



Physico-chemical quality characteristics and shelf-life study of aloe gel and sea buckthorn berry beverage

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Abstract: For centuries the *aloe vera* plant has been known for its medicinal properties. It is proposed to be of benefit for the digestive and immune systems, as well as having healing effects on the skin. Chemical constituents of sea buckthorn berries are under research due to positive effect in inflammatory disorders, cancer mechanisms and on bone marrow after chemotherapy. The present study was undertaken to develop a ready to serve (RTS) beverage using *Aloe vera* gel and sea buckthorn berries. *Aloe vera* gel (25%), sea buckthorn berry juice (30%), sugar (25%), and citric acid (0.1%) were mixed to prepare the blend with TSS of 13°Brix. The product was bottled, pasteurized, and stored at room temperature. The quality characteristics and storage stability of the berry juice aloe gel beverage (BJAB) was compared with berry juice (BJ). Time-dependent analysis was carried out up to four months for various physicochemical parameters, antioxidant profile, microbial quality and sensory acceptability. The BJAB exhibited superior quality characteristics compared to BJ when compared as in fresh condition and in stored samples. The BJ was acceptable up to three months whereas BJAB for four months. The results indicate that nutraceutical rich aloe gel and berry juice can be utilized to develop nutritional and tasty functional fruit beverage with improved quality.

Key words : Sea buckthorn berry, Beverage, Nutraceutical, Aloe vera, Functional food

Introduction

Sea buckthorn is an herb. The leaves, flowers, and fruits are used to make medicine. *Hippophae*, the sea buckthorns, are deciduous shrubs in the family Elaeagnaceae. It is also referred to as sandthorn, sallowthorn or seaberry as given by Lu (1992). More than 90% or about 1,500,000 ha (5,800 sq mi) of the world's natural sea-buckthorn habitat is found in China, Russia, northern Europe and Canada where the plant is used for soil, water and wildlife conservation purposes and for consumer products as reported by Jeppsson (1999), Swenson *et al.* (2006). The female plants produce orange berry-like fruit 6–9 millimetres (0.24–0.35 in) in diameter, soft, juicy and rich in oils as found by Zeb (2004). Nutrient and phytochemical constituents of sea buckthorn berries are under basic research in inflammatory disorders, cancer mechanisms or positive effect on bone marrow after chemotherapy, or other diseases. The chemical and nutritional composition of sea buckthorn berries vary considerably between different subspecies, with the origin, climate, time of harvesting and method of processing Yang *et al.* (2001). George and Cenkowski (2005) specified that the physical characteristics of sea buckthorn berry were influenced by the harvest time whereas bioactive compounds did not vary significantly with respect to harvest time. Fruit of the plant has a high vitamin C content in a range of 114 to 1550 mg per 100 grams with an average content (695 mg per 100 grams) about 15 times greater than oranges (45 mg per 100 grams) placing sea-buckthorn fruit among the most enriched plant sources of vitamin C was studied by Lu (1993). Bernath and Foldesi (1992) reported that the vitamin E content in sea buckthorn is higher than that

found in wheat embryo, safflower, maize and soybean. They are also rich in several other vitamins, including B1, B2, K and bioflavonoids were studied by Bekker & Glushenkova (2001). Sea buckthorn fruits are rich in pigments and lipoproteins located in membranes and the fleshy mesocarp. Carotenolipoprotein complexes are located particularly in fruit membranes where polar lipids may function as bridge compounds between the polar (protein) and non-polar (carotenoids) moieties Pintea *et al.* (2001). The fruit also contains dense contents of carotenoids, amino acids, dietary minerals, β -sitosterol and polyphenols Kallio *et al.* (2002), Zaib (2004) and Zeb (2004).

Aloe vera (*Aloe Barbadosensis*) is the most well known of the several hundred species of aloe. Other members of the species are prized as ornamental herbs, but none have the reputation of *aloe vera* with regard to medical benefit. The succulent plant is native to Africa, but can be cultivated in warmer areas of Europe, America and Asia, where it grows as a rosette of thick, stiff, grey-green leaves at ground level (Boudreau and Beland, 2006). The plant produces a thicker, jelly like preparation, termed aloe gel. It is to this gel that the majority of health benefits of *aloe vera* are attributed. It is produced by parenchymal cells in the central portion of the leaf, and is a rich mixture of a variety of substances Rodriguez *et al.* (2010). The gel comprises over 70 constituents, and it is currently unclear to which of these the medicinal effect is attributed. Vitamins, amino acids, sugars, minerals and salicylic acids are all found in the gel, and may contribute to the possible therapeutic effects of the preparation (Eshun and He, 2004). Physical and biochemical properties of Aloe vera gel has been extensively investigated and well characterized by Ni *et*

al. (2004), Rodríguez-González *et al.* (2011), Sharrif and Sandeep (2011). New products combining the unpalatable Aloe gel juice with other health foods particularly antioxidant rich fruits such as peaches, pineapple, orange and their extracts has been produced but its blend with sea buckthorn berries however remains to be explored.

