**IJPSR (2012), Vol. 3, Issue 07** **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 02 March, 2012; received in revised form 17 April, 2012; accepted 24 June, 2012

# INFLUENCE OF RIPENING ON ANTIOXIDANT ACTIVITY OF CELL WALL POLYSACCHARIDES IN *PRUNUS ARMANIACA* LINN.

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**Keywords**:
*Prunus armaniaca* Linn,
Pectin,
Reducing power,
Scavenging of hydrogen peroxide

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## ABSTRACT

Cell wall polysaccharides have been of great importance to society and mankind over the years. In the present study Cell wall polysaccharides were extracted from different stages viz- Immature green, mature green and ripe stages of Prunus *armaniaca Linn*. A decrease in the yield of pectin was observed during ripening.The pectin Extracted from various stages of fruit ripening was further analysed for in vitro antioxidant activity by different assays. During the ripening of fruit it was seen than the pectin extracted showed increase in total antioxidant, reducing power and scavenging of hydrogen peroxide which can be related to galacturonic acid content by further studies and can be explored as a novel potent antioxidant.

**INTRODUCTION:** Pectin is the family of complex cell wall polysaccharides that constitute approximately one third of the dry weight of higher plant cell walls [1]. It has been reported that polysaccharides in general have strong antioxidant activities and can be explored as novel potential antioxidants [2]. Pectins are among the cell-wall components whose collective ability to contain the turgor pressure of the cell wall determines its growth [3].

Chemically, pectins are a family of complex heteropolysaccharides comprised by a diversity of carbohydrate residues. Like most other plant polysaccharides, pectins are polydisperse in composition and molecular size, that is, they are heterogeneous with respect to both chemical structure and molecular weight. Their composition varies with the source and conditions of extraction, location, and other environmental factors [4].

The main pectin chain is composed of a (1 -4) linked d-galacturonic acid residues. In the past few years, there has been a renewed interest in evaluating the antioxidant content and distribution patterns of fruits and vegetables. Currently, there is overwhelming evidence indicating that free radicals cause oxidative damage to lipids, proteins and nucleic acids. Free radicals may lie at the heart of the etiology or of the natural history of a number of diseases, including cancer and atherosclerosis [5]. Fruits and vegetables contain many different antioxidant components

**MATERIALS AND METHODS:** The fruits of different stages of *Prunus armaniaca* L. were deseeded and subjected to the preparation of AIS. After Preparation of the AIS it was treated with Phenol: acetic acid: water in the ratio of 2:1:1(W/V/V) to degrade the endogenous enzyme activity [6].

The AIS Powder was processed to pectin extraction by the method of Merk KGA Darmstadt, Germany. About 1g of the AIS Powder of each stage of *Prunus armaniaca* was taken and treated with 85% ethanol (25%. w/v) four times for 20 minutes (70°C). The powder was then filtered with miracloth and the residue was treated with oxalic acid/ammonium oxalate, pH 4.6 and the whole mixture was agitated for 1 hour (85°C). The reaction mixture was then filtered and the filtrate was treated with 3 volumes of ethanol (96%) followed by centrifugation (14, 500g/10min). The precipitates were washed with ethanol (70% and 96%) respectively. The extracted pectin was then oven dried at 50°C.

**Determination of yield of Extracted Pectin:** The extracted pectin from different staged was dried and dessicated and yield was measured.

**Determination of Antioxidant Activity:**

1. **Total Antioxidant Activity:** The total antioxidant activity was determined by a slight modification in the method of Pan [7]. Different aliquots (10-100µg/ml) 0.1 ml were combined with 1ml of reagent solution (0.6M sulphuric acid. 28 mM sodium phosphates and 4mM Ammonium molybdate). The tubes were capped and incubated at 95 degree Celsius for 90 minutes. After the samples were cooled to 25 degree Celsius, the absorbance was measured at 695 nm against blank having no test samples but only 1ml of the reagent solution. The total antioxidant activity was expressed as the absorbance value at 695nm. The higher absorbance value indicates greater antioxidant activity. Ascorbic acid at various concentrations was used as standard.

