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Proteomic Quantitative UV Absorption Spectrum Analysis of Effect of Heat Stress on Protein Extract from Cowpea Seed (*Vigna unguiculata* (L) Walp)

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Authors' contributions

This work was carried out in collaboration between all authors. Author AO designed the study, performed the statistical analysis and prepared the draft manuscript, author AAO carried out the cluster analysis comparison of absorbance spectra optical density values, author OAO carried out the bench work, author TOO prepared the final manuscript, authors OBA, AA and JAF carried out the comparison of absorbance spectra of protein with different heat treatments. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Proteomic quantitative UV absorption spectrum analysis was used to study the effect of heat stress on protein extract from cowpea seeds (*Vigna unguiculata* (L) Walp).
Study Design: Protein extracts were obtained from 9 cowpea accessions obtained from GeneBank of International Institute of Tropical Agriculture in January 2014. Each protein extract was divided into four batches out of which three batches were subjected to different temperature treatments and incubation at 37°C, 60°C and 100°C for 1 hour and the remaining one batch served as control. Protein content in each control protein extract and 37°C, 60°C and 100°C treated protein extracts

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from each of the 9 cowpea samples were determined at 280 nm using bovine serum albumin standard curve.
Place and Duration of Study: Biochemistry Unit, Department of Chemical Sciences, Afe Babalola University Ado Ekiti, Nigeria between January 2014 and June 2014.
Methodology: A_{200}-A_{960} UV wavelengths absorption spectrum analysis was carried out on control protein extract and 37°C, 60°C and 100°C treated protein extracts respectively from each of the 9 cowpea samples. In order to establish the relationship between protein extracts (control) and protein extracts heat treated (37°C, 60°C, and 100°C), cluster analysis of optical density (OD) data was carried out using numerical taxonomy and multivariate analysis system.
Results: The protein content (control) in *Vigna unguiculata* (L) Walp was between 8.4 and 10.8 mg/ml (10.5-13.5%) in seed, while protein content (heat treated) in *Vigna unguiculata* (L) Walp was between 8.9 and 9.5 mg/ml (11.2-11.9%), 8.7-9.5 mg/ml (10.9-11.9%), 9.0 and 11.8 mg/ml (11.3-14.7 %) in heat treatments of 37°C, 60°C, and 100°C respectively. The protein UV absorption spectra of control protein extract and 37°C, 60°C and 100°C treated protein extracts from each cowpea accession were generally different due to differential UV wavelength protein absorption. Cluster analysis of absorbance spectra optical density values revealed five clusters (cluster 1, cluster 2, cluster 3, cluster 4, and cluster 5) among control protein extracts and protein extracts heated at 37°C, 60°C, and 100°C. Cluster1 was made up of protein extracts heated at 37°C and 60°C, while cluster2 and cluster3 constituted closely related protein extracts heated at 37°C and 100°C respectively. Cluster4 was typical of control protein extracts, while cluster5 was made up of distinct protein extracts heated at 100°C.
Conclusion: Heating protein extracts at 37°C, 60°C, and 100ºC has altered proteomic diversity in different cowpea accessions and this could make protein extraction more difficult with implications on protein properties.

Keywords: Cowpea; proteomic; spectrum; absorbance; multiwavelength.

1. INTRODUCTION

Cowpea [*Vigna unguiculata* (*L.*) Walp.] is an important crop in many countries of tropical Africa, Asia and South America. It is one of the most important food legume crop grown in semi-arid tropics covering Africa, Latin America, West Indies, India, South-east Asia and the southern United States [1] with an annual production of more than five million metric tons world-wide [2]. West Africa produces about 80% of the world's total cowpea production, with Nigeria, Niger and Senegal as the principal producers [3]. In Nigeria, the grain legume is mostly grown in the drier climate of the North than in the humid climate of the South, where high humidity causes diseases and drying problems [4].

Cowpea grains and leaves are edible products that are rich and cheap sources of high-quality protein. They supplement to the lower quality cereal or root and tuber protein commonly consumed in tropical Africa [5,6]. Cowpeas are mainly consumed as a favorite food-stuff in the form of dried seeds, either as flour or split [7,8]. They are a good source of carbohydrates, vitamins, and protein, providing more than half of plant protein in human diets in some areas of the semi-arid tropics [9,10]. On average cowpea grains contain 23-25% protein and 50-67% starch in dry bases [11]. The seeds make up the largest contributor to the overall protein intake of several rural and urban families hence [12] regarded cowpea as the poor man's major source of protein. Their amino acid complements those of cereals [13-15]. Cowpea is a most versatile African crop, it feeds people, their livestock and because of its ability in nitrogen fixation, it improves soil fertility, and consequently helps to increase the yields of cereal crops when grown in rotation and contributes to the sustainability of cropping systems [16].

Cowpea [*Vigna unguiculata* (L) Walp.] is one of the important leguminous crops, which belongs to the family *Leguminosae*, subfamily Faboideae, tribe *Phaseoleae*, genus *Vigna*, subgenus *Ceratotropis* and species *unguiculata* [17]. It is subdivided into four cultivar groups (cv. gr.): unguiculata (the common cowpea), biflora (the catjang), sesquipedalis (the yard-long bean) and textilis (used for fibers) [18,9]. It is primarily originated in West Africa [19]. It has a number of common names which includes crowder pea, black eyed pea and southern pea [20]. It is generally called beans in Nigeria.

Apart from its protein content (about 25%), cowpea also contains some vitamins and minerals and it's also an excellent source of fibre, calcium, folic acid and vitamin A [21]. As with most legumes, they are poor in some of the sulphur-containing amino acids needed for a complete protein. Several methods are commonly used for the determination of protein concentration. Each of these methods depends on the source of the protein, amount of protein present, the specificity of the method, the presence of interfering substances, and the amino acid composition of the protein. Most of these methods rely either on colorimetric assays or the use of UV absorbance spectroscopy [22]. A number of methods have been devised to measure protein concentration, which are based on UV-visible spectroscopy. These methods use either the natural ability of proteins to absorb (or scatter) light in the UV-visible region of the electromagnetic spectrum, or they chemically or physically modify proteins to make them absorb (or scatter) light in this region. The basic principle behind each of these tests is similar. First of all a calibration curve of absorbance (or turbidity) versus protein concentration is prepared using a series of protein solutions of known concentration. The absorbance (or turbidity) of the solution being analyzed is then measured at the same wavelength, and its protein concentration determined from the calibration curve. The main difference between the tests are the chemical groups which are responsible for the absorption or scattering of radiation, e.g., peptide bonds, aromatic side-groups, basic groups and aggregated proteins.

Temperature treatments include the use of freeze–thaw and heat treatments. Freeze–thawing uses the effect of ice crystal formation in the tissue during the freezing process. Lysis of the cells or tissues is usually achieved by flash-freezing the cells in liquid nitrogen and homogenizing in a mortar with a pestle. Examples of this process are found in the analysis of leaves [23], fruits [24], and seeds [25-27]. [28] developed a very efficient cell disruption method for grape berry clusters, which were pulverized frozen with dry ice using a stainless steel blender. The use of heat is common in protein processing. Heating protein solutions usually improves their solubility, emulsifying, and foaming properties, but it makes protein extraction more difficult and alter proteomic diversity in grains [29,30,27]. The aim of the present study was to compare the effect of heat stress at various temperatures on protein extract from seeds of *Vigna unguiculata* (L) Walp accessions using UV absorption spectrum analysis.

2. MATERIALS AND METHODS

2.1 *Vigna unguiculata* (L) Walp Seed

The *Vigna unguiculata* (L) Walp seeds used in this study were obtained from International Institute of Tropical Agriculture (IITA), Ibadan in January 2014.

2.2 Protein Extraction

A total of 9 seed samples (Table 1) were used for protein extraction. The protein extraction procedure used was according to Barbarino and Lourenco [31] and Onasanya et al. [27]. 1 g of seed was grinded in 5 ml of 0.1 M NaOH using mortar and pestle. The homogenate was transferred into 2.5 ml Eppendorf tubes and incubated at room temperature for 12 hours. After incubation, the homogenate was centrifuged at 4ºC at 12,000 rpm for 20 min using refrigerated centrifuge. The supernatant was collected as crude protein extract. The collected protein extract was stored at 4ºC for use in heat treatment and UV absorbance spectrum analyses.

Table 1. List of *Vigna unguiculata* (L) Walp seeds used for the study

S/N	Cowpea Code	Cowpea Seed Name	Color
1	V1	IT99K-573-2-1	Brown
2	V2	T Vu – 8775	Brown
3	V3	T Vu-7846	Brown
4	V4	T Vu – 15636	Red
5	V5	TVu- 13965	Red
6	V6	T Vu-8586	Red
7	V7	IT98K-205-8	White
8	V8	T Vu – 3947	White
9	V9	T Vu-702	White

2.3 Heat Treatment of Protein Extract

The procedure used for heat treatment was according to Hiller et al. [32] and Rees and Robertson [33] with slight modification. The protein extracts were divided into four batches out of which three batches were was subjected to different temperature treatments and incubation at 37ºC, 60ºC and 100ºC respectively for 1 hour and the remaining one batch served as control (Table 2).

