



Effect of different drying methods on the water soluble total phenolics and flavonoids contents, and antioxidant activities of sunchoke (*Helianthus tuberosus* L.) powder

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Abstract

The effect of different drying methods on the water soluble total phenolics, flavonoids contents and antioxidant activities in sunchoke (*Helianthus tuberosus* L.) powder were investigated. Three different drying methods were shade (49 h), sun (49 h) and oven at 60 °C (12 h). The *Helianthus tuberosus* L. powder (2 g) was extracted by water as extracting solvent with control 80 °C for 45 min. Total phenolics contents, total flavonoids contents and antioxidant capacity were measured using DPPH free radical scavenging activity, total antioxidant capacity, ABTS free radical scavenging activity, reducing power and ferrous ion chelating effect. Total phenolics contents (1.463 ± 0.005 mg GAE g⁻¹ powder), total flavonoids contents (0.341 ± 0.003 mg QE g⁻¹ powder), DPPH free radical scavenging activities (0.488 ± 0.006 mg AAE g⁻¹ powder), total antioxidant capacities (68.073 ± 0.521 mg AAE g⁻¹ powder) and ABTS free radical scavenging activities (1.473 ± 0.066 mg AAE g⁻¹ powder) in the shade dried *Helianthus tuberosus* L. powder were higher than the sun and oven dried powder. Reducing power (OD 0.221 ± 0.003) and ferrous ion chelating effect ($82.852 \pm 0.727\%$) in the oven dried *Helianthus tuberosus* L. powder were higher than sun and shade dried powder.

Keywords: drying method; antioxidant capacity; total phenolics; total flavonoids; *Helianthus tuberosus* L. powder

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1. Introduction

Free radicals had been reported to be responsible for the pathophysiological of several diseases like cancer, diabetes, and cardiovascular diseases [1]. Antioxidant has capacity to dissolve the free radicals cells in human bodies [2]. In recent years, several plants had been studied to contain phytochemicals that can protect a human system against different diseased. However, the structures of these phytochemicals made them scavenge free radical cells [3, 4].

Helianthus tuberosus L. known as “sunchoke”, “jerusalem artichoke” and “earth apple” in English is one of the commonly consumed plants in Thailand as food and medicine. They are biennial plants, food storage root crop, not smoothy skin. They are active ingredients include inulin, vitamin A, vitamin B₁, vitamin B₂, vitamin B₆, vitamin B₉, calcium, magnesium, phosphorus and others. They do make an appetizing, immune in bodies, weight control, protect high blood fat, risk reduction on heart disease and vascular diseases, reduction of blood sugar level, protect the diabetes and others. In the present, sunchoke was processing to many products include sunchoke powder, capsule, herbal tea, coffee mix, herbal drink and others. Especially, sunchoke was often processed to herbal tea. The herbal tea processing base on the drying method to reduce the moisture content (<10% of basis weight) to protect the growth of

mold and pathogenic microorganism [5]. There are many drying method include sun [6, 7], under shade [8, 9], vacuum [8, 10], oven [10, 11], microwave [12], and freeze drying [13, 14]. In this study, they focus on shade, sun and oven at 60 °C drying method due to easy to do, safe, save, reduce production costs and appropriate drying method for community enterprise, small and medium enterprises (SMEs) and startup companies.

In addition, the high quality herbal tea must have a high of the active ingredient compounds [5, 15] and antioxidant activities [15 – 18]. Therefore, the antioxidant property of the *Helianthus tuberosus* L. powder was affected by different drying methods (shade, sun, oven at 60 °C) using DPPH scavenging activity, ABTS radical scavenging activity, total antioxidant capacity, reducing power and ferrous ion chelating effect. The water soluble total phenolics and flavonoids contents of the different drying method were studies.

2. Materials and methods

Reagents and standards

DPPH was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid, ammonium molybdate, sodium phosphate, sulphuric acid, gallic acid, FeCl₃, K₃Fe(CN)₆, Sodium carbonate and Folin-Ciocalteu reagent (FCR) were purchased from Merk (Darmstadt, Germany). All the other chemicals and solvents used were of analytical grade.

Plants materials

Sunchoke (*Helianthus tuberosus* L.) was collected in Sri-Udom Koana community enterprise, Amphoe Thung Si Udom, Ubonratchathani province of Thailand (Fig. 1). Samples were dried with three different drying methods as I) sun-drying (49 h), II) shade drying (49 h) and III) oven-drying (60 °C for 12 h). The moisture content was reduced to less than 10% with the three different drying methods. The dried sunchoke was ground and sieved pass through the sieving to 60 mesh. The powder was divided to two parts, first part was analyzed the moisture content and the second part was stored at 4 °C for total phenolics, total flavonoids, radical scavenging activities determinations.



