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ENZYME ACTIVITIES IN RELATION TO SUGAR ACCUMULATION IN TOMATO

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Abstract: Enzyme activities in tomato juice of five different varieties viz. Ratan, Marglove, BARI-1, BARI-5 and BARI-6, in relation to sugar accumulation were investigated at different maturity stages. The highest amount of invertase and β-galactosidase was found in Marglove and the lowest in BARI-6 at all maturity stages. Total soluble sugar and sucrose contents were highest in BARI-1 and lowest in BARI-6. The activity of amylase was maximum in Ratan and minimum in Marglove. Protease activity was highest in Ratan and lowest in BARI-6. BARI-1 contained the highest cellulase activity and the lowest in BARI-5. The amount of total soluble sugar and sucrose increased moderately from premature to ripe stage. The activities of amylase and cellulase increased up to the mature stage and then decreased drastically in the ripe stage. The activities of invertase and protease increased sharply from the premature to the ripe stage while the β-galactosidase activity decreased remarkably. No detectable amount of reducing sugar was present in the premature stage in all cultivars of tomato but increased thereafter upto the ripe stage. The highest reducing sugar was present in BARI-5 in all of the maturity stages.

Keywords: Tomato enzymes, invertase, galactosidase, protease, amylase, cellulase, soluble sugar, sucrose, reducing sugar, maturity stages

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is a popular, delicious and nutritious vegetable. It is one of the most important and widely cultivated vegetables in Bangladesh. It plays a vital role in providing a substantial quantity of vitamin C and other nutrients in human diet [1]. There are many varieties of tomato in our country but their nutritive values are not known clearly. It was found that the nutritional quality of tomato flesh is greatly affected with changes in maturity. The enzymes present in the tomato flesh may play some role in the nutrient changes in tomato flesh at different maturity stages. It has been reported that proteolytic and hydrolytic enzymes play some physiological roles during maturation and senescence of fruits [2,3]. Dilley [4] suggested that the dramatic physical and chemical changes attending the process of ripening occur as a result of catabolic and anabolic events. The data available on the biochemical parameters of tomato, produced in Bangladesh, are quite scanty. Only limited work has been done on the physico-chemical properties of the different local varieties of tomato. Some work is going on at Bangladesh Agricultural Research Institute with emphasis on the higher production and development of new varieties and control of diseases. No detailed work has been done on changes in various

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enzyme activities in tomato. The present investigation was undertaken with a view to making comparative and detailed studies on biochemical changes in different cultivars of tomato at various maturity levels. Enzyme activities of amylase, invertase, cellulase, protease, methyl-β-D-galactosidase, were assayed at premature, mature and ripening stages in relation to the accumulation of the total soluble sugar, reducing sugar and sucrose.

Materials and Methods

Collection of tomato varieties

Five different varieties of tomato viz. Ratan, Marglove, BARI-1, BARI-5, and BARI-6 were collected during premature, mature and ripening stages. These were collected from Bangladesh Agricultural Development Corporation (Garden), Naodapara, Rajshahi. The days required for the fruit to set were 8 to 11, 13 to 17 and 24 to 27 for premature, mature and ripening stages, respectively.

Preparation of crude enzyme extract

About 50 g of tomato were cut into small pieces, crushed thoroughly using mortar and pestle and homogenized well in cold buffer. For amylase, invertase and cellulase 0.1M sodium acetate-acetic acid buffer was used at pH 6, pH 5 and pH 5.2, respectively. For methyl-β-D-galactosidase, 0.1M sodium citrate buffer was used at pH 4.1, and for protease, it was 0.1M phosphate buffer at pH 7.0. The extracts were filtered through cheese cloth and further clarified by centrifugation at 6000 r.p.m. for 15 minutes at 4°C. The clear supernatant was collected and used as crude enzyme extract.

Measurement of enzyme activities

Amylase, invertase and cellulase activities were assayed following the method of Mahadevan and Sridhar [5]. Methyl-β-D-galactosidase activity was determined by the modified method of Lazan *et al.* [6]. The amount of galactose released was estimated by dinitrosalicylic acid (DNS) method [7]. The protease activity was measured following the method of Kunitz [8].

