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RESEARCH **P**APER

Studies on polyphenol oxidase in pomegranate (*Punica granatum* L.)

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The present investigation was undertaken to study properties of polyphenol oxidase and monitor changes in polyphenal oxidase activity in Ganesh cultivar of pomegranate during storage. The characterization of polyphenol oxidase in pomeqranate fruit extract with respect to effect of enzyme concentration and substrate concentration was studied . for studying changes in polyphenol oxidase activity during storage, the pomegranate fruits at half yellow stage were freshly harvested and stored at ambient temperature and analyzed after 0, 2, 4, 6, 8 and 10 days of storage. The polyphenol oxidase activity has increased up to 8th day of storage and thereafter, decreased slightly on 10th day.

Key words : Ambient, Half yellow stage, Substrate, Enzyme

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INTRODUCTION

Pomegranate (*Punica granatum* L.) belongs to family punicaceue and is one of the favourite table fruit of tropical and subtropical region. In India it is a minor horticultural crop. However, it has become commercially important horticultural crop in Maharashtra due to its hardy nature. Low cost of cultivation and good returns to farmers (Purohit, 1986), better keeping quality, very good export potential and introduction of high yielding varieties like Musical, Ganesh, E-137 and Mrudula. In Maharashtra area under cultivation is out 30,000ha (Mote *et al.,* 1992).

Pomeqranate fruit is good source of carbohydrates (14.5%), proteins (1.9%), fat (0.1%) and minerals (0.7%) including calcium (0.10mg/100g), magnesium (12mg/100g), phosphorous (70mg/100g) and iron (0.3mg/100g). Pomegranate supplies vitamins like thiarnine riboflavin and nicotinic acid. They are however, a poor source of vitamin C (Stood *et al.,* 1992). The edible portion of pomegranate fruits contains 77 to 88 per cent moisture, 5.1 per cent crude fibre and 0.66 tp 0.76 per cent ashwjthenerge value 65Kcal/100mg (El-Nemar *et al.,* 1990).

Browning of fruit tissues has generally been attributed to oxidative processes (Joslyn and Ponting, 1951), Enzymatic oxidation of phenols is catalysed by polyphenol oxidase (Mayer and Harel, 1981). Therefore, it was felt necessary to study the changes in polyphenol oxidase during different stages of development and storage. The proposed investigation was undertaken with following objectives.

– To study properties of polyphenol oxidase in pomegranate fruits.
– To study changes in polyphenol oxidase during storage of pomegranate fruit at ambient temperature.

RESEARCH METHODOLOGY

The present investigation entailed ‘study on polyphenol oxidase in pomegranate’ was conducted at college of Agricultural Biotechnology, Loni, Amednagar district, Maharashtra. Pomegranate fruits of Ganesh cultivar were harvested from the experimental plot of Department of Horticulture, Mahatma Phule Krishi Vidyapith, Rahuri, Ahmednagar (M.S.).

For storage studies, about 5kg physiologically matured fruits (half yellow 130 days after flowering) were harvested from each of five plants. The fruits were pooled together and divided into 5 sets of triplicate, Eachbt contained 3 fruits. These fruits were stored in plastic plates at ambient

temperature. One set of each treatment was used for analysis after 0, 2, 4, 6, 8 and 10 days of storage.

Collection of samples :

The fruits were collected from Mahatma Phule Krishi Vidyapith, Rahuri during year 2013.

Extraction of polyphenol oxidase :

Extraction and assay of polyphenol oxidase were carried out with slight modification of method given by Kumar and Khan (1932). Ten grams of freshly harvested pomegranate arils were macerated with 20ml of 0.05M phosphate buffer (pH 7.0) in prechilled mortar and pestle. The homogenate was centrifuge at 15000rpm at 4°C for 30min and supernatant was used as source of enzyme. To 1ml of catechol reagent, 3ml of phosphate buffer (pH 7.0) and 1ml of enzyme extract wasadded and content were mixed, absorbance recorded at 420nm with control.

Table A : Extraction fo enzyme and analysis

Sr. No	Time (see)	Catechol (ml)	Phosphate buffer (ml)	Enzyme extract (ml)	Absorbance O D at 420nm
1.	0	1	3	1	0.08
2.	30	1	3	1	0.10
3.	60	1	3	1	0.12
4.	90	1	3	1	0.13
5.	120	1	3	1	0.15
6.	150	1	3	1	0.16
7.	180	1	3	1	0.18
8.	210	1	3	1	0.20
9.	240	1	3	1	0.22
10.	270	1	3	1	0.23
11.	300	1	3	1	0.25

The protein content of enzyme extract was determined by using Folin-ciocalteau reagent (Lowry *et al.,* 1951).

