# FUNCTIONAL AND PASTING PROPERTIES OF PUMPKIN (*Cucurbita pepo*) SEED PRODUCTS

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## ABSTRACT

Proteins of plant origin form an alternative to animal proteins for food and non food use. The use of oil seed proteins for non-food applications show growing potential but the protein products are limited at present and are mostly marketed for the food industry as they confer desirable functional properties when added to food. This work focused on the functionality of pumpkin (*Cucurbita pepo*) seed flour, protein concentrate and isolate in relation to food applications. Pumpkin seed was processed into defatted flour (CPF), which was further processed into protein concentrate (CPC), and protein isolate (CPI) using alkaline water/isoelectric precipitation. The functional properties of these products were evaluated. All samples exhibited a broad range of minimum protein solubility at pH 6-8 and maximum at pH 10. Defatted pumpkin seed flour had significantly ($p<0.05$) higher water absorption capacity (1.60ml/g) than the protein concentrate (1.20ml/g) and isolate (1.25ml/g). The flour was a better emulsifier with emulsion capacity of 22.0% compared to the protein concentrate and isolate with emulsion capacity of 5.0% and 1.0%, respectively. The most stable emulsions were obtained at pH 12 and at 0.1%, 0.5% and 1.0% sample concentrations. Pumpkin seed protein concentrate and isolate showed poor foaming ability. Maximum foaming capacity was obtained at pH 2.0 in all the samples. Protein isolate had the least and better gelation capacity. Peak viscosity ranged from 32.83-44.25 RVU and was significantly different while the breakdown value and pasting temperature (ranging from 1.75-3.16 RVU and 93.60-94.20$^{o}$C respectively) showed no significant differences ($p>0.05$). This work revealed that pumpkin seed has great potentials as a food ingredient. The flour and protein concentrate exhibited better functionality and may be used as functional agents in the formulation of a wide range of new food products or as possible replacement for animal protein in conventional foods.

**Keywords:** Pumpkin Flour, Protein concentrate, protein isolate, Functional properties, pasting properties.

## INTRODUCTION

Recent development in human nutrition demands sourcing for cheap and abundant protein foods. Proteins are commonly employed as food ingredients on the bases of their importance in human diet. The best proteins belong to animal sources since they have a suitable functionality. However the high cost of animal protein makes vegetable protein the main dietary component for most of the world's population. In the industry, the production of plant protein isolate is of growing interest because of the increasing application of plant proteins in food and non-food markets [1]. Rich and valuable protein found in oil seeds could be made available in form of protein concentrate and isolate. Proteins in these forms are necessary ingredients in many food processes where they perform specific functions. Nevertheless, the application of protein in these forms in food trade is almost limited to proteins from soybean seeds while proteins from other plants could be utilized. The use of plant proteins in the formulation of new food products or in

conventional foods has been the focus of much research in recent years [2] . To develop plant proteins for use as food ingredients, their physicochemical and functional properties must be evaluated [2]. In the food industry, selected protein display a wide range of functional properties which are closely related to their structure [3]. The effective utilization of proteins entails matching a wide variety of functional and nutritional characteristics to the complex needs of manufactured products. The objectives of this study are to evaluate the functional and pasting properties of *Cucurbita pepo* seed flour, protein concentrate and isolate.

## MATERIALS AND METHODS

### Sample preparation

Defatted pumpkin seed flour was produced as described by Atuonwu and Akobundu [4] and protein concentrate and protein isolate were produced from the defatted seed flour [5].

**Functional Properties:** The solubility properties of protein in the samples were investigated according to the method described by Akobundu *et al*. [6] with slight modifications. Protein was determined for sample suspensions of 1.0% prepared in de-ionized water and 0.1mol/L, 0.5mol/L and 1.0mol/L sodium chloride solutions respectively after adjusting the pH to 4, 6, 8 and 10 using either 0.5mol/L hydrochloric acid or 0.5mol/L Sodium hydroxide with continuous stirring at room temperature, 40$^{o}$C, 60$^{o}$C and 80$^{o}$C. One gram of sample was suspended in 80ml of each solution, adjusted to the desired pH and allowed to stand for 5 minutes at the respective temperatures to solubilize the proteins. All extractions were brought to the final volume of 100ml after pH adjustment and the suspensions were centrifuged at 1500rpm for 30minutes. The supernatants were filtered and the protein content determined the proteins of the supernatants were determined by mixing 5.0ml of the reagent mixture and 1.0ml of the supernatant and allowing to stand for 10minutes. Folin Ciocalteau reagent (0.5ml) was added and the content was mixed and allowed to stand for 30minutes before reading the absorbance at 750nm in UV spectrophotometer against the blank. With the absorbance values, the protein concentration of each supernatant was estimated by extrapolation on the BSA standard curve. The soluble protein was calculated as milligram soluble protein per milliliter of supernatant.

