http://www.medscidiscovery.com

OPEN ACCESS JOURNAL ISSN: 2148-6832

MSD Medical Science and Discovery
2019; 6(11):301-4

Research Article Doi: 10.36472/msd.v6i11.326

# The comparison of antioxidant enzyme activities in Quince Fruit (Cydonia Oblonga) grown in Van, Ankara and İzmir

**Halit Demir[1]*, Canan Demir[2]**

## Abstract

**Objective:** In this study, it was aimed to determine the activity of some antioxidant enzymes in the quince fruit (Cydonia Oblonga) which grown in different regions of Turkey.

**Materials and Methods:** For this study, firstly, quince fruit grown in different provinces such as Van, Ankara and İzmir was obtained for this study. Then, extracts of quince fruit were prepared and antioxidant enzyme activities were determined by spectrophotometric method. The findings were analyzed using statistical methods and the results were interpreted.

**Results:** The difference between the means in terms of Superoxide Dismutase, Catalase and Glutathione Reductase (SOD, CAT and GR) enzyme levels was found to be statistically significant for quince fruit grown in Van, Ankara and İzmir ($p < 0.05$). Accordingly, CAT, SOD and GR levels in quince fruit grown in İzmir were significantly higher than other regions.

**Conclusion:** As a result, the antioxidant property of quince fruit seems to be very important. The consumption of quince fruit especially in winter can be protective against some diseases, especially winter diseases. Further research on quince fruit should be done.

**Key words:** Quince, catalase, superoxide dismutase.

## Introduction

Quince (Cydonia oblonga Miller) tree drops leaves in winter. Quince is a sturdy fruit. It has been reported to be hard and acidic when the chemical and physical properties are examined. In the studies conducted in Eastern and Far East countries, quince fruit has been recommended to be consumed against diseases such as colds and coughs. Flavonoids are abundant in fruits and vegetables. They are also phenolic (1). In different studies, colorful fruits and vegetables have been determined to be rich in phenolic substances (2).

Free radicals are substances that contain uncombined electron pairs. When free radicals are over-synthesized, they can cause some damage to living organisms. Antioxidant enzymes serve as a shield against these damages (3, 4).

The studies conducted have focused on dust and extract in quince seeds. The above-mentioned substances have begun to be used in the cosmetic industry, espacially for beauty and skin care purposes (5). In literature studies, some antioxidant parameters of quince fruit seeds have been investigated (6).

This study aims to determine the antioxidant enyzme activities in quince fruit grown in different regions of Turkey.

## Material and Method

### Determination of superoxide dismutase (SOD) activity

SOD activity was determined using the method recommended by Sun et al. (7).

### Preparation of Reagent Solution:

1. 0.3 mM Xanthine: 4.56 mg of xanthine (Sigma X7375) was first dissolved in a few drops of 1N NaOH and dissolved in 100 ml of bi-distilled water.

2. 0.6 mM EDTA: 4.46 mg EDTA was dissolved in 20 ml bi-distilled water.

3. 150 mg / L NBT: 12.3 mg NBT (Sigma N6876) was dissolved in 100 ml bi-distilled water.

4. 400 mM $Na_2CO_3$: 2.544 gr Na 2 CO 3 was dissolved in 60 ml bi-distilled water.

**Received** 12-11-2019 **Accepted** 26-11-2019 **Available Online** 28-11-2019 **Published** 30-11-2019
1 Van Yuzuncu Yil University, Faculty of Science, Biochemistry of Department, Van, TR
2 Van Yuzuncu Yil University, Vocational School of High of Health Services, Van, TR
*** Corresponding Author:** Halit Demir **E-mail:** halitdemi@gmail.com

http://dx.doi.org/10.36472/msd.v6i11.326

5. Bovine serum albumin (1 g/L): 12 mg BSA (Sigma A2153) was dissolved in 12 ml bi-distilled water.

**Preparation of the reagent solution**

40 ml of xanthine solution, 20 ml of EDTA solution, 20 ml of NBT solution, 12 ml of $Na_2CO_3$ solution and 6 ml of BSA were mixed.

-16 µl of xanthine oxidase (167/l/L) (Sigma X1875) was taken and dissolved in 1 ml 2 M $(NH_4)2SO_4$.

