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Original Article

Long-term Effect of Drying Method on the Alkaloid of *Atropa belladonna* L. Leaves During Storage

Mohaddeseh Amiri[1*], Akbar Arabhosseini[1] and Mahboubeh Zare Mehrjerdi[2]

[1]*Department of Agricultural Engineering, University of Tehran, Tehran, Iran*
[2]*Department of Horticulture, University of Tehran, Tehran, Iran*

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ABSTRACT

Atropa belladonna L. is a perennial plant belonging to the Solanaceae family. The plants of Solanaceae family are the best-known source of tropane alkaloids primary hyoscyamine and scopolamine that act on the Parasympatic nervous system. The purpose of the present study was to determine the effect of storage on the alkaloid content of *A. belladonna* L. leaves dried by different traditional drying methods such as shade drying, sun drying, and also hot air drying at temperature of 80 °C and oven drying at temperature of 80 °C. Alkaloid compounds were measured after 1, 15, 30, and 60 days of storage. The alkaloids were determined by high-performance liquid chromatography (HPLC) method immediately after drying and during storage. Analysis of the experimental data revealed that the amount of total alkaloid, atropine, and scopolamine was significantly different ($p< 0.01$) for different drying methods and storage time. The total alkaloid was significantly at the lowest level in sun-dried samples while it was at the highest level in shade-dried samples. The atropine and scopolamine contents increased significantly just after one-day storage in the samples dried by hot air drying at 80 °C. Storage caused fluctuation in the amount of alkaloids compounds. The atropine content decreased in the oven drying method at 80 °C on the first day of storage and increased after 60 days of storage. The scopolamine content increased in the oven drying method at 80 °C after 15 days of storage.

INTRODUCTION

Atropa belladonna L. is an annual plant of the Solanaceae family. It is a wild-growing plant, which is a local source of tropane alkaloids, including anticholinergic drugs atropine and scopolamine [1,2]. Medicinal plants have been one of the first and most accessible resources used for remedying sickness. With a growth in development of research on herbal medicinal effects and their uses, caused increasing in production and supply of new herbal treatments [3,4]. The tropane alkaloids, isolated from the plants for therapeutic purposes, are highly toxic and should be dosed carefully [5,6]. Alkaloids of *A. belladonna* are used as anti-emetics, anti-spasmodic, in therapy (in ophthalmology, cardiology, gastroenterology, etc.) and also used to treat Parkinson's diseases because of its naturally occurring alkaloids, atropine, and scopolamine in the late 1800s [7,8]. Hyoscyamine is usually the main alkaloid in many of these plants like the genus *Atropa*, *Datura*, *Duboisia*, *Hyoscyamus* and *Scopolia*. In contrast, scopolamine is often produced in small amounts [9,10]. Scopolamine is more valuable and desirable to Hyoscyamine in the pharmaceutical market because of its higher physiological activity and fewer side effects [11,12]. Most recently, there is a tenfold higher commercial request for scopolamine than for both Hyoscyamine and atropine [12,13]. Medicinal plants should have certain moisture content before analysis to prevent damage to the active chemical components during the drying process. In order to reduce damages from microbial invasion, the moisture content of the dried material should be kept as low as possible regarding to WHO guidelines (2013) [14]. Drying process is one of the factors that can influence on the chemical

[*]Corresponding author: Department of Agricultural Engineering, University of Tehran, Tehran, Iran
Email Address: amiri.mohaddeseh@alumni.ut.ac.ir

analysis on the final concentrations of the alkaloids and different drying methods has a significant effect on the concentration of alkaloids [15]. In order to maintain the quality and improve the efficiency of the dried medicinal plants especially the roots, the harmful substances are removed because of pre-processing operations like chopping and cleaning [16]. Drying is essentially defined as the process for decreasing of plant moisture content, that affect the physical and chemical properties of plants [17]. Also, the first step in many postharvest processes, is removal of water that is also drying [18]. Large volumes of fresh harvested medicinal plants in transportation and storage period causes problems such as reproduction of microorganisms and plant tissue degradation. For this reason, these changes can be minimized by appropriate design of the drying process [19,20,21]. Drying is a complex process because medicinal plants are often dried and stored for a long time until they use in manufacturing several types of products, which affects the quality of medicinal plants in many ways [21,22]. Drying of medicinal plants can be classified into natural and artificial drying methods based on heat source or energy utilization [19]. Since long time ago, the natural drying methods such as shade or sun drying have been used as a popular method and easiest way for drying of medicinal plants. In many natural drying methods, evaporation of the water from the product is done using solar energy and the energy of the wind [16,23]. The shade and sun drying methods are cheap and do not need any extra thermal energy. The disadvantage of this method is its dependence on favorable weather conditions and long drying time [19]. Several drying processes are used to dry such plants, including oven drying, microwave-drying, hot air drying, far infrared drying, vacuum drying, and spray drying [21,24]. The advantages of the oven drying method can be the short drying time and temperature control, because the long drying time can negatively influence on the quality and quantity of compositions in medicinal plants [25]. Chemical changes during and after the drying process are important and can be influenced by the remained moisture content in the medicinal plants at the end of the drying [18]. The main objective is to reduce the metabolic activity of medicinal plants in order to prevent deterioration during storage. Most of the drugs with moisture content about 10–14% can be stored for a long time without any microbial damage of the plants and also without using any chemical additives [16,18].

