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Study of biochemical changes and microbial load of custard apple RTS beverage for better human health and nutrition

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Abstract
Custard apple – one of the “super fruits” of 21st century in India is not only a delicious fruit but also possesses great nutritive and medicinal value. It contains many nutrients, anti oxidants and minerals that are beneficial for a healthy life. The fruit exhibits potent anti-diabetic, anti-obese, anti-microbial and anti-cancer properties. However, one of the major drawbacks of the fruit is that it is characterized with high perishability and a short shelf life. The post-harvest losses are substantial due to heavy losses in firmness at room temperature. In these circumstances, preparation of Ready-To-Serve (RTS) beverage is one technique that fulfills the requirement of standard technique to reduce the post-harvest losses. The present study investigates the effect of acidity and ascorbic acid changes and effect on microbial load of on the storage of custard apple RTS beverage. Acidification of custard apple RTS from 0.3% to 0.61% and storage at ambient temperature resulted in marked reduction in total colony count during 6 month of storage; however, ascorbic acid degradation might be due to oxidation or irreversible conversion of L-ascorbic acid into dehydro ascorbic acid oxidase (ascorbimase). The results also indicated that relatively high concentration of vitamin C were present in custard apple RTS beverage containing high population of microorganisms.

Keywords: biochemical changes, custard apple RTS beverage, human health and nutrition.

Introduction

Custard apple is one of the most important fruit crops of tropical and dry land India and possesses numerous nutritional and medicinal benefits. In India, custard apple is grown in Maharashtra, Gujarat, Andhra Pradesh, Uttar Pradesh, Madhya Pradesh, Bihar, Assam, Rajasthan, Orissa and Tamil Nadu. Its cultivation in India has been estimated to be 44,000 hectares with an annual production of 367 thousand million tonnes (Annon, 2017). The large aggregate fruits are composed of peel, pulp and seeds. Pulp is cream coloured, custard like, granular, sweet with pleasant flavour and mild aroma. Fruit contain 45% edible portion, 100 g of which has a composition of 70.5 g moisture, 23.5 g carbohydrates, 16 g protein, 0.4 g fat, 17 mg niacin, 37 mg vitamin and gives 104 k calories of energy (Singh, 1995) [10]. The fruit contains significant quantities of vitamin C, thiamine, potassium, magnesium and dietary fibre. Despite its high sugar content, the glycemic index of custard apple is low (54) and the glycemic load moderate (10.2). Custard apple appears to possess potent bioactive principles in most of its plant parts (fruit, seed and leaves). The fruit exhibits potent anti-diabetic, anti-obese, anti-microbial and anti-cancer properties (George, 2018) [3]. The ripe fruit of this tree possesses astringent, cooling, febrifugal and pectoral properties. It works as a tonic for the blood and improve muscular strength. It is excellent for the digestive system and is prescribed for vomiting, diarrhoea, dysentery and vertigo.

The dried, powdered, unripe fruits are considered effective in healing ulcers, and are used as an insecticide, particularly for lice. Both the ripe pulp and crushed leaves are mixed with salt and applied as a poultice to hasten suppuration of boils and to relieve pains and swellings (www.indianetzone.com/e-magazine.html).

However, due to its high perishability and short production season, it requires careful and efficient post-harvest handling and management skills. The short shelf life of this fruit coupled with the absence of appropriate on farm preservation and value addition, leads to its glut in market during on season, resulting in low prices, ultimately causing losses to the producers. In these circumstances there is need to develop standard techniques to reduce those post-harvest losses. Preparation of Ready-To-Serve beverage is one technique that fulfills the requirement of standard technique to reduce the post-harvest losses, but it needs proper standardization of juice extraction method with suitable recipe.

Materials and methods

Preparation of RTS

Fresh matured custard apple fruit were purchased from the college farm and were brought to Department of Horticulture, JAU, Junagadh, where the research was carried out used for experimentation. The fruits were washed and cleaned thoroughly then peeling was done manually by hand. Two method of juice extraction were used for preparation of the product.

