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RESEARCH ARTICLE

Utilization of karonda (*Carissa carandas*) juice in *Lassi* for enhancement of antioxidant activity

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Abstract: Karonda (*Carissa carandas*) an underutilized berry is known for rich source of bioactive compounds. The karonda juice (KJ) was extracted manually and analysed for physico-chemical characteristics, color and antioxidant activity. The juice had TSS (18.23 °Brix), acidity (0.5 % citric acid), pH (4.79), ascorbic acid content (24.56 mg/100 g) and TPC (7.88 µmol GAE/g). The color parameters measured as *L, a* and *b* values were recorded as 4.45, 8.55, and 1.38, respectively. The KJ exhibited antioxidant activity of 6.36 and 4.66 µmol TE/g as measured by ABTS and DPPH scavenging assay, respectively. The analysis of KJ using LC-MS QTOF revealed the presence of 13 phenolic acids, 19 flavonoids and 4 anthocyanins. The sweetened lassi was prepared with (3, 4, 5 and 6%) or without addition of KJ and assessed for changes in physio-chemical characteristics and microbiological quality during storage. The addition of juice to lassi resulted a decrease in the lightness with L* value from 77.00 to 63.52 and yellowness with b* value from 4.63 to -2.95 while increased the redness with a* values from -2.10 to 6.97 as measured by colorimeter. Incorporation of 5 % KJ was adjudged as best by the sensory panel. The storage study revealed that the product could be stored upto 21 days without any adverse changes in the sensory and antioxidant activity at 5±2°C. The results suggest that KJ can be utilized as ingredient in the development of functional fermented milk products with enhanced antioxidant properties.

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Introduction

Around the globe fruits grown locally possess distinct nutrients and unique flavors specific to their region are often ignored and underutilized (Atasoy, 2009). Many of these fruits have potential to play crucial role in enhancing the nutritional security and wellbeing of individuals. Furthermore, the cultivation and consumption of these fruits can support local economies, preserve biodiversity and foster cultural heritage. Karonda (*Carissa karandas)* is a clustered berry-producing shrub widely cultivated as a biofence in gardens and orchards across various regions of India and Southeast Asia. The berries are naturally sour when unripe but become sweeter when ripened and color of fruit varies from purple to blackish brown. Karonda is commonly consumed in the form of pickles and candies (Chinmayi *et al.* 2024). The fruit is exceptionally rich in bioactive compounds such as flavonoids, phenolic acids and anthocyanins and also good source of minerals (Azeez *et al.* 2016). Traditionally, the fruits have been used in alternative medicines to improve digestive condition, stimulation of immune system and control urinary tract infections. The phytochemicals present in karonda can contribute to its antioxidant, anti-inflammatory and anticancer properties (El-desoky *et al.* 2018). However despite of abundant bioactive compounds these berries are facing challenges to achieve high economic value.

In recent years there has been increased interest in functional foods that confer health benefits beyond regular nutritional properties. Fermented dairy products, especially yoghurt, lassi or stirred yoghurt, cultured butter milk is regularly consumed as part of human daily diet. Therefore, emphasis has been on these products as carriers for delivery of natural functional ingredients to improve the immunity of human being (El-Said *et al.* 2014). New varieties of lassi with unique flavor and aroma and bioactive compounds increase sales and are liked more by consumers (Shah *et al.* 2016). More specifically the additions of fruits or their derivatives which are rich source of several phytochemical have been explored for development of commercial lassi and stirred yoghurt varieties. Consequently numerous studies have investigated production of varieties of lassi with these additives

such as aloevera (Kumar et al. 2021), orange juice (Jadhav *et al.* 2014), pomegranate juice (Cakmakci et al. 2014), tomato pomace (Kumar et al. 2023), amaranthus (Patel et al. 2020) and sapota fruit (Khateeb et al. 2025).

Although the utilization of various fruit juices in lassi and other fermented milk products has already been extensively studied, very limited studies have investigated the potential of Karonda fruit. Lassi has mass consumption and high bioavailability of nutrients, make it suitable as functional ingredient for formulation of products for health conscious consumers. Therefore, the objective of this study was to determine the bioactive compounds in Karonda and to assess the possibility of incorporation into lassi with karonda juice (KJ) as a functional ingredient with the attention focused on its effects on sensory attributes, physicochemical parameters and antioxidant properties of lassi.

