Analysis of Anthocyanins, Carotenoids and Colour Degradation of European Blackberry (*Rubus fructicosus* L.) of Kashmir Valley

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Abstract
European Blackberry (*Rubus fructicosus* L.) is a prickly, sarmentose shrub found in a variety of habitats including woodlands, hedgerows, gardens, roadsides, wastelands, etc. The objective of the present study was to evaluate the dye yielding potential of the fruits of European Blackberry of Kashmir valley. The stability and colour quality of pigments (anthocyanins and carotenoids) were assessed by quantifying the pigments and recording their colour quality at 10 days interval at 0, 10, 30, 40, 50, 60, 70, 80 and 90 days after collection under ambient and refrigerated storage conditions. Results revealed that the total anthocyanin content (mg/100 g) and total carotenoid content (mg/100 g) were in a range of 521.04 to 1109.97 mg/100 g and 0.125 to 0.943 mg/100 g. Highest pigment content was recorded during 0 days of storage. Under ambient conditions plant material perished due to fungal attack. With increase in days of storage pigment content decreased in both plant material as well as extract. Pigment degradation was more in the plant material as compared to the plant extract. Colour quality of the plant species changed with increase in days of storage. From the present study it was concluded that European Blackberry fruits contained superior levels of anthocyanin content, thus it can be used as a raw material for extraction of reddish to pink edible food colours.
Keywords: Anthocyanin, blackberry, carotenoids, hunter lab, *Rubus fructicosus* L.

Introduction
European blackberry (*Rubus fructicosus* L.) is a prickly, sarmentose shrub found in a variety of habitats including woodlands, hedgerows, gardens, roadsides, wastelands, etc. Stem is arched, obtusely angled, channeled with stout prickles which are scattered and hooked. Branches are robust with alternate 3 or 5 orbicular obovate or elliptic obtuse or acute leaflets. Its flowers are in dense thiyrsoid terminal panicles with obovate petals and are white or pinkish white in color. Fruits are fleshy, black drupes crowded on receptacle **(Figure 1)**. The blackberry is a highly appreciated fruit due to its intense acid-sweet taste and color ranging from dark red to black, it has a high content of phenolic compounds mainly anthocyanins (Wang *et al.*, 2008; Koca and Karadeniz, 2009; Acosta-Montoya *et al.*, 2010). Among the anthocyanins, the gallic and ellagic acids stand out (Sellappan *et al.*, 2002) and in smaller amounts the epicatechin (Hassimotto *et al.*, 2008) and quercetin (Wang *et al.*, 2008; Hassimotto *et al.*, 2008). Blackberry also possesses high antioxidant capacity (Wang and Jiao, 2000; Halvorsen *et al.*, 2002). Blackberry fruits are

significant sources of polyphenolic compounds in the human diet (Sellappan *et al.*, 2002; Zheng and Wang, 2003).

The colour of a food substance indicates freshness and safety that are indices of good aesthetic and sensorial values (Pritam *et al.*, 2008). In the ancient times wide varieties of food colourants were derived from natural sources-plant, animal or mineral. The British chemist Sir William Henry Perkin created the first synthetic dye, mauveine, in 1856 by oxidizing aniline. By the end of the century, eighty synthetic dyes were produced. With the discovery of synthetic dyes the use of natural food dyes declined in the middle of nineteenth century. Industrialization of the food system, including a rise in food processing, has increased the use of food additives including food dyes as synthetic food dyes are less expensive and have intense hue than natural edible dyes but have general toxicity (Carvalho, 1992). Synthetic food dyes and other additives may contribute to hyperactivity and other disturbed behavior in children (Feingold, 1975; Pollock and Warner, 1990; Weiss, 1980). Interest in anthocyanin-rich foods and extracts has intensified because of their possible health benefits. Anthocyanins are potent antioxidants and may be chemoprotective. The increased stability of these pigments together with their added value due to potential beneficial effects opens a new window of opportunities for use of these extracts in a variety of food applications. Anthocyanins represent an option for use, with the potential to substitute artificial red dyes, since they present brilliant, attractive colors, besides possessing several recognized functional properties (Ribeiro *et al.*, 2005). In view of harmful impact of synthetic dyes on human health and environment and beneficial effect of natural food colourants, the present investigation was carried out to study the natural dye yielding potential of European Blackberry (*Rubus fructicosus* L.) of Kashmir valley **(Figure 3).**

