Comparative study of different drying methods regard to the phenols and flavonoids content of dried *Citrus aurantium* L. leaves

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**ABSTRACT**

**Objective**: To determine the effects of different thermal drying technologies on the total phenol and flavonoid contents (TPC) and total flavonoids (TFC) in sour orange (*Citrus aurantium* L.) leaves.

**Design/methodology/approach**: Solar drying was carried out in outdoor sunny conditions using two direct solar dryers; one with natural convection, the other with forced convection. The total phenol and flavonoid contents in gallic acid equivalents (GAE) and quercetin (Q), respectively, of ethanolic extracts of *C. aurantium* were assessed with spectrophotometric techniques.

**Results**: The results demonstrated maximum phenol values for the direct natural convection solar dryer (161.4 mg EAG/g MS) and minimum values for shade drying (61.43 mg EAG/g MS). As for flavonoids, the highest values were obtained in the direct forced convection solar dryer (32.22 ± 1.6 mg EQ/g MS), while the lowest was registered in the open air sun (11.72 mg EQ/g MS).

**Conclusions**: Direct solar dryers are technologies effective for maintaining the phenols and total flavonoids content in dried leaves of *C. aurantium*.

**Keywords**: *Citrus aurantium*, sour orange, flavonoids, phenols, solar drying.

**INTRODUCTION**

Plants are both agrochemical and food additive sources, as well as pharmaceuticals, yet, their adequate use constitutes a challenge for current pharmacology. Worldwide, there is a wide variety of plants with no researched pharmacological effects which could be crucial in diseases treatment (Ghasemzadeh *et al.*, 2016).
The properties in the leaves of the sour orange tree (*Citrus aurantium* L.), for example, have been scarcely studied; however, their availability year-round is greater than that of the fruits and can even be waste materials from pruning processes, which facilitates using them in obtaining extracts with pharmacological purposes.

*Citrus aurantium* L. belongs to the *Citrus* genus, which inhabits tropical and subtropical areas (Arendse *et al*., 2014). It is a thorny small tree of green color; its very fragrant green leaves have an oval limb of complete edges; of white and highly fragrant flowers; spherical fruit with a thick-skinned peel, that protects the edible and juicy parts (Capeccka *et al*., 2005). It is widely used in southeastern Mexico because of its anticarcinogenic, anxiolytic, antiobesity, antibacterial, antioxidant, pesticidal, and antidiabetic properties (Aiello *et al*., 2020).

Among the bioactive compounds in *Citrus* are the flavonoids, present in thousands of different structural forms in a variety of plants, which provide citrus with antioxidant activity (Pellati *et al*., 2002, Suryawanshi, 2011). The major flavonoids found in citrus include flavanones (hesperetin and naringenin), polymethoxylated flavones (PMF) (nobiletin and tangeretin) (Horowitz and Gentili, 1977), and glycosylated flavanones such as naringin. At the same time, Naringin is widely used in traditional medicine to have anti-apoptotic, anti-osteoporosis, anti-ulcer, antioxidant, anti-inflammatory, and anticancer properties (Cui *et al*., 2012).

Remedies made from bitter orange leaves are often consumed in infusions, either from the dried or fresh leaves; yet, drying, extraction, storage, and processing have a significant impact on the quality of the plants (Kunle *et al*., 2012). That is why they should be dried under specific conditions, to preserve their nutritional quality and avoid contamination, deterioration and protect their phytochemical efficiency (Ghasemzadeh *et al*., 2016).

Worldwide, several methods are used for plant drying; from those using conventional fuels, such as gas or electricity, to open-air solar drying or via solar thermal technologies. The latter has great advantages over the others, both from an environmental perspective and on the final quality of the dried products (Fonseca *et al*., 2002).

Given the potential therapeutic value of *Citrus aurantium* and the scarce knowledge about the impact of different drying methods, including solar drying, on the phenolic compounds, the objective of this research was to determine the effect of different thermal drying technologies on the total phenols and flavonoids content of sour orange leaves; to understand the changes during drying for obtaining the required quality characteristics for consumption.

