Full Paper

FUNCTIONAL, THERMAL AND STRUCTURAL PROPERTIES OF CONOPHOR (*TETRACARPIDIUM CONOPHORUM*) ALBUMIN

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ABSTRACT

The functional, thermal and structural properties of conophor albumins under various medium conditions were investigated with a view to elucidating its behaviour under these conditions for improved utilization as functional food ingredients. Conophor albumins were isolated by dialysis of the supernatant obtained after 1.0 M NaCl extraction of the defatted flour and samples were lyophilized. Protein solubility, emulsifying activity index (EAI) and emulsion stability index (ESI), foaming capacity (FC) and foaming stability (FS) under the influence of pH and NaCl were investigated. Thermal stability and structural properties as measured by Differential Scanning Calorimetry (DSC) and Circular Dichroism (CD) respectively were also investigated under various environmental conditions. Minimum protein solubility in water (44.36%), 0.5 M (41%) NaCl and 1.0 M (38.91%) NaCl occurred at pH 6. At pH 2-6, EAI decreased with increasing pH from 34.89 ± 5.68 to 9.00 ± 1.09 (m^2g^{-1}) and thereafter, increased with increasing pH. FC and FS ranged between 7.00 - 20.00% and 40.00 - 60.71%, respectively with the maximum value obtained at pH 10 for FC and pH 6 for FS. Decreases in enthalpy (ΔH) and denaturation temperature (T_d) were observed under the influence of highly acidic and alkaline pHs. The progressive increase in T_d (70.95 – 77.20 °C) with increase in salt concentration (0.00M – 0.10 M) suggests a more compact conformation for conophor albumin with higher thermal stability. Far-UV-CD spectra showed that the secondary structure was stable to changes in pH (3-9), ionic strength and temperature. The tertiary structure was found to be more sensitive to change in temperature as indicated by near-UV-CD spectra.

Keywords: *Protein solubility, foaming properties, DSC, near-UV-CD spectra, far-UV-CD spectra*

1. INTRODUCTION

Plant proteins remain a very cheap and alternative source of proteins for human consumption in developing countries owing to the high cost of animal proteins. Increased utilization has necessitated the quest for non conventional sources of vegetable proteins in place of commonly used ones such as legumes and soybeans. Conophor nut (*Tetracarpidium conophorum*) is an oilseed with great potentials for increased utilization. The whole nut contains 40% oil, 5.9% crude fibre, 2.8% ash and 20 – 24% protein and amino acid analysis shows that all the essential amino acids are present in adequate amounts for human nutrition [1, 2]. The food processing industry utilizes protein ingredients to impart specific functional properties in a wide range of formulated foods. However, the ultimate success of utilizing any plant protein as food ingredients depends largely on its functional and nutritional properties. The functional properties of proteins, such as protein solubility, foaming properties and emulsifying activities, are in turn highly dependent on many factors such as pH and type and amount of salt present. Vegetable proteins such as legumes and oilseeds undergo various thermal treatments such as blanching, steaming, roasting and cooking during processing to eliminate anti-nutrients and therefore improve its nutritional quality and palatability. However, uncontrolled or excessive heating could cause undesirable changes in proteins, such as lowering of their digestibility and bioavailability [3]. Hence, thermal analysis, such as DSC, is particularly useful in evaluating effects of heat treatment on protein quality in foods [4]. In addition to DSC characteristics, analyses such as hydrophobicity, amino acid composition and secondary and tertiary structures are keys to understanding the nature of these proteins and thus their suitability for various functional purposes [5]. Currently there is a dearth of information on functional, thermal and structural properties of conophor albumin under various medium conditions. The results are expected to assist in the evaluation of conophor albumins as functional food ingredients.

2. MATERIALS AND METHODS

Fresh mature conophor nuts were obtained from a local market in Modakeke-Ife, Osun State, Nigeria. The nuts were immediately washed under tap water to remove adhering dirt and mud. The cleaned nuts were then packaged in polythene bags and stored at -20^{0}C for further use. All chemicals used were obtained from Fisher Scientific (Oakville, ON, Canada) and Sigma Chemicals (St. Louis, MO).

2.1. Preparation of defatted flour

Defatted Conophor flour was prepared using a modified method of [6]. Whole uncooked conophor nuts were shelled manually and rinsed with tap water. The shelled nuts were comminuted using mortar and pestle to obtain coarse flour. The coarse flour obtained was then ground using Marlex Excella grinder (Marlex Appliances PHT, Daman) and the resulting flour was subsequently defatted with cold (4 $^{\circ}$C) acetone (flour to solvent ratio 1:5 w/v) with constant magnetic stirring provided. The slurry was then filtered through a filter (Whatman No 1) paper. The residue was re-extracted twice in a similar fashion. The defatted flour was desolventized by drying in a fume hood and the dried flour was finally ground in a Marlex Excella grinder (Marlex Appliances PVT., Daman) set at maximum speed to obtain homogenous defatted flour. The defatted flour obtained was stored in an air-tight plastic bottle and kept at 4^{0}C until further use.