Material and Methods

The experimental material, comprising of the fruits of European Blackberry (*Rubus fructicosus* L.) were collected from the local forest areas of Srinagar, Kashmir.

Total anthocyanin content (mg/100g)

Anthocyanins were calculated as per the method of Rangana (1986).

Calculations

The total anthocyanin content was calculated using following equations:

$$\textbf{Total optical density} = \frac{\textbf{Optical density X Volume made}}{\textbf{Weight of sample}} \times 100$$

$$\textbf{Total anthocyanin content (mg/100 g)} = \frac{\textbf{Total optical density}}{\textbf{98.20}} \times 100$$

Total carotenoid content (mg/100g)

Total carotenoids were estimated as per Arya (1981). To remove the interference of the chlorophyll during carotenoid estimation, the filtrate obtained at the end of the above procedure was evaporated to dryness and the residue was dissolved in the minimum quantity (10 ml) of ethanol. Afterwards, 60% of

Figure 1: Blossom and fruit of European Blackberry (*Rubus fructicosus* L.)

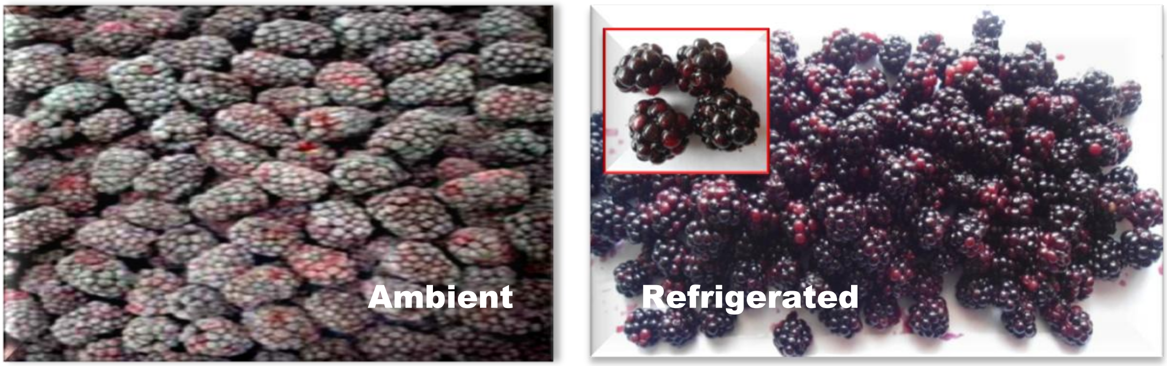

Figure 2: European Blackberry (*Rubus fructicosus* L.) under ambient (12-28 ^{o}C) and refrigerated (4 ^{o}C) storage conditions