Fig. 1 The *Helianthus tuberosus* L. fresh (A) and powder (B)

Preparation of extracts

In accordance with the objective to produce sunchoke tea, the water-soluble effective components were extracted with modified by M. Zheng et al., 2015. Two grams of the dried *Helianthus tuberosus* L. powder were extracted for 45 min at 80 °C using 50 mL of water [5]. The *Helianthus tuberosus* L. powder water extracted solution was filtered pass through the filter paper NO.102 and then used to analyze the contents of the water-soluble effective components, namely, total phenolics, total flavonoids, as well as radical scavenging activities.

Total phenolics contents

TPC in the *Helianthus tuberosus* L. powder was determined by the Folin-Ciocalteu colorimetric method [5]. A 1-mL sample extract obtained from the *Helianthus tuberosus* L. powder and 1 mL of 1.0 – 60 mg L⁻¹ gallic acid standard solutions were put into a series of 15-mL vial with 0.50-mL Folin-Ciocalteu reagent, respectively. After sitting at room temperature for 5 min, the mixture was added with 5-mL 5% Na₂CO₃, and place for 60 min at room temperature. The absorbance of the sample and standards were measured spectrophotometrically at 750 nm. TPC was calculated by the absorbance of sample according to standard curve as milligrams of gallic acid equivalents per gram of powder (mg GAE g⁻¹ powder).

Total flavonoids contents

The flavonoids contents of the *Helianthus tuberosus* L. powder water extracts were assessed using the method based on the formation of a complex flavonoid-aluminium [15]. Briefly, 1 mL of water extract was mixed with 1 mL of 2% (w v⁻¹) aluminium chloride methanolic solution, after incubation for 10 min at room temperature. The absorbance was measured at 430 nm and the total flavonoids contents was expressed in milligrams of quercetin equivalents per gram of powder (mg QE g⁻¹ powder).

DPPH scavenging activity

The DPPH radical scavenging activity of the *Helianthus tuberosus* L. powder water extracts was determined following the method reported by N. Tlili et al., 2015. Aliquot (0.50 mL) of the water extract was added to 3.80 mL of a 6 µM methanol solution of DPPH. After being energetically stirred, the mixture was left in the dark at room temperature for 30 min. Then, the absorbance was measured at 517 nm [16]. The percentage of inhibition (I%) of free radical DPPH was calculated using the formula : $I\% = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$. DPPH scavenging capacity was expressed as milligrams of ascorbic acid equivalents per gram of powder (mg AAE g⁻¹ powder).

ABTS radical scavenging activity

ABTS radical cation decolorization activity was assayed by the method of J. Oh et al., 2013. ABTS radical cations were generated by reacting 7.4 mM ABTS with 2.45 mM potassium persulfate (1:1, v v⁻¹). The mixture was left to stand for 12-16 h in the dark at room temperature. The ABTS radical cation solution was then diluted with ethanol to give an absorbance of 1 – 1.20 at 734 nm [18]. The *Helianthus tuberosus* L. powder water extract and ascorbic acid as a standard solution (200 µL) were mixed with diluted ABTS radical cation solution (1 mL). The mixture was vortexed and left to stand at room temperature for 1 h. The absorbance of the resulting solution was measured at 734 nm using a spectrophotometer. Results were shown as milligrams of ascorbic acid equivalents per gram of powder (mg AAE g⁻¹ powder).

Total antioxidant capacity

The assay was based on the reduction of Mo (VI) to Mo (V) and subsequent formation of a green phosphate/Mo(V) complex in acid pH with modified by N. Chahmi et al., 2015. A 0.30 mL of water extract was added to 3 mL of reagent solution (0.6 mol L⁻¹ sulphuric acid, 28 mmol L⁻¹ sodium phosphate and 4 mmol L⁻¹ ammonium molybdate, 1:1:1). The mixtures were incubated at 95 °C for 90 min and then cooled to room temperature. The absorbance was measured at 695 nm [15]. The total antioxidant activity was expressed as milligrams of ascorbic acid equivalents per gram of powder (mg AAE g⁻¹ powder).

