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Enhancement of antioxidant properties of *Triticum durum* obtained by traditional spontaneous fermentation in underground silos

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ABSTRACT. Fermented foods have made important contributions to human diets for thousands of years and continue to do so. Their health-promoting benefits are attracting increasingly attention. The present study was conducted to evaluate the impact of natural fermentation on antioxidant properties of traditionally fermented wheat (*Triticum durum*) compared to unfermented samples. Initially, the samples were submitted to traditional spontaneous fermentation. Subsequently, an aqueous extract was obtained and used to determine polyphenolic and flavonoid contents. Moreover, the antioxidant potential was also measured through the determination of the scavenging ability against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, reducing power and hydrogen peroxide scavenging activity. The results showed that the total phenolic and total flavonoid contents were significantly increased in fermented wheat. Moreover, the antioxidant activity was more effective in fermented than in unfermented wheat. Thus, natural fermentation can enhance natural antioxidants in wheat and transform it into a healthy food or ingredient with multi-functional properties which can be used in the food industry.

Keyword: *Triticum durum*; natural fermentation; antioxidant activity; phenolic contents; flavonoids.

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Introduction

The oxidative stress is the major cause of pathological disorders and diseases, including cardiovascular diseases, hypertension, diabetes, cancer and carcinogenesis, inflammation, and aging. Whatever the case is, the risk is increased with the accumulation of reactive oxygen species (ROS), eventually leading to the activation of dangerous enzymatic cascades which damage organic molecules (Afanas'ev, 2010; Ishibashi, 2013; Petrie, Guzik, & Touyz, 2018; Rodrigues, Lima, Melo, & Trindade 2019). The oxidative stress occurs when the balance between ROS formation and detoxification favors an increase in ROS levels, leading to disturbed cellular function (Birben, Sahiner, Sackesen, Erzurum, & Kalayci, 2012).

Antioxidants protect the human organism by neutralizing the free radicals interactively and synergistically (Taghvaei, & Jafari, 2015). Natural antioxidants have been proposed to have beneficial effects on health and on different disease states (Pohl & Lin, 2018). Plants are rich sources of free radical scavenging molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains and other metabolites, whose antioxidant activity is outstanding (Aiyegoro, & Okoh, 2010; Skała et al., 2016). These antioxidant agents prevent damage caused by free radicals (Medhe, Bansal, & Srivastava, 2014; Tułodziecka & Szydłowska-Czerniak, 2016). Thus, a diet containing various healthy foods with antioxidant properties is still the best strategy to benefit from antioxidants and the many other dietary bioactive components (Jamshidi-Kia et al., 2020).

Fermented foods are considered prominent constituents of the human diet because of their content in health-promoting compounds (Şanlier, Gökcen, & Sezgin, 2019). It has been established that micro-organisms start to modify plant constituents during fermentation (Tangyu, Muller, Bolten, & Wittmann, 2019). Such fermentation reduces carbohydrates and non-digestible polysaccharides and oligosaccharides and increases some amino acids as well as vitamins of group B. When cereals are fermented with lactic acid bacteria, anti-nutrients such as tannin and phytic acid decrease, resulting in increased iron absorption and

reduced non-nutritive compounds which negatively affect the digestibility, absorption, and solubility of minerals. In the end, fermented cereals showed increased anti-oxidant activities (Đorđević, Siler-Marinkovic, & Dimitrijevic-Brankovic, 2010).

The storage of durum wheat in underground silos is a traditional method used by Algerian farmers to prepare traditionally fermented wheat, leading to a special product locally named 'Mzeyet' or 'Elhamoum'. Such method is based on the use of underground holes or silos built near the farm at generally high places. They are called 'Matmours'. The method gives to durum wheat brown color and very strong acid odor, following the natural fermentation due to native micro-organisms. It was found that this fermentation was lactic dominant (Gourchala, Hobamahoro, Mihoub, & Henchiri, 2014).

To the best of our knowledge, no studies have already considered the use of natural fermentation (in underground silos) to enhance the functional properties of durum wheat. This study is aimed at clarifying the role of spontaneous fermentation in increasing the antioxidant features of fermented wheat, with the perspective of producing a functional ingredient or dietary supplement.

