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NİĞDE ÖMER HALİSDEMİR ÜNİVERSİTESİ

T.C.
NİĞDE ÖMER HALİSDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF PLANT PRODUCTION AND TECHNOLOGIES

INVESTIGATION OF BIOCHEMICAL DIFFERENCES BETWEEN COMMERCIAL CULTIVAR DEEP PURPLE AND LOCAL GENOTYPES OF BLACK CARROTS

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MUHAMMAD YASIR NAEEM

Master Thesis

Supervisor

Assistant Professor Dr. Şenay OZGEN

July 2017

Muhammad Yasir Naeem tarafından **Yrd. Doç. Dr. Şenay Ozgen** danışmanlığında hazırlanan **"Investigation of Biochemical Differences between Commercial Cultivar Deep Purple and Local Genotypes of Black Carrots"** adlı bu çalışma jürimiz tarafından Niğde Ömer Halisdemir Üniversitesi Fen Bilimleri Enstitüsü **Bitkisel Üretim ve Teknolojileri** Ana Bilim Dalı'ndaYüksek Lisans tezi olarak Kabul edilmiştir.
(The study titled **"Investigation of Biochemical Differences between Commercial Cultivar Deep Purple and Local Genotypes of Black Carrots"** and presented by **Muhammad Yasir Naeem** with the help of supervisor **Asst. Prof. Dr. Şenay Ozgen,** has been found as Master thesis by the jury at the Department of **Plant Production & Technologies** of Nigde Ömer Halisdemir University Graduate School of Natural and Applied Sciences.)

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THESIS CERTIFICATION

I certify that the thesis has been written by me and that, to the best of my knowledge and belief. All information presented as part of this thesis is scientific and in accordance with the academic rules. Any help I have received in preparing the thesis, and all sources used, have been acknowledged in the thesis.

Muhammad Yasir NAEEM

ÖZET

DEEP PURPLE (F1) SİYAH HAVUÇ ÇEŞİDİ VE YEREL GENOTİPLERİN KİMYASAL FARKLILIKLARININ BELİRLENMESİ

NAEEM, MUHAMMAD YASIR

Niğde Ömer Halisdemir Üniversitesi

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Türkiye de havuç üretimi Orta Anadolu da (Konya, Karaman and Ankara), Akdenizsahilinde (Burdur, Antalya, Mersin and Hatay), Egebölgesinde (İzmir, Aydin and Manisa) ve Güney Marmara da yaygın bir şekilde yapılmaktadır. Ancak, siyah havuç üretimi Konya ve Ereğli de oldukça yaygındır. Üretimin çoğu sanayiye yöneliktir. Bu bölgede çiftçiler kendi tohumlarını kendileri üretmektedirler. Havuç kökleri arasındakişekil, renk ve aroma bakımından homojenlik endüstriye yönelik yetiştiricilik yapıldığından çok önemli kriterler değildir. Turuncu havuçlarla karşılaştırıl dığında tat, aroma ve tekstür açısından, aynı zamanda, yerken elde bıraktığı renkten dolayı taze tüketimde talep edilmemektedir. Bu bölge de bulunan üreticiler sanayiye yönelik yetiştiricilik yapmalarına ragmen Hollandalı Bejo firmasının Deep Purple (F1) çeşidinin yetiştiriciliğine başlamışlardır. Bu çalışmada Konya vecivarında yetiştirilen genotiplerden kök örnekleri ile F1 çeşidinden kök örnekleri toplanmış ve -80˚C de depolanmışlardır. Genotipler farklı tohum kaynaklarından üretilmiş 16 farklı genotipten alınmıştır. Deep Purple (F1) çeşidinden ise 3 farklı alanda yetiştirilmiş köklerden alınmıştır. Analizler için her genotipten 3 veya 5 tekkerrurde ayrı ayrı analizler yapılarak genotipler arasındaki farklılıklar belirlenmiştir.

Anahtar Sözcükler: Daucus carota, antioksidan, fenolik, radikalsüpürmegücü, β-karoten, trolox, DPPH, protein analizi, total RNA analizi, antosiyanin, CUPRAC

SUMMARY

INVESTIGATION OF BIOCHEMICAL DIFFERENCES BETWEEN COMMERCIAL CULTIVAR DEEP PURPLE AND LOCAL GENOTYPES OF BLACK CARROTS

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Carrot production in Turkey is widely in the region of Central Anatolia (Konya, Karaman and Ankara), Mediterranean (Burdur, Antalya, Mersin and Hatay), Aegean (Izmir, Aydin and Manisa) and South Marmara (Anonyms 2009). However, most of the black carrot production is in Konya-Eregli region. The production is mainly for black carrot industry. The region has its own seed sources where growers are producing seeds from their root production fields. Uniformity of the color, root shape or aroma is not very important since black carrot is being grown for industry. Due to taste, texture and undesirable color stay on consumer hand black carrot is not suitable for fresh table production. Although, in Konya region some growers already started to use Deep Purple (F1) black carrot seeds that is breed in Holland, company Bejo. In this study, we collected the roots coming from domestic genotypes and Deep Purple to compare root quality in term of the chemical composition. We collected them from the domestic genotypes according to different seed sources. Root of the Deep Purple and 16 genotypes was collected from Konya for the analysis. On the basis of differences developed among root material, grower will decide which seed to use depending on target of their production. As it's known that hybrid seeds are much more expensive than domestic seed sources.

Keywords: Daucus carota, antioxidant, phenolic, radical scavenging activity, β-carotene, trolox, DPPH, protein analysis, total RNA analysis, anthocyanin,

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SYMBOLS AND ABBREVIATIONS

Symbols	Description
Na	Sodium
K	Potassium
Ca	Calcium
Cu	Copper
Fe	Iron
Mg	Magnesium
Mn	Manganese
P	Phosphorus
Se	Silicon
Zn	Zinc

Abbreviations	Description
TSS	Total Soluble Solıd
TEAC	Trolox Equivalent Antioxidant Capacity
FRAP	Ferric Reducing Ability of Plasma

CHAPTER I

INTRODUCTION

Carrot (*Daucus carota L.*) belongs to parsley family. Member of parsley family have generally small umbrella like inflorescence containing flowers called umbel (Essig, 2013). Almost 5000 years ago, carrots were firstly cultivated in Afghanistan, Pakistan and Iran and spread out towards the region of Mediterranean (USDA, 1989). Until the mid of 15th century carrots were spread out to different regions of world i.e. North Africa, Middle East China and Europe. Major carrot yield comes from California.

Carrot is multipurpose vegetable easily available in the form of frozen, canned products and as a fresh vegetable. It was the first vegetable that commercially canned. The role of fruit and vegetables in our diet is important contribution to the nutritional value of vitamins and minerals that make up the protective nutrients (Okpanachi, 2005). Carrot is placed at 10th position on the basis of nutritive value among the different 39 fruits and vegetables (Acharya et al.,, 2009).Carrot is the basic source of antioxidants, vitamin A and C and also free from fat, cholesterol and low in sodium (Pool-Zobelet al., 1998). Currently, it has been reported that initial cancer can be treated with the help of fresh carrot juice (El-Abasy et al., 2012). It has high nutritive value, vitamin B-complex (B6) help in the production of different hormones and chemicals as melatonin and serotonin that are important in sleeping and stability conditions. Also accomplish the function of immune system and metabolic process (Wood-Moen, 2011). Also, carrot juice helps tooth growth in children (Capstone, 2005).

World production of carrots in different countries is around 37,226,640 tons per annum (Figure 1). Turkey produce 569,855 tons in 25,901 ha area in the world which Turkey is the 10th major carrot producing country (FAO, 2013).

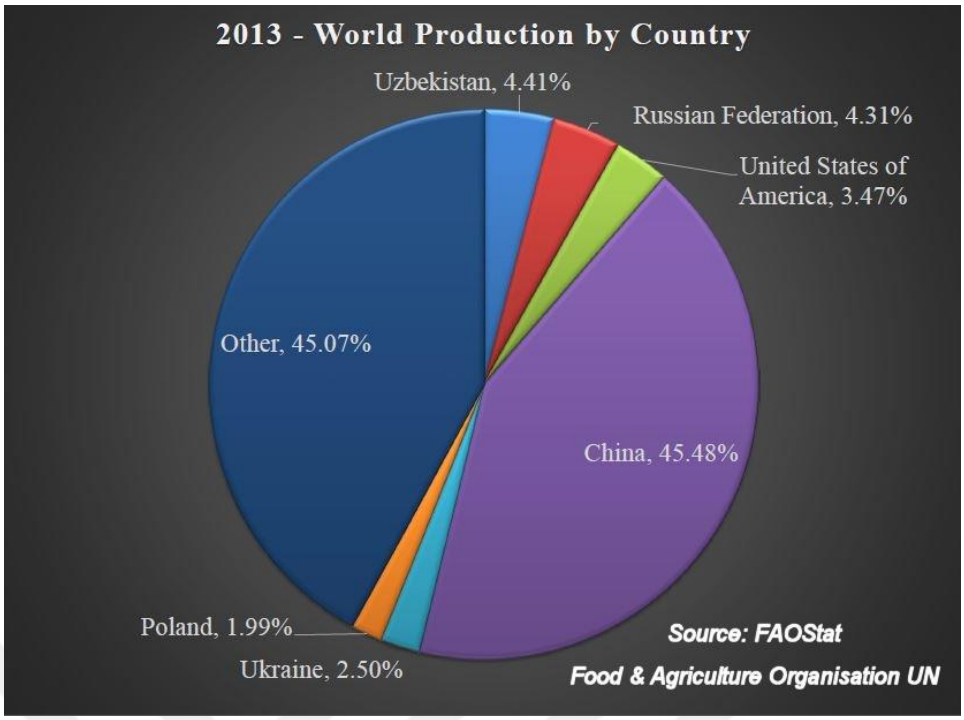

Figure 1.1. Carrots producing countries.

There are several carotenoid pigments that are found in the root fragments of the carrot which give different colors. These carotenoids are split into different pigments as Xanthophyll (golden color), lycopene (red color), anthocyanins (purple or black color), and orange (due to pigment called lutein), White carrot lacks these color pigments.

Black carrots contain many important nutrients like folates, niacin, thiamine, riboflavin, vitamins, carotenoids, anthocyanin etc. as presented in Table 1.1.

Table 1.1. Percentage of nutrients in black carrots (per 100 g, w/w,USDA National Nutrient Data Base)

Principle	Nutrient Value	Percentage of RDA
Energy	41 Kcal	2%
Carbohydrates	9.58 g	7%
Protein	0.93 g	1.5%
Total Fat	0.24 g	1%
Cholesterol	0 mg	0%
Dietary Fiber	2.8 g	7%
Vitamins	**Vitamins**	**Vitamins**
Folates	19 µg	5%
Niacin	0.983 mg	6%
Pantothenic acid	0.273 mg	5.5%
Pyridoxine	0.138 mg	10%
Riboflavin	0.058 mg	4%
Thiamin	0.066 mg	6%
Vitamin A	16706 IU	557%
Vitamin C	5.9 mg	10%
Vitamin K	13.2 µg	11%

Black carrot is an important nutrient source due to the presence of 1750 mg/kg high anthocyanin content (Khandare et al., 2011). Black carrot has been cultivated in Turkey for at least 3000 years which shows that black carrot has an origin from Turkey and Middle East (Montilla et al., 2011). Black and red carrots have been cultivated in Europe since middle age and continue until 18th century. In that time one of the leading varieties of carrot was orange varieties. In recent years black carrots found cultivation in many countries as Pakistan, Turkey, Afghanistan and Far East.(Kammereret al., 2003). Today's, orange carrot is more mutual but the consumption of black carrot and its by products are also increasing day by day (Algarra et al., 2014).

