JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

EFFICIENT EXTRACTION AND CLARIFICATION OF FRUIT JUICES USING CONCURRENTLY PRODUCED XYLANO-PECTINOLYTIC ENZYMES

Nancy Sikodia, Bindu Battan, Sulekha Chahal, Jitender Sharma*

Address(es):
Department of Biotechnology, Kurukshetra University, Kurukshetra, India.

*Corresponding author: bbattan@kuk.ac.in

https://doi.org/10.55251/jmbfs.10260

ARTICLE INFO

Received 7. 6. 2023
Revised 2. 2. 2024
Accepted 5. 2. 2024
Published 1. 4. 2024

Regular article

OPEN ACCESS

ABSTRACT

The purpose to effectuate this research work is to investigate the efficiency of crude xylano-pectinolytic enzymes in extraction and clarification of fruit juices, so as to improve their physical (clarity, viscosity and soluble solid), functional (total polyphenolic content) and sensory characteristics (acceptability). In this study, various conditions like enzyme dose, treatment time and stirring speed have been optimized. The extraction and clarification efficiency were found to be optimum at enzyme dose between 2:8 and 4:16 IU/g pulp, treatment time between 30 to 60 min and stirring speed of 50 and 60 rpm for different fruit juices. Enzymatic treatment enhanced the physicochemical, organoleptic and nutritional properties and generated juice with improved yield and clarity. After xylano-pectinolytic enzymatic treatment of various fruit juices, maximum increase in yield (95%), filterability (40%) and maximum decrease in viscosity (58%) was found in case of *Tamarindus indica.* Maximum increase in clarity (39%) and polyphenolic content (37%) was observed in case of *Aegle marmelos*. Maximum increase in reducing sugars was found in case of *Fragaria ananassa* along with other properties. All these attributes of xylano-pectinolytic enzymes indicate an adequate prospect for bio-industrial research.

Keywords: Bio-extraction, filterability, polyphenolic content, reducing sugars, titrable acidity, yield

INTRODUCTION

As awareness about the human health is taking primary seat in today's lives, the health benefits of fruit juices are being recognized and the demand of fresh, crystal clear, less viscous and less turbid juice is increasing day by day. Fruit juice is a rich source of vitamins, dietary fibers and minerals (**Mark *et al*., 2018)**. They contain antioxidants, which aid in the elimination of free radicals and the reduction of oxidative stress in the body. Fruit juice keeps our body hydrated, activate the metabolism and helps in the detoxification of body.

Fruits are more sensitive to microbial activity, which make them highly putrescible with a short shelf life **(Hmar *et al*., 2018)**. Preservation of these fruits for a longer duration is not only energy consuming but also costly. In addition to this, Food and Agriculture Organization (FAO) revealed that approximately 45% of fruits are wasted every year because of post-harvesting spoilage or are repudiated due to lower quality. To overcome this, contemporary techniques are implemented to convert them into superior quality products such as juices, which is simple and easy while maintaining their functional, physical, sensory and organoleptic properties.

Fruit juices obtained by conventional methods such as diffusion, extraction and decanter centrifuges are cloudy, viscous and turbid due to the presence of polysaccharides (Starch, pectin, cellulose and hemicellulose), thus do not meet the consumer expectations and also there is high risk of cross- contamination **(Sharma *et al*., 2017)**. These conventional methods have several disadvantages such as high initial and ongoing cost and low yield **(Vaillant *et al*., 2001; Sulaiman *et al*., 1998)**. As crystal clear and less viscous juice is important to consumers, researchers working in this area are looking for alternative methods to improve this feature **(Nachal *et al*., 2023)**. In many studies, the use of pectinase and xylanase enzyme for clarification of fruit juices has been presented as an efficient alternative to improve yield and clarity **(Kashyap *et al*., 2001; Landbo *et al*., 2007; Pandey *et al*., 2022; Panda *et al*., 2021; Saxena *et al*., 2015)**. These enzymes degrade pectin and xylan in the cell wall, reducing viscosity and facilitating separation by centrifugation and filtration. Consequently, the juice is clearer, with more concentrated flavor and color **(Abdullah *et al*.,2007)**. These enzymes soften plant tissue, thereby causing the release of cell contents, which can be recovered in larger quantities **(Sreenath *et al*., 1984)**.

Xylano-pectinolytic enzymes have emerged as effective concoction for fruit juice extraction and clarification, capable of degrading hemicellulosic polysaccharides, resulting in reduced cloudiness and viscosity, as well as increased nutritional value. A bacterial isolate, *Bacillus pumilus,* that co-produces xylanase and pectinase enzymes, was employed in this investigation. There have been few reports of a microbial isolate co-producing xylano-pectinolytic enzymes under submerged fermentation like *Aspergillus niger F3* **(Rodriguez *et al*., 2018)**, *Aspergillus flavus* **(Mellon, 2015; Pradal-Velázquez *et al*., 2018)**, *Aspergillus fumigatus* MS16 **(Zehra *et al*., 2020)** etc. The co-production of xylano-pectinolytic enzymes using low-cost agro-waste medium will lower the cost of enzyme production and make the process more cost-effective.

The purpose of this research was to demonstrate the application of concoction made of crude xylanase and pectinase enzymes in extraction and clarification of fruit juices. The impact of enzyme concoction on yield, clarity, pH, TSS, TDS, reducing sugars, polyphenolic content, acidity, viscosity and filterability of various fruit juices have been studied by optimizing various conditions such as enzyme dose, treatment time and stirring speed. **Haile *et al*. (2022)** observed clarification in apple, lemon and mango juice and improvement in other qualities when treated with crude pectinase enzyme from *Serratia marcescens.* **Santana *et al*. (2022)** reported improvement in various physico-chemical properties of guava fruit after treatment with crude multi-enzyme treatment from *Rhodotorula mucilaginosa, Rhodotorula orizycola*, and *Pseudozyma* sp.

