Fronteiras: Journal of Social, Technological and Environmental Science
http://periodicos.unievangelica.edu.br/fronteiras/
ISSN 2238-8869

Article

Mechanical Drying of *Azadirachta indica* A. Juss. Seeds: a Tool to Keep Quality of this Eco-Friendly Bio-Insecticide

Joelma Abadia Marciano de Paula [1] *, Gabriel Luis Castiglioni [2], Lucas Ferreira Brito [3], Aline Mar Rodrigues da Silva [4], Debborah Gonçalves Bezerra [5], Edemilson Cardoso da Conceição [6]

[1] Doutora (Universidade Estadual de Goiás); ORCID: 0000-0003-0737-2600, E-mail: joelma.paula@ueg.br

[2] Doutor (Universidade Federal de Goiás); ORCID: 0000-0001-6941-148X, E-mail: gabrielcastigli@gmail.com

[3] Graduado (Universidade Paulista); ORCID: 0000-0003-3741-762X, E-mail: lucas.ferrbrito@gmail.com

[4] Mestre (Universidade Federal de Goiás); ORCID: 0000-0003-3012-9027, E-mail: alinemarr@gmail.com

[5] Doutora (Universidade Estadual de Goiás); ORCID: 0000-0002-5450-9940, E-mail: debborah.b@gmail.com

[6] Doutor (Universidade Federal de Goiás); ORCID: 0000-0003-4113-2686, E-mail: ecardosoufg@gmail.com

*Correspondence: joelma.paula@ueg.br

ABSTRACT

Products based on *Azadirachta indica* A. Juss. (Meliaceae) seeds are worldwide renowned as eco-friendly bio-insecticides. However, the improper processing of the seed may cause significant losses in product effectiveness, as the contents of azadirachtin – its main component – may be affected. This paper aims to assess the degradation level of azadirachtin found in *A. indica* seeds during the kiln drying process under different temperature and airflow conditions. In order to do so, samples of *A. indica* fresh seeds were crushed and subjected to 11 drying assays in temperatures ranging from 55 °C to 65 °C and airflows of 0.015 – 0.023 $m^3.kg^{-1}.s^{-1}$, according to the central composite rotatable design 2^2, with triplicate on the central point. The contents of azadirachtin in both dried and fresh samples were defined through High-Performance Liquid Chromatography (HPLC). Temperatures and airflows had no significant effects on the contents of azadirachtin.

Keywords: sun drying, physical-chemical quality, processed seeds.

RESUMO

Produtos oriundos das sementes de *Azadirachta indica* A. Juss. (Meliaceae) são mundialmente reconhecidos como bioinseticidas "ecofriendly". Entretanto o processamento inadequado das sementes pode ocasionar perdas significativas na efetividade dos produtos, uma vez que os teores de azadiractina, seu principal constituinte, podem ser afetados. O objetivo deste trabalho foi avaliar o grau de degradação da azadiractina presente nas sementes de *A. indica* durante o processo de secagem em estufa sob diferentes condições de temperatura e vazão de ar. Para tanto, amostras de sementes frescas foram trituradas e submetidas a 11 experimentos de secagem em temperaturas de 55 - 65 °C e fluxos de ar de 0,015 – 0,023 $m^3.kg^{-1}.s^{-1}$, conforme delineamento composto central rotacional 2^2, com triplicata no ponto central. Os teores de azadiractina nas amostras dessecadas e frescas foram determinados por Cromatografia a Líquido de Alta Eficiência (CLAE). Não houve efeito significativo das temperaturas e fluxos de ar sobre os teores de azadiractina.

Submissão: 06/03/2020 Aceite: 29/03/2022 Publicação: 29/04/2022

v.11, n.1, jan. - abr. 2022 • p. 39-44. • DOI http://dx.doi.org/10.21664/2238-8869.2022v11i1.p39-44.

Palavras-chave: secagem ao sol; qualidade físico-química; sementes processadas.

