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Effect of enzyme concentration and treatment time on sapota juice clarity, sedimentation and overall acceptability

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Abstract
The experiment was conducted to developed a methodology to standardize the process for preparation of sapota juice, and to study the storage stability of the sapota juice. The sapota juice was prepared using twelve treatment combinations comprised of three levels of pectinase enzyme treatment [Pectinase @ 0.05% (P_1), Pectinase @ 0.10% (P_2), Pectinase @ 0.15% (P_3) and four levels of treatment time [1 hour (T_1), 2 hours (T_2), 3 hours (T_3) and 4 hours (T_4)]. The results of investigation indicated that sapota juice extracted by treating sapota pieces with 0.15 per cent pectinase for 1 hour juice observed to have higher juice clarity, minimum sedimentation and remained shelf stable on the basis of sensory quality upto six months storage when packed in 200ml glass bottles and heat processed at 95 ±1°C in boiling water for 30 minutes followed by cooling and storage. The cost of production per 200 ml bottle of sapota juice worked out Rs. 94.37. Thus, prepared juice can commercially be explored by food processing industry to ensure better returns to growers, processors and consumers as well.

Keywords: Pectinase, treatment time, clarity, Sedimentation, Overall acceptability, juice

Introduction
Sapota (*Manilkara achras* (Mill) Fosberg) belongs to family sapotaceae and is a popular tropical fruit commercially grown in India. Sapota is native of Tropical America and probably originated from southern Mexico or Central America. In South Mexico, Guatemala and other countries, it is commercially grown for the production of chickle which is a gum like substance obtained from the latex and is mainly used for the preparation of chewing gum. However, in India, it is cultivated extensively for table purpose owing to its fruit value. Among the tropical fruits, sapota is the fifth popular fruit crop in both production and consumption next to mango, banana, citrus and grape. Sapota is mainly grown in India, Philippines, Malaysia, Indonesia, Florida, Guatemala, Mexico and Sri Lanka. India is the largest producer of sapota in the world and cultivated under 0.10 million hectare area with the production of 1.22 million MT and 9.9 MT/ha productivity (Anon., 2019-20). According to Chadha (1992) [5], sapota was first introduced in Gholwad village in Maharashtra during the year 1898, from where it has been spread to other parts of the country. It is commercially grown in India in the states Maharashtra, Gujarat, Andhra Pradesh, Karnataka, Tamil Nadu, Kerala, Uttar Pradesh, West Bengal, Punjab, Haryana and some humid part of Rajasthan. In Gujarat, the area under sapota cultivation is 27.83 thousand ha with production of 3.1 lakh tones and productivity of 11.06 MT/ha. While in south Gujarat, the area under sapota cultivation is 13.03 thousand ha with 1.58 lakh tones of annual production wherein its cultivation is concentrated mainly in Navsari, Valsad and Surat districts (Anon., 2019-20). In India, Gujarat possess second rank in both area and production after Karnataka. Sapota fruit is known for its sweet delicious taste and possesses a delicate characteristic aroma when fully ripe. The fruit is a good source of digestible sugar (12 to 18%) and appreciable source of protein, fat, fiber and minerals like calcium, phosphorous and iron (Chadha, 2001) [6]. Among the 41 varieties grown all over India, Kalipatti is an outstanding variety of sapota and popularly cultivated in Gujarat due to its excellent taste and aroma, soft and mellow flesh with less number of seeds, high productivity, continuous fruiting throughout the year, very little incidence of insect-pest and diseases and free from physiological disorders which otherwise very common in other major fruits like mango, citrus, etc. It is well known fact that in Gujarat, this crop has played a significant role in socio-economic upliftment of both marginal and big farmers.

Being an assured and regularly paying crop, and better marketing facilities provided by co-operative societies; farmers from South Gujarat tend to grow sapota for commercial fruit production.

