Studies on phyto-chemicals of 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 (1750 mg/kg) and extraordinary quality parameters. Their anthocyanin pigment is acylated with p-coumaric, ferulic, p-hydroxy benzoic and sinapic acids and thus more resistant to hydration, light and food pH. The analysis of phytochemicals has shown varied results among the treatments, T_9 {Black carrot juice (BCJ) 50 % + Water 40 %} possessed highest antioxidant activity (76.07 %) total phenol (230.73 mg/100ml), tannin (18.81 mg/100ml). And total flavonoids (81.57 mg/100ml) in T_8 50% BCJ+ES (Edible sugar) +40% Water.

Index Terms: Phytochemicals, Antioxidant activity, Total phenol, Tannin and Total flavonoids

Carrot constitutes a valuable source of health promoting components like carotenes, phenols and flavanoids thus is important in human nutrition. The annual production of carrot is 1379 million tonnes (Anon, 2017) with an area of 87 ha. Orange carrot varieties account for the majority of the area, where as black or purple carrot are still not very well known in the western world, even though they are thought to be much older than orange carrot varieties and are still traditionally grown and consumed in orientel countries such as Turkey, Afghanisthan, Egypt, Pakisthan, India and the Far East (Berry *et al*, 1989). Recently, black carrots (*Daucus carotas* sp *sativus*) have been in focus because of their high anthocyanin content (1750 mg/kg) and extraordinary quality parameters (Kirca *et al*., 2007). Their anthocyanin pigment is acylated with p-coumaric, ferulic, p-hydroxy benzoic and sinapic acids and thus more resistant to hydration, light and food pH. Black carrot extract provides an excellent bright strawberry red shade at acidic pH values and is a good choice for coloring fruit juices and nectars, soft drinks, preserves, jellies and confectionary. Processing of black carrots into products like juice, concentrate, pickle, preserve, cake, and halwa (Sharma *et al*., 2012) are some of the methods to make this important vegetable available throughout the year. Hence, the present study fascinated to investigate physico-chemical and phyto-chemicals of black carrot RTS beverage

Materials and methods

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

Total antioxidants (%)

The results in Table 1 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).

In present data (Table. 1) it was found that black carrot RTS was recorded the highest antioxidant activity (76.07 %) in the treatment T_9 (BCJ 50% + SE 0.2 % + GE 1% +LE 5%+ HBE 3%+ AE 1% +Water 40%). 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 treatment. This result will be anticipated attending to the significantly higher amounts of total phenolics present in the black carrot extracts including anthocyanins that have been widely reported to have good free radical scavenging capacities (Azevedo *et al*., 2010). Also the various medicinal plants and their extracts used in the present study also contributed to the antioxidant activity because of the presence of high phenols in them (Kumar et al., 2010). Also, All grains are rich in antioxidants, phenolic compounds, phytate, phytoestrogens such as lignan, plant stanols and sterols, vitamins and minerals (Teradal *et al*., 2017).

Total phenol (mg /100 g)

The data pertaining to total phenol content of black carrot RTS as influenced by treatments is presented in Table 1. 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. Teradal *et al*., 2017 studied the better biochemical outcomes predict a health promoting role of the combination of ingredients that were used in the wholesome grain based functional foods. Here in the present study total phenol content of RTS varied significantly among treatments, the maximum phenol content of 230.73 mg GAE/g was recorded in the treatment T_9 (BCJ 50%+ SE 0.2 %+GE 1%+LE 5%+HBE 3%+AE 1% +Water 40%: 230.73mg GAE/g) this was in confirmation with total phenolic contents in the raw black carrot presented by Sezen *et al*. (2014) and was found to be 2526±45 mg GAE per 100 g dry weight this is because of increased concentration of raw black carrot juice has increased phenol content, this difference is partly due to the presence of anthocyanins that occur in large amounts in black carrot extracts (Algarra *et al*., 2014) . Harish *et al*., 2022 study in confirm with results that the total phenol content and per cent antioxidant activity of the cookies was increased with the increasing level of incorporation of pomegranate seed powder.

Table 1: 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 2) 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). Increased tannin is might be due to presence of polyphenols in black carrot. Tannins are an important component of berry fruits. In berry fruits, the largest quantity of condensed tannins with a high degree of polymerization is found in the chokeberry and small quantities of tannins are found in the honeyberry and the blackberry (Seeram *et al*., 2002).

Table 2: 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	

Total flavonoids (mg/100 ml)

The data (Table 2) 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 %). The total flavonoids were supported by the study by (Mehriz *et al*., 2013) where raw black carrot juice was noted to be 118±12 mg catechin/100 mL juice. Flavonoids were found high in black carrot flavoured yoghurts and 22.14 ± 1.32 mgQUE/100g flavonoid was noticed in black carrot by Frond *et al*. (2019).

Conclusion: The analysis of phytochemicals has shown varied results among the treatments, T_9 {Black carrot juice (BCJ) 50 % + Water 40 %} possessed highest antioxidant activity (76.07 %) total phenol (230.73 mg/100ml), tannin (18.81 mg/100ml). And total flavonoids (81.57 mg/100ml) in T_8 50% BCJ+ES (Edible sugar) +40% Water.

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