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ORIGINAL ARTICLE

Effect of karwanda (*Carissa congesta* Wight) and sugar addition on physicochemical characteristics of ash gourd (*Benincasa hispida*) and bottle gourd (*Langenaria siceraria*) based beverages

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Abstract There is a clear trend towards increasing consumption of juices as they can reduce imbalance of redox potential and provide necessary health benefits to consumers. Levels of karwanda (*Carissa congesta* Wight) and vegetable juices were varied to prepare nine different formulations of ash gourd-karwanda (AgK) and bottle gourd-karwanda blends (BgK) of higher nutritive, sensory qualities and storability. Total polyphenols (TP), antioxidant activity (AOA), total soluble solids and acidity were increased significantly ($p \leq 0.05$) with addition of karwanda. AgK blend (35:35) and BgK blend (35:30) were selected based on their higher overall acceptability, TP and AOA. AgK blends had higher α-amylase (31%) while BgK blends had higher α-glucosidase (43%) inhibitory activities. Concentration of TP and anthocyanins decreased significantly ($p < 0.05$), AOA remained unchanged and anti-inflammatory activities decreased (33–38%) in AgK and BgK blends during accelerated storage at 50 °C for 12 days. Addition of sugar in BgK blend decreased stability of TP (11%), flavonoids (31%) and anthocyanins (8%). During in vitro gastrointestinal digestion, TP, flavonoids and anthocyanins reduction rate was significantly higher for BgK blend with sugar.

Keywords Karwanda · Blended beverage · AgK · BgK · TP · AOA

Introduction

Last century's realization that majority of modern world chronic diseases are result of over consumption of metabolites and imbalance of redox potential led to significant increase in use of plant foods. Results indicate that when vegetable juices are taken before a starch-based diet meal, carbohydrates digest slowly thus resulting in lower glycemic load (Tiwari 2014). In fact, increased occurrence of diabetes in Indian subcontinent attributed to higher consumption of calorie rich foods may be managed by multifaceted pharmaceutical approach of vegetables. For this reason, Indian cuisine always promoted use of vegetables alongside with meals.

Ash gourd [*Benincasa hispida* (Thunb.) COGN.] has been used for treatment of hypertension, inflammation and to cure diabetic complications in Chinese and Korean medicines (Majumdar et al. 2012). Ash gourd has been recommended as anti-ulceric agent in Ayurveda. Bottle gourd [*Langenaria siceraria* (Mol.) Standl.], also known as Doodhi or Lauki, belonging to *Cucurbitaceae* family like ash gourd, is a good source of nutrients, minerals and polyphenols such as choline. Bottle gourd is known for its anti-inflammatory, anti-hyperlipidemic, anti-stress, cardiotonic and diuretic properties as well as antidote for certain poisons (Ghule et al. 2006). Karwanda (*Carissa congesta* Wight) is an underutilized shrub naturally grown all over India. Unripe karwanda fruits which are berry sized, are commonly used in pickles whereas sweet, reddish-black ripe fruit, are consumed as such or can be processed to make jelly, jam and squash. Karwanda being a good source

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of iron and fair source of vitamin C, has been used in treatment of anemia, anorexia and skin problems. Karwanda has traditionally been used as medicinal plant for its analgesic, anti-inflammatory, anti-cancer, chemoprotective, cardio tonic and free radical scavenger effects (Singh and Uppal 2015).

There is a clear trend towards increasing consumption of juices as they are considered as an excellent medium for delivering nutraceuticals such as soluble fiber, vitamins, phenolic compounds and carotenoids. These functional beverages not only provide taste and satisfaction, but also necessary health benefits to consumers. Despite having significant nutritive value, ash gourd and bottle gourd juices are difficult to preserve and utilize due to their high pH and having poor sensory qualities. Karwanda is an acidic and underutilized fruit and only seasonally available. Blended juices are good alternative for development of new products and utilization of juices as they have higher nutritional and sensory qualities and better shelf life than pure juices (Raj et al. 2011; Jayachandran et al. 2015). Besides, it is reported that concentration of bioactive compounds in fruit juices exceeds that of intact fruits after consumption (Rodríguez-Roque et al. 2013; Ribnicky et al. 2014). In this sense, many studies have been focused on bioavailability of these compounds. As in vivo trials are expensive, time consuming and have intersubject variability in addition to effect of food matrix on these compounds, in vitro gastrointestinal digestion has been used as a fast and simple methodology to measure release of bioactive compounds from food matrix (Rodríguez-Roque et al. 2013). Hence in current study, ash gourd-karwanda and bottle gourd-karwanda blended beverages were developed and quality evaluation and storage study of these beverages were conducted. In addition, effect of added sugar on stability and bioaccessability of phenolic compounds was also analyzed.