Determination of sugar content in tomato flesh

Total soluble sugar content in tomato flesh was determined colorimetrically by the anthrone method [9]. Reducing sugar content of the tomato flesh was determined by dinitrosalicylic acid (DNS) method [7]. Sucrose content of tomato flesh was calculated by the following formula [10].

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

Results and Discussion

Amylase activity and its relation to sugar compositions in tomato juices at different maturity stages

Amylase (also called diastase) has physiological, commercial and historical significance. It is found in both plants and animals. Payen and Persoz [11] were the first to become aware of enzymatic starch hydrolysis; they found that malt extract converted starch (a complex of amylase and amylopectin) to sugar. Amylase is a hydrolytic enzyme, which hydrolyzes starch to yield monomeric carbohydrates. Starch breaks down during the germination of cereal seeds. This results from the action of hydrolytic enzymes, and it is generally accepted that phosphorylases are not involved in this process, while α-amylase plays a major role during the degradation of native starch granules [12,13,14]. The concerted action of α-amylase, β-amylase, a debranching enzyme, and α-glycosidase is essential for the complete hydrolysis of starch [15,16]. In order to make use of the carbon and energy stored in it, the polymer must be first broken down to smaller assimilable sugars before it can be converted to its individual basic glucose units.

The activity of amylase and sugar composition in the five different varieties of tomato juices at different maturity stages are shown in Table 1.

The activities of amylase at all stages were found to be the highest in Ratan and the lowest in Marglove. The activities varied from 16 to 56.89 U/ml at the premature stage, from 58.69 to 71.11 U/ml at the mature stage and from 21.33 to 53 U/ml at the ripening stage. The amylase activities in all varieties increased significantly up to the mature stage but decreased abruptly from the mature to the ripening stage. Similar trends were reported by Desai and Despande [3], Mao and Kinsella [17] and Garica and Iajolo [18] in banana. On the other hand, total soluble sugar and sucrose content in all varieties increased from the premature to the ripening stage (Table 1). These observations indicate that the increased amount of total soluble sugar and sucrose up to mature stage is directly proportional to amylase activities and from mature to ripen stage the amount of amylase is inversely proportional to total soluble sugar and sucrose content. Recent evidence associating amylase with sugar content and sucrose transport implies that amylase control is a potential means of sugar regulation [19,20]. Some past studies [21,22] have also shown that amylase level has an inverse relationship to sucrose forming activity.

Reducing sugar was absent in the juice of all varieties of tomato at the premature stages but it increased thereafter up to the ripening stage (Table 1). This is consistent with the increased amount of total soluble sugar and sucrose from the premature to the ripening stage.

Invertase activity and its relationship to sugar composition in tomato juice at different maturity stages

Invertase (β-fructofuranoside; EC 3.2.1.26) was first isolated from sugarcane more than a century ago [23]. The enzyme occurs widely in plant, microbial and animal products [24,25,26]. Invertase 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. Sucrose serves as an important reserve carbohydrate in plants, especially in such storage organs as tuber, root and seed. During germination, sucrose is a readily degradable source of energy [27]. The activities of invertase and the sugar composition in different varieties of tomato juice at different maturity stages are shown in the Table 1.

Invertase activities varied between 21 and 64 U/ml in the premature stage, 26.67 and 72.00 U/ml in the mature stage and 37.68 and 82.32 U/ml in the ripening stage. Its activities in all stages were found to be the highest in Marglove and the lowest in BARI-6. The activities were the lowest in all varieties at the premature stage, thereafter increasing remarkably up to the ripening stage. This is in good agreement with the increasing amount of total soluble sugar and sucrose from the premature to the ripening stage. The amount of invertase was directly proportional to the total soluble sugar and sucrose content (Table 1). Reducing sugar was absent in the tomato juice of all varieties at the premature stage but it increased thereafter up to the ripening stage. Sarah *et al.* [28] reported that invertase activities were much lower in the green than in the red tomato. In *L. esculentum* a marked increase of invertase activity [29] and invertase protein was found during fruit development. High sucrose levels could be of potential importance in improving tomato yield and flavor in the cultivated tomato varieties. Peter and Stephanie [30] showed that invertase activity is positively correlated with reducing sugar accumulation in the two studied varieties of tomato.

