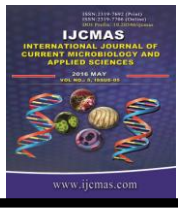
Int.J.Curr.Microbiol.App.Sci (2016) *5*(5): 293-306

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 5 (2016) pp. 293-306**
Journal homepage: http://www.ijcmas.com



Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.505.032

Utilization of Microwave Treatments for Germination and α-amylase Characteristics in Some Cereals

Firdaws A. Al Mashhdani* and Shno Salam Muhammed

Food Technology-Agriculture College-Salahaddin University, Iraq
**Corresponding author*

Keywords

Oat, Microwave irradiation, α-amylase enzyme. Wheat, Barley.

Article Info

Accepted:
15 April 2016
Available Online:
10 May 2016

A B S T R A C T

In the present study, the effect of the biophysical microwave irradiation with different exposure times, on the development and germination of Wheat (*Triticum* spp), Barley (*Hordeum vulgare* L.) and Oat (*Avena sativa* L) after steeping, seeds in Arbil city was evaluated, the study also include comparison of α-amylase yield produced by the treated and untreated cereals, partial purification and characterization of the enzyme from the best source of enzyme treated malted oat. The specific activity of α-amylase from (Wheat, Barley and Oat) seeds in microwave treatment at the best time of exposure (10sec for wheat and 30sec for both barley and oat) compared to control were (1690.86, 2931.91 and 3817.20) and (1053.52, 2019.87and 2203.34) IU/mg protein respectively. The best concentrations of ammonium sulfate used for α-amylase precipitation for both control(untreated) and treated malted oat were 50% saturation, which achieved a higher specific activity of 5537.37and 7123.78 IU/mg protein, 30.78% and 32.90% yield with increase 2.56 and 1.84 in purification folds respectively. Enzyme purification by dialysis and gel filtration by using Sephadex G-75 column chromatography for control and treated malted oat had a specific activity of 33696.66, 84793.67IU/mg with 15.58, 22.002 and 13.52, 22.69% purification fold and yield respectively.With50°C optimal temperature for α-amylase activity, optimum PH activity for the catalytic reaction at pH 5. The kinetic parameters Km and Vmax of α-amylase from control and treated malted oat were 3.75 and 3.25 mg/ml respectively. Vmax was 250µmol/min. The molecular weight of the purified α-amylase from malted oat treated and untreated using SDS-PAGE electrophoresis was44KD.

Introduction

α-Amylases (EC 3.2.1.1) are endoenzymes that catalyze the cleavage of α-1,4-glycosidic bonds in the inner part of the amylose or amylopectin chain and related polysaccharide. The end products of α-amylase action are oligosaccharides, with an α-configuration and varying lengths, and α-limit dextrins, which are branched oligosaccharides (Van der Maarel *et al*., 2002). These enzymes can be obtained from cereal, fungal, bacterial and biotechnologically altered bacterial sources. (Synowiecki *et al*., 2007).

α-Amylases, are among the most valuable enzymes, starch depolymerization which is basis for several industrial processes, which generally require α-Amylases with high activity profile. Thus much research effort to increase the α-Amylases in germination processes of cereal were performed.

The use of physical methods for plant growth stimulation attracts more and more the attention of agricultural producers as an alternative of chemical amelioration, which offers ways to improve food quality without impairing its safety (Aladjadjiyan, 2011). Most perspective factors are the treatment with ultrasound and microwave. The interest in the use of physical methods of plant growing stimulation has increased (Carbonell *et al*., 2005 and Soltani *et al*., 2006). All living processes are highly dependent on energy exchange between the cell and the environment In the case of physical treatment the energy introduced in the cell creates conditions for molecular transformations and as a result, the necessary substances are provided for the cell. This is the core concept in "quantum agriculture" that has been intensively discussed in the last years (Aladjadjiyan, 2007).

The treatment with microwave radiation can cause transitions of electrons between rotation sublevels. Transitions between vibration levels of organic molecules are in near Infrared (IR) regions, and those between rotation levels are in far IR regions and near microwave regions of the electromagnetic spectrum (Mullin, 1995).

The usage of these methods in the agriculture production will enable intense and more quantitative production of enzymes, protein….etc as well as the protection of the environment, but seeds exposed to high level of radiation will not germinated. Seed exposed to intermediated level of radiation may actually exhibit higher growth rates and yield of enzymes. Rajagopal (2009) was reported that exposure to 650 W, 2.45 GHz microwaves for about 30 s is sufficient to ensure a high rate of germination by some mechanism that is not as yet fully understood.

Aladjadjiyan (2010) has stated that the magnetic field intensity and seeds temperature increased progressively with microwave pretreatments of 5, 10, 15, 20 and 25 sec compared with controls and an increase in α-amylase activity. Studies on the effect of electromagnetic microwave irradiation of (2.45 GHz) on wheat were conducted by (Bhaskara *et al*., 1995 and Khalafallah *et al*., 2009) who also detected increased germination.