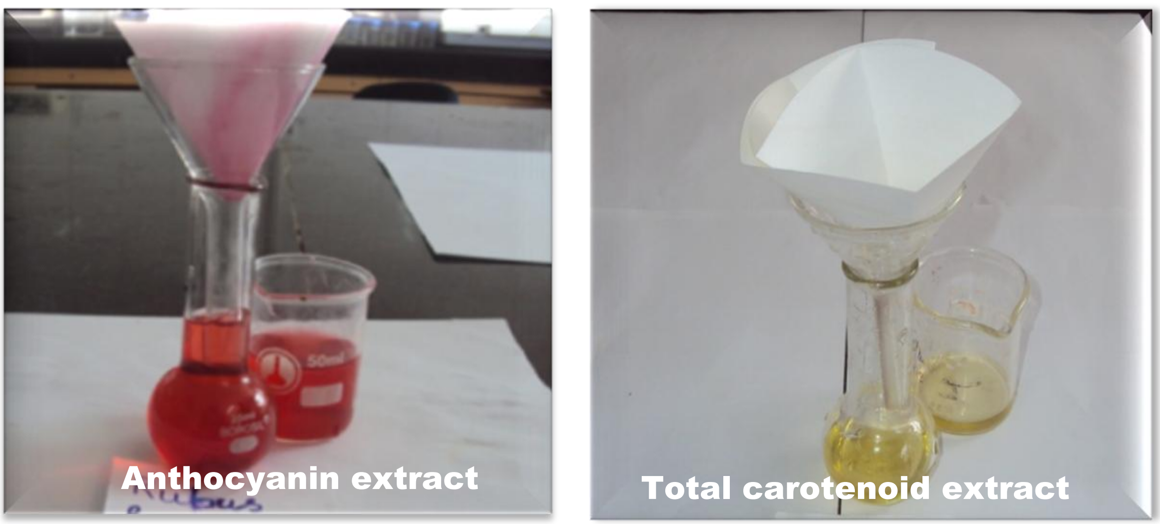

Figure 3: European Blackberry (*Rubus fructicosus* L.) extracts; Analysis of anthocyanins & carotenoids and colour degradation of European Blackberry (*Rubus fructicosus* L.) of Kashmir valley

KOH (1ml for 10ml) was added to it. The whole mixture was boiled for about 5-10 minutes. The equal amount of distilled water was added to it and the solution obtained was partitioned twice with petroleum ether. The combined ether layers were evaporated and the residue obtained was dissolved in 25 ml hexane. A pinch of anhydrous sodium sulphate was added to it and resultant mixture was filtered through Whatman No.1 filter paper and the optical density (O.D) at 449 nm was recorded using hexane as blank.

Calculations

The total carotenoid content was calculated using following equation:

$$\textbf{Total carotenoid content (mg/100 g)} = \frac{\textbf{Optical density x Volume made}}{\textbf{250 x Weight of sample}} \textbf{ x 100}$$

Evaluation of CIE L*a*b* values

CIE L*a*b* values of the dyed and undyed fabrics was determined by chromometer (Model CR-2000, Minolta, Osaka, Japan) equipped with 8 mm measuring head and AC illumination (6774 K) based on CIE system (International Commission on Illumination). The meter was calibrated using the manufacturer's standard white plate. L*, a* and b* coordinates, Chroma (C*) and hue angle (H^o) values were calculated by the following equations:

Chroma = $(a^{*2} + b^{*2})^{1/2}$

Hue (h°) = $\tan^{-1}(b^*/a^*)$

Total color change was calculated from the L*, a* and b*coordinates by applying the following equation:

Total Color change (ΔE) = $[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$

Where, ΔL^* = L* sample - L* standard

Δa^* = a* sample - a* standard

Δb^* = b*sample - b*standard

Results and Discussion

Anthocyanin content (mg/100g)

Anthocyanin content in European Blackberry (*Rubus fructicosus* L.) ranged between 521.04 to 1109.97 mg/100g (**Table 1 and Figure. 4 A**). Highest pigment content (1109.97 mg/100g) was recorded on day 0 of storage which is lower than the value 2199.00 mg/100 g reported by Bowen-Forbes *et al.* (2010) in *Rubus* sp. Further, Torre and Barritt (1977) reported anthocyanin contents of 0.23-0.59 mg/g in Red Raspberries (*Rubus idaeus* L.) and 1.09 mg/g in Marionberries (*Rubus ursinus* Cham and Schldl.). Under ambient conditions plant material perished due to fungal attack, however under refrigerated conditions pigment content ranged between 521.04 to 1109.97 mg/100g **(Figure 2).** In the extract of the species the pigments ranged between 526.13 to 1109.97 mg/100g under ambient storage conditions and 578.74 to1109.97 mg/100g under refrigerated storage conditions. Highest (1109.97 mg/100g) pigment content was recorded during 0days of storage and lowest (521.04 mg/100g) in plant material stored for