**MATERIALS AND METHODS**

**Samples**

The studied vegetal material was collected in a rural plantation located in the municipality of Huimanguillo, Tabasco, Mexico, in November 2016. The sampling site locating at 18° 01' 32.0” N and 93° 39’ 05.7” W, 10 m a.s.l. All the material was washed by jet water to remove external impurities, and the leaves were grouped following the following characteristics: maturity, color, and freshness (Castillo *et al*., 2017). Plant identification was performed by Dr. Eustolia García López of the Colegio de Postgraduados, H. Cárdenas, Tabasco, and a sample was deposited in the departmental herbarium.
Drying

The fresh leaves of *C. aurantium* (20 g) were dried in the facilities of the Solar Drying Laboratory at the Facultad de Ingeniería, Universidad Autónoma de Campeche. The following methods were used: drying in shade, drying in direct solar dryers, open-air drying, and drying in an electric oven with controlled temperature.

a) Shade drying: It took place during 21 d at environmental temperature, in a dark and well-ventilated room, at an average temperature of 30 °C and 60% relative humidity.

b) Direct solar drying: Solar drying was performed in two prototype non-commercial direct solar cabinet dryers (Cuernavaca, Morelos, Mexico); one operating with forced convection and the other with natural convection. Both dryers, which have holes to extract humid air, were designed at the Instituto de Energías Renovables (IER) at the Universidad Nacional Autónoma de México (UNAM) and manufactured by Manufacturas Plásticas de Cuernavaca S.A de C.V in Cuernavaca, Morelos.

c) Open-air drying. In this process, *C. aurantium* leaves were placed on a suitable piece of plastic. The leaves were placed without overlapping and left until sunset. If by then these were not completely dry, they were collected and stored indoors. This method was daily repeated until the drying process was complete. The total duration was 31.3 h (hours of sun exposure).

d) Oven drying at controlled temperature: done in a conventional dryer consisting of a non-convective electric oven, Riossa brand (Morelos, Mexico), capable of a 50 °C to 220 °C temperature range, with a ±2 °C sensitivity. Drying was carried out at 55 °C.

During the drying process, the following variables were assessed: internal temperature, weight, and size of the samples, solar irradiance, relative humidity, air temperature (Castillo *et al.*, 2017). The dried samples were then stored at −20 °C.

Moisture

The moisture was determined before and after each drying method, using two thermobalances with a moisture analyzer, brand Ohaus MB45 (New Jersey, USA), with ±0.01%/0.001 g accuracy. The evaluations were performed on 1 g of sample, following the procedure by Castillo *et al.* (2017).

Once the desired moisture values were obtained, extracts were prepared in two stages. A grinding stage of the materials in an industrial mill, Thomas-Wiley Laboratory Mill, Thomas Scientific model (New Jersey, USA), and another of extraction by maceration. A successive cold maceration with 85% ethanol was carried out at a rate of 1 g of plant material for every 10 mL of solvent. They were previously covered with aluminum foil and placed in an IKA shaking equipment, model KS 260 Basic (Wilmington, USA), for 7 d at environmental temperature for subsequent filtration in a Büchner funnel with filter paper attached to its bottom. Then the Büchner funnel was placed on a Kitasato flask connected to a vacuum pump (Stage Vacuum Pump Model RS-4, Zhejiang, China), for solvent removal. Subsequently, the solvent was removed by concentrating with a Rotavapor, Model RE52, Lanphan RE-52 (Zhengzhou, Henan, China) at 40 °C.
Total phenols quantification
The total phenolic content in the ethanolic extracts of *C. aurantium* was assessed following the Folin-Ciocalteu assay (Fonseca et al., 2002). Four hundred $\mu$L of the extract (1 mg/mL) were introduced into test tubes. Then, 1 mL of Folin-Ciocalteu reagent (1:10) was added, followed by 1 mL of sodium carbonate (20%). Subsequently, the test tubes were allowed to stand in the dark for 90 min at environmental temperature, before measuring their absorbance using a UV-Vis spectrophotometer (Velab brand, Model VE-5600UV, EU) at 760 nm.

