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İ Ç İ N D E K İ L E R / C O N T E N T S

Determination of Antioxidant Activity of Sunflower Growing in Hayrabolu District of Tekirdağ Province

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Sunflower, apart being the plant of cooking oil, is grown as an ornamental plant in parks and gardens. Protein, carbohydrate and fat is found in sunflower pulp utilized as animal feed apart being used as cooking oil. While its seeds are being used as snacks, its stems and slabs are used in the paper industry. In this study, sunflower widely cultivated in Tekirdağ province, Tunca MR (5580) sunflower variety grown in the basic soils of Banarli Village in Hayrabolu District and antioxidant activities of plant extracts during physiological and harvest periods were researched with β-carotene Linoleic Acid Method and it was compared with Butylated Hydroxy Toluene (BHT), which is a synthetic antioxidant, and Trolox as a standard. Total antioxidant activity was determined with β-carotene Linoleic Acid Method. In this study, however, antioxidant capacities of sunflowers were compared by considering their seeds in terms of soil and climate.

Keywords: Antioxidant activity, β-carotene Linoleik Asit, Sunflower, Tekirdağ.

Tekirdağ İli Hayrabolu İlçesinde Yetişen Ayçiçeği Bitkisinin Antioksidan Aktivitesi Tayini

Ayçiçeği yemeklik yağ bitkisi olması yanında süs bitkisi olarak park ve bahçelerde yetiştirilir. Yemeklik yağ dışında hayvan yemi olarak değerlendirilen ayçiçeği küspesinde protein, karbonhidrat ve yağ bulunur. Tohumları ülkemizde çerez olarak kullanılırken sap ve tablası kağıt sanayiinde kullanılır. Bu araştırmada, Tekirdağ İli Banarlı Köyü Hayrabolu ilçesinde yaygın olarak ekilen ayçiçeği bitkisinin Tunca MR(5580) ayçiçeği çeşidi, bitkileri özütlerinin fizyolojik ve hasad dönemindeki antioksidan etkinliği β-karoten Linoleik Asit Yöntemi ile araştırılmış ve standart olarak sentetik bir antioksidan olan butillenmiş hidroksi toluen (BHT), ve Troloks ile karşılaştırılmıştır. Toplam antioksidan aktivitesi, β-karoten Linoleik Asit Yöntemi ile belirlenmiştir. Bu çalışmada ayrıca ayçiçeği tohumlarının toprak, iklim açısından da göz önünde bulundurularak antioksidan kapasiteleri karşılaştırılmıştır.

Anahtar kelimeler: Antioksidan aktivite, β-karoten Linoleik Asit, Ayçiçeği, Tekirdağ.

Introduction

Sunflower, apart being the plant of cooking oil, is grown as an ornamental plant in parks and gardens. Protein, carbohydrate and fat is found in sunflower pulp utilized as animal feed apart being used as cooking oil. While its seeds are being used as snacks, its stems and slabs are used in the paper industry. Sunflower (*Helianthus annuus*) is one of the most important oil plants of today.

Sunflower oil occupies the first place among the preferred vegetable oils in terms of cooking quality. Sunflower is a hoe plant. It leaves clean and an aired soil to the plant that is cultivated after itself. The cultivation time for sunflower is spring. It should be cultivated in the months of March-April right after the cultivation of grain and sugar beet in spring when the soil temperature became 8-9 °C. Cultivation must be completed in Marmara and Thrace regions by April 15. Its sensitivity increases against temperature below -5 °C after the period of 4-6 leaves.

But sunflower downy mildew disease occurs in damp and cold climates. Taken from the perspective of soil, it grows well in deep, well-cultivated, airy, most and humus soils whose changing structure physically ranges from sand to clay. Amounts of nutrient it draws from the soil are very high. It exploits especially potassium and lime, and, for this reason, it develops quite well in light and calcareous alluvial soils found in the shores of the river. Although sensitive to acid soils, its favorable soil reaction is pH:6,0 -7,5. Soil

needs to be prepared in a way to be tight and moist enough. Field, where sunflower will be cultivated, needs to be ploughed at a depth of 18-25 cm. Leveling tasks of the soil is performed in spring and if it is settled goosefoot will be used to loosen it, and if it is bulging it will be harrowed when soil is in opportune moment. Plowing with goosefeet and rake is applied in 8-10cm in depth. Surface cultivation as well as the leveling process are completed at once by rake and harrow attached one after another. Seed-bed should be moist and adequately tight, and weeds must be destroyed (Esendal, 2008; Kacar and Katkat, 2007).

