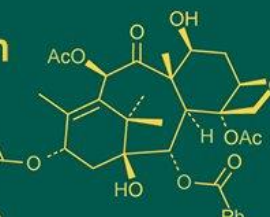
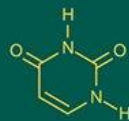


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Abha Thakur

Dietician, Military Hospital
Palampur, Holta Camp,
Kangra, Himachal Pradesh,
India

Ranjana Verma

Department of Food Science,
Nutrition and Technology,
College of Community Science,
Chaudhary Sarwan Kumar
Himachal Pradesh Krishi
Vishwavidyalaya (CSK
HPKV), Palampur, Himachal
Pradesh, India

Nilakshi Chauhan

Department of Food Science,
Nutrition and Technology,
College of Community Science,
Chaudhary Sarwan Kumar
Himachal Pradesh Krishi
Vishwavidyalaya (CSK
HPKV), Palampur, Himachal
Pradesh, India

Kanchan Thakur

Department of Food Science,
Nutrition and Technology,
College of Community Science,
Chaudhary Sarwan Kumar
Himachal Pradesh Krishi
Vishwavidyalaya (CSK
HPKV), Palampur, Himachal
Pradesh, India

Corresponding Author:**Kanchan Thakur**

Department of Food Science,
Nutrition and Technology,
College of Community Science,
Chaudhary Sarwan Kumar
Himachal Pradesh Krishi
Vishwavidyalaya (CSK
HPKV), Palampur, Himachal
Pradesh, India

Physicochemical, antioxidant and sensory evaluation of a beetroot juice-based functional energy drink

Abha Thakur, Ranjana Verma, Nilakshi Chauhan and Kanchan Thakur

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Abstract

Beetroot (*Beta vulgaris L.*) is a rich source of betalains, phenolic compounds, flavonoids and antioxidants which contribute to improved health and physical performance. The present study aimed to develop a beetroot juice based energy drink fortified with litchi juice, pear juice and *ashwagandha* extract and to evaluate its sensory, chemical and antioxidant properties. Different formulations containing varying levels of *ashwagandha* extract were prepared and evaluated using a nine-point hedonic scale. The formulation containing 5% *ashwagandha* extract (E2) was found to be the most acceptable among the blended treatments. The developed beverage showed appreciable antioxidant potential with total betalains of 39.32 mg/100 ml, total phenols of 9.43 mg/100 ml, total flavonoids of 6.23 mg/100 ml and DPPH scavenging activity of 33.52%. The study demonstrated the potential of beetroot juice as a functional ingredient for development of nutritionally enriched energy beverages.

Keywords: Functional beverages, *ashwagandha*, betalain, energy drink, beetroot juice

Introduction

The innovation and advancement over the past several years has facilitated a plethora of functional beverages. A functional beverage can be defined as “any non-alcoholic drink that provides additional health benefits due to the inclusion of any bioactive component from a plant, animal, marine or microorganism source” (Gayathry & John, 2021) [5]. Functional beverages can be classified as dairy-based beverages (including probiotics and mineral enriched drinks), vegetable and fruit beverages, sports and energy drinks (Corbo *et al.* 2014) [4]. Functional beverages are gaining popularity due to increasing consumer awareness regarding health promoting foods rich in bioactive compounds. Beetroot is recognized as an excellent source of betalains, phenolic compounds, flavonoids, minerals and dietary fibre (Chhikara *et al.* 2019) [3]. Beetroot juice contains biologically accessible antioxidants and inorganic nitrates which have been associated with improved cardiovascular health, enhanced exercise performance and reduced oxidative stress (Wootton *et al.* 2011) [15]. Litchi and pear juices contribute desirable flavour and additional nutrients, whereas *ashwagandha* is traditionally known for its adaptogenic and energy enhancing properties. Development of a beetroot based energy drink incorporating these ingredients provides an opportunity to formulate a novel functional beverage with improved nutritional quality and consumer acceptability.

Material and Methods**Procurement of beetroot**

Fresh and optimally mature beetroot, *ashwagandha*, litchi and pear were purchased from the local market of Palampur, Himachal Pradesh, ensuring they were free from any bruises or damage.

Development of beetroot juice

Beetroots were kept in freezer for one day for (Freezing process of fresh beetroots could have led to higher degree of tissue damage resulted in an increased extraction of betalains reported by Bianchi *et al.* 2021). After peeling it was cut in pieces and crushed in mixer grinder to extract juice.