1. Introduction

Azadirachta indica A. Juss. (Meliaceae) is a native tree from the Indian sub-continent, known worldwide as "neem" and for its unique insecticide properties (Roychoudhury 2016) and benefits to human health (Kumar & Navaratnam 2013; Alzohairy 2016). Therefore, it is considered to be a multiuse plant, including for agriculture, medicine, cosmetics, among others (Tiwari et al. 2014). In most of the tropical and sub-tropical regions of the world it is commonly cropped for reforestation purposes and production of raw material for natural insecticides and medicines (Hiwale 2015). Its main active constituent is the azadirachtin – a tetranortriterpenoid limonoid mainly found in the plant's seeds (Kumar & Navaratnam 2013; Alzohairy 2016) with proven action against several insect-pests. It works as antifeedant, in growth interruption and as ovicidal (Tiwari et al. 2014).

Azadirachtin-based insecticides used in the Integrated Pest Management (IPM) have proved their efficacy against typical agrochemicals, besides being considered less pollutant, with low residual power and lower intoxication risk for mammals and birds (Kizilkaya et al. 2012; Roychoudhury 2016).

The immediate applications as eco-friendly or biodegradable biopesticide increase the demand by industries for raw materials from *A. indica* seeds, rich in azadirachtin. States in all regions of Brazil, notably the Northeast and South regions, have invested in cash crops of *A. indica* (Neves & Carpanezzi 2008).

To maximize the use of production and thus reduce losses in the azadirachtin contents, when the *A. indica* fruits are reaped these should undergo the pulping process and the sorted seeds should be dried (Immaraju 1998; Gahukar 2014). Seed drying increases the storage time to market and the use in production, since low water content hampers the degradation of chemical constituents and microbiological proliferation (WHO 2003; WHO 2011; Gahukar 2014; Tanuja et al. 2012).

Among the several drying methods one of the most popular among farmers is the sun or open air drying, due to its low cost (Gahukar 2014). However, this type of drying may entail significant disadvantages, such as dependence on weather conditions, longer time of drying, labor force and loss of photodegradable chemical constituents (Chen et al. 2013; Kara & Doymaz 2015).

The azadirachtin, main active ingredient of the *A. indica* seed, is a molecule prone to rearrangements in acid, basic, photolytic and environmental conditions because of its several reactive functional groups (Kumar et al. 2012). Studies have shown its photodegradability including with reduction of its field effectiveness (Yakkundi et al. 1995; Pitta 2010). In order to improve the quality of *A. indica* processed seeds, sun or open air drying techniques that expose the material to ultraviolet radiations should be replaced by less aggressive drying methods, such as drying in kiln with air circulation.

Considering the required maximization of stability and preservation of the chemical integrity of the neem-based products, this paper aimed at evaluating the degradation level of the azadirachtin found in *A. indica* seeds during the drying process in kiln under different temperature and airflow conditions.

2. MATERIAL AND METHODS

The *A. indica* ripe fruits were reaped in January 2015 at *Embrapa Arroz e Feijão,* located in the city of Santo Antônio de Goiás, state of Goiás (S16º 30' 26.0994"; O 49º 16' 58.8720"; Altitude: 821 m). To identify the botanic material, voucher species were assembled and deposited at the Herbarium of the Federal University of Goiás, under the number UFG-48590. After harvest, the fruits were packed in low-density polyethylene (LDPE) plastic bags, promptly carried to the plant processing lab of the School of Agronomy – Food Engineering of the Federal University of Goiás (UFG) and cooled in cold chamber at 4 °C. Previously to the drying process, the *A. indica* seeds where removed from the pulps in pulper (model Bonina 0.25 df A8, of the brand Itametal) and crushed in Poli 8-liter multiprocessor (brand Siemsen Ltda) to allow drying on trays.

The drying assays were performed in tray dryer (1.90 m high for 0.80 m wide, with capacity for five metallic trays of 55 x 57 cm each) with forced ventilation. The independent variables were temperature (°C) and airflow ($m^3\ kg^{-1}\ s^{-1}$) (Table 1). A central composite rotatable design (CCRD) 2^2 was proposed, with triplicate on the central point, totaling 11 trials (Barros Neto et al. 2010). Variables

were selected to define the process conditions to minimize losses of azadirachtin, responsible for the functional traits of the *A. indica* seeds. Temperatures lower than those used in this trial would require longer drying time and, therefore, greater energetic output with no significant physical-chemical quality gains to the plant material. Samples with 115 g of crushed fresh seeds were kept in the conditions pre-established by the design (Table 1) to evaluate the drying process results. The drying time required for each sample to reach 10.0% (m/m) of humidity was defined based on the polynomial adjustment of the results to minimize errors.

