

IMPACT OF MICROWAVE AND OVEN TREATMENT ON PROTEIN PROFILE OF *NIGELLA SATIVA* (KALONJI)

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ABSTRACT

Black cumin seeds (*Nigella sativa*) have been widely used for the treatment and prevention of various diseases. It is a multidimensional herbal medicine known for its high protein quality. This study shows the effect of microwave and oven treatments on the protein quality of black cumin seeds analyzed by SDS-PAGE. Microwave treated samples resulted in high protein yield as compared to oven treated samples. Highest protein yield in microwave treated sample T16 (43.9125 mg/ml) while 0 mg/ml in oven treated samples T10, T11, T12. The best overall results for protein quality, high molecular weight protein bands by SDS- PAGE and enzymatic activity were obtain by samples treated at 50°C for 1, 2 and 3 hr (T1, T2, T3 respectively).

KEY WORD: *Nigella sativa*, microwave heating, SDS-PAGE, oven heating, protein analysis, enzymatic activity.

1. INTRODUCTION

Religious and traditional utilization of *Nigella sativa* in daily life in past and present is immense. The use of black seed for good health has been central theme in teachings of Prophet Muhammad (SAW). According to one of the saying of Hazrat Prophet Muhammad (SAW) saying; "Hold on to use of the black cumin seed, for it has a remedy for every illness except death" (Bukhari, 1985). There are multiple pharmaceutical importances of black seeds as formerly used to treat liver, kidney, bladder, circulatory, immune, stomach, respiratory,

intestinal dysfunctions and overall health. (Baser *et al.*, 1986; Handa, 1998; Deliorman *et al.*, 2002; Malhotra, 2006). Hypcholestermic (Bamosa *et al.*, 2002), hypoglycemic (Bamosa *et al.*, 1997) and antioxidant properties were reported previously as the functional role of *Nigella sativa* (Kanter *et al.*, 2003).

Functional constituents of plants are gaining popularity as result of studies on their phytochemistry (Tapsell *et al.*, 2006). Black cumin (*Nigella sativa*) locally called Kalonji and commonly known as black seeds. (Gilani *et al.*, 2004; Black *et al.*, 2006). The compositional analysis of kalonji revealed; 3.8-7.0 % moisture, 22.0-40.35 % oil, 20.85-31.2 % proteins, 3.7-4.7 % ash and 24.9-40.0 % carbohydrates (Takruri and Dameh, 1998; Atta, 2003).

Nigella sativa has reported to play important role in addressing cardio vascular diseases. It has hypolipidemic effect by lowering cholesterol level, LDL, VLDL and triglyceride and enhances the amount of HDL in human and animal subjects. (Tissera *et al.*, 1997; Najmi *et al.*, 2008; Qidwai *et al.*, 2009). Research has proved that two constituents volatile oil nigellone and thymoquinone of *Nigella sativa* plays an important role in prevention of heart diseases. (Gad *et al.*, 1963; Babayan *et al.*, 1978). Antioxidant property thymoquinone's protect against high level of homocysteine results in reduction of atherosclerosis. (El-Saleh *et al.*, 2004). Thymoquinone (TQ) and *ter*-butylhydroquinone (TBHQ) act as super anion scavenger (Badary *et al.*, 2003). Essential oils of black seed are reported to sustain the integrity of B-cell and insulintropic properties that helps to arbitrate diabetes mellitus. (Mansi, 2005, 2006; Kaleem *et al.*, 2006). Thymoquinone and oil of black cumin boosts immune, anti-inflammatory and immunomodulatory effects. (Abbas *et al.*, 2005; Tekeoglu *et al.*, 2006, 2007).

Microwave energy is considered as superior alternative to many heating methods. The food industry in late 1970s began to use dielectric and microwave heating in analytical application (B. Ramanadhan, 2005). The mechanism of microwave rests on conversion of radio waves to heat at frequency approximately 300 MHz to 300 GHz. (Singh and Heldman, 2001). Utilization of microwave has successfully done in pasteurization, oil extraction, and baking, cooking, sterilization, drying, thawing, and blanching of multiple food products and brought ease during process. (C. P. Tan, 2001). Studies have revealed that heat treatment of different medium influences the composition of food. It may affect nutritional, functional and destroy toxic substances in food. Hernandez and coworker (1998) reported loss of antinutritional material in legumes while no impact on the protein quality of most of the legumes with

exception to common beans. When the effect of cooking and warm-holding in kidney, chick peas and lentil was examined it concluded that the protein in kidney beans, chick peas and the amino acid amount was reduced due to cooking. Warm-holding had no effect on chick peas and kidney but affect the attributes of lentil. (Montserrat Candela *et al.*, 1997).