Materials and Methods

Extraction of Karonda juice

The fresh ripened Karonda (*Carissa carandas*) fruits of dark brown color were harvested from the Horticultural farm of SDAU, Sardarkrushinagar. The ripened fruits obtained were sorted, cleaned and surface dried using muslin cloth. Using a lemon juice extractor, the juice was extracted by manual pressing. The collected juice was heat treated (90°C/5 min) and immediately cooled. The juice thus obtained was filled into plastic bottles and were stored at -18 °C for further use.

LC/MS profiling of phenolic compounds

For the chemical profiling of Karonda juice, liquid chromatography system coupled with a quadrupole time-of-flight mass spectrometer (LC-QTOF-MS; Agilent 6545, USA), equipped with a Dual Agilent Jet Stream electrospray ionization (ESI) source, was employed following the methodology described by Suleria et al. (2020). Chromatographic separation was performed on a reversed-phase C18 analytical column (Zorbax Eclipse Plus, 100 mm×2.1 mm, 1.8 µm particle size) maintained at 35 °C. The mobile phase system comprised two solvents: solvent A-water with 0.1% formic acid, and solvent B-acetonitrile with 0.1% formic acid. A gradient elution was implemented as follows: 0 min, 90% A; 1 min, 95% A; 3 min, 60% A; 5 min, 35% A; 8 min, 20% A; held until 9 min; 10 min, 50% A; 12 min, 95% A; and 15 min, 90% A. Ionization was achieved using electrospray ionization (ESI) in positive mode, with optimized parameters including a drying gas temperature of 325 °C, drying gas flow rate of 10 L/min, nebulizer pressure of 35 psig, sheath gas temperature of 350 °C, and sheath gas flow rate of 11 L/min. The flow rate was maintained at 0.3 mL/min, and the injection volume was fixed at 5 µL. The obtained MS data was processed using Agilent Mass Hunter workstation software.

Antioxidant activity

For ABTS (2,2'-azino-bis, 3-ethylbenzothiazoline-6-sulfonic acid) assay the method described by (Re *et al.* 1999) was followed. A 0.2 ml of sample was mixed with 2ml of ABTS solution and the absorbance was taken at 734 nm using UV-VIS spectrophotometer (Model-SL210, Elico equipment, India). A standard curve was plotted for percentage inhibition of absorbance as a function of concentration of Trolox (25-200 µM), and results were expressed as mM Trolox equivalents (TE)/g of sample fruit or lassi.

The antioxidant activity of fruit and lassi samples was assessed using the DPPH assay (Brand-Williams *et al.* 1995). A 0.1 ml sample was mixed with 3.9 ml DPPH solution and incubated at 37°C for 120 min. Absorbance was measured at 515 nm using a UV-VIS spectrophotometer using as the blank. Following, same procedure a Trolox standard curve (100–1000 µM) was plotted, and results were expressed as mM Trolox equivalents (TE)/g of sample.

Total phenolic content

To determine the total phenolic content Folin Ciocalteu method was followed (Amirdivani and Baba, 2011). Briefly, 0.4 ml of diluted sample was mixed with 2 ml of Folin-Ciocalteu reagent (0.2 N) and kept undisturbed for 3 min. Then, 1.6 ml of 7.5% sodium carbonate and contents were mixed well and let stand in the dark for 30 min. Finally, the absorbance was recorded at 765 nm using a UV-VIS spectrophotometer (Model-210, Elico equipment, India). Results were expressed as µmol of gallic acid equivalent (GAE) per gram of sample.

Preparation of experimental lassi

The lassi preparation was done based on the procedure of Patidar and Prajapati (1998) with minor modifications. Homogenized milk containing 3% fat and 8.5% SNF was added with sugar (12%), heat treated at 90 °C for 10 min followed by immediate cooling to 45°C. The milk was inoculated with 0.02% w/v of Yomix® starter culture (*St. thermophilus* and *Lb. bulgaricus*) and incubated for 4 to 5 hours at 41±1 °C. The obtained curd was cooled by storing in the refrigerator for 6 hours. The cooled curd was broken using domestic hand blender and different levels of KJ were added, mixed and filled in pre-sterilized PET bottles and stored at 5±2 °C. The control product was prepared without addition of KJ.

Physico-chemical analysis

The pH was monitored using a digital pH meter (Labman, India) fitted with glass electrode. The titratable acidity was determined by titration against 0.1N NaOH solution using phenolphthalein indicator and the results were expressed as percent lactic acid per gram (FSSAI, 2015). The total soluble solids (°Brix) were measured at 20±1°C using a hand refractometer. Instrumental color measurement for the lassi and juice samples were performed

where *L* (lightness-darkness), *a* (greenness-redness) and *b* (blueness-yellowness) values were determined using a Lovibond colorimeter. Ascorbic acid was measured by the method of AOAC (2005).