The cost of producing enzymes is a critical factor in determining whether or not they can be applied in industries. The co-production of enzymes by a single bacterial isolate, as well as use of agricultural waste as a production substrate, can reduce production costs **(Kaur *et al*., 2019)**. Concurrent production of enzymes can save energy and sustaining expenses. Thus, this co-production of xylano-pectinolytic enzymes by a bacterial isolate appears to be acceptable for use in fruit juice industry, lowering the cost of enzyme production and increasing the feasibility of using these enzymes for extraction and clarification. Both xylanase and pectinase enzymes used in this study are produced extracellularly by *B. pumilus* AJK, and no expensive materials were used in their production. This pectinase enzyme preparation also contains a blend of seven enzymes (Pectin esterase, polymethylgalacturonate lyase, polygalacturonate lyase, exo-polymethylgalacturonase, exo-polygalacturonase, endo-polymethylgalacturonase and endo-polygalacturonase) that have been reported to give significant results **(Sharma *et al*., 2019)**. Due to simultaneous extracellular enzyme synthesis using low-cost substrates, the approach is commercially viable for fruit juice industry. This study looks into the treatment of various fruit pulps with crude xylano-pectinolytic enzymes under optimal conditions to evaluate if these enzymes are suitable for extraction and clarification of fruit juices.

MATERIALS AND METHODS

Fruits and chemicals

All the fresh and ripened fruits were purchased locally. Pectin and birchwood xylan were purchased from Sigma, USA. Other chemicals used were of analytical grade and purchased from Himedia, India.

Microorganism and enzymes production

Bacillus pumilus AJK (MTCC Accession No. 10414) was used to co-produce xylano-pectinolytic enzymes. The crude enzymes concoction was produced under submerged fermentation **(Kaur *et al*., 2010)**. Erlenmeyer flasks (250 mL) containing 50 mL of medium (peptone, 0.5 %; MgSO4, 10 mM, pH 7.0), was supplemented with 2% of washed and dried wheat bran and 2% citrus peel. The media was inoculated with 2% of 21-hour-old bacterial culture and the flasks were incubated at 37°C for 60 hours under shaking conditions at 150 rpm. After incubation, crude enzymes were obtained as clear supernatant by centrifugation at 10,000 rpm for 15 minutes, and stored at 4°C until further use. The bacterium produced 270 IU/mL of xylanase and 70 IU/mL of pectinase.

Enzymes assay conditions

Xylanase and pectinase enzyme activities were determined using 3, 5-dinitrosalicylic acid and the reducing sugars liberated from birchwood xylan (2%, prepared in 0.1 M glycine-NaOH buffer, pH 8.5) and polymethylgalacturonic acid (1%, prepared in 0.1 M glycine-NaOH buffer, pH 9.0) were measured (Miller, 1959).

Pulp preparation

All the fruits were washed with tap water, deseeded manually and blended using food processor with appropriate amount of distilled water. No dilution was done for *Citrullus lanatus* because it already contains 92% of water.

Evaluation of extraction and clarification conditions

To determine the best conditions for extraction and clarification of fruit juices, pulp of various fruits was treated with crude xylano-pectinolytic enzymatic concoction. The pH of fruit pulps was adjusted to 6.0 and all the experiments were performed at 50°C temperature. For efficient removal of xylan and pectin, conditions such as enzyme dose (1:4 – 5:20), treatment time (40-80 min), and stirring speed (40-80 rpm) were optimized for maximum yield, clarity, and enhanced qualities. Following each enzymatic treatment, the suspension was immediately cooled in an ice water bath kept at 4ºC to stop the enzymatic reactions before being centrifuged at 2000 g for 5 minutes. The obtained supernatant (juice) was tested for yield, clarity and other quality characteristics. Simultaneously control experiments were also carried out.

Analysis of physicochemical parameters

Various analyses were carried out for each juice sample in order to evaluate the parameters like yield, clarity, pH, reducing sugars, TSS, TDS and polyphenolic content. Other parameters like viscosity, acidity, and filterability were also analyzed.

Determination of yield, pH, TSS and TDS

The volume of supernatant obtained following pulp treatment with enzymes was measured to calculate the yield of the juice, which is stated in terms of mL per 100 g of pulp and pH was measured using digital pH meter (Eutech). On the other hand, total soluble solids (TSS), expressed in ºBrix and TDS were measured using a hand-held refractometer and TDS tester (Eutech), respectively.

Determination of clarity

The clarity was determined by measuring percentage transmittance of juices at a wavelength of 660 nm using UV-vis spectrophotometer taking distilled water as blank.

Determination of reducing sugars

Following the enzymatic treatment, the amount of reducing sugar in the juices was measured using DNS method **(Miller, 1959)**. The results were represented as the amount of reducing sugar in g per 100 g of pulp using a standard galacturonic acid curve.

Determination of polyphenolic content

Total phenolic content was determined using the Folin-Ciocalteu reagent **(Lin and Tang, 2007)**. Fruit juice (0.1 mL) was diluted appropriately and combined with 2.8 mL of deionized water, 2 mL of sodium carbonate (2%), and 0.1 mL of 50% Folin-Ciocalteau reagent followed by incubation at room temperature for 30 minutes. After that, absorbance of reaction mixture was measured at 750 nm against deionized water used as a blank. Gallic acid was employed as the benchmark. The total phenolic content of fruits was assessed in triplicates using a six-point standard curve (0-200 mg/L), and the results are shown as mg gallic acid equivalents (GAE)/100 g of pulp.

Determination of viscosity and filterability

The viscosity of each juice was measured using a glass capillary viscometer, also known as an Ostwald viscometer and expressed in centipoise (cp). Suction mechanism was applied to draw the juices into the upper bulb, and they were then allowed to flow through the capillary into the lower bulb. Using a stopwatch, the length of time it takes for the juice level to pass between the two markings (one above and one below the upper bulb) is proportional to the kinematic viscosity. On the other hand, the filterability (sec^{-1}) was calculated from the reverse of the time taken to filter juice through a whatman filter paper No. 1 under vacuum.