The evaluation of linear or quadratic effects of drying temperature and airflow, as well as its effects on the azadirachtin contents, was performed through analysis of variance (ANOVA), where values of $p \leq 0.05$ were considered significant. The Response Surface Methodology (RSM) was used to estimate the best drying conditions. All statistical analyses used the software Statistic 12.0.

The azadirachtin contents were determined through High-Performance Liquid Chromatography (HPLC) on fresh seeds samples (triplicate) and after being submitted to each drying trial. Analyses were performed in a chromatographic system of the brand Waters®, model HPLC Alliance® with e2695 separation module, diode arrangement detector (PDA) 2998 and Empower 2.0 data processing system. Chromatographic run was performing according to Paula et al. (2016). A Zorbax Eclipse Plus Agilent C18 (250 mm X 4.6 mm, 5 μm) column with Security Guard Cartridges Phenomenex C18 (30 mm X 4 mm, 4 μm) were used. The mobile phase (isocratic) was constituted by acetonitrile-water (40:60), 1 $mL.min^{-1}$ flow, 214nm detection wave length, 10 μL samples injection volume and the temperature of the column kiln was maintained at 30 °C. The mobile phases were filtered through Durapore PVDF membrane with 0.45 μm pore (Merck®). Both fresh and dried seeds samples were crushed and extracted with methanol HPLC grade at the ratio of 0.15 $g.mL^{-1}$ and 0.05 $g.mL^{-1}$, respectively, in ultrasonic bath for 30 minutes at ambient temperature (25-35 °C). The resulting extracts were filtered through filter paper and then in Millipore membrane with 0.45 μm pore and stowed in the vials.

The azadirachtin contents were calculated based on the average calibration curve and equation of the line resulting from the linear regression of the azadirachtin standard (Sigma). To that, analyses in HPLC were performed for six concentration levels of the azadirachtin standard (1000, 500, 250, 125, 62.5 and 31.25 $\mu g.\ mL^{-1}$) in methanol. Each point was prepared in triplicate and the calibration curve was constructed based on the correlation between the peak areas and the standard concentration. The linear regression coefficients (r) were calculated. The contents (% m/m) of azadirachtin were calculated through the ratio between the azadirachtin concentration in the sample ($\mu g.mL^{-1}$) and the sample concentration ($\mu g.mL^{-1}$), multiplied by 100.

3. RESULTS AND DISCUSSION

The average azadirachtin content in the *A. indica* seeds submitted to drying trials was 1.44% (±0.37) (m/m), ranging from 1.00% - 2.08% (m/m) (Table 1).

Table 1. Actual matrix used in the Central Composite Rotatable Design (CCRD) used to assess the azadirachtin stability during the drying process

Trial	Temperature (°C)	V ($m^3.kg^{-1}.s^{-1}$)	Azadirachtin (%, w/w, average ±SD)
1	55	0.016	2.06 (±0.10)
2	65	0.016	2.08 (±0.13)
3	55	0.022	1.20 (±0.17)
4	65	0.022	1.49 (±0.07)
5	60	0.019	1.71 (±0.08)
6	60	0.019	1.24 (±0.02)
7	60	0.019	1.51 (±0.04)
8	52.9	0.019	1.16 (±0.12)
9	60	0.023	1.26 (±0.06)
10	67.1	0.019	1.12 (±0.03)
11	60	0.015	1.00 (±0.04)

V: airflow; SD: standard deviation

The average content of azadirachtin in neem fresh seeds was 0.72% (±0.13) (w/w). However, considering the average mass loss of the samples after drying (58.18% ±2.24), the azadirachtin content in the fresh sample in dry weight would be around 1.73% (m/m). According to Roychoudhury (2016), the contents of azadirachtin range from 0.05% to 4.24% in seeds from different geographic regions in India. Contents may also vary depending on the seed's maturity stage and the year's season. The average content of azadirachtin in neem seeds in India is 3.5%. This variation results from the fact that the development process of *A. indica* trees is influenced by numerous factors, namely geographic area, climate, genetic variability, agronomic conditions, plant morphology and physiology, collection and storage of plant material (Fernandes et al. 2019).

