Assessment of Physico-chemical and Phyto-chemical Analysis in Black Carrot (*Daucus carotas* sp *sativus*) RTS beverage

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Abstract

Recently, black carrots (*Daucus carotas* sp *sativus*) have been in focus because of their high anthocyanin content. The present study deals about assessment of physico-chemical and phytochemicals in black carrot RTS . Analysis of physico chemical and phytochemicals in black carrot RTS has shown varied results among the treatments, the mean maximum total phenol (230.73 mg/100ml), tannin (18.81 mg/100ml) and total antioxidants (76.07 %) were found in T_9 (50% BCJ+ Aqueous Stevia Extract+40% Water) except total flavonoids (81.57 mg/100ml) in T_8 (50% BCJ+ Edible Sugar+40% Water). Whereas, A total soluble solid was maintained in all the concentration when sugar used as sweetener (15.47^0 B) and titratable acidity was found to be non-significant.

Index terms: Black carrots, Phytochemicals, RTS, Black carrot pomace, Total flavonoids, Total phenols and Total antioxidants.

Introduction

According to research, fruits and vegetables are a promising source of polyphenols since they have a number of health-promoting qualities. These plants' components have the power to reduce oxidative stress and inflammation, increase lipid profiles, and speed up glucose metabolism. Additionally, polyphenols protect against UV radiation and typically give bitterness, astringency, color, flavor, odor, and oxidative stability to a variety of meals. They are secondary metabolites of plants (Beckman, 2000). Furthermore, black carrot contains relatively higher content of flavonoids as compared to orange or red carrots. These flavonoids are important in terms of their biochemical and pharmacological role as anti-oxidant, anti-inflammatory, anti-atherosclerosis, antiplatelet aggregation, antitumor, antimicrobial, and anti-allergic agents. Similarly, the presence of a numerous bioactive compounds such as carotenoids, polyacetylenes, falcarindiol, falcarindiol-3-acetate, and anthocyanins (cyanidin, delphinidin, petunidin, peonidin, malvidin and pelargonidin) has been reported in black carrot. Black carrots are potential source of anthocyanin pigment and their high antioxidant activity has lately been recognized for its high nutraceutical application (Kaur and Kapoor, 2002). The anthocyanin pigment from black carrots is Kosher and does not require E number labelling on food labels.

Anthocyanins available in black carrots have been extensively investigated and products such as black carrot RTS and pomace spice balls were prepared and the present study assessed phytochemicals present in the black carrot RTS and pomace spice balls.

Materials and methods:

Total soluble solids (°Brix)

Total soluble solids were estimated at ambient temperature using hand refractometer (0-32%) Erma, Japan and the values were expressed as per °Brix**.**

Titratable acidity (%)

Acidity of the sample was measured by potentiometric titration against 0.1 N NaOH solution and phenolphthalein as indicator. Titratable acidity was determined by titrating the known value of 0.1 N sodium hydroxide along with phenolphthalein as indicator (Ranganna, 1997). One or two ml sample was taken for estimation. Volume was made up to 100ml with distilled water and filtered. 5ml aliquot was taken and 1-2 drops of phenolphthalein indicator was added, it was titrated against NaOH to faint pink end colour. The results were expressed as per cent citric acid.

$$\text{TA (\%)} = \frac{\text{Titre value x Noramlity of alkali used x Volume made x eq. weight x 100}}{\text{Aliquot taken x Weight of sample (g) x 1000}}$$

Antioxidant (%)

The capacity of the extracts to scavenge the stable 2, 2'-diphenyl-2-picrylhydrazyl (DPPH) free radical was measured as per Singleton *et al*. (1999). To 0.2 ml extract, 0.2 ml DPPH solution was added and incubated for 30 min under dark condition. The absorbance of the reaction mixture was measured at 517 nm. DPPH radical-scavenging rate was calculated according to the equation as given below.

DPPH radical scavenging activity (%) = (Ab control- Ab sample) / Ab control

Where, Ab is Absorbance value

Total phenol (mg/100g)

For the determination of total phenol content of the sample, folin-ciocalteu reagent method was followed using gallic acid as a standard. A known volume of 0.2 and 1ml samples were taken and volume was made up to 3ml with water and 2.5 ml of 10 per cent v/v folin-ciocalteu reagent was added. After 5 min, 2 ml of 20 per cent sodium carbonate was added and incubated the tubes at room temperature for 15 min. Absorbance of the colour developed was measured at 765 nm and the total phenol content was expressed as mg/100g extract (Madaan *et al.*, 2011).

Tannins (mg/100g)

For the determination of tannin content of the sample folin- ciocalteu reagent method was followed using gallic acid as standard. A known volume of 0.2 and 1 ml samples were taken and the volume was made up to 3 ml. To each tube 2.5 ml of 10 per cent v/v folin-ciocalteu reagent was added and incubated for 5 min before addition of 2 ml of 20 per cent sodium carbonate. The reaction mixture was incubated at room temperature for 15min. Absorbance of the colour developed was measured at 725 nm and tannin contents was expressed as mg /100g extract (Madaan *et al.*, 2011).

