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Summary

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Comparative evaluation of the drying characteristics and quality attributes of Sarpagandha roots of various maturity stages using hot air, solar, sun and shade drying

Vergleichende Bewertung der Trocknungseigenschaften und Qualitätsmerkmale von Sarpagandha-Wurzeln verschiedener Reifestadien unter Verwendung von Heißluft-, Solar-, Sonnen- und Schattentrocknung

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Sarpagandha (Rauwolfia serpentina) root of Grade 1 (G1) and grade 2 (G2) of 6, 12 and 18 months maturity stages were dried using four different drying methods viz. sun drying (SD), shade drying (SHD), solar drying (SLD) and hot air drying (HAD 50°C, HAD 60°C, HAD 70°C). Drying characteristics for different drying methods, root grade and maturity stage have been explored and their influence on moisture ratio versus drying time was assessed using an ANN model. The phytochemicals such as total phenolic content (TPC), flavonoid, ascorbic acid and tannin as well as the antioxidant activity of all the dried samples have been evaluated. Drying time requirement for sarpagandha roots to achieve final moisture content of about 4.0 to 6.95% d.b. varied between 150-750 mins for HAD and 4-15 days for traditional drying (SD, SHD, SLD) methods also Grade 1 samples took more time than grade 2 samples irrespective of drying methods and maturity stage. The ANN model using logsig and transig transfer functions could best predict the moisture ratio vs drying time (R²=0.999) for HAD and (R²=0.995) for traditional methods respectively. The study revealed that the samples of 18 months maturity possessed maximum amount of all the bioactive compounds and antioxidant activities. Among the hot air drying, the samples dried at 60°C and among the traditional methods, the shade dried samples could retain significantly higher amount of most of the phytochemicals and antioxidant properties.

Keywords: Drying, maturity stage, kinetics, artificial neural network, phytochemicals

Introduction

Rauwolfia serpentina (R. serpentina), also commonly known as Sarpagandha has been pronounced as a "Wonder drug of India", is an endangered medicinal plant, of family Apocynaceae (De and Dey, 2010; Singh et al., 2010a). Use of its root as a medication in Ayurveda for management of hypertension, nervous disorders, cardiovascular disorders, psychiatric disorders, rheumatism, insomnia, eczema, fever, anxiety epilepsy and snake bites are well documented (Manuchair, 2002; Negi et al., 2014). Although the roots are the major source of active principles, leaves, stem, fruits, seeds and flowers are also being utilized to treat different diseases (De and Dey, 2010). It has proven to be an effective antihypertensive and is known to be the world's first anti-hypertensive drug (Singh et al., 2015). R. serpentina comprises of numerous bioactive chemicals such as flavonoids, tannins, phenols, minerals, vitamins and more than 50 different alkaloids such as Reserpine, Ajmaline, Ajmalicine, Ajmalidine, Yohimbine etc. (Gao et al., 2012). Presence of high quantity of total polyphenolic compounds in R. serpentina shows significant antidiabetic and hypolipidemic properties (Azmi and Qureshi, 2013). Tannins possess stringent properties whereas flavonoids have antioxidant, anticancerous and anti-inflammatory activity (Harisaranraj et al., 2009). Usually, the roots of R. serpentina plant are used in extraction of the above mentioned phyto constituents as it is rich in them as compared to other parts of the plant. For instance, of all the alkaloids present, reserpine is the most important indole alkaloid and 72% of it is present in the roots, 25 and 3% are present in stem and leaves of the plant respectively (Panda et al., 2010; Hazra et al., 2018). However, due to high demand but low availability of R. serpentina roots (as the plant is endangered), its roots have been found to be adulterated with other species such as Ophiorrhiza mungos, red and white flowered Clerodendrum and Tabernaemontana divaricate (Suliman et al., 2020). This has led to degradation in quality of finished herbal products. Moreover, the problem is compounded by the fact that the root of R. serpentina plant has a short shelf-life. To extend the shelf-life of R. serpentina roots, appropriate form of preservation is needed. Drying is one of the oldest and most widely used method of food preservations. Since long, it has been a common and fundamental way to preserve the quality of aromatic and medicinal plants (Müller and Heindl, 2006). However, several published studies have revealed that drying alters the phytoconstituents and antioxidant properties of the different components of medicinal herbs (Ademiluyi, 2018; Barimah et al., 2017; Kumar et al., 2018; Nguyen et al., 2016; Shaw et al., 2016). Moreover, the biological activities of the plants are also dependent on the stage of growth of plant. Findings of Singh et al. (2010b) revealed that the stage of plant development contributes to the phytochemical contents. Hence, standardization of maturity stage is equally vital to identify the stage of the plant root which can provide maximum yield of certain health promoting phytoconstituents. The preliminary studies further indicated that the roots of sarpagandha plants vary in its girth sizes when harvested. Therefore, it becomes imperative to categorize them in to suitable grades based on size in order to achieve uniform drying which is very essential to maintain the quality attributes. Studies investigating the effects of grade, stage of maturity and different drying methods on health promoting bioactive chemicals present in R. serpentina roots as well as the drying characteristics of the plant roots are lacking.

The study was aimed to investigate the effect of stage of maturity and selected drying methods (sun, shade, solar and hot air oven drying) on the health-promoting properties of *R. serpentina* roots. First, the drying characteristics such as drying air temperature, relative humidity, moisture depletion, moisture ratio and drying time for different drying methods, root grade and maturity stage have been explored. Next, the drying kinetics in terms of moisture ratio versus drying time using an ANN model has been assessed. Further, the influence of drying methods and stage of maturity on phytochemicals (total phenol, flavonoid, ascorbic acid and tannin) and antioxidant activity was evaluated.