The comparison between the average values of content of azadirachtin in dry samples (1.44%) against the expected value in samples not submitted to drying (1.73%) discloses a drop of nearly 17%. For some trials analyzed individually that drop was not even observed.

The ANOVA using the CCRD data for the neem seeds drying in kiln with air circulation showed that the model was significant (p = 0.008513). Table 2 shows the ANOVA results calculated based on pure error. The lack of adjustment of the model was not significant (p>0.05) evidencing its suitability to accurately predict the variation.

Table 2. Analysis of variance (ANOVA) of the contents of azadirachtin as a function of temperature and airflow used in the central composite rotatable design (CCRD) model.

Factor	Sum of squares	Degrees of freedom	Mean squares	*F* value	*p*
x_1	0.007981	1	0.007981	0.143458	0.741295
x_2	0.161119	1	0.161119	2.896090	0.230903
x_1^2	0.003371	1	0.003371	0.060596	0.828516
x_2^2	0.000693	1	0.000693	0.012454	0.921332
x_1x_2	0.018225	1	0.018225	0.327591	0.624843
Lack-of-fit	1.076603	3	0.358868	6.450585	0.137160
Pure error	0.111267	2	0.055633		
Sum of squares total	1.378691	10			

x_1= Temperature (°C); x_2= Airflow ($m^3.kg^{-1}.s^{-1}$)

According to data in Table 2 the temperature and airflows have no significant linear or quadratic effect on the contents of azadirachtin. Likewise, the interaction of both factors (temperature and airflow) has not significantly affected the contents of azadirachtin. However, the response surface (Figure 1) showed that higher contents of azadirachtin were obtained from lower airflows used in trials, i.e., between 0.015 and 0.017 ($m^3.kg^{-1}.s^{-1}$).

