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Effect of Three Drying Methods on Antioxidant Efficiency and Vitamin C Content of *Moringa oleifera* Leaf Extract

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Abstract*—Moringa oleifera* is a plant containing many nutrients that are mostly concentrated within the leaves. Commonly, the separation process of these nutrients involves solid-liquid extraction followed by evaporation and drying to obtain a concentrated extract, which is rich in proteins, vitamins, carbohydrates, and other essential nutrients that can be used in the food industry. In this work, three drying methods were used, which involved very different temperature and pressure conditions, to evaluate the effect of each method on the vitamin C content and the antioxidant efficiency of the extracts. Solid-liquid extractions of *Moringa* leaf (LE) were carried out by employing an ethanol solution (35% v/v) at 50 °C for 2 hours. The resulting extracts were then dried *i*) in a convective oven (CO) at 100 °C and at an atmospheric pressure of 750 mbar for 8 hours, *ii*) in a vacuum evaporator (VE) at 50 °C and at 300 mbar for 2 hours, and *iii*) in a freeze-drier (FD) at -40 °C and at 0.050 mbar for 36 hours. The antioxidant capacity (EC_{50}, mg solids/g DPPH) of the dry solids was calculated by the free radical inhibition method employing DPPH˙ at 517 nm, resulting in a value of 2902.5 ± 14.8 for LE, 3433.1 ± 85.2 for FD, 3980.1 ± 37.2 for VE, and 8123.5 ± 263.3 for CO. The calculated antioxidant efficiency (AE, g DPPH/(mg solids·min)) was 2.920×10^{-5} for LE, 2.884×10^{-5} for FD, 2.512×10^{-5} for VE, and 1.009×10^{-5} for CO. Further, the content of vitamin C (mg/L) determined by HPLC was 59.0 ± 0.3 for LE, 49.7 ± 0.6 for FD, 45.0 ± 0.4 for VE, and 23.6 ± 0.7 for CO. The results indicate that the convective drying preserves vitamin C and antioxidant efficiency to 40% and 34% of the initial value, respectively, while vacuum drying to 76% and 86%, and freeze-drying to 84% and 98%, respectively.

***Keywords*—**Antioxidant efficiency, convective drying, freeze-drying, *Moringa oleifera*, vacuum drying, vitamin C content.

I. Introduction

MORINGA oleifera is a tree native to India that grows best in tropical or subtropical climates and is cultivated worldwide. It has been used as a nutritional plant due to the presence of macro and micronutrients within several parts of the plant, especially the leaves and seeds [1]. *Moringa oleifera* leaves have been reported to be a good source of natural antioxidants, such as ascorbic acid, carotenoids, flavonoids and phenolic compounds and the highest amount of vitamin C within the plant is concentrated in the fresh leaves [2].

Moringa oleifera nutrients from leaves have been extracted with several processes, the most common involves solid-liquid extraction with a mixture ethanol-water followed by evaporation and a drying process [3]. The concentrated extract of *Moringa oleifera* leaves might be a useful source of nutrients for several processes in food industry; however, extraction and drying operation conditions may damage many of the nutritional constituents from the raw material.

In this work, three drying methods: convective, vacuum and freeze-drying, were used to study the effect of different conditions of drying temperature and pressure on vitamin C content and antioxidant efficiency of the extract of *Moringa oleifera* leaves.

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II. Materials and Methods

A. Plant Material

Moringa oleifera leaves were collected from several trees grown within the National University of Engineering, Managua, Nicaragua. Leaves, excluding petioles, were washed with distilled water and drained for experimentation.

B. Solvents and Reagents

The following reagents were used: Ascorbic acid, Methanol HPLC grade and 2,2-Diphenyl-1-picrylhydrazyl (DPPH˙) were purchased from Sigma-Aldrich Co. (St Louis MO, USA), Ethanol absolute for analysis EMSURE® ACS and Sulfuric acid ACS were purchased from EMD Millipore Co. (Billerica MA, USA). Ethanol absolute for lixiviation step was purchased from J. T. Baker® Chemical Co. (Center Valley PA, USA). All of the reagents used were of analytical quality.