The calibration curve was prepared using aqueous gallic acid with 0.02 mg/mL to 0.1 mg/mL, with an $r^2$ of 0.999. The results were expressed in milligram gallic acid equivalents per gram of dry material (mgEAG/gMS). Standard gallic acid monohydrate (>99%; CAS registry number 5995-86-8) was used; the coefficient of variation was between 2% to 5% in all cases. Each sample was run in triplicate.

Quantification of total flavonoids
Total flavonoid content was measured using the aluminum trichloride colorimetric method described by Liu and Zhu (2007) with some modifications. One mL of solution containing flavonoids was mixed with a NaNO$_2$ solution (0.3 mL, 5%); after 6 min of incubation at 25 °C, 0.3 mL of AlCl$_3$ solution (2%) was added. The mixture was stirred and allowed to stand for 6 min. Subsequently, 1 M NaOH (0.2 mL) solution was added to each extract and incubated for 10 min at environmental temperature. The absorbance was determined at 510 nm against a blank. Quercetin dihydrate (>98%, CAS registry number 6151-25-3) was used as a standard to prepare the calibration curve ($r^2 = 0.9924$). The results were expressed as milligram quercetin equivalents per gram of dry mass (mgEQ/gDM).

The hydroalcoholic extract sample was prepared at 0.2 mg/mL from each sample dissolved in 70% ethanol, and One mL of reactive grade methanol was added to 1 mL of the sample. All samples were done in triplicate.

Statistical analysis
The Statistical analysis was performed using the OriginPro 2018 software. Data are presented as mean ± standard deviation (SD) and a $P<0.05$ was considered as statistically significant. An analysis of variance (one-way ANOVA) was used to compare the means of the phenol and flavonoid content between the different drying methods for multiple comparisons. Holm Sidak’s=0.05 test was used to determine significant differences between two groups.

RESULTS AND DISCUSSION
Drying time and moisture content
Table 1 shows that the drying time varied from one method to another, solar drying with natural convection being the shortest (5 h), followed by solar drying with forced convection (6.7 h), and drying in an electric oven at 55 °C (7.7 h). In contrast, the traditional methods of open-air and shade drying showed longer drying durations (31.3 h and 504 h, respectively).
No significant differences were observed between drying methods for the moisture content ($P<0.001$). The highest moisture loss was obtained in the forced convection and natural convection methods (89% each) and the lowest in shade drying (85%). The moisture values achieved in this study were between 8% and 12%, which corresponds to previously established values (9% to 10%) for the commercialization of dried plants such as wormwood, marigold, thyme, sage, oregano, lavender, mint, chili, among others (Ceballos and Jimenez, 2012).

**Phenols and flavonoids quantification**

Figure 1 shows the concentration in mgEAG/gDM for each of the drying methods. It is observed that phenolic compounds are present in each of them, but with significant differences according to the analysis of variance ($P<0.001$).

![Figure 1](image_url)

**Figure 1.** Effect of different drying methods on total phenolic content. Data represent means ± standard deviation of triplicate measurements. $P<0.001$. S- Shade drying; CA- Open-air drying; SCF- Solar drying with forced convection; SCN- Solar drying with natural convection; HTC- Oven drying with controlled temperature.

| Drying methods                  | Drying time (h) | Moisture (X±SD) (%) |
|---------------------------------|-----------------|---------------------|
|                                 |                 | Initial          | Final           |
| Hot air oven drying (55 °C)     | 7.7             | 80.68±1.10        | 8.57±0.30       |
| Direct natural convection solar dryers | 5.0             | 83.67±1.20        | 8.85±0.50       |
| Direct forced convection solar dryers | 6.7             | 80.73±1.10        | 8.68±0.16       |
| Open sun drying                 | 31.3            | 81.20±0.35        | 9.11±0.18       |
| Shade drying                    | 504 (21 days)   | 80.50±1.15        | 12.00±0.20      |

