
Effect of Superfine Grinding on the Phytochemicals and Antioxidant Activities of Mulberry Leaves

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Abstract: In this study, two species of mulberry leaves were used as materials and four different size powders (about 20-115 μm , 15-20 μm , 10-15 μm and <10 μm) were prepared. The effects of superfine grinding (SG) on the extraction of active components from mulberry leaves (MLs) and their antioxidant activities were investigated. The results indicated that the contents of the active component such as polyphenol, polysaccharides, alkaloids were increased markedly with the decreasing in the particle size, but there was a bit difference in flavonoids. Additionally, the antioxidants properties of MLs treated on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing antioxidant power (FRAP) were also increased, but the scavenging activity in hydroxyl radical scavenging assay (HAS) decreased in the size of D10. These results indicated that SG could improve the extraction efficiency of active components from MLs and make its antioxidant activity enhance, implying that SG can be an effective way to improve the quality and functionality of MLs in foodstuff and pharmacological field.

Keywords: Mulberry Leaves, Superfine Grinding, Active Components, Antioxidant Activities

1. Introduction

Mulberry, which belongs to the *Morus* genus, *Moraceae* family [1], was domesticated thousands of years ago and adapted to a wide area of tropical, subtropical, and temperate zones in the world. This genus has twenty-four species with one subspecies, and 100 varieties are known to date [2]. The mulberry tree is deciduous and fast growing with medium to large sized tree (10-20 m). Their leaves are alternatively arranged, simple and mostly lobed on the margin. Various functions of mulberry leaves (MLs) have been broadly discussed in prior studies [3-5], and its importance has been also highlighted. Due to the highest content of its active components, MLs are widely applied in pharmacological and food industry. Its fruits, bark, leaves, branches and roots have been used for various health benefits and ailments [6]. MLs proved to be rich in minerals, dietary fiber, vitamins as well as proteins, are considered as good source of nutrients. For example, it was generally used in noodles, cakes and tea as

nutraceutical supplements [7]. Besides, it also contains various bioactive components including flavonoids, polyphenols, polysaccharides, and alkaloids, and so on. Hence, MLs is also found to possess a good potential of antioxidants. Nowadays, these components have been regarded as an important quality control parameter for function evaluation. Many studies indicated that MLs plays many important roles in antioxidant, antiviral, anti-inflammatory hypo-lipidemic, anti-hyperglycemic, neuro-protective [8], anti-HIV, anti-hypotensive and anti-cytotoxicity [9], reduce blood pressure, anti-obesity, anti-diabetes [10]. Most of the Asian countries have mainly grown the mulberry for different purposes. For instance, China uses its leaves to feed the silkworm (*Bombyx mori* L.) [11, 12], in Korea it is for consumption purpose, and in Japan it is utilized for diabetes mellitus patients [13]. Hence, MLs are increasingly consumed as a functional food, and their

utilization in pharmacological factory also have a higher potential value.

Superfine grinding (SG) is a new technique, which is revealed to be a useful tool for making superfine powders. Zhang defined that superfine powder as small size solid particles from 4 μm to 200 μm [14]. It is usually used in different fields such as food industry. Compared with ground traditional powders, it bears good physical properties such as dispersibility and solubility [15]. Superfine powder obtained can easier to release its activity components, and is possible for improving the quality and nutritional value of food products [16]. Moreover, it is also easier to be incorporated into food structure than those obtained through traditional methods [17]. To date, the SG technique has shown the high potential in many commercial applications [18]. It possesses good stability, high reliability, simple structure, and easy to operate. It was found as well that it does not consume more energy than the traditional mechanical grinding [19], and can considerably improve the efficiency of the extraction from superfine powders and fits the environment conditions. Despite the fact that there is scarce information on the quality evaluation of MLs through superfine grinding, it is highly relevant to our present study to develop the agricultural products and food products.

Several recent articles are accessible on the extraction of the active components and antioxidants in mulberry leaves, fruits, barks and roots [20, 21]. For this purpose, we review several techniques to determine and analyze these active components. Even, the light consideration literature is clear, but a little information has found on the extraction active components and antioxidant from plant materials with the superfine grinding process such as mulberry leaves.

In order to analyze the effect of SG on the extraction of phytochemical components, the content changes of active components from MLs of two species in three different seasons were determined in the present study. Then, it is also focused on the effect of SG on particle size and antioxidant activity.