2.2. Preparation of albumins

The procedure described by [6] was employed with some modifications. Defatted flour (50g) was extracted with 1 M NaCl at room temperature for 4 h with constant magnetic stirring. The sample was centrifuged (4500g, 28 ^{0}C, 30 min) and the supernatant obtained was extensively dialysed against distilled water at 28 ^{0}C (48 h, five changes, 4 litres each). The molecular weight (MW) cut-off of the dialysis tubing (Spectrum Laboratories Inc., CA) was 6000-8000. Samples were then centrifuged (4500g, 28 ^{0}C, 30 min) and the supernatant and precipitate collected. The precipitate was redissolved in a minimum amount of 1M NaCl, re-precipitated with 4 volumes of distilled water and centrifuged (4500g, 28 ^{0}C, 30 min). This process was repeated two more times under the same conditions. The supernatant obtained was dialyzed against distilled water as above and the combined supernatant was freeze dried and kept at -4°C for further use.

2.3. Chemical analyses

2.3.1. pH

The pH was measured by making a 1% w/v suspension of the sample in distilled water. The suspension was mixed thoroughly and the pH was measured with a Combo pH meter (Model HI 98129, Hanna Instrument, Italia).

2.3.2. Bulk density

Bulk density was determined according to the modified method of [7]. A 10 ml graduated cylinder, previously tared, was gently filled with the sample. The bottom of the cylinder was gently tapped on a laboratory bench several times until there was no further diminution of the sample level after filling to the 10 ml mark. Bulk density was calculated as weight of sample per unit volume of sample (g/ml).

2.3.3. Water absorption capacity (WAC)

Water absorption was determined by a modification of the method described by [8]. A sample (200 mg) was transferred into a weighed centrifuge tube and 10 ml of distilled water added. Using a glass stirring rod, the sample and water were mixed thoroughly for 30 s every 10 min over a period of 30 min. The flour particles adhering 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 4500g 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 expressed as percentage increase of the sample weight.

2.3.4. Oil absorption capacity (OAC)

Oil absorption capacity was determined following a modification of the method described by [9]. A 200 mg sample was weighed into a tared 50 ml centrifuge tube and 10 ml of pure Gino oil (commercial cooking oil) was added. The mixture was mixed with a glass stirring rod for 30 s, allowed to stand for 30 min and then centrifuged (MSE Harrier 15/80, Sanyo, UK) at 4500g for 20 min. The supernatant was decanted and the tubes were allowed to drain at a 45º angle for 30 min before being weighed. Oil absorption capacity was expressed as percentage increase of the sample weight.

2.3.5. Effect of pH on emulsifying activity index

To study the effect of pH on emulsifying activity index, the method described by [10] was followed with some modifications. The protein sample (200 mg) was dispersed in 30 ml of distilled water and gently stirred to disperse the sample. The pH of the protein solution was then adjusted to the desired pH with either 1 N HCl or 1 N NaOH. 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. Fifty microlitres of the aliquot of the emulsion were 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 (Unican Heλios, UV-Visible Spectrophotometer, Australia). The EAI was expressed as interfacial area per unit weight of protein (m^2g^{-1})

2.3.6. Emulsion stability (ES)

The emulsions were allowed to stand for 10 min at room temperature and the EAI determined as described above, and expressed as a percentage of the initial EAI [11].

2.3.7. Effect of pH on Foam capacity and stability

Foam capacity and foam stability as influenced by pH were determined by a modification of the method described by [12]. A-25 mg protein sample was dispersed in 250 ml of distilled water and the pH of the protein solution was then adjusted to the desired pH with either 1 N HCl or 1 N NaOH. The solution was then 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 [13]. Foam stability was expressed as percentage of the volume of foam remaining after 30 min of quiescent period.

2.3.8. Differential scanning calorimetry

The thermal properties of conophor albumins under different pH and sodium chloride concentrations were investigated using a DSC Q200 thermal analyzer (TA Instruments, New Castle, DE). The method described by [4] was employed for this analysis. Approximately 1 mg sample (protein basis) was weighed into the empty aluminium pan, and 10 µl of either 0.1 M phosphate buffer, pH 3-9 or 0 – 1.0 M sodium chloride was added. The pan was hermetically sealed and heated from 30 to 140°C at a rate of 10°C/min. A sealed empty pan was used as a reference. Onset temperature (T_m), peak transition temperature or denaturation temperature (T_d) and enthalpy of denaturation (ΔH) and cooperativity, represented by the width at half- peak height ($\Delta T_{1/2}$),

were computed from the thermograms by the Universal Analysis Program, Version 1.9 D (TA Instruments).

