FOOD SCIENCE RESEARCH JOURNAL;
Volume 1 Issue 2
(October, 2010) Page : 137-141

RESEARCH ARTICLE

Accepted : *July, 2010*

Studies on inactivation profile of polyphenol oxidase (PPO) from custard apple (*Annona squamosa* L.) pulp by heat treatment and its effects of sensorial quality

V.N. PAWAR, W.G. KARDILE AND SYED IMRAN HASHMI

ABSTRACT

Custard apple (*Annona squamosa* L.) fruit pulp has got many food applications as flavour enhancing ingredient in various desserts 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. Browning is one of the major constraints responsible of underutilization of this highly valuable dry land fruit. In present investigation, efforts were made to study the heat inactivation profile of polyphenol oxidase in custard apple pulp which was further correlated with changes in sensorial quality (browning and discoloration). Efforts were also made to analyze the effect of ascorbic acid addition on discoloration and organoleptic characteristics of pulp. The heat treatment was given to fruit pulp by steaming, at the range of temperatures with different periods of time. The results revealed that steam heating of custard apple pulp facilitated at linearly increasing temperatures exhibited accelerated inhibition of PPO activity leading to 100 % inhibition at 83°C temperature for 2 minutes while complete inhibition of PPO activity was also observed at the temperature of 82°C for 5 minutes. However, heat treatment resulted in decrease in consumer acceptability of pulp in terms of sensorial characteristics. Addition of 2000 ppm of ascorbic acid without heat treatment showed highest sensorial properties without discoloration compared to heat treated samples.

Key words : Custard apple, Heat treatment, Fruit pulp.

INTRODUCTION

The custard apple (*Annona squamosa* L.) fruit is mostly used as a dessert for its delicious taste and nutritive values. The custard apple pulp has been reported to contain 73.5 per cent moisture, 23.9 per cent carbohydrates, 1.6 per cent proteins, 0.3 per cent fat with calcium, phosphorus and iron to the level of 0.02, 0.04 and 0.01 per cent, respectively. It is also a good source of vitamin A and C (Gopalan *et al*., 1991). The fruit yields about 40 per cent pulp having 26.4 Brix (TSS), 5.5 pH and 0.5 per cent tannins. Skin of fruits is high in phenols and causes rapid browning and strong off flavour during storage and processing of pulp. The processed products and byproducts of custard apple are nutritionally important. Fruit pulp of custard apple is of pleasant taste, texture and flavour. It is sweet and slightly acidic.

The period of availability of the custard apple fruits commences in August and continues up to December notifying October and November as peak period. Climatic and highly perishable fruits of custard apple can not be stored for longer period. Cold storage is not promising as the fruits are chilling sensitive at 15.5^{0}C or below. The ripe fruits can be stored for 6 weeks at 4.4^{0}C but the skin becomes brown, black and unattractive and result in lose of market value. Moreover, if fruit is allowed to remain on the tree for prolonged period, the pericarp splits to open facilitating deterioration.

Custard apple fruit pulp can be used in preparation of various value added food products like ice cream, etc. (Bray, 1981). However, the rapid discoloration of fruit during processing and storage is one of the major constraints in utilization of this valuable fruit (Venkatasubbaiah and Mathew, 1970). Due to which custard apples have to be disposed off in local market while glut in market leads to lower prices. If fruits pulp is to be in the form of pulp during seasonal glut without any

Pawar, V.N., Kardile, W.G. and Imran Hashmi, Syed (2010). Studies on inactivation profile of polyphenol oxidase (PPO) from custard apple (*Annona squamosa* L.) pulp by heat treatment and its effects of sensorial quality, *Food Sci. Res. J.*, **1** (2) : 137-141.

discoloration, it will help in better utilization of custard apple pulp. The browning or discoloration of fruit pulp is majorly due to activity of polyphenol oxidase (PPO) enzyme which can be inactivated by heat treatment (Cayvela *et al.*, 1988). While during heat treatment, it is also necessary to monitor the changes in organoleptic properties which ultimately are responsible for commercial value of fruit pulp. It finds worthwhile to monitor PPO inactivation and delaying of discoloration in pulp, Hence, in present investigation efforts were made to study the PPO inhibition profile in custard apple pulp with its effects of organoleptic characteristics of the pulp.

MATERIALS AND METHODS

Fruits:

Custard apple fruit were obtained from Marathwada Agricultural University Orchard, Parbhani (MS) india. The critical stage of harvesting was judged from the gap and colour between aerols on the surface. Fruits were graded and controlled ripening of fruit was carried out by keeping at the temperature of 27^{0}C for 2 days at 85 per cent relative humidity.