Table 2. List of *Vigna unguiculata* (L) Walp protein extracts and different temperature treatments

S/N	Cowpea Seed Name	Protein Extract Code	Heat Treatment (Temperature^{o}C)
1.	IT99K-573 2-1	V1	Control
2.	IT99K-573-2-1	V1-37C	37
3.	IT99K-573-2-1	V1-60C	60
4.	IT99K-573-2-1	V1-100C	100
5.	T Vu – 8775	V2	Control
6.	T Vu – 8775	V2-37C	37
7.	T Vu – 8775	V2-60C	60
8.	T Vu – 8775	V2-100C	100
9.	T Vu-7846	V3	Control
10.	T Vu-7846	V3-37C	37
11.	T Vu-7846	V3-60C	60
12.	T Vu-7846	V3-100C	100
13.	T Vu – 15636	V4	Control
14.	T Vu – 15636	V4-37C	37
15.	T Vu – 15636	V4-60C	60
16.	T Vu – 15636	V4-100C	100
17.	TVu- 13965	V5	Control
18.	TVu- 13965	V5-37C	37
19.	TVu- 13965	V5-60C	60
20.	TVu- 13965	V5-100C	100
21.	T Vu-8586	V6	Control
22.	T Vu-8586	V6-37C	37
23.	T Vu-8586	V6-60C	60
24.	T Vu-8586	V6-100C	100
25.	IT98K-205-8	V7	Control
26.	IT98K-205-8	V7-37C	37
27.	IT98K-205-8	V7-60C	60
28.	IT98K-205-8	V7-100C	100
29.	T Vu – 3947	V8	Control
30.	T Vu – 3947	V8-37C	37
31.	T Vu – 3947	V8-60C	60
32.	T Vu – 3947	V8-100C	100
33.	T Vu-702	V9	Control
34.	T Vu-702	V9-37C	37
35.	T Vu-702	V9-60C	60
36.	T Vu-702	V9-100C	100

2.4 UV Absorption Spectrum Analysis of Protein Extract

The procedure used for UV absorption spectrum analysis of protein extracts was according to Onasanya et al. [27] and Grimsley and Pace [34]. 0.4 ml of crude protein extract was added to 1.6 ml of 0.1 M NaOH, mixed well, and the optical density (OD) absorbance value was taken from A_{200}-A_{960} UV wavelengths using Spectronic 20 spectrophotometer. All the 36 protein samples were analyzed (Table 2).

2.5 Graphical Analysis of Optical Density (OD) Data

The procedure used for the graphical analysis of optical density (OD) data was according to Mattley and Garcia-Rubio [35] and Pavokovic et al. [36]. A Standard Curve was prepared by plotting the optical density (OD) absorbance valuesatA_{280} UV wavelengths against the concentrations of protein standard (bovine serum albumin). The concentration of each protein extracts was obtained using the linear equation generated from the protein Standard Curve. A

plot of optical density (OD) absorbance values against A_{200}-A_{960} UV wavelengths was also carried out to generate a combined UV absorbance spectra profile for protein extracts (control) and protein extracts heat treatments (37ºC, 60ºC, and 100ºC).

2.6 Cluster Analysis of Optical Density (OD) Data

In order to establish the relationship between protein extracts (control) and protein extracts heat treated (37ºC, 60ºC, and 100ºC), cluster analysis of optical density (OD) data was carried out using numerical taxonomy and multivariate analysis system (NTSYS-PC), version 2.1 [37]. OD data were first converted to pairwise distance matrices using the Jaccard coefficient of similarity (Jaccard, 1908) present in NTSYS-PC 2.1 and dendrogram cluster was created by Unweighted Pair Group Method with Arithmetic mean (UPGMA) cluster analysis [38].

3. RESULTS

3.1 Concentration of Protein Extracts

The final concentrations of protein standard (bovine serum albumin) used were 1.0 mg/ml, 2.0 mg/ml, 3.0 mg/ml, 4.0 mg/ml and 5.0 mg/ml respectively. A plot of absorbance values at 280 nm against concentrations of the standard produced a highly significant (P≤0.01) standard linear curve (Fig. 1). A standard linear equation (y = 0.3549x – 0.0925) was generated from the plot where y and x represented absorbance value at 280 nm and protein concentration in mg/ml respectively (Fig. 1). The standard linear equation (y = 0.3549x – 0.0925) was used to deduce the concentration of the protein extracts. The protein content (control) in *Vigna unguiculata* (L) Walp was between 8.4 to 10.8 mg/ml (10.5-13.5%) in seed, while protein content (heat treated) in *Vigna unguiculata* (L) Walp was between 8.9 to 9.5 mg/ml (11.2-11.9%), 8.7-9.5 mg/ml (10.9-11.9%), 9.0 to 11.8 mg/ml (11.3-14.7%) in seed protein extracts heat treatments of 37ºC, 60ºC, and 100ºC respectively (Table 3). Protein extract control (V1) from seed of IT99K-573-2-1 has the highest protein content of 10.8 mg/ml (13.5%), while protein extract control (V2) from seed of TVu–8775 has the lowest with 8.4 mg/ml (10.5%) protein content (Table 3).

The protein content (heat treated) in IT99K-573-2-1 was 11.9% (37ºC), 11.7% (60ºC), 14.3% (100ºC), while 11.4% (37ºC), 11.6% (60ºC), 14.4% (100ºC) were obtained in TVu –8775 (Table 3). Moreover, in TVu-7846, protein content (heat treated) was 11.3% (37ºC), 12.4% (60ºC), 14.7% (100ºC), while 11.3 % (37ºC), 11.5% (60ºC), 13.1% (100ºC) were obtained in

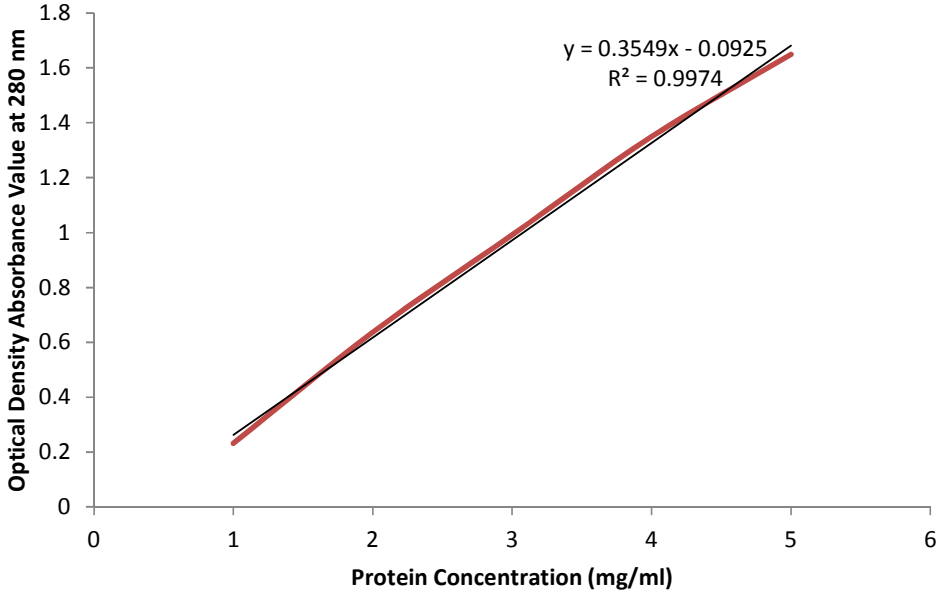

Fig. 1. Protein standard curve using bovine serum albumin

*Standard linear curve equation: y = 0.3549x – 0.0925; R: Regression value; ** = highly significant (P≤0.01)*

Table 3. Comparison of protein content with different heat treatments in *Vigna unguiculata* (L) Walp