Material and methods

Sample collection

Three different samples of traditionally fermented wheat (TFW) were collected from Mila, a region situated in Eastern Algeria. The samples were obtained after being subjected to traditional spontaneous fermentation in underground silos (called Matmours) during approximately 10 months in rural areas. Unfermented wheat was purchased from a market in Jijel, Algeria.

Extract preparation

The wheat grain powder was extracted in distilled water (1/2) (W/V) for 10 min at room temperature. The extract was filtered using Whatman N°1 filter paper (Talbi, Boumaza, El-mostafa, Talbi, & Hilali, 2015). The filtrate of aqueous extract was freshly used for further assessment in *in vitro* assays.

Phenolic compounds content

The total phenolic content (TPC) was investigated using the Folin-Ciocalteau assay (Othman, Ismail, Ghani, & Adenan, 2007). Briefly: 0.2 mL of the traditionally fermented wheat extract (TFWE) was mixed with 1.5 mL of Folin-Ciocalteau reagent. After 5 min, 1.5 mL of 7% sodium carbonate solution was added and the mixture was incubated for 90 min, then the absorbance was measured at 750 nm. The TPC was expressed as mg gallic acid equivalents per g of dry weight (mg GAE g^{-1}).

Flavonoid content

To determine the total flavonoid content (TFC), aluminum chloride complex forming assay was used according to Djeridane et al. (2006). In this test, 1.5 mL of the sample was added to 1.5 mL of 2% aluminum chloride solution. The mixture was allowed to stand in darkness for 30 min. The absorbance of this reaction mixture was recorded at 430 nm and the results are expressed as mg quercetin equivalents per g of dry weight (mg QE g^{-1}).

Antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activity of the fermented wheat extracts was measured as scavenging free radical potential in methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH), as described by Mansouri, Embarek, Kokkalou, and Kefalas (2005). 100 µL of TFWE were added to 1300 µL of 0.004% DPPH methanolic solution freshly prepared. After incubation for 30 min. at room temperature and in darkness, the absorbance was recorded at 517 nm and the antiradical activity was calculated as percentage of DPPH discoloration compared to the control using the following formula:

$$\% \text{ inhibition} = [(A - B) / A] \times 100 \quad (1)$$

where (A) is the absorbance of pure DPPH in oxidized form and (B) is the absorbance of the sample.

Ferric reducing antioxidant power (FRAP) assay

The ability of the extracts to reduce ferric iron (Fe^{+3}) into ferrous iron (Fe^{+2}) was assessed by the method described by Costa et al. (2010): 2.5 mL of the wheat extract were mixed with 2.5 mL of 200 mmol L^{-1} sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After the addition of 2.5 mL of 10% trichloroacetic acid (w v^{-1}), the mixture was centrifuged at 650 rpm for 10 min. The upper layer (5 mL) was mixed with 5 mL of deionized water and 1 mL of 0.1% ferric chloride, and the absorbance was measured at 700 nm. The results are expressed as absorbance values. The increased absorbance of the reaction mixture indicates an increase in reducing power.

Hydrogen peroxide scavenging assay

The ability of fermented wheat extracts to scavenge hydrogen peroxide was determined according to the method of Ruch, Cheng, and Klaunig (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The extracts in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both fermented wheat extracts and the standard compound were calculated:

$$\% \text{ Scavenged } [H_2O_2] = [(A_C - A_S)/A_C] \times 100 \quad (2)$$

where A_C is the absorbance of the control and A_S is the absorbance in the presence of the samples of TFWE or standards.

Statistical analysis

All data were performed using SPSS software version 22.0 for windows. All experiments were performed in duplicate and the data obtained from the analysis are expressed as the mean ± standard (SD). Statistical differences were analyzed by one-way analysis of variance (ANOVA) at $p < 0.05$ and Tukey's posthoc test. Correlation analysis between some parameters was performed using Pearson correlation at $p < 0.05$.