Chemicals and processing equipments:

Most of the chemicals used in this investigation were of analytical grade. The equipments were obtained from the Department of Animal Products Technology, College of Food Technology, M.A.U., Parbhani

Extraction of pulp and treatments:

Ripe fruits 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 the following methods:

Cold hand press after heat treatment (T_1):

Known quantities of custard apple fruits were taken. The fruits were opened into two halves and scooped by using spoon to separate skin. The seeds were separated by pressing each sack by hand. The seeds and pulp were obtained separately. The weight of seeds, pulp and skin were recorded.

Steaming of fruit pulp at range of temperatures for different period of time
↓
Seed separation (manually)
↓
Addition of citric acid to maintain acidity (0.5%)
↓
Packaging of pulp in HDPE pouches

Cold hand press before heat treatment (T_2):

Known quantities of custard apple fruits were taken. The fruits were opened into two halves and scooped by using spoon to separate skin. The mass obtained was allowed to heat at specific temperature and pressure under steam. The mass then transferred to cooling trays and seeds were separated by pressing each sack by hand. The seeds and pulp were obtained separately. The weight of seeds, pulp and skin were recorded.

Seed separation (manually)
↓
Steaming of fruit pulp at range of temperatures for different period of time
↓
Addition of citric acid to maintain acidity (0.5%)
↓
Packaging of pulp in HDPE pouches

Cold hand press after ascorbic acid without heat treatment (T_3):

Known quantities of custard apple fruits were taken. The fruits were opened into two halves and scooped by using spoon to separate skin. The mass obtained is added with ascorbic acid at the rate 2000 ppm and then followed by packaging in HDPE bags

Seed separation (manually)
↓
Addition of ascorbic acid (2000 ppm)
↓
Addition of citric acid to maintain acidity (0.5%)
↓
Packaging of pulp in HDPE pouches

Storage of pulp:

The pulp packaged in HDPE bags immediately transferred at storage at the temperature of -18^{o}C

Analysis of pulp:

Custard apple pulps processed by above stated methods were analyzed for moisture content, acidity, pH, ascorbic acid, and TSS using standard chemicals and or physical procedure (AOAC, 1995).

Colour measurement:

1 g of sample mixed with 2 ml of ethanol. The content filtered through filter paper. The content then analyzed for absorbance at 420 nm. One colour unit was expressed as 0.1 units of absorbance (Lee and Smith, 1979).

PPO activity measurement:

Preparation of crude PPO extract:

Pulp was homogenized and a 60 g sample was immediately chilled in 120 ml of 0.067 M K_2HPO_4 buffer solution at pH 7.0. The sample and buffer solution were mixed uniformly. The macerate was immediately filtered through Whatman (No.4) paper into an Erlenmeyer flask placed in ice. The filtered extract was used immediately. No brown colour was observed in the extract.

Assay for PPO activity:

PPO activity was determined using a colorimetric method based on the increase in absorbance at 420 nm (Lee and Smith, 1979). A buffer solution 20ml of 0.01 M K_2HPO_4, pH 7.0 was pipetted into a 50 ml conical Earlinmayer flask and 2 ml of the 0.5 M catechol solution added. One ml crude enzyme extract was added, mixed and the portion rapidly transferred into a 1.0 cm path length cuvette and the optical density was measured immediately. Each sample was assayed in duplicate. The instrument was standardized using the same solution without enzyme. One unit of PPO enzyme was defined as the amount of enzyme extract causing a change in absorbance of 0.001/ min in 20 ml buffer solution with 2ml substrate solution added.

Sensory analysis:

The sensory evaluation of cakes was carried out by a 10 member trained panel comprised of postgraduate students and academic staff members of the faculty who had some previous experience in sensory evaluation of fruit and vegetable products. The panel members were requested in measuring the terms identifying sensory characteristics and in use of the score. Judgments were made through rating products on a 9 point Hedonic scale with corresponding descriptive terms ranging from 9 'like extremely' to 1 'dislike extremely' (Amerine *et al.*, 1965).

RESULTS AND DISCUSSION

The results obtained from the present investigation are presented below :

Physical characterization of custard apple fruits:

The physical characteristics of custard apple fruits used in the present investigation are given in Table 1.

The custard apple fruits were spherical to heart shaped and greenish in colour. The average length and diameter of the fruits were 6.9 cm, 6.70 cm, respectively. The average number of seeds per fruit was 47. The custard apple fruits contained on an average 8.2% seeds, 46.8 % skin or peel and 43.70 % pulp.

