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EFFECT OF THERMAL AND CHEMICAL PRE-TREATMENTS ON OXIDATIVE ENZYME ACTIVITIES OF BANANA AND APPLE SLICES

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ABSTRACT

In this study, effect of thermal (water and steam blanching) and chemical (adding of ascorbic acid, citric acid and $Na_2S_2O_5$) pre-treatments on oxidative enzyme activities of banana and apple slices was investigated. High degree of inactivation of oxidative enzymes has been achieved in the first 2 min until 5min of water and steam blanching treatment. The best used concentration of ascorbic acid was found to be 2.0%, hence it improved the final acceptability and inhibited PPO activity for various fruit slices, especially banana and apple. Also, the best concentration of CA was 2.0% to inhibit PPO in banana and apple slices. However, it could be noticed that addition of $Na_2S_2O_5$ at 0.2% in the soaking solution took place as the inhibition of PPO increased. Generally, the result showed that utilization of CA, $Na_2S_2O_5$ and steam blanching heated samples did not cause any browning for all banana slices compared with AA, water blanching and untreated samples. The apple slices pretreated with $Na_2S_2O_5$, steam and water caused the highest reduction of oxidative enzyme activities followed by CA, while the pretreated samples with AA and untreated ones showed the lowest reduction of oxidative enzyme activities.

INTRODUCTION

Enzymatic browning is one of the most studied reactions in fruits, vegetables and sea foods. Researchers in the fields of food science, horticulture, plant and postharvest physiology, microbiology, and even insect and crustacean physiology have studied this reaction because of the diversity of its impact in these systems. Prevention of browning in the apple and banana slices is difficult to achieve because of rapidity of the enzymatic oxidation of phenolic substrates (Annese *et al.*, 1997 and Kim *et al.*, 1993). A common approach to prevent the enzymatic browning is the use of antibrowning agents that act primarily on enzymes or reacts with substrates and/or products of enzymatic catalysis, so that the pigment formation is inhibited (McEvily *et al.*, 1992). The prevalent use of sulfites as inhibitors of enzymatic browning in foods has been restricted by the Food and Drug Administration (Anon, 1987) due to allergic reactions produced some times in individuals with respiratory ailments. Some natural agents can be used as effective replacements for sulfur dioxide (SO_2). Pineapple and lemon juices have been reported to prevent discoloration of cut surfaces of fruits and vegetables (Bennion, 1990 and Patricia *et al.*, 1993). Steam blanching is widely used in the industry of fruit and vegetable products. It helps to inactivate the polyphenoloxidase (PPO) activity responsible for flavor

alteration and tissue softening (Ponne *et al.*, 1994). The thermal and chemical pre-treatments primary preservation of fruits is one of the growing food industries in Egypt in the last decade. Local apple and banana have been used in this research. Most recent reviews have concentrated on the action mechanism of some enzymes and particularly on their function. Therefore the present work was designed to study the enzyme activities of the oxidative enzymes under different conditions. Also, the scope of the investigation was concentrated on effect of the conventional thermal (water and steam blanching) and chemicals (ascorbic acid, citric acid and sodium meta-bisulphite) pretreatments on the oxidative enzyme activity of the apple and banana slices.

MATERIALS AND METHODS

Preparation of fruit material:

Banana (*Musa sapientum*) and Apple *(Anna delicious)* samples representing common cultivars were obtained from local food stores and stored briefly at 4°C until needed. One hour prior to use, fruits were removed from the refrigerator and equilibrated to room temperature. Each apple fruit was rinsed with water, sectioned to slices at least 1.0 cm from the skin end (to exclude the effects of bruising), exposing fresh surface. Each Banana fruit was simultaneously peeled and sliced to 0.5 -1.0 cm thick slices, then immediately placed in glass beakers. The selected fruits had a good maturity, color, free from any undesirable odor, free from any spoil part by microorganisms or enzymes or accidents from transporting process and/or premature or have increasing in maturity. Banana and apple slices were subjected to thermal and chemical pretreatments as follows:

Thermal pretreatments:

Banana and apple slices were loaded in aluminum blanching basket and subjected to two different blanching pretreatments for 1, 2 and 5 minutes as follows:

Water blanching at 100°C.

Steam blanching of the slices was carried out in a beaker which was held in a retaining basket using pressure-cooking pan in the range 100°C. After blanching the fruit slices samples were cooled by immersion in cold water, drained and immediately evaluated or subsequent analysis (enzyme activities of PPO, POD and CAT). Each pretreatment was analyzed similarly to the initial control. Control samples were dipped in distilled water (Beveridge and Weintraub, 1995).

