Pyrethrum (*Chrysanthemum cinerariifolium*) Flowers’ Drying Conditions for Optimum Extractable Pyrethrins Content

H. O. Otieno¹, D. K. Kariuki¹ & J. M. Wanjohi¹

¹Department of Chemistry, School of Physical Sciences, University of Nairobi, Nairobi, Kenya

Correspondence: H. O. Otieno, Department of Chemistry, School of Physical Sciences, University of Nairobi, Nairobi, Kenya. E-mail: hillotow@gmail.com

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Abstract

Pyrethrum flowers of the genus *Chrysanthemum cinerariifolium* are grown in Kenya by small scale farmers for extraction of pyrethrins, a natural insecticide’s active ingredient. Pyrethrins are classified in two groups, Pyrethrins I and II, and are degradable when exposed to air, moisture and high temperatures. The contents and ratio of Pyrethrins I:II determine the efficacy of the insecticide. Therefore, drying of the pyrethrum flowers should be optimized in order to attain maximum extractable pyrethrins content and optimum ratio. The aim of this research was to optimize the drying temperatures, time and moisture content of pyrethrum flowers. The flowers were harvested and dried at varying temperatures of 40, 50, 60 and 70 °C to total dryness. Another set of flowers were harvested and dried in the oven at the same temperatures for a maximum period of 18 hrs. Moisture content was determined at each temperature, at intervals of one hour. The dried flowers were then ground into fine powder and extracted using Soxhlet extraction method with hexane. The extracts were refined and analyzed by Mercury reduction and High Performance Liquid Chromatographic methods. Pyrethrum flowers were found to achieve maximum moisture loss, at varying times and temperature with 70 °C recording the shortest time of 18 hrs. The yield of pyrethrins obtained on drying the flowers to constant weight at 40 °C was 0.90% while drying for 18 hrs yielded 0.79%. Extractable Pyrethrins II were found to reduce by 8.6% when the drying temperature was raised from 50 to 60 °C and by 11.3% from 60 to 70 °C. Extractable Pyrethrins I were found to reduce by 6% when the drying temperature was raised from 50 to 60 °C and by 5% from 60 to 70 °C. The total pyrethrins obtained from the flowers dried at 50 °C were found to be 1.37% at 18 hrs and 1.44% to constant weight drying. The pyrethrins I:I ratio was found to vary over the temperature range 40-70°C. The optimum temperature and time for drying pyrethrum flowers was found to be 50 °C for 21 hrs.

Keywords: *Chrysanthemum cinerariifolium*, pyrethrins I:II ratio, drying, temperature, time

1. Introduction

Pyrethrum is a plant genus classified as *Chrysanthemum* or *Tanacetum* and is commercially cultivated for its flowers that are used to produce natural insecticides. The natural insecticide extracted from the dried flowers of *C. cinerariifolium* or *C. coccineum* are referred to as pyrethrins. *Chrysanthemum cinerariifolium* is the genus grown in Kenya and has white flowers, with yellow center that sprout from the stiff stems. The stems have bluish-green leaves and can grow to about 46-100 cm tall (Elliott, 2007). The growing of pyrethrum flowers in Kenya is of great economic value as it is an income generating activity for small scale farmers and also earns the country foreign exchange through the export of pyrethrins. Processing the flowers to extract pyrethrins is a long process involving many actors, such as agronomists, farmers, chemical engineers, chemists and entomologists. Processing starts with picking of the mature flowers with horizontal petals. The flowers are then dried at optimum conditions to total dryness and then ground into fine particles before extraction. Finely ground flower powder is better suited for use as an insecticide but the coarsely ground have long shelf life and deteriorates less. The dried flowers are then transported to the processing factories for extraction of pyrethrins which are the active components and sold as oleoresin (Ang’endu, 1994).