90 days under refrigerated storage conditions while as in extract lowest pigment content to the tune of 526.13 mg/100g was recorded under ambient and 578.74 mg/100g under refrigerated storage conditions. Decreasing trend was recorded in **Table 1** and every storage interval is significantly different from eachother. Blackberries are rich source of anthocyanins solely cyanidine-based compounds major anthocyanin being cyanidine-3glucoside. However pigment quantity varies to variation in cultivar, maturity, etc. Siriwohran *et al.* (2004) reported variation in anthocyanin content from 131.00 to 256.00 mg/100g FW in 11 blackberry cultivars. Under ambient conditions plant material of European Blackberry (*Rubus fructicosus* L.) perished due to fungal attack. The results were in consistence with the findings of MinJung *et al.* (2011) who revealed that the shelf life of blackberries is relatively short, 2-3 days at 0°C. Pigments present in the plant material and extract stored under refrigerated conditions lasted longer than the pigments present in the plant material and extract stored under ambient storage conditions. In European Blackberry plant material perished under ambient storage, however anthocyanins (521.04 mg/100g) were detected even on 90th day of storage under refrigerated conditions.

The drastic decrease of anthocyanins was because of rapid degradation. Anthocyanin molecules are unstable and very sensitive to technological processing particularly when heat is involved. Waskar and Khurdiya (1987) have reported degradation of anthocyanin in Phalsanector, concentrate, squash and crush during entire period of storage. Forni *et al.* (1993) reported anthocyanin loss in thermal processing and storage in osmodehydrated and pasteurized cherries. Similar findings have been reported by Siddiq *et al*. (1994) in plum juice, Uygan and Acar (1995) in cherry nectar, Iversen (1999) in black currant nectar, Gimenez *et al.* (2001) in strawberry jam, Kmiecik *et al.* (2001), in bilberry and strawberry jam, Kim and Pandilla (2004) in cherry, plum and raspberry. Anthocyanin degradation during refrigerated storage is believed to be due to native enzymes, particularly polyphenoloxidase.

Total carotenoid content (mg/100 g)

In fruits of European Blackberry (*Rubus fructicosus* L.) the total carotenoid content ranged between 0.125 to 0.943 mg/100g. Ferreira *et al.* (2010) reported low total carotenoid contents of 86.5 µg/100 g or 0.086 mg/100g in *Rubus* spp. grown in Brazil. The highest total carotenoid content (0.943 mg/100g) was recorded during 0 days of ambient storage. Under ambient storage conditions the plant material got perished due to fungal attack. Under refrigerated storage conditions it ranged between 0.125 to 0.943 mg/100g. In the extract of the species the pigments ranged between 0.213 to 0.943 mg/100g under ambient storage conditions and 0.288 to 0.943 mg/100g under refrigerated storage conditions. Highest (0.943 mg/100g) pigment content was recorded during 0 days of storage and lowest (0.125mg/100g) on 90th day of refrigerated storage while as in extract lowest pigment content to the tune of 0.213 mg/100g was recorded under ambient and 0.288 mg/100g under refrigerated storage conditions on 90th day of storage (**Table 1 and Figure 4B**). Decreasing trend was recorded in Tables and every storage interval was significantly different from each other.

Color parameters (L*, a*, b*, C* and H°)

The L* value of European Blackberry (*Rubus fructicosus* L.) ranged between 1.68 to 21.79. The L* value showed the increasing trend with increase in storage time in both ambient as well as refrigerated conditions, showing lightening of colour with storage. The a* value ranged between 27.33 to 31.17.