2. Experimental

2.1. Collection of Mulberry Leaves (MLs)

Fresh MLs were collected from Shanghai University (Longitude 121°23'44"E, Latitude 31°19'22"N). MLs of two species named MLs1 and MLs2 were used for this present investigation. MLs were picked in three different seasons including spring season (in May) named MLs1.5 and MLs2.5, summer season (in August) named MLs1.8 and MLs2.8, and autumn season (in October) named MLs1.10, MLs2.10.

2.2. Chemicals and Reagents

Analytical grade reagents were used throughout this work. Folin-Ciocalteu's phenol reagent, rutin, glucose, ascorbic acid, potassium ferricyanide, ferrous sulfate, ferric chloride, sodium phosphate buffer (0.2N, pH 6.6) and sodium

salicylate were purchased from Shanghai Biotechnology Co. Ltd. Sulfuric acid, methanol, ethanol, acetone, sodium nitrate, sodium bicarbonate, aluminum chloride, sodium hydroxide, bromocresol green (BCG), sodium dihydrogen phosphate, citric acid, gallic acid, anthrone, sulfuric acid, trichloroacetic acid (TCA), hydrogen peroxide, chloroform, coomassie Brilliant Blue (G-250) were purchased from Sinopharm-Chemical Reagent Co. Ltd. Matrine was purchased from Beijing huagong Co. Ltd. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma-Aloricha, Co. Ltd.

2.3. Preparation of Samples

After collection, MLs were washed with running tap water, followed by rinsing with deionized water, and consequently chopped into small pieces, and then placed in a vacuum drying machine at 50°C, until completely dried. The dried leaves were grounded coarsely by using a disc-mill, and then screened through a 100-mesh net. Finally, the MLs powders are divided into four parts, one part was kept as control, and the other three parts were milled with a superfine grinder mill): (FDV, Shanghai Instruments Co. Ltd) to obtain the different size particles. All samples obtained were kept at 4°C in a refrigerator until further use.

2.4. Measurement of Particle Size Powder

The particle sizes of MLs powder were obtained by using a microscopy. The process could be described as follows. First, Minimum amounts of MLs powders was placed on a glass slide, and 1 to 2 drops of distilled water was dropped on it, and then covered with a coverslip. The observation was performed by using the microscopy (Nikon Eclipse 80i, Leica DM1400B) with eyepiece (10 \times) and an objective lens (40 \times). In this study, thirty particles for each sample have been measured in order to get the mean diameter (D) of MLs powder.

2.5. Extraction of Phytochemicals

One gram of plant MLs powder was transferred into a bottom flask and with 20 mL of solvent (70% ethanol, 80% methanol, 85% acetone, distilled water). The mixture was incubated in a water bath at 70°C for 1.5 hours (for ethanol and water solvent), and the other two solvents were directly incubated at room temperature for 24 hours. Then, the mixture was centrifuged at 5000 \times g for 20 min to obtain the supernatant; the residue was re-extracted two times with a proper volume. The resulting supernatant was combined for each sample and adjusted to 50 mL (20 mg dry powder /mL), and then stored in a refrigerator at 4°C further used.

2.6. Phytochemical Analysis

2.6.1. Determination of Flavonoids Contents

Total flavonoids content in the extract of 70% ethanol was measured by using the aluminum chlorometric method [22]. Rutin was used to make the calibration curve. The results were expressed in terms of mg rutin equivalents (RE)/g of

MLs powder. The absorbance of the reaction mixture was measured at 510 nm. All measurements were carried out in a triplicate experiment.

2.6.2. Determination of Polyphenol Contents

Total polyphenol content in the extract of 70% ethanol was determined by using the Folin-Ciocalteu's reagent, according to the method [23]. Gallic acid was used to make the calibration curve. The polyphenol content was expressed in terms of mg Gallic acid equivalents (GAE)/g of MLs powder. The absorbance of the resulting solution was measured at 765 nm. All tests were performed in triplicate.

2.6.3. Determination of Polysaccharides Contents

The polysaccharide content in the extract of distilled water was determined by enthrone-sulfuric acid method [24]. The standard curve was made with glucose. The content of polysaccharide was expressed in terms of mg glucose equivalents per gram of MLs powder. The absorbance of reaction solution was measured at 620 nm. All samples and standard were carried out in triplicate.

2.6.4. Determination of Alkaloid Contents

Crude alkaloid content in the methanol extract was determined by the method [25] with slight modification. Matrine was used to make the calibration curve. The content of alkaloids was expressed in terms of mg matrine equivalents per gram of MLs powder. The absorbance was measured at 422 nm. All measurements were carried out in triplicate.