Water and oil absorption capacities were assessed using the method outlined by Beuchat [7]. Emulsifying capacity and stability were determined using the method of Neto *et al*. [8]. Foaming capacity and stability were determined by the method of Eke and Akobundu [9]. Bulk density was determined according to the method of Okezie and Bello [10], wettability by Onwuka [11] and gelation capacity according to Coffman and Garcia [12].

### Effect of pH and sample concentration on emulsion capacity and stability

The method described by Bilgi and Çelik [13] was adopted. Emulsifying properties of *Cucurbita pepo* flour, protein concentrate and isolate at concentrations of 0.1%, 0.5% and 1.0% (w/v) in distilled water were examined at pH values of 2-12. The sample suspension (5ml) was homogenized with 5ml vegetable oil for 60 seconds to form an emulsion. The emulsion was centrifuged at 3000rpm for 25 minutes. The height of the total content in

the tube was measured. The emulsifying capacity and stability were calculated using the formulae below [8].

$$\text{Emulsifying Capacity (\%)} = \frac{\text{Height of emulsified layer in the tube}}{\text{Height of the total content in the tube}} \times 100$$

$$\text{Emulsifying Stability (\%)} = \frac{\text{Height of emulsified layer after heating}}{\text{Height of emulsified layer before heating}} \times 100$$

**Effect of sodium chloride concentration on emulsion capacity and stability**

Two grams of each sample and 100ml of varied concentrations (0.20, 0.40, 0.60, 0.80 and 1.0%) of sodium chloride solution was blended for 30 seconds in a blender (Super Intermet Japan Magic blender SI 462 model). The sample suspension (5ml) was homogenized with 5ml vegetable oil for 60 seconds to form an emulsion. The emulsion was centrifuged at 3000rpm for 25 minutes [13] . The emulsifying capacity and stability were calculated using the formulae above [8].

**Effect of sodium chloride concentration on foaming capacity and stability**

Two grams of each sample and 100ml of different concentrations of sodium chloride (0.20, 0.40, 0.60, 0.80, and 1.0%) was whipped vigorously for 5 minutes and allowed to stand for 20-120 minutes at room temperature. Foaming capacity and stability were calculated as percentage volume of foam after mixing and over a period of 20-120 minutes [12] .

**Effect of pH on foaming capacity and stability**

The influence of pH on foaming properties was investigated using the method of Coffmann and Garcia [12]. Two grams of each sample was dissolved in 100ml of distilled water and the pH was in each case adjusted to the desired value (2.0, 4.0, 6.0, 8.0 and 10.0 ) using either 1.0M hydrochloric acid or 1.0M sodium hydroxide. Each mixture was whipped vigorously and allowed to stand for 20 – 120 minutes at room temperature. Foaming capacity and stability were calculated as percentage volume of foam after mixing and over a time period of 20-120 minutes, respectively.

**Pasting properties**

Pasting characteristics were determined with a Rapid Visco Analyzer (RVA) (Model RVA 3D +, Newport Scientific, Australia). Each sample was weighed (2.5g) and mixed with 25ml of distilled water in a canister. The canister was fitted into the RVA as recommended and the slurry was heated from 50$^{o}$C to 95$^{o}$C with a holding time of 2 minutes followed by cooling to 50$^{o}$C. The heating and cooling were at a constant rate of 11.25$^{o}$C/ min. Pasting properties were read from the pasting profile with the aid of thermocline for windows software connected to a computer.

**RESULTS AND DISCUSSION**

**Protein solubility:** *Cucurbita pepo* seed protein isolate exhibited a higher solubility compared to the flour and protein concentrate in distilled water at various pH values. Solubility was quite high at pH 10 (Fig.1). The poor solubility of the protein concentrate may be attributed to the higher content of lipids associated with the protein concentrate. *Cucurbita pepo* seed flour, protein concentrate, and isolate exhibited a broad range of minimum solubility at pH range of 6-8 in distilled water and at different temperatures

(Fig.1 and 2). Solubility increased between pH 4-5 and 8-10, which suggest the suitability of pH10 and 4.5 for protein extraction and precipitation during the preparation of protein concentrate and isolate from *cucurbita pepo* seed flour. *Cucurbita pepo* seed flour was more soluble than protein concentrate and isolate at 40$^{o}$C and pH 4 (Fig. 2). The flour, protein concentrate and isolate exhibited their highest solubility at pH10; 80$^{o}$C, pH 10; 60$^{o}$C and pH 10; 80$^{o}$C, respectively. *Cucurbita pepo* seed flour with low solubility in water was 1.5 times more soluble in 0.1% sodium chloride concentration and had the highest solubility at 1.0% at pH10 (Fig. 3). Protein concentrate and isolate were more soluble at 0.5% sodium chloride concentration. The effect of sodium chloride concentration on the flour, protein concentrate and isolate was to increase their solubility around the iso-electric point. The increase in salt concentration up to 1.0% decreased the solubility of both protein concentrate and isolate. These observations were also similar to those obtained for canola [14], and cowpea [15]. El-Adawy *et al*. [16] reported an increase in protein solubility as sodium chloride concentration increased up to 1.0M for lupin seed flour which later decreased beyond 1.0M concentrations. Generally, protein solubility is known to increased with moderately increasing salt concentration due to salting-in effect and at a higher salt concentrations, the protein solubility does not increase as it is then likely to undergo salting-out [16] .