Materials and Methods

In this study, Tunca MR (5580) species belonging to Sunflower (*Helianthus annuus*) plant widely-grown in Hayrabolu District of Tekirdag were collected. Antioxidant activity was looked at the seedy part of sunflower collected during physiological and harvesting period. Then, extraction and evaporation processes of sunflower with soxhlet apparatus and rotary evaporator were carried out in the laboratory. Anti-oxidative properties of sunflower seed oil's active materials were determined by using T80+UV-VIS Spectrophotometers. Determination of pH, Lime amount, Organic matter amount, available phosphorus, exchangeable Potassium, Calcium and Magnesium in the soil samples of research area were determined according to Sağlam (2008). Texture in soil samples was determined according to (Demiralay, 2003) and some available micro elements in the soil such as Fe, Cu, Zn and Mn with DTPA method was determined according to (Kacar, 2009) in ICP-OES.

β-Carotene Linoleic Acid Method

Total anti-oxidant activity of the extracts was determined by β-carotene-linoleic acid method which is based on the measurement of inhibition of conjugated diene hydroperoxides resulting from linoleic acid oxidation (Dapkevicius et al., 1998). β-carotene solution was prepared by solving 2 mg of β-carotene in 10 ml of chloroform. 25 µl linoleic acid and 30 µl Tween 20 were added to 1 ml of this solution. Chloroform was mixed with 100 ml of distilled water after being vaporized in rotary evaporator. 3ml of this emulsion and 250 µl extract solution containing sample were added to the available test tubes. 250 µl solvent (methanol, ethanol) was put into the test tube instead of extract for control. The initial absorbance was measured at 470 nm by using spectrophotometer as soon as emulsion was added into test tubes. Tubes were left for incubation at 50°C. Incubation process continued until the color of β-carotene disappeared (180 minutes). Total antioxidant activity was calculated by using the following equation.

$R = \ln (a / b) / t$

Here, R: β-carotene color expansion rate, ln: natural logarithm, a: initial absorbance,

B: absorbance after 180 minutes of incubation,

Antioxidant Activity (AA) was calculated according to the following equation.

$AA = [(R_{control} - R_{sample}) / R_{control}] \times 100$

Results and Discussion

Some physical and chemical properties of the soils

Sunflower mostly grows at places that annually receive 500 mm of rainfall. However, excessive rain with the condition of being experienced during a period of drought towards the end of the season increases the productivity. It is very important for the rainfall to show a regular distribution within the growing season. It exploits extra potassium and lime in particular (Esendal, 2008).

Soil plays a crucial role in the antioxidant capacity of the plant of such an important plant. Soil sample in the research area had basic property and its saltless, low-lime, loamy-texture, low organic matter content, sufficient available phosphorous content, exchangeable potassium, calcium and magnesium contents as well as its available iron, copper, zinc and manganese contents were at sufficient level (Table 1).

Nitrogen, one of the macro nutrition elements, is one of the important elements that affect productivity as the basic building block and necessary for the synthesis of proteins in plant cells. Nitrogenous fertilizer decreases the oil proportion of the seed as well as increases the protein proportion. It was found that increased dose of nitrogen reduces the oil rate of the seed. It is the most needed within the nutrients taken from the soil by high plants.

Table 1. Some physical and chemical properties of the research area soil.

Village Name	BANARLI
pH	7,88
Salt %	0,10
Lime %	1,63
Texture	Loam
Org. Mat. %	1,04
Total N %	0,05
P (ppm)	44,68
K (ppm)	197,99
Ca (ppm)	3670,17
Mg (ppm)	366
Fe (ppm)	4,77
Cu (ppm)	1,08
Zn (ppm)	0,59
Mn (ppm)	13,42

Because plants are in need of large quantities of nitrogen in order for them to develop and to provide maximum productivity and they consume Phosphorus, takes part in numerous physiological events that require energy including protein synthesis through its energy-rich pyrophosphate bonds. It does not affect the oil proportion alone. However, it was discovered that the oil proportion either increased or did not change when phosphorus was given together with potassium. Fe reception of plants from the soil was also negatively affected by high pH and high P and Ca concentrations in the environment. When excessive lime is added to the soil, Mn transforms into useless forms by being exposed to oxidation with the increase in pH (Karaman et al., 2012). Zinc becomes useless in alkaline soils with chemical bonding. pH is the most important factor in affecting zinc availability. Zinc compounds, which are difficult to dissolve, will be formed with the rise of pH when availability of zinc reduces and zinc hydroxides precipitate in the form of $Zn(OH)_2$ (Mengel and Kirkby, 1978).

