BIOCHEMICAL STUDIES OF ANNATTO COLOURED GUAVA BEVERAGES ON STORAGE

Abstract

A study was carried out on biochemical studies of guava beverages during storagein Laboratory of the Department of Post-harvest Technology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya during 2018-19. Pink or redguava is generally used in processing industry due to its attractive pulp colour, making a significant contribution to food industry. Colouris an integral part of any processed food productsince colour makes the processed food productattractive. In the present experiment,different amounts of annatto seed were used for adding color to white fleshed guava beverages, *i.e.*, RTS and Squash. The results reveal that 0.8 g annatto seeds were found the most appropriate quantity for colouring one-litre RTS and 2 g annatto seeds for coloring one litre squash.It was also observed that the guava RTS and squash retained normalannatto color for eight months, thereafter, both the beverages started showing non-enzymatic browning during storage.The extract of annatto seedswas also found to act as preservative forboth the processed products of guava.

Keywords: *RTS, squash, guava, annatto*

Introduction

Guava (*Psidium guajava* L.) is one of the most important fruit crops. Because of the biological activities and medicinal applications, guava is considered as the *Poor Man's Apple* or *Apple of the Tropics*. It is often marketed as *Super Fruit*, which has a considerable nutritional importance in terms of C vitamin and A.Guava, which is pleasantly sweet and refreshing in flavour, is normally consumed fresh as dessert. However, its fruits can be processedas value added durable products, like jelly, jam, RTS, squash, toffees, *etc*. Hence, there is a great demand of red-fleshed guava in the international market. Beverages prepared from white fleshed guava are very good in flavour but scarce in attractive colour, whereas, pink or red-fleshed guava beverages are more attractive in colour. The pink colour of guava is due to the presence of naturally occurring pigments called carotenoids. Theunavailability of pink or red-fleshed guava in the market is due to less area under guava crop, which is not enough to fulfill the demand of beverage industries. Therefore, synthetic colour is used to make the guava beverages appealing and attractive. However, the consumers have now become aware of the hazards caused by synthetic food additives, thus looking for food with natural ingredients.

Annatto (*Bixa orellana* L.) is a tall shrub or small tree of Bixaceae family. Aril portion of its seeds is orange-red in colour, which is one of the oldest known sources of natural colour. Annatto seed extract can be used as a colorant in the formulation of lipstick preparation(Indriana and Salman, 2022)Due to the colour producing property, it is also known as *lipstick tree*. Annatto is often used to impart colour in processedproductsand it isused for its aroma and flavour. The extract of annatto seeds is now widely used on industrial scale as a colouring agent in many processed food products. The colour in annatto is due to the presence of carotenoids, *i.e.*, Bixin ($C_{25}H_{30}O_4$) and Norbixin ($C_{24}H_{28}O_4$), and addition of its extract may improve nutritional value of the beverages. Therefore, keeping in view theabove facts, theexperiment on *annatto enriched guava beverages* was plannedto study the chemical composition of guava pulp and the changes in annatto enriched guava beverages during storage.

Materials and Methods

The experiment comprising of five treatments,*i.e.*,(i) 10% guava pulp + acidity 0.25% + annatto seed nil + sodium benzoate 100 ppm, (ii) 10% guava pulp + acidity 0.25% + annatto seed 0.4g + sodium benzoate 100 ppm, (iii) 10% guava pulp + acidity 0.25% + annatto seed 0.8g + sodium benzoate 100 ppm, (iv) 10% guava pulp + acidity 0.25% + annatto seed 1.2g + sodium benzoate 100 ppm and (v) 10% guava pulp +

