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Ultrasonic vs. Microwave Extraction Intensification of Active Principles from Medicinal Plants

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Microwave and ultrasonic fields, biochemically safe, were used to extract stevioside glycosides from Stevia rebaudiana Bert. Distilled water in 1/10 (w/v) ratio was employed as solvent. The active principle was quantified by HPLC. A comparison with classical extraction results is provided.
Both intensive extraction techniques of active compounds seem to be economically promising (simple and efficient), still care must be taken to avoid local over-exposure.

Keywords: Ultrasound assisted extraction, Microwave extraction, Stevioside glycoside, Medicinal plants.

1. Introduction

Stevia rebaudiana, native from Paraguay, is used as herbal sweetener for over 1500 years. Extracts of Stevia rebaudiana are part in weight-loss programs because of its ability to reduce the cravings for sweet and fatty foods, to treat the diseases diabetes, hypoglycaemia, candidasis, high blood pressure, skin abrasions and inhibiting growth and reproduction of bacteria-like plaque. Stevia's greatest appears to be a natural alternative to artificial sweeteners (such as aspartame or sodium saccharin). The sweetness in *Stevia rebaudiana* is mainly attributed to two glycoside compounds: stevioside (3-10% of dry leaf weight) and rebaudioside A (1-3%) which can be up to 250 times sweeter than sucrose (Duke, 2006). The glycosides of Stevia rebaudiana leaves have been extracted using classical techniques: maceration or thermal extraction, either requiring long processing time and low efficiency, in case of maceration, or facing thermal degradation, in case of infusion and decoction (Vinatoru, 2001).
In order to increase the productivity, several intensification techniques like ultrasonic waves, supercritical fluids or microwaves were associated with extraction of plant's compounds to improve the yield and quality of extracted products (Wang, 2006). From these, ultrasound assisted and microwave extractions emerged as two promising techniques from an economical point of view, being inexpensive, simple and efficient.

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These procedures increase at least one of the major parameters governing extraction: the kinetic, through the partial mass transfer rates, the interfacial area or the driving force (Cravotto, 2008).

Ultrasound waves were employed to extract active compounds such saponins, steroids and triterpenoids from *Chresta spp*. about three times faster than with the traditional extraction methods (Schinor et al., 2004).

The ultrasonic field enables generation, locally, of micro-cavitations in the liquid surrounding the plant material. The effects are twofold: mechanical disruption of the cell's wall releasing its content and local heating of the liquid, increasing the extract diffusion. The kinetic energy is introduced in the whole volume following the collapse of cavitation bubbles at or near walls or interfaces thus improving the mass transfer across the solid-liquid interface. The mechanical effects of ultrasounds induce a greater penetration of solvent into cellular membranes walls, facilitating the release of contents of the cells and improve mass transfer (Kiel, 2007).

The applications of microwave assisted extraction to natural compounds such glycosides, alkaloids, carotenoids, terpenes, essential oils has been reviewed in (Kaufmann, 2002).

The use of microwave energy for the extraction of active substances from plant materials results in more effective heating, faster energy transfer, reduced thermal gradients, selective heating, reduced equipment size, faster response to process heating control, faster start-up and increased production. During absorption, the microwaves' energy is converted into kinetic energy, thus enabling the selective heating of the microwave-absorbent parts of the plant material. The volume increased in this way makes cells explode, releasing their content into the liquid phase. When the liquid phase absorbs the microwaves, the kinetic energy of its molecules increases, and consequently, the diffusion rate increases too (Mandal, 2007).

2. Materials and Methods

2.1. Reagents and plant material

Stevioside (S3572, assay ≥98% - HPLC - form: solid , colour: white, free soluble in water and ethanol, solubility H2O: >20 mg/mL, storage temperature: 2-8^{0}C, chemical name: 4α-13-[(2-O-β-D-Glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid β-D-glucopyranosyl ester, chemical formula: C38H60O18, molecular weight: 804.87) was used as standard chemical for HPLC analysis.

Chromatographic grade – distilled water, HPLC grade acetonitrile, were utilized as extraction solvents and mobile phase.

The plant material consists of dried leaves of Stevia rebaudiana purchased from the Paraguay Medicine Market, harvested in 2008. They were stored in dark hermetic tight bags to protect them from humidity and light and, before each bunch of experiments, they were cut into pieces of the appropriate equivalent diameter.

2.2. Experimental setup

An extractor equipped with an ultrasonic horn transducer (Model 750W, Sonics & material Inc., USA) working at 20kHz frequency and 750W input power with amplitude range and sample temperature being monitored up to 100^{0}C and a microwave enclosed

extractor (Model Initiator 2.0, Biotage) working at 2.45GHz frequency, in the range of powers from 0 to 400W and temperatures between 40 and 2500C were employed.
For stirring and heating a TK-22 Magnetic stirrer with heating was used and the temperature in case of classic thermal extraction was controlled with a fiber optic sensors for temperature and pressure (Model Pico Power Sens 6.2, Opsens, Canada).
The filtrate was separated from the residual material by Vacuum Captiva filtration system. For chromatographic measurements, a HPLC system Agilent Technologies 1200 series model with UV-VIS detector was used. After the extraction process, irrespective of the method, the filtrate was separated from the residual plant material by vacuum pump filtration.

