Effects of drying process on total phenolics, antioxidant activity and flavonoid contents of common Mediterranean herbs

Taha M. Rababah[1*], Muhammad Al-u'datt[1], Mohammad Alhamad[1], Majdi Al-Mahasneh[2], Khalil Ereifej[1], Juan Andrade[3], Bayan Altarifi[1], Ali Almajwal[4], Wade Yang[5]

(1. *Faculty of Agriculture, Jordan University of Science and Technology, Irbid 22110, Jordan*;
2. *Faculty of Engineering, Jordan University of Science and Technology, Irbid 22110, Jordan*;
3. *Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana Illinois 61801, USA*;
4. *Department of Community Health Sciences, College of Applied Medical Sciences, King Saud University, Riyadh 11451*, *Saudi Arabia*;
5. *Department of Food Science and Human Nutrition, University of Florida, Gainesville Florida 32611, USA*)

Abstract: Four different fresh and dried herb species (sage, thyme, mint and lemonbalm) were evaluated to study the effect of drying process on the total phenolics, antioxidant activity, flavonoid contents and color properties. Fresh mint had the highest contents of total phenolics (335.4 mg GAE/100 g), whereas lower levels were respectively found in sage (316.4 mg GAE/100 g) followed by lemonbalm (303.2 mg GAE/100 g) and thyme (299.2 mg GAE/100 g). Dry processing significantly decreased the phytochemical contents of all investigated herbs. Compare to air drying, total phenolics, antioxidant activity and flavonoids content in herbs decreased apparently by oven dried. Fresh mint had the highest antioxidant activity (87.46%) and flavonoids content (298.51 mg CE/100 g), while the fresh sage, thyme and lemonbalm were found to have antioxidant activity values of 86.81%, 86.56% and 85.26%, respectively. Results showed that air drying herbs contained more total phenolics, antioxidant activity, and flavonoids than oven drying herbs.

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1 Introduction

Large numbers of medicinal herbs were identified in Jordan. These herbs, leaves or seed, are used as a source of medicinal products either by native people or at industrial scale[1]. The benefit of these medicinal herbs are related to the secondary metabolites produced by these plants as a defense mechanism against injury and infection, but also found to be valuable for human health including curing human diseases such as malaria, eczema, cancer and cardiovascular disease[2]. These substances and secondary metabolites include among others: phenolic acids, flavonoids and their derivatives (essential oils, tannins, minerals and vitamins)[3]. The content of active substances in herbs can vary among others due to plant species, the country of origin, its growth phase and seasonal changes (biotic factors – alleopathy, vermins, diseases; and abiotic factor, soil, climate, fertilization)[4].

In general, plants produce phenolic compounds in

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Biographies: Muhammad Al-u'datt, PhD. Research interest: food chemistry, Email: malodat@just.edu.jo. **Mohammad Alhamad**, PhD. Research interest: natural resources, Email: malhamad@just.edu.jo. **Majdi Al-Mahasneh**, PhD. Research interest: food engineering, Email: mmajdi@just.edu.jo. **Khalil Ereifej**, PhD. Research interest: food chemistry, Email: ereifej@just.edu.jo. **Juan Andrade**, PhD. Research interest: nutritional sciences, Email: jandrade@illinois.edu. **Bayan Altarifi**, Master. Research interest: food processing, Email: bayanal_86@yahoo.com. **Ali Almajwal**, PhD. Research interest: clinical nutrition, Email: aalmajwal@ksu.edu.sa. **Wade Yang,** PhD. Research interest: food processing engineering, Email: wade.yang@ufl.edu.
***Corresponding author: Taha M. Rababah**, PhD. Research interest: food processing engineering. Tel.: +962-2-7201000; Fax: +962-2-7201087. Email: trababah@just.edu.jo, ebabah@gmail.com.