Phenolic compounds are secondary metabolites, abundant in fruits and vegetables and due to their antioxidant properties and potential beneficial health effects are considered

a vital portion of the human food. These compounds vary from a modest phenolic molecule to complex high molecular weight polymers. There are many reports that intake of a diverse range of phenolic compounds found in diets may give less the hazard of human health complaints as of their antioxidant activity. The phenolic profilesare present in the food where they control rancidity development, reduced formation of toxin products, prolong the shelf-life of products and regulate nutritional quality (Shahidi and Ambigaipalan, 2015). They are synthesized by plants to play role producing chemicaldefense against herbivoresand/or participate in reproduction and in plant-plant interaction (Velderrain-Rodríguez et al., 2014).

Recently, phenolic compounds have receivedsignificantimportanceowing to the fact thattheir intake can be associatedwithlowerincidenceof many chronic diseases, such as Alzheimer's, cancer, and heart diseases (Gutiérrez-Grijalva et al., 2016).Phenolic compounds are sub divided into synthetic and natural based on their origin. Synthetic phenolic compounds are currently registered for use in food is butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propylgallate (PG) and tertiary-butylhydroquinone (TBHQ). These synthetic phenolic antioxidants prevent or delay the onset of lipid oxidation during storage and processing of lipid containing food (Makahleh et al., 2015;Saad et al., 2007). Natural phenolic antioxidants are found in fruits and vegetables produce in all parts of the plant. The naturally occurring compounds are terpenes, tocopherols, phospholipids, lignans and phenolic acids (Wanasundara et al., 1996, Blekas et al., 1995). Carrots are rich in natural dietary components, are well known for their high phenolic contents (Uzel, 2016). The most important phenolic compounds are chlorogenic acid,ferulic acid, p-hydroxybenzoic acid and caffeic acid (Alasalvar et al., 2001). Among them, chlorogenic acid is an important hydroxycinnamic acid which signifies 42.2-61.8% of total phenolic compounds existing in various carrots.The phenolic profiles in various tissues of carrot accounted in peel, phloem and xylem 54.1%, 39.4% and 6.4% respectively (Zhang and Hamauzee, 2004).

Nowadays, natural food colorant is gaining popularity in food industry (Kammerer et al.,2003). Anthocyanins, the rich group of pigments, are responsiblee for the different colors of fruits, flowers and vegetables (primarily red, blue, and purple). Anthocyanins, phenolic compound often found in the plant kingdom which found to be the main set of

water-soluble pigments commonly related by red fruits are also found in vegetables, cereals and roots. (Maza and Minati, 1993, Kammerer et al.,2004a). Intrest in anthocyanins increased due to their part in decreasing the incidence in various diseases like atherosclerosis, cancer, diabetes and neurodegenerative disorders (Wrolstad, 2004). Black carrots have attracted researchers for their maximum anthocyanin contents and astonishing quality valueparameters (Kirca et al., 2006).The Anthocyanins pigment contains *p*-coumaric, *p*-hydroxybenzoic, ferulic and sinapic acids and hence less prone towards light, hydration and pH of food (Kammerer et al.,2004b). Anthocyanin in black carrot can be used as a source of different natural colorants like blue, purple and red pigments owing to their pH, heat and light stability (Khandare et al., 2011; Cevallos-Casalset al., 2004, Reinetal, 2004, Bakowska-Barczak, 2005). Many experiments have been performed which directly shows the importance of these chemicals related to health benefits and biological effects all of these are good source of awareness for scientists. Total 539 anthocyanins has been reported that insulated from plants but six are very important like as;delphinidin, cyanidin,pelargonidin, malvidin.petunidin and peonidin(Andersen and Jordheim, 2006). The foremost anthocyanins are derivative from cyanidin, being basically acylatedin black carrots. (Schwarz et al., 2004).Different extraction techniques from black carrots have received significant attentions substituents to artificial colorants because of the function of more pH and heat stable, less toxic food colorants. Natural additives, extracted from plant sources also known as anthocyanins are replacing artificial additives in food industries (Kirca et al., 2006).

Carotenoids are produced secondary metabolites during metabolism of lipids .They play crucialroles in plants are important compounds from nutritious point of view (Etohet al., 2000; Grobushet al., 2000). It receive great intrest because they projected antioxidant assets and as ordinary pigments (primarily orange-red)rich in most vegetables and fruits, which make an key quantity of the human diet (Edge et al., 1997; Meléndez et al., 2014). An intensive study on carotenoids explored the role of their pigments, which play an key role against disorders related with aging, including cancer,heart disease, (Bowen et al., 2015, Bermudez et al., 2005,Pantavos et al., 2015).Black carrots enriched with important nutrients, contain the carotenoids, like α-carotene, lutein and β-carotene (Riccioni,2009; Habibiet al.,2011; Wanget al.,2013).

The extraction of different compounds like total phenolic contents, antioxidants, β-carotene, anthocyanin and other nutrients has been broadly documented from various vegetables as well as from fruits also but still there exist a wide gap of information regarding the extraction of these compounds from black carrots. Therefore for the first time we are conducting a study to determine the differences between Deep Purple (F1) and local black carrot genotypes. The hypothesis underlying this study is that it will explain the chemical, transcriptional and translational differences among various parameters such as β-carotene, total phenolic profile, total RNA and protein assays, anthocyanin and other different nutrients from both local genotypes and Bejo (F1) black carrots. In addition, growers cultivated black carrots from their own seeds as it is the important part of industry. Deep Purple (F1) is a cultivar of black carrot that is recently cultivated in Konya and Eregli areas. The findings of the study are beneficial for both growers and industry equally and also explains the visible difference between international seeds sources.

CHAPTER II

REVIEW OF LITERATURE

During 2004, Kammerer and his co-workers evaluated color arrangement of 15 black carrot cultivars using HPLC-MS. From seven cyanidin glycosides, five were selected for acylation withhydroxybenzoics acids and hydroxycinnamic acids, followed by identification and quantification in roots using HPLC-DAD. Biochemical analysis of separate compounds revealed significant variances among the anthocyanin accumulation amongvarious cultivars and also among the carrots of the similar cultivars. Totalanthocyanin contents were found in range between 45.4 mg/kg to 17.4 g/kg dry matter. It was the initial study concerning quantification of different types of anthocyanins invarious black carrot roots cultivars. Anthocyanins from black carrot roots can be used as natural food colorants also for little acid food possessions as it was proven by evaluation of color properties in the extracts under various pH, while a significant color loss was observed at nearly neutral pH conditions. Additionally, higher carbohydrate matters remained observed in approximately each cultivar that can be harmful while coloring essences are removed from black carrot roots.

Lavelli et al. (2005), worked onexcellence of slightly treated carrots during storage at 4.5to10°C. They evaluated microbial directoriestotal bacterial count (TBC), coliforms, whiteness index (WI), yeasts; the physicochemical indices chlorogenic acid, carotenoids, sugars, and lactic acid bacteria, sensory attributes, namely: fruity aroma, colour (whitening), and off odor. Correlation among sensual perception of unwanted alterations and the kinetics of quality indices difference was calculated. Carotenoid degradation, microbial impurity was thresholds and physicochemical indices were also studied towards exploration of most important factors playing role in carrot stability during storage. A pseudo-first order and zero-order kinetics triggered the chlorogenic acid concentration and WI increase, respectively. Threshold concentration was approached by TBC and total coliforms in relatively smaller time than the other microbial groups (3 days at 10°C and 7 days at 4°C). Carrots suffered degradation while there was no carotenoid damage throughout storage at (4 to 10°C). Off-odor was observed after 2 days while color and reduction in fruits aroma was found by a skilled

sensual board of evaluators 5 days post storage at 5°C, when carrots reached the microbial contamination threshold. There is no any correlation among the other microbial parameters and physicochemical and sensory attributes. TBC along with the total coliforms permitted assessment of its microbial value while the WI was found to be the maximum sensitive indicator of sensory quality of treated carrot.

Kirca et al. (2005), worked on the effect of temperature (70–90°C) and storage at (4 to 37°C) on stability of carrot anthocyanins in different matrices offruit juices (grape, orange, grapefruitapple, tangerine and lemon) and nectars (apricot, peach and pineapple). First-order reaction kinetics was observed in degrading anthocyanin poverty, in every colored juices and nectars. Apple juice depicted more stability rather than citrus juices on heating, when colored with black carrot anthocyanin. Additionally, higher level of stability was also found in peaches and apricot nectars at higher temperature (70–90°C). However, in orange juice, anthocyanins of black carrots were least stable through together warming and storing. Throughout storing, rapid decreases of anthocyanins werefound at 37°C, particularly in pineapple nectars. Stability increased in all refrigerated samples stored at 4°C. Among samples undergoing degradation of anthocyanins, activation energy in colored juices and nectar ranged from 65.9–94.7 kJ/mol at about 37°C which decreased to 42.1 to 75.8 kJ/mol at higher temperature (70–90°C).

In 2006, Ersus and Yurdagel worked onthe acidified ethanol extracts of carrots. Series of maltodextrins (Stardri 10 (10DE), Glucodry 210 (20–23DE) and MDX 29 (28–31 DE)], at three different air temp with continuous feed solid content 20%high were used to spray dry black carrots containing anthocyanin contents. More anthocyanin contents loss was recorded during spray drying at higher inlet/outlet air temperatures. Further characterization was carried out on the basis of anthocyanin content, antioxidant capacity, L*, a*, b*, C* and H-values, dehydrated matter content and hygroscopicity after their production at optimum drying temperatures (160°C). The Glucodry 210, when used as wall substantial, yielded the best dried pigment containing powder. To monitoring the size (3 to 20 lm) and structures of the powders, scanning electron microscope was utilized. Purees were kept at changed temperatures (4 to 25°C) and

light illumination (3000 lx) to determine the half-life period and stability of microencapsulated pigments.

Marxen and his co-workers at 2007worked on spectrophotometric method with some modification to utilize the 2, 2- diphenyl-1-picrylhydrazyl (DPPH) radical and its specific absorbance capability in a wiser manner. Antioxidants significantly reduce the radicals which in turn decreases the absorbance. Unlike previous studies, the absorbance capability was observed at 550 nm wavelength owing to the fact that this wavelength made the calculation free of micro-algal pigments interference. Two different DPPH concentrations were measured by methanolic microalgae extracts. A linear decline resulting in a saturation region was obtained by the variations in absorbance measured when compared to the amount of the methanol extract. Antioxidative potentials of microalgal methanol extracts independent of the employed DPPH concentrations was measured by linear regression analysis of DPPH reduction vs. extract concentration. Significant differences viz. 6 to 34 μmol DPPH g^{-1} extract concentration was observed among the single different species of microalgae in their capability to reduce the DPPH radical. The independency of a signal has enabled the DPPH concentration an appreciated improvement over the calculation of EC50value.