Determination of acidity

Using starch solution (0.5%) as an indicator, 5 mL of diluted juice (1:4, juice: distilled water) was titrated with 0.005 mol/L iodine solution to estimate the overall acidity. Completion of titration process was indicated by appearance of blue color. A comparison was made between the amount of iodine used to titrate each juice and the amount used to titrate ascorbic acid at 0.001% (w/v). The outcomes are given in mg ascorbic acid equivalent/100 g of pulp.

RESULTS

Enzymatic extraction and clarification strategy was tried for increasing the yield, clarity and enhancing various physico-chemical parameters of fruit juices with the concept that xylanase and pectinase enzymes hydrolyse xylan and pectin contents respectively, present in the cell wall of fruits and ultimately would help in better release of crystal clear and less viscous juice with enhanced qualities. So, the commercial preferability and acceptability of fruit juices would increase. The results obtained are discussed under the following heads:

Optimization of extraction and clarification conditions

In order to achieve the best yield and quality of fruit juices, various conditions were examined (Table 1). The enzymatic treatment result showed that the pectinase: xylanase (IU/g) enzyme dose of 3:12 for *Actinidia chinensis* and *Fragaria ananassa*, 2:8 for *Pyrus communis* and *Citrullus lanatus* and 4:16 for *Manilkara zapota*, *Aegle marmelos* and *Tamarindus indica* was found to be optimum. A treatment time of 50 minutes and 30 minutes was found to be optimum for *Actinidia chinensis* and *citrullus lanatus*, respectively, whereas for the rest of the fruits duration of 60 minutes was found to be optimum. A stirring speed of 60 rpm was found to give good results for all the fruits except *Citrullus lanatus* for which 50 rpm was optimum in our studies.

Table 1 Optimum enzyme dose, treatment time and stirring speed for extraction and clarification of different fruit juices

S.No.	Fruits	Enzyme dose (IU/g) Pectinase: xylanase	Treatment time (Min)	Stirring speed (rpm)
1	*Actinidia chinensis*	3:12	50	60
2	*Pyrus communis*	2:8	60	60
3	*Manilkara zapota*	4:16	60	60
4	*Citrullus lanatus*	2:8	30	50
5	*Aegle marmelos*	4:16	60	60
6	*Fragaria × ananassa*	3:12	60	60
7	*Tamarindus indica*	4:16	60	60

Effect of enzymatic treatment on various parameters

To appraise the potential of enzymatic extraction and clarification, both control and enzyme treated samples of fruit juices were tested for various parameters (Table 2 and 3).

Table 2 Comparison of yield, pH, clarity, TSS and reducing sugars before and after enzymatic treatment

S.No.	Parameters		Fruits						
			Actinidia chinensis	***Pyrus communis***	***Manilkara zapota***	***Citrullus lanatus***	***Aegle marmelos***	***Fragaria × ananassa***	***Tamarindus indica***
1	Yield (mL/100 g pulp)	C	68±2.72	62±3.28	59±2.36	76.4±3.04	64±2.56	68±2.72	60±2.4
		T	102±4.08	107±4.28	113±4.52	92.9 ±3.71	121±4.84	99±3.96	117±4.68
2	pH	C	6.00 ±0.24	6.00 ±0.24	6.00 ±0.24	6.00 ±0.24	6.00 ±0.24	6.00 ±0.24	6.00 ±0.24
		T	5.80±0.23	5.82 ±0.23	5.76 ±0.23	5.91 ±0.23	5.80±0.232	5.86±0.234	5.75±0.230
3	Clarity (%T at 660 nm)	C	9.4 ±0.38	8.01 ±0.32	1.78 ±0.07	14.8 ±0.59	6.60 ±0.26	14.65±0.58	8.6±0.34
		T	12.47±0.4	11.01±0.43	4.15 ±0.08	18.09±0.72	9.22±0.36	18.76±0.75	10.9±0.43
4	TSS (°Brix)	C	7.7 ±0.31	12.45 ±0.4	14.6 ±0.58	5.02 ±.0.20	33.11 ±1.3	10.2±0.40	35.9±1.44
		T	10.4 ±0.41	13.31±0.57	21.6±0.67	6.6 ±0.26	37.11 ±1.4	13.2±0.52	39.0±1.51
5	Reducing sugar (g/ 100 g of pulp)	C	7.3 ±0.28	7.6 ±0.3	10.7 ±0.43	3.4 ±0.144	3.3 ±0.128	4.0±0.324	5.1±0.20
		T	9.8 ±0.39	9.8 ±0.396	15.6 ±0.61	4.9 ±0.196	4.8 ±0.188	6.3±0.368	7.4±0.28

C=Control, T=Test

Table 3 Comparison of polyphenolic content, TDS, viscosity, acidity and filterability before and after enzymatic treatment

S.No.	Parameters		Fruits						
			Actinidia chinensis	***Pyrus communis***	***Manilkara zapota***	***Citrullus lanatus***	***Aegle marmelos***	***Fragaria × ananassa***	***Tamarindus indica***
1	Polyphenolic content (mg GA/100g pulp)	C	60.4 ±2.42	17.7 ±0.70	350.6±14.0	72.5±2.88	15.59 ±0.6	110.9±4.46	270.0±10.8
		T	73.6 ±2.94	23.8 ±0.95	421.±16.84	90.4±3.61	21.39 ±0.8	135.8±5.42	320.6±12.81
2	TDS (ppt)	C	2.71±0.109	1.52 ±0.060	1.95 ±0.079	1.32 ±0.052	2.78 ±0.110	1.53±0.060	1.50±0.06
		T	3.30±0.13	2.03±0.080	2.58 ±0.10	1.68 ±0.06	3.38 ±0.13	1.99±0.079	1.86±0.072
3	Viscosity (cp)	C	1.80 ±0.07	4.0 ±0.15	4.16 ±0.16	1.93 ±0.07	4.26 ±0.16	3.53±0.142	1.9±0.076
		T	1.24±0.04	2.95 ±0.11	3.89 ±0.15	1.52±0.060	3.46 ±0.13	2.92±0.118	1.2±0.064
4	Acidity (mg/ 100g pulp)	C	89.9±3.60	5.4 ±0.212	13.4 ±0.52	7.7±0.31	17.8 ±0.71	89.8±3.58	160.2±6.40
		T	94.9 ±3.79	7.5 ±0.292	15.4 ±0.60	9.1 ±0.36	20.4±0.81	95.0±3.72	199.6±7.96
5	Filterability (Sec^{-1})	C	0.0239±0.009	0.0529±0.0021	0.03948±0.0015	0.019±0.0007	0.04575±0.001	0.0519±0.0020	0.0496±0.001
		T	0.0344±0.013	0.0688±0.0027	0.05162±0.0020	0.025±0.0010	0.05949±0.002	0.0668±0.0026	0.0698±0.002