The present study was conducted to investigate the effect of heat treatment on protein profile of kalonji and the effect of treatment on enzymatic activity of kalonji. Microwave heating and conventional oven heating at specific temperature for particular time interval was analyzed. The oven treated samples were preserved using chemical preservative. The protein profile was analysed using Mini Protein 3 cell Gel- Electrophoresis from Bio-Rad power PAC 300.

2. MATERIAL AND METHOD

2.1 Chemicals and Equipments

For the determination of proteins in treated kalonji with unlike medium of heat following chemicals were available for analysis via gel electrophoresis; NN'-Methylenebisacrylamide BDH Electran England lot No. K33932617 533, sodium dodecyl sulphate BDH England lot No. L55075692 601, ammonium peroxodisulphate Omicron Sciences limited- London UK. lot No. A 23001, TEMED reagent grade Scharlau 3440 batch No. 12229801Glycine Biochemical BDH England lot No. K32421913 441, Trizma R Base from Sigma-Alorich Life Science USA lot No. 031M5414V, acrylamide BDH Electran England lot No. K33851990 501. The standard protein marker was from Sigma SDS6H2-1VL 027K6063.

For the purpose of destaining and staining these materials were involved; acetic acid Lab Scan Ireland Batch No. 07080039, methanol RCI Labscane limited. Batch No. 0910 0326 Thailand, Coomassie brilliant-G 250 Merk Germany.

Following equipments were used for analysis of kalonji protein; weight balance from Sartorius, pH meter from Mettler Toledo MP220, Mini Protein 3 cell Gel- Electrophoresis from Bio-Rad power PAC 300 (serial No. 67S/ 06917, Voltage Limit 600 VDC, Power Limit 15W, Bio-Rad Laboratories, Hercules, CA), Binder Microwave from Binder series –Nr. 05-81106, Microwave from Dawlance, model No. DW-125C Input: 230V ~50 Hz 1400W (micro), output: 900W 2450MHz, dimension: 300mm (H) x 539mm (W) x446mm (D).

2.2 Sample Preparation

For protein analysis on SDS- Gel the samples was taken from pre-treated kalonji with microwave (2 min, 4 min, 6 min, 8 min and 10 min) and oven heated samples at temperature 50 °C, 100 °C, 150 °C, 200 °C for 1, 2, 3 hours. 1g sample was properly mixed with 10 ml Tris buffer using hand crusher. 0.5ml of each protein samples and sample diluting buffer was centrifuged at 6,000 rpm for 10 minutes. The supernatant was separated and residue was discarded. 0.5 ml of sample (supernatant) and 0.5ml of sample diluting buffer was heated for 1 to 2 minute at 100 °C in eppendorfs. After heating 0.5 ul sample was poured into well of gel to determine division protein based on molecular weight.

2.3 Protein Quantification by Bradford Method

Protein quantification was determined using Bradford method. Dye stock and assay reagent was prepared according to Bradford procedure. Modification was done while taking absorbance on spectrometer. Sample was diluted four times before its absorbance was noticed at 595 nm.

2.4 Electrophoresis

2.4.1. Protein Resolution

Kalonji protein was electrophoretically analyzed using Laemmli method 1970. Treated kalonji proteins of quantity 5 µL were poured into the wells vertically at 10 %, 12.5 % polyacrylamide gel slab along 2 cm stacking gel. The proteins were separated in a vertically using Bio-Rad mini-Protean 3 Cell System (serial No. 67S/ 06917, Voltage Limit 600 VDC, Power Limit 15W, Bio-Rad Laboratories, Hercules, CA) at constant current of 120 V for 4 hr.

2.4.2 Staining and destaining of the gel.

For staining 0.2 % (0.2gm) Commassie Blue, 75 % (7.5ml) acetic acid and 5 % (5ml) methanol make up with 100 ml deionized water was used. The stained gel was left over night. The next morning the stained gel was first washed with deionized water than with destaining solution. The ratio of destaining solution was; 50 ml (10 %) acetic acid, 150 ml (30 %) methanol with 500 ml with deionized water. The gel was slightly heated on heater to remove commassie dye colour. Repeatedly the gel was washed with destaining solution till clear bands of protein kalonji were seen.

