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Effect of enzymes on clarified aonla (*Embilica officinalis* Gaertn) juice with respect to sensory quality

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Abstract
The present investigation entitled "Standardization of Process for juice clarification from aonla (Embilica *officinalis* Gaertn) fruits" was aimed to standardize formulation for the preparation of nutritional as well as medicinal drink by juice clarification of juice. In the clarified aonla juice sensory parameters were found decreasing trend during six months of storage. C_0P_2 *i.e.* (pectinase: cellulase, 0:1) was rated best treatment on the basis of higher sensory scores in colour, taste, flavour, aroma and Overall acceptability. The cost of production of clarified aonla juice were found maximum in C_2P_2 *i.e.* (pectinase: cellulase, 1:1) with high benefit: cost ratio, while low cost of production in C_0P_0 *i.e.* (pectinase: cellulase, 0:0) with low benefit: cost ratio. The best quality clarified aonla juices can be obtained from crushed aonla fruits given enzyme treatment with 1.0 per cent pectinase for 2 hours followed by pressing to get higher juice recovery with better quality.

Keywords: aonla, clear juice, enzymes, pectinase, cellulose, colour, taste, flavor

Introduction
The Indian gooseberry (*Emblica officinalis* Gaertn) also known as "Amla" or "Aonla". It is one of the most important indigenous fruit of the Indian origin. It belongs to the family Euphorbiaceae. It was found mention in the *Vedas*, *Ramayana*, *Charak Samhita*, *Sushruta samhita*, and literature of *Kalidas* and *Kadambari* further it occupies a sacred place in Indian mythology. Aonla trees thrive well throughout the tropical and sub-tropical parts of India. It can be grown successfully in dry regions owing to its hardy nature. It is spreading rapidly in the semi-arid regions of Gujarat, Maharashtra, Rajasthan, Andhra Pradesh, Karnataka, Tamil Nadu and some parts of Haryana, Punjab and Himachal Pradesh. In India area of aonla is 105 million hectares and production of aonla is 1257 MT. (Anon., 2015) [3]. However, in Gujarat it is commercially cultivated in Anand, Kheda, Panchmahal, Mehsana, Patan, Sabarkantha, Banaskantha, Bhavnagar, Rajkot and Ahmadabad.

Aonla is the second richest source of vitamin-C among fruits after Barbados cherry (*Malpighia glabra* L.). Content found to be superior in aonla pulp which is 20 times higher than the orange juice. It is also rich in lysine and phenol content. The pectin and minerals like iron, calcium, and phosphorus are also found abundantly in the fruit. The aonla is a very powerful anti-inflammatory herb. The fresh fruits are generally not consumed as it highly acidic and astringent and therefore it is not so popular table fruit. But, it has got great potential in processed forms. Moreover, 17 per cent or more of the produce fruits are lost during transport, storage and marketing therefore modern technologies are needed to reduce the losses. The fruits are made into various processed products *i.e.* murabba, squash, dried chips, tablets, jam, jelly, pickle, toffees, powder, juice, pulp, *mukhwas*, chocolate, candy, drops swarasa, mouthwash, *kulfi*, biscuit, *Shikhanda*, *Ghanvati*, Pan masala, *Amchoor*, *Churan*, *Chywanprash*, mouth freshener, *Laddoo*, Chutney, *Sabzi*, ice-cream, Supari, cold drinks, capsules, and aonla pak (Singh *et al*., 2006) [18]. Among these, juice is the preferred product. Moreover, antioxidants present in aonla juice in the form of polyphenols and vitamin-C have been shown to provide a cardio protective effect (Pathak, 2003) [13].

As interesting trend in the use of "food as medicine" is the emergence and classification of some lesser known fruits as "super fruits", with benefit that go far beyond nourishment alone. Aonla fruit enjoys a special place in Ayurveda, as a nurturing food, that is credited with a

number of health benefi ts. In the Ayurvedic tradition, the fruit forms an integral part of medicinal preparation that are used to supports wellness and healthy aging (Majeed *et al.*, 2009) [10].