response to stress as a defense mechanism[5]. Phenolics compound belong to the very important group of plant antioxidants. They are always substituted phenolics compound with one to three hydroxyl groups on the aromatic ring in different positions. Carboxylic acid group may also be present as the main substituent or another ring may be linked to the aromatic ring. The antioxidant effect depends mainly on the number and position of hydroxyl groups and the identity of the main substituents. Fresh herbs (sage, thyme, mint and lemonbalm) are an excellent source of phenolic compounds (flavonoids, phenolic acid and alcohols, tocopherols, tocotrienols, stilbenes), ascorbic acid and carotenoids which have been reported to show good antioxidant activity[6]. Drying is basically defined as a process of water removed and decreasing of herbs moisture content, aimed at preventing microbial and enzymatic activity, consequently preserving the product for extend shelf life. Also, it results in reduction of the weight and volume of the plant with positive consequences for transport and storage[7]. Consumers are now more concerned about healthy lifestyle, the demands for natural and safe herbs are increasing. Thus, the search for potential keeping the phytochemical in these herbs has gained increasing interest among researchers. Thus far, very little is known about the phytochemical contents of the most common herbs using different drying methods. Therefore, the basic aim of this research was to determine the effect of drying process on total phenolics, antioxidant activity and flavonoids content of different common herbs in Mediterranean area.

2 Materials and methods

2.1 Plant species

Four fresh herbs species, Mint (*Mentha Cpipata*), Sage (*Salvia officinalis*), Lemonbalm (*Melissa Officinalis*) and Thyme (*Thymus vulgaris*), were collected from north of Jordan.

2.2 Determination of moisture content

Moisture content was evaluated according to the methods described by AOAC[8] and McClements[9].

2.3 Drying the herbs

The obtained herbs materials were separated into two divisions; one kept as fresh and the other subjected for two drying methods: air drying at room temperature (24°C) and oven drying at 40°C. Air drying method was conducted as reported by Hossain et al.[10] with some modification (drying time and temperature). One kilogram of each investigated herb at initial moisture content ranged from 70%-83.6% (w.b.). Drying air temperature was determined with a T-type thermocouple fixed at the exhaust duct. Herbs were carried out using tray dryer made of wood strip (60 cm×40 cm×20 cm), with small pores to prevent leaves loss, tray was placed in a dry place. Herbs were removed every 30 min and weighed. The test was completed in triplicate and the average of drying time was around 48 h. The oven drying method was conducted as reported by Anwar et al.[11] One kilogram of fresh harvested herbs was spread on punched foil to allow for moisture filtration, and placed in an oven set at 40°C with the relative humidity of 60%, then dry to a constant weight. Dried herbs were packaged and stored in a dark place further to use.

2.4 Preparation of plant extract

Both fresh and dry herbs from the same sampling time were subjected to the same analysis. Five grams from fresh plant sample were ground and two grams of dried plant sample were crushed to powder, mixed with 50 mL methanol at room temperature and heated in water shaker at 60°C for 1 h. The extract was filtered by qualitative filter paper (type Whatman No. 3), and stored in refrigerator until use.

2.5 Determination of the total phenolics assay

The total phenolic content of the dry herbs was determined with the Folin-Ciocalteau assay[12].

2.6 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH with 2,2-Azinobis (3-ethyl-benzothiazoline-6-sulfonic acid), commonly used as antioxidant assessment methods, was used to determine the antioxidant activity[13]. The radical scavenging activity was expressed as percentage of inhibition[14] and was calculated as following equation:

$$\text{Inhibition (\%)} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100 \quad (1)$$

where, A_{blank} is absorbance of the blank; A_{sample} is

absorbance of the sample.

2.7 Determination of total flavonoid assay

The total flavonoid content was determined with aluminum chloride colormetric assay[12]. The data of flavonoid content expressed of milligram of catechin equivalent (mg CE/100 g FW), all samples were analyzed triplicated.

2.8 Color assessment

Fresh and dry sample color was measured by hand held tristimulus reflectance colorimeter (Model CR-200b, Minolta Corp., Ramsey, NJ, USA). Color was recorded by using the *L*a*b** uniform color space. In addition, total color difference (ΔE) and chroma were calculated using following equations:

$$\Delta E=(\Delta a^2+\Delta b^2+\Delta L^2)^{1/2} \quad (2)$$

$$\text{Chroma}=(\Delta a^2+\Delta b^2)^{1/2} \quad (3)$$

2.9 Statistical analysis

Data on total phenolics, antioxidant activity, flavonoids and colors of fresh and dried sage, thyme, mint and lemonbalm were analyzed using the general linear model (GLM) procedure with JMP statistical package (JMP Institute Inc., Cary, NC). Means were separated by LSD analysis at a least significant difference of 0.05 *p*-value.