2.3.9. *Circular dichroism (CD)*

Far- and near-UV-CD spectra were measured using a JASCO model J-815 spectropolarimeter (JASCO Corporation, Tokyo, Japan) at 25 °C unless otherwise stated. The far-UV-CD spectrum was measured on the 0.4 mg/ml protein sample in 0.1 M phosphate buffer at 190-240 nm, using a quartz cell with path length of 0.5 mm. The pH of the sample buffer was adjusted to between 3 and 9 with either 1 M HCl or 1 M NaOH to determine the pH effect on the structures. The effect of sodium chloride (0.1 M) and pH was also determined by dispersing protein sample in 0.1 M phosphate buffer containing 0.1M NaCl and the pH was subsequently adjusted to between 3 and 9. The effect of calcium ions (up to 50 mM $CaCl_2$) and temperature sweeps (30-90 °C) were carried out at pH 7. The near-UV spectrum was measured at 250-320 nm under the same condition as above but using 2 mg/ml protein concentration and a quartz cell path length of 1 mm. For near-UV spectrum the effect of calcium ions and temperature sweeps were carried out at pH 5. Molar ellipticity (Ø), (deg cm^2 $dmol^{-1}$) was calculated from the formula (Ø) = Ø/(10Cl), where Ø is the measured ellipticity (deg), C is the molar concentration of albumin solution (mol L^{-1}) and l is the cell path length (cm).

2.4. Statistical analysis

All samples were analysed in triplicates. Data are reported as means ± standard deviation. Where appropriate, analysis of variance (ANOVA) was performed and differences in mean values were evaluated using Tukey`s test at $p < 0.05$. One-way ANOVA with Turkey`s post test procedures of GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego California USA) was employed.

3. Results And Discussion

3.1. Physicochemical and functional characteristics

The results of bulk density and pH as well as functional properties (oil absorption, emulsifying activity, emulsion stability, foam capacity and stability) of conophor albumins are presented in Table 1. Conophor albumins exhibited very low bulk density (0.18 g/ml) when compared to its isolates (0.66 g/ml) and globulin (0.50 g/ml). This implies that smaller quantity of sample may be packed within a constant volume. According to [14] 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 solution of albumin in water showed a pH value (6.90) that was slightly acidic to neutral. The pH value of proteins in water are important since some functional properties such as solubility [12, 15], whippability [16] and emulsion properties [17] are affected by pH changes. The protein sample exhibited very high oil absorption capacity (340%) which may be related to its relatively high proportion of hydrophobic amino acids. Oil binding capacity may determine whether the protein materials will perform well as meat extenders or analogs [7]. Conophor albumins exhibited low foaming capacity (16%) in water when compared to other plant proteins such as beach pea proteins 128-143%; [12], green pea (170-185%) and grass pea (151-175%). The low foam capacity of conophor albumins may be due to inadequate electrostatic repulsions, and hence, excessive protein-protein interactions to form aggregates that are detrimental to foam formation. The proteins however demonstrated appreciable ability (71%) to hold and maintain the foam. [18] and [19] have suggested that, in foams, the ability to hold water in the protein film surrounding the air particle and presence of electrostatic repulsions are important for their stability. The emulsifying activity index (21.08 m^2g^{-1}) and emulsion stability index (49.80%) of conophor albumins were low compared with the EAI reported for defatted cashew nut protein (24.63 m^2g^{-1}), but higher than those of cashew nut concentrate (13.68 m^2g^{-1}) and isolate (12.48 m^2g^{-1}) reported by [20].

3.1.1. *Effect of pH and NaCl concentration on protein solubility*

Protein solubility of conophor albumins as a function of pH and NaCl concentration is shown in Fig. 1. Generally, in the absence or presence of NaCl, minimum protein solubility was obtained at pH 6. On either side of this pH protein solubility increased. Alkaline pH was however, more effective in solubilizing conophor albumins than acidic pH. The protein solubility of albumins in the presence of NaCl was generally lower than solubility in the absence of NaCl at all pH values and the solubility decreased with increasing NaCl concentration. According to [21], the occurrence of minimum solubility near the isoelectric point is due primarily to both the net charge of peptides, which increase as pH moves away from the isoelectric point, and surface hydrophobicity that promotes the aggregation and precipitation via hydrophobic interactions. The presence of NaCl exerted `salting-out` effect causing aggregation or precipitation of protein molecules, i.e. a reduction in protein solubility. At pH lower than the isoelectric pH, the reduction of solubility for conophor albumin could be explained by the predominant electrostatic screening of the positively charged protein and/or by adsorption of chloride ions by the protein [22]. Similar observations were reported for peanut proteins [23], cashew nut proteins [20] and beach pea protein isolates [12].