C. Experimental Apparatus

Solid-liquid extraction of leaves was carried-out in a Julabo® Shaking Water Bath model SW-23. For convective drying at normal pressure, a Fisher Scientific™ Isotemp™ model 825F oven was used. Vacuum drying was carried-out in a Büchi Rotavapor model R-124 connected with a Büchi Vac® model V-500 Vacuum Pump. For the freeze-drying process, a Labconco Freezone Console 12 L Freeze Dry System along with a Labconco Rotary Vane Vacuum Pump with a 195 L/min capacity, were used.

Determination of vitamin C content was carried-out using High-Pressure Liquid Chromatography (HPLC) in a KONIK® HPLC 560 System, while a Hach® DR 5000™ UV-Vis Laboratory Spectrophotometer was used to measure absorbance for the free radical inhibition method, employing DPPH at 517 nm to determine antioxidant efficiency.

D. Experimental Procedure

Two hundred grams of leaves were introduced into two flasks containing 1000 mL each of a mixture of 35% ethanol (v/v) in distilled water. Solid-liquid extraction of leaves was carried-out at 50°C for two hours. After the lixiviation process, the Moringa leaf (LE) extract was separated from the solids using a Büchner flask and funnel along with a vacuum pump.

The concentration of solids in this LE extract was calculated and found to be 10.40 mg/mL after vacuum drying of a sample in the rotary evaporator.

The ethanol in the lixiviate was distilled at 40°C and 185 mbar using a rotary evaporator. Ethanol was needed to be removed before the freeze-drying process.

The aqueous extract was then separated into three volumes of 300 mL to be dried using the three different drying methods mentioned. An aliquot of 300 mL was taken for the vitamin C content and Antioxidant Efficiency Assays.

The convective drying at normal pressure (1000 mbar) was carried-out in a convective oven at 100°C. An amount of 300 mL was placed in a 500 mL Beaker and put inside the oven for 8 hours, when the solid was completely dried (CO).

The vacuum drying was performed in a rotary evaporator (VE) at 50°C and 300 mbar for 2 hours.

For the freeze-drying process, the 300 mL aliquot was frozen at -12°C during 12 hours in a freeze-drying flask. Then, the flask was connected to the freeze-dryer operating at a condenser temperature of -50°C and an absolute pressure of 0.050 mbar. The sample was dried completely after 36 hours of operation (FD).

E. Antioxidant Efficiency Assay

To evaluate the antioxidant efficiency of the samples, a procedure based on the methodology described by Sánchez-Moreno et al. [4] was employed. A 0.0250 g/L solution of DPPH· (2,2-diphenyl-1-picrylhydrazyl) in ethanol absolute was prepared daily for analysis.

A calibration curve of DPPH concentration against absorbance was carried-out to calculate DPPH concentration in reaction medium.

The correlation determined by linear regression for the DPPH calibration curve shown at Fig. 1 was:

$$A_{517\,nm} = 62 \times [DPPH^{\bullet}]_t + 0.0122 \quad (1)$$

where $[DPPH^{\cdot}]_t$ was expressed as g/L with $r^2 = 0.9996$.

To perform a comparative analysis, antioxidant efficiency of ascorbic acid (AA) as a standard antioxidant was calculated. Several solutions at different concentrations of ascorbic acid from 50 mg AA/g DPPH· to 200 mg AA/g DPPH· were prepared and the curves of DPPH· absorbance at 517 nm through time were obtained.

The percentage of remaining DPPH (% $DPPH^{\cdot}_{REM}$) was calculated as follows [4]:

$$\%DPPH^{\bullet}_{REM} = [DPPH^{\bullet}]_t / [DPPH^{\bullet}]_{t=0} \quad (2)$$

