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ENHANCING THE AQUEOUS EXTRACTION OF STEVIA GLYCOSIDES FROM *STEVIA REBAUDIANA* LEAVES UNDER THE ACTION OF ELECTRIC DISCHARGE PRETREATMENT

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ABSTRACT: The Extraction of the natural sweetening glycosides from *Stevia rebaudiana* leaves (Steviosides) can be processed by several methods such as hot water, alcohols (methanol or ethanol) or supercritical fluid extraction. Water is an environmentally friendly and economically preferable alternative to harmful organic solvents in spite of its inferior efficacy. Therefore, different high voltage electric discharge (HVED) treatments (Intensity 40 KV cm-1 and 50, 125, and 200 pulses) were used to enhance the water extraction of Stevioside. The extraction kinetics was studied at two temperatures (20 and 55°C) with agitation during 60 minutes. Experimental data were fitted by simplified empirical model. The extract quality was determined by spectrum scanning at wavelength range 190-700 nm for all treatments. The results indicated generally that the HVED treatments accelerated the extraction process of glycosides with a better quality comparing with a control at the end of extraction time.

Key words: High voltage electric discharge, stevia, stevioside, sweetener, extraction.

INTRODUCTION

Stevia rebaudiana is a plant native of South America and its active constituents are considered, by Food Science researchers, as the "sweeteners of the future". Stevia sweetener is a low calorie, heat stable, intensely sweet (300 times sweeter than sucrose at 0.4% concentration), proven safe by many years of continual use in Japan since its introduction to the market in 1970. The consumption of steviosides in Japan has accounted for more than 20 percent of low calorie sweeteners. Interest in this low calorie sweetener was renewed in North America during the 1990s because of export opportunities and positive nutritional properties (Zhang *et al.,* 1999).

Stevia leaf has several sweetener glycosides such as stevioside, rebaudioside A, B, C, D, E and dulcosides A and B. Stevioside represents the highest proportion (5-10%) followed by rebaudioside A (2-4%), rebaudioside C (1-2%); while other constituents are present in smaller concentrations (Shi *et al.,* 2002).

Several processes of extraction of the sweetening substances from Stevia presented in literature follow approximately the same methodology. First, extraction from the leaves of Stevia is made with water or alcohols (ethanol or methanol); the obtained extract is in the form of a solution loaded with colloidal particles of dark brown colour, containing all the active principles, pigments of the leaf, soluble polysaccharides and other impurities.

Some primary processes remove the greases, essential oils, chlorophyll and other non polar principles from the leaves with solvents such as chloroform or hexane (Zhang *et al.,* 2000).

Supercritical fluid extraction (SFE) employing CO_2 as a medium for extraction is faster than the previous methods. However, pure CO_2 does not have sufficient solvation power for polar stevioside and therefore it requires the addition of polar cosolvent. Investigated cosolvents have included methanol, water and/or ethanol, a mixture of methanol and water (Choi *et al.,* 2002). Methanol showed better extraction ability for isolation of stevioside from *Stevia rebaudiana* leaves than water within the range 110–160 °C.

Water represents the green alternative to methanol, but thermal degradation of stevioside was equally observed in both used solvents within a temperature range of 70 –160 °C (Pol *et al.,* 2007). Different ways (mechanical, biological, thermal, and electrical) may be applied as pretreatments before and/or during extraction (El-Belghiti *et al.*, 2005a). High-pulsed electric field as a new nonthermal method of foods preservation has been intensively studied (Barbosa-Ca´ novas *et al.*, 1998). Successful application of pulsed electric fields encouraged the studies focusing on electrical extraction technologies (Vorobiev *et al.*, 2005).

The treatment by high voltage electrical discharges (HVED) was also proposed for different physical effects as shock waves, cavitation and particles breakage (Vitkovitsky, 1987). (Barskaya *et al.*, 2000) used the HVED for acceleration of aqueous extraction from the dry crop products (peat, tea, soya beans). Also, the extraction kinetics was 40 to 50 times faster than the traditional infusion and the obtained yield was about 95%.

The improvement of the aqueous extraction by HVED has been shown for the linseeds oil after pressing. Three successive treatments were done (40 KV, 300 impulses and 0,5 Hz) and almost all the oil quantity was extracted in less than 40 minutes, whereas in absence of HVED, the kinetics were rather slow. More than 300 minutes were required to extract only half of oil extracted with HVED water extraction (Gros *et al.*, 2003, Gros *et al.*, 2004).

