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Effect of heat treatments on polyphenol oxidase (Ppo) activity of custard apple (*Annona squamosa* L.) pulp

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Abstract
Custard apple (Annona squamosa L.) fruit pulp has got many food applications as flavour enhancing ingredient because of its delicious taste and flavour. However, the pulp has limited shelf life due to polyphenol oxidase activity which causes discoloration or browning that result in deterioration of commercial quality of pulp.
Hence, the study on effect of PPO activity by application of different heat treatment combination with pressure and time.
custard apple fruits were harvested at physiological stage of maturity. Fruits were graded, washed and dried under fan and extract pulp by using custard apple deseeding pulp machine. After these pulp treated with different degree of temperature range between 59 to 92^{0}C and maintain steam pressure between 0.20 to 0.80 (Kg/sq cm) with manage processing period about 1-5 minutes and processed pulp was analyzed for polyphenol oxidase activity. The results showed that as processing temperature and pressure goes on increasing the rate of polyphenol oxidase activity inhibition continuously. The heat treatment was given to fruit pulp by steaming, at the range of temperatures with different period of time. The results revealed that steam heating of custard apple pulp facilitated at linearly increasing temperatures exhibit accelerated inhibition of PPO activity leading to 100 % inhibition at 830C temperature for 2to 5 minutes.

Keywords: Polyphenol oxidase (ppo), Custard apple, Pulp extraction, Temperature

Introduction
Custard apple (*Annona squamosa* L.) is one of the finest fruit gifted to India by Tropical America. Custard Apple is also known as Sugar Apple. Custard apples are climacteric and have a very short storage life due to their fast ripening after harvest. The fruit is an excellent source of energy as it high in carbohydrate. The fruit contains vitamins-C and minerals such as calcium, phosphorus potassium.
Custard apple is one of the most delicious and highly perishable fruit. It has its delightful taste, flavor, moderate price in markets and a high nutritional status. Overall the importance of fruits in domestic and export market as a fresh fruits and processed products. Under ordinary condition, fruits can keep well only for 3-4 days after harvest. The physiological changes in fruit occur continuously after harvest. By reducing these changes, the shelf-life of mature fruits can be effectively increased. The cold storage is not feasible for custard apple because at low temperature, the blackening or discolouration of fruits is increased.
The major and foremost constraints of custard apple processing are development of bitterness and change in colour. Heating of pulp beyond 65 ^{o}C impairs pulp flavor considerably and often results in the development of bitterness and unpleasant repulsive off flavor in the pulp. Bitterness precursors are present in the pulp, but upon pectin enzyme treatment of pulp, the clarified pulp is free from bitterness. Preliminary studies on freeze drying the pulp have shown encouraging results.
The visual appearance is the first quality impact that leads the consumer to accept a product. The enzymatic browning reaction catalyzed by polyphenol oxidase (PPO) affects the preservation of pulp. Control of enzymatic browning during processing and storage is important to preserve the sensorial quality of fruit pulp. Till today several methods have been used to facilitate the inhibition of *in vivo* PPO activity in fruits and vegetables. Addition of chemicals, pH adjustment, and exclusion of oxygen (deaeration), refrigeration and thermal treatments are among the most effective methods. The injured tissues of fruit on exposure to air rapidly get darkened, due to the conversion of phenolic compounds to brown melanin, in presence of PPO. The most responsible substrate for this enzyme is diphenols and monophenols. Flavonoids and tannins also act as substrates.

Enzymatic browning is the discoloration that results when monophenolic compounds of plants or shellfish, in the presence of atmospheric oxygen and polyphenol oxydase (PPO), are hydroxylated to o-diphenol, and the later are oxidized to o-quinones. The quinines condense and react non-enzymatically with other phenolic compounds, amino acids, etc., to produce dark brown, black or red pigment of indeterminate structure. However, the pulp has limited shelf life due to polyphenol oxidase activity which causes discoloration or browning that result in deterioration of commercial quality of pulp. Considering all above constraints, custard apples have to be disposed off in local market. Glut in market leads to lower prices. If fruits are preserved in the form of pulp during seasonal glut, it will help in better utilization of custard apple fruits. Hence, it is necessary to study on effect of heat treatments on polyphenol oxidase (ppo) activity of custard apple pulp

Material and Method

A present investigation on "effect of heat treatments on polyphenol oxidase (PPO) activity of custard apple pulp." conducted at Department of Horticulture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani and framed in Complete Randomized design (CRD).

