Extraction of rebaudioside-A by sonication from *Stevia rebaudiana* Bertoni leaf and decolorization of the extract by polymers

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Abstract Optimization of steviol glycosides extraction from *Stevia rebaudiana* Bertoni leaf was carried out by investigating the effects of isopropyl alcohol concentration (60 %, v/v), time (6–24 min), temperature (30 °C) and sonic power (300–480 W) on extraction of rebaudioside A from *Stevia rebaudiana* leaves and decolorization of the extract by polymer (Separan AP30 and Resin ADS-7). The results showed that isopropyl alcohol was suitable for the extraction of rebaudioside A from *Stevia rebaudiana* leaves and the yield of rebaudioside A achieved 35.61 g/100 g when the output power was 360 W and treatment time was 18 min. The sonication had influence on the particle size of stevia leaf and the color of the extracted solution. As the sonication intensity increased, the particle size decreased. The colour of differently treated stevia solutions were significantly different ($P < 0.05$). Separan AP30 and adsorption resin ADS-7 were performed to remove the colour impurity. The results showed that more than 65 % of the coloured impurity was removed by Separan AP30 combined with Calcium oxide (CaO).

Keywords Rebaudioside A · Sonication · Extration · Decolorization · Separan AP30 · Resin ADS-7

Introduction

Stevioside (S) and rebaudioside A (RA) are two major steviol glycosides in the extracts produced from the leaves of *Stevia rebaudiana* Bertoni (Puri et al. 2011). The stevia extracts, recently approved by the US FDA as GRAS (generally recognized as safe), have been widely used as a natural sweetener or a dietary supplement in various food and beverage products. Stevioside tastes 250–300 times sweeter than sucrose but exhibits a significant bitter aftertaste (DuBois and Stephenson 1985). 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 and Weller 2006). Stevia has been used as a low-calorie sweetener for years in South America, Asia, Japan, and China, and in some countries of the European Union. Currently, Japan and Korea are the largest markets for stevia. However, China is the largest stevia grower in the world, and about 80 % of their production is exported. Stevia is also being cultivated by Indian farmers since the last decade, and is used as raw dry leaf or as a processed sweetener. In Brazil, Korea and Japan, stevia leaves and highly refined extracts are used as a low-calorie sweetener (Kim et al. 2002; Mizutani and Tanaka 2002). According to the International Diabetes Federation (IDF) and the Madras Diabetes Research Foundation, India had 62.4 million people with type 2 diabetes in 2011, compared with 50.8 million in 2010 (Shetty 2012). In the USA, powdered stevia leaves and refined extracts from leaves have been used as a dietary supplement since 1995 (Geuns 2003). Therefore, the worldwide demand for *Stevia rebaudiana* is expected to increase. The leaf yield and concentration of active compounds depend on the cultivar, growing conditions and agronomic practices.

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The increasing demand for herbal care for diabetes calls for intense farming of *Stevia rebaudiana* to increase the production of its low-calorie sweetening glycosides. Studies reveal that the content of these glycosides, especially stevioside, greatly depends on the total biomass produced, which in turn depends on agricultural practices for cultivation of *Stevia* plants (Geuns 2003). In pursuit of high production of glycosides, researchers have adopted modern agro-techniques (Das et al. 2008), water management (Fronza and Folegatti 2003) and fertiliser applications (Rahi et al. 2010). In addition, tissue culture techniques and cultivation of *Stevia* plants in bioreactors have also been tested (Madan et al. 2010). However, in the above techniques, the main shortcomings are the high costs and low in-field practicability. For example, chemical fertilisers constitute a few mineral nutrients and create an imbalance in the whole mineral pattern of the plant body by hindering the uptake of other useful nutrients (Das et al. 2008). Nowadays, due to the growth of the health food industry reduction of sucrose content of food products by full or partial replacement of sucrose using alternative sweeteners have become a viable option for producing low calorie/zero calorie foods. Low calorie food products of good quality can be made by incorporating combinations of non-caloric and carbohydrate sweeteners (Nabors 2012).