S/N	Cowpea Seed Name	Protein Sample	Heat Treatment (Temperature°C)	Protein Content[α] (mg/ml)	% Protein[α]
1	IT99K-573-2-1	V1	Control	10.8ns	13.5ns
2	IT99K-573-2-1	V1-37C	37	9.5	11.9
3	IT99K-573-2-1	V1-60C	60	9.3	11.7
4	IT99K-573-2-1	V1-100C	100	11.4	14.3
5	T Vu – 8775	V2	Control	8.4*	10.5*
6	T Vu – 8775	V2-37C	37	9.1	11.4
7	T Vu – 8775	V2-60C	60	9.3	11.6
8	T Vu – 8775	V2-100C	100	11.5	14.4
9	T Vu-7846	V3	Control	8.7*	10.9*
10	T Vu-7846	V3-37C	37	9.0	11.3
11	T Vu-7846	V3-60C	60	9.9	12.4
12	T Vu-7846	V3-100C	100	11.8	14.7
13	T Vu – 15636	V4	Control	8.5**	10.6**
14	T Vu – 15636	V4-37C	37	9.1	11.3
15	T Vu – 15636	V4-60C	60	9.2	11.5
16	T Vu – 15636	V4-100C	100	10.5	13.1
17	TVu- 13965	V5	Control	8.7***	10.9***
18	TVu- 13965	V5-37C	37	9.1	11.4
19	TVu- 13965	V5-60C	60	9.3	11.7
20	TVu- 13965	V5-100C	100	9.3	11.6
21	T Vu-8586	V6	Control	8.7***	10.9***
22	T Vu-8586	V6-37C	37	9.3	11.7
23	T Vu-8586	V6-60C	60	9.3	11.6
24	T Vu-8586	V6-100C	100	9.1	11.4
25	IT98K-205-8	V7	Control	8.8**	11.1**
26	IT98K-205-8	V7-37C	37	8.9	11.2
27	IT98K-205-8	V7-60C	60	9.2	11.5
28	IT98K-205-8	V7-100C	100	9.4	11.8
29	T Vu – 3947	V8	Control	8.6**	10.7**
30	T Vu – 3947	V8-37C	37	9.1	11.4
31	T Vu – 3947	V8-60C	60	9.5	11.9
32	T Vu – 3947	V8-100C	100	9.3	11.6
33	T Vu-702	V9	Control	8.8ns	11.1ns
34	T Vu-702	V9-37C	37	9.0	11.3
35	T Vu-702	V9-60C	60	8.7	10.9
36	T Vu-702	V9-100C	100	9.0	11.3

*[α]T-test used to compare the mean value of protein content of control protein extract with that of heat treated protein extracts (37ºC, 60ºC and 100ºC) and the significant difference level (between control protein extract and heat treated protein extracts) was indicated on each control protein extract; ns=no significant difference. *=significant difference at p≤0.10. **=significant difference at p≤0.05. ***=significant difference at p≤0.01.*

TVu–15636 (Table 3). Protein content (heat treated) in TVu-13965 was 11.4% (37ºC), 11.7% (60ºC), 11.6% (100ºC), and 11.7% (37ºC), 11.6% (60ºC), 11.4% (100ºC) in TVu-8586. Also protein content (heat treated) in IT98K-205-8 was 11.2% (37ºC), 11.5% (60ºC), 11.8% (100ºC), while TVu–3947 was 11.4% (37ºC), 11.9% (60ºC), 11.6% (100ºC). In TVu-702, protein content (heat treated) was 11.3% (37ºC), 10.9% (60ºC), 11.3% (100ºC) (Table 3).

3.2 Comparison of absorbance spectra of protein with different heat treatments in IT99K-573-2-1

There was comparative difference between the absorbance values of protein with different heat treatments in IT99K-573-2-1. At the same wavelength of 280nm, the protein concentration is more in IT99K-573-2-1 protein extract heated at 100ºC (V1-100C) than in the control (V1), protein extract heated at 37ºC (V1-37C), and

protein extract heated at 60ºC (V1-60C) respectively (Fig. 2). At other wavelength (300-960 nm), there was presence of other protein molecules of even higher concentration at different peaks in IT99K-573-2-1 protein extract heated at 100ºC (V1-100C) than in the control (V1), protein extract heated at 37ºC (V1-37C), and protein extract heated at 60ºC (V1-60C) respectively (Fig. 2). Comparing absorbance spectra, IT99K-573-2-1 protein extract heated at 100ºC (V1-100C) has two peaks at 320 nm and 460 nm respectively with maximum absorbance peak at 320 nm, both the control (V1) and protein extract heated at 60ºC (V1-60C) have two peaks at 320 nm and 440 nm respectively with maximum absorbance peak at 320 nm, while protein extracts heated at 37ºC (V1-37C) has two peaks at 320 nm and 440 nm respectively with maximum absorbance peak at 440 nm (Fig. 2).

3.3 Comparison of Absorbance Spectra of Protein with Different Heat Treatments in TVu – 8775

Difference between the absorbance value of protein with different heat treatments in TVu–8775 was compared. TVu–8775 protein extract heated at 100ºC (V2-100C) has more protein concentration at the wavelength of 280 nm than in the control (V2), protein extract heated at 37ºC (V2-37C), and protein extract heated at 60ºC (V2-60C) respectively (Fig. 3). Other protein molecules are found along other wavelengths (300-960) at even higher concentrations in TVu–8775 protein extract heated at 100ºC (V2-100C) than in the control (V2), protein extract heated at 37ºC (V2-37C), and protein extract heated at 60ºC (V2-60C) respectively (Fig. 3). Comparing absorbance spectra of control and heat treated protein extracts, TVu–8775 protein extract

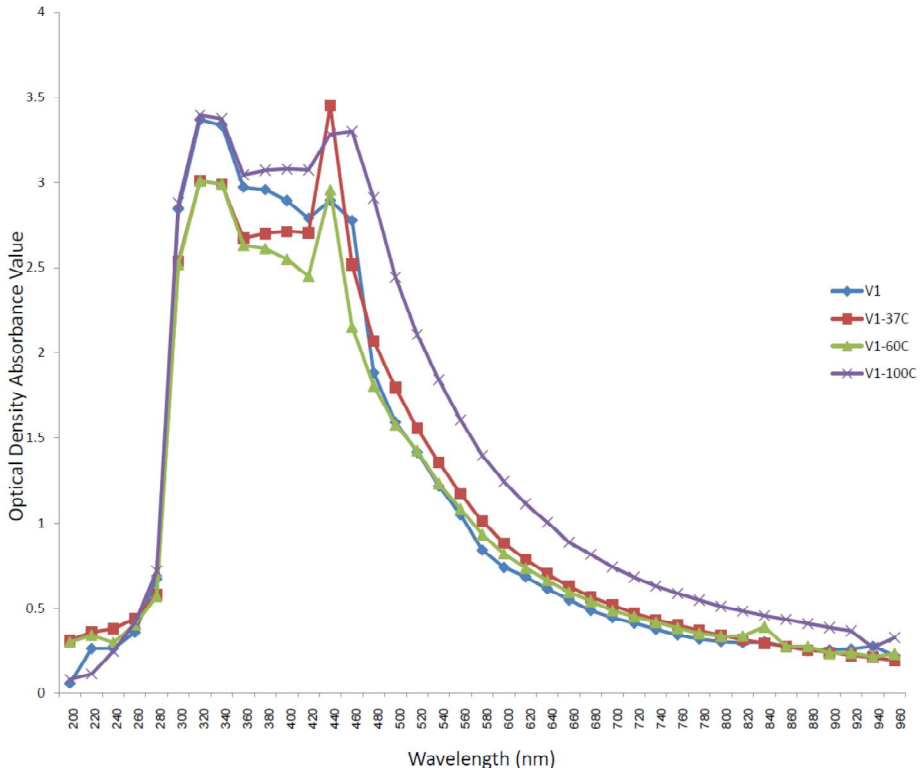

Fig. 2. Comparison of absorbance spectra of protein with different heat treatments in IT99K-573-2-1

V1= IT99K-573-2-1(Control); V1-37C= IT99K-573-2-1 Protein extract heated at 37ºC for 1 hr; V1-60C= IT99K-573-2-1 Protein extract heated at 60ºC for 1 hr; V1-100C= IT99K-573-2-1 Protein extract heated at 100ºC for 1hr

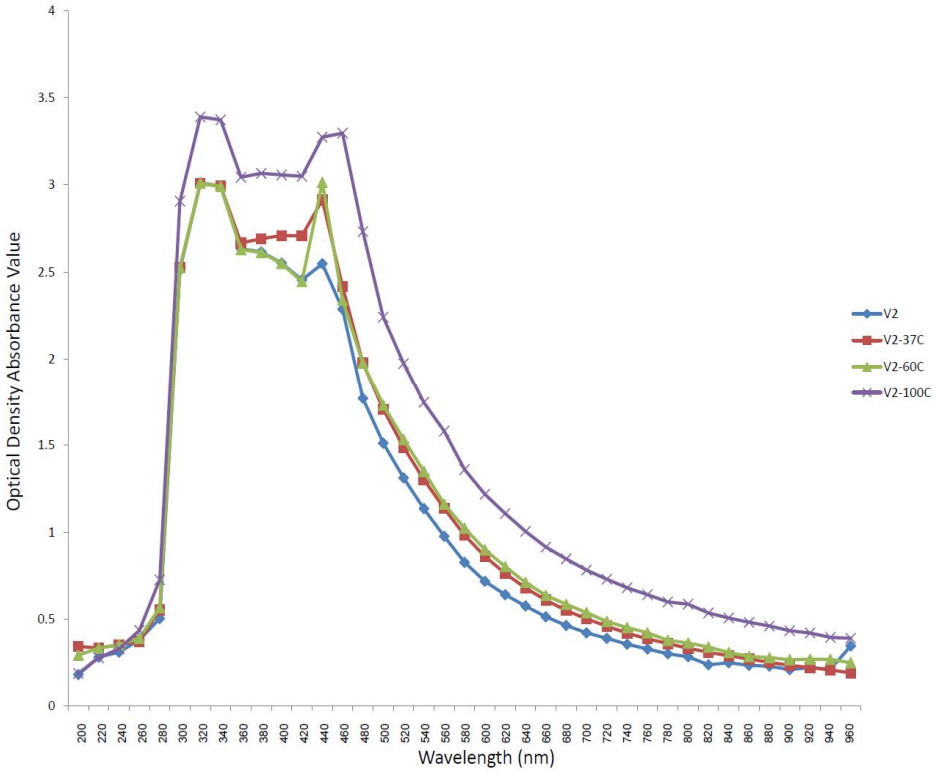

Fig. 3. Comparison of absorbance spectra of protein with different heat treatments in TVu – 8775

