HortScience 56(2):242–243. 2021. https://doi.org/10.21273/HORTSCI15500-20

Influence of Postharvest Drying Temperatures on Alkaloid Levels in Goldenseal (*Hydrastis canadensis* L.)

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Additional index words. alkaloid, medicinal plant chemistry, drying, quality control

Abstract. **Goldenseal (*Hydrastis canadensis* L.) is a medicinal forest herb native to Appalachia. Its roots and rhizomes are used as an antimicrobial and for the treatment of intestinal ailments. Three alkaloids–berberine, hydrastine, and canadine–are recognized as the major bioactive constituents in goldenseal. One important postharvest processing step for goldenseal is drying; however, it is not known how drying temperature influences the concentrations of these alkaloids. In this study, pre-emergent (dormant) goldenseal samples were freeze-dried or air-dried at six different temperatures (26.7 to 54.4 °C) to determine the relationship between drying temperature and alkaloid content in the rhizome and roots. High performance liquid chromatography analysis showed that berberine and hydrastine levels were unaffected by drying temperature, while canadine levels decreased as temperature increased (0.55% w/w on average when samples were freeze-dried, down to 0.27% w/w on average when dried at 54.4 °C). While canadine is the least abundant alkaloid of the three, it is known to have key antibacterial properties. Developing a more standardized drying protocol for goldenseal could lead to a more predictable phytochemical profile.**

Goldenseal (*Hydrastis canadensis* L., Ranunculaceae) is a medicinal herb indigenous to eastern North American forestlands. It is included in many formulations used to treat intestinal infections and digestive ailments, and it is known to have antimicrobial, anticancer, and immunostimulatory properties (Predny and Chamberlain, 2005). The medicinal properties of goldenseal are widely attributed to three major isoquinoline alkaloids (Supplemental Fig. 1A): berberine, hydrastine, and canadine (Abidi et al., 2006; Brown et al., 2008; Mahady and Chadwick, 2001; Scazzocchio et al., 2001).

Most of the commercially traded goldenseal is still wild harvested (Oliver and Leaman, 2018). While drying is an important postharvesting step for goldenseal (Lloyd and Lloyd, 1884), no standardized protocol exists, and various drying methods are used (Personal observation; Upton, 2001). Current "folk" drying methods include drying the rhizomes in the sun and/or shade, in small drying sheds, and in forced air dryers (Davis and Persons, 2014; Personal observation). The most recent recommendations for goldenseal rhizome suggest drying at 35 to 37.7 °C to prevent mold growth and the decay of the rhizome (Davis and McCoy, 2000). Davis and Persons (2014) state that rhizomes will lose 70% of their weight as water evaporation during drying, and temperatures should be kept as low as possible (ideally between 29.4 and 37.8 °C). The American Herbal Pharmacopoeia has identified that determining the optimal drying conditions for goldenseal is an area that needs further research (Upton, 2001). Developing a more standardized drying protocol for goldenseal could lead to more predictable health applications and outcomes by preserving the alkaloids found in the plant. The goal of this study was to determine the influence of postharvest drying temperature on alkaloid levels in goldenseal roots and rhizomes.

Received for publication 5 Oct. 2020. Accepted for publication 4 Dec. 2020.
Published online 12 January 2021.
This research was supported by the Pennsylvania State University College of Agriculture Graduate Student Competitive Grants Program (to G.H.Z.) and was supported in part by USDA Hatch Project PEN 4565 and the Azzara Family Food Bioactives Research Endowment (to J.D.L.).
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Materials and Methods

Plant material. In this study, goldenseal samples were removed from three spatially distinct (e.g., at least 20 m apart) colonies within a wild population located in central Pennsylvania. Fourteen ramets were harvested from each plot in early April while plants were dormant. Voucher specimens were collected and deposited at the Pennsylvania State University Herbarium (PAC).

Processing conditions. The underground portion of the plant consists of a horizontal rhizome roughly 1–1.5 cm in diameter, with multiple fibrous rootlets extending from the rhizome. For this study, the roots and rhizomes (subsequently referred to simply as rhizomes) were processed and analyzed together. This was done because industry typically does not differentiate between these parts. Following harvest, rhizomes were cleaned on a screen by hand under running water to remove any soil.

Rhizome samples were dried using seven different drying conditions (6 samples/drying temperature). Samples were dried until the dried mass was 30% of the fresh weight, and the rhizomes could be broken cleanly (Davis and Persons, 2014). Supplemental Table 1 shows the final mass of the dried samples and the mass of moisture lost. Air-dried samples were dried in an adjustable Lindberg/Blue M 260 Mechanical Oven (Model number MO1490C-1; Thermo Scientific, Asheville, NC), with an air flow rate of 25.5 L/min and specific humidity of 8.9 g H_2O/kg air at 26.7, 32.2, 37.8, 43.3, 48.9, or 54.4 °C (nominal uniformity of 3.5% of the setpoint). The drying temperatures selected cover the range of current recommendations and reach the lower limits that can be obtained while still using a drying oven. One set of samples was freeze-dried using a VirTis Genesis Freeze Dryer (SP Industries, Warminster, PA) to provide a baseline of alkaloid concentrations for comparison. After being dried, samples were stored in air-tight containers at 4 °C until analysis.