The electric discharge treatment with 40 KV (HVED) was found to accelerate the extraction of aqueous solutions of two dry products (dried leaves of black tea and mint). Solution Brix was raised and the extraction kinetics were accelerated by HVED treatment (El-Belghiti, 2005).

The objective of this investigation was to study the effect of HVED treatment (0, 50, 125, 200 pulses) on the kinetics of solute extraction from stevia leaves and on the extract quality. Extraction experiments were performed at two different temperatures: 20 and 55 °C.

MATERIALS AND METHODS

Stevia rebaudiana leaves, planted under organic conditions, dried in shade at temperature 25°C for 24 hours to get a ~7 % moisture content, were supplied from Kato Aromatic Company (Giza, Egypt).

Electric Discharge Pretreatment

The generator (Polytechnic University of Tomsk, Russia) provided 40 kV discharges during a few microseconds in a treatment cell with 1 L capacity (Fig. 1).

These HVED were applied directly to the dried stevia leaves immersed in water with a ratio (*r*) 1:16 respectively at two temperatures (20 °C and 55 °C). Electrical discharges were applied with a 0.5 Hz frequency, which was imposed by the generator.

Aqueous Extraction of Glycosides

The HVED - pretreated mixture was transferred to the extraction unit. Extraction was effected under gentile rotation at 170 rpm, and two temperatures: ambient (20°C) and 55 °C for 60 min. The extraction unit was closed to avoid evaporation. All extracts were filtered with filter paper Rotilabo 601P, Ø 8 µm (Carl Roth, Germany).

Quantitative and Qualitative Analyses of Extract

The soluble solids (°Brix), as an indication of glycosides extraction, were determined by a refractometer Atago- PR-101 (Brix 0-53% ± 0.10) (Sciencelab.com, Houston, USA). The yield of extraction (Y%) versus time was calculated as:

$$Y = (°Brix / °Brix_{\infty}) \times 100$$

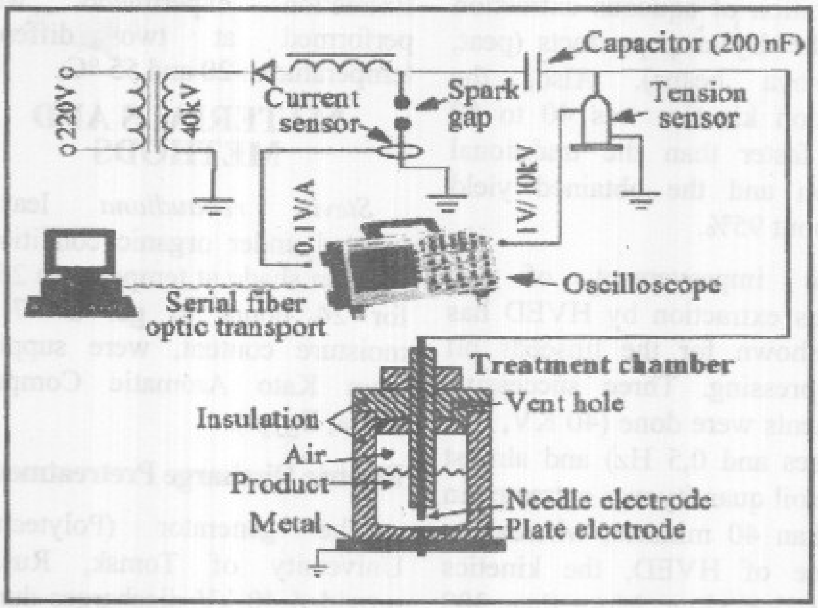

Fig. 1. Scheme of the high voltage electric discharge generator

where $°Brix_\infty$ was calculated as $°Brix_\infty \cong °Brix_0/(r+1)$ and $°Brix_0$ is the concentration of solute in leaves tissue in g solute per 100g juice (El-Belghiti *et al.,* 2005b). The crude extract of stevia contains impurities (protein, pectin, pigments and flavonoides) which are practically soluble in water. So, the wavelength ranged between 190 and 900 nm used to determine the extract quality (with the precision of ±1 nm) and recorded by Secomam Anthelie Advanced spectrophotometer (Domont, France).

Empirical Model for Data Fitting

The following empirical equation fits rather well both pressure and solute extraction data (Bouzrara and Vorobiev, 2003):

$$m = \frac{t}{1/v_0 + at} \quad \text{........ (Eq.1)}$$

Where m is the mass of solute (kg) extracted in time t (s), v_0 is the initial velocity of solute extraction (kg/s); a=1/ m_∞, where m_∞ is the mass of solute (kg) extracted after the infinite time.

