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Antioxidant Properties Of Goji Berries

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ANTIOXIDANT PROPERTIES OF GOJI BERRY

by

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THESIS

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Approved by:

Advisor

Date

DEDICATION

This thesis is dedicated to my parents, my father Zhi-yong Zhang and my mother Kuai-le Gao, who support and love me forever. To my good friends, Anna Winegarden, Yu-chen Xie, Yi zhen-Wu and He Xie, who encourage me all the time. My achievement is also dedicated to my co-workers and my friends, XiuXiu, Corene, and Hoda who give me constant instruction and help.

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Introduction

Free Radicals and Antioxidants

Human metabolism is a type of oxidation, that is, human bodies will generate some “rust” which is called free radicals in medical science [2]. Free radicals are molecules with an unpaired electron. They are very unstable; besides, they can convert stable molecules into free radicals through a series chain reactions [1,2]. After the reaction repeats with time, there will be plenty of active and restless free radicals accumulated in the body [3].

Under normal situations, the free radicals are harmless to life and our bodies have a comprehensive antioxidant system to destroy them [1,3]. Unfortunately, there are many factors that increase the concentration of free radicals greatly in our environment, such as smoking, air pollution, water pollution, radiation (X-rays, ultraviolet rays), pesticides, life stress, excessive exercise, etc. Accumulation of excessive free radicals could cause chronic diseases such as diabetes, heart diseases and cancer. [4].

An antioxidant is a substance capable of inhibiting the oxidation reaction of free radicals, and its mechanism may be through playing a direct role in radical scavenging, or indirectly through destroying consumed substances that easily generate free radicals, thus preventing the occurrence of further reactions [5].

There are two types of antioxidants, natural and synthetic antioxidants.

Natural antioxidants, including vitamin E, vitamin C, and β -carotene, are naturally present in fruits, vegetables and plants, such as mangostana, tomato, nuts, blueberries, grapefruit, etc [1-7]. Synthetic antioxidants are produced chemically, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ) [8,9]. These synthetic antioxidants have been widely used in foods to prevent oxidation. However, the use of synthetic antioxidants can be harmful to human health due to their potential toxicity and carcinogenicity [9]. This has generated an interest in obtaining antioxidants from natural foodstuff.

Goji Berry

Since the beginning of the 21th century, wolfberries (*Lycium barbarum*, Solanaceae) a traditional food and medicine in East Asia have become increasingly popular in Europe and North America [25]. Numerous products are commercialized under the relatively new name goji berry on the health food market [25].

Goji berry is a type of fruit that is rich in antioxidants. Goji plants are widely grown in East Asia, specifically in the western part of China, Tibet and Mongolia [10]. They are members of the nightshade (*Solanaceae*) family [12]. Goji berries are usually found dried. They are shriveled red berries that look like red raisins. Raw fresh fruit (100 g) is found to contain approximately 4.49 g, 2.33 g, 9.12 g, 7.83 mg, 0.23 mg, 0.33 mg, and 1.7 mg of protein, total lipid, total carbohydrate, carotene, thiamine, riboflavin and niacin, respectively [11, 13]. In general, goji

berry is consumed in the form of jellies, milk, juices, and ginger tea whereas the fresh fruit is the most nutritious [14].

Goji berries are rich in nutrients, namely various vitamins, minerals, antioxidants, betaine, physalene, and other special nutrients, which contribute to its extraordinary health benefits [16,18]. They also contain 21 trace minerals including: copper, germanium, iron, selenium, and zinc [17,19]. Goji berries also contain 18 amino acids including all of the essential amino acids [20,21].

Goji berries can be used in a variety of ways [6,17,19]. Traditionally in Chinese medicine, they are used in teas or alcoholic beverages [8,18,20]. They are also consumed in soups and trail mixes, as porridge with rice and added to numerous meat and vegetable dishes [22,25]. The fruits, which are harvested from August to October, are usually dried, but they may also be consumed fresh, like the young leaves which are a valued vegetable [25]. Meanwhile goji berry is also found in conventional food products such as yoghurts [25]. Goji berries play an important role in traditional Chinese medicine (TCM), where they are believed to enhance immune system function, help eyesight, protect the liver, boost sperm production, and improve circulation, among other effects [18,19]. In TCM terms, goji berries are sweet in taste and mild in nature that is good for most people; they act on the liver, lung, and kidney channels [23,24].