Now-a-day the enzymes are commonly used in much industrial application and demand for more stable, highly active and specific enzymes is growing rapidly. According to a recent publication, the industrial enzymes have already reached a market of 1.6 billion US dollars (Demain, 2000) [5]. Fruits are amongst the first food items know to human beings. Fruits, whether fresh or dried, have always formed a part of staple diet of human beings. The reason for this is that they are rich in nutrients and provide some of the essential minerals, vitamins, and the like, to our body. Apart from that, they also help in curing a number of diseases. 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. 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.

Enzymes are biocatalyst of tertiary or quaternary globular protein structure and are formed by long chains of amino acids with peptide bonds. They may react under moderate conditions of temperature and pH and are present in microorganisms, animals and plants. Actually enzymes are applied in different areas: medical, food, textiles, chemical, pulp and paper, to name a few (Mussato *et al.*, 2007) [12]. Profound increases in industrial applications of food grade enzymes laid down a mile stone to assess biotechnological potential in fruit and vegetable processing industries. The enzymes are used in processing agricultural and agro-industrial waste, clarification of fruit juices and wines, extraction of vegetable oils, reduction of viscosity of concentrates, fermentation of coffee and tea, production of paper, treatment of natural fibers (linen and remie fibers) and degumming of plant fibers (Jin and Masako, 2001) [7]. Pectinases have extensive applications in fruit juice industries in order to improve fruit juice yield and clarify. The use of liquefying enzymes for mash treatment results in improvement of free flowing juice flow which leads to a lower press time even in absence of suitable pressing aids. At the same time pectin is broken down into such an extent that the viscosity of mash is considerably reduced (Sartoglu *et al.*, 2001) [14].

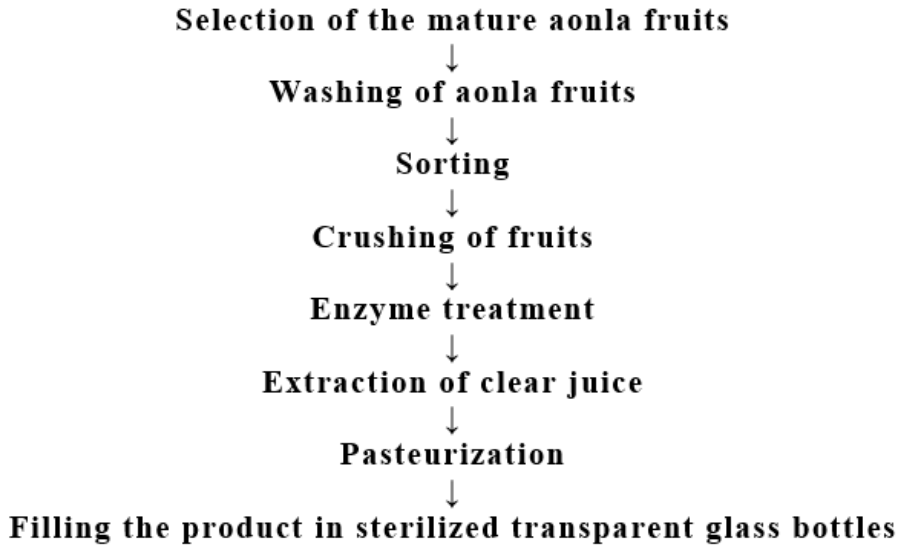
Enzymes are expensive products and clearly juice manufacturers would wish to minimize their costs by using the enzymes at their optimum conditions and therefore maximizing their effectiveness and re-using the enzymes (Blandino, 2001) [4]. Clarification is a beneficial step in juice processing and improvement of consumer acceptability. It is often achieved through enzymatic treatment, membranes filtration, or using clarifying aids. The use of commercial pectin enzymes is common in fruit juice processing. The advantages of pectin enzyme use have been to increase the flow of juice, clarity, improve juice yield, and facilitate filtration (Alkorta *et al.*, 1998) [2]. Therefore the present investigation was under taken with following objectives:

Material and method

Mature fruits of aonla were used for extraction of Clarified juice. The fruits were washed in running water to remove adhering dirt and dust particles. Then Clarified juice was extracted by manually. The procedure of juice was described in figure 1. Total nine treatments were used for preparation of juice using different concentration of enzymes.

