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CHEMICAL SCIENCES

Ultrasound combined with microwave hydrodiffusion and gravity for enhancement of caffeine extraction from guarana powder

DÉBORA P. DE MORAES, DANIELE F. FERREIRA, ALEXANDRE JOSÉ CICHOSKI, MILENE T. BARCIA & JULIANO S. BARIN

Abstract: The extraction of valuable compounds from dried fruits and vegetables by microwave hydrodiffusion and gravity (MHG) requires previous hydration of the plant material. In this work, ultrasound was used to speed up the hydration of guarana powder before MHG extraction and increase caffeine recovery. The humidification step was speeded up with ultrasound taking only 15 min over 60 min without ultrasound. Water and 50% (v/v) ethanol were evaluated as green solvents for humidification, with a higher concentration of caffeine obtained for the hydroalcoholic solution. Ultrasound pretreatment allowed guarana extracts from MHG with two times more caffeine for both solvents evaluated. Therefore, ultrasound can be used in the hydration step before MHG extraction to reduce time and increase caffeine recovery from guarana powder.

Key words: caffeine, ultrasound, MHG, green extraction, GRAS solvents, microwave.

INTRODUCTION

Guarana (*Paullinia cupana*) has stimulating properties due to its high caffeine content (Schimpl et al. 2013), representing 2 to 6% of the weight of the guarana seed. In addition to the stimulant action of guarana, products from this plant are consumed worldwide due to their beneficial effects in improving cognitive function and increasing energy expenditure (Santana & Macedo 2018). In this sense, guarana seed extracts have attracted the interest of the pharmaceutical and food industries.

The choice of extraction method is crucial because it directly influences the quality of extracts (Marques et al. 2019). The commonly used processes for caffeine extraction are time- and energy-consuming, requiring high volumes of solvent. Thus, developing new alternatives to recover caffeine faster and greener is attractive. In this sense, some emerging technologies, such as those based on microwaves and ultrasound, can be used for process intensification, reducing extraction time and energy consumption, and providing high-quality extracts by preserving the target compounds (Cvjetko Bubalo et al. 2018, Santana & Macedo 2019).

Microwave hydrodiffusion and gravity (MHG) is an innovative technology for the green extraction of fresh plant materials without solvents using in situ water. For dried materials, a solvent is required for humidification, often performed by soaking the material in water until the liquid absorption and swelling. Therefore, the proportion of solvent/sample for the extraction in MHG is much lower than that used in solvent extraction (e.g., in maceration). Thus, changes in the humidification process can influence MHG extractions (Ferreira et al. 2020). Other solvents than water can be used to improve MHG extractions, but there are only a few studies in this regard (Confortin et al. 2021, Farias et al. 2022).

Ultrasound has been extensively used in solvent-based extractions, facilitating the penetration of liquids in the plant, and the swelling of the plant, leading to improved mass transfer and higher yields (Mason 2015). Despite the limitations of ultrasound-assisted extraction related to the recovery of compounds bound to complex matrices (Carreira-Casais et al. 2021), for guarana seeds, a significant increase in caffeine extraction yields was reported by using ultrasound (Carciochi et al. 2021). However, ultrasound has not yet been used as a pretreatment before MHG extraction, and it was limited to removing compounds from the residual dried matrix after MHG processing (Boukroufa et al. 2015, Ravi et al. 2018, Sanz et al. 2020).

For these reasons, combining ultrasound with MHG results in an attractive alternative for the extraction of natural products, which has not yet been studied. Thus, ultrasound was used as a pretreatment to speed up the humidification and improve MHG extraction of caffeine from guarana seeds powder. Water and ethanol solvents with and without ultrasound were used. An additional extraction step was also investigated, and the results for the proposed ultrasound-assisted humidification for MHG extraction (US-MHG) were compared with a maceration with solvents.

Therefore, this study aimed to verify if MHG technology is efficient for obtaining an extract rich in caffeine from guarana, considering the effect of ultrasound as a pre-treatment step to improve humidification and caffeine extraction.

MATERIALS AND METHODS

Samples and reagents

Dried guarana (*Paullinia cupana)* seed powder samples were kindly provided by Duas Rodas Industrial (Jaraguá do Sul, SC, Brazil). The samples were previously ground in a knife mill (Model MA 630/1, Marconi, Brazil) to improve the homogeneity (particle size lower than 1 mm).

The extractions were performed using ethanol (99.5%) from Dinâmica (Jóia, Brazil) and distilled water. For mobile phase preparation, chromatographic grade acetonitrile was purchased from J. T. Baker (Phillipsburg, USA). Double-deionized water was used after treatment in a Milli-Q system (Millipore, Bedford, MA, USA). The caffeine (≥99%) standard was purchased from Sigma-Aldrich (Jurubatuba, Brazil).

