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Effects of drying process on total phenolics, and flavonoids content of thyme vulgaris extract

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Abstract : The present study investigated the changes in total phenols (TPC), flavonoids (FC) content of thyme vulgaris (in Continuous infusion, Infusion (without boiling)) after three drying (air-shade, air sun and oven-drying) and compared to fresh. The Folin-Ciocalteu method was used to determine TPC, while FC was determined by the aluminum chloride method. . The results showed that total phenolics and flavonoids in continuous infusion are higher than infusion without boiling. Fresh thyme have the highest contents of total phenolics (291.23, 322.12 mg GAE/100 g) and flavonoids (210.11, 296.22 mg QE/ 100 g), whereas the lowest levels were found in oven dried (200.10, 231.30 mg GAE/100 g ,100.11, 111.42 mg QE/100g). Dry processing significantly decreased the phytochemical contents of thyme . The air shade drying contained more total phenolics, and flavonoids than air –sun drying of thyme.
Keywords: total phenols , drying process, flavonoids , Continuous infusion, Infusion (without boiling), Thyme vulgaris.

Introduction

Herbs are a valuable source of active substances beneficially influencing human body. These substances include among others: flavonoids, phenolic acids and their derivates, tannins, essential oils, vitamins or minerals [34, 14]. The content of active substances in herbs can vary among others due to plant species variability, its growth phase, the country of origin, and seasonal environmental variability (biotic factors, vermins, alleopathy, diseases; and abiotic factors , climate, soil, fertilization) Crude extracts of herbs , spices, and other plant materials rich in phenolics are of increasing interest in the food industry ,because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [38].

Studies show that herbs generally contain higher levels of antioxidant content than fruits, vegetables, and nuts [15, 39]. More and more people have realized the health benefits of herbs.and the use of herbs is increasing [13].

The drying of spices has been used for disinfestations, microbial decontamination, and long-term preservation [32]. Although reports are limited, studies indicate that drying conditions can impact the chemical and biological activities of herbs [6,16]. The Mint family, Lamiaceae, is a group of 210 genera and some 3500 species. Many members of the family have high phenolic and antioxidant content such as basil (Ocimum basilicum), lemon balm (Melissa officinalis), sweet marjoram (Origanum majorana), oregano (Origanum vulgare), peppermint, rosemary, sage (Salvia officinalis), and thyme (Thymus vulgaris) [9,37].

Thymus vulgaris is a flowering plant in the mint family Lamiaceae. It is growing upto 15-30 cm tall by 40 cm wide. Thyme is cultivated in most of the European countries, together with France, Svizzera, Spain, Italy, Bulgaria, Portuguese Republic and Ellas [7]. Thyme has been thought of to be antiseptic, antimicrobial,

medication, astringent, anthelmintic, medicinal drug, carminative, disinfectant, medicinal drug and tonic. Thyme is incredibly useful in cases of assorted intestinal infections and infestations, like hookworms, ascarids, gram-positive and gram-negative bacterium, fungi and yeasts as well as Candida albicans. Its active constituent, thymol, is active against enterobacteria and cocci bacteria. Thyme may also improve liver functioning, and act as an appetite stimulant. It will be used in treatment of cartilaginous tube, bronchial and urinary infections. Used as a gargle, Thyme is helpful in treatment of laryngitis and inflammation[10].

The present study showed that the extract of *Thymus vulgaris* plant contain high amount of flavonoids, and exhibited antioxidant and antibacterial activity. So, thyme can be used as an easily available source of natural antioxidants and antibiotics in food products and drugs. The dried product should be processed to get rid of the leaves from the stems, and so sieved to get rid of dirt and to provide a consistent product. Many strategies exist from sun to classy driers. The employment of sun-drying strategies leads to poor quality of the volatile oil. Artificial drying strategies permit higher management of product quality. A forced air-flow drier could be a appropriate system to dry better-quality leaves. Thyme should to be dried at temperatures not up to 40°C to cut back loss of flavor through volatilisation of volatile oil, and to keep up a decent inexperienced color. once drying, the leaves should be separated clear of the stems, sieved and hierarchic. Fresh turn out has got to be clean of foreign material and looking out recent and tender with a decent color and flavor [27]. The objectives of this study were to evaluate the Effects of drying process on total phenolics, and flavonoid contents of Continuous infusion and Infusion thyme vulgaris extract.