Fikselová et al. (2008), explains carotenes extraction as of carrot by various situations like, treatment of samples, solvents (ethanol, 2-propanol) and different temperatures. Roots of carrot were examined for yield extraction of carotenes on various temp 20°C, 40°C, and 60°C, the purees were tested later harvest, after cold storagestored at 5°C, and freezing–18°C. It is noted that carotenoids concentration from carrot root was recorded that production rate and solubility depends on time of extraction and temp. Best extraction rate was noted with freeze samples for 2 to 4 hours at 60°C. Maximum temp produced an increase in the carotenoid concentrations. Black carrot variety and the extraction at temp 60°C were used for the model production of carotenoid concentrate.

Özen et al. (2009), conducted aresearch on local Turkish delight (lokum) that was coloured with the black carrot juice concentrate, while storing at altered temp (10, 20 and 30°C) for the period of 5 month, later it was read via spectrophotometer and high performance liquid chromatography (HPLC). Examination of kinetic data recommended a first-order reaction forthe decrease of carrot anthocyanins inlocal lokum. Dilapidation

rates of anthocyanins of black carrot roots increased by increasing temperature. It was noted that the degradation rate of black carrotroots anthocyanins during the storage period at 12°C increased quicker than other temp (20 and 30°C). The kinetic values for 10, 20 and 30°C were recorded to be 6.91 ¥ 10-3, 4.21 ¥ 10-3 and 9.21 ¥ 10-3/day, respectively. Effects of pH on the thermal stability of black carrot anthocyanins were also determined.Results presented that the stability of anthocyanins reduced as the pHwhile, lightness (L*) increased.

During 2011, Khandare et al., verified carrot as a remarkable basis of anthocyanin pigments with maximum antioxidant ability. They evaluated the result of prepress softening treatment on antioxidant activity in black carrot juice with various amounts of cell wall corrupting enzyme pectinase. The antioxidant activity of juice extracted from black carrots were significantly enhanced by enzyme-assisted processing ($p< 0.05$). An overall up-surge i.e. 27% total phenolics, 46% total flavonoids and 33% in juice yield was observed. Approximately double quantity of total anthocyanins in black carrot juice was observed. For black carrot sap,enzyme-assisted processing was used to get theantioxidant activity (30.0 μmol TE/mL) in ferric reducing antioxidant power (FRAP) and 62.0 μmol TE/mL in case cupric reducing antioxidant capacity (CUPRAC) assays was observed *in vitro*. Juice ofblack carrot obtained through enzyme over straight pressed juice depicted 30% increase in total antioxidant activity. Altering of enzymes' blends can give antioxidant rich juice products. They concluded that novel useful drinks by better aesthetic color and more antioxidant activity can be prepared by developing a novel approach for extracting phenolic rich juices from black carrot.

Turkyılmaz et al. 2012, evaluated the impact of pasteurization and clarification on anthocyanins of black carrot juice,Anthocyanin profiling, monomeric anthocyanin and percent polymeric color were calculated during processing of BCJ. Bentonite treatments and depectinisation resulted in 7% and 20% increases in monomeric anthocyanin contents of BCJ, respectively. While pasteurization and gelatine–kieselsol treatment resulted in 10% and 3–16% decrease. Percent polymeric color declined after clarification and gradually started increasing in samples when exposed to heat. Identification of anthocyanin contents in BCJ samples was carried out by HPLC–MS. Major anthocyanin in unclarified BCJ was cyanidin-3-galactoside- xyloside-glucoside-

ferulic acid tracked by cyanidin-3-galactoside-xylosideglucoside- coumaric acid and cyanidin-3-galactoside-xyloside-glucoside. 02 more anthocyanins named cyanidin-3-galactoside-xyloside and cyanidin-3-galactoside-xyloside-glucoside-sinapic acid were also recognized. Concluding, bentonite treatment and depectinisation depicted positive impact on color of BCJ, while pasteurization and gelatin–kieselsol treatment depicted undesirable result.

Algarra et al. (2013),worked on the anthocyanin profiling of two different cultivars of black carrots linked with Antonina and Purple Haze cultivars, from Cuevas Bajas (Ma'laga, Spain) and some of their antioxidant features. The main types of anthocyanins detected by LC–MS were found in association with five cyanidin-based anthocyanins: cyanidin 3-xylosylgalactoside, cyanidin 3-xylosylglucosylgalactoside and the sinapic, coumaric and ferulic acid derivative of cyanidin 3-xylosyl glucosylgalactoside. The anthocyanins in the black carrots were acylated and their contents were found to be 25% in case of Purple Haze and 50% in case of Antonina cultivars of the total phenolic contents. Moreover, the radical scavenging ability (17.6±9.0 and 240.0±54.0 mM TE/100 g fw) and reducing ability of the two black carrot roots extracts (86.4±8.0 and 182.0±27 mM TE/100 g fw) depicted in Trolox equivalents units were determined. The antioxidant features of the orange carrots used for these experiments were significantly lower than those of black carrot extracts.

During 2013,Damian and Oroian conducted experiments on carrotpurée to measure groups of bio-active compound, total antioxidant activity and instrumental color via thermal treatment (70°C/2 min). Synthetic chemical 2,6-diclorophenolindophenol was utilized in the technique used to the dose of ascorbic acid. Folin-Ciocalteau method was used for the determination of total phenols (TP) while antioxidant activity was calculated via DPPH free radical method. Hunter-Lab color meter was used to determine the color of the samples. A sharp decline in ascorbic acid was observed due to heat treatment. While there was no effect of heat on phenolic contents. Heat treatment significantly affected the color parameters. Their experiments provided a useful tactic in understanding the impact of processing on color variation of carrot purée in a broad spectrum.

Assous and his co-workers (2014) extracted,derived and identified anthocyanin contents from purple carrots by using HPLC. Around 168.7 mg anthocyanin/100 g on fresh-weight base was found in purple carrots. Other main elements include cyanidin-3-xylosyl-glucosyl-galactoside acylated by ferulic acid (33.65%) charted by cyanidin-3-xylosyl-glucosyl-galactoside acylated with coumaric acid (29.85%) and cyanidin-3-xylosyl-galactoside (28.70%) when calculated via HPLC. Dextrin, cellulose, soluble starch and glucose were the main transporter for purple carrots anthocyanin pigment followed by respectively. Instead, higher stability in pigment of anthocyanin derivative of purple roots was found at pH values ranging between 1.0 and 4.0 and temperature ranging from 40 and 80°C, while at 100°C the degradation ratio of anthocyanin was 15% of total pigments after 180 minutes. Antioxidant activity of anthocyanin from purple carrots was calculated at 60°C by determiningperoxide value on sunflower oil during 7 days. Lower peroxide value (7.90) was observed in sunflower oil containing 1000 ppm of purple carrots' extract instead of using 200 ppm synthetic antioxidant (BHT) (8.38) meq/kg. Statistical study of processed hard sweetie and sweet jelly depicted that no significant differences among hard candy (0.30%) and sweet jelly (0.20%) along with anthocyanins pigments from purple carrot.

During 2015, Gras and his colleagues were of the view thatlight red hues are added to diets viz. beverages, fruit preparations, and confectionaries via anthocyanin contents from black carrots. According to them, highly efficient techniques can be contributory for producers of food colorants and plant breeders should select more pigmented cultivars of carrot. Keeping in view its significance, they developed and validated an efficient but exhausting ultrasound-assisted procedure for anthocyanins' extraction. Thereby, inactivation of rancidity causing enzymes i.e. peroxidase and polyphenol oxidase was firstly carried out. Yielding coefficients of variation (CVs) for intra-day, inter-day repeatability of ≤4.4 and 4.7% for only 60 seconds were basic requirements of extraction. An efficient UHPLC-PDA technique was established and validated for quantitation of post-extractive black carrot anthocyanin contents. Separation time UHPLC was 4.5 min which was 8.2 times faster with 92% less solvent consumption in contrast to HPLC separation using a core-shell-column. Boundaries of quantitation and detection were quite satisfactory. For one day room temperature, stability of non-

acylated pigments utilized as position compounds were suitable and pigment concentration was undisturbed in 4.5 months when stored at -20°C and -80°C.

During 2015 Bystrickáet al., verifiedthat carrot are rich basis of vitamins (A, B, C, E and H), beta carotene, folic acid and pantothenic acid. They also concluded that carrot are important source of trace elements like K, Na, Ca, Mg, P, S, Mn, Fe, Cu and Zn. Carrots' Consumption can aid in improving eyesight and digestion, on other hand they lower cholesterol. They comparatively worked on ß-carotene, total polyphenols and antioxidant activity in five cultivars of carrot viz. Jitka, Kardila, Katlen, Rubína and Koloseum. Analyses were carried out after homogenizing the samples in 50 ml ethanol (80 %) and keeping them to stay for 16 hours. The Folin-Ciocalteu reagent (FCR) was used to measure total polyphenols contents. The content of ß-carotene was determined spectrophotometricaly at 450 nm. Spectrophotometer was used to measure the antioxidant activity using a compound DPPH˙ (2.2-diphenyl-1-picrylhydrazyl) at wavelength of 515.6 nm. ß-carotenes were ranging between 24.58±2.38 mg/kg and 124.28 ± 3.54 mg/kg while total polyphenols were ranging between 81.25±13.11 mg/kg to 113.69±11.57 mg/kg. Antioxidant activity in selected carrot cultivars varying between 6.88±0.92% and 9.83±0.62% were also evaluated and compared. Significantly higher value of total polyphenols was determined in Koloseum (113.69 ± 11.57 mg/kg). Significantly higher ß-carotenes (124.28 ± 3.54 mg/kg) and antioxidant activity (9.83±0.62 %) was also calculated in this cultivar.

During 2015 Guleret al., found thatcarrot flavor is affected by sugar and acid contents along with Volatile organic compounds (VOCs). For this purpose, they compared VOCs in 11 different cultivars of carrot. For bio-chemical analysis of VOCs, gas chromatography/mass spectrometry utilizing static headspace method was used.A range of 17 to 31 was recorded regarding number of VOCs per sample. With the exception of Yellow Stone cultivar, primary VOCs in raw carrots were terpenes, ranging between 65 to 95%. Sesquiterpenes ranging from 2 to 15% were significantly lower than the monoterpenes with values ranging between 31 and 89%. Main VOC in extracts of nine carrot cultivars were Monoterpene α-terpinolene (ranging between 23 and 63%) and (-)-α-pinene (26%). Out of 16 monoterpenes identified so far, 60% of total VOCs was constituted by 7 monoterpenes (-)-α-pinene, (-)-β-pinene, β-myrcene, d-limonene, γ-

terpinene, α-terpinolene and p-cymene in carrots including “Atomic Red”, “Nantes”, “Cosmic Purple”, “Red Samurai”, “Eregli Black”, “White Satin”, “Parmex” and “Baby Carrot”. Concluding, these cultivars can be attractive for carrot breeders due to the remarkable effects of terpenes, especially monoterpenes on human health.

Uzel(2016) found that subcritical water can be used for extraction of phenolic in the presence or absence of secondary co-solvent from ethanol compounds anthocyanins along with their by-products from highly pigmented garden vegetables. They focused on the research of bioactive compound outline of black carrots. Subcritical water was used in the new capable system for extraction in combination with powdered particle production as well as for the extraction of phenolic compounds in powdered form. At temperatures ranging between 40 to 100°C, water was extracted at 10-20 MPa. Comparison of different methods was also carried out to evaluate the efficacy of different methods in terms of anthocyanin recovery with respect to the extraction parameters used and the product was served to air assisted particle formation system, the influence of $T^0$and pressure on the subsequent particle.