Great interest was taken in the various methods used in drying the flowers and the thermal effect on the stability of the active components of the flowers. Small scale farmers always spread the flowers on polythene sheets in direct sunlight or in a shade for about two weeks. More advanced farmers always use solar driers or roasters to hasten the drying process due to the quantity of the produce. In Japan, the flowers are hung upside down to dry
which increases pyrethrin concentration. In the recent past due to the technological developments, use of oven was adopted as a faster and more efficient method of drying the flowers. During this process, drying temperature and moisture content of the flowers are of great significance and should be monitored. Research shows that pyrethrins are thermally and photochemically unstable. Heat causes rearrangements of the pyrethrins structure to form iso-pyrethrins which are insecticidally inactive. There are high chances that the resonance conjugation of the unsaturated side chain with the cyclopropane ring encampasses the disappearance of the vital activated methylene next to the ring and so reduces biological activity in iso-pyrethrins results (Wang, 2017). Pyrethrins are classified in two groups, Pyrethrins I and II. Pyrethrins I is composed of pyrethrin 1, jasmolin 1 and cinerin 1 while Pyrethrins II is composed of pyrethrin 2, jasmolin 2 and cinerin 2. The structures of the pyrethrins are shown in Figure 1.

Pyrethrins “knockdown” and “kill” insects by delaying the closure of voltage gated sodium ion channels in the nerve cells. Insecticidal and insect repellant properties of these compounds have been known for millennia and chrysanthemum species have long been cultivated for this purpose. Pyrethrins I are responsible for the knockdown and pyrethrins II for killing the insects. The knowledge of the ratio of pyrethrins I to pyrethrins II is of great importance since it helps in determining the efficacy of the insecticide. The highest efficacy is achieved with a pyrethrins I: II ratio of 1:1 (Wagner, 2000).

Pyrethrins as an insecticide is always applied in places where animals and human are present and therefore their toxicity must be considered. Pyrethrum as a mixture of cinerin, pyrethrin and jasmolin can be inhaled by people in their workplaces, it can get into the eye or the skin or it can be swallowed. A legal limit for pyrethrum exposure to humans has been set as 5mg/m³ over an 8 hours workday (Costa, 2015). Pyrethrum becomes dangerous to health at the levels of 5000 mg/m³. Symptoms as pruritus (itching), dermatitis, papules, erythema, rhinorrhea, sneezing and asthma can be experienced by people exposed to pyrethrum. Safety equipment should be put on by people using pyrethrum (Mader, 2012). Due to the increasing use of pyrethrins in agricultural and consumer products, there is a need for improved analytical techniques both to assure product quality and to monitor the fate of pyrethrins in the environment. The degradation of pyrethrins in pyrethrum flowers with respect to the moisture content and drying rates is also of great importance. Pyrethrins degrade when exposed to the environment hence insects are unable to develop resistance to the insecticide. This partly explains the insecticide’s continuous use and efficacy for more than a century. It does not pose threat to human beings and animals since it does not persist in the environment (Power et al, 2007).

![Figure 1. Structures of pyrethrins](Source: Casida, 1995)
Processing of pyrethrins begins with the picking and drying of pyrethrum flowers. Mature pyrethrum flowers that are ready for picking are those that have petals lying horizontally. Flowers are picked and stored in dry, well-ventilated bags for transport to drying sites or rooms. Drying of the flowers is an essential post-harvest activity that determines the extractable pyrethrins content. Farmers dry the flowers in the open, on materials such as mats, polythene sheet spread on the ground for a minimum of 4 days depending on sunlight (Wang, 2017).

2. Materials and Methods

Sampling
Once the flowers had horizontal petals, they were taken to have matured. The flowers were hand-picked ensuring that no part of the flower stock is taken. The flowers were picked in aerated baskets to avoid accumulation of heat generated by the picked flowers that may have led to rotting. The fresh flowers were weighed and then taken to the oven for drying.

Drying of pyrethrum flowers at regulated known temperatures
The harvested flowers were put on trays and then the trays were put in the oven. The temperatures of the oven were regulated between 30-70 ºC at intervals of 10 ºC but at constant time of 18 hrs. The moisture contents of the dry flowers were then tested and the flowers were then taken for analysis.

Grinding
The dried flowers were kept in the room for two days at ambient temperature before grinding, using a pestle and mortar. After grinding, the powder was sieved using a BS 410 mesh and put in labeled brown paper bags awaiting extraction.

Reagents
Dinige’s reagent, potassium iodate standard solution, iodine mono-chloride all prepared using AOAC method 936.05, n-hexane, sodium hydroxide, barium chloride and methanol.

Extraction Procedures

Step 1 Extraction
The dried flowers were then placed into an extraction thimble. Extraction at varying temperatures was done using soxhlet extraction for 10 hours using 1000 ml n-hexane in a 2000 ml Erlenmeyer conical flask containing a few anti-bumping chips. After extraction, the n-hexane was removed through a rotor vapour to reduce extracts volume to about 50 ml.

