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Detection of some antioxidant enzyme activities in apricot fruit grown in Van region from Turkey

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ABSTRACT

In this study, it was aimed to determine antioxidant enzyme activity in apricot fruit. The antioxidant property of this fruit is quite high. Its structure contains many vitamins, minerals and enzymes. For this purpose, it is aimed to determine the activity of superoxide dismutase (SOD) which is one of the enzyme activities thought to be present in apricot fruit. Furthermore, oxidative stress indicator and the level of malondialdehyde (MDA) were determined. SOD and MDA values of the apricot samples were determined to be 0.076 U/ml and 1.579 mmol/l, respectively (($p \leq 0.05$). In this study, antioxidant enzyme activity and lipid peroxidation were determined by spectrophotometric method. Then, the results obtained were analyzed by using multidimensional statistical methods and the results were discussed in a multidimensional manner.

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1. Introduction

The reactive molecules formed during the transformation of nutrients into energy form provided that they use oxygen are called free radicals. Oxygen is a quite necessary molecule for the maintenance of life and also forms intermediates that act as sources of free radicals and are highly reactive. These molecules formed cause damage to protein, lipid and DNA-like cell groups. Antioxidant defense systems have been developed to control the formation of free radicals in aerobic structures and to prevent the bad effects of these molecules [1,2]. Sometimes, antioxidant defense systems cannot completely prevent the harmful effects of free radicals, which results in oxidative stress [3]. In some studies that have been recently carried out in pathogen-plant interaction, it has been shown that catalase, ascorbate peroxidase, superoxide dismutase-like enzymes that destroy the toxic side effects of reactive oxygen molecules have effects [4].

In this study, the fact that the antioxidant properties of the above-mentioned enzymes are quite high is clearly seen in our analysis results. The inoculation of plants with pathogens or the application of microbial signals of cell culture leads to the rapid generation of hydrogen peroxide, which is one of the reactive oxygen species [5]. The organism structure tries to keep free radicals occurring as the normal product of the physiological activities of plants at a level as the oxidant-antioxidant balance with a very sensitive structure that the plant first gain. The disruption of this balance structure leads to oxidative stress. The biggest barrier to oxidative damage is the big difference between the oxygen concentration in the atmosphere (150mmHg) and the oxygen concentration in the tissue structure (30mmHg). In addition to this advantage, endogenous antioxidant systems and antioxidants which are called exogenous are also available [6,7].

Fruits are a rich source of phenolic and polyphenolic compounds that are responsible especially for their protection against oxidative stress. The close interest in the possible protective effects of dietary antioxidants against human degenerative diseases has led to the investigation of food components [8]. Apples, blueberry and apricot are the members of ***ericaceae*** family. Apricot that can be very easily distinguished from other fruits with the help of its different colors is grown in many regions in Turkey and in many regions in America and northern Europe in the world. That's exactly for this reason apricot fruit with many different species is called by different names according to its types in different regions. It is also of great importance to distinguish well according to this type of fruit which is called by the names Şekerpare, Iğdır, Malatya, Hacıhaliloğlu, Kabaaşı and Soğancı apricot to take advantage of its benefits. Some of the other apricot varieties grown in Turkey are Hasanbey, Çataloğlu, Çöloğlu, Alyanak, Şalak (Aprikoz), Şekerpare, Tokaloğlu-

Erzincan, Tokaloğlu-Yalova, Tokaloğlu-Konya Ereğli, Şam, Turfanda İzmir, İri, Bitirgen, İmrahor, Karacabey, Çiğli, Sakıt 2, Mahmudun Eriği, Adilcevaz-5, Turfanda Eskimalatya, Çekirge 52, Hacıkız, İsmailağa, Ethembey, Kuru Kabuk varieties. There are also some foreign apricot varieties which can be listed as Paviot, Canino, Stark Early Orange, Hungarian Best, Cafona, Precoce de Colomer, Polonais, San Castrese, Boccuccia, Wilson Delicious, Luizet, Fracasso, Royal, Perfection. Furthermore, apricot fruit contains antioxidants that fight cancer. Strong antioxidants in apricot fruit decrease the risk of developing various types of cancer. Antioxidants protect your cells against the damage of free radicals and environmental pollutants. This is very important to prevent the growth of tumors. Apricot fruit also contains folic acid. They are nutrients that help repair and synthesize DNA and also prevent the mutation of malignant cells. They strengthen the bones and teeth, protect the heart health, and in addition to these, they are also perfect for improving circulation and oxygenation of cells. They are also known to be good for diabetes, eye health and kidney disorders. When all these characteristics are taken into consideration, apricot fruit is a very important plant for human health. [9,10]