Determination of Total Antioxidant Activity

Total antioxidant activities, their absorbance values against time and % percentage of antioxidant activity values of extract oils and standard antioxidants of Tunca MR (5580) belonging to sunflower (*Helianthus annuus*) during physiologic and harvesting period determined by β-carotene-linoleic acid system are provided in Figure 1 and Figure 2.

nitrogen from soil at a higher amount (Adiloğlu et al., 2010).

In β-carotene color expansion method, linoleic acid creates hydroperoxides as being free radicals during incubation in aforementioned temperature. The presence of antioxidant materials found in sample extracts causes β-carotene to undergo oxidation at minimum level by hydroperoxides. Degradation rate of β-carotene depends on antioxidant activities of the extracts due to neutralization of hydroperoxides formed in this system by antioxidant materials in extracts. There is a relationship between β-carotene color expansion and degradation power, and this is extract's, found in the environment where the lowest β-carotene degradation rate is observed, having the highest antioxidant activity (Othman et al., 2007). High absorbance value indicates that so-called extract has high antioxidant activity (Amin, 2002).

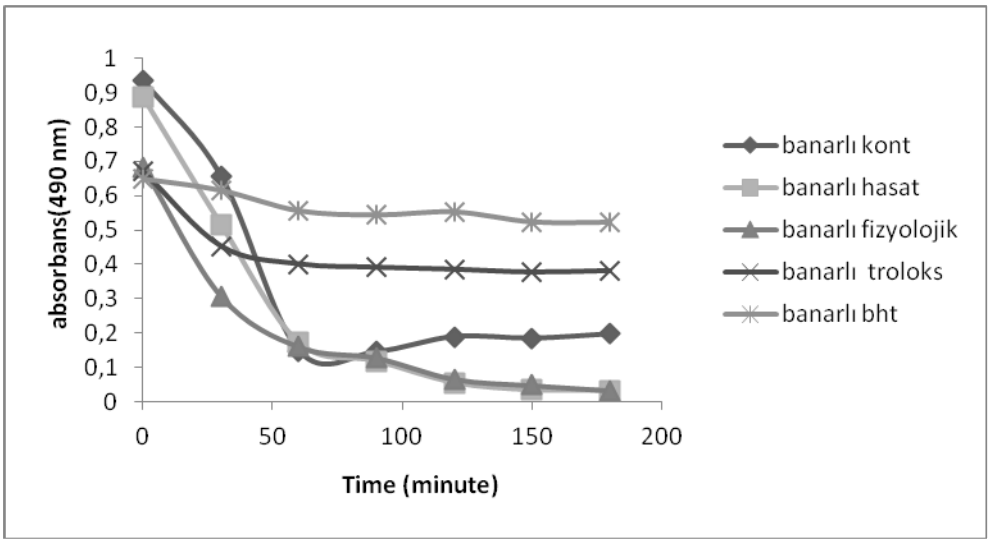

Figure 1. Absorbance values against time of sunflower (*Helianthus annuus*) plant by β-carotene Linoleic acid.

In this method, compounds, which are located in the body of extracts and have antioxidant effects, can reduce the degradation degree of β-Carotene (expansion of characteristic yellow color) by neutralizing linoleate and other free radicals that are formed in the system. Thus, degradation rate of β-Carotene changes depending on the power of antioxidant activity of the extracts. Among β-carotene's color expansion and degradation rate, the extract, having the lowest β-carotene degradation rate, is determined to have the highest antioxidant activity (Othman et al., 2007).

