The Pharma Innovation Journal 2022; 11(9): 1844-1847

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(9): 1844-1847

www.thepharmajournal.com
Received: 01-06-2022
Accepted: 05-07-2022

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Non-enzymatic browning of white guava fruit bar (cv. Allahabad Safeda) during storage: Effects of Antibrowning agents

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Abstract
The use of different antibrowning agents on the intensity of non-enzymatic browning in white guava fruit bars (cv. Allahabad Safeda) was evaluated. The purpose of the study was to examine the efficacy of novel antibrowning agents on browning index and non-enzymatic browning (OD values at 440 nm) during the storage period of three months. Initially, there was no significant difference among the different anti-browning agents for the moisture content (%) and water activity, while the highest browning index and non-enzymatic browning were found in the guava fruit bar incorporated with 1% ascorbic acid. During the three months of storage irrespective of antibrowning agents, the moisture content (%) and water activity decreased gradually while the browning index, and non-enzymatic browning significantly increased. In general, this paper brings out the antibrowning capabilities of novel anti-browning agents in non-enzymatic browning of guava fruit bars.

Keywords: Antibrowning agents, browning index, guava fruit bar, non-enzymatic browning

1. Introduction
Guava (*Psidium guajava* L.) is one among important fruit crop of tropical and subtropical regions and its commonly known as the Apple of the tropics. It's delicious and nutritious fruit with refreshing flavor and aroma. It's has four times higher concentration of vitamin C when compared to orange (Medina and Pagano, 2003) [10]. It's also a rich source of vitamin A (retinol), vitamin B_1 (thiamine), vitamin B_2 (riboflavin), vitamin B_3 (niacin), and vitamin B_6 (pyridoxine) (Kumari *et al*., 2017) [8]. In addition, it has high content of dietary fiber 5.2 g/100 g (Ramulu and Udayasekhara, 2003). The bioactive components contribute to the functional properties that help to prevent many degenerative diseases (Blancas-Benitez *et al*., 2018) [4]. Essential oils, phenols, triterpenes, flavonoids, and fatty acids all are present in fair amounts (Mehta *et al*., 2018) [11]. The pectin ranges from 0.5 to 1.9 per cent (Salunkhe and Desai, 1984). Minerals such as phosphate, calcium, and iron are also found abundant in the fruit (Kumari *et al*., 2017) [8].

The guava is processed into a diversity of products such as puree, juice, concentrate, jam, jelly, cheese, toffee, fruit flakes, squash, syrup, nectar, powder, wine, and vinegar, as well as ready-to-eat snacks, drinks, and dehydrated and canned products (Kumari *et al*., 2017) [8]. Fruit bars are dehydrated and shelf-stable products made by pureeing and reconstructing the natural fruit structure into dried sugar-acid-pectin gels. Fruit leathers are also useful for repurposing overripe fruits (Ruiz *et al*., 2012) [17]. The reduction in quality and color, particularly owing to browning, has been a major challenge in the production of white guava fruit bars (Singh *et al*., 2019) [17]. Normally, the consumer evaluates the quality of a processed product based on color. Browning not only reduces the aesthetic appeal (color) of the guava fruit bar but also degrades the natural fruit flavor and simultaneously aids in the formation of off-flavor or off-odor compounds (Van Boxstael *et al*., 2014) [19]. Fruit bars made from white pulp guava quickly lose their qualitative characteristics *viz*., color, resulting in a shorter shelf life during storage (Bons *et al*., 2011) [20]. Therefore, efforts were made to minimize the browning of white guava fruit bar using different anti browning agents.

2. Materials and Methods

2.1. Raw Material

This study involves firm ripe guava (*cv*. Allahabad Safeda) fruits produced from a guava orchard at the ICAR-Indian Institute of Horticultural Research in Bengaluru.

