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ANTIOXIDANT POTENCY OF WATER KEFIR

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ABSTRACT

Reactive oxygen species (ROS) have strong relationship with several diseases. Many fermented foods were reported to be important sources for antioxidant compounds. Antioxidant activity of water kefir never reported in the scientific literature. The objective of this study was to detect and investigate the antioxidant potency of water kefir. Water kefir was prepared by fermentation of sugar solution with kefir grains for 24h. Antioxidant activity of fresh water kefir drink and its extract with (0.125–5 mg/ml) was evaluated using 2,2,-diphenyl-1-pricrylhydrozyl (DPPH) scavenging method, and inhibition of ascorbate autoxidation and the reducing power of water kefir were determined, Butylated hydroxyanisole (BHA) and ascorbic acid were used for comparison. Water kefir demonstrated great ability to DPPH scavenging ranged (9.88-63.17%). And inhibit ascorbate oxidation by (6.08-25.57%) increased in consequent with concentration raising. These results prime to conclude that water kefir could be promisor source of natural antioxidants with good potency in health developing.

Keywords: Water kefir, antioxidant, kefir grains, fermentation

INTRODUCTION

Kefir was defined in different sources according to its characteristics, contains, microorganisms and the manner of its production. Here we will adduce the more common and comprehension definitions. Kefir is defined as a beverage produced by the action of lactic acid bacteria (LAB) (*lactobacilli, lactococci, leuconostocs*), yeasts, and acetic acid bacteria (aceterobacteria) on milk (**Farnworth and Mainville, 2008; Halle´ *et al.*, 1994**). It is a refreshing fermented milk beverage, a viscous pourable liquid, with a smooth, slightly foamy body and whitish color. It is yeasty, acidic, mildly alcoholic, refreshing, slightly effervescent, and believed to contain many functional substances **(Vikram Mistry2004; Gilliland, 1990)**.

Kefir is produced in large amounts, particularly in Russia, It is also well known in Sweden, Norway, Finland, Hungary, Poland, Germany, Greece, Austria, Brazil, Argentina,Taiwan, Portugal, Turkey, France and Iran. In addition to that it is as a new product for wide-scale introduction into the United States, and it is also sold in Japan (**Iconomopoulou and Psarianos, 2004; Farnworth, 2005**). Kefir has been produced using milk from cows, sheep, goats, and buffalo, milk whey, soy milk, soy whey, rice milk, (**Cais-Sokolinska *et al.*, 2008; Farnworth, 2008; Athanasiadis *et al.*, 2001; Monajjemi *et al.*, 2012; Kesenkaş *et al.*, 2011 ; Sirirat and Jelena, 2010**).

Recently kefir can be made from other medias of fermentation as Carbohydrates solutions (**Harta *et al.*, 2004; Bergmann *et al.*, 2010**), cheese whey, and a lactose-rich waste of negligible cost (**Papapostolou *et al.*, 2008**). This kefir which based on carbohydrates is called sugary kefir or water kefir.

Water kefir is hazy and gluey beverage with a smooth, streamlined, fizzing texture and blond to yellowish color, it is acidic and yeasty, slightly alcoholic and refreshing taste with feeble sweetness. It is home-produced drink made by adding of kefir grain to sugar solution in water and incubating this mixture at 20-25 °C for at least 12 h, and then separation of kefir grain to other production. Pieces of fresh or dried fruit can be added for flavor and removed in the end of fermentation period. Water Kefir varies from milk kefir according the substrate and kefir grain, color, shape, microbial composition at the level strains. There is no sufficient scientific literatures specified for water kefir, its origin, microbial and chemical structure, and the standard method of preparation. The few findings that have been obtained about water kefir focused on the microflora and chemical structure of sugar kefir drink and , sugary Brazilian kefir, brown sugar kefir, sugary kefir grain (gingerbeer plant) in Frence, tibico (sugary kefir) and water kefir in Germany. These studies and that of milk kefir and kefir grains indicated that both kefir grains contain the same common groups of microorganisms (lactic acid, acetic acid bacteria, and yeasts) (**Garrote *et al.*, 2001; Gulitz *et al.*, 2001; Pidoux 1989; Franzetti *et al.*, 1998; Galli *et al.*, 1995; Chen *et al.*, 2009; Magalhães and Pereira, 2010; Waldherr *et al.*, 2010; Maria *et al.*, 2011**)

