Food Research 1 (2) : 39 - 42 (March 2017)
Journal homepage: *http://www.myfoodresearch.com*

FOOD RESEARCH

FULL PAPER

Hydrogen peroxide scavenging activity of grape *(Vitis vinifera)* methanolic extract

Benmeziane, F.*

Department of Agronomy, Laboratory of Animal Health, Plants Production, Environment and Food Safety, University of El-Tarf. PB 73. El-Tarf 36000. Algeria

Article history:
Received : 24 January 2017
Received in revised form : 2 March 2017
Accepted : 3 March 2017
Available Online : 10 March 2017

Keywords:
Grape
Hydrogen peroxide
Scavenging activity

DOI:
http://doi.org/10.26656/fr.2017.2.005

Abstract

Grape is a fruit used since antiquity by man for its therapeutic virtues due to its richness in antioxidant molecules that can replace synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) and *tert*-butylhydroquinone (TBHQ) which are dangerous for human health. Antioxidant activity of methanolic grape extract was evaluated using the hydrogen peroxide (H_2O_2) scavenging activity. The high percentages of H_2O_2 scavenging activity were obtained for black varieties (Gros noir and Muscat noir) with 72.86 and 65.72, respectively. The white variety (Victoria) has exhibited a percentage of 38.57 which is close to that obtained for Cardinal 2 (38.64) and higher than that obtained for Cardinal 1 (30.64) which is red variety. These results suggest that grape extracts may serve as a potential source of natural antioxidant for food preservative and pharmaceutical application even black, red or white varieties.

1. Introduction

Antioxidants can terminate or retard the oxidation process by scavenging free radicals. Recent epidemiological studies have revealed the associations between the consumption of antioxidant-rich foods and the prevention of oxidative-stress-related diseases (Sies, 1997). However, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ) are restricted by legislative rules and have recently been reported to be dangerous for human health due to the doubts over their toxicity and carcinogenicity in many countries (Gupta et Sharma, 2006; Hao *et al.*, 2007). Thus, the search for effective, non-toxic natural compounds with antioxidant activity has been intensified in recent years. In order to protect foods and human beings against oxidative damage, considerable attention has been paid to explore the natural and safer antioxidants, which could be used for human consumption. Among the dietary antioxidants, phenolic compounds, secondary metabolites occurring in plants, are the most abundant natural antioxidants (Gupta and Sharma, 2006; Pandey and Rizvi, 2009). Hydrogen peroxide (H_2O_2) is a non-radical reactive oxygen species (ROS) in living organisms and has the ability to penetrate cell membranes, inactivate enzymes by oxidation of thiol groups, and initiate lipid peroxidation (Zhang *et al.*, 2011). H_2O_2 is produced inside cells both enzymatically and non-enzymatically by cellular metabolism. In the presence of metal ions, especially iron and copper, it causes mutations by producing hydroxyl radicals through the metal-catalyzed Fenton reaction (Halliwell and Gutteridge, 1999). Methanolic extracts from five Algerian *Vistis vinifera* varieties, Gros noir, Muscat noir (black grape), Cardinal 1, Cardinal 2 (red grapes) and Victoria (white grapes) were tested for their scavenging activity against H_2O_2.

2. Materials and methods

2.1 Grape samples

The work was carried out on four different varieties of table grape, namely: Cardinal (red variety), Gros noir, Muscat noir (blue-black varieties), Victoria (white varieties) (harvest year 2012) grown at four different sites (Cardinal grapes were harvested in two different sites 1 and 2) in the region of El-Tarf located in northern Algeria (36° 45' 00" N; 81 ° 1 0' 00" E). Samples were harvested at maturity (as determined by the owners of the vineyards), which was confirmed later by measuring the sugar content (°Brix). The sampling of grapes was done meticulously and berries were collected randomly from top, bottom, sun exposed and unexposed clusters on each side of the vine. Samples were placed in clean, dry, plastic boxes and quickly transported and stored until analysis. Table 1 summarizes the main characteristics of the vineyards and sample plots.

*Corresponding author.
Email: *benmezianefarida@yahoo.fr*

eISSN: 2550-2166

Table 1. Characteristics of the vines and topography of the plots.