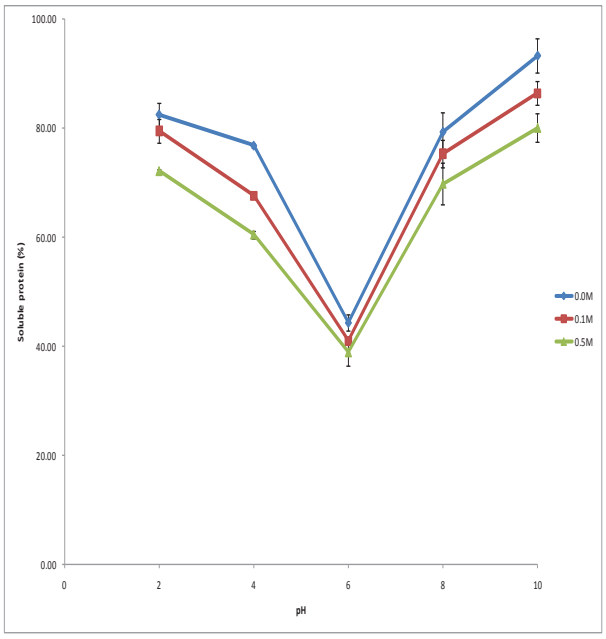

Fig. 1: Solubility profiles of conophor albumin as a function of pH and NaCl concentration.

3.1.2. *Effect of pH on functional properties*

The results of emulsifying activity index (EAI), emulsion stability index (ESI), foam capacity (FC) and foam stability (FS) measured as a function of pH are presented in Table 2. At low pH (2-6), EAI decreased with increasing pH with the lowest EAI obtained around the isoelectric regions (pH 6) and thereafter, increased with

increasing pH. The results show that EAI was pH dependent. Generally, the observed trend of decrease and increase is in agreement with the pH dependent solubility of proteins. pH has pronounced effect on emulsifying activity because emulsifying activity of soluble proteins depends upon the hydrophilic-lipophylic balance [24]. Proteins generally have been shown to be poor emulsifiers at isoelectric pH, owing to their low solubility, poor hydration and lack of electrostatic repulsive forces [25]. The emulsion stability index (ESI) of conophor albumins in water improved sharply as pH approaching the isoelectric pH (pI), and a maximum ESI of approximately 74% was attained at the pI. These results are in agreement with literature values for cashew nut flour, protein concentrate and isolate [20].

At low pH, foam capacity decreased with increasing pH with minimum foam capacity (7.00%) obtained at pH 6 which is the point of minimum protein solubility. At pH values above pI, foam capacity exhibited significant ($p < 0.005$) increase. The results further corroborated earlier findings that foam formation is influenced by protein solubility [26, 27]. The high foaming capacity at extreme alkaline and acidic pH was attributed to increase in the net charge of the protein molecules, which weakens hydrophobic interactions and increases protein flexibility [27]. On the other hand maximum foam stability was obtained around the pI and on either side of this pH the stability decreased. Protein adsorption and viscoelasticity at an air-water interface is maximal near or at isoelectric pH because protein is not strongly repelled. In addition, the protein possesses low net charge near isoelectric pH, which may contribute to the formation of stable molecular layers in the air-water interface, a development that improves foam stability [27].

3.2. Differential scanning calorimetry

3.2.1. *Effect of pH*

The thermal transition properties of conophor albumin as influenced by pH (3-11) and NaCl concentrations are shown in Tables 3 and 4, respectively. The denaturation temperature (T_d) and enthalpy (ΔH) values ranged between 72.12 – 99.08 °C and 2.48 – 5.13 J/g protein, respectively. The highest T_d value was obtained at pH 5 and on either side of this pH there were significant decreases which are indicative of reduced thermal stability. The lowering of ΔH at the extreme acidic or alkaline conditions suggests partial denaturation since partially denatured proteins require less energy for complete denaturation. Similar observations have been reported for red bean globulin [4] and flaxseed proteins [28]. The pH – induced changes in the conformation resulted in a less cooperative system as indicated by the decrease in $\Delta T_{1/2}$ values on either side of pH 5. Most proteins achieve stability at a narrow pH range normally near the isoelectric pH, when the repulsive forces are quite low and therefore the proteins remain in the native state. At high or low pH, large net charges are induced and repulsive forces increase, resulting in unfolding of proteins [29]. The unfolding of proteins at extreme pHs may also be attributed to rupture of hydrogen bonds and a breaking of hydrophobic interactions [30].