Free radicals inducing lipid peroxidation, causes the deterioration of foods, and may also cause oxidative damage by oxidizing biomolecules leading to cell death and tissue damage, and atherosclerosis, cancer, emphysema, cirrhosis, and arthritis are correlated with oxidative damage (**Duthie, 1993; Kehrer, 1993; Jacob, 1994**). Recently, the increased interest in natural antioxidants has given rise to the screening of microbial sources for compounds to replace the synthetic compounds currently in use as food antioxidants. Natural oxidants are presumed to be safer for human beings (**Gazi *et al.*, 2001**). Natural antioxidants can also be used in nutraceutical applications as supplements (**Nishino and Ishikawa, 1998**). The ingestion of antioxidative supplements, or foods containing antioxidants, may reduce the oxidative damage on the human body (**Lin and Yen, 1999**). Antioxidation activity is increased by fermentation process of foods and the additions of microorganisms. *Lactobacillus*strains could be useful as starter cultures to provide antioxidants in food and that fermented milk may serve as a delivery vehicle for antioxidative, probiotic lactobacilli from non-dairy sources (**Osuntoki and Korie, 2010**). There is increasing in antioxidative activities of soymilk after fermentation with lactic acid bacteria and *bifidobacteria* (**Wang *et al.*, 2006**). And Probiotic Yeast and Bacteria (**Rekha and Vijayalakshmi, 2008**). Kefir could play an antioxidant role and its effectiveness depended on the dosage and time of application in Coruh trout (**Can *et al.*,2012**). Milk-rice kefir displayed high significantly greater of antioxidant activity comparing with BHA as standard (**Sirirat and Jelena, 2010**). Milk-kefir and soymilk-kefir possess significant antimutagenic and antioxidant activity (**Liu *et al.*, 2005a**). There are variations among antioxidant properties of kefir samples produced from different cow/soy milk mixtures related to soymilk ratio in kefir milk (**Kesenkaş *et al.*, 2011**). Fermented soy whey extracts exhibited a potentially antioxidant activities (**Monajjemi *et al.*, 2012**).

Water kefir is the results of fermentation of sugar solution by kefir grain which contains of lactic acid, acetic acid and yeasts, which produce important molecules such as polypeptide, polysaccharide , organic acid, and other compounds. Thus and according to the previous findings about kefir and other fermented foods, water kefir can provide benefit microorganisms and bioactive molecules, and help in health improving.

The objective of our study was to investigate the antioxidant activity of water kefir as the first study in this subject.

MATERIAL AND METHODS

Chemicals

The 2,2,-diphenyl-1-pricrylhydrozyl (DPPH), butylated hydroxyanisole (BHA), ascorbic acid , Sodium phosphate buffer, potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrogen peroxide (H2O2 30%) was obtained from Mallinckrodt Baker (Phillipsburg, NJ, USA). HPLC grade methanol and ethanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). All reagents were of analytical grade.

Kefir grains and Preparation of water kefir

Kefir grains abstained from laboratory of microbiology of the university of Evry (France). Table sugar and apples fruit were purchased from the local market of Telemcen city (Algeria).

Water kefir prepared by inoculation sugar solution (6.5% w/v) in mineral drinking water with 5% (w/v) of water kefir grains, 5g/l of fresh apple pieces was added, the mixture was placed in glass bottle with plastic cover (not closed completely), incubated at 21 °C for 24 h, and was stirred and mixed in intervals of 5h. Apples pieces were deducted and kefir grain were sieving after fermentation period by with plastic siever, to be used in other process, and the water kefir drink was stored at 4 °C until being used to evaluate its antioxidant activity.

Preparation of water kefir sample

Samples of water kefir were then filtered with Whatman No. 1 filter paper, The resulting supernatants were then dried under reduced pressure by rotary evaporator At 45 °C, The dry matter then collected, weighted, dissolved in distilled water, and stored at 4 °C until their analysis.