2.5 Enzyme Activity

The enzyme activity of alpha amylase and trypsin was checked on black cumin seed. For alpha amylase plate was prepared using 1.5 g agar and 1.5g starch, while for trypsin agar 1.5 g and casein 1.5 g in 75 ml deionized water were heated for 3 and 2 minutes. The solutions were mixed and poured into plate. When the gel solidified **3(diameter)** holes were made by the help of browed. Standard of both enzyme and samples were poured into hole incubated for 24 hrs at 37 °C. Next day the plate was washed with iodine solution (1.5 gm potassium iodide with 0.3 gm iodine in 100 ml deionized water) and zone activity of enzyme was observed.

3. RESULT AND DISCUSSION

The impact of temperature and time was checked on the protein profile of kalonji using SDS-PAGE. For the analysis, kalonji protein treated in oven and microwave at different temperature and time. Following treatments were used; T0 controlled, Oven treated; T1 50 °C for 1 hr, T2 50 °C for 2 hr, T3 50 °C for 3 hr, T4 100 °C for 1 hr, T5 100 °C for 2 hr, T6 100 °C for 3 hr, T7 150 °C for 1 hr, T8 150 °C for 2hr, T9 150 °C for 3 hr, T10 200 °C for 1hr, T11 200 °C for 2 hr, T12 200 °C for 3 hr. Microwave treated; T13 2 min, T14 4 min, T15 6 min, T16 8 min and T17 10 min.

3.1. Effect of Microwave and Oven Treatments on Sensory Attributes of Black Cumin Seeds

Color and aroma of black seed was studied in sensory attributes. No specific changes in sensory characteristics were observed in microwave treated black cumin seeds. The color remained the same and no off odor was detected. This indicates that the microwave treatments did not cause any adverse effect to the sensory characteristics of samples. While in the case of oven treated samples alterations in sensory characteristics were observed. The color and odor changed with increase in temperature and time of treatments. T1, T2 and T3 (50 °C 1 hr, 2 hr and 3 hr respectively) were darker in shade as compared to T10, T11 and T12 (200 °C 1 hr, 2 hr and 3 hr respectively). Also burned odor was detected in T10, T11 and T12. This observation indicates that high temperature in oven can cause adverse effect to the colour and to some extend to the aroma of black cumin seeds. In a research by Kiralan M (2012), the effect of microwave heating and conventional roasting was analyzed on volatile compounds of black cumin seed which revealed loss and increase of specific volatile compounds with elevation in time of roasting.

3.2. Effect of Treatments on Protein Extraction of Black Cumin Seeds

Protein quantification by Bradford method (Table. 1) shows that amount of protein extracted were more in microwave treated samples on comparison with oven treated samples. High temperature causes protein loss due to denaturation of proteins. Also mailard reaction between carbohydrates and protein in oven treated samples resulted in low protein extraction that's might be the case in samples with 200 °C treatments for 1 hour, 2 hour and 3hour (see table). Protein extraction were higher 43.925 mg/ml in 8 minute microwave treated sample, second highest 41.85 mg/ml in 100 °C for 1 hour treated samples and then 41.675 mg/ml in 50°C for 2 hour treated samples (see Table). From the result it was concluded that heating with microwave helps in preserving protein profile of black cumin seeds also concluded by M. Hernández-infante *et.al* that microwave heating destroys the hemagglutinins and trypsin inhibitors without affecting the protein quality of most legumes seeds.

3.3.Effect of SDS-PAGE on Microwave and Oven Treated Kalonji Protein

The SDS-PAGE profile of kalonji protein treated in microwave and oven at specific temperature for particular time are shown in Fig.1 (10 % gel), Fig.2 (10 % gel), Fig.3, (12 % gel) and Fig.4 (12 %gel).

In lane 1 standard of protein (Protein Marker Sigma SDS 6H2-1VL027K6063) was run in both gel%. Standard contains a lyophilized mixture of the six following proteins: 1) Carbonic Anhydrase, Bovine 29 kDa, 2) Albumin, Egg 45 kDa, 3) Albumin, Bovine 66 kDa, 4)Phosphorylase B, Rabbit 97 kDa, 5) β -Galactosidase, *E. coli* 116 kDa, 6)Myosin, Rabbit muscle 200 kDa.

The controlled treatment T0 given no heat treatment showed cluster of bands which shows different proteins of various molecular weight. But heat treatment (T1 to T17) shows an upper lighter zone, middle darker zone and lower lighter zone of protein bands. These three zones are prominent in all the treated samples of kalonji protein. This showed the impact of heat treatment on the protein profile of kalonji.

