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Enzymatic extraction of wood apple (*Feronia* limonia Swingle) pulp

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Abstract
Wood apple pulp was extracted by treating pulp with water @ 1:2 containing enzymes *viz.*, Pectinase (0.1%), cellulose (0.1%) and combination of pectinase and cellulose (0.1%), incubated for 2 hours at 40 and 50 ºC temperature by maintaing constant 4.5 pH. Among the different methods of enzymatic extraction of wood apple pulp, maximum pulp recovery, pulp per 100 mL of extract, TSS, ascorbic acid, brix: acid ratio, total sugars and reducing sugars were obtained by soaking the wood apple pulp in water containing pectinase + cellulase enzyme @ 0.1 per cent and incubated for 2 hours at 50 ºC (81.11%, 40.55%, 6.60ºB, 3.18 mg/100 mL, 3.95, 5.23% and 3.62%, respectively). The highest score for colour and appearance (7.83), flavour (8.33), taste (7.50), mouth feel (8.17) and overall acceptability (7.96) was also recorded soaking pulp in water + pectinase and cellulase (0.1%) at 50 ºC. Combination of pectinase and cellulase enzymes was found effective from the present study. But the incubation temperature plays an important role in obtaining maximum recovery and quality of pulp.

Keywords: wood apple, enzymes, pectinase, cellulase

Introduction
Wood apple (*Feronia limonia* Swingle) belongs to the family Rutaceae, is commonly found in dry deciduous forests. In India, it is known by several names like elephant apple, curd apple, monkey fruit, kavat, curd fruit and kath bel (Mazumder *et al.*, 2006) [17]. The wood apple is native to India and Sri Lanka. Its cultivation as a fruit tree is rare, but fruits of naturally occurring trees in community lands, forests and on road sides are used for chutney and pickle making. Importance of wood apple fruit lies in its curative properties, which makes the tree as one of the useful medicinal plants of India. The fruits and the leaves are used in herbal preparations. The fruit is used in India as a liver and cardiac tonic, when unripe, as an astringent means of halting diarrhea and dysentery (Singh, 2001) [24], effective treatment for hiccups, sore throat and diseases of the gums. The pulp is poultice onto bites and stings of venomous insects (Kirtikar and Basu, 1935) [13]. Due to its high religious, cultural, nutritional and medicinal values, this is one of the fruits awarded with "Shree" title. In Sanskrit language, its name is "Shree Phalam" or "Amrit Phal".

Wood apple is known for its excellent flavour and high nutritional value. Average weight of fruit is 350 g with pulp weight of 224 g. Wood apple fruits have higher content of both acidity and pectin, hence the most suited for jelly making (Singh *et al.*, 1999) [25]. People consume the raw fruit pulp as such with or without sugar/jaggery, or as a beverage after blending it with other ingredients. The pulp is also suitable for making food products such as juice, nectar, jam, fruit bar, wine, chutneys, sherbet, pulp powder *etc.*

Because of its excellent flavour and nutritive value this fruit has a great potential for value addition especially in beverage industry. Srivatsava and Vatsya (1986) [27] investigated that the wood apple beverage produces cooling effect in the same way as Bael fruit. Fruit juices are refreshing and retain characteristic taste and aroma even after a few months of their preparation into beverage. Compared to several other fruit products, fruit juices and ready-to-serve beverages are increasingly gaining popularity throughout the country (Chakraborthy *et al.*, 1993) [5]. Wood apple is not a succulent fruit to yield a free flowing juice by the usual methods of extraction as does the oranges, nor is it pulpy like the mango in which pulp can be separated from the seeds and skins by simple means. Extraction of pulp from wood apple is the major bottle neck in beverage industry mainly due to its compact, fibrous and mucilaginous flesh with numerous seeds.

Enzymatic extraction process has many advantages over mechanical and thermal process. In particular, the use of pectinases and cellulases has been an integral part of modern fruit processing technology involving treatment of fruit mashes as they not only facilitate easy pressing and increase in juice recovery but also ensure the highest possible quality of end products (Sreenath *et al.*, 1994) [26]. The enzymes thus are important as a processing aid for production of high quality fruit juices and concentrates (Albrecht, 1996) [1]. Therefore, the present study was undertaken to standardize the method of extraction of wood apple pulp using pectinase, cellulose enzyme.

Material and Methods

An experiment was carried out during 2019-20 in the Department of Post-harvest Technology, KRC College of Horticulture, Arabhavi (UHS, Bagalkot), Karnataka. The fully ripe fruits at edible stage were purchased from local market, opened by breaking against the hard surface. The pulp along with the seeds and fiber was separated with the help of stainless steel spoon from the hard shell. The pulp was homogenized and its weight was recorded.