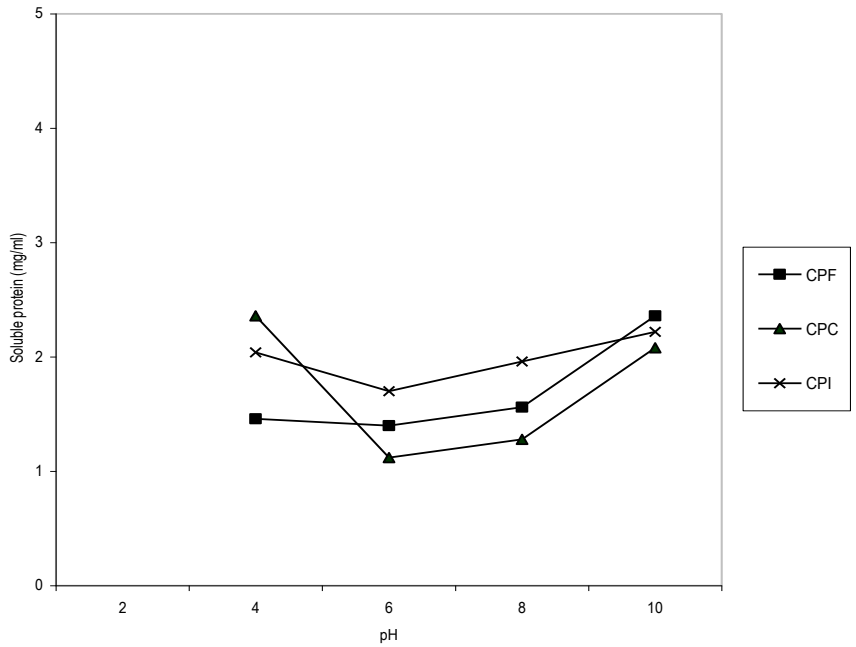


Fig. 1: Solubility profile of protein in *Cucurbita pepo* seed flour (CPF), protein concentrate (CPC) and isolate (CPI) in distilled water

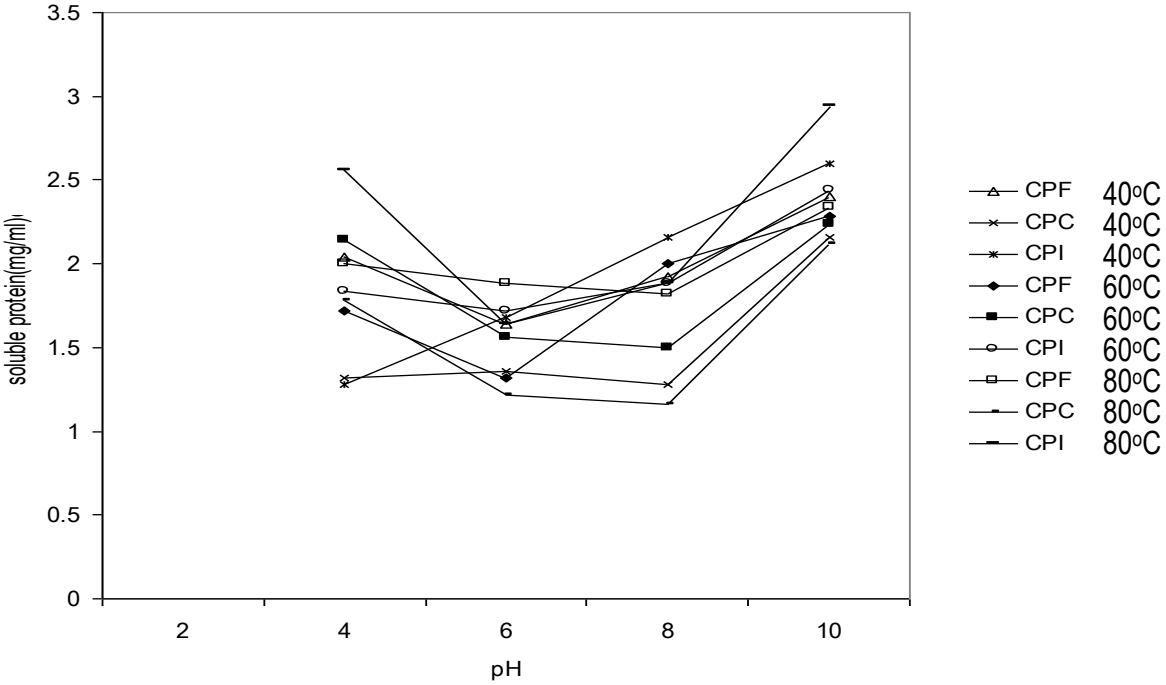


Fig. 2: Solubility profile of *Cucurbita pepo* seed flour, protein concentrate and isolate as a function of pH and temperature

