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Effect of storage conditions on the residual polyphenol oxidase (PPO) activity of raisins

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ABSTRACT

Polyphenol oxidase (PPO) activity profile of various morphological parts of grape was considered as an index to specify its suitability to process in the form of raisins. In the present investigation the presence of residual PPO in different morphological parts of grapes and the effects of pH, temperature and storage conditions on the PPO activity were studied. The raisins stored at refrigerated condition significantly retained the golden yellow colour, as compared to raisins stored at room temperature. Residual PPO activity during processing and storage was found to be an important factor affecting the changes in colour and external appearance of raisins. The PPO activity of the skin (108) was found more than the flesh (55) which indicates that the PPO is located in skin than flesh. Also the PPO activity was found maximum at pH 5.4 and at 25ºC. Raisin sample prepared in the laboratory by Australian cold dip method and stored in low temperature was best with respect to colour.

Key words : Grapes, Raisins, Polyphenol oxidase (PPO), Browning, Storage.

INTRODUCTION

The browning of foods and beverages is cosmetic discoloration which has negative impact on appearance, consumer acceptability, commercial value and sensorial quality. In many edible plants products formation of several shades of colour ranging from pink to bluish black, generally termed as "Browning".

The browning of raw fruits, vegetables and beverages is a major problem in food industry. Enzymatic browning is initiated by the activity of copper containing enzyme polyphenol oxidase. This is also known as tyrosinase, phenol oxidase, mono-phenol oxidase or cresolase. Endogenous PPO activity present in fruits is responsible for oxidative browning (Mc Evily *et al.,* 1992).

PPO is present universally in all types of plants but its optimum activity is confined in fruits and vegetables containing high phenolic compounds. Diversified intracellular concentration and PPO in edible plant and tissue established a fact that its distribution within the specified morphological part is exclusively uneven. The browning induced undesirable change in fruits and vegetable is mostly associated with the activity of PPO (Mathew and Parpia, 1971). Production of colored pigments was originated by PPO action lead to enzymatic browning in fruits and vegetable.

The fundamental step in enzymatic browning of food materials is oxidation of phenolic compounds into o-quinones. The o-quinones polymerize to form high molecular weight compounds, which react with amino acids and proteins enhancing the production of brown colour pigments (Vamos Vigyazo, 1980 and Yoruk ans Marshall, 2003). Browning being an oxidative reaction can be retarded by elimination of available oxygen from damaged surfaces of the fresh fruits and vegetable. However, this method of preventing browning is expensive. The most common and precise approach for prevention of browning in food beverages is the use of anti browning agents.

Grape is one of the most important horticultural crops in India. The grapes are highly perishable in nature owning their short ripening period. Dehydration of grapes during peak season results in better economic returns. The demands of conventionally processed raisins have declined in market because of discolorations during storage, which is one of the important notified sensory parameters. Grapes contains a large amount of different phenolic compounds in skins, pulp and seeds, that are partially extracted during winemaking (Orak, 2006 and Revilla and Ryan, 2000). The grapes show the higher level PPO activity (Kimberly *et al*., 1981). This enzyme is highly heat sensitive and its activity reduced during the dehydration process. The residues of this enzyme interfere with the discoloration of the raisins during storage. However, this factor has also adversely affected the export quality of raisins, besides its sensory and nutritional quality attributes. It is important to know the characteristics of the PPO associated with raisins in order to control browning during storage. Therefore, the present investigation was carried out to study the effect of browning due to residual PPO during storage conditions of raisins.

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MATERIALS AND METHODS

Fully matured and well-ripened grapes of Thompson seedless variety were procured from local market of Parbhani, Maharashtra state (M.S.). The raisins were prepared in the laboratory as per Australian Cold dip methods (Deoreyappa and Gowda, 1998). Commercially prepared fresh raisins of same variety were obtained from the different locations *viz.*, Tasgaon, Akola and Nasik (M.S.). The high quality chemicals were used for chemical analysis. The freshly prepared raisins and raisins collected from different markets were packed in 150 gauge LDPE bags. Then these raisins were stored for the period of 2 months at low temperature (5 $\pm 2^0$C, RH 85 ± 5 %) and ambient temperature (35 ± 2^0C, RH 65 ± 5 %), respectively. These raisins were analyzed for their residual PPO activity the regular intervals of 10 days during storage.