Development of beetroot-based energy drink

Beetroot juice was the main ingredient along with other functional ingredient. The energy drink was prepared by

blending beetroot juice with litchi juice, pear juice and ashwagandha extract in different ratios as per details below:

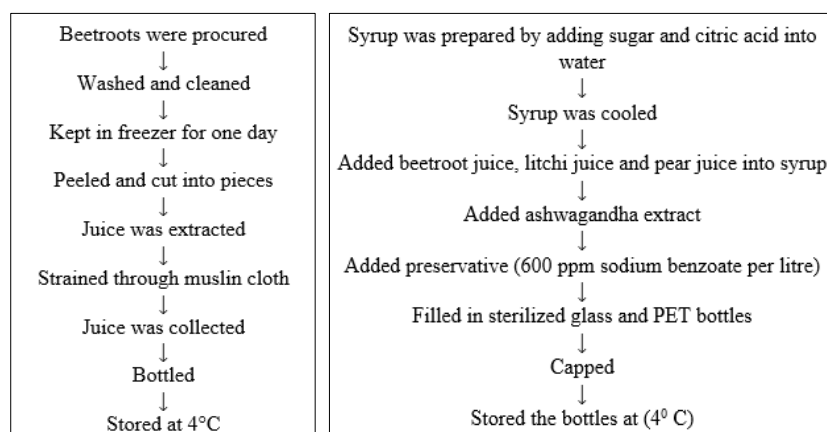


Fig 1: Steps involved in development of beetroot juice, **Fig 2:** Flowsheet of beetroot-based energy drink

Proportion				Ingredients			
BJ:LJ:PJ:AE	Beetroot juice (ml)	Litchi juice (ml)	Pear juice (ml)	Ashwagandha extract (ml)	Sugar (g)	Citric acid (g)	Water (ml)
10:10:10:0 E ₀	10	10	10	-	7	0.2	60
10:10:10:2.5 E ₁	10	10	10	2.5	7	0.2	60
10:10:10:5 E ₂	10	10	10	5	7	0.2	60
10:10:10:7.5 E ₃	10	10	10	7.5	7	0.2	55
10:10:10:10 E ₄	10	10	10	10	7	0.2	50

*BJ- Beetroot juice; LJ- Litchi juice; PJ- Pear juice; AE- Ashwagandha extract

Note: Ashwagandha extract was prepared by boiling 20g of ashwagandha leaves in 100ml of water until the volume reduced by half, resulting to 50ml.

Sensory evaluation of developed energy drink

A group of 10 sanctioned judges from the Department of Food Science, Nutrition, and Technology at COCS, CSKHPKV, Palampur conducted sensory evaluation of different functional beverages made with beetroot as the main ingredient. Each judge received one control sample and four other treatments E₁, E₂, E₃, E₄ for energy drink. The control treatment followed a standard recipe, while the other four treatments involved blending of functional ingredients such as litchi juice, pear juice and ashwagandha in varying proportions with the standard recipe. To ensure impartial evaluation, the samples were given numerical codes. Panellists were instructed to rate the products based on various attributes like colour, appearance, consistency, flavour, taste, and overall acceptability using a scorecard that used a nine-point hedonic scale, following the guidelines described in Larmand, 1977.

Nutritional analysis: Moisture, crude fat, crude protein, crude fibre, ash content, and total carbohydrate contents were determined according to the standard procedures of AOAC (2010) [1] for proximate composition analysis. Total soluble solids (TSS) and titratable acidity were estimated following the methods described by Rangana (2014). Ascorbic acid content was determined using the method of Rangana (2007). Total sugars and reducing sugars were estimated by the Lane and Eynon (1923) method as reported by Rangana (2014) [16].

[17, 18], while non-reducing sugars were calculated from the difference between total sugars and reducing sugars. Total phenolic content and total flavonoid content were determined according to the methods of Singleton *et al.* (1999) and Zhishen *et al.* (1999) [11, 14], respectively. Antioxidant activity was evaluated using the DPPH radical scavenging assay described by Brand-Williams *et al.* (1995) and total betalain content was determined following the method of Stintzing *et al.* (2003) [12, 13], whereas total carotenoid content was estimated using the procedure described by Rangana (2014). The colour characteristics of the samples were measured in terms of L*, a*, and b* values using a Colour Flex meter with 45°/0° geometry and standard illuminant C (Hunter Lab Colour Flex 150, Hunter Associates Inc., USA). The energy value of the samples was calculated according to the method proposed by O'Shea and Maguire (1962) [9].

Statistical Analysis: The data analysis involved the use of OPSTAT software to calculate the average and variation in different attributes of beetroot based energy drink. To compare the chemical and antioxidant properties of the newly developed products with their corresponding control samples, an independent sample t-test was performed. When examining the sensory attributes, a one-way ANOVA test was employed and compared at 5 percent level of significance to identify any level of significant.