Whey separation in lassi was assessed by storing 100 ml sample in a graduated cylinder in the refrigerator. Volume of whey separated was measured and recorded as ml/100ml at different storage intervals (Hussain *et al.* 2014).

Sensory Analysis

The developed lassi was subjected to the sensory evaluation by expert panelist having adequate knowledge about sensory evaluation process as well as product characteristics. The temperature of lassi (50 ml) was brought to 10°C and served in odorless, disposable polystyrene cups with lids. The 9-point hedonic scale was used to adjudge the product for flavor, colour and appearance, body and texture and overall acceptability, where, 1=dislike extremely, 2 =dislike very much, 3=dislike, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like, 8=like very much and 9=like extremely.

Microbiological Analysis

The microbiological counts of total lactic acid bacteria (ISO 7889:2003/IDF 117), coliforms (IS 5401 (Part 1):2002) and yeast molds (IS 5403: 1999) were enumerated in the lassi by pour plate method. The counts were taken in triplicates and results were expressed as colony forming unit (CFU)/gram of lassi sample.

Statistical Analysis

The obtained data were analyzed for statistical significance by analysis of variance (ANOVA) using SPSS software. The significant differences between the means were determined at $p<0.05$. The experiments were carried out in triplicates and the results are presented as mean of the obtained values along with the standard deviation.

Results and Discussion

Characteristics of Karonda juice (KJ)

The various physicochemical characteristics of KJ used in this study-are presented in the Table 1. The sourness of any given fruit plays important role as its overall taste is typically determined by the balance between the sourness and sweetness. The titratable acidity of KJ studied was found to be 0.5±0.02 percent citric acid. The pH of KJ was 4.79. These values are dependent on type of fruit and its composition. Juices with lower acidity tend to have a less sour taste.The citrus fruit pH ranges from 2.5 to 4.82 and titratable acidity upto 0.96 % citric acid mainly due to organic acids found in fruit juices include citric acid, malic acid, ascorbic acid and tartaric acids(El-Said *et al.* 2014; Ning *et al.* 2021). The juices are good source of natural pigments which can potentially enhances the sensory acceptance of lassi. The color parameters measured as *L, a,* and *b* values were recorded as 4.45±0.13, 8.55±0.53, and 1.38±0.07, respectively. This makes KJ particularly suitable for incorporation into mildly acidic foods such as lassi (Jadhav *et al.* 2014; Kumar *et al.* 2023). In addition to this KJ showed TSS (18.23±0.25 °Brix), total phenolic (7.88±0.1µmol GAE/g), and antioxidant capacity of 6.36/ ±/ 0.04 and 4.66/ ±/ 0.04 µmolTE/g as measured by ABTS and DPPH assays, respectively.

The plant biomolecules are of great interest in recent decade as they have been identified as potential functional ingredients. The metabolites especially phenolic compounds are primarily known for antioxidant activity in the fruit juices. It is important to have a clearer understanding of the phenolic acids, flavonoids and anthocyanins that contribute to antioxidant activity of KJ examined. A qualitative characterization of phenolic metabolites from KJ was achieved using the technique LC-QTOF/MS. Table 2 summarize the phenolic compounds including their respective formulas, retention times, and masses annotated through LC-QTOF/MS based Agilent Mass Hunter database as suggested by Rahim et al. (2022). This has resulted 13 phenolic acids, 19 flavonoids and 4 anthocyanins. The phenolic acids were mainly of derivatives of benzoic acid, cinnamic acid, quinic acid, ferulic acid, caffeoylquinic acid and salicylic acid derivatives. Notably, 2,4-Dihydroxybenzoic acid and m-Coumaric acid are known for strong antioxidant properties and presence of compounds such as 3-O-caffeoyl-4-O-methylquinic acid and 5Z-caffeoylquinic acid have been associated with health benefits including anti-inflammatory and anticancer properties. The LC-MS screening also resulted of 19 flavonoids in this analysis compounds were identified as derivatives of quercetin, apigenin, epicatechin, kaemferol and quercetin. Anthocyanins are group of phenolic compounds water soluble and contribute to color of the fruit. The analysis has shown presence of 4 anthocyanins in KJ as Cyanidin 3-(6''-succinyl-glucoside), Pelargonidin 3-(2G-