Superoxide dismutase (SOD, EC 1.15.1.1) is an enzyme that catalyses the conversion of the superoxide (O_2^-) radical to oxygen (O_2) and hydrogen peroxide (H_2O_2). Superoxide is a by-product of oxygen metabolism and, if not regulated orderly, leads to formation of cell damage [11]. H_2O_2 is also harmful for living cells and can be degraded by other enzymes. Therefore, SOD is an important antioxidant defense in nearly all living cells. One exception is Lactobacillus plantarum and related lactobacilli, which use a different mechanism to prevent damage from reactive O_2^-. Irwin Fridovich and Joe McCord at Duke University discovered the enzymatic activity of superoxide dismutase in 1968. SODs were previously known as a group of metalloproteins with unknown function; for example, CuZnSOD was known as erythrocuprein (or hemocuprein, or cytocuprein) or as the veterinary anti-inflammatory drug "Orgotein". Likewise, Brewer (1967) identified a protein that later became known as superoxide dismutase as an indophenol oxidase by protein analysis of starch gels using the phenazine-tetrazolium technique [12].

Free radicals that occur during normal metabolism or pathologically cause many damages in cells and tissues. Since oxidative damage caused by free oxygen radicals affects biomolecules such as protein, lipid and nucleic acid, some clinical tests for oxidative products of these biomolecules are used to demonstrate oxidative stress [13]. Hydroperoxides are the first stable products formed during the peroxidation of unsaturated lipids. The decomposition of lipid hydroperoxides forms a complex mixture of secondary peroxidation products such as hydrocarbon gases (ethane, pentane) and aldehydes (malondialdehyde, MDA). It is reported that MDA increases the permeability of cell membranes, affects ion exchange of membranes, disrupts intracellular ion balance, causes enzyme activities to deteriorate, breaks DNA structure. It is known that it may cause endothelial dysfunction and consequently atherosclerosis, causing disruption between antioxidants and free radicals [14,15].

In this study, superoxide dismutase (SOD), the antioxidant enzyme of apricot fruit and the level of malondialdehyde (MDA), which is the end product of lipid peroxidation, were examined.

2. Material and method

In this study, fruit samples were taken for the processes performed to determine antioxidant enzyme activity, and for the methods and antioxidant enzyme activities. Then, the processes of obtaining extracts were performed for antioxidant enzyme analyses. Apricots samples (***Prunus armeniaca***) were collected in Van region from Turkey.

2.1. Preparation of Apricot Extracts;

Apricot samples were cut into small pieces, frozen in liquid nitrogen and crushed in a blender. The apricot samples obtained were subjected to extraction with three different solvents (diethyl ether, ethanol, water) with different polarities (4,3; 24,3; 78,5 dielectric constant, respectively). For this purpose, 10 g of fruit samples was taken and extracted for 400 minutes with 250 mL diethyl ether in soxhlet apparatus. After extraction, diethyl ether was removed at 40 °C' in a rotary evaporator (ether extract). After the fruit residues obtained at the end of diethyl ether extraction were treated for eight times with 150/min revolution in the stirrer and one hour periods until the solution became colourless with 50 mL ethanol in the dark, they were filtered, and the filtrates were combined. Ethanol in the sample was removed at 40 °C' in a rotary evaporator (ethanol extract). Fruit residues from ethanol extraction were dried and then boiled for 10 minutes by mixing with distilled water (200 mL) with a volume of up to 20 times of it, and the filtrate was frozen after the filtration process. The frozen samples were kept in freeze dryer at 0.04 mbar -50 °C for 96 hours, and then the water was removed (water extract). Furthermore, after 10 grams of crashed fruit samples were boiled with distilled water (200 mL) with a volume of up to 20 times of them for 10 minutes and filtered, they were frozen and lyophilized (hot water extract). All extracts obtained were stored at +4 °C. The samples were preserved at -18 °C in cases where they were not used for a long time.