Reducing power

The assay was estimated according to the report of S.C. Liu et al., 2017. An aliquot of each *Helianthus tuberosus* L. powder extract (125 μ L) mixed with 125 μ L of sodium phosphate buffer (0.20 M, pH 6.6) and 125 μ L of 1% $K_3Fe(CN)_6$ was incubated at 50 $^{\circ}$ C for 20 min. After adding 125 μ L of 10% TCA, the mixture was centrifuged at 3750xg for 10 min. Briefly, the supernatant solution (100 μ L) was mixed with 100 μ L of distilled deionized water and 20 μ L of 1% $FeCl_3$ and reacted for 10 min. The absorbance was read at 700 nm [17].

Ferrous ion chelating effect

The ferrous ion chelating effect of the *Helianthus tuberosus* L. powder extracts was investigated with a method slightly modified from that of J. Oh et al., 2013. The water extract and control (1 mL of distilled deionized water) were reacted with 2 mM of ferrous chloride (100 μ L) for 10 min, and 5 mM ferrozine (100 μ L) was added. After 10 min, the solvent of the sample (3 mL) was mixed with the mixture and reacted for another 10 min. The absorbance of the mixture was measured at 562 nm using a spectrophotometer [18]. Ferrous ion chelating capacity was calculated by the follow equation.

$$\text{Chelating effect (\%)} = [1 - (A_{\text{sample}}/A_{\text{control}})] \times 100 \quad (1)$$

3. Results and discussion

Moisture contents of powder

Sunchoke (*Helianthus tuberosus* L.) was dried by three different drying methods, including a) shade drying (49 h) b) sun drying (49 h) and c) oven drying (60 $^{\circ}$ C, 12 h) and then ground for analysis of the moisture contents of the *Helianthus tuberosus* L. powder (Table 1).

Table 1 Moisture contents of the *Helianthus tuberosus* L. powder from three different drying methods

Drying method	Moisture content (% ; n = 5)
shade drying	6.08 \pm 0.07
sun drying	6.96 \pm 0.04
oven drying	4.97 \pm 0.06

The moisture contents in the *Helianthus tuberosus* L. powder from three different drying methods were showed in Table 1. The results indicated the temperature and time of drying method had effected on the moisture contents [9]. The oven dried *Helianthus tuberosus* L. powder (4.97%) had the moisture content lower than the shade (6.08%) and the sun dried powder (6.96%), due to the oven drying method used oven which control a stable temperature at 60 $^{\circ}$ C for 12 h, a long time for the *Helianthus tuberosus* L. drying and got the lowest moisture content in the *Helianthus tuberosus* L. powder. The shade and sun drying method used to get the *Helianthus tuberosus* L. in the open of the sunlight which not control a stable temperature. The *Helianthus tuberosus* L. touch the sunlight directly and got the energy from the sunlight uncertainty. In addition, the *Helianthus tuberosus* L. may be absorbed by the moisture content in the air. The drying time for the shade and sun drying method was longer than the oven drying method. The shade dried *Helianthus tuberosus* L. powder had the moisture contents lower than the sun dried *Helianthus tuberosus* L. powder because the *Helianthus tuberosus* L. was covered with clear plastics and open the vents, led to temperature inside the clear plastic be higher than the outer a little. They tough the outer air a little compared with the sun dried *Helianthus tuberosus* L. powder.

Total phenolics (TPC) and flavonoids contents (TFC)

The contents of the water soluble phenolics and flavonoids in the *Helianthus tuberosus* L. powder by three different drying methods were showed in Table 2. The highest contents of the water soluble phenolics and flavonoids existed in the shade dried *Helianthus tuberosus* L. powder (1.463 ± 0.005 mg GAE g^{-1} powder, 0.341 ± 0.003 mg QE g^{-1} powder) among the sun dried (1.326 ± 0.006 mg GAE g^{-1} powder, 0.205 ± 0.001 mg QE g^{-1} powder) and oven dried powder (1.047 ± 0.004 mg GAE g^{-1} powder, 0.200 ± 0.002 mg QE g^{-1} powder) because the *Helianthus tuberosus* L. was covered with clear plastics and open the vents, led to temperature inside the clear plastic be higher than the outer a little and do not touch the sunlight directly while the sun dried *Helianthus tuberosus* L. tough the sunlight directly led to the water soluble phenolics and flavonoids were decomposed. In addition, the oven dried *Helianthus tuberosus* L. powder was the least of the water soluble phenolics and flavonoids. The oven drying method used oven which control a stable temperature at $60\text{ }^{\circ}\text{C}$ for 12 h, a long drying time led to the lowest moisture content in the *Helianthus tuberosus* L. powder may be the water soluble phenolics and flavonoids volatiled together with moisture contents in drying process [6]. The high temperature and longer drying time resulted in the degradation of some the water soluble phenolics and flavonoids, corresponding to M. Zheng et al. (2015) reported that using the high temperature drying method led to reduce total phenolics and flavonoids contents in loquat flower tea [5].