Cellulase activity and its relationship to sugar compositions in tomato juice at different maturity stages

Cellulolytic enzymes, a group of hydrolytic enzymes including cellulase, are capable of hydrolyzing cellulose to glucose. There are at least three major types of cellulolytic enzymes produced by fungi: endoglucanases, cellobiohydrolases and cellobiases [31]. These are produced by a number of microorganisms such as fungi and bacteria [32,33]. Many plant pathogens are also known to produce, either adaptively or non-adaptively, proteolytic, cellulolytic enzymes and various polysaccharides [33]. They are used to perform various functions including removal of cell wall or crude fiber to release valuable components (flavors, enzymes, polysaccharides and other proteins) from the plant cells to improve nutritional value of animal feeds or to prepare plant protoplasts for genetic research [34]. The cellulase activities and sugar

Table 1. Activity of amylase, invertase, cellulase and relationship to sugar accumulation in five different varieties of tomato.

Varieties	Stages	Activity of Enzyme (Units/ml)			Composition (g %)		
		Amylase	Invertase	Cellulase	Total soluble sugar	Reducing sugar	Sucrose
Ratan	Premature	56.89 ± 0.02	28.44 ± 0.1	8.32 ± 0.021	1.25 ± 0.033	0.00	1.18 ± 0.09
	Mature	71.11 ± 0.021	35.56 ± 0.11	16.63± 0.012	1.88 ± 0.023	0.42 ± 0.021	1.39 ± 0.087
	Ripening	53.00 ± 0.021	46.42 ± 0.2	9.10 ± 0.022	3.37 ± 0.041	0.78 ± 0.03	2.46 ± 0.056
Marglove	Premature	16.00 ± 0.032	64.00 ± 0.09	7.50 ± 0.053	1.20 ± 0.021	0.00	1.14 ± 0.041
	Mature	58.69 ± 0.025	72.00± 0.086	14.88 ± 0.023	1.55 ± 0.022	0.30 ± 0.004	1.19 ± 0.033
	Ripening	21.33 ± 0.005	82.32 ± 0.07	9.0 ± 0.03	2.38 ± 0.032	0.41± 0.005	1.87 ± 0.035
BARI-1	Premature	53.30 ± 0.004	40.00 ± 0.012	8.52 ± 0.07	1.75 ± 0.035	0.00	1.66 ± 0.022
	Mature	58.70 ± 0.021	50.22 ± 0.022	17.15 ± 0.09	2.25 ± 0.032	0.35 ± 0.011	1.80 ± 0.023
	Ripening	35.56 ± 0.02	62.25 ± 0.034	9.23 ± 0.10	3.85 ± 0.044	0.82 ± 0.02	2.88 ± 0.030
BARI-5	Premature	53.33 ± 0.01	21.53 ± 0.008	4.40 ± 0.012	1.05 ± 0.041	0.0	0.99 ± 0.045
	Mature	69.33 ± 0.015	27.00 ± 0.005	9.35 ± 0.023	1.63 ± 0.047	0.43 ± 0.01	1.24 ± 0.03
	Ripening	32.00 ± 0.03	38.54 ± 0.006	5.03 ± 0.02	2.20 ± 0.054	0.83 ± 0.021	1.35 ± 0.043
BARI-6	Premature	54.77 ± 0.008	21.00 ± 0.05	4.88 ± 0.015	1.00 ± 0.011	0.0	0.95 ± 0.033
	Mature	71.00 ± 0.005	26.67 ± 0.004	10.05 ± 0.008	1.40 ± 0.035	0.33 ± 0.005	1.02 ± 0.021
	Ripen	22.22 ± 0.01	37.68 ± 0.003	5.11 ± 0.01	1.80 ± 0.021	0.66 ± 0.011	1.08 ± 0.20

composition of five different varieties of tomato juice at different maturity stages are presented in the Table 1. Among these varieties, the activity of cellulase was the highest in BARI-1 (8.52 U/ml at premature stage, 17.15 U/ml at mature stage and 9.23 U/ml at ripening stage) and the lowest in BARI-5 (4.4 U/ml at premature stage, 9.35 at mature stage and 5.03 U/ml at ripening stage) (Table 1). Cellulase activities in all varieties increased remarkably up to the mature stage and decreased abruptly from the mature to the ripening stage.