Chemical calibration standards. Berberine hydrochloride (purity > 98%) and canadine (tetrahydroberberine) (purity > 98%) were purchased from Quality Phytochemicals LLC (East Brunswick, NJ); (-)-β-Hydrastine (purity > 99%) was purchased from ChromaDex (Irvine, CA).

High-performance liquid chromatography analysis. Dried rhizomes were ground using a mortar and pestle. Fifty mg (±10%) of ground material was combined with 4 mL of extraction solvent (70% water, 29.9% acetonitrile, 0.1% phosphoric acid). Tubes were vortexed to mix, sonicated for 10 min at room temperature, and centrifuged for 8 min at 3220 g_n. The supernatant was diluted 1:4 with 10% aqueous acetonitrile and filtered through 0.2-µm nylon filters.

High-performance liquid chromatography (HPLC) analysis was performed using a Shimadzu HPLC system (Shimadzu Co., Columbia, MD) equipped with two LC-20AD pumps, a SIL-20AC HT refrigerated auto injector, a column oven maintained at 24 °C, and an SPD-20AV ultraviolet/Vis detector. A binary gradient of water containing 0.1% formic acid (solvent A) and methanol containing 0.1% formic acid (solvent B) with a flow rate of 0.75 mL/min was used. The initial mobile phase was 20% B. The concentration of B increased linearly for 15 min to 45%, and this was held at the concentration for 5 min. The mobile phase was then returned to 20%, and the HPLC was re-equilibrated for 7 min. Analytes were separated using a Zorbax Eclipse XDB-C18 column (4.6 × 150 mm, 3.5 µm particle size, 80 Å pore size). Eluent was monitored at 280 nm.

Statistical analysis. All statistical analysis was completed in R-studio version 3.5.1 (RStudio Team, 2015). Analysis of variance was calculated among the different drying conditions for each of the three major alkaloids, and Tukey's honestly significant difference was used for post hoc analysis. Level of significance was set at $\alpha = 0.05$.

Results and Discussion

Hydrastine, canadine, and berberine had retention times of 9 min, 14.5 min, and 16 min, respectively (Supplemental Fig. 1B) and were clearly resolved by our gradient conditions (Supplemental Fig. 1B). HPLC analysis showed that neither berberine nor hydrastine content were influenced by increasing drying temperatures (Supplemental Fig. 2). Canadine levels were significantly lower in samples dried at temperatures above 30 °C (Supplemental Fig. 2), which is lower than the current recommendation for drying goldenseal (Davis and McCoy, 2000). When samples were dried at 26.7 °C, average canadine levels were 0.46% w/w, compared with 0.34% w/w when dried at 32.2 °C. Canadine levels in samples dried at 54.4 °C were less than 50% of those in freeze-dried samples, going from an average of 0.55% w/w when freeze-dried to 0.27% w/w when dried at 54.4 °C (Supplemental Fig. 2).

One explanation for the difference in findings between the alkaloids is that berberine and hydrastine are both end products of biosynthetic pathways, while canadine is an intermediate step in berberine biosynthesis by the action of the enzyme tetrahydro-protoberberine oxidase (Mander and Liu, 2010). As such, an increase in the enzymatic activity of the oxidase would result in a decrease in canadine levels; and it is known that as temperature increases, enzyme activity increases up to the point of enzyme denaturation (Daniel and Danson, 2013; Elias et al., 2014). The results for canadine are consistent with this hypothesis.

While canadine is the least abundant of the three alkaloids, it is still potentially an important contributor to the overall efficacy of goldenseal. When isolated, canadine has been found to have significant activity against numerous strains of bacteria, and it is the only one of the three major alkaloids found to be active against *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Abbasoglu et al., 1991; Scazzocchio et al., 2001). Further, canadine possesses significant antioxidant properties (Correché et al., 2008), and it has also been identified as effective at upregulating low-density lipoprotein receptor expression (Abidi et al., 2006). Additional research is needed to determine if the lower canadine alkaloid reductions we obtained (because of higher drying temperatures) is of any significance to human efficacy or health benefits.

This study provides a phytochemical basis for guidance that temperatures less than 30 °C results in a superior product by preserving three of the key alkaloids currently of importance to product quality: berberine, hydrastine, and canadine.

