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Screening of some enzymes and nutrients in radish (*Raphanus sativus* L.) root

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ABSTRACT

Activity of various enzymes in three different varieties of radish viz. BARI Mula-1, BARI Mula-2 and BARI Mula-4 available in the local area was assayed. BARI Mula-1 showed the highest β-amylase, invertase, cellulase and peroxidase activity, whereas BARI Mula-2 and BARI Mula-4 showed highest β-galactosidase and protease activity, respectively. The amount of total protein and water soluble protein along with other nutrients like carbohydrates viz. total soluble sugar, reducing sugar, starch, sucrose and fats were also investigated. The highest amount of total protein was found in BARI Mula-4 and the lowest in BARI Mula-2. The maximum amount of water soluble protein was in BARI Mula-2 followed by BARI Mula-1 and BARI Mula-4. The variety BARI Mula-1 contained highest amount of total soluble sugar (TSS) and reducing sugar, while BARI Mula-4 possessed highest amount of starch and sucrose. Among the varieties, the BARI Mula-1 was found to contain the highest amount of lipid. © 2011 Trade Science Inc. - INDIA

KEYWORDS

Radish;
Raphanus sativus;
Enzyme;
Enzyme activity;
Nutrients.

INTRODUCTION

Radish (*Raphanus sativus* L.) is widely cultivated for their edible roots. It has a hot, sharp, bitter taste and plays a vital role in providing a substantial amount of vitamins, minerals and other nutrients in our diet. It is well reputed in the folkloric system of medicines for the treatment of gastrointestinal (GIT) and cardiovascular disorders[1]. Many varieties are cultivated differing greatly in size, shape and color of the root on commercial scale[2]. Commonly, Radish is a winter crop but can be grown to some extent in summer and is greatly preferred by the people for its nutritive value and relatively low price rate. Data available on the biochemical parameters of radish produced in Bangladesh are quiet scanty. Only limited work has been done on the physiochemical properties of different varieties of radish. Some works have been going on at Bangladesh Agricultural Research Institute (BARI) with emphasis on the higher production and development of new varieties and control of the diseases. No detail work has been done on the chemical composition and enzyme activities in radish. Enzymes have become a powerful catalytic tool in a wide variety of chemical processes[3]. Higher plants play an important role as a source of many useful enzymes[4]. Extensive use of enzyme activity de-

termination as an aid to the diagnosis in hospital and laboratories all over the world is one of dramatic developments in modern medicine.

The present study is, therefore, designed to study the activities of different enzymes and to estimate the amount of major nutrients viz. carbohydrate, protein and lipid in radish root.

EXPERIMENTAL

Material

Three different varieties of radish viz. BARI Mula-1, BARI Mula-2 and BARI Mula-4 were collected directly from the cultivating field of the local area in the winter season. Soluble potato starch, Glucose, BSA and Dinitrosalicylic acid (DNS) were purchased from Sigma Chemicals Ltd., USA. All other chemicals were of analytical grade and were used without further purification.

Preparation of the crude enzyme extract

Fresh healthy radish roots were washed thoroughly with distilled water, cut into small pieces and ground with distilled water and sand in a mortar at 4ºC. The extracts were filtered with few layers of cheese cloth and further clarified by centrifugation at 5500 r.p.m. for 15 minutes at 4ºC. The clear supernatant was collected and used as crude enzyme extract.

Ammonium sulphate fractionation

The crude extract was saturated to 30-50% by the addition of solid ammonium sulphate under constant and gentle stirring at 4°C. The resulting precipitate was collected by centrifugation, dissolved in minimum volume of pre-cold distilled water and dialyzed against distilled water for 24 hours at 4°C. The dialyzed solution was then centrifuged in a refrigerated centrifuge machine at 5500 r.p.m. for 15 minutes to remove the insoluble materials. The clear supernatant thus obtained was designated as "crude enzyme solution".