During 2004, Kammerer and his co-workers evaluated 15 black carrot cultivars using HPLC-MS for their pigment composition. From seven cyanidin glycosides, five were selected for acylation with hydroxycinnamic and hydroxybenzoic acids, followed by identification and quantification in the roots using HPLC-DAD. Biochemical analysis of individual compounds revealed significant differences among the potential of anthocyanin accumulation between different cultivars and also among the carrots of the same cultivar. Totalanthocyanin contents were found in range between 45.4 mg/Kg to 17.4 g/kg dry matter. It was the first research regarding quantification of different types of anthocyanins in roots of various black carrot cultivars. Black carrot anthocyanins can be used as natural food colorants also for low acid food commodities as it was proven by evaluation of color properties in the extracts under various pH, while a significant color loss was observed at nearly neutral pH conditions. Additionally, higher carbohydrate contents were observed in approximately all cultivars which can be disadvantageous when coloring essences are extracted from carrot roots.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

This research was carried out in the laboratory of the Faculty of Agricultural Sciences and technologies, department of plant production and technologies, Omer HalisDemir University (Nigde University) during 2016-17. Roots of the Deep Purple F1 and 16 genotypes will be collected from Konya for the furtheranalysis.Total 17 Roots of the black carrots (Bejo 3 samples and 14 local samples of genotypes) were collected from Konya region for the further analysis.

3.2 Methods

Roots sample were first washed with tape water to remove the soil particles. After washing the roots samples were dried at room temperature in the laboratory.

3.3 Color Measurement

Color intensity was measured with a Minolta color measuring device (Chroma Meter, CR-300, Japan) based on three-dimensional color measurement in carrot samples. Measurements were taken from various parts of sample from epidermis, cortex and vascular tissue.Before color reading, the device was calibrated with the standard calibration scale.

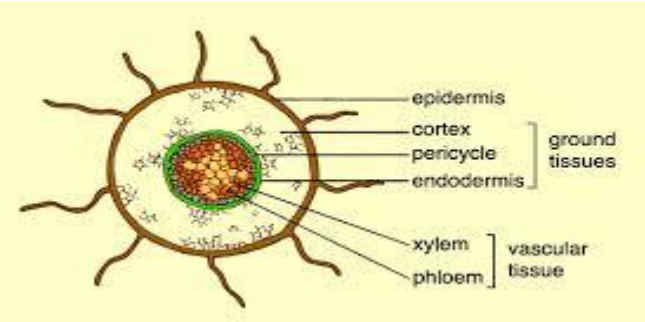

Figure 3.1. Cross section of root of carrot

3.4 Sample Preparation

Roots of the carrots were trimmed from the top and bottom and then chopped into pieces and stored in -80˚C until analysis. Pieces of the roots were homogenized using waring blender.

3.4.1 Total Soluble Solid (TSS)

The puree of TSS was measured using a handheld digital temperature-compensated refractometer and expressed as %TSS in roots.

3.4.2 Determination of total phenolic (TP)

TP content was measured according to Ozgen and Sekerci (2013). 1 grams of puree samples were taken and incubatedfor 24 h by buffer containing acetone, water and acetic acid. Samples were incubated in the dark at 4˚C. Then 0.5 ml of the sample extract and Folin-Ciocalteus, phenol reagent with 4.5ml of distilled water mixture was incubated for 8 min followed by adding sodium carbonate. Then samples were incubated for couple of hour. The absorbance was measured at 750 nm by UV-vis spectrophotometer. The outcomes were stated as µg garlic acid equivalent fresh weight basis.

3.4.3 Total antioxidant activity (TAC)

TAC was assessed using two standard actions; FRAP (The ferric reducing ability of plasma) and TEAC (Trolox equivalent antioxidant capacity) assays.

1gram of grinded carrot tissue was taken was mixed with 10ml Methanol and HCl(99:1 v/v) solution. 1ml of sample was taken and followed FRAP procedure as Benzie and Strain (1996) described. According to the assay, buffers were prepared by mixing 0.1 mol/L acetate (pH 3.6), 10 mmol/L TPTZ, and 20 mmol/L ferric chloride solutions (10:1:1). Aliquot from sample were mixed with FRAP reagent prior to analysis. After 30 min, the absorbance of the reaction mixture was determined at 593 nm by spectrophotometer.

Sample was prepared by taking 6 grams of carrot root tissues and was added 15ml methanol and HCl(99:1 v/v) solution. 1ml of sample was taken and followed TEAC procedure as Ozgen and Sekerci (2013) described. ABTS (2,2-Aniso-bis 3-ethylbenzothiazoline-6-sulfonic acid) was mixed with potassium bisulfate and allowed to stand in the dark for 12-16 hours and aliquots from the sample was mixed and incubated. After 10 min, 2 mL of the prepared buffer was added to 1 mL of the sample extract, followed by measurement at a wavelength of 734 nm in the spectrophotometer.The antioxidant capacities of the samples were calculated using Trolox standard graph and Trolox equivalent/g as an example.

3.4.4 Anthocyanin concentration

Anthocyanin content was measured as per methodology described by Ozgen et al.,(2014). According to the procedure, 0.1 gram of puree of the roots was extracted with 10ml acidified methanol (1:99 v/v) solution. After incubation at 4˚C for 24h, the liquid fraction was transferred to spotless cuvette to bring volume to 3ml with acidified methanol. Reading of absorbance was taken at 515 nm using spectrophotometer. Standard curves was developed using solution containing laboratory grade cyanidin 3-glucoside chloride.

3.4.5 β-carotene concentration

Purees of the roots were placed in to freeze drier at -80˚C to evaporate the water. Methods of De Ritter and Purcell (1981) were followed for the determination of β-

carotene. The mixture of hexane, acetone and ethanol were added to 0.02g freeze dry samples. After incubation,absorbance wascarried out at 450 nm. Pure β-carotene (Sigma-Aldrich)was purchased to calculate β-carotene content of the samples.

3.4.6 DPPH free radical scavenging activity assay

The free radical-scavenging activity of roots was studied using the method described by Brand-Williams et al., (1995). Ethanol solution of the sample extracts were added to 0.06 mM methanol solution of DPPH and allowed to stand for 30 min at 25°C. The absorbance of the samples was measured at 517 nm against to blank samples. 0.1 mM solution of DPPH in methanol will be used as control, whereas ascorbic acid will be the reference standard.

3.4.7 Cupric reducing antioxidant capacity (CUPRAC)

The CUPRAC method was performed according to Apak (2004). The CUPRAC method is based on the reduction of copper I to copper II by antioxidants. 10^{-2} M Cu (II) solution was prepared and 1mL Cu (II), Nc, and NH_4Ac (pH: 7) buffer solution was added to test tubes. The tubes were incubated 30 min, then, absorbance at 450 nm (A_{450}) wasrecorded against blankreagent. The molar absorptivity for each antioxidant pertaining to CUPRAC method was calculated with the absorbance.

3.4.8 Determination of reducing power

The reducing power of the puree was determined according to the method of Oyaizu (1986). Puree of black carrots of 25µl were taken and 25µl ethanol was added, mixed with 2500µl of 0.2M phosphate buffer (pH 6.6), then 2500µl of 1% potassium ferricyanide solution was added and incubated at 50°C for 20 min. After incubation, 2500µlTCA (10%) was added in the reaction mixture and then vortex. Samples were centrifuge at 4000 rpm for 10 min.2500µl supernatant was taken, mixed with 2500µl distilled water and 500µl iron chloride was added. Then the absorbance of the reaction mixture was measured at 700 nm. Ascorbic acid will be used as positive control.

3.4.9 β-Carotene bleaching assay

Antioxidant activity was assessed using the β-carotene linoleate model system with a slight modification according to He (2012). β-carotene was dissolved in chloroform, and 1 mL of this solution was pipetted into a round-bottom 250 mL flask containing 40 μL linoleic acid and 500 μL Tween-20. Chloroform was removed using a rotary evaporator; distilled water was added slowly to the mixture with vigorous agitation to form a stable emulsion. Aliquots from the samples and the emulsion were mixed to measure absorbance at 470 nm. Vitamin C was used as a standard for comparison.

3.4.10 Total RNA analysis

Total RNA analysis was performed according to Chomzynski and Sacchi (1987). RNA was separated from DNA after extraction with acidic solution containing guanidinium thiocyanate, sodium acetate, phenol and chloroform followed by centrifugation. Quantity and purity of the extracted RNA was obtained spectrophotometric readings at wavelengths of 260 nm and 280 nm. The reading at 260 nmallows us calculation of the concentration of RNA in the sample. An optical density of 1 corresponds to approximately 40 μg/ml of single-stranded RNA. The ratio of the absorbance at 260 nm and 280 nm (A260/A280) was provided an estimate of the purity of RNA.

3.4.11 Protein assay

Total protein was quantified by the colorimetric method of Lowry et al., (1951) using BSA as the standard. 0.5 ml of sample was transferred in a 10ml glass tube. 0.7 ml of Lowry solution was added to glass tubes. Cover glass tubes with cap and mixed well by using vortex (vortex speed 3 to 4). Samples were incubated for 20 min at room temperature in dark. Diluted Folin reagent of 0.1 ml was added to glass tubes. Again samples were caped and vortex. Samples were incubated once more for 30 mins in dark at room temperature. Samples were briefly vortex after 30 mins. Samples were transferred to 1.3ml disposable cuvettes and absorbance was noted at 750nm.

CHAPTER IV

RESULTS

4.1 Statistical Analysis

The collected data were analyzed statistically by the method and procedure of Jandel Scientific (1991) using Statistic, 2000 package.

4.2 Total Soluble Solid (TSS)

The data from the TSS values shows in (Table 2)highly significantdifferentamong genotypes. TSS Brix values range from 15.10 to 8.90 in different genotypes of black carrots.The maximum TSSvalue (15.10) was recorded in G07 while the minimum TSS value (8.90) was found in G10.The Deep Purple **F1** gave TSS values (13.33) which were statistically nonsignificant with genotypes **G1**(12.63), **G2** (13.83), **G7** (15.10), **G13**(13.56) and **G15** (13.10). However, all of these genotypes were significantly different from G3 (9.63), G8 and G10.

Table 4.1. The TSS values from different genotypes of black carrots.

