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FREE-RADICAL SCAVENGING ACTIVITY OF SOME FRESH AND REFRIGERATED FRUITS

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ABSTRACT

The antioxidant and free-radical scavenging activities of five different fruits (*Malus sylvestris*, L., *Psidium gujava*, L., *Citrus aurantium*, L., *Punica granatum*, L., *Achras sapota*, L.,) were studied to examine the impact of refrigeration. Two sets of selected fruits were examined for fresh and refrigerated conditions under 10˚C for 20 days. In the present investigation, it was found that the antioxidant activity was higher in fresh *Punica granatum*, (61.2 mg/g) and the free-radical scavenging activity was higher in fresh *Citrus aurantium*, (86.6%). Whereas, all the refrigerated fruits exhibited a drastic decrease in the antioxidant and free-radical scavenging activities ranging from 33.9 to 57 mg/g and 33 to 80% respectively. Therefore, our study clearly indicates, low temperature exposure of fresh fruits for longer time may destroy the nutritive compounds, especially antioxidant, a source that neutralize free-radicals damage.

KEYWORDS: Antioxidant activity, free-radical scavenging activity, fresh fruits, refrigerated fruits, nutritive compounds.

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INTRODUCTION

A fruit, when exposed to extreme temperatures (either high or low) can be envisaged that some reactions may be accelerated or retarded to excess and the result will be that the general ripening pattern will be permanently disturbed, by the production of substances which may be toxic[11]. Refrigeration or cold storage has relatively adverse effects on taste, texture, nutritive value and permits exchange of flavours between fruits and other foods stored along with it, which affects the other attributes of fruits. Another common change in fruit during refrigerated storage involves loss of firmness and crispness[18]. An antioxidant is a substance that retards or prevents deterioration, damage or destruction by oxidation[4]. It is a classification of several organic substances, including vitamins C and E, vitamin A (which is converted from β-carotene) [13]. A free radical is a compound with one or more unpaired electrons in its outer orbital[12]. Such unpaired electrons make these species very unstable and therefore quite reactive with other molecules due to the unstable conditions[15]. Also, they pair their electrons and generate a more stable compound. The most dangerous free-radicals are the atomic and molecular varieties of oxygen, which is known as Reactive Oxygen Species (ROS). While ROS are not technically free-radicals, there are highly reactive with the molecules around them[21]. ROS is a collective term, which includes not only the oxygen radicals (O_2 and OH) but also some non radical derivatives of oxygen including hydrogen peroxide (H_2O_2), hyprochlorous acid (HOCl) and ozone (O_3)[22]. Free-radicals are formed continuously as normal byproducts of oxygen metabolism during mitochondrial oxidative phosphorylation. Thus, the mitochondrion is the main source of free-radicals[6, 20].The human body is composed of many different types of cells. Cells are composed of many different types of molecules. Molecules consist of one or more atoms of one or more elements joined by chemical bonds. Normally, bonds don't split in a way that leaves a molecule with an odd, unpaired electron[8]. But when weak bonds split, free-radicals are formed[15]. Free-radicals are very unstable and react quickly with other compounds, trying to capture the needed electron to gain stability. Generally free-radicals attack the nearest stable molecule, "stealing" its electron. When the "attacked" molecule loses its electron, it becomes a free radical itself, beginning a chain reaction and can be "thousand of events long"[7]. Once the process is started, it can cascade, finally resulting in the disruption of a living cell[12].The role of free-radicals in many disease conditions has been well established. Several biochemical reactions in our body generate reactive oxygen species and these are capable of damaging the crucial biomolecules. If they are not effectively scavenged by cellular constituents, they lead to disease condition[9, 10]. Antioxidants can take the form of enzymes in the body vitamin supplements or industrial additives. They are routinely added to metals, oils food stuffs and other materials to prevent free radical damage. To neutralize these free-radicals, antioxidant plays an important role[23].Commercially fruit are preserved to avoid ripening either under low temperature or using any chemicals/hormones. In recent years, there is a practice of storing fruits for longer duration in the refrigerator and made available throughout the year. While doing so, the fruits are exposed to several factors to control their normal metabolism process. Antioxidant activity is one such process that adds to the nutritive value of fruits. Therefore, an experiment was conducted to study the impact of refrigeration on antioxidant activity and free-radical scavenging activity of five different fruits (*Malus sylvestris*, L., *Psidium gujava*, L., *Citrus aurantium*, L., *Punica granatum*, L., *Achras sapota*, L.,) under 10°C for 20 days.

MATERIALS AND METHODS

Fruits selected for the study were, *M. sylvestris* of Rosaceae, *P. gujava* of the family Myrtaceae, *C. aurantium* of the family Rutaceae, *P. granatum* of Punicaceae, *A. sapota* of Sapotaceae. Fresh fruits mentioned

above were purchased from the local market. The fresh fruits were analyzed for the antioxidant and free-radical scavenging activity. One set of each fruit was wrapped in polythene bags and stored in the household refrigerator at 10°C for 20 days. After 20 days, the refrigerated fruits were examined for both the activities.