2. **Scavenging of Hydrogen peroxide:** The hydrogen peroxide scavenging activity was determined by slight modification in the method of Ruch and colleagues [8]. A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). Pectin extracts (10-100µg/ml) were added to hydrogen peroxide solution. Absorbance of hydrogen peroxide at 230 nm was determined after ten minute against a blank solution containing in phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of pectin extract and standard compounds was calculated using the following equation:

$$\text{Percent scavenged } [H_2O_2] = [(A_o - A_t)/ A_o] \times 100$$

Where $A_o$ was absorbance of control and $A_t$ was the absorbance of pectin extract and standard respectively.

3. **Reducing Power Assay:** The reducing power assay of various stages of pectins was determined by the method of Oyaizu [9]. A solution of potassium ferricyanide was prepared (1%) in phosphate buffer. Various concentrations of the extracts (10-100µg/ml) 1ml were mixed with the potassium ferricyanide solution. The mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (10%) (2.5ml) were added to the mixture. 2.5 ml of the above solution was taken and freshly prepared ferric chloride solution was added to it (1%) 0.5ml. The absorbance was measured at 700nm. Ascorbic acid at various concentrations was used as standard. A blank was prepared without adding extract. Increase in absorbance of the reaction mixture indicated increase in reducing power. The antioxidant activity was Expressed as $EC_{50}$ and compared with standard. The % increase in reducing power was calculated by the following formula-

$$\% \text{ increase in reducing power} = [(A_{test}/A_{blank})-1] \times 100$$

**RESULTS & DISCUSSION:**

**Yield of Pectin Extracted:** There was a decrease in the yield of pectin during ripening (**fig. 1**).

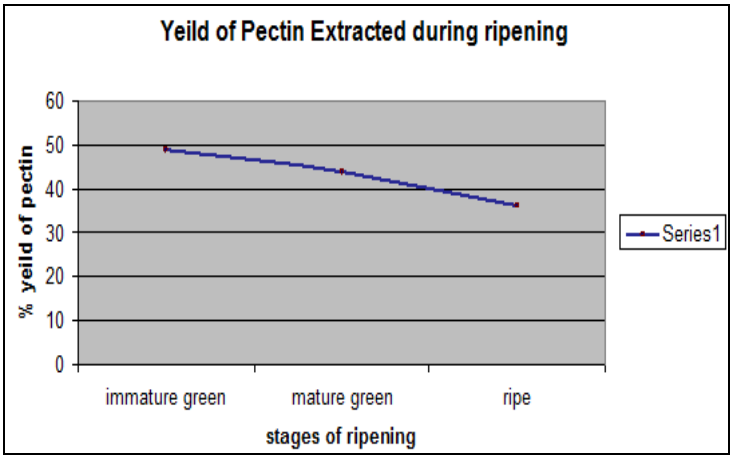


**FIG. 1: YIELD OF PECTIN EXTRACTED**

1. **Total Antioxidant activity:** The pectin extracted from immature green showed the lowest absorbance values whereas the pectin extracted from the ripe fruits showed the highest values. Ascorbic acid showed the maximum absorbance values (**fig. 2**).

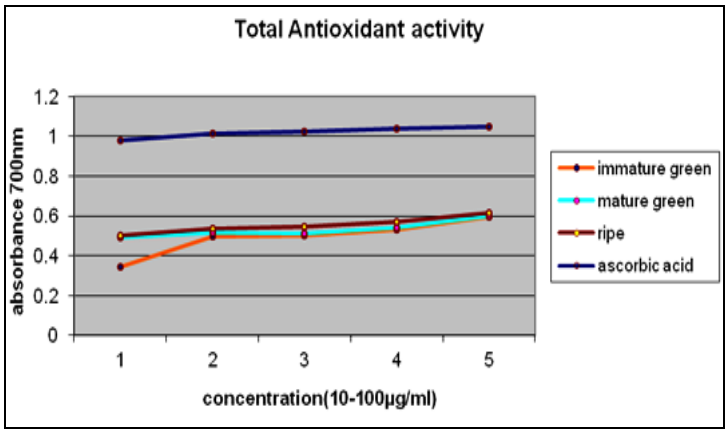


**FIG. 2: TOTAL ANTIOXIDANT ACTIVITY**

2. **Scavenging of Hydrogen Peroxide:** Similar results were seen in this assay, the hydrogen peroxide scavenging activity was increased during ripening, though there was considerably low difference in scavenging activities of pectin from immature green, and mature green but an increase was seen in case of pectin extracted from ripe fruits (**fig. 3**).