3.2.2 *Effect of NaCl concentration*

The influence of NaCl on the thermal transition of conophor albumin is demonstrated in Table 4. Increasing concentration of NaCl from 0 – 1.0 M raised the T_d from 70.95 – 77.20 °C and ΔH increased from 29.00 – 35.81 J/g protein indicating the stabilizing effect of NaCl on conophor albumin. The heat stability of proteins is controlled by the balance of polar and nonpolar residues [31], such that the higher the proportion of nonpolar residues, the greater is the stability to heat, (i.e., higher temperature required for denaturation) [32]. Protein conformation can be perturbed by the addition of salts which influence the electrostatic interaction with the charged groups and polar groups, and affect the hydrophobic interactions via a modification on the structure of water [33, 34]. The stabilizing effect of NaCl solution at lower concentration (< 1.0 M) was attributed to the electrostatic response or induction of alteration of water structure around the protein, which enhanced the hydration of the protein molecule [35, 33]. At higher concentration of NaCl (> 1.0 M), the solubility of protein decreased, due to a "salting –out" phenomenon, which caused the aggregation or precipitation of protein molecules due to lack of water molecules in the competition between the proteins and ions for water [29], [4]. In this case a more compact protein conformation was formed with increased thermal stability (higher T_d and ΔH). The decrease in $T_{1/2}$ values indicated that the transition was highly cooperative requiring more energy for complete denaturation as the concentration of NaCl increased.

3.2.2. *Circular dichroism (CD) spectra*

The influence of pH on conophor albumins secondary structure as measured by far-UV-CD data is presented in Fig. 2. Analysis of far-UV-CD data has proven to be a good method to elucidate changes in secondary structure of proteins and enzymes, including relative contents of α-helix, ß-sheet, ß-turn and random coil [36, 37]. The CD spectrum at all pHs revealed 2 positive peaks at around 192 nm and 230 nm; one major negative peak at 203 nm with a shoulder at 209 nm. The observed spectrum was typical of proteins in which α-helical structures predominate [38]. The positive peak at 230 nm may be due to the presence of non albumin proteins since the sample used was not completely pure. On either side of pH 5, the intensity of positive peaks was reduced while those of negative peaks were increased. In spite of this there appeared to be no major changes in the backbone secondary structure of conophor albumins under the influence of pH. The effect of 0.1 M NaCl on the CD spectrum (Fig. 3) was more pronounced resulting in a shift of positive peaks maximum around 192 to the negative region of the spectrum which is indicative of possible change in structure in the presence of NaCl.

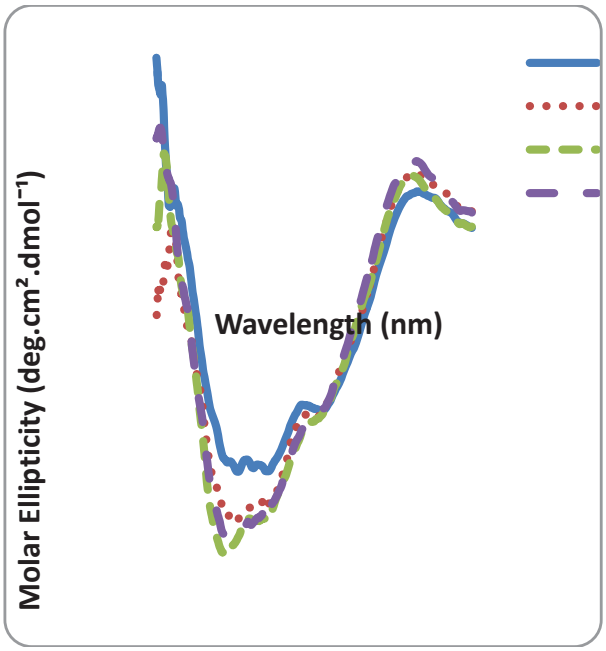

Fig. 2: Effects of pH on far-UV-CD spectra of conophor albumins.

Fig. 4 shows the effect of calcium chloride at different concentrations on the CD spectrum. A noticeable change in

spectrum was observed only at higher concentration of $CaCl_2$ (50 mM) where there was a shift in negative peaks maximum at 203 nm (0 mM) to 200 nm (50 mM) with a decrease in molar ellipticity (Ø) from -22914 deg cm^2 $dmol^{-1}$ (0 mM) to 11155 deg cm^2 $dmol^{-1}$ (50 mM). The effect of calcium did not, however, translate into significant changes in the fraction of secondary structures.