2.2. Production of guava fruit bar

Using potable water, the chosen guava fruits were carefully washed. Fruits were manually peeled, sliced in half, and the pulp and seeds were extracted using a laboratory-grade Pulper and sieve, respectively. The extracted pulp without pasteurizing was incorporated with three antibrowning agents at two different concentrations (0.5% Ascorbic acid, 1.0% Ascorbic acid, 10 ppm L-Cysteine, 20 ppm L-Cysteine, 5 ppm Hexylresorcinol, 10 ppm Hexylresorcinol), and as a common treatment 15% sugars, 0.3% citric acid and 700 ppm potassium metabisulphite was incorporated to maintain the desirable sugar acid blend in final product. Further, the mixture was stirred well. The mixes were equally distributed on a tray, and a cabinet dryer dried them at 50±5 °C. The drying process continued till the moisture content reached about ~15%. Further, the guava bar sheets were cut into 8 x 4 cm bars, packed in Metalized polyester polyethylene and later was subjected to biochemical studies for the period of 3 months.

2.3. Physico-chemical analysis

The samples' moisture content was measured gravimetrically in a thermo-ventilated oven to get a constant weight over the course of three measurements spaced 12 hours apart. An electric water activity meter (Rotronic Hydrolab, UK) was used to detect water activity at a temperature of 25±2 °C. The samples were immersed in 60% ethanol overnight to detect non-enzymatic browning, and the OD values at 440 nm were then read (Ranganna, 1986) [15]. Using a colorimeter, the color (*L* a* b**) was determined (Model: Colour Reader, CR-10, Konica Minolta, Japan). Based on *L*a*b** coordinates obtained using a colorimeter, the browning index was derived. To condense this variance into a single index that is related to the color brown, the browning index is created using the equation below (Pathare *et al*., 2013) [14].

$$BI = 100\ \frac{(X-0.31)}{0.17}$$

$$X = \frac{(a* + (1.75 \times L)) \times a*}{((5.645 \times L) + a* - (3.012 \times b))}$$

2.4. Statistical analysis

The analysis was done in triplicates and the results were presented in mean of three replicates. The experiment was carried out by using Factorial Complete Randomized Design (FCRD) at α = 0.05 level of significance of using R software.

3. Results and Discussion

3.1. Moisture

The moisture contents ≤15 per cent, it is said to be safe with respect to microbiological activity and adverse deteriorative biochemical reactions (Suna *et al*., 2014) [23]. Irrespective of the storage period, though moisture content among different antibrowning agents was significant it was very linear and this may be due to variation in the drying temperature and batch effect. Meanwhile, irrespective of the antibrowning agents, maximum moisture content was observed at 0 MAS (14.68%) and minimum at 6 MAS (12.75%). The decrease in the moisture content of guava fruit bar during the storage is due to the impermeable properties of packaging material (Bhatt and Jha, 2015) [3]. Because of evaporation, moisture content has decreased in guava fruit bar (Panja *et al*., 2016) [12]. The interaction effect between antibrowning agents and storage period was significant and the control treatment reported the highest (14.76%) which was on par with 20 ppm L-Cysteine (14.77%) during the storage the moisture content decreased and found to be least in 10 ppm Hexylresorcinol (14.60%) and 1% ascorbic acid (14.67%).

3.2. Water Activity

Water activity is one of the most crucial quality factors for long-term storage (Diamante *et al*., 2014) [13]. The water activity of the guava fruit bar followed a similar trend as that of the moisture content. Though there was a significant difference in the water activity among different antibrowning agents the difference was very narrow. This variation may be due to the batch effect and variation in drying temperature during product development. However, the highest was observed in 1.0% Ascorbic acid (0.578) which was on par with 5 ppm Hexylresorcinol (0.574) and Control (0.571). During storage, water activity dropped dramatically regardless of antibrowning agents. The highest was observed at 0 MAS (0.582) and the least at 3 MAS (0.558). A similar pattern in water activity during storage was found in guava leather (Singh *et al*., 2019) [18] and papaya-guava leather (Singh *et al*., 2020) [22]. As the storage time progressed the reduction in water activity is due to a reduction in moisture content (Huang and Hsieh, 2005) [7].