-2M $(NH_4)2SO_4$: 2.643 g $(NH_4)2SO_4$ was completed to 10 ml with distilled water (stored at +4 °C).

-0.8 mM $CuCl_2$ $2H_2O$ 13.6 mg of $CuCl_2$ $2H_2O$ was prepared and completed to 100 ml with distilled water.

**Table 1.1.** Method of determination of SOD activity.

| Blind | Sample | |
|---|---|---|
| **Reaktif** | 1.425 µl | 1.425 µl |
| **Sample** | - | 50 µl |
| **Bi-distilled** | 100 µl | - |
| **Xanthine oxidase** | 25 µl | 25 µl |
| **It was kept for 20 minutes at room temperature of 25˚C** | | |
| **$CuCI_2$** | 50 µl | 50 µl |

After pipetting as indicated in Table 1.1, the absorbances in blind and sample tubes were determined versus bidistilled water at 560 nm.

Activity Account:

% inhibition: [(Blind OD - Sample OD) / Blind OD]x100

1 Unit SOD: The enzyme activity that inhibits NBT reduction by 50%.

Activity= (% inhibition)/(50x0.1)

Activity; Calculated in U/ml.

**Determination of catalase (CAT) activity**

In this study in which hydrogen peroxide was used as substrate, the catalase activity was determined through Aeibi method. The activity was carried out as follows: 1.4 ml of 30 mM $H_2O_2$ was added to the blinded tube and 0.1 ml of phosphate buffer was added. 1.4 ml of 30 mM H2O2 was added to the sample tube. 0.1 ml of enzyme was added and mixed with vortex. Absorbances at 240 nm were determined twice at 30 second intervals, and thus activity was prescribed (8).

**Solutions used:**

1. Preparation of 30 mM $H_2O_2$: 34 ml of 30% $H_2O_2$ was added into 10 ml of bidistillated water (25.8 ml of 35% $H_2O_2$).

2. Preparation of 50 mM Phosphate Buffer: 6.81 gr of $KH_2PO_4$ and 7.1 gr of $Na_2HPO_4$ were dissolved in bidistillated water, the pH of the buffer was adjusted to 7.4 with 1N NaOH and the volume was completed to 1 litre.

**Activity Account:**

E.Ü. = (2,3 / Δx) x [(log A1 / log A2)] Activity; Calculated in U / L.

Δx = 30 seconds

2,3 = the optical density given by 1 olmol $H_2O_2$ in 1 cm of light path.

Determination of glutathione reductase (GR) activity:

**Solutions used:**

1. 100 mM $Na_2HPO_4$ buffer with a pH of 8 was prepared. 7.1 gr of $Na_2HPO_4$ was dissolved in 400 ml of bidistilled water, a few drops of 1N NaOH were added, the pH was adjusted to 8 and the total volume was completed to 500 ml with the remaining 100 ml of bidistilled water.

**2. Daily buffer:** Prepared by dissolving 0.12 mM NADPH (Sigma N7505) and 1 mM GSSG (Sigma G4626) in buffer. Buffer solutions were stirred magnetically and the indicated amount of NADPH and GSSG was melted.

50 tests (must be prepared daily in buffer): 0.0050 gr of NADPH was dissolved in 50 ml of buffer, 0.0328 g of GSSG was dissolved in the same buffer.

**Experimental Procedure:**

1-) 100 µl of purified water and 900 µl of daily buffer were put in the blind tube.

2-) 900 ul daily buffer and 100 seruml serum were added to the sample tube and the tubes were vortexed.

3-) Tubes were incubated at 37 C for 10 minutes.

4-) Absorbances were determined at 340 nm in 0th and 5th minutes versus blind tube

**Activity Calculation**

(U/ml): (ΔOD/6.22) x (Vt/V0)

ΔOD = absorbance change over time.

Vt=Total volume.

V0=Sample volume.

6.22 = OD value given by 1 nmol NADPH in 1 cm of light path (9).