Materials and methods

Sample Collection

Fresh and healthy Sarpagandha (R. serpentina) roots of three different maturity stages (6, 12 and 18 months) were harvested and collected in the early morning in the month of December from the medicinal garden of All India Coordinated Research Project on Medicinal and Aromatic Plants, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India. Very fine hairs of roots and adhered small lumps of soil attached to the surface were removed easily without scratching the bark. Post dry cleaning, gentle wash was carried out by tap water after dipping the roots in water for few minutes. Then extra water on the surface of the roots was wiped out with the help of tissue paper. During preliminary experiments of drying, it was observed that the process could not be conducted uniformly because of heterogeneity in the girth of roots. There was a large variation depending on stage of maturity and the location of roots. Therefore, it was essential to classify them into grades of some range. Root samples were cut into small pieces ranging between 5-10 cm sizes carefully using a stainless steel knife. Then for the cut section of roots grading was done as grade 1 (G1), grade 2 (G2) depending on the girth of the roots (Table 1). Then all the samples of G1 and G2 were dried separately using different drying methods. Initial moisture content of fresh Sarpagandha roots was determined by standard method (AOAC, 2005).

Chemicals

The analytical grade chemicals used for this study were purchased from Himedia Laboratories, Mumbai, India; Sisco Research Laboratories, Mumbai, India and Sigma Aldrich, Bengaluru, India.

Drying processes

Experiments were conducted using four different drying methods for drying of Sarpagandha roots. Sun drying (SD), shade drying (SHD), solar drying (SLD) and hot air drying (HAD) at 50, 60 and 70°C were considered to select the best method based on the quality characteristics. A hot air convective cabinet dryer and solar dryer was used for drying purpose. To achieve and enhance steady conditions before the start of drying experiment, the drying system was run for about 30 minutes. Initial weight of roots was noted in each case. Subsequent observations on weight were taken at 30 minutes interval in case of hot air drying (50, 60 and 70°C) whereas for sun, solar and shade drying subsequent observations on weight were taken initially at 1 h then 3 h and after that at 5 h interval. A digital balance (accuracy: ± 0.001 g) was used to record moisture loss du-

ring the drying process at each interval. Temperature and relative humidity was recorded using digital thermometer. Drying was carried out until the consecutive weight readings were recorded indicating that the final moisture content reached an equilibrium conditions. Grinding of dried Sarpagandha roots was done in the mixer grinder to get the powder in the form of fine particle (60 mesh) and was packed in plastic bottle with proper label.

Sun and Shade drying

In both these methods, aluminum trays $(75\times60 \text{ cm}^2)$ were used to carry out drying of Sarpagandha roots. In sun drying, root samples of 6, 12 and 18 months (grade 1 and 2) were spread uniformly in a single layer on the aluminum trays and placed 10 cm above the concrete floor under direct sun light from 9.00 am to 5.00 pm. In shade drying, a single uniform layer of roots was put over the aluminum trays and placed 10 cm above the concrete floor inside a room throughout. At different time intervals, the air temperature and RH (%) were measured and the average values were reported.

Solar drying

The solar dryer developed at CAET, OUAT, Bhubaneswar was used for the present investigation. The choice of the solar dryer was based on its requirement of fuel or electrical energy and suitability for summer and winter days. The chosen solar dryer requires none and is all weather suitable. The solar dryer consisted of a drying chamber with two racks, metal surface inside and a glass lid on the top. Once subjected to sunlight, solar radiation enters the box from the top and heats up the air inside the box.

The drying experiments were carried out at a site with full exposure to sunlight. Solar drying experiments were usually started at 9 am and ended at 5 pm. For thermal stabilization of the solar dryer, it was run empty for about half an hour. Then, the root samples of 6, 12 and 18 months (grade 1 and 2) were spread in a single layer on the top rack of solar dryer for the dehydration process to begin. Similar to the sun and shade drying methods, the air temperature and RH (%) of the air inside the cabinet of solar dryer was measured.

In all these traditional methods, subsequent observations on weight were taken at 1, 3 and 5 h interval. Moreover, plastic covers were used to keep the samples during the night hours.

Hot air drying

Hot air drying experiments under controlled conditions were conducted for Sarpagandha roots at 50, 60 and 70°C using a hot air convective tray dryer (IIC, Model TD-12). The hot air dryer consists of four major components, namely centrifugal blower, electrical resistance air heating section, measurement sensors and displaying unit. The sample tray was placed and removed through a door at the front of the chamber. As mentioned above with solar drying method, thermal stabilization was achieved at the start in this method by letting the dryer run with no load for about thirty minutes. The air velocity was measured throughout the processing an anemometer (Lutron AM-4201). Once the desired drying air temperature was attained, root samples of 6, 12 and 18 months (grade 1 and 2) were kept into the drying trays in a single layer. Next, weight loss measurements were taken every 30 minutes interval until the weights of the samples became constant. The drying rates were computed and drying characteristics curves were plotted using the observed experimental data.