Materials and methods

Chemicals and reagents

Plate Count Agar and Potato Dextrose agar (PDA) were obtained from HiMedia, Mumbai. Folin–Ciocalteu reagent, Gallic acid, Quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), 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 a-D-glucopyranoside (PNPG) were procured from SRL, Mumbai. Bovine Serum Albumin (BSA), 3,5-Dinitrosalicylic acid (DNS), Potessium Metabisulphite (KMS) and other chemicals and solvents were acquired from SDFCL, Mumbai.

Preparation and processing of juices

Ash gourd, bottle gourd and karwanda (Online Resource 1) were purchased from Matunga, Mumbai. Ash gourd was peeled, deseeded and cut into cube shape and rinsed into 0.1% KMS solution for 5 min to deactivate enzymes. Ash gourd cubes were pulped in a commercial mixer with addition of distilled water. Bottle gourds were peeled, cut into ring shape and rinsed into 0.1% KMS solution for 5 min. Bottle gourd rings were pulped in a commercial mixer with addition of 0.1% KMS solution to prevent browning. Karwanda were washed, deseeded and pulped in a commercial mixer with addition of water. Ash gourd, bottle gourd and karwanda pulp were then strained through a double layered muslin cloth to get juices.

Ash gourd-karwanda (AgK) blends were prepared by mixing different proportions of 25, 30 and 35 mL ash gourd juice with 15, 25 and 35 mL karwanda juice. Similarly, bottle gourd-karwanda (BgK) blends were prepared by mixing different proportions of 20, 27.5 and 35 mL of bottle gourd juice with 15, 22.5 and 30 mL karwanda juice. Sugar (5 g) was added as sweetener, salt (0.5 g) was added as taste enhancer, gum acacia (1 g) was added as emulsifier and volume was adjusted to 100 mL by addition of distilled water. These beverages were pasteurized at 95 °C for 15 min and stored in sterile screw cap glass bottles at 50 °C for 12 days to simulate conditions for 70 days storage at room temperature (Pérez-ramírez et al. 2015). Sample blends were optimized based on sensory score. Total soluble solids (TSS), pH, acidity, total phenolic content and DPPH radical scavenging activity of blends were evaluated and optimized beverages were further studied for their quality and storage stability. Beverages were also prepared without addition of sugar to measure their α-amylase and α-glucosidase inhibition capacity.

Sensory evaluation

Sensory evaluation of beverages was performed by 20 untrained panelists (11 females and 9 males) consisting of graduate students aged between 20 and 30 years having prior knowledge of consumer preference using 9-point hedonic scale (1 = extreme dislike; 9 = extreme like). Samples (50 mL) were served chill (10 °C) individually to panelists in coded identical transparent 150 mL cups with white illuminating background at 11 am. Samples were evaluated for colour, flavor, taste, mouthfeel and overall acceptability using 9-point hedonic scale (Temiz and Kezer 2015).

Microbiological assay

For evaluation of microbial safety of samples during accelerated storage, number of aerobic viable cells were estimated by total viable count and total yeast and mold count methods (Evrendilek et al. 2000). Number of colony forming units (CFU) were counted for total viable count and yeast and mold count after incubation at 37 °C for 48 h and 72 h respectively.

TSS, 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 titratable acidity was determined by titration against 0.1 N NaOH and expressed as % citric acid.

Total phenolic, flavonoid and anthocyanin content

Water extracts of beverages prepared by centrifugation (5000 rpm/15 min) were used for evaluation of functional properties of beverages. Total polyphenols were determined by slightly modified Folin–Ciocalteu method (Dewanto et al. 2002): 100 g/L Sodium Carbonate (2 mL) and Folin–Ciocalteu reagent (2.5 mL) were added to beverage extract (0.5 mL) and mixture was incubated for 1 h in darkness. Absorbance was measured at 760 nm and results were expressed as Gallic acid equivalents (GAE/mL). Appropriate blank and standard solutions were prepared.

Total flavonoid content was determined by modified $AlCl_3$ method (Sravani et al. 2017). Briefly, aliquot of beverage extract (1 mL) and 5% Sodium Nitrite (0.3 mL) were mixed and diluted to 5 mL with distilled water. After 5 min, 10% $AlCl_3$ (3 mL) and further after 6 min, 1 M NaOH (2 mL) were added to complete the reaction. The change in colour was measured at 510 nm and results were expressed as Quercetin equivalents (QE/mL).

Total monomeric anthocyanins of beverages were determined by pH differential method of Sonawane and Arya 2015 and results were expressed as cyanidin-3-glucoside (C3GE/mL) equivalents.