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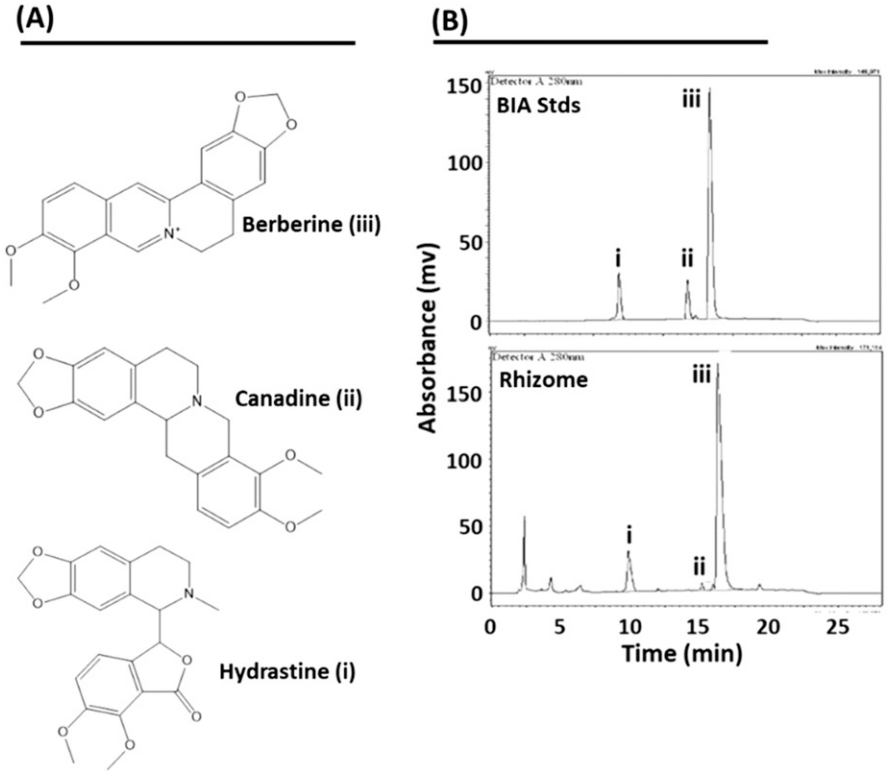
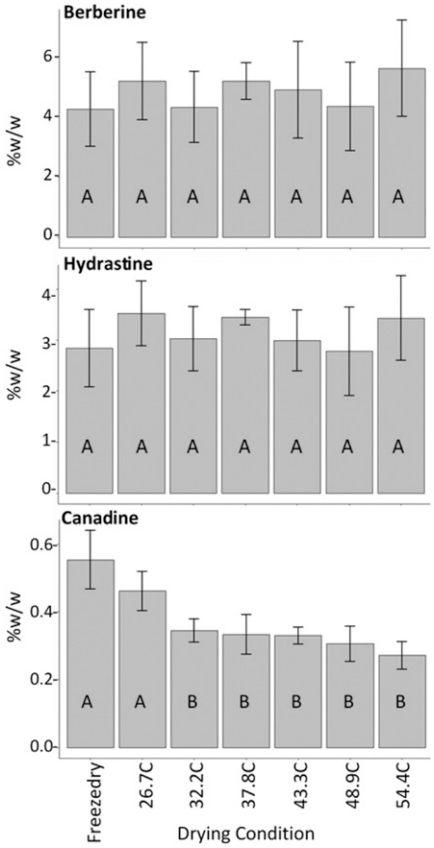
Supplemental Table 1. Drying conditions and sample metrics of goldenseal rhizome samples.[z]

Drying temp	Drying time (h)	Fresh weight (g) Mean ± SD	Dry weight (g) Mean ± SD	Moisture removed (% fresh weight) Mean ± SD
Freeze-dry	-	3.21 ± 2.08	0.94 ± 0.64	71.4 ± 3.3
26.7 °C	74	3.79 ± 1.89	1.07 ± 0.53	71.7 ± 0.8
32.2 °C	55	3.74 ± 2.17	1.12 ± 0.74	71.0 ± 2.8
37.8 °C	41	3.60 ± 0.63	1.03 ± 0.24	71.7 ± 3.2
43.3 °C	42	3.90 ± 1.40	1.08 ± 0.42	72.5 ± 1.4
48.9 °C	30	3.40 ± 1.05	0.98 ± 0.34	71.4 ± 2.1
54.4 °C	14	3.82 ± 1.13	1.15 ± 0.39	70.4 ± 2.2

[z]Forced air drying was done with an air flow rate of 25.5 liters per minute and specific humidity of 8.9 grams H_2O per kilogram of air (n = 6).



Supplemental Fig. 1. HPLC analysis of the major benzylisoquinoline alkaloids (BIA) in goldenseal (*Hydrastis canadensis*). (**A**) Chemical structures of berberine, canadine, and hydrastine. (**B**) Representative of HPLC-DAD chromatogram of alkaloid standards and of whole rhizome goldenseal sample. Key: i = hydrastine, ii = canadine, iii = berberine.



Supplemental Fig. 2. Effect of drying condition on alkaloid concentrations in goldenseal rhizomes. Data are expressed as the percent that each alkaloid is, in relation to dry weight. Data are presented as the mean of n = 6 biological replicates; error bars represent 95% confidence. Within each alkaloid, bars with different letters are significantly different by one-way analysis of variance with Tukey's honestly significant difference post-test ($P < 0.05$).