In recent years, ultrasonic treatment has found numerous applications in the food industry (Mason 1998). Ultrasound can be used either as a diagnostic tool or as a source of energy. For each of these applications, different ultrasound frequency ranges should be used (Mulet et al. 2002). Power ultrasound has additional potential applications, including enzyme inhibition, hydrogenation of oils, crystallisation control, extraction of protein and enzymes, inactivation of microorganisms and improvement of heat and mass transfer (Mason et al. 1996; Condón et al. 2005). However, scaling up these potential applications requires more research. The major mechanism of power ultrasound is the generation and subsequent destruction of cavitation bubbles. When ultrasonic waves propagate into a medium, a series of compression and rarefaction waves are induced in the molecules of the medium through which it passes. During these cycles, small bubbles form and expand due to gas diffusion (Neppiras 1984; Henglein 1987). The main focus of this work is to maximize the yield of rebaudioside A and investigate the color removal of *Stevia rebaudiana* Bertoni solution treated by two types of polymer (Separan AP30 and Resin ADS-7).

Materials and methods

Materials

Green *Stevia* leaves were obtained from the Yancheng Xiaguang Stevioside Trading Company (Jiangsu, China), a

high-speed blender (25000/min), type WK – 1000A (Qingzhou Jing cheng Machinery Co., LTD- Shandong - China), pH-meter FE20 Mettler-Toledo Instruments (Shanghai, China), Centrifuge CT14D Shanghai Techomp Bio-Equipment LTD (Shanghai, China). Polymeric adsorbent ADS-7 was obtained from the Tianjin Nankai Hecheng S&T Co., Ltd (Tianjin, China). Sodium hydroxide (H₂SO₄), Hydrochloric acid (HCL), solvents and others chemical were obtained from Sigma Chemical Co. (Shanghai, China). A Minolta spectrophotometer (CR-400, Konica Minolta Sensing, Tokyo, Japan). Microtrac Bluewave S3500 SIA (Microtrac Inc. FL., USA) with Microtrac Flex 10.5.4 software

Sample pretreatments

The fresh green leaves of *Stevia rebaudiana* Bertoni (Fig. 1) were allowed to dry using direct sun light for about 5 days. The dried leaves were then blended to powder using a high-speed blender (25000/min). The powder samples were stored in polyethylene bags at 4 °C until used (Gasmalla et al. 2014).

Ultrasound apparatus

An ultrasonic generator (JY98-III DN, Nanjing FeiQi Industry &Trade Co., LTD. Nanjing- China) was used for extraction (shown in Fig. 2). The immersion stainless steel transducer was of the horn-type with a length of 300 mm and a diameter of 20 mm. delivering a maximum power of 1200 W at 20 kHz it was equipped with a thermometer to measure the reaction

Fig. 1 *Stevia rebaudiana* Bertoni leaf (Mondaca et al. 2012)

Fig. 2 Schematic diagram of experimental apparatus used for rebaudioside A extraction under ultrasound irradiation

temperature and inserted in the slurry to introduce ultrasound field. A circulating water bath (HH-2 Guohua Wiring Company, Shanghai, China) was adopted to keep the reaction temperature at a constant. A magnetic stirrer was used to suspend the particles in the reactor and speed up heat transfer.

Rebaudioside A extraction

Dry and ground stevia leaves samples (10 g) were suspended and extracted in 100 mL of 60 % isopropanol, with stirring. The pH value was controlled with 0.01 M pH 7sodium phosphate. The sample was put in a 100 mL cylindrical glass reactor of standard geometry thoroughly mixed and the ultrasound generator probe inserted immediately. The tip of the probe was immersed about 1.5 cm into the slurry. The sonication experiments were carried out at a frequency of 20 kHz and rated power output of 300–480 W. The solution was processed with the ultrasound radiation for different irradiation times (6, 12, 18 and 24 min) while the temperature inside was kept constant at 30 °C. The extracted solution was centrifuged and filtered off through 0.45 µm microporous membrane; the filtrate was taken for total rebaudioside A content analysis. The extraction yield of total rebaudioside A content was defined by the HPLC analysis. After extraction optimized, the crude extracts were treated by polymers.

Rebaudioside A analysis

Standard rebaudioside A, 1 g was accurately weighed and transferred to a 25 mL volumetric flask and the volume was made with water. Solutions of 25, 50, 100, 150, 200, 250, and 300 µg/L were made by transferring an aliquot from stock solution and the volume was made with water in each case. Further standard solutions were prepared

freshly each day by appropriate dilution of stock solution with water for intraday as well as interday analysis. 200 µg/L of solvent extract was accurately weighed and transferred to a 25 mL volumetric flask and the volume was made by distilled water. Then 10 µL of the stock solution was subjected to HPLC analysis and the concentration of rebaudioside A was calculated based on the calibration curve equation ($y=36781x+2887.7$, $R=0.9993$).