Step 2 Dewaxing
Normal Hexane was added to the sample extracts in a flask at a ratio of 3:1 v/v solvent to sample. The extracts were then kept in the refrigerator at 2 ºC for a period of 12 hrs for dewaxing. After 12 hrs, the extracts were filtered through cotton wool into conical flasks. The extracts were then evaporated to dryness using a rotor vapour.

Step 3 Saponification
A volume of 20 ml alcoholic NaOH was then added into extracts and refluxed for 1 hour for saponification of the fatty acids. The extracts were then transferred into 250 ml beakers after saponification.

Step 4 Evaporation
Distilled water was added to the extracts to a volume of 200 ml and evaporated on a hot plate up to a volume of 150 ml to remove the alcohol. The solution was then cooled to room temperature using tap water.

Step 5 Fatty acid removal
After cooling the solutions, they were transferred into 250 ml volumetric flasks and 1.0g of filter-Celite was added to each solution. A volume of 10 ml of 10% BaCl₂ was added to the extracts and topped up to the mark with distilled water. The mixtures were then shaken vigorously to ensure that the fatty acids were removed by the BaCl₂. Yellowish-orange Barium fatty acid salt precipitate was then formed. 200 ml of the extracts were then filtered into 250 ml beakers and three drops of phenolphthalein indicator was added. The filtrates were then neutralized with excess 20% sulphuric acid to precipitate the remaining BaCl₂. White precipitate of BaSO₄ formed was then filtered off through whatmann filter paper no. 1 coated lightly with a suspension of filter-Celite on a Buchner funnel aspirator. The precipitates were then washed severally with distilled water.

Step 6 Separation of Chrysanthemic and Pyrethric acid
The filtrates obtained in step 5 above were then transferred into a 500 ml separating funnel and extracted twice with two 50 ml portions of petroleum ether. The petroleum ether layers were transferred into a 250 ml separating funnel and washed twice with 5 ml of distilled water to remove aqueous portions that were emptied into 250 ml beakers. The Chrysanthemic acid is less polar than the pyrethric acid formed and therefore, the pyrethrins I are contained in the petroleum ether layer while the aqueous layer contain pyrethrins II. The main goal achieved in this step was the separation of the two acids subsequently used in quantifying the pyrethrins I and pyrethrins II. The aqueous layer was then evaporated to 50 ml for 1 hour. The mixture was then cooled to room temperature using tap water.

Analytical Methods

Titrimetric Method of Determination of Pyrethrins I

The petroleum ether layers were extracted twice using 5 ml 0.1M NaOH solution. Chrysanthemic acid was mixed with 0.1M NaOH and placed in 100 ml beakers formed a basic extract, while the petroleum ether layers were discarded.

Mercury Reduction

A volume of 10 ml of Dinige’s reagent was added to the basic extract then kept in the dark for one hour in a water-bath at 25 ºC. The colour of the solution changed gradually to purple-light blue-deep blue-green which is characteristic of Chrysanthemic acid on adding Dinige’s reagent. The intensity of these colours depend on the concentration of the acid. The mixture was then removed from the dark and immediately 3 ml of saturated NaCl solution was added followed by 20 ml amyl alcohol. The saturated NaCl precipitates Mercurous Chloride (HgCl). The precipitate was boiled, filtered and a further 10 ml amyl alcohol added to precipitate remaining HgCl. The white precipitate of HgCl was filtered and the filter paper washed twice with 10 ml of distilled chloroform, so as to remove the alcohol. The beaker was washed with 50 ml of 60% HC1 aqueous solution and the piece of cotton wool into the 200 ml conical flask containing the mixture washed with 50 ml of 60% HC1aqueous solution and the piece of cotton wool into the 200 ml conical flask containing the filter paper and HgCl(s). Further 20 ml of CHC13 was put into the 100 ml beaker and added to the 200 ml conical flask contents. Indicator iodine mono chloride (IC1) 1 ml, was added into the solution and titrated with 0.01M KIO3 solution carried out. The titration was carried out with constant shaking until the pink colour in the chloroform phase just disappeared.