X: average; SD: standard deviation
The highest phenol content was recorded in SCN (161.40±3.38 mgEAG/gMS) and the lowest content in shade drying, with 69.43±3.48 mgEGA/gMS. It is important to note that shade drying was not only the method that yielded the lowest phenol content, but also the one that showed the longest drying time. Regarding the flavonoid content, the obtained results show differences (P<0.05) between the different drying methods, with a minimum value of 11.72 mgEQ/gMS in open-air drying and a maximum of 32.23 mgEQ/gMS in SCF (Figure 2).

From the obtained results and those published in previous studies, it is to be noted that the observed decrease in the total phenol content in shade drying concurs with Ghasemzadeh et al. (2016). This could be attributed to the degradation caused by the Polyphenol Oxidase (PPO) enzyme because the sample’s drying process was completed at environmental temperature for 21 d. However, in previous studies, drying lemon, oregano, and mint at temperatures between 25 °C and 32 °C for 10 days, both significant increases and decreases in antioxidant activity were observed, which was related to the phenol content (Capecka et al., 2005).

Likewise, Norhidayah et al. (2013) reported that freeze-drying of ginger (Zingiber officinale Rosc.) at −40 °C, showed lower phenol content (P<0.05) compared to cabinet drying at a 60 °C temperature, but not in the flavonoids, which, although higher in cabinet drying, were not significant. However, for blackberry (Morus alba L.) leaves drying, no differences were found in different drying methods (Katsube et al., 2009). Whereas, for Chinese chasteberry (Vitex Negundo Linn), hot air oven drying was not the best method to preserve antioxidant compounds (Rabeta and Vithya, 2013).

Enzyme degradation and loss of antioxidant activity, related to the decrease of total phenols, have also been reported due to sun drying (Chan et al., 2009). Therefore, it is
important to examine the correlation between the content of certain constituents and their biological activity (Ghasemzadeh et al., 2016). Such information could help researchers to establish suitable conditions and techniques to improve these featured compounds. Therefore, it is important to understand how they vary in the C. aurantium case with each drying method to predict which would be better.

This is the first study that evaluates the variation of the total phenols and flavonoids content of C. aurantium leaves by different drying methods. The results indicate that the solar convection drying method, either natural or forced, preserves the highest content of total phenols and flavonoids, being a suitable method for drying and preserving bioactive compounds of this plant. Also, it is an environmentally friendly method, which contributes to maintaining the pharmacological properties by preserving their secondary metabolites.

The results here are promising and will contribute to broadening the scientific knowledge on the extracts of sour orange leaves, due to their content of phenolic compounds. However, further studies on the chemical composition of these extracts and their relationship with antioxidant activity should follow.

The temperature-controlled electric oven drying method showed values of the pharmacological properties of C. aurantium, which are intermediate between those of direct solar drying and the shade and open-air methods. Taking into account this result and the requirement of electrical energy for its operation, which also comes mostly from fossil resources, the viability of using the solar resource for drying medicinal plants is reinforced.

Further analysis of this plant and its effects on humans, for the treatment of cardiac and nervous system disorders, which could be due to the high content of phenolic compounds, would be suitable. Finally, it is desired that the present study serves as a basis for further research aimed at the complete characterization of sour orange leaves.

**CONCLUSIONS**

These results demonstrated that solar drying by direct dryers is superior compared to other drying methods for preserving the total phenols and flavonoids content in dried C. aurantium leaves. The maximum values for phenols were obtained with the direct solar dryer with the natural convection method, while shade drying was the least favorable method. As for flavonoids, the highest values were obtained in the direct solar dryer with forced convection, followed by the solar dryer with natural convection; the lowest values were observed in the open-air drying method.

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