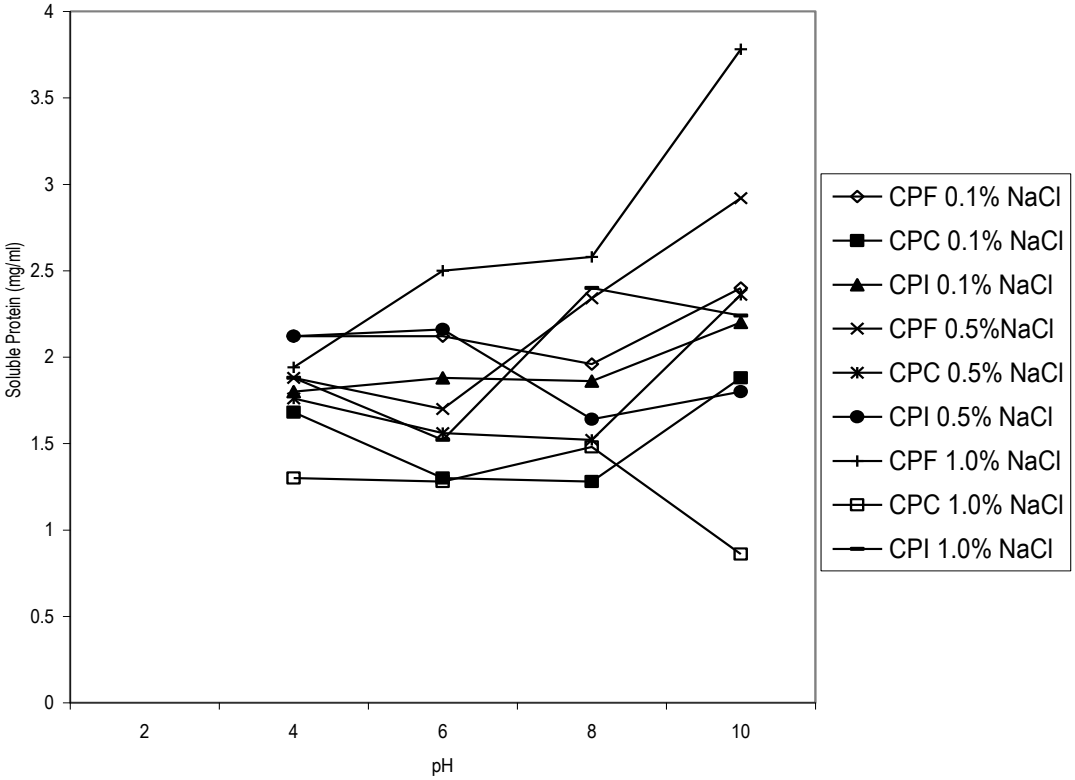


Fig.3: Solubility profile of *Cucurbita pepo* seed flour(CPF), protein concentrate (CPC) and isolate (CPI) as a function of pH and sodium chloride concentration

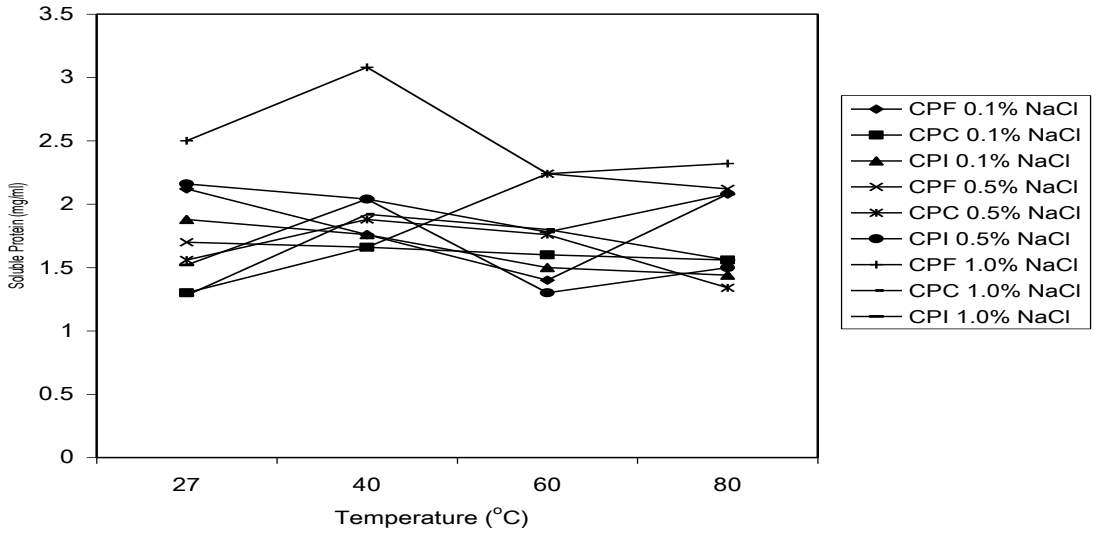


Fig. 4: Solubilty Profile of *Cucurbita pepo* Seed Flour(CPF), Protein Concentrate(CPC) and Isolate (CPI) as a Function of Temperature and Sodium Chloride Concentration