Chemical pretreatments:

Banana and apple slices were dipped in different chemical solutions at 60-65°C for 10 minutes. All chemical pretreatments were carried out under atmospheric pressure with different concentrations of ascorbic acid (0.5, 1 or 2%), citric acid (0.5, 1 or 2%) or sodium metabisulphite ($Na_2S_2O_5$) - (0.05, 0.1 or 0.2%).

At the end of each chemical pretreatment, banana and apple samples were drained and immediately evaluated or subsequent analysis

(enzyme activities of polyphenol oxidase (PPO), peroxidase (POD) and catalase (CAT)). Each pretreatment was analyzed similarly to the initial control. Control samples were dipped in distilled water (Ding *et al.*, 2002).

Extraction of different enzymes under investigation:

Extraction of polyphenoloxidase (PPO, E.C. 1.14.18.1.) peroxidase (POD, E.C. 1.11.1.7) and catalase (CAT, E.C. 1.11.1.6) was carried out using the method described by Galeazi *et al.* (1981). Crude enzymes extracts were prepared from the tested samples by extracting with sodium phosphate buffers as follows: 10 g of fresh juices were mixed for 30 sec. with 100 ml of 0.2 M sodium phosphate buffer at pH 7.0, the suspension was centrifuged at 4°C for 15min at 5000 rpm, HERMLE Z 323 K Germane. The enzyme activity remained in the supernatant as a crude of different enzymes.

Assay of polyphenoloxidase (E.C. 1.14.18.1) enzyme activity:

The enzyme activity was assayed according to the method described by Oktay *et al.* (1995). Where, PPO enzyme activity was determined by measuring the increase in absorbance at 420 nm at 25°C with, Spectrophotometer UVD-3500, Labomed, USA. The sample cuvette contained 2.0 ml of 0.1M catechol in sodium phosphate buffer (pH 7.0) with 1.0 ml of the crude enzyme extract. The optical density at 420nm of the mixture was recorded using Spectrophotometer, UVD-3500, Labomed, USA.

Assay of peroxidase (E.C. 1.11.1.7) enzyme activity:

The enzyme activity was assayed according to the method described by Olmos *et al.* (1997). Where, POD enzyme activity was determined by measuring the increase in absorbance at 450 nm at 25°C with, Spectrophotometer UVD-3500, Labomed, USA. To a clean dry cuvette of a spectrophotometer 1.0 ml of crude enzyme extract (from different samples) was added and mixed with 5 ml sodium phosphate buffer solution (pH 7.0), 0.5 ml of 2% O-phenylene diamine, 0.5 ml of 0.3% H2O2 and 1ml redistilled water. The optical density at 450nm of the mixture was recorded using Spectrophotometer, UVD-3500, Labomed, USA.

Assay of catalase (E.C. 1.11.1.6) enzyme activity:

Catalase (CAT) enzyme activity was measured by titrimetric method as described by Aebi (1983).

RESULTS AND DISCUSSION

Effect of heat treatments on different extracts of polyphenol oxidase (PPO), peroxidase (POD) and catalase (CAT) activities of banana and apple slices:

Blanching of slices banana and apple were carried out by two different methods; boiling water at 100°C and steam at atmospheric pressure. Residual activities of PPO, POD and CAT in the blanched banana and apple samples were determined and recorded in Tables (1 and 2).

Table (1): Effect of thermal treatments on oxidative enzymes activity of banana.

Oxidative enzymes	Control (units/mg)	Residual activities (units/mg)					
		Thermal treatments					
		Water blanching (100°C)			Steam blanching (100°C)		
		1 min	2 min	5 min	1 min	2 min	5 min
Polyphenol oxidase (PPO)	3.30	0.440	0.220	0.082	0.700	0.093	0.072
% Inhibition		86.67	93.33	97.52	78.78	97.18	97.81
Peroxidase (POD)	0.45	0.035	0.032	0.027	0.032	0.028	0.026
% Inhibition		92.22	92.88	94.00	92.88	93.77	94.22
Catalase (CAT)	0.63	0.29	0.250	0.21	0.28	0.24	0.20
% Inhibition		53.96	60.31	66.66	55.55	61.40	68.25

Table (2): Effect of thermal treatments on oxidative enzymes of apple.