Phytochemical analysis and antioxidant activity

The phytochemical parameters studied were total phenolics (TPC), flavonoid, ascorbic acid and tannin. Root powder of G1 and G2 (1:1) of each maturity stages was mixed and then used for analysis for each drying method. During the preliminary experiments the quality analysis was made separately for G1 and G2. No significant difference of quality parameters was observed between these two grades. So in the final experiments no discrimination was made on quality analysis.

Total phenolic content and antioxidant activity measurement

For preparation of extract, 1 g of root powder of all 18 samples was weighed and 80% methanol was prepared. The powder was then soaked into 10 ml methanol (80%) for 24 hours and filtered with filter paper. Total phenolic content of each dried sample was determined by Folin-Ciocalteau method with slight modification using Gallic acid as a standard. Initially, each sample extract (0.5 ml) was mixed with distilled water (7.5 ml) and 1 ml of Folin-Ciocalteu reagent was added to it. 1 ml of 20% sodium carbonate (Na₂CO₃) was then added after 2 minutes and shaken well. The colour was developed and after 45 min reaction at room temperature in dark, absorbance was measured at 750 nm in UV-Visible Mini1240 spectrophotometer (Shimadzu Co., Japan). Results were expressed as mg GAE/100g of dry matter using the formula for equivalent:

Total phenolic =
$$\frac{c}{1000} \times \frac{10}{x} \times \frac{10}{w} \times 100$$
 (1)

where, c = concentration, x = Aliquot taken (ml) and W = Weight of sample taken (g)

Determination of antioxidant activity (of sample) was done by DPPH inhibition method (Nishino et al., 2000). To 3 ml of each sample extract, 3 ml of DPPH (2, 2- diphenyl-1-picrylhydrazyl) solution was added. After the mixtures were shaken properly, they were incubated at room temperature for 45 minutes in dark. For the control sample, 3 ml of methanol was mixed with 3 ml of DPPH solution and pure methanol was taken as blank. The reduction in colour of the solution caused by free radicals (DPPH) was measured at 515 nm using a spectrophotometer. The capability of samples to reduce DPPH was determined by sample colour reduction effect with control using following equation:

Radical scavenging activity (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$ (2)

where, $\mathbf{A}_{\text{control}}$ = absorbance of the control, $\mathbf{A}_{\text{sample}}$ = absorbance of the sample.

Determination of flavonoid

To prepare the sample extract, 10 ml of ethanol was mixed with 1g of root powder, kept for 48 hrs and filtered (Whatman no. 1 filter paper). Next, 5 ml ethanol was added and filtrate was collected. First, 3 ml of aliquot of each sample was mixed with 8.2 ml of distilled water. Then, 0.4 ml of 10% aluminum chloride (AlCl₃) was mixed and after 2 minutes, 0.4 ml potassium acetate (CH₃COOK) was mixed and shaken well. Absorbance was measured at 415 nm in a spectrophotometer after 30 minutes incubation. 100 ppm quercetin was used as a standard for determining the flavonoid content. Flavonoid content was expressed as mg flavonoid/100 g of sample and calculated by the formula:

$$Flavonoid = \frac{c \times 12 \times 15}{10 \times x \times w}$$
(3)

where, c = concentration, x = Aliquot taken (ml) and W = Weight of sample taken (g)

Tannin content determination

In a 250 ml conical flask, 0.25 gm of root powder was added along with 30 ml of distilled water. Next, the flask was gently heated and the mixture boiled for 30 min. It was then centrifuged (2000 rpm) for 20 minutes and the supernatant was collected. Before transferring in test tube it was filtered with the help of filter paper.

For the determination of tannin content, a mixture of root extract (0.5 ml) and distilled water (8 ml) was prepared with Folin-Denis reagent (0.5 ml) added to it later. Next, 1 ml of sodium carbonate (20%) was added to the mixture and shaken well. Absorbance was measured at 700 nm using a UV-Visible Mini 1240 spectrophotometer (Shimadzu Co., Japan) after 30 minutes incubation.100 ppm of tannic acid was taken as standard. Tannin content was expressed mg tannic acid/100 g of sample and calculated by the formula:

$$Tannin = \frac{c \times 10 \times 50 \times 100}{1000 \times x \times w}$$
(4)

Where, C =concentration, x =aliquot taken (ml) and W = weight of sample taken (g)

Ascorbic acid content

Visual titration method using 2, 6-dichlorophenolindophenol dye (AOAC, 2004) was used to determine the ascorbic acid content. For standardization of dye, a mixture of standard ascorbic acid solution (5 ml) and HPO₃ (5 ml, 3%) was prepared. A titration with the dye solution to a pink colour (persisted ~15 seconds) was performed. The dye factor was determined as follows:

Dye factor = 0.5/titre value

2.5 gm root powder was weighed and mixed with 25 ml of 3% meta phosphoric acid (HPO₂). It was then centrifuged at 2000 rpm for 15 minutes. The supernatant was filtered in a conical flask and volume made to 25 ml. 10 ml of aliquot was taken for titration. Ascorbic acid content was then determined as follows:

Ascorbic acid =
$$\frac{T \times DF \times V \times 100}{x \times W}$$
(5)

Where, T = titre, DF = dye factor, V = volume made up, x = aliquot of extract taken for estimation and W = weight of sample taken for estimation

Regression analysis through ANNModeling

ANN is an effective tool to compute relation between input and output variables. There are several types of networks of ANN such as feed-forward network (perceptron network) and feedback network (recurrent network). The feed-forward network is commonly used with an error correction algorithm such as back-propagation. A Back-Propagation Artificial Neural Network (BPNN) is a non-linear processing system operating in parallel that is composed of neurons which between them can be used for mapping input and output data. The BPNN is a single computational processor, which has four steps:

1) Assembly of the data set, defining the input and output data

- 2) Deciding the network architecture
- 3) Training (network learning)
- 4) Simulating the network response to new inputs

The most widely used transfer functions to solve linear and non-linear regression problems are the linear transfer function (purelin), log-sigmoid transfer function (logsig) and tan-sigmoid (tansig) transfer function (Hagan et al., 1996). The outputs of neurons were computed by these transfer functions which can be written as:

$$S(x) = \frac{1}{1 + \exp(-x)} (LOGSIG)$$
(6)

$$S(x) = \frac{1}{1 + \exp(-2x) - 1} (TANSIG)$$
(7)

$$S(x) = (x)(PURELIN)$$
(8)

Training algorithm (Levenberg-Marquardt (Trainlm)) was used for updating the network weights. Four input variables, *i.e.* root grade, temperature, maturity and drying time as input variables and moisture ratio (MR) were as output variable with ten nodes in the hidden layer.

Since drying time in HAD was limited to some hours but in traditional drying (Sun, Shade and Solar drying) it was in days, so two models were developed. One for HAD and another for traditional drying. In this case, drying method was one of the input variables instead of temperature.

For ANN model building, the available data was randomly divided into training (70%), validation (15%), and testing (15%) sets. A total 286 data points for HAD and 15 data points for traditional methods were auto distributed by the software into three sets: training, validation and testing. The neural network toolbox of the MATLAB software (Math Works, Natick, Massachusetts, USA) was used in this study with purelin, log-sigmoid (logsig) and tan-sigmoid (tansig) transfer functions (Hagan et al., 1996). Training algorithm (Levenberg-Marquardt (Trainlm)) was used for updating the network weights. The best training performance of the neural network was then chosen based on highest regression coefficient (R²) and minimization of root mean square error (RMSE).

$$RSME = \sqrt{MSE} = \sqrt{\frac{1}{N}} \sum_{i=1}^{N} (y_i - \tilde{y})^2$$
(9)

$$R^{2} = 1 - \frac{\Sigma(y_{i} - \tilde{y})^{2}}{\Sigma(y_{i} - \bar{y})^{2}}$$
(10)

Where, = \tilde{y} predicted value of y and \bar{y} = mean value of y

A probability plot between predicted MR values obtained from ANN modeling and mathematical modeling versus observed MR was plotted to verify the validation of ANN and the performance was confirmed.

Statistical analysis

To see the effect of drying methods and maturity stages on phytochemical parameters, one-way analysis of variance (ANOVA) was used. Different conditions (groups) were defined based on the drying methods used and maturity stages of the samples. Total 18 conditions were defined based on 6 drying methods and 3 maturity stages. An ANOVA with p-value of <0.05 was considered significant to see which specific combination of drying method and stage of maturity differed significantly. For each sample, the results are presented as the mean±standard deviation (SD) for the three replicates. Based on these statistically significant differences, inferences were drawn for effect of different drying methods and maturity stages on the quality content.

Results and discussion

Initial moisture content of Sarpagandha roots

Sarpagandha roots were brought from the garden in two slots. Initial moisture content of 6, 12 and 18 months old roots of first slot were measured as 82.48, 103.25 and 96.07% (d.b.) respectively. For second slot initial moisture content of 6, 12 and 18 months old roots were measured as 94.17, 127.27 and 104.08% (d.b.), respectively. Highest initial moisture content was observed for 12 months matured root and the least was for 6 months old root sample indicating an increasing trend initially with growth of plants up to a certain maturity and then reducing with further growth. This is mostly due to the rapid developmental process during initial period of growth (Walker et al., 2003).

Drying characteristics

Drying air temperature and relative humidity

Sun drying (SD), shade drying (SHD), solar drying (SLD) and hot air drying (HAD) at 50, 60 and 70°C were used to study drying characteristics. For HAD, the air velocity was 1.8 ± 0.1 m/s. On the other hand, the other three drying methods (SD, SHD and SLD) was effected under natural convection current. Drying air temperature varied by 13°C (15-28°C) in SD, 11°C (12-23°C) in SHD, 17°C (27-44°C) in SLD and were fixed 50°C in HAD₅₀, 60°C in HAD₆₀ and 70°C in HAD₇₀ drying. The drying air temperature inside solar dryer was higher by 12 to 16°C than that of sun drying atmosphere and that of shade drying was minimum throughout the drying period (Morad et al., 2017; Orphanides et al., 2016). A variation of 19% (48-67%) in SD, 17% (56-73%) in SHD and 15% (37-52%) in SLD was observed for RH of drying air. RH for HAD varied between 28 to 48% through the experimental range of drying air temperature.