In Fig. 1 at gel 10 % T1, T2, T4, T5, T6, T7, T8 for 1hr to 3hr between 50 °C to 150 °C showed middle darker colour bands showing higher molecular weight while T3 revealed lighter bands. All treatments have middle darker, lower lighter and upper very lighter representing the amount of proteins remained after heating in oven. T12, T10, T11 (lane 2, 3, 4) have no bands of protein present meaning proteins are denatured. T9 (lane 5) has lighter

middle bands while T13 to T17 in microwave treated the middle zone of proteins showed higher molecular weight are present in lane 6 to 10 in Fig. 2.

At 12 % gel oven treated T0 showed clustered of protein bands, T1 (50 °C) reveals dense amount of protein bands, which became lighter in T2 T3, T4, T5, T6 (lane 3 to 8 fig. 3) temperature range from 50 °C to 100 °C but T7 and T8 (lane 9 & 10) the middle zone is more darker and prominent shows denser molecular weight along upper zone lightly darker compared to the rest of the treatment's bands. On the other hand in T9 upper and middle zone are visible but lower zone has no bands at 150 °C for 3 hrs (Fig. 4 lane 2). At 200 °C for 1hr (T10) and 2hr (T11) lighter bands is visible in the middle zone only but no protein bands can be seen for 3hr (T12) showing denaturation of protein bands at high temperature for longer time period (Lane 3, 4, 5. Fig.4 respectively). Whereas the results in microwave heating showed darker middle zone in T15, T16 and T17 heated for 6, 8 and 10 min. (Lane 8, 9, 10. Fig.4). T13 (lane 6) was lighter to T14 (Lane 7) for 2 and 4 min respectively in Fig. 4.