For cold method of juice extraction, the pulp was extracted by scooping and kept in sterilized stainless steel container. The skin and seeds were separated and recorded. Then, juice was filtered through a fine meshed silk cloth. This juice containing some pulp particles was allowed to keep in refrigerator at low temperature overnight in airtight condition to settle down the coarse particle at bottom.

For hot method of juice extraction, the crushed pulp with half quantity of water heated up to 80 °C for softening. The heated mass is passed through fine meshed silk cloth to extract the juice. The same process was employed to separate the pure juice content as in case of cold method.

For the preparation of RTS of different recipe, sugar syrup was prepared by adding water and citric acid. For this purpose required quantity of sugar was added to measured quantity of water and boiled it to dissolve the sugar. During heating the required quantity of citric acid and potassium metabisulphite was added. The prepared syrup was strain through coarse muslin cloth and then according to recipe required quantity of custard apple alone or blended with lime was added. The prepared RTS beverage filled in clean, sterilized bottles of 200 ml and air tight by crown corking machine. The filled bottles of RTS beverage bottle were stored in dried place at ambient temperature which ranged from 10.60 °C (minimum) to 28.84 °C (maximum).

Twelve treatment combinations consisting of two levels of juice extraction method (cold method (M_1) and hot method (M_2)), six levels of recipe (15% juice + 15% TSS + 0.3% acidity (R_1), 20% juice + 15% TSS + 0.3% acidity(R_2), 25% juice + 15% TSS + 0.3% acidity (R_3), 15% blended juice of custard apple and lime (3:1) + 15% TSS + 0.3% acidity (R_4), 20% blended juice of custard apple and lime (2:1) + 15% TSS + 0.3% acidity (R_5), 25% blended juice of custard apple and lime (1:1) + 15% TSS + 0.3% acidity (R_6) were used in the study.

Microbial load/count

Microbiological contamination by bacteria and fungi was determined by serial dilution method on standard NAA media and PDA media, respectively. The determination of the total microbial contamination of the pulp samples was performed after 30 days until six months. In serial dilution, 1 ml juice sample is taken and is mixed in 9 ml sterile water. This will make total volume of tube 10 ml and the solution will be 1:100 time diluted. Similarly, if the solution is diluted six time this will give 10^{-6} fold dilution of the sample. Hundred micro liters to one ml of the solution is taken and spread on the Petri plate filled with the desired media. Plates are incubated in inverted position for 24 hours at approximately 37 °C temperatures in incubator and total bacterial colony and total fungal colony was calculated by using colony counter.

Result and Discussion

Acidity is an important quality parameter of the product. Therefore, maintenance of acidity of product during storage assumed special significance. The data presented in Table 1 reveals the acidity of custard apple RTS beverage during storage increased with the advancement of storage period. However, the rate of increase in acidity of the product was significantly influenced by various juice extraction methods during entire storage period. The increase in acidity of custard apple RTS beverage during 180 days of storage may be due to formation of organic acids by ascorbic acid degradation (Kumar, 1990) [5]. Another possible reason of increase acidity is degradation of pectic substances into soluble solids which might have contributed toward increase in the acidity of product (Pandey and Singh, 1999) [7]. The treatment R_4 and treatment R_6 both recorded the maximum acidity, which might be due to blending of custard apple with lime juice. Attri *et al*. (1998) [2] reported that the acidity was found to increase by blending apricot with plum juice, whereas it got reduced with apple juice and apple concentrate after six month of storage at ambient temperature.