2.6.5. Determination of Chlorophyll Contents

The chlorophyll content in the extract of 80% acetone was estimated according to the method [26]. The chlorophyll content present in MLs was expressed in mg/g of MLs powder. The absorbance was measured at 663 nm and 645 nm, separately. All experiments were carried out in triplicate. The amount of chlorophyll was calculated using the following equations:

$$\text{Chlorophyll a (mg/g)} = [12.7 \times (A_{663\text{nm}}) - 2.69 \times (A_{645\text{nm}})] \times V / (1000 \times W) \quad (1)$$

$$\text{Chlorophyll b (mg/g)} = [22.9 \times (A_{645\text{nm}}) - 4.68 \times (A_{663\text{nm}})] \times V / (1000 \times W) \quad (2)$$

$$\text{Total Chlorophyll (mg/g)} = [20.2 \times (A_{645\text{nm}}) + 8.02 \times (A_{663\text{nm}})] \times V / (1000 \times W) \quad (3)$$

Where, A=Absorbance at a specific wave length; V= Final volume of chlorophyll extract at 80% acetone; W= Weight of MLs powder extracted.

2.7. Determination of Antioxidant Activity

In this study, the DPPH, FRAP, and HAS radical scavenging activity were used for evaluating the antioxidant activity of MLs. The supernatant was prepared with 1mg/mL of 70% ethanol. Ascorbic acid (V_C , 100 $\mu\text{g/mL}$) was used as reference standard in all experiments.

2.7.1. DPPH Radical Scavenging Activity

Free radical scavenging activity was measured according to the method [27]. The stock solution (40 $\mu\text{g/mL}$) of DPPH radical was prepared with 70% ethanol. 2.95 mL of DPPH solution and 0.05 mL sample were mixed well and reacted in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. The radical scavenging activity was calculated by using the following equation:

$$\text{DPPH radical scavenging (\%)} = \left(\frac{A_C - A_S}{A_C} \times 100 \right) \quad (4)$$

Where, A_S =Absorbance of the sample obtained after 30 min; A_C =Absorbance of control under the same condition.

2.7.2. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP radical scavenging from different sized power was estimated by the method [28]. Briefly, 100 μL of each sample was mixed with 2.5 mL sodium phosphate buffer (0.2 mol/L pH 6.6), 2.5 mL 1% of potassium ferricyanide, 2.5mL 10% of trichloroacetic acid (TCA, v/v), then, after adding 2.5 mL distilled water of the mixture, 0.5 mL ferric chloride (0.1%, v/v). The reaction mixture was kept at room temperature for 10 min and the absorbance was measured at 700 nm. All samples and positive control were carried out in triplicate. FRAP radical of MLs powder was calculated by using the following formula:

$$\text{FRAP radical assay (\%)} = \left(1 - \frac{A_S - A_I}{A_C} \times 100 \right) \quad (5)$$

Where, A_S = Absorbance of the sample, A_I = Absorbance from tested of solvents, A_C = Absorbance obtained from test without samples (control).

2.7.3. Hydroxyl Radical Scavenging Assay (HSA)

The HSA activity was determined by using the method [29] with slightly modification method [30]. The reaction mixture (3 mL) containing 1mL of Iron (II) FeSO_4 (9 mM), 0.3 mL of Sodium salicylate ($\text{C}_7\text{H}_5\text{NaO}_3$, 9 mM), 0.7 mL of Hydrogen peroxide (H_2O_2), (8.8 mM) and 1 mL of each sample were add. After incubation for 45 min at 37°C in a water bath, the absorbance of the reaction mixture was measured at 510 nm. HSA ability of MLs powder was calculated as following formula:

$$\text{HSA radical scavenging (\%)} = \left(1 - \frac{A_S - A_I}{A_C} \times 100 \right) \quad (6)$$

Where, A_C = Absorbance obtained from the test without sample (Control), A_I =Absorbance of solvents tested and A_S =Absorbance obtained from tested samples.

2.8. Statistical Analysis

In order to make sure the accuracy of experimental data, all measurements were executed at least in triplicate and the results were express as mean \pm standard derivation. Statistical analysis was calculated with software SPSS 16.0, using one-way analysis of variance (ANOVA). $P < 0.05$ was considered to be significant by using Duncan's test.