2. Materials and methods

2.1. Plant Sample

Fresh leaves of Thymus vulgaris were collected on October 2015 in the afternoon [2].

2.2. Chemical materials

Folin–Ciocalteu, gallic acid (97.5-102.5 per cent) and quercetin (98 per cent) were purchased from Sigma; anhydrous sodium carbonate (99 per cent) was purchased from Tekkim; anhydrous aluminum chloride (98 per cent) was obtained from Merck; anhydrous sodium acetate (99-101 per cent), glacial acetic acid (99.5-100.5 per cent) and methanol (HPLC grade, 99.99 per cent) were obtained from Panreac; and de-distillated water was used to prepare all extracts.

2.3 Drying methods

To study the effect of drying method, three methods of drying, (sun-drying, shade-drying and oven drying at 40°C: for 15 days) were investigated. The shade-drying occurred at room temperature $(23 \pm 2)c^0$ [19] .

2.4. Determination of moisture content

Fresh and dried thyme (0.5 g) were ground and spread on aluminium foil. drying was applied: at temperature 105±2 °C, constant value if the weight difference was less than 2 mg for 30 s. Samples were measured in triplicate and the average was calculated. Moisture content was evaluated according to the methods described by [1,5,20].

2.5. Preparation of plant extract

Both fresh and dry thyme from the same sampling time were subjected to the same analysis. **Infusion types preparation:** For continuous infusion, boil 100 ml of de-distillated water (100°C), then add 5 g of the leaves of the plant and continue boiling for 10 min. For infusion, add 100 ml of boiled de-distillated water to 5 g of the leaves and leave them for 10 min without boiling, each sample was replicated three times. All extracts are filtered to a measuring flask the calibration of size up to 100 ml with de-distillated water is completed and then stored at -20°C until the time of analysis [22, 23].

2.6. Determination of total phenol content

Procedures have been applied according to Maksimovic *et al.[17]* , with some modifications; 0.05 ml aliquots of aqueous infusions were transferred into the test tubes and their volumes made up to 0.5 ml with distilled water. After addition of 0.25 ml of Folin-Ciocalteu reagent, wait three minutes and then add 1.25 ml of aqueous sodium carbonate solution (7 per cent). After 40 min at room temperature (23 ± 2°C) in dark, absorbance of blue-colored mixtures was recorded at 764 nm against a blank by using a T80 _ UV/VIS spectrometer from PG Instruments Ltd. Using a temperature stabilizer (PTC-2 Peltier temperature controller), all measurements have been done at 24°C.Concentration values were extrapolated by a calibration curve prepared from different concentrations (from 20 to 300 mg/L) of gallic acid solutions in de-distillated water (y=0.0033x + 0.0044, *R*2 _ 0.9995). Total phenol content (TP) was expressed as mg gallic acid equivalent (GaE)/100g dried leaves.

2.7. Determination of total flavonoid content

Procedures have been applied according to Portmann *et al.* [25]. Aliquots (0.1 ml) of each extract were added to 1.4 ml of de-distillated water and 0.50 ml of reactive flavonoid: 133 mg crystalline aluminum chloride and 400 mg of crystalline sodium acetate dissolved in 100 ml of extracting solvent (140 ml methanol, 50 ml water and 10 ml acetic acid). After 30 minutes at room temperature (23 ± 2°C) in the dark, the absorbance was recorded at 415 nm against a blank, as indicated in the previous paragraph. Concentration values were extrapolated by a calibration curve constructed from different concentrations (from 5 to 300 mg/L) of quercetin solutions in 70 per cent methanol (y = 0.0031x + 0.0016, *R*2 _ 0.9992). Total flavonoid content (TF) was expressed as mg quercetin equivalent (QE)/100g dry leaves.