The pH optima for the PPO activity was determined in the pH range from 3.6 -5.6 and 5.8 -8.0 by using 0.01M phosphate buffer at 27^0 C. The optimum temperature for the enzyme activity was determined by maintaining buffer and substrate concentration constant in water bath at various temperatures range 10^0 – 70^0C.

The thermal stability of crude PPO was determined by adding 10 ml of crude enzyme extract into an equilibrated pre-warmed flask in water bath that was set up at respective temperature. A 1 ml aliquot of heated enzyme was withdrawn at specific intervals of time followed by cooling in a pre chilled test tube placed in ice bath. It was immediately assayed for PPO activity as described earlier. Five different temperatures (65, 70, 75, 80 and 85^0C) were tested with seven independent timings (1, 2, 3, 4, 5, 10, 20 and 30 min) to assay residual activity.

The extraction of crude extract and assay of the residual PPO activity was carried out with slight modification in the method described by Lee and Smith (1979). Grape /raisin (60g) was chilled in 120 ml of 0.067M K2HPO4 buffer solution at pH 7.0. Then the whole mass was homogenized for 3 min in prechilled (0^0 – 4^0C) waring blender jar. The macerates was filtered through Whatman filter paper (No.4). The filtered extracts was used as crude enzyme (CE). The reaction mixture consists of 0.01M Sodium acetate buffer 20 ml (5.6 pH) and 0.5M catechol solution as a substrate. The enzyme reaction was initiated by adding 1ml of CE extract. The change in absorbance was monitored by noting constant change in the absorbance with respect to time. One unit of PPO was defining as the amount of enzyme extract causing a change in absorbance of 0.001/ml. The data obtained in the present study were statically analyzed as suggested by Panse and Sukhatme (1989).

RESULTS AND DISCUSSION

The different morphological parts of fresh grapes (peel and flesh) were analyzed for PPO activity and the data obtained are presented in Table 1. The data indicated that the concentration of PPO was found more predominant in peel of berry (108 units) as compared to the flesh (55 units). Therefore, the activity of enzyme was more in the skin, which affects color of the skin and imparts undesirable brown color to the raisins. These findings were also consistent with earlier reports of Dandwate (1996), Miller *et al*. (1990), Zowistowski *et al*. (1986) and Rathjen and Robinson (1992).

Table 1: Polyphenol Oxidase (PPO) activity of crude extract of various morphological parts of grapes*

Morphological Part	PPO activity (Units/min/ml)
Grapes (Whole)	147
Peel/Skin	108
Flesh/Pulp	55

*Each value represents the average of three determinations

The PPO activity as a function of pH was studied over wide range of pH 3.6 to 8.0. The data on the effect of pH on PPO activity was depicted in Fig. 1. It was noticed that the remarkable decrease in PPO activity was observed below pH 5.2 and above 6.4 underlined the pH zone of maximum activity. The liner increase in enzyme activity as a function of pH upto 5.6 followed by remarkable decrease in activity notified pH optima as 5.6. This concludes that grape PPO activity is characterized by rapid decrease in enzyme activity at both acidic and alkaline pH range. The results were in close agreement with earlier reports of Kimberly *et al*. (1981), Park *et al*. (1980), Cash *et al*. (1976) and Reyes and Luh (1960).

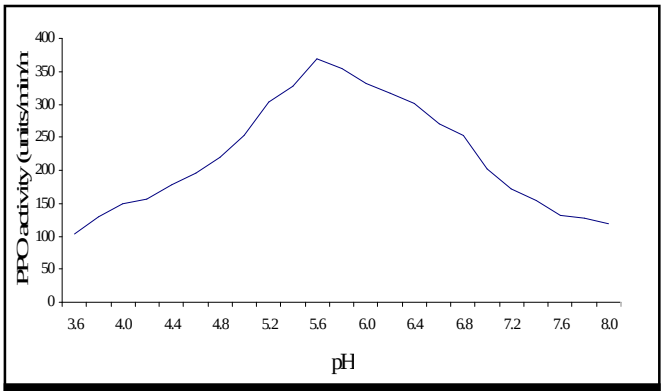

Fig. 1 : Effect of pH on the PPO activity

The effect of temperature on PPO activity was presented in Fig. 2. The enzyme activity as a function of temperature increased linearly up to 25^0C and then