The coefficients v_0 and a of Eq. (1) can be found after the data linearization:

$$\frac{t}{m} = \frac{1}{v_0} + at \quad \text{........... (Eq.2)}$$

Statistical Analysis

The results reported in this work are the average of at least three measurements. The data presented in tables and figures represents mean values ± standard deviation (n=3). Significance levels (p<0.05) were evaluated using statistical package of Microsoft Office software.

RESULTS AND DISCUSSIONS

Effect of HVED Pretreatments on the Extraction Kinetics

The extraction kinetics at ambient temperature (20°C) presented in Fig. 2, shows changes in the yield of solute after HVED pretreatments followed by the time of maceration (60 min) at ambient temperature. There were noticeable changes between the control (without HVED) and the treated samples in the extraction kinetics. Also, the yield increased by increasing the pulses number. After 30 min, the extraction extent if the control reached 78% of the T.S.S against 85, 88, 90% for the pretreatments 50, 125, 200 pulses respectively, i.e., the HVED pretreatments enhanced the extent of extraction by 1.09, 1.13, 1.15 times.

After 60 min, the extraction extent of the control reached 87% of the T.S.S against 90, 93, and 93% for the pretreatments 50, 125, 200 pulses respectively. So, the HVED pretreatments slightly enhanced the extent of extraction by 1.03, 1.06, 1.06 times. It may be noticed that time factor has maximized the extraction yield either for control or pretreated samples. For control, the extraction extent increased by 14 times after 1hr while for the HVED treatments (50, 125, 200 pulses) it increased only by 1.9, 1.5, and 1.3 times respectively. It can also be observed that the HVED can decrease the time required for high extraction yield. It rests to compare the constitution of the obtained extracts in each case to determine the impact of this pretreatment.

The data introduced in Fig. 3, explains the effect of thermal extraction (55°C) with mixing time (60 min) in addition to HVED treatments on the yield kinetics. Applying heat treatment (55°C) to the used extraction system has only slightly increased the extraction extent during 1hour.On the other hand, the extraction extent of the HVED pretreated samples increased only by 6, 5.5 and 4.7% when subjected to 55°C after 30 min compared to the corresponding non heated treatments.

It can also be observed that the influence of pretreatment reached its maximum (92.5%) with 125 pulses at 20°C after 60 min of extraction. The higher HVED pretreatment (200 pulses) was associated with higher extraction extent (99.7%) compared to the pretreatment with 125 pulses as it attained only 98.1% extraction yield. This relative increase in the extraction yield for the 200 pulses pretreated samples may be due to the possible release of different soluble compounds probably other than the targeted constituent.

Inserting the thermal treatment accelerated the yield kinetics (Eshtiaghi and Knorr, 2002), and it was faster comparing with ambient temperature. It was reached 65% and 85% respectively with pretreatment by 50 pulses. After 10 min of maceration, the effect of pulses number was less than the effect of thermal extraction during the time of maceration and the HVED pretreatments were nearly the same kinetics. These results from the temperature dependence upon the solute diffusivity (El-Belghiti *et al.*, 2005b, Jemai and Vorobiev, 2003).

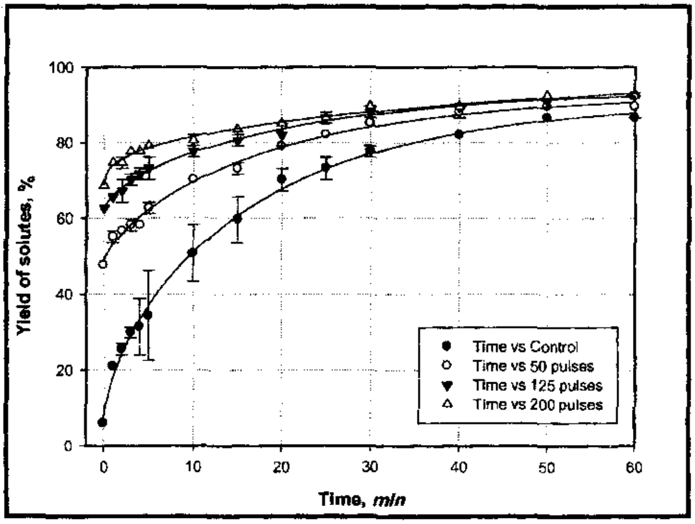

Fig. 2. Yield of extraction during 60 min at 20 °C from stevia leaves treated with HVED at different pulses (50, 125, and 200)

