Anadolu Tarım Bilimleri Dergisi,
Anadolu Journal of Agricultural Sciences

e-ISSN: 1308-8769, ANAJAS February 2023, 38 (1): 199-208

Characterization of Catalase Enzyme from Leaf Tissue of Aronia (Aronia melanocarpa) Plant

Aronia (Aronia Melanocarpa) Bitkisinin Yaprak Dokusundan Katalaz Enziminin Karakterizasyonu

Ömer TAŞ[1], Betül MİTROVİCA[2], Deniz EKİNCİ[3]

[1]Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Biotechnology, 55139, Samsun
• omer.tas@omu.edu.tr • ORCİD > 0000-0003-1782-8210

[2]Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Biotechnology, 55139, Samsun
• betullmitrovica@gmail.com • ORCİD > 0000-0002-6229-6172

[3]Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Biotechnology, 55139, Samsun
• deniz.ekinci@omu.edu.tr • ORCİD > 0000-0001-7849-4117

Makale Bilgisi / Article Information

Makale Türü / Article Types: Araştırma Makalesi / Research Article
Geliş Tarihi / Received: 27 Aralık / December 2022
Kabul Tarihi / Accepted: 25 Ocak / January 2023
Yıl / Year: 2023 | **Cilt – Volume:** 38| **Sayı – Issue:** 1 |**Sayfa / Pages:** 199-208

Atıf/Cite as: Taş, Ö., Mitrovica, B. ve Ekinci, D. "Characterization of Catalase Enzyme from Leaf Tissue of Aronia (Aronia melanocarpa) Plant" Anadolu Journal of Agricultural Sciences, 38(1), February 2023: 199-208.

Sorumlu Yazar / Corresponding Author: Deniz EKİNCİ

https://doi.org/10.7161/omuanajas.1225053

CHARACTERIZATION OF CATALASE ENZYME FROM LEAF TISSUE OF ARONIA (ARONIA MELANOCARPA) PLANT

ABSTRACT

Aronia is among the most antioxidant containing plants which is found commonly around the world. Aronia cultivation started in Turkey for the first time in 2012 at the Atatürk Central Research Institute of Garden Cultures, and a plantation was constructed in the experimental area. Since antioxidants help to preserve food by blocking oxidation processes and contributing to the health promotion provided by numerous dietary supplements, nutraceutical and functional food additives, antioxidant capacity of these plants should be well characterized. To assess and evaluate the antioxidant content of foods and plant products, many approaches are utilized. In this study, catalase (CAT) enzyme was partially purified from aronia plant leaf tissue and characterization was carried out. Purification process consisted of homogenate preparation, ammonium sulfate precipitation and dialysis. The optimal ionic strength, pH, substrate concentration and enzyme quantity were examined. These values were found to be 300 mM Tris, pH:8.0, 12 mM H_2O_2 and 75 µl, respectively, for the catalase enzyme of the Aronia plant leaf tissue. This study is the first in the literature dealing with the characterization of antioxidant enzyme from Aronia plant.

Keywords: Antioxidant, Aronia, Catalase, Characterization.

ARONIA (ARONIA MELANOCARPA) BİTKİSİNİN YAPRAK DOKUSUNDAN KATALAZ ENZİMİNİN KARAKTERİZASYONU

ÖZ:

Aronia, dünya çapında yaygın olarak bulunan en çok antioksidan içeren bitkilerden biridir. Türkiye'de ilk kez 2012 yılında Atatürk Bahçe Kültürleri Merkez Araştırma Enstitüsü'nde Aronia yetiştiriciliğine başlanmış ve deneme alanına ağaçlandırma yapılmıştır. Antioksidanlar, oksidasyon süreçlerini bloke ederek ve çok sayıda diyet takviyesi, nutrasötik ve fonksiyonel gıda katkı maddesi tarafından sağlanan sağlığın önemine katkıda bulunarak gıdaların korunmasına yardımcı olduklarından, bu bitkilerin antioksidan kapasiteleri iyi karakterize edilmelidir. Gıdaların ve bitkisel ürünlerin antioksidan içeriğini değerlendirmek ve belirlemek için birçok yaklaşım kullanılmaktadır. Bu çalışmada aronia bitkisinin yaprak dokusundan katalaz (CAT) enzimi kısmen saflaştırılmış ve karakterizasyonu yapılmıştır. Saflaştırma işlemi, homojenat hazırlama, amonyum sülfat çökeltme ve