Reducing sugar determination

The samples were analyzed for reducing sugar concentration using a modified dinitrosalicylic acid (DNS) colorimetric method (Miller 1959). A sample volume of 1 ml was mixed thoroughly with 1.5 mL of DNS reagent. The solution was heated to 100 °C for 5 min and then cooled down in an ice bath. The absorbance of the samples was measured at a wavelength of 520 nm using a spectrophotometer (MAPADA-Model YM1208PTS2, Shanghai, China). Reducing sugar concentrations were calculated from the standard calibration curve obtained using standard solutions of D -glucose and the DNS reagent.

Effect of sonication on the total soluble substance

A refractometer was used to determine the total soluble substance of the samples according to the method reported by Kimball (Kimball 1999).

Effect of sonication on the color

Hunter a, b and L parameters of stevia solution were determined with a Minolta spectrophotometer (CR-400, Konica Minolta Sensing, Tokyo, Japan) in the reflection mode. The

instrument was standardized with a white ceramic plate ($L=99.50$, $a=-0.01$, $b=-0.12$). The hue (H), chroma (C) and browning index (BI) were calculated as follows:

$$H = \tan^{-1}[b/a] \quad (i)$$

$$C = a^2 + b^2 \quad (ii)$$

$$BI = [100(x - 0.31)]/0.172 \quad (iii)$$

Where:

$$x = (a + 1.75L)/(5.645L + a - 3.012b) \quad (iv)$$

The browning index (BI) represents the purity of brown color and is reported as an important parameter in processes where enzymatic or non enzymatic browning takes place (Buera et al. 1986; Guerrero et al. 1996; Castañón et al. 1999).

Effect of sonication on the particle size

The particle size distributions of stevia dispersions were determined at room temperature with a Microtrac Blue wave S3500 SIA (Microtrac Inc. FL., USA) with Microtrac Flex 10.5.4 software.

Decolorization

The absorbance A of the stevia solution was measured with a visible spectrophotometer (722E, Shanghai China) at 420 nm as an index of the coloured impurity. The decolorization efficiency R was calculated by determining the percentage decrease of the absorbance of the solution using the following equation:

Where A_0 and A_t were the absorbance of the samples at 420 nm before and after adsorption by polymer, respectively.

$$R (\%) = (A_0 - A_t) / A_0 \times 100 \quad (v)$$

Statistical analysis

All experiments described above were made in triplicate for each sample. The data presented were the means and standard deviations of each experiment. The experimental data were analyzed using the ANOVA and Duncan's multiple range tests by the SPSS 17.0 (SPSS Inc., Chicago, USA) software. Unless otherwise noted in the text, a $P<0.05$ level was used where values were considered as being significantly different.

Table 1 Process parameters studied and their proposed conditions

Experimental parameters	Proposed conditions
Extraction Time (min)	6,12,18 and 24
Sonic Power (W)	300,360,400 and 480
Temperature (°C)	30
pH	7
Solvent amount (ml)	100
Sample amount (gm)	10

Results and discussion

Effect of sonication on the rebauidioside A extraction yield

The process parameters were studied and their levels were shown in Table 1. Ultrasonic power and extraction time are two important factors that affect the extraction of total steviolglycoside, the effect of isopropyl alcohol extraction on the extraction yield of total rebauidioside A was showed in Table. 2. The average of rebauidioside A content Increased with more sonic power from 300 to 360 W but decreased at still higher sonic level at 400 to 480 W which is referred to degradation of the extracted rebauidioside A. Alupului et al. (2009) determined the benefits of applying high intensity ultrasound of the amplitude 50, 80 and 100 % and input power of 750 W as a method for the extraction of stevioside from the stevia dry leaves. The increase of the ultrasonic field's power

Table 2 Effect of sonication on the extraction of total soluble substance and rebauidioside A from the stevia leaves

