The Pharma Innovation Journal 2023; 12(11): 2327-2331

www.ThePharmaJournal.com

The Pharma Innovation

**ISSN (E): 2277-7695**
**ISSN (P): 2349-8242**
**NAAS Rating: 5.23**
TPI 2023; 12(11): 2327-2331

www.thepharmajournal.com
Received: 02-09-2023
Accepted: 09-10-2023

**AS Ghorband**
College of Food Processing Technology & Bioenergy, Anand Agricultural University, Anand, Gujarat, India

**BH Joshi**
College of Food Processing Technology & Bioenergy, Anand Agricultural University, Anand, Gujarat, India

**Corresponding Author:**
**AS Ghorband**
College of Food Processing Technology & Bioenergy, Anand Agricultural University, Anand, Gujarat, India

# Effect of enzyme treatment on yield, TSS, ascorbic acid and viscosity of dragon fruit juice

**AS Ghorband and BH Joshi**

**Abstract**
The effect of enzyme concentration, incubation time and incubation temperature on extract yield, TSS, ascorbic acid and viscosity of dragon fruit (*Hylocereus polyrhizus*) juice was studied. Pectinase assisted enzymatic extraction was carried out at three different levels of concentrations, incubation time and temperatures ranging from 38-380 IU, 90-150 min. and 30-60 °C respectively. The clarified juice samples were analyzed for extract yield, TSS, ascorbic acid and viscosity by using completely randomized design (CRD) using software design expert 13. The optimum conditions for pectinase enzyme treatment for dragon fruit juice was found 380 IU of enzyme concentration, 45 °C incubation temperature and 120 min of incubation time. The result showed the significant increase in extract yield, whereas TSS, total phenol increased marginally. The reduction in viscosity was observed significant while ascorbic acid was decreased in small amount.

**Keywords:** Dragon fruit, pectinase, extract yield, TSS, viscosity and ascorbic acid

## Introduction
Dragon fruit (*Hylocereus polyrhizus*) is part of the Cactaceae family and order of Caryophyllales which is originally grown in southern Mexico and South and Central America (Britton and Rose, 1963; Mizrahi *et al*., 1997) [2, 7]. In early 19th century, the French brought it to Southeast Asia. More than 93% of the world's dragon fruit production is produced by three major countries: Vietnam, China, and Indonesia. *Hylocereus polyrhizus* (red flesh with red peel dragon fruit), *Selenicereus megalathus* (white flesh with yellow peel dragon fruit) and *Hylocereus undatus* (white flesh with red peel dragon fruit) are the three different species of dragon fruit. Producing juice from red dragon fruit is a cost-effective technique to create value added products and boost the profitability of fruit sector. Enzyme is a crucial component of juice processing, both in terms of quality and cost. In the preparation of red dragon fruit juice, commercial pectinolytic enzymes are used. The use of the enzyme pectinase resulted in a juice with greater protein content and phenolic levels of up to 15% (Nur Aliaa, 2010) [8]. Enzyme addition enhances the release of different phenolic and other nutritionally significant components in the juice, in addition to improving juice extraction (Kumar, 2015) [5]. Enzymatic degradation of substrate is depending upon the type of enzyme, use of different combinations of enzymes, enzyme concentration, incubation time, incubation temperature, agitation, pH etc. The application of enzymes like pectinases, amylases and cellulases alone and their combination claim to increase juice yield, TSS, clarity and decrease the viscosity and turbidity. *Aspergilus niger or Aspergilus aculeatus* is used for industrial production of pectolytic enzymes. Pectic enzyme treatments vary depending on the type of juice (Wagh *et al*., 2022) [14]. Hence keeping these points in mind present investigated was done to optimize enzyme extraction of dragon fruit juice.

## Materials and Methods
The dragon fruits (*Hylocereus polyrhizus*) uniform, ripened were procured from R K farm and Nursery, Nakhatrana, Dist. Kutch-Bhuj Gujarat. Pectinase enzyme (Enzyme activity > 3800 IU) was purchased from Sigma Aldrich, Co., 3050 spruce street, St. Louis, MO 63103 USA. Polyethylene terephthalate (PET) bottles of 200 ml capacity were purchased from Axar blow plast, GIDC, Anand, Gujarat, India. The enzyme treatment was optimized using different levels of enzyme concentration, incubation time and incubation temperature as per treatment given in table.

The selected and sorted dragon fruits were washed with tap water to remove soil and dust particles. The fruits were cut into half, peel was removed manually and small pieces were made by using stainless steel Kinfe. After cutting operation thick mass of pulp was passed through stainless steel sieve to remove seeds.