Genotype Code	TSS (%)
G1	12.63 abcd
G 2	13.83 ab
G 3	9.63 e
Deep Purple F1 (4)	11.13 bcde
(5)	10.36 cde
G 6	11.76 bcde
G 7	15.10 a
G 8	10.16 de
Deep Purple F1 (9)	13.33 abc
G 10	8.90 e
Deep Purple F1 G (11)	11.13 bcde
G 12	11.46 bcde
G 13	13.56 ab
G 14	10.56 cde
G 15	13.10 abcd
G 16	11.86 bcde
G 17	10.33 de

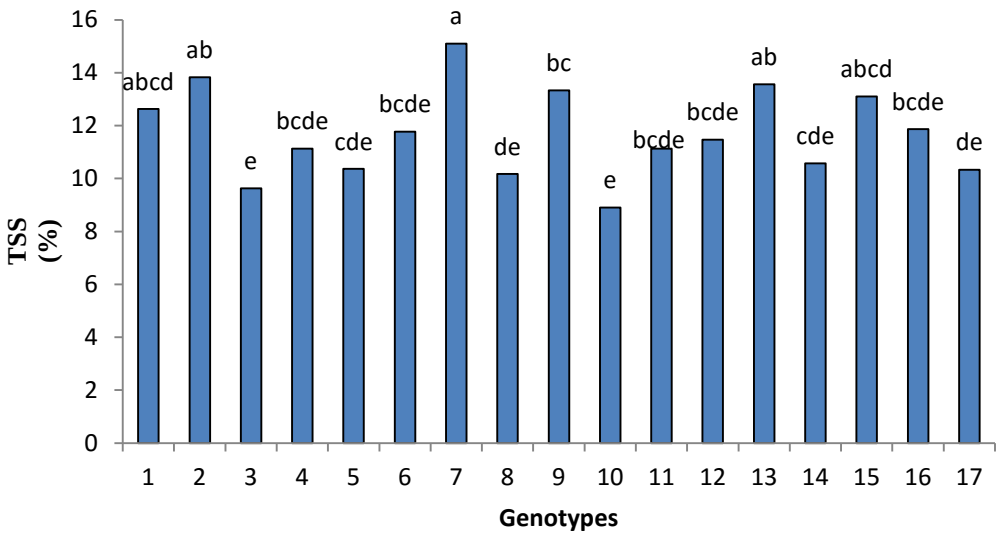

Figure 4.1. Results of TSS from various black carrots genotypes

4.3 Color Reading

Color reading was measured from three regions of black carrots genotypes. That are as cortex, epidermis and vascular tissues

Cortex

All the data are homogenize. However, highest value was recorded in “L” in genotype 06 while in “a” and “b’ highest value was found in G7.

Table 4.2. The Vascular tissue values from different black carrot genotypes

Genotype codes	L*	a*	b*
G1	40.73 a	3.94 a	2.34 a
G2	42.75 a	3.54 a	2.21 a
G3	42.14 a	3.75 a	1.64 a
G4	42.07 a	3.64 a	1.41 a
G5	41.95 a	3.84 a	1.58 a
G6	42.92 a	3.77 a	2.34 a
G7	42.56 a	4.21 a	2.87 a
G8	41.80 a	3.81 a	2.46 a
G9	42.40 a	3.40 a	2.01 a
G10	42.74 a	3.90 a	1.90 a
G11	42.21 a	3.26 a	2.59
G12	40.75 a	4.08 a	2.07 a
G13	41.65 a	3.43 a	0.80 a
G14	41.10 a	3.89 a	1.48 a
G15	41.10 a	3.35 a	0.86 a
G16	41.10 a	3.55 a	0.89 a
G17	41.65 a	3.56 a	0.78 a

Epidermis

The data of epidermis was analyzed and found no significant difference among the "L" values where maximum and minimum values were recorded in **G9** and **G11** respectively. Where as in "a" and "b" values a slight significant difference was found. The high values were observed in "a" and "b" in **G14** (1.0793) and (2.8480) while low value was of **G6** (0.654) and (1.9760) respectively. This shows significant difference in "a" with genotypes **G4**, **5** and **6**. Where as in "b" it shows significant difference only with **G6**.

Table 4.3. The epidermis values from different black carrot genotypes

Genotype codes	L*	a*	b*
G1	46.25 a	0.816 ab	2.38 ab
G2	45.43 a	0.73 ab	2.63 ab
G3	45.80 a	0.77 ab	2.35 ab
G4	45.57 a	0.69 b	2.76 ab
G5	45.73 a	0.89 ab	2.83 a
G6	45.94 a	0.65 b	1.80 b
G7	45.33 a	0.705 b	2.34 ab
G8	45.93 a	0.84 ab	2.82 a
G9	46.98 a	0.73 ab	2.67 ab
G10	45.18 a	0.86 ab	2.52 ab
G11	46.15 a	0.80 ab	2.71 ab
G12	46.77 a	0.93 ab	2.83 a
G13	46.46 a	0.94 ab	2.53 ab
G14	46.67 a	1.08 a	2.85 a
G15	47.30 a	0.81 ab	2.59 ab
G16	46.089 a	0.90 ab	2.48 ab
G17	45.673 a	0.78 ab	2.47ab

Vascular Tissue

The endodermis region data was analyzed and noted a significant difference among the "L" values where maximum and minimum values were recorded in **G1** (45.438) and **G10** (35.510) respectively. This shows that **G1** is significantly different from **G10** where no significant difference was found in rest of genotypes. Where as in "a" and "b" values was slight significant difference from each other. The maximum values were observed in "a" and "b" in **G5** (6.4200) and **G16** (5.8480) while minimum values was of **G13** (3.2000) and (0.3500) respectively. This shows significant difference in "a" with genotypes **G10**. Where as in "b" it shows no significant difference with **G01, G08, G10** and **G16.**

Table4.4.The endodermis values from different black carrot genotypes

Genotype codes	L*	a*	b*
G1	45.44 a	4.56 ab	4.23 ab
G2	41.60 ab	5.49 ab	0.61 b
G3	41.81 ab	5.21 ab	0.81 b
G4	42.20 ab	5.70 ab	1.04 b
G5	41.63 ab	6.42 a	0.71 b
G6	40.90 ab	4.73 ab	0.37 b
G7	42.79 ab	6.05 ab	0.72 b
G8	44.54 a	4.59 ab	2.25 ab
G9	40.74 ab	4.01 ab	0.38 b
G10	35.51 b	3.60 ab	1.48 ab
G11	40.23 ab	3.85 ab	0.49 b
G12	40.43 ab	4.03 ab	0.35 b
G13	39.91 ab	3.20 b	0.46 b
G14	41.81 ab	5.60 ab	0.67 b
G15	40.90 ab	4.04 ab	0.77 b
G16	45.18 a	3.76 ab	5.85 a
G17	40.71 ab	4.04 ab	0.51 b

4.4 Determination of Total Phenolic (TP)

The analyzed data of total phenolic (TP) in different genotypes is given in Table 6. Significant changes were recorded in the TP of different black carrots genotypes. Total Phenolic contents in fresh weigh was recorded from G10 (505.54) to G15 (355.03). The TP value of G10 (505.54) is significantly different from F (387.811), G14 (376.28) and G15(355.03) but it does not indicate any significant differences from the rest of genotypes mentioned in the table 6.

Table 4.5. The Total phenolic values from different black carrot genotypes

Genotype Code	Total Phenolic (µg GAE/g FW)
G1	422.55 ab
G 2	428.92 ab
G 3	457.90 ab
Deep Purple F1 (4)	421.19 ab
Deep Purple F1 (5)	467.92 ab
G 6	452.44 ab
G 7	444.70 ab
G 8	453.81 ab
Deep Purple F1 (9)	387.81 b
G 10	505.54 a
G 11	414.36 ab
G 12	393.72 ab
G 13	453.96 ab
G 14	376.28 b
G 15	355.03 b
G 16	470.04 ab
G 17	468.07 ab

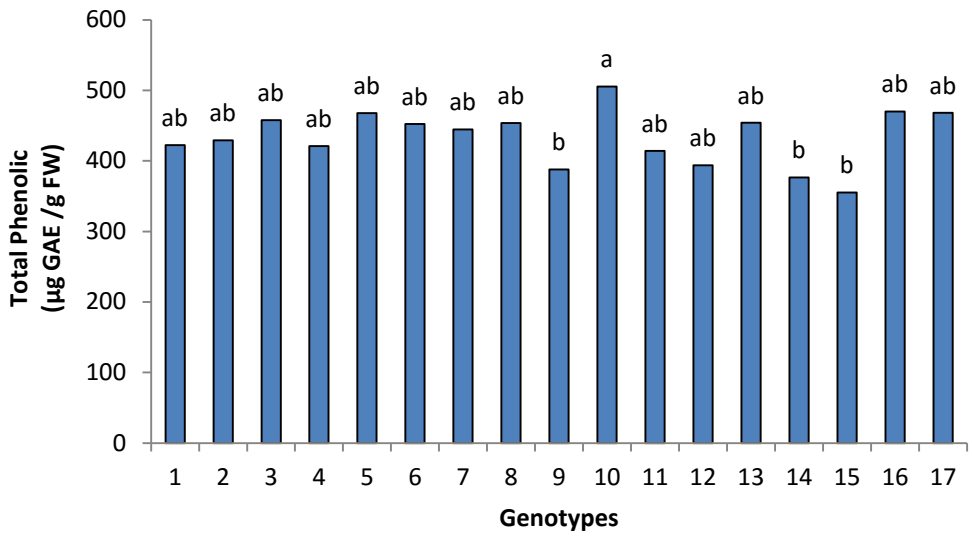

Figure 4.2. Results of phenolic concentration of black carrot genotypes showing significant differences

4.5 Total Antioxidant Activity (TAC)

TAC was estimated using two standard procedures; FRAP (The ferric reducing ability of plasma) and TEAC (Trolox equivalent antioxidant capacity) assays.

4.5.1 FRAP (The ferric reducing ability of plasma)

The data of total antioxidant activity (TAC) in several genotypes analyzed by FRAP is labeled in table 7. Significant changes were recorded in the TAC of different black carrots genotypes. The FRAP values was ranged from Deep Purple F1 (1.01) to (G17 0.60).A maximum value of FRAP was indicated of TAC inDeep Purple F1(1.01), whereas the lower value was confirmed in G 17 (0.60). The TAC values of Deep Purple F1 was found significantly difference with **G2** (0.70), **G5** (0.70),**G12** (0.89)0.60 (0.67) and**G17** (0.60) but it does not indicate any significant difference with the rest of genotypes mentioned in the table 7.

Table 4.6. The FRAP values from different black carrot genotypes

Genotype Code	FRAP (mg Trolox/g FW)
G1	0.77 abc
G 2	0.70 bc
G 3	0.83 abc
Deep Purple F1 (4)	0.91 ab
Deep Purple F1 (5)	0.70 bc
G 6	0.84 abc
G 7	0.76 abc
G 8	0.77 abc
Deep Purple F1 (9)	1.01 a
G 10	0.81 abc
G 11	0.81 abc
G 12	0.89 ab
G 13	0.76 abc
G 14	0.74 abc
G 15	0.74 abc
G 16	0.67 bc
G 17	0.60 c

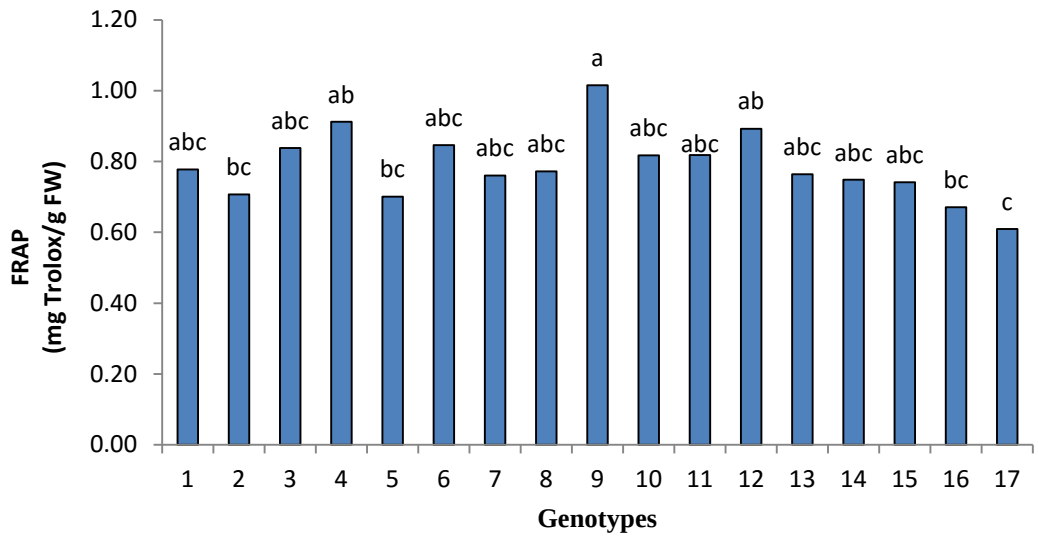

Figure 4.3. Results of FRAPof black carrot genotypes showing significant differences

4.5.2 TEAC (Trolox equivalent antioxidant capacity)

The total antioxidant activity as analyzed by TEAC in several genotypes of black carrots is depicted in table 8. The values were found in the range of 0.13 to 1.8 which were of G11 and Deep Purple F1 respectively. Maximum value of TAC was found in G 6 (1.8) while its minimum value was noted in G 11 (0.13). Deep Purple F1 was found significantly different from G11and G17 (0.13) and (0.15) respectively. While there was no significant differences in rest of genotypes with Deep Purple F1.