Results and discussion

Phenolic and flavonoid contents

Phenolic compounds play a role in free radical scavenging capacities. They are one of the most effective antioxidative constituents that contribute to the antioxidant activity (Boo, 2019; Pawlowska, Szczepanska, Koskela, Kaarniranta, & Blasiak, 2019; Zhou et al., 2019). According to Figure 1A, the TPC of the samples varied significantly ($p < 0.001$ ***). The highest TPC was obtained in fermented wheat extract of sample WM2 (1.12 ± 0.007 mg GAE g^{-1}). The fermented wheat extracts present increased values compared to the control. These results are in perfect agreement with those found by Gourchala et al. (2014). The same results were got by Zhang, Guoying, Pan, Fan, Soccol, and Pandey (2012). A previous research has suggested a relationship between free phenolic content and β-glucosidase and amylase activities in fermented food substrates (Xiang, Apea-bah, Ndolo, Katundu, & Beta, 2019; Stojiljkovi´c, Arsi´c, & Tadi´c, 2016). These endogenous enzymatic activities play an important role in starch degradation, which is considered as source of fermentable sugars, leading to an increase in the total phenolic content (Gänzle, 2014).

The same Figure 1B shows TFC in the samples of TFW ($p < 0.001$ ***). The sample coded WM2 has also the highest value (0.30 ± 0.007 mg QE g^{-1}) and the control presents the lowest value. Zhang et al. (2012) and Sandhu, Punia, and Kaur (2016) proved that fermentation using fungus species promotes the increase of total polyphenols and flavonoid contents. Đorđević, Šiler-Marinković, and Dimitrijević-Branković (2010) found that fermentation using lactic acid bacteria and yeast can enhance polyphenol and flavonoid contents, which is confirmed by our results. According to Blandino, Al-aszari, Pandiella, Cantero, and Webb (2003), natural fermentation involves mixed cultures of yeasts, bacteria and fungi. Some microorganisms may act simultaneously, while others act sequentially with a changing dominant flora during fermentation. Spontaneous fermentation in the fermented wheat samples involves all of these microorganisms which act to increase the level of the antioxidant compounds.

A positive correlation was noted between phenolic and flavonoid contents (r = 0.996 **, $p < 0.001$ ***).