Effect of drying methods, root grade and maturity stage on moisture depletion

Under all the drying methods, final moisture content (MC) achieved by Sarpagandha roots was approximated under the prevailing drying conditions of respective methods. The final MCs reached by G1 and G2 samples of 6, 12 and 18 months old in SD, SHD, SLD, HAD₅₀, HAD₆₀ and HAD₇₀ ranged between 4 to 5% in HAD and 5.5 to 6.95% in traditional drying. The lowest final MC (db) was observed for 6 months old G1 sample in HAD₇₀ experiment. A possible explanation could be that the high temperature of air reduced the relative humidity of drying chamber and promoted the moisture removal at a higher rate. In general, the FMC of all the samples dried by traditional methods (SD, SHD and SLD) had higher values than those of the samples dried by HAD. It might be due to less amount of moisture removal at lower temperature and higher RH of atmosphere during the drying process. The samples achieved EMC at higher values. Moreover, the fluctuations in temperature and RH of the drying air in all types of traditional methods were very high round the clock throughout the drying period. It was observed that moisture removal at the initial period of drying was high in all drying methods, however, the final moisture content of the samples differed in both the groups. HAD drying was superior (with respect to moisture removal rate) followed by SLD, SD and SHD. Also the moisture removal rate of G2

was faster than G1. It may be due to lesser girth diameter of G2 as compared to that of G1 for which the radial diffusion of moisture from centre of roots up to the surface was faster in G2 than in G1. Trend of moisture removal in G1 and G2 was almost similar and followed the falling rate period only. However, an interesting phenomenon with respect to the drying behavior of roots as affected by maturity stage of roots was revealed. The moisture depletion was fastest in the roots of 6 months old followed by roots of 12 and 18 months. This variation was found to be very less between 12 and 18 months old sample than that between 6 to 12 months samples. This phenomenon was visible in drying curves of all the samples irrespective of drying methods and grade types of roots. This is possibly due to the presence of larger proportion of roots having smaller girth size in 6 months old samples.

Effect of maturity stage, grade and drying methods on drying time

Figure 1 shows the effect of maturity stage, grade and HAD methods on total drying time whereas Figure 2 shows the effect of maturity stage, grade and traditional drying methods on total drying time. Total drying time for HAD methods and traditional drying methods was taken in hours and days respectively. It was observed that drying time for traditional drying method (4 to 15 days) was much longer in comparison to HAD methods (2.5 to 12.5 hour). Similar findings have been reported by Yuan et al. (2015) where sun-drying and shade-drying had drying times of 12, 24 day respectively and 12 to 1 hour by oven drying as temp increased from 40 to 120°C. Also G1 of each maturity stage sample took more time to dry than that of G2 sample and with increase in stages of maturity the total drying time in-



FIGURE 1: Effect of maturity stage, grade and drying temperature on drying time.



FIGURE 2: Effect of maturity stage, grade and drying method on drying time.

creased. This is attributed to the higher drying rates from roots of smaller girth size and to the presence of higher quantum of these roots in 6 months old samples and G2 samples.

The least drying time (2.5 h) was taken by 6 months, G2 sample at HAD 70° C (Fig. 1). It was due to the obvious reason that high temperature increased the moisture evaporation rate and subsequently reduced the drying time. Also 6 months old sample was less matured than 12 and 18 months old sample, so the girth diameter was also less as compared to other maturity stage samples. The longest drying time (15 days) was taken by 18 months, G1 sample in shade drying (Fig. 2). Lower drying temperature and higher maturity stage with larger proportion of thicker roots may be the reason for this.

Effect of different drying methods and different maturity stage on moisture ratio of Sarpagandha roots

Figures 3 and 4 shows the variation of moisture ratio, a dimensionless parameter of G1 and G2 roots with drying time for different maturity stage of roots and HAD temperature. It was observed that with increase in maturity stages value of MR increased relatively where as it decreased with increase in temperature. Also, it was observed that for any given temperature the MR curves for 12 and 18 months was closer to that of 6 months. Trend of G1 (Fig. 3) and G2 (Fig. 4) root sample was almost same, only total drying time for both grade roots was different.



FIGURE 3: Variation of MR of G1 roots with drying time for different maturity stage and HAD temperature.



FIGURE 4: Variation of MR of G2 roots with drying time for different maturity stage and HAD temperature.



FIGURE 5: Variation of MR of G1 roots with drying time for different maturity stage and traditional drying.



FIGURE 6: Variation of MR of G2 roots with drying time for different maturity stage and traditional drying.

Figures 5 and 6 show the variation of moisture ratio of G1 and G2 roots with drying time for different maturity stage and traditional drying methods. It was observed that the sample dried in solar dryer took lesser time as compared to sun and shade drying irrespective of maturity stage and grade of roots. It is attributed to high temperature maintained in solar dryer as mentioned earlier. Also the stage of maturity maintained almost similar trend as that obtained in HAD but here the curves were not very regular and smooth as compared to HAD. Fluctuation in the environmental conditions during the drying period brought in irregular drying rates leading to uneven decreasing MR with drying time where as in HAD, uniform drying condition was provided, so the drying curves obtained were more smooth.

Drying kinetics through ANN modelling

Moisture ratio, as already discussed, a dimensionless drying parameter eliminate the effect of variation in initial moisture content (IMC) and describes the drying behavior of material more precisely. Therefore, the kinetics of drying is better modeled through variation of MR with drying time. But at the same time the drying process of biological materials is also dependent on the factors like the age of the plant, growth conditions and drying methods (Ascrizzi, 2018). The present investigation aimed at assessing the influence of drying method, stage of maturity and root size on MR versus drying time. Conventional method of empirical modelling does not accommodate

more than one input variable for which a number of equations are generated for MR versus drying time to explain the behaviour of other factors each with different set of constants and coefficients. In the present case, 18 sets of experiments are involved for which conventional modelling generates 18 sets of equations making it bulky and complicated for prediction of MR reflecting all the four input variables. Therefore, ANN modelling was decided to be conducted

TABLE 1: Girth diameter of roots (G1 & G2) of different maturity stages.