3.3. Browning Index

The degree of enzymatic and non-enzymatic browning will be expressed by the browning index (Costa *et al*., 2006) [1]. Among different anti browning agents tested the highest browning index was found in 1.0% ascorbic acid (100.48) followed by 0.5% ascorbic acid (97.47) and the lowest in 10 ppm Hexylresorcinol (80.02). This increased browning index in the ascorbic acid sample is due to the degradation of ascorbic acid to dehydroascorbic acid. As the storage period increased browning index also increased and was found to be maximum at 3 MAS (100.48). This is a result of the ascorbic acid degradation into a furfural complex, which gives the product its brown hue during storage. With respect to interaction, the lowest browning index (80.02) was observed in 10 ppm Hexylresorcinol throughout the storage period and the highest was in 1.0% ascorbic acid (111.63). This increase in browning is due to complex biochemical changes occurring in the guava fruit bars. Similar observations were made in dehydrated potato slices (Nascimento and Canteri, 2019) [6] and apple leather (Demarchi *et al*., 2013) [5].

3.4. Non-Enzymatic Browning

Non-enzymatic browning (NEB) is an evaluation of the quality and color modifications that occurred during storage as a result of chemical interactions (Bharate and Bharate, 2014) [2]. Among different antibrowning agents tested the highest non-enzymatic browning was found in 1.0% ascorbic acid (0.232) and the lowest in 10 ppm Hexylresorcinol (0.185). This increased non-enzymatic browning in the ascorbic acid sample is due to the degradation of ascorbic acid. As the storage period increased non-enzymatic browning

also increased and was found to be maximum at 3 MAS (0.334) and minimum at 0 MAS (0.100). As ascorbic acid is a sensitive bioactive compound degradation of it and also Maillard reaction occurring between reducing sugars and amino acids have increased non-enzymatic browning during storage (Bharate and Bharate, 2014; Kutzli *et al*., 2021) [2, 9]. Among the interaction, the lowest non-enzymatic browning (0.089) was observed in 10 ppm Hexylresorcinol at 0 MAS and the highest was in 1.0% ascorbic acid (0.395). This decrease in non-enzymatic browning in Hexylresorcinol treated guava fruit bar is due to a mixed type of inhibition.

Table 1: Physicochemical composition of guava pulp (cv. Allahabad Safeda)

Colour	***L****	**61.32**
	*a**	0.20
	*b**	18.48
Moisture (%)		84.55
Water activity		0.836
TSS (° Brix)		10.6
Titratable acidity (%)		0.47
Reducing Sugar (%)		4.42
Total Sugar (%)		6.35
Non - Reducing Sugar (%)		2.31
Ascorbic Acid (mg/100g)		241.13

Table 2: Effect of anti-browning agents and their concentration on moisture content (%) of guava (cv. Allahabad Safeda) fruit bar during storage

	0 MAS	1 MAS	2 MAS	3 MAS	Mean A
Control	14.76	14.30	13.92	12.13	13.78
0.5% Ascorbic acid	14.67	14.34	13.05	12.71	13.69
1.0% Ascorbic acid	14.61	14.23	13.19	13.09	13.81
10 ppm L-Cysteine	14.66	14.32	12.22	12.13	13.33
20 ppm L-Cysteine	14.77	14.12	13.16	12.71	13.69
5 ppm Hexylresorcinol	14.63	14.29	13.17	13.08	13.80
10 ppm Hexylresorcinol	14.60	14.18	13.18	13.11	13.80
Mean B	14.68	14.25	13.11	12.75	
Factors	C.D.	SEm±			
Factor (A)	0.10	0.04			
Factor (B)	0.09	0.03			
Factor (A X B)	0.18	0.06			

MAS: Months after Storage

Table 3: Effect of anti-browning agents and their concentration on water activity of guava (cv. Allahabad Safeda) fruit bar during storage