2.2. Determination of Superoxide Dismutase (SOD) Activity (Manual Method)

To determine the SOD activity of the apricot samples, the reactive solution sample was prepared according to literature [16]. The sample volume and blind volume were adjusted as indicated Table 1. After it was waited at a room temperature of 25˚C for 20 minutes, 50 µl of blind and 50 µl of samples from $CuCl_2$ were added to it. After pipetting was performed as indicated in 1, blind and sample tubes were read

spectrophotometrically against bidistilled water at 560 nm. The SOD activity of the sample was calculated accordingly to equation given below:

Inhibition, %: [(Blind OD – Sample OD) / Blind OD] x 100

1 Unit SOD: is the enzyme activity inhibiting Nitroblue tetrazolium reduction by 50%.

Activity = (inhibition, %) / (50 x 0.1)

Activity was calculated in U/ml [16].

Table 1. *SOD Activity Determination Method:*

	Blind	Sample
Reactive	1.425 µl	1.425 µl
Sample	-	50 µl
Bidistilled	-	100 µl
Xanthine oxidase	25 µl	25 µl

2.3. Determination of Malondialdehyde (MDA) Level

To determine the MDA level of the samples, the reactive solutions were prepared according to published procedure [17]

2.4. Experimental Procedure

200 ul of sample was taken in a tube. 800 µl of phosphate buffer and 25 µl of BHT solution and 500 µl of 30% TCA were added to it. The tubes were mixed in vortex and kept in the ice bath for 2 hours after their caps were closed. The tubes were cooled to room temperature. Then, after the caps of the tubes were removed, they were centrifuged at 2000 rpm for 15 minutes. 1 ml of the supernatant obtained from centrifugation (filtrate) was taken and transferred to other tubes. 75 µl EDTA, 25 µl TBA were added to filtrates 1 ml of which was taken. The tubes were mixed in vortex and kept in hot water bath at (70˚C) for 15 minutes. Then, it was cooled to room temperature, and their absorbance was read in UV/Vis spectrophotometer at 532 nm.

Calculation of Malondialdehyde (MDA) level:
C= F x 6.41 x A
C= concentration
F= dilution factor
A= absorbance
Level calculation; it was calculated as µmol/L [18].

3. Results

The experimental results for SOD and MDA are given in the Table 2. The trials were given according to statistical value as a result of 5 different groups and 3 repeated analyses ($p \leq 0.05$). Duncan and ANOVA statistical methods were used. Based on these results obtained, it was observed that apricot fruit had quite high values in terms of antioxidant enzyme activity compared to many other fruits (Apple, Pear, Quince Cranberry. Strawberry, etc.).

Table 2. *In this study, SOD activity values, the antioxidant enzyme apricot contains, and MDA values, which are the lipid peroxidation products, are given below.*

	Result
SOD (Superoxide Dismutase)	0.060 U/mI
MDA (Malondialdehyde Acid)	1.579 mmol/I

4. Discussion

The active oxygen derivative has at least 4 different roles in the plant.

1. Active oxygen first causes hypersensitive cell death.
2. Active oxygen has a direct lethal effect against the disease factor.
3. It plays a role in lignification. It is important for strengthening the cell wall.
4. Active oxygen acts as a stimulant molecule in the plant [19].

Hydrogen peroxide formed in the plant cell after the infection of the cause of disease or other active oxygen species have the signal effect on the plant's endurance mechanism.