Calculations of Pyrethrins I concentration

The % (w/w) Pyrethrins I was calculated using equation 1,

\[
\% \text{Pyrethrins I} = 0.7125 \times \frac{V_1}{W_1}
\]

where:

- \(V_1\) - Titre volume (volume of 0.01M KIO3 used) (cm3)
- \(W_1\) - Weight of sample (g)

0.7125 - Stoichiometric factor for pyrethrins I

Determination of Pyrethrins II

After evaporating the aqueous layer containing Pyrethrins II in Step 6 above to about 50 ml and cooling to 20 ºC, the solution was transferred into a separating funnel containing a saturated solution of NaCl. The sodium chloride solution supersaturates the aqueous layer and reduces the solubility of pyrethric acid in the aqueous layer. The aqueous layer was then extracted three times with diethyl ether and 10 ml portions of saturated NaCl solution to remove traces of HC1. The diethyl ether layer was then filtered through a cotton plug and the filtrate evaporated to dryness before putting it in an oven at 100 ºC for ten minutes. On removing the aliquot from the oven, a current of compressed air was blown into the flask to remove HCl fumes. The aliquot was then dissolved in 2 ml of neutral alcohol and 20 ml of water was added. The neutral alcohol and water were prepared by neutralizing both with dilute sodium hydroxide to pH 7. The aliquot was titrated against with 0.02N NaOH with phenolphthalein indicator.

Calculation of Pyrethrins II concentration

The percentage (w/w) Pyrethrins II = 0.4675 x \(\frac{V_2}{W_2}\) x TF  

where:

- \(V_2\) = Volume of 0.02N NaOH (cm3)
- \(W_2\) = Weight of Sample extracted (g)
- TF = Titration factor due to the hygroscopic alkali base NaOH

0.4675 = Stoichiometric factor for Pyrethrins II
Ultra-High Performance Liquid Chromatography (UHPLC) Analysis

Samples
One gram sample of the grist was each extracted in triplicates using n-hexane in volumetric flasks. The extract was then left to settle for three hours before injection into the UHPLC instrument. This procedure was repeated the following day for reproducibility.

Instrument
A Varian Model 5000 liquid chromatograph equipped with a UV-100 detector, a flow cell 4.5µl and an integrator model 4400 was used.

Operational conditions
Detector settings – lambda max 230nm; Absorbance range = 0.5AuF
High pressure pump: multi-head reciprocating type capable of minimizing pulsation
Flow rate – 0.8ml/min
Integrator settings: chart speed = 1cm/min
Attenuation = 8; Peak threshold = 6
Pressure: 14-16 atmospheres
Syringe; 10µl Hamilton syringe
Solvents: Acetonitrile – HPLC grade, water: methanol (10:90).
Column: a Varian micropak CN-5 nitrile, 30cm × 4mm id and ambient column temperature.
Aliquots of 20 microliters were injected into the pot to be analyzed which is connected to the column through the sample injection system. The compounds in the aliquot mixture exhibit different flow rates through the adsorbent material in the column resulting in separation and quantification. The stationary phase is made of silica adsorbent material while the mobile phase is the pressurized mixture of solvents, water, methanol and acetonitrile. At the end of each analysis, the data recorded in the computer was passed through a “smoothing” routine to remove random electronic noise and then saved as a digital spectrum. The system was then ready for the next analysis.

The peaks of interest were identified by comparing with those of known standards and where there was ambiguity internal standardization was carried out.

Analysis was carried out in triplicates and the average peak area worked out. All the analyses were done at a wavelength of 230 nm.

Percentage pyrethrins content was calculated as follows:

\[ \text{Percentage of Pyrethrins (w/w)} = \frac{C_{\text{std}}}{C_{\text{std}}} \times \frac{C_{\text{s}}}{C_{\text{std}}} \times \% \text{ Purity of pyrethrin standard} \]

Where
\( H_s = \text{Average peak height of sample} \)
\( H_{\text{std}} = \text{Average peak height of pyrethrin standard} \)
\( C_s = \text{Concentration of sample (gm/1)} \)
\( C_{\text{std}} = \text{Concentration of pyrethrin standard} \)

3. Results and Discussion

Drying at different temperatures at a constant time

The logarithm of moisture content lost at given temperatures was plotted against time.
The graphs above show that much water is lost in the first one hour of drying across all the drying temperatures. After drying for about 12 hrs, there is insignificant change in moisture content loss. This shows that once the moisture content of the flowers is below 10%, high quantity of pyrethrins is obtained. However, when the drying is done for a longer period at temperatures above 60 ºC, this leads to a negative change in the quantity of the pyrethrins due degradation.