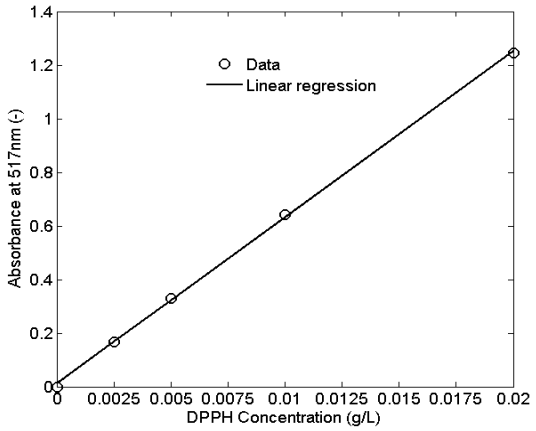

Fig. 1 Linear correlation between DPPH· concentration and Absorbance at 517 nm

Then, the percentage of remaining DPPH against time was plotted to find the ascorbic acid concentration that decreased the initial DPPH· concentration by 50% (EC_{50}). The time needed to reach a steady state to EC_{50} ($t_{EC_{50}}$) was calculated graphically.

Antioxidant Efficiency (AE) defined by [4] was calculated with the equation:

$$AE = 1/(EC_{50} \times t_{EC_{50}}) \quad (3)$$

For the leaves extract (LE) using the calculated concentration of solids, several solutions were prepared diluting LE to obtain solutions from 2700 mg solids/ g DPPH· to 3600 mg solids/g DPPH·. For the dried solid from CO, solutions from 3600 mg solids/g DPPH· to 18000 mg solids/ g DPPH· were prepared.

In the case of the dried solid from VE, solutions from 3060 mg solids/g DPPH· to 5400 mg solids/ g DPPH· were prepared. For the dried solid from FD, solutions from 2340 mg solids/g DPPH· to 3420 mg solids/ g DPPH· were prepared.

The antioxidant efficiency of the aqueous leaves extract and the dried solids obtained from each drying method was then calculated similarly using (1)–(3). All the experiments were run in triplicate.

F. Vitamin C Content Determination

High-performance liquid chromatography (HPLC) technique was used to measure vitamin C content in the samples. A ZORBAX Eclipse XDB C-8 Column was used with a UV Visible 254 nm detector. The operation parameters were 1 mL/min flow with a mobile phase of methanol (50%), 0.05 M sulfuric acid (40%) and deionized water (10%). A vitamin C quantification method was established in the equipment and a calibration curve using concentrations from 10 mg/L to 60 mg/L prepared with a vitamin C standard was developed initially. From each dried solid sample, 1.2500 grams was taken and diluted with distilled water to obtain a 20 mL solution.

One milliliter of each solution was taken and diluted to 100

mL with distilled water. Subsequently, a small amount of this solution was filtered with a Nylon membrane filter of 0.22 µm pore size. One hundred microliters of this filtered solution for each sample were taken with a syringe and injected to the equipment. All the injections were done in triplicate.

III. Results and Discussion

A. Antioxidant Efficiency Assay

The kinetic behavior of ascorbic acid (AA) with DPPH˙ is shown in Fig. 2. Several runs at different concentrations of AA were developed and the antioxidant capacity was found at 170 mg AA/ g DPPH. The time to reach steady state was found graphically at 1.0 min.

Fig. 3 shows the kinetic plots for the different concentrations of dried solids vs. time. The antioxidant capacity, EC_{50}, was estimated from these plots when the remaining DPPH˙ reached a 50% value at steady state. The estimated time to reach steady state was also found graphically with these plots.

The values of antioxidant capacity (EC_{50}, mg solids·g^{-1} DPPH˙), time to reach steady state ($t_{EC_{50}}$, min) and antioxidant efficiency (g DPPH˙·mg^{-1} solid·min^{-1}) of vitamin C, leaves extract (LE) and the dried solids are shown in Table I. This was calculated using (1)–(3).