Measurement of enzyme activities

Amylase activity was assayed following the method as described by Jayaraman[5]. Invertase and cellulase activity was measured following the method described by Mahadevan and Sridhar[6]. Cellulase activity was measured by estimating the reducing sugar released from the substrate using DNS method[7]. Methyl-β-D-galactosidase activity was assayed by the modified method of Lazan *et al.*,[8] using β-galactopyranoside as a substrate. The protease activity was measured following the method of Kunitz[9]. The peroxidase activity was measured following the method of Anonymous[10].

Determination of total and water soluble protein content

Total protein content of radish was determined by the method of Micro-Kjeldahl[11], while water soluble protein content was determined by method of Lowry[12].

Determination of sugar content

Total soluble sugar content in radish root was determined calorimetrically by the anthrone method[5]. Reducing sugar content of radish root flesh was determined by dinitrosalicylic acid method[7]. Sucrose content of radish root was calculated by the following formula[13].

% of sucrose or non-reducing sugar = (% of total sugar - % of reducing sugar) X 0.95.

The starch content in radish root was determined by the anthrone method as described in Laboratory Manual in Biochemistry[5].

Determination of lipid content

Lipid content of radish root was determined by the method of Bligh and Dyer[14].

RESULTS AND DISCUSSION

Activity of amylase in radish root

Amylase and invertase play a major role in carbohydrate metabolism in several plant tissues[15]. Though it has physiological, commercial, and historical significance, the physiological roles of β-amylase (α-1, 4-glucan maltohydrolase, EC 3.2.1.2) in plant cells are not well understood. Traditionally, β-amylase has been associated with starch degradation[16]. Although the entire pathway of starch degradation has not been conclusively established in any plant tissue[17], it has long been attributed to various combinations of activities of α- amylase, β-amylase, starch debranching enzyme, starch phosphorylase, and α-glucosidase[18]. The ac-

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tivity of β-amylase in the three varieties of radish is given in the TABLE 1. Among the three varieties, the β-amylase activity was found to be highest in the BARI Mula-1 (2.75 U/ml) and the lowest in the BARI Mula-4 (2.02 U/ml).

Activity of invertase in radish root

Invertase occurs widely in plants, microbial and animal sources[19-21]. The enzyme plays an important role in the hydrolysis of sucrose to glucose and fructose in higher plants, especially in the storage organs. Sucrose is an early product of photosynthetic reaction and is the most abundant transportable free carbohydrate in the plant kingdom. It serves as an important reserve carbohydrate in plants, especially in such storage organs as tuber, root and seed. The activity of invertase in the three varieties of radish root is given in the TABLE 1. The highest activity of invertase was found in the BARI Mula-1 (1.65 U/ml) and lowest in the BARI Mula-2 (0.27 U/ml)

Activity of cellulase in radish root

Cellulolytic enzymes are group hydrolytic enzymes (cellulases) capable of hydrolyzing cellulose to glucose. There are at least three major types of cellulolytic enzymes produced by fungi: endoglucanases, cellobiohydroses and cellobias[22]. These are produced by a large number of microorganisms like fungi and bacteria[22-24]. Many plant pathogens are also known to produce either adaptively or non-adaptively proteolytic, cellulolytic, and various polysaccharides[23]. They are used to perform various functions including removing cell walls or crude fiber to release valuable components (flavors, enzymes, polysaccharides and other proteins) from plant cells to improve nutritional value of animal feeds or to prepare plant protoplast for genetic research[25]. The activity of cellulase was examined in the varieties of radish root. The highest activity was in the BARI Mula-1 (0.87 U/ml) followed by BARI Mula-2 (0.71 U/ml) and BARI Mula-4 (0.65 U/ml).

Activity of β-galactosidase in radish root

β-Galactosidases (EC 3.2.1.23) are widely distributed in various plant tissues[26]. This carbohydrate splitting enzyme plays a significant role in plant tissues specifically after maturation of fruits. The enzymic properties, multiple forms and specificities relevant to structural studies on glycoproteins have been investigated[26-28]. Recently, interest in this enzyme has been focused on its *in vivo* functions concerning the degradation of such galactose-containing cell wall polysaccharides as galactan-pectin polymers and xyloglucan in relation to cell growth[29], fruit ripening[30], and seed germination[31]. The activity of β-galactosidase in the three varieties of radish root is presented in the TABLE 1. Among the three varieties, the β-galactosidase activity was found to be highest in the BARI Mula-2 (0.37 U/ml) and the lowest in the BARI Mula-1 (0.02 U/ml).