Table 2 Total phenolics and flavonoids contents of the *Helianthus tuberosus* L. powder from three different drying methods

Drying method	Total phenolics contents (TPC) (mg GAE g^{-1} powder; n = 5)	Total flavonoids contents (TFC) (mg QE g^{-1} powder; n = 5)
shade drying	1.463 ± 0.005	0.341 ± 0.003
sun drying	1.326 ± 0.006	0.205 ± 0.001
oven drying	1.047 ± 0.004	0.200 ± 0.002

DPPH scavenging activity

DPPH radical is a stable free radical that can donate hydrogen when reacts with antioxidant compounds and reduce to diphenyl picrylhydrazine. These showed the ability of extracts to neutralize free radicals which possess unpaired electrons [4]. The DPPH free radical scavenging activity in the *Helianthus tuberosus* L. powder by three different drying methods was showed in Table 3. The highest contents of the DPPH free radical scavenging activity existed in the shade dried *Helianthus tuberosus* L. powder (0.488 ± 0.006 mg AAE g^{-1} powder) among the sun dried (0.406 ± 0.006 mg AAE g^{-1} powder) and oven dried *Helianthus tuberosus* L. powder (0.291 ± 0.002 mg AAE g^{-1} powder), corresponding to O.R. Alara *et al.* (2017) reported that the DPPH scavenging activities were in the order: shade drying > sun drying > oven drying, it was depending to phenolic contents of *Vernonia amygdalina* leaf extract [4]. An increase in the DPPH inhibition caused by antioxidant might be due to the scavenging ability of radicals by hydrogen donation. It can also be seen that the water extract was active with relation to the water soluble phenolics and flavonoids in the *Helianthus tuberosus* L. powder. Therefore, it can be deduced that drying methods have effected on the antioxidant activity of the *Helianthus tuberosus* L. powder resulting in different values of antioxidant inhibitions [4].

Table 3 DPPH free radical scavenging activity, total antioxidant capacity and ABTS free radical scavenging activity of the *Helianthus tuberosus* L. powder from three different drying methods

Drying method	DPPH free radical scavenging activity (mg AAE g ⁻¹ powder; n = 5)	Total antioxidant capacity (mg AAE g ⁻¹ powder; n = 5)	ABTS free radical scavenging activity (mg AAE g ⁻¹ powder; n = 5)
shade drying	0.488 ± 0.006	68.073 ± 0.521	1.478 ± 0.066
sun drying	0.406 ± 0.006	60.781 ± 1.302	0.866 ± 0.057
oven drying	0.291 ± 0.002	41.771 ± 0.939	0.582 ± 0.057

Determination of total antioxidant capacity

The phosphomolybdenum was a quantitative assay. Since the antioxidant activity was expressed as number of ascorbic acid, the method was based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate Mo (V) complex at acid pH [15]. The total antioxidant capacity in the *Helianthus tuberosus* L. powder by three different drying methods was showed in Table 3. The highest contents of total antioxidant capacity existed in the shade dried *Helianthus tuberosus* L. powder (68.073 ± 0.521 mg AAE g⁻¹ powder) among the sun (60.781 ± 1.302 mg AAE g⁻¹ powder) and oven dried powder (41.771 ± 0.939 mg AAE g⁻¹ powder), corresponding to L.F. Ballesteros et al. (2017) reported that an increase in the total antioxidant capacity caused by antioxidant might be due to the phenolics and flavonoids contents [10]. Therefore, total antioxidant capacities in the *Helianthus tuberosus* L. powder from three different drying methods were depend on the water soluble phenolics and flavonoids contents.