The extraction of dragon fruit juice was carried out by using three different levels of pectinase concentrations (38-380 IU), incubation temperature (30-60 $^{o}$C) and time (90-150 min) respectively. The incubation process was carried out in shaker incubator at 100 rpm. After incubation, centrifugation and filtration was carried out. The enzymes in the extracted clarified juice were inactivated by heating at 90 °C, 5 min and then cooled at room temperature and stored in 200 ml PET bottles for further use. Principal steps used for enzymatic extraction of dragon fruit juice are in Flow chart 1.

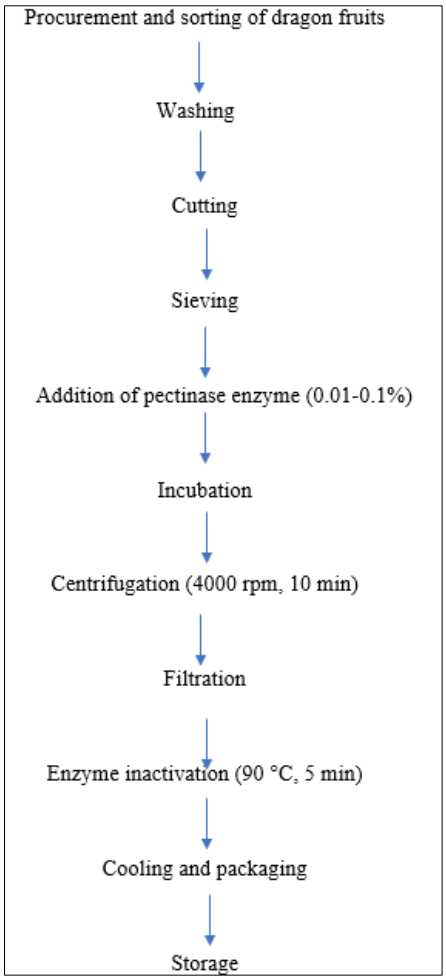


**Fig 1:** Flow chart for production of clarified juice from dragon fruit juice

## Results and Discussion

The perusal of data containing the enzyme assisted extraction of dragon fruit juice and its effect on extract yield, TSS, ascorbic acid and viscosity was presented in Table 1.

**Table 1:** Optimization of enzyme concentration, incubation time and temperature for dragon fruit juice extraction

| Run No. | Independent parameters | | | Dependent parameters | | | |
|---|---|---|---|---|---|---|---|
| | Enzyme concentration (IU) | Incubation time (min) | Incubation temp (°C) | Extract yield (%) | TSS (°Bx) | Viscosity (cP) | Ascorbic acid (mg/100 ml) |
| 1 | 380 | 120 | 60 | 75.36 | 11.80 | 5.65 | 18.57 |
| 2 | 190 | 90 | 45 | 76.41 | 11.82 | 5.45 | 21.04 |
| 3 | 38 | 150 | 30 | 72.31 | 11.90 | 5.91 | 20.18 |
| 4 | 380 | 90 | 45 | 77.40 | 11.89 | 5.20 | 19.80 |
| 5 | 38 | 120 | 60 | 72.30 | 11.72 | 5.91 | 19.12 |
| 6 | 190 | 90 | 30 | 69.45 | 11.50 | 6.15 | 21.12 |
| 7 | 190 | 120 | 45 | 77.58 | 12.00 | 4.71 | 20.40 |
| 8 | 380 | 90 | 60 | 71.72 | 11.67 | 6.01 | 19.18 |
| 9 | 38 | 90 | 30 | 68.15 | 11.42 | 6.34 | 21.22 |
| 10 | 38 | 90 | 45 | 74.28 | 11.71 | 5.79 | 21.23 |
| 11 | 380 | 150 | 30 | 75.46 | 11.90 | 5.60 | 20.00 |
| 12 | 38 | 90 | 60 | 68.73 | 11.46 | 6.29 | 19.23 |
| 13 | 380 | 120 | 30 | 75.02 | 11.84 | 5.68 | 21.10 |
| 14 | 190 | 90 | 60 | 69.91 | 11.55 | 6.11 | 19.13 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 15 | 190 | 120 | 60 | 73.85 | 11.80 | 5.76 | 19.02 |
| 16 | 380 | 150 | 45 | 79.95 | 12.10 | 4.55 | 19.29 |
| 17 | 38 | 150 | 60 | 72.58 | 11.90 | 5.89 | 19.27 |
| 18 | 380 | 150 | 60 | 75.70 | 11.92 | 5.57 | 18.18 |
| 19 | 380 | 90 | 30 | 71.23 | 11.62 | 6.04 | 21.40 |
| 20 | 38 | 120 | 45 | 75.27 | 11.92 | 4.98 | 20.25 |
| 21 | 38 | 150 | 45 | 75.50 | 11.97 | 4.99 | 20.12 |
| 22 | 190 | 150 | 30 | 73.94 | 11.94 | 5.81 | 20.24 |
| 23 | 380 | 120 | 45 | 79.54 | 12.14 | 4.50 | 19.18 |
| 24 | 190 | 150 | 45 | 77.43 | 12.01 | 4.76 | 20.11 |
| 25 | 190 | 150 | 60 | 74.28 | 11.87 | 5.74 | 16.56 |
| 26 | 38 | 120 | 30 | 71.96 | 11.68 | 5.96 | 21.12 |
| 27 | 190 | 120 | 30 | 73.41 | 11.78 | 5.80 | 21.36 |