diyalizden oluşmaktadır. Optimum iyonik kuvvet, pH, substrat konsantrasyonu ve enzim miktarı incelenmiştir. Bu değerler Aronia bitki yaprak dokusunun katalaz enzimi için sırasıyla 300 mM Tris, pH:8.0, 12 mM H_2O_2 ve 75 µl olarak bulunmuştur. Bu çalışma Aronia bitkisinden elde edilen antioksidan enzimin karakterizasyonu ile ilgili literatürdeki ilk çalışmadır.

Anahtar Kelimeler: Antioksidan, Aronya, Katalaz, Karakterizasyon.

1. INTRODUCTION

A native plant of North America, Aronia melanocarpa (aronia) is commonly known as black chokeberry and is now cultivated worldwide. The aronia plant, which belongs to the Rosaceae family, is very rich in anthocyanins and other phenolic compounds and has a dark purple color. With its high total polyphenol and anthocyanin content and DPPH radical scavenging activity, Aronia is known to have powerful antioxidant features compared to many other fruits (Jakobek et al., 2007; Benvenuti et al., 2004).

Aronia has a wide range of phenolic chemicals, including phenolic acids, flavonols, anthocyanins, and flavan-3-ols (Taheri et al., 2013). So far, it has been shown that aronia berry decreases cystolic blood pressure and cholesterol levels (Hawkins et al., 2021), which reduces the risk of chronic illnesses (Jurikova et al., 2017) and provides strong antioxidant protection (Kardum et al., 2014).

O_2 is generally not reactive to most cellular components, but ROS (reactive oxygen species) causes oxidation of lipids, proteins, RNA, DNA and many small molecules in the cell. ROS have a high reactivity to these biological components due to their changed chemistry as compared to O_2, which permits them to donate an electron or transfer an excited energy state to an acceptor molecule (Halliwell and Gutteridge, 2015). Hydrogen peroxide (H_2O_2), superoxide (O_2), singlet oxygen ($^1O_2$), the hydroxyl radical (HO.) and different types of organic and inorganic peroxides are the principal forms of ROS in cells, which vary widely in their characteristics and chemical reactivity (Halliwell and Gutteridge, 2015; Mittler, 2017; Waszczak et al., 2018; Smirnoff and Arnaud, 2019; Sies and Jones, 2020). Since ROS is very reactive and is produced independently in nearly all cell compartments, its levels must be controlled to prevent undesired cellular oxidation.

A group of polyphenolic compounds commonly found in fruits, vegetables and other food products and produced as secondary metabolites in plants are called flavonoids. In addition to other bioactivities (eg, anti-inflammation, anti-aging), flavonoids have beneficial biochemical effects on certain diseases (eg, anti-glaucoma, anti-cancer) by affecting certain enzymes (Guven et al., 2019; Williamson et al., 2018; Gentile et al., 2018). Their principal biological function is antioxidant

protection. Flavonoid antioxidant activity can protect against free radical damage by scavenging reactive oxygen species, activating antioxidant enzymes, inhibiting oxidases (e.g., xanthine oxidase [XO], cyclooxygenase [COX], lipoxygenase and phosphoinositide 3-kinase [PI3K]), and reducing α-tocopheryl radicals. To decrease oxidative stress, flavonoid antioxidant activity can raise uric acid levels, metal-chelating activity, and low-molecular-weight antioxidant activity (Williamson et al., 2018).

Antioxidants have been promoted as helpful agents in improving plant stand and minimizing the impacts of biotic and abiotic stressors.

Plants have many enzymatic and non-enzymatic defensive strategies against oxidative stressors caused by ROS. The antioxidant enzymes of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) have an important place in the enzymatic defense systems of plants to remove ROS (Azevedo Neto et al., 2004). SOD is the primary O_2.- scavenger and results in the formation of H_2O_2 and O_2 by enzymatic reaction. The H_2O_2 generated is subsequently removed by CAT (Azevedo Neto et al., 2006). Catalase (H_2O_2 : H_2O_2 oxidoreductase E.C.1.11.1.6) is a kind of antioxidant enzyme found in all aerobic organisms. It is known that H_2O_2 is converted into water and oxygen in the cell in the presence of catalase with the realization of environmental stress. Catalase is found in all major locations of H_2O_2 generation in higher plants' cellular environments (such as peroxisomes, mitochondria and cytosol) (Sharma and Ahmad, 2014).