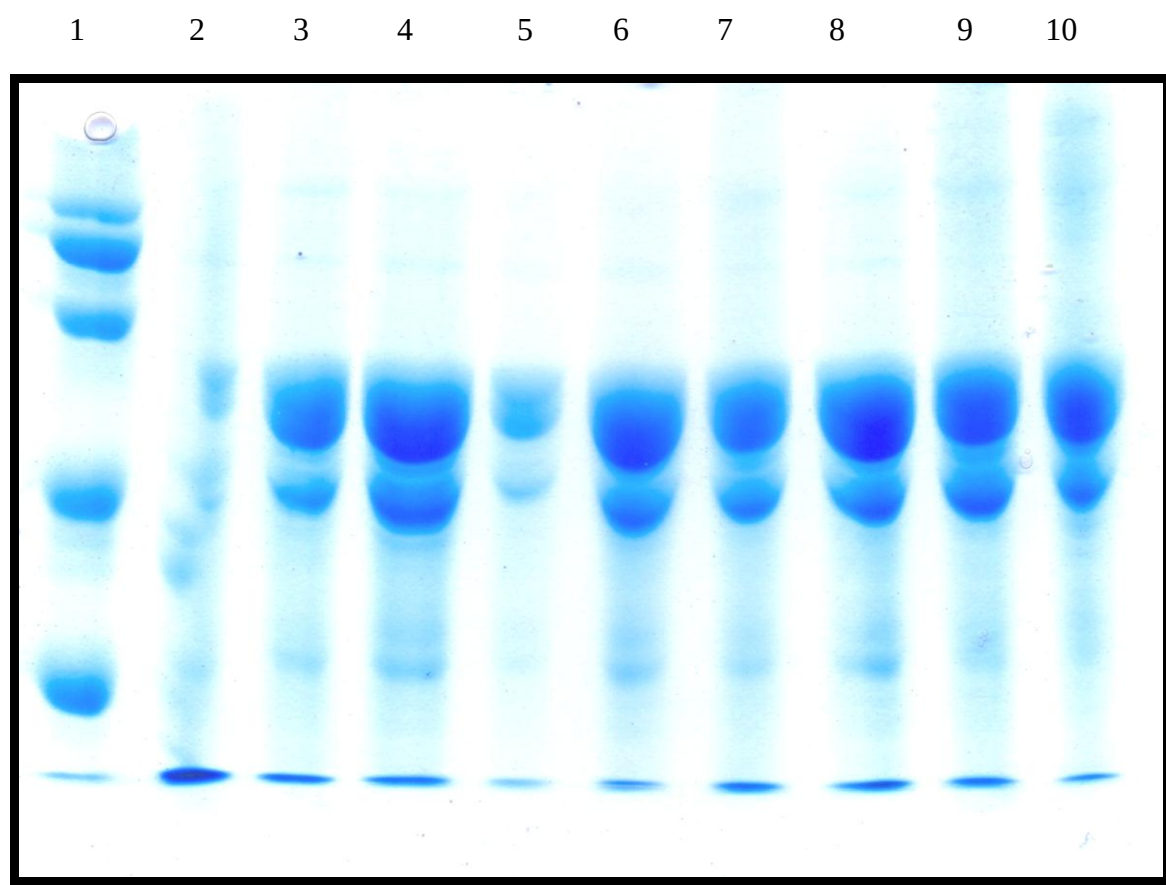


Figure 1, The SDS-PAGE pattern of the kalonji protein on 10% gel. (1) standard; (2) zero treatment; oven treated; (3) 50 °C 1 hr, (4) 50 °C 2 hr, (5) 50 °C 3 hr, (6) 100 °C 1 hr, (7) 100 °C 2 hr, (8) 100 °C 3 hr, (9) 150 °C 1 hr, (10) 150 °C 2 hr.

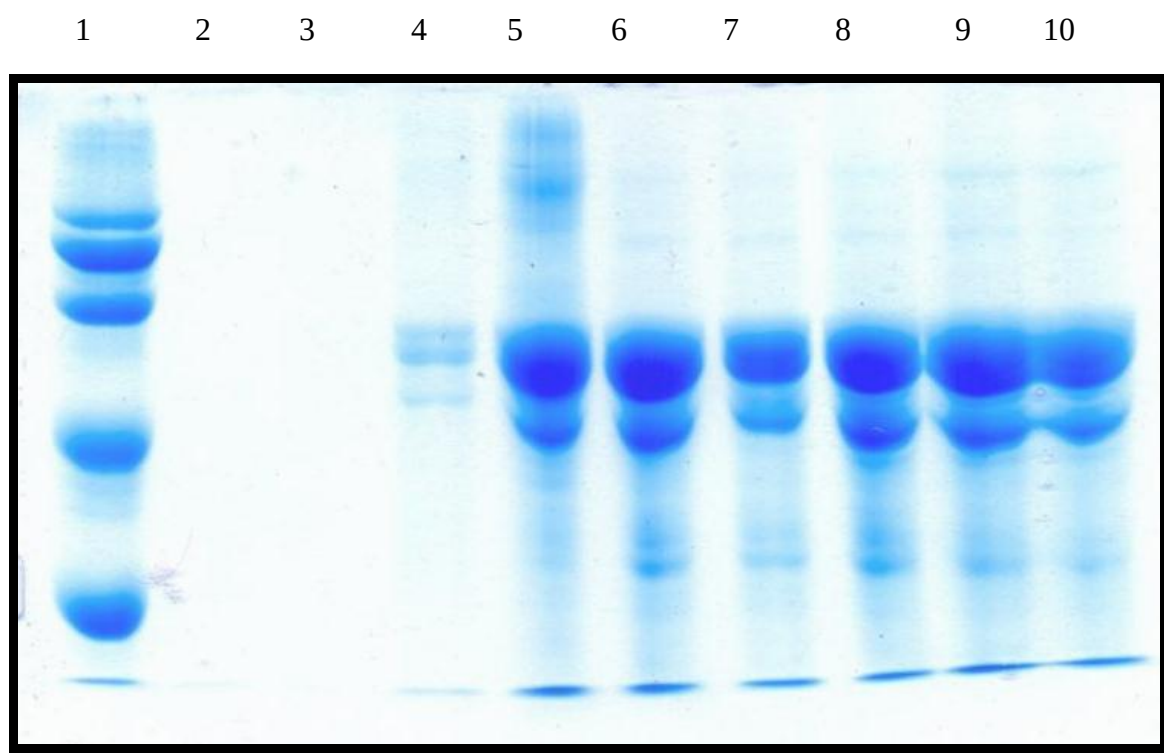


Figure. 2: The SDS-PAGE pattern of the kalonji protein on 10% gel. (1) Standard, oven treated, (2) 200 °C 3 hr, (3) 200 °C 1 hr, (4) 200 °C 2 hr, (5) 150 °C 3 hr; microwave treated (6) 2 min, (7) 4 min, (8) 6 min, (9) 8 min, (10) 10 minutes .

