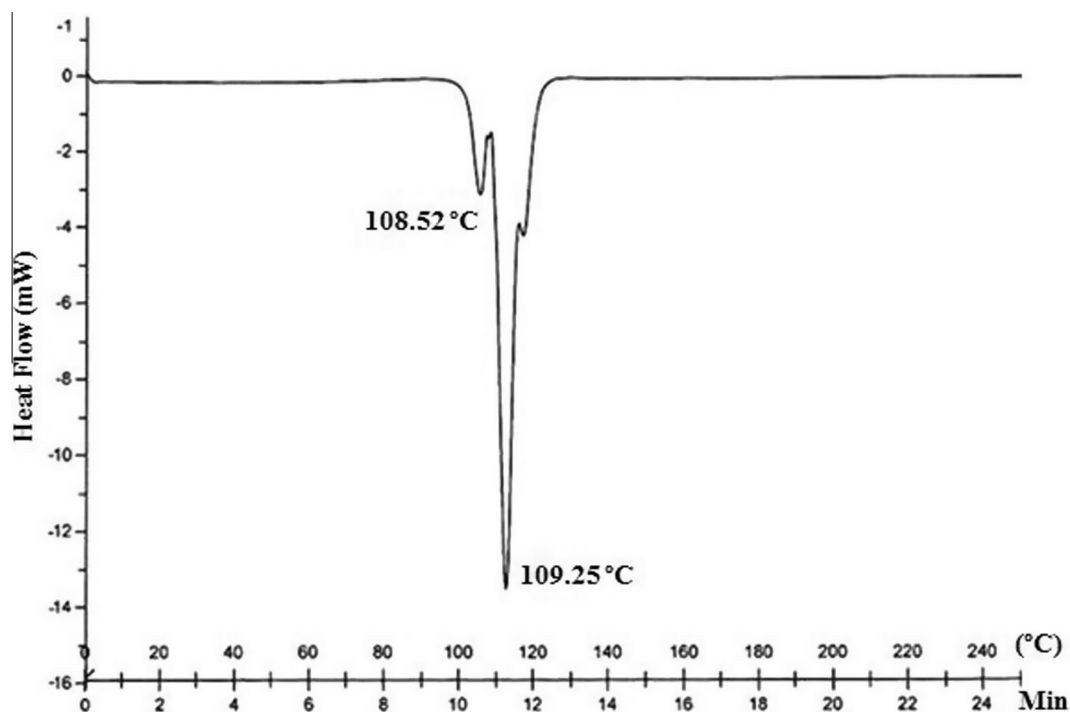
Fig. 4. Typical DSC thermograms of *K. alvarezii* protein concentrate.

Fig. 4 shows typical DSC thermograms of *K. alvarezii* protein concentrates in 0.06 M phosphate buffer (pH 7.0). The protein sample was heated from 0 to 300 °C at 10 °C/min. The PC exhibited two observable endothermic peaks; the minor endothermic peak temperature (T_m) at about 108.52 °C and the major one at 109.25 °C (T_M) and the enthalpy of the thermal denaturation was ΔH – 5.3 J/mg. It has been demonstrated that the enthalpy change (ΔH) reflects the status of ordered conformation of food proteins (Koshiyama, Hamano, & Fukushima, 1981). Therefore, the "net" ΔH indicates cumulative effects of endothermic events, such as the breakdown of hydrogen bonds and exothermic phenomena such