Oxidative enzyme	Control (units/mg)	Residual activities units/mg . 10^{-2}					
		Thermal treatments					
		Water blanching (100°C)			Steam blanching (100°C)		
		1 min	2 min	5 min	1 min	2 min	5 min
Polyphenol oxidase (PPO)	0.097	0.029	0.027	0.022	0.043	0.036	0.00
% Inhibition		70.10	72.16	77.31	55.67	62.88	100.00
Peroxidase (POD)	0.470	0.120	0.080	0.05	0.19	0.09	0.06
% Inhibition		74.46	82.97	89.36	59.57	80.85	87.23
Catalase (CAT)	0.065	0.041	0.023	0.00	0.041	0.023	0.00
% Inhibition		36.92	64.61	100.00	36.92	64.61	100.00

From these tables, it could be seen that the activity of the oxidative enzymes in the control (un-blanched banana and apple slices samples) was 3.30, 0.45 and 0.63 units/mg) and (0.097, 0.470 and 0.065 units/mg) for PPO, POD and CAT, respectively. A one minute blanching of samples in water at 100°C and steam was not sufficient for inactivation of PPO and POD enzymes, since the residual activities were (0.44 and 0.035 units/mg) and (0.029 and 0.12 units/mg), respectively. CAT enzyme was totally inactivated after 2 and 5 min blanching at 100°C in water and steam respectively. Steam blanching of fruit samples was less effective than boiling in water with regard to enzyme inhibition, as seen in Table (2).

The residual activities of peroxidase (POD) were at all treatments relatively higher than those of PPO which prove the assumption that POD enzyme is the most heat resistant enzyme during blanching treatment.

Blanching of banana and apple slices was carried out in boiling water (100°C) and steam at 90°C for 30 sec. Residual activity of PPO enzyme in the blanched fruit slices were given in Tables (1 and 2)

From these tables, it could be seen that blanching treatment of 5 min in water at 100°C was sufficient to inhibit the PPO enzyme activity to 97.52% in all banana slices.

On the other hand, steam blanching gave the same effect of boiling in water with respect to PPO enzyme inhibition. However, at 5 min steam blanching was sufficient to inhibit the PPO enzyme activity to 97.81% in all banana slices.

From these results, it could be seen that a high degree of inactivation has been achieved in the first 2 min until 5 min of water and steam blanching

treatment. These results are in agreement with those reported by Eissa (1992).

The treatment conditions were drastic and produced a slight over cooking of final product, but this treatment is necessary to inhibit the color changes deterioration of stored fruit slices when the simultaneous addition of chemical preservatives showed to be avoided as indicated by Siddiq *et al.* (1992). It was confirmed that plum PPO of banana and apple seems are relatively heat sensitive when compared to PPO of some other fruit (Mohamed, 1998).

From these results, it could be concluded that the blanching of fruit slices for 5 min in boiling water was sufficient to reduce the activity of PPO enzyme to 97.52% in banana slices. The residual activity achieved after 5 min of steam blanching was 97.81-77.31% in banana and apple slices.

The purpose of chemical treatments was to study the activity and inhibition pattern of some chemical compounds on the oxidative enzymes of banana and apple fruits (Tables 2 and 3). The samples were soaked in different mono-component solutions for a period of 10 min. at 60°C, after which they have been tested for enzyme activity (PPO, POD and CAT). The applied chemical components were: ascorbic acid (AA), citric acid (CA) and sodium metabisulfite ($Na_2S_2O_5$). Therefore, AA (0.5, 1.0 and 2.0%), CA (0.5, 1.0 and 2.0%) and $Na_2S_2O_5$ (0.05, 0.1 and 0.2%) was used as dipping solutions for banana and apple slices fruits.

Effect of different anti-browning agents on the activity of PPO, POD and CAT in banana slices

From these results, it is clear that the effect of ascorbic acid (AA) on iron enzymes (POD and CAT) was more pronounced than its effect on copper containing enzyme PPO. Dipping in 2% AA solution reduced the POD and CAT activities to 55.20-75.0% of their original activity, while the corresponding value for PPO was 48.87%. It could be also observed, that AA was non effective inhibitor for PPO enzyme which is the most critical enzyme in banana and apple processing because it's browning effect.

The use of citric acid (CA) was effective in the inhibition of the three tested oxidative enzymes to an acceptable level for banana and apple processing. Increasing the concentration of CA in the dipping solution from 0.5 to 2% reduced the residual activity of POD from 14.58% to 44.79%. The corresponding values for CAT were 26.19 to 73.81%. Inhibition of PPO was not to much high as POD and CAT, while the increase in citric acid concentration from 0.5 to 2% reduced the PPO activity to 40.47% (Table, 3).