Protein concentrate showed maximum solubility at 0.1% NaCl concentration at 80°C while at the same temperature the isolate was more soluble at 1.0% NaCl concentration. Generally the result revealed that at lower salt concentrations, a higher temperature is required to enhance protein solubility.

**Water and oil absorption capacities**

Defatted *Cucurbita pepo* seed flour had significantly ($p<0.05$) higher water absorption capacity than the protein concentrate and isolate (Table 1). This result was similar to that reported by Monteiro and Prakash [17] for defatted peanut meal and isolated proteins. The water absorption capacity of *Cucurbita pepo* protein isolate was 4.17% higher than the protein concentrate. Water binding capacity of proteins is a function of several parameters such as size, shape, conformational characteristics, hydrophilic-hydrophobic balance of amino acids in the protein molecules as well as lipids, carbohydrates and tannins associated with proteins [18]. The differences in water binding capacities of *Cucurbita pepo* seed protein concentrate and isolate may be due to the protein concentration and possibly their conformational characteristics. *Cucurbita pepo* seed flour gave a significantly higher oil absorption capacity (37.0%) compared to the protein concentrate and isolate. Similar observations were reported by Mahajan *et al*. [19] for defatted heat treated sesame (*Sesamum indicum*) and rapeseed (*Brassica campestris*). The low fat-binding capacity of *Cucurbita pepo* seed protein concentrate and isolate compared to the flour suggests the presence of a large proportion of hydrophilic to hydrophobic groups on the surface of the protein molecules. However, low oil absorption capacity may be desirable in some food applications such as those involving deep frying of legume-base products.

**Gelation capacity**

Gelation capacity was significantly different in all the samples with the protein isolate having the lowest value (2.00%). Lawal *et al*. [20] reported that lower least gelation

capacity means better gelation capacity and increase in ionic strength enhance gelation properties. Hydrogen ion concentration affects gelation reactions by influencing the balance of the polar and non-polar residues. Thus at pH values close to isoelectric point, protein-protein interactions are generally favoured because the net surface charge is close to zero, which reduces the repulsive interactions between protein molecules thus enhancing gelation, while at pH far from isoelectric point, the surface charge on the protein is large and significant repulsive forces prevent protein-protein interactions [20] . The latter observation suggests the reason for the low least gelation capacity of the *Cucurbita* seed proteins compared to the flour (Table 1). Protein gelation is vital in the preparation and acceptability of many food products.

**Emulsifying properties**

The formation and stability of emulsion is very important in food systems such as salad dressing. Proteins are composed of charged amino acids, non-charged polar amino acids and non-polar amino acids, which make protein a possible emulsifier, the surfactant possessing both hydrophilic and hydrophobic properties and able to interact with both water and oil in food system [21]. Defatted *Cucurbita pepo* seed flour was a better emulsifier with emulsion capacity of 22.0% compared to the protein concentrate and isolate with emulsion capacity of 5.0% and 1.0% respectively, (Table 1). This effect may probably have resulted from the high protein solubility of the *Cucurbita pepo* seed flour which exposed more hydrophobic groups to water and oil interface leading to increased emulsion capacity and stability. Highly insoluble proteins are not good emulsifiers and can generate coalescence [22]. Keto and Nakai [23] also reported that emulsifying properties show a good correlation with the presence of hydrophobic residues in the protein surface which are unstable in the oil-water interface. This observation was also in agreement with the result of Yu *et al*. [21] who reported increase in emulsion capacity of defatted peanut flour as a result of increased surface hydrophobicity and decrease in solubility of the peanut proteins. The emulsions formed by *Curcubita pepo* seed flour and protein isolate (with emulsion stability of 50% each) were more stable than that of the protein concentrate as shown in Table 1. This observation may also be attributed to the increase in protein solubility and the exposure of more hydrophobicity group to water and oil interface. Resolubilization of *Curcubita* protein isolate at pH 7.0 may also have contributed to its improved emulsification activity. Thus preparation conditions of the samples affected their emulsifying properties.

