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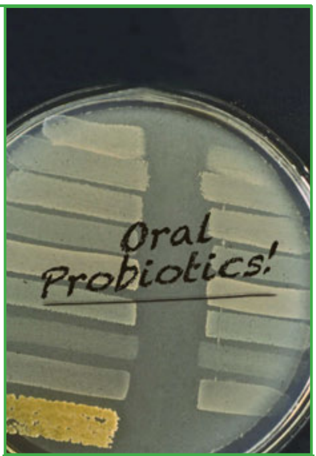

Studies on the Physicochemical and Nutritional Characteristics of Ash Gourd-Carrot Juice

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Abstract

The formulation and storage stability of ash gourd-carrot juice was investigated. Cloud stability is an important criterion for ash gourd-carrot juice and was achieved with the addition of 0.35% pectin. The effects of heat treatment, physicochemical properties, microbiological count and nutrient loss of β-carotene and total polyphenols were determined. The processing temperature was optimised at 95°C for 10 min before the juice was bottled. Nutrient content and physicochemical, microbiological and organoleptic characteristics were evaluated before and after 8 weeks of storage at 4°C and 28°C, respectively. The pH, total soluble solids (TSS), titratable acidity, reducing sugar content and sensory score did not alter significantly during storage at either temperature. However, cloud stability decreased by 20% and 32%, while juice lightness increased by 23% and 31% at 4°C and 28°C, respectively. Nutrient content decreased significantly during storage. The results indicated that the quality of juice stored for up to 8 weeks at both 4°C and 28°C was acceptable. Therefore, ready-to-drink ash gourd-carrot blended juice is nutritious, healthful and can be considered for commercialization.

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Introduction

Ash gourd (*Benincasa hispida*) belongs to the Cucurbitaceae family and is a familiar crop cultivated and valued for its nutritional and medicinal properties, especially in Asian countries [1]. The fruit of the ash gourd contains triterpenoids, flavonoids, glycosides, saccharides, proteins, carotene, vitamins, minerals, β-sitosterin, uronic acids, lupeol and sterols [2] and hence has been widely used for therapeutic treatments. It has several functional properties such as anti-ulcer [3], anti-angiogenic [4], antihistaminic [5], anti-inflammatory [6], antioxidant [7], anorectic [8] and hypoglycaemic [9] activity.

Ash gourd juice has little taste, colour or flavour, so various attempts have been made to produce processed juice blended with carrot (*Daucus carota*).

Carrot is acceptable worldwide because of its high nutritional value. It is an important dietary source of carotenoids such as α and β-carotene, zeacarotene, lutein and lycopene [10] and has perceived health benefits in human disorders associated with vitamin and dietary fibre deficiency [11]. It improves immunity, helps to heal injuries, reduces the risk of heart disease, lowers blood pressure, benefits the liver by improving fat and bile excretion and combats anaemia [10]. It is also a healthy low-calorie and nutritious juice as demanded by consumers.

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The consumer expects homogenous distribution of cloud particles in juice with no sedimentation during storage and rejects products with a clear phase above the sediment. The addition of hydrocolloids, in addition to homogenization, has been suggested for cloudy juice products [12-14]. Hydrocolloids such as pectin are mostly used as gelling and thickening agents and maintain the texture of many foods such as dairy products, beverages, jams, jellies, bakery products and confectionery. Hydrocolloids change the structure and texture of products, and can modify the flavour profile and/or perception [12]. Pectins are the most complex class of plant cell wall polysaccharides and comprise two families of covalently linked polymers: galacturonans and rhamnogalacturonans. Based on the degree of esterification, pectins are divided into two categories: low methyl (LM) pectin that contains less than 50% methyl esters, and high methyl (HM) pectin which consists of more than 50% methyl esters. In this study pectin was used for stabilizing the clouding particles in juice. Pectin is used as stabilizer in the food industry due to its deformation behaviour and polarity properties. The clouding of fruit juice is stabilized when pectin is added because the viscosity of the juice increases, which in turn retards the movement of clouding particles, with subsequent sedimentation avoidance [15].
Consumers purchase health drinks which provide instant energy as well as boosting the immune system, restoring vitality and improving time to recovery from illness [10,11]. Fruit and vegetable juices provide the body with the vitamins and minerals required for a healthy immune system. In light of the therapeutic importance of the ash gourd fruit, the food industry could focus on ash gourd-based juice and make it commercially available. The aim of this study was to develop an ash gourd-carrot blended juice and evaluate storage stability in order to provide maximum storage and nutritional benefits to consumers.