Literature in this field of study indicated that microwave radiation had a positive effect on some plants and had an adverse effect on others; which suggested that microwave radiation effects may depend on: radiation frequency, exposure period and the environmental conditions (Khalafalah *et al*., 2009 and Aladjadjian, 2012).

A process for the isolation of amylase rich fraction from malted cereals was developed (Nirmala and Muralikrishna, 2002). α-amylase has been purified and characterized from different sources and different cereals using conventional as well as classical method., These methods include dialysis, ion exchange, affinity chromatography, sephacryl-S-200 gel filtration, glycogen-Sepharose and chromatofocusing can be used to purify enzymes. (Muralikrishna and Nirmala, 2005).

The present study aimed to:-

Investigate the effect of microwave

irradiation treatments on the germination of some different cereals (Wheat, Barley and Oat).

Figure out the best source of treated cereals to produced α-amylase with the highest specific activity to be used further throughout the study.

Partial purification of α-amylase produced from the richest source, and to study its properties and its kinetic parameters.

Materials and Methods

Raw Materials

Type of Grains

Barley (Hordeum vulgare L.), Wheat (Triticum spp) and Oat (Avena sativa L) grain sample with moisture content of (11.8, 11.7 and 10% (for barely, wheat, and oat respectively were taken from Research centre in Erbil, it was pre stored in dry place at 20 ºC.

Microwave Treatment

The influences of microwave irradiation with wavelength 12 cm on seeds have been investigated. A magnetron 1042GL with frequency of radiation 2.45 GHz and maximum output power 900 W according to supplier's data has been used as microwave source. Maximum density of irradiation has been estimated at 45kW/m3. The estimation has been obtained by dividing the output power of the device (900 W) to the working volume having dimensions 510 mm (W) ×310mm (H) ×404 mm (D).Seeds have been soaked in distilled water, presuming that the imbibed water plays an important role in the absorption of the energy of microwave radiation. Seeds for the experiment have been distributed in 5 replicates each containing 20 seeds. The variants differ by the time of exposure to the microwave radiation. Seeds have been exposed to the microwave radiation for 0 s (control), 10s, 20s, 30 s, 40s, 50s, 60 s, 90 s. a modifications of output powers of magnetron as 450 W, corresponding to intensities of 22.5kW/m3, have been applied.

Extraction of Enzymes from Malt

In this research commonly 50 mM potassium-phosphate buffer with pH = 7 was used as the best extraction solution (Osman, 2002). Approximately, 0.75 g malt flour was weighed into centrifuge tubes and 4 ml extraction solution was added with mixing. Extraction was performed for 30 min at 30 ºC with regular vortexing for 5 s at 5 min intervals and was terminated by centrifugation for 10 min at 3000g. This experiment was carried in duplicate.

Enzyme Activity Assay

α-amylase was assayed by measurement of maltose related as a result of α-amylase action on starch according to the procedure of (Bernfeld, 1955). This method measured the activity of α-amylase by performing reaction using 3, 5-dinitrosalicylic acid (DNSA).

Protein Determination

The method of Lowery *et al* (1951) was used for the estimation of protein concentration. Bovine Serum Albumin (BSA) was used as standard

Partial Purification of α-amylase:

Ammonium Sulphate Precipitation:

The enzyme was precipitated with ammonium sulphate at its 30, 40, 50, 60, 70 and 80% of saturation (w/v) and centrifuged

at 10000 rpm at 4 ºC for 15min. Then the pellets were dissolved in 10ml of potassium phosphate buffer 0.05M with pH 7 and stirred at 500rpm. The α-amylase activity and protein concentration was determined.

Dialysis

Active fraction of enzyme from precipitation step with 50% saturation was dialyzed against (0.01M, Potassium phosphate pH 7.0 buffer) for 24hr at 5 ºC and the enzyme activity and protein content was determined.

Gel-filtration Chromatography

Preparation of the gel column and the fractionation procedures was determined as previously mentioned by Ammar (1975). For this purpose, column (1.6×65 cm) and Sephadex G- 75 (Pharmacia fine chemicals)"practical size 200 μ" had been used, flow rate of (18ml/hr). Fraction of 2ml was collected by fraction collector at 5 ºC. The protein content in all fractions were monitored spectrophotometrically at 280nm. Active fraction were pooled, assayed for total enzyme activity and protein.

Characterization of α-amylase

Effect of Temperature on α-Amylase

α-Amylase activities were determined at a temperature range of 20-70°C (with an interval of 5°C) with 1% soluble starch as substrate. The relative activity at different a temperature was calculated, taking the maximum activity obtained as 100%.

Effect of pH on α-amylase activity

α-Amylase activities were determined at various pH values using different buffers ranged from 3.0 to 10.0 with the same ionic strength of 0.2M

Determination of Kinetic Constants

The activity of the enzyme was assayed in 0.05M sodium acetate buffer (pH 5.0) with varying substrate concentrations ranging from 0.2 to 2.0 mg/ml. *Km* and *V*max were calculated from Linerweaver and Burk plot (Lineweaver and Burk**,** 1934).