**Table 2:** The optimum solution for extraction of dragon fruit juice by pectinase treatment

| Parameter | Optimum value |
|---|---|
| Enzyme concentration (IU) | 380 |
| Incubation time (min) | 120 |
| Incubation temperature (°C) | 45 |

**Table 3:** Responses for optimized dragon fruit juice by enzymatic treatment

| Parameters | Mean ± SD |
|---|---|
| Yield (%) | 79.66 ± 0.80 |
| TSS (°Bx) | 12.14 ± 0.06 |
| Ascorbic acid (mg/100 ml) | 20.43 ± 0.26 |
| Viscosity (cP) | 4.42 ± 0.02 |

Each value is replication of the three observations

**Effect of enzyme concentration, incubation time and temperature on yield of dragon fruit juice**

The yield of clarified juice was varied from 68.15 to 79.95% upon macerating with the enzyme pectinase for juice extraction. Maximum yield was obtained at enzyme concentration, incubation time and temperature of 380 IU, 150 min and 45 °C (Table 1). This increase in yield may be due to hydrolysis of pectic substances present in pulp which results in reduction in water holding capacity of pectin hence free water is released (Lee *et al*., 2006) [6]. Extraction yield was significantly increased ($p$<0.05) by 16.71% compared with untreated sample from i.e. 68.15 to 79.54% in first 2 h of incubation at 45 °C when enzyme used at concentration of 380 IU/100 ml and afterwards there was no significant difference (Fig 2) in yield upon further increment in incubation temperature and time was increased. Present studies confer the observation independently reported by Truong *et al*. (2016) [12] and Jiang *et al*. (2020) [4] about the application of hydrolytic enzymes for enhancing the yield of dragon fruit juice.

**Effect of enzyme concentration, incubation time and temperature on TSS of dragon fruit juice**

TSS (Total soluble solids) of dragon fruit juice ranged from 11.42 to 12.14°Bx (Table 1). Maximum TSS was observed at 380 IU of enzyme concentration, 120 min of incubation time and at 45 °C temperature; whereas lowest TSS was observed at 38 IU of enzyme concentration, 90 min of incubation time and at 30 °C temperature. TSS of enzymatically treated dragon fruit juice was increased after pectinase treatment. This may be due to higher breakdown of tissue which releases more substances like sugars those contribute to soluble solids. there was significant difference within three independent variables i.e. enzyme concentration, incubation time and temperature (Fig 3). The combined effect of enzyme concentration and incubation temperature as well as incubation time and temperature had significant effect ($p$<0.05) on total soluble solids whereas there was no combined significant difference ($p$>0.05) among enzyme concentration and time and among three variables. The result obtained for total soluble solids was in close agreement with and Yusof and Ibrahim (1994) [11] and Ghorband *et al*. (2020) [3].

**Effect of enzyme concentration, incubation time and temperature on viscosity of dragon fruit juice**

Viscosity of extracted dragon fruit juice ranges from 4.5 to 6.34 cP. Maximum viscosity was found at enzyme concentration, incubation time and temperature of 38 IU, 90 min and 30 °C respectively whereas minimum viscosity was found at enzyme concentration, incubation time and temperature of 380 IU, 120 min and 45 °C respectively. Decrease in viscosity trend was observed after enzymatic treatment. This was observed due to hydrolytic action of enzymes on cellulosic and pectic substances in the juice.

There was significant effect ($p$<0.05) of enzyme concentration, incubation time and temperature on viscosity of dragon fruit juice within the treatment (Fig 4). There was also significant difference ($p$<0.05) found among effect of enzyme concentration and incubation time as well as incubation time and temperature on the viscosity reduction of dragon fruit juice. But there was non-significant effect of enzyme concentration and incubation temperature as well combined three independent process parameters on viscosity reduction.