V2= TVu – 8775 (Control); V2-37C= TVu – 8775 Protein extract heated at 37ºC for 1 hr; V2-60C= TVu – 8775 Protein extract heated at 60ºC for 1 hr; V2-100C= TVu – 8775 Protein extract heated at 100ºC for 1hr

heated at 100ºC (V2-100C) has two peaks at 300 nm and 460 nm respectively with maximum absorbance peak at 300 nm, both the control (V2) and protein extracts heated at 37ºC (V2-37C) have two peaks at 300 nm and 440 nm respectively with maximum absorbance peak at 300 nm, while protein extract heated at 60ºC (V2-60C) has two peaks at 320 nm and 440 nm respectively with maximum absorbance peak at 440 nm (Fig. 3).

3.4 Comparison of Absorbance spectra of Protein with Different Heat Treatments in TVu-7846

Comparative studies revealed that TVu-7846 protein with different heat treatments showed difference in protein concentration across the same wavelength of 280 nm and at different wavelengths, other protein molecules with even higher protein concentration are present (Fig. 4). The protein concentration in TVu-7846 protein extract heated at 100ºC (V3-100C) at the wavelength of 280 nm was higher than in the control (V3), protein extract heated at 37ºC (V3-37C), and protein extract heated at 60ºC (V3-60C) respectively (Fig. 4). Comparing absorbance spectra of control and heat treated protein extracts, both the control (V3) and TVu-7846 protein extract heated at 100ºC (V3-100C) have two peaks at 300 nm and 460 nm respectively with maximum absorbance peak at 300 nm, while both protein extracts heated at 37ºC (V3-37C) and at 60ºC (V3-60C) have two peaks at 300 nm and 440 nm respectively with maximum absorbance peak at 300 nm (Fig. 4).

3.5 Comparison of Absorbance Spectra of Protein with Different Heat Treatments in TVu–15636

TVu–15636 control protein extract (V4), and protein extract heated at 37ºC (V4-37C), at 60ºC (V4-60C) and at 100ºC (V4-100C) respectively showed contrasting absorbance spectra. Comparative studies showed that TVu–15636 protein extract heated at 100ºC (V3-100C) has more protein concentration than TVu–15636

control protein extract (V4), protein extract heated at 37ºC (V4-37C) and at 60ºC (V4-60C) respectively at wavelength of 280nm (Fig. 5). In the absorbance spectra graph, other proteins were found to be present and were absorbed at different wavelengths. Comparing absorbance spectra of control and heat treated protein extracts, TVu–15636 control protein extract (V4) has two peaks at 320 nm and 480 nm respectively, protein extract heated at 37ºC (V4-37C) has two peaks at 320 nm and 460 nm, while protein extract heated at 60ºC (V4-60C) has three peaks at 320 nm, 440 nm and 480 nm, and protein extract heated at 100ºC (V4-100C) produced five peaks at 320 nm, 440 nm, 500 nm, 580 nm and 620 nm respectively (Fig. 5).

3.6 Comparison of Absorbance Spectra of Protein with Different Heat Treatments in TVu-13965

In the comparison of absorbance spectra of TVu-13965 protein with different heat treatments, it was found that protein extract heated at 37ºC (V5-37C) has it maximum absorbance at 460 nm unlike the protein absorbance in the protein extract control (V5), protein extract heated at 60ºC (V5-60C), and protein extract heated at 100ºC (V5-100C) respectively (Fig. 6). Other forms of protein are found in the protein extract control (V5), protein extract heated at 37ºC (V5-37C), protein extract heated at 60ºC (V5-60C), and protein extract heated at 100ºC (V5-100C) respectively which were absorbed at different wavelengths (Fig. 6). Comparing absorbance spectra, protein extract control (V5) has three peaks at 320 nm, 440 nm and 480 nm, while protein extract heated at 37ºC (V5-37C) produced three peaks at 320 nm, 400 nm and 460 nm. However, protein extract heated at 60ºC (V5-60C) has three peaks at 240 nm, 320 nm and 460 nm, while protein extract heated at 100ºC (V5-100C) produced five peaks at 320 nm, 440 nm, 480 nm, 520 nm and 580 nm respectively (Fig. 6).

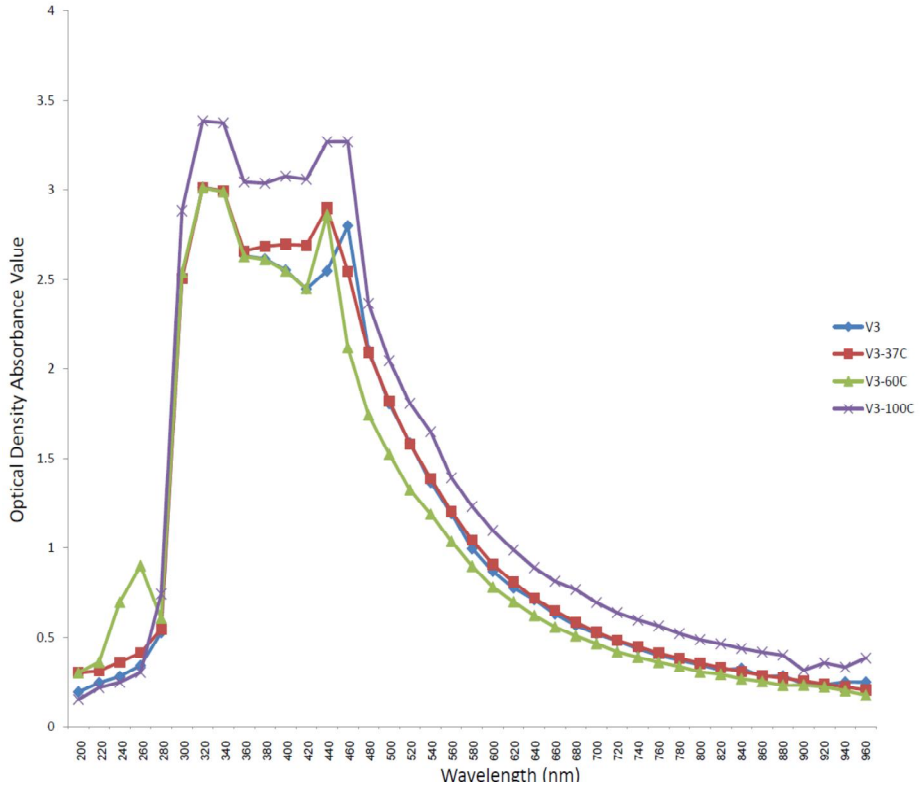

Fig. 4. Comparison of absorbance spectra of protein with different heat treatments in TVu-7846
V3= TVu-7846 (Control); V3-37C= TVu-7846 Protein extract heated at 37ºC for 1 hr; V3-60C= TVu-7846 Protein extract heated at 60ºC for 1 hr; V3-100C= TVu-7846 Protein extract heated at 100ºC for 1hr

