Physicochemical and functional properties of ash gourd/bottle gourd beverages blended with jamun

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Abstract This paper reports the formulation and storage stability of Ash gourd (*Benincasa hispida*) and Bottle gourd (*Lagenaria siceraria*) juice blended with the Jamun (*Syzygium cumini*). Both the beverages found to be rich in polyphenols, flavonoids, and anthocyanins. The Ash gourd–Jamun (AGJ) and Bottle gourd–Jamun (BGJ) beverages showed significant bio-accessibility of polyphenols, flavonoids, and anthocyanins. Moreover, the addition of sugar was found to enhance the bioaccessibility of these fractions in both the beverages. Further, the biochemical attributes such as physiochemical and functional properties of Ash gourd–Jamun and Bottle gourd–Jamun blended juice were evaluated during the accelerated storage. The total soluble solids and acidity and the sensory score did not change significantly during the storage period. The AGJ exhibited a 35%, 73%, 34% and 35%, whereas BGJ shows 32%, 65%, 35% and 20% decrease in total polyphenol, anthocyanin, DPPH and inflammatory activity during the 2 months of storage period respectively. However, the reduction was less in Bottle gourd–Jamun beverage. Results of the study are promising and add to the necessity and potential of gourd family based functional food development.

Keywords Gourd family · Antioxidant activity · Anthocyanin · Functional food

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Introduction

During the last decade, the importance of dietary antioxidant components has been attracted great attention due to their ability in the prevention of chronic diseases including cancer and heart diseases. Further, various studies show that consumption of fruits and vegetables reduces the risk of diseases related to oxidative damage (Zheng et al. 2017). The reason for this might be the ability of phytonutrients, such as vitamins, carotenoids, and phenolic contents, to protect the human body against damage from reactive oxygen and nitrogen species. Utilization of fruits and vegetables in a processed juices and beverages helps in providing more options to the consumers. Moreover, since the processed food products have better shelf-life compared to fresh fruits and vegetables, processing enhances the economic value of the produce (Pinho et al. 2014). Many recent findings indicate that vegetable juices are rich in antioxidants and other health-promoting nutrients. Therefore, daily intake of juices are recommended and are considered as an integral part of a healthy diet.

The gourd family (*Cucurbitaceae*) includes hundreds of vine species bearing coiled, climbing tendrils. Vegetables belonging to gourd family are very beneficial from the health point of view. Among the various gourds, ash gourd and bottle gourd are well known for their therapeutic values and therefore are used widely in traditional medicines. Ash gourd (*Benincasa hispida*) is an annular trailing or climbing herb and is cultivated widely in India. In Ayurveda, it is recommended for treating peptic ulcer, urinary tract infections, diabetes mellitus, epilepsy and other nervous system disorders (Gill et al. 2010; Palamthodi and Lele 2014). Further, ash gourd is proved to have an excellent prebiotic activity (Sreenivas and Lele 2013). Bottle gourd contains all the essential amino acids and

nutrients required for the normal human health (Rahman et al. 2003). Traditionally, this fruit is used as a cardi tonic and general tonic (Deshpande et al. 2008). The antioxidant, anti-inflammatory, anti-cancer and diuretic properties of bottle gourd also been reported (Palamthodi and Lele 2014).

The major hurdle associated with vegetable-based beverages is preservation. Due to high pH of the vegetable juices, they are highly prone to microbial contamination. Moreover, vegetables like ash gourd and bottle gourd produce colorless and flavorless juices. Thus, it is ideal to blend these juices with some tropical colored fruit juices. This will enhance the shelf-life as well as the aesthetic properties of the beverage. Further, various studies showed that blending enhances the sensory as well as the nutritional quality of the beverages (Curi et al. 2017). Therefore, it is important to study the characteristics of the blended beverages and their optimization. The optimization can be achieved through response surface methodology (RSM). RSM is an empirical modeling method using mathematical and statistical tools, whose objective is to establish the relationship between a response and different variables to yield an optimum level of the best system performance. Zhu et al. (2010) applied RSM to optimize the microwave-assisted extraction of astaxanthin from *Phaffia rhodozyma* and provides some fundamental information for using RSM in food processing.