Results and Discussion

Sensory evaluation of beetroot-based energy drink

Various energy drink formulations were prepared with varying ratios of beetroot juice (BJ), litchi juice (LJ), pear

juice (PJ), and ashwagandha extract (AE). The treatments are denoted as E0, E1, E2, E3, and E4, each with a specific ratio of the ingredients. The sensory evaluation was conducted based on four attributes: colour, taste, consistency, and overall impression (Table 1). Colour refers to the visual appearance of any product. In all the energy drink formulations the colour scores ranged from 8.00 to 5.50, with higher scores indicating a more visually appealing colour. Colour and consistency scores of the blended energy drink differed non-significantly in case of control (E0) energy drink and the first two formulations of energy drink (E1 and E2) but a significant decrease was observed in these two attributes in E3 and E4 formulations of energy drinks. On the other hand, when it comes to taste, the scores in all the formulations including the control ranged from 8.60 to 4.80, with higher scores representing a more favourable taste.

Table 1: Sensory scores of beetroot-based energy drink

Treatments (BJ:LJ:PJ:AE)	Colour	Taste	Consistency	Overall
(10:10:10:0) Control E ₀	7.90	8.60	7.90	8.60
(10:10:10:2.5) E ₁	7.70	7.50	7.70	7.50
(10:10:10:5) E ₂	8.00	7.80	8.00	7.80
(10:10:10:7.5) E ₃	6.50	5.50	6.50	5.50
(10:10:10:10) E ₄	5.50	4.80	5.50	4.80
C _(p<0.05)	0.50	0.60	0.50	0.60

*BJ (beetroot juice):LJ (*litchi* juice):PJ (pear juice):AE (*ashwagandha* extract)



Control (E₀) Blended (E₂)

Plate 1: Appearance of beetroot-based energy drink

The control (E0) energy drink had a significantly higher taste scores than blended energy drink (E1), but no significant difference in taste scores was observed in E1 and E2 energy drink with taste scores of 7.50 and 7.80 respectively. Subsequently, there was a significant decrease in taste with increase in proportion of ashwagandha extract in different formulations. Overall acceptability which provides an overall impression of the energy drink based on colour, taste, and

consistency ranged from 8.60 to 4.80, with higher scores in control energy drink suggesting a better overall sensory experience followed by E2 treatment. Based on these observations, it was concluded that blended energy drink (E1 and E2) did not exhibit any significant differences in terms of colour, taste, and consistency. Therefore, treatment E2, which contained a 5 percent of ashwagandha extract was taken further for analysis in comparison with the control (E0) energy drink. Jairajpuri *et al.* (2012)^[6] reported that orange juice blended with 6 percent ashwagandha extract was the most acceptable by a panel of judges for sensory parameters.

Chemical parameters of beetroot-based energy drink

Table 2 depicts that the total soluble solid content of control (E0) and blended energy drink (E2) was 11.00 and 10.00 brix whereas the acidity content of both the formulations of energy drink was 0.26 and 0.25 percent respectively. Further scrutiny of data reveal that there was no significant difference in the brix acid ratio between the control (E0) energy drink (42.35%) and blended (E2) energy drink (40.49%). Similarly, a non-significant variance in the levels of total sugars, reducing sugars, and non-reducing sugars between the control energy drink (23.64%, 12.83%, and 10.81%, respectively) and blended (E2) energy drink (22.41%, 11.94%, and 10.47%, respectively).

Table 2: Chemical parameters of beetroot-based energy drink

Parameters	Control (E ₀)	Blended (E ₂)	t-value (p value)
TSS (⁰ Brix)	11.00	10.00	1.26 (0.14)
Acidity (%)	0.26	0.25	1.84 (0.20)
Brix Acid Ratio	42.35	40.49	3.02 ^{NS} (0.05)
Total sugars (%)	23.64	22.41	1.16 ^{NS} (0.3)
Reducing sugars (%)	12.83	11.94	1.54 ^{NS} (0.19)
Non-reducing sugars (%)	10.81	10.47	0.66 ^{NS} (0.54)
Energy (kcal/100ml)	35.60	39.20	8.60 (0.009)

Significance at 5% level of significance ($p < 0.05$) NS- Non significant

The blended energy drink (E2) exhibited a significantly higher energy content (39.20 kcal/100ml) compared to the control energy drink (35.60 kcal/100ml) with a t-value of 8.60, indicating a significant difference ($p = 0.009$) between both treatments. This variation in energy content could be attributed to the inclusion of ashwagandha extract, which is a known source of energy. Ashwagandha extract was intentionally added to create an energy drink based on beetroot juice. Ashwagandha is known as energy boosting tonic and memory enhancer. It is mainly grown in India that is why also called Indian ginseng (Javed *et al.* 2023)^[7]. This value of energy closely resembled to value reported by Ashraf *et al.* (2022)^[2] who reported that beetroot energy drink contained an energy content of 35.223 Kcal/100ml.