Ripe fruit with firm texture, uniform in size and maturity were used for the experiment. After cleaning the fruits, preliminary trials were conducted to standardize the methods of extraction of pulp for this purpose. The pulp was extracted by using custard apple deseeding pulp machine developed by DR. PDKV, Akola. After these pulp treated with different degree of temperature range between 59 to 92^{0}C and maintain steam pressure between 0.20 to 0.80(Kg/sq cm) with manage processing period about 1-5 minutes. Steam heating of pulp was done for different combinations of time, temperature and pressure, followed by cooling in chilled water and then analyzed for the activity of PPO.

Result and Discussion

The experimental result were obtained by processing particular quantity of custard apple pulp at specific temperature and pressure whereas the time of processing was kept constant for the same trial. The number of trials was carried out for continuously increasing processing time and then processed pulp was analyzed for PPO activity.

The results showed that as processing temperature and pressure goes on increasing the rate of inhibition continuously.

when processing time kept constant at 1 min, the rate of inhibition of PPO is increases linearly with the increase in temperature and pressure (Table 1) from 57.43 to 99.95 per cent. There is no significant change in the rate of inhibition when temperature and pressure goes beyond 83 ^{o}C and 0.55 kg/cm2 and there is no complete inhibition of PPO even at 92 ^{o}C at 0.8 kg/cm2 for 1 min. Secondly there is significant loss of sensorial qualities of the pulp as the temperature and working pressure goes beyond 83 ^{o}C and 0.55 kg/cm2.

Table 1: Effect of processing for 1 minutes at varying time and temperature on PPO activity of custard apple pulp.

Temperature (^{0}c)	Steam pressure (Kg/sq cm)	Initial PPO activity (0.0001OD/min)	Final PPO activity (0.0001OD/min)	% Inhibition
		Mean value	Mean value	Mean value
59	0.20	164.20	69.90	57.43
64	0.25	163.50	49.40	69.79
75	0.40	171.90	51.30	70.16
80	0.50	222.00	44.90	79.77
81	0.51	201.20	35.60	82.31
82	0.53	222.00	9.90	95.54
83	0.55	213.00	1.90	99.11
84	0.56	213.00	1.20	99.44
85	0.60	196.80	0.90	99.54
90	0.70	196.80	0.40	99.80
92	0.80	201.20	0.10	99.95

Table 2: Effect of processing for 2 minutes at varying time and temperature on PPO activity of custard apple pulp

Temperature (^{0}c)	Steam pressure (Kg/sq cm)	Initial PPO activity (0.0001OD/min)	Final PPO activity (0.0001OD/min)	% Inhibition
		Mean value	Mean value	Mean value
59	0.20	164.20	52.60	67.97
64	0.25	163.50	45.50	72.17
75	0.40	171.90	45.10	73.76
80	0.50	222.00	7.90	96.44
81	0.51	196.80	7.30	96.29
82	0.53	196.80	4.30	97.82
83	0.55	201.20	0.00	100.00
84	0.56	201.20	0.00	100.00
85	0.60	222.00	0.00	100.00
90	0.70	213.00	0.00	100.00
92	0.80	213.00	0.00	100.00

Table 3: Effect of processing for 3 minutes at varying time and temperature on PPO activity of custard apple pulp.

Temperature (^{0}c)	Steam pressure (Kg/sq cm)	Initial PPO activity (0.0001OD/min)	Final PPO activity (0.0001OD/min)	% Inhibition
		Mean value	Mean value	Mean value
59	0.20	164.20	41.30	67.97

64	0.25	163.50	29.90	72.17
75	0.40	171.90	16.20	73.76
80	0.50	222.00	5.10	96.44
81	0.51	196.80	4.30	96.29
82	0.53	196.80	0.70	97.82
83	0.55	201.20	0.00	100.00
84	0.56	201.20	0.00	100.00
85	0.60	222.00	0.00	100.00
90	0.70	213.00	0.00	100.00
92	0.80	213.00	0.00	100.00

Table 4: Effect of processing for 4 minutes at varying time and temperature on PPO activity of custard apple pulp.

