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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(9): 2515-2519

www.thepharmajournal.com
Received: 03-07-2022
Accepted: 04-08-2022

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Studies on clarification techniques of Bael fruit Pulp

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Abstract
Bael fruit (*Aegle marmelos*) is a seasonal fruit which belongs to *Rutaceae* family. This fruit is an underutilized subtropical fruit which mainly available in the month of February to May. In traditional systems, each part of the bael tree such as fruit, seed, bark, flowers and leaves have been used for curing various diseases. The bael juice has cloudy or sedimentation in nature and the consumers also feel unappealing with lower shelf life. To develop attractive and clear bael juice, bael pulp was clarified by various techniques. The best clarification was found with pectinase enzyme 0.02% enzyme concentration, along with optimal conditions which includes incubation @ 40 °C for 210 min in shaker incubator followed by centrifuged @ 4000 rpm for 10 min and filtration using muslin cloth and enzyme denaturation by heating @ 90 °C for 5 min.

Keywords: *Aegle marmelos*, bael fruit, bael pulp, clarification, enzymes, sub-tropical

1. Introduction
Bael (*Aegle marmelos*) is one of the most useful medicinal plants of India. It is also known as golden apple or Bengal quince, which belongs to family *Rutaceae*. All the parts of this tree including stem, bark, root, leaves and fruit at all stages of maturity have been used in the indigenous medicine for a long time (Kaur *et al.*, 2017) [10]. India produces 81.9 tonnes of bael (APEDA, 2021) [17]. Orissa produces maximum bael (45.29 tonnes) and it contributes the 55.31% of total production of bael, in India. The Jharkhand produces the second maximum i.e. 33.38 tonnes and it produces 40.76% of total production of bael. The states of Madhya Pradesh, Haryana, Chhattisgarh and Himachal Pradesh are producing about 2.01, 0.78, 0.43 and 0.01 tonnes of bael, respectively.

The bael fruit consists of various nutritional elements such high vitamins, minerals, different fatty acids, amino acids, higher amounts of fibre, glucose and sugar with larger group of phyto-chemicals with organic acids gives nutritious and improved health benefits. The problems of gastrointestinal, diseases such diabetes & cardiac and diseases related to inflammation are cured by the bael fruit (Sarkar *et al.*, 2020) [13]. The different parts of bael are used for various therapeutic purposes, such as for treatment of asthma, anaemia, fractures, healing of wounds, swollen joints, high blood pressure, jaundice, diarrhoea healthy mind and brain typhoid troubles during pregnancy (Sharma *et al.*, 2014) [14]. The unripe dried fruit is astringent, digestive, stomachic and used to cure diarrhea and dysentery. Sweet drink prepared from the pulp of fruits prevents soothing effect on the patients who have just recovered from bacillary dysentery. The ripe fruit is a good and simple cure for dyspepsia (Bhuiyan *et al.*, 2013) [6]. The bael fruit is one of the underutilized fruit and it is a valuable nutritious health house. Utilization of bael has great nutritional, environmental as well as commercial importance. So, the bael is converted into various value added products from pulp such as juice, jam, candy, toffee, leather and nectar etc. The main aim is to conduct this experiment to develop systematic approach for development of clarified juice from bael fruit.

Clarification is a process which “broken” the semi-stable emulsion of colloidal insoluble cloud material supported carbohydrates of a freshly pressed juice such that the viscosity is dropped and the opacity of the cloudy juice is changed to a transparent look. This can be carried out by both enzymatic and non-enzymatic methods (Sharma *et al*., 2017) [15]. Enzymes are an integral component of modern fruit juice manufacturing processes. Their main functions are to increase extraction of juice from raw material, and processing efficiency (pressing, solid settling or removal), with clear and visually attractive final product generation. Juice extraction can be done by using various mechanical processes, which may be achieved through diffusion extraction, decanter centrifuge, screw type juice extractor, fruit pulper and also different types of presses.

Enzymatic treatment for juice extraction and clarification is most common now-a-days. Enzymatic treatment prior to mechanical extraction significantly improves juice recovery compared to any other extraction process. Enzymatic hydrolysis of the cell wall increases the extraction yield, reducing sugars, soluble dry matter content and galacturonic acid content and titrable acidity of the products. Enzymatic degradation of the biomaterial depends upon the type of enzyme, incubation time, incubation temperature, enzyme concentration, agitation, pH and use of different enzyme combinations. The use of the enzymes like cellulases, pectinases, amylases alone and their combination can give better juice yield with superior quality of the fruit juice (Sharma *et al*., 2014) [14]. Fruit contains pectin and other polysaccharides. So, it may lead to fouling during filtration through membrane. Enzymatic treatment leads to degradation of pectin. Enzymatic clarified juice resulted in viscosity reduction and cluster formation, which facilitates separation through centrifugation or filtration results a higher clarity, as well as more concentrated flavour and colour (Abdullah *et al*., 2007) [2].