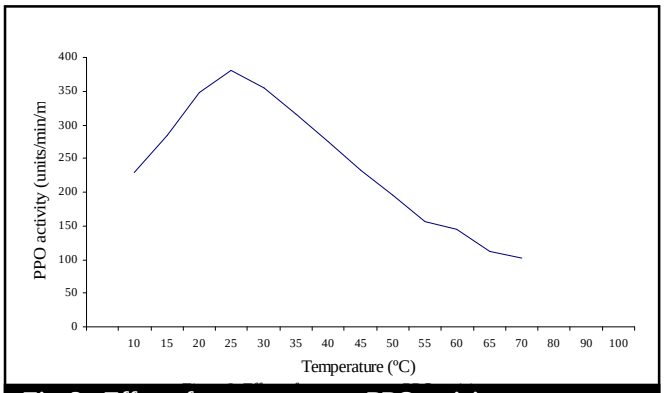

Fig. 2 : Effect of temperature on PPO activity

decreased linearly up to 70^{0}C. The findings on the effect of temperature on enzyme activity were in good confirmation with earlier reports of Olusola *et al*. (1992) and Valero *et al*. (1980).

Thermal inactivation of grape PPO is presented in Table 2. From the results it was observed that reduction in residual PPO activity was a function of temperature at a specified inactivation time. The inactivation time was longer for low temperatures and shorter for high temperatures. Complete inactivation of PPO was noticed at 65^{0}C for 30 min, 70^{0}C for 20min, 75^{0}C for 10 min, 80^{0}C for 5 min and 85^{0}C for 3min. These results are in agreement with earlier results of Dimick *et al*. (1951).

The raisins prepared in the laboratory and raisins procured from different markets were analyzed for their residual PPO activity at different storage conditions. The data pertaining to the enzyme activity were presented in Table 3. It was revealed from the data that the raisins prepared in the laboratory and stored at low temperature was found significantly superior than the raisins of Akola market, followed by Nasik and Tasgaon market stored at low and ambient temperature, respectively. The decreasing trend of enzyme activity was observed as the storage periods increased. Similar results are being reported by Dandwate (1996). The residual PPO enzyme activity of raisins stored at ambient temperature showed linear decrease as compared to low temperature.

Conclusion :

The PPO activity was higher in skin than the flesh of the grapes. The optimum PPO activity was found at pH 5.6 and temperature 25^{0}C. The reduction in PPO activity was a function of time and temperature combination. The raisins prepared in laboratory by an Australian cold dip method and stored at low temperature was best with respect to colour among all the samples. The activity of residual PPO interferes with change in colour, sensorial quality and deterioration of raisin quality. Residual PPO activity during processing and store was found to be an important factor affecting the changes in

Table 2 : Thermal inactivation of PPO as a function of temperature and time. [a,*]

Time (min)	PPO activity (Units/min/ml) Temperature(ºC)				
	65	70	75	80	85
0	389 (0)	389(0)	389 (0)	389(0)	389 (0)
1	218 (43.96)	205 (47.31)	142 (63.50)	72 (81.50)	40 (89.72)
2	180 (53.73)	142 (63.50)	67 (82.78)	52 (86.64)	12 (96.92)
3	153 (60.67)	112 (71.21)	43 (88.95)	40 (89.72)	8 (97.95)
4	131 (66.33)	85 (78.15)	37 (90.49)	20 (94.86)	na
5	75 (80.72)	55 (85.87)	23 (94.09)	13 (96.66)	na
10	67 (82.78)	32 (91.78)	12(96.92)	na	na
20	20 (94.86)	15 (96.15)	na	na	na
30	8 (97.95)	na	na	na	na

* = Each value is average of three determinations
a = Each figure in parenthesis include the % Inactivation of PPO activity na = Not detected

Table 3: Effect of storage conditions on residual PPO activity of raisins

Storage period (days)	PPO enzyme activity (Units/min/ml) A		B		C		D	
	RT	LT	RT	LT	RT	LT	RT	LT
0	74	74	45	45	62	63	49	49
10	70	74	43	45	79	63	45	48
20	67	73	41	44	59	62	41	48
30	65	73	40	43	57	61	38	46
40	62	71	37	42	53	61	33	43
50	60	71	33	42	52	60	30	42
60	58	70	31	40	51	59	28	41
S.E. ±	2.95		2.44		6.04		3.33	
C.D. (P = 0.05)	7.24		5.98		14.79		8.17	

A = Laboratory sample B = Akola sample
C = Nasik sample D = Tasgaon sample
RT = Room temperature storage LT = Low temperature storage

colour and external appearance of raisin.