3. Results and Discussions

3.1. Particle Size of MLs Powder

Fig. 1 showed the morphological feature of fragmented MLs powder. The results of measurement showed the sizes of MLs powder were about 113 μm for control (D115), and about 20.24 μm for (D20), 15 μm for (D15) and finally, 9.92 μm for (D10) for four stages, separately (Fig. 1). The result

showed that the particle size of MLs powder obviously become small after SG, which could have a significant impact on phytochemical and the antioxidant activity. These results were in agreement with those reported Hu *et al.* [31], who studied the effect of SG on the quality and antioxidant from green tea powder, that particle sizes were arranged from 180 to 6.3 μm .

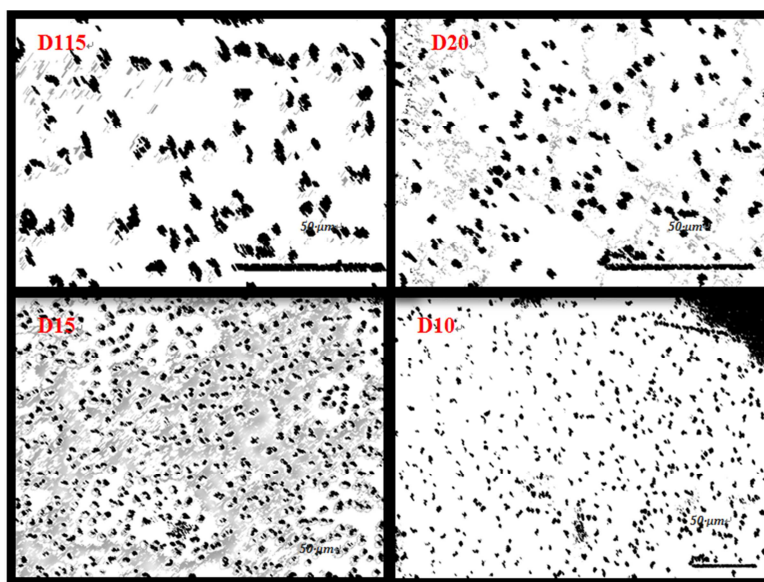


Fig. 1. The morphological feature of MLs powder. (D115: 113 μm ; D20: 20.24 μm ; D15: 15 μm ; D10: 9.92 μm).

3.2. Effect of Superfine Grinding (SG) on Active Components from Mulberry Leaves

3.2.1. Effect of SG on Flavonoids Contents

Flavonoids, the largest subgroup of plant phenolic substances, have attracted much interest of food chemists, nutritionists, as well as clinical researchers. Table 1 showed the flavonoids content in the four different sizes from MLs of two species, harvested in three different seasons. The results showed that the flavonoids content of MLs1 without SG were 24.58 ± 0.11 mg/g (MLs1.5); 30.94 ± 0.64 mg/g (MLs1.8) and 12.63 ± 0.07 mg/g (MLs1.10), with significant difference ($p < 0.05$). A similar trend was observed for MLs2. The

flavonoids content in MLs2 was higher than that in MLs1.

The flavonoids content of MLs harvested in August was higher than that in the other 2 seasons. These results are in line with previous researches [32, 33]. The flavonoids content of MLs increased in D20 after treated with SG, comparing without SG. However, the flavonoids content in D15 and D10 markedly decreased with the decreasing in MLs size. These results are in agreement with prior published reports [34, 35]. The result implied that it is helpful to extract the flavonoid from MLs treated with SG, but the treatment of over superfine grinding makes the flavonoid content decrease.

Table 1. Effect of SG on the flavonoids content of from MLs (mg/g).

Samples	D115	D20	D15	D10
MLs1.5	24.58 ± 0.11^c	30.93 ± 0.241^b	15.67 ± 0.08^{ab}	11.36 ± 2.88^a
MLs1.8	30.94 ± 0.64^d	38.03 ± 3.86^c	22.74 ± 0.99^b	15.94 ± 2.09^a
MLs1.10	12.63 ± 0.07^b	26.62 ± 1.37^c	12.14 ± 0.38^{ab}	11.06 ± 0.63^a
MLs2.5	28.80 ± 2.48^d	36.29 ± 3.08^{bc}	21.71 ± 1.44^{ab}	11.58 ± 1.69^a
MLs2.8	36.94 ± 2.33^d	41.15 ± 2.76^c	27.33 ± 1.07^{ab}	15.07 ± 2.48^a
MLs2.10	25.89 ± 0.85^d	38.67 ± 1.11^c	14.96 ± 0.32^b	14.90 ± 1.48^a

