

ASSESSMENT OF QUALITY CHARACTERISTICS UPON ENZYMES ASSISTED JUICE EXTRACTION FROM PLUM

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ABSTRACT

The aim of this work was to optimize type and concentrations of commercial enzymes for the treatment of the plum to improve the juice yield with colour enhancement. Pectinase enzyme at a concentration 10 units/mL was found to give juice yield upto 86.33 %. However, juice quality with respect to TSS reading and phenol content could be enhanced by treating the fruit mash with enzymes cellulase. Maximum TSS (13.07°B) was observed on treating fruit juice pulp with cellulase at 4 units/ml concentration whereas cellulase @ 6 units/mL gave superior result in terms of phenol content (481.45 mg/100mL). Enhanced extraction of ascorbic acid (541.54 mg/100mL), anthocyanin (6.08 mg/kg) and % DPPH radical scavenging activity (89.85%) was found on treating fruit mash with enzyme hemicellulase (@ 3 units/mL, 6 units/mL and 9 units/mL) respectively. Treating fruit mash with Hemicellulase @ 9 units/mL is the best for obtaining maximum antioxidant compounds and % DPPH radical scavenging activity whereas cellulase @ 6 units/mL is found to produce juice with maximum value for best colour (37.35 L*, 5.61 b*, 40.68 dE and 20.73 H) properties.

INTRODUCTION

Plum (*Prunus domestica* L.) is relatives of the peach, nectarine, plum and almond is considered as one of the most popular fruit found in Himalayan region of Darjeeling and Sikkim. Traditionally plums are growing in Darjeeling and Sikkim and mostly produced plums are used for fresh consumption, but very small quantities are processed into juices. On the market citrus juices are the most popular following by apple juice, but plum juices are very rare. Despite reports of plum benefits to human health, consumption remains low, which has been attributed to unavailability of this fruit for consumer in fresh state during whole year. Moreover, due to high acidity consumption of fresh fruit is often limited (Szajdek and Borowska, 2008) and normally prevent the marketing of 100% natural juices (Robin *et al.*, 2013). However, in the processed form they may be available year-round. To increase consumption of plums in off season it is important to produce more attractive processed products.

The plum fruit is a good source of vitamins, minerals, fiber and enzymes that are good for the digestive system and positively associated with nutrient intake, improves anthropometric measurements and reduced risk of hypertension (Beals *et al.*, 2005). In recent years, there is an increasing interest in finding antioxidant phytochemicals (Terao and Piskula, 1997), because plants like *P. ferrugineum* containing high total phenol and flavonoid contents can be considered as a medicinal source for the treatment and

prevention of many free radical related diseases (Chanda *et al.*, 2013). Besides various sugars, acids, pectins, tannins and enzymes, plum also polyphenolic compounds (Walkowiak-Tomczak *et al.*, 2008) featuring a high antioxidant capacity (Kahkonen *et al.*, 1999). Fortunately, compounds other than polyphenolic compounds, such as ascorbic acid, may also contribute to the total antioxidant activity of plums (Walkowiak-Tomczak *et al.*, 2008). Anthocyanins contribute greatly to the antioxidant properties of certain colourful foods, such as grapes and cranberries (Wang *et al.*, 1996). As pigments, they are almost exclusively responsible for the red, blue and purple colours in fruits. Even the use of apple-pomace fruit based medium for pigment production by bacteria isolates found to be optimum at pH 7.5 for maximum bio pigment production (Deb and Madhugiri, 2012).