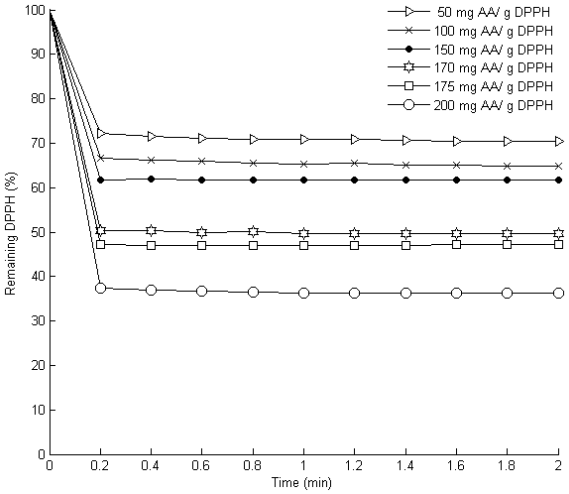

Fig. 2 Kinetic plot for DPPH˙ free radical scavenging activity of ascorbic acid standard

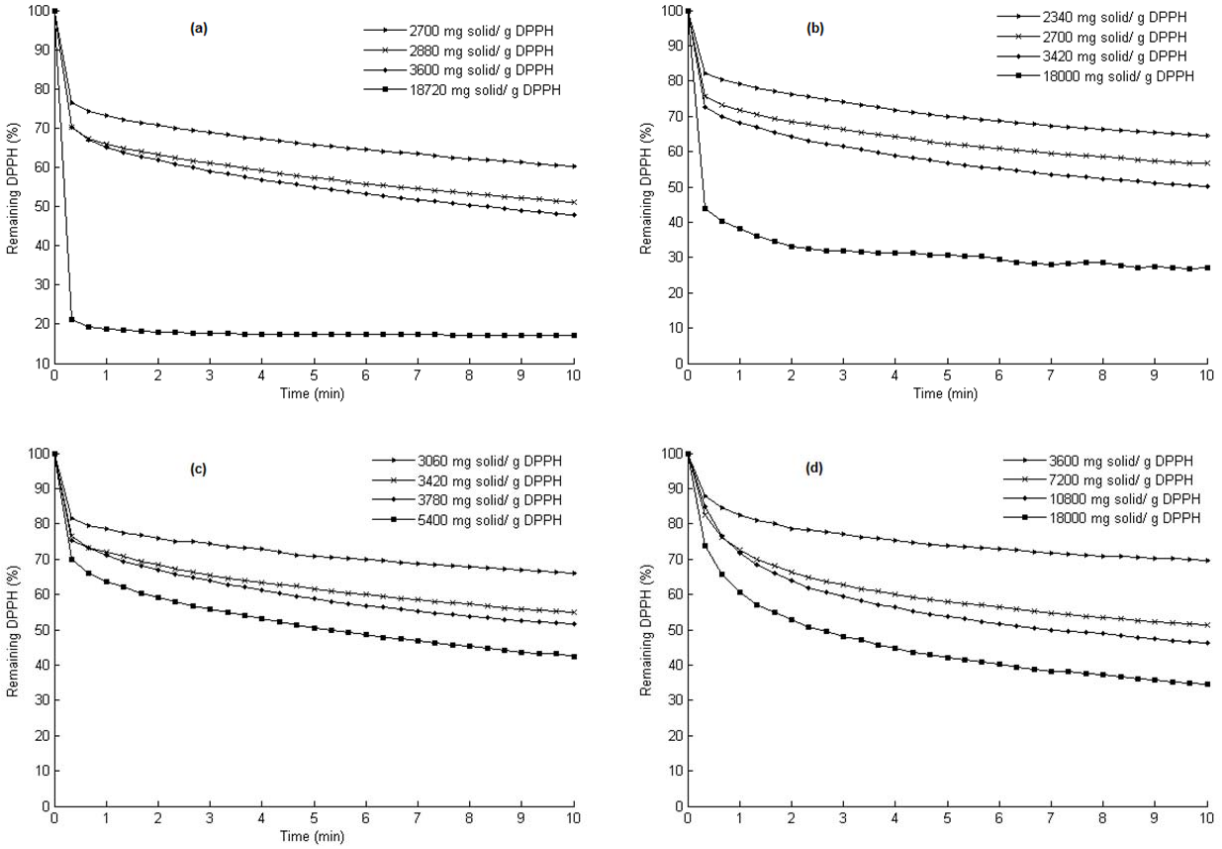

Fig. 3 Kinetic plots for determination of EC_{50} and $t_{EC_{50}}$ values. a) Leaves extract (LE), b) Freeze-dried extract (FD), c) Vacuum dried extract (VE) and d) Convective dried extract (CO)