Emulsifying capacity of CPF (3.33%) and CPI (12.5%) were highest at 0.4% salt concentration, respectively. The emulsifying capacity of CPF and CPI followed a similar pattern and reduced with further increase in salt concentration. CPC had the same emulsion capacity (0.5%) at all levels of salt concentration except at 1.0% salt concentration with no emulsion. Emulsions of CPF, CPC and CPI were most stable at 0.8%, 0.6% and 0.8% salt concentrations respectively. CPF and CPI gave emulsions with fair stability irrespective of varying concentrations while CPC exhibited poor emulsion stability. This observation could be attributed to the protein structure and conformation, hydrophobicity/ hydrophilicity and solubility [2].

The emulsion capacity of CPF, CPC and CPI were highest at pH 12 and sample concentration of 0.1%, 0.5% and1.0%, respectively. The increase in emulsion capacity

with increase in pH may suggest that droplet size decreases with increase in pH beyond the isoelectric point [2]. The most stable emulsions were obtained at pH 12 for all the samples at different concentrations. Emulsifying properties decreased towards pH values close to the isoelectric point (4-6) and increased towards the basic pH values (8-12). The emulsion properties of CPC were least at pH 6. Similar result was obtained for barley protein concentrate [13]. The low emulsifying properties at decreased pH value may probably be as a result of the increased protein-protein interaction resulting in low surface hydrophobicity, decreased net charge and solubility of proteins [24]. Increased protein solubility will probably result in increased protein concentration at the interface, which in turn would enhance the formation of interfacial films and improve emulsion properties [13].

**Table 1: Functional properties of *Cucurbita pepo* seed flour (CPF), protein concentrate (CPC) and protein isolate (CPI).**

| Functional Properties | Samples | | |
|---|---|---|---|
| | CPF | CPC | CPI |
| Water Absorption Capacity (ml/g) | $1.60^{a} \pm 0.0424$ | $1.20^{b} \pm 0.1131$ | $1.25^{b} \pm 0.0566$ |
| Oil Absorption Capacity (ml/g) | $3.70^{a} \pm 0.2687$ | $0.80^{b} \pm 0.0566$ | $0.80^{b} \pm 0.0141$ |
| Gelation Capacity (%) | $14.00^{a} \pm 0.0000$ | $8.00^{b} \pm 0.0000$ | $2.00^{c} \pm 0.0000$ |
| Emulsion Capacity (%) | $22.00^{a} \pm 1.4142$ | $5.00^{b} \pm 0.1414$ | $1.00^{c} \pm 0.0707$ |
| Emulsion Stability (%) | $50.00^{a} \pm 0.7071$ | $20.00^{b} \pm 0.2828$ | $50.00^{a} \pm 3.1112$ |
| Bulk density (g/ml) | $0.4902^{a} \pm .0141$ | $0.5823^{b} \pm .0236$ | $0.5682^{b} \pm .0301$ |
| Wettability (s) | $61.00^{a} \pm 2.8284$ | $718.00^{b} \pm .2426$ | $98.00^{c} \pm 1.4142$ |
| Foaming Capacity (%) | $20.00^{a} \pm 2.8284$ | $0.00^{b} \pm 0.0000$ | $0.00^{b} \pm 0.0000$ |
| Foaming Stability (%) | $37.50^{a} \pm 3.5355$ | $0.00^{b} \pm 0.0000$ | $0.00^{b} \pm 0.0000$ |

Different letters indicate statistically significant differences among samples within the same row ($p<0.05$). Data are means ± standard deviation of duplicate determinations.

**Foaming properties:** Foam capacity requires that protein should solubilize in the aqueous phase and rapidly unfold to form a cohesive layer of protein around gas/air droplet [25] . Data in Table 1 suggests that *Cucurbita pepo* seed protein concentrate and isolate are not good foaming agents. *Cucurbita pepo* seed flour had a better foaming capacity (20%) and stability (37.5%) which may be due to increased solubility, rapid unfolding at the air-water interface, limited intermolecular cohension and flexibility of the surfactant molecules [26]. Solubility has an important influence on the foaming behaviour of proteins [27]. Proteins forming continuous intermolecular polymers enveloping the air bubbles help to form stable foams [25].

Foaming capacity and stability of CPF, CPC and CPI were affected in a similar manner between 0.4% - 1.0% sodium chloride concentration (Fig.5 and 6). *Cucurbita pepo* seed protein isolate was not stable at all with 0.2% - 1.0% sodium chloride concentration. Foam stability could be related to the amount of native protein which has been shown to give higher stability than denatured protein.