According to applied technology three main groups of juices is known: clear, cloudy and pulpy juices. Processes of juice separation from fruit cell are different due to type of juice and for the first two juices are usually obtained by pressing. All fruits are not suitable for all type of juices, e.g. fruit with the pigments insoluble in water is not suitable for producing clear juices. Red colour of plum skin origin from anthocyanins, pigment soluble in water, so plum fruit is suitable for all type of juices (Lovric and Pilizota, 1994). In case of cloudy apple juice production, the enzymation and clarification stages are omitted. This process is not justified for berry and stone fruits containing a large amount of pectic substances (Hilz *et al.*, 2005), which have an impact on the structure of raw fruit and

juice yield during mash pressing (Grassin *et al.*, 2005). The extraction of plum juice on large scale bases includes pressing of juice from comminuted solids of plum. The residual pulp remaining after juice extraction still contains valuable extractable material such as particulate, flavour, soluble solids, etc., which would improve the final quality of the juice. By adding cell wall liquefying enzymes, it is possible to further extract valuable juice components from pulp (Robin *et al.*, 2013).

The use of fungal enzyme in fruit juice extraction had shown significant increase in juice recovery as compared to cold and hot extraction methods (Joshi *et al.*, 1991). The enzymes, mainly pectinases, and cellulases assist in pectin and cellulolytic hydrolysis respectively, which cause a reduction in pulp viscosity and a significant increase in juice yield (Pilnik and Voragen, 1993). Purpose of pectinolytic enzymes addition is degradation of protopectin and partly pectins from primary cell wall and middle lamella (Kashyap *et al.*, 2001) and this process is responsible for release of juice from the cells as well as the pigments from the plum skin cells (Will and Dietrich, 2006). Many authors studied usage of various enzymes in juice production concern to better yield and colour extraction e.g. Chang *et al.* (1994) and Will and Dietrich (2006) in plum juices, Landbo and Meyer (2004) and Pap *et al.* (2010) in black current juices, and Landbo *et al.* (2007) in elderberry juices. Its effectiveness depends on type of applied enzyme.

Most of commercial enzyme preparations contain pectinases, cellulases and hemicellulases in various ratios, which act in different way (Mieszcakowska-Frac *et al.*, 2012). The enzymatic hydrolysis of pectic substances depends on several processing variables and one of them is type of enzyme and their concentration (Baumann, 1981) and these parameters need to be optimized for maximal juice recovery and enhancement of phenol and anthocyanin extraction. Therefore the present study was undertaken with objective to optimize type and concentrations of commercial enzymes for the treatment of the plum pulp to improve the juice yield with optimum colour acceptability.

MATERIALS AND METHODS

The investigation was carried out to evaluate different enzymes on plum juice extraction in the Departmental Laboratory of Pomology and Post Harvest Technology, Pundibari, West Bengal during the year 2009-2011. The fruit samples of Santa Rosa are purchased from the local farmers. The fruit pulp was extracted using Electric Mixer Grinder. Immediately, a known quantity of fruit pulp was poured into 250 mL conical flasks and covered with aluminium foils. The different enzymes like Pectinase and Cellulase were purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai-93, India. Hemicellulase was purchased from Sigma Aldrich Co., St. Louis, USA. The enzymes obtained were dissolved in double distilled water to prepare a enzyme solution of known concentration (units/mL) and then were added to fruit pulp as specified in treatment details like T₁: Control (no enzyme); T₂: Pectinase-3.33 units/mL; T₃: Pectinase-6.66 units/mL; T₄: Pectinase-10 units/mL; T₅: Cellulase-2 units/mL; T₆: Cellulase-4 units/mL; T₇: Cellulase-6 units/mL; T₈: Hemicellulase-3 units/mL; T₉:

Hemicellulase-6 units/mL; T₁₀: Hemicellulase-9 units/mL were put into the conical flask containing extracted fruit pulp. Then the enzymes treated fruit juice pulp was put in the Temperature Controlled Shaking Incubator at 120 rpm for 2hrs at 45°C. The experiment was carried out in Completely Randomized Block Design using 10 numbers of treatments with 3 replications for each treatment. Juice yield in %, was calculated as the ratio of the weight of extracted juice to the total weight of the extracted juice and the residual products after extraction and mathematically expressed as described by Tressler and Joslyn (1961). Total soluble solids (°Brix) content of fruit was determined with the use of a hand refractometer calibrated in 0°Brix at 20°C with the help of a temperature correction correlation chart (Mazumdar and Mazumdar, 2003). Ascorbic acid was estimated as described by Mukherjee and Choudhury (1983). The amount of phenol in the extract was quantified using a spectrophotometer at 650 nm and the values were expressed as milligrams of phenol per gram of fruit juice (Mallick and Singh, 1980). Anthocyanin content was determined using the pH differential method in which absorbance of anthocyanin extracts at pH 1.0 and 4.5 are determined (Rodriguez-Saona and Wrolstad, 2001). Monomeric anthocyanins are highly coloured at pH 1.0 and colourless at pH 4.5 (Wrolstad *et al.*, 2005). Since the visible spectrum showed the anthocyanin maximum absorbance to be at 512 nm, the difference in absorbance (ÅA) was calculated from (A_{512 nm pH 1.0} - A_{700 nm pH 1.0}) - (A_{512 nm pH 4.5} - A_{700 nm pH 4.5}). Absorbance at 700 nm was used to account for any turbidity in the samples. Total anthocyanin content was expressed as cyanidin-3-rutinoside on a fresh weight basis, mg kg⁻¹ and calculated using its molar absorbance coefficient of 26900 L⁻¹ M⁻¹ cm⁻¹ (Wrolstad *et al.*, 2005). The antioxidant activity of the extracts was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH, according to the method (Blios, 1958). Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated (Formula: % DPPH radical scavenging activity = (Control OD - Sample OD) / Control OD X 100) as described by Mohamed (2008). Hunter colorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA) based on three colour coordinates, namely L*, a* and b* was used for determination of colour of plum juice. Procedures were carried out and values were expressed as described by Edward *et al.* (1966) and Ahmed (2004) for juice colour determination. Mean values were used for calculating chroma (dC), colour intensity (dE) and hue angle (H) were calculated according to the equations as described by Mohammadi *et al.* (2008).

RESULTS AND DISCUSSION

Quality characteristics of enzyme treated plum juice

The enzymatic treatment significantly influenced the physico-chemical properties of the plum juice and presented in Table 1. Pectinase (at 10 units/mL fruit juice pulp) treated fruit mash were found to score the maximum juice percentage (86.33%) as compare to hemicellulase and cellulase. This might be due to the type of enzymes aided for juice yield as the main factor determining high juice is appropriate selection of enzyme (Buchert *et al.*, 2005; Landbo and Mayer, 2004) and the success

of the liquefaction methods greatly depends on the ability of the enzymes to liquefy cell wall materials (Will *et al.*, 2002). The results are supported by the findings of Kaur *et al.* (2009), who reported that the maximum juice yield from guava is obtained by pectinolytic enzyme treatment of pulp. The increase in juice yield with increasing pectinase enzyme concentration is also supported by Pilnik and Voragen (1993) who reported that pectinases degrade pectic substances leading to increase in juice yield. Cellulase treated fruit juice showed the highest TSS content and maximum TSS (13.07°B) was observed in T₆ (Cellulase @ 4 units/mL fruit juice pulp). The obtained results are in accordance with literature data of Shah (2007) on litchi pulp and Levaj *et al.* (2012) on plum juice who investigated the influence of enzyme treatment exhibited an increase in total soluble solids. Higher cellulase dosages were the most effective in increasing TSS. This may be due to the enzymatic action thereby increase in TSS and decrease in viscosity (Sharma *et al.*, 2005). The raise could be partially accounted for by increase in soluble sugars, which may result from the action of cellulase on cellulose to produce soluble sugars (Schobinger *et al.*, 1981) or this increase may be due to hydrolysis of some carbohydrates into sugars (Satkar *et al.*, 2013). High concentration aided Hemicellulase treatment representing T₁₀ (Hemicellulase @ 9 units/ml fruit juice pulp) gave maximum ascorbic acid (541.54mg/100mL) content as compared cellulase treated fruit mash. The obtained results are in accordance with findings of Ali and Essa (2002) who investigated the ascorbic acid content increased with