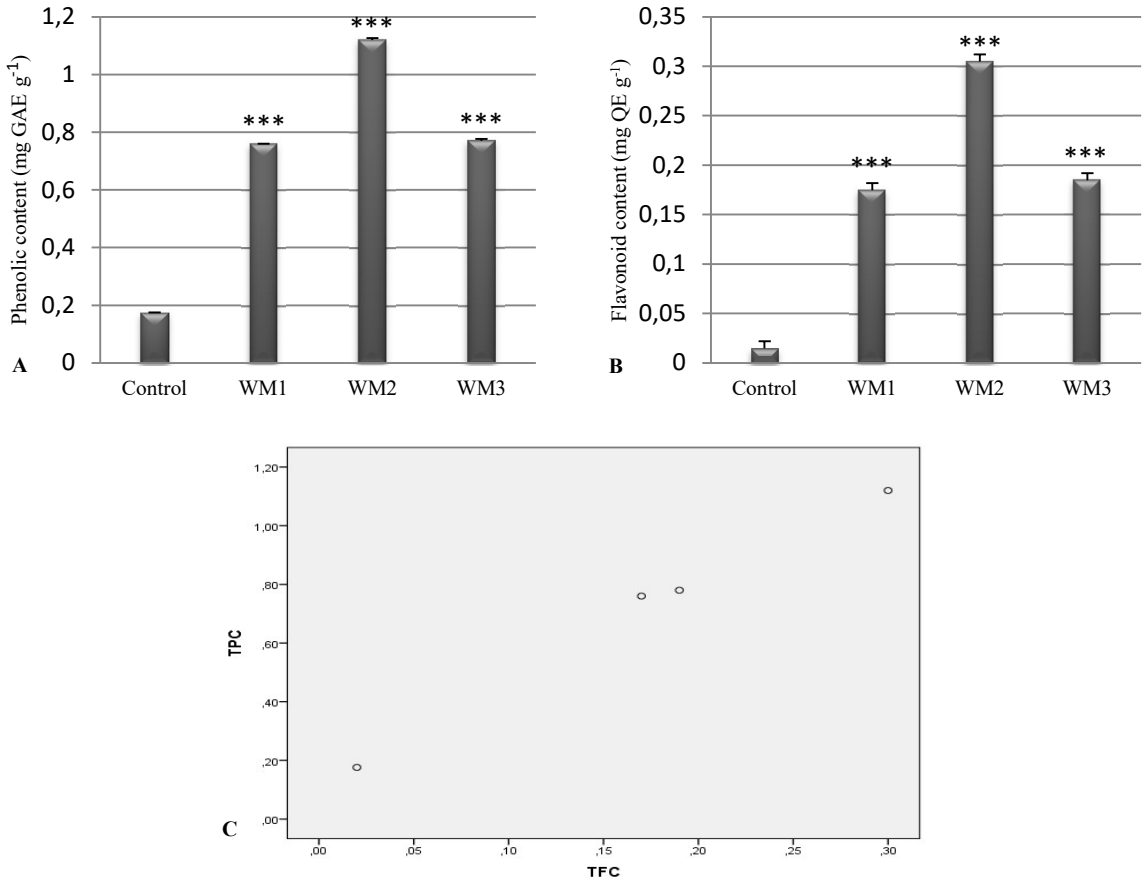

Figure 1. Phenolic (A) and flavonoid (B) contents in fermented and unfermented wheat samples. (C): Correlation between TPC and TFC. WM1, WM2 and WM3: Fermented wheat samples, TPC: Total phenolic content, TFC: Total flavonoid content.

Antioxidant activity of fermented wheat extracts

The fermented wheat extracts were screened for antioxidant property of DPPH radical, hydrogen peroxide (H_2O_2) and the FRAP assay method.

DPPH assay

Figure 2 shows the DPPH antioxidant activity of TFWE samples ($p < 0.001$). The decrease in the absorbance of DPPH radicals at 517 nm induced by antioxidants determines its reduction capacity. During the radical scavenging assay, the DPPH radical without the extracts of TFW was stable over the time. However, in the presence of several concentrations of TFWE, the DPPH radical is reduced to non-radical DPPH-H. This reduction depends on the used concentrations. It was found that the DPPH scavenging effect of TFWE increased with the increase of their concentration.

According to Figure 2, the highest DPPH antioxidant capacity was attributed to WM1 fermented wheat sample (28.64 ± 1.85%) followed by WM2 (26.75 ± 1.98%), against only (3.51 ± 0.99%) for the control sample.

The result of DPPH scavenging activity assay indicates that fermented wheat was potently active, and that the TFWE contain compounds that are capable of donating hydrogen to a free radical in order to eliminate the odd electron, which is responsible for the radical's reactivity (Aiyegoro & Okoh, 2010).

DPPH antioxidant activity is effective in the traditionally fermented wheat. This can be explained by the enhancement of antioxidants like polyphenols and flavonoids through fermentation. According to Zhang et al. (2012); DPPH antioxidant activity was more effective in wheat fermented using *Cordyceps militaris* than in unfermented wheat. Higher antioxidant activity was due to high total phenolic content (Duan, Zhang, Zhao, Chang, & Guo, 2020).

It has been demonstrated that fermentation has a positive influence on the TPC and the antioxidative activity of cereals, and that the degree of this influence depends on the microorganism species. In fact, an earlier study confirmed that lactic acid bacteria are involved in spontaneous fermentation of durum wheat (Gourchala, Hobamahoro, Mihoub, & Henchiri, 2014). All of these findings support the above results.