3 Results and Discussion

3.1 Determination of total phenolics, antioxidant activity, flavonoids and colors of fresh sage, thyme, mint and lemonbalm

Total phenolics, antioxidant activity, flavonoids and colors of fresh sage (*Salvia officinalis*), thyme (*Thymus vulgaris*), mint (*Mentha scapita*), and lemonbalm (*Melissa officinalis*) were investigated. The phenolics content were significantly different and ranged from 299.2 mg GAE/100 g FW (thyme) to 335.4 mg GAE/100 g FW (mint) (Table 1). Similar results were reported in previous researches[15-17] which found total phenolics were 399.8-515 mg/100 g in mint, 532 mg/100 g in sage, 452-530 mg/100 g in thyme and 820.3 mg/100 g in lemonbalm, respectively.

Antioxidant activities and flavonoids contents in plant are shown in Table 1. The inhibition values varied significantly and ranged from 85.3% (Lemonbalm) to 87.5% (mint). The similar results found by Grzeszczuk and Zheng et al.[18-19] who reported an inhibition value up to 90.60% in mint (*Mentha x piperita L. var. officinalis* Sole f.) followed by thyme, peppermint, lemonbalm and sage respectively. High content of phenolic compounds may responsible for their strong antioxidant activity. Our results consistent with other researcher's results[20]. Flavonoids content are varied significantly and ranged from 298.5 mg CE/100 g FW in mint to 252.9 mg CE/100 g FW in lemonbalm.

Table 1 Total phenolics, antioxidant activity, and flavonoids content of fresh sage, thyme, mint, and lemonbalm

Phytochemicals	Sage	Thyme	Mint	Lemonbalm
Total phenolics/ mg GAE per 100 g FW	316.4 ± 0.7^{b}	299.2 ± 1.2^{d}	335.4 ± 1.7^{a}	303.2 ± 1.2^{c}
Antioxidant activity	86.8 ± 0.2^{b}	86.6 ± 0.1^{b}	87.5 ± 0.2^{a}	85.3 ± 0.4^{d}
Flavonoids content/ mg CE per 100 g FW	273.5 ± 0.3^{b}	260.3 ± 0.6^{c}	298.5 ± 1.2^{a}	252.9 ± 1.2^{d}

Note: All values are calculated as fresh weight and means of three replicates. Means ±SD in the same row with the same letters are not significantly different ($p\leq0.05$). The same below.

3.2 Effect of drying on total phenolics content (TPC)

Compared with oven dried, the level of TPC decreased and significantly ($p\leq0.05$) in herbs with air dried (Tables 2-5). The minimum reduction of TPC after air drying treatment was 31.7% in sage (Table 2), whereas, lemobalm showed the maximum phenolic content reduction of 49.1% by oven drying (Table 5). The TPC of fresh samples were corrected for moisture content before experiment, the moisture content of samples were 70.00%-83.60% while lemonbalm having the highest and sage having the lowest moisture content. During drying period, metabolically active plants lose moisture slowly and might have sensed the moisture loss as drought-stress. TPC loss after air drying may be caused by enzymatic processes. Air drying did not immediately deactivate degraded enzymes such as polyphenol oxidases; therefore, they are able to degrade phenolic compounds before the plant materials are completely dry. Oven drying at 40°C was shown to rapidly inactivate polyphenol oxidases present in herb materials; however, some of their initial activities may have occurred earlier and degraded some polyphenols[21]. Felipe et al. reported that drying process led to loss of 30% of polyphenols in total phenol content[22]. Various

researches found that degradation of phytochemicals related to thermal process, the reduction of total phenols were between 72%, 66% and 65%, respectively[23].