Table 1: Physicochemical characteristics, instrumental color, total phenolic content and antioxidant activity of Karonda juice

Constituent	Values
Physicochemical properties	
Total Soluble solids (°Brix)	18.23±0.25
Titratable acidity (% citric acid)	0.5±0.02
pH	4.79±0.02
Ascorbic acid (mg/100 g)	24.56± 0.41
Instrumental Color	
L	4.45±0.13
a	8.55±0.53
b	1.38±0.07
Antioxidants	
Total phenolic content (µmol GAE**/g)	7.88±0.1
Antioxidant capacity ABTS (µmol *TE/g)	6.36±0.04
Antioxidant capacity DPPH (µmol *TE/g)	4.66±0.04

Table 2: Phenolic compounds profile from Karonda Juice by LC/MS (Q-TOF)

Name	Formula	RT (min)	Ionization ESI^-/ ESI^+	Mass	Theoretical (m/z)	Observed (m/z)	Error (ppm)
			Phenolic acids				
2,4-Dihydroxybenzoic acid	$C_7H_6O_4$	6.102	$[M-H]^-$	154.0267	153.0194	153.0194	0.39
3,4-Methylenedioxybenzoic acid	$C_8H_6O_4$	6.762	$[M-H]^-$	166.0265	165.0192	165.0192	-0.94
3-Methoxy-4,5-methylenedioxybenzoic acid	$C_9H_8O_5$	6.327	$[M-H]^-$	196.0371	195.0298	195.0298	-0.19
3-O-Caffeoyl-4-O-methylquinic acid	$C_{17}H_20O_9$	5.659	$[M-H]^-$	368.1109	367.1036	367.1036	0.34
5-Hydroxyferulate	$C_{10}H_{10}O_5$	5.520	$[M-H]^-$	210.0529	209.0455	209.0455	0.41
5Z-Caffeoylquinic acid	$C_{16}H_{18}O_9$	5.224	$[M-H]^-$	354.0950	353.0878	353.0878	-0.16
Dihydroferulic acid 4-O-glucuronide	$C16H_{20}O_{10}$	5.614	$[M-H]^-$	372.1057	371.0984	371.0984	0.26
Glucocaffeic acid	$C_{15}H_{18}O_9$	1.859	$[M-H]^-$	342.0947	341.0875	341.0875	-1.11
Homogentisic acid	$C_8H_8O_4$	6.123	$[M-H]^-$	168.0422	167.0349	167.0349	-0.22
Isoferulic acid	$C_{10}H_{10}O_4$	6.646	$[M-H]^-$	194.0577	193.0504	193.0504	-1.22
m-Coumaric acid	$C_9H_8O_3$	6.005	$[M-H]^-$	164.0474	163.0401	163.0401	0.12
Salicylic acid beta-D-glucoside	$C_{13}H_{16}O_8$	3.432	$[M-H]^-$	300.0843	299.0770	299.0770	-0.62
trans-p-Coumaric acid 4-glucoside	$C_{15}H_{18}O_8$	4.926	$[M-H]^-$	326.1000	325.0928	325.0928	-0.38
			Anthocyanins				
Cyanidin 3-(6"-succinyl-glucoside)	$C_{25}H_{25}O_{14}$	6.090	$[M-H]^-$	549.1233	549.1225	549.1225	-2.14
Pelargonidin 3-(2G-xylosylrutinoside)	$C_{32}H_{39}O_{18}$	5.395	$[M-H]^-$	711.2136	711.2131	711.2131	-0.09