Table 1: detail of Treatment formulation used for aonla clarified juice

No. of Treatments	Treatments Combination	Details of treatment combination
T_1	C_0P_0	Control
T_2	C_0P_1	Cellulase 0 % + Pectinase 0.5 %
T_3	C_0P_2	Cellulase 0 % + Pectinase 1%
T_4	C_1P_0	Cellulase 0.5 % + Pectinase 0 %
T_5	C_1P_1	Cellulase 0.5 % +Pectinase 0.5 %
T_6	C_1P_2	Cellulase 0.5 % + Pectinase 1 %
T_7	C_2P_0	Cellulase 1 % + Pectinase 0 %
T_8	C_2P_1	Cellulase 1 % + Pectinase 0.5 %
T_9	C_2P_2	Cellulase 1 % +Pectinase 1 %



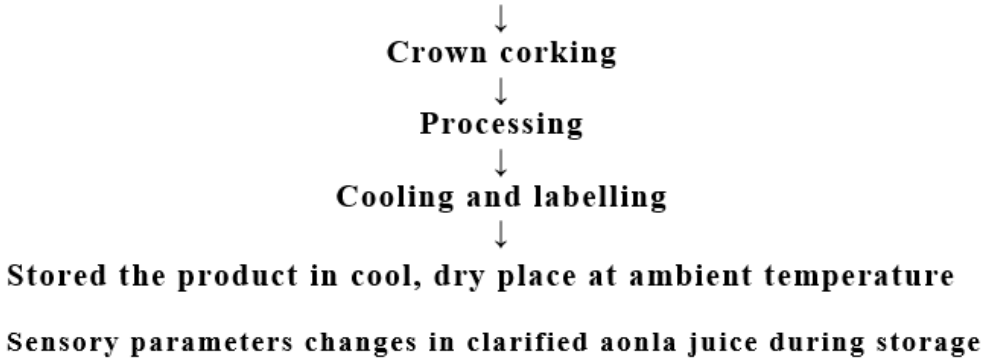


Fig 1: Principal step of juice clarification of aonla juice

Results and Discussion

Colour (out of 9 points)

The score of colour in clarified aonla juice was found significant. The colour contain was found highest in C_0P_2 i.e. (pectinase: cellulase, 1:0) which was at par with C_0P_1 i.e. (pectinase: cellulase, 0.5:0) and while significantly lowest in C_0P_0 i.e. (pectinase: cellulase, 0:0). The colour score of the product was decreased significantly irrespective of the treatment up to the end of the six months storage. Moreover, the highest colour score was recorded in C_0P_2 i.e. (pectinase: cellulase, 1:0) may be due to the enzyme like pectinase breakdown the particles, hence improve the sensory qualities in terms of colour. The lowest colour score was recorded in C_0P_0 i.e. (pectinase: cellulase, 0:0) which could be mainly due to the enzyme like cellulase it's give dark colour. The pattern of decline of colour during storage might be due to the oxidation, which was responsible for increase the production of black compounds resulting in browning of product during long term storage and thus it's adversely affected on colour acceptance. These observations were also similar to finding of Sin *et al*. (2006) [17] clarification of sapodilla juice, Vaidya *et al*. (2009) [19] in enzymatic extraction of kiwifruit juice, Joshi *et al*. (2011) [8] in pectinase enzyme on clarification of apple juice, Akesowan and Choonhahirun (2013) [1] pectinase enzyme on clarification of guava juice, Egwim *et al*. (2013) [6] clarification of aonla juice, Kadam *et al*. (2014) [9] in effect of pectinase enzyme on clarification of grape juice, Sharma *et al*. (2014) [15] in enzymatic extraction and clarification of juice from various fruits, Sherpa *et al*. (2014) [16] in enzymes assisted juice extraction from plum.

