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# Nutraceutical composition of *ber* (*Zizyphus mauritiana* Lamk.) juice: effect of enzyme-assisted processing

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## ABSTRACT

**An investigation was undertaken to study the effect of pre-press maceration treatment with cell-wall degrading enzyme, pectinase, on antioxidant composition of *ber* juice, during 2011-2012. Enzyme-assisted processing significantly ($p<0.05$) improved antioxidant composition of *ber* juice. *Ber* juice extracted using pectinase had richer nutraceutical composition than in the Control. There was an overall increase of 43% in juice yield, 30% in total phenolics and 37% in total flavonoids with use of pectinase. *In vitro* total antioxidant activity (AOX) in *ber* juice was 19.58µmol Trolox/ml in Ferric Reducing Antioxidant Power (FRAP) and 13.44µmol Trolox/ml in Cupric Reducing Antioxidant Capacity (CUPRAC) assay. There was 41-65% increase in total AOX of *ber* juice extracted with the enzyme over-straight pressed juice. Results indicated that tailoring of the enzyme can yield antioxidant-rich juice products.**

**Key words:** *Ber*, enzyme assisted processing, pectinase and antioxidant activity

## INTRODUCTION

*Ber* (*Zizyphus mauritiana* Lamk.) grows excellently in arid and semi-arid regions of the world and is considered the poor man's apple, being an excellent source of several polyphenols including caffeic acid, *p*-hydroxybenzoic acid, ferulic acid and *p*-coumaric acid (Dahiru and Obidoa, 2008). As phenolics are known for their wide ranging health-protecting properties as anti-atherogenic, anti-inflammatory and anti-microbial, commercial processing of *ber* into juice rich in phenolics could prove useful. However, extraction of juice from *ber* is difficult and protracted because of its pulpy nature and high pectin content. Enzyme-assisted processing using pectinolytic enzyme is an effective approach for degrading pectineous material to yield free-flowing juice. In addition, the enzyme-catalyzed degradation also helps release phenolics and flavonoids that would otherwise be lost in press residues (Sowbhagya and Chitra, 2010). Several researchers have reported pectinase and cellulase enzyme treatments to significantly enhance recovery of phenolics and to improve functional properties of the juice. In view of the enormous potential of *ber* as a source of phenolics, the current study was undertaken to examine the effect of enzyme-assisted processing on nutraceutical composition of *ber* juice.

## MATERIAL AND METHODS

The present study was carried out on antioxidant composition of *ber* juice as affected by enzyme-assisted processing, during the year 2010-2011. Mature, ripe fruits of *ber* (cv. Umran), free from blemishes and mechanical injury were obtained from the local market of Parbhani and processed at Post Harvest Technology Laboratory of Department of Horticulture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. Fruits were washed thoroughly in tap water to remove any adhering dirt or dust. Whole fruits were then subjected to hot-breaking at 90°C for 20 min to soften them. These were then macerated in a Waring blender and, subsequently, passed through a laboratory-scale pulper for extracting a homogeneous pulp and to separate seeds. Pulp samples were weighed out into 500ml glass bottles and the enzyme preparation (pectinase EC 3.2.1.1 from *Aspergillus niger,* 1 U/mg from *Aspergillus* sp.) was added at four levels of dose: 0.10, 0.15, 0.20 and 0.25% E/S. Control (straight-pressed) juice samples were incubated without the enzyme under the same conditions. For each concentration, 500ml pulp was taken in three replicates. The bottles were capped and incubated at 50°C in a thermostatically controlled water bath for 1 h. The macerate was then pressed using a hydraulic press with a nylon filter

bag to extract the juice. Juice yield was determined by weighing the juice extracted, which was subsequently heat-processed at 90°C for 1min, and packed in clean, sterilized glass bottles, upturned and sealed. This juice was then used for analysis.