Jamun (*Syzygium cumini*) also known as Black plum, underutilized fruit grown widely in western regions of Maharashtra India. Jamun fruit yields an anthocyanin-rich, dark-purple fleshy pulp. It is greatly recognized as an adjuvant therapy in type-2 diabetes (Swami et al. 2012). In addition, reports state that Jamun has significant pharmacological properties such as antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, cardioprotective, antiallergic and chemopreventive activities (Sehwag and Das 2014).

In the present study, efforts were made to develop gourd vegetable-based beverages comprising their functional properties. Further, to overcome the hurdles associated with vegetable juice processing and preservation, blending of ash gourd and bottle gourd juices was considered. Jamun (*Syzygium cumini*) was chosen for blending as it is expected to extend the shelf life through lowering the pH. Further, this could enhance the functional properties of the beverages since it is rich in bioactive compounds.

Material and method

Chemicals and reagents

Folin–Ciocalteu reagent, gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), potassium metabisulfite (KMS), cellulose dialysis membrane (molecular weight cutoff of 12,000 Da) and pancreatic α -amylase (from porcine) were purchased from Sigma-Aldrich, Bangalore. Intestinal α -glucosidase (from *Saccharomyces cerevisiae*) and *p*-nitrophenyl α -D-glucopyranoside (PNPG) were procured from SRL, Mumbai. Bovine Serum Albumin (BSA), 3, 5-dinitrosalicylic acid (DNS) and other chemicals and solvents were acquired from SDFCL, Mumbai. Plate count agar and potato dextrose agar (PDA) were obtained from HiMedia, Mumbai.

Preparation and processing of juices

Ash gourd (*Benincasa hispida*), bottle gourd (*Lagenaria siceraria*), and Jamun (*Syzygium cumini*) were purchased from local market in Matunga, Mumbai. Ash gourd was cut into cube-shaped pieces after peeling and de-seeding. The pieces were given a dipping treatment in 0.1% KMS solution for 5 min to reduce the enzyme activity. Ash gourd cubes were pulped in a commercial mixer with the addition of distilled water. Similarly, bottle gourd was peeled, cut into pieces and dipped in 0.1% KMS solution for 5 min. Juice was extracted from the pre-treated pieces in a commercial mixer with the addition of 0.1% KMS solution to prevent browning. Jamun was washed, deseeded and pulped with added water. All the pulps were then strained through a double-layered muslin cloth to get juices.

Ash gourd–Jamun (AGJ) and bottle gourd–Jamun (BGJ) beverages were prepared by mixing different proportions of the individual juices. The process was optimized by RSM, using central composite rotatable design to fit a polynomial model by least square technique (Design expert 6.0, Stattease Inc. Minneapolis, USA). A set of combinations were obtained on the basis of independent factors (Ash gourd/bottle gourd and jamun juice concentration). The parameters that influenced the product quality, acceptability and the shelf-life (pH, titratable acidity, TSS, total phenolic content and antioxidant activity) were taken as responses. Beverages were also prepared without addition of sugar to measure their α -amylase and α -glucosidase inhibition capacity. All the final products were pasteurized at 95 °C for 15 min (Supplementary data).

Sensory evaluation

Sensory evaluation of beverages was performed by 20 trained panelists (11 females and 9 males) using 9-point hedonic scale (1 = extreme dislike; 9 = extreme like) (Chen et al. 2010). The panelists were graduate students aged between 20 and 30 years selected such a way that all have prior knowledge of consumer preferences. Samples were served chilled (10 °C) individually with male and female panelists in coded identical cups with a white illuminating background at 11 am. Samples were evaluated for color, flavor, taste, mouthfeel and overall acceptability using a 9-point hedonic scale.

Storage studies

The shelf-life of the beverages was evaluated at an accelerated temperature of 50 °C. The storage at the latter temperature for 12 days corresponds to a shelf-life of 70 days at 20 °C (Pérez-Ramírez et al. 2015). The samples were drawn aseptically from bottle and analyzed at 4 days intervals.

Microbiological analysis

The microbial safety of beverages during the accelerated storage period was done by estimating the number of aerobic viable cells using total viable count and total yeast and mold count methods (Evrendilek et al. 2000). Total plate count agar and potato dextrose agar was used to evaluate the bacterial and fungal count respectively. The number of colony forming units (CFU) was calculated for the bacterial and fungal count after incubation at 37 °C for 48 h and 72 h respectively.