Determination of Molecular Weight A-Amylase by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (Sds-Page)

Uniformity and molecular weight of purified α-amylase were detected by denaturing SDS-PAGE method according to Laemmli method (1970) with little modification.

Statistical Analysis

The data were analyzed using statistical program (SAS 2002, 2003) analysis system depending on (general liner mode.).

Results and Discussion

The Effect of Microwave Treatment with different Exposure Time on malted

Wheat, Barley and Oat α-amylases Activities

Table (2) showed that microwave treatment at 10sec for wheat and 30sec for barely and oat (1699.90, 2913.15)

The best specific activity was with oat seeds were irradiated by microwave irradiated at 30sec from the time of exposure and 450W energy.

Precipitation of the Enzyme

Precipitation of α-amylase from untreated oat seeds and microwave treated oat seeds

by ammonium sulfate at different saturation, the best concentration for ammonium sulfate saturation was 50% for control and microwave treatment which gave a high specific activity of 5538.37 and 7123.78U/mg respectively increase 2.54 and 1.84 in purification fold respectively and yield 30.78 and 32.90 respectively.

Purification of α-amylase

The active portion the precipitate of 50% enzyme was dissolved in 8ml potassium phosphate buffer and dialyzed against (0.01M, PH 7.0 potassium phosphate buffers for 24h at 4°C) as showed in table 18 and 19, this process gave specific activity of 6697.92 and 12592.47 U/mg protein 3.07 and 3.26 in purification fold and yield 15.54 and 19.69 for control and MW treatment respectively.

Kumar *et al.*, (2005) succeeded in purification of α-amylase from malted sorghum using two steps include ion exchange chromatography on DEAE cellulose then gel filtration on sephadex G-75 with 24.7 fold f purification and about 17.1% yield. Purification method used by Machaiah and Vakil, (1981) for α-amylase from gamma irradiation wheat was performed by precipitation of enzyme from the crude extract and isolated as enzyme-glycogen complex. several researchers also used sephadex G-75 for purification α-amylase from microorganisms and the results were similar in salting out and chromatography procedures, for Rhizupas microspores Vijayaraghavan *et al.*, (2011)

This can be explained by the fact the activity of an enzymatic reaction depended on the temperature, with increasing temperature the kinetic energy for each of the enzyme and substrate which leads to increase the possibility of collisions between their molecules up to the optimum temperature which promote potential binding between them (Segel, 1976), the enzyme activity decrease by increasing temperature above the optimal temperature due to denaturation process of the protein part of the enzyme and the loss of its natural properties(Maciunska and Synowiecki, 1998 and Zhou and Chen, 2001).

α-amylase properties

Effect of Temperature on Activity

The effects of temperature on α-amylase activity of oat (treated and untreated) seeds was studied by determining the activity between 20-70°C, as exposed in figure 12 and 13 the enzyme activity increase gradually with temperature rising up to 35°C, at 50°C the enzyme showed maximum activity, above 50°C decreasing in enzyme activity was observed, and the activity was dropped to less than 37% and 35% at 70°C for control and microwave irradiation respectively.

Machaiah and Vakil, 1981 reported that the low dose of irradiation had no appreciable effect on physiochemical properties of α-amylase. They found no significant difference between treated wheat seeds and untreated seeds.

The enzyme activity was optimal at 50°C. This value is similar to the one reported for ragi α-2 amylase (Nirmala and Muralikrishna, 2003b) and in local malted barley by (Majeed *et al*., 2011) in wheat (*Triticum Aestivum*) by (Mohamed *et al*., 2009). However, slightly higher temperature optima 55°C was reported for amylases from pearl millet (Adelaide and Varriano, 1981), malted barley, malted sorghum ((MacGregor, 1978 and Kumar *et al*., 2005).

Effect of PH on α-amylase Activity

The effect of PH on oat seed purified α-amylase activity for control and 30sec irradiated sample, using the different PH ranged from 3 to 9 at 40°C for 10min. From fig (16) and fig (17), the optimum PH was found to be 5 for α-amylase of untreated and treated seeds. Also aboard PH for enzyme activity was noticed which ranged from 4 to 7.

The results of the present study were agree with many researchers (Tkachuk and Kruger, 1974; Beers and Duke, 1990 and Nirmala and Muralikrishna, 2003b) reported a similar acidic pH range 4.5 to 6.5 for activity of α-amylase from wheat, shoots and cotyledons of pea (*Pisum sativum L.*) seedlings and finger millet respectively. Also Majeed *et al.*, (2011) observed that the enzyme purified from malted barley exhibited optimum activity at PH 5.