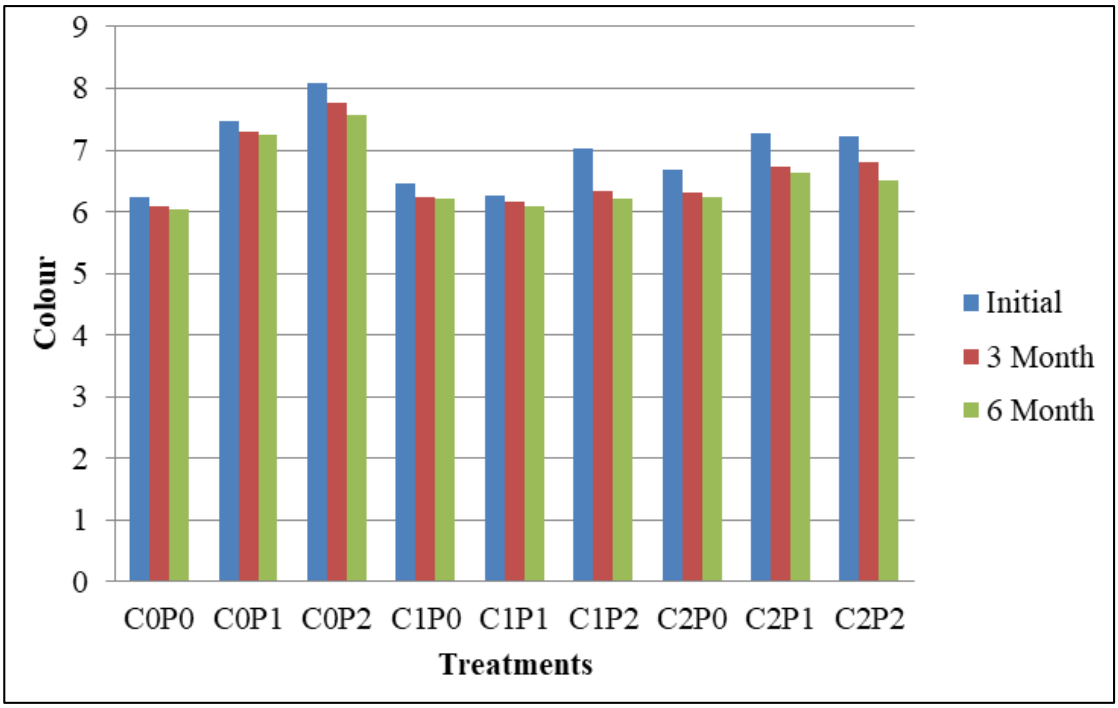


Fig 1: Effect of enzyme on Colour of clarified aonla juice during storage

Taste (Out of 9 points)

The score of taste in clarified aonla juice was found significant. The maximum taste score was recorded in C_0P_1 i.e. (pectinase: cellulase, 1:0) which was at par with C_2P_2 i.e. (pectinase: cellulase, 1:1) and minimum score in C_1P_0 i.e. (pectinase: cellulase, 0:0.5). The taste score of the product was decreased significantly irrespective of the treatment up to the end of the six month storage. Moreover, the highest taste score was recorded in C_0P_1 i.e. (Pectinase: cellulase, 0.5:0) may be due to the pulp particles contain air and later on results in the development of oxidative changes. The lowest taste score was recorded in C_1P_0 i.e. (pectinase: cellulase, 0:0.5) may be due to the enzyme like cellulase its give bitter taste. The pattern of decline of taste during storage might be due to the biochemical changes like increase in TSS, sugars and acidity as well as decrease ascorbic acid during storage. These observations were also similar to finding of Sin *et al*. (2006) [17] clarification of sapodilla juice, Vaidya *et al*. (2009) [19] in enzymatic extraction of kiwifruit juice, Joshi *et al*. (2011) [8] in pectinase enzyme on clarification of apple juice,

Egwim *et al*. (2013) [6] clarification of aonla juice, Kadam *et al*. (2014) [9] in effect of pectinase enzyme on clarification of grape juice, Sherpa *et al*. (2014) [16] in enzymes assisted juice extraction from plum.