In present study the ascorbic acid content in custard apple RTS beverage was decreased as the storage period increased (Table 2). It is evident from the data that the custard apple blended with lime juice exhibited higher initial ascorbic acid content. The decrease in ascorbic acid ascorbic acid content was significantly influenced by different recipe treatment. At the end of storage, the maximum retention of ascorbic acid content of 4.87 mg/100 ml RTS was recorded at R_6 treatment (25% blended juice of custard apple and lime (1:1) + 15% TSS + 0.3% acidity) as compared to R_1 (15% juice + 15% TSS + 0.3% acidity) i.e. 3.38 mg/100 ml RTS (Table 2). The decrease in ascorbic acid in RTS during storage might be due to oxidation or irreversible conversion of L-ascorbic acid into dehydro ascorbic acid oxidase (ascorbimase). Similar reduction in ascorbic acid content has also been reported in guava beverage (Pandey and Singh, 1998) [8]. Singh (2000) [9] found continuous decrease in the level of ascorbic acid in RTS and squash of carambola fruit during six months of storage. In the present study, the higher ascorbic acid content at different recipe treatment having custard apple juice blend with lime juice is due to the additional ascorbic acid content received from the lime juice. On 180th day of storage, the minimum reduction of ascorbic acid content from 7.76 mg/100 ml to 4.36 mg/100 ml RTS was recorded at M_2R_4 treatment, whereas, the maximum reduction might be due to complementary effect of these two factors.

In case of microbial load the data recorded that bacterial load and fungal load on custard apple RTS beverage were increased from 0 day upto 30 days then decreased with in advancement of storage period up to 180 days. However, the rate of increased and decreased in bacterial colony load (Table 4) and fungal colony load (Table 5) and graphically depicted affected by various treatment i.e., juice extraction methods, recipe and their combinations. Similar finding was reported by Goyle and Ojha (1998) [4] studies the effect of microbial load and sensory attributes of orange pulp and reported that the total bacterial counts increased in the first weak and then decreased considerably from the second to the fourth week of storage. In the first week, the plate counts had increased from the initial value of 52 x 10^2 to 131 x 10^2cfu /ml.

The data revealed that the bacterial and fungal growth pattern at various time intervals and the systemic increased in dilution of the custard apple RTS beverage samples, corresponding to the storage time. The growth was found to be highest at the 30th day of the storage period which may be attributed to the chemical changes, specifically alteration in the pH of the system that would take place resulting from the presence of the chemical preservatives in the samples. Similar finding was reported by Li *et al*., (1989) [6] studied the effect of acidification, combination of acidification and low temperature and sorbates on the storage of orange juice and reported that relatively high concentrations of vitamin- "C" were present in orange containing high population of microorganisms. Thakur *et al*., (2000) [11] also studies on physico-chemical and microbiological qualities of Debittered Kinnow Juice and conclude that low microbial count was detected in the concentrate at the beginning, which increased with the advancement of storage. Bacteria had lowest counts, whereas, osmophilic yeasts recorded highest counts after 6 months of storage.

Table 1: Effect of juice extraction methods and recipe on Acidity content (%) of custard apple RTS beverage during storage.

Treatment	Storage periods (days)						
	0	**30**	**60**	**90**	**120**	**150**	**180**
M_1	0.30	0.36	0.41	0.44	0.50	0.54	0.61
M_2	0.30	0.36	0.41	0.44	0.51	0.54	0.59
S.Em±	-	0.001	0.02	0.001	0.001	0.001	0.005
C.D. at 5%	-	NS	0.04	0.004	0.004	NS	0.016
R_1	0.30	0.35	0.39	0.43	0.48	0.52	0.56
R_2	0.30	0.36	0.40	0.43	0.49	0.53	0.56
R_3	0.30	0.36	0.40	0.43	0.49	0.54	0.58
R_4	0.30	0.38	0.43	0.46	0.52	0.55	0.63
R_5	0.30	0.37	0.42	0.45	0.51	0.55	0.61
R_6	0.30	0.36	0.43	0.46	0.52	0.56	0.63
S.Em±	-	0.002	0.003	0.002	0.002	0.002	0.009
C.D. at 5%	-	0.004	0.007	0.007	0.006	0.005	0.027
CV %	-	1.03	1.52	1.31	1.05	0.76	3.84
Interaction	NS	NS	NS	NS	NS	NS	NS

Table 2: Effect of juice extraction methods and recipe on ascorbic acid content (mg/100 ml) of custard apple RTS beverage during storage.