Maturity stages	Grade 1 (G ₁) cm	Grade 2 (G ₂) cm
6 M	0.9 - 1.0	0.4 - 0.8
12 M	0.9 – 1.2	0.4 - 0.8
18 M	0.9 – 1.4	0.5 – 0.8

for easier, more precise and faster prediction (Amin et al., 2019).

The neural network model consisted of three layers, namely input, hidden and output. The input layer had four nodes which corresponded to grade, temperature, maturity stage and drying time. The output layer has 1 output i.e., MR. The method of computation and the algorithm for minimization of error used were back propagation and Levenberg-Marquart. 1000 epochs were used with 6 iterations for network training with a goal of minimum error (Vogl et al., 1988). The transfer functions used were hyperbolic tangent (tansig), sigmoid (logsig) and linear purelin. The number of neurons in each hidden layer was 10.

Results indicated that best fitting with training data set were obtained with only one hidden layer and 10 neurons were sufficient to minimize the error (Table 2). For HAD the R² was 0.928-0.999 with RMSE values of 0.010 to 0.076 for different transferfunctions respectively. For traditional drying the R^2 was 0.797-0.995 with RMSE values of 0.021 to 0.137 for different transfer functions respectively. Among all transfer functions logsig was found to be the best for HAD whereas for traditional drying, the most suitable function was tansig. The results of the testing model on new trials showed excellent

agreement between the actual and predicted data with high coefficient of determination more than 0.99 (Table 2). The plotting of predicted MR obtained in both drying methods with those of experimental MR has been illustrated in Figures 7 and 8. It revealed a high accuracy of the constructed ANN model by close placing of predicted vs observed data points to 45° line. Arslan et al. (2008), Arslan et al. (2010) and Arslan et al. (2012) conducted drying experiments on rosemary, peppermint and savory leaves respectively using traditional and microwave drying. The drying kinetics were adequately fitted with Midilli and Kucuk models for sun drying, hot air oven and microwave drying.

However, the proposed predictive model using ANN can have on-line predictions of moisture kinetics with different using a single model. The developed single ANN model can better predict the drying kinetics for different drying methods and drying time. This demonstrated that ANN modeling can remove the dependence on empirical modelling which is cumbersome and time consuming.

TABLE 2: Modelling of MR Vs DT using different transfer functions of ANN

Drying methods	Training algorithm	Transfer function	No of neurons in hidden layer	R ²	RMSE	Epochs (iteration)
HAD	Trainlm	Tansig	4-10 ⁻¹	0.998	0.012	6
	Trainlm	Logsig	4-10 ⁻¹	0.999	0.010	6
	Trainlm	Purelin	4-10 ⁻¹	0.928	0.076	6
Traditional	Trainlm	Tansig	4-10 ⁻¹	0.995	0.021	6
	Trainlm	Logsig	4-10 ⁻¹	0.990	0.031	6
	Trainlm	Purelin	4-10 ⁻¹	0.797	0.137	6

TABLE 3: Analysis of Variance showing the significant difference among quality parameters.

Drying method	Maturity stages (months)	Total Pheno- lics (mg GAE/ 100g sample)	ANTI- OXIDANT (% R.S.A)	FLAVO- NOID (mg/100g)	TANNIN (mg tannic acid/100g)	ASCORBIC ACID (mg/100g)
Solar drying	6 12 18	154.68 ± 4.8^{fg} 163.88 ± 6.2^{ef} 166.16 ± 6.5^{de}	56.19 ± 1.90 ^f 57.72 ± 1.65 ^{ef} 60.14 ± 2.86 ^{de}	65.48 ± 2.63 ^{gh} 78.09 ± 2.76 ^e 84.19 ± 2.06 ^d	0.752 ± 0.016^{e} 0.756 ± 0.007^{d} 0.764 ± 0.021^{cde}	23.89 ± 0.90 ^{ij} 26.54 ± 0.74 ^{gh} 34.62 ± 1.75 ^c
Sun drying	6 12 18	122.37 ± 3.2^{k} 143.20 ± 5.5^{hi} 144.60 ± 6.3^{ghi}	48.03 ± 1.60 ⁹ 56.29 ± 1.74 ^f 57.87± 1.48 ^{ef}	60.33 ± 1.34 ^h 71.33 ± 0.90 ^f 77.90 ± 1.45 ^e	$\begin{array}{l} 0.772 \pm 0.019^{bcde} \\ 0.780 \pm 0.007^{bcde} \\ 0.782 \pm 0.020^{bcd} \end{array}$	28.14 ± 1.18 ^{fg} 33.14 ± 1.10 ^{cd} 35.20 ± 2.64 ^c
Shade drying	6 12 18	143.64 ± 4.7 ^{hi} 166.54 ± 7.1 ^{de} 183.70 ± 6.7 ^c	59.44 ± 1.77 ^e 63.77 ± 1.97 ^c 65.72 ± 2.34 ^c	54.87 ± 2.31^{i} 69.99 ± 3.46^{fg} 70.24 ± 1.62^{g}	$\begin{array}{c} 0.768 \pm 0.070^{bcde} \\ 0.776 \pm 0.018^{bcde} \\ 0.779 \pm 0.020^{bcde} \end{array}$	35.41 ± 1.42 ^c 38.05 ±1.57 ^b 43.60 ± 3.19 ^a
HAD ₅₀	6 12 18	179.29 ± 9.6° 181.00 ± 4.7° 195.82 ± 11.0 ^b	$\begin{array}{c} 62.99 \pm 1.22^{cd} \\ 64.56 \pm 1.10^{c} \\ 70.44 \pm 1.40^{ab} \end{array}$	62.43 ± 2.83^{h} 78.92 ± 0.98^{e} 85.39 ± 3.57^{d}	$\begin{array}{c} 0.776 \pm 0.020^{bcde} \\ 0.782 \pm 0.003^{bcd} \\ 0.789 \pm 0.004^{abc} \end{array}$	22.79 ±1.21 ^{ijk} 29.42 ± 0.64 ^{ef} 31.64 ± 1.42 ^{cde}
HAD ₆₀	6 12 18	176.20 ± 6.0^{cd} 201.94 ± 6.1 ^b 213.92 ± 8.0 ^a	64.74 ± 1.34 ^c 69.28 ± 2.85 ^b 73.65 ± 1.36 ^a	72.15 ± 3.60 ^f 84.97± 2.38 ^d 98.63 ± 3.33 ^c	$\begin{array}{l} 0.778 \pm 0.030^{bcde} \\ 0.792 \pm 0.011^{abc} \\ 0.812 \pm 0.052^{ab} \end{array}$	22.64 ± 0.99^{jk} 27.92 \pm 0.69 ^{fg} 30.12 \pm 0.85 ^{ef}
HAD ₇₀	6 12 18	128.80 ± 4.8 ^{jk} 139.37 ± 4.0 ^{ij} 151.76 ± 3.8 ^{gh}	$\begin{array}{l} 57.16 \pm 2.42^{\text{ef}} \\ 60.47 \pm 3.44^{\text{de}} \\ 62.99 \pm 1.22^{\text{cd}} \end{array}$	88.00 ± 6.77 ^d 106.33± 4.20 ^b 122.97 ± 3.90 ^a	$\begin{array}{c} 0.790 \pm 0.008^{abc} \\ 0.800 \pm 0.015^{ab} \\ 0.820 \pm 0.025^{a} \end{array}$	21.17 ± 1.39 ^k 25.30 ± 1.53 ^{hi} 28.06 ± 1.97 ^{fg}