REFERENCES

Cash, J.N., Sistrunk, W.A. and Stutte, C.A. (1976). Characteristics of concord grape polyphenoloxidase involved juice colour less. *J. Fd. Sci.*, **41**: 1398 – 1402.

Dandwate, M. (1996). "Studies on Polyphenol oxidase profile of grapes and raisin." M. Tech. Thesis, Marathwada Agricultural University, Parbhani (M.S.).

Deoreyappa, I.N. and Gowda (1998). Advances in improvement of raisin quality. *Indian Fd. Ind.*, **17**(4): 218 – 223.

Dimick, K.P., Ponting, J.D. and Makower, B. (1951). Heat inactivation of polyphenolase in fruits purees. *Food Technol.*, **5** : 237

Kimberly, W., Wissemann, W. and Lee, C.Y. (1981). Characterization of polyphenol oxidase from ravat 51 and Niagara grape. *J. Fd. Sci.*, **46**: 506-508 and 514.

Lee, C.Y. and Smith, N.L. (1979). Blanching effect on polyphenol oxidase activity in table beets. *J. Fd. Sci.*, **44**(1): 82 -83 & 86.

Mathew, A.G. and Parpia, H.A.B. (1971). Food browning as a polyphenol reaction. *Adv. Food Res.*, **19**: 75-141.

McEvily, A.J., Iyengar, R. and Gross, A.T. (1992). Inhibition of polyphenol oxidase by phenolic compounds. *American. Chem. Soc.*, : 318-325.

Miller, A.R., Kelley, T.J. and Mujer, C.V. (1990). Anodic peroxidase isoenzymes and polyphenol oxidase activity from cucumber fruit, tissue and substrate specificity. *Phytochem.*, **29**:705 -709.

Olusola, Lamikanra, Kirby Sharon, D. and Musingo Mitby, N. (1992). Muscadine grape polyphenoloxidase: Partial purification by high pressure liquid chromatography and some properties. *J. Fd. Sci.*, **57**: 686 - 689

Orak, H. (2006). Total antioxidant activities, phenolics, anthocyanins, polyphenoloxidase activities and its correlation of some important red wine grape varieties which are grown in turkey. *Electronic J. Polish Agric. Univ.*, **9**(1):18.

Panse, V.G. and Sukhatme, P.V. (1989). "*Statistical Methods For Agricultural Workers*" Publication and Information division, Indian Council of Agricultural Research, New Delhi.

Park, Y.K., Sato, H.N., Almeida, T.D. and Moretti, R.H. (1980). Polyphenol oxidase of mango (*Mangifera indica) J. Fd. Sci.*, **45**: 1619 – 1621.

Rathjen, A. and Robinson, S.P. (1992). Aberrant processing of polyphenol oxidase in a variegated grapavine mutant. *Plant Physiol.*, **19**: 43-54.

Revilla, E. And Ryan, M. (2000). Analysis of several phenolic compounds with potential antioxidant properties in grape extracts and wines by high-performance liquid chromatography photodiode array detection without sample preparation. *J. Chromatogr. A*, **881**: 461-469.

Reyes, P. and Luh, B.S. (1960). Characteristics of browning enzyme in Fay Elberta freestone peaches. *Food Technol.*, **14**: 570.

Valero E., Ramon, V. and Garmona, F. (1988). Characterization of polyphenol oxidase from airen grapes. *J. Fd. Sci.*, **53**: 1482 – 1485.

Vamos Vigyazo, L. (1981). Polyphenol oxidase and peroxidase in fruits and vegetables. *Crits. Rev. Fd. Sci. Nutri*., **15**: 49.

Yoruk, R., Marshall, M.R. (2003). Physicochemical properties and function of plant polyphenol oxidase: A review. *J. Food Biochem.*, **27**: 361-422.

Zawistowski, J., Blank, G. and Murray, E.D. (1986). Polyphenol Oxidase activity in jeruselem artichokes (*Helianthus tuberosus* L.). *Canadian Inst. Fd. Sci. Tech. J*., **19**: 210 - 214.

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