Now-a-days the enzymes are commonly used in much industrial application and demand for more stable, highly active and specific enzymes is growing rapidly. Fruit juice can be extracted from a wide variety of fruits. This can be done by simply squeezing the fruits but it is more common to use enzymes to increase the volume of juice produced and speed of extraction. Due to pulpy nature of sapota fruits, it is not possible to extract sapota juice by simple squeezing process without using enzymes. Enzyme breaks down the cell walls within the fruits and release liquids and sugars. The fruit juice companies utilize a variety of different treatments and enzymes to maximize the yield of juice (Demain, 2000) [7].

Materials and Methods

Experiment was carried out in Centre of Excellence on Post Harvest Technology, Department of Post Harvest Technology, ASPEE College of Horticulture and Forestry, N.A.U., Navsari. The selected ripe sapota fruits were washed with tap water to remove the adhering dirt and dust particles. After washing, the fruits were peeled, cut into small pieces (cuboids 5-7 mm) by using sharp stainless steel (SS) knife and seeds were removed. The extraction of sapota juice in this experiment was carried out using different combination of pectinase concentration (Factor 1) and treatment time level (Factor 2). The enzyme treatments were given as per treatment combination and then juice was extracted manually followed by pre-pasteurization heating at 95±1 °C temperature for 5 minutes. The pre-pasteurized juice was filled into pre-sterilized glass bottles (200 ml) and sealed air tight with crown caps. The juice was then heat processed (pasteurized) at 95±1 °C in boiling water for 30 minutes followed by cooling and storage at room temperature. Principal steps used for preparation of sapota juice in Fig.1.

Selection of the ripe sapota fruits
↓
Washing
↓
Sorting
↓
Removal of peel and seeds
↓
Cutting of fruits
↓
Enzyme treatment (As per treatments)
↓
Extraction of clear juice and filtration
↓
Pre-pasteurization heating (95 ±1 °C for 5 minutes)
↓
Filling the product in pre-sterilized transparent glass bottles (Bottling method)
↓
Crown corking
↓
Heat processing (95 ±1 °C for 30 minutes)
↓
Cooling and labeling
↓
Stored the product in cool and dry place at ambient temperature

Fig 1: Principal steps used for preparation of sapota juice

Results and Discussion

Clarity

The perusal of data pertaining to effect of pectinase concentration and treatment time during six months storage on clarity of sapota fruit juice has been presented in Fig.2.

Effect of pectinase: Data shows that among different concentration of pectinase, the mean clarity (P) of sapota juice varied significantly from 0.609 to 0.635 OD_{420nm} absorbance, with maximum clarity (0.635 OD_{420nm} absorbance) in sapota juice when sapota tit bits were treated with 0.15 per cent pectinase (P_3) and minimum clarity (0.609 OD_{420nm} absorbance) in sapota juice treated with 0.05 per cent pectinase (P_1). Brown and Ough (1981) [4] reported increase in grape juice by using pectinase enzymes juice clarity and filterability by 100%. Abdullah *et al*. (2007) [1] in their study on clarification of carambola fruit juice using a commercial enzyme observed the clarity of fruit juice was maximum of 0.019 Abs at optimized conditions of incubation time 20 min, temperature 30 °C and pectinase concentration of 0.10%.

Effect of treatment time: It was observed that mean clarity (T) of sapota juice varied significantly from 0.600 to 0.644 OD_{420nm} absorbance when sapota tit bits were treated with different concentration of pectinase for different treatment time, with maximum clarity in sapota juice when sapota tit bits were treated with pectinase for 1 hour (T_1) and minimum clarity in sapota juice when sapota tit bits were treated with pectinase for 4 hours (T_4). Similar results were found by Rai *et al*. (2003) [12] are reported that clarity of mosambi juice decreases with time up to 90 min and increases thereafter. Similarly at constant time and temperature, the clarity decreases with increase in enzyme concentration.