Goji Berry As a Dietary Source of Natural Antioxidants

Research has shown that polysaccharides, beta-carotene, vitamin E,

phenolic, zeaxanthin and flavonoids are the main antioxidant components in goji berry [10,25,26]. In the study, polysaccharides (Lycium barbarum polysaccharides, LBP), one of the bioactive ingredients of goji berry fruit peel, enhances bone marrow function and cellular immune indicators [23]; it could significantly improve the active content of superoxide dismutase (SOD) in the body's blood, liver and muscle tissue, which is conducive to the scavenging of free radicals [21]. Research has even shown that LBP protected frog eggs from oxidative damage by free radicals [13,14] and increased the number of antibody-forming cells to improve antibody effect [20,21].

In another study, goji flavonoids or TFL (total flavonoids of Lycium) were shown to protect red blood cells and mitochondria from oxidative damage [11]. Similarly, TFL were also shown to protect certain white blood cells from oxidative damage [12]. Zeaxanthin, one of the antioxidants that goji berry contains, may even help promote eye health [15]. Studies have certainly shown that zeaxanthin from goji is well absorbed in humans [10,15].

Goji berry contains phenolic constituents which helping in retarding oxidation of lipids and thereby improve quality and nutritional value of food [22]. Phenolics are natural antioxidants which have powerful effects in inhibiting the oxidation process [23]. Antioxidant action of phenolic compounds is due to their tendency to chelate metals [24,27].

According to the literature, most of studies on goji berry that have been carried out and fully explored are about its medical value [25,29]. This study was conducted to gain more information about goji berries especially antioxidants it

contains. The study could help to determine the antioxidant properties and antioxidant activity of goji berries such as total phenolic content and radical scavenging activity [10,28,30].

Objective of the Study

The objective of this study is to investigate the antioxidant properties and activity of goji berry in order to provide more information for future research. Specifically, antioxidant properties of the berries are evaluated by determining their total phenolic content (TPC), oxygen radical absorbance capacity (ORAC) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity.

Materials and Methods

Chemicals and Apparatus

Gallic acid, Folin-ciocalteu's phenol reagent and sodium carbonate (Na_2CO_3) were purchased from Sigma-Aldrich (St. Louis, MO). Acetone and methanol were obtained from Fisher (Fair Lawn, NJ). Hydrochloric acid (HCl), fluorescein was acquired from Fluka (Buchs, Switzerland). 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Aldrich (Milwaukee, WI). Trolox was purchased from ACROS (Geel, Belgium). Ethanol was acquired from Decon labs (King of Prussia, PA). Sodium phosphate monobasic and sodium phosphate dibasic dehydrate, were obtained from Sigma (St. Louis, MO). 2, 2'-Azobis (2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako (Richmond, VA).

Goji berry was purchased from faculty at Virginia Polytechnic Institute and State University, Blacksburg, VA. Sample was extracted by Hyun Chung who is a former Phd student in our lab. The sample was extracted with 50% acetone and then freeze-dried to yield HC 17, an extract of the fruit in a powder form. Total phenolic content (TPC) assay was evaluated using a Beckman DU 640 spectrophotometer (Beckman Coulter, Fullerton, CA). Both 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and Oxygen radical absorbance capacity (ORAC) assay were analyzed using an HTS 7000 Bio Assay Reader (Perkin Elmer, Norwalk, CT).

Sample and Standard Preparation

The sample HC 17 has stored in the refrigerator. It was treated with different solvents in three assays. Samples were dissolved with 50% acetone in the TPC and ORAC assay while it was dissolved with 100% ethanol in the DPPH radical scavenging assay.

Total Phenolic Content (TPC) Assay

TPC was evaluated by using Folin-ciocalteu's phenolic reagent. HC 17 samples were diluted to 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml and 50 mg/ml with 50% acetone. Gallic acid was used as a standard to prepare the standard curve (0.1, 0.2, 0.3, 0.4, 0.5 mg/ml in 50% acetone). All samples/standards were run in triplicates. Each test tube was filled with 25 μ L of sample/standard and 250 μ L distilled water. Then, 750 μ L of 0.2 N Folin-Ciocalteu's phenol reagent was added to each tube and mixed thoroughly using a vortex mixer. Next, 500 μ L of 20% sodium carbonate was added to each tube and the solution was vortexed to mix well. Last, samples and standards were incubated for 2 hours in the dark, at room temperature. Absorbance was detected at 765 nm using a spectrophotometer and the total phenolic content was expressed as gram of gallic acid equivalents (GAE) per 100 gram of dry weight.