	0 MAS	1 MAS	2 MAS	3 MAS	Mean A
Control	0.583	0.576	0.566	0.559	0.571
0.5% Ascorbic acid	0.582	0.573	0.562	0.554	0.568
1.0% Ascorbic acid	0.591	0.586	0.573	0.563	0.578
10 ppm L-Cysteine	0.582	0.569	0.562	0.556	0.567
20 ppm L-Cysteine	0.579	0.571	0.567	0.555	0.568
5 ppm Hexylresorcinol	0.584	0.579	0.571	0.560	0.574
10 ppm Hexylresorcinol	0.572	0.568	0.564	0.562	0.567
Mean B	0.582	0.575	0.566	0.558	
Factors	C.D.	SEm±			
Factor (A)	0.10	0.04			
Factor (B)	0.09	0.03			
Factor (A X B)	0.18	0.06			

MAS: Months after Storage

Table 4: Effect of anti-browning agents and their concentration on Browning index of guava (cv. Allahabad Safeda) fruit bar during storage

	0 MAS	1 MAS	2 MAS	3 MAS	Mean A
Control	84.09	90.43	92.68	98.59	91.45
0.5% Ascorbic acid	87.92	95.68	98.42	107.87	97.47
1.0% Ascorbic acid	90.81	98.46	101.03	111.63	100.48
10 ppm L-Cysteine	90.27	91.29	94.92	103.70	95.04
20 ppm L-Cysteine	89.00	91.78	94.48	100.17	93.86
5 ppm Hexylresorcinol	85.00	94.53	102.16	106.46	97.04
10 ppm Hexylresorcinol	80.02	85.77	89.40l	92.53	86.93
Mean B	84.38	90.10	94.01	100.48	
Factors	C.D.	SEm±			
Factor (A)	1.69	0.60			
Factor (B)	1.07	0.38			
Factor (A X B)	3.38	1.20			

Table 5: Effect of anti-browning agents and their concentration on non-enzymatic browning (OD at 440 nm) in guava (cv. Allahabad Safeda) fruit bar during storage

	0 MAS	1 MAS	2 MAS	3 MAS	Mean A
Control	0.095	0.153	0.212	0.307	0.192
0.5% Ascorbic acid	0.101	0.171	0.241	0.357	0.218
1.0% Ascorbic acid	0.106	0.177	0.250	0.395	0.232
10 ppm L-Cysteine	0.102	0.166	0.235	0.337	0.210
20 ppm L-Cysteine	0.104	0.165	0.230	0.297	0.199
5 ppm Hexylresorcinol	0.101	0.165	0.254	0.358	0.220
10 ppm Hexylresorcinol	0.089	0.145	0.220	0.284	0.185
Mean B	0.100	0.163	0.235	0.334	
Factors	C.D.	SEm±			
Factor (A)	1.69	0.60			
Factor (B)	1.07	0.38			
Factor (A X B)	3.38	1.20			

MAS: Months after Storage

4. Conclusion

This study revealed the effects of different antibrowning agents (0.5% Ascorbic acid, 1.0% Ascorbic acid, 10 ppm L-Cysteine, 20 ppm L-Cysteine, 5 ppm Hexylresorcinol, 10 ppm Hexylresorcinol) and their influence on non-enzymatic browning in white guava fruit bar (cv. Allahabad Safeda). The application of antibrowning agents during the product development and storage affected the colour which was expressed in terms of Browning in index and Non enzymatic browning (OD at 440 nm). The use of ascorbic acid at higher concentration i.e., ≥ 0.5% induced the browning while the use of 10 ppm Hexylresorcinol had the least browning among the tested. During the storage moisture content and water activity of white guava fruit bar decreased while the browning index and non-enzymatic browning (OD at 440 nm) increased significantly. This is due to degradation of ascorbic acid and occurrence of Maillard reaction (reaction of biomolecule with free carbonyl groups) during the storage. Therefore, from this study it was evident that use of ascorbic acid at higher concentration favoured the browning while hexylresorcinol was effective in minimizing it to some extent in white guava fruit bar (cv. Allahabad Safeda).

5. Acknowledgement

The support received from Ministry of Tribal Affairs, GOI through NFST for carrying out the work is duly acknowledged.

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