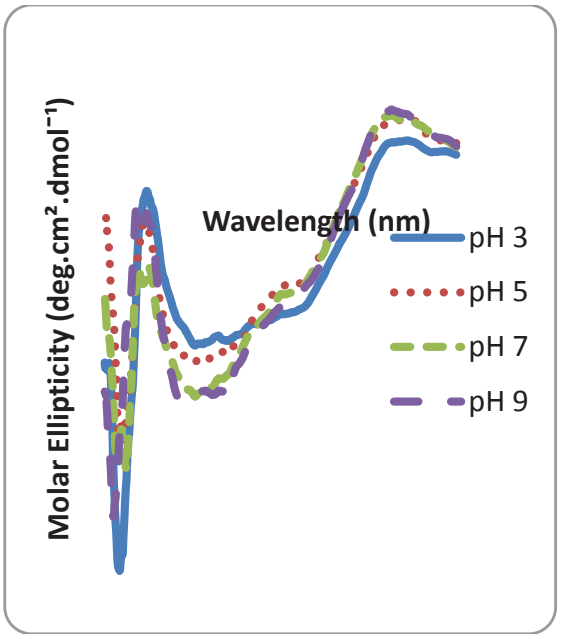

Fig 3: Effect of 0.1 M sodium chloride on far- UV-CD spectra of conophor albumins prepared at different pH

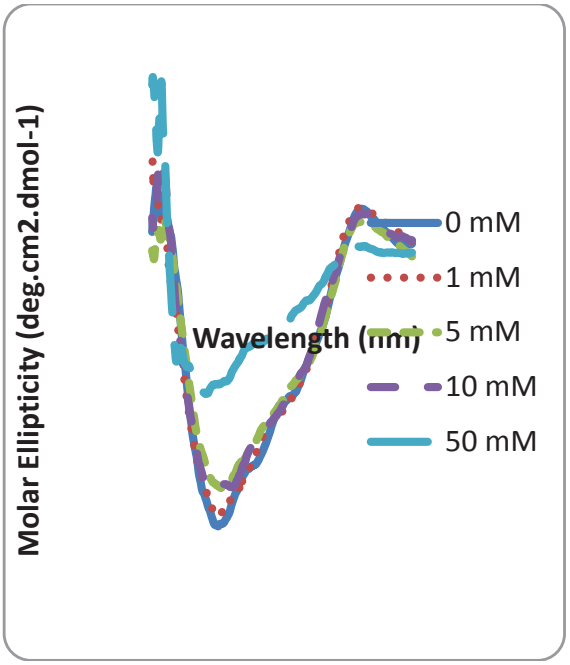

Fig. 4: Effect of calcium chloride concentrations on far UV-CD spectra of conophor albumins.

Fig. 5 shows the result of temperature sweeps at 220 nm and 195 nm. Changes at 220 nm are normally used as indicators of α-helix structure while changes at 195 nm are usually indicative of changes in ß-sheet structure. The results show that at pH 7, α-helix was relatively stable up to about 60 °C compared to the ß-sheet structure which exhibited poor stability at virtually all temperatures. The relative poor stability exhibited by conophor fractions at high temperatures may likely be due to the fact that the analysis was conducted at pH 7 which is different from its isoelectric pH at which point the protein is likely to maintain a very rigid structural conformations.

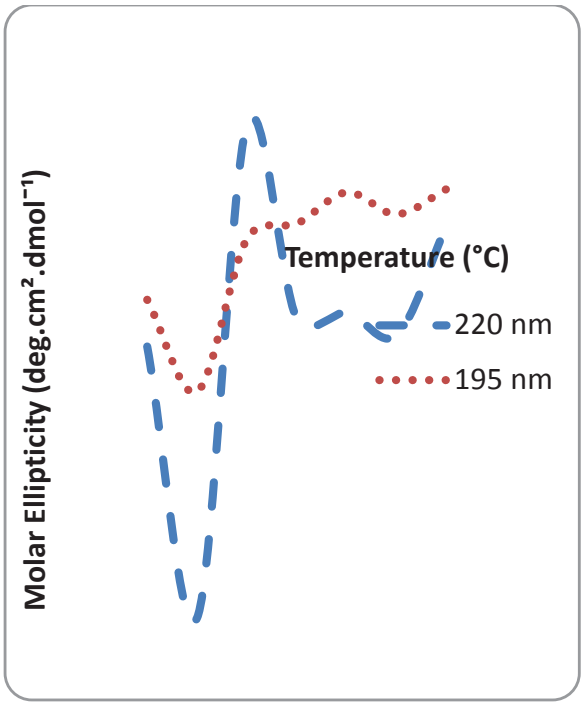

Fig. 5: Effect of temperature on molar ellipticity of conophor albumins.