Table.1 The Effect of Microwave Treatments on A-Amylase Activity with different Exposure Time in Malted Wheat, Barley and Oat Seeds

Type	Tool	Time	Enzyme activity IU/ml	Protein concentrate mg/ml	Specific activity IU/mg
Wheat	Microwave	Control	1168.6^{r,s}	1.11^{r}	1052.79^{u}
		10sec	1835.9^{q}	1.08^{r}	1699.90^{p}
		20sec	1730.3^{q}	1.11^{r}	1558.82^{q}
		30sec	1659.4^{q}	1.13^{r}	1468.99^{q,r}
		40sec	1356.4^{r}	1.10^{r}	1233.09^{s}
		50sec	1185.0^{r,s}	1.00^{s}	1185.01^{t}
		60sec	890.7^{t}	0.98^{s}	908.87^{v}
Barley	Microwave	Control	3332.5^{m}	1.65^{m,n}	2019.69^{n}
		10sec	4141.9^{k}	1.68^{l,m}	2465.41^{i}
		20sec	4380.0^{j}	1.81^{k}	2419.13^{i}
		30sec	5360.2^{h}	1.84^{j,k}	2913.15^{f}
		40sec	3577.1^{l}	1.93^{i}	1853.55^{o}
		50sec	3049.1^{n}	1.98^{h}	1539.94^{q}
Oat	Microwave	Control	4605.0^{i}	2.09^{f}	2203.34^{l,m}
		10sec	6007.3^{f,g}	2.03g	2949.26^{f}
		20sec	6236.7^{e}	2.08^{f,g}	2998.41^{d,e,f}
		30sec	8054.3^{a}	2.11^{e,f}	3817.20^{a}
		40sec	6929.2^{c}	2.32^{a}	2986.72^{e,f}
		50sec	5284.P5^{h}	2.17^{c,d}	2435.06^{i}
		60sec	4375.6^{j}	2.04^{g}	2144.90^{m}

Table.2 Purification of Untreated Oat Seeds α-amylase

Purification Steps	Volume Ml	Enzyme activity IU/ml	Protein concentrate mg/ml	Total activity IU	Specific activity IU/mg	Purification fold	Yield %
Crud enzyme	50	4605.1	2.13	230255	2162.01	1	100
Ammonium sulfate 50%	8	7089.29	1.28	56714.32	5538.5	2.56	24.63
Gel filtration	22	1415.26	0.042	31153.72	33696.66	15.58	13.52

Table.3 α-amylase in Oat Seeds Purification Table (microwave treatment)

Purification Steps	Volume Ml	Enzyme activity IU/ml	Protein concentrate mg/ml	Total activity IU	Specific activity IU/mg	Purification fold	Yield %
Crud enzyme	50	8054.44	2.03	402722	3853.79	1	100
Ammonium sulfate 50%	8	13250.24	1.86	106001.92	7123.78	1.84	26.31
Gel filtration	22	4154.89	0.049	91407.58	84793.67	22.002	22.69

Fig.1 Standard Graph for Maltose

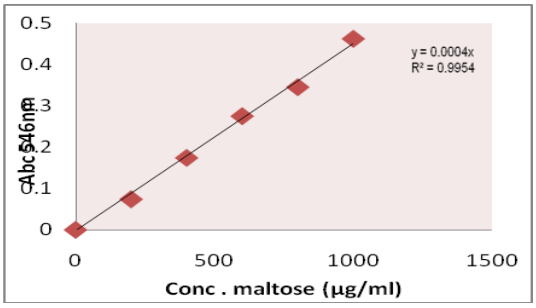


Fig.2 Standard Graph for Bovine Serum Albumin

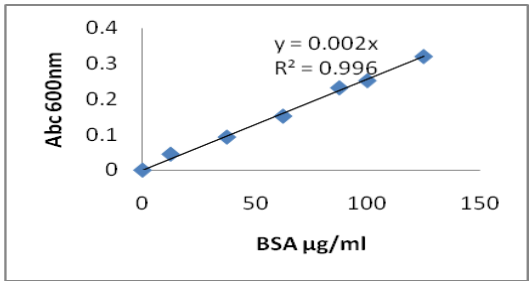


Fig.3 Gel Filtration Chromatography for A-Amylase in Oat Seeds Treated by Microwave on Sephadex G-75(1.6*65cm) Column Eluted with Potassium Phosphate Buffer (0.1m: Ph 7.0) with A Flow Rate of (18 Ml/Hr) (Protein Activity)

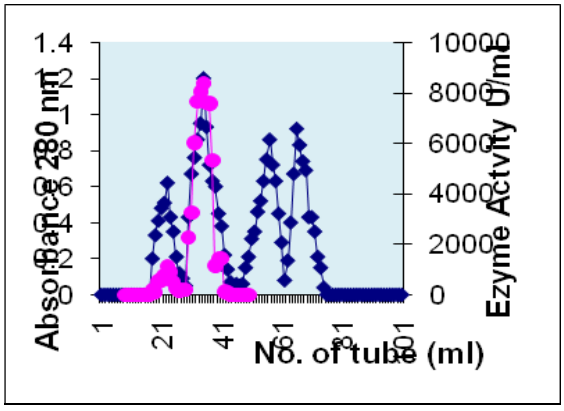


Fig.4 Gel Filtration Chromatography for A-Amylase from Untreated Oat Seeds on Sephadex G-75(1.6*65cm) Column Eluted with Potassium Phosphate Buffer (0.1m: Ph 7.0) with A Flow Rate of (18 Ml/Hr) (Protein, Activity)