Table 2 Total phenolics, antioxidant activity, flavonoids content, Δ*E* and chroma of sage after drying process

Phytochemicals	Treatment		
	Fresh	Air-sun dried	Oven dried
Total phenolics/ mg GAE per 100 g FW	316.4±0.7^a	216.0±0.6^b	192.1±0.3^c
Antioxidant activity	86.8±0.2^a	85.2±0.2^b	83.8±0.1^c
Flavonoids content/ mg CE per 100 g FW[1]	273.5±0.3^a	103.0±0.5^b	101.3±0.2^c
L	50.8±0.7^a	49.6±0.7^b	45.6±0.5^c
a	-6.9±0.1^b	-1.6±0.1^a	-1.6±0.2^a
b	27.1±0.5^a	19.2±0.5^b	15.5±0.0^c
Δ*E*	57.4±0.3^a	53.1±0.3^b	48.1±0.5^c
chroma	27.9±0.6^a	19.2±0.6^b	15.6±0.0^c
Moisture content/%	70.00	7.50	6.80

Table 3 Total phenolics, antioxidant activity, flavonoids content, Δ*E* and chroma of thyme after drying process

Phytochemicals	Treatment		
	Fresh	Air-sun dried	Oven dried
Total phenolics/ mg GAE per 100 g FW	299.2±1.2^a	195.2±0.3^b	168.6±0.1^c
Antioxidant activity	86.6±0.1^a	84.5±0.2^b	83.9±0.2^c
Flavonoids content/ mg CE per 100 g FW	260.3±0.6^a	109.7±2.0^b	104.7±0.2^c
L	49.2±0.5^a	49.9±0.0^a	48.5±0.8^a
a	-7.7±0.8^b	-1.3±0.2^a	-1.8±0.4^a
b	24.4±0.2^a	19.5±0.7^b	15.5±0.4^c
Δ*E*	55.5±0.4^a	53.6±0.3^b	51.2±0.9^c
chroma	25.6±0.1^a	19.8±0.7^b	15.6±0.5^c
Moisture content /%	80.00	7.20	7.10

Table 4 Total phenolics, antioxidant activity, flavonoids content, Δ*E* and chroma of mint after drying process.

Phytochemicals	Treatment		
	Fresh	Air-sun dried	Oven dried
Total phenolics/ mg GAE per 100 g FW	335.4±1.7^a	181.0±0.5^b	137.9±0.2^c
Antioxidant activity	87.5±0.2^a	85.8±0.2^b	84.0±0.1^c
Flavonoids content/ mg CE per 100 g FW	298.5±1.2^a	113.8±0.9^b	90.6±0.6^c
L	42.0±0.1^a	42.0±0.4^a	33.2±0.2^b
a	-5.3±0.1^c	4.4±0.1^b	-1.1±0.0^a
b	23.5±0.6^a	23.5±0.1^a	16.2±1.1^b
Δ*E*	48.4±0.4^a	48.4±0.3^a	36.9±0.3^b
chroma	25.6±0.1^a	19.8±0.7^a	15.6±0.5^b
Moisture content/%	82.00	7.60	6.80

Table 5 Total phenolics, antioxidant activity, flavonoids content,*ΔE* and chroma of lemonbalm after drying process

Phytochemicals	Treatment		
	Fresh	Air-sun dried	Oven dried
Total phenolics/ mg GAE per 100 g FW	303.2±1.2^a	175.2±0.0^b	154.4±1.0^c
Antioxidant activity	85.3±0.4^a	84.2±0.2^b	82.9±0.2^c
Flavonoids content/ mg CE per 100 g FW	252.9±1.3^a	103.6±0.5^b	97.8±1.2^c
L	44.4±0.0^a	43.7±1.0^a	37.6±0.0^b
a	-4.0±0.2^b	-2.2±0.4^a	-2.2±0.1^a
b	27.3±1.0^a	22.1±0.4^b	17.4±1.4^c
Δ*E*	52.3±0.5^a	49.0±0.6^b	41.5±0.6^c
chroma	27.6±0.9^a	22.2±0.5^b	17.5±1.4^c
Moisture content/%	83.60	6.50	6.80