Table 4.7. The TEAC values from different black carrot genotypes

Genotype Code	TEAC (mg Trolox/g FW)
G1	0.18 ab
G 2	0.17 abc
G 3	0.18 ab
Deep Purple F1 (4)	0.17 abc
Deep Purple F1 (5)	0.18 abc
G 6	0.18 a
G 7	0.17 abc
G 8	0.18 abc
Deep Purple F1 (9)	0.18 a
G 10	0.17 abc
G 11	0.13 d
G 12	0.16 abcd
G 13	0.17 abc
G 14	0.15 bcd
G 15	0.18 abc
G 16	0.16 abc
G 17	0.15 cd

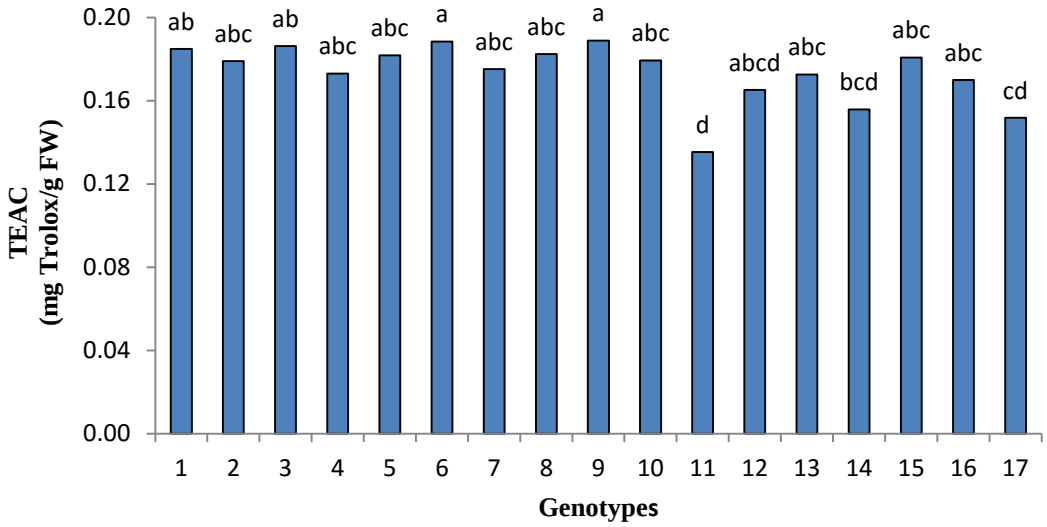

Figure 4.4. Results of TEAC of black carrot genotypes showing significant differences

4.6. β-carotene

The statistical study of beta carotene in several genotypes was found significantly different and is labeled in table 9. The data ranges from (35.75) to (143.58) in various local genotypes along with Deep Purple F1. Maximum value of β-carotene was found in G1 (143.58) while its minimum value was recorded in G12 (35.78). The Deep Purple F1 give value (96.21) which was found statistically non-significant with the local genotypes. However, the local genotypes were recorded statistically highly significant among themselves. Local genotypes **G1** (43.58) and **G2** (141.87) are significantly different from **G7** (40.46) and **G12** (35.78) .

Table 4.8..The β-carotene standards from different black carrot genotypes

Genotype Code	β-carotene (µg/g DW)
G1	143.58 a
G 2	141.87 a
G 3	119.15 ab
Deep Purple F1 (4)	96.21 abcd
Deep Purple F1 (5)	78.09 abcd
G 6	76.46 abcd
G 7	40.46 d
G 8	63.17 bcd
Deep Purple F1 (9)	69.93 abcd
G 10	104.31 abcd
G 11	70.52 abcd
G 12	35.78 d
G 13	57.38 bcd
G 14	78.17 abcd
G 15	76.31 abcd
G 16	108.61 abcd
G 17	113.51 abc

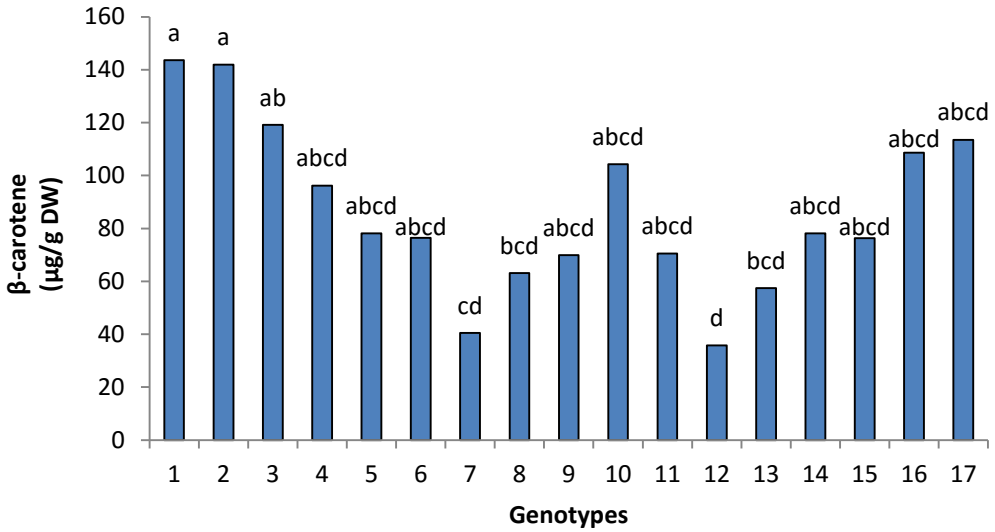

Figure 4.5 Results ofβ-carotene of black carrot genotypes showing significant differences

4.7 Anthocyanin Concentration

The data from the anthocyanin concentration was found significantly different among genotypes. The anthocyanin concentration range from 5.54 to 14.34 in different genotypes of black carrots. The maximum anthocyanin value (14.34) was recorded in Deep purple (F1) while the minimum anthocyanin value (5.54) was found in G1. The Deep Purple **F1** gave anthocyanin concentration values (14.34) which were statistically nonsignificant with genotypes **G2** (10.73), **G3** (11.28), **G5** (12.66), **G6** (11.47), **G7** (11.15), **G8** (10.50), **G10** (12.56), **G11** (13.49), **G12** (9.32), **G13** (9.61), **G14** (8.916) and **G15** (7.86). However, all of these genotypes were significantly different from **G1** (5.56), **G16** (6.38) and **G17** (5.64). On the other hand local genotypes like as **G2** (10.73), **G3** (11.28), **G6** (11.47), **G7** (11.15), **G8** (10.50), **G12** (9.32), **G13** (9.61), **G14** (8.916) and **G15** (7.86) were found no significant difference among themselves.

Table 4.9. The anthocyanin concentration values from different genotypes of black carrots.

Genotype Code	Anthocyanin
G1	5.56 d
G 2	10.73 abcd
G 3	11.28 abcd
Deep Purple F1 (4)	7.44 bcd
Deep Purple F1 (5)	12.66 abc
G 6	11.47 abcd
G 7	11.15 abcd
G 8	10.50 abcd
Deep Purple F1 (9)	14.34 a
G 10	12.56 abc
G 11	13.49 ab
G 12	9.32 abcd
G 13	9.61 abcd
G 14	8.916 abcd
G 15	7.86 abcd
G 16	6.38 cd
G 17	5.64 d

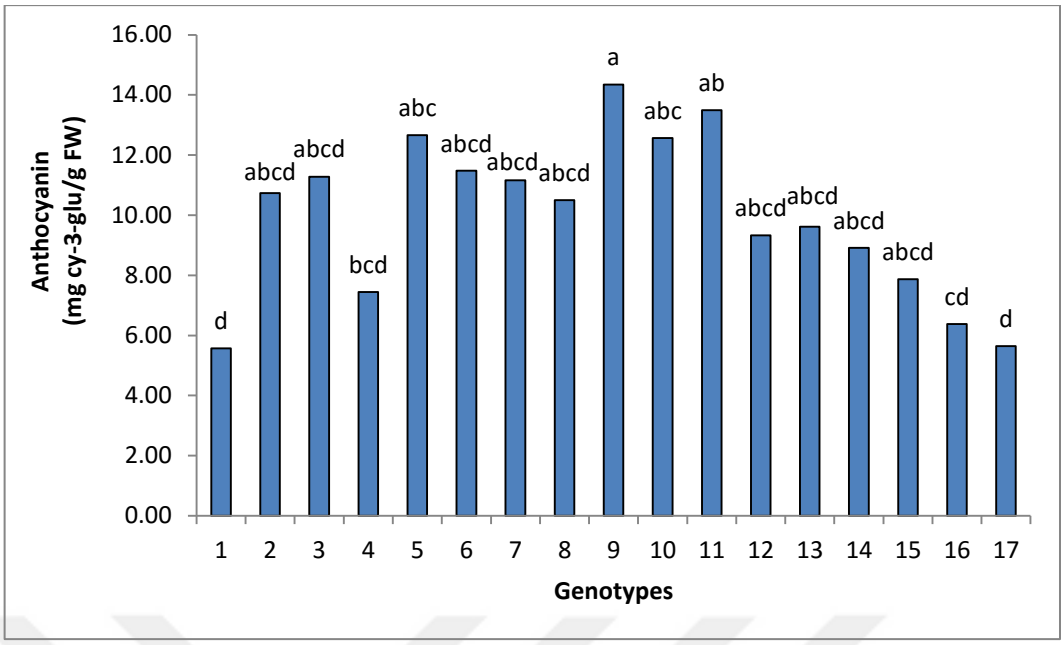

Figure 4.6 Result of Anthocyanin concentration of black carrot genotypes showing significant differences

4.8 DPPH Free Radical Scavenging Activity Assay

The statistical conclusion of DPPH in different genotypes is mentioned in table 11. It is indicated in the table that the maximum value of DPPH was observed in G11 and the theminimum value was recorded in G14. It is almost equivalent to other genotypes except G14, which shows no significant difference with others genotypes treatments. On the other hand, the mentioned genotypes are significantly different from the rest of genotypes.

Table 4.10. The free radical scavenging activity values from different black carrot genotypes included in the study.

