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SCREENING OF THE INFLUENCE OF DRYING METHODS ON THE TOTAL PHENOLIC CONTENT OF *AMARANTHUS CAUDATUS*

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Abstract: *Plants are an inexhaustible source of bioactive compounds that play an essential role in human diet, nutrition and health. Therefore, researchers' studies are increasingly focusing on improving the ways of obtaining bioactive compounds from plants. The aim of this research paper is to investigate the effects of drying methods on bioactive compounds such as phenols from "Amaranthus caudatus (L.)" aerial parts waste. In this investigation, various drying methods including freeze-drying, oven drying, microwave drying and air-convection drying were applied. Moreover, depending on the target bioactive compound, one of the investigated drying methods can be chosen.*

Key words: *"Amaranthus caudatus", drying, total phenolic content (TPC).*

1. Introduction

Amaranthus caudatus is a grain species native to South America. It grows in Ecuador, Peru, Bolivia and Argentina in temperate areas and interandean valleys from sea level up to approximately 3000 m above sea level [10]. It is one of the oldest pseudo cereals originating from South America, being an important alternative to traditional cereals; a major advantage is that it is gluten free, so an important alternative for celiac people' diet. Among other properties, it is an excellent source of high-quality protein and lipids, with a high content of dietary fibres, as well as minerals [9].

Amaranthus caudatus is an often-encountered plant in Romania, which, due to its nutritious and healthy properties, is

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used in the diet and treatments for humans and animals.

In the last years, special attention has been paid to maintaining the quality of the plants during the drying stage, because preparing the samples proved to be an important step in increasing the content of bioactive compounds from the extraction process. Moreover, the drying process increases the shelf life by slowing or stopping microorganism's growth and preventing certain unfavourable biochemical reactions. Every widely used drying method has advantages and disadvantages.

Natural drying and oven drying are still the most widely used methods because of their lower cost, but they require a long drying time, and this eventually translates into high costs. Air-convection drying and microwave drying reduce the drying time of vegetal materials without quality degradation. Freeze drying is one of the most advanced drying methods, expensive and requiring a long drying time.

1.1. Composition

Starch is the major part of *Amaranth caudatus,* carbohydrates being about 48–69% of its total dry weight. Starch granules are extremely small, ranging in dimensions from 0,8 to 2,5µm and degradable by α-amylases. The total dietary fibre content (soluble and insoluble) in grains of *Amaranthus caudatus*, ranges between 9,8 and 14,5% [9].

The composition of *Amaranthus caudatus* shows that the plant is a source of protein with an excellent biological and nutritional value [5]. Amaranth has about 16%–18% protein, as compared with values of 14% or less in other cereals [9].

Amaranth leaves contain 27.8–48.6% dry matter as crude protein [11].

It has a balanced amino acid pattern with leucine as a limiting amino acid [7], [16]. Lysine content is 3–3,5 times that of corn and 2–2,5 higher than in wheat [6].

The amaranth seeds have about 7% content of fat. Around 8% of this fat is squalene, a highly regarded compound by the pharmaceutical industry [6]. The lipids content is generally more than twice as high as that of traditional cereals and amaranth lipids are characterized by a high proportion (more than 75%) of unsaturated fatty acids; it is particularly rich in palmitic acid as saturated fatty acid, oleic acid and linoleic acid. Sterols, phospholipids, triglycerides and tocopherols [9] were also identified. Using fixed experimental parameters, a squalene concentration of 80,60–82,95% (wt/wt) with a yield η_{sq}=80,88–74,65% [8], [15], [17] was obtained.

Amaranthus caudatus leaves are a good source of Ca, K, Mg, P, Zn, Na, Fe, Mn, S and Vitamin C and a source of bioactive compounds such as phenols, flavonoids and betalains [6], [18]. Minerals such as P, Fe, Mg, and Ca exist in high concentrations, giving a total of 33,1–40,7% of ash content. Vitamins C and A are in concentrations of nutritionally significant levels, ranging between 420 and 250 ppm, respectively [11].