***Antioxidant activity*[19]**

The total antioxidant activity of the extract was evaluated by phosphomolybdenum assay method, which is based on the reduction of Mo (IV) to Mo (V) by the extract and subsequent formation of a green phosphate-Mo (V) complex in acidic condition.

Sample extraction

Ethanolic extract

Hundred milligrams of the fruit sample was homogenized with 5ml of 80% ethanol and centrifuged at 2000rpm for 10 min. The pellet was extracted again with the same solvent and centrifuged again. The supernatant were pooled.

Assay

To 0.2 ml of the extract, 3 ml of reagent solution (0.6M sulphuric acid, 28mM Sodium phosphate & 4mM Ammonium molybdate) was added and the reaction mixture was incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695nm using UV-visible spectrophotometer against a reagent blank after cooling at room temperature. The antioxidant activity was expressed as the no. of gram equivalents of ascorbic acid /gram fresh tissue.

***Free radical scavenging activity*[1]**

DPPH radical scavenging is considered a good *in vitro* model and is widely used to assess antioxidant efficacy. In its radical form, DPPH free radical has an absorbance at 517m which disappears when DPPH is reduced by an antioxidant compound or a radical species to become a stable diamagnetic molecule. As a result, the colour changes from purple to yellow. This colour change is taken as an indication of the hydrogen donating ability of the tested compounds.

Sample extraction

Methanolic extract

Hundred milligrams of fruit tissue were ground with 3 ml of methanol and the extract was centrifuged at 2000rpm for 10min. The supernatant was used for the assay.

Assay

To 3.0 ml methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH-20µg/ml), 2.0 ml sample was added. The mixture was incubated in dark at room temperature for 30 min. and the absorbance was measured at 517nm using UV spectrophotometer.

The degree of free- radical scavenging activity was expressed as,

$$\%\ radical\ scavenging\ activity = \frac{absorbance\ of\ blank - absorbance\ of\ sample}{absorbance\ of\ blank} \times 100$$

RESULTS AND DISCUSSION

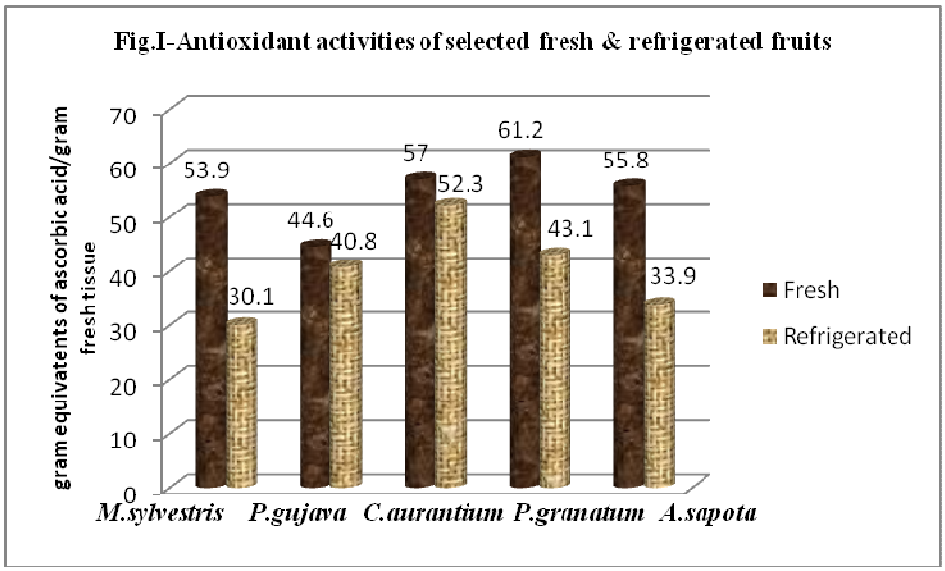
Antioxidant content

Antioxidant is a beneficial biochemical component available in fresh vegetables and fruits in nature itself. It is noteworthy to indicate that, the fruits selected in the present study revealed a significant quantity of antioxidant. In our study, the antioxidant content in fresh fruits exhibited a range of about 44.6 (*P. gujava)* to 61.2 gram equivalents of ascorbic acid /gram fresh tissue (*P. granatum*). Among the other selected fresh fruits, the antioxidant content was found to have an insignificant variation in *C. aurantium*, *A. sapota* and *M. sylvestris* with a range of 57, 55.8 and 53.9mg/g respectively. The variation in the antioxidant content of selected fruits in two different conditions is

shown in Table-I and in Figure-I. After refrigeration, the antioxidant content showed a marked reduction in all the selected fruits. The reduction in the amount of antioxidant content was very high and ranged between 33.9 (*A. sapota*) to 52.3 mg/g (*C. aurantium*). Also a range of about 38.1, 40.8, and 43.1mg/g of antioxidant content was noticed in *M. sylvestris*, *P. gujava* and *P. granatum.* Thus, our study reveals that, the antioxidant content was declined due to refrigeration where phenol compounds play an important role. The high phenol and polyphenolic compounds such as flavanoids are widely found in citrus fruits and food products derived from plants sources have been shown to possess significant antioxidant activities[11]. Whereas, the antioxidant activity of citrus fruits significantly increase with the presence of high concentrations of total polyphenol content[14]. The antioxidant activity of *Randia dumetoum*, was due to inhibiting the formation of radicals or scavenge the formed radical and the presence of phenolics compounds[17]. Thus, the observation of our experiment regarding the antioxidant content suggests that, the activity of individual compounds may depend on structural factors such as the number of phenolic, hydroxyl or methoxyl groups, flavones hydroxyl, keto groups, free carboxylic groups and other structural features.