Due to the importance of catalase in plant defense system, we aimed in this study to purify the enzyme from Aronia melanocarpa leaves for the first time and to determine optimum buffer, optimum ionic strength, optimum pH and optimum substrate amount in order to find new potential natural antioxidant sources.

2. METHODS

2.1. Chemicals

Sigma-Aldrich supplied the chemicals utilized in the purifying process Aldrich (St. Louis, Mo, USA). Merck supplied the other compounds used (Darmstadt, Germany).

2.2. Preparation of the Homogenate and in Vitro Enzyme Assay

Leaf tissue was obtained from the aronia plant. The leaves were crushed into small pieces and then thoroughly crushed and homogenized with liquid nitrogen (approximately -196°C), then 300 mM Tris (2-Amino-2-(hidroksimetil)-1,3-propandiol) buffer was added and centrifuged at 4°C, 15.000 xg. After centrifugation

step, the supernatant was filtered and enzyme activity was measured. The activity was measured at 240 nm using a Shimadzu UV-1800 spectrophotometer.

Hydrogen peroxide (H2O2) was used as the substrate for catalase. 100 µl of H2O2 in 0.3 M Tris buffer (pH 8.0) was transferred to the cuvette. The enzymatic reaction was initiated by adding 100 µl of supernatant containing catalase enzyme to the cuvette, and the final volume was reduced to 1 ml with distilled water; the absorbance value at the commencement of the reaction was then measured in a spectrophotometer at 240 nm.

2.3. Ammonium Sulfate Precipitation and Dialysis

Ammonium sulfate precipitation was performed for the prepared homogenate. Accordingly, the homogenate was adjusted at different intervals with solid ammonium sulfate at a salt concentration of 0-100%. The precipitate was dissolved with a minimal amount of Tris buffer 0.3 M, Tris. The maximum enzyme activity was found at concentrations ranging from 20-40%.

To remove salts from protein solutions, dialysis was performed. A dialysis bag with a semi-permeable membrane, typically composed of cellulose acetate and having a porous structure, is utilized for this procedure. The prepared solution was placed in this bag and slowly mixed by passing it through the suitable buffer. Small molecules go across the membrane until the osmotic pressure was adjusted. The buffer outside the membrane was altered multiple times during this procedure.

2.4. Characterization of the Enzyme With Kinetic Parameters

To characterize the enzyme, different pH, substrate, and ionic strength parameters were examined. The enzyme's characterization parameters were calculated as optimum ionic strength: 300 mM Tris, pH: 8.0 and substrate concentration: 12 mM.

3. RESULTS AND DISCUSSION

Catalase enzyme was partially purified and characterized from aronia plant leaf tissue in this work. The high antioxidant content of the aronia plant and its favorable effects on human health highlight the significance of our work. Throughout history, people have used the leaves of many plants to make herbal tea. These teas contain high levels of phytochemicals derived from polyphenols, flavonoids and chlorophyll. Phytochemicals are secondary metabolites found in plants that have been extensively studied in terms of different bioactivities such as anticancer, anti-inflammatory and antibacterial, and so on (Gawron-Gzella et al., 2012; Do Thi and Hwang, 2014). Quercetin, rutin, and chlorogenic acid are the most abundant polyphenols in aronia leaves (Pirvu et al., 2014). Polyphenols are the most abun-

dant antioxidant chemicals in plants which can reduce inflammation, cancer, and aging by quenching reactive oxygen species (Tsuda, 2012).

The characterization research is critical for determining and selecting the ideal values for the importance and antioxidant characteristics of the aronia plant-derived catalase enzyme. As previously stated, in addition to the relevance of the aronia plant's leaf content, the characterisation of the catalase is critical for both the plant and the enzyme.

Because of the strong antioxidant content of aronia, the purification and characterisation of the catalase enzyme demonstrates the study's uniqueness.