Data analysis of near-UV-CD spectra were used to monitor changes in tertiary structure of conophor albumins. The effects of pH and NaCl on the near-UV-CD spectra are presented in Figs. 6 and 7, respectively. CD bands in the near-UV region depend on changes to the aromatic amino acids, tyrosine, tryptophan and phenylalanine, which have been found to be present in conophor albumins (data not shown).The CD spectrum (Fig. 6) shows only one negative band maximum around 278 nm and this was common for all the pHs. However, reducing the pH from 5 to 3 or raising it from 5 to7 or 9 decreased the intensities of the peaks. This negative peak (278 nm) falls between responses for phenylalanine (255 -270 nm) and tyrosine (275 -280 nm) [39], [40]. This result indicated that aromatic residues were probably confined to a particular location and that changes in pH of the aqueous environment did not cause major changes in the tertiary structure of conophor albumins. Under the influence of NaCl (Fig. 7) the CD spectrum obtained was similar to that of Fig. 6 except that part of the peaks (pH 3, 5 and 7) shifted upwards into the positive region. A minor positive (pH 9) and negative (pH 3 and 7) peaks maximum around 306 nm were also observed. Figs. 8 and 9 show the influence of $CaCl_2$ and temperature on the near-UV-CD spectra of conophor albumins, respectively. The effect of $CaCl_2$ did not result in appreciable changes in the tertiary structure of the albumins at lower concentrations. At higher concentration (50 mM), however, there was an upward shift of band to the positive region. The single peak maximum around 278 nm was still maintained indicating no major changes to the tertiary structure of the proteins. The effect of temperature (Fig. 9) was more dramatic showing both positive and negative bands but with the majority of the bands shifting upward to negative region but with different intensities and the peaks maximum around 278 nm. At 90 °C the negative peak maximum equally around 278 °C but with very high

intensity. The results indicate temperature- dependent increased interactions of the phenylalanine and tyrosine residues with each other or with adjacent aromatic residues.

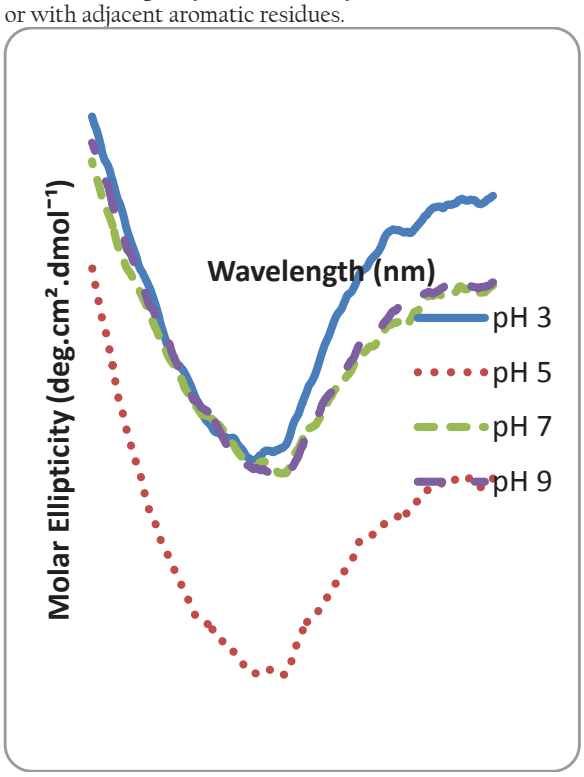

Fig. 6: Effects of pH on near-UV-CD spectra of conophor albumins

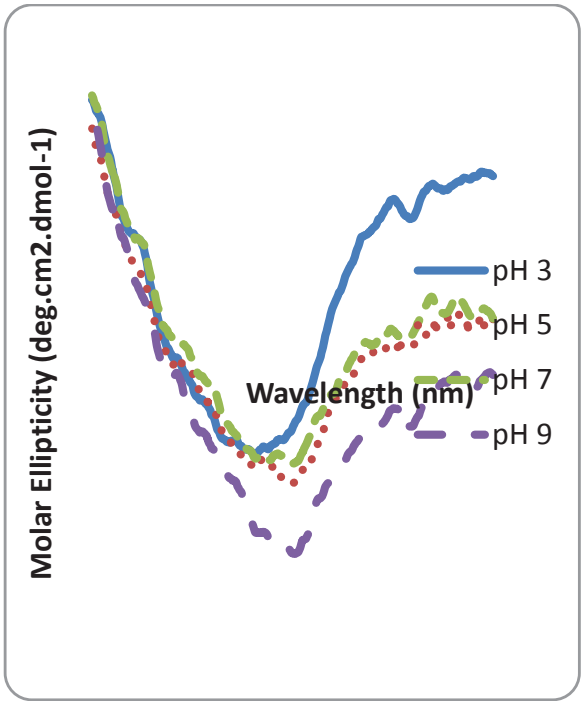

Fig 7: Effect of 0.1 M sodium chloride on near- UV-CD spectra of conophor albumins prepared at different pH