6-Hydroxydelphinidin 3-glucoside	$C_{21}H_{21}O_{13}$	1.868	$[M-H]^-$	481.0973	481.0977	481.0977	-1.93
4'-O-Methyldelphinidin 3-O-beta-D-glucoside	$C_{22}H_{23}O_{12}$	1.847	$[M-H]^-$	479.1190	479.1183	479.1183	0.13
			Flavonoids				
3-O-Methylquercetin	$C_{16}H_{12}O_7$	7.098	$[M-H]^-$	316.0579	315.0505	315.0505	-1.27
6-C-Glucopyranosylepicatechin	$C_{21}H_{24}O_{11}$	5.011	$[M-H]^-$	452.1314	451.1241	451.1241	-1.08
Apigenin 7-(6"-crotonylglucoside)	$C_{25}H_{24}O_{11}$	6.249	$[M-H]^-$	500.1316	499.1243	499.1243	-0.52
Apigenin 7-methyl ether 4'-glucoside	$C_{22}H_{22}O_{10}$	6.323	$[M-H]^-$	446.1215	445.1142	445.1142	0.47
Apigenin 7-rhamnosyl-(1->2)-galacturonide	$C_{27}H_{28}O_{15}$	5.657	$[M-H]^-$	592.1431	591.1356	591.1356	0.39
Epicatechin 7-O-glucuronide	$C_{21}H_{22}O_{12}$	5.026	$[M-H]^-$	466.1111	465.1038	465.1038	-0.13
Epigallocatechin 3-O-p-coumarate	$C_{24}H_{20}O_9$	6.280	$[M-H]^-$	452.1109	451.1036	451.1036	0.46
Isorientin 4'-O-glucoside 2"-O-p-hydroxybenzoagte	$C_{34}H_{34}O_{18}$	5.216	$[M-H]^-$	730.1722	729.1643	729.1643	-3.21
Kaempferol 3-(3"-acetyl-alpha-L-arabinopyranosyl)-(1->6)-glucoside	$C_{28}H_{30}O_{16}$	5.749	$[M-H]^-$	622.1548	621.1436	621.1436	2.27
Kaempferol 3-apiosyl-(1->2)-galactoside	$C_{26}H_{28}O_{15}$	5.322	$[M-H]^-$	580.1412	579.1330	579.1330	-2.74
Kaempferol 3-O-β-D-glucosyl-(1->2)-β-D-glucoside	$C_{27}H_{30}O_{16}$	5.582	$[M-H]^-$	610.1534	609.1460	609.1460	-0.01
Kaempferol 7-O-glucoside	$C_{21}H_{20}O_{11}$	5.027	$[M-H]^-$	448.1006	447.0932	447.0932	0.06
Myricetin 3-(3'''-6'''-diacetylglucosyl)	$C_{35}H_{38}O_{21}$	1.817	$[M-H]^-$	794.1919	793.1839	793.1839	1.64
Myricitrin	$C_{21}H_{20}O_{12}$	5.741	$[M-H]^-$	464.0957	463.0884	463.0884	0.50
Naringenin 4'-O-glucoside	$C_{21}H_{22}O_{10}$	5.704	$[M-H]^-$	434.1217	433.1144	433.1144	0.91
Quercetin	$C_{15}H_{10}O_7$	5.752	$[M-H]^-$	302.0426	301.0354	301.0354	-0.02
Quercetin 3-(2'''-feruloylsophoroside)	$C_{37}H_{38}O_{20}$	1.837	$[M-H]^-$	802.1960	801.1886	801.1886	0.39
Quercetin 3'-xyloside	$C_{20}H_{18}O_{11}$	5.862	$[M-H]^-$	434.0844	433.0773	433.0773	-1.19
Quercetin 7-(6"-acetylglucoside)	$C_{23}H_{22}O_{13}$	5.826	$[M-H]^-$	506.1060	505.0985	505.0985	-0.12