*Values have been calculated moisture free basis and are given as means of 3 replicates \pm standard deviation.

*Values having same superscripts along a column indicated no significant difference. HAD: hot air drying

Total phenol content

The effect of the various drying methods and maturity stages on total phenolic content of the roots samples was studied and the result is presented in Table 3. A variation in the phenolic content was observed in all root samples. Total phenolic content increased with the maturity stage and it was highest for 18 months old rootsamplewhereas it was lowest for 6 months root sample irrespective of drying methods. Pandey and Das (2014) reported that the harvest of 18 months old sarpagandha plants had highest yield of roots having maximum alkaloid content. Oszmiański et al. (2017) also found immature fruits to have lowest level of antioxidants and polyphenolic compounds, which increased with maturity in cranberry fruits. Total phenolics content of the samples of present study varied from 122.37±3.2 mg GAE/100g dry matter for 6 months, SD sample to 213.92±8 mg GAE/100g dry matter for 18 months, HAD 60°C sample respectively. Table 3 revealed that there was significant difference in TPC of samples of different maturity. However, the sun dried and solar dried samples of 12 and 18 months old did not exhibit any significant difference.



1 0.9 0.8 0.7 0.6 0.7 0.6 0.5 0.4 0.3 0.2 0.1 0 0 0.5 1 Moisture ratio (observed)

HAD

FIGURE 7: Predicted vs observed value of MR for all samples of traditional drying.

In traditional drying total phenolic content was higher for shade drying in comparison to that of SD and SLD. In HAD it increased till 60°C and then decreased at 70°C. These findings can be explained by the longer time required at lower drying temperature, thus the roots had longer exposure to heat, which resulted in degradation of TPC. It is recognized that higher temperatures improve the solubility of phenolic compounds as the hot air facilitates cell structure breakdown, which leads to the release of phenolics from macromolecules (Klava et al., 2018; Guido and Moreira, 2017). At HAD 70°C, TPC decreased, which could be explained by thermal degradation during high temperature treatment. Also decline in TPC resulting from shade drying in comparison to HAD (50 and 60°C) could be due to enzymatic degradation as the process was carried at room temperature and took several days to dry. Saifullah et al. (2019) found similar findings in case of lemon myrtle dried leaves when it retained higher TPC in HAD followed by shade drying and sun drying. Rosemary, peppermint and savory leaves were dried by Arslan et al. (2008), Arslan et al. (2010) and Arslan et al. (2012) respectively using sun, oven drying (50°C) and microwave drying (700 watt). They inferred that microwave drying preserved the colour values of leaves better whereas the mineral content retention was more in ovendried samples. Sun dried samples lost both colour and mineral content to a greater extent than the samples dried matching with the present findings. However, Juhaimi et al. (2018) reported better preservation of total phenolics of apricot kernels with microwave drying at 720 watt as compared to control samples.

Antioxidant activity (DPPH method)

The antioxidant activities of eighteen root samples were evaluated by DPPH methods. Results are presented in Table 3. The antioxidant activity varied from 48.03 ± 1.60 (% R.S.A) to 73.65 ± 1.36 (% R.S.A). It was noted that antioxidant activity was highest for 18 months, HAD 60° C sample and was lowest for 6 months, SD sample. In HAD antioxidant activity increased with increasing temperature till 60° C but it decreased at 70° C. In traditional drying it was highest for shade drying.