C=Control, T=Test

Effect on yield

The results showed that under best enzymatic conditions, maximum increase in yield was observed in case of *Tamarindus indica* (95%) followed by *Manilkara zapota* (91.52%), *Aegle marmelos* (89.06%), *Pyrus communis* (72.58%), *Actinidia chinensis* (50%), *Fragaria ananassa* (45.58%), and least increase in yield was observed in case of *Citrullus lanatus* (21.59%).

Effect on pH

Our results demonstrated that 1- 4% decrease in pH was observed in different fruit juices. Maximum decrease in pH was observed in case of *Tamarindus indica* (4.34 %) followed by *Manilkara zapota* (4.16), *Actinidia chinensis* and *Aegle marmelos* (3.44%), *Pyrus communis* (3.09%), *Fragaria ananassa* (2.38%) and least decrease in pH was observed in case of *Citrullus lanatus* (1.5%).

Effect on TSS

The results showed that an increase of nearly 47.94% TSS was observed in case of *Manilkara zapota* followed by 35.06% in case of *Actinidia chinensis*, 31.47% in case of *Citrullus lanatus*, 29.41% in case of *Fragaria ananassa*, 15.14% in case of *Pyrus communis*, 12.08% in case of *Aegle marmelos* whereas least increase in TSS was observed in case of *Tamarindus indica* (8.03%).

Effect on TDS

In our study, after enzymatic treatment, maximum increase in TDS was observed in case of *Pyrus communis* (33.55%) followed by *Manilkara zapota* (32.30), *Fragaria ananassa* (30.0%), *Citrullus lanatus* (27.27), *Tamarindus indica* (24.0%) while least increase was recorded for *Actinidia chinensis* and *Aegle marmelos* (21.0%).

Effect on clarity

The results showed that under optimized enzymatic conditions, maximum increase in clarity was observed in case of *Aegle marmelos* (39.69%) followed by *Pyrus communis* (37.45%), *Actinidia chinensis* (32.65%), *Fragaria ananassa* (28.05%), *Tamarindus indica* (26.74%), *Citrullus lanatus* (27.2) and least increase in clarity was noticed in case of *Manilkara zapota* (20.57%).

Effect on reducing sugar

Our studies showed that maximum release of reducing sugars was observed in case of *Fragaria ananassa* (57.5%) followed by *Manilkara* zapota, *Aegle marmelos*, *Citrullus lanatus* and *Tamarindus indica* (44- 45%), *Actinidia chinensis* (34.24%) and least increase in reducing sugars was found in case of *Pyrus communis* (28.94%).

Effect on polyphenolic content

The results showed that after enzymatic treatment, maximum increase in polyphenolic content was observed in case of *Aegle marmelos* (37.20%) followed by *Pyrus communis* (34.46%), *Citrullus lanatus* (24.68%), *Fragaria ananassa* (22.45), *Actinidia chinensis* (21.85%), *Manilkara zapota* (20.25%) while least increase was observed in case of *Tamarindus indica* (18.74%).

Effect on viscosity

Maximum decrease in viscosity after xylano-pectinolytic treatment, was noticed in case of *Tamarindus indica* (58.33%) followed by *Actinidia chinensis* (45.16%), *Pyrus communis* (35.59%), *Aegle marmelos* (23.12%), *Fragaria ananassa* (20.89%) and least decrease was observed in case of *Manilkara zapota* and *Citrullus lanatus* (12.0%- 14.0%).

Effect on filterability

The studies demonstrated that maximum increase in filterability was observed in case of *Tamarindus indica* (40.36%) followed by *Actinidia chinensis* (38.21%) while *Citrullus lanatus*, *Fragaria ananassa*, *Pyrus communis*, *Manilkara zapota* and *Aegle marmelos* showed a similar level of increase in filterability i.e., in the range of 28.0% to 30.0%.

Effect on acidity

The results showed that maximum increase in acidity was obtained in case of *Pyrus communis* (38.88%) followed by *Tamarindus indica* (24.59%), *Manilkara* zapota, *Citrullus lanatus* and *Aegle marmelos* (14.0%- 18.0%) whereas least increase in acidity was noticed in case of *Fragaria ananassa and Actinidia chinensis* which was only 5 to 6%.

Absorption spectra of all samples were taken from 200-800 nm in order to determine the release of various phenolics or other related components. Several peaks were observed in the region of 320-380, signify the release of several phenolics compounds (Fig 1-7).

