

## Biochemical components and sensory acceptance of a ready-to-drink from sea buckthorn berries

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### Article history:

Received: 26 April 2022

Received in revised form: 27 June 2022

Accepted: 30 June 2022

Available Online: 13

September 2023

### Keywords:

Sea buckthorn,  
Biochemical compounds,  
Sensory evaluation,  
Ready-to-drink

### DOI:

[https://doi.org/10.26656/fr.2017.7\(5\).235](https://doi.org/10.26656/fr.2017.7(5).235)

### Abstract

Sea buckthorn (*Hippophae rhamnoides*), also known as Chharma as well as a member of the Elaeagnaceae family, naturally occurs in Himachal Pradesh's high-altitude highlands amid harsh weather circumstances. This current research detailed the processing of sea buckthorn fruit juice into a ready-to-drink (RTD) drink, as well as variations throughout the beverage's condition improves after preservation about three months. During the September quarter, when the fruits were fully mature, sea buckthorn berries were picked from the Lahaul valley. The pulp was removed from the berries using a fruit pulper. Variable quantities of pulp and sugar were used in the beverage compositions to create ready-to-drink beverages. The ascorbic acid level rose considerably with increasing pulp percentage and declined with increasing curing time. In contrast, a growing tendency in decreasing sugars were found with increasing pulp and sugar concentration as well as storage time. A beverage composition combining 10%, 12% and 15% pulp with total soluble solids (TSS) 11°, 15° and 15°Bx respectively of zero-day prepared drink were voted the most acceptable by a panel of judges on a nine-point hedonic scale. It was determined that adding value to sea buckthorn can help to establish a commercial sector for such a natural berry, that has been ignored and underused in Himachal Pradesh.

## 1. Introduction

Sea buckthorn (*Hippophae rhamnoides*), a nitrogen-fixing plant of the Elaeagnaceae group, occurs abundantly in the arid subtropical Himalayan. This occurs at high elevations (2500-4500 m) in Himachal Pradesh, J and K, Gangtok and Utrakhhand in India. Sea buckthorn grows on the riverbanks and solar hills of Lahaul-Spiti, as well as in sections of Chamba, Kinnaur, Kullu, and Shimla in Himachal Pradesh. From April to November, the plant produces leaves, and even from semi-August to April, it produces red, yellow, and orange berries. Berries are harvested between September and early October. Dwivedi *et al.* (2006) indicate that sea buckthorn berry oil is high in omega-3 fats. Pre-clinical research using sea buckthorn has discovered uses in oxidation protection (Luntraruru *et al.*, 2022), cancer prevention (Shah *et al.*, 2021), cardiovascular protection (Kumari and Sharma, 2011; Guo *et al.*, 2017), and diabetes prevention (Gao *et al.*, 2017). Sea buckthorn berries are high in antioxidants, organic acids, vitamins B, E, and K, as well as carotene and flavonoids (Dong *et al.*, 2021).

Sea buckthorn is proven to have industrial, medicinal, cosmetic, and nutraceutical value. It contains various extravagant chemical compounds which makes it useful as unconventional provenance in the global market. Also nutritionally, fruits have a substantial amount of carbohydrates, protein, organic acids, amino acids, and vitamins. Moreover, it also contains bioactive compounds like carotenoids, dietary minerals, antioxidants, flavonoids, vitamin C, minerals, monosaccharides, organic acids, tannins, etc. Sea buckthorn plant parts are becoming one of the most useful and promising nutraceuticals for the food industry and all over the world.

Because of this, sea buckthorn seems to be an excellent choice for the health food, cosmetic, and pharmaceutical sectors. The fruit's enormous advantages must be realised through its application in the creation of valuation goods. This has the potential to improve the economic position of Himachal Pradesh's indigenous people as well as the country as a whole. As a result, the current study was done to evaluate the drink growth prospects of Sea buckthorn berries.