The objective of this study is to develop a beverage of exotic fruits such as sea buckthorn berries and Aloe vera that has high nutritional and medicinal properties for health conscious and nutritionally deficient society. In the present study, a sea buckthorn berry juice aloe vera beverage (BJAB) was developed and its physicochemical characteristics, antioxidant potential, microbial quality, and sensory acceptability were evaluated.

Materials and Methods

Uniformly ripened sea buckthorn berries of uniform size and shape and *aloe vera* leaves were procured from a local supplier. Other raw materials like food grade citric acid, sugar, and other materials were procured from Mereck pvt. Ltd.

Preparation of sea buckthorn berry juice : Berries (*Hippophae rhamnoides*) were washed with Millipore water and dried. The seeds were separated by pressing the berries resulting in juice with three different phases: an upper floating particulate phase, a center liquid portion, and sinking particulate sediment. Ultracentrifugation was carried out with molecular weight cutoff of 100,000. The oil was removed as an upper ring and the coarse sediment settled to the bottom of the bowl Beveridge *et al.* (1999). After removal of the upper oily layer and lower coarse bed the middle layer of clear juice was obtained. The juice was then prepared by taking undiluted berry juice 30% and sugar syrup of 25%. Citric acid was also added at 0.1% along with coloring and propylene glycol 0.05% each. The resultant juice was again blended. The berry juice was then hot filled into presterilized glass bottles, screw capped and pasteurized for about 15 minutes at 80-90°C followed by immediate cooling. This was termed as the control beverage.

Development of berry juice aloe gel beverage blend (BJAB): The fresh green *Aloe vera* leaf was taken for the extraction of *Aloe vera* gel. The Aloe gel was removed carefully by the hand filleting method from the leaf. The gel was washed 3-4 times in Millipore water, hand crushed, blended, and filtered through muslin cloth. Similar procedure was followed for the preparation BJAB as the control beverage, except that in BJAB, Aloe gel juice obtained was added at different levels of 15%, 25% and 35% to prepare different samples. This was followed by addition of other ingredients; hot filling, pasteurization, cooling, and storage. The developed beverages were initially evaluated for sensory acceptability in terms of color, taste, texture and overall acceptability. The Aloe gel enriched beverage found best in terms of the above mentioned parameters was selected for further storage analysis, along with the control beverage. The selected beverages were stored at room temperature (28°C) in dark upto four months. Analysis for various parameters was carried out on 0 days and after 30 days, 60 days, 90 days and 120 days of storage. The beverage was evaluated for physicochemical characteristics, antioxidant profile and microbial and sensory quality.

Analysis of physicochemical characteristics: The samples were analyzed for various physicochemical parameters. The pH of the samples was read with a calibrated pH meter (Yokogawa Analytical).

Table-1: Sensory Acceptability of Berry juice fortified aloe gel beverage (BJAB)

Sample	Color	Taste	Texture	Overall Acceptability
Berry juice	4.75±0.18	4.47±0.23	4.68±0.22	4.53±0.31
15% BJAB	4.86±0.21	4.77±0.20	4.76±0.31	4.82±0.27
25% BJAB	4.94±0.19	4.84±0.34	4.95±0.24	4.92±0.22
35% BJAB	4.90±0.36	4.01±0.22	4.56±0.31	4.60±0.34

*Results are expressed as Mean±SD for triplicates using ANOVA test.

Titrate acidity was measured using 0.1N NaOH and expressed as percentage citric acid as recommended by Anonymous 1a (2015); total soluble solids (in °Brix) determined using digital hand refractometer (Rudolph Research Analytical) and total sugars were determined by the using Fehling's reagent. All these properties were analyzed according to methods as described by Ranganna (1986), Nielsen (2010). Total polysaccharide content was calculated from the standard curve generated from D (+) glucose analysis as described by Vega-Gálvez *et al.* (2012). Polysaccharide analysis was performed in duplicate and measured in triplicate, and the respective mean values were reported.