Studies have shown that stems and leaves of amaranth contain polyphenols and flavonoids which thus endow with the protective and curative properties in virtue of the desirable strong antioxidant activity. The phyto-chemical analysis of the aerial parts of various *Amaranthus* spp. has established a presence of active constituents like alkaloids, flavonoids, glycosides, phenolic acids, steroids, saponins, amino acids, vitamins, minerals, terpenoids, lipids, betaine, catechuic tannins and carotenoids [1].

Phenolic compounds can be found free or bound, with their sum representing total phenolics. In *Amaranthus* leafy vegetables, phenolics were found mainly in the free form, at levels from 51% to 73% of the total phenolic compounds. *Amaranthus acanthochiton* and *Amaranthus blitum* were the species with the highest concentrations of total phenolics (5,5 and 4,9mg GAE/g FW, respectively). *Amaranthus* species have statistically concentrations of total phenolics ($p<0,05$) (3,4–4,5mg GAE/g FW), and these values were like those of the spider flower. For *Amaranthus caudatus* there were 2,9 ± 0,84 (free phenols) and 4,1 ± 1,1 (total phenols) [18].

Other studies offered different results, depending on being native or germinated for content of TP: native: 17,61 ± 0,16; germinated: 58,55 ± 0,11 mg gallic acid equivalents (GAE)/100g sample [19].

The wide variation of the total polyphenols content in vegetables could result from the variety of the plant, stage of ripening, place of cultivation, climate condition, fertilisation, sample collection, transportation, sample preparation, and methods of analysis (e.g. *Amaranthus caudatus*: 158,33mg GAE/100g) [12]. There are also differences in TPC considering different plant parts; TPC seeds: 1,55 ± 0,06; stalks: 1,89 ± 0,08; leaves: 10,47 ± 0,36; flowers: 4,58 ± 0,14; sprouts: 1,70 ± 0,08 (mg GAE/g DW) [3].

1.2. Utilisation

As food ingredient, *Amaranthus caudatus* is used in a large variety of traditional recipes in many countries, e.g. ‘alegria’ and ‘atole’ in Mexico, ‘al-boroto’ in Guatemala, ‘bolos’ in Peru, ‘Chapati’ in the Himalayas, ‘laddoos’ in India and ‘sattoo’ in Nepal [9].

Amaranthus caudatus has also been used for treatments in traditional medicine, especially in Latin America, India and Africa, where an indigenous healing system is still important. In traditional medicine, different parts of the plant such as the root, stem and seeds, have been utilized to treat constipation, pulmonary problems, urinary disorder, gastrointestinal complications, dermatological diseases, and as a diuretic to mention only a few benefits [1].

The results of the pharmacological evaluation of seeds and leaf extracts of amaranth revealed the potency of its bioactive compounds in suppressing terminal diseases like diabetes, hyperlipidaemia, diarrhoea, coupled with antioxidant, anti-helminthic, antimalarial, anti-inflammatory and antifungal characteristics [1].

In clinical studies it was observed that *Amaranthus caudatus* possesses an antioxidant potential, anti-bacterial and anti-microbial activity, a hypocholesterolemic but also an antipyretic effect, a central and peripheral anti-nociceptive potential, anti-carcinogenic properties and an anti-diabetic, anti-atherosclerotic effect and an anti-inflammatory activity in mouse models [1].

Oil from *Amaranthus caudatus* including squalene is used for oncological treatments, sclerosis, malfunction of the brain, immunodeficient states, skin, stomach and liver diseases, wounds, bedsores, and ulcers [6].

Also, *Amaranthus caudatus* is traditionally used to treat infections. Studies reveal that *Amaranthus caudatus* is efficient to treat urinary tract illnesses like urolithiasis, in which infection is an element which influences stone formation and kidney pain. Moreover, it is used to increase diuresis and its anti-inflammatory effect increases bacterial clearance in the urinary tract and relieves pain. In addition, *Amaranthus caudatus* is well known for the antibacterial (Gram-negative) activity [2].