The most effective treatment for the inhibition of oxidative enzymes in banana and apple fruits was dipping in solutions containing sodium metabisulfite ($Na_2S_2O_5$). Application of 0.2% solution of sodium metabisulfite ($Na_2S_2O_5$) reduced the enzyme activity to an acceptable level for processing. At this level of concentration, 87.50% of POD, 96.74 of PPO and 100% of CAT activities were inhibited after a 10 min. of dipping treatment.

Effect of different anti-browning agents on the activity of PPO, POD and CAT in Egyptian apple slices:-

The slices of apple were soaked in different solutions for a period of 10 min at room temperature. The applied chemical compounds were ascorbic acid (AA), citric acid (CA) and sodium metabisulfite ($Na_2S_2O_5$).

Effect of dipping apple slices in ascorbic acid (AA):-

For this purpose apple fruit slices were dipped in solution containing different concentrations (0.5, 1.0 and 2.0%) of ascorbic acid as a browning inhibitor after each treatment oxidative enzymes activities were assayed and the results were given in Table (3).

Table (3): Effect of chemical treatments on oxidative enzymes activity of banana.

Oxidative enzymes	Control (units/mg)	Residual activities (units/mg)								
		Chemical treatments								
		Citric acid CA			Ascorbic acid AA			Sodium metabisulphite		
		0.5	1.0	2.0	0.5	1.0	2.0	0.05	0.1	0.2
Polyphenol oxidase (PPO)	0.892	0.662	0.574	0.531	0.623	0.546	0.453	0.577	0.066	0.029
% inhibition		25.78	35.65	40.47	30.15	38.78	48.87	35.31	92.60	96.74
Peroxidase (POD)	0.096	0.082	0.074	0.053	0.074	0.054	0.043	0.047	0.023	0.012
% inhibition		14.58	22.91	44.79	22.91	43.75	55.20	51.04	76.04	87.50
Catalase (CAT)	0.089	0.062	0.042	0.022	0.059	0.041	0.021	0.021	0.012	0.000
% inhibition		26.19	50.00	73.81	29.76	51.19	75.00	75.00	85.71	100.00

Table (4): Effect of chemical treatment s on oxidative enzymes activity of apple.

Oxidative enzyme	Control (units/mg)	Residual activities (units/mg)								
		Chemical treatments								
		Citric acid CA			Ascorbic acid AA			Sodium metabisulphite		
		0.5	1.0	2.0	0.5	1.0	2.0	0.05	0.1	0.2
Polyphenol oxidase (PPO)	0.156	0.067	0.056	0.039	0.065	0.054	0.038	0.087	0.061	0.022
% inhibition		57.05	64.10	75.00	58.33	65.38	75.64	44.23	60.89	87.17
Peroxidase (POD)	0.523	0.404	0.341	0.232	0.109	0.094	0.090	0.197	0.025	0.014
% inhibition		22.75	34.79	55.64	79.15	82.02	82.79	62.33	95.21	97.32
Catalase (CAT)	0.712	0.573	0.346	0.233	0.534	0.334	0.213	0.517	0.325	0.122
% inhibition		19.52	51.40	67.27	25.00	53.08	70.08	27.38	54.35	82.86

The effect of ascorbic acid on polyphenoloxidase activity and the ratio of inhibition browning were studied.

From these results following observation could be considered. The effect of AA on PPO enzyme was more efficient in banana and apple. Dipping in 2.0% AA solution reduced the PPO enzyme activity to 48.87 and 75.64% in banana and apple slices respectively of their original activity. It could be also observed that AA was non-high effective inhibitor for PPO enzyme, which is the most critical enzyme in banana and apple processing, because of its browning effect and penetration of the AA solution to banana and apple tissues. Therefore, it could be concluded that an increase of AA concentration to 2.0% would be of a great benefit for the inactivation of PPO enzyme

activity in fruit slices. These results are in agreement with the results obtained by Nasr (1994) and Mohamed (1998).

Effect of dipping apple slices in citric acid (CA):

For this purpose banana and apple fruit slices were dipped in solution containing different concentration (0.5, 1.0 and 2.0%) of citric acid as a browning inhibitor after each treatment PPO enzyme activity was assayed and the result were given in Tables (3 and 4).

The use of citric acid was effective in the inhibition of the PPO enzyme to an acceptable level for fruit slices processing. Increasing concentration of CA in the dipping solution to 2.0% reduced the residual PPO activity to 40.47 and 75.00% in banana and apple, respectively. It could be also observed that CA was non-high effective inhibitor for PPO enzyme, which is the most critical enzyme in apple and banana.