Activity of protease in radish root

Proteases are important enzymes of plant metabolism and are instrumental in regulating senescence progress[32]. They are responsible for the degradation of the proteins. Proteolytic enzymes are used extensively in industrial and medical applications[33]. Many useful proteases were isolated and characterized from microorganisms[34]. Protease activities have also been detected in many higher plant species. The activity of protease in the three varieties of radish root is shown in the TABLE 1. The highest activity of protease was found in the BARI Mula-4 (1.02 U/ml) and lowest in the BARI Mula-2 (0.95U/ml)

Activity of peroxidase in radish root

Peroxidase (EC 1.11.1.77) belongs to the class oxidoreductase, is an iron-porphyring ring containing enzyme that catalyzes the redox reaction between hydrogen peroxide (H_2O_2) as an electron acceptor and sub-

TABLE 1 : Activities of β-amylase, invertase, cellulase, β-galactosidase, protease and peroxidase in three different varieties of radish root.

	Activity of Enzymes (Units/ml)					
	β-amylase	**Invertase**	**Cellulase**	**β-galatosidase**	**Protease**	**Peroxidase**
BARI Mula-1	2.75±0.01	1.65±.05	0.87±.01	0.02±0.04	1.0±0.03	0.26±0.02
BARI Mula-2	2.23±0.03	0.27±0.01	0.71±0.04	0.37±0.03	0.95±0.04	0.18±0.02
BARI Mula-4	2.02±0.04	0.97±.02	0.65±0.03	0.07±0.01	1.02±0.02	0.23±0.05

TABLE 2 : Amount of total protein, soluble protein, total soluble sugar (TSS), reducing sugar, sucrose, starch and lipid present in different varieties of radish root.

Varieties	Amount (gm %)						
	Total Protein	Soluble Protein	TSS	Reducing Sugar	Sucrose	Starch	Lipid
BARI Mula-1	0.69±0.03	0.16±0.02	1.70±0.03	1.60±0.02	0.10±0.03	0.30±0.05	0.18±0.01
BARI Mula-2	0.64±0.01	0.17±0.02	1.65±0.01	1.56±0.03	0.09±0.04	0.45±0.01	0.13±0.02
BARI Mula-4	0.70±0.01	0.15±0.01	1.50±0.01	1.20±0.05	0.30±0.04	0.50±0.01	0.10±0.01

strates by means of O_2 liberation[35]. The enzyme is present naturally in plants e.g. soybean, radish, horse-radish, tomato, potato, carrot, turnip, wheat, dates, beats, and strawberry etc.[36-37]. Peroxidase has wide applications in health sciences as a diagnostic tool. Autoantibodies directed the thyroid peroxidase are widely used to diagnose human autoimmune thyroid disease[38]. A variety of enzymes including peroxidase, alkaline phosphatase, urease and β-galactosidase etc have been used in ELISA kits. Among which peroxidase is widely used to prepare "antibody-enzyme" or "anti-antibody-enzyme conjugates" for ELISA due to its turn over rate, rapid availability, ease of conjugation and better sensitivity[39-40]. The peroxidase activity in the BARI Mula-1 and BARI Mula-4 were 0.26 U/ml and 0.23 U/ml respectively while 0.18 U/ml was in the BARI Mula-2.

Total and Water soluble protein content in different varieties of radish root

Total and water soluble protein content of the three different varieties of radish is presented in TABLE 2. Among the varieties studied, the highest amount of total protein was found in BARI Mula-4 (0.70 gm %) and the lowest in BARI Mula-2 (0.64 gm %). The maximum amount of water soluble protein was in BARI Mula-2 (0.17 gm %) followed by BARI Mula-1 (0.16 gm %) and BARI Mula-4 (0.15 gm %).