The reduction in viscosity of dragon fruit juice may be due to hydrolysis of pectin substance which possess a high water holding capacity and develops a cohesive network structure. Pectin hydrolysis by the action of enzymes results in reduction in water holding capacity and ultimately water is released into system which is responsible for reduction in viscosity. Urlaub (1996) [10] as well as Abdullah *et al*., (2007) [1] also found reduction in viscosity of carambola juice upon treatment of 0.1% enzyme.

**Effect of enzyme concentration, incubation time and temperature on ascorbic acid content of dragon fruit juice**

Ascorbic acid content of dragon fruit juice obtained by pectinase treatment was in the range of 21.40 mg/100 g to 16.56 mg/100 g. Maximum ascorbic acid was retained at 380 IU enzyme concentration for 90 min incubation time at 30 °C temperature; while, minimum ascorbic acid was retained at 190 IU enzyme concentration for 150 min incubation time at 60 °C temperature. Ascorbic acid content was decreased as

enzyme concentration and incubation time increased when compared to untreated sample having 21.4 mg/100 g of ascorbic acid. The decrease in ascorbic acid may be due to oxidation during clarification and higher exposure time to pectinase treatment. Vaidya *et al*. (2009) [13] also observed decrease in ascorbic acid of kiwi fruit after pectinase treatment. Both incubation time and temperature have significant effect ($p<0.05$) showing decrease in ascorbic acid content within the treatments, whereas enzyme concentration have non significant effect on ascorbic acid of dragon fruit juice. This may be due to oxidation of ascorbic acid upon exposure to temperature. The combined effect all the independent parameters on ascorbic acid was non significant ($p>0.05$). Similar trend in result was observed showing retention of 83.10 to 79.30% ascorbic acid during enzymatic clarification of various juices carried out by Singh *et al*., (1993) [9].