2.3. Classic extraction
Thermal extraction was used as reference for comparisons with the ultrasound and microwave assisted extraction methods. Classic extraction was performed with stirring and with 1/10 ratio (w/v) sample of Stevia rebaudiana dry leaves with 0.315mm particle sizes weight to solvent (distilled water) volume, in small flasks. The mixture was heated at 90^0C for 1, 2, 3, 5 minutes.

2.4. Ultrasound assisted extraction
The mixture with the same composition was placed into the ultrasound assisted extractor and sonicated for 1, 2, 3, 5, minutes at 50%, 80% and 100% amplitude of output power, without stirring.

2.5. Microwave assisted extraction
The mixture with the same composition was placed into the closed microwave assisted extractor and irradiated for 1, 2, 3, 5, minutes at 70^0C, 90^0C, 100^0C and 110^0C, with stirring.

2.6. Extractive values
According to the Romanian Pharmacopoeia (10th edition) approximately 2g (2 ml) of extract was placed into a flat-bottomed glass dish (36 mm diameter and 28 mm height) covered to prevent evaporation of solvent before weighting. After weighting, the extract was dried in oven at 103^0C for 3h. The content of extractive substances in the plant material was calculated from the mass of dry extract and the initial mass of plant subject to experiment. The concentration of extractive substances in the liquid extract was calculated from the mass of dry extract and the volume of liquid extract. The extractive value of the soluble compounds from the extract was calculated as a mass percentage of dry residue (g/100g extract).

2.7. Determination of steviol glycosides percentages using HPLC
The HPLC method was applied to quantify the stevioside in the extracts obtained from Stevia rebaudiana dry leaves.
Standard series in the concentration range of 0.5-10mg/mL were prepared in the mobile phase consisting of mix HPLC grade acetonitrile and bi-distilled water (80:20, v/v) from the stock solution. The mobile phase was used as solvent for all HPLC studies. The HPLC analysis conditions were performed by isocratic elution with a flow rate of 0.5 mL/min. All solvents were filtered through a 0.22µm Millipore filter. Volumes of 5µL extracts prepared from each sample were directly injected into HPLC then the peak areas at the characteristic wavelength of the steviol glycosides were measured. The UV-

VIS detector was set to 210nm and peak areas were integrated automatically using Agilent software. Separation was carried out using a Supelcosil LC-NH2 or equivalent (length: 15cm; inner diameter: 3.9 - 4.6mm) and flow rate was set to 0.5 mL/min for an isocratic elution at 35^0C as column temperature. The instrument was calibrated pumping mobile phase through it until a drift-free baseline is obtained. The Agilent software recorded the chromatograms of the sample standard solution.
All the computations concerning the quantitative analysis were performed with external standardization of the measured peak areas. The results were obtained as the mean value of three separate injections. The measurements of stevioside from samples of Stevia rebaudiana were done according to stevioside standard. At flow rate of 0.5 mL/min the retention time for stevioside was 1.075min.

3. Results and Discussions

The results of different extraction methods employed being presented in Figures 1 to 3.
Although not unexpected, the results from Figure 1 show a remarkable dependency of concentration in stevioside upon the temperature and type of the waves used to intensify the mass transfer. The supplemental kinetic energy introduced from outside into the liquid phase through heating, wave energies and stirring, modifies the extraction process rate and without altering the thermodynamic equilibrium.

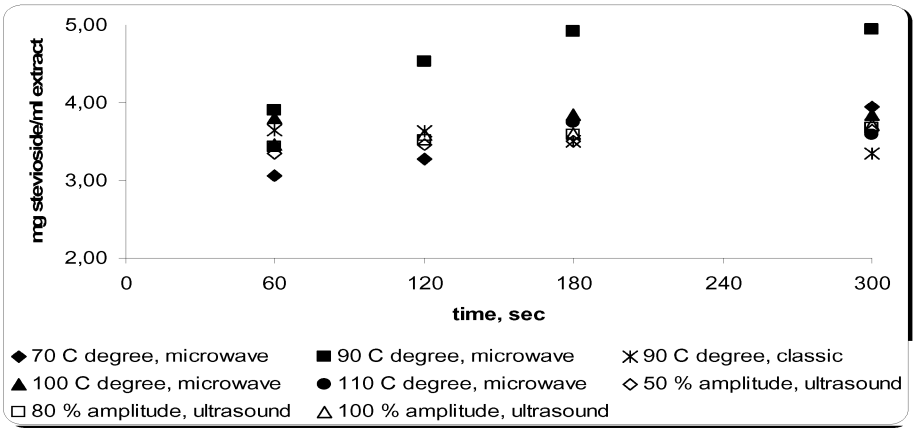

Figure 1. Influence of temperature and amplitude of ultrasound power upon the stevioside content in time