TABLE I
ESTIMATED DPPH· ASSAY PARAMETERS

Sample	EC_{50} ($mg\ solid\ g^{-1}\ DPPH^{\cdot})^a$	$t_{EC_{50}}$ $(min)^a$	AE ($g\ DPPH^{\cdot}\ mg^{-1}\ solid \cdot min^{-1}$)	Kinetic Classification[b]	AE Classification[b]	AE Degradation[c]
Vitamin C	169.38 ± 6.4	1.0 ± 0.1	5.903 E-3	Rapid	High	-
LE	2902.5 ± 14.8	11.8 ± 0.5	2.920 E-5	Intermediate	Low	-
FD	3433.1 ± 85.2	10.1 ± 0.3	2.884 E-5	Intermediate	Low	1.2 %
VE	3980.1 ± 37.2	10.0 ± 0.2	2.512 E-5	Intermediate	Low	14.0 %
CO	8123.5 ± 263.3	12.2 ± 0.8	1.009 E-5	Intermediate	Low	65.4 %

[a]Each value is the mean ± standard deviation. [b]Classification according to [2]. [c]Calculated considering leaves extract (LE) as reference. Values with the same letter (A, B, C) are not significantly different ($p < 0.05$), between samples.

TABLE II
RESULTS OF ASCORBIC ACID CONTENT DETERMINATION

Sample	Ascorbic acid (*mg/L*)	Degradation Percentage[a]	Drying Process Affectation[b]
LE	59.0 ± 0.3	-	-
FD	49.7 ± 0.6	15.8 %	Low
VE	45.0 ± 0.4	23.7 %	Moderate low
CO	23.6 ± 0.7	60.0 %	High

[a]Calculated considering leaves extract (LE) as reference. [b]Considering reported degradation values of vegetables after drying process at several temperatures [5]. All values were significantly different ($p < 0.05$), between samples.

A one factor ANOVA test with $p \leq 0.05$ and Duncan's multiple range test demonstrated that antioxidant capacity, EC_{50}, and ascorbic acid content of the dried solids were statistically significant different from each other for each drying method and from the leaves lixiviate. The antioxidant efficiency and vitamin C content of the solid dried with the convective oven method was remarkable lower than vacuum drying and freeze-drying.

Freeze-drying was an excellent method for antioxidant efficiency conservation. Antioxidant efficiency after Freeze-drying was not significantly different ($p < 0.05$) from leaf lixiviate before drying. Solids after the Vacuum drying showed a small loss in antioxidant efficiency.

B. Vitamin C Content Determination

The content of vitamin C (mg/L) determined by HPLC is shown in Table II. The degradation percentage after each drying process was calculated from the initial leaves lixiviate (LE).

In the case of vitamin C loss, both vacuum drying and freeze-drying exhibited a relative low degradation of ascorbic acid. Compared to both, solids dried from the convective oven suffered a considerable affectation due to the high temperature and prolonged time.

Considering both antioxidant efficiency and conservation of vitamin C after drying, the best method for preservation of these properties is freeze-drying. Taking into account the high cost of freeze-drying equipment and long time of the process, vacuum drying is a good alternative for conservation of both vitamin C and antioxidant efficiency along with many of the nutritional components in *Moringa oleifera* leaves.

IV. CONCLUSIONS

The different drying conditions inherent to the three drying methods (temperature, pressure and time) were found to have a considerable effect on the ascorbic acid and antioxidant efficiency degradation. The convective drying at 100°C, normal pressure and eight hours damaged significantly the ascorbic acid and antioxidant efficiency compared to the leaves lixiviate. The most effective method for conservation of both parameters was freeze-drying with a remarkable conservation of antioxidant efficiency compared to other methods. Nevertheless, the vacuum drying method showed a good performance in conservation of vitamin C and antioxidant efficiency and is also recommended for industrial processing of Moringa leaves extracts.

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