Antioxidant groups are divided into two groups as enzymatic and non-enzymatic. We can define the substances that can prevent or delay the oxidation of substances with oxidization potential such as lipid, protein, DNA and carbohydrate, which are contained in the living cell, as antioxidants. They are present in fewer amounts in the tissues compared to oxidizable substrates [20,21]. In this study carried out on apricot fruit, it was found that antioxidant enzyme SOD (Superoxide Dismutase) was 0.06 U/mI and MDA was 1.579 mmol/I. The systems that prevent the formation of ROS may be different. The antioxidants that capture and neutralize ROSs such as mitochondrial cytochrome oxidase, which reduces ROSs are the systems detoxifying radicals such as flavonoids, alpha tocopherol, ascorbic acid, methionine, uric acid, beta carotene, reduced glutathione, mucus [22]. In accordance with these explanations in this study, it is seen that the increase in the consumption of apricot fruit according to the values of SOD and the values of the lipid peroxidation product MDA will be beneficial (due to high antioxidant enzyme activities) with respect to decreasing oxidative stress and removing radicals from the body or reducing their harmful effects for human health.

In apricot fruit, it was determined that SOD (Superoxide Dismutase) was 0.06 U/ml and MDA (Malondialdehyde acid) was 1.579 mmol/I. These values mean that the antioxidant values of apricot fruit are quite high compared to many other plants and fruits. This also shows the richness of the fruit in terms of the antioxidant which is effective in the removal of

radical compounds resulting from metabolic activities in living creatures, and that it is also an important factor especially in reducing the risk of cancer. On the other hand, it is also known to be effective on the prevention of the formation of Alzheimer's disease, which has a significant effect on brain function [23]. In the literature studies carried out, it is seen that apricot fruit protects the body against high levels of oxidative stress when it is considered that the DPPH Radical removal activity of blueberry is 52.5% and its reducing power value that is an indicator of antioxidant activity is 0.170 [10]. Recently, the tendency to the use of plant-derived natural antioxidants has gained importance in the treatment of many diseases [24]. In another study, total antioxidant capacity in apricot juice was determined by using FRAP method and it was reported to have higher antioxidant properties than orange, peach and cherry. In the literature studies, in both plant and human studies, it was reported that superoxide dismutase (SOD) enzyme activity increased in some plants, however, it decreased in various diseases in people [25].

5. Conclusion

In conclusion, it is seen that the investigation of apricot samples in Turkey in terms of the antioxidant substances, they contain natural nutrition in terms of human health are important and will help in fighting against diseases and adding value to national economy.

References

[1] Tosun İ., Yüksel S., "Üzümsü meyvelerin antioksidan kapasitesi", Gıda, 28(3), (2003), 305-311

[2] Akkuş İ., "Serbest Radikaller ve Fizyopatolojik Etkileri", Mimoza Yayınları, Konya, 1995, 68

[3] Yılmaz İ., "Antioksidan içeren bazı gıdalar ve oksidatif stress", İnönü Üniversitesi Tıp Fakültesi Dergisi, 17(2), (2010), 143-153

[4] Derviş E., "Oral antioksidanlar. Dermatoz", 2(1), (2011), 263-267

[5] Güler E., Dedeakayoğulları H., Kılınç A., Yalçın A.S., "Oksidatif stresin belirlenmesinde yeni bir yaklaşım", 23.ulusal biyokimya kongresi, 2011

[6] Mavi A., "İnsan eritrosit ve lökositlerinden süperoksit dismutaz enzimininsaflaştırılması ve bazı ilaçların enzim üzerine etkilerinin incelenmesi", Ph.D. dissertation, Institute of Natural Sciences, Atatürk University, 2005.