TSS, titrable acidity and pH

Total soluble solids (°Brix) was measured using a hand-held refractometer (ERMA-58-92E, Erma Inc., Japan). pH was determined using a digital pH meter (S90528, Fisher Scientific Education™ pH meters, Fisher Scientific Inc., MA, USA) and titrable acidity was determined by titration against 0.1 N NaOH and expressed as % citric acid.

Total phenolic, flavonoid and anthocyanin content

Extracts of beverages prepared by centrifugation at 5000 rpm for 15 min were diluted 100 times and used for evaluation of functional ingredients and properties. Total polyphenols were determined by slightly modified Folin-Ciocalteu method (Dewanto et al. 2002): About 2 mL sodium carbonate (100 g/L) and 2.5 mL Folin–Ciocalteu reagent were added to 0.5 mL beverage extract and the

mixture was incubated for 1 h in darkness. Absorbance was measured at 760 nm and results were expressed as gallic acid equivalents (GAE/mL).

Total flavonoid content was determined by the modified AlCl_3 method (Zhishen et al. 1999). Briefly, aliquot of beverage extract (1 mL) and 5% sodium nitrite (0.3 mL) were mixed and diluted to 5 mL of distilled water. After 5 min, 10% AlCl_3 (3 mL) was added and after 6 min, 1 M NaOH (2 mL) was added to complete the reaction. The absorbance was measured at 510 nm and results were expressed as quercetin equivalents (QE/mL).

Total monomeric anthocyanins of beverages were determined by the pH differential method and results were expressed as cyanidin-3-glucoside (C3GE/mL) equivalents (Lee et al. 2005).

Antioxidant capacity

The change in color of DPPH solution from purple to yellow due to the addition of ascorbic acid or sample was measured through a calorimetric method (Vaz et al. 2011; Kadam and Lele 2017) and DPPH radical scavenging activity was measured as ascorbic acid equivalent antioxidant capacity (AAE/mL).

Antioxidant activity was also measured as ferric reducing antioxidant power (FRAP) and was determined according to Abeyasinghe et al. (2007) and the results were expressed as AAE/mL. About 100 times diluted samples were used for the assay.

Anti-inflammatory activity

Measurement of in vitro anti-inflammatory activity was done by inhibition of albumin denaturation method (Reshma et al. 2014). Beverage extracts were diluted 100 times in 0.2 M phosphate buffer (pH 7.4). Test solution (1 mL) and 1% albumin solution (1 mL) in 0.2 M phosphate buffer (pH 7.4) were mixed and incubated at 37 °C for 20 min. The mixture was then heated to 51 °C for 20 min and cooled to room temperature. Absorbance was measured at 660 nm and percent inhibition of albumin denaturation was calculated. Diclofenac sodium (15 µg/mL) used as a standard drug.

In-vitro gastrointestinal digestion

In vitro gastrointestinal digestion was carried out by undertaking samples through two simulated sequential digestion phases: gastric stage at pH 2 with pepsin and intestinal digestion stage including dialysis at 7 via pancreatin-bile mixture (Rodríguez-roque and De Ancos 2015). After completion of the digestive process, aliquots of the digested samples were collected from the dialyzed

Table 1 TPC ($\mu\text{g GAE/mL}$), DPPH ($\mu\text{g AAE/mL}$), Overall acceptability (0.1%) as a function of Ash gourd–Jamun (AGJ) and Bottle gourd–Jamun (BGJ) independent variable