Materials and Methods

Raw material
Ash gourd (*Benincasa hispida*) and carrot (*Daucus carota*) were procured from a local market in Matunga, Mumbai, India.

Processing of ash gourd-carrot juice
The ash gourd was cleaned with water, peeled, cut into pieces and blanched in boiling water for 2 min, and the juice extracted using a food processer. The carrots were washed with tap water, peeled using sodium hydroxide (40 g/l) at 95ºC for 1 min and then washed again in tap water [11]. This was followed by blanching in citric acid solution (60 g/l) at 95ºC for 5 min and cooling in iced water to inactivate endogenous enzymes and soften the carrots. Finally, the carrots were sliced and grounded with addition of distilled water 1:1 (v/w) and filtered using cheese cloth under vacuum to extract fresh juice. The ash gourd:carrot ratio in filtrate was optimised at 80:20 and the filtrate adjusted to have total soluble solids of 10.2 ºBrix with sucrose for standardisation. The ash gourd-carrot juice was subsequently mixed with 0.25%, 0.3%, 0.35% or 0.4% (w/v) pectin. The juice was vigorously homogenized at room temperature. Then 200 ml of juice was poured into glass bottles and pasteurized in hot water at 75°C, 85°C and 95°C for 5, 10 and 15 min, and microbial counts and changes in the physicochemical characteristics of the juice determined.

Microbiological Analysis
Microbiological analysis of ash gourd-carrot fruit juice was performed using the serial dilution agar plate method. Microbiological analyses of coliforms, yeast, moulds and spores were carried out using standard procedures [16].

Analytical evaluation
Total acidity was determined by titration with sodium hydroxide standard solution and expressed as citric acid [17]. Total soluble solids were determined with an ATAGO refractometer (0–32 ºBrix). pH values were measured with a pH meter [18]. Total, non-reducing and reducing sugars were determined according to the method of Lane and Eynon [17]. β-Carotene content was determined by a column chromatography method [17]. All measurements were carried out in triplicate.

Measurement of juice stability
During storage, aliquots (10 ml) of juice were drawn from the upper portions of the bottles. The cloud stability of the juice was determined before

and after centrifugation at 4200 × g for 15 min, in a 1 cm path cuvette cell at 660 nm using a UV spectrophotometer [12].
The resistance to clarification (cloud stability) was calculated from the relative turbidity:

$$(T\ \%): T\ \% = (T_c/T_o) \times 100$$

where T_o and T_c are juice turbidity before and after centrifugation, respectively.

Viscosity measurement
The viscosity of ash gourd-carrot juice was measured using a Brookfield Viscometer (Model LVDV-II; Brookfield Engineering Laboratories). A 200 ml sample was used in a 250 ml glass beaker for all experiments. The measurement range of an LVDV-II Brookfield Viscometer between 10 % and 100 % full scale torque was adjusted by the selection of a specific spindle (S-61) and its rotational speed (100 rpm).

Colour analysis
The HunterLab parameters *L** (whiteness or brightness), *a** (redness/greenness) and *b** (yellowness/blueness) of ash gourd-carrot juice were determined using a Lab Scan XE (LX17375) spectrocolorimeter (HunterLab, USA).

Determination of total phenolic contents
Total phenolic contents were determined using the Folin–Ciocalteu reagent [19]. A 2 ml sample of ash gourd-carrot juice was stirred with 8 ml of 100 % ethanol for 15 min, and centrifuged at 2000 × g for 15 min. Then, 0.5 ml of supernatant was added to 2.5 ml of 10 % Folin–Ciocalteu reagent and allowed to react for 5 min. Subsequently, 2 ml of saturated sodium carbonate solution was added to the mixture and held for 2 h at room temperature. The apparent blue colour complex was determined at 765 nm. Total phenolic contents were expressed as mg gallic acid equivalent per 100 ml sample (mg GAE/100 ml).