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## 2. Materials and methods

### 2.1 Sample procurement

A sample of 5 kg berries were subjected to the extraction process at the Fruit Canning Unit, and sea buckthorn pulp was obtained using a pulper. The pulp was then acquired from Vital Herbs in New Delhi, India. The pulp recovery and seed and skin proportions were recorded and analysed for significant chemical components. Pulp was used in the manufacture of a ready-to-drink beverage.

### 2.2 Preparation of drink

The ready-to-drink was made by modifying the total soluble solids (TSS) to three different levels (9°, 11°, and 15°Bx) adding cane sugar using a manual refractometer and utilizing three different levels of sea buckthorn pulp (10%, 12%, and 15%). In beverage compositions, citric acid was also utilized. Despite sea buckthorn berries being a high source of ascorbic acid, it is lost throughout preparation. To compensate for that loss, 20 mg/100 mL of ascorbic acid was added to beverage Freshly processed drinks and then quickly poured (70°C) into 200 mL glass containers, capped using crowns stoppers, thermally processed by dipping in hot water (75°C for 20 mins), cooled, and kept at room temperature for 3 months before being tested for biochemical and assortments.

### 2.3 Parameters analyzed

Standard procedures were used to determine the moisture, protein, crude fat, and ash levels (AOAC, 1990). The pigments were also estimated from whole berries, peel and seed. For lycopene, 5 g of crushed sample of berries were used by employing acetone using mortar and pestle. By using a separating funnel having 10 mL of petroleum ether it has been separated. By transferring the lower phase (acetone) in a volumetric flask, it was extracted by petroleum ether. Extraction was continued until it became colorless. For anthocyanin estimation in skin and pulp, 5 g of sample was homogenized by using appropriate ethanolic HCl and making up the volume. After keeping it at 4°C overnight it was filtered and analyzed (Srivastava and Kumar (2003). TSS was analyzed by hand refractometer. The total sugar present in the sample was calculated by taking 25 mL of filtrate in a volumetric flask. In a 1:1 ratio HCl was added. Keeping in a standing position at

room temperature for 24 hrs, it was neutralized by NaOH. Further titration method was used to estimate ascorbic acid content and titrable acidity (Ranganna, 1991). Brix acid ratios were calculated by dividing the total soluble solids of the fruits by the sample' titrable acidity values. The sensory evaluation was carried out in the manner indicated by Larmond (1977), with respect to its taste, odor, texture, and flavor with a board of ten assessors using a 9-point hedonic scale. With the use of computer tools, the values were statistically analysed.

## 3. Results and discussion

### 3.1 Proximate analysis

The average fruit pulp recovery was 65%, with seed and peel proportions of 9.78 and 25%, as shown in Table 1. Fresh berries comprised 73.5% moisture and 26.5% dry matter, whereas pulp included 74.2% moisture and 25.8% dry matter. Kernel and epidermis had lower moisture levels (41.01 and 47.2%, respectively) and greater dry matter contents (58.99% and 52.8%). The protein concentration of sea buckthorn seeds was found to be the highest (16.7%), followed by the skin (12.1%), entire berry (5.6%) and pulp (2.3%), in that order. The total fat content of kernels was found to be the highest (10.1%), next by skin (6.2%), entire fruit (7.1%) and pulp (5.9%), in that order. The average ash concentration of sea buckthorn fruits ranged between 1.5 to 2.1% (Figure 1). The investigation of different anatomical components (whole berries, peel, seed) for pigments like lycopene and anthocyanin revealed that the entire berry has 44.02 mg/100 g, 33.01 mg/100 g and 2.09 mg/100 g respectively while the anthocyanin concentrations ranged from 5.01 to 7.98 mg/100 g.