Site	1	2	3	4
Culture system	Royat Cordon	Royat Cordon	Royat Cordon	Pergola
Fertilization treatment	No	No	Yes (foliar nitrogen)	Yes (nitrogen fertilizers and phosphoric plus trace elements (Ca, K) by fertigation)
Vineyard age (year)	32	32	39	5
Distance x spacing (m)	3.0 x 1.0	3.0 x 1.0	3.0 x 1.0	3.0 x 2.5
Topographical situation	Plain	Plain	Plain	Plain
Soil type	Silty sandy	Silty sandy	Silty sandy clay	Silty sandy
Irrigation	Non irrigated	Non irrigated	Noni rrigated	Dropwise
Varieties	Cardinal	Cardinal Gros noir	Muscat noir	- Victoria
Sampling date	15/07/2012	15/07/2012 (Cardinal) 04/08/2012 (Gros noir)	04/08/2012	04/08/2012

2.2 Sample preparation and extraction for scavenging activity analysis

Frozen grapes were washed with distilled water, dried with a cloth and crushed in a domestic mixer. A mixture of 10 g of crushed grapes, 20 ml of extraction solvent (methanol 80%) and 0.1 ml/10 ml of solvent (v/v) of concentrated HCl (to avoid oxidation of the phenolic compounds) was placed in a water bath with stirring. After agitation, the liquid extract was separated from the solid residues by centrifugation at 3 000 rpm/15 min; the extraction procedure was repeated three times. The final extract, which consisted of a mixture of three supernatants, was transferred into opaque vials and stored in a fridge (4°C) until further analysis. The extraction procedure was performed twice.

2.3 Scavenging activity against hydrogen peroxide

The scavenging capacity of grape extracts on hydrogen peroxide was determined according to the method of Atmani *et al.* (2009). Test tubes were prepared with 2.0 ml of various grape extracts and a solution of H_2O_2 (1.2 ml, 40 mM) in phosphate buffer (pH 7.4). A blank solution was prepared in the same way but without H_2O_2. After incubation of the mixture for 10 min, the absorbance was recorded at 230 nm. The scavenging activity was calculated using the following formula: % scavenging activity = [(Ac–At)/ Ac] 100, where Ac is the absorbance of the control and At is the absorbance of the extract. All data are reported as mean ± standard error of two replicates.

3. Results and discussion

A wide variety of in vitro methods have been set up to assess radical scavenging ability and antioxidant activity. Antioxidant capacity is widely used as a parameter for medicinal bioactive components. Different artificial species have been used such as 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (Brand-williams *et al.*, 1995; Mariutti *et al.*, 2008), 1,1 diphenyl- 2-picrylhydrazyl (DPPH) (Sharma *et al.*, 2013; Arkoub-Djermone *et al.*, 2015), OH radicals scavenging activity (Mathew and Abraham, 2006), hydrogen peroxide scavenging activity (H_2O_2) (Baydar *et al.*, 2007), Ferric reducing-antioxidant power (FRAP) (Derradji-Benmeziane *et al.*, 2014), superoxide anion radical scavenging activity (O.2-)(Gulcin *et al.*, 2010) and many others methods. In this study, the antioxidant activity of the grape extract has been evaluated in vitro tests namely: hydrogen peroxide scavenging activity.

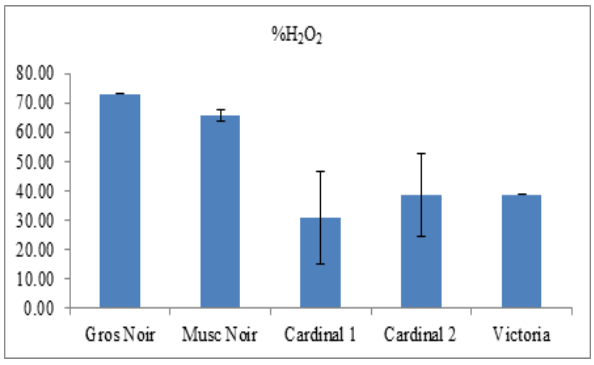

Figure 1. H_2O_2 scavenging activity of methanolic grape extract.

Values are means of duplicate determinations (n=2) ± standard deviation.