The data are mean \pm SD ($n=3$) of three-replicated analysis. Means with the different letter in the same index are significantly difference ($p < 0.05$)

3.2.2. Effect of SG on Polyphenol Contents

Table 2 showed the result of polyphenol content. The content of polyphenol for MLs in D115 in the summer season was the highest, and up to 37.37 mg/g and 39.26 mg/g for

MLs1 and MLs2, separately, following by the autumn and spring season. These results were in agreement with prior reports [36, 37]. The contents of polyphenols in MLs1 gradually increased with the decreasing of particle size. A similar trend was obtained in MLs2. These results were

consistent with the results [34]. In addition, the content of polyphenol in MLs1 was a bit lower than that in MLs2. The

result also indicated that it is helpful to extract the polyphenol from MLs through the SG treatment.

Table 2. Effect of SG on the polyphenols contents from of MLs (mg/g).

Samples	D115	D20	D15	D10
MLs1.5	8.02±0.09 ^a	11.15±0.53 ^{ab}	13.79±0.39 ^c	21.27±0.98 ^d
MLs1.8	37.37±3.34 ^a	39.33±3.27 ^b	41.61±1.30 ^{bc}	48.59±6.50 ^c
MLs1.10	8.43±0.50 ^a	13.38±0.76 ^b	14.43±0.97 ^b	16.26±0.03 ^c
MLs2.5	11.97±0.15 ^a	17.30±0.01 ^b	20.87±0.03 ^c	26.61±0.04 ^d
MLs2.8	39.26±1.00 ^a	43.70±1.18 ^b	46.83±1.58 ^c	52.30±0.98 ^d
MLs2.10	16.70±0.56 ^a	17.72±0.09 ^{ab}	23.28±1.24 ^c	26.28±2.64 ^d

The data are mean ±SD (n=3) of three-replicated analysis. Means with the different letter in the same index are significantly different (p < 0.05).

3.2.3. Effect of SG on Polysaccharides Contents

The polysaccharide content significantly (p<0.05) for MLs1 increased from 66.82±1.02 mg/g to 96.86±0.22 mg/g for three different seasons (May, August, and October) separately, shown in Table 3. A similar trend was obtained for MLs2, but its content in polysaccharide had a little lower when compared with MLs1. These results were in correspondence with those previously reported [38]. As shown in Table 3, the content of polysaccharides showed MLs1.10 and MLs1.8 treated with SG increased considerably, with different significant value p<0.05 from 96.86 mg/g (D115) to 157.68 mg/g (D10). A similar trend was obtained for MLs2. Hu et al, indicates that green tea polysaccharides increased after the SG process [31]. The result also suggested that the SG helpful for extracting the content of polysaccharide from MLs.

Table 3. Effect of SG on the polysaccharides contents from MLs (mg/g).

Samples	D115	D20	D15	D10
MLs 1.5	66.82±1.02 ^a	73.11±0.72 ^b	81.15±2.22 ^c	96.13±1.67 ^d
MLs1.8	83.27±0.44 ^a	112.00±9.61 ^d	127.13±0.44 ^{bc}	148.55±1.15 ^d
MLs1.10	96.86±0.22 ^a	119.38±0.32 ^b	127.05±1.88 ^b	157.68±2.79 ^c
MLs2.5	51.54±0.32 ^a	59.36±0.51 ^a	63.02±1.51 ^b	68.06±1.48 ^c
MLs2.8	76.54±2.39 ^a	87.99±3.00 ^b	102.13±0.76 ^c	107.17±0.63 ^c
MLs2.10	84.58±0.22 ^a	91.24±0.29 ^{ab}	98.03±4.12 ^c	122.45±6.73 ^d

The data are mean ±SD (n=3) of three replicated analysis. Means with the different letter in the same index are significantly different (p < 0.05).

3.2.4. Effect of SG on Alkaloids Contents

The contents of alkaloids in MLs collected in three different seasons were observed in Table 4. It was the highest in the summer season for MLs1, up to 3.18±0.24 mg/g, and the lowest in the autumn season, just to 1.29±0.04 mg/g. However, the MLs2 was different from MLs1. It was the highest in autumn season for MLs2, up to 2.29±0.02 mg/g, and the lowest in the spring season just to 1.09±0.45 mg/g. Li [39] also found the similar amounts of alkaloids contents in MLs. After the SG, the alkaloids content markedly increased with the decreasing in particle size of MLs. The alkaloids content of ML1 in D10 has 3.83±0.25 mg/g, 4.40±0.32 mg/g and 3.34±0.13 mg/g over three different seasons were three were increased when compared with the results obtained from D115, and the alkaloids content of ML2 in D10 has 22.22±0.27 mg/g, 3.3±0.21 mg/g and 4.15±0.08 mg/g for

three different seasons also increased when compared with the D115 results. It is to say; the result suggested that the SG is useful for extracting of alkaloids in MLs.