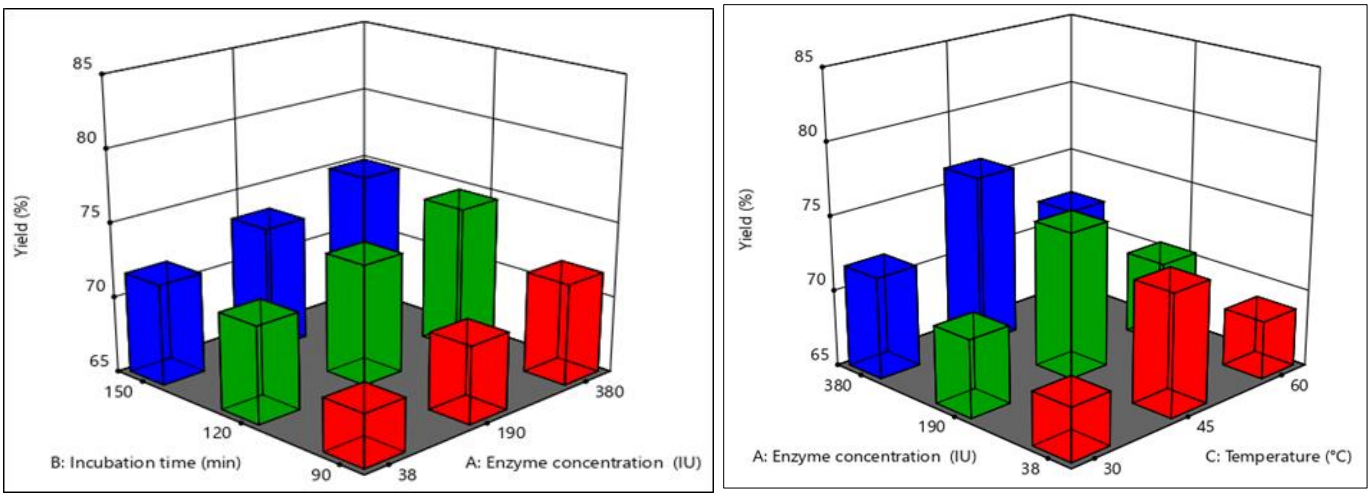


**Fig 2:** Effect of enzyme treatment on extract yield of dragon fruit juice

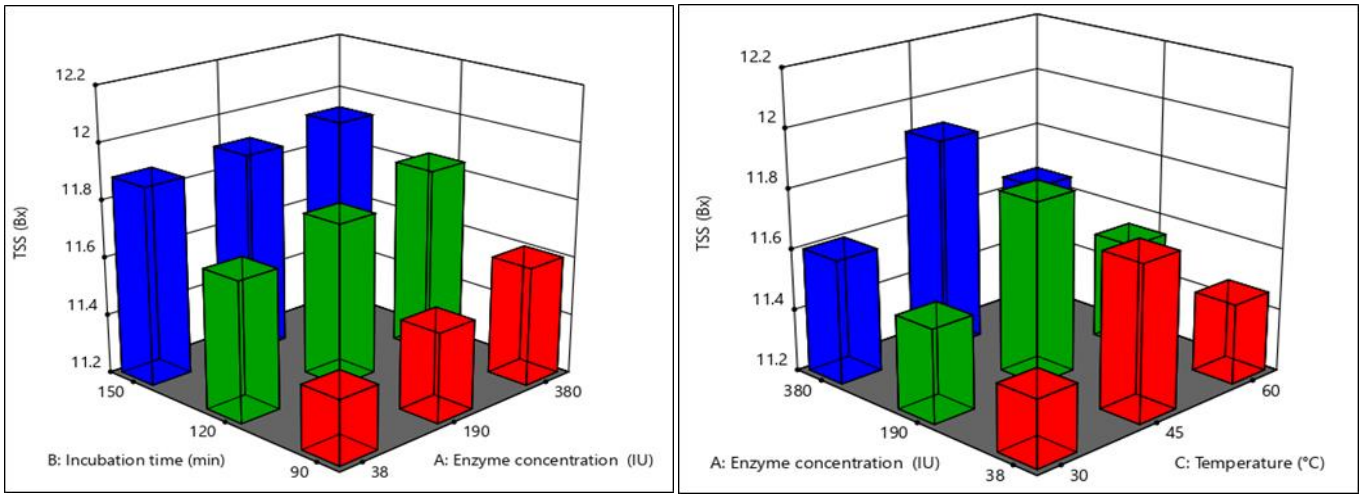


**Fig 3:** Effect of enzyme treatment on the TSS of dragon fruit juice

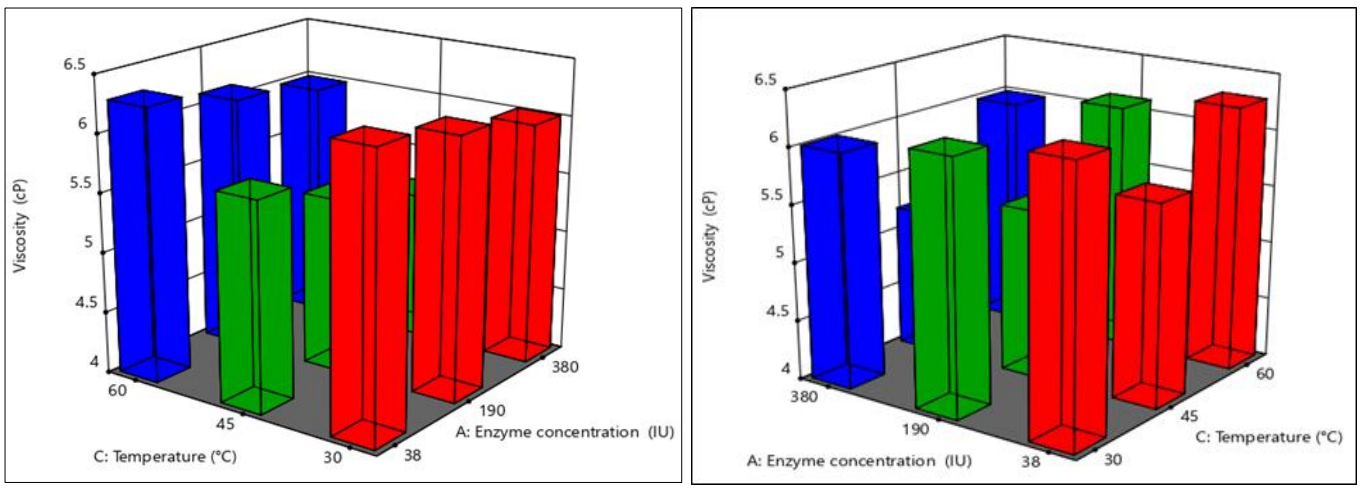


**Fig 4:** Effect of enzyme treatment on viscosity of dragon fruit juice

## Conclusion

The findings above indicate that extractability and quality of fruit juice can be improved by use of enzyme at 380 IU concentration, 120 min of incubation time and 45 °C of incubation temperature. Thus, enzyme applications have paved the way in fruit juice processing. Pectinase treatment results in clarification of fruit juice by reducing cloudiness and resulting in to more consumer acceptability of product prepared from it. It is also beneficial to increase yield and TSS and reduction in viscosity and sugar content in extracted juice which ultimately reducing the total cost of juice production.

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