**Statistical analysis**

Mean, standard deviation, standard error, minimum and maximum values were used in descriptive statistics of the data. Univariate analysis of variance was used in cases where normal distribution condition was provided, and Kruskal Wallis test statistic was used in cases where normal distribution condition was not provided. The statistical significance level was taken as $p<0.05$ and the SPSS statistical software version 19.0 (SPSS Inc, Chicago, III, USA) pack has used for analyses.

http://dx.doi.org/10.36472/msd.v6i11.326

## Results

**Table 1.** Descriptive statistics and comparison results of parameters CAT, SOD and GR

| | | n | Mean±Std. Dev. | Std. Error | Minimum | Maximum | p |
|---|---|---|---|---|---|---|---|
| CAT (U/L) | Van | 20 | 2,555±0,22118 | 0,04946 | 2,20 | 2,90 | 0.001 |
| | Ankara | 20 | 1,225±0,11180 | 0,02500 | 1,10 | 1,40 | |
| | İzmir | 20 | 3,7±0,14510 | 0,03244 | 3,50 | 3,90 | |
| SOD (U/L) | Van | 20 | 4,54±0,25005 | 0,05591 | 4,20 | 4,90 | 0.001 |
| | Ankara | 20 | 2,53±0,17800 | 0,03980 | 2,30 | 2,80 | |
| | İzmir | 20 | 5,06±0,19029 | 0,04255 | 4,80 | 5,30 | |
| GR (U/L) | Van | 20 | 7,1655±0,07571 | 0,01693 | 7,05 | 7,32 | 0.001 |
| | Ankara | 20 | 6,109±0,04745 | 0,01061 | 6,04 | 6,19 | |
| | İzmir | 20 | 8,119±0,08855 | 0,01980 | 7,96 | 8,25 | |

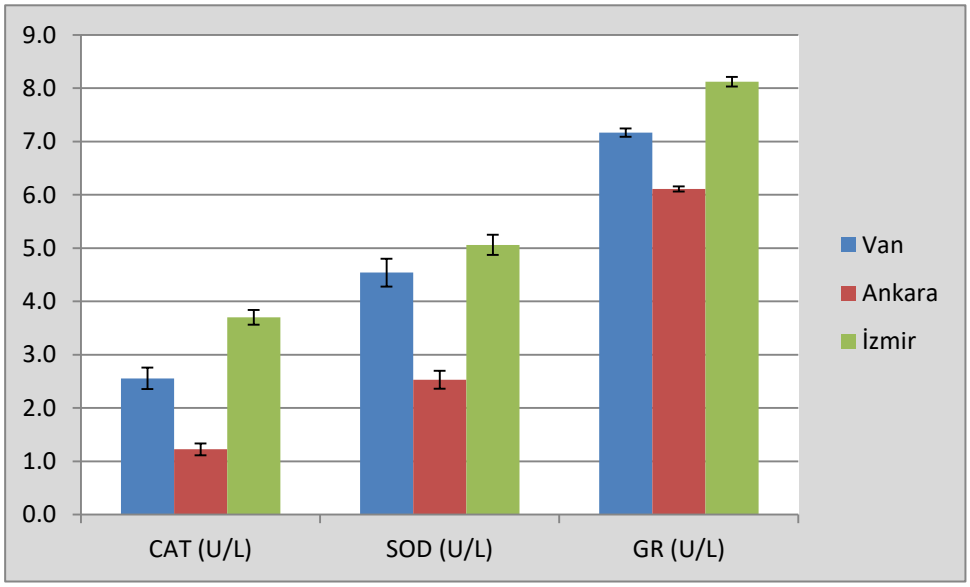


**Figure 1.** CAT, SOD and GR levels of quince fruit by provinces

Descriptive statistics and comparison results for CAT, SOD and GR are given in Table 1. When Table 1 was examined, the difference between the means in terms of CAT, SOD and GR levels was found to be statistically significant for quince fruit grown in Van, Ankara and İzmir ($p < 0.05$). CAT levels were found to be 2,555 in the quince fruit grown in Van, 1,225 in the quince fruit grown in Ankara, and 3.7 in the quince fruit grown in İzmir. SOD levels were found to be 4.54 in quince fruits grown in Van, 2.53 in quince fruits grown in Ankara, and 5.06 in quince fruits grown in Izmir. GR level was found to be 7,1655 for quince fruits grown in Van, 6,109 for quince fruits grown in Ankara, and 8,119 for quince fruits grown in İzmir. CAT, SOD and GR levels in quince fruit grown in İzmir province were significantly higher than other regions (Figure 1).