Table 4. Effect of SG on the alkaloids content from of MLs (mg/g).

Samples	D115	D20	D15	D10
MLs1.5	2.79±0.01 ^a	3.48±0.07 ^b	3.51±0.02 ^b	3.83±0.25 ^c
MLs1.8	3.18±0.24 ^a	3.58±0.24 ^a	4.09±0.14 ^b	4.40±0.32 ^b
MLs1.10	1.29±0.04 ^a	2.29±0.11 ^b	3.05±0.07 ^b	3.34±0.13 ^b
MLs2.5	1.09±0.45 ^a	1.62±0.37 ^{ab}	2.17±0.43 ^b	2.22±0.27 ^b
MLs2.8	1.86±0.34 ^a	2.29±0.10 ^b	3.14±0.18 ^c	3.32±0.21 ^c
MLs2.10	2.29±0.02 ^a	3.35±0.03 ^b	3.57±0.31 ^b	4.15±0.08 ^c

The data are mean ±SD (n=3) of three-replicated analysis. Means with the different letter in the same index are significantly different (p < 0.05).

3.2.5. Effect of SG on Chlorophylls Contents

Table 5 indicated the content of chlorophyll in the MLs extract. The chlorophyll content obtained for MLs1 in D115 was 3.94±0.07 mg/g in spring season (MLs1.5), 3.58±0.03 mg/g in summer season (MLs1.8), and 2.79±0.13 mg/g in autumn season (MLs1.10). The chlorophyll content for MLs1 was higher than that for MLs2. Our results were in agreement with the investigation reported [39, 40]. The total content of chlorophyll was also evaluated after treated SG. The result showed that the more chlorophyll could be extracted through the treatment of SG from D115 to D15, but the chlorophyll content a bit decreased when the particle size was up to 10 µm. A similar result was detected in MLs2. The result implied that SG was to a certain extent more effective for extracting chlorophyll than MLs powder.

Table 5. Effect of SG on the total chlorophyll content from of MLs (mg/g).

Samples	D115	D20	D15	D10
MLs1.5	3.94±0.07 ^b	4.07±0.03 ^{bc}	4.20±0.01 ^c	3.19±0.09 ^a
MLs1.8	3.58±0.03 ^b	3.64±0.03 ^b	3.85±0.04 ^c	3.21±0.07 ^a
MLs1.10	2.79±0.13 ^b	3.07±0.01 ^c	3.29±0.06 ^d	2.59±0.04 ^a
MLs2.5	2.68±0.10 ^a	2.79±0.06 ^{ab}	3.30±0.03 ^b	2.95±0.20 ^c
MLs2.8	2.19±0.06 ^b	2.26±0.01 ^b	2.51±0.04 ^c	2.07±0.05 ^a
MLs2.10	1.23±0.01 ^b	1.49±0.04 ^c	1.66±0.04 ^d	0.89±0.02 ^a

The data are mean ±SD (n=3) of three-replicated analysis. Means with the different letter in the same index are significantly different (p < 0.05).

3.3. Effect of SG on the Antioxidant Activity of MLs

3.3.1. Effect of SG on the Antioxidant Activity Analyzed by DPPH

The effect of SG on the antioxidants was analyzed with DPPH[•] radical. The results found the antioxidant activity (53.75%) of MLs1.8 in D115 possessed higher value than those of MLs1.5 and MLs1.10, which was comparable with V_C as a standard antioxidant, shown in Fig. 2. A similar pattern was observed between MLs1 and MLs2, but the

antioxidant activities in MLs2 revealed were a bit higher than that of MLs1. These results were consistent with the results of the antioxidant activity of MLs in [20]. The scavenging activity of MLs1 gradually increased with decreasing of size particles (from D115 to D10). A similar result was observed in MLs2. The DPPH radical scavenging activity was increased after SG, which is in agreement with previous studies reported [34, 35].