xylosylrutinoside), 6-Hydroxydelphinidin 3-glucoside, and 4'-O-Methyldelphinidin 3-O-beta-D-glucoside. Similar qualitative assay were carried out using LC-MS technique to determine phenolic compounds profile in blueberry, herbs, leaves extracts of *Alstonia angustiloba,* and cherries leaves and fruits biomolecules (Suleria *et al.* 2020; Ali *et al.* 2021; Ngcobo *et al.* 2024;). These studies have also observed presence of diverse variety of phenolic compounds. Notably, higher numbers of flavonoids were observed than the phenolic acids which is in agreement with the study conducted by Suleria et al. (2020) and Ali et al. (2021). However, the phenolic compound profile may vary significantly depending on the plant species, seasonal changes, climate conditions, and location of cultivation (Stein-Chisholm *et al.* 2017). Identification of anthocyanin particularly by mass spectra remains challenging due to complex structure and sugar moiety, further analysis coupled with quantitative technique can provide complete identification and characterization of anthocyanins.

Effect of karonda juice addition on lassi

The effects of different levels of KJ addition on the sensory attributes, pH and instrumental color parameters of lassi are presented in Table 3. The incorporation of KJ upto 5% level did not affect the pH of lassi. However, at 6% juice the pH values of lassi were significantly different. The addition of fruit juices beyond certain threshold level results in pH reduction likely due to organic acids causing undesirable flavor changes and could also affect the gel structure of yoghurt (Dimitrellou *et al.* 2020). Consistent with these findings, in our study sensory scores of lassi with 6% KJ was declined as sensory panelists perceived a higher acidity which affected the taste and aroma of the lassi. Accordingly, in contrast with control addition of KJ at 3 to 5% did not significantly change the flavor, body and texture, color and appearance and overall acceptability. Based on hedonic scale the aroma, color and acceptability characteristics the lassi containing 65 % KJ scores were significantly different as compared to control due to acidic taste. Therefore, lassi with 5% KJ was selected for further analysis. The perception of acidic taste in foods often connected with the pH and organic acids in it. Previous studies (Atasoy, 2009; Cakmakci *et al.* 2014) have also reported that the overall acceptability of yoghurt was highest when carob fruit and carrot juices were incorporated at moderate levels between 5% and 7% without causing excessive acidity or flavor changes. Ning et al. (2021) reported in their study that addition of passion fruit juice at 5% to yoghurt was adjudged with higher sensory scores and was able to retain the gel structure of yoghurt.

Color is one of the first noticeable attribute of food product that influence the sensory acceptance there by making it critical characteristics for marketability of food and beverages. The addition of juice to lassi resulted a decrease in the lightness with L* value from 77.00 to 63.52 and yellowness with b* value from 4.63 to -2.95 while increased the redness with a* values from -2.10 to 6.97 (Table 3). The change in color values were significant which can be attributed to the natural pigments present in the juice and color difference noticeable to human eye. Similar results have been reported in previous studies where addition of aronia, blueberry and grape juices increased the redness of yoghurt (Dimitrellou *et al.* 2020). In this study, specifically due to addition of KJ made the lassi appear darker, redder and less yellow compared to the control sample.

Changes during storage of experimental lassi

Whey separation or syneresis visible at the surface of yoghurt is often regarded as defect by the consumers due to change in appearance, body and texture. During the 21 days storage period whey separation was observed in all the samples (Table 4). The addition of KJ on the other hand, resulted in decrease in syneresis in lassi indicated better water holding capacity. According to Guénard-Lampron et al. (2020), the whey separation in stirred yoghurt is affected by various factors such as pH, stabilizer, storage time, total solids and hydrocolloids. The lesser whey separation in Karonda added lassi may be due to higher water holding capacity contributed by juice constituents including fibers. Such behavior has been reported in yoghurts with different

Table 3: Effect of different concentrations of Karonda juice on sensory properties of lassi

Parameter		Level of Karonda juice				
		Control	3%	4%	5%	6%
Sensory Attributes	Flavor	7.96±0.43^{a}	8.00±0.01^{ac}	7.90±0.32^{a}	7.92±0.33^{a}	7.49±0.45^{b}
	Body and Texture	7.93±0.53^{a}	8.00±0.01^{a}	7.95±0.16^{a}	7.97±0.29^{a}	7.80±0.50^{a}
	Colour and appearance	7.83±0.51^{a}	7.80±0.26^{a}	7.80±0.26^{a}	7.92±0.33^{a}	7.95±0.44^{a}
	Overall acceptability	7.91±0.51^{a}	7.80±0.26^{a}	7.75±0.35^{a}	7.91±0.33^{a}	7.48±0.43^{b}
pH		4.65±0.05^{a}	4.57±0.08^{ab}	4.44±0.08^{b}	4.35±0.07^{bc}	4.25±0.02^{c}
Color	*L*	77.00±0.01^{a}	69.06±0.27^{b}	66.62±0.26^{c}	65.78±0.08^{d}	63.52±0.18^{e}
	a	-2.10±0.02^{e}	2.44±0.23^{d}	4.22±0.10^{c}	4.92±0.03^{b}	6.97±0.15^{a}
	b	4.63±0.02^{a}	-0.85±0.14^{b}	-1.98±0.09^{c}	-2.41±0.03^{d}	-2.95±0.13^{e}

fruit and vegetable juices (Cakmakci *et al.* 2014; Dimitrellou *et al.* 2020; Kumar *et al.* 2023; Ning *et al.* 2021).

During the storage of lassi samples, the pH values decreased significantly. The initial pH 4.47 in control sample decreased to 3.98 as compared to juice added lassi reached from pH 4.34 on 0 day to 3.87 at the end of storage period ($p<0.05$). The decrease in pH observed in this study is similar with the findings reported by Atasoy (2009). A slightly lesser pH value 4.34 observed in lassi with juice on 0 day could be due to acidic environment as a result of added fruit juice. A similar decrease in pH values of yoghurt due to fruit juice addition was earlier reported by (Cakmakci *et al.* 2014) in carrot juice added yoghurt during 21 days storage.