Humidification step

Conventional humidification

The guarana seed powder was placed in a 2 L glass beaker and moistened with water or 50% (v/v) ethanol at room temperature. The concentration of the hydroalcoholic solution was chosen based on the studies performed by Hu et al. (2016). Preliminary tests were carried out to choose the volume of solvent to be added to 200 grams of sample. The volume chosen (150 mL) allowed the swelling of the entire sample mass in 1 hour of humidification (Lucchesi et al. 2004). The same was done for the second extraction cycle with MHG, using 125 mL, which was the volume of solvent that allowed the entire sample to swell in 1 h of humidification.

Ultrasound-assisted humidification

In the same way as conventional hydration, 200 g of sample were mixed with 150 mL of solvent. For the second extraction 125 mL was used. The flask was placed in the center of the ultrasonic bath (model TI-H-10, Elma®, Germany, 750 W, 8.6

L) with a fixed frequency of 25 kHz, and 175 W of power. Preliminary tests were performed to determine the humidification time (data not shown). The complete absorption of solvent by the sample in a shorter period was used as a criterium. It was found that 15 min at 80% amplitude was the most suitable experimental condition.

Extraction with MHG

The equipment for MHG extractions (NEOS-GR, Milestone, Bergamo, Italy) was equipped with a 1.5 L glass container with a polytetrafluoroethylene (PTFE) cap and sample holder and a glass condenser, which was connected to an ultra-thermostatic bath at 7 ºC. The extraction was performed using 2 W/g (400 W for 200 g of dry sample) for 16 minutes (Benmoussa et al. 2018). The guarana extract was collected in a round-bottomed flask and then transferred to a graduated glass to verify the volume obtained. The extracts were immediately prepared for the determination of caffeine.

Maceration with water or hydroalcoholic solution

For maceration, 3 grams of samples were weighed in 50 mL polypropylene tubes and mixed with 30 mL of water or 50% ethanol (v/v). The samples were mixed in an orbital shaker for 4 h (250 rpm; Q225 M, Quimis, Brazil) at room temperature. After the maceration time, the samples were filtered for further analysis (Ferreira et al. 2020).

Determination of caffeine by high-performance liquid chromatography (HPLC)

The identification and quantification of caffeine were performed using HPLC-UV/Vis equipment (ProStar 210, Varian, San Diego, EUA). Separation was performed in the isocratic mode at room temperature for 5 min using a Hypersil Gold C18 column (5 µm of particle, 4.6 mm, 250 mm) (Musilová & Kubíčková 2018). The mobile phase was a mixture of 30% acetonitrile and 70% water. The identification of caffeine was performed by comparing retention time and analysis of the sample's peak spectrum compared to the standard. A calibration curve at 270 nm was prepared with the caffeine standard, which was composed of seven points in the range of 0.1 to 12.1 mg/L (R^2 = 0.9973). The results were expressed in mg of caffeine/mL of extract.

Statistical analysis

The data was evaluated using analysis of variance (one-way ANOVA) followed by Duncan's test, with a significance level of 5% using the R (version 4.3.2) software. Graphs showing the data obtained in the study were plotted using GraphPad Prism 5 software.

RESULTS AND DISCUSSION

Influence of solvent and ultrasound pretreatment in MHG extraction

The humidification of guarana powder was performed with water and 50% (v/v) ethanol, and the efficiency of these solvents for recovering the caffeine was evaluated. The results were presented in Figure 1, which shows that the humidification with hydroalcoholic solution provided extracts with a larger volume and richer in caffeine than those obtained with water.

The ethanolic solution solubilized the caffeine efficiently and showed a greater capacity to recover the volume of solvent added compared to water. With the ethanolic solution, it was allowed to recover 62% of the added solvent, while with water, it was possible to recover only 35%. The ability of a hydroalcoholic solution to penetrate the guarana structure and remove the caffeine makes this solvent the most efficient solvent for extracting caffeine from guarana with MHG. These results are in agreement with the studies of Hu et al. (2016), which proved that