Catalase enzyme was partially purified from aronia plant and characterized in this work for the first time. After homogenization, the enzyme's precipitate saturation with solid (NH4)2SO4 was determined to be 20-40%. This finding demonstrates that the purification process is consistent with previous investigations and will serve as a model for future research. Both potassium phosphate and Tris buffer measurements were made for optimum ionic strength optimization. The ideal ionic strength was evaluated between 10 mM and 600 mM Tris buffer as a result of the optimization experiments, and the optimum ionic strength was identified in 300 mM Tris (Table 1).

Table 1. Activity measurements of aronia fruit leaf tissue catalase enzyme optimal ionic strength TRIS buffer

[mM]	10	20	50	100	150	200	300	400	500	600
%Activity	22.8	74.8	64.5	58.2	84	70,8	100	53,7	74,8	63,4

pH was adjusted between 5.0 and 8.5 to find the optimal pH, which was determined to be 8.0 (Table 2).

Table 2. Activity measurements of aronia fruit leaf tissue catalase enzyme optimal pH value Tris (300 mM) buffer

pH	5.0	6.0	6.5	7.0	7.5	8.0	8.5
%Activity	95.2	62.8	56.2	68.8	82.0	100	90.4

In addition, the optimum substrate concentration was measured between 3 and 15 mM and the optimum substrate concentration was found to be 12 mM H2O2 (Table 3).

Table 3. Optimum substrate concentration 300 mM TRIS (pH=8) buffer activity measurements for aronia fruit leaf tissue catalase enzyme

H_2O_2 (mM)	3	6	9	12	15
%Activity	25	50	75	100	125

Many studies on the inhibition of CAT and other antioxidant enzymes have been done in the literature, and the results showed similar results with our study.

In a study by Dinçler and Aydemir, catalase enzyme was purified from the chard plant. As a result of the study, a wide optimum pH range was found to be 6.0-8.0. At the same time, the precipitation range of ammonium sulfate was found to be 45% (Dinçler and Aydemir, 2001).

In another study, catalase enzyme was purified from sprouted black gram (*Vigna mungo*) seeds and optimum pH and temperature were found to be 7.0 and 40 °C, respectively (Kandukuri et al., 2012).

In the purification and characterization study of the catalase enzyme carried out in Turkey Van apple, the optimum pH value was found to be 5.0 and the optimum temperature was 50 °C (Yoruk et al., 2005).

In the study of partial purification of catalase enzyme from red cabbage, the optimum pH was found to be 7.0 at an optimum temperature of 30 °C (Adnan et al., 2018).

Agaricus bisporus is a well-known and extensively consumed mushroom. The best pH value for the purification and characterisation of the catalase enzyme from this fungus was determined to be 7.5, while the optimum ammonium sulfate precipitation range was 45-90% (Susmitha et al., 2013).

In another study, the catalase enzyme from the seaweed *Porphyra yezoensis* was characterized and the optimum pH value was found in the range of 6.0 to 11.0 at an optimum temperature of 30 °C (Nakano et al., 1995).

Catalase enzyme is found not only in plants but also in animals and the purification process takes place. Purification and characterization studies from different animals and different tissues have also found similar results with our study.

In the study on the purification and partial characterization of catalase from chicken erythrocytes, the optimum pH was 7.0 and the optimum temperature was 25 °C (Aydemir and Kuru, 2003).

The optimal pH and temperature in the Purification and Properties of Liver Catalase in Water Buffalo (*Bubalus bubalis*) investigation were determined to be 7.5 and 30 C, respectively (Nadeem et al., 2015).

In a research comparing purification and characterisation of liver catalase with normal dog liver catalase in the acatalasemic beagle dog, the activities of wild type and acatalasemic dog liver catalases revealed distinct pH profiles in the pH range of 3.0 to 11.0. Catalase activity purified from wild type dog liver did not alter significantly across a large pH range, although it did demonstrate activity even at pH 11.0. Catalase activity isolated from acatalasemic dog liver, on the other hand, was only stable in a restricted pH range of 6.0-9.0 (Nakamura et al., 2000).

In a study, it was found that the aronia plant has high antioxidant activity. These results demonstrated the high antioxidant activity of chokeberry berries with a large variation ranging from 127.45 (juice) to 301.89 (pomace) for DPPH radical equivalents per µM Trolox/100 g dry weight and from 314.05 (juice) for ABTS radical to 779.58 (pomace) µM Trolox/100 g dry weight (Oszmiański and Wojdylo, 2005).