Total soluble sugar content

Free sugars, particularly glucose, are important in the nervous systems, muscles and many other tissues. Combined with proteins as glycoproteins, the sugars play a role in secretion and external recognition properties of cell membranes. Total soluble sugar contents of the three different varieties of radish available in our country are listed in TABLE 2. Among them, the highest amount of total soluble sugar was found in the BARI Mula-1 (1.7 gm %) followed by the BARI Mula-2(1.65 gm %) and the BARI Mula-4 (1.5 gm %).

Reducing sugar content

The reducing sugar content of the three different varieties of radish namely BARI Mula-4, BARI Mula-1 and BARI Mula-2 was examined. Data obtained from the experiment are given in the TABLE 2. Among the three different varieties, the amount of the reducing sugar was highest in the BARI Mula-1 (1.6 gm %) and lowest in the BARI Mula-4 (1.20 gm %).

Sucrose content

Sucrose is as an important reserve carbohydrate in plants, especially in such storage organs as tuber, root and seed. Sucrose and glucose contents are high during tuber bulking. As the tuber size and the crop matures, sucrose and glucose decrease to a lower level often referred to as 'chemical maturity', dry matter accumulation is at a maximum and the crop is generally ready for harvest. The sucrose content is therefore, a good measure of crop maturity. Sucrose content of the three different varieties of radish is presented in the TABLE 2. It was found that the BARI Mula-4 contained the highest amount of sucrose (0.3 gm %) while the BARI Mula-2 contained the lowest amount (0.09 gm %).

Starch content

Starch is the most important reserve polysaccharide found in plants. Starch synthesize and degradation take place in many plant cells at some point in their development- as a major function in storage organs such as tubers, roots, and embryos, as a transient phase in meristem and organ development, and on a diurnal basis in leaves. Starch content of the three different varieties of radish is represented in the TABLE 2. The BARI Mula-4 contained the highest amount of starch (0.50 gm %) while the BARI Mula-1 contained the lowest amount (0.03 gm %).

Lipid content

All the roots exhibit very low lipid content. The radish is also not an exception. Lipids contribute to the

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palatability of the tubers. Amount of lipid in different cultivars of radish was determined by solvent extract process and is shown in TABLE 2. The result shows that the radishes (BARI Mula-1, BARI Mula-2, and BARI Mula-4) contained 0.18 gm %, 0.13 gm % and 0.10 gm % lipid, respectively.