Antioxidant properties of beetroot-based energy drink

The ascorbic acid, betacyanins, betaxanthins, total betalains, TPC, TFC, antioxidant activity as assessed by DPPH assay of beetroot blended energy drink has been presented in Table 3.

Table 3: Antioxidant properties of beetroot-based energy drink

Parameters	Control (E ₀)	Blended (E ₂)	t-value (p value)
Ascorbic acid (mg/100ml)	4.76	4.73	0.26 ^{NS} (0.80)
Betacyanin (mg/100ml)	30.32	30.03	1.1 ^{NS} (0.33)
Betaxanthin (mg/100ml)	9.90	9.30	2.61 ^{NS} (0.12)
Total betalain (mg/100ml)	40.23	39.32	2.0 ^{NS} (0.1)
Total Phenols (mg/100ml)	8.50	9.43	0.09 ^{NS} (0.9)
Total Flavonoids (mg/100ml)	5.40	6.23	0.3 ^{NS} (0.7)
DPPH (%)	31.68	33.52	1.59 ^{NS} (0.08)

Significant at 5% level of significance ($p < 0.05$) NS- Non significant

A perusal of the data reveal that there were no significant difference between the control energy drink and blended energy drink (E₂) in all these components. The ascorbic acid content in both control energy drink (4.76 mg/100ml) and blended (E₂) energy drink (4.73 mg/100ml) did not show a significant difference. Similarly, there were no significant variations in betacyanins, betaxanthins, and total betalains levels in control energy drink with values of 30.32, 9.90 and 40.23 mg/100ml, respectively and blended (E₂) energy drink with values of 30.03, 9.30 and 39.32 mg/100ml, respectively. The total phenols and flavonoids contents in the control energy drink (8.50 mg/100ml) differed non-significantly to blended (E₂) energy drink (9.43 mg/100ml). Similarly, the antioxidant activity in the control (E₀) energy drink (31.68%) did not significantly differ from that in blended (E₂) energy drink (33.52%). These results suggested that the formulation changes made in the blended (E₂) energy drink did not significantly alter the antioxidant properties of the beetroot energy drink when compared to the control energy drink.

Colour analysis of beetroot-based

The degree of lightness, or L* value, was found to be significantly lower in the blended (E₂) energy drink (19.15) in this analysis when compared to the control (E₀) energy drink (17.44), suggesting that the control energy (E₀) drink appeared darker (Table 4).

Table 4: Colour analysis of beetroot-based energy drink

Parameters	Control (E ₀)	Blended (E ₂)	t-value (p-value)
L*	17.44	19.15	93.66 (<0.001)
a*	23.13	10.52	613.6 (<0.001)
b*	10.76	13.62	23.93 (<0.001)

Significance at 5% level of significance ($p < 0.05$)

The redness intensity, or a* value, was found to have significantly decreased in the blended (E₂) energy drink (10.52) as compared to the control (E₀) energy drink (23.13), suggesting a change towards a less intense red colour. Furthermore, there was a significant decrease in the control energy (E₀) drink (10.76) yellowness (b* value) when compared to the blended (E₂) energy drink (13.62),

indicating a reduction in the beverage's yellow tones. Key information about how the treatment affects the beverage's aesthetic appeal is provided by the significant variations in colour parameters that were found between both the samples. These outcomes are significant for the thesis research because they provide important information about potential shifts in consumer preferences and sensory perception regarding the colour of the beetroot energy drink. The a* values were significantly higher in control energy drink as compared to blended energy (E₂) drink. This significant difference in L*, a*, b* values might be due to the blending of 5 per cent ashwagandha extract in E₂ energy drink.

Conclusion

The study concluded that beetroot juice can successfully be utilized for the development of a functional energy drink. Incorporation of 5% ashwagandha extract along with litchi and pear juice produced a beverage with good sensory acceptability and enhanced energy value without adversely affecting antioxidant properties. The developed beverage exhibited appreciable levels of betalains, phenolics, flavonoids and antioxidant activity, indicating its potential as a natural functional beverage for health-conscious consumers.

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