Fermented milk consumption is beneficial due to the lactic acid bacteria present in it and also inhibit growth spoilage and harmful microorganisms. Table 4 shows the changes in the total lactic acid bacteria (LAB), yeast and mold & coliform count in lassi samples during storage at 5±2 ºC. The initial LAB counts in lassi with juice (8.98±0.07 log cfu/g) were comparable to the control sample (8.92±0.07 log cfu/g). A gradual decrease in counts was observed in both the samples over time and the reduction was significant in first and third week of storage. A similar trend of reduction of numbers of lactic acid bacteria in lassi and yoghurt has been reported previously by Ning et al. (2021). Particularly when fruit juices are added the lactic acid bacteria face acidic stress due to low pH result in low viable cell counts. In the present study, addition of KJ after fermentation process, the numbers in both lassi sample after 21 days storages was more than Log 7 CFU/g cells. In a previous study (Dimitrellou *et al.* 2020) addition of grape and berries juices into yoghurt was able to maintain starter culture cell counts upto more than 7 log CFU/g at the end of 28 days. Yeast and molds count on the other hand can grow in acidic products such as lassi and are indicators of process hygiene. In both the control and KJ added lassi samples, yeast and mold counts began to increase from 7 days and reached 1.86 and 1.72 log CFU/g, respectively at the end of 21 days storage. However, the yeast and mold counts in lassi with juice was less than 1 log CFU/g and increased at low rate probably due to presence of antimicrobial compounds in KJ. As the storage period increased,

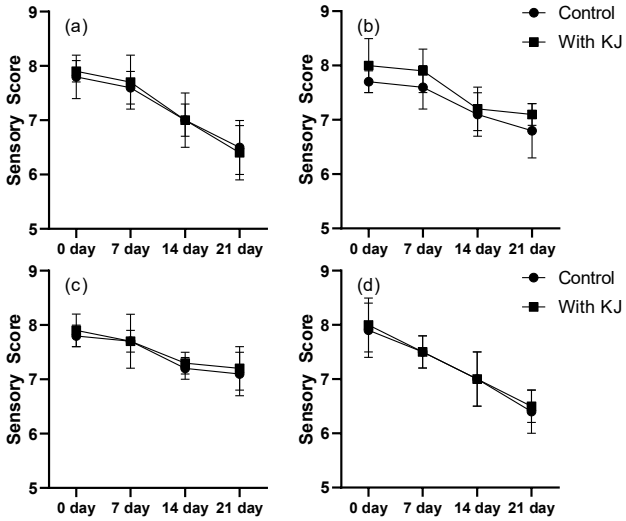

Fig. 1 Changes in sensory scores of control lassi ( ) and lassi with KJ ( ) during storage at 5±2 ºC (a) Flavor (b) Color and appearance (c) body and texture (d) Overall acceptability.

the depletion in the nutrients, built up of lactic acid that create acidic environment favor less to growth of lactic acid bacteria and more to growth of undesirable spoilage microorganism (Ning *et al.* 2021).

Sensory analysis was carried out for both control and 5% KJ added lassi samples during storage at 5±2 ºC. No significant differences were observed for taste, aroma, color and overall acceptability for control and KJ added lassi (Figure 1). In general the sensory scores on 0 day remained similar for both samples and were significantly decreased as the storage days were increased. Decrease in sensory scores during storage at low temperatures has been reported by several researchers (Guénard-Lampron *et al.* 2020; Kumar *et al.* 2023). In this study, by the end of storage period the overall acceptability score of control sample

Table 4: Changes in pH, whey separation and microbiological characteristics of lassi during storage at 5±2 ºC