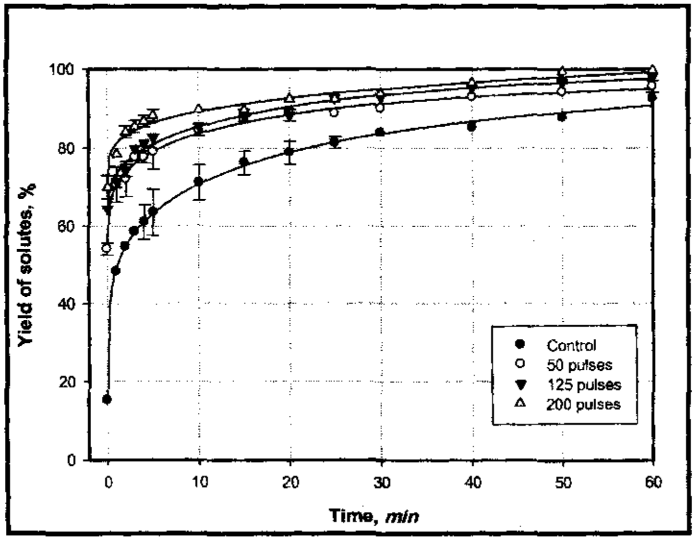

Fig. 3. Yield of extraction during 60 min at 55 °C from stevia leaves treated with HVED at different pulses (50, 125, and 200)

The Empirical Model

Fig. 4 represents a straight line in co-ordinates t/m versus t which gives the values of coefficients $1/v_0$ and a. The curve $m(t)$ at $t \to \infty$ approaches to the constant value of $m_\infty = 1/a$.

This value characterizes a maximal mass of liquid, which can be extracted from stevia leaves at given conditions. Using the 55°C increased the m_∞ value compared with the extraction at ambient temperature; as can be seen from Fig. 4 there is an increase of v_0 by the pulses number and also by increasing the temperature during the time. This can be explained by destruction of most cells after the combined electrical and mechanical treatment.

Pigmented and Other Component Matter

The crude water extract were scanned by UV/Visible light absorption to reveal the interference of the contaminants possibly released during the extract course of the different treatments. The results in Fig 5 show generally that all samples contained different species of contaminants at the wave length of 250-400 nm. Heat treatment has increased the extent of contaminants. The HVED pretreatment (200 pulses) has relatively reduced the relative content of contaminants especially when the extraction was conducted at room temperature. Comparing the different HVED pretreatments show that all the used pulses (50-200) have significantly reduced the total content of the contaminants when the extraction was conducted at 20°C, without significant differences between the pretreatments, however carrying out the extraction at 55°C has pronouncedly limited the reducing effect of the pretreatment (50-200) pulses on the contaminants.

The different treatments absorbance at the maximum wavelength (A_{max}=340 nm), are presented in Fig. 6. The ambient temperature extracts were lower than the thermal treatment referring to the effect of heat which raised the amount of impurities at this wavelength. The effect of HVED with different pulses number at ambient temperature was noticeable for decreasing the impurities extraction comparing with the control.

In thermal extraction, the effect of heat was higher than HVED effect resulting a very slight