Our values are slightly less than those reported by Patras *et al.* (2009) who reported a* values of 32.24 in the unprocessed purees of strawberry. The a* value showed the decreasing trend with storage in both storage conditions, showing decrease in redness with storage. The b* value ranged between -3.86 to 1.80. The b* value showed the decreasing trend with increase in storage time in both storage conditions. The H^o ranged between 3.31 to 351.96^o. Further the C* ranged between 27.60 to 31.22 showing the decreasing trend reflecting the transformation of the more vivid berries to dull ones in both the storage conditions. ΔE indicating the total colour difference between two samples ranged between 76.05 to 96.16, it showed the decreasing trend with storage. The total colour difference (ΔE) between plant material stored under refrigerated conditions was smaller than that stored under ambient conditions. Under ambient storage conditions the plant material perished. The decline in a* values in blackberries is due to anthocyanin degradation because of enhancement of the activity of PAL (Phenylanaline ammonia- Lyase) and flavonoid glucosyltransferase (GT). Same finding was reported by Given *et al.* (1988).

Trend of hue angle (H^o) changes

The hue angle (H^o) of the selected plant species during 0 and 90 days of storage under ambient and refrigerated are shown in **Table 2**. The less changes in hue angle (H^o) were recorded under refrigerated conditions as compared to the ambient storage conditions in selected plant species. Thus, among the two storage conditions, the refrigerated condition is the best condition for maintaining the hue angle (H^o).

Table 1: Changes in anthocyanin content (mg/100 g) and total carotenoid content (mg/100 g) at different storage intervals of plant material and extract of European Blackberry (*Rubus fructicosus* L.) under ambient (12-28 ^{o}C) and refrigerated (4 ^{o}C) storage conditions

Storage intervals (days)	European Blackberry (*Rubus fructicosus* L.)							
	Anthocyanin content (mg/100 g)				Total carotenoid content (mg/100 g)			
	Plant material		Extract		Plant material		Extract	
	Ambient	Refrigerated	Ambient	Refrigerated	Ambient	Refri-gerated	Ambient	Refri-gerated
D_0	1109.98	1109.97 (33.33)	1109.97 (33.33)	1109.97 (33.33)	0.943 (1.393)	0.943	0.943	0.943
D_{10}	-	1081.12 (32.89)	1093.00 (33.08)	1103.19 (33.23)	0.000 (1.000)	0.818	0.848	0.878
D_{20}	-	1052.27 (32.45)	1055.66 (32.51)	1070.94 (32.74)	0.000 (1.000)	0.748	0.792	0.812
D_{30}	-	1006.45 (31.74)	1025.11 (32.03)	1037.00 (32.22)	0.000 (1.000)	0.632	0.703	0.753
D_{40}	-	940.25 (30.68)	953.83 (30.90)	967.41 (31.12)	0.000 (1.000)	0.510	0.622	0.682
D_{50}	-	858.79 (29.32)	854.70 (29.23)	868.97 (29.50)	0.000 (1.000)	0.420	0.497	0.603
D_{60}	-	795.99 (28.23)	807.87 (28.44)	826.54 (28.77)	0.000 (1.000)	0.338	0.417	0.525
D_{70}	-	734.89 (27.13)	745.07 (27.31)	765.44 (27.68)	0.000 (1.000)	0.257	0.348	0.460
D_{80}	-	638.15 (25.28)	643.24 (25.38)	665.31 (25.81)	0.000 (1.000)	0.183	0.283	0.365
D_{90}	-	521.04 (22.85)	526.13 (22.96)	578.74 (24.08)	0.000 (1.000)	0.125	0.213	0.288
C.D ($p \leq 0.05$)	-	**0.326**	**0.189**	**0.161**	**0.004**	**0.037**	**0.033**	**0.034**