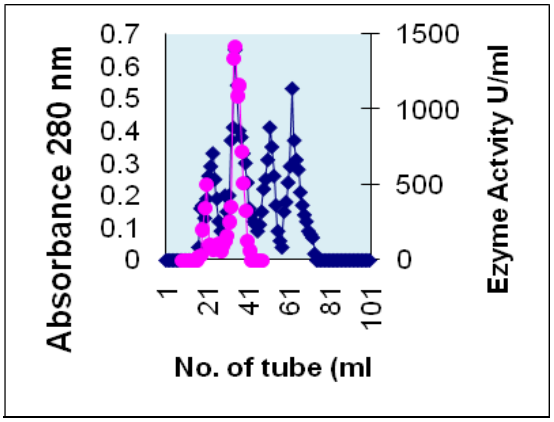


Fig.5 Effect of Temperature on A-Amylase Activity Treated Oat Seed

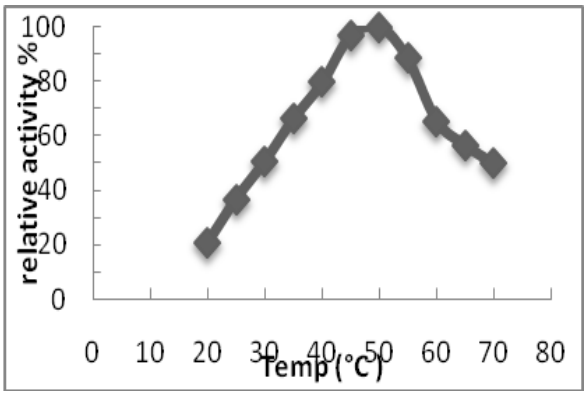


Fig.6 Effect of Temperature on A-Amylase Activity from Untreated Oat Seed

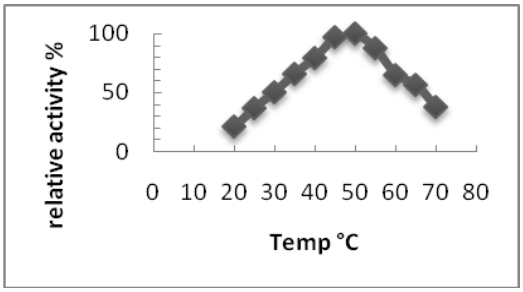


Fig.7 Effect of Ph on A-Amylase Activity in Untreated Oat Seeds

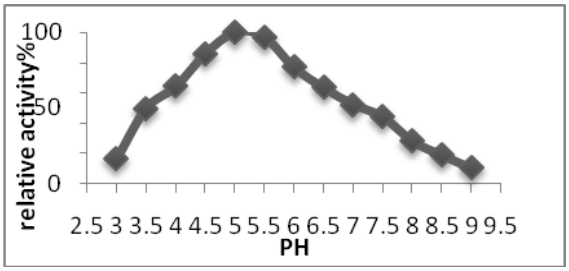


Fig.8 Effect of Ph on A-Amylase Activity in Oat 30sec Microwave Irradiated Seeds

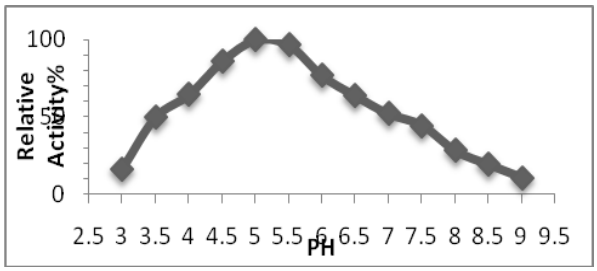


Fig.9 Linewear – Burk Plot for Untreated Oat Seeds

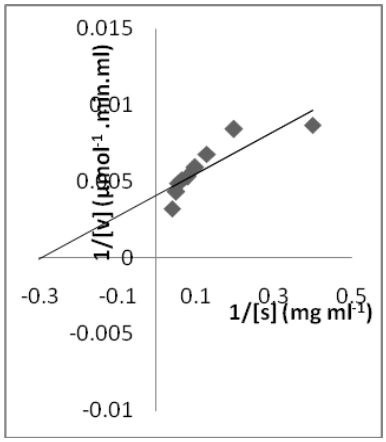


Fig.10 Linewear – Burk Plot for Treated Oat Seeds

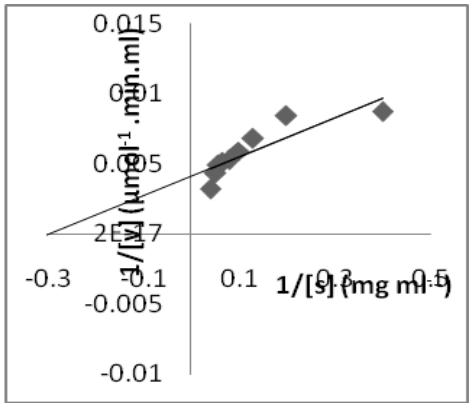


Fig.11 Standard Curve for Proteins Molecular Weight Determination

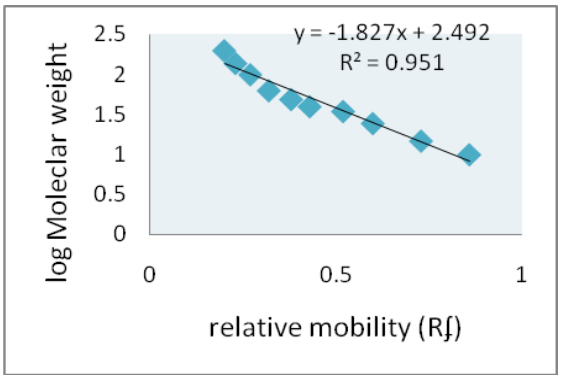
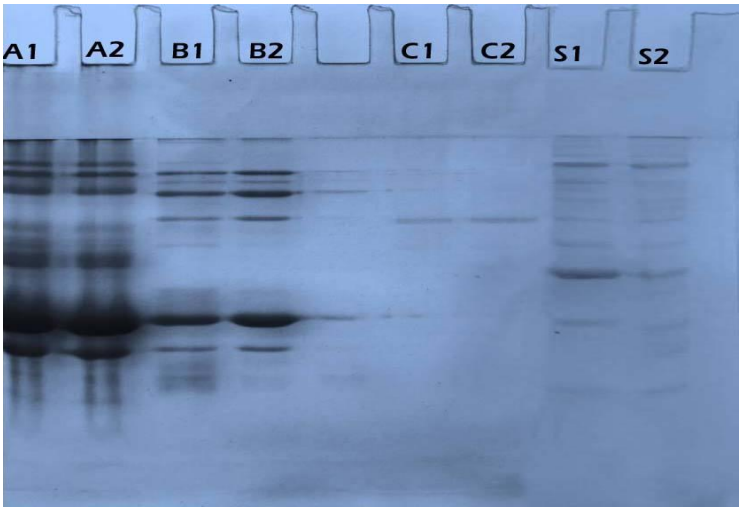


Fig.12 Photograph of Sds-Page of Alpha Amylase And Mobility of Standard Molecular Mass Marker Proteins Obtained From Sigma (A1 Crud Enzyme Untreated, A1 Crud Enzyme Treated, B1 Ammonium Sulfate Fraction 50% for Untreated Oat, B2 Ammonium Sulfate Fraction 50% For Treated Oat, C1 Pure Enzyme Untreated Oat, C2 Pure Enzyme Treated And S1,2 Molecular Weight Marker)



The optimum PH for enzyme activity in present study was different from that of P. *erosus* tuber α-amylase, which expressed a maximum activity at PH of 7.3(Noman *et al.*, 2006), furthermore Valaparla, (2010) reported the broad activity range (6.8-8.3) for α-amylases from *Acremonium Sporosulcatum*.