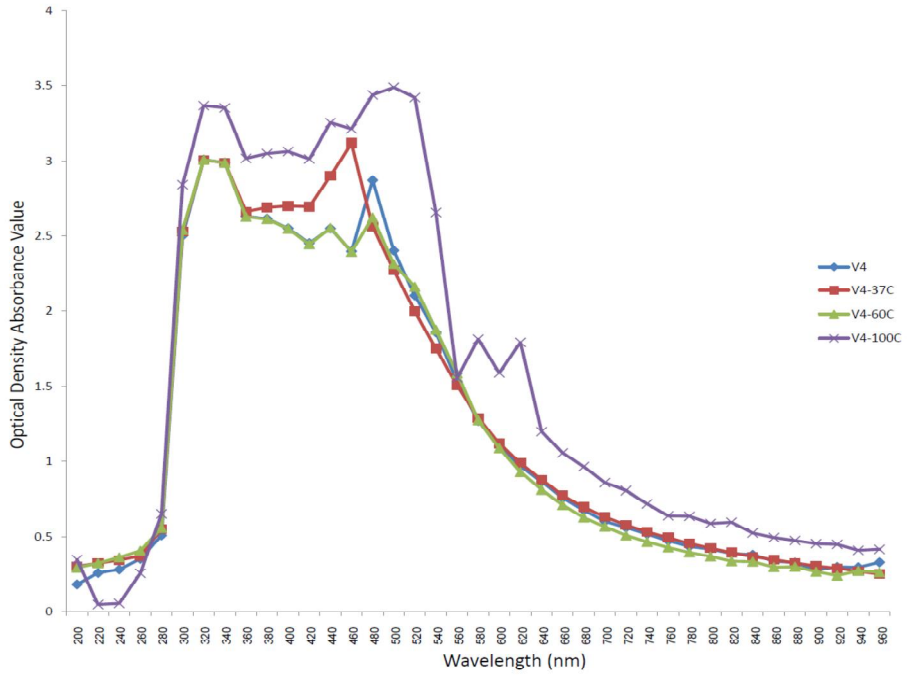

Fig. 5. Comparison of absorbance spectra of protein with different heat treatments in TVu – 15636

V4= TVu –15636 (Control); V4-37C= TVu –15636 Protein extract heated at 37°C for 1 hr; V4-60C= TVu –15636 Protein extract heated at 60°C for 1 hr; V4-100C= TVu –15636 Protein extract heated at 100°C for 1hr

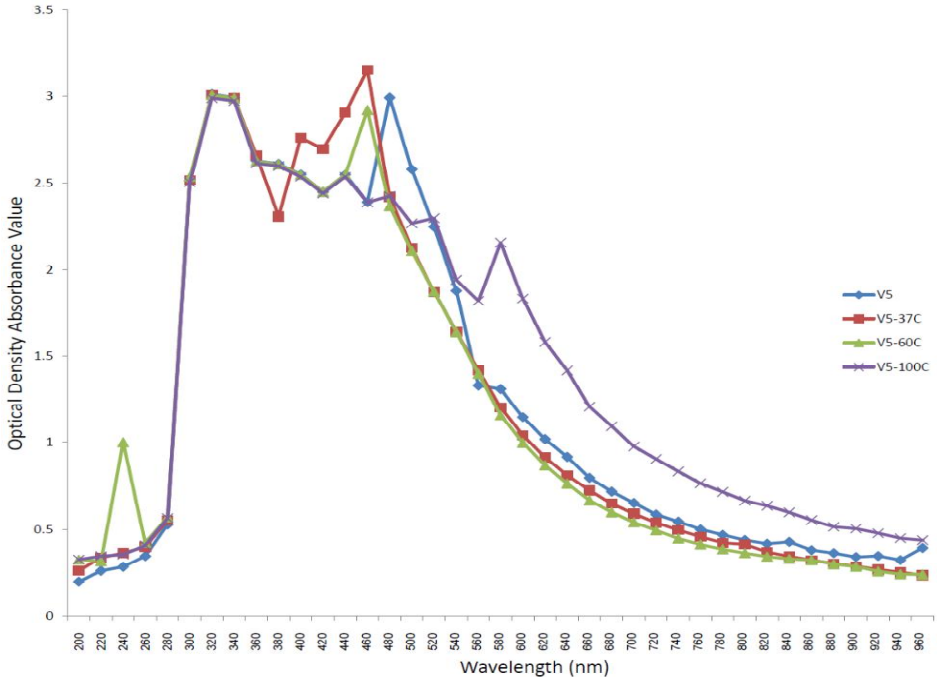

Fig. 6. Comparison of absorbance spectra of protein with different heat treatments in TVu-13965

V5= TVu- 13965 (Control); V5-37C= TVu- 13965Protein extract heated at 37ºC for 1 hr; V5-60C= TVu-13965Protein extract heated at 60ºC for 1 hr; V5-100C= TVu- 13965Protein extract heated at 100ºC for 1hr

3.7 Comparison of Absorbance Spectra of Protein with Different Heat Treatments in TVu-586

Given the comparison of absorbance spectra of TVu-586 protein with different heat treatments, it was observed that the absorbance value of the protein extract heated at 37ºC (V6-37C) has it maximum absorbance at 460 nm unlike the protein absorbance in the protein extract control (V6), protein extract heated at 60ºC (V6-60C), and protein extract heated at 100ºC (V6-100C) respectively (Fig. 7). Comparing absorbance spectra of TVu-586 protein with different heat treatments, protein extract control (V6) has three peaks at 320 nm, 440 nm and 480 nm, while protein extract heated at 37ºC (V6-37C) produced two peaks at 320 nm and 460 nm. However, protein extract heated at 60ºC (V6-60C) has three peaks at 320 nm, 440 nm and 480 nm, while protein extract heated at 100ºC (V6-100C) produced four peaks at 320 nm, 440 nm, 480 nm and 560 nm respectively (Fig. 7).

3.8 Comparison of Absorbance Spectra of Protein with Different Heat Treatments in IT98K-205-8

It was observed that the protein extract heated at 37ºC (V7-37C), protein extract control (V7), protein extract heated at 60ºC (V7-60C), and protein extract heated at 100ºC (V7-100C) respectively have it maximum absorbance at 320 nm (Fig. 8). Comparing absorbance spectra of IT98K-205-8 protein with different heat treatments, protein extract control (V7) has one peak at 320 nm, while protein extract heated at 37ºC (V7-37C) produced two peaks at 320 nm and 420 nm. However, protein extract heated at 60ºC (V7-60C) has two peaks at 320 nm ane 440 nm, while protein extract heated at 100ºC (V7-100C) produced four peaks at 320 nm, 440 nm, 480 nm and 560 nm respectively (Fig. 8).

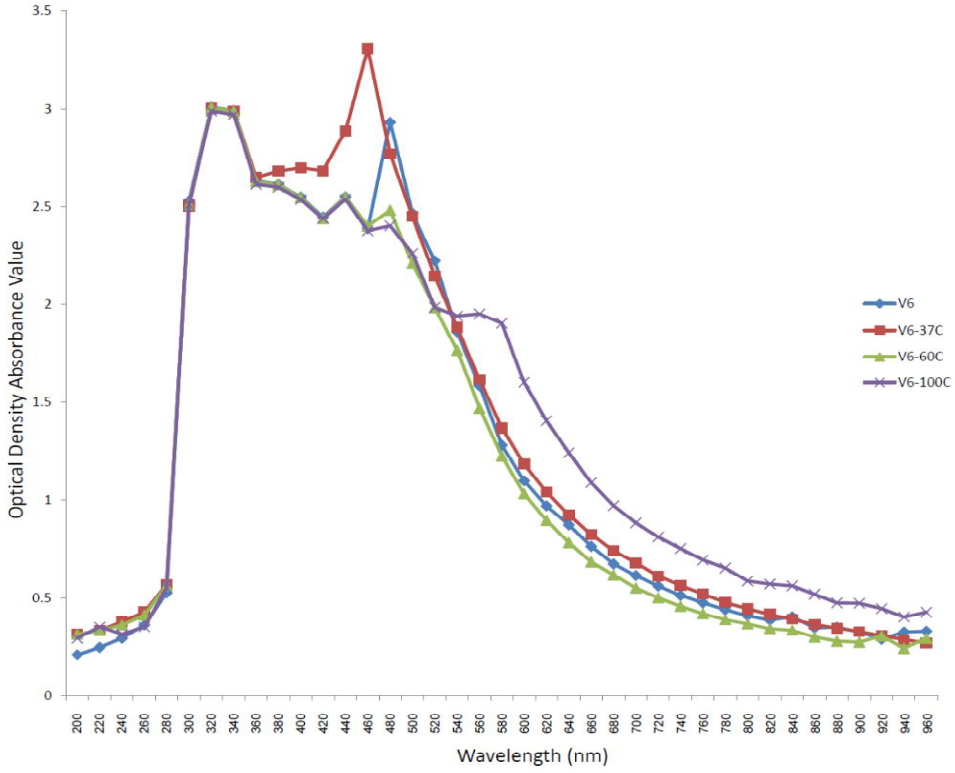

Fig. 7. Comparison of absorbance spectra of protein with different heat treatments in TVu-8586
V6= TVu-8586 (Control); V6-37C= TVu-8586 Protein extract heated at 37ºC for 1 hr; V6-60C= TVu-8586 Protein extract heated at 60ºC for 1 hr; V6-100C= TVu-8586 Protein extract heated at 100ºC for 1hr

3.9 Comparison of Absorbance Spectra of Protein with Different Heat Treatments in TVu – 3947

Given the comparison of absorbance spectra of TVu–3947 protein with different heat treatments, it was observed that the absorbance value of the protein extract heated at 37ºC (V8-37C) has it maximum absorbance at 420 nm unlike the protein absorbance in the protein extract control (V8), protein extract heated at 60ºC (V8-60C), and protein extract heated at 100ºC (V8-100C) respectively (Fig. 9). Comparing absorbance spectra of TVu–3947 protein with different heat treatments, protein extract control (V8) has one peak at 320 nm, while protein extract heated at 37ºC (V8-37C) produced two peaks at 320 nm and 420 nm. However, protein extract heated at 60ºC (V8-60C) has two peaks at 320 nm and 440 nm, while protein extract heated at 100ºC (V8-100C) produced four peaks at 320 nm, 440 nm, 480 nm and 560 nm respectively (Fig. 9).