Determination of Antioxidative Activity

Determination of DPPH radical-scavenging activity

The antioxidant activity of the water kefir samples was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH **(Brand-Williams, Cuvelier and Berset *et al.*, 1995)**. A methanolic solution (50 µl) of the water kefir samples at various concentrations (0.125–5 mg/ml) which was placed in a cuvette, and 2 ml of 6×10^{-5} M methanolic solution of DPPH was added, the mixture was incubated in the dark for 30 min at ambient temperature. After that the decrease in absorbance at 517 nm was determined by spectrophotometer for all samples. Methanol and samples was used to zero the spectrophotometer. And Ascorbic acid and BHA were used as positive reference standards. The absorbance of the DPPH radical without kefir samples used as the control. The inhibitory percentage of DPPH by the kefir samples was calculated according to the following equation:

$$\text{Scavenging effect}(\%) = \frac{Ac - As}{Ac} \times 100\%$$

where Ac is the absorbance of the DPPH solution and As is the absorbance of the DPPH solution after the addition of samples. All tests were operated in triplicate.

Measurement of inhibition of ascorbate autoxidation

Inhibition of ascorbate autoxidation was measured by the method described by **Mishra and Kovachich (1984)**. A 0.1 ml of water kefir samples (0.125–5 mg/ml) was added to 0.1 ml of ascorbate solution (5.0 mM) and 9.8 m phosphate buffer (0.2 M, pH 7.0). The mixture was incubated at 37°C for 10 min and then the absorbance was measured at 265 nm. A 0.1 ml of distilled water used as control. And BHA was used as positive reference standard. All tests were operated in triplicate and the ascorbate autoxidation inhibition rate of kefir samples were calculated as follows:

$$\text{Inhibition effect}(\%) := \left[\frac{Absorbance_{(sample)}}{Absorbance_{(control)}} - 1\right] \times 100\%$$

Measurement of reducing power activity

The reducing power of water kefir samples was determined according to the method of **Oyaziu (1986).**Various concentration of kefir samples (2.5 ml) (0.125–5 mg/ml) were mixed with an equal volume of sodium phosphate buffer (200 mM; pH 7) and 1% potassium ferricyanide solution. The mixture was incubated at 50°C for 20 min. After that, an equal volume of 1% trichloroacetic acid was added to the mixture, which was then centrifuged at 5000 rpm for 10 min. The upper layer (5 ml) was diluted with 5 ml of distilled water and 1 ml of a 0.1% ferric chloride solution, and then the absorbance of the solution was measured at 700 nm. Ascorbic acid was used as positive reference standard. Increased absorbance of the reaction mixture indicates greater reducing power of the kefir sample. All samples were assayed in triplicate.

Statistical analysis

Experimental results recorded were the means ± standard deviation (SD) of triple determinations. The obtained data were subjected to analysis by One-way ANOVA and the differences between means were at the 5% probability level using Duncan's new multiple range tests using SAS software V. 8 eds. (**SAS, 2001**).

RESULTS AND DISCUSSION

Antioxidant activities of water kefir drink

Antioxidant activities of fresh water kefir drink were measured using DPPH scavenging, Inhibition of Ascorbate autoxidation and reducing power methods to determine the antioxidant effects of fresh water kefir drink. The results are exposed in (Tab 1). Where the DPPH scavenging was 11%, and Inhibition of Ascorbate autoxidation was 7.5%, while the reducing power was 0.756 as the absorbance at 700nm. These results exhibit the ability of fresh water kefir drink to scavenge the radical, and inhibit oxidation of ascorbate, and it contains reducing compounds.

Table 1 Antioxidative activities of fresh water kefir drink

	Antioxidant activity of water kefir (Means)	±SD
DPPH scavenging %	11	0.797
Inhibition of Ascorbate autoxidation %	7.5	0.203
Reducing power (A.700 nm)	0.756	0.15

Results are mean values of triplicate determination ±S.D.

DPPH radical-scavenging activity

DPPH radical is organic compound containing nitrogen and has proton free radical. DPPH. solutions show a strong absorption at 517-250 nm appearing a deep violet colour. The absorption vanishes and the resulting decolourization is stoichiometric with respect to degree of reduction. (**Blois, 1958; Yamaguchi, 1998**). Hence, this stable DPPH radical is a widely used as a substrate to evaluate the free radical scavenging ability or antioxidant properties of various samples (**Ebrahimzadeh *et al.*, 2008; Brand-Williams *et al.*, 1995; Yen & Duh, 1994**). Figure 1illustrates the results of scavenging of DPPH by water kefir that produced by incubation of kefir grains with sugar solution at 21 °C for 24 h. in comparison with those of common synthetic antioxidants, ascorbic acid and BHA. As revealed in Figure 1, the DPPH radical scavenging effect of water kefir increased as the concentration of the water kefir increased.Water kefir samples at a concentration of (0.125–5 mg/ml) exhibited (9.88 – 63.17%) scavenging activity of DPPH radical.