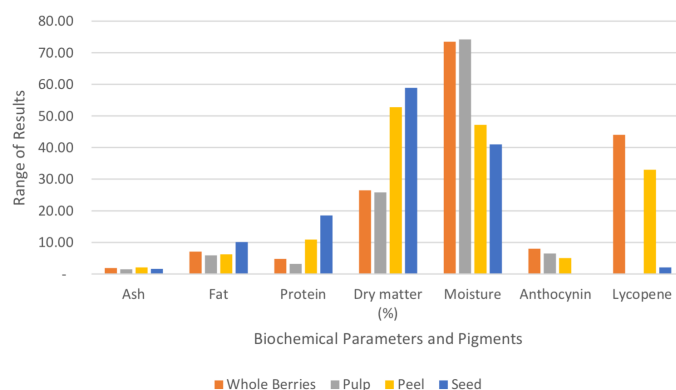


Figure 1. Estimation of biochemical and pigments present in sea buckthorn berries.

Table 1. Pigments and biochemical composition in sea buckthorn berries and its other parts

Parts of berries	Ash (%)	Fat (%)	Protein (%)	Dry matter (%)	Moisture (%)	Anthocyanin (mg/100 g)	Lycopene (mg/100 g)
Whole Berries	1.9	7.1	4.8	26.5	73.5	7.98	44.02
Pulp	1.5	5.9	3.2	25.8	74.2	6.49	-
Peel	2.1	6.2	10.9	52.8	47.2	5.01	33.01
Seed	1.6	10.1	18.5	58.9	41.01	-	2.09

### 3.2 Effect of storage on ascorbic acid

The ascorbic acid level rose dramatically as the quantity of pulp in ready-to-drink beverage formulations increased and dropped as preservation time increased (Table 2). Nevertheless, this rise in TSS seemed to have no influence on the ascorbic acid concentration of the juice beverage. There is a decrease in ascorbic acid content as the storage days proceed due to the concentration of oxygen in its surroundings, significant loss of ascorbic acid during storage may be attributed to oxidation of ascorbic acid to dehydro-ascorbic acid throughout preservation.

Initially, drinks having 10% pulp content contained 55.0 mg/100 g ascorbic acid which increased to 68.0 mg/100 g - 69.0 mg/100 g as the pulp concentration increased to 12%. For 15% mass concentration of pulp the content of ascorbic acid ranges between 76.0 mg/100 g - 78.0 mg/100 g ascorbic acid on 0 day of preparation. The mean value of initial ascorbic acid content is 67.11 mg/100 g which decreases to 55.18 mg/100 g on 90<sup>th</sup> day of storage.

### 3.3 Effect of storage on total soluble solids

The TSS of drink compositions, originally set at 3 distinct levels, were reduced marginally but substantially over an additional storage period. The associations among pulp, sugar, and storage length were determined to be non-significant. The average initial TSS value of all samples was 11.6°Bx, which fell to 11°Bx after 90 days of storage. Similarly, following storage, the mean values for alternative treatments ranged from 8.7 to 14.1°Bx, compared to previously corrected TSS values ranging from 9 to 15°Bx. Increases in pulp level and preservation length reduced increased TSS of the beverage formulations marginally, which might be attributed to chemical interactions between the organic ingredients of the beverage (Ghorai and Khurdiya, 1998).

### 3.4 Effect of storage on acidity

The acidity of the juice beverage originally ranged from 0.367 - 0.588 for all pulp concentrations 10%, 12% and 15% and reduced somewhat after 90 days of storage as mentioned in Table 2. The total mean acidity levels of the fresh and preserved beverage samples ranged from 0.467 to 0.441%. The titratable acidity of the drink types differed significantly and improved as the quantity of sea buckthorn pulp in the beverage grew from 10% to 15%. It could be ascribed in proportion to the intrinsic acid naturally present in sea buckthorn pulp and in part to the citric acid purposefully added to the drink during processing.