The results of the scavenging ability of the grape extracts on H_2O_2 are shown in Figure 1. H_2O_2 scavenging ability of the grape extracts was not dependent on the variety. The percentage of H_2O_2 scavenging activity of fresh grape extract varied between 72.86 ± 0.00 in Gros noir and 30.64 ± 15.9 in Cardinal 1, with intermediate values for the other three verities: 65.72± 2.0; 38.64 ± 14.1 and 38.57 ± 0.00 in Muscat noir, Cardinal 2 and Victoria, respectively. The ability of H_2O_2 of the extracts decreased in order of Gros Noir>Muscat Noir>Victoria>Cardinal

eISSN: 2550-2166

2>Cardinal 1. Thus, the variety Gros noir showed the highest scavenging activity when comparing with other grape varieties, the result was of 72.86% which is important. This variety had presented the highest total polyphenols, total flavonoïds and reducing power (data not shown in this work).

In their work, Baydar *et al.* (2007), found a percentage of H_2O_2 scavenging ability of 77.8% and 72.9% of grape seed acetonic and methanolic extract of Narince variety, respectively, results which are closer to those obtained in this study. H_2O_2 scavenging activity varied from 4.6 to 18.6%, at a concentration of 1 mg in 50 µl grape skin and pulp extracts depending on grape cultivar and extract (Singha and Das, 2015). Rekha and Bhaskar (2013) found in their study that A 200 µg/ml of ethanolic grape seed extract and Vitamin C exhibiting 75.67% and 79.27% inhibition, respectively and the concentration of ethanolic grape seed extract needed of 50% of inhibition was found to be 88.15 µg/ml and 62.51 µg/ml was needed for Vitamin C.

Scavenging of H_2O_2 by the plant extracts may be attributed to their phenolics, which donate electron to H_2O_2, thus reducing it to water. These results suggested that phenolic compounds in grape with electron-donating capacities may govern the H_2O_2 scavenging activities of the tested extracts. Although H_2O_2 is not a highly reactive molecule, it can sometimes be toxic to cells and food systems because it may give rise to hydroxyl radicals and singlet oxygen by reacting with transition metal ions (Halliwell and Gutteridge, 1999). Significant correlation between the scavenging activities of grape extracts with phytochemicals, such as total phenolics and flavonoids, were observed in many previous studies (Caillet *et al.*, 2006; Lutz *et al.*, 2011; Weidner*et al.*, 2012; Singha and Das, 2015). In fact, phenolics are one of the major classes of natural antioxidants found in plants that remove such free radicals. Polyphenols are able to neutralize free radicals, scavenge singlet and triplet oxygen, and to break down peroxides. It is clearly shown that the number of phenolic-OH groups present in the structure of an antioxidant molecule isn't always the only factor determining its antioxidant activity. Positions of phenolic-OH groups, presence of other functional groups in the whole molecule, such as double bonds and their conjugation to (-OH) groups and ketonic groups, also play important roles in antioxidant activities (Gordon, 1990). It is noteworthy that the winery residues (skin and grape) could be an alternative source for obtaining natural antioxidants, and are considered completely safe in comparison with synthetic ones such as BHA and BHT, compounds used widely in the food industry now with undesirable effects on the enzymes of human organs (Gupta and Sharma, 2006). Hence, scavenging H_2O_2 by natural antioxidants sources is important for protection of biological systems. The measurement of H_2O_2 scavenging activity can be one of the useful methods determining the ability of antioxidants to decrease the level of prooxidants such as H_2O_2 (Pazdzioch-Czochra and Widenska, 2002). Although H_2O_2 itself is not very reactive as cited above, it together with superoxide radical anion can damage many cellular components. This study affirms the in vitro antioxidant potential of crude methanolic grape extract.

References

Arkoub-Djermone, L., Boulekbache–Makhlouf, L., Zeghichi-Hamri, S., Bellili, S., Boukhalfa, F. and Madani, K. (2015). Influence of thermal processing on the physicochemical propreties and the antioxidant activity on of a solanaceae vegetable: eggplant. *Journal of Food Quality,* 39(3), 181-191.

Baydar, N.G., Ozkan, G. and Yasar, S. (2007). Evaluation of the antiradical and antioxidant potential of grape extracts. *Food Control,* 18, 1131-1136.

Brand-Williams, W., Cuvelier, M.E. and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science Technology,* 28, 25-30.