Drying of potato peels, shoots and berries significantly increased the steroidal alkaloids content compared to the fresh samples [26]. Alkaloids concentration of Khat (*Catha edulis* forsk) leaves was much lower in the samples dried with sun drying method than the other methods [27]. The cathinone concentration of Khat (*Catha edulis* forsk) resulted in about 57% in hot air drying and 42% in sun-drying method [27]. Alkaloids appeared to be affected by mechanical drying. The effects of drying methods on *Catha edulis* plant for protection of cathinone was investigated by Chappell and Lee [28]. The product was dried in a room at an ambient temperature of 20 °C, convection oven drying at 55 °C and microwave oven drying at 100 °C and the amount of alkaloids at these drying methods were 5.233, 5.771, and 5.070 (mg/g dry weight) respectively. Alkaloids concentration was lower in the samples dried by convection oven at 55 °C than the microwave oven at 100 °C [28].

The amount of alkaloids from Ponderosa Pine were significantly affected by storage time and drying methods such as air drying, freeze-drying, and oven drying. When pine tree samples were stored for 3 to 5 weeks, the concentrations of dehydropinidinone and euphococcinine were significantly affected by the drying method and storage time [29]. Storage conditions (temperature, light, and time) affected the glycoalkaloid content of potato tubers and sprouts. During storage, the level of glycoalkaloids increased, and the amount of glycoalkaloid was more in the samples stored under light than those stored in the darkness [30].

The drying process is essential for alkaloid compounds because of the changes in their concentrations during drying and storage. Yet, no study has documented a suitable drying method to preserve alkaloid compounds in dried *A. belladonna* L. during storage time. In this research, the relation between the drying process and the amount of *A. belladonna* L. compounds is studied. Therefore, the objectives of this study were to investigate the impacts of drying methods and storage time on the amount of major compounds in *A. belladonna* L. leaves.

MATERIALS AND METHODS

Sample Collection

The *A. belladonna* L. leaves were collected from a natural habitat in the forests of Ramiyan in the latitude of 50° 36' north and 55° 13' longitude in the Golestan province, Iran, at the end of May 2017, when the plants started flowering. Subsequently, the samples were transferred to the postharvest laboratory, and then the plant leaves were chopped into equal pieces and dried by different drying methods.

Drying methods

The pieces of leaves with almost identical dimensions were dried in the shade, under direct sunlight (sun drying) and also by hot air and oven drying methods. The drying procedure was carried out until the moisture content of the samples reached to about 13% (w.b.) in all drying treatments. The weight loss was measured using an analytical balance with an accuracy of 0.001 g (Mettler Toled (AB204-S/FACT), Switzerland). All treatments were replicated three times.

Shade-drying (SH)

The shade-drying method was carried out in a dark and dry room with appropriate ventilation. The temperature of the room was 25 ± 2 °C, and air humidity ranged from 33 to 44% during the drying period.

Sun-drying (SD)

In sun-drying method, a piece of cloth was laid between plant material and ground to avoid plant material infection. Plant materials were distributed on the cloth in thin layer for raising the speed of drying [14]. The maximum daily temperature during sun-drying was between 38.5 °C and 42 °C.

Oven drying

In the oven drying method, the samples were dried at 80 °C. The *A. belladonna* L. leaves were distributed in an aluminum container, and the weight of the specimens was measured every 15 minutes in the first hour and every hour after that, until a constant weight was obtained [27].

Hot air drying

A laboratory dryer set was used for the hot air drying of the samples. In the hot air drying method, the samples were dried at 80 °C. The dryer was equipped with some devices to control the temperature and airflow velocity. The weight of the specimens was measured every 5 minutes in the first hour, and then every 15 minutes until a constant weight was obtained.

Storage conditions

The dried leaves were covered with foil sheets and then packed in plastic bags. The samples were stored in a refrigerator at 4 °C to evaluate the quality of the samples during storage. Alkaloid compounds were measured after 1, 15, 30, and 60 days of storage. All experiments were repeated three times.