In T_1 treatment *i.e.* control, wood apple pulp was soaked in two parts of normal water and homogenized hand crushing and kept in room temperature for 2 hours. In treatments T_2 to T_7 wood apple pulp was soaked in two parts of normal water and immediately homogenized by hand crushing. The pH of the pulp was adjusted to 4.5 by addition of buffer. Afterwards, immediately the pectinase enzyme 0.1 per cent in T_2 and T_3, cellulose enzyme 0.1 per cent in T_4 and T_5 and combination of these enzymes 0.05 per cent each in T_6 and T_7 were added and incubated for 2 hours at different temperatures. T_2, T_4 and T_6 were incubated at 40 ^oC whereas, T_3, T_5 and T_7 at 50 ^oC. At the end of treatment period the pulp was extracted by passing through strainer and pulp was pasteurized at 75 ^oC for 5 minutes in-order to inactivate the enzyme, then cooled and filled in clean sterilized glass bottles for estimation of physicochemical and sensory quality parameters.

The pulp recovery percentage was calculated by using the following formula and pulp

$$\text{Pulp Recovery (\%)} = \frac{\text{Weight of flesh taken for Pulp extraction (g)} - \text{Left out pulp after extraction (g)} \times 100}{\text{Weight of flesh taken for pulp extraction (g)}}$$

Per 100 mL of extract *i.e.* the actual quantity of pulp present in the water used for the extraction was measured and expressed in per cent.

The TSS was measured by using an 'Erma' make hand refractometer and expressed as ^{o}Brix after necessary corrections. Acidity was estimated by titration method and expressed in terms of citric acid as per cent titrable acidity. Ascorbic acid (mg/100ml) content was estimated titrimetrically using 2, 6-dichlorophenol indophenol dye as per the modified procedure of AOAC (Anon., 1984) [3]. The pH of wood apple pulp was measured by using pH meter ((Model: Analog research, USA). Reducing sugars was estimated as per the Dinitro - salicylic acid method (Miller, 1972) [18]. The total sugars content was estimated by the same method as in case of reducing sugars after inversion of the non-reducing sugars using dilute hydrochloric acid (Anon., 1984) [3]. The per cent non-reducing sugars were obtained by subtracting the values of reducing sugar from that of total sugar. Pectin was estimated as calcium pectate and expressed in percentage (Carre and Haynes, 1922) [4]. Brix: acid ratio was calculated by dividing of TSS (%) by the respective value of titratable acidity (%) of the particular sample.

The organoleptic characters like colour and appearance, flavour, taste, mouth feel and overall acceptability were evaluated by a panel of semi- trained judges consisting of teachers and post-graduate students of KRC College of Horticulture, Arabhavi, on a nine point hedonic scale and the mean scores given by panelist were used for statistical analysis (Ranganna, 2010) [23]. The data recorded on the physico-chemical and organoleptic parameters were subjected to statistical analysis in Completely Randomized Design (CRD). The interpretation of data was carried out in accordance with Panse and Sukhatme (1985) [19]. The level of significance used in 'F' test was p=0.01.

Table 1: Treatment details

T_1 – Control (Soaking pulp in water at room temperature)
T_2 – Soaking pulp in water + Pectinase (0.1%) at 40 ^oC
T_3 – Soaking pulp in water + Pectinase (0.1%) at 50 ^oC
T_4 – Soaking pulp in water + Cellulase (0.1%) at 40 ^oC
T_5 – Soaking pulp in water + Cellulase (0.1%) at 50 ^oC
T_6 – Soaking pulp in water + Pectinase and Cellulase (0.1%) at 40 ^oC
T_7 – Soaking pulp in water + Pectinase and Cellulase (0.1%) at 50 ^oC

Result and Discussion

Effect of methods of extraction on recovery of wood apple pulp

The recovery of pulp is most important from the point of cost effectiveness of the product. In the present investigation significantly highest pulp recovery per cent (81.11%) and pulp per 100 mL of extract (40.55%) was recorded in pulp treated with pectinase + cellulase enzyme @ 0.1 per cent and incubated at 50 ^oC (T_7) with fixed pH of 4.5 while, the minimum per cent was recorded in control (56.39 and 28.20%, respectively). This is mainly due to the complete removal of pulp adhering to the seeds by heat and enzymatic treatment as the partial or complete degradation of the cell wall and middle-lamella, pectin, other polysaccharides and cell substances (Dorreich, 1996) [8] thus increasing press capacity which results in increased pulp yield and dry matter content of the product (Demir *et al.*, 2001) [7]. Pectinase and cellulase hydrolyse the pectin and cellulose, respectively in the plant cell walls and increase extraction efficiency. Further, the incubation temperature and pH of the pulp plays an important role in getting maximum pulp recovery due to activity of enzymes which depends on these factors. The highest recovery at 50 ^0C incubation temperature and 4.5 pH may be attributed to maximum activity of pectinase + cellulase enzyme at this temperature and pH. Similar findings have also been reported by Gowda (2017) in wood apple.