### Determination of total amount of phenolics, flavonoids and total antioxidant activity

Total phenolic content of the juice (80% ethanol extract) was estimated spectrophotometrically using Folin–Ciocalteu reagent, as per Singleton *et al* (1999). Results were expressed as mg gallic acid equivalents (GAE/100ml). Total amount of flavonoids was estimated by the method of Zhishen *et al* (1999) and the results were expressed as catechin equivalents/100 ml. Antioxidant activity was measured using two *in vitro* assays: ferric-reducing antioxidant power (FRAP), and cupric-reducing antioxidant capacity (CUPRAC). FRAP assay was performed as per Benzie and Strain (1996), and CUPRAC assay was performed as per Apak *et al* (2004). Results were expressed in mmol Trolox/ml (TE/ml).

### Statistical analysis

Each experimental unit was replicated three times. Data were subjected to Analysis of Variance, using Completely Randomized Design.

## RESULTS AND DISCUSSION

### Juice yield

Data on effect of pectinase enzymes at different doses (0.1–0.25%) on *ber* juice yield is presented in Fig. 1. The pulpy macerate of *ber* was highly viscous and difficult to press. With conventional straight-pressing (Control), the yield averaged 27%, while, with increasing concentrations of pectinase enzyme, juice-yield increased to 70%. Enzyme-assisted processing accelerated liquefaction of the pulpy macerate, resulting in an 43% increase in juice yield.

### Total amount of phenolics, flavonoids and antioxidant (AOX) composition of *ber* juice

Enzyme-assisted processing had a significant impact on recovery of total phenolics and flavonoids too in *ber* juice. Compared to the Control, percentage increase in recovery of total phenolics was higher in pectinase treatments. Total phenolics content increased to 314.36mg GAE/100ml at 0.25% pectinase, from an initial 240.48mg GAE/100ml (Fig. 2). Phenolics contained in the vegetable and fruit matrix appear to be entangled with the plant cell wall polysaccharides via tight hydrophilic and hydrophobic bonds. The release of those phenolics can be enhanced via enzyme catalyzed degradation of the cell wall polysaccharides. Enzyme facilitated polysaccharide helps in exposing possible cell wall sites for phenolics, resulting in enhanced recovery (Pinelo and Meyer, 2008).