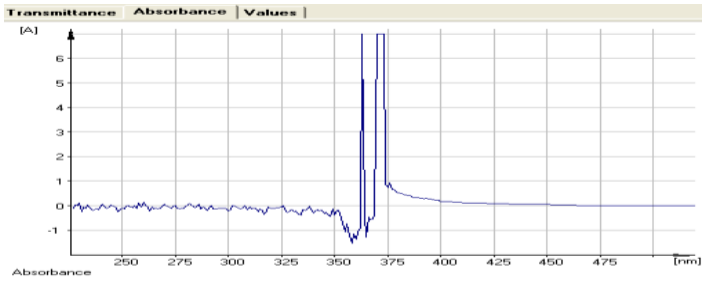

Figure 1 Absorption spectra of *Actinidia sinensis* control verses enzymes treated fruit juice

Figure 2 Absorption spectra of *Pyrus communis* control verses enzymes treated fruit juice

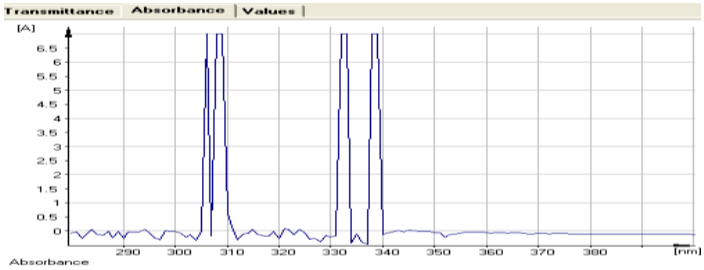

Figure 3 Absorption spectra of *Manilkara zapota* control verses enzymes treated fruit juice

Figure 4 Absorption spectra of *Citrullus vulgaris* control verses enzymes treated fruit juice

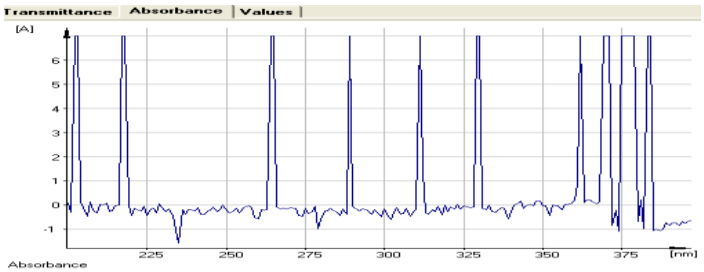

Figure 5 Absorption spectra of *Aegle marmelos* control verses enzymes treated fruit juice

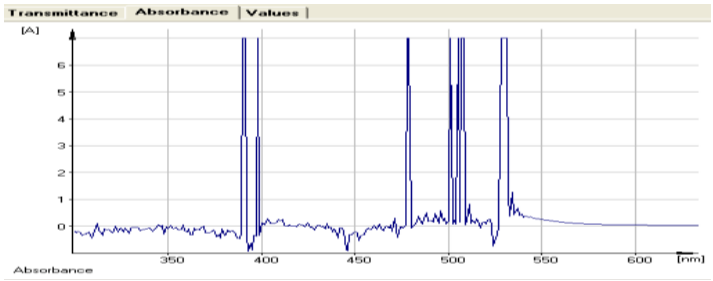

Figure 6 Absorption spectra of *Fragaria ananassa* control verses enzymes treated fruit juice

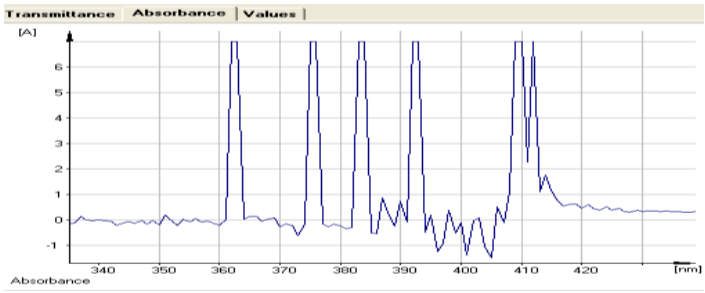

Figure 7 Absorption spectra of *Tamarindus indica* control verses enzymes treated fruit juice

DISCUSSION

In order to achieve the best yield and quality of fruit juices, various conditions (enzyme dose, treatment time and stirring speed) were examined and best conditions were found after experimentations. Different fruits showed different optimization values for various parameters. Pectinase: xylanase dose between 2:8 to 4:16 was found to be optimum and enzymatic dose beyond optimized values did not further increase the efficiency of extraction and clarification of fruit juices. Higher amount of positively charged protein was produced when the enzyme concentration was increased. This resulted in the aggregation of larger particles because it lessened the electrostatic attraction between cloud particles. However finally, these particles settled down **(Kaur *et al*., 2011)**. The enzyme dose of 3 U/ml of polygalacturonase from *Aspergillus japonicas* (PGAj) was found to be optimum for clarification of Palmer and Tommy mango varieties, white guava, banana and apple by **Guimaraes *et al*. (2022)**. **Azar *et al*. (2020)** have reported that 10 IU/ml of purified polygalacturonase enzyme from *Calonectria pteridis* was found to be optimum for apple juice clarification.

Time period in the range of 30 to 60 minutes was found to be best for enzymatic extraction of different fruits. Optimum time period is required for effective enzymatic action on the fruit pulp, beyond which, it may affect the sensory qualities of juice. **Amin *et al*. (2021)** have also reported that treatment time period of 60 min was found to be optimum for extraction of apple, peach and grapes juices with exo-polygalacturonase from *Penicillium fellutanum*.

Stirring speed of 50 to 60 rpm was found to be best for extraction of all the fruits. Stirring during the extraction and clarification process enables the ambient oxygen to be mixed in the reaction medium. This mixing increases the amount of dissolved oxygen in the juice, which raises the amount of substrate for the oxidation of polyphenols, which would have contributed to browning by encouraging mono-phenolase activity **(Ninga *et al*., 2021)**.

The results showed that enzymatic treatment of fruit pulp resulted in enhancement of yield up to 95% and minimum being 21.5%. This is because treatment of xylanase and pectinase enzymes break down the xylan and pectin respectively, loosening the cell wall and releasing the juice maximally which is entrapped in the vacuole. Other workers have reported less increase in yield after treatment with different enzymes or their concoctions. **Azar *et al*. (2020)** treated apple fruit with purified polygalacturonase from *Calonectria pteridis* that resulted in 10.65% increase in volume. The extraction of kiwi fruit juice using pectinase, amylase and mesh enzymes concoction resulted in 20.02% increase in yield **(Vaidya *et al*., 2009)**. **Joshi *et al*. (2011)** revealed that in case of pear fruit, 12% increase in yield was obtained when treated with pectinase enzyme. Extraction of bael fruit juice using pectinase enzyme resulted in 17.5% increase in juice yield **(Singh *et al*., 2012)**. **Joshi *et al*. (2012)** observed 92.4% juice recovery in case of tamarind fruit when treated with pectinase enzyme but this again is lesser as compared to our results in case of tamarind, which is 95%.