Oxygen Radical Absorbance Capacity (ORAC_{FL}) Assay

ORAC was performed as described by Zhou *et al* [31]. Seventy-five mM phosphate buffer (pH 7.4) was prepared for making 0.01 mM fluorescein and

0.72 M AAPH, which could produce peroxy radicals. Serial dilutions of standard (trolox) were performed to achieve concentrations of 0, 20, 40, 80, 100, and 200 μM in 50% acetone. HC 17 samples were diluted to concentrations of 1 mg/ml, 1.5 mg/ml, 2 mg/ml and 2.5 mg/ml with 50% acetone. All samples/standards were run in triplicates. Two hundred μL of fluorescein and forty μL of samples or standards were placed in each well of a 96-well microplate. The plate was incubated for 15 minutes at 37°C. AAPH (35 μL) was then added to each sample and fluorescence was measured at 37°C in a plate reader. Fluorescence was recorded every 5 minutes for 120 minutes using an excitation wavelength of 485 nm and an emission wavelength of 520 nm. Radical absorption capacity was calculated using a standard curve established with various concentrations of trolox. Results are presented as μmol trolox equivalent (TE) per gram dry weight.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The protocol of Brand-William *et al* [32] was modified. DPPH radical is a free radical that can be reduced by antioxidants. This assay therefore measures the ability of our sample to reduce DPPH radicals. 0.8 mM solution of DPPH in 100% ethanol was prepared. Samples were diluted to 10 mg/ml, 20 mg/ml, 40 mg/ml and 50 mg/ml by 100% ethanol and then centrifuged at 7200 rpm for 15 minutes to eliminate residues. One hundred μL of each sample was mixed with 150 μL of DPPH solution in each well of a 96-well microplate and absorbance was measured at room temperature every 5 minutes for 2 hours at 500 nm, using a plate reader. All samples were run in triplicates. The percent scavenging capacity was calculated using the following equation: Scavenging effect

$$(\%) = \left\{ \frac{[\text{Abs}_{\text{control}} - (\text{Abs}_{\text{sample}} - \text{Abs}_{\text{sample background}})]}{\text{Abs}_{\text{control}}} \right\} \times 100.$$

Statistical Analysis

Antioxidant data executed from the above-performed assays were analyzed via Microsoft Excel. Mean and standard deviation (SD) were calculated for each parameter. Data are therefore reported as mean \pm SD. Outcomes were compared using $p < 0.05$ as a cutoff point for statistical significance.

Results

Total Phenolic Content (TPC) Assay

The standard curve of gallic acid was shown in the **Figure 1**. The TPC of the berry was 1.06 ± 0.03 g GAE/100 g dry weight.

Oxygen Radical Absorbance Capacity (ORAC_{FL}) Assay

The assay was developed to measure peroxy radical scavenging capacity. Trolox is used as the standard, as presented in **Figure 2**. We used the standard curve to calculate and convert our sample to trolox equivalents. The ORAC value of the berry extract was 188.52 ± 1.3 $\mu\text{mol TE/g}$ dry weight.

DPPH Radical Scavenging Assay

Antioxidant capacity was further determined by DPPH radical scavenging assay. As shown in **Figure 3**, the scavenging effect of goji berry extract increased with increasing concentrations (10 mg/ml, 20 mg/ml, 40 mg/ml, 50 mg/ml). The remaining capacity was 70.58%, 65.21%, 59.94% and 52.99%, respectively.

Discussion

Antioxidant assays

Regarding TPC experiment development. In the preliminary assay development, the activities of sample were not detected because the absorbance of sample could not appear in the standard curve's area. For this reason, the concentrations of sample have to be raised. Based on the standard curve, various concentrations of sample were tried and finally confirmed by 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml, 50 mg/ml.

As for the ORAC, the first step was to establish an optimal concentration of fluorescein. Due to the 10 μ M fluorescein was shown to be optimal after several trials. AAPH concentration was also enhanced to 0.72 M.

Antioxidant properties estimated by three antioxidant assays (TPC, ORAC and DPPH) showed significant differences among different concentrations. Evaluation of TPC in samples is a widely used method to determine the amount of antioxidant in the samples [33,34]. Therefore, according to our TPC results, the higher the concentration of sample, the higher total phenolic contents, which demonstrates that the samples of higher concentration seem to effectively enhance the amount of antioxidants (phenolic compounds) or the anti-oxidative property of goji berry [51]. Studies of different fruits have shown varying results when comparing with goji berry [35,36,37]. The total phenolic content of dry fruits showed a wide range, with values ranging from 0.099 to 0.959 g GAE/100 g, the highest content being in walnuts (0.959 g GAE /100 g) and the lowest in piyal

seeds (0.099 g GAE /100 g), shown in **Table 1** [36]. Comparing these data with goji berry result (1.06 ± 0.03 g GAE/100 g), we can conclude that goji berries have a high amount of antioxidants (phenolic compounds).