Effect of storage: Data depict that storage of sapota juice resulted significant decrease in mean clarity (S) from initial value of 0.580 to 0.651 OD_{420nm} absorbance during six month storage. Brown and Ough (1981) reported increase in grape juice by using pectinase enzymes juice clarity and filterability by 100%. Abdullah *et al*. (2007) [1] in their study on clarification of carambola fruit juice using a commercial enzyme observed the clarity of fruit juice was maximum of 0.019 Abs at optimized conditions of incubation time 20min, temperature 30°C and pectinase concentration of 0.10%.

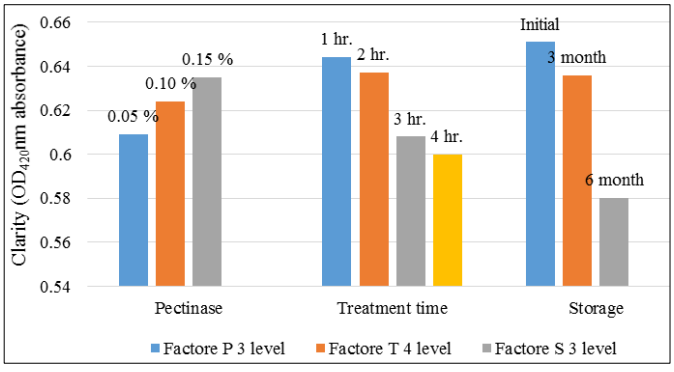

Fig 2: Effect of enzyme concentration and treatment time on clarity of clarified sapota juice during storage

Effect of interactions

Interaction of pectinase concentration and treatment time depicted variation in juice clarity from 0.585 to 0.657 OD_{420nm}

absorbance, with minimum clarity (0.585 OD_{420nm} absorbance) in sapota juice when sapota tit bits were treated with 0.05 per cent pectinase for 4 hours (P_1T_4) and maximum clarity (0.657 OD_{420nm} absorbance) in sapota juice when sapota tit bits were treated with 0.15 per cent pectinase for 1 hour (P_3T_1). Interaction of pectinase concentration and storage depicted variation in clarity from 0.563 to 0.662 OD_{420nm} absorbance. Interaction of treatment time and storage resulted variation in clarity of sapota juice from 0.554 to 0.670 OD_{420nm} absorbance, with minimum decrease in clarity from 0.670 to 0.605 OD_{420nm} absorbance (T_1S_1 to T_1S_3) in sapota juice when sapota tit bits were treated with 0.15 per cent pectinase for 1 hour (T_1). Whereas, maximum decrease in clarity was observed from 0.632 to 0.554 OD_{420nm} absorbance (T_4S_1 to T_4S_3) in sapota juice when sapota tit bits were treated with 0.05 per cent pectinase for 4 hours. Further, interaction of pectinase, treatment time and storage depicted variation in sapota juice during six month storage from 0.535 to 0.680 OD_{420nm} absorbance, with minimum decrease from 0.680 to 0.620 OD_{420nm} absorbance in sapota juice when sapota tit bits were treated with 0.15 per cent pectinase for 1 hour ($P_3T_1S_1$ to $P_3T_1S_3$) and maximum decrease from 0.620 to 0.535 OD_{420nm} absorbance in sapota juice when sapota tit bits were treated with 0.05 per cent pectinase for 4 hours ($P_1T_4S_1$ to $P_1T_4S_3$). Despite minimum changes in P_3T_1, the treatment combination P_3T_1 were found to possess highest clarity after six months storage. However, the all interactions were found to have non-significant effect.

Sedimentation

The perusal of data pertaining to effect of pectinase concentration and treatment time during six months storage on sedimentation of sapota juice has been presented in Fig. 3.

Effect of pectinase: Data shows that among different concentration of pectinase, the mean sedimentation (P) of sapota juice varied from 4.33 to 4.81 per cent, with maximum sedimentation in sapota juice when sapota tit bits were treated with pectinase 0.05 per cent (P_1) and minimum sedimentation in sapota juice when sapota tit bits were treated with 0.15 per cent pectinase (P_3). Similar results were found by Sreekantish *et al.* (1968) [15] reported that minimum sediment in enzymatic juice clarification in banana, guava and litchi fruits.