Statistical analysis completed by CRD method (Haplin and Lee, 1987 and Panse and Sukhatme, 1967) formula :

$$\text{Specific enzyme activity} = \frac{\text{Enzyme activity}}{\text{Conc. of protein}}$$

Properties of polyphenol oxidase :

Effect of enzyme concentration :

The effect of enzyme concentration was studied by using different concentrations of enzyme (0, 0.5, 1, 1.5, 2, 2.5ml) in reaction mixture of 3ml of phosphate buffer of 0.05m pH 7.0 and 1ml of catechol solution(0.1M) as a substrate.

Effect of substrate concentration :

For studying effect of substrate concentration on the activity of polyphenol oxidase, different concentrations of catechol (0, 0.02, 0.04, 0.06, 0.08, 0.1mM) were used. The volume adjusted with phosphate buffer. The rest of procedure was same as described above.

Changes in polyphenol oxidase during storage :

The selected half yellow fruits (130 days after flowering) were stored open at ambient conditions one set of each sample was used for analysis after 0, 2, 4, 6, 8 and 10 days of storage.

RESEARCH FINDINGS AND ANALYSIS

The changes in the polyphenol oxidase were studied during storage of pomegranate fruits of Ganesh variety. The effect of enzyme concentration and substrate concentration on activity of polyphenol oxidase were also studied. The obtained results are given below properties of polyphenol oxidase.

Effect of enzyme concentration :

The polyphenol activity increased gradually up to 2ml of enzyme concentration but thereafter rate of increase was not proportional to enzyme concentration.

Table 1: Effect of enzyme concentration on polychenol oxides activity

Enzyme concentration (ml)	Specific Enzyme activity (units/mg of soluble protain/min)
0.5	1.17 ± 0.02
1.0	1.60 ± 0.06
1.5	2.43 ± 0.12
2.0	3.26 ± 0.06
2.5	3.15 ± 0.03

One unit of polyphenol ozidase activity is defined as the change in absorbance by 0.01 at 420nm formula :

$$\text{Specific enzyme activity} = \frac{\text{Enzyme activity}}{\text{Total protain concentration}}$$

Effect of subtract concentration :

The enzyme activity increased as the substrate concentration increased from 0.02 to 0.08mM. Further increased in substrate concentration did not increased the enzyme activity.

Table 2 : Effect of substrate concentration on polyphenol oxidase activity

Substrate concentration (mM) catechol	Specific Enzyme activity (units/mg of soluble protain/min)
0.02	0.99 ± 0.015
0.04	1.50 ± 0.025
0.06	1.71 ± 0.020
0.08	1.81 ± 0.035
0.10	1.81 ± 0.035

Changes in polyphenol oxidase during storage of pomegranate at ambient temperature :

It was seen that the activity of polyphenol oxidase activity increased progressively up to 8th of storage followed by slight reduction during subsequent storage.

Table 3 : Changes in polyphenol oxidase activity during storage of pomegranate fruit at ambient temp

Storage period	Enzyme activity Units/mol/min	Specific enzyme activity Units/mg of soluble protein/min
0.	3.20 + 0.05	1.28 + 0.08
2.	4.37 + 0.15	1.75 + 0.13
4.	4.60 + 0.08	1.84 + 0.13
6.	5.30 + 0.07	2.12 + 0.13
8.	7.07 + 0.05	2.83 + 0.08
10.	6.07 + 0.019	2.35 + 0.05
S.E. ±	0.05	C.D. (P = 0.05) 0.18

The studies on polyphenol oxidase in pomegranate (*Punica granatum* L.) arils extract was carried out during the year 2012-13 Polyphenol oxidase enzyme is one of enzyme associated with browning in many fruits. It was there fore proposed to study changes in polyphenol oxidase during growth and storage of pomegranate with the aim of having better understanding of postharvest behavior of the pomegranate fruits.

For storage study, pomegranate fruits were harvested at pale green stage and stored at room temperature which were analyzed on 0,2,4,6,8, and 10 days of storage. For characterizing the polyphenol oxidase extract was prepared by harvesting pale green fresh fruits with phosphate buffer.

The polyphenol oxidase activity increased progressively up to 8th day during storage then a slight reduction was noticed at 10th day of storage Gradual increase in polyphenol oxidase activity may be responsible for early and quick browning. Similar findings are supported by Jaiswal *et al.* (2010).

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