as aggregation of food proteins due to hydrophobic interactions (Murray, Arntfield, & Ismond, 1985). The appearance of the minor endothermic peak shoulder might be due to the presence of carrageenan and the second big transition peak presumably represents the denaturation of a high percentage of *K. alvarezii* PC. A similar trend has been observed in a mixture of whey protein isolate (WPI) and the hydrocolloid *i*-carrageenan, where the thermogram showed two transition peaks at 52 and 78.5 °C (Ibanoglu, 2005); however, in that report the first transition peak observed in the WPI + *i*-carrageenan mixture appeared in the presence of carrageenan, while the second big transition peak in both thermograms
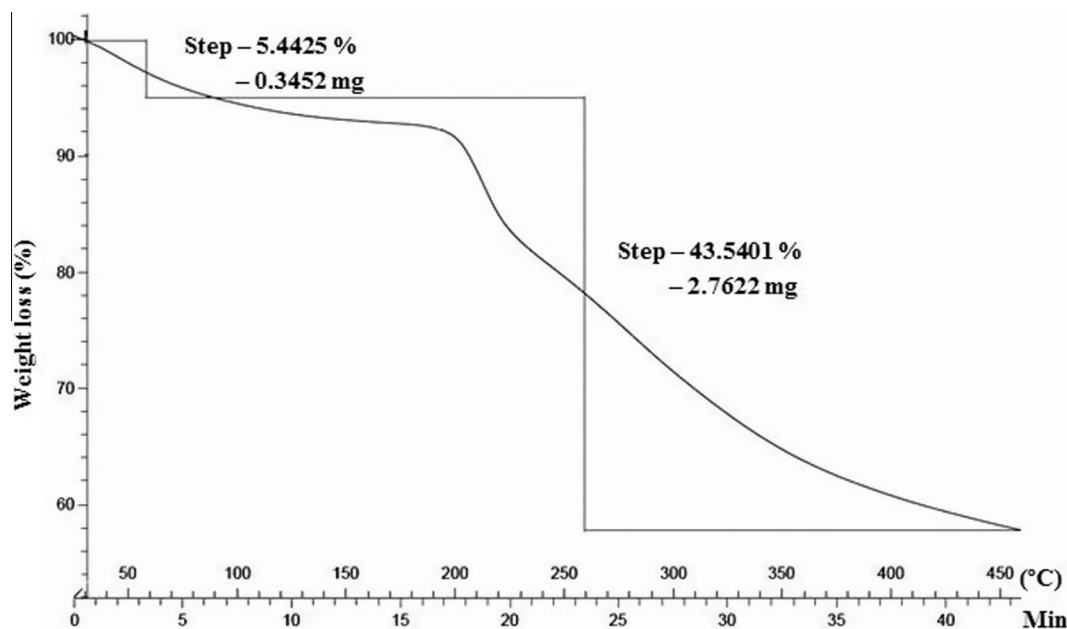

Fig. 5. Typical TGA thermogram of *K. alvarezii* protein concentrate.
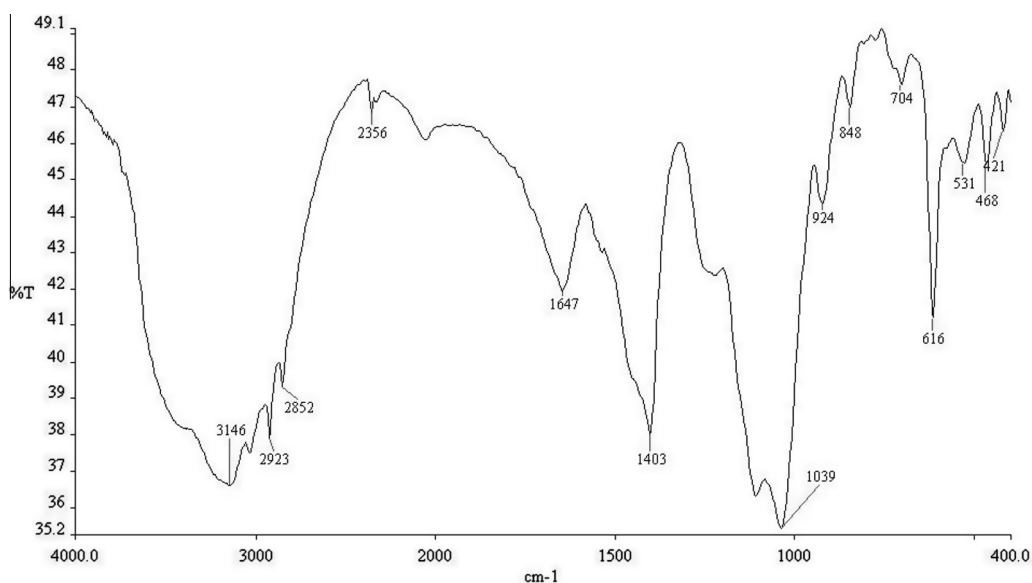
Fig. 6. FT-IR Spectrum of *K. alvarezii* protein concentrate.

probably represented the denaturation of a high percentage of this protein (Ibanoglu, 2005).

TGA carried out dynamically between weight loss vs. temperatures have been elucidated in Fig. 5. TGA shows that degradation of *K. alvarezii* protein concentrate takes place in two steps: here, the 5.4% weight loss of total PC was recorded from 30 to 100 °C, which could be due to moisture content, thereafter a second phase of degradation (43.5%) was observed with maximum weight loss at 260 °C. However, the total weight loss of PC occurred on further increase of temperature. This is chiefly associated with degradation of the major protein component of *K. alvarezii* PC.

3.7. FT-IR spectroscopy

Proteins are frequently referred to as having a certain fraction of structural components (α-helix, β-sheet, etc.); however, the secondary structural composition is some of the most important information for a structure-unknown protein. Therefore estimation of protein secondary structure is one of the major applications of the FT-IR technique (Kong & Yu, 2007). FT-IR spectra provides information about the structural composition of proteins. The spectrum of the PC (Fig. 6) showed a band at 616 cm^{-1} which could be due the presence of phosphate group, while a stretching band at 704 cm^{-1}, reveals out of plane N–H bending (El-Bahy, 2005; Jung, Stuehr, & Ghosh, 2000). Kumar and Kaladharan (2007) discretely describe *K. alvarezii* to contain numerous amino acids including 0.08% histidine; however, they further emphasize on the utility of seaweeds as a potential low-cost source of protein for fish.