Sample extraction

The Kamada *et al.*, method was used to extract the tropane alkaloids of the collected plants leaves [31]. An amount of 0.5 g *A. belladonna* leaves was powdered and then sonicated with 40 ml solvent contains $CHCl_3$, methanol and ammonia-25% (15:5:1) (v/v/v) for 10 min by using an ultrasound device [32,33]. The extraction solution was stored at room temperature for one hour, then purified by passing the solution through a paper filter, washed twice with 1 ml $CHCl_3$. The solvent was evaporated to dryness under vacuum (Hei-VAP Value R-280, Germany) at 40 °C and the residue was dissolved in 5 ml $CHCl_3$ and 2 ml 1 N H_2SO_4. The $CHCl_3$ fraction was removed and adjusted until the pH reached to 10 with ammonia 28% on ice [34]. The extraction was done once with 2 ml $CHCl_3$ and then twice with 1 ml $CHCl_3$. Dehydration of the extract was done with dry sodium sulfate and the extract was purified by filter paper and washed the residue with 2 ml $CHCl_3$. Finally, the evaporated of $CHCl_3$ was dissolved in 2 ml of methanol [35,36].

Analytical determinations

UV-Vis Spectrophotometry

The total alkaloid content was determined by spectrophotometer. The UV-Vis spectra of all the extracts were recorded in the region of 258 nm with a spectrophotometer (Photonix Ar 2015, Iran). The measured aliquots (1, 2, 4 and 6 mg/ml) of various concentrations of atropine (Sigma Aldrich) was plotted at 258 nm [37,38].

HPLC Analysis

The atropine and scopolamine contents in the sample extracts were determined by HPLC. The HPLC device was equipped with Eurospher C18 column (25 cm × 4 mm i.d., RP) and a UV detector [11]. Elution was monitored at 210 nm. The mobile phase was made up of methanol, phosphate buffer, and

acetonitrile 2:45:53 [33]. Sample injection was 20 µl, and the analysis was performed at a flow rate of 1.0 ml/min for 10 min. Atropine and scopolamine from Sigma-Aldrich were prepared in methanol at different concentrations (25, 50, 100, 300, 400 ppm). A 20 µL volume of each standard solution was injected onto the HPLC column [35,39].

Statistical analysis

The design of the experiments was split plot design based on a completely randomized design. All experiments were repeated three times for 1gr sample total alkaloid (mg/gdw). The data was analyzed by Statistical Analysis System software package (SAS Institute, Cary, NC, USA). For assessing a significant difference among treatments, Duncan's multiple range tests were performed to determine the differences among the mean values.

RESULTS AND DISCUSSION

Effect of Drying Methods on *A. belladonna* L.

Drying methods significantly ($p< 0.01$) affected the amount of total alkaloids of *A. belladonna* L. leaves (Table 1). The total alkaloid content was at highest level in shade drying process. According to the research of Atlabachew *et al*. [27], the amount of total alkaloid decreased in the samples dried by the sun-drying method. The hot air drying method was better than the oven-drying method at temperature of 80 °C. The hot air drying method is a slow process, losing moisture while the tissues are still biologically active could be considered as stress for the tissues. Degradation might had been taken place during the oven drying method before the moisture level and temperature of the sample got unfavorable for metabolic processes [26].

Among the constituents of *A. belladonna* L. the most vital constituents are atropine and scopolamine, which are valuable in the pharmaceutical market and their concentrations were significantly affected by the drying methods. The amount of atropine varied from 0.82 to 2.26 (mg/g dry weight) at different drying methods (Table 1). The amount of alkaloid compounds changed by changing the drying method. Drying methods significantly affected the amount of alkaloids [26,27,29]. In sun-dried samples, the amount of total alkaloid was in lowest level while atropine content was in average level at similar drying conditions.

The amount of scopolamine varied from 0.05 to 0.28 (mg/g dry weight) at different drying methods (Table 1). The amount of scopolamine increased in hot air-dried samples at 80 °C. Also, the scopolamine content was in the average level, when the samples were dried under sun light. These results are similar to the amount of atropine at the same conditions.

Effect of Storage Time on *A. belladonna* L.