Total antioxidant capacity

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

Antioxidant activity measured as ferric reducing antioxidant power (FRAP) was determined by a previous method (Abeysinghe et al. 2007) and results were expressed as ascorbic acid equivalent antioxidant activity (AAE/mL).

In vitro gastrointestinal digestion

The in vitro gastrointestinal digestion was carried out according to methodology described by Rodríguez-Roque et al. 2013. In this method, samples underwent two simulated sequential digestion phases: gastric and intestinal digestion (including dialysis). To estimate changes after gastrointestinal digestion, aliquot of dialyzed sample were taken and stored at − 20 °C until analyzed. Bioaccessability of total phenolic, flavonoids and anthocyanins content were determined from following equation:

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

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.

α-Glucosidase and α-amylase inhibition activity

The α-glucosidase inhibition activity was measured according to a previous method (Oboh et al. 2015) with some modification. Briefly, aliquot of beverage extract (50 µL) was added to 0.9 mg/mL α-glucosidase (10 µL) solution in 0.1 M phosphate buffer (pH 6.8). The mixture was allowed for incubation at 37 °C for 15 min after addition of 400 µL 0.1 M phosphate buffer (pH 6.8). Then 20 mM PNPG (10 µL) solution in 0.1 M phosphate buffer (pH 6.8) was added and mixture was incubated at 37 °C for 15 min. To stop the reaction, 0.1 M Sodium Carbonate (400 µL) was added and amount of 4-nitrophenol was measured at 405 nm. The control sample contained all reagents and enzyme without beverage extracts and percent inhibition was calculated.

Beverage extract (25 µL) and porcine α-amylase (0.5 mg/mL) solution in 20 mM pH 6.9 phosphate buffer (25 µL) were incubated at 25 °C for 10 min. Then, 0.5% starch solution (25 µL) in 20 mM pH 6.9 phosphate buffer was added and mixture was allowed to incubate at 25 °C for 10 min. The reaction was stopped by addition of 96 mM 3, 5-dinitrosalicylic acid reagent (50 µL) and the microplate was incubated at 100 °C for 5 min. After immediately cooling to room temperature, absorbance was measured at 540 nm. The α-amylase inhibition activity was measured by percent inhibition (Subramanian et al. 2008). The uninhibited enzyme was taken as control and appropriate blanks were used for all reactions.

Anti-inflammatory activity measurement by inhibition of albumin denaturation

Measurement of in vitro anti-inflammatory activity was based on inhibition of albumin denaturation method (Reshma and Brindha 2014). Beverage extracts and standard drug were diluted 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. The mixture was incubated at 37 °C for 20 min and then heated to 51 °C for 20 min. After cooling to room temperature, the absorbance was measures at 660 nm and percent inhibition of albumin denaturation was calculated. Diclofenac sodium (15 µg/mL) used as standard drug.

Data analysis

IBM SPSS statistics 20 (IBM Corporation, NY) was used to analyze results by one-way analysis of variance (ANOVA; $p < 0.05$) and the Tukey means comparison test ($p < 0.05$). Pearson's linear regression model was applied for determination of correlation.

Results and discussion

Total soluble content of ash gourd, bottle gourd and karwanda juice were 2.7, 3.0 and 15.0°Brix respectively. The titratable acidity value (expressed as % citric acid) of ash gourd, bottle gourd and karwanda juice were 0.04, 0.04 and 0.68 respectively.

Sensory characteristics

As ash gourd and bottle gourd juices have very distinct raw vegetable taste and flavor, the overall acceptability score were low for high proportion of these juice. Addition of karwanda to these beverage added desired colour, flavour and taste to these beverages. In general, BgK blends scored higher on overall acceptability value than AgK blend due to its less distinctive vegetable flavour. Addition of higher quantity of karwanda juice in blends negatively affected sensory qualities as it increased acidity of blends which is also undesirable. The objective of present study was to select blends with maximum proportion of ash gourd and bottle gourd juices without affecting sensory qualities. Though blends with higher proportion of karwanda than ash gourd or bottle gourd were desirable (Tables 1 and 2), they were not preferred as karwanda is a seasonal fruit and lower in supply. However in future, beverages having higher proportions of karwanda can be prepared to have additional health benefits. Based on this criteria, AgK blend (35:35) and BgK blend (35:30) were selected for further studies. The selected blends also had higher concentration of polyphenols and high antioxidant activity measures as % DPPH inhibition compared to similar or higher level of ash gourd or bottle gourd juice (Tables 1 and 2).

Microbiological safety

Microbiological safety of selected beverages was evaluated during accelerated storage condition. Total viable count and yeast and mold count were nil (< 1 CFU/mL) during storage at 50 °C for 12 days, hence blended beverages were microbiologically safe during storage period. Microbiological safety was also evaluated for beverages stored at 25 °C after 90 days. Total viable count and yeast and mold count for this period were less than 2 CFU/mL. These results confirm that selected beverages were microbiologically safe during storage period.