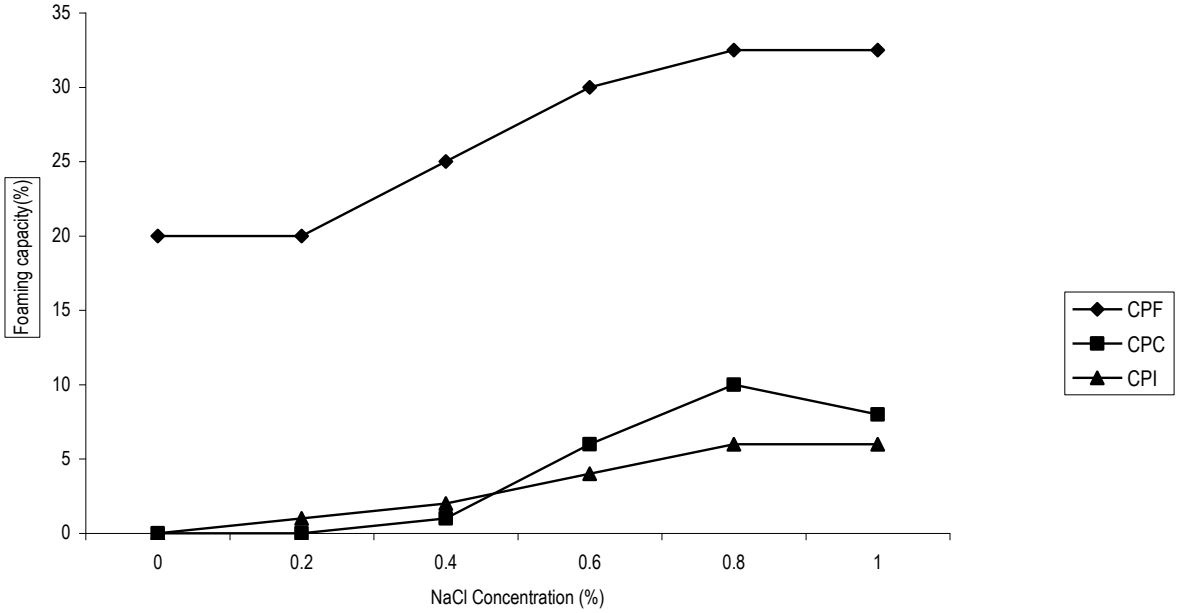


Fig.5: Effect of NaCl concentration on foaming capacity of *Cucurbita pepo* seed flour (CPF), protein concentrate (CPC) and isolate (CPI)

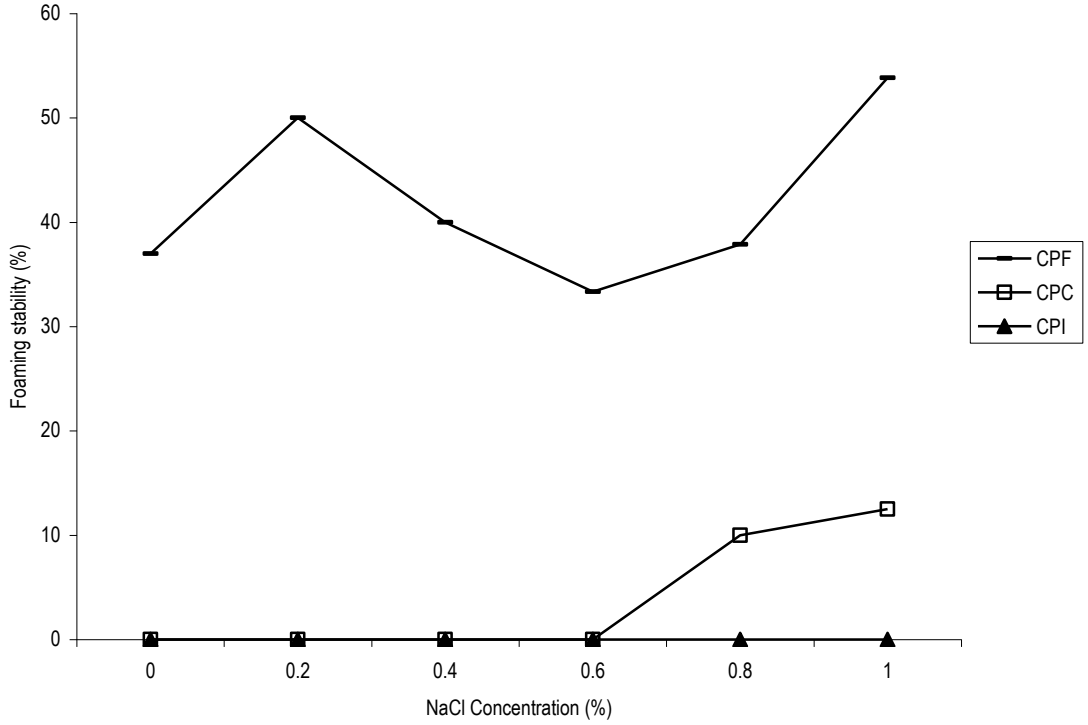


Fig. 6: Effect of NaCl concentration on foaming stability of *Cucurbita pepo* seed flour (CPF), protein concentrate (CPC), and isolate (CPI)