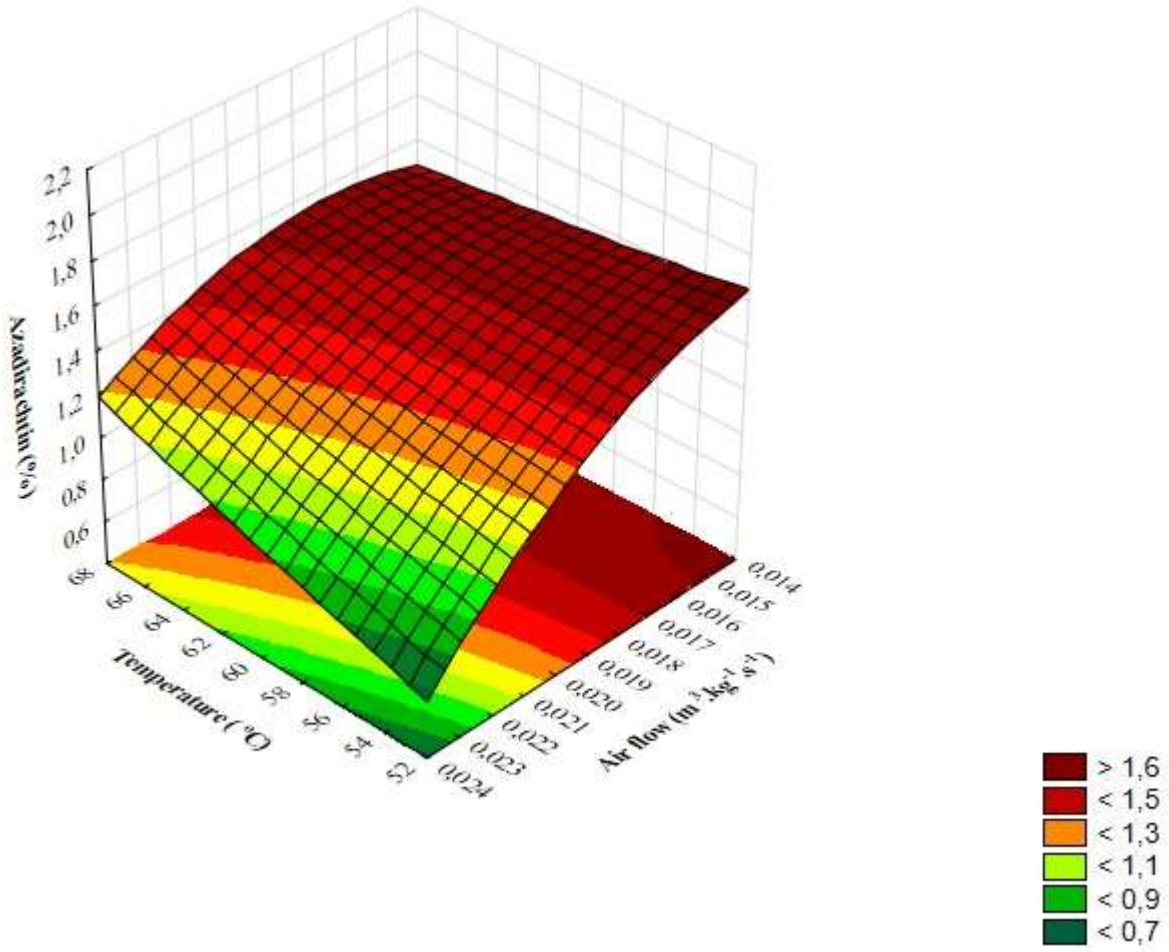

Figure 1. Response surface of the contents of azadirachtin as a function of temperature and airflows used during the drying trials

These finds suggested that the drying temperature and airflow range selected to the Central Composite Rotatable Design (CCRD) not only led to proper drying of the vegetal material (maximum humidity: 10.0% p/p) but also led to loss of the main active constituent within the expected levels. In the monographs described in the Farmacopeia Brasileira (Brazil 2017) most vegetal materials present humidity contents ranging from 8% to 12% (p/p), considered suitable to the physical-chemical and microbiological preservation of these materials. Literature data has demonstrated the basic skeleton of azadirachtin to be remarkably stable under the influence of high temperatures (Fernandes et al. 2019). According to Gahukar (2014) the content of azadirachtin in the seeds or powder of the inner core of the seeds is generally reduced in only 20% under proper drying and storage conditions. If drying and storage conditions expose the material to sunlight for excessively long periods, in turn, the content of azadirachtin may drop up to 45% during four months of storage (Fernandes et al. 2019; Yakkundi et al. 1995). Thus, the prior processing of the fruits in pulper, followed by the drying of the resulting material in mechanical dryers is recommended today, since the exposure of the material to sunlight, in open air drying, can cause considerable loss of the azadirachtin contents. These founds corroborate the study conducted by Tajane et al. (2017) about the characterization of a fine powder formulation of whole *A. indica* fruits. In this study, the authors figure out that azadirachtin content in the formulation suffers minor alteration along 15 days of exposition at UV light and temperature of 50 °C. At 100 °C, it was observed that degradation of azadirachtin was slow in the initial period (up to 3 days) after which, it increased suddenly.

4. CONCLUSION

Proper conditions for processing and drying the *A. indica* seeds could ensure longer storage time of plant materials that are not immediately used by the industry, with better usage of the annual harvest of the fruits. This paper showed that drying up to the constant weight of the *A. indica* seeds processed in kiln with air circulation, at temperatures ranging from 55 °C to 65 °C and airflow

between 0.015 and 0.017 ($m^3.kg^{-1}.s^{-1}$), results in materials with residual humidity within the limits considered acceptable for good conservation during storage (10.0% p/p) and lower loss of azadirachtin against the fresh material.

ACKNOWLEDGMENT

To the Fundação de Amparo à Pesquisa do Estado de Goiás for the financial support.

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