[7] Becker A., "Controlling als Praxis: Eine structurationsTheoretische Perspektive auf Controlling", In E. Scherm & G. Pietsch (Hrsg.), Controlling: Theorien und Konzeptionen. 2004, pp. 753-777

[8] T.C Orman ve Su İşleri Bakanlığ Orman Genel Müdürlüğü Yayınları Maviyemiş Likapa Eylem Planı 2015-2019

[9] Uruk T., Kahraman S., "Yaban Mersini (Vaccinim myrtillus) Antioksidan aktivitesinin araştırılması", Iğdır.Üniversitesi Fen Bilimleri Enst.Der., 71(4), (2017), 77-83

[10] Mot C.A., Dumitrescu S.R., Sarbu C.,."Rapid and effective evaluation of the antioxidant capacity of propolis extracts using DPPH bleaching kinetic profiles, FT-IR and UV-VIS spectroscopic data", Journal of Food Composite and Analysis, 24, (2011), 516-522

[11] Hayyan M., Hashim M.A., Al Nashef I.M., "Superoxide Ion, Generation and Chemical Implications", Chem. Rev., 116 (5), (2016), 3029–3085.

[12] Brewer G.J., "Achromatic regions of tetrazolium stained starch gels: inherited electrophoretic variation", American Journal of Human Genetics., 19 (5), (Sep 1967), 674–80.

[13] Therond P., Bonnefont-Rousselot D., Davit-Spraul A., Conti M., Leqrand A., "Biomarkers of oxidative stress: an analytical approach", Curr Opin Clin Nutr Metab Care, 3, (2000), 373-84

[14] Sargowo, D., Ovianti, N., Susilowati, E., Ubaidillah, N., Nugraha, A. W., Proboretno, K. S., Failasufi M., Ramadhan F., Wulandari H., Waranugrah Y., Putri, D. H. "The role of polysaccharide peptide of Ganoderma lucidum as a potent antioxidant against atherosclerosis in high risk and stable angina patients", Indian heart journal, 70(5), (2018), 608-614

[15] Erçin U., Bilgihan A., Erkan A.F., Yücel, H.б "New Parameters of Coronary Artery Diseases: Oxidative Stress Markers", Journal of Turkish Clinical Biochemistry, 17(1), (2019), 48-55

[16] Sun. Y., Oberley LW., Li Y., "A simple method for clinical assay of superoxide dismutase", Clin Chem. Mar, 34(3), (1988), 497-500

[17] Gutteridge J.M., "Lipid peroxidation and antioxidants as biomarkers of tissue damage", Clin Chem. Dec;41(12 Pt 2), (1995), 1819-28.

[18] Bergmeyer J., Grabl M., "Methods of Enzymatic Analysis (Third Edition), Germany, 1983, 190-302

[19] Stadtman E.R., "Importance of individuality in oxidative stress and aging", FreeRadical Biology and Medicine, 33, (2002), 597-604

[20] Çaylak E, "Hayvan ve bitkilerde oksidatif stres ile antioksidanlar. Biyokimya", AD, Çankırı Karatekin

Üniversitesi 18200 Tıp Araştırmaları Dergisi, 9(1) , (2011), 73-83

[21] Gutteridge J.M.C.,"Lipid peroxidation and antioxidants as biomarkers of tissue damage", Clin. Chem., 41, (1995), 1819-1828

[22] Rice-Evans C., , Re R, Pellegrini N., Proteggente A., Pannala A., Yang M., "Antioxidant activity applying an improved ABTS radical cation decolorization assay", Free Radic. Biol. Med., 26, (1999), 1231-1237

[23] Skrovankova S., Sumezynki P., Mlcek J., Jurikova T., Sochor J., "Bioactive Compounds and Antioxidant Activity in Different Types of Berries", Int.J.Mol.Sci.16, (2015), 24673-24706

[24] Haznedaroğlu M.Z., Öztürk T. Konyalıoğlu S., "Salvia smyrnaea boiss. uçucu yağının antioksidan ve antimikrobiyal aktivitesi", Bitkisel İlaç Hammaddeleri Toplantısı, Bildiriler, 2002. Eskişehir.

[25] Tosun İ., Üstün Ş., "An investigation about antioxidant capacity of fruit nectars", Pakistan Journal of Nutrition, 2(3), (2003), 167-169