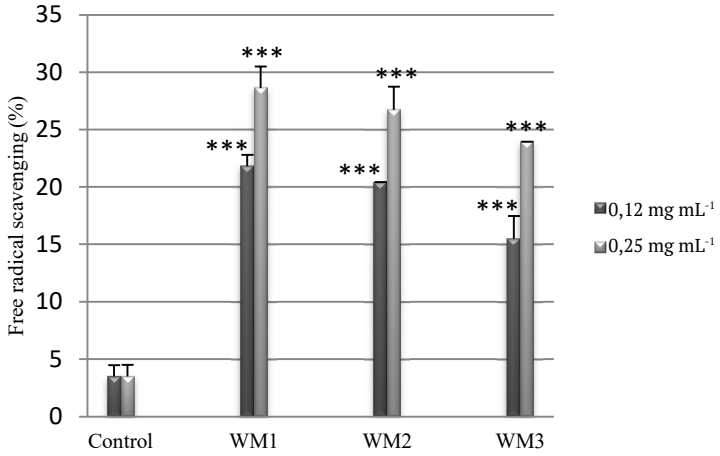

Figure 2. DPPH scavenging activity of different concentrations of TFW and control extracts. WM1, WM2 and WM3: Fermented wheat samples.

FRAP assay

Figure 3A shows the reducing power potentials of the aqueous extract of TFW in comparison to the control at 700 nm ($p < 0.001$ ***).

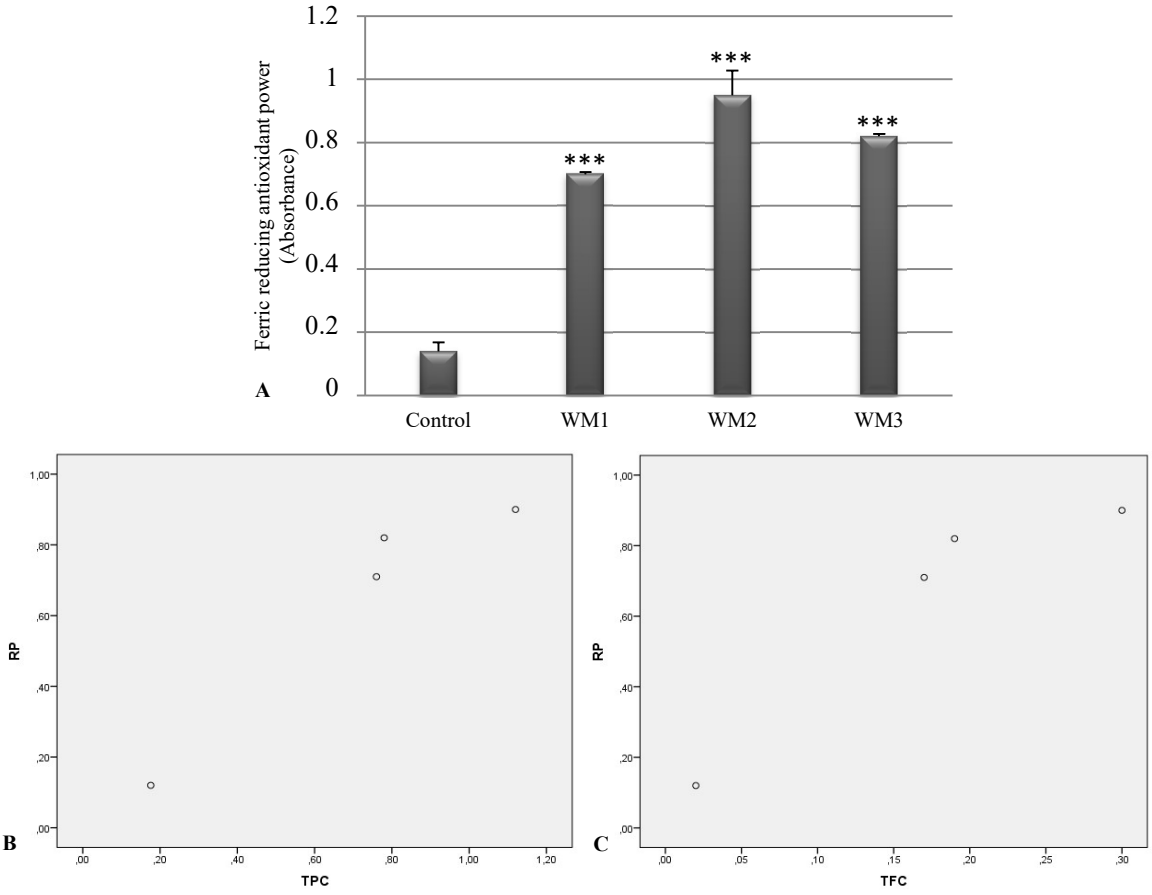

Figure 3. Reducing power of TFW and control extracts (A), (B): Correlation between FRAP and TPC, (C): Correlation between FRAP and TFC. WM1, WM2, WM3: Fermented wheat samples, RP: Reducing power, TPC: Total phenolic contents, TFC: Total flavonoids content.