Storage of the samples showed different effects on alkaloid compounds for the samples, dried with different drying methods. Drying methods showed no change in the amount of total alkaloid after 30-days storage whereas shade-drying showed an increase in the amount of total alkaloid at first day of storage and a decrease after 30-days storage. After 60 days, the samples dried by shade-drying method showed an increase in the amount of total alkaloid (Table 2). The oven drying at 80 °C showed an increase in the amount of atropine after 60-days storage (Table 2). After first day of storage, the hot air drying method at 80 °C showed an increase in the amount of scopolamine. It should be noted that the hot air drying method at 80 °C for the scopolamine content was lower than that observed for the atropine content after 60-day storage (Table 2). Alkaloid compounds were not stable during 60 days of storage, and during this time significantly ($p< 0.01$) increased or decreased (Table 2). The fluctuations of the alkaloid compounds during the storage may be because of two different aspects. One of them is the degradation which decreased the amount of the alkaloid compounds [29]. The other one is the establishment of the low molecular weight alkaloid compounds due to the degradation of the high molecular weight alkaloid compounds [40]. The physiological and chemical processes in tissue plant during storage, through active metabolism caused fluctuation in the amount of alkaloids compounds. Decrease the amount of alkaloids compounds with drying can occur by enzymatic degradation [29]. In previous studies, alkaloid yields were most stable for the oven-dried samples amongst all samples. Although the reason for the stable concentrations was not clear for oven-dried samples, alkaloid yields in the different drying methods used in this study was different than the previous reports, which was fluctuated for 8 weeks.

The process of changing the total alkaloids is almost decreased for 30 days of storage. On the other hand, there was an almost increasing trend in the next month (Table 2). The amount of alkaloids compounds increased by increasing the storage time,

while the results of this research showed that the amount of alkaloids compounds increased at the first day of storage and after that decreased and increased during the 60 days of storage [29,30]. In all drying processes, the intercellular spaces of tissues collapse, liberating more bioactive secondary metabolites such as alkaloids [41]. Sun *et al*. [25] stated that synthesis or decomposition of enzyme activity, were caused an increase in compounds concentration in the second week, decrease in the third week and increased again in the fourth week of storage. Lin *et al*. [21] also found that a considerable level of degradation occurred in samples stored under high relative humidity (>80% RH) because of re-uptake of moisture. Therefore, the observed degradation of dried samples might be resulting from increased moisture content during long-term storage.

CONCLUSION

In this study, the alkaloid compounds of fresh as well as dried *A. belladonna* L. leaves was studied just after drying and during the storage. Based on the obtained results, drying temperature affects the quality and concentration of compounds in medicinal plants. The shade drying was found to be the best method among the examined drying methods for retention of alkaloid compounds. The total alkaloid reached to the highest level (21.18 mg/g dry weight) after 60 days of storage in the samples dried in shade-drying. The highest level of scopolamine content (0.28 mg/g dry weight) was obtained in the samples dried with hot air drying method at 80 ℃ after one day of storage. The highest level of atropine content was recorded in the samples dried with hot air drying method at 80 ℃ after one day of storage.

Table 1 Effect of drying methods on the amount of alkaloid compounds in *A. belladonna* L. leaves after one day storage

Drying method	Compounds (mg/g dry weight)		
	Total alkaloids	Atropine	Scopolamine
Oven 80 °C	14.84 **	0.82 **	0.05 **
Hot air 80 °C	15.02 **	2.26 **	0.28 **
Sun drying	14.04 **	1.45 **	0.17 **
Shade	15.80 **	0.93 **	0.12 **

An asterisk (**) on a column denotes significant differences (p< 0.01); Different letters (a, b, c, d) on each column represent statistically significant differences (p< 0.01) between the samples evaluated.

Table 2 Effect of drying methods during storage times on the amount of alkaloid compounds in *A. belladonna* L. leaves

Drying method	Storage times (day)	Total alkaloids (mg/g dry weight)	Atropine (mg/g dry weight)	Scopolamine (mg/g dry weight)
Oven 80 °C	1	14.84 **	0.82 **	0.05 **
	15	12.99 **	1.09 **	0.04 **
	30	11.03 **	1.17 **	0.11 **
	60	18.37 **	1.74 **	0.11 **
Hot air 80 °C	1	15.02 **	2.26 **	0.28 **
	15	12.44 **	1.27 **	0.03 **
	30	14.27 **	1.66 **	0.14 **
	60	13.28 **	1.29 **	0.06 **
Sun drying	1	14.04 **	1.45 **	0.17 **
	15	12.25 **	1.21 **	0.04 **
	30	12.41 **	0.97 **	0.005 **
	60	18.56 **	0.48 **	0.10 **
Shade drying	1	15.80 **	0.93 **	0.12 **
	15	12.22 **	0.98 **	0.04 **
	30	10.17 **	0.85 **	0.02 **
	60	21.18 **	0.47 **	0.06 **

An asterisk (**) on a column denotes significant differences (p 0.001); Different letters (a, b, c, …) on each column represent statistically significant differences (p 0.01) between the samples evaluated.

The findings of this research indicated that selection of appropriate drying method, proper to drying temperature and storage time are very important for obtaining the maximum alkaloid compounds of *A. belladonna* L. leaves. However, plant biosynthesis in different drying methods and during storage time are complex, and this mechanism needs to be studied further.

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