Antioxidant activity : Twenty five µl of adequate dilution (in sodium phosphate buffer solution, PBS, 75 mM, pH 7.4) of berry juice (control and fortified samples) were placed in polystyrene black plates with 96 round bottom wells (Costar, USA). A microplate reader (Synergy HT; Bio-Tek, USA) was used to automatically dispense 150 µl of 1µM fluorescein. After 30 mins of incubation at 37 °C, 25 µl of 153 M 2, 22 -Azobis (2-methylpropionamide) dihydrochloride was dispensed. Fluorescence (485/20 nm excitation wave length and 528/20 nm emission wavelength) was measured during 1 h/37 °C by Tabart *et al.* (2009). ORAC values were expressed as µmol trolox equivalents/100 g of berry juice in wet basis (µmol TE/100 g).

Microbial analysis : Total bacterial count (TBC) and yeast and mold counts (YMC) of the samples were determined by standard plate count method and expressed as log CFU ml⁻¹. Analyses were carried out using standard method as recommended by Anonymous 1b (2015)

Sensory acceptability : The sensory acceptability of the samples was evaluated as method suggested by Ranganna (1986). A group of 21 untrained male and female panel members between 18 and 30 years of age, selected from the university. The samples were randomly coded and served at 20°C in 15ml aliquots to the panelists. They were asked to rate the samples for various sensory attributes, namely, color, taste, texture and overall acceptability, on a 5-point hedonic rating scale with 5 and 1 indicating the highest and lowest scores, respectively as suggested by Juyun (2011). The samples were evaluated at the end of each storage interval and the mean scores were reported.

Statistical analysis: Data obtained for the various parameters were expressed as mean values ± standard deviations of at least three replications. Results were statistically evaluated by ANOVA test.

Results and Discussion

Raw material analysis : Sea buckthorn berry and fresh Aloe gel juice used were first analyzed. Berry juice was found to have moisture content of 70% and TSS of 10.12°Brix. Aloe gel recorded a higher moisture content of 95% and low TSS of 0.10°Brix. Acidity of juice was also higher (0.98%) compared to Aloe gel (0.04%). The berries were found to contain a good amount of vitamin C (200

mg/100 gms). For Aloe gel, the vitamin C content was found to be 18mg/100gms. Berry juice recorded total sugars as 10% and the corresponding value for Aloe gel was 2%. The various blends of Aloe gel and berry juice formulated were initially evaluated for sensory acceptability (Table 1). The overall acceptability as well as color, taste and texture of different blends of BJAB had a higher score as compared to the control beverage (berry juice). Also within the aloe gel blends the highest score in all parameters was obtained for 25% blend (color: 4.94, taste: 4.84, texture: 4.95, overall acceptability: 4.92). As the percentage of gel was increased to 35%, the scores decreased especially for taste (color: 4.90, taste: 4.01, texture: 4.56, overall acceptability: 4.60). A major problem with commercial Aloe gel juice and beverages is that as the amount of gel increases the bitter taste surfaces out, which is exactly what had happened as the concentration of gel was increased from 25% to 35%. Till 25% blend the fruity and tangy flavor of berry juice was able to cover the bitterness of the gel but beyond that the fruity flavor did not work. Since the addition of aloe gel at 25% was most acceptable and also it is the value which falls in the range of RDA for aloe gel juice consumption (Williamson *et al.*, 2011) thus 25% BJAB was chosen for further time-dependent studies.

Shelf-life studies: The control beverage and 25% BJAB were periodically analyzed for various parameters on storage. The results are summarized in Table 2. The initial pH of the samples, both the control beverage and BJAB was found to be 3.76 and 4.63 respectively. With an increase in storage time, the acidic nature of the samples was found to increase this could be attributed to the decomposition of fermentable substrate, especially carbohydrates in the fruits and added sugar, thereby increasing the acidity as reported by Fasoyiro *et al.* (2005). Control beverage was found to exhibit a higher increase in acidity as compared to BJAB after 120 days of storage. In fact on 120 days of incubation the control beverage showed highly acidic pH making the sample unpalatable. Similarly TSS and total sugars showed an increase with progression of incubation time. The increase in both the parameters was more in the control beverage as compared to BJAB. The TSS and total sugars on initial day were 10.12 °Brix and 20.1% respectively for control beverage and for BJAB the values were 10.14 °Brix TSS and 19.7% for total sugars. But on 120 days these values increased markedly for control beverage (TSS: 15.03 Brix, Total sugars: 25.2%) and less intensely for BJAB (TSS: 13 °Brix, Total sugars: 22.2%). The increase in TSS observed on storage could be due to the conversion of polysaccharides to sugars. This could have also led to an increase in total