All sample extracts showed reducing power potential, but WM2 proved to be more active. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Increasing absorbance indicates an increase in reductive ability. The results show that there has been an increase in reducing power in the samples. The reducing capacity of fermented wheat is more significant than the reducing capacity of the control. This result indicates that fermented wheat is rich in antioxidant

compounds that act as electron donors and can reduce the oxidized intermediates of lipid peroxidation processes (Medhe, Bansal, & Srivastava, 2014). The same result was found by Zhang et al. (2012) in fermented wheat using *Cordyceps militaris*.

The capacity of the extracts to reduce Fe^{+3} into Fe^{+2} is due to their antioxidant molecules contents, such as phenolic compounds and flavonoids. The findings are proved by positive correlation between these parameters. The results of the reducing power test correlate positively with polyphenolic contents (r = 0.972 **, $p < 0.001$ ***) and with flavonoid contents (r = 0.958 **, $p < 0.001$ ***).

Hydrogen peroxide scavenging assay

The sample extracts showed appreciable scavenging activity on hydrogen peroxide, compared to the control ($p = 0.003$ **). The highest value was attributed to sample WM3 (84.6% ± 7.00) following by WM1 (81.90% ± 8.90) and WM2 (77.4% ± 0.00), while the control represented only the value 18.19% ± 0.00 (Figure 4).

Hydrogen peroxide is an important reactive oxygen species because it is able to penetrate biological membranes and it may be toxic if converted to hydroxyl radical in the cell (Pagano et al., 2014; Zhao et al., 2016; Lv, Booz, Fan, Wang, & Roman, 2018).

According to Figure 4, TFWE can scavenge H_2O_2. This ability to scavenge H_2O_2 may be attributed to their antioxidant compounds such as phenolics, which donate electrons to H_2O_2, thus reducing it to water (Aiyegoro & Okoh, 2010).

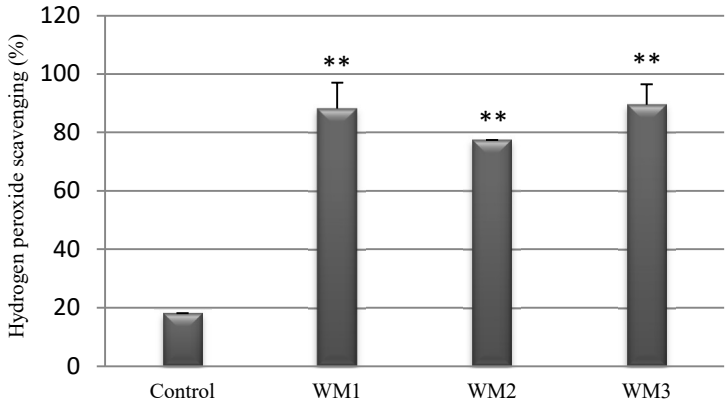

Figure 4. Hydrogen peroxide scavenging activity of TFW and control extracts. WM1, WM2, WM3: Fermented wheat samples.

Conclusion

In conclusion, the results of this study demonstrate that spontaneous fermentation of durum wheat can enhance the contents of total polyphenols and flavonoids as well as the antioxidant activity of wheat. A correlation between the total phenolic content, flavonoid content and reducing power can also be deduced. Therefore, spontaneous fermentation can be applied as a way to transform wheat in particular and cereals in general into a healthy food or ingredient in food industry and then increase their antioxidant content. These antioxidants can be healthy when added to the food especially when they come from natural and healthy treatments. However, high concentrations could be harmful rather than beneficial for health because they may act as pro-oxidants.

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