From Figure 1, we can observe an important downside of classic extraction - the thermal degradation of the valuable compound which starts manifesting with increasing the temperature at values higher than 100^0C or using long extraction time (higher than 3 minutes).
When using ultrasound and microwave fields as intensification technique, the extraction rate increases and the stevioside content attaining its maximum value for less than five minutes (see Figure 1 and 2 for details). This is the effect of the sharp increase of the local turbulences in case of ultrasound extraction and of the effective heating with the

disruption of weak hydrogen bounds promoted by the dipole rotation of the molecules, in case of microwave, which increase the mass transfer through diffusion inside the solid and also faster mixing of the liquid, thus maintaining the highest possible driving force.

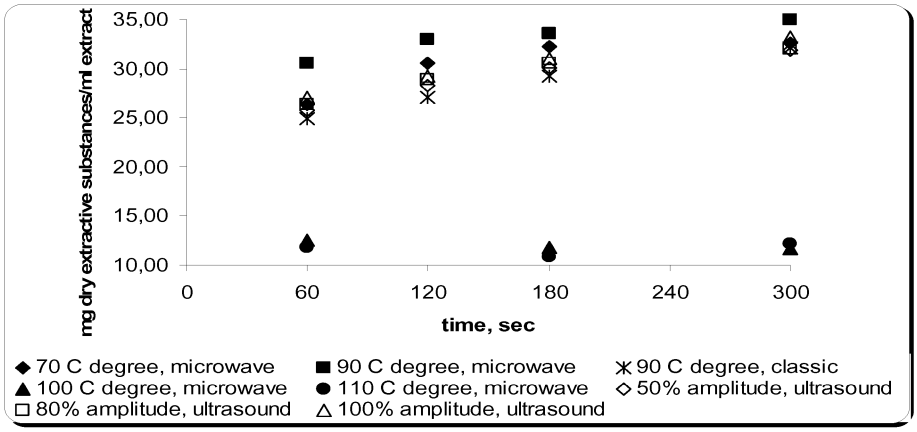

Figure 2. Influence of temperature and amplitude of ultrasound power upon the dry extractive substances content in time

Increasing the power of the ultrasonic field has no visible effect (Figures 1 and 2) which means that the field density is already high for the quantities at hand. The main drawback is the danger of overexposure, when the valuable species ends up being destroyed (see Figure 1, the decrease in the concentration of stevioside for long times).

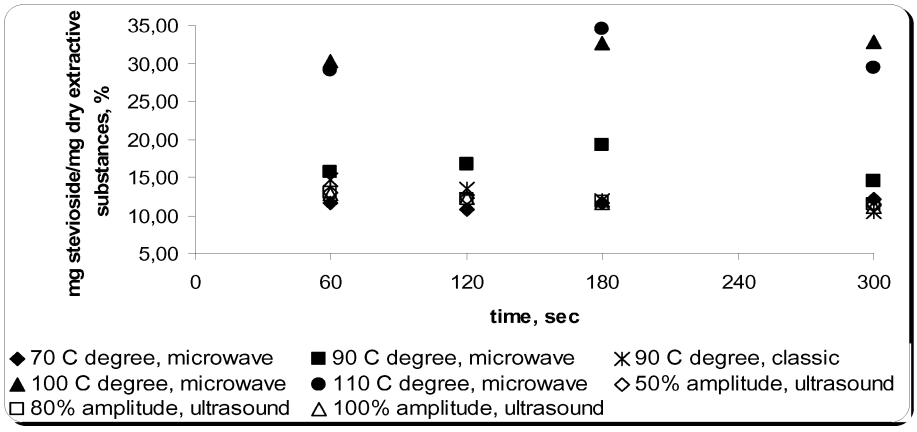

Figure 3. Influence of temperature and amplitude of ultrasound power upon the purity of the extracts in time

Compared to thermal extraction, when the extract is subject to thermal degradation through overheating, these two intensification techniques ensure higher purity values in

active substances (see Figure 3) together with a much lower possibility of overexposure, i.e. the waves' field could be cut off rapidly, contrary to the thermal field.

4. Conclusions

In order to increase process productivity, yield and quality of products, microwave or ultrasonic fields were associated with extraction of plant's active compounds.
The purpose of the experiment was to compare the intensifications techniques with classical extraction method like thermal extraction, studying the influence of temperature and output power to determine the optimum domain in reliable extraction protocols.
In comparison with thermal extraction, the intensification techniques emerged as secure and worthy methods to improve either a rather time consuming or an energy intensive (far from optimal conditions) process.
Like classic extraction, intensification methods of extraction needs special equipment to be functional, which means higher investments, and electricity to produce the ultrasonic and microwave waves. So, a soundly economic analysis should be done, in order to choose the best extraction procedure.

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