TSS, acidity and pH

Addition of karwanda increased acidity and decreased pH of blends whereas addition of ash gourd and bottle gourd increased acidity but had no significant difference on pH (Tables 1 and 2). pH value not only depends on titratable acids but also on concentration of minerals which interfere with concentration of free protons and organic acids which account for titratable acidity but contribute very little for pH value (Barnuud et al. 2014). TSS content of selected beverages increased gradually during the storage period (Fig. 1) which might be due to hydrolysis of polysaccharides into monosaccharides and oligosaccharides (Raj et al. 2011). BgK blend showed less variation in TSS and acidity than AgK blend during storage period which is desirable (Fig. 1). There was little change in acidity during initial days but during later days it increased significantly. TSS to titratable acidity ratio was same or increased slightly during initial days but declined significantly during later days.

Total phenolic content, flavonoids and anthocyanins

Total phenolic content as determined by FC method for both beverages decreased over storage period though AgK blend had more phenolic content than BgK blend. Concentration of phenolic compounds depends on many factors such as type of fruit, ripening stage, growing location, environment and storage period (Morales-de la Peña et al. 2010). The decreasing trend may be attributed to susceptibility of polyphenols to high temperature during processing (Morales-de la Peña et al. 2010). These polyphenols contribute to antioxidant properties of fruits thus good correlation ($r^2 = 0.84$) can be found between

Table 1 Overall acceptability, TSS, TPC, acidity, pH and DPPH scavenging activity of AgK blends

AG (% v/v)	KD (% v/v)	OA	TPC (μg GAE/mL)	DPPH inhibition[1]	TSS (°Brix)	pH	Acidity (%)
25	15	$6.00 \pm 0.10^{b,c,d,e}$	142.92 ± 0.92^{c}	834.37 ± 52.14^{a}	8.20 ± 0.03^{b}	$3.94 \pm 0.02^{c,d}$	0.25 ± 0.02^{b}
	25	$6.80 \pm 0.30^{e,f}$	176.42 ± 0.62^{d}	1045.59 ± 35.34^{c}	9.00 ± 0.05^{c}	4.01 ± 0.05^{d}	0.30 ± 0.02^{c}
	35	6.85 ± 0.30^{f}	212.26 ± 0.30^{g}	1191.81 ± 44.66^{d}	10.10 ± 0.10^{e}	3.66 ± 0.04^{a}	0.45 ± 0.01^{d}
30	15	$5.20 \pm 0.20^{a,b}$	104.25 ± 0.74^{a}	809.99 ± 24.78^{a}	7.30 ± 0.03^{a}	3.89 ± 0.05^{c}	0.15 ± 0.01^{a}
	25	$5.70 \pm 0.20^{b,c}$	266.51 ± 1.36^{i}	850.61 ± 17.32^{a}	9.00 ± 0.06^{c}	4.29 ± 0.04^{e}	0.45 ± 0.02^{d}
	35	$6.40 \pm 0.30^{c,d,e,f}$	233.49 ± 0.87^{h}	1009.03 ± 56.47^{c}	11.10 ± 0.11^{f}	3.70 ± 0.02^{a}	0.60 ± 0.03^{e}
35	15	$5.95 \pm 0.20^{b,c,d}$	124.06 ± 0.62^{b}	809.99 ± 35.98^{a}	8.00 ± 0.02^{b}	$3.68 \pm 0.03^{a,b}$	0.15 ± 0.0^{a}
	25	$6.40 \pm 0.40^{c,d,e,f}$	190.57 ± 1.04^{e}	$1122.76 \pm 82.14^{c,d}$	9.50 ± 0.09^{d}	$3.74 \pm 0.03^{a,b}$	0.30 ± 0.02^{c}
	35	$6.55 \pm 0.40^{d,e,f}$	201.42 ± 0.15^{f}	$1088.40 \pm 22.46^{c,d}$	10.20 ± 0.08^{e}	3.77 ± 0.02^{b}	0.30 ± 0.02^{c}
100	0	4.60 ± 0.20^{a}	197.86 ± 6.64^{f}	1057.26 ± 38.52^{c}	2.70 ± 0.03^{g}	5.10 ± 0.05^{f}	0.04 ± 0.01^{f}
0	100	$6.05 \pm 0.30^{c,d,e,f}$	734.17 ± 16.80^{j}	2397.30 ± 41.10^{e}	15.00 ± 0.10^{h}	3.20 ± 0.0^{g}	0.68 ± 0.03^{g}