The enzymatic treatment showed up to 4% decrease in pH for different fruit juices. This may be due to enhancement in galacturonic acid monomer liberation during hydrolysis of pectin **(Wang *et al*., 2007)**. The reduction in pH may also be due to ascorbic acid liberation during pectin hydrolysis may also be the reason of reduction in pH **(Akesovan *et al*., 2013)**. The release of carboxylic groups (acid groups) during pectin hydrolysis may potentially be responsible for pH drops after enzyme treatment.

After enzymatic treatment, significant increase in TSS (up to 47%) was observed. This might be explained by the breakdown of pectin, which liberates galacturonic acid monomers that can be absorbed to glucose molecules and boost the TSS. The enhancement in TSS depict the tissue breakdown at higher degree, resulting in release of compounds such as sugars, which contribute to soluble solids **(Chang *et al*., 1995)**. **Yosuf *et al*. (1994)** observed that treatment of soursoup with commercial pectinex ultra-SPL enzyme significantly enhanced TSS from 6.8 °Brix to 7.3 °Brix. **Joshi *et al*. (2011)** have reported that treatment of plum, peach, pear and apricot with pectinase enzyme resulted in increase in TSS from 12.20 °Brix to 14.10 °Brix, 9.0 °Brix to 14.27 °Brix, 10.17 °Brix to 12.20 °Brix and 8.60 °Brix to 11.20 °Brix, respectively.

Approximately 21% to 33.5% increase in TDS was observed after enzymatic extraction of different fruit juices. This is due to release of Ca^{2+} and Mg^{2+} ions when xylano-pectinolytic enzymes break down Ca and Mg pectate, and TDS meter primarily count these ions by monitoring the current flowing between the electrodes.

A significant increase in clarity (up to 39.6%) was observed after our enzyme treatment, this may be because cloud particles are made up of positively charged protein-carbohydrate cores encased in negatively charged pectin molecules. Pectin delays aggregation by covering the particles and charging them sufficiently to cause strong electrostatic attraction between them. Due to their small size, mutual charge repulsion between them and the shielding effect of pectin, these turbidity-causing particles are thus kept in suspension. Enzyme catalysis results in electrostatic destabilization leading to creation of ions, which then causes electrostatic aggregation increasing the size of the particles. The larger particle size causes a floc to form, which settles out and allows the clarified juice to be retrieved **(Yamasaki *et al*., 1964)**. **Guimaraes *et al*. (2022)** have reported that treatment of different varieties of mango (Tommy and Palmer variety), apple, banana and white guava with polygalacturonase from *Aspergillus japonicus*, resulted in 41%, 65%, 9.4%, 11% and 40 % increase in transmittance.

Enzymatic treatment of various fruit pulps resulted in up to 57.5% enhancement in amount of reducing sugars. The release of more solid components, including sugars, and a larger degree of tissue disintegration may be linked to the higher value of reducing sugars. **Azar *et al*. (2020)** have reported that apple juice clarification with purified polygalacturonase from *Calonectria pteridis* resulting in 40.93% increase in reducing sugars.

Results also showed 37.20% enhancement in polyphenolic content, which may be due to their more bioaccessiblity when enzymes are employed. The release of cell wall-bound phytonutrients, particularly polyphenols from the fruit matrix is enhanced by the breakdown of pectic and cellulosic polysaccharides in the cell wall and middle lamella.

Viscosity is frequently regarded as being crucial to the quality of liquid foods. Pectin, a substance with a high degree of hydroxylation, is hydrolyzed, releasing oligomers of galacturonic acid, an acid with a lower degree of hydroxylation. The release of water molecules would result in a reduction of viscosity since hydrolysis would be accompanied by a decrease in the system's ability to retain water **(Akesowan *et al*., 2013)**. A significant decrease in viscosity (up to 58.33%) of different juices was observed due to breakdown of xylan and pectin in the cell wall by the action of our enzymes. Enzymatic hydrolysis of polymeric carbohydrates increases the degree of liquefaction and reduces the viscosity of fruit juices. As a result, the enzyme treated juices require less time to filter via a Whatman filter paper. Up to 40.3% increase in filterability was also obtained after enzymatic treatment. **Sin *et al*. (2006)** reduced viscosity by exerting a hydraulic action on the cellulose and pectin present in the juices. **Singh *et al*. (2012)** have reported 20.7% decrease of viscosity in case of bael fruit. **Ninga *et al*. (2021)** have revealed 91% reduction in viscosity after hydrolysis of guava pulp with pectinase enzyme.

Enzymatic treatment of various fruit pulps resulted in up to 38.8% increase in titratable acidity. This could be due to better release of ascorbic acid during the hydrolysis of cell wall.

CONCLUSION

The result obtained in this study showed that pre-treatment of fruit pulp with xylano-pectinolytic enzymes enhanced the juice yield, clarity and various quality characteristics. This study concluded that enzymatic treatment is an effective strategy for the production of crystal clear and less viscous juice along with enhanced qualities. Further, as the enzymes have been derived from the microbial source produced on readily available waste lignocellulosics, the technology becomes more cost-effective and sustainable. This study shows that, xylano-pectinolytic enzymatic method for juice extraction and clarification can be used by juice industry for better quality juices.

Acknowledgement: Authors are thankful to Kurukshetra University, Kurukshetra, India for providing space and research facilities.

FUNDING

The authors gratefully acknowledge the financial support given by Council of Scientific and Industrial Research (CSIR) to Ms. Nancy in the form of JRF.