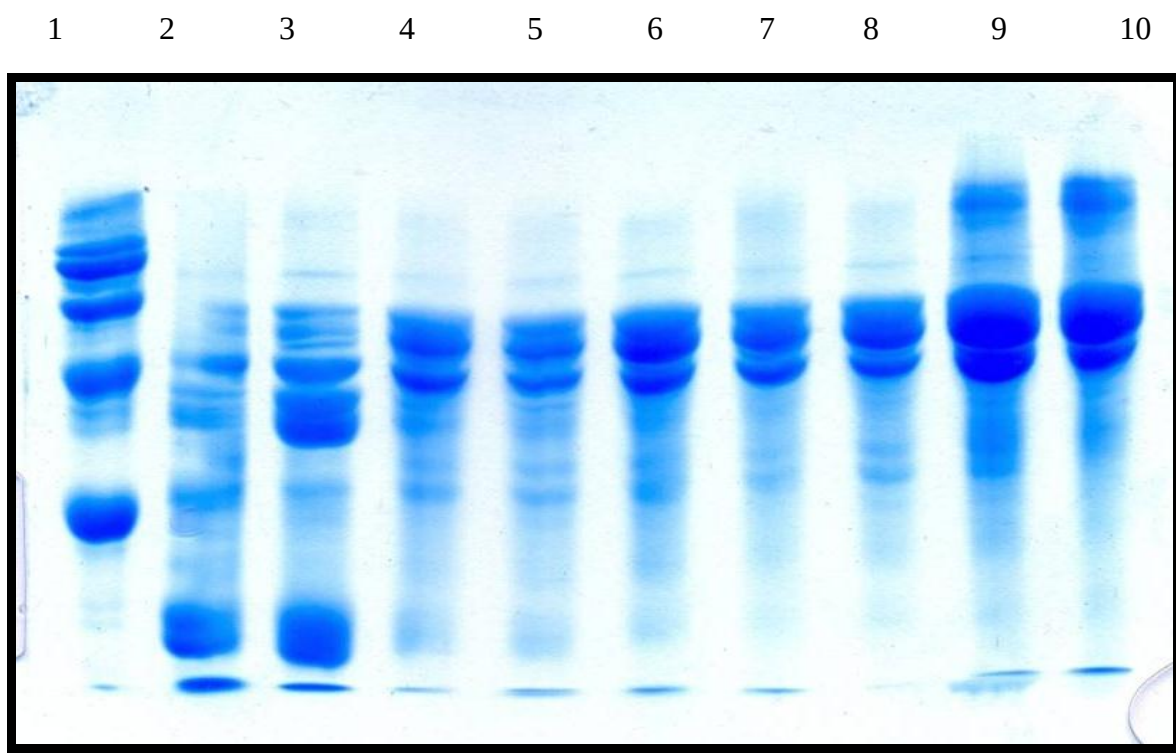


Figure 3: The SDS-PAGE pattern of the kalonji protein on 12 % gel. (1) standard, (2) zero treatment; oven treated (3) 50 °C 1 hr, (4) 50 °C 2 hr, (5) 50 °C 3 hr, (6) 100 °C 1 hr, (7) 100 °C 2 hr, (8) 100 °C 3 hr, (9) 150 °C 1 hr, (10) 150 °C 2 hr.