Treatment	Storage period (days)						
	0	**30**	**60**	**90**	**120**	**150**	**180**
M_1	7.49	6.77	5.64	4.99	4.64	4.39	3.98
M_2	7.46	6.75	5.15	4.95	4.59	4.34	3.94
S.Em±	0.02	0.01	0.05	0.03	0.01	0.03	0.01
C.D. at 5%	NS	NS	0.13	NS	0.04	NS	0.04
R_1	7.15	6.48	5.20	4.29	3.77	3.53	3.38
R_2	6.92	6.54	4.86	4.33	4.08	3.91	3.43
R_3	7.47	6.63	5.17	4.47	4.17	4.05	3.60
R_4	7.76	6.88	5.45	5.36	5.06	4.96	4.40
R_5	7.31	6.87	5.28	5.32	5.04	4.74	4.10
R_6	8.23	7.17	6.41	6.05	5.58	5.01	4.87
S.Em±	0.03	0.02	0.08	0.05	0.02	0.05	0.02
C.D. at 5%	0.08	0.06	0.23	0.13	0.07	0.14	0.07
CV %	0.89	0.71	3.56	2.28	1.19	2.64	1.38

Table 3: Interaction effect of pulp/juice extraction method and recipe on ascorbic acid content of custard apple RTS during storage (mg/100 ml RTS).

Treatment	Storage Periods (days)						
	0	**30**	**60**	**90**	**120**	**150**	**180**
M_1R_1	7.16	6.43	5.50	4.29	3.82	3.55	3.39
M_1R_2	7.18	6.56	5.14	4.33	4.09	3.94	3.43
M_1R_3	7.26	6.60	5.14	4.44	4.19	4.07	3.60
M_1R_4	7.76	6.89	5.83	5.45	5.09	4.97	4.43
M_1R_5	7.24	6.87	5.77	5.37	5.07	4.75	4.12
M_1R_6	8.32	7.28	6.48	6.08	5.61	5.06	4.93
M_2R_1	7.15	6.53	4.90	4.29	3.73	3.50	3.37
M_2R_2	6.65	6.51	4.57	4.33	4.07	3.88	3.42
M_2R_3	7.67	6.66	5.20	4.50	4.16	4.02	3.59
M_2R_4	7.76	6.88	5.07	5.27	5.04	4.95	4.36
M_2R_5	7.38	6.87	4.80	5.27	5.01	4.73	4.09
M_2R_6	8.13	7.07	6.35	6.03	5.55	4.96	4.81
S.Em.±	0.04	0.03	0.11	0.07	0.03	0.07	0.03
C.D. at 5%	0.11	0.08	0.32	NS	NS	NS	NS
C.V. %	0.89	0.71	3.56	2.28	1.19	2.64	1.38

Table 4: Interaction effect of pulp/juice extraction method and recipe on bacterial colony of custard apple RTS during storage.

Treatment	Storage periods (days)						
	0	30	60	90	120	150	180
M_1R_1	19 x 10^3	36 x 10^3	12 x 10^3	6 x 10^3	3 x 10^3	2 x 10^3	1 x 10^3
M_1R_2	17 x 10^3	34 x 10^3	11 x 10^3	3 x 10^3	2 x 10^3	-	-
M_1R_3	18 x 10^3	32 x 10^3	10 x 10^3	2 x 10^3	2 x 10^3	1 x 10^3	-
M_1R_4	8 x 10^3	14 x 10^3	10 x 10^3	3 x 103	2 x 10^3	-	
M_1R_5	10 x 10^3	22 x 10^3	9 x 10^3	2 x 10^3	2 x 10^3	2 x 10^3	2 x 10^3
M_1R_6	12 x 10^3	24 x 10^3	8 x 10^3	2 x 10^3	1 x 10^3	-	-
M_2R_1	12 x 10^3	13 x 10^3	10 x 10^3	5 x 10^3	2 x 10^3	-	-
M_2R_2	8 x10^3	16 x 10^3	8 x 10^3	3 x 10^3	1 x 10^3	1 x 10^3	-
M_2R_3	6 x 10^3	14 x 10^3	9 x 10^3	2 x 10^3	-	-	-
M_2R_4	8 x 10^3	11 x 10^3	7 x 10^3	4 x 10^3	1 x 10^3	-	-
M_2R_5	3 x 10^3	11 x 10^3	6 x 10^3	3 x 10^3	-	-	-
M_2R_6	4 x 10^3	12 x 10^3	8 x 10^3	2 x 10^3	-	-	-