Table: 1 : Physical parameters of custard apple fruits

Sr. No.	Particulars	Range	Average
1.	Height (cm)	5.3 to 8.1	6.90
2.	Diameter(cm)	5.1 to 7.6	6.70
3.	Weight (g)	115 to 182	153.00
4.	No of seeds	45 to 55	47.00
5.	Seeds (%)	7.5 to 8.6	8.20
6.	Peel (%)	46 to 48.3	46.80
7.	Pulp (%)	42.3 to 46.3	43.70

Effect of heat treatments on activity of the Polyphenol oxidase (PPO):

The data 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 numbers of trials were 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 increases continuously. Similar results were obtrain for enzyme which shows that enzymes are relatively heat liable (Whitaker and Martinez, 1995). Heat inactivation of PPO is feasible by applying temperatures of more than 50°C but may produce undesirable colours, flavours and undesirable change in texture. Heat treatment of more than 60°C for 3 min sometimes used to heat treat red grapes to inactivate the PPO before vinification.

The data presented in Table 2 reveal that when processing time kept constant at 1 min, the rate of inhibition of PPO increased linearly with the increase in temperature and pressure from 57.43 to 99.95 per cent. There was not significant change in the rate of inhibition when temperature and pressure went beyond 83°C and 0.55 kg/cm^2 and there was no complete inhibition of PPO even at 92°C at 0.8 kg/cm^2 for 1 min. Secondly there was significant loss of sensorial qualities of the pulp as the temperature and working pressure went beyond 83°C and 0.55 kg/cm^2.

Table 2 also shows 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°C temperature at 0.55 kg/cm^2 for 2 min was the critical processing condition for the complete inhibition of the PPO of custard apple pulp. The rate of inhibition went on increasing with increased temperature and pressure from 67.97 to 100 per cent from 59 to 83°C and 0.2 to 0.55 kg/ cm^2 temperature and pressure, respectively. If the processing temperature and pressure go beyond the critical processing conditions, there is the loss of sensorial qualities like taste, flavour, appearance, texture.

Table 2 : Effect of processing at varying time, temperature and pressure on PPO activity of Custard apple pulp

Sr. No.	Temperature (^oC)	Steam pressure (kg/sq cm)	% Inhibition (1 min)	% Inhibition (2 min)	% Inhibition (3 min)	% Inhibition (4min)	% Inhibition (5 min)
1.	59	0.20	57.43	67.97	74.85	74.85	75.46
2.	64	0.25	69.79	72.17	81.71	94.37	96.09
3.	75	0.40	70.16	73.76	90.58	96.92	97.85
4.	80	0.50	79.77	96.44	97.70	98.60	99.28
5.	81	0.51	82.31	96.29	97.98	99.11	99.58
6.	82	0.53	95.54	97.82	99.64	99.80	100.00
7.	83	0.55	99.11	100.00	100.00	100.00	100.00
8.	84	0.56	99.44	100.00	100.00	100.00	100.00
9.	85	0.60	99.54	100.00	100.00	100.00	100.00
10.	90	0.70	99.80	100.00	100.00	100.00	100.00
11.	92	0.80	99.95	100.00	100.00	100.00	100.00

When there was increase in processing time beyond 2 min there was no significant change in the rate of inhibition of the PPO of custard apple pulp. It has been observed that there was no complete inhibition of PPO at temperatures less than that of 83°C and working steam pressure of 0.55 kg/cm^2. It has been also observed that there was complete inhibition of the PPO at 82° C and 0.53 kg/cm^2 pressure for 5 min processing time but that could lead to more loss of sensorial properties of the pulp.

Chemical characterization of the fresh custard apple pulp with respect to different treatments:

The chemical composition of the finished custard apple pulp for various treatments reveled that there was complete inhibition of the PPO activity in each treatment. Increase was in density of colour gradually from treatment T_1 to T_3, This might be due to the action of PPO during the time of seed separation which is omitted in the T_1 treatment.

There is no remarkable difference in the titratable acidity, pH, TSS and Moisture content of the pulp for different treatments. Ascorbic acid content was found to increased in T_3 this is due to the addition of the ascorbic acid as antioxidant to monitor the activity of the PPO.

Sensorial characterization of the fresh custard apple pulp with respect to different treatments:

The data representing the effect of different treatments on sensorial quality of the custard apple pulp are presented in Table 4. The pulp of custard apple subjected to treatments T_1, T_2 and T_3, respectively and evaluated for sensorial properties by a trained pannel. The scores for different sensorial characters were given on a 9 point hedonic scale and mean values are presented.

