EVALUATION OF CHEMICAL AND FUNCTIONAL PROPERTIES OF PROTEIN ISOLATES FROM *Basella alba* AND *Senecio biafrae* LEAVES

Supported by tetfund TERTIARY EDUCATION TRUST FUND

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Received: November 20, 2018 **Accepted:** January 26, 2019

Abstract: The study focused on the preparation of protein isolates from leaves of *Basella alba* (amunututu) and *Senecio biafrae* (worowo) leaves with the view to determining their in-vitro digestibility and functional properties. Fresh leaves of the vegetables were processed to obtain protein isolates by combination of solubilization, precipitation, centrifugation and freeze drying. The proximate analysis of the leaf meal and functional properties (bulk density, foaming properties, emulsifying ability and capacity, water and oil absorption capacity and in-vitro digestibility) of the isolate obtained from the leaves were determined using standard method. The protein contents of the protein isolates of *Basella alba* and *Senecio biafrae* were 89.75 and 67.50%, respectively; Water absorption capacity ranged from 240-305% for *Basella alba* and 200-285% for Senecio biafrae while oil absorptions were 140 and 190%, respectively; the least foaming capacities for the two isolates (30 and 40%, respectively) were obtained at pH 4 with poor stability at all pH. The lowest Emulsifying Activity Index was observed at pH 4 having values of 19.35 and 71.25 m^2/g, respectively and the % in-vitro protein digestibility values were high (86.34 and 82.86%) for *Basella alba* and *Senecio biafrae*, respectively. The study concludes that leaves of *Basella alba* are good sources of protein and the proteins are easily digested by gastro-intestinal enzymes. The good functional properties exhibited by the isolates are indications that they could be used as food ingredients.

Keywords: *Basella alba*, *Senecio biafrae*, protein isolates, *in-vitro* digestibility

Introduction

Leafy vegetables are important items of diet in many Nigerian homes and they are valuable sources of nutrients especially in rural areas where they contribute substantially to protein, mineral, vitamins, fiber and other nutrients which are usually in short supply in daily diets (Mosha and Gaga, 1999). They have the cheapest and most abundant sources of protein (Fasuyi, 2006) and add flavour, variety, taste, colour and aesthetic appeal to diet (Mepba *et al*., 2002). In Nigeria and many African countries of the tropics; vegetables are very abundant immediately after the rains but becomes scarce late in rainy season and more so in dry season (Ihekoronye and Ngoddy, 1985). Among the traditional vegetables in Nigeria are *Solanium nodiflorum* (ogumo), *Senecio biafrae* (worowo), *Talinium triangulare* (water leaf), *Celosia orgentea* (soko), *Vemonia amygdalia* (bitter leaf), *Teifaira occidentalis* (ugu), *Basella alba* (amunututu), *Occimum graticimum* (efinrin, scent leaf), *Corchorus olitorus* (ewedu), *Cnidoscolus chayamansa* (iyana ipaja), *Beilschmedia manni* (tete), *Grongromena ratifolia* (utazi leaf), *Papilionoideae soyauxii* (oha leaf) and *Piper guineenses* (uziza leaf) (Adebooye, 2004).

Basella alba (Plate 1) commonly called Ceylon or Malabar or Indian spinach is a tropical perennial vine widely used as leafy vegetable. It is known as "amunututu" in Western part of Nigeria. *Basella alba* is the green variety and is commonly grown in the Southern part of Nigeria, usually at the back of bathroom or any water outlet or channel in the house. It grows best in sandy loam soils rich in organic matter with pH ranging from 5.5 to 8.0. *Basella alba* is a fast-growing, soft-stemmed vine, reaching 10 m in length. It's thick, semi-succulent, heart-shaped leaves have a mild flavour and mucilaginous texture. Typical of leaf vegetables, Indian spinach is high in vitamin A, vitamin C, iron, and calcium. It is low in calories by volume, but high in protein per calorie (Grubben & Denton, 2004). Leaves of *Basella alba* are used for the treatment of hypertension by Nigerians in Lagos, and malaria in Cameroonian folk 2medicine. The plant has been reported for its antifungal, anticonvulsant, analgesic, anti-inflammatory and androgenic activities and for the treatment of anaemia (Kumar, 2010).