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REFERENCES

[1] P.Sipos, K.Hasgymasi, A.Lugasi, E.Feher, A.Blazovies; Phytother.Res., **16**, 677-679 **(2002)**.
[2] S.Murty, S.S.VA, S.N.Subrabmanyam; 'A Text Book of Economic Botany', Wiley Eastern Ltd., New Delhi, India, 649-650 **(1989)**.
[3] J.S.Dordick; Enzyme.Microb.Technol., **11(4)**, 55-56 **(1989)**.
[4] S.T.El-Sayed, E.W.Jwanny, M.M.Rashad, A.E.Mahmoud, N.M.Abdallah; Appl. Biochem. Biotechnol., **55**, 219-230 **(1995)**.
[5] J.Jayaraman; 'Laboratory Manual in Biochemistry', 1st edition Wiley Eastern Ltd. New Delhi, India, **(1981)**.
[6] A.Mahavdevan, R.Sridhar; 'Method in physiological plant pathology', Sivakami Publication, Madras, 316, **(1982)**.
[7] G.L.Miller; Anal.Chem., **31**, 426-428 **(1972)**.
[8] H.Lazan, Z.M.Ali, J.S.Soh, Z.Talkah; Acta.Hortic., **341**, 500-509 **(1993)**.
[9] M.Kuntiz; J.Gen.Physiol., **30**, 291-310 **(1947)**.
[10] Anonymous; Phytopathology, **62**, 100-110 **(1969)**.
[11] T.Wong; J.Biol.Chem., **55**, 427 **(1923)**.
[12] O.H.Lowry, N.J.Rosenbrough, A.L.Fann, R.J.Randall; Biol.Chem., **183**, 265-275 **(1951)**.
[13] S.Rangama; 'Manual of analysis of fruits and vegetable products', Tata McGraw-Hill Publishing Company Ltd., New Delhi, **(1979)**.
[14] E.G.Bligh, W.J.Dyer; Can.J.Biochem.Physiol., **37**, 911-917 **(1959)**.
[15] WHO: Energy and protein requirements. Report of a joint FAO/WHO/UNU Expert consolation. WHO Tech. Rep. Ser. 724: Geneva, WHO, 1-206, **(1985)**.
[16] P.A.Lizotte, C.A.Henson, S.H.Duke; Plant Physiol., **92**, 615-621 **(1990)**.
[17] K.Subbaramaiah, R.Sharma; J.Biochem. Biophy.Methods, **10**, 315-320 **(1985)**.
[18] E.Beck; The degradation of transitory starch granules in chloroplasts, pg. 27-44, in R. Health, J. Preiss, Eds. 'Regulation of Carbon Partitioning in Photosynthetic Tissue', American Society of Plant Physiologists, Rockvile, MD, **(1985)**.
[19] H.Nakagawa, Y.Kawasaki, N.Ogura, H.Takehada; Agric.Biol.Chem., **36**, 18-26 **(1971)**.
[20] H.B.Krishan, J.T.Blanchette, T.W.Okita; Plant Physiol., **78**, 241-245 **(1885)**.
[21] H.B.Hirayama, N.Sumi, H.Hidaka; Agric.Biol.Chem., **53**, 667-673 **(1989)**.
[22] A.A.Klysov; Biochemistry, **29**, 10577-10585 **(1990)**.
[23] T.M.Enari; Microbial cellulases, pg. 83-223, in W.F. Rofgaty Ed. 'Microbial enzymes and Biotecgnology', Applied Sciences publishers, London, **(1983)**.
[24] R.K.S.Wood; Ann.Rec.PL.Physiol., **2**, 299-332 **(1960)**.
[25] M.Mandels; Biochemical Society Transaction, **13**, 414-416 **(1985)**.
[26] P.M.Dey, K.D.Campillo; Adv.Enzymol., **56**, 141-249 **(1984)**.
[27] M.Arakawa, S.Ogata, T.Muramatsu, A.Kobata; J.Biochem., **75**, 707-714 **(1974)**.
[28] S.C.Li, M.Y.Mazzotta, S.F.Chien, Y.T.Li; J.Biochem., **250**, 6786-6791 **(1975)**.
[29] M.Edwards, Y.J.L.Bowman, I.C.M.Dea, J.S.G.Reid; J.Biochem., **263**, 4333-4337 **(1988)**.
[30] H.Koono, Y.Yamasaki, K.Katoh; Plant Physiol., **68**, 46-52 **(1986)**.
[31] R.Pressey; Plant Physiol., **71**, 132-135 **(1983)**.
[32] C.Lauriere; Physiol.Veg., **21**, 1159-1177 **(1983)**.
[33] T.Sanna, El-Sayed; Pak.J.Biol.Sci., **4(5)**, 564-568 **(2001)**.
[34] S.A.El-Aassar; Biotechnol.Leu., **17**, 943-951 **(1995)**.
[35] A.S.Brill; Peroxidase and catalase comprehensive. Biochemistry, Vol. XIV, Elsevier Pub. Amsterdam, 447-479, **(1996)**.
[36] G.Reed; Oxidoreductase: Enzymes in food processing. Academic Press, USA., 216, **(1975)**.
[37] S.Ambreen, K.Rehman, M.A.Zia, F.Habib; Pak.J.Agric.Sci., **37(3-4)**, **(2000)**.
[38] F.F.Nord; Interscience Pub., **15**, 156-158 **(1953)**.
[39] D.M.Kemery, S.J.Challancombe; ELISA and other solid phase immunoassays. John Wiley and sons, USA, 1-16, **(1989)**.
[40] A.Zia, M.Yaqub, K.Rehman, T.Mahmood; Pak.J.Biol.Sci., **3**, 1716-1718 **(2000)**.