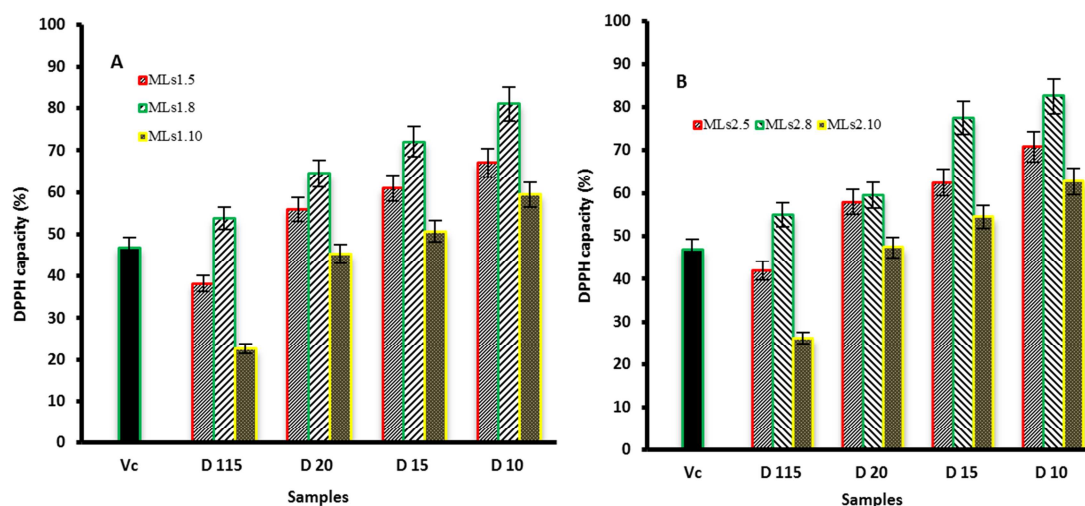


Fig. 2. DPPH radical scavenging activity (%) of MLs (A= MLs1; B= MLs2).

3.3.2. Effect of SG on the Antioxidant Activity Analyzed by FRAP

The FRAP assay was used to measure the antioxidant capacity in terms of extracts with the ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) state. Antioxidant capacities of MLs1 in D115 were changed from $29.21 \pm 1.59\%$ to $49.57 \pm 4.31\%$ with the change of season. Antioxidant activities in MLs1 were a bit higher than that in MLs2 (Fig. 3). As we know, V_C

is a standard antioxidant, which could be utilized for evaluating the antioxidant of MLs extract. These results were in agreement with those reported by [21], who indicated the phenolic and antioxidant activity from mulberry leaves. After SG treatment, the reducing capacities were also obviously improved for different SG powder as shown in Fig. 3. However, there is no information have been the study of the effect of SG treated by FRAP.

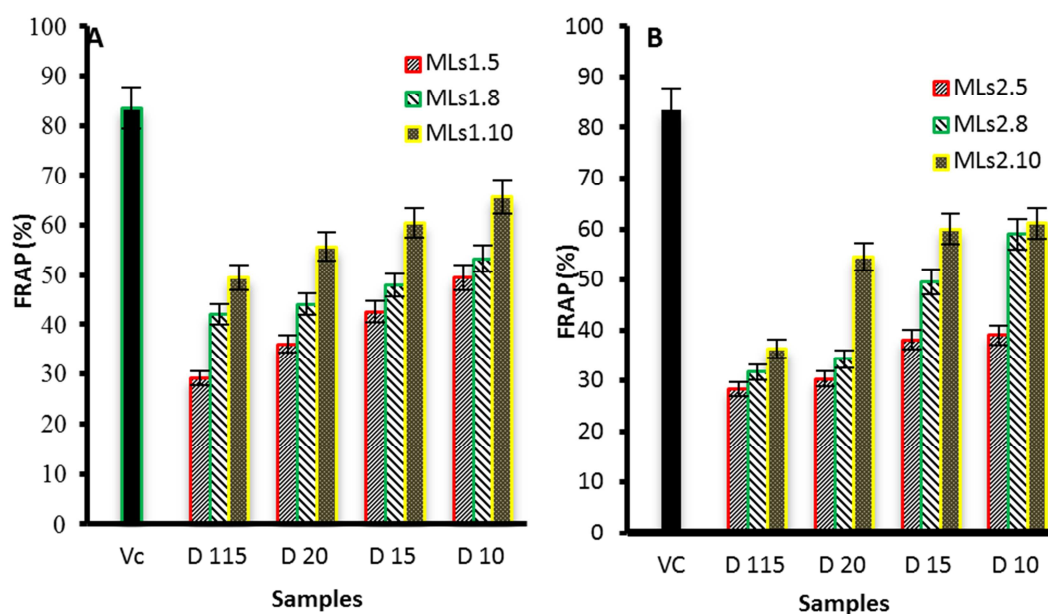


Fig. 3. FRAP radical scavenging (%) of MLs (A=MLs1; B= MLs2).

