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Research Paper

INFLUENCE OF INVERTASE AND PEROXIDASE ACTIVITY ON FRUIT QUALITY UNDER FRESH AND REFRIGERATED CONDITIONS

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Abstract

An experiment was conducted to study the impact of refrigeration on some common fruits (Malus sylvestris, L., Psidium gujava, L., Citrus aurantium, L., Punica granatum, L., Achras sapota, L.,) under 20°C after 20 days. The present investigation was aimed to study the enzyme activity (invertase and peroxidase), of some fresh and refrigerated fruits. Among the selected fruits, the invertase activity was found to be high in refrigerated apple (Malus sylvestris, L.,) (1800µg/glucose released/h/g) which is responsible for breakdown of carbohydrate, it leads to accumulation of more amount of glucose. Among the selected fruits under two different conditions (fresh and refrigerated), a significant change was noticed in fresh sapota (Achras sapota, L.,) which exhibited a highly remarkable peroxidase activity (306 od units/h/g fresh tissue). As an antioxidant enzyme it confers resistance against pathogens. Exposure of fruits at low temperature (20ºC), exhibited a drastic increase in invertase activity and decrease in the peroxidase activity, that affects the nutrient and physical nature of the fruits.

Key Words: Antioxidant, invertase, peroxidase, fresh fruits & refrigerated fruits

Introduction

Refrigeration or cold storage has relatively few adverse effects on taste, texture, nutritive value and permits exchange of flavours between fruits and other foods, which affects the other attributes of fruits. Another common change in fruit during refrigerated storage involves loss of firmness and crispness. The fruits to be stored must be stabilized against enzymatic changes during frozen condition. The principal enzymatic changes that are objectionable in the case of refrigeration are oxidation, which cause darkening of colour and alternations in flavour. An important colour change is enzymatic browning of lighter coloured fruits such as apples, peaches and bananas. This is due to oxidation of pigment precursors, often referred to as catechol – tannin substrates, by enzymes of the group known as phenol oxidases and polyphenol oxidases. Refrigeration preserves perishable fruits for days or weeks. Too low temperature can cause damage called “chill injury” to fruits when those are not physically damaged by freezing. In case of bananas and tomatoes, at storage temperatures of below 13°C can slow down the activities of natural ripening enzymes and results in poor colours (Norman and Joseph, 1996).Invertase which occur in higher plant tissues are mostly

extracellular or in soluble form. Invertases have been detected, isolated and characterized from several higher plant tissues that are engaged in growth, development and sugar storage. In plant tissues, invertases are usually classified as acid, neutral or alkaline depending on the basis of the pH range required for their maximum activity (Dahot and Hanif, 1996). They are also classified as soluble and bound invertases on the basis of their intracellular localization. This enzyme hydrolyses sucrose and related sugars, thereby providing hexoses which can be utilized for the energy and carbon requirements of the cell (Habibur Rahman et al., 2001). The role peroxidases play in the living plant tissues is not completely understood, although they have been associated with cell wall synthesis, response to injury, disease resistance and wound repair. They normally increase in activity and number during ripening. Peroxidases consist of a family of isozymes (Shanon et al., 1966) that catalyse the same or similar functions. All of these enzymes contain identical heme groups but differ in the precise composition of glycoprotein (Gasper et al., 1982). Commercially fruits are subjected to several storage conditions by the use of chemicals or exposure to extreme low temperature (cold storage) before it reaches the public and also, fruits are allowed for house hold storage in refrigerator before consumption. This storage practice is responsible for degradation of nutrient quality in fruits. So, an experiment was designed to study the impact of refrigeration of some common edible fruits (Malus sylvestris, L., Psidium gujava, L., Citrus aurantium, L., Punica granatum, L., Achras sapota, L.,) under 20°C for 20 days on the enzyme activity (invertase and peroxidase).

Materials and methods

Samples and sample preparation

Fruits selected for the study were, Malus sylvestris L. (apple) of Rosaceae, Psidium gujava L. (guava) of the family Myrtaceae, Citrus aurantium L. (orange) of the family Rutaceae, Punica granatum L. (pomegranate) of Punicaceae, Achras sapota L. (sapota) of Sapotaceae.Fresh fruits mentioned above were purchased from the local market and were analyzed for the invertase and peroxidase activity at two different conditions. One set of each fruit was wrapped in polythene bags and left in the refrigerator for 20 days at 20°C. After 20 days the refrigerated fruits were biochemically examined for both the activities.

Invertase activity (Pressey, 1966)

Invertase is an enzyme which hydrolyses the disaccharide sucrose into two monosaccharides such as glucose & fructose.