Reduction of blood glucose by plant extracts recommend their use in the treatment of diabetes mellitus [14].

Due to these nutritional and pharmaceutical properties of *Amaranthus caudatus*, namely due to the bioactivity of the specifically high concentration of phenolic acids, polyphenols, saturated and unsaturated fatty acids, proteins, soluble peptides, flavonoids, squalene and beta-carotene in the seeds and other parts, *Amaranthus* is a promising plant for food and pharmaceutical industries [13].

2. Objectives

The aim of the present work is to study the drying effects on such bioactive compounds as phenols from *Amaranthus caudatus* waste.

In this investigation, four drying methods (freeze-drying, oven drying, microwave drying and air-convection drying) were applied. In order to obtain a favourable extraction efficiency, the solvent extraction method on the dried vegetal material was modified by the application of the microwave effect.

3. Material and Methods

The plant material is the waste resulted after an aqueous extraction of *Amaranthus cauatus* aerial parts.

The waste material was dried through four different methods such as:

a) oven drying was performed in an oven UN110 Memmert, at 40°C for 25 hours;
b) microwave drying was performed in a microwave oven (Samsung, 2900W, 2450 MHz) at 450W for 16 minutes;
c) air-convection drying was made in a food dehydrator (Excalibur) at 40°C for 240 minutes;
d) freeze-drying was realized in a freeze-dryer (Alpha 2-4 LCSplus, LyoCube 4-8, Christ) using the Christ Standard program, at -15°C for 22 hours.

Humidity was measured periodically during the drying process.

The drying process of the plant material following the four drying methods was considered completed when the material had a moisture below 9% and a constant weight.

Humidity was measured using a thermo-balance (Partner MAC 50).

The dried waste material was grinded and extracted with 80% ethanol, using a Microwave assisted extractor (Ethos Easy Milestone), 600W, 70°C, 40min.

The extracts were filtered and diluted (1:100).

The total phenolic content (TPC) was measured by means of the spectrophotometric method. The phytochemical quantification procedures were used for the determination of the total phenolic content in the extract. The assays: 1mL diluted extract, 5mL Folin-Ciocalteau reagent, after 8 minutes, 4mL saturated sodium carbonate; the probes were incubated for 60 min at room temperature; the absorbance was measured at 765nm. The obtained results were expressed as mg gallic acid equivalents per gram of extract (mg GAE g^{-1} extract) [4].

All results were expressed as mean ± SD of six repetitions.

One-way analysis of variance (ANOVA) was used to compare the means. Differences were considered significant at $p < 0,05$.

4. Results and Discussions

The variation in time of the moisture of the probes is presented in the next four figures. The moisture of the samples starts above 80% and ends below 8%. The drying time varies between 25h in case of the oven drying method to 16min for the microwave drying method.

In Figure 1 the longest drying method is represented. Because the moisture values were not measured at equal time intervals, a polynomial trendline was used.

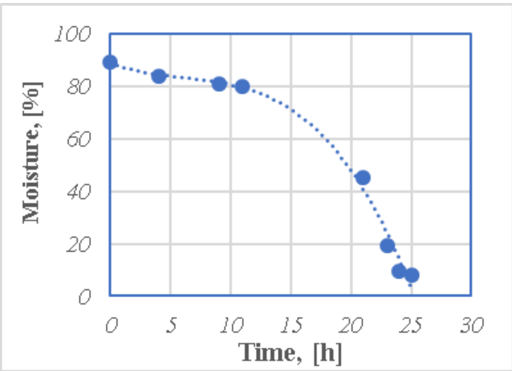

Fig. 1. *Oven drying*

In Figure 2 the representation is not comparable with other methods in point of evolution of the moisture contents in the probe; only the onset and the end value of the moisture can be compared and evaluated in relation to other three types of drying methods. This result is due to the drying program, which does not allow the analysis of intermediate values and it does not allow shorter time for the process either.