Catalase enzyme has been isolated from many tissues of both plants and mammals, as observed in our work and other investigations, and characterisation tests have been performed. We think that the results obtained from our study will contribute to catalase enzyme purification and characterization studies to be carried out in the future.

4. CONCLUSIONS

As a result, catalase enzyme was characterized from leaf tissue of aronia plant. This study is the first to reveal the partial purification and characterization of the catalase enzyme of aronia, which is an economically important plant and has high antioxidant value in both the leaf and fruit part. Our findings will help to promote the emergence of novel aronia plant characteristics as well as the consumption of the plant.

Conflict of Interest:

The authors declare that there is no conflict of interest.

Ethics:

This study does not require ethics committee approval

Author Contribution Rates:

Design of Study: ÖT (%40), BM (%20), DE (%40)

Data Acquisition: ÖT (%40), BM (%20), DE (%40)

Data Analysis: ÖT (%40), BM (%30), DE (%30)

Writing Up: ÖT (%30), BM (%30), DE (%40)

Submission and Revision: ÖT (%40), BM (%20), DE (%40)

REFERENCES

Adnan, A. M., & GhalebAL-Dabbagh, R., 2018. Examination Of Catalase Enzyme In Green Cabbage And Some Characters Of It. *European Journal of Sport Sciences and Public Health,* 5, 1.

Aydemir, T., Kuru, K., 2003. Purification and partial characterization of catalase from chicken erythrocytes and the effect of various inhibitors on enzyme activity. *Turkish Journal of Chemistry,* 27(1), 85-98.

Azevedo Neto, A. D. D., Prisco, J. T., Enéas-Filho, J., Lacerda, C. F. D., Silva, J. V., Costa, P. H. A. D., & Gomes-Filho, E., 2004. Effects of salt stress on plant growth, stomatal response and solute accumulation of different maize genotypes. *Brazilian Journal of Plant Physiology,* 16, 31-38.

Benvenuti, S., Pellati, F., Melegari, M. A., & Bertelli, D., 2004. Polyphenols, anthocyanins, ascorbic acid, and radical scavenging activity of Rubus, Ribes, and Aronia. *Journal of food science,* 69(3), 164-169.

de Azevedo Neto, A. D., Prisco, J. T., Enéas-Filho, J., de Abreu, C. E. B., & Gomes-Filho, E., 2006. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environmental and Experimental Botany*, 56(1), 87-94.

Dinçler, A., Aydemir, T., 2001. Purification and characterization of catalase from chard (Beta vulgaris var. cicla). *Journal of enzyme inhibition*, 16(2), 165-175.

Do Thi, N., Hwang, E. S., 2014. Bioactive compound contents and antioxidant activity in aronia (Aronia melanocarpa) leaves collected at different growth stages. *Preventive nutrition and food science,* 19(3), 204.

Gawron-Gzella, A., Dudek-Makuch, M., & Matlawska, I., 2012. DPPH radical scavenging activity and phenolic compound content in different leaf extracts from selected blackberry species. *Acta Biologica Cracoviensia. Series Botanica*, 54(2).

Gentile, D., Fornai, M., Pellegrini, C., Colucci, R., Blandizzi, C., & Antonioli, L., 2018. Dietary flavonoids as a potential intervention to improve redox balance in obesity and related co-morbidities: a review. *Nutrition Research Reviews*, 31(2), 239-247.

Guven, H., Arici, A., & Simsek, O., 2019. Flavonoids in our foods: a short review. Journal of Basic and *Clinical Health Sciences*, 3(2), 96-106.

Halliwell, B., & Gutteridge, J. M., 2015. Free radicals in biology and medicine. Oxford university press, USA.

Hawkins, J., Hires, C., Baker, C., Keenan, L., & Bush, M., 2021. Daily supplementation with aronia melanocarpa (chokeberry) reduces blood pressure and cholesterol: A meta analysis of controlled clinical trials. *Journal of dietary supplements*, 18(5), 517-530.

Jakobek, L., Šeruga, M., Medvidović-Kosanović, M., & Novak, I., 2007. Antioxidant activity and polyphenols of Aronia in comparison to other berry species. *Agriculturae Conspectus Scientificus*, 72(4), 301-306.