pectinase treatment in guava juice, but decreased in plum juices. This might be due to improved stability of vitamins on hemicellulase treatment of fruit mash. Miller and Rice-Evans (1996) suggested that the phenolic antioxidants of fruit juices protect the vitamin C content from oxidative degradation. Phenolics are important because of their contribution to the sensory quality of fruits (colour, astringency, bitterness and flavour), which may be affected during the technological processes used for obtaining the juices and other transformation products (Ramadan and Moersel, 2007). In general, it seems that higher enzyme concentration at higher temperature resulted with increase of concentration of total phenolics (Will and Dietrich, 2006; Rop *et al.*, 2009). Highest phenol content (481.45 mg/100mL) was recorded on fruit mash treated with high concentration of cellulose representing T₇ (Cellulase 6 units/mL fruit juice pulp). The results are supported by the findings of Sowbhagya and Chitra (2010), who reported that the enzyme assisted carrot juice extraction helps to release phytochemicals thus helping in greater recovery of phenols (Sowbhagya and Chitra, 2010). Anthocyanins are located mainly in the skin of the fruit and during juice pressing it is important to transfer into the juice (Mieszczakowska-Frac *et al.*, 2012). Plum gets its characteristic and bright red colour from anthocyanin and enzyme assisted plum juice extraction may helps to release phytochemicals thus helping in greater anthocyanins. Maximum (6.08 mg/kg) anthocyanin content was observed in T₈ (Hemicellulase 3 units/mL fruit juice pulp). This may be due to action of enzymes

Table 1: Quality characteristic of plum juice upon enzyme assisted juice extraction

Treatments	Yield (%)	TSS (°Brix)	Ascorbic acid (mg/100mL)	Phenol(mg/100mL)	Anthocyanin (mg/kg)	DPPH radical scavenging activity (%)
T ₁	57.33	10.70	167.58	292.01	1.71	45.49
T ₂	71.33	12.17	197.43	364.29	1.47	56.51
T ₃	82.67	11.43	228.45	389.80	2.88	85.59
T ₄	86.33	11.30	253.34	452.24	2.87	88.76
T ₅	71.33	12.97	201.68	350.94	2.49	70.42
T ₆	75.33	13.07	224.24	367.47	4.72	88.78
T ₇	71.67	13.00	455.63	481.45	3.97	89.43
T ₈	72.00	11.67	210.04	323.32	6.08	86.34
T ₉	77.33	11.40	418.89	383.21	5.42	88.26
T ₁₀	80.00	11.87	541.54	418.56	5.40	89.85
SEm (±)	2.19	0.26	19.80	23.85	0.21	2.79
C.D. at 0.05	6.46	0.78	58.41	70.36	0.61	8.23

Table 2: Colour properties of plum juice upon enzyme assisted juice extraction

Treatments	L*	a*	b*	dE	dC	H
T ₁	34.53	18.15	4.11	39.22	18.61	12.76
T ₂	34.42	17.57	5.08	38.98	18.29	16.11
T ₃	30.10	16.34	3.55	34.43	16.73	12.23
T ₄	28.65	12.84	3.11	31.55	13.22	13.62
T ₅	35.42	16.77	3.76	39.37	17.19	12.63
T ₆	36.46	17.34	4.64	40.64	17.95	14.96
T ₇	37.35	15.12	5.61	40.68	16.13	20.73
T ₈	32.59	19.49	3.67	38.16	19.83	10.66
T ₉	30.26	17.60	3.28	35.16	17.90	10.54
T ₁₀	31.80	19.40	4.37	37.50	19.88	12.68
SEm (±)	0.14	0.14	0.12	0.14	0.15	0.34
C.D. at 0.05	0.41	0.41	0.37	0.42	0.45	1.00