[1]μg AAE/mL. *AG* Ash gourd, *KD* Karwanda, *OA* Overall acceptability

Means followed by same letter within same column are not significantly different (n = 3, $p < 0.05$)

Table 2 Overall acceptability, TSS, TPC, acidity, pH and DPPH scavenging activity of BgK blends

BG (% v/v)	KD (% v/v)	OA	TPC (μg GAE/mL)	DPPH inhibition[1]	TSS (°Brix)	pH	Acidity (%)
20	15	$6.15 \pm 0.30^{a,b,c}$	142.93 ± 0.26^{b}	554.09 ± 45.10^{b}	8.00 ± 0.02^{a}	$4.14 \pm 0.05^{c,d}$	0.15 ± 0.01^{a}
	22.5	$6.65 \pm 0.40^{a,b,c}$	137.26 ± 0.47^{a}	318.50 ± 33.42^{a}	8.90 ± 0.06^{b}	4.01 ± 0.01^{b}	0.15 ± 0.01^{a}
	30	7.30 ± 0.30^{c}	185.38 ± 0.21^{f}	$448.48 \pm 86.50^{a,b}$	9.20 ± 0.02^{c}	3.93 ± 0.02^{a}	0.30 ± 0.0^{b}
27.5	15	$5.85 \pm 0.20^{a,b,c}$	158.96 ± 1.41^{c}	$854.67 \pm 24.81^{c,d}$	8.00 ± 0.05^{a}	4.20 ± 0.0^{d}	0.30 ± 0.02^{b}
	22.5	$6.30 \pm 0.30^{a,b,c}$	169.34 ± 1.29^{d}	$915.60 \pm 35.01^{c,d}$	9.20 ± 0.08^{c}	4.01 ± 0.01^{b}	0.45 ± 0.03^{c}
	30	$7.00 \pm 0.20^{b,c}$	198.59 ± 0.77^{h}	$929.30 \pm 71.16^{c,d}$	10.00 ± 0.04^{e}	$3.94 \pm 0.03^{a,b}$	0.60 ± 0.02^{d}
35	15	$6.00 \pm 0.30^{a,b,c}$	181.60 ± 0.98^{e}	838.43 ± 15.27^{c}	9.50 ± 0.03^{d}	4.22 ± 0.04^{d}	0.45 ± 0.01^{c}
	22.5	$6.25 \pm 0.20^{a,b,c}$	201.42 ± 0.15^{i}	$964.35 \pm 43.17^{c,d}$	9.00 ± 0.06^{b}	4.10 ± 0.04^{c}	0.45 ± 0.03^{c}
	30	$6.65 \pm 0.40^{a,b,c}$	188.68 ± 0.90^{g}	$949.31 \pm 36.87^{c,d}$	9.90 ± 0.08^{e}	4.01 ± 0.03^{b}	0.60 ± 0.02^{d}
100	0	4.50 ± 0.20^{d}	218.85 ± 0.28^{j}	981.41 ± 29.36^{d}	3.00 ± 0.02^{f}	5.31 ± 0.03^{e}	0.04 ± 0.01^{e}

[1]μg AAE/mL. *BG* Bottle gourd, *KD* Karwanda, *OA* Overall acceptability

Means followed by same letter within same column are not significantly different (n = 3, $p < 0.05$)

polyphenols and antioxidant properties of these beverages (Tables 1, 2, Fig. 1). Similar positive relationship between polyphenols and antioxidant properties was found in a previous study on Indian fruits and vegetables (Singh et al. 2016). Total flavonoids content as determined by $AlCl_3$ method, were higher in BgK blend than AgK blend (Table 3). During storage, total monomeric anthocyanins, as determined by pH differential method, declined gradually (Fig. 1). This can be explained as anthocyanins are known as being susceptible to light and oxidation, which might have occurred during processing and storage.

Total antioxidant capacity

Antioxidant capacity, measured as percent DPPH inhibition, like total polyphenols, was higher for higher proportions of karwanda (Tables 1 and 2). Antioxidant capacities measured as DPPH and FRAP assay, were higher for AgK blend than BgK blend. As karwanda is known to have high antioxidant potential (Patil et al. 2012), this effect may be due to higher proportion of karwanda in AgK blend. Though Ash gourd and bottle gourd also exhibited antioxidant capacities as can be inferred from Tables 1 and 2.