Process variable		Level									
		Low		Medium		High					
X1 (Ash gourd)	Y1 (Bottle gourd)	– 1		0		+ 1					
X2 (Jamun)	Y2 (Jamun)	– 1		0		+1					
Run	AGJ		BGJ		TPC ($\mu\text{g GAE/mL}$)		DPPH ($\mu\text{g AAE/mL}$)		Overall acceptability		
	X1	X2	Y1	Y2	AGJ	BGJ	AGJ	BGJ	AGJ	BGJ	
1	35.00	22.07	35	15	173.11 $\pm$ 0.34 ^h	165.09 $\pm$ 0.04 ^f	1229.36 $\pm$ 23.2 ^d	1346.9 $\pm$ 24.2 ^c	7.04 $\pm$ 0.02 ^{bcd}	6.7 $\pm$ 0.14 ^{cd}	
2	35.00	15.00	35	15	165.09 $\pm$ 0.37 ^g	165.09 $\pm$ 0.11 ^f	1280.19 $\pm$ 19.1 ^{de}	1346.9 $\pm$ 17.14 ^c	6.9 $\pm$ 0.1 ^{bc}	6.7 $\pm$ 0.1 ^{cd}	
3	35.00	15.00	35	15	165.09 $\pm$ 0.13 ^g	165.09 $\pm$ 0.08 ^f	1280.19 $\pm$ 15.5 ^{de}	1346.9 $\pm$ 12.09 ^c	6.9 $\pm$ 0.23 ^{bc}	6.7 $\pm$ 0.17 ^{cd}	
4	40.00	10.00	30	20	148.58 $\pm$ 0.17 ^b	183 $\pm$ 0.05 ^h	895.29 $\pm$ 11.3 ^b	1570.17 $\pm$ 26.3 ^f	7.09 $\pm$ 0.19 ^{cd}	7.9 $\pm$ 0.13 ^e	
5	35.00	15.00	40	10	165.09 $\pm$ 0.11 ^g	146.23 $\pm$ 0.12 ^c	1280.19 $\pm$ 19.2 ^{de}	962.95 $\pm$ 14.11 ^a	6.9 $\pm$ 0.2 ^{bc}	5.9 $\pm$ 0.11 ^a	
6	42.07	15.00	35	8	160.85 $\pm$ 0.14 ^f	124.53 $\pm$ 0.24 ^a	1280.19 $\pm$ 21.4 ^{de}	1071.03 $\pm$ 16.17 ^b	5.45 $\pm$ 0.1 ^a	5.8 $\pm$ 0.08 ^a	
7	35.00	15.00	35	15	165.09 $\pm$ 0.21 ^g	165.09 $\pm$ 0.17 ^f	1280.19 $\pm$ 13.3 ^{de}	1346.9 $\pm$ 28.21 ^c	6.9 $\pm$ 0.14 ^{bc}	6.7 $\pm$ 0.18 ^{cd}	
8	27.93	15.00	30	10	158.02 $\pm$ 0.18 ^c	141.04 $\pm$ 0.13 ^b	1058.69 $\pm$ 19.6 ^c	911.33 $\pm$ 12.14 ^a	7.45 $\pm$ 0.19 ^{de}	6.1 $\pm$ 0.13 ^{ab}	
9	40.00	20.00	35	15	165.57 $\pm$ 0.9 ^g	165.09 $\pm$ 0.09 ^f	1719.55 $\pm$ 23.1 ^f	1346.9 $\pm$ 25.34 ^c	7.23 $\pm$ 0.17 ^{cde}	6.7 $\pm$ 0.24 ^{cd}	
10	35.00	15.00	42	15	165.09 $\pm$ 0.13 ^g	165.09 $\pm$ 0.15 ^f	1280.19 $\pm$ 17.3d ^e	1419.16 $\pm$ 27.29 ^d	6.9 $\pm$ 0.11 ^{bc}	6.4 $\pm$ 0.19 ^{bc}	
11	35.00	7.93	28	15	138.21 $\pm$ 0.11 ^a	160.01 $\pm$ 0.17 ^e	721 $\pm$ 9.12 ^a	1513.27 $\pm$ 31.07 ^{de}	6.59 $\pm$ 0.1 ^b	6.8 $\pm$ 16 ^{cd}	
12	30.00	20.00	35	22	151.89 $\pm$ 0.19 ^d	162.74 $\pm$ 02 ^f	1320.13 $\pm$ 18.19 ^e	1464.15 $\pm$ 23.25 ^{ef}	7.59 $\pm$ 0.18 ^e	7.8 $\pm$ 0.13 ^e	
13	30.00	10.00	40	20	150.95 $\pm$ 0.11 ^c	158.49 $\pm$ 0.12 ^d	935.24 $\pm$ 9.4 ^b	1304.44 $\pm$ 21.04 ^c	7.14 $\pm$ 0.12 ^{cde}	7.0 $\pm$ 0.1 ^d	