Effect of treatment time: It was observed that mean sedimentation (T) of sapota juice significantly varied from 4.15 to 4.97 per cent when sapota tit bits were treated with pectinase for different treatment time, with maximum sedimentation in sapota juice when sapota tit bits were treated with pectinase for 4 hours (T_4) and minimum sedimentation in sapota juice when sapota tit bits were treated with pectinase for 1 hour (T_1). Similar results were found by Sreekantish *et al.* (1968) [15] reported that minimum sediment in enzymatic juice clarification in banana, guava and litchi fruits.

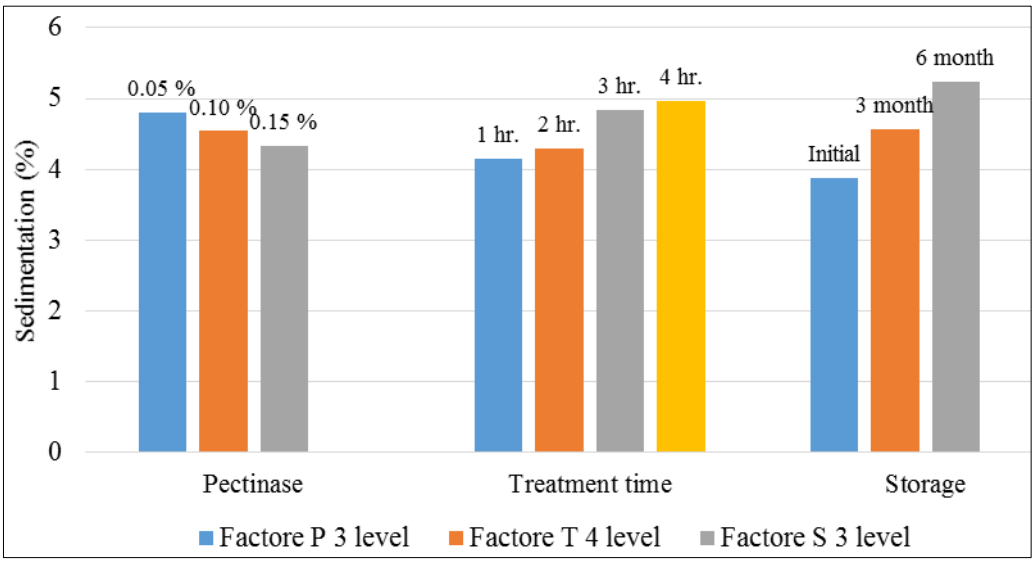

Fig 3: Effect of enzyme concentration and treatment time on sedimentation of sapota juice during storage

Effect of Storage: Data depict that storage of sapota juice resulted significant increase in mean sedimentation (S) from initial value of 3.88 to 5.23 per cent during six month storage. Similar results were found by Sreekantish *et al.* (1968) [15] reported that minimum sediment in enzymatic juice clarification in banana, guava and litchi fruits.

Effect of interactions: Interaction of pectinase concentration and treatment time depicted variation in sedimentation from 3.91 to 5.27 per cent, with minimum sedimentation (3.91%) in sapota juice when sapota tit bits were treated with 0.15 per cent pectinase for 1 hour (P_3T_1) and maximum sedimentation (5.27%) in sapota juice when sapota tit bits were treated with 0.05 per cent pectinase for 4 hours (P_1T_4). Interaction of pectinase concentration and storage depicted variation in sedimentation from 3.77 to 5.57 per cent. Interaction of treatment time and storage resulted variation in sedimentation of sapota juice from 3.69 to 5.79 per cent, with minimum increase of sedimentation from 3.69 to 4.68 per cent (T_1S_1 to T_1S_3) in sapota juice when sapota tit bits were treated with pectinase for 1 hour. Whereas, maximum sedimentation observed from 4.07 to 5.79 per cent (T_4S_1 to T_4S_3) in sapota juice when sapota tit bits were treated with pectinase for 4 hours. Further, interaction of pectinase concentration, treatment time and storage depicted variation in sapota juice during six month storage from 3.58 to 6.20 per cent, with minimum increase from 3.58 to 4.36 per cent ($P_3T_1S_1$ to $P_3T_1S_3$) in sapota juice when sapota tit bits were treated with 0.15 per cent pectinase for 1 hour (P_3T_1) and maximum increase from 4.20 to 6.20 per cent ($P_1T_4S_1$ to $P_1T_4S_3$) in sapota juice when sapota tit bits were treated with 0.05 per cent pectinase for 4 hours (P_1T_4). However, the interaction of