The yield of juice can be increased by using various extraction methods such as, cold, hot and enzymatic extraction. The clarification of juice by enzymatic treatment increased the juice recovery than cold and hot extraction, significantly. The amylases and pectinases are integral part of fruits. So, it does not give any hazards or chemical residues. The enzyme helps for softening the plant tissue and lead to the release of cell contents which facilitates easy pressing and high amount of juice recovery with quality end products.

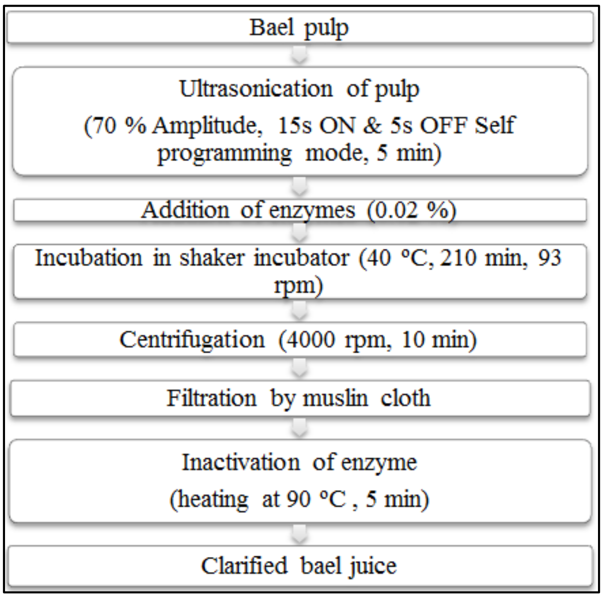

Fig 1: Flowchart for the production of clarified juice from bael pulp

Fig 2: Bael fruits

Fig 3: Enzymes

2. Materials and Methods

The process flowchart for production of clarified juice from bael pulp is presented in Fig. 1. The ripened bael fruits (Fig. 2) (Rajasthani local variety) were procured from ICAR-Directorate of Medicinal and Aromatic Plants Research, Boriavi farm, Anand (Gujarat). The clarification process includes unit operations like ultrasonication, addition of enzyme (Fig. 3) to pulp (Fig. 4), mixing, incubation, centrifugation and filtration. Total eight treatments were carried out for bael pulp clarification. The clarification parameters were selected as per review (Reddy *et al*., 2020; Singh *et al*., 2012) [12, 16].

Where

T_1: Control (no treatment)
T_2: Ultrasonication treatment
T_3: Pectinase treatment
T_4: Amylase treatment
T_5: Pectinase and amylase treatment
T_6: Combined sonication and pectinase treatment
T_7: Combined sonication and amylase treatment
T_8: Combined sonication and pectinase & amylase treatment

The bael pulp was subjected to the ultrasonication treatment using probe type ultra sonicator (20 kHz and 300W Sonics Vibra ®cell) with maximum amplitude of 70% for 5 min (Self-programming mode of 15s ON and 5s OFF) followed by addition of enzyme (0.02%), incubation at 40 ºC for 210 min in shaker incubator with 93 rpm (Saif surgical & scientific equipment, Anand) and then centrifuged @ 4000 rpm for 10 min. The clarified juice was obtained by filtered through muslin cloth cum denaturizing of enzyme.

Fig 4: Bael pulp

Fig 5: Clarified bael juice

The clarified juice (Fig. 5) was subjected for analysis of yield, clarity, total soluble solids, total phenols, ascorbic acid and acidity using standard methods to optimize the best clarification technique of bael.

2.1 Clarified juice yield
The percentage, ratio of clarified juice to the bael pulp is defined as the clarified juice yield (%).

$$\text{Clarified Juice Yield (\%)} = \frac{\text{Clarified juice}}{\text{Bael pulp}} \times 100$$

2.2 Clarity (%T)
Clarity of juice was determined by measuring the absorbance at 660 nm using UV-Visible spectrophotometer and the distilled water was used as a reference.

2.3 Total soluble solids
The total soluble solids of extracted bael pulp, clarified juice and clarified RTS beverage were estimated by using pocket hand refractometer-PAL-1(ERMA, Japan) having measuring range 0-90 °B.

2.4 Total acidity (% citric acid)
The titrable acidity of bael pulp, clarified juice and clarified RTS beverage were estimated by normal titration method. The sample about 1 ml was diluted with distilled water and volume was made up to 10 ml by using volumetric flask. 10 ml of diluted sample was taken for the titration against the 0.1 N NaOH using few drops of 1% phenolphthalin solution as an indicator. Note the titre value. The percentage titrable acidity was calculated by formula given in Ranganna, (2004) [11].