Similar trends were observed for the effect of pH on foaming capacity of the three samples at pH 2.0 and 4.0. Foaming capacity was highest at pH 2.0. This effect may be due to increase in the net charge of the protein molecules, which weakens hydrophobic interactions and increases protein flexibility/solubility, allowing the molecules to spread to the air interface more quickly thus encapsulating air molecules and increasing foam formation. Foam stability increased within the region of isoelectric point (4.0) mainly in *Cucurbita pepo* seed flour and protein isolate which may be attributed to the formation of stable molecular layers in the air-water interface of the foam. Protein adsorption and viscoelasticity at an air-water interface is maximal near or at isoelectric pH because protein is not strongly repelled and it possesses low net charge which contributes to the formation of stable molecular layers in the air-water interface thus improving foam stability. This result agrees with the observation of Lawal *et al*. [20] on foaming capacity of bambarra groundnut protein concentrate

## Bulk density

Bulk density signifies the behaviour of a product in dry mixes and it varies with the fineness of particles. The bulk density of *Cucurbita pepo* seed protein concentrate and isolate showed no significant differences (Table 1), but differ significantly from the seed flour which had the least bulk density (0.4902g/ml). High bulk density is disadvantageous for the formulation of weaning foods, where low bulk density is required. The bulk

densities of all the samples were very low compared to casein (0.89g/ml) as reported by Chandi and Sogi [28].

## Wettability

*Cucurbita pepo* seed flour wetted faster than the protein concentrate and isolate. This could be as a result of increased carbohydrate content which allows easy absorption of water. It took a longer time for the protein concentrate to get wetted and this may be attributed to the conformation and hydrophobic nature of the protein.

## Pasting properties

The peak viscosity developed after heating, ranged from 32.83 to 44.25 RVU with protein concentrate having the highest value at 93.65°C (Table 2). There was significant difference in the peak viscosity of all the samples ($p<0.05$). *Cucurbita pepo* seed flour had a higher breakdown viscosity. Adebowale *et al*. [29] reported that the higher the breakdown viscosity, the lower the ability of the sample to withstand heating and shear stress during cooking. The breakdown values for all the samples showed no significance differences ($p>0.05$). The final viscosity indicates the ability of the sample to form a viscous paste/gel after cooking and cooling [30]. The samples differ significantly ($p<0.05$) in the final viscosity. Protein concentrate exhibited the highest final viscosity. This may be attributed to partial protein denaturation which is said to increase protein viscosity due to the expanded hydrodynamic surface produced by protein unfolding [31]. The setback viscosity of the flour differed significantly ($p<0.05$) from the protein concentrate and isolate. The least and highest setback value was observed in *Cucurbita* flour and protein concentrate respectively. There was no significant difference ($p>0.05$) in the pasting temperature for all the samples. The pasting time was least in the flour and highest in the protein concentrate.

**Table 2: Pasting properties[d] of *Cucurbita pepo* seed flour, protein concentrate and isolate.**

| Samples | Peak Viscosity (RVU) | Trough Viscosity (RVU) | Breakdown Viscosity (RVU) | Final Viscosity (RVU) | Setback value (RVU) | Peak time (min) | Pasting Temp. (°C) |
|---|---|---|---|---|---|---|---|
| CPF | $32.83^{a}$±0.24 | $29.17^{a}$ ±0.81 | $3.16^{a}$ ±0.57 | $78.67^{a}$ ±2.97 | $49.50^{a}$ ±3.78 | $5.40^{a}$±0.14 | $94.20^{a}$±0.71 |
| CPC | $44.25^{b}$±0.35 | $41.92^{b}$ ±1.87 | $2.33^{a}$ ±1.51 | $107.33^{b}$±4.43 | $65.41^{b}$ ±6.29 | $7.00^{b}$ ±0.71 | $93.65^{a}$ ±1.70 |
| CPI | $38.75^{c}$±2.19 | $37.00^{b}$ ±2.83 | $1.75^{a}$ ±0.64 | $94.08^{c}$ ±2.67 | $57.08^{ab}$±0.16 | $6.31^{ab}$±0.13 | $93.60^{a}$ ±2.40 |

[d]Mean± standard deviation of duplicate determinations; Mean values with different superscript in same column are significantly different ($p<0.05$). CPF = *Cucurbita pepo* Seed Flour,CPC = *Cucurbita pepo* seed protein concentrate, CPI = *Cucurbita pepo* seed protein isolate.

## CONCLUSION

The importance of oilseed proteins in human diet is based not only on the nutritional quality but also on the functional properties. *Cucurbita* seed protein concentrate exhibited good functional properties and may be used as a functional agent in the formulation of a

wide range of new food products or as a possible replacement for animal proteins in conventional foods. The protein isolate with fairly good functionality could also be used as a food supplement but has limitations as a result of its poor colour quality. *Cucurbita* seed flour could be suitable for preparation of baked and meat products because of its high water and oil absorption and could also be used in foods in which the emulsion properties are important such as creams.

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