All data are expressed as a mean $\pm$ standard deviation. Mean with the different superscript letter in a column are differ significantly ($P < 0.05$)

fraction and followed by rapid cooling by placing in a cold water bath for 10 min. The changes after gastrointestinal digestion were evaluated to calculate the bioaccessibility of total phenolics, flavonoids and anthocyanins content.

$$\text{Bioaccessibility}(\%) = \frac{BC_{\text{Dialyzed}}}{BC_{\text{Nondigested}}} \times 100$$

where BC_{Dialyzed} is the concentration of bioactive compounds after gastrointestinal digestion and $BC_{\text{Nondigested}}$ is the concentration of bioactive compounds before digestion per mL of sample.

Statistical analysis

All the analysis were done in triplicates. Analysis of variance was used to examine data at the 95% confidence level. IBM SPSS version 19 software was used. Results are presented as means and standard deviation (SD).

Results and discussion

Ash gourd, bottle gourd, and Jamun juices were analyzed and the attributes were recorded before blending. The total soluble content was higher in Jamun juice

(16.5 $\pm$ 0.12 °brix) compared to ash gourd (2.7 $\pm$ 0.1 °brix) and bottle gourd (3.0 $\pm$ 0.23 °brix) juices. The titrable acidity values were expressed as % citric acid and that of ash gourd, bottle gourd, and jamun juices were 0.05 $\pm$ 0.001, 0.05 $\pm$ 0.002 and 1.9 $\pm$ 0.03 respectively. The antioxidant activity of juices of ash gourd, bottle gourd, and jamun was found as 630.45 $\pm$ 11.05, 830.07 $\pm$ 4.6 $\mu\text{g AAE/mL}$ respectively. However, Table 1 clearly indicate that the blending of gourd juice with jamun found increases the antioxidant potential of the beverages.

Optimization of beverages by RSM

The concentrations of the gourd and Jamun juices in the beverages were optimized using design expert 7. The experiments were designed considering the juice concentrations as independent variables and the total phenolic content, DDPH free radicle scavenging activity and overall acceptability as responses (Table 1).

Equations generated in terms of coded factors (Ash gourd: Jamun (X_1X_2) and bottle gourd: Jamun juice (Y_1Y_2)), for the significant responses in the products, were as follows;

For Ash gourd–Jamun beverage (AGJ)

Table 2 Analysis of variance (ANOVA) for the response surface quadratic model of the TPC

Source	Sum of square		DF		Mean square		f-value		P value	
	AGJ	BGJ	AGJ	BGJ	AGJ	BGJ	AGJ	BGJ	AGJ	BGJ
TPC										
Model	933.89	2363.08	5	5	186.78	472.62	8.61	26.57	0.0067	0.0002
X ₁	29.31	18.77	1	1	29.31	18.77	1.35	1.06	0.2833	0.3384
X ₂	565.93	1464.94	1	1	565.93	1464.94	26.08	82.37	0.0014	< 0.0001
X ₁ X ₂	64.40	220.52	1	1	64.40	220.52	2.97	12.40	0.1286	0.0097
X ₁ ²	92.81	0.20	1	1	92.81	0.20	4.28	0.011	0.0774	0.9182
X ₂ ²	213.51	651.86	1	1	213.51	651.86	9.84	36.65	0.0165	0.0005
Residual	151.91	124.50	7	7	21.70	17.79				
Lack of fit	151.91	124.50	3	3	50.64	41.50				
Pure Error	0.000	0.000	4	4						
Cor Total	1085.80	2487.58	12	12						

AGJ: R² = 0.8601; C.V.% = 2.92; BGJ: R² = 0.9500; C.V.% = 2.65**Table 3** Analysis of variance (ANOVA) for the response surface quadratic model of the DPPH