ORAC is the standard test, adopted by the US Department of Agriculture, to measure the potency of antioxidants in food. Although ORAC and DPPH are both used to measure radical scavenging, they also have some diversity, such as the type of radical produced, scavenging method and measurement. AAPH produces peroxy radicals that are to be scavenged by antioxidants found in our fruit samples [41]. Fluorescein, a molecular probe, is under peroxy radical attack. The more antioxidants, the more peroxy radicals are scavenged and the less fluorescence is detected (fluorescein is protected by antioxidants from radical attack) [38].

Higher Trolox equivalents calculated from a sample correspond to better ability of scavenging AAPH radicals. The higher concentrations (2 mg/ml and 2.5 mg/ml) show a higher peroxy radical scavenging capacity than their lower concentration counterparts, which further assures the previously discussed results (TPC). Similarly, studies of different fruits have shown varying results when comparing with goji berry [35,37]. As shown in the **Table 2**, the ORAC scavenging capacity of dry fruits represents a broad range from 9.0 to 153.6 TE $\mu\text{mol TE/g}$, the highest capacity belongs to strawberry while the lowest capacity belongs to banana [35]. From the above results, we can draw a conclusion that goji berries have a good capacity to scavenge free radicals (188.52 ± 1.3 μmol

TE/g).

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) [7]. DPPH radicals are frequently utilized in antioxidant studies [39]. DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods [7]. Antioxidants in a sample can scavenge the DPPH radicals. A gradual reduction in absorbance is observed, which implies that DPPH radicals are being scavenged. Through adding samples, which are rich in antioxidant, to a DPPH solution. Therefore, the percentages we have presented pertain to DPPH radical scavenging capacity which in turn is directly proportional to antioxidant capacity [51]. As shown in the **Figure 3**, goji berries have a significant capacity to scavenge DPPH radical, which shows a good dose dependency. Our results indicate that in the concentrations of 40 mg/ml and 50 mg/ml, our samples showed higher remaining ability (59.94% and 52.99%, respectively) and therefore higher antioxidant capacity than the lower concentrations 10 mg/ml and 20 mg/ml (70.58% and 65.21%, respectively), which further reinforce the TPC and ORAC findings that have been discussed. These results may also indicate that the higher phenolic content in the samples (according to TPC) might be correlated to their higher potency in scavenging DPPH radicals [51]. Again, studies have shown varying results when comparing the DPPH radical scavenging capacities of various samples of fruits [37,40].

As aforementioned, goji berries have important health benefits by providing potentially higher antioxidant ability. The stability of antioxidant capacity has been analyzed in other fruits, for instance, chokeberries, gooseberries, blueberries, strawberries, cranberries, blackberries, dates, plumes, walnuts and so on [36-37,42-44]. For this reason, more and more researches have been studied on goji berry with antioxidants activities [49,50]. So, although eating a large amount of antioxidants is always a plus, it is important to eat a variety of healthy foods, not only for their antioxidant levels, but for their other nutritional properties as well.

In agreement with the above mentioned studies, we report that goji berry is a remarkable source of antioxidant compounds when compared to other fruits. Research supports deep colored fruits as potent antioxidant sources. Berries and dried fruit compose a relatively small part of the average diet, but they are important antioxidant sources. Highly pigmented berries have the highest antioxidant activity. Such fruits are rich in antioxidant compounds that are known for their enhanced stability and bioaccessibility [47,48]. Based on our findings and the cited literature, we can suggest that goji berry be listed among red fruits that provide antioxidants.

Conclusion

Goji berry contains significant amounts of phenolic compounds and exhibits scavenging activities against different free radicals. Therefore, goji berry could be used as a dietary source of natural antioxidants and be worthy of development and utilization. Moreover, antioxidant activity and mechanisms of goji berry should be a focus of future research. It would be interesting in the future to identify and characterize the specific antioxidants that exist in a goji berry fruit in high amounts.