which facilitate the extraction of anthocyanin to a greater extent (Sowbhagya and Chitra, 2010). Khandare *et al.* (2011) also reported that enzymatic treatment of black carrot resulted in 27% increase in total phenolics and 33% in overall juice yield. Hemicellulase treated samples got the highest anthocyanin contents as compared to cellulase and pectinase treated fruit juices and this might be due to specificity of each of three commercial enzymes in hydrolysis of cell wall and starch (Kaur and Sharma, 2013). Maximum (89.85% DPPH radical scavenging activity) was recorded under T₁₀ (Hemicellulase 9 unit/ml fruit juice pulp) which was followed by T₇ (89.43% DPPH radical scavenging activity, (Table 1). But lowest antioxidant percent (45.49% DPPH radical scavenging activity) was observed in T₁ (without enzymatic treatment of fruit mash). Sun and Tang (2007) worked on asparagus juice also reported a significant increase in the free radical scavenging activity of juice extracted with viscozyme. Results are also in agreement with the findings of Landbo and Meyer (2004).

Colour properties of enzyme treated plum juice

Total anthocyanin generally increased in plum with addition of enzymes (Rommel *et al.*, 1992) and influenced the appearance of plum juice. Colour is an important parameter as it is associated with the anthocyanin content of plum juice. Colour measurement by standardized instrumental method corresponds to a visual assessment of the colour and is important for determination of conformity of juice quality. Colour parameters were expressed as L*, a*, b*, colour intensity (dE), chroma (dC) and hue angle (H). L* indicates the colour lightness and a* and b* are the chromaticity coordinates and dE were calculated from a*, and b* values. The lightness value L* of enzyme assisted plum juice and control samples is presented in Table 2. Maximum lightness (L*) value 37.35 was observed in cellulase (Cellulase @ 6 units/mL fruit juice pulp) treated plum juice representing T₇. ANOVA revealed the significant difference (P<0.05) in the L* value of enzymatically treated samples. Sims *et al.* (1993) also reported that enzymatic extraction improves the colour of carrot juice. Highest a* value (19.49) was recorded under T₈ (Hemicellulase 3 units / ml fruit juice pulp) while lowest a* value was observed in T₄ (Pectinase 10 units/ml fruit juice pulp) (12.84). The increase of a* value of enzyme treated plum juice might be due to release of more colour compounds during enzymatic hydrolysis as colour compounds remained intact and were released with cell wall materials to the juice during enzymatic liquefaction (Mohammad *et al.*, 2010). The values of b* obtained from the experimental data on enzymatic treatments and significantly maximum (5.61) b* value was observed in T₇ (Cellulase 6 units / ml fruit juice pulp). The total colour intensity (dE) is a colorimetric parameter extensively used to characterize of colours intensity on enzymatic treatments. Maximum dE (40.68) was observed in T₇ (Cellulase 6 units / ml fruit juice pulp). Increase in b* value and slight dE value may be due to addition of anthocyanin from fruit cells, because colour of the fruit juices increased apparently due to release of red coloured anthocyanin from cells (wong and Thomas, 1989). Chroma and hue angle were calculated by using equations as described by Mohammadi *et al.* (2008) and the results are illustrated in Table 2. The values of chroma and

hue angle increased on application of enzyme for juice extraction. The maximum chroma and hue angle value of enzymatically treated samples was found to be 19.88 under T₁₀ (Hemicellulase 9 units/ml fruit juice pulp) and 20.73 under T₇ (Cellulase 6 units/ml fruit juice pulp) respectively.

CONCLUSION

Enzymatic treatment caused significant increase in yield of juice and maximum juice yield was obtained treating the fruit mash with pectinase whereas maximum TSS and phenol content extraction was found when the fruit mash was treated with cellulase. With respect to ascorbic acid, anthocyanin and % DPPH radical scavenging activity (H), it was found to have an enhanced extraction of these compounds on treating mash with hemicellulase. The effect of enzymatic treatment on colour properties of juice was found to be statistically significant and maximum L*, b*, dE and H values were recorded with cellulase. Considering all parameters studied hemicellulase @ 9units/ml and cellulase @ 6units/ml could be recommended to produce plum juice with enhanced quality attributes and best colour properties respectively.

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