***Figures in parentheses are square root transformed means**

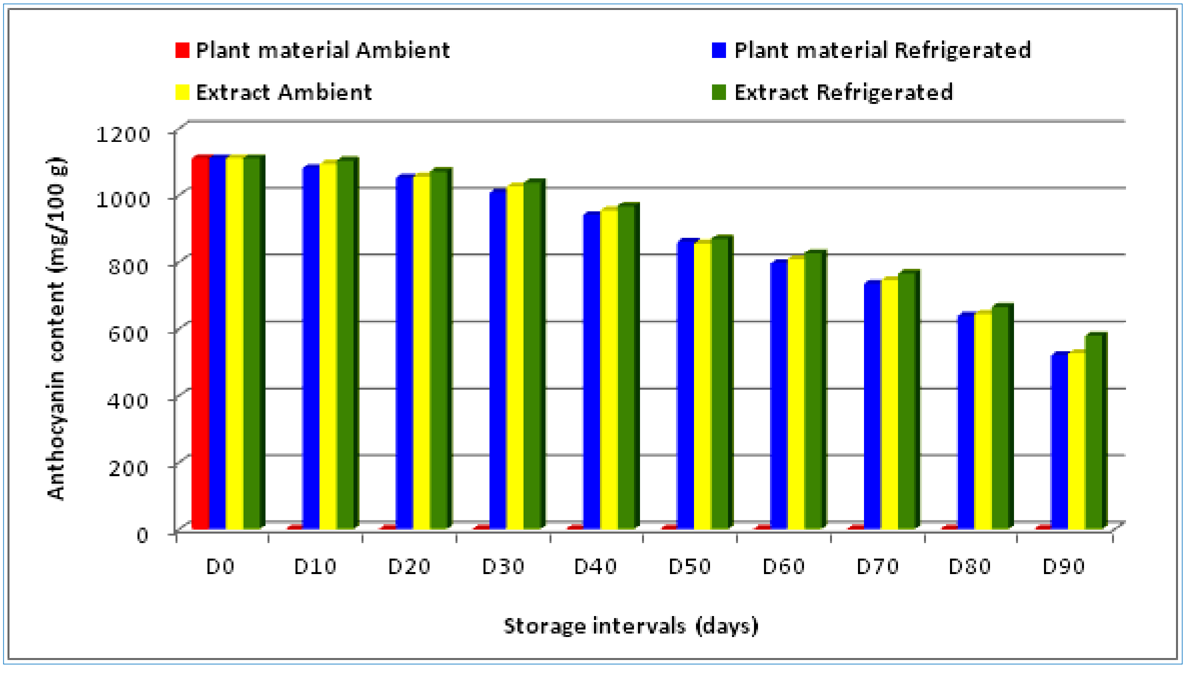

Figure 4. A: Changes in anthocyanin content (mg/100 g) at different storage intervals of plant material and extract of European Blackberry (*Rubus fructicosus* L.) under ambient (12-28^{o}C) and refrigerated (4^{o}C) storage conditions