Source	Sum of square		DF		Mean square		f-value		P value	
	AGJ	BGJ	AGJ	BGJ	AGJ	BGJ	AGJ	BGJ	AGJ	BGJ
DPPH										
Model	6.635E+005	410,694	5	5	1.327E +005	82,139	12.81	6.01	0.0021	0.0179
Y ₁	56,568.74	187,091	1	1	56,568.74	187,091	5.46	13.69	0.0521	0.0076
Y ₂	4.647E+005	9862	1	1	4.647E+005	9862	44.87	0.72	0.0003	0.4237
Y ₁ Y ₂	48,261.50	18	1	1	48,261.50	18	4.66	0.00	0.0677	0.9717
Y ₁ ²	2528.35	213,636	1	1	2528.35	213,636	0.24	15.64	0.6364	0.0055
Y ₂ ²	93,920.92	4207	1	1	93,920.92	4207	9.07	0.31	0.0196	0.5962
Residual	72,497.33	95,638	7	7	10,356.76	13,663				
Lack of fit	72,497.33	42,869	3	3	24,165.78	14,290		1.08		0.4515
Pure error	0.000	52,768	4	4	0.000					
Cor total	7.360E+005	506,331	12	12						

AGJ: R² = 0.9015; C.V.% = 8.50; BGJ: R² = 0.8111; C.V.% = 9.78

$$TPC = -18.11 + 8.2 \times X_1 + 2.7 \times X_2 + 0.16 \times X_1X_2 - 0.14 \times X_1^2 - 0.22X_2^2$$

$$DPPH = 295.3 + 4.29 \times X_1 + 33.8 \times X_2 + 4.39 \times X_1X_2 - 4.64 \times X_2^2$$

For Bottle gourd–Jamun beverage (BGJ)

$$TPC = -117.6 + 24.9 \times Y_2 - 0.29 \times Y_1Y_2 - 0.39 \times Y_2^2$$

$$DPPH = -8632.8 + 153.7 \times Y_1 + 25.88Y_2 - 7.11Y_1^2$$

The analysis of variance (ANOVA) presented that the model is highly significant with a *P* value (*P* < 0.05) (Tables 2 and 3). The model also demonstrated a high value of correction coefficient *R*², adjusted *R*² and predicted *R*², highlighting a good correlation between

predicted and experimental value of the response (Tables 2 and 3). Moreover, the lack-of-fit value which was not significant showed that the model can adequately fit the experimental data.

The 3D response surface plots of these models have been plotted as a function of two variables. The initial points for optimization of both the beverages are given in Table 1. The variations in the total phenolic content, DPPH free radicle scavenging activity responses with a change in levels of the juices are given in (Tables 2 and 3). In the case of ash gourd–Jamun beverage, the change in levels of ash gourd juice and jamun juice did not influence the overall acceptability. Whereas, the overall acceptability of bottle gourd–jamun beverage increased with increasing jamun juice concentration. Moreover, the total phenolic content was found to significantly increase (*P* < 0.05) with

Fig. 1 Effect of gourd–Jamun concentration on total phenolic content (μg GAE/mL) and DPPH radical scavenging activity (μg/AAE/mL)

Table 4 Physiochemical and functional properties of ash gourd/bottle gourd beverages

Parameter	Ash gourd–jamun (35:20)	Bottle gourd–jamun (30:20)
TSS (°brix)	9.5 ± 0.68 ^a	9.9 ± 0.13 ^b
Acidity (% citric acid)	0.53 ± 0.02 ^a	0.525 ± 0.04 ^b
Total polyphenols (μg GAE/mL)	267.45 ± 1.2 ^a	191.04 ± 4.7 ^b
Total flavonoids (μg QE/mL)	16.50 ± 0.12 ^a	35.31 ± 0.53 ^b
Total anthocyanins (μg C3GE/mL)	8.85 ± 0.5 ^{ab}	8.18 ± 0.3 ^{ab}
DPPH assay (μg AAE/mL)	1727.2 ± 23.78 ^a	1527.2 ± 13.42 ^b
FRAP assay (μg AAE/mL)	736.29 ± 10.21 ^a	736.3 ± 11.35 ^{ab}

All data are expressed as a mean ± standard deviation. Mean with the different superscript letter in a column are differ significantly ($P < 0.05$)

an increase in the level of jamun juice in both the beverages. Moreover, the DPPH free radicle scavenging activity increased with the rise in jamun juice concentration in ash gourd–jamun beverage and remained unaffected in bottle gourd–jamun beverage.

