Extraction Intensification Using Ultrasounds

Case study: Glycosides from Stevia rebaudiana Bert

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The paper shows the benefits of ultrasound techniques particularization at extraction of some active principles from some plants like Stevia rebaudiana Bert. leaves. Ultrasound assisted extraction proved to be a biochemically safe procedure, unless time overexposure. The objective of experimental works was to find out some optimum extraction conditions for a fast and reliable ultrasound assisted extraction protocol and to compare the results with the classical extraction methods. The efficiency of Stevia rebaudiana Bert. glycosides extraction was investigated with respect to process factors like solvent type, state and concentration of discrete phase in extraction system and the time of extraction. The optimum parameters for the ultrasound assisted extraction are: water as solvent, 1/10 (g/L) as solid concentration in extraction media and 20 min for extraction time.

Keywords: Stevia rebaudiana Bert., Glycoside, extraction enhancement, ultrasound-assisted extraction

Medicinal and aromatic plants provide an inexhaustible resource of row materials for the pharmaceutical, cosmetic, and food industries [1]. *Stevia rebaudiana* is normally used as a natural herbal sweetener to appease otherwise unpalatable medicinal drinks. Other uses of the plant and its extracts are in weight-loss programs because of its ability to reduce the cravings for sweet and fatty foods, in treating diseases like diabetes, hypoglycemia, candidasis, high blood pressure, skin abrasions or inhibiting growth and reproduction of bacteria-like plaque [2]. Stevia’s greatest economic potential is as a natural alternative to artificial sweeteners such as aspartame or sodium saccharin. The sweetness in *Stevia rebaudiana* is attributed mainly to two glycoside compounds: stevioside (3-10% of dry leaf weight) and rebaudioside A (1-3% of dry leaf weight) which can be up to 250 times sweeter than sucrose. The stability at higher temperatures and for a pH range of 3-9 is another advantage of stevioside over artificial sweeteners [3].

The preparation of row extracts from vegetable tissues could be considered a starting point for the isolation and purification of chemical constituents present in medicinal plants. Thus the extraction process is useful in removing the compounds of interest; increasing its selectivity is the best way to separate them from other extractives which could be harmful latter [4]. Typically, the glycosides of *Stevia rebaudiana* leaves have been extracted using classical techniques: maceration, infusion or decoction. Usually, these traditional procedures require either long extraction times together with low efficiencies (maceration), or increased temperatures (thermal extraction – infusion and decoction), which have the inconvenient that many natural products could suffer degradations since they are thermally unstable [1, 3, 5-7].

The glycosides compounds contain many hydroxyl groups thus conferring them a high solubility in water or alcohols. The toxicity restriction imposes as safe solvents only water and lower alcohols (methanol, ethanol) [1, 3, 5].

Ultrasound assisted extraction is an inexpensive, simple and efficient alternative to conventional extraction techniques. The main benefits of using ultrasounds in solid-liquid extraction are the increase of the extraction yield and faster kinetics. Ultrasounds can also reduce the operating temperature allowing the extraction of thermolabile compounds [8]. Nowadays, ultrasound assisted extraction has been employed to extract, three times faster than traditional extraction methods, active compounds such saponins [9], steroids and triterpenoids from Chresta spp. [4].

Ultrasound produce cell membrane walls disruption, particle size reduction leading to a greater interfacial area between solid and liquid phases and faster access of solvent to cell components, compared with traditional methods [10].

In solid-liquid extraction where solid is from plants, the mechanical effects of ultrasounds induce a higher penetration of solvent into cellular membranes walls, facilitating the release of contents of the cells and improve mass transfer [11]. So the use of ultrasounds increases component extraction in a shorter time and at lower temperatures [10].

Practically the ultrasounds dissipation in extraction media strongly increases the rate of the process steps: first, dissolution of the soluble constituents on or near surface of solid plant particles and second, mass transfer of soluble constituents from the plant material into the solution by diffusion and osmotic process [12, 13].

The present study focuses on proving experimentally the advantages of using ultrasound assisted extraction instead of several classical methods thorough a carefully designed experiment. The purpose of the experimental plan is to vary, in a controlled manner, the current parameters of extraction: sugar solvent type, solid concentration in system given by its mean ratio \( x_p \), temperature, and specific power of ultrasound dissipation and to determine the optimum extraction conditions.

**Experimental part**

**Reagents and plant material**

Aqueous ethanol and distilled water was used as extraction solvents. Dried leaves of *Stevia rebaudiana* stored in dark bags to protect them from humidity and light (that prevents the degradation of the compounds with biologic activity).
**Apparatus:** Two types of ultrasound-assisted extractors were employed: an extractor equipped with a 2 mm ultrasonic horn transducer (Model UP50H, Hielscher Ultrasonics, Germany) working at 30 kHz frequency and 50 W input power and an ultrasonic bath (Model 2510 Branson, USA) working at 40 kHz frequency and 100 W output power.

**Extraction methods:** Before experiment, dried leaves of *Stevia rebaudiana* Bert. were cut into pieces then only the 4 mm fraction was retained for the experiment. After the extraction process, irrespective of the method used, the filtrate was separated from the residual plant material by vacuum pump filtration and collected for analysis.

**Classical extraction methods:** The conventional extraction methods like maceration and infusion was used as a reference for comparison with the ultrasound-assisted extraction method.

**Maceration:** Maceration was performed using 5 g of dried leaves from *Stevia rebaudiana* Bert. and different solvents: distilled water and water/ethanol mixtures (35% and 50%) with different volume of solvent to weight sample ratios: 1/10, 1/8, 1/6. The mixture was left at room temperature for 12 and 24 h in closed Erlenmeyer flasks.