Jurikova, T., Mlcek, J., Skrovankova, S., Sumczynski, D., Sochor, J., Hlavacova, I., Snopek, L., & Orsavova, J. 2017. Fruits of black chokeberry Aronia melanocarpa in the prevention of chronic diseases. *Molecules*, 22(6), 944.

Kandukuri, S. S., Noor, A., Ranjini, S. S., & Vijayalakshmi, M. A., 2012. Purification and characterization of catalase from sprouted black gram (Vigna mungo) seeds. *Journal of Chromatography B*, 889, 50-54.

Kardum, N., Takić, M., Šavikin, K., Zec, M., Zdunić, G., Spasić, S., & Konić-Ristić, A.. 2014. Effects of polyphenol-rich chokeberry juice on cellular antioxidant enzymes and membrane lipid status in healthy women. *Journal of Functional Foods,* 9, 89-97.

Mittler, R., 2017. ROS are good. *Trends in plant science,* 22(1), 11-19.

Nadeem, S. M. S., Khan, J. A., Murtaza, B. N., Muhammad, K., & Rauf, A., 2015. Purification and properties of liver catalase from water buffalo (Bubalus bubalis). *South Asian Journal of Life Sciences,* 3(2), 51-55.

Nakamura, K., Watanabe, M., Sasaki, Y., & Ikeda, T., 2000. Purification and characterization of liver catalase in acatalasemic beagle dog: comparison with normal dog liver catalase. *The International Journal of Biochemistry & Cell Biology*, 32(1), 89-98.

Nakano, T., Watanabe, M., Sato, M., & Takeuchi, M. 1995. Characterization of catalase from the seaweed Porphyra yezoensis. *Plant Science*, 104(2), 127-133.

Oszmiański, J., & Wojdylo, A., 2005. Aronia melanocarpa phenolics and their antioxidant activity. *European Food Research and Technology,* 221(6), 809-813.

Pirvu, L., Hlevca, C., Nicu, I., & Bubueanu, C., 2014. Comparative studies on analytical, antioxidant, and antimicrobial activities of a series of vegetal extracts prepared from eight plant species growing in Romania. *JPC-Journal of Planar Chromatography-Modern TLC,* 27(5), 346-356.

Sharma, I., Ahmad, P., 2014. Catalase: a versatile antioxidant in plants. In Oxidative damage to plants (pp. 131-148). Academic Press.

Sies, H., Jones, D. P., 2020. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nature reviews Molecular cell biology*, 21(7), 363-383.

Smirnoff, N., Arnaud, D., 2019. Hydrogen peroxide metabolism and functions in plants. *New Phytologist,* 221(3), 1197-1214.

Susmitha, S., Ranganayaki, P., Vidyamol, K. K., & Vijayaraghavan, R., 2013. Purification and characterization of catalase enzyme from Agaricus bisporus. *International Journal of Current Microbiology*, 2(12), 255-263.

Taheri, R., Connolly, B. A., Brand, M. H., & Bolling, B. W., 2013. Underutilized chokeberry (Aronia melanocarpa, Aronia arbutifolia, Aronia prunifolia) accessions are rich sources of anthocyanins, flavonoids, hydroxycinnamic acids, and proanthocyanidins. *Journal of agricultural and food chemistry,* 61(36), 8581-8588.

Tsuda, T., 2012. Dietary anthocyanin-rich plants: biochemical basis and recent progress in health benefits studies. *Molecular nutrition & food research,* 56(1), 159-170.

Waszczak, C., Carmody, M., & Kangasjärvi, J., 2018. Reactive oxygen species in plant signaling. *Annual review of plant biology*, 69, 209-236.

Williamson, G., Kay, C. D., & Crozier, A., 2018. The bioavailability, transport, and bioactivity of dietary flavonoids: A review from a historical perspective. *Comprehensive Reviews in Food Science and Food Safety, 17(5), 1054-1112.*

Yoruk, I. H., Demir, H., Ekici, K., & Sarvan, A., 2005. Purification and properties of catalase from Van Apple (Golden Delicious). *Pakistan Journal of Nutrition*, 4(1), 8-10.

https://doi.org/10.7161/omuanajas.1225053