Free radical scavenging activity

The antioxidant activities of selected fruits were investigated by DPPH free radical scavenging assay. The model system of scavenging DPPH free-radicals is a simple and acceptable method to evaluate the antioxidative activity of antioxidants. It is accepted that DPPH free radical scavenging by antioxidants is due to their hydrogen donating ability[3]. The free radical scavenging activities of all the fruits was substantially higher in fresh condition than after refrigeration. The activity was highly remarkable in fresh fruits and registered to be the highest in fresh *C. aurantium* (87%) followed by the fresh fruit of *P. gujava* (77%). An equal amount of free radical scavenging activity of 70% was registered in *M. sylvestris* and *A. sapota*. The minimal activity was found in *P. granatum* (63%). Comparatively, there was a moderate level of decrease in the free radical scavenging activity in all the fruits due cold storage at 10°C. The range of activity was noticed to be similar to the activity of fresh fruits showing a one-fold difference between *C. aurantium* L. and *P. gujava* of about 80% and 70% respectively. Also the order of series of variation in the activity was found to be in *A. sapota, M. sylvestris* and *P. granatum* with 60, 53 and 33% respectively. In *P. granatum,* the free radical scavenging activity was found to have a two-fold decrease after refrigeration. Our experiment has a direct correlation between antioxidant content and free radical scavenging activity. In the fruit of *Zizyphus mauritiana,* a relationship between DPPH free radical scavenging ability associated with high phenolics and antioxidant content was revealed[2]**.** In *Cucumis sativus* the presence of flavanoids and tannins being responsible for free radical scavenging and analgesic activities[16]. Also, in *Ziziphus nummularia* fruits exhibit their antioxidant potential via free radical scavenging and electron donation[5]. The results of free radical scavenging activity are shown in Table-I and in Fig-II.



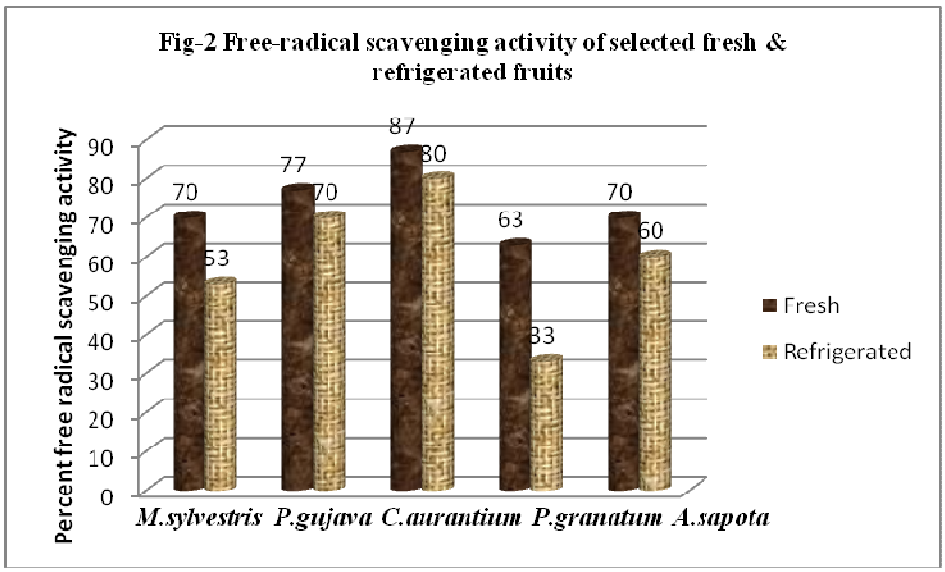


Table I

Antioxidant & free radical scavenging activity of selected fresh & refrigerated fruits

Fruits	Antioxidant (gram equivalents of ascorbic acid /gram fresh tissue)		Free radical scavenging activity (%)	
	Fresh	Refrigerated	Fresh	Refrigerated
M. sylvestris	53.9	38.1	70	53
P. gujava	44.6	40.8	77	70
C. aurantium	57	52.3	87	80
P. granatum	61.2	43.1	63	33
A. sapota	55.8	33.9	70	60

CONCLUSION

Our study is an evident that selected fruits contain high concentrations of antioxidants which have a beneficial role in building up immune system and fight against free-radicals in human body. These compounds may be destroyed, when fruits are exposed to low temperature. Refrigeration may be responsible for the decrease in the nutrient quality of fruits due to few adverse effects on its other attributes also. Therefore, this study suggests that consumption of fresh fruits is good for health and provide an excellent source of nutrients, especially antioxidants that helps to protect free radical damage in human body system.

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