Determination of antioxidant capacity
2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was determined as described by Chaikham et al [19]. A 2 ml sample of ash gourd-carrot juice was mixed with 100 % methanol for 15 min and centrifuged at 2000 × g for 15 min. Subsequently, 1.6 ml of supernatant was mixed with 0.4 ml of 1.5 µM DPPH methanol solution and allowed to stand for 30 min at room temperature. Absorbance of the solution was measured at 517 nm. A control was prepared using 1.6 ml methanol.
Percentage inhibition of DPPH radicals was calculated as follows:

$$\%\ \text{DPPH radical scavenging activity} = (1 - (Abs_{sample}/Abs_{control})) \times 100.$$

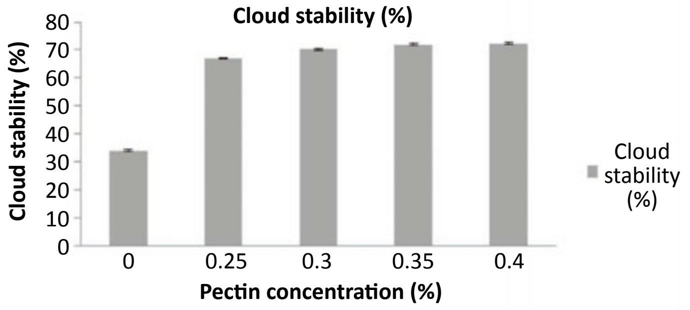
Juice shelf life study
For shelf life studies, sterilized glass bottles (200 ml capacity) were filled with hot ash gourd-carrot blended juice and sealed by corking. The bottles were stored at ambient temperature (28 ± 2°C) or refrigerated (4 ± 2°C) for 2 months and the juice examined for sensory and nutritional changes at regular intervals for shelf life analysis.

Sensory analysis
At baseline and periodically thereafter, samples were evaluated by a panel of 15 testers for colour, flavour, taste, mouth feel and overall acceptability of the juice. The tests were performed using a nine-point hedonic scale, where 9 was 'like extremely' and 1 was 'dislike extremely'.

Statistical analysis
Analysis of variance was used to examined data at the 95 % confidence level. IBM SPSS version 19 software was used. Results are presented as means and standard deviation (SD)

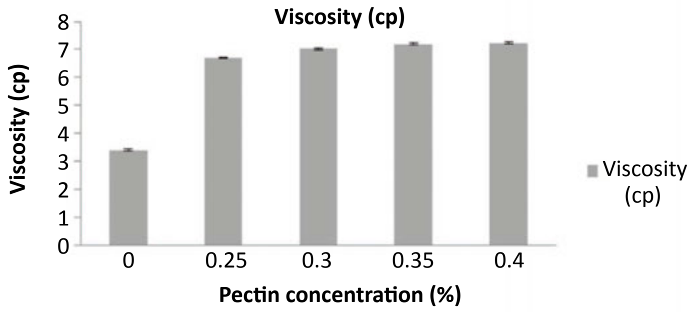
Result and Discussion

Cloud stability and viscosity
The effect of pectin addition on cloud stability, viscosity and overall acceptability of ash gourd-carrot juice was examined; the results are presented in Figs. 1, 2 and 3. The addition of pectin considerably increased the cloud stability of ash gourd-carrot juice as compared to the control sample. The cloud stability of ash gourd-carrot juice increased with the addition of hydrocolloids, reaching stability with the addition of 0.35% pectin. Ibrahim et al [12] reported the positive effect of pectin on the cloudiness of apple juice. They added 0.1–0.5



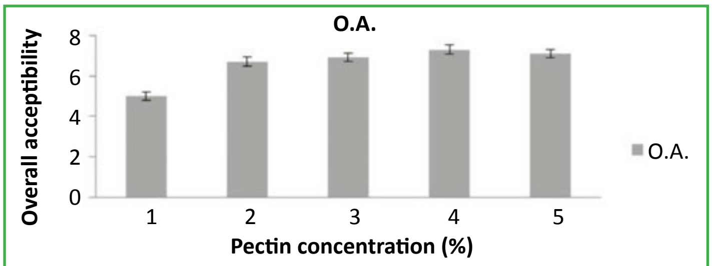
All values are mean ± SD (n=3)