During quantitative estimation of cellulase enzyme in *Botryodiplodia theobromae* in culture, the activity of the enzyme was found to increase up to the mature stage as reported by Chakrabarti and Nandi [35]. Nehemiah and Despande [36] found that cellulase production and decomposition of cotton fabric increase in *Alternania brassicae* as well. The present findings demonstrate that the activities of cellulase and the amount of total soluble sugar and sucrose increase up to the mature stage. Beyond this stage, the amount of cellulase reduces drastically but the total soluble sugar and sucrose contents increase.

β-galactosidase activity and its relationship to sugar composition in tomato juice at different maturity stages

β-galactosidase (EC 3.2.1.23) is widely distributed in plants, animals and micro-organisms. It is a carbohydrate splitting enzyme, plays a significant role in plant tissues, specifically after maturation of fruits. Wallenfels and Weil [37] have reviewed the extensive literature on the enzymology of β-galactosidase. The activities of β-galactosidase and sugar composition in the juice of different varieties of tomato at different maturity stages are shown in Table 2. Of the varieties studied, Marglove contained the highest and BARI-6 contained the lowest β-galactosidase activity. β-galactosidase activities ranged from 6.15 to 40.25 U/ml at different maturity stages in all varieties. All of these varieties contained the highest β-galactosidase activiy and the lowest reducing sugar at the premature stage, but the lowest β-galactosidase activity and the highest reducing sugar was detected at the ripening stage. The total soluble sugar and sucrose contents increased sparingly from the premature to the ripening stage (Table 2). It is thus clear that the activities of β-galactosidase decrease rapidly and the amount of total soluble sugar, reducing sugar and sucrose increase moderately with advancement of maturity.

Protease activity and its relationship to sugar composition in tomato juice at different maturity stages

Proteases are protein hydrolytic enzymes. These act on proteinacious substances of the cell wall, releasing amino acids and amides. The activities of protease and the sugar composition in the juice of different varieties of tomato at different maturity stages are illustrated in Table 2. Of the above mentioned varieties, protease activity at all stages was found to be the highest in Ratan (2.25 U/ml at premature stage, 4.40 U/ml at mature stage and 7.50 U/ml at ripening stage) and the lowest in BARI-6 (1.50 U/ml at premature stage, 3.35 U/ml at mature stage and 5.54 U/ml at ripening stage). The activity of protease was the lowest in all varieties at the premature stage but then increased sharply up to the ripening stage. The amount of total soluble sugar and sucrose increased moderately from the premature to the ripening stage (Table 2). These results indicate that the activity of protease and the amount of total soluble sugar and sucrose in the juice of different varieties of tomato increases with changes in maturity stage. Hashinaga *et al.* [2] reported that protease activity (KFP-1) increases in Kiwifruit flesh during ripening. Increased protease activity has also been observed in passion fruit juice during maturation [38]. High protease activity during the ripening stage may be attributed to protein catabolism,

Table 2. Activity of β-galactosidase, protease and relationship to sugar accumulation in five different varieties of tomato.