During storage, antioxidant capacity, as measured by DPPH inhibition, was to a great extent stable tough there were fluctuations during storage period (Fig. 1). The results are in agreement with several previous studies on fruits (Ayala-Zavala et al. 2004; Kevers et al. 2007). Kevers et al. 2007 observed that during storage of most of fruits and vegetables, anthocyanins content decreased whereas total flavonoids increased. Anthocyanins and other phenolic

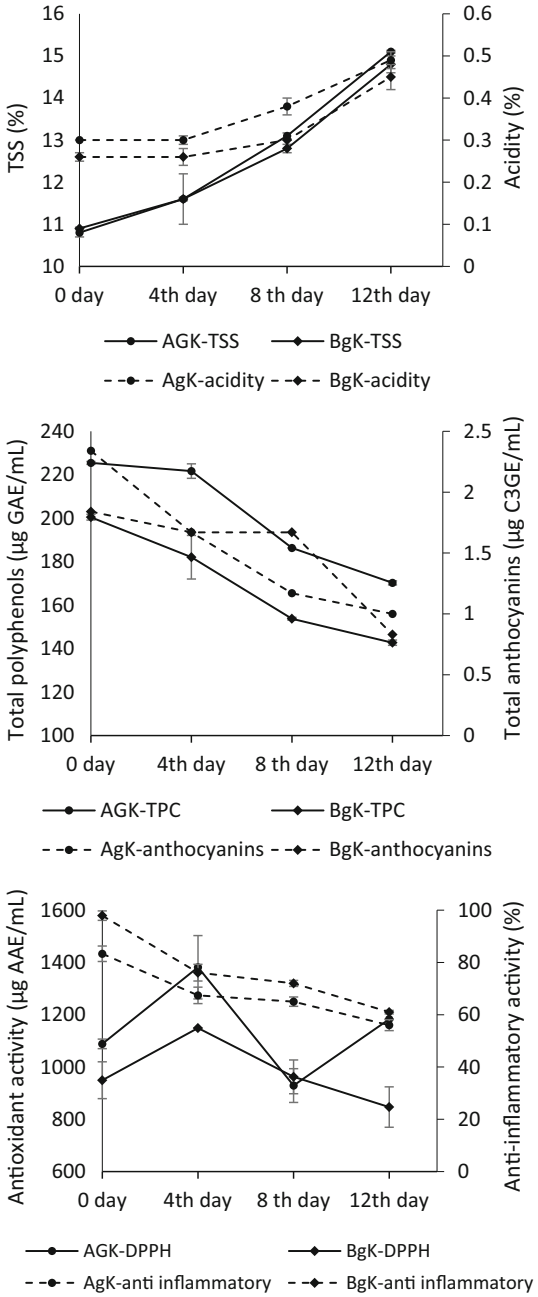

Fig. 1 Results of accelerated storage study (50 °C for 12 days) of AgK (35:35) and BgK (35:30) blended beverages. Error bar represent the standard deviation of mean (n = 4)

compounds might have been degraded to thermally degradation products which have high scavenging capacity leading to higher 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 intermediate state (Sharma et al. 2010) but addition of beverage extracts exhibited to preserve native state of BSA (Fig. 1). BgK blends preserved native state of BSA better than AgK blends even though anti-inflammatory activity decreased progressively during accelerated storage (Fig. 1). Secondary metabolites present in ash gourd, bottle gourd and karwanda such as flavones, flavonoids and phenolic compounds are known to possess anti-inflammatory activity (Tiwari 2014; Singh and Uppal 2015). Decrease in anti-inflammatory activity during accelerated storage might be associated with decrease in concentration of these compounds (Fig. 1). Diclofenac Sodium drug (15 µg/mL) used as positive control prompted 40% inhibition of albumin denaturation.

Table 3 Physiochemical properties (TSS and acidity), total phenolics, total flavonoids, anthocyanins content, antioxidant activity (measured by DPPH and FRAP assay) and α-amylase and α-glucosidase inhibitory activity of selected beverages

Parameter	AgK	BgK
TSS (°Brix)	10.80 ± 0.10	10.90 ± 0.50
Acidity (% citric acid)[b]	0.30 ± 0.0	0.26 ± 0.0
Total polyphenols (µg GAE/mL)[b]	225.47 ± 0.67	200.47 ± 1.33
Total flavonoids (µg QE/mL)[b]	18.69 ± 0.06	22.83 ± 1.11
Total anthocyanins (µg C3GE/mL)	2.34 ± 0.44	1.84 ± 0.08
DPPH assay(µg AAE/mL)[b]	1088.40 ± 18.44	949.31 ± 70.36
FRAP assay (µg AAE/mL)	921.30 ± 8.08	883.40 ± 23.23
α-amylase inhibition (%)[ab]	31.05 ± 1.20	26.22 ± 1.57
α-glucosidase inhibition (%)[ab]	26.03 ± 3.88	42.81 ± 3.39

[a]α-amylase inhibition (%) and α-glucosidase inhibition (%) were measured for beverages without added sugar

[b]Means within same row are significantly different (n = 4, $p < 0.05$)

α-Amylase and α-glucosidase inhibitory activity

Hydrolysis of starch into glucose and disaccharides is catalyzed by pancreatic α-amylase and intestinal α-glucosidase. Therefore, inhibition of these enzymes contributes to antihyperglycemic activity and management of type II diabetes. Furthermore, reduction of oxidative stress is also known to reduce diet-induced glycemic overload (Pérez-ramírez et al. 2015).