### 3.4.1 Acid brix ratio

The sugar and pulp percentages in ready-to-drink drinks had a massive impact on the Brix acidic ratios of the drinks, but the effects of storage period and associations were determined to be non-significant (Figure 2). In general, the brix acid ratios fell as pulp content rose and increased as TSS increased. The average brix acid ratios ranged from 25.59 to 25.70 across all treatments.

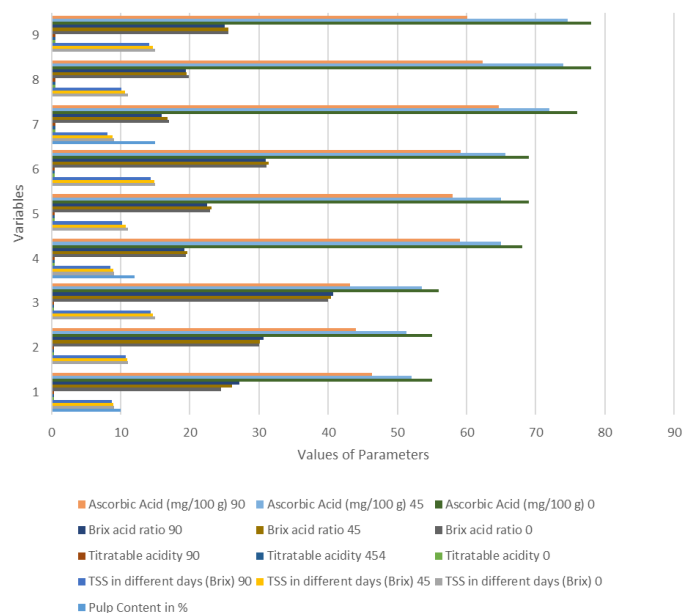


Figure 2. Impact of soluble solids, concentration of pulp of sea buckthorn and preservation time on its titratable acid, TSS, Brix acid ratio, and ascorbic acid content.

The influence of pulp quantities and associations involving pulp, TSS, and prolonged storage on the total sugar content of juice beverages was determined to be negligible. (Table 3). The influence of TSS and preservation period upon variations throughout the total sugar concentration of prepared drinks was statistically meaningful. Originally, the total sugar content of the drinks ranged from 10% to 14% on day 0 among nine distinct preparations. After 90 days of storage, the content ranged from 9.93% to 13.94%.

### 3.5 Effect of storage on reducing and non-reducing sugars

The influence of pulp and sugar levels, and also preservation periods, mostly on lowering the sugar concentration of said fruit drink was shown to be significant. While the associations among pulp, sugar, and preservation time frames on the reducing sugar concentration of said drink were non-significant. On average, the amount of reducing sugars rose as even the amount of pulp and sugars increased. Modest improvements in the reducing sugar content of the drinks

Table 2. Impact of soluble solids, concentration of pulp of sea buckthorn and preservation time on its titratable acid, TSS, Brix acid ratio, and ascorbic acid content.

Pulp Content In %	Adjusted TSS (Brix)	TSS in different days (Brix)			Titratable acidity			Brix acid ratio			Ascorbic Acid (mg/100 g)		
		0	45	90	0	45	90	0	45	90	0	45	90
10	9	9.00	8.90	8.70	0.37	0.34	0.32	24.52	26.09	27.10	55.00	52.00	46.30
	11	11.00	10.90	10.70	0.37	0.35	0.35	30.05	30.10	30.65	55.00	51.30	44.00
	15	15.00	14.60	14.30	0.38	0.36	0.35	40.00	40.44	40.74	56.00	53.50	43.20
12	9	9.00	8.90	8.50	0.46	0.45	0.44	19.43	19.67	19.23	68.00	65.00	59.00
	11	11.00	10.70	10.20	0.48	0.46	0.45	22.96	23.11	22.51	69.00	65.00	58.00
	15	15.00	14.90	14.30	0.48	0.47	0.46	31.05	31.36	30.95	69.00	65.60	59.10
15	9	9.00	8.80	8.10	0.53	0.53	0.51	16.94	16.76	15.88	76.00	72.00	64.70
	11	11.00	10.60	10.10	0.55	0.54	0.52	19.85	19.55	19.38	78.00	74.00	62.30
	15	15.00	14.70	14.10	0.59	0.58	0.56	25.51	25.56	25.04	78.00	74.60	60.10

Table 3. Total sugars, reducing and non-reducing sugars found in sea buckthorn ready-to-drink beverage and hedonic score.