4. Conclusion

Protein concentrates and isolates prevalently used in food industry are mainly derived from dairy, soy or wheat; however, certain reports suggest that these could trigger allergic responses. The nutritional quality of plant proteins is lower than animal protein; moreover, plant PCs could at times possess anti-nutritional factors. Therefore, food manufacturers, as well as consumers, are looking for alternative protein sources which could be economically viable and available all year round. Keeping in mind that seaweeds have been used as a traditional food for many years, it could be stated that seaweeds are probably the best alternative in this regard. It should also be noted that for seaweeds, data regarding anti-nutritional factors are virtually non-existent. In addition to providing nutrition, it is necessary that the proteins being used in food formulations should possess specific functional properties that facilitate processing and serve as the basis of product performance. Thus, in order to demonstrate the applicability of seaweed PCs in the food formulations, in-depth studies need to be carried out in this regard. On this consensus, the *K. alvarezii* protein concentrate studied herein demonstrated admirable functional properties at par with most other protein concentrates; furthermore, its efficacy to be used at varying pH ranges and in the presence of salt indeed qualifies this PC to be incorporated into several value-added food products.

References

Abdel-Aal, E. S. M., Yousif, M. M., Adel-Shehata, A., & El-Mahdy, A. R. (1986). Chemical and functional properties of some legumes powder. *Egyptian Journal of Food Science, 13*, 201–205.

Bencini, M. C. (1986). Functional properties of drum dried chickpea (*Cicer arietinum* L.) flour. *Journal of Food Science, 51*, 1518–1526.

Bera, M. B., & Mukherjee, R. K. (1989). Solubility, emulsifying, and foaming properties of rice bran protein concentrates. *Journal of Food Science, 54*, 142–145.

Boye, J. I., Aksay, S., Roufik, S., Ribéreau, S., Mondor, M., Farnworth, E., et al. (2010). Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. *Food Research International, 43*, 537–546.

Chandi, G. K., & Sogi, D. S. (2007). Functional properties of rice bran protein concentrates. *Journal of Food Engineering, 79*, 592–597.

Cooper, D. G., & Goldenberg, B. G. (1987). Surface-active agents of two *Bacillus* species. *Applied and Environmental Microbiology, 53*, 224–229.

Dickinson, E., Galazka, V. B., & Anderson, D. M. W. (1991). Emulsifying behavior of gum Arabic. Part I—Effect of the nature of the oil phase on the emulsion droplets. *Carbohydrate Polymer, 14*, 373–383.

El Nasri, A. H., & El Tinay, A. H. (2007). Functional properties of fenugreek (*Trigonella foenum graecum*) protein concentrate. *Food Chemistry, 103*, 582–589.

El-Bahy, G. M. S. (2005). FTIR and Raman spectroscopic study of Fenugreek (*Trigonella foenum graecum* L.) seeds. *Journal of Applied Spectroscopy, 72*, 111–116.

Fidantsi, A., & Doxastakis, G. (2001). Emulsifying and foaming properties of Amaranth seed protein isolate. *Journal of Colloids and Surfaces, 21*, 119–124.

Fleurence, J. (1999). Seaweed proteins: Biochemical, nutritional aspects and potential uses. *Trends in Food Science and Technology, 10*, 25–28.

Fleurence, J., Le Coeur, C., Mabeau, S., Maurice, M., & Landrein, A. (1995). Comparison of different extractive procedures for proteins from the edible seaweeds *Ulva rigida* and *Ulva rotundata*. *Journal of Applied Phycology, 7*, 577–582.

Ganesan, K., Kumar, K. S., & Subba Rao, P. V. (2012). Salt- and pH-induced functional changes in protein concentrate of edible green seaweed *Enteromorpha* species. *Fisheries Science, 78*, 169–176.

Gbadamosi, S. O., Abiose, S. H., & Aluko, R. E. (2012). Amino acid profile, protein digestibility, thermal and functional properties of Conophor nut (*Tetracarpidium conophorum*) defatted flour, protein concentrate and isolates. *International Journal of Food Science and Technology, 47*, 731–739.

Gill, P., Moghadam, T. T., & Ranjbar, B. (2010). Differential scanning calorimetry techniques: Applications in biology and nanoscience. *Journal of Biomolecular Techniques, 21*, 167–193.