To assess the fitting of the model, the analysis of variance was calculated for each selected model as well as a response (Tables 2 and 3). Figure 1 depicts the effect of gourd–Jamun juice on TPC and DPPH radical scavenging activity of the beverages. Analysis indicated that 70–40% of ash gourd–jamun and 60–40% bottle gourd–jamun blends yielded the best results and best fitted with the theoretical model (Table 4). These combinations were used for further analysis and storage studies.

Bioaccessibility anthocyanin, phenolic, and flavonoid compound

The type, class and molecular structure of phenolic and flavonoid compound play a significant role in their bioaccessibility and bioavailability. In addition to that their size and pattern of glycosylation taken into consideration during application. Also, there is numerous phenolic compound in plant-based food shows a vast array of chemical property and large diversity in structural form (Ribas-Agustí et al. 2017). Bottle gourd and ash gourd exhibit a higher amount of dietary fiber and lipid content, which causes the interaction between polyphenols associated with dietary fiber and sugar, making them more bio-accessible. This digestible carbohydrate has the ability to potentiate digestion of flavonoids and anthocyanins which improve the bioaccessibility of these compounds.

In this paper, we have studied the percent bioaccessibility of anthocyanins ($\mu\text{g C3GE/mL}$), total polyphenols ($\mu\text{g GAE/mL}$) and flavonoids ($\mu\text{g QE/mL}$) of AGJ and BGJ beverages in with and without sugar. AGJ exhibit 61.50%, 47.67%, and 57.42%, whereas BGJ shows 82.47%, 76.76% and 60.02% exhibit the significant ($P < 0.05$) increase in bioaccessibility of total anthocyanins, phenolic and

flavonoid compound in sugar added beverage respectively (Fig. 2). Increase in bioaccessibility may be due to the glycosylation of native functional compound which is having a great possibility of absorbing by the small and large intestine. Since the anthocyanin is very unstable and sensitive to degradation during the processing and absorption, glycosylation plays a very precise role in the increase in bioaccessibility value in the human body. A similar result was also observed in the stability of anthocyanins in blackberry juices with the addition of sugar (Pérez-Ramírez et al. 2015). Sugar preserves food and prevents the loss of functional ingredients by forming glass, locking in compounds, mimicking the hydrogen-bonding character of water, increasing the surface tension of the bulk solvent, preventing thermotropic phase separations in lipid bilayers, and fusion of membranes (Kopjar et al. 2008; Loncaric et al. 2014). Although, sugar affects the bioavailability of various phenolic compounds differently (Nikkhah et al. 2007). Therefore we select the AGJ and BGJ beverage with added sugar for further formulation and shelf life study.

Storage studies

Storage stability of the optimized beverages was evaluated at accelerated storage conditions (12 days at 50 °C which is equivalent to 70 days at 20 °C). The attributes and functional properties were analyzed at 4 days intervals.

Microbiological safety

Microbiological safety of the beverages was evaluated during accelerated storage studies. Total bacterial count and yeast and mold count were nil (< 1 CFU/mL) during the storage at 4 days interval, making the blended beverages microbiologically safe during the entire storage period (12 days at 50 °C). Total bacterial count and yeast and mold count for this period was less than 2 CFU/mL. These results confirmed the microbial safety of the beverages.

Fig. 2 Bioaccessibility (%) of total polyphenols ($\mu\text{g GAE/mL}$), Anthocyanins ($\mu\text{g C3GE/mL}$) and Flavonoids ($\mu\text{g QE/mL}$) in AGJ and BGJ beverages

Fig. 3 Changes in beverages during storage **a** TSS and acidity; **b** Total polyphenols (μg GAE/mL) and anthocyanin (μg C3GE/mL); **c** Antioxidant activity, measured by DPPH assay (μg AAE/mL) and

anti-inflammatory activity, measured as percent albumin denaturation inhibition (%)

TSS and titrable acidity

TSS did not change considerably with the addition of jamun juice. There was a significant change ($P < 0.05$) in titrable acidity with varying levels of jamun juice, though the pH remained unaffected. This can be explained with the hypothesis that minerals that interfere with a concentration of free protons determine the pH value and organic acids which account for titratable acidity and it contributes very little to pH value (Boulton 1980). Both the beverages showed an increase in the total soluble solid content and decrease in titrable acidity during storage (Fig. 3a). The gradual increase in the TSS content during the storage period might be due to hydrolysis of polysaccharides into monosaccharides and oligosaccharides (Raj et al. 2011; Kadam and Lele 2016).