The variation between these results could be due to the differences in buffers, PH of the solvent which cause ionization of the functional group which is present in the amino acid sequence of the active site, as well as it may causes changes in the ionic state of substrate [S], the complex between enzyme and substrate [ES] and for the enzyme product compound complex [EP], (Crabb and Shetty, 1999).

Also another effect of the PH it may cause a change in their space geometrical structure due to changes in the bonding which founded in the secondary and tertiary structure of protein part of the enzyme (Nielsen *et al.*, 2001).

α-amylase Kinetics

Molecular weight was determined from standard curve of known molecular weight proteins, as shown in fig (22).

The molecular weight of the purified α-amylase was found to be 44.8311KDa.

The two graph gave two kinetic parameters, K_m and V_{max}. The K_m values of untreated and treated malted oats were 3.25 and 3.75 mg respectively, while the V_{max} was 250µmol/min/mg for both, these values were similar to Km values reported for α-amylases from ragi *Eleusine coracana* (5.9-14.3 mg/ml starch) (Nirmala and Muralikrishna, 2003a,b). And tuber *Pachyhizus erosus* (2.9 mg/ml starch) (Noman *et al*., 2006).

Molecular Weight of α-amylase

The molecular weight was determined by SDS-electrophoresis technique.

The molecular weight of α-amylase found in the present study was within the range of several cereal α-amylases such as that obtained for molecular weight of malt cereal enzymes ranged from 42-46 kDa (Greenwood and Milne, 1968).

However, different molecular weight of 52 kDa was reported for amylases from malted barley (MacGregor, 1978) and 52-54 kDa for immature wheat (Marchylo *et al*., 1976).