The average score for appearance of custard apple pulp exposed to different treatments ranged from 7.25 to 8.20; whereas the minimum score 7.25 observed in treatment T2 and may be due to the lag period between extraction of the pulp and processing. The scores for colour, flavour, taste, texture and overall acceptability were observed 7.3 to 8.0, 6.0 to 7.25, 6.0 to 7.75, 6.25 to 7.0 6.30 to 7.50 respectively. The maximum score for each sensorial quality were observed in T3 treatment in which heating was omitted and the activity of PPO is monitored by using ascorbic acid as antioxidant. The superiority of the quality was might be due to the absence of the heat application. Since due to the application of heat there might be change in the chemical composition of the pulp viz. denaturation of protein, evaporation of volatile constituents,

Table 3 : Chemical composition of finished product

Sr. No.	Respective changes	T_1	T_2	T_3
1.	PPO activity (0.001 Change in OD/min at 420nm)	Nil	Nil	Nil
2.	Colour (OD at 440 nm)	0.082	0.089	0.119
3.	Acidity (%)	0.532	0.512	0.525
4.	pH	4.150	4.490	4.220
5.	Ascorbic acid (ppm)	35.640	37.400	2013.000
6.	Degree Brix (%TSS)	26.200	25.400	27.000
7.	Moisture content (%)	72.180	72.370	72.030

Table 4 : Sensory analysis of finished product

Sr. No.	Respective changes	T_1	T_2	T3
1.	Appearance	7.75	7.25	8.20
2.	Colour	7.75	7.30	8.00
3.	Flavour	6.25	6.00	7.25
4.	Taste	6.30	6.00	6.75
5.	Texture	6.25	6.25	7.00
6.	Overall acceptability	6.50	6.30	7.50

* Each value is average of 10 determinations

gelatinization of the starchy materials etc. that may lead to the changed sensorial characteristics of the pulp like increased viscosity, loss of flavour and taste, change in colour etc.

In case of treatment T_1 and T_2 it has been observed that there was development of bitter taste after application of heat. The bitterness developed was temporary since after chilling the pulp there was no bitter taste observed.

Conclusion:

– Polyphenol oxidase activity in custard apple pulp can be completely inhibited by heating the pulp at the temperature of 83^0C for the period of 2 minutes or by heating the pulp at the temperature of 82^0C for the period of 5 minutes. However, complete inhibition of PPO at 83^0C for 2 minutes results in less deterioration of sensorial quality compared to heating at 82^0C for 3 minutes.

– Seed separation after heat treatment resulted in better sensorial quality of pulo compared to seed separation before heat treatment.

– The discoloration of custard apple pulp can also be prevented with addition of 2000 ppm of ascorbic acid without heat treatment which resulted in maximum score for sensorial quality.

REFERENCES

A.O.A.C. (1995). *Official methods of analysis*. Association of Official Analytical Chemist, Wahington, D.C.

Amerine, M.A., Pangborn, R.M., and Rossler, E.A. (1965). *Principles of sensory evaluation of food*. Academic Press, London, 315 pp.

Bray, F. (1981). Use of tropical fruits in ice cream production. *J. Industries Alimentari*., **20** (3) : 171-173.

Cayvela, M.M., De-Medina, L.S., Facus, M.J. and Gil, A. (1988). Cherimoya (*Annona cherimoya* M.). polyphenol oxidase, monopheolase and dihydroxyphenolase activities. *J. Food Sci.*, **53**(4):1991-1996.

Lee, C.Y., and Smith, L.N. (1979). Blanching Effect on Polyphenol Oxidase Activity in table beets. *J. Food Sci.*, **44**(2): 51 -57

Venkatasubbaiah, G. and Mathew, A.G. (1970). Polyphenols of custard apple and their role in pink discoloration. *J. Food Sci. Technol.*, **7**(4):203-205.

Whitaker, J. R. and Martinez, M. W. (1995). The biochemistry and control of enzymatic browning. *Trends in Food Sci. & Technol.*, **6**(6):195-200.

Address for correspondence :
SYED IMRAN HASHMI
Department of Food Trade and Business Management,
College of Food Technology,
Marathwada Agricultural University, PARBHANI (M.S.) INDIA
E.mail : imran.foodresearch@gamil.com

Authors' affiliations :
V.N. PAWAR AND W.G. KARDILE
Department of Food Science and Technology,
College of Food Technology,
Marathwada Agricultural University, PARBHANI (M.S.) INDIA
E.mail : vasantraopawar@rediffmail.com