ABTS⁺ radical scavenging activity

The antioxidant activity of the *Helianthus tuberosus* L. powder from three different drying methods using ABTS radical assay was generated by potassium persulfate in order to determine its hydrogen donation ability. Likewise, hydrogen peroxide is a weak oxidizing agent that can inactivate a few enzymes directly usually by oxidation of essential thiol (-SH) groups. In the ABTS⁺ radical and H₂O₂ assays, similar trend of DPPH assay were obtained. ABTS⁺ radical inhibition might be due to its ability in donating hydrogen and terminating the oxidation process by converting free radicals into their stable form [4]. The ABTS⁺ radical scavenging activity in the *Helianthus tuberosus* L. powder by three different drying methods was showed in Table 3. The highest contents of ABTS⁺ radical scavenging activity existed in the shade dried *Helianthus tuberosus* L. powder (1.478 ± 0.066 mg AAE g⁻¹ powder) among the sun (0.866 ± 0.057 mg AAE g⁻¹ powder) and the oven dried powder (0.582 ± 0.057 mg AAE g⁻¹ powder). Because, the shade dried *Helianthus tuberosus* L. powder had the highest the water soluble phenolics and flavonoids contents, compared with the sun and the oven dried powder, corresponding to O.R. Alara et al. (2017) reported that ABTS⁺ radical scavenging activity related to the phenolics and flavonoids contents [4]. Therefore, ABTS⁺ radical scavenging activity related to the water soluble phenolics and flavonoids contents in the *Helianthus tuberosus* L. powder from three different drying methods.

Reducing power

Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain via donating a hydrogen atom. In the ferric to ferrous ion reduction assay [15]. The electron donation capacity of the extracts was assessed. The reducing power in the *Helianthus tuberosus* L. powder by three different drying methods was showed in Table 4. The highest of reducing power existed in the oven dried *Helianthus tuberosus* L. powder (OD 0.221 ± 0.003)

among the sun ($OD\ 0.201 \pm 0.002$) and shade dried powder ($OD\ 0.180 \pm 0.002$) in the same concentration. The reductive agents, sensitive by the sunlight in the *Helianthus tuberosus* L. powder including vitamin C, vitamin B and others which may be decomposed by photochemical reaction from tough the sunlight directly led to the sun and shade dried *Helianthus tuberosus* L. powder had reducing power lower than the oven dried powder.

Chelating effect on ferrous ions

Iron is essential for oxygen transport, respiration, and enzyme activity and is a reactive metal that catalyzes oxidative damage in cell. Furthermore, among the transition metals, iron is regarded as the most important pro-oxidant because of its high reactivity [18]. Therefore, ferrous ion chelating ability can be an indicator of antioxidant activity of the *Helianthus tuberosus* L. powder and was monitored by measuring the formation of the ferrous ion-ferrozine complex. Table 4 showed the chelating effect (%) on ferrous ions of the *Helianthus tuberosus* L. powder from three different drying methods. The highest of the chelating effect existed in the oven dried *Helianthus tuberosus* L. powder ($82.852 \pm 0.727\%$) among the sun ($81.599 \pm 1.335\%$) and shade dried powder ($67.823 \pm 0.167\%$) in the same concentration. The reductive agents, sensitive by the sunlight in the *Helianthus tuberosus* L. powder may be decomposed by photochemical reaction led to the sun and shade dried powder, had chelating effect lower than the oven dried *Helianthus tuberosus* L. powder. Chelating effect on ferrous ions had trend to reducing power of the dried *Helianthus tuberosus* L. powder from three different drying methods.

Table 4 Reducing power and chelating effect of the *Helianthus tuberosus* L. powder from three different drying methods

Drying method	Reducing power (40 mg mL^{-1}) (OD_{700})	Chelating effect (40 mg mL^{-1}) (%)
shade drying	0.180 ± 0.002	67.823 ± 0.167
sun drying	0.201 ± 0.002	81.599 ± 1.335
oven drying	0.221 ± 0.003	82.852 ± 0.727

4. Conclusion

The three different drying methods include shade drying (49 h), sun drying (49 h) and oven drying ($60\text{ }^{\circ}\text{C}$, 12 h) method were effected on the water soluble total phenolics and flavonoids contents, and antioxidant activities of the *Helianthus tuberosus* L. powder. The shade dried *Helianthus tuberosus* L. powder had total phenolics contents, total flavonoids contents, DPPH free radical scavenging activity, total antioxidant capacity and ABTS free radical scavenging activity higher than the sun and oven dried powder. The oven dried *Helianthus tuberosus* L. powder had reducing power and chelating effect higher than the sun and shade dried powder. The shade drying was an appropriate preservative method for processing of *Helianthus tuberosus* L. product due to it is easy to do, very safe, save, high quality and get possibility of commercialization as infusion tea and other products for community enterprise, small and medium enterprises (SMEs) and startup companies.

5. Acknowledgement

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6. References

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