Gressler, V., Fujii, M. T., Martins, A. P., Colepicolo, P., Mancini-Filho, J., & Pintoa, E. (2011). Biochemical composition of two red seaweed species grown on the Brazilian coast. *Journal of the Science of Food and Agriculture, 91*, 1687–1692.

Ibanoglu, E. (2005). Effect of hydrocolloids on the thermal denaturation of proteins. *Food Chemistry, 90*, 621–626.

Ju, Z. Y., Hettiarachchy, N. S., & Rath, N. (2001). Extraction, denaturation and hydrophobic properties of rice flour proteins. *Journal of food science, 66*, 229–232.

Jung, C., Stuehr, D. J., & Ghosh, D. K. (2000). FT-infrared spectroscopic studies of the iron ligand CO stretch mode of iNOS oxygenase domain: Effect of arginine and tetrahydrobiopterin. *Biochemistry, 39*, 10163–10171.

Kong, J., & Yu, S. (2007). Fourier transform infrared spectroscopic analysis of protein secondary structures. *Acta Biochimica et Biophysica Sinica, 39*(8), 549–559.

Koshiyama, I., Hamano, M., & Fukushima, D. (1981). A heat denaturation study of the 11S globulin in soybean seeds. *Food Chemistry, 6*(4), 309–322.

Kumar, M., Gupta, V., Kumari, P., Reddy, C. R. K., & Jha, B. (2011). Assessment of nutrient composition and antioxidant potential of Caulerpaceae seaweeds. *Journal of Food Composition and Analysis, 24*, 270–278.

Kumar, V. V., & Kaladharan, P. (2007). Amino acids in the seaweeds as an alternate source of protein for animal feed. *Journal of the Marine Biological Association of India, 49*(1), 35–40.

Meng, G. T., & Ma, C. Y. (2001). Thermal properties of *Phaseolus angularis* (red bean) globulin. *Food Chemistry, 23*, 453–460.

Murray, E. D., Arntfield, S. D., & Ismond, M. A. H. (1985). The influence of processing parameters on food protein functionality. II. Factors affecting thermal properties as analyzed by differential scanning calorimetry. *Canadian Institute of Food Science and Technology Journal, 18*(2), 158–162.

Nath, J. P., & Rao, M. S. N. (1981). Functional properties of guar proteins. *Journal of Food Science, 46*, 1255–1259.

Ogunwolu, S. O., Henshaw, F. O., Mock, H. P., Santros, A., & Awonorin, S. O. (2009). Functional properties of protein concentrates and isolates produced from cashew (*Anacardium occidentale* L.) nut. *Food Chemistry, 115*, 852–858.

Rajasulochana, P., Krishnamoorthy, P., & Dhamotharan, R. (2012). Biochemical investigation on red algae family of *Kappahycus* sp.. *Journal of Chemical and Pharmaceutical Research, 4*(10), 4637–4641.

Rouimi, S., Schorsch, C., Valentini, C., & Vaslin, S. (2005). Foam stability and interfacial properties of milk protein–surfactant systems. *Food Hydrocolloids, 19*, 467–478.

Seena, S., & Sridhar, K. R. (2005). Physiochemical, functional and cooking properties of Canavalia. *Journal of Food Chemistry, 32*, 406–412.

Slaski, R.J., & Franklin, P.T. (2011). A review of the status of the use and potential to use micro and macroalgae as commercially viable raw material sources for aquaculture diets. Report commissioned by Scottish Aquaculture Research Forum (SARF), 94 pp. Available from: http://www.sarf.org.uk/cms-assets/documents/29524-222388.sarf077.pdf.

Sorgentini, D. A., & Wagner, J. R. (2002). Comparative study of foaming properties of whey and isolate soybean proteins. *Food Research International, 35*, 721–729.

Wathelet, B. (1999). Nutritional analyses of proteins and amino acids in beans (*Phaseolus* sp.). *Biotechnology, Agronomy, Society and Environment, 3*, 197–200.

Wong, K. H., & Cheung, P. C. K. (2001). Nutritional evaluation of some subtropical red and green seaweeds. Part II. In vitro protein digestibility and amino acid profiles of protein concentrates. *Food Chemistry, 72*, 11–17.

Wong, K. H., Cheung, P. C. K., & Ang, P. O. Jr., (2004). Nutritional evaluation of protein concentrates isolated from two red seaweeds: *Hypnea charoides* and *Hypnea japonica* in growing rats. *Hydrobiology, 173*, 271–278.

Yun, J. H., Kwon, I. K., Lohakare, J. D., Choi, J. Y., Yong, J. S., Zheng, J., et al. (2005). Comparative efficacy of plant and animal protein sources on the growth performance, nutrient digestibility, morphology and caecal microbiology of early-weaned pigs. *Asian – Australasian Journal of Animal Sciences, 18*, 1285–1293.