Pulp Content In %	Adjusted TSS (Brix)	Total Sugar (%), A			Reducing Sugar (%), B			Non-reducing sugar (%), C=A-B			Hedonic scale		
		0	45	90	0	45	90	0	45	90	0	45	90
10	9	10	9.96	9.93	2.00	2.21	2.29	8.0	7.75	7.62	6	6	7
	11	12	11.97	11.96	2.22	2.34	2.37	9.78	9.63	9.59	8	7	7.5
	15	14	13.93	13.91	2.25	2.39	2.41	11.75	11.54	11.5	7	6	6.2
12	9	10	9.98	9.94	2.45	2.46	2.49	7.55	7.52	7.45	6	5	5.3
	11	12	11.94	11.91	2.56	2.58	2.61	9.44	9.36	9.3	6	5	5.1
	15	14	13.98	13.93	2.61	2.65	2.68	11.39	11.33	11.2	8	7	6.6
15	9	10	9.98	9.94	2.68	2.69	2.71	6.32	7.29	7.23	7	7	6.3
	11	12	11.98	11.94	2.75	2.78	2.82	9.25	9.2	9.12	6	5	4.3
	15	14	13.96	13.94	2.80	2.81	2.84	11.19	11.15	11.1	8	7	6.9

were also detected as preservation time increased. Originally, the reducing sugar level of the drinks ranged from 2% to 2.80% on day 0, rising to 2.29% to 2.84% after 90 days of storage. Reducing sugars rose as preservation frequency increased, which would be due to the acid in the drinks hydrolyzing sucrose to glucose and fructose, resulting in concomitant declines for non-reducing sugars.

Non-reducing sugars were estimated by omitting reducing sugars from total sugars and differed considerably as the pulp, sugar concentration, and storage period increased (Table 3). Associations between such parameters, though, appeared to be non-significant. Non-reducing sugars rose as sugar and pulp content increased but decreased slightly as storage duration rose. The non-reducing sugar content of fresh drinks ranged from 8.00% to 11.19% on day 0, and after 90 days of storage, it ranged from 7.62% to 11.1% (Figure 3).

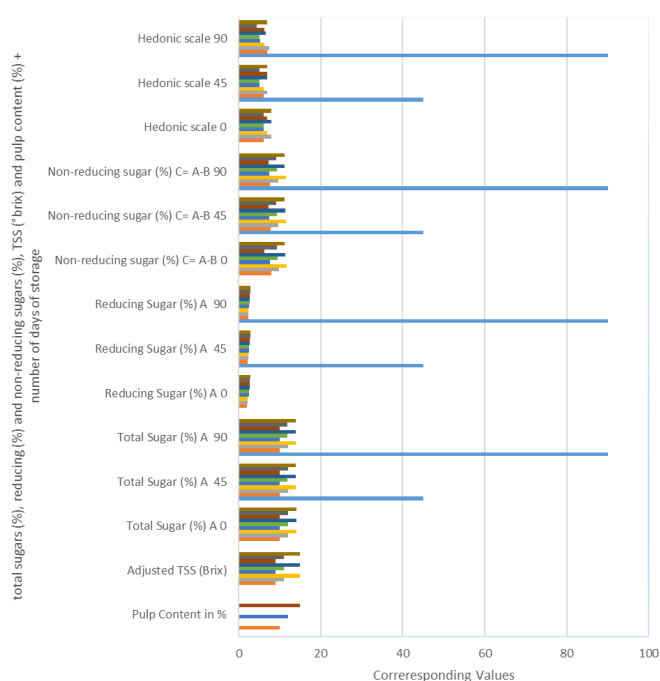


Figure 3. Data depicting for total, reducing and non-reducing sugars.