3.10 Comparison of Absorbance Spectra of Protein with Different Heat Treatments in TVu-702

It was observed that the protein extract heated at 37ºC (V9-37C), protein extract control (V7), protein extract heated at 60ºC (V9-60C), and protein extract heated at 100ºC (V9-100C) respectively have it maximum absorbance at 320 nm (Fig. 10). Comparing absorbance spectra of TVu-702 protein with different heat treatments, both protein extract control (V9) and protein extract heated at 37ºC (V9-37C) have two peaks at 320 nm and 420 nm respectively. However, protein extract heated at 60ºC (V9-60C) has two peaks at 320 nm and 440 nm, while protein extract heated at 100ºC (V9-100C) produced five peaks at 320 nm, 440 nm, 480 nm, 540 nm and 700 nm respectively (Fig. 10).

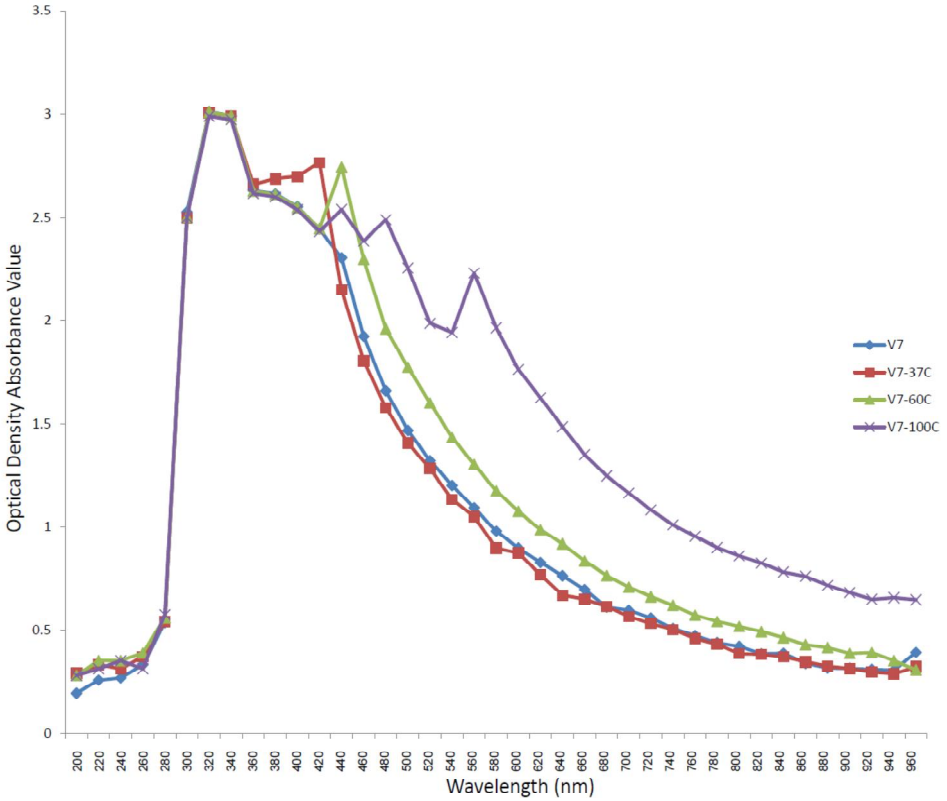

Fig. 8. Comparison of absorbance spectra of protein with different heat treatments in IT98K-205-8

V7= IT98K-205-8 (Control); V7-37C= IT98K-205-8 Protein extract heated at 37°C for 1 hr; V7-60C= IT98K-205-8 Protein extract heated at 60°C for 1 hr; V7-100C= IT98K-205-8 Protein extract heated at 100°C for 1hr

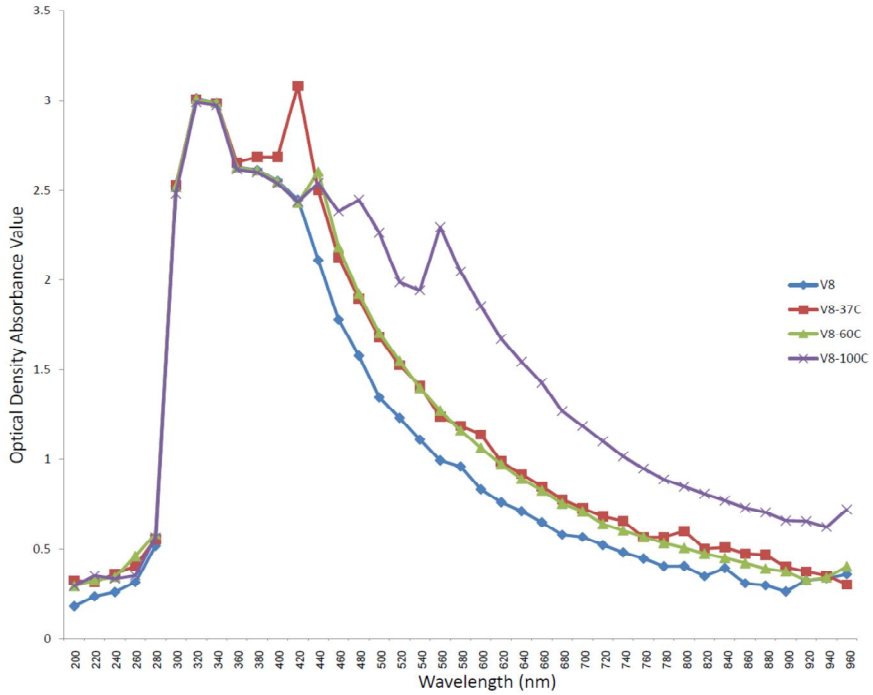

Fig. 9. Comparison of absorbance spectra of protein with different heat treatments in TVu – 3947

V8= TVu – 3947 (Control); V8-37C= TVu – 3947 Protein extract heated at 37ºC for 1 hr; V8-60C= TVu – 3947 Protein extract heated at 60°C for 1 hr; V8-100C= TVu – 3947 Protein extract heated at 100°C for 1hr

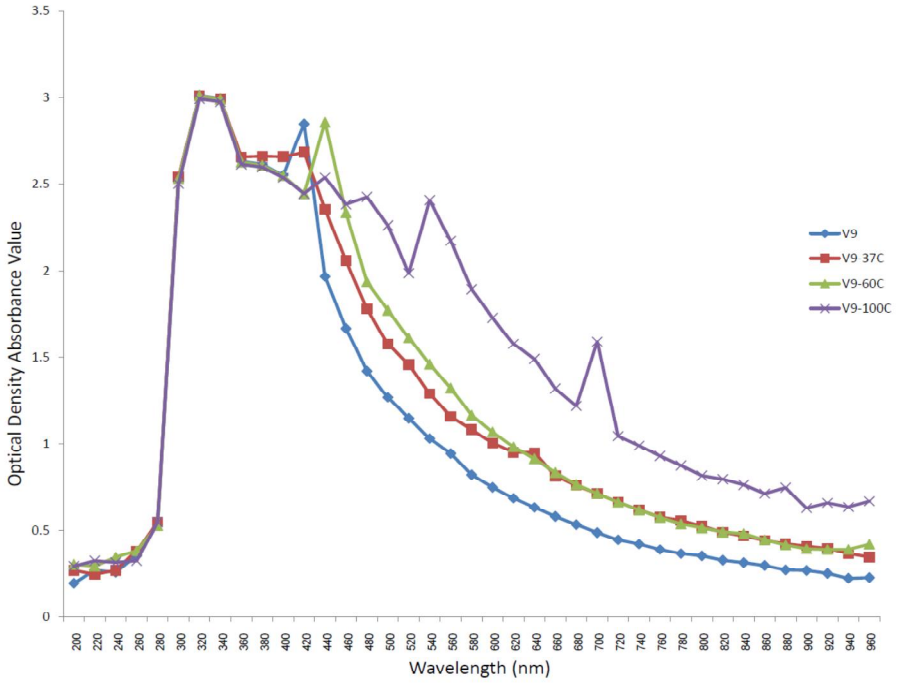

Fig. 10. Comparison of absorbance spectra of protein with different heat treatments in TVu-702

V9= TVu-702 (Control); V9-37C= TVu-702 Protein extract heated at 37°C for 1 hr; V9-60C= TVu-702 Protein extract heated at 60°C for 1 hr; V9-100C= TVu-702 Protein extract heated at 100°C for 1hr

3.11 Cluster Analysis Comparison of Absorbance Spectra Optical Density Values of Protein with Different Heat Treatments

Cluster analysis of absorbance spectra optical density values revealed remarkable differences in the form of clusters among control protein extracts and protein extracts heated at 37ºC, 60ºC, and 100ºC respectively (Fig. 11). Five clusters (cluster1, cluster2, cluster3, cluster4, cluster5) have been identified among control protein extracts and protein extracts heated at 37ºC, 60ºC, and 100ºC. Cluster1 was made up of protein extracts heated at 37ºC and 60ºC, while cluster2 and cluster3 constituted closely related protein extracts heated at 37ºC and 100ºC respectively. Cluster4 was typical of control protein extracts, while cluster5 was made up of distinct protein extracts heated at 100ºC (Fig. 11).