**Infusion:** Infusion was performed using 5 g of dried leaves from *Stevia rebaudiana* Bert. and different water to sample ratios: 1/10, 1/8, 1/6. Water at 40°C was mixed with the dried leaves into Erlenmeyer flasks and the mixture was left for 10 min.

**Ultrasound-assisted extraction:** Samples of 5 g were mixed with the extracting solvent (sample weight to solvent volume ratio of 1/10) and then placed into ultrasound-assisted extractors at the room temperature. The ultrasonic bath was filled with liquid water and the recipients with samples were placed into it and sonicated for 10, 15 and 20 min. The ultrasonic horn transducer was placed into the recipients with samples for 10, 15 and 20 min also. Its ultrasonic vibrations amplitude controller was varied from 60 to 100% of nominal power, and pulsed range was modified from 20% to 80%.

**Dry residue analysis: extractive values:** A gravimetric analysis [5] is used to establish the concentration of total extracted species in the liquid phase. For that about 2 g (2 mL) of separated liquid placed in a flat-bottomed glass dish covered to prevent evaporation of solvent, is analytically weighted. The weighed extract was dried in oven at 103°C for 3 h. The content of all extractive species in the plant material (ωesp) was calculated from the mass of dry extract and the mass of initial plant material. The concentration of extractive species in the liquid extract (ωesp) was calculated from the mass of dry extract and used mass of liquid extract.

**Results and discussions**

The results of experiments carried out to investigate the influence of mentioned process parameters to the state of liquid concentration of all extracted species from leaves of *Stevia* are shown in figures 1-7.

As solvent type, water and ethanol/water mixtures were tested to extract glycosides from dried leaves of *Stevia rebaudiana* Bert. In general, a higher solvent quantity (xp) proved to be beneficial, dissolving the constituents more effectively thus leading to an enhancement of the extraction yield as depicted in figures 1-3.

When the solvent quantity is lower, there is not enough liquid to ensure a proper/complete welling of the cell's membrane, leading to a smaller diffusional flux of extraction. More, the concentration of the extracted species into the liquid phase rises faster to the equilibrium value, decreasing this way the extraction driving force. On the other hand higher values for the solvent volume means lower concentrations with detrimental effects upon the separation/purification steps envisaged for the valuable product. We retained as optimal the sample weight to solvent volume ratio of 1/10.

Temperature is a key factor for classical extraction. This increase changes the kinetic energy of the liquid phase, thus intensifying the diffusion process which is the leading mass transfer mechanism involved in discontinuous
Therefore the extraction efficiency obtained with infusion extraction is higher than that obtained with maceration extraction. According to figures 2 and 3, the temperature has a beneficial effect upon maceration too, but less pronounced, due to the values implied (up to 50 °C). On the other hand, with infusion, although the final extracted substances have almost the same weight, the extraction time reduces considerably (see for comparison fig. 2 and fig. 3), due to the higher temperature involved since the working liquid is boiled water.

The intensification of the extraction process using ultrasonic fields is considerable, not with respect to the quantity of extracted species, but with the rate of extraction. Due to the highly localized kinetic energy introduced by ultrasounds, the mass transfer across the cell membrane is several times faster and likely the dissipation the extracted species into the bulk of the liquid phase. Thus, the yields obtained by ultrasound-assisted extraction after 20 min are comparable with those achieved by maceration after 24 h (see for details figures 4 and 5 with respect to fig.1).

The same behaviour could be noticed when the ultrasonic filed is generated in a bath (ultrasonic assisted batch extraction, fig. 6). The process is almost completed after 20 min; increasing this limit would damage the structure of the extracted species, turning it into a biologically unsafe procedure.

As expected, the amplitude of the ultrasound waves plays an important role in the extraction intensification, lower amplitudes diminishing the extraction time. Analyzing figures 4 and 5, we observe that the amplitude effect is asymptotic, which means that a trade off between the extraction time and the waves amplitude could be found. The way of ultrasounds dissipation into the liquid phase plays also a crucial role as proved by figure 7.

In ultrasonic baths, the waves are generated outside the liquid and act in the whole volume, while when using an ultrasonic horn, the waves are generated locally, in a small volume of the extraction phase, generating a complicate internal circulation which helps the spreading of the extracted species into the bulk liquid. Another advantage of the ultrasonic bath is its wider availability and the possibility of processing many samples in the same time, in contrast to only one at a time with the ultrasonic horn. More, the sonication with the bath is non-intrusive, which eliminates all possible contamination and loss of the extract [15]. On the other hand, the use of a carrier to transmit the waves to the extracting liquid decreases slightly the efficiency of the ultrasonic bath compared to the ultrasonic horn system (fig. 7).

Conclusions

Distilled water seems to be the best choice to extract glycosides from *Stevia rebaudiana* Bert. dried leaves, for its efficiency, low cost, low toxicity and environmental compatibility.

Compared with classical methods like maceration and heat extraction, the utilization of ultrasound-assisted extraction proved to be a much simpler and more effective mean to obtain efficiently extractive species from plants. Also, the ultrasound-assisted extraction can be carried out at lower temperature, which avoids the degradation of thermally unstable compounds. This means not only decreasing the operating costs, due to the economy of energy, but also improving the productivity, a very important industrial issue. In order to discriminate between the use of ultrasonic bath or horn, further economic analyzes should be done since, from the extraction point of view, both proved to have comparable efficiencies.

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