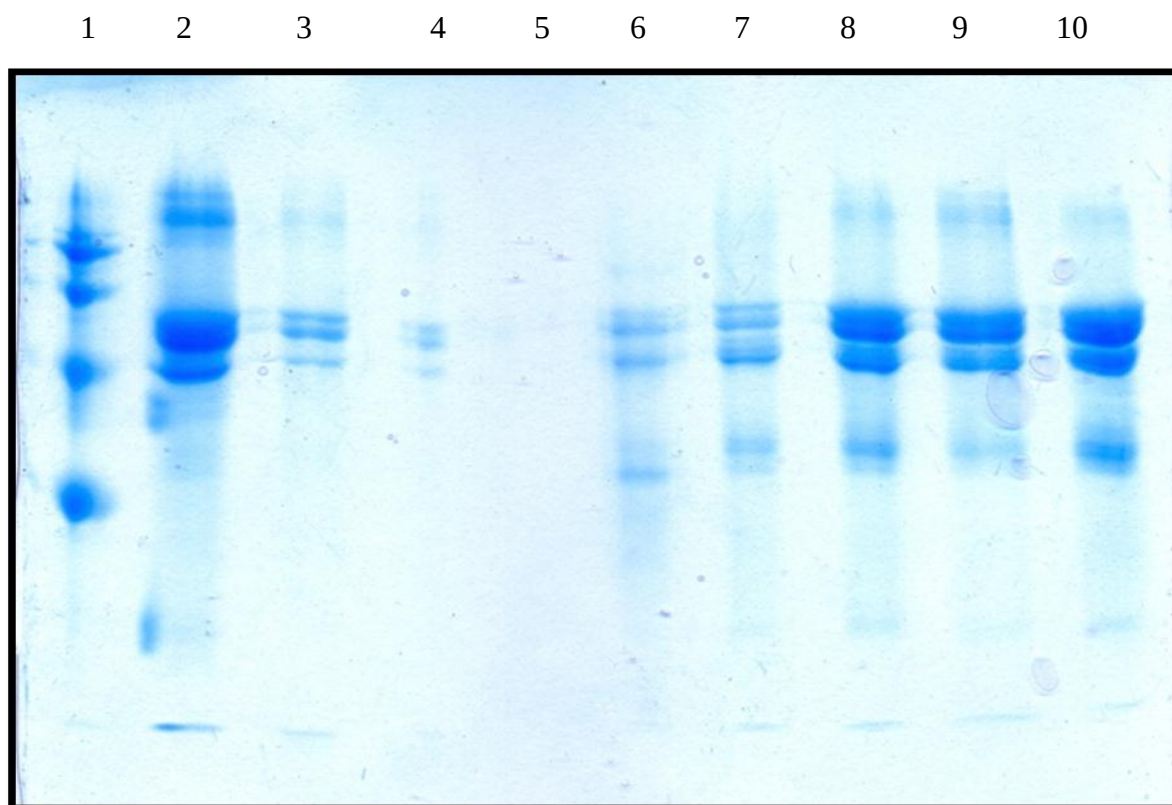


Figure 4 : The SDS-PAGE pattern of the kalonji protein on 12 % gel. (1) standard; oven treated (2) 150 °C 3 hr, (3) 200 °C 1 hr, (4) 200 °C 2 hr, (5) 200 °C 3 hr; microwave treated (6) 2 min,(7) 4 min, (8) 6 min, (9) 8 min, (10) ten min.

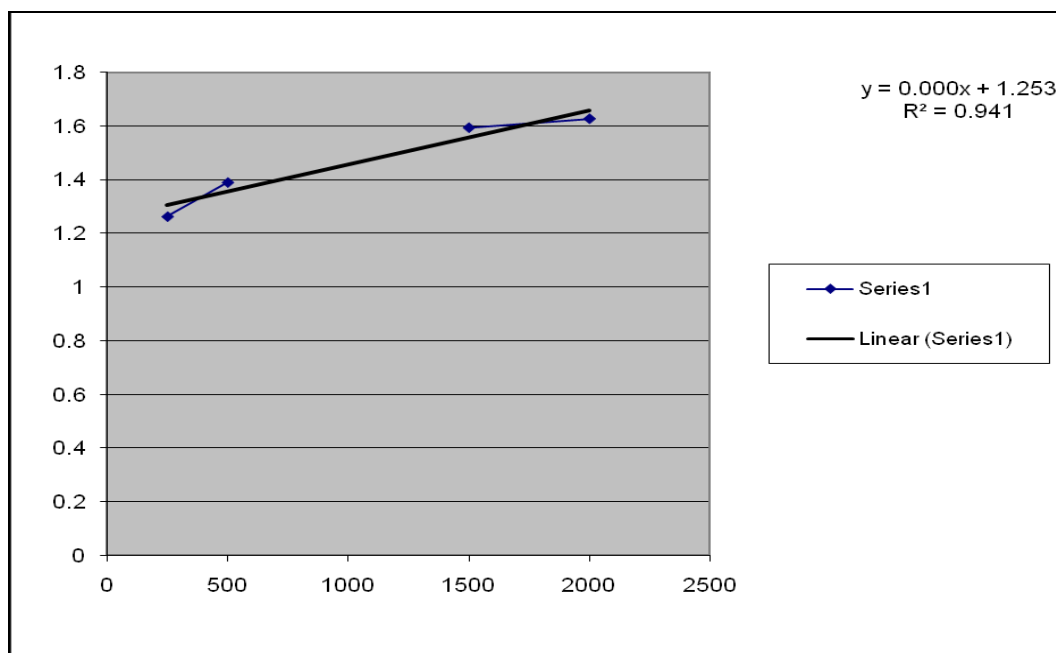


Figure 5: Standard curves for kalonji protein quantification

3.4. Effect of Treatments on Alpha Amylase Extraction and Enzyme Activity of Black Cumin Seeds Protein Samples

Enzymatic activity is directly related to enzymes zone and is affected by temperature and time of treatments. In this study no trypsin activity was observed in all samples while alpha amylase activity was observed in both microwave and oven treated samples. See Fig. 6A and 6B and Table 1.