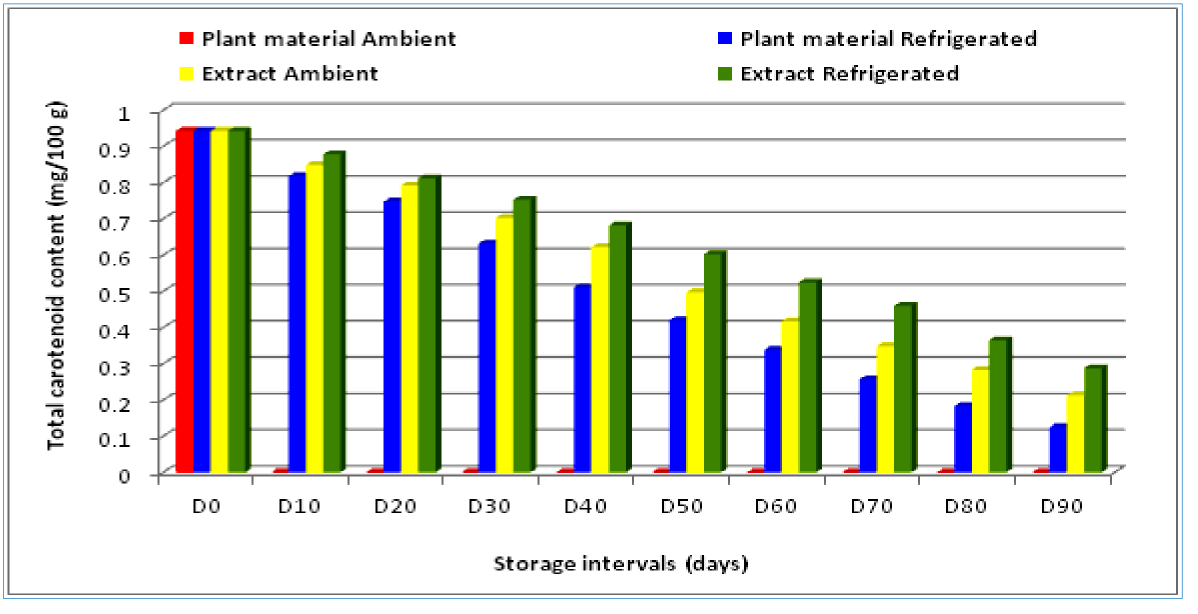

Figure 4. B: Changes total carotenoid content (mg/100 g) at different storage intervals of plant material and extract of European Blackberry (*Rubus fructicosus* L.) under ambient (12-28^{o}C) and refrigerated (4^{o}C) storage conditions

Table 2: Chromaticity of the European Blackberry (*Rubus fructicosus* L.) under ambient (12-28 °C) and refrigerated (4°C) conditions

Storage intervals (days)	European Blackberry (*Rubus fructicosus* L.)											
	Color coordinates						Hue angle (H°)		Chroma (C*)		Total color change (ΔE)	
	L*		a*		b*							
	A	R	A	R	A	R	A	R	A	R	A	R
D_0	1.68 (1.63)	1.68	31.17 (5.67)	31.17	1.80 (1.67)	1.73	3.30	3.31	31.22	31.22	96.16	96.16
D_{10}	0.00 (1.00)	2.32	0.00 (1.00)	30.69	0.00 (1.00)	1.50	0	2.78	0	30.73	0	95.39
D_{20}	0.00 (1.00)	3.96	0.00 (1.00)	30.49	0.00 (1.00)	1.27	0	2.35	0	30.52	0	93.77
D_{30}	0.00 (1.00)	6.19	0.00 (1.00)	30.17	0.00 (1.00)	1.06	0	-0.09 (359.91)	0	30.17	0	91.55
D_{40}	0.00 (1.00)	8.54	0.00 (1.00)	29.89	0.00 (1.00)	1.15	0	-0.17 (359.83)	0	29.89	0	89.25
D_{50}	0.00 (1.00)	11.99	0.00 (1.00)	29.47	0.00 (1.00)	-1.44	0	-3.20 (356.80)	0	29.52	0	85.88
D_{60}	0.00 (1.00)	14.50	0.00 (1.00)	29.00	0.00 (1.00)	-1.85	0	-4.16 (355.84)	0	29.08	0	83.38
D_{70}	0.00 (1.00)	18.76	0.00 (1.00)	28.55	0.00 (1.00)	-2.70	0	-5.56 (354.44)	0	28.69	0	79.27
D_{80}	0.00 (1.00)	20.80	0.00 (1.00)	28.08	0.00 (1.00)	-3.34	0	-6.60 (353.40)	0	28.27	0	77.22
D_{90}	0.00 (1.00)	21.79	0.00 (1.00)	27.33	0.00 (1.00)	-3.85	0	-8.04 (351.96)	0	27.60	0	76.05
C.D($p \leq 0.05$)	**0.021**	**0.177**	**0.007**	**0.129**		**0.169**						

A= Ambient; R = Refrigerated

Conclusion

From the present study it could be concluded that European Blackberry fruits contained superior levels of anthocyanin content, thus it is recommended to be used as a raw material for extraction of reddish to pink edible food colours when stored in extract form under refrigerated conditions. However further research must be done in identifying the new areas of demand and application.

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