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

Halit Demir¹*, Canan Demir²

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, especially 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 enzyme 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 of bi-distilled water.
4. 400 mM Na₂CO₃: 2.544 gr Na₂CO₃ was dissolved in 60 ml bi-distilled water.
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 Na₂CO₃ 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 (NH₄)2SO₄.

-2M (NH₄)2SO₄: 2.643 g (NH₄)2SO₄ was completed to 10 ml with distilled water (stored at +4 °C).

-0.8 mM CuCl₂ 2H₂O 13.6 mg of CuCl₂ 2H₂O was prepared and completed to 100 ml with distilled water.

Table 1.1. Method of determination of SOD activity.

<table>
<thead>
<tr>
<th>Blind</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaktif</td>
<td>1.425 µl</td>
</tr>
<tr>
<td>Sample</td>
<td>1.425 µl</td>
</tr>
<tr>
<td>Bi-distilled</td>
<td>-</td>
</tr>
<tr>
<td>Xanthine oxidase</td>
<td>100 µl</td>
</tr>
<tr>
<td>It was kept for 20 minutes at room temperature of 25°C</td>
<td>25 µl</td>
</tr>
<tr>
<td>CuCl₂</td>
<td>50 µl</td>
</tr>
<tr>
<td></td>
<td>50 µl</td>
</tr>
</tbody>
</table>

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] x 100

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

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

Activity Account: 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 H₂O₂ 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 H₂O₂: 34 ml of 30% H₂O₂ was added into 10 ml of bidistilled water (25.8 ml of 35% H₂O₂).

2. Preparation of 50 mM Phosphate Buffer: 6.81 gr of KH₂PO₄ and 7.1 gr of Na₂HPO₄ were dissolved in bidistilled 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.U. = (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 H₂O₂ in 1 cm of light path.

Determination of glutathione reductase (GR) activity:

Solutions used:

1. 100 mM Na₂HPO₄ buffer with a pH of 8 was prepared. 7.1 gr of Na₂HPO₄ 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.
Results

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

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean±Std. Dev.</th>
<th>Std. Error</th>
<th>Minimum</th>
<th>Maximum</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT (U/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Van</td>
<td>20</td>
<td>2,555±0,22118</td>
<td>0,04946</td>
<td>2,20</td>
<td>2,90</td>
<td>0.001</td>
</tr>
<tr>
<td>Ankara</td>
<td>20</td>
<td>1,225±0,11180</td>
<td>0,02500</td>
<td>1,10</td>
<td>1,40</td>
<td></td>
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<tr>
<td>İzmir</td>
<td>20</td>
<td>3,7±0,14510</td>
<td>0,03244</td>
<td>3,50</td>
<td>3,90</td>
<td></td>
</tr>
<tr>
<td>SOD (U/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Van</td>
<td>20</td>
<td>4,54±0,25005</td>
<td>0,05591</td>
<td>4,20</td>
<td>4,90</td>
<td>0.001</td>
</tr>
<tr>
<td>Ankara</td>
<td>20</td>
<td>2,53±0,17800</td>
<td>0,03980</td>
<td>2,30</td>
<td>2,80</td>
<td></td>
</tr>
<tr>
<td>İzmir</td>
<td>20</td>
<td>5,06±0,19029</td>
<td>0,04255</td>
<td>4,80</td>
<td>5,30</td>
<td></td>
</tr>
<tr>
<td>GR (U/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Van</td>
<td>20</td>
<td>7,165±0,07571</td>
<td>0,01693</td>
<td>7,05</td>
<td>7,32</td>
<td>0.001</td>
</tr>
<tr>
<td>Ankara</td>
<td>20</td>
<td>6,109±0,04745</td>
<td>0,01061</td>
<td>6,04</td>
<td>6,19</td>
<td></td>
</tr>
<tr>
<td>İzmir</td>
<td>20</td>
<td>8,119±0,08855</td>
<td>0,01980</td>
<td>7,96</td>
<td>8,25</td>
<td></td>
</tr>
</tbody>
</table>

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

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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