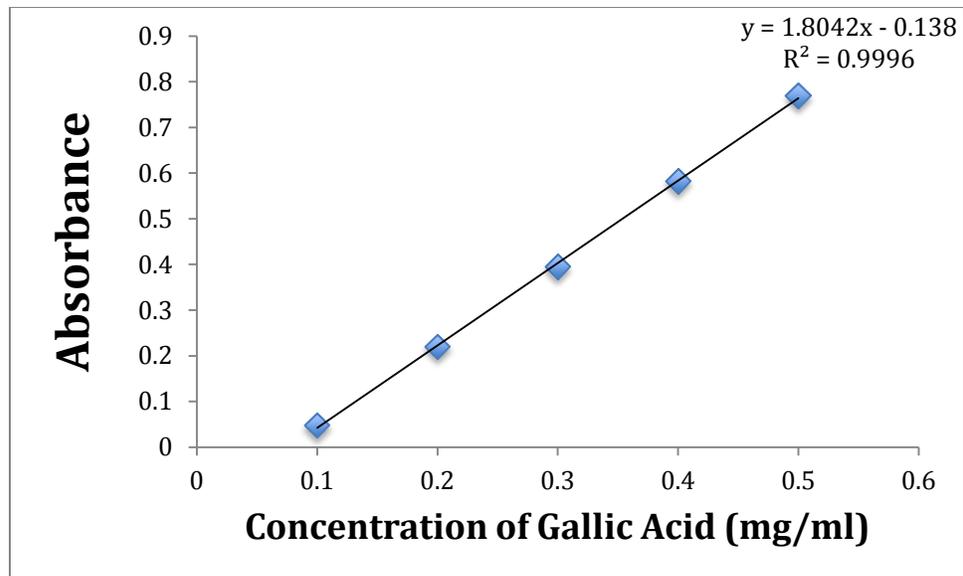


Figure 1 Standard curve of goji berry in Total phenolic content

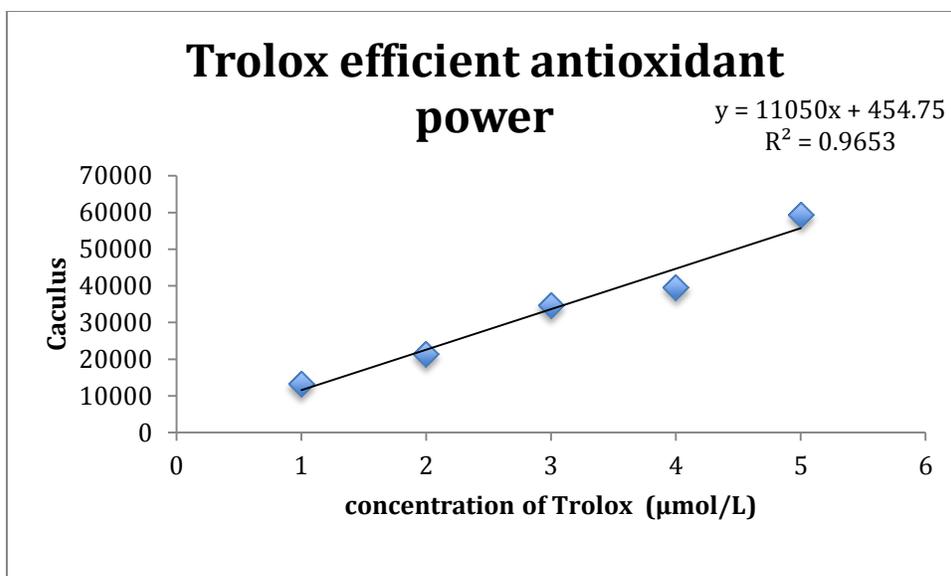


Figure 2 Standard curve of goji berry in ORAC assay

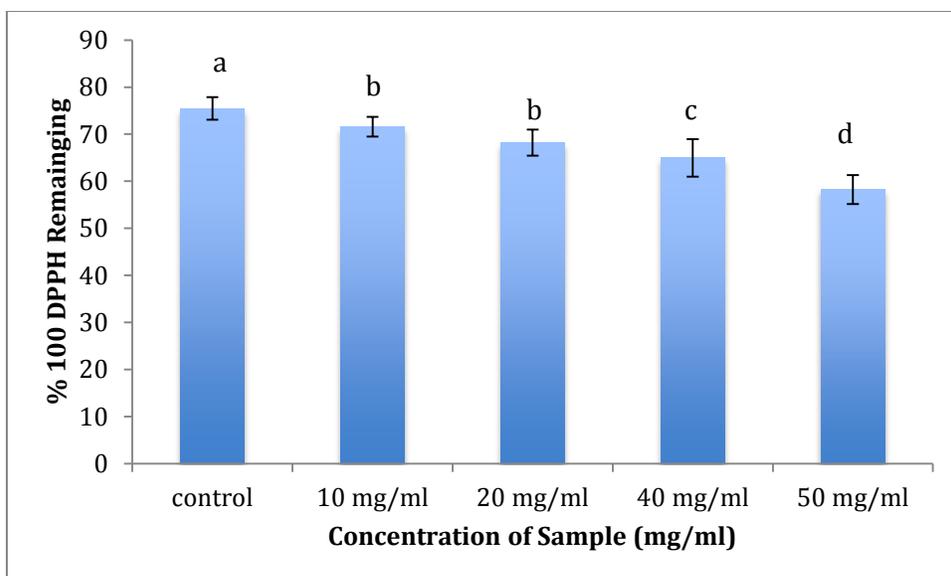


Figure 3 % DPPH radical scavenging by goji berry. Bars with different letters indicate significant difference ($P < 0.05$)

Number	Name of the fry fruit	Total Phenolic content (g GAE/ 100 g dry weight)
1	Almond	0.109 ± 0.016
2	Apricot	0.304 ± 0.039
3	Brown raisins	0.749 ± 0.025
4	Cashew nuts	0.153 ± 0.004
5	Dry dates	0.242 ± 0.050
6	Figs (Anjeer)	0.331 ± 0.051
7	Ground Nut	0.324 ± 0.042
8	Piyal seeds	0.099 ± 0.002
9	Walnuts	0.959 ± 0.078

Table 1 Total phenolic content of commonly consumed dry fruits
(Values are mean ± SD) [36]

Number	Name of Fruits	ORAC value ($\mu\text{mol TE/g}$ of dry weight)
1	Strawberry	153.6 \pm 7.5
2	Plum	79.1 \pm 1.9
3	Orange	51.7 \pm 2.7
4	Grape, red	36.0 \pm 1.1
5	Kiwi fruit	36.5 \pm 1.3
6	Grapefruit, pink	48.3 \pm 0.6
7	Grape, white	26.2 \pm 2.6
8	Banana	9.0 \pm 0.4
9	Apple	13.2 \pm 0.9
10	Tomato	37.8 \pm 0.5
11	Pear	9.6 \pm 0.2
12	Melon	12.9 \pm 0.5

Table 2 ORAC values of commonly consumed dry fruits
(Values are mean \pm SD) [35]

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ABSTRACT

ANTIOXIDANT PROPERTIES OF GOJI BERRY

by

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Antioxidants properties have been discussed for many years. Their benefits, especially for human health, have been more and more recommended and supported by nutritionists. It is necessary to obtain sufficient amounts of these compounds from dietary sources. Goji berries are particularly rich in antioxidants. Due to the roles they play in protecting the human body from oxidative