Plate 1: *Basella alba*

Plate 2: Green stemmed specie of *Senecio biafrae*

Fresh succulent leave of *Senecio biafrae* Plate 2 also known as "Worowo" in Western Nigeria and is a popular leafy vegetable in Sierra Leone, Ghana, Benin, Nigeria, Cameroon, and Gabon (Adebooye, 2004). It is a perennial climbing herb which naturally occurs in African forest zones. Leaves of *Senecio biafrae* contain per 100 g dry matter: crude protein (12.3 g), crude fibre (11.8 g), Ca (342 mg), P (39 mg), Fe (52 mg). The leaves also contain small amounts (less than 0.1 g/100g fresh leaves) of terpenoids, mainly the sesquiterpenegermacrene D (Adebooye, 2000) and various secondary metabolites such as dihydroisocoumarins, sesquiterpenes or amino acids (Tabopda *et al.*, 2009). *Senecio biafrae* is equally known for its therapeutic virtues, notably in Nigeria where it is used in the treatment of diabetes or pulmonary defects (Burkill, 1985; Iwu, 1993; Adebayo, 2009). In Benin, Côte d'Ivoire, Congo, or Cameroon it is used in traditional medicine to treat many diseases such as bleeding from cuts, sore eyes, cough, heart troubles, rheumatic pain, or localized oedemas (Adebooye, 2004). In the Western and North-western Regions of Cameroon, ethno-botanical studies revealed its utilization in the treatment of cases of women infertility (Focho *et al.*, 2009).

Proteins that are utilised in food processing are of various origins, and can be roughly grouped into animal proteins (e.g. gelatine), vegetable proteins (e.g. soya protein, peanut protein and wheat protein), and animal-derivatives protein (e.g. milk proteins). Many of the vegetable proteins require processing to provide a food material having acceptable functional properties, such as emulsification, fat and water absorption, texture modifications, colour control and whipping properties, which are attributed primarily to the protein characteristics. Proteins are important in food processing and food product development, because they are responsible for many functional properties that influence the consumer acceptance of food products. Among the several of vegetable proteins considered as food ingredients are peanut and soybeans. The increased utilization of conventional sources of proteins coupled with rapid population growth has prompted research efforts into finding alternative sources of proteins which are cheap and abundantly available. The utilization of such proteins as food ingredients will however depend on its physico-chemical and functional characteristics.

Although past research works on *Basella alba* have focused mainly on the nutritional benefits (Oboh *et al.*, 2006), medicinal importance, the evaluation of micronutrient composition and also on the nutritional quality of *Senecio biafrae* (Dairo and Adanlawo, 2007), there is dearth of information on the protein isolation and the characteristics of protein isolates in terms of chemical and functional properties hence this study.

Material and Methods

Materials

Leaves of both *Basella alba* and *Senecio biafrae* were obtained from a market in Ile-Ife, Osun State, Nigeria. The leaves were immediately washed under tap water to remove adhering dirt and mud. The cleaned leaves were dried in an oven at 50^0C after removing the stalk. The dried leaves were then milled and sieved through (60-80 mesh) sieves. The milled leaves were packaged as leaf meal in polythene bags and were stored at -20°C for further use. All chemicals used for this work were of analytical grade and was obtained from Fisher Scientific Company, Orlando, FL. or Sigma Chemical Company, St. Louis, MO.

Preparation of protein isolate

A modified method of Gbadamosi *et al.* (2012) was adopted for the preparation of protein isolates from *Basella alba* and *Senecio biafrae* leaves. A leaf meal to water in ratio 1:20 was stirred in a magnetic stirrer for 10 min, and the pH of the medium was adjusted to 10.0 and stirred for 4 h at a constant pH. The slurry was centrifuged at 4,500 g for 30 min at room temperature. The supernatant was collected and the pH was adjusted to 4.5 to precipitate the protein. The mixture was centrifuged at 4,500 g for 30 min and the precipitate obtained was washed twice with distilled water, re-suspended in distilled water and the pH was adjusted to 7.0 and then centrifuged at 4,500 g for 10 min. The precipitate was then lyophilized and the protein isolate obtained was stored at -4^0C in tightly sealed containers in a freezer for further analysis

Proximate analysis

Proximate chemical composition of the protein isolate that was obtained from the two leaves was determined according to standard AOAC (2000) methods.