Assay

To 1ml of 0.1 M sucrose, 1ml of reaction buffer & 1ml of enzyme extracts were added and incubated at 37°C for an hour and then Nelson's alkaline copper reagent was added to stop the reaction. The reaction mixture was boiled for 10 minutes, cooled and mixed with 1ml of arsenomolybdate reagent and made up to 10ml with distilled water. The absorbance of blue colour thus formed was read at 500nm in Spectronic 20- D. The amount of reducing sugars formed was calculated from the reference curve prepared by heating various concentration of glucose with copper reagent followed by the addition of arsenomolybdate reagent.

Calculation

Enzyme activity was expressed in terms of microgram glucose released/minutes/gram tissue**.**

Peroxidase (pod) activity (Malik and Singh, 1980)

Principle

Pyrogallol is used as substrate for the assay of peroxidase.

Pyrogallol + H_2O_2 → oxidized pyrogallol + 2 H_2O

The amount of oxidized pyrogallol formed during the reaction can be measured in a spectrophotometer.

Procedure

Extraction

500 mg fresh fruit tissue was ground with 5ml phosphate buffer (pH 7) and was filtered. The filtrate was centrifuged at 2000rpm at 4°C for 5 minutes in a pre-chilled condition. The supernatant was the enzyme source.

Assay

To 1ml of enzyme extract, 2ml of 0.05M pyrogallol and 1ml of 0.1M H_2O_2 were added, mixed and immediately inserted in to the cuvette of the colorimeter. Readings were taken for 60 seconds at 420nm setting the first reading as zero.

Calculation

The peroxidase activity was expressed as OD $unit^{-1}minute^{-1}gram$ tissue.

Results and discussion

Invertase activity

Invertase is an enzyme that splits sucrose into glucose and fructose. With the progressive increase in the activity of invertase, a parallel decrease in the sucrose concentration takes place. On the whole fresh fruits revealed a reduced invertase activity which is good for health. Among the five fresh fruits selected, Citrus aurantium L. and Achras sapota L. showed an equal activity of 798 µg glucose released/h/g, followed by Punica granatum L. (780 µg glucose released/h/g). The minimum activity of invertase was found in Malus sylvestris L. and Psidium gujava L. with 444 µg glucose released/h/g and 240 µg glucose released/h/g respectively. In refrigerated condition, the enzyme activity was inversely propotional in all the selected fruits, which showed 2 to 3 folds increase than in fresh fruits (fig-I). The maximum invertase activity was observed in Malus sylvestris L. (1800 µg glucose released/h/g) followed by Punica granatum L. (1776µg glucose released/h/g), Achras sapota L. (1620µg glucose released/h/g), Citrus aurantium L. (1512µg glucose released/h/g) and Psidium gujava L. (1464µg glucose released/h/g) under refrigerated condition. From this study, it is evident that, the enzyme invertase plays an important role in fruit maturity. During maturation the enzyme activity is higher and also, it is responsible for large amount of sugar breakdown. Therefore, refrigeration (20°C) enhances the invertase activity and accumulation of higher amount of sugar content. Consumption of such fruits may gradually affect human health and is more harmer for diabetic patients. Similar findings were reported by Albert and John (1983) in Citrus paradisi. Their findings explained that, the enzyme invertase activity reach a maximum towards its maturity and also during cold storage. Dahot and Hanif (1996) studied the properties of invertase in Achras sapota, which showed 6.5% invertase activity at 20°C. Aynul (1998) has also reported that Himsagar mango variety exhibits a higher invertase activity at its maturity. Arnold (1965) inferred that, the soluble invertases of grapes were stable and active under acidic conditions.

Peroxidase activity

The peroxidase activity was measured in terms of OD units/h/gram tissue of the fruit. Among the fresh fruits selected for the study, Achras sapota showed a highly remarkable activity of 306 OD units/h/g fresh tissue. It was many folds higher than all other fruits under investigation. After refrigeration, all the fruits showed a marked decline in the enzyme peroxidase activity. Approximately 2 folds less activity was noticed in Achras sapota L. (169.2 OD units/h/g fresh tissue) during refrigeration than in the fresh condition. In the fresh fruit of Citrus aurantium L. the peroxidase activity was noted to be 68.4 OD units/h/g fresh tissue, which was three-fold higher than refrigerated fruit (21.6 OD units/h/g fresh tissue). Amongst all other fresh fruits,