Total flavonoids (mg/100 ml)

A colorimetric method using aluminium chloride was followed for flavonoids determination. To 0.5 ml of sample extract, 0.3ml $NaNO_2$ solution was added and after 5 min of time 0.3 ml of $AlCl_3$ (10%) was added. After 6 min of reaction time 2 ml of NaOH (1M) was added and allowed to stand at room temperature for 30 min. the absorbance of the reaction mixture was measured at 510 nm. The calibration curve was prepared using rutine as a standard substrate (Madaan *et al.*, 2011).

Results and discussion

Physico-chemical and phyto-chemicals of black carrot RTS beverage

Total Soluble Solids (TSS) (°B)

The TSS content in fresh raw black carrot was found to be 7°B. However, the TSS content of RTS recipes in the present study varied significantly (Table 1). The treatments T_2 (15.43°B), T_5 (15.47°B) and T_8 (15.47°B) had significantly higher TSS than rest of the recipes experimented and they were on par with each other. Significantly minimum TSS was noted in T_1 (1.47°B) and T3 (1.00°B). The TSS value is defined as the amount of sugar and soluble minerals present in fruits and vegetables. Sugars constitute 80-85% of the soluble solids. RTS is a type of fruit beverage which contains at least 10% and 10% TSS besides about 0.30% acidity. Measurement of TSS in a beverage constitutes a rough estimate of sugars present in it. In the present study, recipes of the black carrot beverages contained minimum pulp or juice content required to satisfy the definition of RTS. However, their TSS content varied significantly with some recipes containing less than 10°B (Table 1).

Titratable Acidity (%)

Raw black carrot juice was found to have the titratable acidity (TA) of 0.29 per cent (Annexure-4). The perusal data (Table 1) showed that there was no significant difference among the treatments of black carrot RTS. However, the treatment T_6 (BCJ 40% + SE 0.2 % +Water 50%) and T_7 (50% BCJ+ 50% water) recorded highest 0.14 per cent and followed by T_8 (BCJ 50% + ES 15°B + Water 60%; 0.13 %) and T_9 (BCJ 50% + SE 0.2 % +Water 40%) had 0.13 per cent. Whereas lowest acidity was recorded in T_2 (BCJ 30% + ES 15°B +Water 60%) with 0.09 per cent. The perusal data from Table 1 evoke that titratable acidity among different treatments of RTS had no significant change.

Table 1: Effect of different concentrations of black carrot juice on physico-chemical properties of ready-to- serve beverage

Treatment details	Physico-chemical properties	
	Total soluble solids (°B)	Titratable acidity (%)
T_1 : 30% BCJ+ 70% Water	1.53^{d}	0.10
T_2 : 30% BCJ +ES+60% Water	15.23^{a}	0.09
T_3 : 30% BCJ +ASE+60% Water	1.27^{d}	0.10
T_4 : 40% BCJ+ 60% Water	2.53^{c}	0.12
T_5 : 40% BCJ +ES+50% Water	15.30^{a}	0.12
T_6 : 40% BCJ +ASE+50% Water	2.27^{a}	0.14
T_7 : 50% BCJ+ 50% Water	3.23^{c}	0.14
T_8 : 50% BCJ+ES+40% Water	15.20^{b}	0.13
T_9 : 50% BCJ+ ASE+40% Water	3.33^{b}	0.13
Mean	6.66	0.12
S.Em±	0.16	0.01
CD at 5%	0.48	NS

Note: Columns with different superscripts are significantly different at $p \leq 0.05$ according to Duncan's Multiple Range Test (DMRT)

The above treatment includes the following ingredients in common	Ginger-1ml, Lemon Juice-5ml, Holy Basil Extract -3ml and Aloe Extract -1ml
NS: Non significant, BCJ: Black Carrot Juice, ES: Edible Sugar, ASE: Aqueous Stevia Extract	

Total antioxidants (%)

The results in Table 2 on antioxidant activity of black carrot RTS with different concentration of black carrot juice has shown significant difference among the treatments. The highest antioxidant activity (76.07 %) was found in the treatment T_9 (BCJ 50% + Water 40%) followed by the treatment T_8 (BCJ 50% + Water 60%) and T_7 (BCJ 50% + Water 50%) with 74.13 and 70.60 per cent, respectively. The lowest antioxidant activity of 59.10 per cent was observed in the treatment T_1 (30% BCJ+ 70% water).

Total phenol (mg /100 g)

The data pertaining to total phenol content of black carrot RTS as influenced by treatments is presented in Table 2. Total phenol content of RTS varied significantly among the treatments**.** The maximum phenol content of 230.73 mg/100g was recorded in the treatment T_9 (BCJ 50%+ Water 40%) and it was significantly different from other treatments. The treatment T_5 (BCJ 40% + Water 50%; 215.93 mg/100g) and T_6 (BCJ 40% + Water 50%; 216.17 mg/100g) were found to be on par with each other. The minimum phenol content was found in the treatment T_1 (BCJ 30% + Water 70%) with 188.63 mg/100g of total phenol. To support this Harish *et al*., 2022 studied the effect of incorporation of PSP and DSF on total phenol content and antioxidant value

of cookies was showed significant values and non-significant values among the treatments. Total phenol content and per cent antioxidant activity of the cookies was increased with the increasing level of incorporation of pomegranate seed powder.