Functional properties determination

Water and oil absorption capacity

Water absorption was determined by a modification of the method described by Sathe and Salunkhe (1981). A sample (200 mg) was transferred into a weighed centrifuge tube and 10 ml of distilled water was added. Using a glass stirring rod, the sample and water was mixed thoroughly for 30 s every 10 min over a period of 30 min. The flour particles that adhered to the side of the centrifuge tube were scrubbed down with the stirring rod to prevent it from drying. The suspension was then centrifuged (MSE Harrier 15/80, Sanyo, UK) at 4500 g for 20 min. The supernatant was decanted, and the tubes were allowed to drain at a 45º angle for 10 min and then weighed. Water absorption was then expressed as percentage increase of the sample weight and this was taken at temperature ranging from 60º to 90º.

Foam capacity and foam stability

Foam capacity and foam stability was determined by a modification of the method described by Chavan *et al*. (2001). A 25 mg protein isolate was dispersed in 250 ml of distilled water and homogenised for 3 min using Marlex Excella blender (Marlex Appliances PVT, Daman) set at high speed. The percentage ratio of the volume increase to that of the original volume of protein solution was calculated and expressed as foam capacity or whippability. Foam stability was then expressed as percentage of the volume of foam remaining after 30 min of quiescent period.

$$\%\ \text{Foaming capacity} = \frac{\text{vol. after homogenization} - \text{vol. before homogenization} \times 100}{\text{vol. before homogenisation}}$$

$$\%\ \text{Foaming stability} = \frac{\text{foam volume after time (t)} \times 100}{\text{Initial foam volume}}$$

Emulsifying activity index (EAI)

The protein isolate (200 mg) was dispersed in 30 ml of distilled water and gently stirred to disperse the sample. The protein solution was mixed with 10 ml of pure Gino oil and the mixture was homogenized using a magnetic stirrer (AB Biotech, Sweden) set at speed 10 for 60 s. Five hundred microlitres of the aliquot of the emulsion was transferred from the bottom of the centrifuge tube after homogenization, and mixed with 5 ml of 0.1% sodium dodecyl sulphate (SDS) solution. The absorbance of the diluted solution was measured at 500 nm using spectrophotometer (UnicamHελios α, UV-Visible Spectrophotometer, England). The EAI was then expressed as interfacial area per unit weight of protein (m^2g^{-1}) Pearce and Kinsella (1978).

$$\text{Emulsifying Activity Index (m2/g)} = \frac{2 \times 2.303 \times \text{A}}{0.25 \times \text{Protein weight (g)}}$$

Emulsion stability (ES)

The emulsion was allowed to stand for 10 min at room temperature and the EAI determined as described above, and was expressed using the method described by Pearce and Kinsella (1978);

$$\text{Emulsion Stabilty Index (min)} = \frac{\text{AA} \times \Delta\text{t}}{\text{A} - \text{AA}}$$

Where:

A =Absorbance at 0 min after homogenization

AA = Absorbance at 10 min after homogenization.

In-vitro protein digestibility

In-vitro protein digestibility of proteins was determined using pepsin-pancreatin enzyme systems according to the method of Saunder *et al.* (1973) with minor modifications. A 250 mg sample was suspended in 15 ml of 0.1 M HCl containing 1.5 mg of pepsin, which was followed by gentle shaking for 1 h at 37^0C. The resultant solution was then neutralized with 0.50 M NaOH and treated with 4 mg pancreatin (from porcine pancreas, activity equivalent to 4 × US Pharmacopeia) in 7.5 ml of phosphate buffer (0.1 M, pH 8.0). The mixture was shaken for 24 h at 37^0C in a water bath shaker, and the undigested solid was separated by centrifugation washed with distilled water and air dried. The undigested residue was then extracted with 0.1 N NaOH, and the soluble protein content was determined. Protein digestibility was obtained using the following equation:

$$\%\ In-vitro \text{ digestibility} = \frac{(I - F)}{I} \times 100$$

Where I is the protein content of the sample before digestion and F is the protein content of the sample after digestion.

Statistical analysis

All experiments were conducted in triplicates. Data reported were averages of three determinations. Analysis of variance (ANOVA) was performed and differences in mean values were evaluated using Tukey`s test at $P \leq 0.05$.