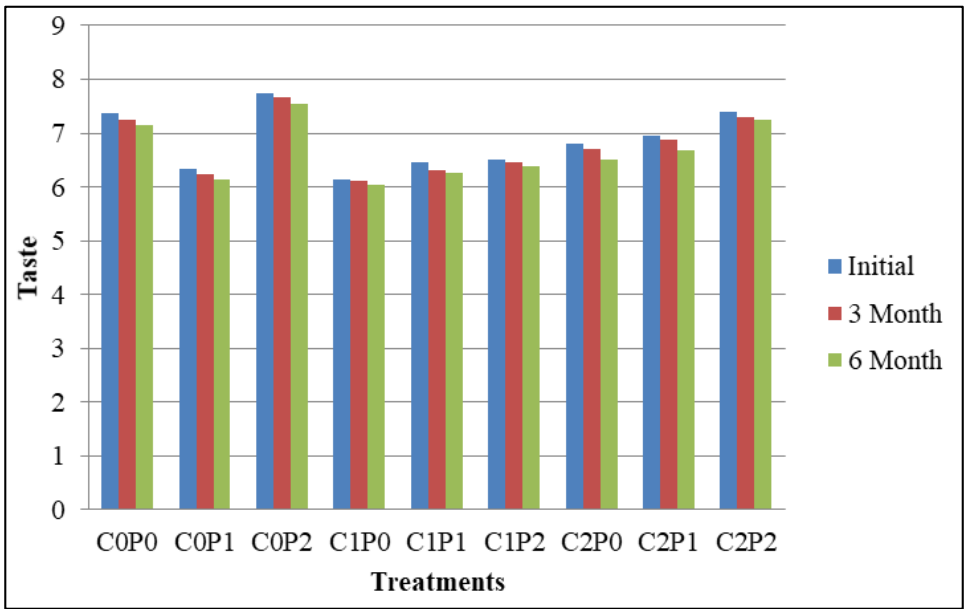


Fig 2: Effect of enzyme on Taste of clarified aonla juice during storage

Flavour (out of 9 points)

The score of flavour in clarified aonla juice was found significant. The flavour contain was found significantly highest in C_0P_1 *i.e.* (pectinase: cellulase, 0.5:0) which was at par with C_2P_2 *i.e.* (pectinase: cellulase, 1:1) and minimum score in C_1P_0 *i.e.* (pectinase: cellulase, 0:0.5). The flavour score of the product was decreased significantly irrespective of the treatment up to the end of the six months storage. Moreover, the highest flavour score was recorded in C0P1 i.e. (pectinase: cellulase, 0.5:0) may be due to the pulp particles contain air and later on results in the development of oxidative changes. The lowest flavour score was recorded in C_1P_0 *i.e.* (pectinase: cellulase, 0:0.5) may be due to enzymes concentration was zero. The pattern of continuously decrease in flavour score during storage might be due to the loss of highly volatile aromatic compound which is very sensitive to high storage temperature. Similar types of results were also in accordance with Sin *et al*. (2006) [17] clarification of sapodilla juice, Vaidya *et al*. (2009) [19] in enzymatic extraction of kiwifruit juice, Joshi *et al*. (2011) [8] in pectinase enzyme on clarification of apple juice, Egwim *et al*. (2013) [6] clarification of aonla juice, Kadam *et al*. (2014) [9] in effect of pectinase enzyme on clarification of grape juice, Sherpa *et al*. (2014) [16] in enzymes assisted juice extraction from plum.