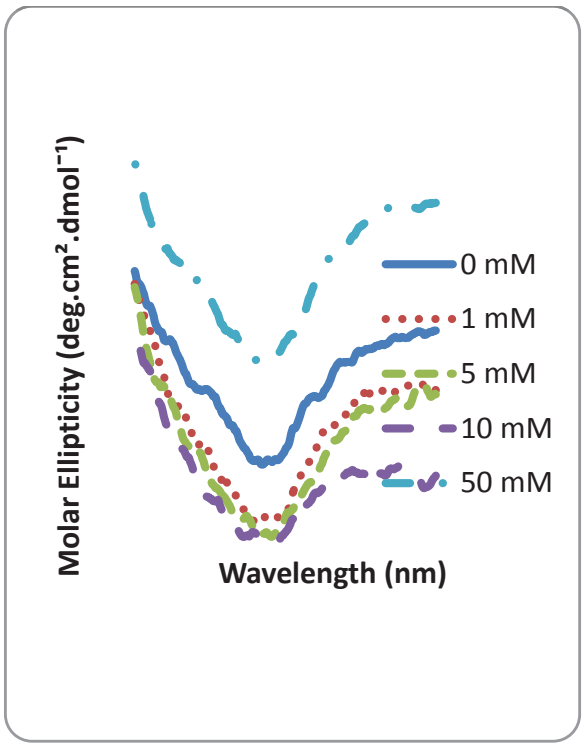

Fig. 8: Effect of calcium chloride concentrations on near UV-CD spectra of conophor albumins.

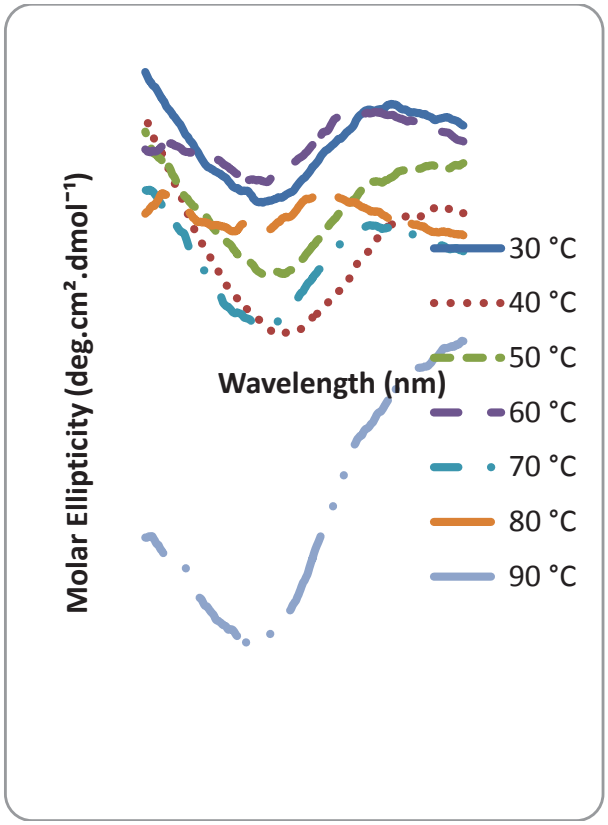

Fig. 9: Effect of temperature on near UV-CD spectra of conophor albumins.

4. Conclusion

The study demonstrated the influence of pH, ionic strength and temperature on the functional, thermal and structural characteristics of conophor albumins. Protein solubility, emulsifying and foaming properties were pH-dependent and NaCl reduced protein solubility. The DSC characteristics of conophor albumins were influenced by environmental factors such as ionic strength and pH. The results suggest that hydrophobic interactions, hydrogen bonds and ionic interactions play an important role in stabilizing the conformation of the protein molecule. Circular dichroism study revealed that conophor albumins` secondary structure was possibly dominated by α-helix which exhibited relative stability under the influence of pH, NaCl, $CaCl_2$ and temperature. The tertiary structure was however, more susceptible to changes in temperature suggesting that further studies on its utilization as a functional food should be conducted under these conditions.

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