Results and Discussions

Proximate and physical properties of Basella alba and Senecio biafrae

The results of the proximate composition of leaves of *Basella alba* and *Senecio biafrae* are shown in Table 1. Moisture content of *Basella alba* and *Senecio biafrae* (8.67 and 7.33%, respectively) were in conformity with the moisture contents of 8.5 and 6.4% reported by othe investigators (Oboh *et al.*, 2006; Dairo and Adanlawo, 2007). Iheanacho and Udebuani (2009) reported that the moisture content provides for greater activity of water soluble enzymes and co-enzymes needed for metabolic activities of these leafy vegetables. A moisture content of less than 10% indicates that the dried leaves could be stored for long period without spoilage. The leaves of *Basella alba* and *Senecio biafrae* had protein contents of 44.15 and 34.15%, respectively; while their isolates had respective values of 89.75 and 69.50% according to the results of this investigation. The crude protein of the leaf meals were higher than what was reported for some other leafy vegetables such as *Momordica balsamina* (11.29%), *Moringa oleifera* (20.72%), *Lesianthera africiana* leaves (13.10-14.90%) and *Leptadenia hastate* (19.10%) (Balogun and Olatidoye, 2012). Therefore, the leaves can serve as a better source of protein. Their basic function as a nutrient is to supply adequate amount of required amino acids. Protein deficiency causes growth retardation, muscle wasting, oedema, abnormal swelling of the belly, etc. (Murray *et al*., 2000). Consumption of leaves of *Basella alba* and *Senecio biafrae* or their isolates could help in solving protein deficiency.

Dried leaves of *Basella alba* and *Senecio biafrae* have crude fibre contents of 15.2 and 17.9%, respectively according to the results of this investigation. This fall within the range of values (8.5- 20.90%) reported for some Nigerian vegetables (Isong & Idiong, 1997). Dietary fibre helps to prevent constipation, bowel problems and piles. It also plays an important role in the maintenance of internal distention for a normal peristaltic movement of the intestinal tract (Balogun and Olatidoye, 2012). It also involves in preventing colon cancer and constipation (Muhammad and Ajiboye, 2010; Ahmed, 1972; Bingham *et al*., 2003; Park *et al*., 2005). Furthermore, dietary fibre decreases the absorption of cholesterol from the gut in addition to delaying the digestion and conversion of starch to simple sugars, which is an important factor in the management of diabetes. Dietary fibre also functions in the protection against cardiovascular disease, colorectal cancer and obesity (Eleazu and Okafor, 2012). Ash content of dried leaves of *Basella alba* and *Senecio biafrae* had ash contents of 12.10 and 5.12%, respectively. These high ash contents were due to the removal of moisture during drying of the fresh leaves. In addition, the ash content gives an indication of the presence of inorganic elements in the leaves. The results suggest that the leaves could serve as potential sources of both macro and micro mineral elements. Meanwhile the crude fat contents of dried leaves of *Basella alba* and *Senecio biafrae* are 3.69 and 5.12%, respectively as shown in Table 1. The percentage of fat were in conformity with values, ranging from 3.51 – 14.02%, in Bushbuck and *Amaranthus hybridus*, respectively (Adebooye, 2004). These values were fairly high when compared with values reported for some other leafy vegetables such as *Ocimum bassillium*, *Ocimum viride* and *Piper guineens* (Udosen, 1995). The carbohydrate values obtained for *Basella alba* and *Senecio biafrae* leaves (16.24 and 26.78%) fell below the amount of carbohydrate for *Momdrica balsamina* (39.05%) and similar to that of grains (Sanni & Olaofe, 1998). These low carbohydrate contents in the samples could be due to high content of protein.

Table 1: Proximate composition (%) of dried of leaves and protein isolates both *Senecio biafrae* and *Basella alba*

sample	Moisture content	Ash content	Crude fibre	Protein content	Crude fat	Carbohydrate
Basella alba leaves	8.67	12.10	15.2	44.1	3.69	16.24
*Senecio biafrae*leaves	7.33	8.72	17.9	34.15	5.12	26.78
Basella alba protein isolate	-	-	-	89.75	-	-
Senecio biafrae protein isolate	-	-	-	69.50	-	-

Physical properties and in-vitro digestibility Basella alba and Senecio biafrae protein