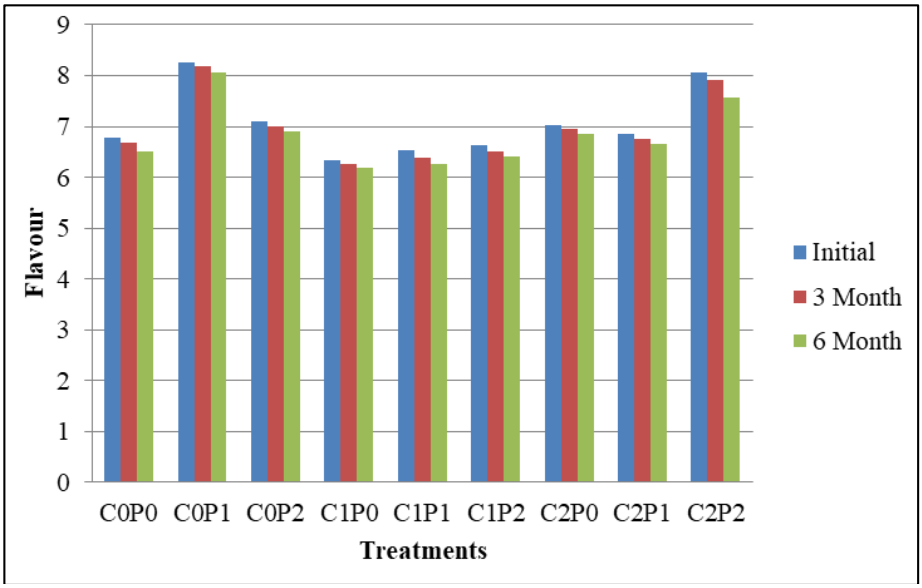


Fig 3: Effect of enzyme on Flavour of clarified aonla juice during storage

Aroma (out of 9 points)

The score of aroma in clarified aonla juice was found significant. The aroma (out of 9 points) contain was found significantly highest in C_2P_2 i.e. (pectinase: cellulase, 1:1) which was at par with C_0P_2 i.e. (pectinase: cellulase, 0.5:0) and minimum score in C_1P_0 i.e. (pectinase: cellulase, 0:0.5). The aroma score of the product was decreased significantly irrespective of the treatment up to the end of the six months storage. Moreover, the highest aroma score was recorded in C_2P_2 i.e. (pectinase: cellulase, 1:1) may be due to the pulp particles contain air and later on results in the development of oxidative changes. The lowest aroma score was recorded in

C_1P_0 i.e. (pectinase: cellulase, 0:0.5) may be due to enzymes concentration was zero. The pattern of continuously decrease in aroma score during storage might be due to the loss of highly volatile aromatic compound which is very sensitive to high storage temperature. Similar types of results were also in accordance with Sin *et al*. (2006) [17] clarification of sapodilla juice, Vaidya *et al*. (2009) [19] in enzymatic extraction of kiwifruit juice, Joshi *et al*. (2011) [8] in pectinase enzyme on clarification of apple juice, Egwim *et al*. (2013) [6] clarification of aonla juice, Kadam *et al*. (2014) [9] in effect of pectinase enzyme on clarification of grape juice, Sherpa *et al*. (2014) [16] in enzymes assisted juice extraction from plum.

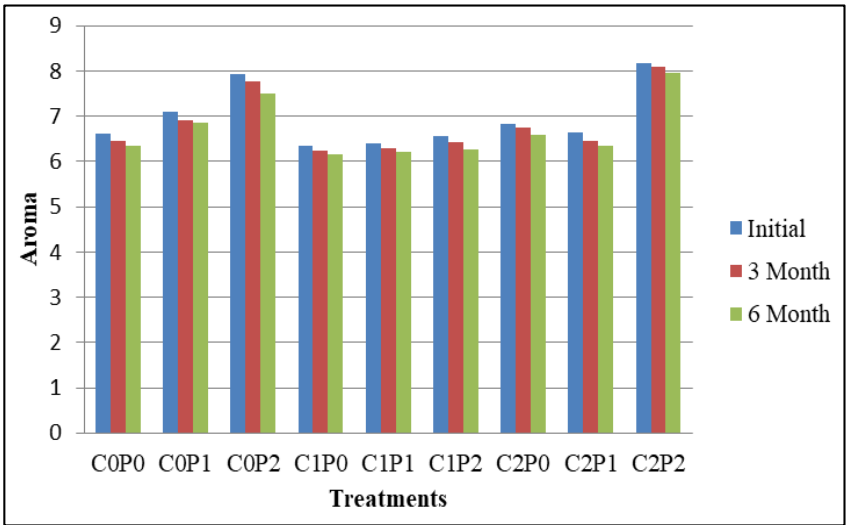


Fig 4: Effect of enzyme on aroma of clarified aonla juice during storage

Overall acceptability (out of 9 points)