**Drying to total dryness at different temperatures and time**

When the flowers were dried to total dryness at different temperatures, they dried at different times.

Table 1. Drying to total dryness at different temperatures and time

| Temperature (ºC) | Time (Hrs) | Weight Lost (%) | Log Weight Lost |
|------------------|------------|-----------------|----------------|
| 40               | 26         | 71.2            | 1.85           |
| 50               | 21         | 75.3            | 1.88           |
| 60               | 19.5       | 75.8            | 1.88           |
| 70               | 18         | 77.1            | 1.89           |

Pyrethrum flowers were dried to total dryness at varying temperatures of 40, 50, 60 and 70 ºC. The logarithm of loss of moisture content was found to marginally increase over the temperature range. The moisture content loss was stable at 50-60 ºC range.

Table 2. Percentage (%) of Pyrethrins on drying at different temperatures for 18 hours

| Temperature (ºC) | Pyrethrins I | Pyrethrins II | Total Pyrethrins | Ratio (PI:PII) |
|------------------|--------------|---------------|------------------|----------------|
| 40               | 0.46         | 0.33          | 0.79             | 1:0.72         |
| 50               | 0.79         | 0.58          | 1.37             | 1:0.73         |
| 60               | 0.77         | 0.53          | 1.30             | 1:0.69         |
| 70               | 0.73         | 0.47          | 1.20             | 1:0.64         |

Total extractable pyrethrins content were found to vary with drying temperature. At 40 ºC, the extractable pyrethrins content was recorded as 0.79% and 1.37% at 50 ºC. However, the total extractable pyrethrins content as well as the Pyrethrins I : II ratio was found to reduce with increase in temperature beyond 50 ºC.

Table 3. Percentage of Pyrethrins on drying to a constant weight

| Temperature (ºC) | Pyrethrins I | Pyrethrins II | Total Pyrethrins II | Ratio (PI:PII) |
|------------------|--------------|---------------|---------------------|----------------|
| 40               | 0.53         | 0.37          | 0.90                | 1:0.70         |
| 50               | 0.83         | 0.61          | 1.44                | 1:0.73         |
| 60               | 0.78         | 0.56          | 1.34                | 1:0.72         |
| 70               | 0.74         | 0.49          | 1.23                | 1:0.66         |

Total extractable pyrethrins content was found to vary with drying temperature, with a peak at 50 ºC and lowest at 70 ºC. At 40 ºC, the pyrethrins content was found to be 0.90% and 1.44% at 50 ºC, implying that, there was loss of pyrethrins at low temperatures. Thus, there were more extractable pyrethrins I and II when flowers were dried at 50 than 40 ºC. However, beyond 50 ºC, both extractable pyrethrins I and II were found to reduce, hence
affecting the ratio and total pyrethrin content. Extractable pyrethrins II were found to reduce by 8.6% when the drying temperature was raised from 50 to 60 °C and by 11.3% from 60 to 70 °C. Extractable pyrethrins I were found to reduce by 6% when the drying temperature was raised from 50 to 60 °C and by 5% from 60 to 70 °C. Extractable pyrethrins II were found to reduce at a higher rate with increase in temperature beyond 50 °C compared to pyrethrins I, thereby affecting the ratio.

High Performance Liquid Chromatographic analyses was carried out on the pyrethrin extracts. The chromatograms attained are presented in Figures 3 and 4. Figure 4 show chromatograms for pyrethrins dried at 50 °C to total dryness.

Results obtained from flowers dried at 50 °C to constant weight are presented in table 4.
Table 4. % Pyrethrins obtained using HPLC

| Temperature(°C) | Pyrethrins I (%) | Pyrethrins II (%) | Total Pyrethrins (%) | Ratio (PI:PII) |
|-----------------|------------------|-------------------|----------------------|---------------|
| 50              | 0.85             | 0.61              | 1.46                 | 1:072         |

4. Conclusion

Total extractable pyrethrins content from pyrethrum flowers was highest at 1.44% and pyrethrins I:II ratio of 1:0.73, when dried at 50 °C at a time of 21 hrs, thereby presenting the optimum drying temperature and time. The flowers were dried to a constant moisture content of less than 10%. Drying flowers at a higher temperature of 70 °C resulted to lower yields of 1.20% and pyrethrins I:II ratio of 1: 0.64 while at 40 °C resulted in 0.79% which is 0.65% lower compared to that of 50 °C.

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