3. Result and discussion

3.1. The moisture content

The results(Table.1) showed that all drying methods tested reduce the moisture content .The moisture content was 80.51% for fresh leaves of thyme and 12.84% by oven drying methods, while sun and shade air drying lowered the water content of thyme to 13.15% and 20.61% respectively.

Table 1. Moisture content of Thymus vulgaris L after drying process

Drying process	*Moisture content %
Fresh	80.51±2.21
Air- sun dried	13.15±1.02
Air – shade dried	20.61±1.11
Oven dried	12.84±1.01

Notes:Results were expressed as mean ±SD, (n = 5)

3.2. Comparison of Temperature of soaking

Temperature of soaking play an important role in the proportion of liberated phenols and flavonoids. As shown in Table 2, TP and F in continuous infusion are higher than infusion without boiling, and this corresponds with the study [3,23] for (white, green and black) tea, and lemonverbena, according to method of infusion, because the high temperature during the preparation of continuous infusion (100°C) increases swelling cells in plant tissue and thus frees the largest amount.

Table 2. Total phenolics (TPC), and flavonoids(FC) content of Thymus vulgaris L after drying process

Extract	drying process	Total phenolics content / mg GaE/100g dried leafs	Flavonoids content/ mg QE/100g dried leafs
infusion	Fresh	291.23±3.23	210.11±2.09
	Air- sun dried	231.21±3.22	114.31±1.01
	Air – shade dried	240.11±2.30	184.12±1.12
	Oven dried	200.10±2.42	100.11±2.12
Continuous infusion	Fresh	322.12±4.33	296.22±3.03
	Air- sun dried	243.20±3.21	176.32±1.98
	Air – shade dried	259.34±3.28	195.21±1.99
	Oven dried	231.30±2.61	111.42 ±1.00

Notes:Results were expressed as mean ±SD, (n = 5)

3.3.Comparison of fresh and dried herbs

The results confirmed higher TPC and FC in fresh thyme . There are many different enzymatic and non-enzymatic reactions taking place during the drying process as well as tissue decomposition. As a result of these processes, different substances (including phenolics) are created. A statistically significant difference between dried and fresh herbs was also confirmed by [12,24,28,29,33,36] investigated the influence of drying on the antioxidant activity .