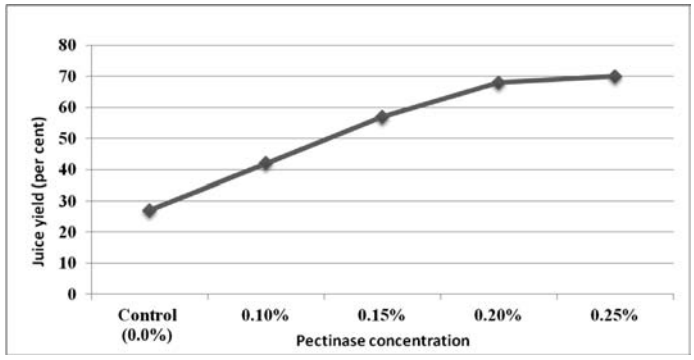


**Fig 1. Effect of pectinase treatment on juice yield in *ber* cv. Umran**

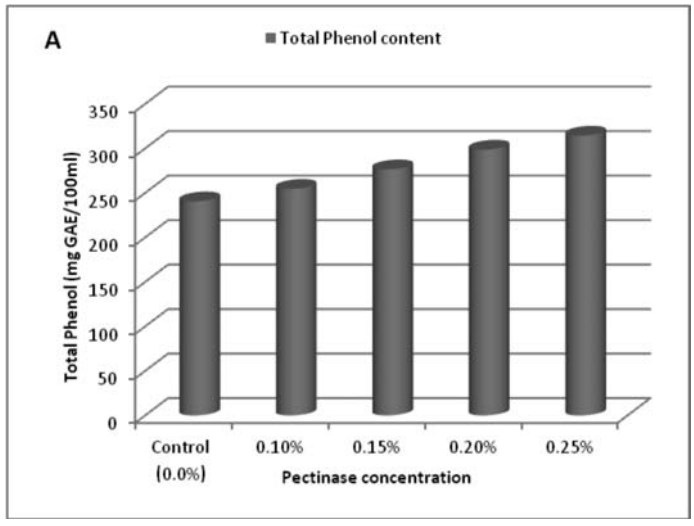


**Fig 2. Effect of pectinase treatment on total phenol content in *ber* juice cv. Umran**

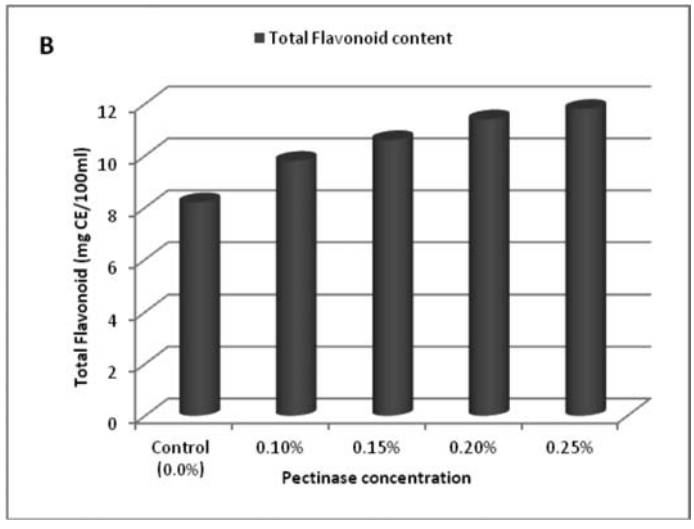


**Fig 3. Effect of pectinase treatment on total flavonids in juice of *ber* cv. Umran**

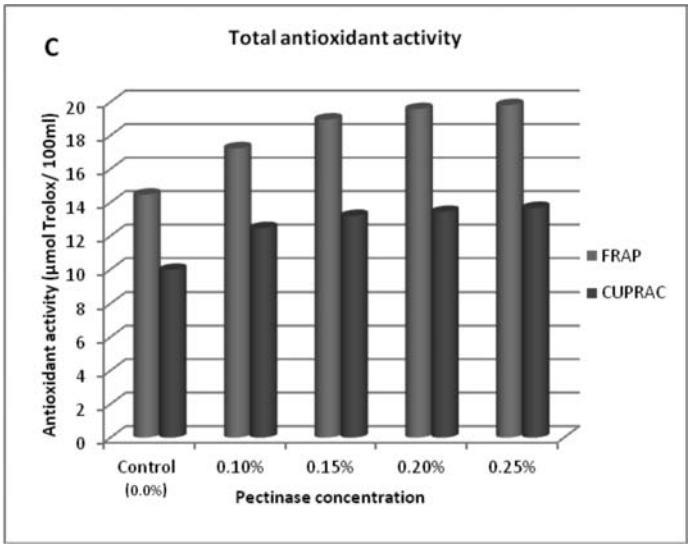


**Fig 4. Effect of pectinase treatment on antioxidant activity in juice of *ber* cv. Umran**

Total flavonoids content in juice also showed progressive increase with various pectinase treatments (Fig. 3). Antioxidant activity of *ber* juice, too, improved dramatically upon enzyme-assisted processing. Values for this ranged from 14.47 to 19.82mmol/ml, respectively, in the Control and in the juice treated with the enzyme pectinase (Fig. 4). Overall, there was a significant increase in total AOX in the juice over Control. An almost identical trend was observed in CUPRAC assay (Fig. 4). High AOX in enzyme-assisted juice may be attributed to a high recovery of phenolic compounds observed in the juice. Similar results on high phenolic content and antioxidant activity have been reported in bilberry by previous workers (Puupponen-Pimia *et al*, 2008).

Enzyme-assisted processing of *ber* significantly enhanced nutraceutical composition of the juice, in contrast to straight-pressing. These results could lead to tailoring of the enzyme for obtaining optimum levels of antioxidants in the juice products. The study also indicated that *ber*, a fruit rich in nutrceuticals, can be commercially processed into juice rich in phenolics.

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