Therefore, it could be concluded that an increase of CA concentration to 2.0% would be of a great benefit for the inactivation of PPO enzyme activity in fruit slices, especially banana and apple slices. This could be attributed to the inhibition of PPO by soaking and as the percentage inhibition increased the degree of browning decreased.

These result are in agreement with Eissa (1992) and Abd El-Wahab 1999), who published that dipping of the fruit slices in citric acid and sodium chloride solution caused a great inhibition of polyphenoloxidase.

Effect of dipping apple slices in sodium metabisulfite ($Na_2S_2O_5$)

For this purpose banana and apple fruit slices were dipped in solution containing different concentration (0.05, 0.1 and 0.2%) of sodium metabisulfite as a browning inhibitor after each treatment PPO enzyme activity was assayed and the result were given in Tables (3 and 4).

The most effective treatment for the inhibition of PPO enzyme in fruit slices was dipping in solution containing sodium metabisulfite. Application of a 0.2% solution of sodium metabisulfite reduced the enzyme activity to an acceptable level for processing. At this level of concentration 96.74 and 87.17% of PPO activity was inhibited in banana and apple slices, respectively. Therefore, it could be concluded that an increase of $Na_2S_2O_5$ concentration to 0.2% would be of a great benefit for the inactivation of PO enzyme activity in fruit slices, especially banana slice.

These results are in agreement with the results obtained by Siddiq *et al.* (1992), Nasr (1994) and Mohamed (1998). They confirmed that increasing concentration of $Na_2S_2O_5$ and dipping time enhanced the inactivation of banana, papaya and pear slices.

From the previous results, it could be concluded that the best concentration of ascorbic acid (AA) was 2.0% to improve final acceptability and to make inhibition of PPO activity in various fruit slices, especially for banana and apple. Also, the best concentration of CA was 2.0% to inhibit PPO in banana and apple. However, it could be noticed that addition of $Na_2S_2O_5$ at 0.2% in the soaking solution took place as the inhibition of PPO increased. The results indicated that the blanching of fruit slices for 5 min in boiling water was sufficient to reduce the activity of PPO enzyme to 100% in all fruit slices. The residual activity achieved after 5 min of steam blanching was 100% in banana and apple slices.

CONCLUSIONS

There are numerous compounds capable of reducing the enzymatic browning; therefore the use of thermal and chemical pre-treatments is still stimulated to meet the demands for production of healthy fruit products having high quality. Therefore, studying and evaluating the efficiency of thermal and chemical pre-treatments to inhibit the enzymatic browning (PPO, POD and CAT) in both banana and apple slices were carried out. Also, the most fruits that have high ratio of the enzymatic browning (PPO, POD and CAT) should be treated with safety thermal and chemical pre-treatments to inhibit the enzymes without any efficient on sensory properties in fruits for consumer.

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تأثير المعاملات الحرارية و الكيميائية المبدئية على نشاط الانزيمات المؤكسدة فى شرائح الموز و التفاح

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تم فى هذه الدراسة معرفة تأثير المعاملات الحرارية (مثل السلق بالبخار أو الماء) و الكيميائية (مثل اضافة حمض الاسكوربيك أو حمض الستريك أو الصوديوم ميتا باى سلفيت) على نشاط الانزيمات المؤكسة فى شرائح الموز و التفاح حيث أوضحت النتائج ان درجة التثبيط العالية للثلاث انزيمات المؤكسدة (PPO, POD and CAT) كانت لمدة٢-٥ دقائق بمعاملة السلق بالبخار أو بالماء. و كان أفضل المعاملات هو استخدام تركيز ٢% من حمض الاسكوربيك أو ٢% من حمض الستريك فى شرائح الفاكهة خاصة الموز و التفاح وكما لوحظ ان اضافة الصوديوم ميتا باى سلفيت بتركيز ٠,٢% تعمل على زيادة تثبيط انزيم البولى فينول اوكسيديز فى شرائح الموز و التفاح. هذا و قد ثبت ان العينات المعاملة بالصوديوم ميتا باى سلفيت و حمض الستريك و السلق بالبخار لا تعطى اى تلون انزيمى بنى مقارنة بالعينات المعاملة بحمض الاسكوربيك و السلق بالماء و العينات الغير معاملة فى شرائح الموزو بينما فى شرائح التفاح المعاملة بالصوديوم ميتا باى سلفيت و السلق بالماء أدت الى أعلى تثبيط للانزيمات المؤكسدة الثلاثة تلاها المعاملة بحمض الستريك بينما المعاملة بحمض الاسكوربيك و العينات الغير معاملة كانت أقل تثبيطا للانزيمات المؤكسدة الثلاثة.