3.4. Effect of drying on total phenolics content (TPC)

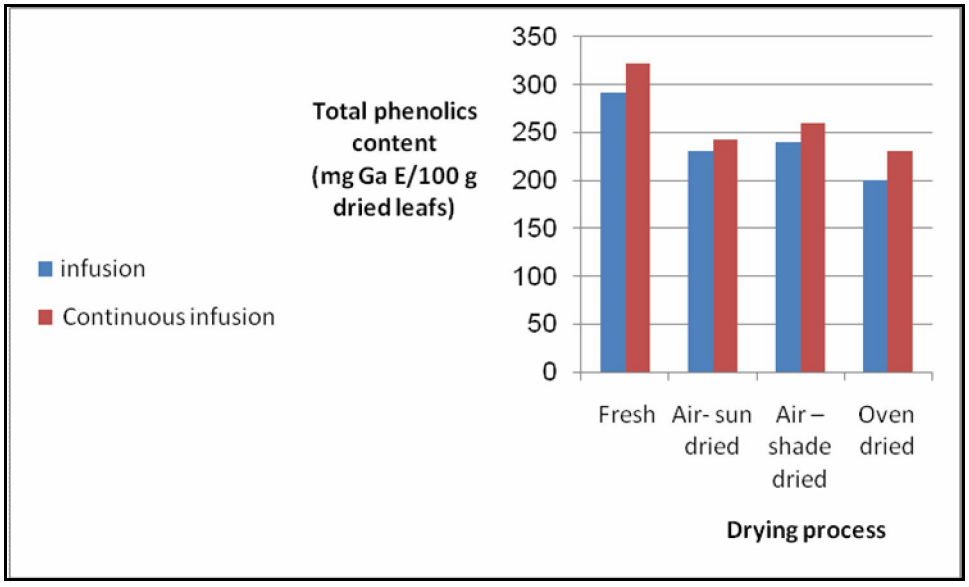

Figure 1. Total phenolics content of Thymus vulgaris L after drying process

Compared with fresh sample, the level of TPC decreased in thyme with drying (Table 2 , figure.1). The minimum reduction of TPC after oven dried treatment was(200.10, 231.30 mg GE/ 100 g), The TPC of air shade in infusion and Continuous infusion is higher than air sun drying, (240.11, 259.34, 231.21, 243.20 mg GE / 100 g) respectively. The TPC of fresh samples were corrected for moisture content before experiment, the moisture content of samples were 80.51%. 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± $2C^0$ 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.[11] reported that drying process led to loss of 30% of polyphenols in total phenol content. Various researches found that degradation of phytochemicals related to thermal process[18].

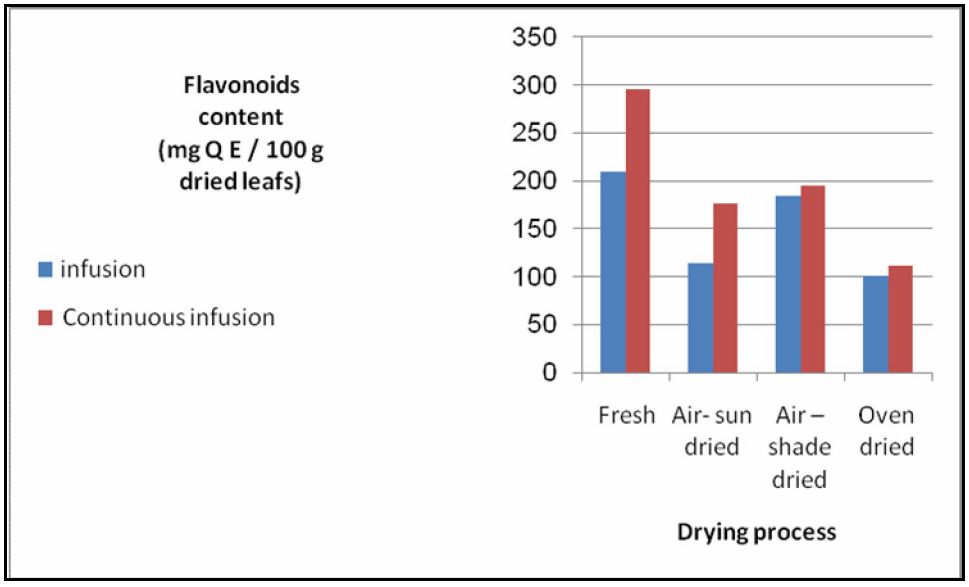

Figure 2 . flavonoids content of Thymus vulgaris L after drying process

3.5. Effect of drying on flavonoids content

The results showed that the destruction of flavonoids had varied significant differences between fresh and thyme dried (Table. 2, figure. 2). The highest amount of flavonoids content of thyme was found in infusion and Continuous infusion fresh sample (210.11, 296.22 mg QE/ 100g), the higher reduction of flavonoids content were after oven drying (100.11, 111.42 mg QE/ 100 g). The reduction amount of flavonoids content was (114.31, 176.32 mg QE/ 100 g) after air sun drying, while (184.12, 195.21 mg QE/ 100 g) after shade drying. The loss of flavonoids was found to be less in oven drying than air and sun drying. These losses may due to temperature [31]. 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 [8]. Increasing pre-heating temperature decreased the enzyme activity of flavonoids to degrading enzyme such as polyphenoloxidase [26], which resulted in an increase in flavonoid content [35].

4. Conclusions

Total phenolics and flavonoids of the investigated fresh and dried thyme were varied significantly. Fresh thyme have higher amounts of total phenolics, and flavonoids contents. Air shade - drying was a better drying method for keeping photochemical contents compared to oven and air sun drying method. Furthermore, air drying treatment kept higher amounts of total phenolics, and flavonoids than oven drying. Total phenolics and flavonoids content in continuous infusion are higher than infusion without boiling.

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