Figure 1 - Effect of pectin content on cloud stability



All values are mean ± SD (n=3)

Figure 2 - Effect of pectin content on viscosity



All values are mean ± SD (n=3)

Figure 3 - Effect of pectin content on overall acceptibility

% pectin to apple juice and concluded that juice cloud stability increased as the pectin concentration increased [20]. Pectin also increases cloud stability by forming a protective layer around the pulp containing fruit juice particles and maintaining the colloidal particle in suspension [21].

Hot/cold break processes have also been used for controlling the enzymes and cloud stability of juices. Srinivasan et al [22] described the process for preparing tomatoes. The hot process comprises a rapid heating of tomatoes to >85–90ºC to inactivate enzymes activity. This preserves the pectin level which promotes viscosity and consistency and stabilizes the cloudiness of the juice. Hydrocolloid addition has been recommended to maintain the cloud stability of juice products.

The addition of up to 3 g/l of pectin is been permitted under German law for juices and nectars made from pineapple and passion fruit as reported by Neidhart et al [23] at an international symposium.

Unlike most polysaccharides, pectin in solution exhibits non-Newtonian pseudoplastic behaviour. Mirhosseini et al [24] studied the emulsion stabilizing influence of pectin and its effect on viscosity, pseudoplastic behaviour and/or negatively charged ζ-potential. Apparent viscosity (η) is a rheological property which is calculated as $\eta a= {}^{\tau}/_{\gamma}$ (τ: shear stress, γ: shear rate). In the present study, the optimized pectin concentration also demonstrated non-Newtonian fluid with shear thinning behaviour without substantially affecting its other properties.

Effect of processing on the physicochemical property of the juice

Processing time and temperature are the most important parameters affecting shelf life and nutrient content. The intensity of heat treatment has a direct effect on the nutritional and organoleptic quality of food [25]. The effects of processing temperature (75°C, 85°C and 95°C) and time (5, 10 and 15 min) on microbial count were investigated. The optimum parameters were 95°C at 10 min (Table 1), which resulted in no microbial load and may increase the storage stability of the juice. The effects of time and temperature on total soluble solids (TSS), acidity, reducing sugar content and total sugar content were also examined (Table 1).

The TSS of the juice increased from 10.2 % to 11.6 % with increased temperature and time, possibly due to the evaporation of water by heat processing resulting in a reduction in the volume of juice. Similar findings were observed by Dar et al [26] in apple juice where an increase in TSS during storage was due to the hydrolysis of polysaccharides (starch) into monosaccharides (sugars), dehydration and the degradation of pectic substances in soluble solids. A similar pattern of increase in TSS

Temperature (°C)	Time (min)	TSS (%)	Acidity (%)	Reducing sugar (%)	Total sugar (%)	β-Carotene (µg/100 ml)	TPP (µg GAL/ml)	DPPH (% inhibition)	Standard plate count (log CFU/ml)	Yeast and mould (CFU/ml)
Control	0	10.2±0.01	0.25±0.01	2.19±0.01	8.5±0.01	3421±76	183±1.1	14.43±0.54	5.88±0.04	3.1±0.08
75	5	10.8±0.02*	0.25±0.01	2.21±0.01*	8.6±0.03*	3401±45*	176±1.2	12.26±0.75*	4.24±0.02*	2.53±0.06*
	10	10.9±0.01*	0.24±0.01	2.24±0.01*	8.7±0.02*	3346±50*	173±1.1	10.87±0.86*	3.15±0.02*	1.86±0.4*
	15	10±0.02	0.23±0.01	2.25±0.02*	8.9±0.04*	3310±62*	167±1.3*	9.21±0.94*	2.90±0.03*	1.46±0.03*
85	5	10±0.01	0.23±0.02	2.27±0.01*	9.0±0.03*	3266±86*	169±0.9	9.34±0.87*	2.95±0.02*	2.1±0.02*
	10	11.2±0.02*	0.22±0.01	2.29±0.01*	9.11±0.01*	3228±73*	167±0.7*	8.92±1.1*	2.44±0.01*	1.2±0.01*
	15	11.5±0.01*	0.21±0.01*	2.31±0.02*	9.19±0.02*	3174±39*	163±0.8*	8.62±1.23*	2±0.01*	ND
95	5	11.4±0.01*	0.20±0.01*	2.30±0.01*	9.18±0.01*	3096±85*	161±1.2*	8.59±0.89*	1.31±0.02*	ND
	10	**11.6±0.02***	**0.19±0.01***	**2.32±0.02***	**9.26±0.01***	**3086±57***	**152±1.3***	**8.12±0.97***	**ND**	**ND**
	15	11.9±0.01*	0.17±0.01*	2.35±0.01*	9.3±0.02*	2923±67*	147±1.1*	6.42±1.25*	ND	ND