COMPLIANCE WITH ETHICAL STANDARDS

CONFLICT OF INTEREST- The authors declare that they have no conflict of interests.

STATEMENT ON THE WELFARE OF ANIMALS- This article does not contain any studies involving animals or human participants performed by any of the authors.

AUTHORS' CONTRIBUTION

Nancy Sikodia: Writing – original draft, all experimental work mentioned in this manuscript and also whole manuscript written by first author under the supervision of corresponding author. **Bindu Battan**: Conceptualization and Supervision. **Sulekha Chahal**: Data analysis. **Jitender Sharma:** Manuscript editing

REFERENCES

Akesowan, A., & Choonhahirun, A. (2013). Effect of enzyme treatment on guava juice production using response surface methodology. Journal of Animal and Plant Sciences, 23, 114–120.

Amin, F., Arooj, T., Nazli, Z., Bhatti, H. N., & Bilal, M. (2021). Exo-polygalacturonase production from agro-waste by *Penicillium fellutanum* and insight into thermodynamic, kinetic, and fruit juice clarification. Biomass Conversion and Biorefinery, 13, 11141–11151. https://doi.org/10.1007/s13399-021-01902-2

Cavalieri de Alencar Guimaraes, N., Glienke, N. N., Silva Galeano, R. M., Ruller, R., Zanoelo, F. F., Masui, D. C., & Giannesi, G. C. (2022). Polygalacturonase from *Aspergillus japonicus* (PGAj): Enzyme production using low-cost carbon source, biochemical properties and application in clarification of fruit juices. Biocatalysis and Agricultural Biotechnology, 39, 102233. https://doi.org/10.1016/j.bcab.2021.102233

Chang, T. S., Siddiq, M., Sinha, N. K., & Cash, J. N. (1995). Commercial pectinases and the yield and quality of Stanley plum juice. Journal of Food Processing and Preservation, 19(2), 89–101. https://doi.org/10.1111/j.1745-4549.1995.tb00280.x

Dreher, M. L. (2018). Whole fruits and fruit fiber emerging health effects. Nutrients, 10(12), 1833. https://doi.org/10.3390/nu10121833

Haile, S., Masi, C., & Tafesse, M. (2022). Isolation and characterization of pectinase-producing bacteria (*Serratia marcescens*) from avocado peel waste for juice clarification. BMC Microbiology, 22(1), 145. https://doi.org/10.1186/s12866-022-02536-8

Hmar, B. Z., Mishra, S., & Pradhan, R. C. (2018). Design, fabrication, and testing of a pulper for Kendu (*Diospyros melanoxylon Roxb.*). Journal of Food Process Engineering, 41(1), e12642. https://doi.org/10.1111/jfpe.12642

Joshi, A. A., Kshirsagar, R. B., & Sawate, A. R. (2012). Studies on standardization of enzyme concentration and process for extraction of tamarind pulp, variety Ajanta. Journal of Food Processing and Technology, 3(2), 1–3.

Joshi, V. K., Parmar, M., & Rana, N. (2011). Purification and characterization of pectinase produced from apple pomace and evaluation of its efficiency in fruit juice extraction and clarification. Indian Journal of Natural Products and Resources, 2(2), 189–197.

Kashyap, D. R., Vohra, P. K., Chopra, S., & Tewari, R. (2001). Applications of pectinases in the commercial sector: A review. Bioresource Technology, 77(3), 215–227. https://doi.org/10.1016/s0960-8524(00)00118-8

Kaur, A., Mahajan, R., Singh, A., Garg, G., & Sharma, J. (2010). Application of cellulase-free xylano-pectinolytic enzymes from the same bacterial isolate in biobleaching of kraft pulp. Bioresource Technology, 101(23), 9150–9155. https://doi.org/10.1016/j.biortech.2010.07.020

Kaur, A., Singh, A., Dua, A., & Mahajan, R. (2017). Cost-effective and concurrent production of industrially valuable xylano-pectinolytic enzymes by a bacterial isolate *Bacillus pumilus* AJK. Preparative Biochemistry and Biotechnology, 47(1), 8–18. https://doi.org/10.1080/10826068.2016.1155059

Kaur, A., Varghese, L. M., & Mahajan, R. (2019). Simultaneous production of industrially important alkaline xylanase-pectinase enzymes by a bacterium at low cost under solid-state fermentation conditions. Biotechnology and Applied Biochemistry, 66(4), 574–585. https://doi.org/10.1002/bab.1757

Kaur, S., Sarkar, B. C., Sharma, H. K., & Singh, C. (2011). Response surface optimization of conditions for the clarification of guava fruit juice using commercial enzyme. Journal of Food Process Engineering, 34(4), 1298–1318. https://doi.org/10.1111/j.1745-4530.2009.00414.x

Ladeira Azar, R. I. S., da Luz Morales, M., Piccolo Maitan-Alfenas, G., Falkoski, D. L., Ferreira Alfenas, R., & Guimarães, V. M. (2020). Apple juice clarification by a purified polygalacturonase from *Calonectria pteridis*. Food and Bioproducts Processing, 119, 238–245. https://doi.org/10.1016/j.fbp.2019.11.013