Genotype Code	DPPH (mg Trolox/ml)
G1	44.96 ab
G 2	44.32 ab
G 3	43.27 ab
Deep Purple F1 (4)	44.80 ab
Deep Purple F1 (5)	44.28 ab
G 6	44.69 ab
G 7	44.69 a
G 8	42.04 ab
Deep Purple F1 (9)	41.18 ab
G 10	38.92 ab
G 11	45.02 ab
G 12	38.15 ab
G 13	37.44 ab
G 14	34.02 b
G 15	36.95 ab
G 16	37.40 ab
G 17	42.25 ab

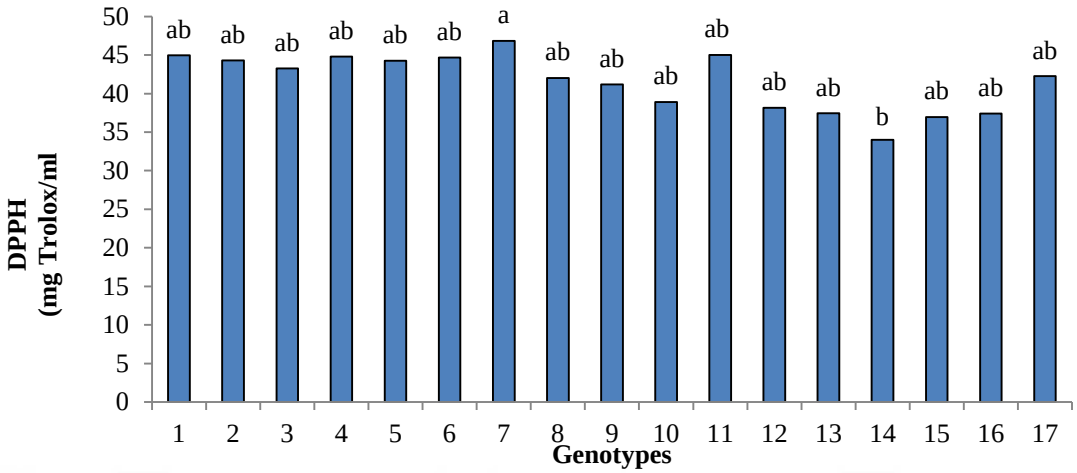

Figure 4.7 Results of DPPH of black carrot genotypes showing significant differences

4.9 Cupric Reducing Antioxidant Capacity (CUPRAC)

The statistical analysis of CUPRAC values were mentioned in Table11. The maximum value was found in G6 (80.11) while minimum value was recorded in G7 (22.98). G6 is significantly different from rest of genotypes except Deep Purple F1 (68.16), and G14 (65.29). While G1, (32.89) G11 (34.29), and G13 (25.84) are non-significant from each other. The genotypes G3, 10, 17, 15, 12 and 10 are non-significant with each other.

Table 4.11. The CUPRAC values from different black carrot genotypes included in the study

Genotype Code	CUPRAC (mg Trolox/ml)
G1	32.89 defg
G 2	23.047 g
G 3	40.53 cdef
Deep Purple F1 (4)	45.68 cd
Deep Purple F1 (5)	39.50 cdefg
G 6	80.11 a
G 7	22.98 g
G 8	23.81 fg
Deep Purple F1 (9)	68.16 ab
G 10	44.79 cd
G 11	34.29 defg
G 12	42.22 cde
G 13	25.84 efg
G 14	65.29 ab
G 15	48.32 cd
G 16	53.46 bc
G 17	45.54 cd

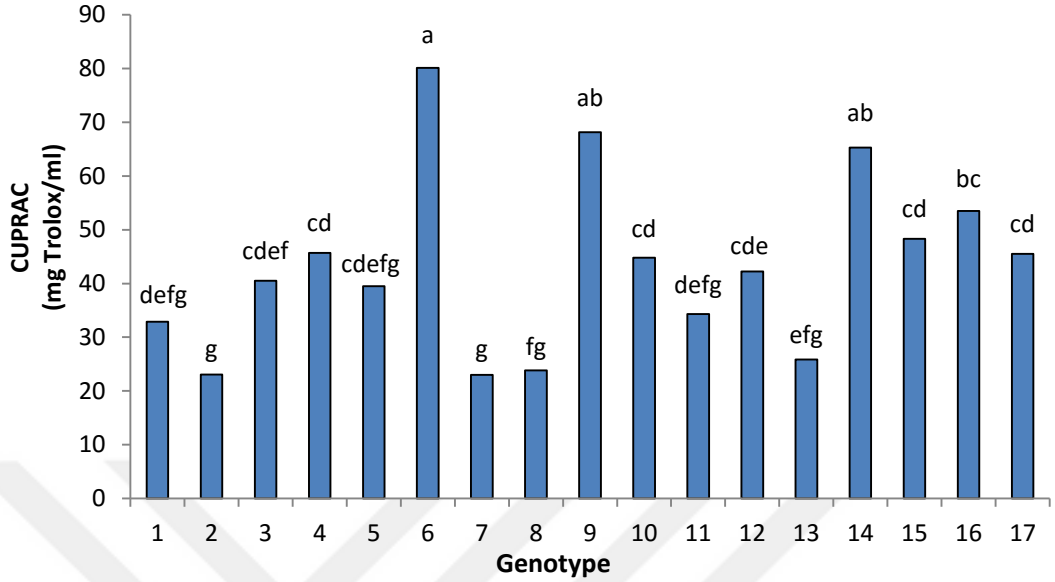

Figure 4.8 Result of CUPRAC of black carrot genotypes showing significant difference

4.10 Determination of Reducing Power

The values of reducing power in various genotypes are described in table 13. It is shown in the table that high value of reducing power was detected in G 16 and lower was recorded in in genotype 04 and 09 deep purple. This is nearly equal to genotypes such as G1, G2, G6, G7, G8, G10, G12, G14 and G15, with no significant difference among themselves. Meanwhile, these genotypes are significantly changed from the rest of genotypes.

Table 4.12. The reducing power values from different black carrot genotypes

Genotype Code	The abs. in 450 nm
G1	0.2970 ab
G 2	0.2630 ab
G 3	0.2390 b
Deep Purple F1 (4)	0.2296 b
Deep Purple F1 (5)	0.2454 b
G 6	0.2938 ab
G 7	0.3148 ab
G 8	0.2806 ab
Deep Purple F1 (9)	0.2272 b
G 10	0.2562 ab
G 11	0.2410 b
G 12	0.2864 ab
G 13	0.2474 b
G 14	0.2514 ab
G 15	0.2606 ab
G 16	0.3376 a
G 17	0.2522 ab

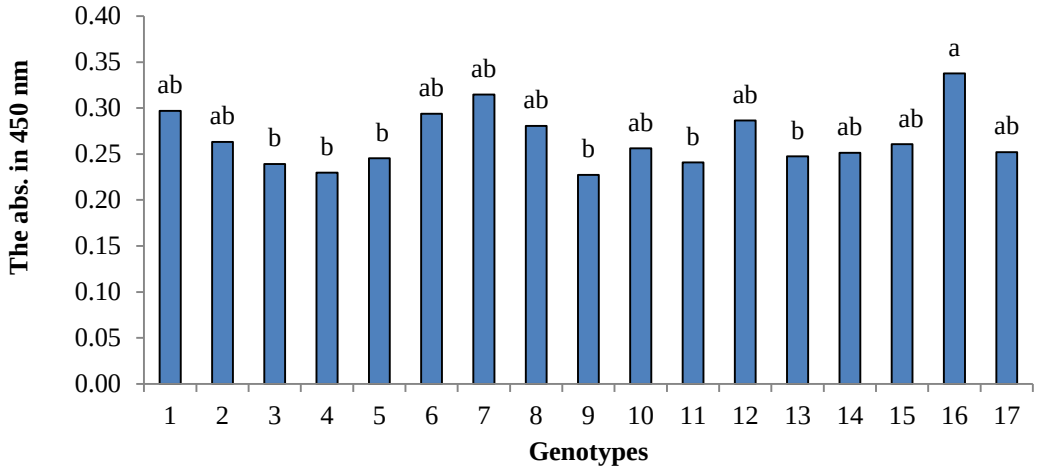

Figure 4.9 Results of reducing power of black carrot genotypes showing significant difference

4.11 β-Carotene Bleaching Assay

The statistical data of beta- Carotene is highly homogeneous, showing no significant differences among the various genotypes in table 4.12.

Table 4.13. The Beta-carotene bleaching Assay values from different black carrot genotypes

Genotype Code	The abs. in 490 nm
G1	0.5838 a
G 2	0.6488 a
G 3	0.5536 a
Deep Purple F1 (4)	0.4342 a
Deep Purple F1 (5)	0.5108 a
G 6	0.4460 a
G 7	0.3982 a
G 8	0.4364 a
Deep Purple F1 (9)	0.5608 a
G 10	0.6132 a
G 11	0.6022 a
G 12	0.5960 a
G 13	0.6238 a
G 14	0.5200 a
G 15	0.4980 a
G 16	0.6224 a
G 17	0.5496 a

4.12 RNA Extraction

RNA extraction was carried out manually according to Chomzynski and Sacchi (1987). RNA was extracted from the roots of 17 different genotypes, twice. The first extraction did not produce significant amount of RNA, therefore extraction was conducted twice to smoothly run the studies. The RNA concentration of the 2nd extraction run was considered for all the procedures selected in the study. The RNA was successfully extracted during the 2nd run from all of the genotypes; however the concentration and quality were varied. The RNA concentration varied from 76 ng/µl to 986 ng/µl. The highest RNA concentration was extracted from the genotype 07, whereas the lowest RNA concentration was obtained from the Deep Purple F1 04. The RNA concentration obtained from each of the genotype is presented in Table.

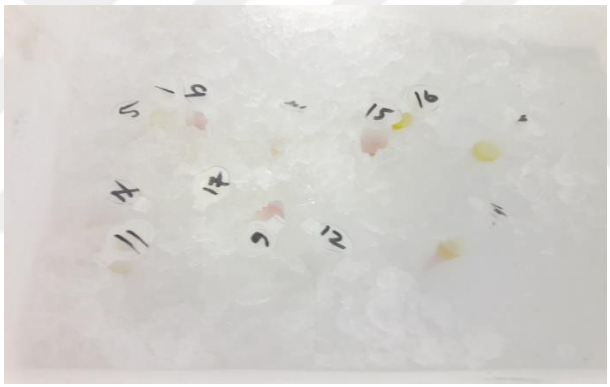

Figure 4.10 Carrot samples during RNA extraction

Table 4.14. The concentration of extracted RNAs with 260(abs)/280(abs) ratio values from different black carrot genotypes included in the study.