Malus sylvestris L. showed 30.6 OD units/h/g fresh tissue and in Punica granatum L., 21.6 OD units/h/g fresh tissue was registered. The least amount peroxidase activity was noticed in Psidium gujava L., which showed 18 OD units/h/g fresh tissue. After refrigeration, the enzyme activity was reduced to some extent in Malus sylvestris L., Psidium gujava L. and Punica granatum L. which showed 27 OD units/h/g fresh tissue, 16.2 OD units/h/g fresh tissue and 10.8 OD units/h/g fresh tissue in respective fruits (fig-II). This observation indicates that, the reduction in peroxidase activity could be attributed to cold storage at 20°C for 20 days, which was considerably a long duration that brings about a drastic change in the nutritive compounds of fresh fruits. It is evident that, Achras sapota L. has an intense power of breaking down of the substrate H_2O_2 in our experiment. Hence, consumption of fresh Achras sapota L. fruit might help in detoxification of the H_2O_2 that accumulates in the cell due to metabolism in human beings. Our results find supportive evidences from the report of Jacek Patykowski et al. (2007). They reported that peroxidase is a membrane bound enzyme. High enzyme activity during the experimental period, limited the harmful effect of H_2O_2 and slowed down the senescence of the apple fruit at 4°C storage for 5 months. Hulme (1970) stated that, the fruits with increase in peroxidase activity are not suitable for cold storage. High peroxidase activity in fresh fruits attributes to its superior quality with respect to its detoxifying of H_2O_2 in human system.

Figure- 1

Invertase activity of selected fresh & refrigerated fruits

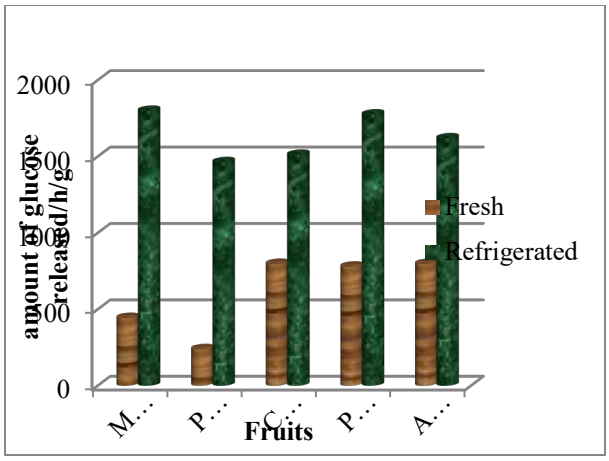

Figure -2

Peroxidase activity of selected fresh & refrigerated fruits

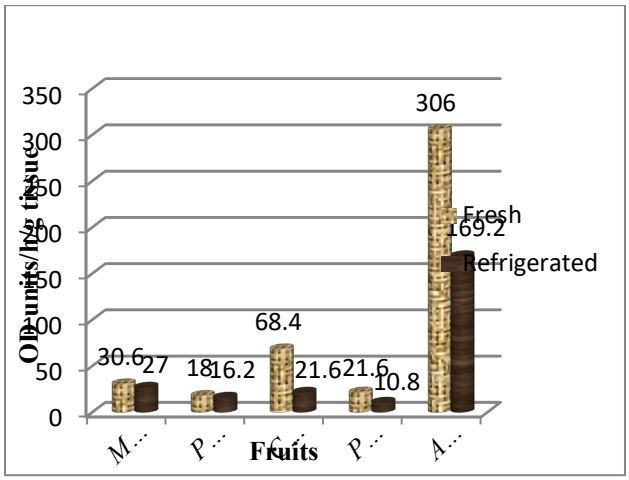

Table I: Invertase & Peroxidase activity of selected fresh & refrigerated fruits

Fruits	Invertase Activity (amount of glucose released/hr/g)		Peroxidase Activity (OD units/hr/g tissue)	
	Fresh	Refrigerated	Fresh	Refrigerated
Malus sylvestris L.	444	1800	30.6	27
Psidium gujava L.	240	1464	18	16.2
Citrus aurantium	798	1512	68.4	21.6

L.				
Punica granatum L.	780	1776	21.6	10.8
Achras sapota L.	798	1620	306	169.2

Conclusion

From this study, it is concluded that the fresh fruits are an excellent source of nutrients. The increase in the activity of the enzyme invertase during refrigeration reduced the complex polymers in to simpler forms and interferes with the shelf- life and in turn the palatability of the fruits. Also, the activity of the enzyme peroxidase was maximum in fresh fruits which are responsible for supreme quality and confer resistance against microbial pathogens. Therefore, it is recommended that the consumption of fresh fruits is good for health and in unavoidable circumstances, the duration of refrigeration could be minimized.

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