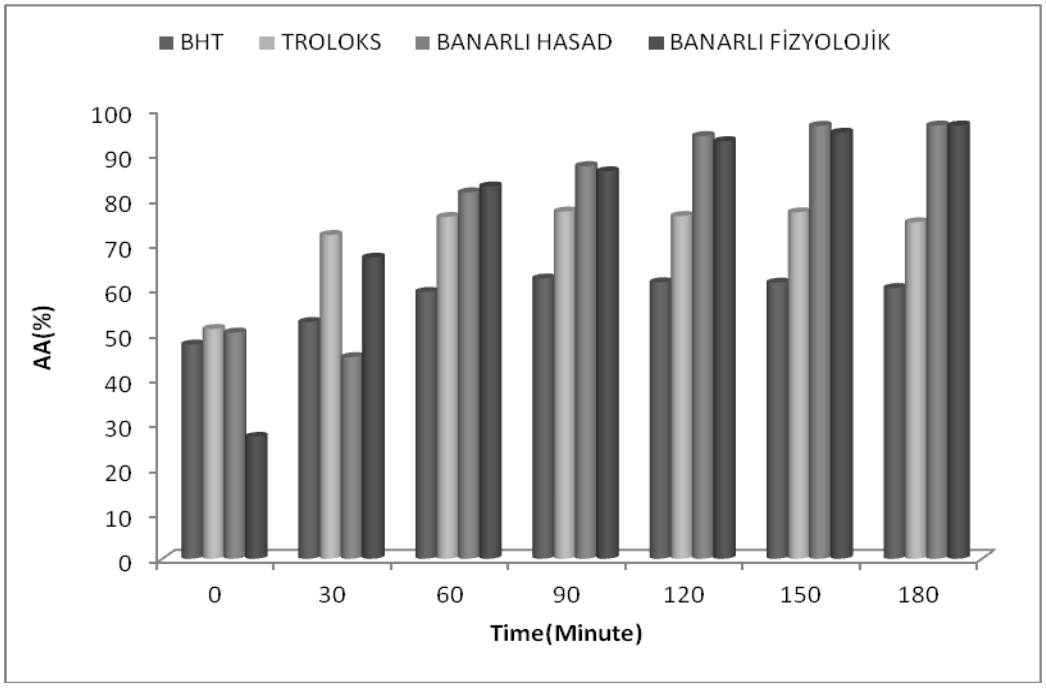

Figure 2. Time and % percentage antioxidant activity in β-carotene Linoleic acid.

As a result of activity operations, various mechanisms of antioxidants, such as stopping radical chain reaction, binding transition metal ions, separating peroxides, reduction power and radical removal, depend on reaction conditions such as pH, temperature and solvent (Diplock, 1997). Antioxidant activity, at the same time, depends on the polarity of extraction solvent and type, isolation techniques and the purity of active compounds (Meir et al., 1995). While antioxidant activity of a compound was determined, it was observed that compound had a powerful antioxidant in one method, and was a pro-oxidant in another (Von Gadow et al., 1997). Since they also keep hydroxyl groups in plants, they are the most important plant components in phenols due to their capacity of radical destruction (Hatano et al., 1989). At the same time, phenol compounds can directly contribute to the antioxidant activity (Duh et al., 1999).

Conclusion

Total antioxidant activities, their absorbance values against time and % percentage of antioxidant activity values of extract oils and standard antioxidants of Tunca MR (5580) belonging to sunflower (*Helianthus annuus*) during physiologic and harvesting period determined by β-carotene-linoleic acid system are provided in Figure 1 and Figure 2. *β*-Carotene degradation rate and total antioxidant activity results of sunflower extracts and synthetic antioxidants analyzed by the application of *β*-carotene linoleic acid model system are given in Figure 1 and 2 respectively.

When we look at the values of degradation rate given in Figure 3.1, we see that the decrease in the values of degradation rate thence in absorbance values of the sample slowly takes place when incubation time increases. The realization of this decrease in speed values, as expressed in Eryiğit (2006), Amin (2002), Makasci Afacan et al. (2010), Nagai et al., (2003), Gülçin et al., (2004) and many other studies conducted in literature related to this topic, can be caused by the presence of compounds (amino acids, phenolic acids, flavanoids, pyrocatechol, catechin, etc.) with antioxidant effect and phenol structure found in the extracts.

As shown in the graph of time and % percentage antioxidant activity in the method of β-carotene linoleic acid at Figure 2, our extracts of harvesting and physiologic period was compared with BHT and trolox. As a result of this comparison, while it initially showed antioxidant activity almost at the same amount with standard compounds, it was observed that it gradually showed more antioxidant activity than it others.

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