damage, studies of the antioxidant absorption are more and more popular in recent years. In this study, the stability of antioxidant properties of goji berries was investigated. Sample of goji berry was tested for antioxidant properties via total phenolic content (TPC) assay, oxygen radical absorbance capacity (ORAC) assay, and DPPH radical scavenging assay. Results from this study implied that goji berries contain a higher amount of phenolic contents and higher scavenging capacity against different free radicals than other fruits from TPC, ORAC and DPPH. Moreover, the phenolic contents and scavenging capacity indicate a well dependency relationship in does. In conclusion, goji berries could effectively prevent human bodies from oxidative damage. They

could be used as a dietary source of natural antioxidants and be worthy of development and utilization. It would also be interesting to identify and characterize the antioxidants that exist in a goji berry fruit.

AUTOBIOGRAPHICAL STATEMENT

Jie Zhang received her bachelor of Food Science and Technology from Shanghai Ocean University (SHOU) in June 2011. In September 2011, she joined Wayne State University (WSU) and is currently completing her graduate studies towards the accomplishment of a master of Nutrition and Food Science degree.

During her undergraduate studies, Jie Zhang was a member of a food science institute in the Shanghai Ocean University from 2007 to 2010 as well as a volunteer in the Food Volunteers Association from 2008 to 2009. She also received the SHOU People Scholarship of Academic Excellence throughout her course of study for every academic year. During her graduate studies, Jie Zhang was a member of Dr. Zhou's lab and she worked in the lab from 2012 to 2013.