3.3.3. Effect of SG on the Antioxidant Activity Analyzed by HSA Radical Scavenging

HSA activities were fluctuated in the two species of MLs, as shown in Fig. 4, the free radical scavenging activity of MLs1 extract in D115 were observed as $63.08 \pm 1.17\%$, $55.00 \pm 2.31\%$ and $54.46 \pm 0.19\%$ for different seasons. A similar down ward trend was observed for MLs2, but the scavenging activity in MLs2 was stronger than that in MLs1. The antioxidant activity obtained by using HSA radical of MLs varied considerably. Our present result was in agreement with the report published [21], who also investigated the antioxidant from MLs and the results were from 50.9 ± 8.6 to $72.8 \pm 6.8\%$. Moreover, Bruno *et al.* also

reported the value of HSA radical scavenging from mulberry [41]. HSA scavenging effect of MLs extract was also comparable with Vc. After SG treatment, HSA scavenging effect of MLs extract was at a certain extent improved, comparing to the sample in D115. However, HSA scavenging effect of MLs extract rapidly decreased in D10. The reasons for the decline could be attributed to the manufacturing process, which could produce heat and make active components deactivate. By contrast, the HSA radical scavenging activity was increased in certain times, after that it slowly decreased. This circumstance was in agreement with that report described by [31].

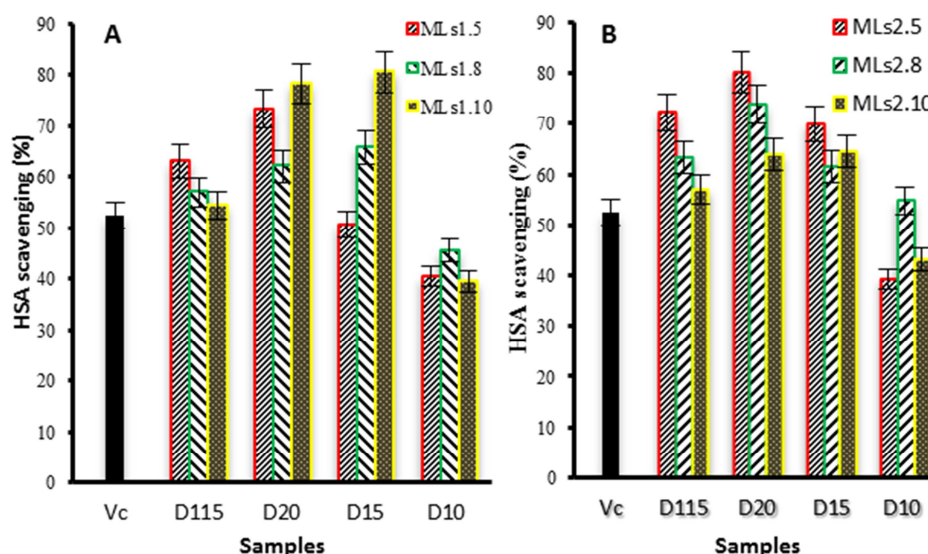


Fig. 4. HSA radical scavenging (%) of MLs (A= MLs1; B= MLs2).

4. Conclusions

In the present study, SG was used to refine the size of MLs powder, up to 10 μm , and which made the content of active components such as polyphenol, polysaccharide, and alkaloids, chlorophyll considerably increased with the decreasing in size of MLs powder. After SG treatment, the antioxidant activity of ethanol extract in DPPH scavenging activity and the reducing capacity was evidently improved, owing to the increasing in the content of active components such as polyphenol; polysaccharide existed in the MLs extract. The variance of the result in several indexes between MLs1 and MLs2 may be related to the physiological characteristics of MLs. MLs1 have different phenotypes with MLs2. The mulberry tree for MLs1 can bear fruit, and the maturation period of its leaves is earlier than MLs2. The leaves of the mulberry tree for MLs2 are large and thick, and its leaves period is longer than MLs1. Our results are well supported by these features. The experimental results implied that SG could be an effective way to improve the quality and functionality of MLs in foodstuff and pharmacological field.

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