The score of overall acceptability in clarified aonla juice was found significant. The overall acceptability contain was found highest in C_0P_2 i.e. (pectinase: cellulase, 1:0) which was at par with C_2P_2 i.e. (pectinase: cellulase, 1:1) and minimum in C_1P_0 i.e. (pectinase: cellulase, 0:0.5).The overall acceptability score of the product was decreased significantly irrespective of the treatment up to the end of the six months storage. In overall acceptability of juice considering the colour, taste, flavour and aroma of the nectar, the treatment C_0P_2 i.e. (pectinase: cellulase, 1:0) was found more acceptable. It may be due to the enzyme like pectinase breakdown the particles, hence improve the sensory qualities in terms of sensory. The lowest overall acceptability score was reported in C_1P_0 i.e. (pectinase: cellulase, 0:0.5) may be due to the enzyme like cellulase which give bitter taste. And dark colour which was responsible for lowest acceptance in the overall acceptability of juice. The pattern of continuously decrease in overall acceptability score during storage might be due to the decline the all sensory parameters like colour, taste, flavour and aroma with increasing storage period. Such identical findings were also revealed by Sin *et al*. (2006) [17] clarification of sapodilla juice, Vaidya *et al*. (2009) [19] in enzymatic extraction of kiwifruit juice, Joshi *et al*. (2011) [8] in pectinase enzyme on clarification of apple juice, Egwim *et al*. (2013) [6] clarification of aonla juice, Kadam *et al*. (2014) [9] in effect of pectinase enzyme on clarification of grape juice, Sherpa *et al*. (2014) [16] in enzymes assisted juice extraction from plum.

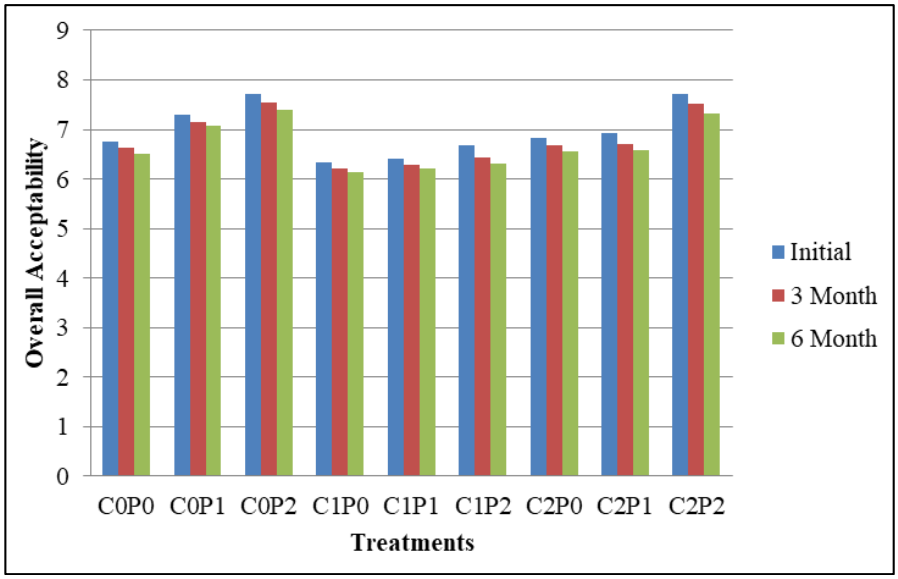


Fig 5: Effect of enzyme on Overall Acceptability of clarified aonla juice during storage

Conclusion

The overall data concluded that sensory evaluation in respect to colour, taste, flavour, aroma and overall acceptability C_0P_2 *i.e.* (pectinase: cellulase, 1:0). Aonla fruits given enzyme treatment with 1.0 per cent pectinase for 2 hours followed by

pressing to get higher number of score in sensory quality of clarified juice.

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