P×S and T×S were found to have significant effect while effect of interaction P×T and P×T×S were found non-significant.

Overall acceptability

The perusal of data pertaining to effect of pectinase concentration and treatment time during six months storage on overall acceptability score of sapota juice has been presented in Fig. 4.

Effect of pectinase

Data shows that among different concentration of pectinase, the mean overall acceptability score (P) of sapota juice varied from 7.34 to 7.78, with maximum overall acceptability in sapota juice when sapota tit bits were treated with 1.5 per cent pectinase (P_3) and minimum overall acceptability score in sapota juice when sapota tit bits were treated with 0.05 per cent pectinase (P_1). Similar observation made by Akesowan and Choonhahirun (2013) [2] for guava juice, Egwim *et al.* (2013) [9] for aonla juice, Kadam *et al.* (2014) [11] for grape pulp, Sharma *et al*. (2014) [13] in enzymatic extraction from various fruits.

Effect of treatment time: It was observed that mean overall acceptability (T) of sapota juice varied from 7.19 to 7.92 when sapota tit bits were treated with pectinase for different treatment time, with maximum overall acceptability score in sapota juice when sapota tit bits were treated with pectinase for 1 hour (T_1) and minimum overall acceptability score in sapota juice when sapota tit bits were treated with pectinase for 4 hours (T_4). However, effect of treatment time on overall acceptability score of sapota juice found to have significant effect. Similar observation made by Akesowan and Choonhahirun (2013) [2] for guava juice, Egwim *et al.* (2013) [9] for aonla juice, Kadam *et al.* (2014) [11] for grape pulp, Sharma *et al*. (2014) [13] in enzymatic extraction from various fruits.

Effect of storage: Data depict that storage of sapota juice resulted significant decrease in mean overall acceptability score (S) from initial value of 7.98 to 7.20 during six month storage. Sin *et al*. (2006) [14] reported decrease in overall acceptability score of sapodilla juice, Devina *et al*. (2009) [8] for kiwifruit juice, Joshi *et al*. (2011) for apple juice, Akesowan and Choonhahirun (2013) [2] for guava juice during storage.