Genotype Code	RNA concentration	
	ng/µL	260/280
G1	83.01	1,39
G 2	291.60	1,54
G 3	460.50	1,17
Deep Purple F1 (4)	76.38	1,13
Deep Purple F1 (5)	854.50	1,39
G 6	155.71	1,17
G 7	952.91	1,44
G 8	742.38	1,22
Deep Purple F1 (9)	763.78	1,17
G 10	187.21	1,26
G 11	81.77	1,04
G 12	391.95	1,49
G 13	407.16	1,50
G 14	109.95	1,51
G 15	797.06	1,22
G 16	238.13	1,12
G 17	363.53	1,48

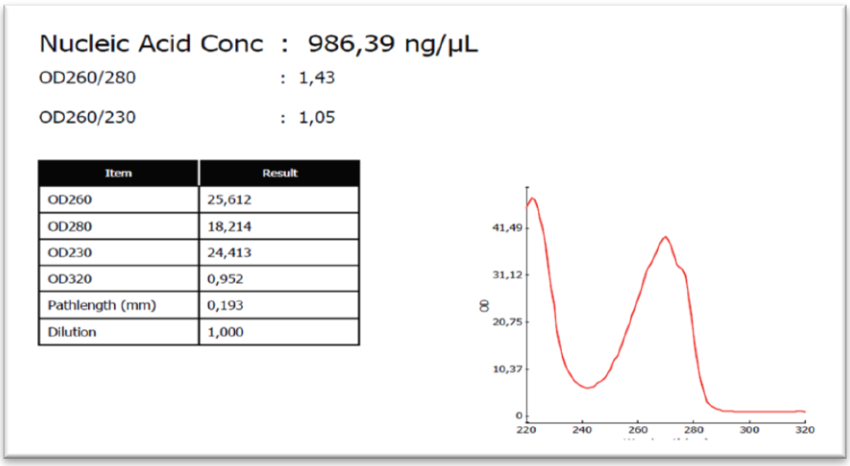

Figure 4.11 Spectrophotometer analysis results of extracted nucleic acids from genotype 07

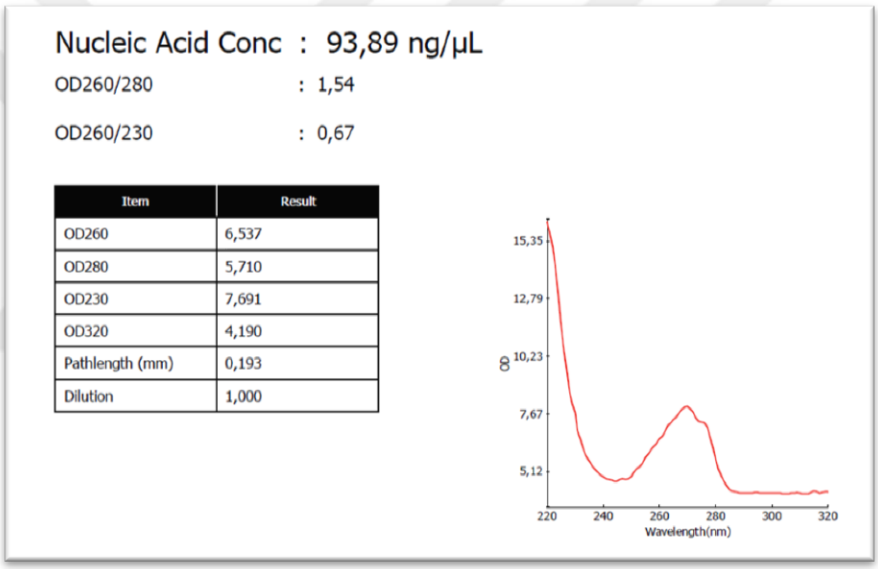

Figure 4.12 Spectrophotometer analysis results of extracted nucleic acids from genotype 02

4.13 Protein Assay

The statistical analysis of protein contents in different genotypes is summarized in table 16. It is obvious from the table that the higher value of protein was observed in G14. It is almost equivalent to other genotypes like as G3, G6 and G13, which shows no significant difference among themselves. On the other hand, these genotypes are significantly different from the rest of other genotypes. Similarly the minimum value of protein contents was recorded in genotype in deep purple F1.

Table 4.15. The protein percentage values from different black carrot genotypes included in the study.

Genotype Code	Protein (%)
G1	23.97 d
G 2	30.77 bcd
G 3	37.29 abcd
Deep Purple F1 (4)	23.97 d
Deep Purple F1 (5)	29.09 cd
G 6	42.89 abc
G 7	28.57 cd
G 8	32.23 bcd
Deep Purple F1 (9)	22.11 d
G 10	26.65 d
G 11	24.49 d
G 12	25.10 d
G 13	46.27 ab
G 14	53.07 a
G 15	33.07 bcd
G 16	36.27 bcd
G 17	25.98 d

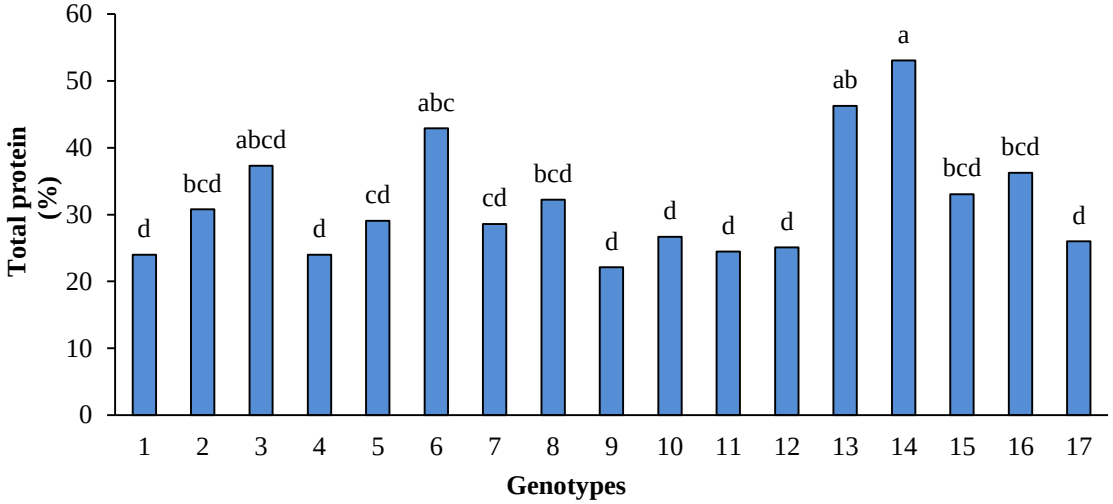

Figure 4.13 Results of % Protein of black carrot genotypes showing significant difference

CHAPTER V

DISCUSSION

In this study we determined importance of black carrots because it is widely grown for use both fresh and specially for industrial processing and it provides an excellent source of anthocyanin mainly food colorant now a days in food industries and beta carotene (Vitamin A) in diet. Globally, carrot ranks among the ten most important vegetables, exceed by potato, lettuce, tomato, onion, celery and sweet corn. In nature, the wild carrot has an annual or winter annual life cycle. In this study, we conducted various analysis of local genotypes and Deep Purple (F1) of black carrot, such as Color Measurement, Total Soluble Solid (TSS), Determination of Total Phenolic (TP), Total Antioxidant Activity (TAC), β-carotene Concentration, Anthocyanin Concentration, DPPH Free Radical Scavenging Activity Assay, Cupric Reducing Antioxidant Capacity (CUPRAC), Total RNA Analysis and total protein assay. The successful large scale use of black carrot depends on the choice of cultivars that are well-adapted to soil and environmental factors at the cultivation site and meets the consumer acceptance (Pereira et al., 2015). The coloring in the inner part showed root differences among different genotypes of black carrots.

The color characteristics such L*, a* and b* values were recorded in various genotypes of black carrots, it was observed that the maximum value of L*, a* and b* were existed in the cortex of all black carrots examined by digital Minolta in laboratory. Moreover, the L*, a* and b* of black carrots cortex observed for each genotype along with Deep Purple were not significantly different from each other and were existed in the close proximity among their selves. Similarly, L*, a* and b* values of epidermis and vascular tissue in all genotypes of black carrots along with Deep Purple showed that they contain maximum L*, a* and b* values in each genotypes but on the other hand we did not observed any significant difference for L*, a* and b* values recorded in the epidermis and vascular tissue between the genotypes of black carrots along with Deep Purple. These results are in agreement with the findings of Malien-Aubert et al. (2001) who also studied color measurement L*, a* and b* values in various black carrots.

TSS mainly contains the total sum of sugar acids (mainly malic and citric acid) and other various round about 310 constituents, such as minerals, pectin, amino acids, phenols, and Vitamin C (Balibrea et al., 2006; Kader, 2008). The TSS results recorded in all local genotypes of black carrots along with Deep Purple shows that it contain some amount of TSS values, that is high in local genotypes which showing no significant different with deep purple Kader, 2008).

The local genotypes and Deep Purple black carrots contained high levels of pigments. Colored carrots are significant spring of dietary nutrients existed as plant pigments, such as anthocyanins, carotenoids, phenolic and other flavonoids. These compounds also play a good role in health, like protection against various forms of cancer, decreasing the risk of cardiovascular diseases, and scavenging of free radicals (reviewed by van den Berg et al. 2000 and Stintzing and Carle 2004), have activated the interest of users in natural products having carotenoids and anthocyanins. This gives an importance to our local genotypes along with Deep Purple used in this study.

The parameters like CUPRAC DPPH, total phenolic and β-carotene recorded in local genotypes of black carrots with Deep Purple showed that they contain different quantities in all genotypes observed in laboratory. The values of all these parameters show that there are some significant differences among themselves local genotypes compared with Deep Purplepurple carrots had more than twice the α- and β-carotene are present in carrots and were perceived by consumers to be sweeter than orange carrots, although total sugars and calculated relative sweetness was lower for purple than for orange carrots . Alasalvar et al. (2001). Moreover, high antiradical activity of purple and orange carrots was previously studied by Sun et al. (2009), who measured such results via DPPH and ABTS methods. Gajewski et al. (2007) also showed maximum antioxidant capacity in methanolic extracts from purple carrots than in extracts from orange and yellow carrots. This is matching with our results in which purple carrot F1 hybrids contained minimum levels of total phenols and minimum percentages of antioxidant activity comparing to local genotypes of black carrots from Konya, Turkey. Leja et al. (2013) studied various carrot cultivars and found their results that red carrots showed higher antioxidant activity than orange, yellow and white carrots and in the season of lower rainfall they accumulated higher amounts of phenolic compounds.

Likewise, protein assay was also evaluated in local genotypes of black carrots along with Deep Purple, which shows that minimum values of protein was recorded in Deep Purple while its maximum value with a minor fluctuation in local genotypes of black carrots.

CHAPTER VI

CONCLUSION

Black carrots have high levels of anthocyanin with bluish-purple color which are being used as a natural food colorant in food industries, medicinal purposes and cosmetic industry due to its high heat, light, and pH stability. They are mainly being used for industrial purposes and are processed into common by products like as juices, candies, confectionery, shalgam and fermented beverages that are good alternative to synthetic colorants like Red 40.4 and 40.5. Since it has been used for industry phenotypic appearance of roots are not critical issue.

The aim of our study was to compare the Deep Purple (F1) with the black carrot local genotypes that were collected from Konya region Turkey, which was mainly found similar in various biochemical parameters such as antioxidant activity, anthocyanin concentration, beta carotene concentration and protein assay etc. There was no significant difference was found among these local and Deep Purple (F1) black carrots. On the basis of these results growers can decide what to select. On the other hand growers in Konya region are mostly depend on their own sources for seed production, which can be cost saving and easily managed, while for (F1) the seeds can be purchased from the international seed companies which can bring a burden on their shoulders.

The results of the study showed that total RNA and protein analysis of local genotypes and Deep Purple (F1) were not different. Further comprehensive studies need to be established to confirm genotypic differences among the local genotypes and Deep Purple (F1).

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