Table 5: Interaction effect of pulp/juice extraction method and recipe on fungal colony of custard apple RTS during storage.

Treatment	Storage periods (days)						
	0	30	60	90	120	150	180
M_1R_1	56 x 10^6	120 x 10^6	32 x 10^6	12 x 10^6	12 x 10^6	8 x 10^6	6 x 10^6
M_1R_2	82 x 10^6	208 x 10^6	46 x 10^6	28 x 10^6	24 x 10^6	12 x 10^6	8 x 10^6
M_1R_3	64 x 10^6	172 x 10^6	36 x 10^6	17 x 10^6	17 x 10^6	11 x 10^6	7 x 10^6
M_1R_4	30 x 10^6	88 x 10^6	26 x 10^6	10 x 10^6	9 x 10^6	6 x 10^6	3 x 10^6
M_1R_5	62 x 10^6	160 x 10^6	33 x 10^6	14 x 10^6	14 x 10^6	9 x 10^6	6 x 10^6
M_1R_6	58 x 10^6	112 x 10^6	38 x 10^6	18 x 10^6	18 x 10^6	10 x 10^6	8 x 10^6
M_2R_1	54 x 10^6	116 x 10^6	26 x 10^6	11 x 10^6	11 x 10^6	7 x 10^6	4 x 10^6
M_2R_2	76 x 10^6	152 x 10^6	39 x 10^6	18 x 10^6	18 x 10^6	11 x 10^6	5 x 10^6
M_2R_3	48 x 10^6	104 x 10^6	35 x 10^6	16 x 10^6	16 x 10^6	13 x 10^6	6 x 10^6
M_2R_4	28 x 10^6	76 x 10^6	32 x 10^6	17 x 10^6	11 x 10^6	9 x 10^6	5 x 10^6
M_2R_5	31 x 10^6	92 x 10^6	24 x 10^6	9 x 10^6	6 x 10^6	4 x 10^6	1 x 10^6
M_2R_6	32 x 10^6	96 x 10^6	26 x 10^6	10 x 10^6	10 x 10^6	6 x 10^6	2 x 10^6

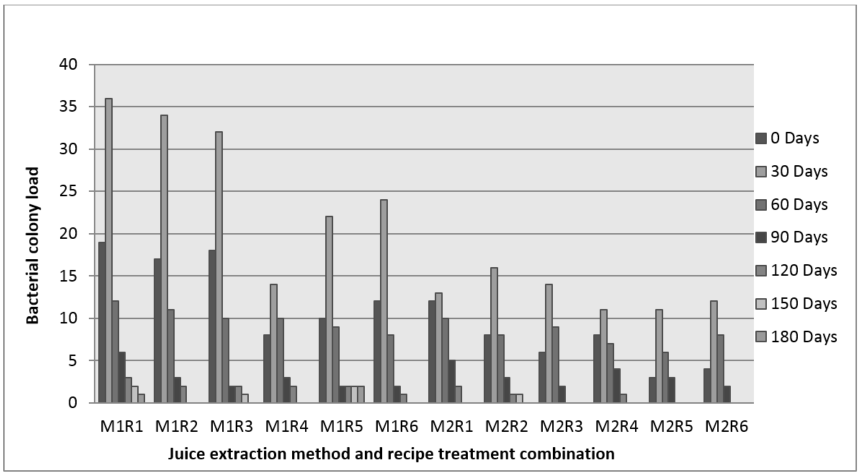

Fig 1: Interaction effect of pulp/juice extraction method and recipe on bacterial colony of custard apple RTS during storage.