All values are mean ± SD (n =3)
*Value significantly different from initial value at the 95% confidence level
DPPH 2,2-diphenyl-1-picrylhydrazyl, *TPP* thiamine pyrophosphate, *TTS* total soluble solids

Table 1 - Effect of temperature on Ash gourd- carrot juice

content with an increase in storage time was observed by Mehta and Bajaj in mandarin, sweet orange and lemon juice [27]. Organic acids appear in foods as a result of biochemical processes, utilization of nutrients (usually sugars), or contamination by microorganisms. The total acidity of the ash gourd-carrot juice decreased with increased temperature and time from 0.25% to 0.17%. Total sugar increased as the temperature increased over time from 8.5% to 9.3%. The increase in total sugars might be due to the hydrolysis of polysaccharides such as pectin, cellulose and starch and their conversion into simple sugars [28].

Effect of processing on microbial count and nutrition loss

Bioactive components such as total polyphenols and β-carotene were significantly reduced by pasteurisation (Table 1). β-Carotene content in ash gourd-carrot blended juice reduced from 3,421 µg/100 ml to 3,086 µg/100 ml, while total polyphenol content decreased from 183 µg/ml to 152 µg/ ml (as compared to gallic acid as reference standard). Pasteurization studies to check microbial content revealed no growth of yeast, bacteria or moulds at 95°C and 10 min, respectively. Nutrients were reduced as expected, but further investigations are required to elucidate possible causes so that such losses can be minimized.

Effect of processing on antioxidant activity

The antioxidant activity of ash gourd-carrot blended juice was evaluated using the DPPH free radical scavenging method and showed a significant decrease (Table 1): initial inhibitory activity decreased from 14.43% to 6.43% after pasteurisation.

Effect of storage temperature on physicochemical changes in juice

pH

pH greatly influences storage stability and impacts on flavour and other juice characteristics. pH decreased with length of storage, but not significantly at storage temperatures of 4°C and 28°C (Table 2). This decrease in pH was attributed to the formation of acidic compounds by degradation of reducing sugars, as discussed by Mubeen et al [29].

Titratable acidity

Acidity contributes tartness and is a major factor in the acceptability of juice. Citric acid also gives the characteristic sourness to the product and enhances the flavour of ash gourd-carrot juice. No significant change in acidity was observed during storage at 4°C and 28°C (Table 2). Any increased acidity was due to the degradation of sugars into carboxyl acids. A similar trend was observed by Mubeen et al [29] in fruit juices.