In Figure 3 the third, in duration, method of drying, with a polynomial trendline is represented. It can be noticed that after 3h the moisture drops below 9%, the value established as reference for considering the drying method completed.

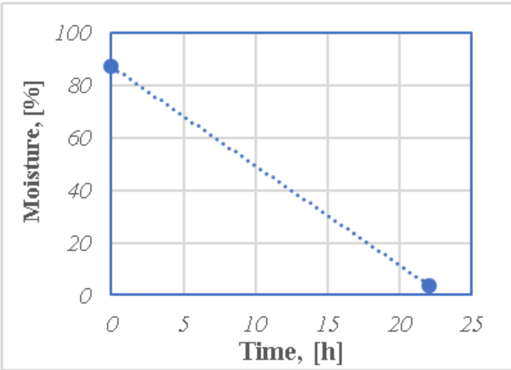

Fig. 2. *Freeze drying*

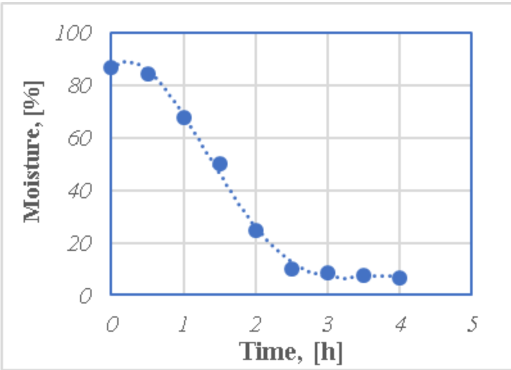

Fig. 3. *Air-convection drying*

The Microwave drying method (Figure 4) is the shortest method, in 15 minutes the moisture of the probe dropping below 9%.

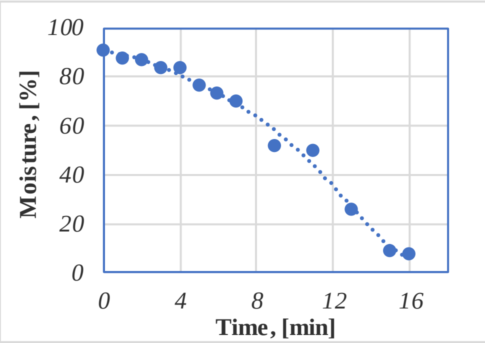

Fig. 4. *Microwave drying*

In Table 1 the values of TPC for the four drying methods considered are presented. The average value is 4,935mg GAE/g, with a deviation below 4%.

Total Phenolic Content (TPC) Table 1

Drying method	TPC [mg GAE/g]
Oven	4.835 ± 0.060
Microwave	4.845 ± 0.074
Air convection	4.941 ± 0.028
Freeze drying	5.118 ± 0.071

5. Conclusions

The results indicated that the *Amaranthus caudatus* plant waste has a satisfactory content of phenols, as compared with the data from the literature regarding TPC from the fresh plant.

The freeze-drying method showed a better TPC as compared with the other three methods which means that it is an optimum method of drying in order to preserve this kind of bioactive compounds.

The lowest TPC content was identified for the air-convection drying method for the studied case.

The statistical analysis performed by Oneway ANOVA indicated a statistically significant difference ($p<0.05$) between all four drying methods, considered as individual groups, regarding the TPC means.

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References

1. Arêas J.A.G., Carlos-Menezes A.C. et al., 2015. Amaranth.
2. Babeanu N., Nita S., Popa O. et al., 2013. Analytical characteristics of amaranth and pumpkin oils from Romania. In: Current Opinion in Biotechnology, vol. 24, S138.
3. Babeanu N., Nita S., Popa O. et al., 2016. Squalene recovery from Amaranth oil by short path distillation. In: Journal of Biotechnology, vol. 231(2), S53.
4. Borneo R., Aguirre A., 2008. Chemical composition, cooking quality, and consumer acceptance of pasta made with dried amaranth leaves flour. In: LWT - Food Science and Technology, vol. 41(10), pp. 1748-1751.