Phenolic content and anthocyanins

Generally, as that of sensory acceptance, the combination of vegetable–fruit helps to develop juices which are nutritionally superior to those prepared with single fruits/vegetables (Curi et al. 2017). AGJ and BGJ beverages as

well aligned with the previous report and found to be rich in phenolics such as anthocyanins. The gradual reduction in phenolics compound during storage was slow and steady compared to the reduction in anthocyanin content (Fig. 3b). The results obtained are in agreement with the reports of (Pinho et al. 2014). Further, the presence of oxidoreductases such as polyphenol oxidase and/or peroxidase is said to enhance the degradation of anthocyanins. In addition, the methoxyl groups and attached sugars in anthocyanins also play a major role in the stability of anthocyanins (Howard et al. 2012).

Total antioxidant capacity

AGJ and BGJ beverages showed good antioxidant potential in terms of DPPH free radical scavenging activity. During storage, antioxidant capacity was to a great extent stable though there were fluctuations during storage period (Fig. 3c). The results are in agreement with several previous studies on fruits (Ayala-Zavala et al. 2004; Kevers et al. 2007; Kadam and Lele 2016). Total phenolic content and anthocyanin levels decreased significantly ($P < 0.05$) during storage. This is similar to Kevers et al. 2007

observation that during storage, anthocyanins content reduces in most of the fruits and vegetables. The reduction in anthocyanins and other phenolic compounds leads to a loss in antioxidant capacity of beverages (Pérez-Ramírez et al. 2015).

Anti-inflammatory activity

Denaturation of protein is an established cause of inflammation. Thus, inhibition of BSA denaturation was regarded as anti-inflammatory activity. During heat treatment at 51 °C, BSA unfolds in an intermediate state (Sharma et al. 2010) but addition of beverage extracts exhibited to preserve the native state of BSA (Fig. 2). As compared to the AGJ beverage, BGJ beverage shows better anti-inflammatory activity in term of preventing the denaturation of BSA during accelerated storage (Fig. 3c). Secondary metabolites present in ash gourd, bottle gourd and jamun such as flavones, flavonoids, and phenolic compounds are known to possess anti-inflammatory activity (Tiwari 2014; Singh and Uppal 2015). The decrease in anti-inflammatory activity during accelerated storage might be associated with a decrease in the concentration of these compounds.

Sensory analysis

The 9-point hedonic scale was used for the sensory score study of AGJ and BGJ beverages during the storage period (Supplementary data). A slight decrease in taste, color, flavor and overall acceptability of AGJ and BGJ beverages was observed as the storage period increased. However, no significant difference was observed ($P > 0.05$). In Ash gourd–jamun the overall acceptability score ranged from 7.1 to 6.5 and bottle gourd–Jamun varied from 7.7 to 6.4 over a period of 12 days at accelerated temperature of 50 °C that corresponds to a shelf-life of 70 days at 20 °C, indicating good acceptability of the product. Similar finding were also observed in shelf life study of Ash gourd–carrot juice (Kadam and Lele 2016). The gradual loss in taste, color, flavor over the storage period might be due to changes in volatile compounds and a possible chemical reaction in the juice that affect the organoleptic attributes (Majumdar et al. 2010).

Conclusion

In this study, AGJ and BGJ beverages were developed with good organoleptic characteristics and acceptance during the storage. The blending of gourd juices with anthocyanin-rich Jamun fruit juice enhanced the aesthetic properties as well as functional properties of the final products. Both the products were microbiologically stable and retained

significant functional characteristics during the storage, for 12 days at 50 °C which is equivalent to 70 days at 20 °C. The bio-accessibility of the products was also promising. It may be concluded that the formulation of AGJ and BGJ blended juice was able to satisfy consumer taste and can be effectively stored for a period greater than 70 days with good acceptability at 20 °C. Further investigations have to be carried out to understand the commercial feasibility of these products.

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Compliance with ethical standards

Conflict of interest Authors have no conflict of interest.

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