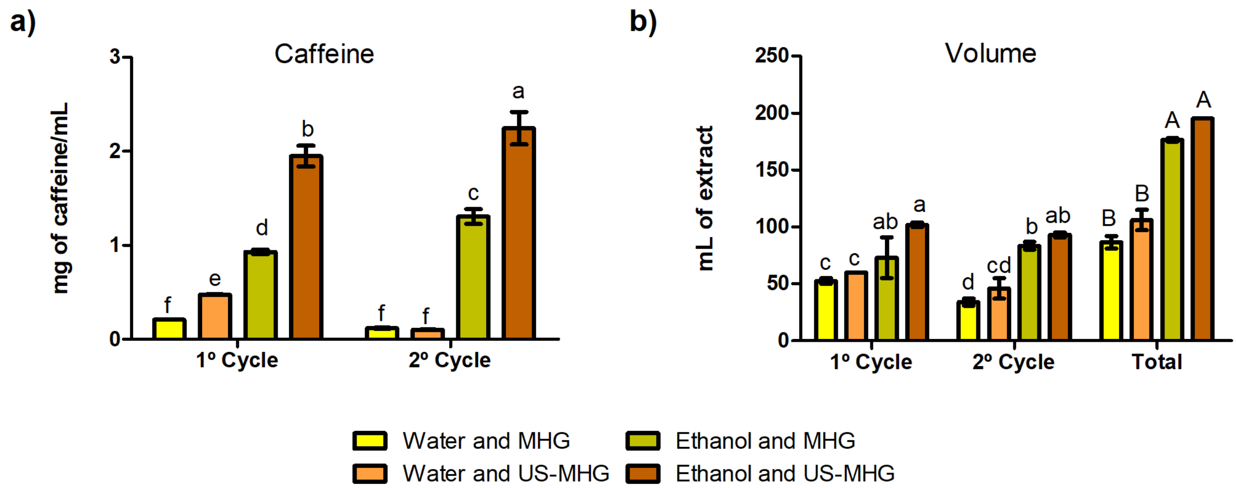

Figure 1. Influence of solvent, ultrasound pretreatment, and the number of cycles on the extraction of caffeine from guarana powder; a) caffeine concentration in guarana extracts; b) volume of extract. The samples from the first and second cycles followed by at least the same lower-case letter do not differ from each other by the Duncan test (p> 0.05). The samples of the total volume of extract followed by the same capital letter do not differ by Duncan test (p> 0.05).

caffeine's solubility increased with the addition of ethanol up to 50% (v/v), being more efficient than pure water. The same authors emphasize the efficiency of 50% (v/v) ethanol solution as a solvent for the extraction of caffeine. In addition to increasing the solubility of caffeine, the hydroalcoholic solution causes a swelling effect due to the water, increasing the yield. For MHG, the use of hydroalcoholic solution in combination with the microwave heating could lead to most significant diffusion of the solvent into the raw material, favoring the penetration of the solvent in the guarana structure and resulting in the most efficient extraction of caffeine.

In the proposed US-MHG the combination of ultrasound for the humidification of guarana powder and subsequent extraction with microwaves promoted an increase in the concentration of caffeine in extracts for both solvents (water and hydroalcoholic solution). The application of ultrasound for humidification provided extracts with higher caffeine content. The use of ultrasound did not affect the volume of the extracts, showing that the difference in volume is related only to the solvent effect. In addition, another advantage presented by the use of ultrasound as a pretreatment was the significant reduction in the humidification time, only 15 min over 60 min without ultrasound (Table I).

Effects of ultrasound on the extraction of natural products have been reported and can be related to the increase in the yields observed in our experiments. Fragmentation, erosion, sonoporation, local shear stress, and destruction of the plant structure, in addition to turbulence and micromixing in the plant cell, result in the overflow of the cellular plant content for the extraction solvent (Chemat et al. 2017). Moreover, the damage caused by ultrasound in cell structure increases the accessibility of the solvent in the plant, improving the solubilization of the intracellular compounds and providing their release from the cell, which resulted in an efficient extraction process (Petigny et al. 2013).

The faster humidification with ultrasound could be related to its influence on the capillary effect. Sonication promoted an increase in the

Table I. Time spent for the different extraction methods evaluated.

	Humidification time (min)	MHG time (min)	Shaking time (min)	Total extraction time (min)
MHG	60	16	-	76
US-MHG	15	16	-	31
Maceration	-	-	240	240

depth and speed of penetration of liquids into the pores of plant material (Mason 2015, Vinatoru 2001). In this way, these effects may also have favored the extraction of caffeine from the guarana using the proposed US-MHG extraction.

The use of a second extraction cycle in MHG allowed the greater use of the raw material to extract target compounds, making it possible to increase caffeine recovery. Thus, the caffeine extraction was performed in this study by two cycles. The results presented in Figure 1 showed that in the second cycle, similar concentrations of caffeine could be obtained for US-MHG with the hydroalcoholic solution. The first extraction with the hydroalcoholic solution could have caused changes in the guarana's structure, possibly favoring the greater solvent penetration and the removal of a higher caffeine content in the second cycle.