### 3.6 Hedonic scale (acceptability)

The first overall acceptability scores of drinks made by altering the pulp content from 10% to 15% and TSS from 9° to 15°Bx varied substantially from 6 - 8 score on day 0 of preparation, with an average score of 6.8 as discussed in Table 3 and detailed discussed in Figure 4. The overall acceptability scores of the beverage decreased slightly but significantly as the storage period increased, and the scores ranged from score 7 - 6.90, with a mean score of 6.13 after 90 days of storage.

The optimum and desirable TSS and pulp quantities have been analyzed as 9°, 11°, and 15°Bx in corresponds to 10%, 12% and 15%, since the drinks produced with

these proportions were deemed preferable to the others in terms of absolute desirability scores by the assessors. Their data indicated it as the pulp amount grew, so did the sugar content in order to hide the astringent taste and flavour provided towards the drinks by the increasing amount of sea buckthorn pulp. The prospective project acceptance evaluations of drinks made by altering the pulp content from 10% to 15% and TSS between 9 to 15°Bx differed significantly ( $P \leq 0.05$ ). With increasing storage duration, there was a minor but significant ( $P \leq 0.05$ ) decline in overall acceptability scores of the beverage, with values ranging from 7.0 to 6.90 after 90 days of storage. The optimal pulp and sugar concentrations were found to be 10, 12, and 15 and 11, 15 and 15°Bx, correspondingly, as the drinks made with these pairings were scored higher than those in terms of absolute acceptance by the assessors. According to the findings, increasing the pulp content requires increasing the sugar content to hide the tannic taste and flavour imparted to the beverages by the increased amount of sea buckthorn pulp.

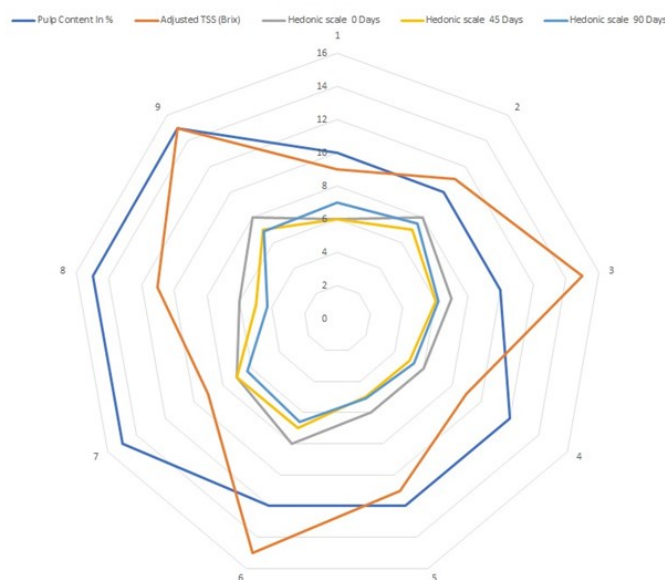


Figure 4. Sensory evaluation of sea buckthorn drink.

## 4. Conclusion

On the basis of the preceding, it can now be concluded that the fruits of sea buckthorn, which grow abundantly across the Himalayan cold deserts, are exceptionally high in antioxidants. This ready-to-drink beverage made from the berry is likewise high in vitamin C as well as other minerals. As a result, it has marketing and public consumption possibilities. Because of the high nutritional worth of sea buckthorn fruit and its RTD, farmers should be encouraged to cultivate it commercially, which will aid in the economic empowerment of the regional economy and environmental standards.

## Acknowledgements

We would like to thank Vital Herbs, New Delhi for procuring berries and conducting a preceding study for this research work.

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