Table-2: Quality characteristics of the control beverage and BJAB

Sample	Storage period (Days)	pH	Total soluble solids (°Brix)	Total sugars (%)	Antioxidant Activity (µmol TE/100 gms)
Control beverage	0	3.76±0.12	10.12±0.6	20.1±0.6	390±32.3
	30	3.30±0.21	10.98±0.3	21.6±0.5	350±18.6
	60	2.62±0.25	12.65±0.2	22.4±0.2	256±22.4
	90	2.11±0.14	13.88±0.5	23.4±0.4	200±16.2
	120	1.77±0.23	15.03±0.3	25.2±0.3	156±28.4
BJAB (25%)	0	4.63±0.24	10.14±0.4	19.7±0.4	496±16.2
	30	4.32±0.17	10.56±0.3	20.3±0.1	473±21.4
	60	4.01±0.12	11.64±0.2	20.5±0.3	430±12.3
	90	4.00±0.16	12.44±0.1	21.3±0.5	410±18.4
	120	3.86±0.14	13.00±0.2	22.2±0.2	396±14.4

*Results are expressed as Mean±SD for triplicates using ANOVA test ($p < 0.05$)

Table-3: Sensory Acceptability of the control beverage and BJAB

Sample	Storage period (Days)	Color	Taste	Texture (%)	Overall Acceptability
Control beverage	0	4.75±0.18	4.47±0.23	4.68±0.22	4.53±0.31
	30	4.60±0.21	4.71±0.33	4.71±0.30	4.62±0.26
	60	4.41±0.22	4.52±0.22	4.60±0.22	4.44±0.33
	90	4.00±0.34	3.87±0.26	4.01±0.36	3.90±0.34
	120	-	-	-	-
BJAB	0	4.94±0.19	4.84±0.34	4.95±0.24	4.92±0.22
	30	4.80±0.21	4.75±0.28	4.76±0.27	4.76±0.23
	60	4.50±0.22	4.55±0.22	4.70±0.33	4.62±0.31
	90	4.48±0.16	4.41±0.16	4.54±0.36	4.50±0.28
	120	4.32±0.23	4.32±0.25	4.48±0.33	4.40±0.14

*Results are expressed as Mean±SD for triplicates using ANOVA test ($p < 0.05$)

sugars content on storage (Yadav *et al.* 2010). The antioxidant activity recorded in the samples on the initial day was 390µmol TE/100 gms for control beverage and 496µmol TE/100 gms for BJAB. A reduction in activity on storage was observed which was more in control beverage (156µmol TE/100 gms) on the 120 day as compared to BJAB (396µmol TE/100 gms). This reduction can be attributed to anaerobic degradation of vitamin C and flavanoids, especially in thermally preserved products (Klimczak *et al.*, 2007). However BJAB samples with increase in storage time showed lesser reduction whereas in control beverage there is exponential decrease in antioxidant activity. This indicates that addition of aloe gel provided stability to the samples in maintaining their antioxidant activity as reported by Hu *et al.* (2003).

The BJAB samples were found to be free from microbial proliferation till the end of storage period in terms of standard plate count and yeast and mold count, thereby indicating its fitness for consumption even after 120 days of storage. This could be attributed to the effective pasteurization treatment, addition of citric acid which acts as a preservative, and also the gel consistency of aloe gel juice used makes the growth of microbes difficult in the samples. Apart from that there are studies which show that aloe gel has good antimicrobial activity Nejat-zadeh-Barandozi (2013), Shelton (1991), Reynolds and Dweck, (1999). The combined beneficial effect of the above mentioned factors could have led to the higher product stability of the beverage blend. No significant differences were observed between the samples initially. However reduction in sensory attributes was witnessed with the progression of storage period. Results

indicated maintenance of good sensory quality in BJBAB for a longer period as compared to the control beverage. In fact with increase in incubation time *i.e.* by 120th day the control beverage sample showed high acidity and precipitation that the sample was declared unfit for consumption and even in 90th day control beverage sample, the overall acceptability had a low score of 3.90 whereas BJBAB had a high score of 4.50. BJBAB maintained its overall acceptability score to 4.40 even on the 120th day demonstrating the beneficial effect of gel addition on maintaining the stability of the developed beverage and hence its sensory quality.

The present study helped in development of a novel sea buckthorn berry juice fortified aloe gel beverage which showed enhanced physicochemical, antioxidant profile, microbial and sensory quality and improved storage stability. The two main ingredients *i.e.* sea buckthorn berries and *aloe vera* gel are well known for their medicinal properties. Thus, the developed beverage blend could offer an attractive means of consuming the adverse tasting aloe gel/juice and also opening new market for the nutritious sea buckthorn berries which are not very commonly consumed. This beverage blend can be promoted as a nutraceutical product with many health benefits to a broad spectrum of consumers.

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