## Discussion

Free oxygen radicals are known to cause many diseases such as diabetic, cancer, arterosclerosis, cardiovascular diseases, malaria, neurodegenerative diseases, kidney disorders, immune system disorder, cataract, DNA damage and many age-associated diseases (10).

Consumption of fruit and vegetables has been reported to be an important protector against many diseases, because many fruits and vegetables contain great amounts of phenolic substances. In the studies, it can be said that the amount of antioxidant also increases if the amount of phenolic substance is high. The fruits are rich in Vitamin E, vitamin C and carotenoids. It has been reported that consuming fruit reduces oxidative stress and cellular damage (11).

Quince fruit is a rich vitamin store. In studies, quince fruit has been observed to have great amounts of trace elements, macro and micro mineral substances and sugar (12).

http://dx.doi.org/10.36472/msd.v6i11.326

In literature, it has been stated that quince fruit has antioxidant, antimicrobial and antiulcerative properties (11, 13).

Descriptive statistics and comparison results for CAT, SOD and GR are given in Table 1. When Table 1 was examined, the difference between the means in terms of CAT, SOD and GR levels was found to be statistically significant for quince fruit grown in Van, Ankara and İzmir ($p < 0.05$). CAT levels were found to be 2,555 in the quince fruit grown in Van, 1,225 in the quince fruit grown in Ankara, and 3.7 in the quince fruit grown in İzmir. SOD levels were found to be 4.54 in quince fruits grown in Van, 2.53 in quince fruits grown in Ankara, and 5.06 in quince fruits grown in Izmir. GR level was found to be 7,1655 for quince fruits grown in Van, 6,109 for quince fruits grown in Ankara, and 8,119 for quince fruits grown in İzmir. CAT, SOD and GR levels in quince fruit grown in İzmir province were significantly higher than other regions (Figure 1).

As a result, the antioxidant property of quince fruit seems to be very important and shows differentiation among the different regions. Further research on quince fruit should be done.

**Conflict of Interest:** The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Author's Contributions:** The correspondent author conceived the idea, carried out the design, and supervised the findings of this work. The correspondent author and co-authors verified the analytical methods and wrote the manuscript. All authors discussed the results and contributed to the final manuscript.

## References


1. Bravo L D. Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. Nutrition Reviews, 1998; 11,317-333.
2. Cieslik E, Greda A, Adamus W. Contents of polyphenols in fruit and vegetables. Food Chem. 2006; 94, 135-142.
3. Gutteridge JMC. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin. Chem. 1995; 41: 1819-28.
4. Güler E, Dedeakayoğulları H, Kılınç A, Yalçın A S. Oksidatif stresin belirlenmesinde yeni bir yaklaşım 23.ulusal biyokimya kongresi. 2011.
5. Silva BM, Andrade PB, Ferreres F, Seabra RM, Oliveira MBPP, Ferreira MA. Natural Product Research. 2005; 19: 3, 275-781.
6. Rice-Evans CA, Miller NJ, Paganga G. Trends in Plant Science. 1997; 2, 152-159.
7. Sun Y, Oberley LW, Lı Y. A simple method for clinical assay of superoxide dismutase. Clin. Chem. 1988;34: 497-500.
8. Aebi H. Catalase.(in) Methods In Enzymology. L. Packer (Editör), Academic Pres, Orlando. 1984;105: 121-126.
9. Jiang M, Zhang J. Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. J Exp Bot 2002; 53(379): 2401-2410.
10. Halliwell B. Antioxidant and human disease: A general introduction. Nutr. Rev. 1997;55(1),44-52.
11. Oliveira PA, Pereira JA, Andrade PB, Valenta P, Seabra MR, Silva BM.Organic acids composition of Cydonia Oblonga Miller leaf. Food Chem. 2008; 111,393-399.
12. Moreira R, Chenlo F, Torres MD, Vallejo N. Thermodynamic analysis of experimental sorption isotherms of loquat and quince fruit. J. of Food Eng. 2008; 88,514-521.
13. Oğraşıcı E. Ayva nektarında biyoaktif bileşenler ve antioksidan aktivitenin depolamada değişimi. Ankara Üniversitesi Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi. 2010. Ankara.


Copyright © 2019 The Author(s); This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), (CC BY NC) which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. International journal of Medical Science and Discovery.