acidity 0.25% + annatto seed 1.4g + sodium benzoate 100 ppm for ready to serve beverage and five treatments, *i.e.*, (i) 25% guava pulp + acidity 1.1% + annatto seed nil + sodium benzoate 600 ppm, (ii) 25% guava pulp + acidity 1.1% + annatto seed 1.0g + sodium benzoate 600 ppm,(iii)25% guava pulp + acidity 1.1% + annatto seed 2.0g + sodium benzoate 600 ppm, (iv)25% guava pulp + acidity 1.1% + annatto seed 3.0g + sodium benzoate 600 ppm and (v) 25% guava pulp + acidity 1.1% + annatto seed 4.0g + sodium benzoate 600 ppm for squash was conducted in Laboratory of the Department of Post-harvest Technology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya during the year 2017-18. Firmly ripened guava fruits of cultivar Lucknow-49and fully mature annatto pods of local germplasmweretaken from Main Experimental Station ofthe College of Horticulture and Forestryin the month of November and May, respectivelyand brought to the Post-harvest Technology Laboratory. Guava fruits were washed under gentle stream of tap water,cut into small pieces and ground in mixture with water in ratio of 1:1 and strained through muslin cloth to obtainpulp.Thereafter,the preservative sodium benzoate was added at the rate of 600mg/litre of pulp and mixed thoroughly. The pulp was filled in bear bottles, which were sealed hermeticallywith crown cork, pasteurized and labeled.The bear bottles containing pulp were stored up to May untilmaturity of the annatto pods.The colourof annatto seeds was extracted by boiling them in 100 ml water for 10 minutesand retaining overnight. Next day, thesolubulizedcolour was obtained by filteringthrough muslin cloth andadded to guava RTS at the rate of 0, 0.4, 0.8, 1.2 and 1.6 g and guava squash at the rate of 0, 1, 2, 3 and 4 g/litre. The syrup was prepared by heating the mixture of sugar,water,annatto colourand citric acidandstraining through muslin cloth. Finally, the pulp was blended with syrup and filled in 200ml bottles leaving 2 cm headspace and then,the bottles were sealed hermetically with crown cork. The same process was replicated thrice. The sealed bottles of RTS were pasteurized for 20 minutes in boiling water and then cooled in air. The bottled RTS and squash were kept for storage studies under ambient room temperature conditions.A panel of 10 semi-trained members carried out the overall acceptance test for the juice by following ninepoints Hedonic scale, where ninepoint on scale represented*Liked Extremely* and one*Dislike Extremely*(Amerine *et al.*,1965). Acidity, ascorbic acid, reducing sugars, non-reducing sugar and total sugars of guava pulp and annatto coloured guava RTS and squash were estimated by titrimetric method (Ranganna, 1986).The total soluble solids(%) were determined directly by usinghand refractometer.The statistical analysis was carried out by using CompleteRandomized Design.

Result

Standardization of annatto seed for colour and quality of RTS

Organoleptic evaluation was done to assess the quality of annatto enriched guava RTS with different amountsof annatto seed colour for quality attributes such as appearance, flavour and overall acceptability. The data on overall ranking of sensory traits reveal that guava RTS enriched with annatto seed 0.8g per litre was rated as *Liked Very Much* with highest point (8.2) as compared to other quantity of annatto seeds. Addition of annatto seeds also prolonged the shelf life of guava RTS. Therefore, annatto seed was selected for storagestudies of guava RTS.

Table 1: Organoleptic quality of guava RTS enriched with different amount of annatto seed colour

Treatments	Amount of annatto seed(g/litre)	Organoleptic quality of guava RTS	
		Score	Rating
T_1	Nil	7.7	LM
T_2	0.4	7.8	LM
T_3	0.8	8.2	LVM
T_4	1.2	7.5	LM
T_5	1.6	6.9	LM

Note: LM- Liked Much; LVM- Liked Very Much

Standardization of annatto seed forcolour and qualityof squash

The guava squash enriched with different amounts of annatto seedwas assessed for quality. The data reveal that the annatto seed 2.0 g per litregave best colourand taste to guava squash, which was rated as*Liked Very Much* with highest point (8.3) as compared to other treatments (Table 2).

Table 2:Overall quality of guava squash enriched with different amount of annattoseed

Treatment	Amount of annatto seed (g/litre)	Score	Rating
T_1	Nil	7.8	LM
T_2	1.0	7.9	LM
T_3	2.0	8.3	LVM
T_4	3.0	7.6	LM
T_5	4.0	7.2	LM