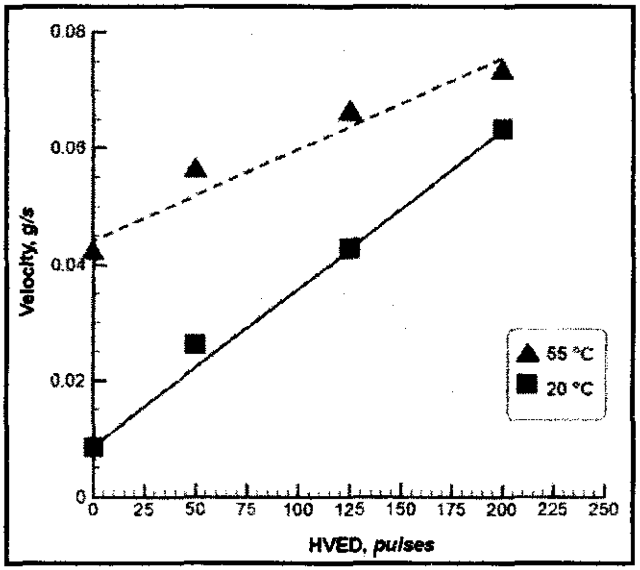

Fig. 4. Zero time velocity (v_0) as a function of pulses number by HVED

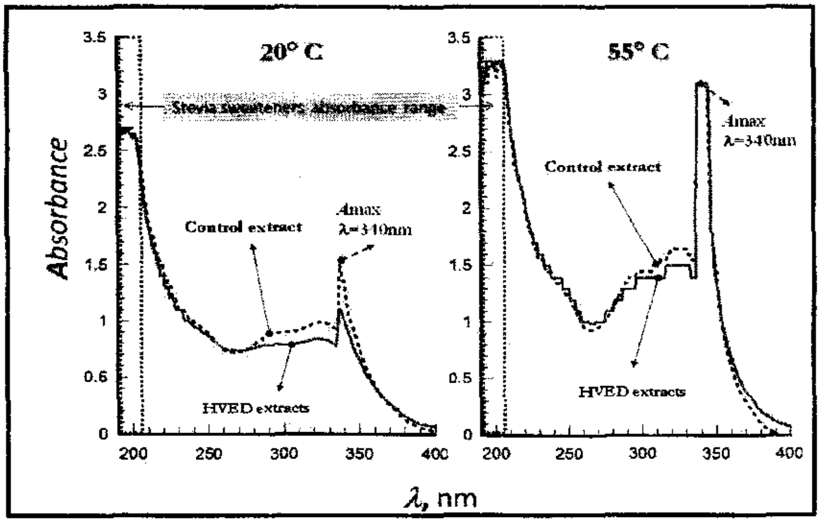

Fig. 5. Spectroscopic scanning of the water extract from Stevia rebaudiana leaves pretreated with HVED (200 pulses) and extracted at 20 °C and 55 °C

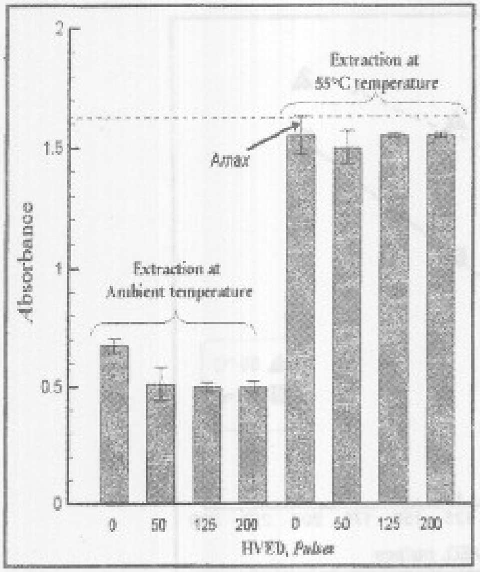

Fig. 6. A*max* 340 nm of the extracted solutes

difference between the treatments in different pulses; these results of the crude extract quality help the further purification procedures to get a clear extract.

Conclusion

HVED remarkably enhanced the yield of extraction containing stevia glycosides with respect to the untreated control along the maceration time. Therefore this treatment could contribute to reduce the duration of the maceration time. Also, it is an environmentally safe comparing with the alcohols and solvents extraction methods.

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زيادة كفاءة الاستخلاص المائي للجليكوسيدات من أوراق نبات الاستيفيا تحت تأثير المعاملة الأولية باستخدام التفريغ الكهربي

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إن عملية الاستخلاص للجليكوسيدات ذات الطعم الحلو من نبات الاستيفيا يمكن أن تجرى بعدة طرق مختلفة مثل الاستخلاص بالماء الساخن، بالكحولات (الميثانول أو الإيثانول) أو باستخدام طريقة استخلاص السوائل عالي الكفاءة *supercritical fluid extraction*، ويعد الماء المذيب الأفضل بيئيا والأرخص سعرا والأقل خطورة مقارنة بالمذيبات العضوية الأخرى على الرغم من انخفاض قدرته على الاستخلاص، ولذلك استخدمت تكنولوجيا التفريغ الكهربي عالي الفولت (شدة التيار ٤٠ كيلو فولت/سم ومن خلال ٥٠، ١٢٥، ٢٠٠ نبضة كهربية) لكي تساعد على رفع كفاءة الاستخلاص بالماء للمحلي الاستيفيوسيد. تم دراسة ميكانيكة الاستخلاص على درجتي حرارة ٢٠°م و ٥٥°م مع التقليب لمدة ٦٠ دقيقة. تم مطابقة النتائج العملية مع النموذج الاختباري البسيط وأيضا شوهدت جودة المستخلص من خلال عملية المسح الطيفي على مدى من الطول الموجي ١٩٠ – ٧٠٠ نانومتر لكل المعاملات. بشكل عام فإن النتائج دلت على إن استخدام التفريغ الكهربي كمعالجة أولية قبل عملية الاستخلاص أدت إلى زيادة سرعة عملية الاستخلاص وبشكل أفضل من ناحية الجودة مقارنة بالعينات المستخلصة مباشرة بالماء وذلك في نهاية وقت الاستخلاص.