3.3 Effect of drying on antioxidant activity

Tables 2-5 indicated that the increase of antioxidant activity by air dried treatments was obviously faster than that by oven dried treatments using all samples. Fresh herbs showed higher antioxidant activity than air-sun dried treatment because fresh herbs exhibited much stronger activity in the DPPH. The antioxidant activity values of sage were 85.2 and 83.8 in air and oven dried samples respectively (Table 2). Similar results were found in other herbs (Tables 3-5). In general drying process resulted in a depletion of naturally occurring antioxidants in raw plant materials[24]. Intense and/or prolonged thermal treatment may be significant effect on most loss of natural antioxidants, but the effect of carotenoids (e.g. lycopene) was not obvious[25]. Furthermore, depletion of antioxidant may a result of peeling, cutting and slicing[21].

According to Lim and Murtijaya's research, highly loss of antioxidant activity was found in oven drying[21], greatly depletion of TPC and antioxidant activity in *Phyllanthus amarus* was discovered after oven drying at 50°C, these depletion induce rapid enzymatic oxidation of natural antioxidant. High temperature/intense thermal process might cause significant loss in antioxidant that found naturally in plants as well as deactivate enzymes and degrade phytochemicals. The effect of drying process could help explain the changing of heat-stable natural antioxidant content such as carotenoid[25]. Furthermore, antioxidant depletion may be due to cutting and peeling process which could induce

rapid oxidation of enzyme. Other researchers reported that air-sun drying caused significant increase of free radical scavenging activity and significant decline of vitamin C[26].

3.4 Effect of drying on flavonoids content

The results showed that the destruction of flavonoids had varied significant differences between fresh samples and dried materials (Tables 2-5). The highest amount of flavonoids content of thyme was found in air dried sample (109.7 mg CE per 100 g FW) (Table 3), the reduction of flavonoids content were 59.8% after oven drying. The reduction amount of flavonoids content was 61.9% after air drying, while 69.6% after oven drying (Table 4). Similar results were found for lemobalm (Table 5).

The loss of flavonoids was found to be less in air drying than oven drying (Tables 2-5). These losses may due to drying time and temperature[27]. Heating may breakdown some phytochemicals which affect cell wall integrity and cause a migration of some flavonoids component. In addition, the loss in flavonoids may due to breakdown or leakage by chemical reactions includes oxygen, enzymes and light[28]. Increasing pre-heating temperature decreased the enzyme activity of flavonoids to degrading enzyme such as polyphenoloxidase[29], which resulted in an increase in flavonoid content[30].

3.5 Effect of drying process on herbs color

The attributes of three color parameters (L^*, a^*, b^*) of the dried herbs were expressed as ΔE, the results of ΔE is shown in Tables 2-5, showed that ΔE values decreased when herbs dried by sun-air treatment. Also, the values of ΔE for herbs after oven dried was small compare to the air-sun dried treatment with all samples. Similar results were found by Sudathip et al.[31], who reported the values of (L^*, a^*, b^*) of Thai red curry powder when using hot-air drying were higher than microwave drying. Compared to air dried, the oven drying was more affected by browning reaction because of more pigment destruction. The results of chroma values (a^* and b^*) showed significant differences between fresh and dried samples, air dried samples also showed higher chroma values than oven dried samples (Tables 2-5). Similar results reported by Sharma and Prasad[32], the pigment decreased and browning color increased with the increment of drying time/temperature. Color changes could be because of chlorophyll pigments were reduced as a result of photooxidation reaction in the cells. In addition, there is competition between peroxidase enzyme and chlorophylase[33-35].

4 Conclusions

Total phenolics of the investigated fresh herbs were varied significantly. Fresh herbs had higher amounts of total phenolics, antioxidant activity and flavonoids contents, also had better color than dried herbs. Air drying was a better drying method for keeping photochemical contents compared to oven dried method. Furthermore, air drying treatment kept higher amounts of total phenolics, antioxidant activity and flavonoids than oven drying. Although the valuable compounds in dried herbs were reduce, drying processing still can retain good amounts of them.

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