The bulk densities of *Basella alba* and *Senecio biafrae* protein isolates as shown in Table 2, were 0.35 and 0.76 g/ml, respectively and the values were higher than the bulk density of protein isolate of cashew nut (0.25 g/ml; Ogunwolu *et al*., 2009) but lower than the bulk density of sesame seed protein isolate (Khalid *et al*., 2003). The bulk densities of the isolates obtained from *Basella alba* and *Senecio biafrae* are comparable to that recorded for soy isolate (0.48 g/ml; Okezie and Bello, 1988). According to Peleg and Bagley (1983), bulk density depends on the combined effects of interrelated factors such as the intensity of attractive interparticle forces, particle size, and number of contact points. The high volume

per gram of protein material is important in relation to its packaging. Increase in bulk densities of *Basella alba* and *Senecio biafrae* isolates are desirable in that it offers greater packaging advantage, as a greater quantity may be packed within a constant volume (Fagbemi, 1999). However, Padmashree *et al*. (1987) reported that higher bulk density is desirable, since it helps to reduce the paste thickness which is an important factor in convalescent and child feeding.

The isolates of *Basella alba* and *Senecio biafrae* in water suspension had pH values of 5.4 and 5.8, respectively (Table 2) and these were about the pH value of 5.37 obtained for conophor protein isolate (Gbadamosi, 2008). The pH values of the isolates of *Basella alba* and *Senecio biafrae* in water suspension are important since some functional properties such as solubility (Chavan *et al*., 2001; Odoemelam, 2003), whippability (Giami, 1993) and emulsion properties (Eke and Akobundu, 1993) are highly affected by pH changes (Khalid *et al*., 2003).

Meanwhile, the result of in-vitro digestibility of protein isolates of *Basella alba* and *Senecio biafrae* with pepsin-pancreatin enzyme systems showed that their respective digestibility values were 86.34 and 82.86% (Table 2). These values are comparable with that of flaxseed protein isolates 90% (Wanasundara and Shahidi, 1997). High digestibility of protein isolates of *Basella alba* and *Senecio biafrae* may be due to decrease in the non-protein compounds especially polysaccharides as well as increase in the availability of the proteins for enzymatic activities.

Functional properties of protein isolates of Basella alba and Senecio biafrae

Water absorption capacity (WAC) as affected by temperature

Protein isolates of *Basella alba* and *Senecio biafrae* were found to possess water absorption capacities (WAC) ranging from 240 – 305 and 200 – 285%, respectively (Fig. 1). These were higher than that of conophor protein isolate (194.1%; Gbadamosi, 2008) and were significantly lower than those of soy isolate (410%) and winged bean isolates (965%) as reported by Okezie and Bello (1988) but were comparable with those of cashew nut protein isolate (220%) and bitter lupin protein isolate (212%) reported by Ogunwolu, *et al*. (2009) and El-Adawy *et al*. (2001), respectively.

Table 2: pH, bulk densities and % *In-vitro* digestibility of protein isolates from *Basella alba* and *Senecio biafrae* leaves

Samples	pH	Bulk density	% *In-vitro* digestibility
Basella alba	5.8	0.35	86.34
Senecio biafrae	5.4	0.76	82.86

It has been reported that water binding capacity of proteins is a function of several parameters, including size, shape, steric factors, conformational characteristics, hydrophilic-hydrophobic balance of amino acids in the protein molecules as well as lipids, carbohydrates and tannins associated with proteins (Chavan *et al*., 2001). The difference in WAC of isolates of *Basella alba* and *Senecio biafrae* and that of soy and winged bean isolates suggests the presence of a large proportion of hydrophobic as compared to hydrophilic groups on the surface of the protein molecules.

The effect of temperature on water absorption capacity is shown in Fig. 1. The water absorption capacity of protein isolates of *Basella alba* and *Senecio biafrae* increased with increase in temperature from room temperature, 30 to 90^{0}C. Increase in water absorption capacity of the isolates as a result of increase in temperature is in conformity with earlier reports for red and white sweet potato (Osundahunsi *et al.*, 2003) and for fermented maize flour (Fasasi *et al.*, 2007). Water absorption capacity ranging from 149.1 to 471.5% is considered critical in the preparation of viscous foods such as soups, gravies (Aletor *et al.*, 2002) hence the isolates of *Basella alba* and *Senecio biafrae* may find use as functional ingredients in soups, gravies and baked products. It could also be used as thickeners in liquid and semi liquid foods since the protein has the ability to absorb water for improved consistency in food particularly at high temperature.