Table 2: Effect of different concentrations of black carrot juice on phyto-chemicals of ready-to- serve beverage

Treatment details	Phyto-chemicals	
	Total phenol (mg /100g)	**Total antioxidant (%)**
T_1 : 30% BCJ+ 70% Water	188.63^{g}	59.10^{f}
T_2 : 30% BCJ +ES+60% Water	200.03^{f}	62.30^{e}
T_3 : 30% BCJ +ASE+60% Water	200.13^{f}	62.40^{e}
T_4 : 40% BCJ+ 60% Water	213.20^{e}	65.53^{bc}
T_5 : 40% BCJ +ES+50% Water	215.93^{d}	68.10^{b}
T_6 : 40% BCJ +ASE+50% Water	216.17^{d}	70.50^{b}
T_7 : 50% BCJ+ 50% Water	218.67^{c}	70.60^{b}
T_8 : 50% BCJ+ES+40% Water	220.63^{b}	74.13^{a}
T_9 : 50% BCJ+ ASE+40% Water	230.73^{a}	76.07^{a}
Mean	**211.57**	**67.64**
S.Em±	0.38	0.76
CD at 5%	1.142	2.26

Note: Columns with different superscripts are significantly different at $p \leq 0.05$ according to Duncan's Multiple Range Test (DMRT)

The above treatment includes the following ingredients in common	Ginger-1ml, Lemon Juice-5ml, Holy Basil Extract - 3ml and Aloe Extract -1ml
BCJ: Black Carrot Juice, ES: Edible Sugar, ASE: Aqueous Stevia Extract	

Tannins (mg/100 g)

The data (Table 3) pertaining to tannin contents in different treatments of black carrot RTS was found to have significant difference, where the treatment T_9 (BCJ 50 % + Water 40 %) with 18.81 mg/100g possessed highest followed by T_8 (BCJ 50% +Water 60%) with 17.73 mg/100g and found to be on par. The lowest score was recorded in treatment T_1 (BCJ 30 % + Water 70 %; 9.74 mg/100g).

Total flavonoids (mg/100 ml)

The data (Table 3) regarding total flavonoids was found to be statistically significant between concentrations of black carrot juice. The highest total flavonoid of 81.57 mg/100 ml was found in T_8 (BCJ 50 % + Water 60 %) followed by T_9 (81.34 mg/100 ml). The lowest total flavonoids of 63.97 mg/100 ml were observed in T_2 (BCJ 30 % + Water 60 %).

This is because intensification of black carrot juice concentration increases the amount of total phenol, total flavonoids and tannins in-turn increasing the number of hydroxyl group which readily donate oxygen atom to free radicals and hence stabilize them, likewise the antioxidant activity is varied among treatments to support this Teradal *et al*, 2017 showed that the consumption of finger millet based composite flour brought about a highly significant reduction in blood glucose levels ($P \leq 0.01$). this may be due to the all grains are rich in antioxidants, phenolic compounds, phytate, phyto-estrogens such as lignan, plant stanols and sterols, vitamins and minerals.

Table 3: Effect of different concentrations of black carrot juice on tannins and flavonoids of black carrot ready-to- serve beverage

Treatment	Tannins (mg/100g)	Flavonoids (mg/100ml)
T_1 : 30% BCJ+ 70% Water	9.74^{g}	64.04^{c}
T_2 : 30% BCJ +ES+60% Water	14.45^{e}	63.97^{c}
T_3 : 30% BCJ +ASE+60% Water	15.20^{de}	64.56^{c}
T_4 : 40% BCJ+ 60% Water	11.01^{f}	71.78^{b}
T_5 : 40% BCJ +ES+50% Water	16.59^{bc}	71.96^{b}
T_6 : 40% BCJ +ASE+50% Water	16.15^{cd}	72.98^{b}
T_7 : 50% BCJ+ 50% Water	11.90^{f}	81.19^{a}
T_8 : 50% BCJ+ES+40% Water	17.73^{ab}	81.57^{a}
T_9 : 50% BCJ+ ASE+40% Water	18.81^{a}	81.34^{a}
Mean	**14.62**	**72.60**
S.Em±	4.58	1.09
CD at 5%	1.146	3.203

Note: Columns with different superscripts are significantly different at $p \leq 0.05$ according to Duncan's Multiple Range Test (DMRT)

The above treatment includes the following ingredients in common	Ginger-1ml, Lemon Juice-5ml, Holy Basil Extract -3ml and Aloe Extract -1ml
BCJ: Black Carrot Juice, ES: Edible Sugar, ASE: Aqueous Stevia Extract	

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