AgK blend (35:35) and BgK blend (35:30) prepared without addition of sugar were estimated for their antihyperglycemic activity. Maximum α-amylase (31%) and α-glucosidase (43%) inhibitory activity achieved for AgK and BgK blends respectively (Table 3). Vegetables are known for their multi-faceted antihyperglycemic activity, such as ash gourd and bottle gourd, also found to inhibit

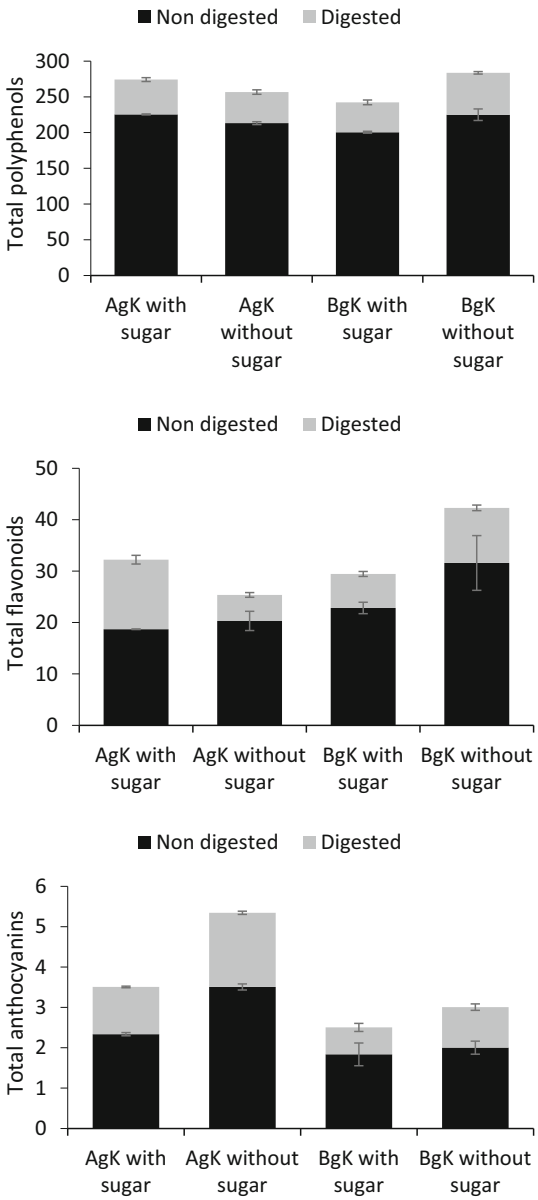

Fig. 2 Changes in concentration of total polyphenols (μg GAE/mL), total flavonoids (μg QE/mL) and anthocyanins (μg C3GE/mL) content of AgK (35:35) and BgK (35:30) blended beverages before and after gastrointestinal digestion. Error bar represent the standard deviation of mean (n = 3)

protein-tyrosine phosphatase 1β enzyme in liver and skeletal muscle, an enzyme responsible to increase insulin resistance (Tiwari 2014). As flavonoids and anthocyanins content of AgK and BgK blends constituted only 9.33% and 12.31% of total polyphenols, which suggests beverages contain many other polyphenols such as alkaloids, saponins, tannins and phenolic acids and degree of polymerization of polyphenols is high. High degree of polymerization is linked with antidiabetic, anti-inflammatory and cytotoxic activity (Itankar et al. 2011).