Temperature (°C)	Days	TSS	pH	Total acidity	Reducing sugar (%)	Non-reducing sugar (%)	β-Carotene (µg/100 ml)	DPPH (% of inhibition)	Cloud stability (%)	Lightness	Total plate count, yeast and mould
Control		11.6±0.01	4.3±0.01	0.19±0.01	2.32±0.02	6.94±0.01	3086±57	8.12±0.97	72±0.27	38.14±0.04	ND
4	15	11.92±0.03	4.3±0.01	0.2±0.01	2.37±0.01	6.87±0.01	2935±12*	7.87±0.7*	68±1.5	40.70±0.04*	ND
	30	12.21±0.02	4.29±0.01	0.21±0.01	2.41±0.04	6.8±0.03	2923±31*	7.31±0.4*	64±2.3*	43.14±0.05*	ND
	45	12.32±0.02	4.29±0.01	0.22±0.01	2.45±0.03	6.74±0.02	2783±17*	6.85±0.9*	61±1.1*	47.62±0.03*	ND
	60	12.36±0.02	4.28±0.01	0.23±0.01	2.48±0.02	6.69±0.02	2715±14*	6.61±0.7*	57±2.4*	49.13±0.03*	ND
28	15	11.75±0.02	4.29±0.01	0.19±0.01	2.42±0.01	6.82±0.01	2913±29*	7.61±0.7*	65±1.1*	41.56±0.03*	ND
	30	11.8±0.03	4.28±0.01	0.2±0.01	2.47±0.04	6.72±0.03	2892±16*	6.91±0.5*	59±1.3*	44.53±0.07*	ND
	45	11.92±0.03	4.27±0.01	0.21±0.01	2.51±0.02	6.66±0.02	2762±12*	5.94±0.3*	54±2.5*	50.32±0.05*	ND
	60	12.1±0.02	4.25±0.01	0.22±0.01	2.54±0.02	6.6±0.02	2689±43*	5.34±0.4*	49±1.8*	55.45±0.04*	ND

All values are mean ± SD (n =3)
*Values significantly different from initial value at 99% confidence level
DPPH 2,2-diphenyl-1-picrylhydrazyl, *TTS* total soluble solids

Table 2 - Shelf life studies of juice stored at 4°C and 28°C

TSS

An increase in TSS in juice compared to the initial content was observed during storage at 4°C and 28°C (Table 2). This increase might be due to the formation of pectic substances from protopectin and of monosaccharides from disaccharides [30]. A similar observation was reported by Kaunjoso and Luh [31] in their study of the canning and storage of oranges and peaches.

Reducing and non-reducing sugars

Reducing sugars increased with increased storage time at different temperature in ash gourd-carrot blended juice with significant variations at all storage intervals. These changes might have been due to inversion of sucrose to glucose and fructose catalysed by acid [32]. Similar results were reported by Babsky et al [33] and Pruthi et al [34] who found that non-reducing sugars were converted to reducing sugars during storage.

The percentages of non-reducing sugars at different temperatures in ash gourd-carrot blended juice are presented in Table 2. Non-reducing sugars decreased with increasing time. However, this decline in non-reducing sugars was slow during initial storage and was not significant. A similar observation was reported by Sandip et al [35] during the storage of passion fruit juice.

Cloud stability

During the 8-week storage period, juice was withdrawn from the upper portions of bottles and assayed before and after centrifugation at 4200 × g for 15 min; the results are given in Table 2. It was observed that the cloud stability of juice declined over the period of storage with less decrease during storage at 4°C compared to 28°C.

Electrostatic charge repulsion is the main mechanism maintaining cloud stability. Pectin carries a negative charge and may form a protective covering around cloud particles, resulting in an overall surface negative charge which causes cloud particles to repel each other. Pectin plays an important role in colloidal stabilization as the hydrated polymer forms a thick layer around the particles [14]. Leroux et al [36] also observed that the relationship between pectin concentration and emulsion stability was most probably due to the protein residues present within the pectin. Pectin molecular weight, degree of acetylation and degree of methylesterification are the main factors affecting the gelling properties and mechanism of action of pectin [37].

Colour and microbial growth

The effects of storage on the colour of ash gourd-carrot blended juice are presented in Table 2. The lightness of the juice gradually decreased over the 8 weeks of storage due to the presence of acid. Muhammad et al [38] also reported a similar loss in colour during the storage of orange squash samples. The ash gourd-carrot blended juice was evaluated for microbiological parameters. No growth of coliforms, spores, yeast or mould was observed over the 2-month storage period. Similar storage studies of sterilized ash gourd juice (with the addition of lemon juice, sugar and salt) over 8 months were carried out by Mujumdar et al [32].

Antioxidant activity

A DPPH assay showed that the scavenging activities of ash gourd-carrot blended juice decreased with prolonged storage time (Table 2). These results are in agreement with the findings of Ibrahim et al [12]. Polyphenols are the main compounds responsible for the total antioxidant capacity as reported by Lee et al [39].