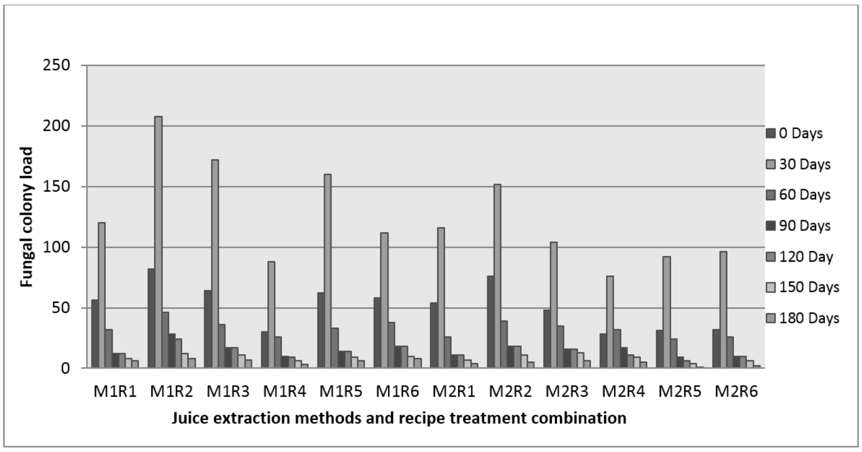

Fig 2: Interaction effect of pulp/juice extraction method and recipe on fungal colony of custard apple RTS during storage.

References

1. Anonymous. Horticulture Statistics- At a Glance-2017. Govt. of India, Ministry of Agriculture & Farmers Welfare, Department of Agriculture & Farmers Welfare, Horticulture Statistics Division, 2017, 142.
2. Attri BL, Lal BB, Joshi VK. Physico-chemical characteristics, sensory quality and storage behaviour of sand pear juice blended with temperate fruit juices/pulp. Indian Food Packer. 1998; 52(6):36-42.
3. George AP. Custard Apple - The New Super Fruit of the 21st Century. Custard apples Australia, 2018.
4. Goyle A, Ojha P. Effect of storage on vitamin C, microbial load and sensory attributes of orange juice. Journal of Food Science and Technology. 1998; 35(4):346-348.
5. Kumar S. Studies on post-harvest technology of papaya fruits. Ph.D. Thesis, NDUA&T, Kumarganj, Faizabad (U.P.), 1990.
6. Li Z, Alli I, Kermasha S. Use of acidification, low temperature, and sorbates for storage of orange juice. Journal of Food Science. 1989; 54(3):674-678.
7. Pandey AK, Singh IS. Studies on preparation and preservation of guava ready-to-serve beverage. Indian Journal of Horticulture. 1999; 56(2):130-132.
8. Pandey AK, Singh IS. Studies on preparation and preservation of guava Ready-to-serve beverage. Indian Journal of Horticulture. 1998; 56(2):130.
9. Singh, J. *Studies on processing of carambola* (*Averrhoea carambola* Linn) *fruit*. M.Sc. Thesis, Narendra Deva University of Agriculture and Technology, Faizabad (U.P.), 2000.
10. Singh SP. Commercial fruits, Kalyani Punblishers, Calcutta, 1995, 289.
11. Thakur NK, Lal Kausal BB, Joshi VK. Effect of different conditions of storage on physico-chemical and Microbiological qualities of debittered kinnow juice concentrate. Journal of Food Science and Technology. 2000; 37(4):415-418.
    www.indianetzone.com/e-magazine.html