Caillet, S., Salmieri, S. and Lacroix, M. (2006). Evaluation of free radical scavenging properties of commercial grape phenol extracts by a fast colorimetric method. *Food Chemistry,* 95, 1-8.

Derradji-Benmeziane, F., Djamai, R. and Cadot, Y. (2014). Antioxidant capacity, total phenolic, carotenoid, and vitamin c contents of five table grape varieties from Algeria and their correlations. *Journal International des Sciences de la Vigne et du Vin,* 48, 153-162.

Gordon, M. (1990). The mechanism of antioxidant action in vitro. In Hudson B. J. F. (Ed.). London: Elsevier Applies Science. Food antioxidants: Gordon pp. 1-18

Gulcin, I., Huyut, Z., Elmastas, M. and Aboul-Enein, H.Y. (2010). Radical scavenging and antioxidant activity of tannic acid. *Arabian Journal of Chemistry,* 3, 43–53.

Gupta, V.K. and Sharma, S.K. (2006). Plants as natural antioxidants. *Natural Products Radiance,* 5(4), 326-334.

Halliwell, B. and Gutteridge, J.M.C. (1999). Reactive species in disease; friends or foes, In Gutteridge J.N.C. (Ed): Oxford University Press. London (UK). Free Radicals in Biology and Medicine: Halliwell, B. and Gutteridge, J.N.C. pp. 511–638: https://books.google.dz/ (consulted on the 11/01/2017).

Halliwell, B. and Gutteridge, J.N.C. (1999). Hydrogen peroxide, in: Halliwell, B. and Gutteridge, J.N.C. (Eds.): Oxford University Press, Oxford. London (UK). Free Radicals in Biology and Medicine: Halliwell, B. and Gutteridge, J.N.C. pp. 30 – 75: https://books.google.dz/ (consulted on the 10/01/2017).

Hao, P.P., Ni, J.R., Sun, W.L. and Huang, W. (2007). Determination of tertiary butylhydroquinone in edible vegetable oil by liquid chromatography/ion trap mass spectrometry. *Food Chemistry,* 105, 1732-1737.

Lutz, M., Jorquera, K., Cancino, B., Ruby, R. and Henriquez, C. (2011). Phenolics and antioxidant capacity of table grape (Vitis vinifera L.) cultivars grown in Chile. *Journal of Food Science*, 76(7), 1088-1093.

Mariutti, L.R.B., Barreto, G.P. de M., Neura Bragagnolo, N. and Mercadante, A.Z. (2008). Free Radical Scavenging Activity of Ethanolic Extracts from Herbs and Spices Commercialized in Brazil. *Brazilian Archives of Biology and Technology,* 51(6), 1225-1232.

Mathew, S. and Abraham, T.E. (2006). In vitro antioxidant activity and scavenging effects of Cinnamomum verum leaf extract assayed by different methodologies. *Food and Chemical Toxicology,* 44, 198-206.

Pandey, K.B. and Rizvi, S.I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity,* 2(5), 270-278.

Pazdzioch-Czochra, M., and Widenska, A. (2002). Spectrofluorimetric determination of hydrogen peroxide scavenging activity. *Analytica Chimica Acta,* 452, 177–184.

Rekha, S.S. and Bhaskar, M. (2013). Screening and identification in vitro antioxidant activities of phytochemical compounds in ethanolic grape (Vitis vinifera) seed extract. *International Journal of Pharma and Bio Sciences,* 4(3), 609-617.

Sharma, R.A., Yadav, A. and Bhardwaj, R. (2013). DPPH free radical scavenging activity of phenolic compounds in Argemone Mexicana LINN. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5(3), 683-686.

Sies, H. (1997). Oxidative stress: Oxidants and antioxidants. *Experimental Physiology,* 82, 291-295.

Weidner, S., Powałka, A., Karamac´, M. and Amarowicz R. (2012). Extracts of phenolic compounds from seeds of three wild grapevines— comparison of their antioxidant activities and the content of phenolic compounds. *International Journal of Molecular Sciences,* 13, 3444-3457.

Zhang, A., Fang,Y., Wang, H., Li, H. and Zhang, Z. (2011). Free-Radical Scavenging Properties and Reducing Power of Grape Cane Extracts from 11 Selected Grape Cultivars Widely Grown in China. *Molecules*, 16, 10104-10122.

eISSN: 2550-2166