Note: LM- Liked Much; LVM- Liked Very Much

The important sensory parameters, *i.e.*, colour, flavour, appearance and overall acceptabilitywererecorded for guava beverages during experimentation. The organoleptic datarecorded on overall ranking of sensory traits reveal that the overall acceptability of RTS and squash wasratedmaximum (Like Very Much)when the annatto seed extract was added @ 0.8 and 2.0 g/litre,respectively. Until now, not much work has been done on the application of annattoseed extractin non-dairy products. Satyanarayana*et al.* (2006) standardized the concentration of norbixin in different fruit and vegetable products, *i.e.*, 12.5 mg/kg in lime squash, 50 mg/kg in orange squash, 150 mg/kg in mixed fruit jam and 50-100 mg/kg intooty-fruity,whereas,Balaswamy *et al*. (2011)found that the water soluble annatto dye sugar powder formulation (WSASF) at a concentration of 5 and 30mg/kg was optimum to obtain required colourin *Jalebi* and *Jagery*,respectively.

Changes in Annatto enriched guava RTS during storage

No significant change was observed in total soluble solids, reducing sugars, total sugars and acidity ofannatto enriched guava RTS up to three months of storage but thereafter,the all four parametersincreased significantly upto the end of experiment. Ascorbic acid in RTS decreased non-significantly up to two months and then, it decreased significantly. No remarkable change was observed in the concentration of non-reducing sugar in RTS up to sixmonths of storage, thereafter, it decreased significantly up to the end of experiment. No non-enzymatic browningwas noticed in annatto-enriched guava RTS up to eight months of storage but thereafter, it started increasing significantly. The organoleptic value of annatto-enriched guava RTS did not change up to three months of storage but thereafter,itdecreased non-significantly up to eight months of storage.

Table 3: Changes in annatto seed enriched guava RTSduring storage

Months	Total Soluble Solids (%)	Acidity	Ascorbic Acid	Reducing Sugar	Non-reducing sugar	Total Sugar	Non-enzymatic browning
0	13.00	0.25	17.53	7.38	3.95	11.33	0.14
1	13.00	0.25	17.11	7.38	3.95	11.33	0.14
2	13.00	0.25	16.80	7.38	3.95	11.33	0.14
3	13.20	0.25	16.35	7.38	3.95	11.33	0.14
4	13.30	0.26	16.05	7.65	3.83	11.48	0.14
5	13.30	0.26	15.46	7.65	3.83	11.48	0.14
6	13.40	0.27	14.94	7.65	3.83	11.48	0.14
7	13.40	0.27	14.56	7.92	3.66	11.58	0.14
8	13.60	0.28	14.18	7.92	3.66	11.58	0.14
9	13.60	0.30	13.61	8.15	3.51	11.66	0.15
10	0.08	0.30	13.09	8.15	3.51	11.66	0.16

SEm±	**13.00**	**0.01**	**0.11**	**0.03**	**0.05**	**0.04**	**0.01**
CD (5%)	**13.0**	**0.02**	**0.32**	**0.09**	**0.16**	**0.13**	**0.01**

Changes in the Annatto enriched guava squash during storage

The total soluble solids, acidity, reducing, non-reducing and total sugars of annatto-enriched guava squashremained unchanged up to fourmonths of storage but thereafter, all the parameters started increasing gradually up to the end of theexperiment except non-reducing sugar. After four months of storage, the total soluble solids of squash increased significantly. The non-reducing sugar decreased non-significantly up to eight month of the storage. Ascorbic acid decreased non-significantly up to two months of storage and thereafter, it decreasedsignificantly. Non-enzymatic browning in annatto enriched guava squash stayedunchanged up to eight months of storage,thereafter, it increasednon-significantly. The organoleptic value of annatto-enriched guava squash stayedunchanged up to four months of storage but after that,the organoleptic value decreased non-significantly. The squash of guava was oranoleptically acceptable until the end of the experiment (ten months)with rating*Liked Very Much*.

Table 4: Changes in the annatto seed enriched guava squash during storages

Months	Total soluble solids	Acidity	Ascorbic acid	Reducing sugars	Non-reducing sugar	Total sugars	Non-enzymatic browning
0	50.0	1.1	43.49	29.16	7.93	37.09	0.37
1	50.0	1.1	43.06	29.16	7.93	37.09	0.37
2	50.0	1.1	42.69	29.16	7.93	37.09	0.37
3	50.0	1.1	42.16	29.16	7.93	37.09	0.37
4	50.0	1.1	41.66	29.16	7.93	37.09	0.37
5	50.3	1.11	41.05	29.94	7.62	37.56	0.37
6	50.6	1.12	40.60	29.94	7.62	37.56	0.37
7	50.6	1.13	40.23	30.73	7.37	38.10	0.37
8	50.8	1.13	39.07	30.73	7.37	38.10	0.37
9	51.0	1.14	38.69	31.23	7.09	38.32	0.39
10	51.2	1.14	38.15	31.23	7.09	38.32	0.41
SEm±	**0.07**	**0.01**	**0.33**	**0.10**	**0.09**	**0.08**	**-**
CD (5%)	**0.20**	**0.01**	**0.97**	**0.30**	**0.27**	**0.24**	**NS**