Effect of methods of extraction on quality of wood apple pulp

In case of processed products yield is not the soul criteria for evaluating the efficiency of a treatment. Quality of product obtained is of prime importance as it is directly related to the consumer acceptability in the commerce. In the present investigation also, it was observed that the variations in physicochemical parameters of the juice were influenced by different treatments. Higher values of TSS, total and reducing sugars were observed in treatments T_7 (6.80^{o}B, 5.23% and 3.62%, respectively) and T_6 (6.77^{o}B, 5.12% and 3.58%, respectively), where pectinase + cellulose enzymes in combination were involved (Table 2 and Table 3). Higher degree of brix level in enzyme treatment may be attributed to the greater degree of tissue breakdown so it releases more components that contribute to soluble solids [Pilnik *et al.* (1975) [21] and Lellan *et al.* (1985) [16]]. Similarly, due to the effect of these enzymes on the cell wall, neutral sugars such as D-arabinose, D-galactose, L-rhamnose and D-xylose, which are bound to pectic substances, are released and become soluble. Thus total sugars as well as reducing sugars were increased, which ultimately increases TSS (Kumar, 2015) [15]. In another way, when fruits treated with enzymes, the cell wall was collapsed, separated and the nutritional components released from the interial of the cells (Qin *et al.*, 2005) [22]. Similar findings were also reported by Sreenath *et al.* (1994) [26] in pine apple; Anastasakis *et al.* (1987) [2] in carrot; Chandana (2016) [6] in wood apple.

Ascorbic acid retention was noticed higher in T_7 (3.18 mg/100mL) fallowed by T_6 (3.12 mg/100mL). With respect to total titratable acidity, higher values were noticed in T_6 (1.78%) followed by T_7 and T_3 (1.72 each). This might be due to maximum pulp per 100 mL of extract and also probably due to release of carboxyl group from the pectin complex during hydrolysis of the pectin by enzymes (Ghosh and Gangopadhyay, 2002) [10]. On other hand, the minimum pH value recorded in the treatment T_6 (3.13) and T_7 (3.17). This might be due to higher acidity content in the hot water enzymatic extracted pulp. It is a general phenomenon that, as the acidity of the product increases the pH value decreases. This finding was in confirmity with the results of Kotecha and Kadam (2002) [14] and Pattar (2012) [20] in tamarind; Chandana, 2016 [6] and Gowda (2017) [11] in wood apple. Higher values of brix: acid ratio, was noticed in T_7 (3.95). This might be due to maximum TSS and acidity content was noticed in these treatments as brix; acid ratio was calculated by dividing the Brix value by per cent acidity. The brix; acid ratio is often better indicator of acceptability than either sugar or acid alone. Several workers (Jayasena and Cameron, 2008 [12]; Fawole and Opara, 2013) [9] are also of the similar opinion in case of estimates as brix: acid ratio in various crops. Pectin content was found lower (0.56) in T_7. This might be due to the higher degree of breakdown of pectin by pectinase enzyme, whose activity was found maximum in this treatment. Lower amount of pectin content signifies maximum breakdown of pectic substances which is advantageous. Tapre and Jain (2014) [28] also reported that enzymatic treatment of fruit pulp results in breakdown of pectin.

The organoleptic scores of wood apple pulp showed difference between the pectinase, cellulose and combination of pectinase and cellulase enzyme treated treatments with respect to all the five parameters (Table 4). The highest scores for organoleptic characters were observed in pulp extracted with combination of enzymes *i.e.* T_7 and T_6 indicates superiority of the juices. This may be due to the presence of more TSS content, total sugar content, acidity content and pulp per 100 mL of extract with less pH value in the pulp treated with pectinase + cellulase enzymatic action. Apart from this, there was a high correlation between consumer acceptance and brix: acid ratio noticed in these treatments T_7 and T_6.