Oven treated sample 50 °C for 2 hour show highest enzymatic activity with 1.8 cm of enzyme zone (fig.6 A). This means that at this temperature and time the protein with alpha amylase property were stable and also this treatment helps in extraction of these proteins. Same case with 150 °C for 1 hour treated sample which resulted in 1.7 cm of enzyme zone (fig. 6 A). 1.2 cm enzyme activity zone of 200 °C for 2 hour treated sample were also observed (fig. 6 B) which explains that the enzyme were stable at high temperature and even active at low concentrations (see Table.1 Bradford). Two microwave treated samples 2 minute and 8 minute resulted in alpha amylase activity with 1.4 cm and 1.2 cm of enzyme zones respectively (fig. 6 B). These results explain that oven treatments support the extraction of proteins with alpha amylase property in our black cumin seeds samples. Multiple research in past has proved process condition has always impact the enzymatic activity of α amylase. Temperature provided for specific process (baking) had influence the α -amylase activity in different cereal products. (Cristina M *et al.*, 2001).

Enzymatic activity of α amylase

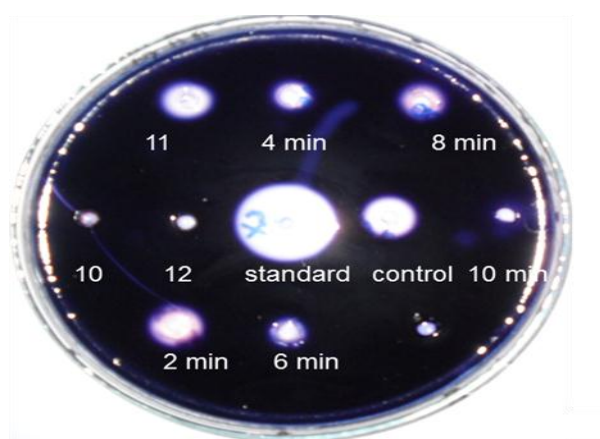


Figure 6: Effect on amylase activity, *Nigella sativa* with different treatment indicating control as 0, 1 as 50 °C 1 hr, 2 as 50 °C 2 hr, 3 as 50 °C 3 hr, 4 as 100 °C 1 hr, 5 as 100 °C 2 hr, 6 as 100 °C 3 hr, 7 as 150 °C 1 hr, 8 as 150 °C 2 hr, 9 as 150 °C 3 hr.

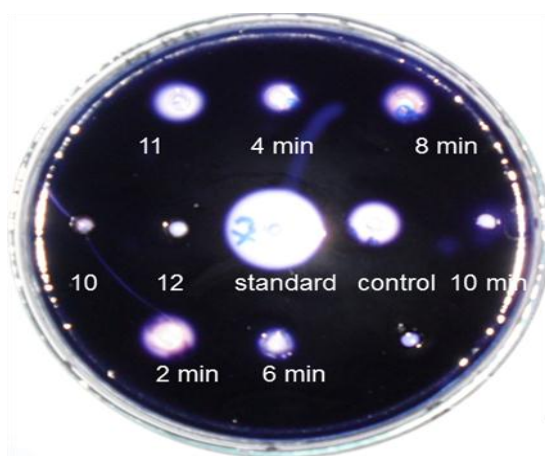


Figure 7: Effect on amylase activity, *Nigella sativa* with different treatment indicating 0 as control, 10 as 200 °C for 1 h, 11 as 200 °C for 2 h, 12 as 200 °C for 3 h and microwave treated sample as 2 min, 4 min, 6 min, 8 min, 10 min. Standard is the purified fungal alpha amylase.

Table 1: Alpha Amylas activity on heat treated Kalonji

	Treatments	Enzyme's Zone of Activity (cm)
Figure 6	Standard	2.5
	T0	1.3
	T1	1.4
	T2	1.8
	T4	1.3
	T6	0.5
	T7	1.7
	T8	0.6
Figure 7	Treatments	Enzyme's Zone of Activity (cm)
	Standard	2.7
	T0	1.2
	T11	1.2
	T13	1.4
	T16	1.2

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