Foaming ability and stability

At their respective natural pH (5.4 and 5.8), the foam capacities of the isolates of *Basella alba* and *Senecio biafrae* were observed to be 17.5 and 60%, respectively. For both samples, the least foam capacities were observed at pH 4 (Fig. 2) and increased beyond and above this pH. The foam capacities of the isolates of *Basella alba* and *Senecio biafrae* were inferior to that of soy isolates (84%; Okezie and Bello, 1988) and were superior to the values of 11.30 and 9% reported for pearl millet flour and quinoa flour respectively (Oshodi *et al*., 1999) and conophor protein isolates (5%; Gbadamosi, 2008).

Foam formation is governed by three factors; including transportation, penetration and re-organisation of the molecule at the air-water interface. Therefore, to exhibit good foaming, a protein must be capable of migrating at the air-water interface, unfolding and rearranging at the interface (Halling, 1981). According to Damodaran (1997), the foam capacity and stability were enhanced by greater protein concentration, because this increases the viscosity and facilitates the formation of a multilayer, cohesive protein film at the interface.

The foam stability of the two samples on the other hand was poor. Foams of both samples were only stable at pH 8 and10. The foam stability is important since the usefulness of whipping agents depends on their ability to maintain the whip as long as possible (Lin *et al*., 1974). The result therefore suggests that protein isolates of *Basella alba* and *Senecio biafrae* may not be suitable as whipping agents in acid foods.

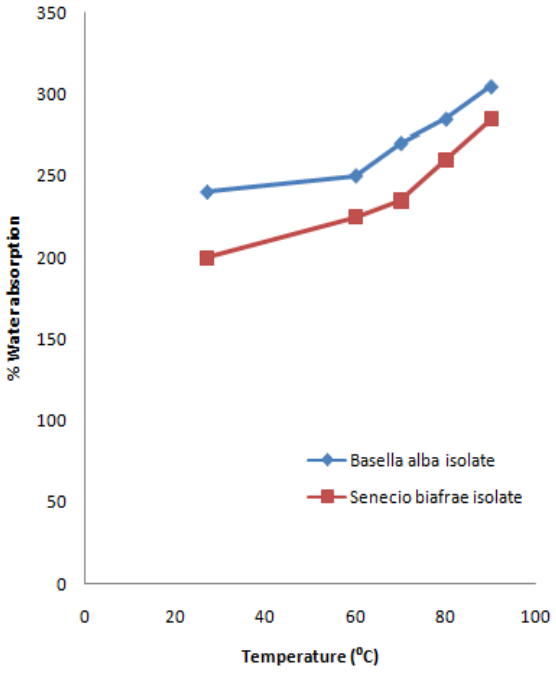

Fig. 1: % Water absorption capacity of isolates of *Basella alba* and *Senecio biafrae*

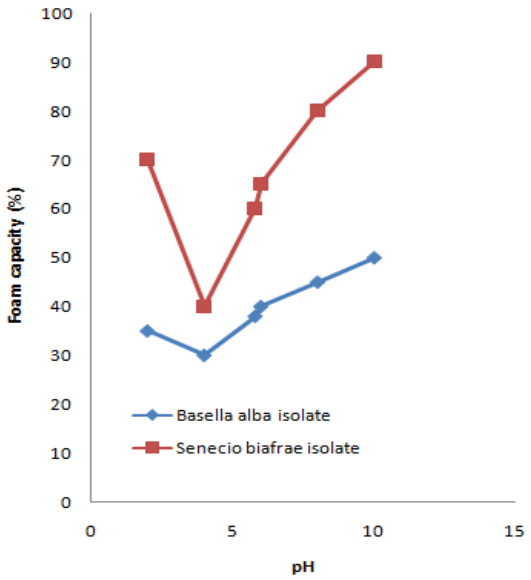

Fig. 2: Foam capacity of isolates of *Basella alba* and *Senecio biafrae*

Effects of pH on emulsifying activity and emulsion stability of isolates of Basella alba and Senecio biafrae

The effect of pH on the emulsifying properties of the isolates of *Basella alba* and *Senecio biafrae* obtained are presented in Fig. 3. The lowest EAIs of isolates of *Basella alba* and *Senecio biafrae* which were also likely to be the isoelectric region, were found at pH 4, and increased at pH values above and below this region. As shown in Fig. 3, the highest EAI of isolates of *Basella alba* and *Senecio biafrae* were found at pH 10.