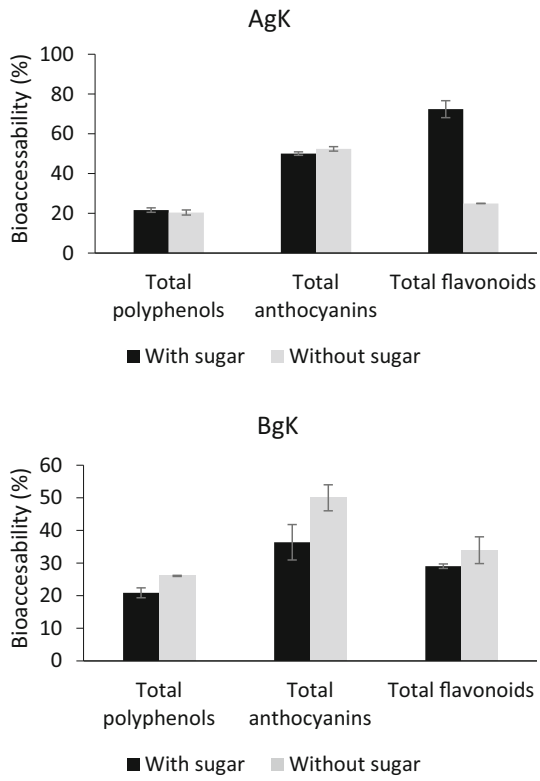

Fig. 3 Bioaccessability (%) of total polyphenols (μg GAE/mL), anthocyanins (μg C3GE/mL) and total flavonoids (μg QE/mL) in AgK (35:35) and BgK (35:30) blended beverages after in vitro gastrointestinal digestion. Error bar represent the standard deviation of mean (n = 3)

Effect of addition of sugar on stability and bioaccessability of phenolic compounds

Addition of sugar to AgK beverage increased total phenolic content slightly (5.8%) while it caused decreased flavonoids (7.9%) and total anthocyanin content (33.4%). On the other hand, addition of sugar to BgK beverage decreased TP (11%), flavonoids (31%) and anthocyanin content (8%) (Fig. 2). Hence, addition of sugar had negative effect on stability of total phenolic content, anthocyanins and flavonoids in both beverages though phenolic content in AgK beverage increased slightly. Similar negative effect was found on addition of sucrose on stability of anthocyanins in blackberry juices (Pérez-ramírez et al. 2015). This effect is result of formation of sugar-degraded products after heating which promote anthocyanin degradation (Hubbermann et al. 2006).

Addition of sugar appeared to have protective effect on bioaccessibility of total phenolics and flavonoids in AgK blends but had no such effect in BgK blends (Fig. 3). As juice is a processed product, effect of food matrix on release of phenolic compounds might be less but it cannot be completely ignored as studies suggest that bioaccessibility

of these compounds in whole food (blueberry polyphenol-enriched defatted soybean flour) was higher than similar studies conducted on individual compound extracts (Ribnicky et al. 2014). Ash gourd has significantly higher dietary fiber and fat content than bottle gourd and this effect might be attributed to interaction between polyphenols such as flavonoids associated with dietary fiber and fat consequently making them more bioaccessible (Ortega et al. 2009; Ribnicky et al. 2014). Furthermore, ability of sugars and digestive carbohydrates to both associate and limit bioaccesibility of phenolic compounds complicate studies on bioaccessiblity analysis. Effect of sugar on bioavailability is different for different phenolic compounds. For example, sugar has negative effect on bioavailabilty of cyanidin-3-glucoside but had no effect on pelargonidin-3-glucoside (Ribnicky et al. 2014). A positive effect was found on absorption of monomeric anthocyanin from grapes and flavan-3-ol from tea when co-ingested with carbohydrate rich food (Serra et al. 2010; Neilson and Ferruzzi 2011). Ability of digestible carbohydrates to potentiate digestion of flavonoids and anthocyanins might improve bioaccessibility of these compounds. Phenolic compounds undergo many structural and chemical composition changes during digestion and only limited number of metabolites were determined in most studies which may leads to underestimating bioavailability.

Bioaccessibility specifically refers to the amount of compounds which are potentially presented to the intestinal brush border for absorption. It differs significantly from bioavailability which refers to the amount of compounds which pass this intestinal border and are available for use. Besides present study omits the colonic phase of the digestive process. Though, bioaccessibility can be used to suggest relative concentration of compounds and infers that if more compounds are presented to the intestinal brush border, more likely they will be absorbed. In a previous study, mice fed with whole blueberry powder or purified blueberry anthocyanins which lacked sugars and lipid components, only later was effective in lowering blood glucose suggesting that the anti-diabetic effects of blueberry anthocyanins and other polyphenols were counteracted by sugars or lipid components in whole blueberries (Ribnicky et al. 2014). Therefore, caution is warranted for increased bioaccessiblity of these compounds in sugar sweetened beverages as increase in potential health benefits are not certain.

Conclusion

Addition of karwanda to these beverage increased overall acceptability, polyphenols, TSS and antioxidant properties while lowering pH of beverages consequently increasing storability of these beverages. Beverages prepared without addition of sugar exhibited significant anti-hyperglycemic activity too, therefore these blends can effectively be used as functional beverages as medium for delivering nutraceuticals and providing necessary health benefits to consumers. Sugar negatively influenced stability of total phenolic, anthocyanins and flavonoids content whereas it appeared to have protective effect on bioaccessability of total phenolics and flavonoids in AgK blends but had no such effect on BgK blends.

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