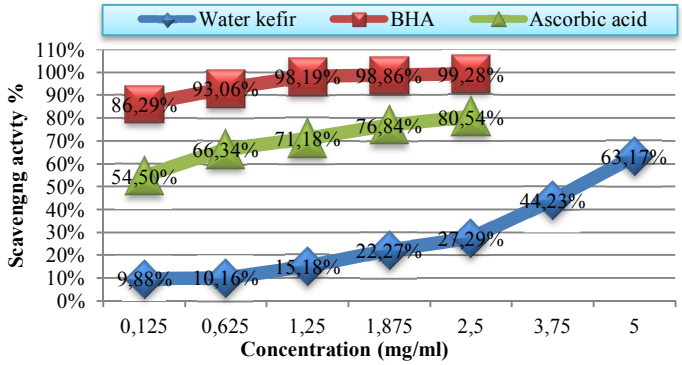

Figure 1DPPH Scavenging effect of Water Kefir

The Immediately increasing of the DPPH radical-scavenging activity of milk and soymilk kefir after kefir grains additions, indicating that some components of the antioxidants contained in the kefir grains were transferred to milk and soymilk, and after the incubation for 32 h, the DPPH radical-scavenging activities of milk-kefir and soymilk-kefir were significantly greater than those of milk and soymilk. (**LIU *et al.*, 2005a**). Recent studies have reported the development of antioxidant activity in whey during fermentation and with lactic acid bacteria, and the antioxidant activity is strain-dependent (**Sun *et al.*, 2010; Osuntoki and Korie, 2010**). Other study demonstrated that the DPPH-scavenging activity of soybean can be enhanced through fermentation with a

certain micro-organism (**Sun *et al.*, 2009**). And the results of **Lin and Chang (2000)** indicate that the radical scavenging ability of the intact cells and intracellular extracts of B. longum and *L. acidophilus* contributes to the antioxidative effect. The scavenging effects % on DPPH radicals of the LAB fermentation product Seaweed oligosaccharide LAB fermentation product (SwOS-LAFP) seaweed and polysaccharide LAB fermentation product (SwPS-LAFP) were in the range of 36.2 ±2.2 to 79.6 ±1.0 (**Wu *et al.*, 2010**). The exopolysaccharides from the corn Stover-containing medium presented significantly strong hydroxyl and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity (**Xiang *et al.*, 2012**).

In this study, we found that Water kefir samples with demonstrated excellent radical-scavenging activity when comparing with those of than of soy whey kefir, cow-milk and goat-milk kefir, rice milk kefir and milk whey kefir which showed respectively (>60,>90,40-90,6-30%) DPPH scavenging ratio, after considering the activity of milks and soy milk that was (80,55-60%) (**Monajjemi *et al.*, 2012; Liu Je *et al.*, 2005b; Sirirat and Jelena, 2010**). That suggested that this activity may be attributable, in part, to the peptides deriving from milk proteins and soybean proteins (**Liu et al., 2005a).**

Inhibition of ascorbate autoxidation

Intracellular cell-free extract of all strains of lactic acid bacteria demonstrated antioxidative activity with inhibition rates of ascorbate autoxidation in the range of 7−12%. Antioxidative mechanisms including metal ion chelating ability, scavenge of reactive oxygen species, enzyme inhibition (**Lin and Yen, 1999**). It was found that the inhibition rate of cow/soy milk kefirs to inhibit ascorbate autoxidation ranged from 8.34–17.00% depending on soymilk ratio. that can explained by intensive effect of intracellular antioxidants of microorganisms (**Kesenkaş *et al.*, 2011**). The development of antioxidant activity was strain-specific characteristic (**Virtanen *et al.*, 2007**). The ability of fermented soymilk to inhibit ascorbate autoxidation with inhibition rate in the range 11.92–16.38%, varied with the starter organism. and the simultaneous of lactic acid bacteria and *bifidobacteria*, exhibited a significantly higher inhibition rate of ascorbate autoxidation (**Wang *et al.*, 2006**).