The results of the present study are in agreement with results obtained by Nirmala and Muralikrisshna, (2003a,b); Majeed *et al.*, (2011) and Usha *et al.*, (2011) who were described that the molecular weight of α-amylases enzyme from ragi *Eleusine coracana*: Malted barley and little millets, were 45, 40 and 46 kDa respectively.

References

Adelaide, B., Varriano, M.E. 1981. Pearlmillet amylases. I. Properties of partiallypurified α-amylase. *Cereal chem*, 58: 433–437.

Aladjadjian, A., Svetleva, D. 1997. Influence of Magnetron Irradiation on Common Bean (Phaseolus vulgaris L.) Seeds. Bulgarian *J. Agri. Sci.*, 3: 741–747.

Aladjadjiyan, A. 2011. Ultrasonic Stimulation of the Development of Lentils and Wheat Seedlings. *Romanian J. Biophys.*, 21(3): 179–187.

Aladjadjiyan, A. 2012. Physical Factors for Plant Growth Stimulation Improve Food Quality, In: Food Production-Approaches, Challenges and Tasks,

Publisher In Tech., 145–168.

Aladjadjiyan, A. 2010. Effect of Microwave Irradiation on Seeds of Lentils (*Lens Culinaris*, Med). *Romanian J. Biophysics*, 20(3): 213–221.

Alfekaiki, D.F. 2013. Extraction, Purification and Characterization of alpha- amylase from germinated Sorghum seeds. *Basra*, 39(1B): 13–26.

Ammar, M.S. 1975. Studies on thermophilic bacteria. Ph.D., Thesis, Botany Department, faculty of science, AL-Azhar University, Cairo, Egypt.

Beers, E., Duke, S. 1990. Characterization of α-amylase from shoots and cotyledons of pea (*Pisum sativum* L.) seedlings. *Plant Physiol.*, 92: 1154–1163.

Bernfeld, P. 1955. Amylases alpha and beta. In: Colowick, S.P., Kaplan, N.O. (Eds.), Methods in enzymology, vol. 1. Academic Press, New York, pp: 149–158.

Bhaskara, R.M.V., Kushalappa, A.C., Raghavan, G.S.V., Stevenson, M.M.P. 1995. Eradication of seed borne *Diaporthe phaseolorum* in Soybean by microwave treatment. *16th Annual meeting Biochem.*, 38: 1599–1616.

Chen, Y.P., Jia, J.F., Han, X.L. 2009. Weak microwave can alleviate water deficit induced by osmotic stress in wheat seedlings. *Planta*, 229(2): 291–298.

Crabb, W.D., Shetty, J.K. 1999. Commodity scale production of sugars from starches. *Current Option in Microbiol*., 2: 252–256.

Fahmy, A.S., Mohamed, M.A., Mohamed, T.M., Mohamed, S.A. 2000. Distribution of α-amylase in the Gramineae. Partial purification and characterization of α-amylase from Egyptian cultivar of wheat Triticum aestivium. *Bull NRC Egypt*, 25: 61–80.

Greenwood, C.T., Milne, E.A. 1968. Studies on starch degrading enzymes. Part VIII. A comparison of α-amylase from different sources: Their properties and action pattern. *Die Starke*, 20(5): 139–150.

Iuliana, C., Caprita, R., Giancarla, V., Ropciuc Sorina, R., Genoveva, B. 2013. Influence of Microwave Treatment on Root Growth Dynamics in Some Genotypes of Barley (*Hordeum Vulgare*). *Ani. Sci. Biotechnol.*, 46: 171–185.

Kumar, R.S.S., Singh, S.A., Rao, A.G.A. 2005. Thermal stability of α-amylases from malted jowar (Sorghum bicolor). *J. Agric. Food Chem*., 53: 6883–6888.

Lineweaver, H., Burk, D. 1934. The determination of enzyme dissociation constants. *J. Amer. Chem. Soc.*, 56: 658–666.

Lowry, O.H., Rosobrough, N., Far, A.L., Randall, R.J. 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem*., 1: 265–270.

MacGregor, A.W. 1978. α-amylase I from malted barley-Physical properties and action pattern on amylose. *Cereal Chem*., 55: 754–765.

Machaiah, J.P., Vakil, U.K. 1981. Purification and physicochemical properties of α- amylase from irradiated wheat. *J. Biosci.*, 3(2): 105–116.

Maciunska, J., Czyz, B., Synowiecki, J. 1998. Isolation and some properties of β-galactosidase from the thermophilic bacterium *Thermus thermophilus*. *Food Chem.*, 63: 441–445.

Majeed, G.H., Sahi, A.A., Alfekaiki, F.D. 2011. Purification and Characterization of alpha-amylase produced from a local Malt Barley. *Basra J. Sci.*, 5B(37): 93–103.

Marchylo, B., Kruger, J.E., Irvine, G.N.