Bioactive component

The β-carotene content of the juice decreased during storage, mainly because β-carotene is sensitive to heat. The initial β-carotene content in ash gourd-carrot juice decreased by up to 12% and 13% at 4°C and 28°C from initial values to the end of storage ($p>0.05$). Losses of β-carotene during the storage of tomato, amla, carrot, carrot-spinach, carrot-pineapple and carrot-beetroot juice have been reported [33].

Sensory analysis

The taste, colour, flavour and overall acceptability of ash gourd-carrot juice pasteurized at 95°C for 10 min were studied. Fig. 4 shows that the mean sensory score of the juice was the same following storage at 4°C and 28°C. However, a slight decline in taste and overall acceptability was observed as the storage period increased. Similar findings were reported by Jain and Khurdiya [40]. The colour changes during storage may be due to the decrease in cloud stability and a possible chemical reaction. The gradual loss in flavour over the storage period was due to changes in volatile compounds in the juice [41]. A gradual loss of flavour in drinks was also reported by Marcy et al [41] and Bezman et al [42].

Conclusion

A nutritious ash gourd-carrot juice blend with good sensory characteristics and a good shelf life was obtained. Juice stability was obtained using pectin. The juice was stable during 2 months of storage with good physicochemical, microbiological and nutritional parameters.

Supplementary data

Optimization of Ash gourd-carrot composition

Juice	Blending ratio	Overall* acceptability	Comment
Ash gourd-carrot	100:0	4.5±0.2	No test, dull colour
Ash gourd-carrot	90:10	5.34±0.18	OK test, light colour
Ash gourd-carrot	80:20	7.2±0.21	Good taste, colour looked good
Ash gourd-carrot	70:30	6.65±0.18	Good taste, lightness increases
Ash gourd-carrot	60:40	6.25±0.23	Masking of carrot
Ash gourd-carrot	50:50	5.62±0.17	Total masking of carrot, no ash gourd flavour
*Values are mean ± SD			

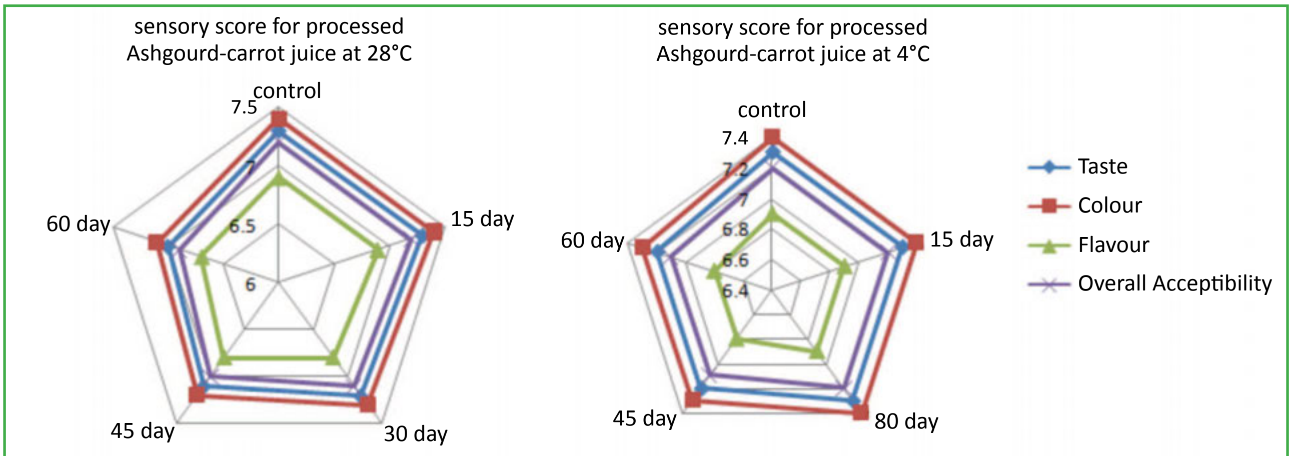


Figure 4 - Sensory analysis of pasteurized ash gourd-carrot juice stored at 4°C and 28°C

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