In this study, water kefir inhibit ascorbate autoxidation with the rate of (6.08-25.57%) as in Figure 2. This results indicate a high ability of water kefir for inhibition ascorbate autoxidation, suggesting that antioxidant activity of water kefir attributed to its contains of lactic acid, acetic acid bacteria, and yeasts, it also can referred to their simultaneously existing and their intracellular and extracellular metabolites.

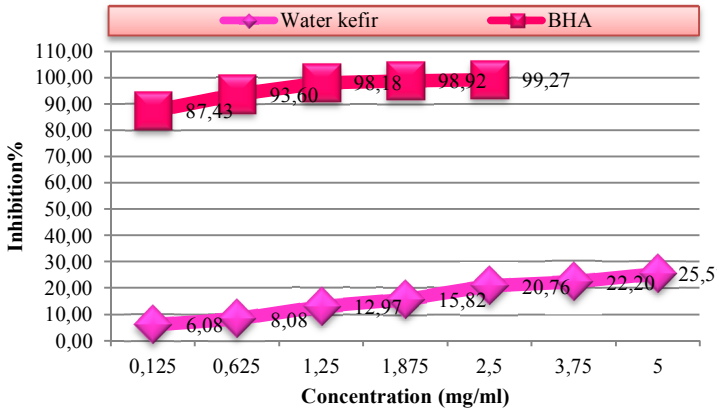

Figure 2 Inhibition of Ascorbic Acid autoxidation by Water Kefir

Reducing power activity

Reducing power of compound have described by certain studies to be an important indicator for its own potential antioxidant activity (**Meir *et al.*, 1995; Liu *et al.*, 2002**). and may be associated with antioxidant activity (**Yen *et al.*, 2000**).

Figure 3 Expose the dose-response curves for reducing power of water kefir samples. Water kefir showed high ability to reduce Fe3+/ferricyanide complex to the ferrous form, reducing power increased as the concentration of the tested samples increased.

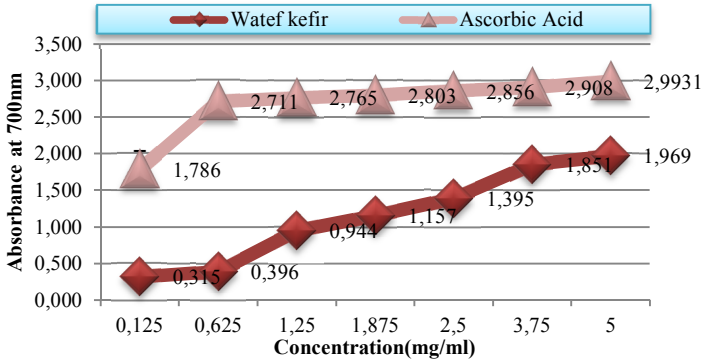

Figure 3 Reducing power activity of water kefir

A number of studies have found that the reducing power of kefir and fermented milk and soymilk were significantly greater than milk and soymilk and suggested that certain metabolites, such as glutathione, that demonstrate superior reducing power might be produced during kefir fermentation and which could react with free radicals to stabilize and terminate radical chain reactions (**Yang *et al.*, 2000; Wang *et al.*, 2006**). Intracellular antioxidants sourced from kefir microflora, soymilk kefir samples (0:100) had approximately 3 times higher reducing activity (**Kesenkaş *et al.*, 2011**). Although the conditions in the gastrointestinal tract are very complicated, the results from the study of **Kaizu *et al.*, (1993)** showed that the intracellular extract was antioxidative in vivo. Our results conducts that water kefir possess great reducing compounds, which are outcome of the fermentation of sugar solution by kefir grains.

CONCLUSION

The results obtained from this study showed antioxidant activity of water kefir and suggesting that this activity attributed to the presence of lactic acid, acetic acid bacteria, and yeasts in water kefir, it also can referred to their simultaneously existing, and their intracellular and extracellular metabolites and also to the products of its cell lysis.

So therefore the study conclude that water kefir can be an interesting source of natural antioxidants with good potential in health improving. Hence, more research is needed to conclusively prove antioxidant activity of water kefir.

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