1976. α-amylase from immature hard red spring wheat. I. Purification and some chemical and physical properties. *Cereal Chem.*, 53: 157–173.

Mohamed, S.A., Al-Malaki, A.L., Kumosani, T.A. 2009. Partial purification and Characterization of Five α-amylases from a Wheat Local Variety (Balady) During Germination. *Australian J. Basic and Appl. Sci.*, 3(3): 1740–1748.

Mullin, J. 1995. Microwave processing. In New Methods of Food Preservation, ed. G.W. Gould. Bishopbriggs, Glasgow: Blackie Academic and Professional, pp. 112–134.

Muralikrishna, G., Nirmala, M. 2005. Cereal α-amylases-an overview. *Carbohydr. Polym.*, 60: 163–173.

Nielsen, J.E., Borchert, T.V., Uriend, G. 2001. The determinants of α-amylase pH – activity profiles. *Protein Engineering*, 14(7): 505–512.

Nirmala, M., Muralikrishna, G. 2002. Changes in starch during malting of finger millet (Ragi, Eleusinecoracana, Indaf-15) and *in vitro* digestibility studies using purified ragi amylases. *European Food Res. Technol.*, 215: 327–333.

Nirmala, M., Muralikrishna, G. 2003a. Properties of three purified α-amylases from malted finger millet (Ragi, Eleusinecoracana,Indaf-15).*Carbohydrate Polymers*, 54: 43–50.

Nirmala, M., Muralikrishna, G. 2003b. Three α-amylases from malted finger millet (ragi,Eleusine coracana, Indaf-15)-Purification and partial characterization. *Phytochem.*, 62: 21–30.

Noman, A.S.M., Hoque, M.A., Sen, P.K., Karim, M.R. 2006. Purification and some properties of α-amylase from post-harvest *Pachyrhizus erosus* L. tuber. *Food Chem.*, 99: 444–449.

Osman, A.M. 2002. The advantages of using natural substrate-based methods in assessing the roles and synergistic and competitive interactions of barley malt starch-degrading enzymes. *J. Inst. Brew.*, 108(2): 204–214.

Rajagopal, V. 2009. Disinfestation of Stored Grain Insects Using Microwave Energy, PhD Thesis, University of Manitoba, USA.

Routray, W., Orsat, V. 2011. Microwave-assisted extraction of flavonoids: a review. *Food Bioprocess Technol.*, 5: 409–424.

SAS. 2002, 2003. Statistical analysis system, user′s guide for personal computer. Version 9.1 SAS Institute Inc. Cary, NC, USA.

Segel, I.H. 1976. Biochemical calculation. 2nd edition, john Wiley and sons. New work.

Soltani, F., Kashi, A., Arghavani, M. 2006. Effect of magnetic field on *Asparagus originalis* L, Seed germination and seedling growth. *Seed Sci. Technol.*, 34(2): 349–353.

Synowiecki, J. 2007. The Use of Starch Processing Enzymes in the Food Industry. In: PolainaJ., MacCabe AP. (eds.) Industrial Enzymes. Sctructure, Function and Applications. Dordrecht: Springer, pp 19–34.

Tkachuk, R., Kruger, J.E. 1974. Wheat α-amylases II. Physical characterization. *Cereal Chem.*, 51: 508–529.

Usha, B., Krishna, V.G., Muni, K.D., Hemalatha, K.P.J. 2011. partial characterization of α-amylase fromgerminating little millets (*panicum sumatrense*). *J. Phytol.*, 3(1): 1–8.

Valaparla, V.K. 2010. Purification and properties of a thermostable α-amylase by*Acremonium*

*sporosulcat*um. *Int. J. Biotech. Biochem.*, 6: 25–34.

Van der Maarel, M.J.E.C., van der Veen, B., Uitdehaag, J.C.M., Leemhuis, H., Dijkhuizen, L. 2002. Properties and applications of starch-converting enzymes of the α-amylase family, *J. Biotechnol.*, 94(2): 137–155.

Vijayaraghavan, P., Remya, C.S., Prakash Vincent, S.G. 2011. Production of α-Amylase by *Rhizopus microsporus* using Agricultural By-products in Solid State Fermentation. *Res. J. Microbiol.*, 6: 366–375.

Zhang, H.F., Yang, X.H., Wang, Y. 2011. Microwave assisted extraction of secondary metabolites from plants: current status and future directions. *Trends Food Sci. Technol.* 22(12): 672–688.

Zhou, Q.Z.K., Chen, X.D. 2001. Effect of temperature and PH on the catalytic activity of the immobilized β-galactosidase from *kluyveromyces lactis*. *Biochem. Engineering J.*, 9: 33–40.

How to cite this article:

Firdaws A. Al Mashhdani, Shno Salam Muhammed. 2016. Utilization of Microwave Treatments for Germination and α-amylase Characteristics in Some Cereals. *Int.J.Curr.Microbiol.App.Sci.* 5(5): 293-306. doi: http://dx.doi.org/10.20546/ijcmas.2016.505.032