Storage period (Days)	Lassi	0 day	7 day	14 day	21 day
pH	Control	4.47±0.04^{a}	4.23±0.05^{b}	4.06±0.13^{c}	3.98±0.08^{c}
	With juice	4.34±0.03^{a}	4.15±0.12^{b}	3.96±0.05^{c}	3.87±0.09^{c}
Whey separation	Control	0.0±0.0^{c}	0.0±0.0^{c}	1.20±0.27^{b}	3.14±0.22^{a}
(ml/100ml)	With juice	0.0±0.0^{c}	0.0±0.0^{c}	1.10±0.42^{b}	2.96±0.15^{a}
LAB counts	Control	8.98±0.07^{a}	8.59±0.35^{a}	8.12±0.02^{b}	7.69±0.27^{c}
(Log CFU/g)	With juice	8.82±0.07^{a}	8.42±0.25^{a}	8.23±0.35^{b}	7.51±0.25^{c}
Yeast and Mold	Control	1.16±0.64^{a}	1.10±0.15^{a}	1.45±0.14^{ab}	1.86±0.08^{b}
(Log CFU/g)	With juice	0.93±0.06^{a}	1.12±0.01^{a}	1.26±0.39^{b}	1.72±0.24^{c}
Coliforms	Control	ND	ND	ND	ND
(Log CFU/g)	With juice	ND	ND	ND	ND

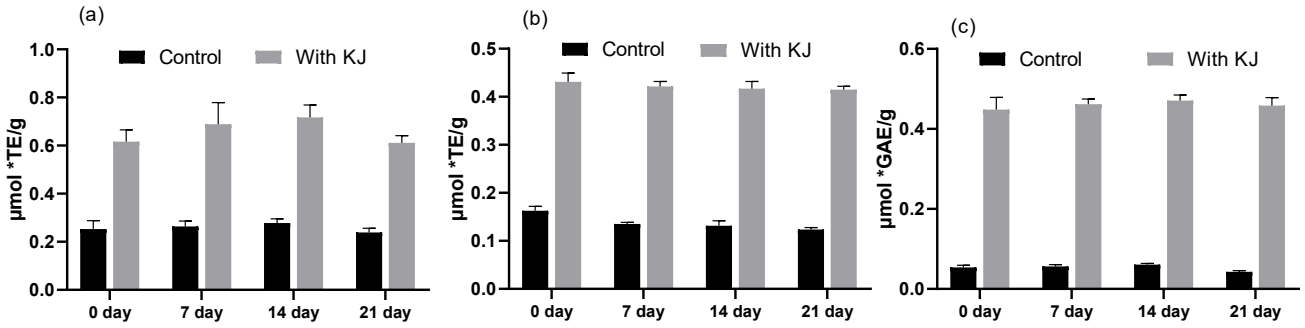

Fig. 2 Antioxidant activity by ABTS assay (a), DPPH assay (b) and total phenolic content (c) of lassi during storage at 5±2 ºC

declined from initial 7.9 to 6.8, while in juice added lassi reduction was from 8.0 to 6.5. A significant decrease in scores was observed in both control and juice added lassi samples from 14 days storage. However, difference between the control and juice added lassi samples were not significant during 21 days storage. These observations are in agreement with the findings of Sharma et al. (2015) on honey-added symbiotic lassi and Patel et al. (2020) on 1% Amaranthus-fortified lassi, where the overall acceptability scores ranged from 6.2 to 6.6 after 21 days of storage at 5±2 °C.The sensory scores chemical and microbiological analysis indicated a shelf life of 21 days.

The antioxidant activity during storage presented in Figure 2 indicate that incorporation of KJ into lassi resulted in significantly higher antioxidant activity by ABTS assay (0.61±0.05µmol TE/g) and DPPH assay (0.43±0.02 µmol TE/g). The values in lassi with KJ were more than two times higher than control samples. Lassi with added KJ showed a higher TPC (0.45±0.01µmolGAE/g) than control lassi as berries or juices are known as good sources of phenolic compounds (Stein-Chisholm *et al.* 2017). Regarding storage time, it was observed that values of antioxidant activity were increased upto 14 days, while it decreased on 21 day of storage. This same behavior was reported by (Erkaya-Kotan, 2020) that during cold storage of yogurts with orange fibre probably due to change in pH, microbial activity and degradation of antioxidant compounds.

Conclusions

In this study we analyzed the phenolic compounds in the KJ. The diverse array of bioactive phenolic compounds in KJ which could be further explored as functional ingredients for development of various functional foods. In this study lassi incorporated with 5% KJ showed sensory acceptability for 21 days during storage at 5±2°C. Furthermore, juice incorporation also significantly enhanced antioxidant activity of lassi. Karonda has limited utilization due to its acidic and astringency. The incorporation of ripened KJ into sweetened lassi showed as a viable approach to promote use of this underutilized fruit. Thus, the karonda has potential for application as functional ingredient in the development of functional fermented products with enhanced antioxidant activity for the advantage of consumer health.

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