5. Campos D., Chirinos R., Gálvez Ranilla L. et al., 2018. Bioactive potential of andean fruits, seeds, and tubers. In: Advances in Food and Nutrition Research, vol. 84, pp. 287-343 (1st Edition, Elsevier Inc.).
6. Corke H., Cai Y.Z., Wu H.X., 2015. Amaranth: Overview. Encyclopedia of Food Grains. 2nd Edition, vol. 1–4, pp. 287-296.
7. Cornejo F., Novillo G., Villacrés E. et. al., 2019. Evaluation of the physicochemical and nutritional changes in two amaranth species (*Amaranthus quitensis* and *Amaranthus caudatus*) after germination. In: Food Research International, vol. 121, pp. 933-939.
8. Fierascu I., Ungureanu C., Avramescu S. et al., 2015. In Vitro Antioxidant and Antifungal Properties of *Achillea millefolium* L. In: Romanian Biotechnological Letters, vol. 20(4), pp. 10626-10636.
9. Fuentes C., Perez-Rea D., Bergenståhl B. et al., 2019. Physicochemical and structural properties of starch from five Andean crops grown in Bolivia. In: International Journal of Biological Macromolecules, vol. 125, pp. 829-838.
10. Furman B.L., Candasamy M., Bhattamisra S.K. et al., 2019. Reduction of blood glucose by plant extracts and their use in the treatment of diabetes mellitus; discrepancies in effectiveness between animal and human studies. In: Journal of Ethnopharmacology, vol. 247, pp. 1-25.
11. Jiménez-Aguilar D.M., Grusak M.A., 2017. Minerals, vitamin C, phenolics, flavonoids and antioxidant activity of Amaranthus leafy vegetables. In: Journal of Food Compozition and Analysis, vol. 58, pp. 33-39.
12. Jimoh M.O., Afolayan A.J., Lewu F.B., 2018. Suitability of Amaranthus species for alleviating human dietary deficiencies. In: South African Journal of Botany, vol. 115, pp. 65-73.
13. Kongkachuichai R., Charoensiri R., Yakoh K. et al., 2015. Nutrients value and antioxidant content of indigenous vegetables from Southern Thailand. In: Foof Chemestry, vol. 173, pp. 838-846.
14. Kraujalis P., Venskutonis P.R., Pukalskas A. et al., 2013. Accelerated solvent extraction of lipids from *Amaranthus* spp. seeds and characterization of their composition. In: LWT - Food Science Technology, vol. 54(2), pp. 528-534.
15. Li H., Deng Z., Liu R. et al., 2015. Characterization of phenolics, betacyanins and antioxidant activities of the seed, leaf, sprout, flower and stalk extracts of three *Amaranthus* species. In: Journal of Food Composition and Analysis, vol. 37, pp. 75-81.
16. López D.N., Galante M., Raimundo G. et al., 2019. Functional properties of amaranth, quinoa and chia proteins and the biological activities of their hydrolysates. In: Food Research International, vol. 116, pp. 419-429.
17. Mohanty S., Zambrana S., Dieulouard S. et al., 2018. *Amaranthus caudatus* extract inhibits the invasion of E. coli into uroepithelial cells. In: Journal of Ethnopharmacology, vol. 220, pp. 155-158.
18. Moronta J., Smaldini P.L., Docena G.H. et al., 2016. Peptides of amaranth were targeted as containing

sequences with potential anti-inflammatory properties. In: Journal of Functional Foods, vol. 21, pp. 463-473.

19. Peter K., Gandhi P., 2017. Rediscovering the therapeutic potential of *Amaranthus* species: A review. In: Egyptian Journal of Basic and Applied Sciences, vol. 4(3), pp. 196-205.