Varieties	Stages	Activity of Enzyme (Units/ml)		Composition (g %)		
		β-galactosidase	Protease	Total soluble sugar	Reducing sugar	Sucrose
Ratan	Premature	32.34 ± 0.05	2.25 ± 0.04	1.25 ± 0.033	0.00	1.18 ± 0.09
	Mature	19.63 ± 0.043	4.40 ± 0.021	1.88 ± 0.023	0.42 ± 0.021	1.39 ± 0.087
	Ripening	7.33 ± 0.045	7.50 ± 0.044	3.37 ± 0.041	0.78 ± 0.03	2.46 ± 0.056
Marglove	Premature	40.25 ± 0.033	2.10 ± 0.045	1.20 ± 0.021	0.00	1.14 ± 0.041
	Mature	22.33 ± 0.045	4.33 ± 0.023	1.55 ± 0.022	0.30 ± 0.004	1.19 ± 0.033
	Ripening	9.21 ± 0.05	7.00 ± 0.033	2.38 ± 0.032	0.41± 0.005	1.87 ± 0.035
BARI-1	Premature	29.99 ± 0.022	1.87 ± 0.042	1.75 ± 0.035	0.00	1.66 ± 0.022
	Mature	16.27 ± 0.032	3.90 ± 0.021	2.25 ± 0.032	0.35 ± 0.011	1.80 ± 0.023
	Ripening	6.66 ± 0.041	6.11 ± 0.03	3.85 ± 0.044	0.82 ± 0.02	2.88 ± 0.030
BARI-5	Premature	35.33 ± 0.012	1.90 ± 0.11	1.05 ± 0.041	0.0	0.99 ± 0.045
	Mature	20.15 ± 0.022	4.00 ± 0.10	1.63 ± 0.047	0.43 ± 0.01	1.24 ± 0.03
	Ripening	8.27 ± 0.013	6.05 ± 0.03	2.20 ± 0.054	0.83 ± 0.021	1.35 ± 0.043
BARI-6	Premature	25.45 ± 0.021	1.50 ± 0.02	1.00 ± 0.011	0.0	0.95 ± 0.033
	Mature	14.82 ± 0.033	3.35 ± 0.01	1.40 ± 0.035	0.33 ± 0.005	1.02 ± 0.021
	Ripening	6.15 ± 0.022	5.54 ± 0.01	1.80 ± 0.021	0.66 ± 0.011	1.08 ± 0.20

which is related to leaf senescence [4]. The reducing sugar was absent in the premature stage, but at later stages its amount increased sharply up to the ripening stage. The highest reducing sugar was present in BARI-5 and the lowest in Marglove at all maturity stages.

Total soluble sugar content in tomato flesh

Total soluble sugar content of the five different varieties of tomato in various stages is shown in Table 1. Among the varieties studied, the highest amount of total soluble sugar was found in BARI-1 (1.75 g %) followed by Ratan (1.25 g %), Marglove (1.20 g %), BARI-5 (1.05 g %), BARI-6 (1.00 g %) in the premature stage. In the mature stage, the total soluble sugar content was maximum in BARI-1 (2.25 g %) and minimum in BARI-6 (1.40 g %), other varieties gave intermediate values. At the ripening stage, the total soluble sugar content ranged from 1.80 g % (BARI-6) to 3.85 g % (BARI-1). Thus according to the present results the total soluble sugar content of the different varieties of tomato increases significantly with change of maturity.

Reducing sugar content in tomato flesh

Reducing sugar present in all varieties of tomato at all maturity stages is shown in Table 1. No detectable amount of reducing sugar was found at the premature stages in all the cultivars of tomato. However, its content increased thereafter up to the ripening stage. At the mature stage, the reducing sugar content was the highest in BARI-5 (0.43 g %) and the lowest in Marglove (0.30 g %). At the ripening stage, the maximum reducing sugar was found in BARI-5 (0.83 g %) followed by BARI-1 (0.82 g %), Ratan (0.78 g %), BARI-6 (0.66 g %) and Marglove (0.41 g %).

Sucrose content in tomato flesh

Sucrose is an early product of photo-synthetic reaction, is the most abundant transport form of sugar and is the major free sugar in many fruits. Sucrose serves as an important reserve carbohydrate in plants, especially in such storage organs as tuber, root, fruit and seed. During germination, sucrose is a readily degradable source of energy. Its content in the different varieties of tomato is presented in Table 1. BARI-1 contained the highest amount of sucrose (2.88 g %), while BARI-6 contained the lowest amount (0.95 g %). Sucrose content in tomato varied between 0.95 - 1.66 g % at the premature stage, 1.02 - 1.80 g % at the mature stage and 1.08 - 2.88 g % at ripening stage. From the present experimental results, it is evident that the sucrose content of the different varieties of tomato increases significantly with changes in maturity.

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