In the first extraction cycle using the US-MHG combination, a high concentration of caffeine was removed, approximately twice the concentration found in the extract without pretreatment with ultrasound. However, the pretreatment with the ultrasound was unable to increase the caffeine content extracted in the second extraction cycle for water as a solvent. Thus, the second cycle of treatment that used water in US-MHG did not differ statistically from the first one.

Although the volume of solvent added in the second cycle was slightly lower, the volume of extract obtained by the second cycle did not differ for all treatments, except for the use of water as a solvent without pretreatment with ultrasound. When analyzing the total volume of the extracts, that is, the sum of the two extraction cycles, it is seen that the hydroalcoholic solution for US-MHG had the highest volume of extract, in addition to being the one with the highest concentration of caffeine. It is important to mention that agglomeration and a lump of guarana powder were observed after the second extraction cycle, which hindered further extractions.

Comparison between extraction methods

The comparison of extraction methods (Figure 2) evidenced that ethanol and US-MHG provided extracts with a higher concentration of caffeine. In addition, the US-MHG allowed extractions in only 31 minutes (including the humidification step), while the maceration lasted 4 hours (Table I).

For extractions using water, a lower caffeine concentration was found even for maceration,

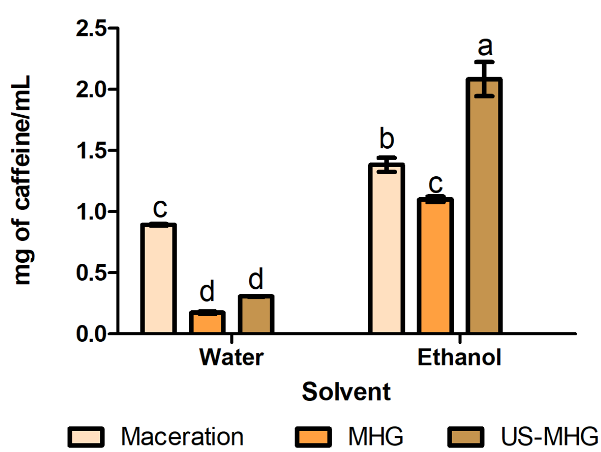

Figure 2. Caffeine concentration of extracts obtained with different extraction methods. The samples followed by the same letter do not differ by Duncan test ($p > 0.05$). The caffeine content of the two extraction cycles was considered and normalized to 1 mL

showing that the hydroalcoholic solution is the best solvent for extracting caffeine from guarana. It is important to mention that like the water, ethanol is considered a green solvent authorized for extraction by Brazilian regulatory bodies (ANVISA 2018).

It is important to mention that this work did not seek to carry out an exhaustive extraction of caffeine from guarana. However, it was possible to obtain concentrated caffeine extracts quickly with a minimum amount of solvent. Removing solvent from the extract is time-consuming and costly, and the proposed method is advantageous in this regard.

CONCLUSION

The ultrasound-assisted humidification reduced the time and improved the extraction of caffeine from guarana powder, mainly using the hydroalcoholic solution. The extraction by MHG with two extraction cycles maximized the caffeine recovery. Compared with maceration, extraction with the proposed US-MHG presented a higher concentration of caffeine and faster extraction. Thus, this study shows that combining ultrasound with MHG is advantageous for caffeine extraction, particularly by speeding up the process and allowing higher yields.

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DÉBORA P. DE MORAES
https://orcid.org/0000-0002-9178-073X

DANIELE F. FERREIRA
https://orcid.org/0000-0002-5481-3790

ALEXANDRE JOSÉ CICHOSKI
https://orcid.org/0000-0002-3996-0143

MILENE T. BARCIA
https://orcid.org/0000-0002-8490-1679

JULIANO S. BARIN
https://orcid.org/0000-0002-8351-3287

Universidade Federal de Santa Maria, Departamento de Tecnologia e Ciência dos Alimentos, Avenida Roraima, 1000, 97105-900 Santa Maria, RS, Brazil

Correspondence to: **Juliano Smanioto Barin**
E-mail: juliano@ufsm.br

Author contributions

DPM- Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Methodology, Validation, Writing - Original Draft; DFF; Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing - Review & Editing; AJC- Formal analysis, Investigation, Supervision, Visualization, Writing - Review & Editing; MTB-Methodology, Validation, Project administration, Supervision, Visualization, Writing - Review & Editing; JSB- Conceptualization, Methodology, Project administration, Supervision, Visualization, Writing - Review & Editing.