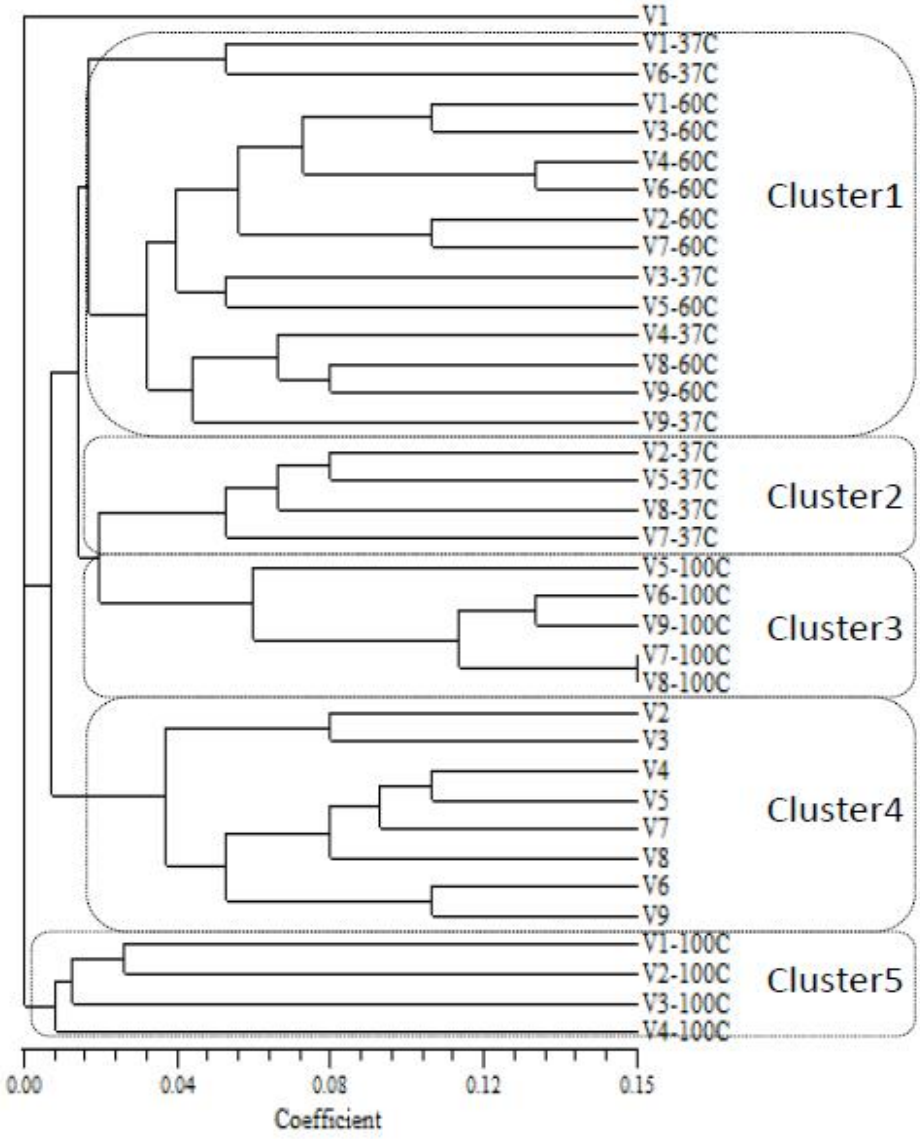

Fig. 11. Cluster analysis comparison of absorbance spectra optical density values of protein extracts with different heat treatments

4. DISCUSSION

Quantitative UV absorption spectrum proteomic study is widely used in biochemistry and molecular biology for quantification of protein extracts from different sources including prokaryotic and eukaryotic cells [39]. The present study used proteomic analysis to study the effect of heat stress on protein extract from cowpea seeds (*Vigna unguiculata* (L) Walp). However, the present results showed that protein content in *Vigna unguiculata* (L) Walp seed was between 10.5-13.5% as compared to 6.6-16.9% previously reported [27]. Protein variability in cowpea population could be as a result of exposures of cowpea seeds to different temperature [29,30]. Previous studies have grouped cowpea varieties on the basis of protein content as high protein lines (above 30%), medium protein lines (20 to 30%), and low protein lines (less than 20%) [40,41]. In the present study, protein extracts exposed to different temperatures (37ºC, 60ºC, 100ºC) gave different protein contents (11.2-11.9%, 10.9-11.9%, 11.3-14.7%) which revealed some levels of protein variability in different cowpea accessions used. In addition, protein content of different accessions of *Vigna unguiculata* (L) Walp seeds tested in this study varied greatly and could possibly depending on different levels of heat tolerance among different chemical composition of the seeds and, in theory, this may lead to differences in the protein content and adsorption spectra [42,29,30].

Large amounts of information are usually generated from single multiwavelength measurement which consequently makes UV absorption spectrum proteomic assay a powerful characterization tool that has several applications [39,43]. The sample information contained in a typical multiwavelength UV spectrum includes cell size, chemical composition and shape [39]. This information is obtained from the spectroscopic analysis of a sample measured over a broad range of wavelengths (200 - 900 nm) [43]. In the present study, absorbance spectra between 200-960 nm UV wavelengths have been used to compare protein content from protein extracts from *Vigna unguiculata* (L) Walp heated at 37ºC, 60ºC, and 100ºC. Within the same *Vigna unguiculata* (L) Walp accession difference protein absorbance spectra were obtained from control protein extract and protein extract heated at 37ºC, 60ºC, and 100ºC. For examples, protein extract from TVu-702 heated at 37ºC have two peaks at 320 nm and 420 nm, another heated at 60ºC has two peaks at 320 nm and 440 nm, while those heated at 100ºC produced five peaks at 320 nm, 440 nm, 480 nm, 540 nm and 700 nm respectively. The difference in protein absorbance spectra could possibly be due to different levels of heat tolerance among protein chemical composition and shape [42,43,29,30]

Cluster analysis comparison of protein multiwavelength absorbance spectra for the characterization of *Vigna unguiculata* (L) Walp revealed the sensitivity and discriminating power of the spectroscopic approach. Cluster analysis of absorbance spectra optical density values revealed five clusters (cluster1, cluster2, cluster3, cluster4, cluster5) among control protein extracts and protein extracts heated at 37ºC, 60ºC, and 100ºC. Heating protein extracts at 37ºC, 60ºC and 100ºC have altered proteomic diversity in different cowpea accessions and this could make protein extraction more difficult with implications on protein properties [29,30].

5. CONCLUSION

The present study used UV absorption spectrum proteomic assay to study the effect of heat stress of protein extract from cowpea seeds (*Vigna unguiculata* (L) Walp). The assay is widely used in biochemistry and molecular biology for quantification of protein extracts from different sources including prokaryotic and eukaryotic cells. Large amounts of information are usually generated from single multiwavelength measurement which consequently makes UV absorption spectrum proteomic assay a powerful characterization tool that has several applications. As shown in this study, cluster analysis comparison of protein multiwavelength absorbance spectra for the characterization of *Vigna unguiculata* (L) Walp revealed five clusters among control and heated protein extracts which further revealed the sensitivity and discriminating power of the spectroscopic approach. The ability to discriminate between crude protein content with different heat-treatment levels from cowpea have been demonstrated with the light scattering and absorption interpretation model and optical properties developed in our laboratory. Heating protein extracts at 37ºC, 60ºC, and 100ºC have altered proteomic diversity in different cowpea accessions and this could make protein extraction more difficult with implications on protein properties. With this level of detail in the analysis, the multiwavelength UV proteomic assay has the potential to provide a sensitive