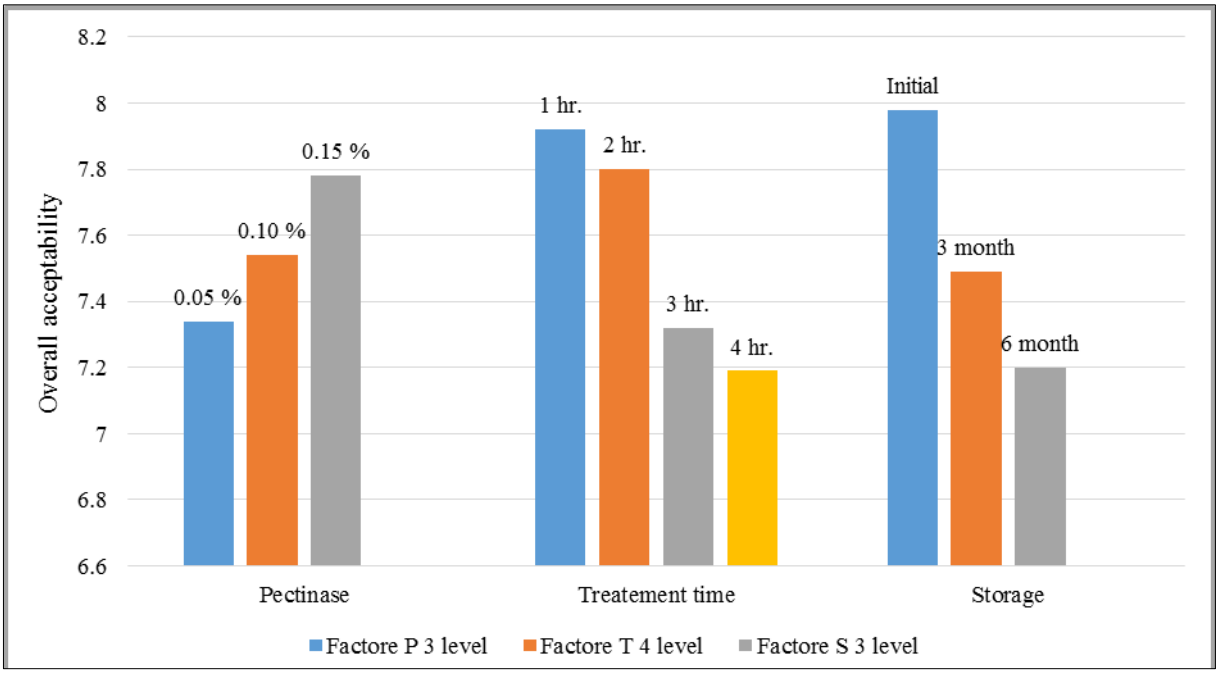

Fig 4: Effect of enzyme concentration and treatment time on sensory overall acceptability of sapota juice during storage

Effect of interactions: Interaction of pectinase concentration and treatment time depicted variation in overall acceptability score from 6.99 to 8.17, with minimum overall acceptability score (6.99) in sapota juice when sapota tit bits were treated with 0.05 per cent pectinase for 4 hours (P_1T_4) and maximum overall acceptability score (8.17) in sapota juice when sapota tit bits were treated with 0.15 per cent pectinase for 1 hour (P_3T_1). Interaction of pectinase concentration and storage depicted variation in overall acceptability score from 6.96 to 8.17. Interaction of treatment time and storage resulted variation in overall acceptability score of sapota juice from 6.80 to 8.28, with minimum decrease in overall acceptability score from 8.28 to 7.60 (T_1S_1 to T_1S_3) in sapota juice when sapota tit bits were treated with pectinase for 1 hour and maximum decrease in overall acceptability score from 7.67 to 6.80 (T_4S_1 to T_4S_3) in sapota juice when sapota tit bits were treated with pectinase for 4 hours. Further, interaction of pectinase, treatment time and storage depicted variation in sapota juice during six month storage from 6.57 to 8.50, with minimum decrease in overall acceptability score 8.50 to 7.88 ($P_3T_1S_1$ to $P_3T_1S_3$) in sapota juice when sapota tit bits were treated with 0.15 per cent pectinase for 1 hour and maximum decrease overall acceptability score from 7.50 to 6.57 ($P_1T_4S_1$ to $P_1T_4S_3$) in sapota juice when sapota tit bits were treated with 0.05 per cent pectinase for 4 hours. Despite minimum changes in P_3T_1 during storage, the treatment combination P_3T_1 were found to possess highest score for overall acceptability 7.88 to 8.50 during storage. However, the all interactions were found to have non-significant effect.

Conclusion

The findings summarized above indicate that during six month storage, sapota juice prepared by using 0.15 per cent pectinase for 1 hours (P_3T_1) observed to have maximum

clarity in sapota juice and minimum sedimentation during six month storage. Overall from the findings of investigation it can be concluded that sapota juice extracted by using of 0.15 per cent pectinase for 1 hour gave clarity in sapota juice, minimum sedimentation and remains shelf stable on the basis of sensory quality upto six month storage when packed in 200ml glass bottles and heat processed at 95 ±1 °C in boiling water for 30 minutes followed by cooling and storage.

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