A number of studies have shown that the pH-emulsifying properties profile of various proteins including soya protein resembles the pH-solubility profile (Aoki *et al*., 1990). According to Damodaran (1997), this is because most food proteins are sparingly soluble at their isoelectric pH, poorly hydrated, and lacking electrostatic repulsive forces, they are generally poor emulsifiers at this pH. These proteins may, however, be effective emulsifiers when moved away from their isoelectric pH. The increase in EAI with pH increase might suggest that droplet size decreased as the pH increased beyond the isoelectric point.

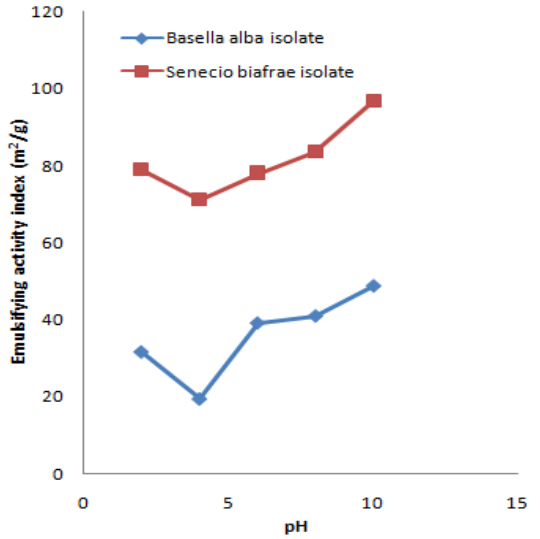

Fig. 3: Emulsifying activity index of isolates of *Basella alba* and *Senecio biafrae*

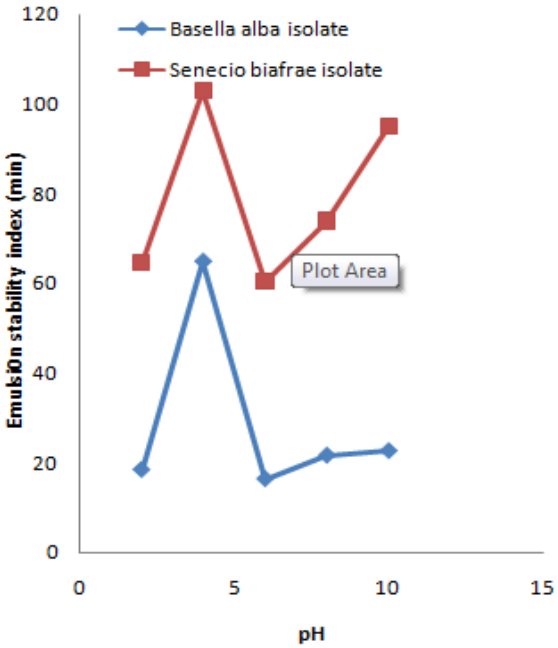

Fig. 4: Emulsion stability of isolates of *Basella alba* and *Senecio biafrae*

The effect of pH on emulsion stability index (ESI) of the isolates of *Basella alba* and *Senecio biafrae* are presented in Fig. 4. The minimum ESIs for the isolates of *Basella alba* and *Senecio biafrae* were obtained at pH 6 and the highest ESI of isolates of *Basella alba* and *Senecio biafrae* were observed at pH 4. The low ESI at low pH has been reported earlier by Chavan *et al*. (2001) and was attributed to increased interactions between the emulsified droplets. As the pH increased, the subsequent increase in Coulombic repulsion between neighbouring droplets and increased hydration of the charged protein molecules may lower interfacial energy and retard droplet coalescence (Kinsella *et al*., 1985; Chavan *et al*., 2001). These results are in agreement with literature values for cashew nut flour, protein concentrate and isolate (Ogunwolu *et al*., 2009).

Conclusion

The result of this investigation revealed that isolates of suitable functional properties could be produced from the leaves of *Basella alba* and *Senecio biafrae* and they can serve as a good source of protein ingredient in food systems.

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