Table 5: Change in organoleptic value of annatto enriched guava beverages

Storage (Month)	RTS		Squash	
	Score	Rating	Score	Rating
0	8.2	LVM	8.3	LVM
1	8.2	LVM	8.3	LVM
2	8.2	LVM	8.3	LVM
3	8.2	LVM	8.3	LVM
4	8.1	LVM	8.3	LVM
5	8.1	LVM	8.2	LVM
6	8.1	LVM	8.2	LVM
7	7.9	LM	8.1	LVM
8	7.9	LM	8.1	LVM
9	7.7	LM	8.0	LVM
10	7.7	LM	8.0	LVM

SEm±	**0.09**	**-**	**-**	**-**
CD (5%)	**0.27**	**-**	**NS**	**-**

Discussion and conclusion

The total soluble solids, acidity, reducing sugars and total sugars of annatto enriched guava beverages increased slightly during storage at ambient room temperature. Similar results have been observed by Jan andMasih(2012) in lime, aonla, mango and pineapple spiced RTS beverage,Bal*etal.* (2014) in guava nectar and Rashid *et al.* (2018) in RTS of bio-coloured guava.The increase in total soluble solids of annatto enriched guava RTS and squash might be possible due to the hydrolysis of polysaccharides and oligosaccharides into monosaccharides (reducing sugars).The acidity in annatto-enriched guava RTS and squash increased during storage due to the presence of pectic substances.An increase in reducing and total sugars might be due to the inversion of non-reducing sugar to reducing sugars and hydrolysis of polysaccharides into monosaccharides.The increase in browning might be mainly due to the non-enzymatic reaction between nitrogenous compounds and sugars, nitrogenous compounds and organic acids, organic acids and sugars and among the organic acids themselves. The carbinol group of a cyclic sugar in combination with basic proteinsand complex of amino acid may cause browning.Ascorbic acid, non-reducing sugar and organoleptic value of annatto enriched guava beverages declined with the progress of storage period. The loss of ascorbic acid, non-reducing sugar and organoleptic quality was also observed byChoudhary*et al.* (2008) in nectar of guava cv.Lucknow-49,Bal*etal.* (2014) in nectar of guava cv. Lalit, Jakhar*et al*. (2012) in guava-barbados cherry blend RTS, Selvi*et al*. (2013) in guava, lime and ginger blended RTS,Sarkar and Bulo (2017) in guava and pineapple blended RTS and Ravi *et al.* (2018) in RTS and squash of different varieties of guava.Ascorbic acid degradation in annatto enriched guava RTS and squash might be due to the transparency of bottles for light since ascorbic acid is very sensitive to light and temperature. Reduction in ascorbic acid content might also be due to the oxidation with tapped oxygen in bottles, which resulted in the formation of dehydroascorbic acid. Gradual decrease in non-reducing sugar of annatto enriched guava RTS and squash during storage might be due to the conversion of non-reducing sugar into reducing sugars.Organoleptic score of annatto enriched guava RTS and squash decreased after 3 and 4 months of the storage, respectively. The annatto enriched RTS was found acceptable up to 10 months of storage, whereas, the annatto enriched squash was acceptableuntil the end of the experiment (upto10 months).In thepresent experiment,it was observed that annatto colour not only improved the appearance but also prolonged the storage life of guava beverages. The delayed changes inbiochemical parameters andprolonged storage life of guava RTS and squash was might be dueto the anti-microbial properties of annatto colour (Yolmeh*et al.*, 2014 ; Najafi*et al.*,2018) though the normal RTS and squash of any fruit had the acceptability only up to 4-6 and 6-8 months respectively.

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