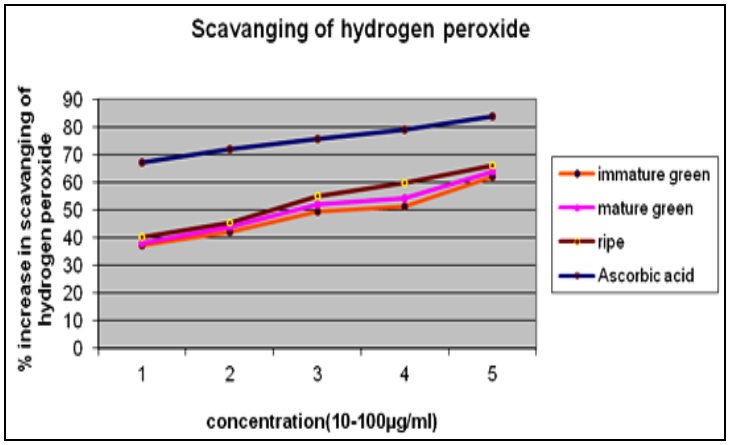


**FIG. 3: SCAVENGING OF HYDROGEN PEROXIDE**

3. **Reducing Power Assay:** The reducing power assay showed maximum values for the pectin extracted from ripe fruits, the mature green showed less reducing power as compared to ripe but increased values as compared to immature green (**fig. 4**).

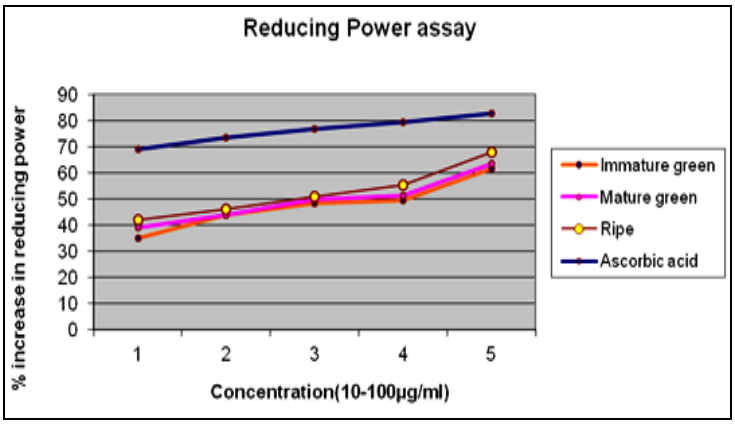


**FIG. 4: REDUCING POWER ASSAY**

It has been reported that fruit ripening influences the antioxidant activity [11]. In the present study, it was seen that there was a significant increase in in vitro antioxidant activity during ripening. The antioxidant activity was minimum in pectin extracted from immature green; it gradually increased in mature green, and was high in pectin extracted from ripe fruits. The total antioxidant activity of pectin were increased in order- immature green (0.343-0.594), mature green (0.492-0.599), ripe (0.504-0.615).The hydrogen peroxide percent scavenging activities of pectins increased in order-immature green (37.2%-62.1%), mature green (38.1%-64%), ripe (40.1%-66.2%).

The reducing power assay also showed a consecutive increase in percentage i.e.-immature green (35%-61.8%), mature green (39.21%-69.4%), ripe (42.2%-67.9%). The increase in total antioxidant, reducing power and hydrogen peroxide radical scavenging activity was observed regardless of the yield of pectin obtained from various stages of fruit ripening. The yield of pectin was observed to be decreased during ripening. Per gram yield of pectin in percent came out to be –immature green (49%), mature green (45%), ripe (39%).

Fruits have long been regarded as having considerable health benefits, due to their main antioxidant compounds. *Prunus armaniaca* L. was not studied in such a way. Therefore pectin extracted from this fruit at different stages of its ripening was investigated in this study as a fruit diet and as an additive to functional foods for prevention of cardiovascular and other diseases as traditional fruits. Fruit ripening in *Prunus armaniaca* Linn. showed decrease in pectin yield and increase in the antioxidant activity.

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**How to cite this article**:
Bhardwaj A, Singhal N, Verma S, Srivastava S and Tripathi K: Influence of Ripening on Antioxidant Activity of Cell Wall Polysaccharides in *Prunus armaniaca* Linn.. *Int J Pharm Sci Res*, 2012; Vol. 3(7): 2082-2085.