A strong correlation was observed in the trend of TPC and antioxidant activity. Here, HAD 60°C sample exhibi-

FIGURE 8: Predicted vs observed value of MR for all samples of hot air drying.

ted significantly (p<0.0) more antioxidant activity than that of SD sample, SLD sample, SHD sample and HAD (70°C). These can be explained by exposure to heat when drying at higher temperature may stimulate degradation of phytochemicals thus reduce in antioxidant capacity (Garau et al., 2007). Also decline in TPC resulting from shade drying in comparison to HAD (50°C and 60°C) could be due to enzymatic degradation as the process was carried at room temperature and took several days to dry. These results corroborate the findings of Nguyen et al. (2016) and Yuan et al. (2015) that the drying processes had significant effects on the contents of bioactive constituents and antioxidant properties. The appropriate drying process was suggested to be oven-dried at 60°C for roots and flower heads.

Flavonoid content

Table 3 shows the effect of various drying methods and maturity stages on Sarpagandha roots. The total flavonoid content was found to vary between 54.87±2.31 mg quercetin/100g dry matter to 122.97±3.9 mg quercetin/100g dry matter. Rajurkar and Hande (2011) also found 130 mg quercetin/100g dry matter in the roots of Sarpagandha plants which is close to the present findings. Result showed that flavonoid content increased with the maturity stage and drying temperature. Unlike TPC and antioxidant, highest significant (p<0.05) amount of flavonoid content was found in 18 months, HAD 70°C sample whereas lowest amount was observed for 6 months, SHD sample. It may be due to the heat resistant quality of quercetin (major flavonoid in Sarpagandha) which increases upto 120°C and then degrades at 150°C (Sharma et al., 2015). As per reports of Orphanides et al. (2016), shade-dried plant material caused significant loss of the functional properties in some types of herbs compared to hot-air drying at 50 to 70 °C. The relative lesser amount of flavonoid retention in case of sun and shade dried samples are possibly due to the higher damage to the epidermal surface and shrinkage during long drying periods.

Ascorbic acid

Table 3 shows the influence of drying methods and maturity stage on ascorbic acid content. Ascorbic acid content was found to vary between 21.17 ± 1.39 mg/100g dry matter

to 43.6±3.19 mg/100g dry matter. Harisaranraj et al. (2009) has also reported similar observation for ascorbic acid content. According to them Sarpagandha roots contained 41.04 mg ascorbic acid/100g dry matter. Highest retention of ascorbic acid was observed in the shade dried sample (43.6 mg/100g dry matter). This may be due to the fact that the roots were not exposed to direct heat as vitamin C is rapidly oxidized on exposure to heat (Ademiluyi et al., 2018). Lowest retention of vitamin C was observed in HAD 70°C sample. This suggests that shade drying has an edge over HAD, SHD and SD in its preservation ability of the vitamin C content which could serve as a good dietary supplement for ascorbicacid.

Tannincontent

The tannin content was found to be very low and there was no substantial difference observed (Table 3) in all the samples after treatment under the various drying methods. Tannin content varied from 0.75 ± 0.016 to 0.82 ± 0.025 mg tannic acid/100g dry matter. Similar result was reported by Bhardwaj and Yadav (2016). They also found 0.85 ± 0.20 mg tannic acid/100g dry matter in sarapagandha root. This might be due to little influence of drying methods on tannin content (Akanji et al., 2003). Kumari and Jain (2012) reported that tannins are usually present in low amounts in plants. Though there was a variation in samples due to maturity stage, no pronounced effect could be visible.

Conclusions

In this study different drying methods were applied to sarpagandha roots of three maturity stages (6, 12 and 18 months) and of two grades. Drying time requirement for Sarpagandha roots varied between 150-750 minutes for HAD and 4-15 days for traditional (sun, shade and solar) drying methods. The order of drying methods based on drying time requirement was as follows: Shade drying > Sun drying > Solar drying > HAD 50°C > HAD 60°C > HAD70°C. Grade 1 samples took more time than grade 2 samples irrespective of drying methods and maturity stage. Drying time increased with increase in maturity stage of roots. And Modeling of drying kinetics was carried out through ANN covering 18 sets of experimental observations with four independent variables (temperature, stage of maturity, root grade and drying time) to predict MR as output variable. The ANN model using logsig and transig transfer functions had the best prediction ($R_2=0.999$) for HAD and $(R_2=0.995)$ for traditional methods respectively. The study demonstrated that the drying methods and stage of maturity significantly affected the phyto-constituents (total phenolics, flavonoids, vitamin C) and antioxidant properties. TPC and antioxidant both were highest in samples of 18 months maturity dried at 60°C and lowest in sun dried samples of 6 months maturity. The highest retention of vitamin C was observed in the 18 months, shade dried sample (43.6 mg/100g dry matter) and lowest in 6 months HAD 70°C sample (21.17±1.39 mg/100g dry matter). However, no significant difference in case of tannin content was observed among samples dried by various drying methods. Overall quality analysis indicated that Sarpagandha roots of 18 months old contained higher amount of most of the phytochemicals. Among hot air drying temperatures, the samples dried at 60°C and among traditional drying methods shade drying could retain higher amount of phytochemicals and antioxidants property.

Conflict of interest

The authors declare that there are no conflicts of interests exist.

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