Titrable acidity (% citric acid) =

$$\frac{\text{Titre (ml)x (Normality of NaOH)} \times \text{Dilution} \times \text{Equivalent weight of citric acid}}{\text{Weight of sample taken} \times \text{Volume taken for estimation} \times 1000} \times 100$$

The equivalent weight of citric acid = 64

2.5 Total phenols
Total phenols were estimated as per the methods described by Henríquez *et al.*, (2010) [8]. 1 ml of sample was added to 20 ml of aqueous methanol (20%, v/v) for 18 h at room temperature. The, 0.5 ml of freshly filtered extract sample was taken and diluted with 8 ml of distilled water. 0.5 ml of folin ciocalteau reagent was added and kept at room temperature for 10 min. 1 ml of sodium carbonate (20%) was added and for kept in dark for one hour. The solutions prepared for total phenols absorbance was read at 650 nm using UV visible Spectrophotometer. The same procedure was repeated for all standard gallic acid solutions and a calibration curve was prepared by plotting concentration vs absorbance using gallic acid as standard. Total phenols in the extract samples were quantified from the calibration curve and were expressed as mg GAE phenols/100g.

2.6 Ascorbic acid
The titrimetric method as described by Ranganna. (2004) [11] was used for the determination of ascorbic acid content of bael pulp. Accordingly, 1 g of sample was transferred to volumetric flask and volume made up to 10 ml by adding 3% metaphosphoric acid solution. 5 ml of aliquot was taken from the prepared sample and titrated against standard dye solution. The titration was continued till the light pink colour persisted for 15 s. Initially the dye solution was standardized by titration against the standard ascorbic acid solution and the dye factor was calculated. Thus amount of ascorbic acid (mg) present in 100 g of juice was calculated as follows:

Dye factor = 0.5/ titre value

Ascorbic acid (mg/100g) =

$$\frac{\text{Titre (ml)x (Normality of NaOH)} \times \text{Dilution} \times \text{Equivalent weight of citric acid}}{\text{Weight of sample taken} \times \text{Volume taken for estimation} \times 1000} \times 100$$

3. Results and Discussion
The results were measured (Table 1) and the data was statistically analysed using completely randomized design. The best clarification technique of bael was optimized on the basis of maximum juice yield, ascorbic acid and total phenols.

Table 1: Effect of treatments on clarification of bael pulp

Treatments	Dependent parameters					
	Yield (%)	TSS (ºB)	Clarity (% T)	Total Phenols (mg GAE/100ml)	Ascorbic acid (mg/100ml)	Acidity (%)
T1	26.93	14.00	11.26	3.55	36.12	0.19
T2	28.75	15.00	13.63	3.86	36.12	0.13
T3	47.50	10.67	20.33	3.97	36.12	0.11
T4	43.83	10.33	25.42	3.95	36.12	0.11
T5	43.33	10.67	24.49	3.96	36.12	0.11
T6	42.33	10.00	20.93	3.75	36.12	0.11
T7	38.50	9.67	19.97	3.55	36.12	0.06
T8	41.67	10.67	16.09	3.93	36.12	0.11
S Em	2.24	0.57	8.81	0.31	-	0.02
CD	6.71	1.70	NS	NS	NS	0.06
CV%	9.85	8.61	80.19	14.17	-	31.46

The yield of clarified bael juice varied from 26.93 to 47.5%. The highest yield of clarified juice from bael pulp was achieved with treatment T3 (pectinase) and the lowest yield was achieved with treatment T1 (control). The variation in yield was due to more amounts of pectin, starch and reducing sugars present in bael pulp. Reddy *et al.*, (2020) [12] was reported the rapid and enhanced liquefaction of mango (*Mangifera indica*) cv. Totapuri pulp using ultrasound-assisted enzyme treatment resulted highest yield in ultrasonication assisted (0.2%) pectinase treatment. The similar result was obtained by Singh *et al.*, (2012) [16]. They reported the enzymatic hydrolysis of bael fruit in Kagazi variety with 0.02% pectinase enzyme. The variation in yield of clarified juice from bael pulp is shown in Fig 6.