biosensor for the detection, identification and enumeration of various biomolecules. Future studies are highly recommended in the areas of developing the necessary interpretation models for more cowpea varieties and investigating the sensitivity of the approach.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Badiane FA, Diouf D, Sane D, Diouf O, Croudiaby N and Diallo N. Screening cowpea varieties by inducing water deficif and RAPD analysis. Afr. J. Biotech. 2004; 3(3):174–178.
2. FAO, 2010. FAO statistical databases. Available:http://faostat.fao.org
3. Ogbonnaya CI, Sarr B, Brou C, Diouf O, Diop NN, Roy-Macauley H. Selection of cowpea genotypes in hydroponics, pots and field for drought tolerance. Crop Sci. 2003;43:1114-1120.
4. Aggarwal KD, N'tare RB, Smithson JB. The relationship among yield and other characteristics of vegetable cowpea and effects of different trellis management system on pod yield. Grain Legume Bulletin. 1982;25:15-20.
5. Kitch W, Boukar O, Endondo C, Murdock LL. Farmer acceptability criteria in breeding cowpea. Experimental Agriculture. 1998;34:475-486.
6. Karikari SK, Molatakgosi G. Response of cowpea (*Vigna unguiculata* (L.) Walp.) varieties to leaf harvesting in Botswana. UNISWA Journal of Agriculture. 1999;8:5-11.
7. Johnson RM, Raymond WD. The chemical composition of some tropical food plants II. Pigeon peas and cowpeas. Tropical Science. 1964;6:68–73.
8. Van Wyk B-E, Gericke N. People's plants: a guide to useful plants of southern Africa. Briza Publications, Pretoria, South Africa. 2000;30.
9. Singh BB, Cambliss OI, Sharma B. Recent advances in cowpea breeding. In: Singh BB, Mohan Raj DR, Dashiell K, Jackai LEN. (Eds) Advances in Cowpea Research. Co-publication of International Institute of Tropical Agriculture (IITA) and Japan International Centre for Agricultural Sciences (JIRCSA), IITA, Ibadan, Nigeria. 1997;30–49.
10. Tuan YH, Phillips RD. Nutritional quality of hard-to-cook and processed cowpea. Journal of Food Science. 1992;68:1371–1374.
11. Quin FM. Introduction. In: Singh BB, Mohan Raj DR, Dashiel KE, Jackai LEN (eds.). Advances in cowpea research. Co-publication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). Ibadan, Nigeria; 1997.
12. Agbogidi OM. Response of six cultivars of cowpea (*Vigna unguiculata* (L.) Walp.) to spent engine oil. African Journal of Food Science and Technology. 2010b;1(6):139-142.
13. Fashakin JB, Ojo FA. Chemical composition and nutritive changes of some improved varieties of cowpea (*Vigna unguiculata* (L.) Walp 2: new breeds of varieties from the IITA Ibadan, Nigeria. Tropical Science. 1988;28:191-199.
14. Fashakin JB, Fasanya JI. Chemical composition and nutritive changes of some improved varieties of cowpea (*Vigna unguiculata* L.): some selected varieties from IITA, Ibadan, Nigeria. Tropical Science. 1988;28:111–118.
15. Asumugha VU. Sensory and functional properties of dry vegetable cowpea product (Akara). In: Ubbanu CN, Eke OS, Uzomah A. (eds.). Proceedings of the 26th Annual Conference of the Nigerian Institute of Food Science and Technology (NIFST) held at the Federal University of Technology, Owerri, Imo State between 4th and 8th of November. 2002;66–67.
16. Agbicodo EM, Fatokun CA, Muranaka S, Visser RGF, Linden-van-der CG. Breeding drought tolerant cowpea: constraints, accomplishments, and future prospects. Euphytica. 2009;167:353-370.
17. Mahalakshmi V, Ng Q, Lawson M, Ortiz R. Cowpea [*Vigna unguiculata* (L.) Walp.] core collection defined by geographical, agronomical and botanical descriptors. Plant Genetic Resources: Characterization and Utilization. 2007;5(3):113–119.
18. Reis CM, Frederico AM. Genetic diversity in cowpea (*Vigna unguiculata*) using

isozyme electrophoresis. Acta Horticulture. 2001;546:497-501

19. Rawal KM. Natural hybridization among wild, weedy, and cultivated *Vigna unguiculata* (L.) Walp. Euphytica. 1975;24(3):699-707.
20. Verdcourt B. Studies in the Leguminosae-Papilionoideae for flora of tropical East Africa. Kew Bulletin. 1970;24:507–569.
21. Li C, Fatokun CA, Ubi B, Singh BB, Scoles GJ. Determining genetic similarities and relationships by microsatellite markers. Crop Sci. 2001;41:18,117.
22. Stoscheck CM. Quantitation of protein. Methods Enzymol. 1990;182:50-68.
23. Wang W, Scali M, Vignani R, Spadafora A, Sensi E, Mazzuca S, et al. Protein extraction for two-dimensional electrophoresis from olive leaf, a plant tissue containing high levels of interfering compounds. Electrophoresis. 2003;24: 2369–2375.
24. Song J, Braun G, Bevis E, Doncaster K. A simple protocol for protein extraction of recalcitrant fruit tissues suitable for 2-DE and MS analysis. Electrophoresis. 2006; 27:3144–3151.
25. Liang XQ, Luo M, Holbrook CC, Guo BZ. Storage protein profiles in Spanish and runner market type peanuts and potential markers. BMC Plant Biol. 2006;6:24–32.
26. Méchin V, Thévenot C, Le Guilloux M, Prioul JL, Damerval C. Developmental analysis of maize endosperm proteome suggests a pivotal role for pyruvate orthophosphate dikinase. Plant Physiol. 2007;143:1213–1219.
27. Onasanya A, Ojo AA, Afolabi A, Adewale OB. Comparative protein UV absorption spectra analysis in seeds and seedlings of *Vigna unguiculata* (L) Walp. International Journal of Applied Research and Technology. 2014;3(3):63–79.
28. Vincent D, Wheatley MD, Cramer GR. Optimization of protein extraction and solubilization for mature grape berry clusters. Electrophoresis. 2006;27:1853–1865.
29. Tang S, Hettiarachchy NS, Shellhammer TH. Protein extraction from heat-stabilized defatted rice bran. 1. Physical processing and enzyme treatments. J Agric Food Chem. 2002;50:7444–7448.
30. Khan SH, Butt MS, Sharif MK, Sameen A, Mumtaz S, Sultan MT. Functional properties of protein isolates extracted from stabilized rice bran by microwave, dry heat, and parboiling. J Agric Food Chem. 2011;59:2416–2420.
31. Barbarino E, Lourenço SO. An evaluation of methods for extraction and quantification of protein from marine macro- and microalgae. J. Appl. Phycol. 2005;17:447-460.
32. Hiller R, Zhou ZH, Adams MWW, Englander SW. Stability and dynamics in a hyperthermophilic protein with melting temperature close to 200°C. Proc. Natl. Acad. Sci. *USA*. 1997;94(21),11329–11332.
33. Rees DC, Robertson AD. Some thermodynamic implications for the thermostability of proteins. Protein Science. 2001;10(6):1187–1194.
34. Grimsley GR, Pace CN. Current protocols in Protein Science. By John Wiley & Sons, Inc. 2003;3.1.1-3.1.9.
35. Mattley YD, Garcia-Rubio LH. Multiwavelength spectroscopy for the detection, identification, and quantification cells, *Proc. SPIE* 4206, Photonic Detection and Intervention Technologies for Safe Food. 2001;64.
36. Pavoković D, Križnik B and Krsnik-Rasol M. Evaluation of protein extraction methods for proteomic analysis of non-model recalcitrant plant tissues. Croat. Chem. Acta. 2012;85(2):177–183.
37. Rohlf FJ. NTSys pc, Version 2.02j, Exeter Software, Setauket, New York; 2000.
38. Sneath PHA, Sokal RR. Numeric taxonomy: the principles and practice of numerical classification. W.H. Freeman; 1973.
39. Garcia-Rubio LH, Lopez-Menacho CA, Grossman S. Characterization of proteins during aggregation II: Use of model molecules for spectroscopy analysis. Chem. Eng. Comm. 1993;122:85-101.
40. Chan CW, Phillips RD. Amino acid composition and subunit constitution of protein fractions from cowpea (*Vigna unguiculata* L. Walp) seeds. J. Agric. Food Chem. 1994;42:1857–1860.
41. Mwasaru MA, Muhammad K, Bakar J, Cheman YB. Effect of isolation technique and conditions on the extractability,

physiochemical and functional properties of pigeon pea (*Cajanus cajan*) and cowpea (*Vigna unguiculata*) protein isolates. J. Food Chem. 1999;67:445–452.

42. Lourenco SO, Barbarino E, De-Paula JC, Pereira LOS, Lanfer Marquez UM. Amino acid composition, protein content, and calculation of nitrogen-to-protein conversion factors for nineteen tropical seaweeds. Phycol. Res. 2002;50:233–241.

43. Mattley Y, Leparc G, Potter R, Garcia-Rubio LH. Light scattering and absorption model for the quantitative interpretation of human blood-platelet spectral data. *Photochem. Photobiol*. 2000;71(5):610-619.

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