Temperature (^{0}c)	**Steam pressure (Kg/sq cm)**	**Initial PPO activity (0.0001OD/min)**	**Final PPO activity (0.0001OD/min)**	**% Inhibition**
		Mean value	Mean value	Mean value
59	0.20	164.20	52.60	67.97
64	0.25	163.50	45.50	72.17
75	0.40	171.90	45.10	73.76
80	0.50	222.00	7.90	96.44
81	0.51	196.80	7.30	96.29
82	0.53	196.80	4.30	97.82
83	0.55	201.20	0.00	100.00
84	0.56	201.20	0.00	100.00
85	0.60	222.00	0.00	100.00
90	0.70	213.00	0.00	100.00
92	0.80	213.00	0.00	100.00

Table 5: Effect of processing for 5 minutes at varying time and temperature on PPO activity of custard apple pulp

Temperature (^{0}c)	**Steam pressure (Kg/sq cm)**	**Initial PPO activity (0.0001OD/min)**	**Final PPO activity (0.0001OD/min)**	**% Inhibition**
		Mean value	Mean value	Mean value
59	0.20	164.20	40.30	75.46
64	0.25	163.50	6.40	96.09
75	0.40	171.90	3.70	97.85
80	0.50	222.00	1.60	99.28
81	0.51	196.80	0.90	99.58
82	0.53	196.80	0.00	100.00
83	0.55	201.20	0.00	100.00
84	0.56	201.20	0.00	100.00
85	0.60	222.00	0.00	100.00
90	0.70	213.00	0.00	100.00
92	0.80	213.00	0.00	100.00

Data presented in Table 2 showed the processing of the pulp at constant time of 2 min with varying heat treatment and working pressure. It could be stated from the data that 83 ^{o}C temperatures at 0.55 kg/cm2 for 2 min is critical processing condition for the complete inhibition of the PPO of custard apple pulp. The rate of inhibition goes on increasing with increased temperature and pressure from 67.97 to 100 per cent from 59 to 83 ^{o}C and 0.2 to 0.55 kg/cm2 temperature and pressure respectively. If the processing temperature and pressure goes beyond the critical processing conditions the loss of sensorial qualities like taste, flavour, appearance, texture.

When there is increase in processing time beyond 2 min there was no significant change in the rate of inhibition of the PPO of custard apple pulp. Table 3rd, 4th and 5th showed that there is no complete inhibition of PPO at temperatures less than that of 83 ^{o}C and working steam pressure of 0.55 kg/cm2. The complete inhibition of the PPO at 82 ^{o}C and 0.53 kg/cm2 pressure for 5 min processing time but that could lead to more loss of sensorial quality of the pulp.

Simillar observation was found by Vamos-Vigyazo (1981) [3] in fruits and vegetables. Galeazzi MA and Sgarbieri VC (1983) in banana. They found that, in most cases, partial or total destruction of the catalytic activity of PPO could be achieved in fruits and vegetables by a short period of exposure at 70 °C to 90 °C. This same enzyme, extracted from banana, was completely inactivated after 5 min at 95 °C respectively.

The results showed that as processing temperature and pressure goes on increasing the rate of inhibition increases continuously. This statement is supported by Whitaker and Martinez, (1995) [4] he reported that the enzyme is relatively heat liable. Heat inactivation of PPO is feasible by applying temperatures of more than 50 ^{0}C but may produce undesirable colours, flavours and undesirable change in texture. Heat treatment of more than 60 ^{0}C for 3 min sometimes used to heat treat red grapes to inactivate the PPO before vinification. Shows that when processing time kept constant at 1 min, the rate of inhibition of PPO is increases linearly with the increase in temperature and pressure from 57.43 to 99.95 per cent. There is no significant change in the rate of inhibition when temperature and pressure goes beyond 83 ^{0}C and 0.55 kg/cm2 and there is no complete inhibition of PPO even at 92 ^{0}C at 0.8 kg/cm2 for 1 min. Secondly there is significant loss of sensorial qualities of the pulp as the temperature and working pressure goes beyond 83 ^{0}C and 0.55 kg/cm2.

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