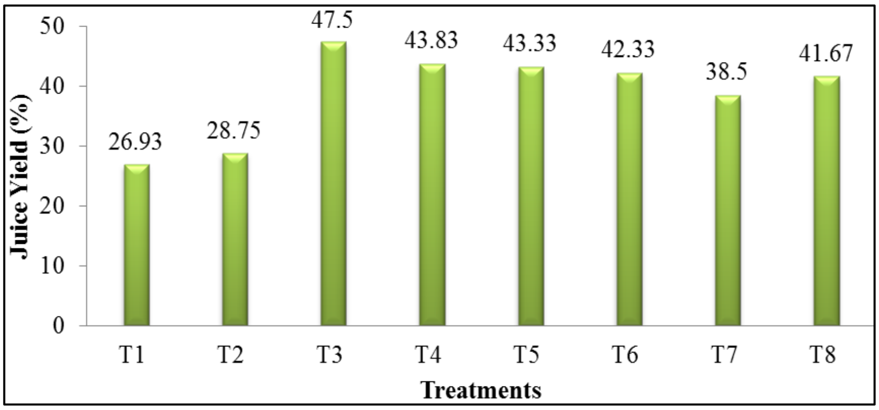

Fig 6: Effect of treatments on yield of clarified bael juice

The TSS of clarified bael juice varied from 14 to 9.67 ºB. T1 (Control) treatment of clarified juice from bael pulp results the highest TSS. The T7 (ultrasonication assisted amylase) treatment of clarified juice from bael pulp resulted lowest TSS. This may be due to higher breakdown of tissue and release of soluble solids. Similar result was reported from Abdullah *et al.*, (2021) [3] in the study of pectinase assisted extraction of cashew apple juice.

The clarity of clarified juice varied from 11.26 to 25.42%T. It was reported that all the eight treatments results non-significant amount of clarity, statistically. This may be due to higher clear juice. Similar result was obtained from clarification of jamun juice using tannase by Ghosh *et al*, (2021) [7]. The variation in clarity of clarified bael juice obtained from bael pulp is shown in Fig 7.

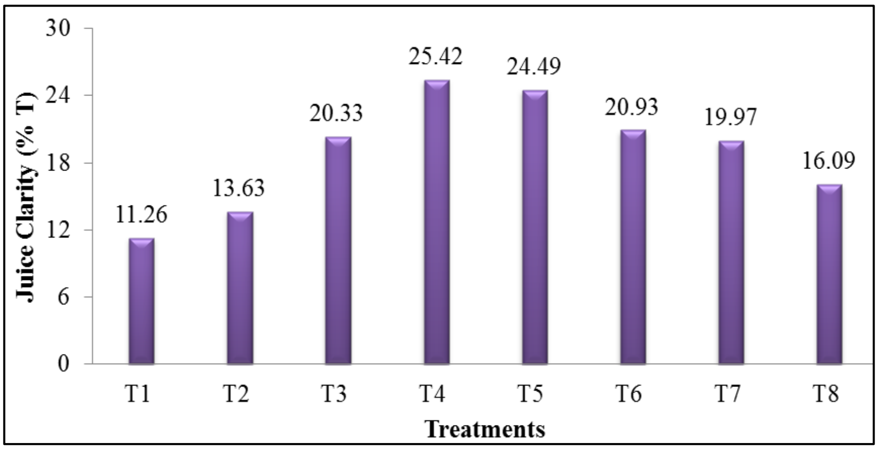

Fig 7: Effect of treatments on clarity of clarified bael juice

The total phenols of clarified juice varied from 3.55 to 3.97 mg/100 ml. It was reported that all the eight treatments results non-significant amount of total phenols, statistically. This may be due to hydrolyzing of bioactive compounds by pectinase. The similar result was reported by Hmid *et al.*, (2016) [9] in the study of clarification of pomegranate juice using protease and pectinase.

The ascorbic acid of clarified juice resulted 36.12 mg/100 ml for all treatments. All eight treatments resulted same amount of ascorbic acid. This may be due to temperature and time combination even though it was sensitive. Similar result was reported from Abdullah *et al.*, (2021) [3] in the study of pectinase assisted extraction of cashew apple juice.

The acidity of clarified juice varied from 0.19 to 0.06%. T1 (Control) treatment results the highest acidity of clarified juice from bael pulp. T4 (ultrasonication assisted amylase) treatment resulted lowest acidity of clarified juice from bael pulp. This may be due to certain acids may be precipitated by flocks development. Similar result was reported from the study of clarification of pomegranate juice by Bayati *et al.*, (2021) [5].

It could be inferred that clarification techniques significantly affects yield, TSS and acidity. Treatment T3 found significantly superior among all for highest yield of clarified juice around 47.5%. So, Treatment T3 was considered best among all eight treatments for clarification of bael pulp. The best clarification was found with pectinase enzyme 0.02% enzyme concentration, along with optimal conditions which includes incubation @ 40 °C for 210 min in shaker incubator followed by centrifuged @ 4000 rpm for 10 min and filtration using muslin cloth and enzyme denaturation by heating @ 90 °C for 5 min.

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