Table 1: Changes in pulp recovery, pulp per 100 mL of extract, TSS, ascorbic acid, acidity and pH of wood apple pulp as influenced by treatments

Treatments	Pulp recovery (%)	Pulp/100 mL of extract (%)	TSS (ºB)	Ascorbic acid (mg/100 mL)	Acidity (%)	pH
T_1 - Control (Soaking pulp in water at room temperature)	56.39	28.20	5.63	2.90	1.54	3.33
T_2 – Soaking pulp in water + Pectinase (0.1%) at 40 ºC	75.12	37.56	6.37	2.83	1.70	3.23
T_3 - Soaking pulp in water + Pectinase (0.1%) at 50 ºC	78.06	39.03	6.57	2.92	1.72	3.20
T_4 - Soaking pulp in water + Cellulase (0.1%) at 40 ºC	67.05	33.53	6.10	2.66	1.62	3.30
T_5 - Soaking pulp in water + Cellulase (0.1%) at 50 ºC	69.40	34.70	6.23	2.68	1.66	3.27
T_6 - Soaking pulp in water + Pectinase and Cellulase (0.1%) at 40 ºC	79.41	39.70	6.77	3.12	1.78	3.13
T_7 - Soaking pulp in water + Pectinase and Cellulase (0.1%) at 50 ºC	81.11	40.55	6.80	3.18	1.72	3.17
Mean	72.36	36.18	6.35	2.90	1.68	3.23
S.Em±	0.123	0.062	0.036	0.026	0.019	0.029
C.D. @ 1%	0.520	0.260	0.150	0.110	0.080	0.122

Table 2: Changes in brix: acid ratio, pectin, reducing, non-reducing and total sugars of wood apple pulp as influenced by treatments

Treatments	Brix: Acid ratio	Pectin (%)	Reducing sugars (%)	Non-reducing sugars (%)	Total sugars (%)
T_1 – Control (Soaking pulp in water at room temperature)	3.65	0.84	2.95	1.14	4.15
T_2 – Soaking pulp in water + Pectinase (0.1%) at 40 ºC	3.74	0.63	3.36	1.48	4.92
T_3 - Soaking pulp in water + Pectinase (0.1%) at 50 ºC	3.82	0.62	3.32	1.64	5.05
T_4 - Soaking pulp in water + Cellulase (0.1%) at 40 ºC	3.77	0.76	3.26	1.10	4.42
T_5 - Soaking pulp in water + Cellulase (0.1%) at 50 ºC	3.76	0.74	3.30	1.21	4.57
T_6 – Soaking pulp in water + Pectinase and Cellulase (0.1%) at 40 ºC	3.79	0.60	3.58	1.46	5.12
T_7 - Soaking pulp in water + Pectinase and Cellulase (0.1%) at 50 ºC	3.95	0.56	3.62	1.53	5.23
Mean	3.78	0.68	3.34	1.37	4.78
S.Em±	0.028	0.009	0.014	0.018	0.016
C.D. @ 1%	0.118	0.036	0.057	0.075	0.065

Table 3: Changes in organoleptic quality of wood apple pulp as influenced by treatments

Treatments	Colour and appearance	Flavour	Taste	Mouth feel	Overall acceptability
T_1 – Control (Soaking pulp in water at room temperature)	6.83	7.17	6.83	7.00	6.96
T_2 – Soaking pulp in water + Pectinase (0.1%) at 40 ºC	7.50	7.83	7.17	7.67	7.62
T_3 - Soaking pulp in water + Pectinase (0.1%) at 50 ºC	7.67	8.17	7.33	7.83	7.75
T_4 - Soaking pulp in water + Cellulase (0.1%) at 40 ºC	7.17	7.17	7.00	7.33	7.17
T_5 - Soaking pulp in water + Cellulase (0.1%) at 50 ºC	7.33	7.33	7.17	7.33	7.29
T_6 – Soaking pulp in water + Pectinase and Cellulase (0.1%) at 40 ºC	7.67	8.17	7.33	8.00	7.79
T_7 - Soaking pulp in water + Pectinase and Cellulase (0.1%) at 50 ºC	7.83	8.33	7.50	8.17	7.96
Mean	7.43	7.74	7.19	7.62	7.50
S.Em±	0.122	0.167	0.104	0.141	0.052
C.D. @ 1%	0.370	0.702	0.440	0.593	0.220

Conclusion

From the above results, enzymatic extraction by using combination of pectinase and cellulase enzymes at 1.0 g per kg of pulp (0.1%) and incubated for 2 hours at 50 ºC temperature by maintaining constant 4.5 pH can be recommended for getting maximum wood apple pulp yield with good quality and consumer acceptability.

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