Landbo, A. K., Kaack, K., & Meyer, A. S. (2007). Statistically designed two step response surface optimization of enzymatic prepress treatment to increase juice yield and lower turbidity of elderberry juice. Innovative Food Science

and Emerging Technologies, 8(1), 135–142. https://doi.org/10.1016/j.ifset.2006.08.006
Liew Abdullah, A. G., Sulaiman, N. M., Aroua, M. K., & Megat Mohd Noor, M. J. (2007). Response surface optimization of conditions for clarification of carambola fruit juice using a commercial enzyme. Journal of Food Engineering, 81(1), 65–71. https://doi.org/10.1016/j.jfoodeng.2006.10.013
Lin, J. Y., & Tang, C. Y. (2007). Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chemistry, 101(1), 140–147. https://doi.org/10.1016/j.foodchem.2006.01.014
Mellon, J. E. (2015). Extracellular xylanolytic and pectinolytic hydrolase production by *Aspergillus flavus* isolates contributes to crop invasion. Toxins, 7(8), 3257–3266. https://doi.org/10.3390/toxins7083257
Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugars. Analytical Chemistry, 31(3), 426–428. https://doi.org/10.1021/ac60147a030
Nachal, N., Pegu, K., & Arya, S. S. (2023). Enhancement of physicochemical stability and reduction in enzyme and microbial activity of apple juice by Hydrodynamic Cavitation processing. Journal of Agriculture and Food Research, 100797. https://doi.org/10.1016/j.jafr.2023.100797
Ninga, K. A., Carly Desobgo, Z. S., De, S., & Nso, E. J. (2021). Pectinase hydrolysis of guava pulp: Effect on the physicochemical characteristics of its juice. Heliyon, 7(10), e08141. https://doi.org/10.1016/j.heliyon.2021.e08141
Panda, G., Vivek, K., Mishra, S., & Pradhan, R. C. (2021). Characterization and optimization of process parameters for enzyme assisted extraction of kendu (*Diospyros melanoxylon Roxb*.) fruit juice. International Journal of Fruit Science, 21(1), 299–311. https://doi.org/10.1080/15538362.2021.1873220
Pandey, C., Sharma, P., & Gupta, N. (2022). Engineering to enhance thermostability of xylanase: For the new era of biotechnology. Journal of Applied Biology and Biotechnology, 11(2), 1–14. https://doi.org/10.7324/JABB.2023.110204
Pradal-Velázquez, M., Martínez-Trujillo, M. A., & Aguilar-Osorio, G. (2018). Xylanases and pectinases of *Aspergillus flavus* cect-2687 on different carbon sources and initial pH values. Revista Mexicana de Ingenieria Quimica, 17(2), 421–431.
Rodríguez-Fernández, D. E., Rodríguez-León, J. A., de Carvalho, J. C., Sturm, W., W., & Soccol, C. R. (2011). The behaviour of kinetic parameters of pectinase and xylanase by solid-state fermentation. Bioresource Technology, 102(22), a10657–a10662.
Santana, M. L., Tavares, I. M. C., Costa, F. S., Teshima, E., de Sena, A. R., Franco, M., & de Assis, S. A. (2022). The improvement of guava (*Psidium guajava*) juice quality using crude multi-enzymatic extracts obtained from yeasts. Biotechnology and Applied Biochemistry. https://doi.org/10.1002/bab.2441
Saxena, S. (2015). Microbial enzymes and their industrial applications. Applied Microbiology, 121–154.
Sharma, D., Sharma, G., & Mahajan, R. (2019). Development of strategy for simultaneous enhanced production of alkaline xylanase-pectinase enzymes by a bacterial isolate in short submerged fermentation cycle. Enzyme and Microbial Technology, 122, 90–100. https://doi.org/10.1016/j.enzmictec.2018.12.008
Sharma, H. P., Patel, H., & Sugandha. (2017). Enzymatic added extraction and clarification of fruit juices–A review. Critical reviews in Food Science and Nutrition, 57(6), 1215–1227. https://doi.org/10.1080/10408398.2014.977434
Sin, H. N., Yusof, S., Sheikh Abdul Hamid, N., & Rahman, R. A. (2006). Optimization of enzymatic clarification of sapodilla juice using response surface methodology. Journal of Food Engineering, 73(4), 313–319. https://doi.org/10.1016/j.jfoodeng.2005.01.031
Singh, A., Kumar, S., & Sharma, H. K. (2012). Effect of enzymatic hydrolysis on the juice yield from bael fruit (Aegle marmelos Correa) pulp. American Journal of Food Technology, 7(2), 62–72. https://doi.org/10.3923/ajft.2012.62.72
Sreenath, H. K., Frey, M. D., Radola, B. J., & Scherz, H. (1984). Degradation of a washed carrot preparation by cellulases and pectinases. Biotechnology and Bioengineering, 26(7), 788–796. https://doi.org/10.1002/bit.260260725
Sulaiman, M. Z., Sulaiman, N. M., & Yih, L. S. (1998). Limiting permeate flux in the clarification of untreated starfruit juice by membrane ultrafiltration. Chemical Engineering Journal, 69(2), 145–148. https://doi.org/10.1016/S1385-8947(98)00062-X
Vaidya, D., Vaidya, M., & Sharma, S. (2009). Enzymatic treatment for juice extraction and preparation and preliminary evaluation of kiwi fruits wine. Natural Product Radiance, 8(4), 380–385.
Vaillant, F., Millan, A., Dornier, M., Decloux, M., & Reynes, M. (2001). Strategy for economical optimization of the clarification of pulpy fruit juices using crossflow microfiltration. Journal of Food Engineering, 48(1), 83–90.
Wang, H. T., Zhang, Y. J., & Mu, Y. J. (2007). Temperature dependence of the absolute rate constant for the reaction of ozone with dimethyl sulfide. Journal of Environmental Sciences, 19(6), 641–643. https://doi.org/10.1016/s1001-0742(07)60106-5
Yamasaki, M., Yasui, T., & Arima, K. (1964). Pectic enzymes in the clarification of apple juice. Part I. Study on the clarification reaction in a simplified model. Agricultural and Biological Chemistry, 28(11), 779–787. https://doi.org/10.1080/00021369.1964.10858304
Yusof, S., & Ibrahim, N. (1994). Quality of soursop juice after pectinase enzyme treatment. Food Chemistry, 51(1), 83–88. https://doi.org/10.1016/0308-8146(94)90052-3
Zehra, M., Syed, M. N., & Sohail, M. (2020). Banana peels: A promising substrate for the coproduction of pectinase and xylanase from MS16. Polish Journal of Microbiology, 69(1), 19–26. https://doi.org/10.33073/pjm-2020-002