Sonication Power [W]	Sonication Time [min]	T.S.S. [%]	Rebauidioside A [mg/g]
300	6	22.00±0.13 ^b	3179±3.52 ^a
	12	17.67±0.76 ^{bc}	2657±0.65 ^{ba}
	18	17.00±0.50 ^d	2979±2.82 ^{cb}
	24	25.00±0.33 ^b	3265±1.57 ^d
360	6	20.00±0.33 ^c	3428±2.58 ^a
	12	19.67±1.26 ^{ba}	3553±3.10 ^a
	18	20.83±0.29 ^b	3561±1.68 ^a
	24	22.33±0.51 ^c	3462±2.26 ^a
400	6	16.33±0.67 ^d	3364±0.51 ^a
	12	17.67±1.04 ^c	3077±2.92 ^{ba}
	18	18.50±0.75 ^c	3312±3.91 ^{ba}
	24	21.50±0.45 ^d	3216±2.23 ^{ba}
480	6	23.00±0.42 ^{bc}	3388±0.50 ^a
	12	24.50±0.29 ^b	3056±1.53 ^a
	18	25.74±0.53 ^b	30.45±2.86 ^a
	24	28.00±1.00 ^a	3136±3.69 ^a

Mean values in the same column with different letters are significantly different ($p\leq0.05$)

in the above mentioned amplitudes did not show any visible effects of the concentration of stevioside, but in the application of higher power ultrasound as the main limiting disadvantage should be considered the risk of degradation of the extracted compound. It also resulted in the changes of some important physical characteristics of isopropyl-alcohol rich phase, such as polarity, viscosity and surface tension. This result may have a significant effect on the sonication activity and the partition behavior of rebaudioside A in aqueous system. Where, the extraction yield changes slightly with the change of isopropyl alcohol extraction as shown in Table 2.

As solvent type 60 % isopropanol was tested to extract rebaudioside A from dried leaves of *Stevia rebaudiana* Berton. Were proved to be beneficial, dissolving the constituents more effectively thus, leading to improvement of the extraction yield. In general, When the solvent quantity is less than the ratio mentioned above, there is not enough liquid to ensure a proper/complete welling of the cell's membrane, leading to a smaller diffusion flux of extraction. Moreover, the concentration of the extracted rebaudioside A into the liquid phase rises faster to the equilibrium value, decreasing this way the extraction driving force. Generally, the extraction efficiency obtained with ultrasonic extraction is 35.61/100 g Table 2, higher than that obtained with maceration extraction which has a beneficial effect as reported by Liu (Liu et al. 2010) whom found rebaudioside A is 24.21 % with classical extraction based method of preliminary experiments. The process was completed after 18 min when we used sonic power 360 W while, the optimum value has been obtained.

On the other hand, the sonication accelerated soluble substance diffusion and gave the higher level of dry matter content as shown in Table 2. In these applications, the power ultrasound increases the surface moisture evaporation rate and causes oscillating velocities and micro streaming at the interfaces, which may affect the diffusion boundary layer and generate rapid series of alternative contractions and expansions of the material, affecting mass transfer. Similar conclusions are presented by (Garcia-Perez et al. 2006; Stasiak 2005).

Effect of sonication on the color

After sample treated with ultrasonic treatment, b^* values (1.76) gradually changed toward a less positive direction in all samples. In addition, a^* values shifted from 1.26 to a slightly positive direction. It was a different direction in color change compared to the color changes observed in stevia solution. A color shift toward negative b^* and a^* directions indicate less red in stevia sonicated solution. The majority of samples showed slight decreases in L^* value after sonication, which indicates a lightening of solution surface color. A small increase in L^* value for stevia solution can probably be attributed to partial precipitation of unstable, suspended particles in solution as described (Genovese et al. 1997). The conditions used

for sonication of stevia solution are listed in Table 1. Significant changes in color of stevia solution were observed during sonication. Untreated samples had lightness (L), yellowness (a) and redness (b) values of 26.95, 1.26, and 1.76 respectively. Three characteristics were significantly influenced by ultrasound, namely Hue, Chroma and Browning Index. Sonication time affected the color parameters of the stevia leaves solutions (Table 3). The L value for each sonication time, the initial color parameters were not different ($p < 0.05$).

Effect of sonication on the particle size

Various conditions for the preparation of stevia slurry particles are summarized in Table 4. Effect of different sonication times, that is, 6, 12, 18 and 24 min on the particles are shown in Fig. 3. There was a significant decrease in the particle size with an increased in sonication time from 6 to 24 min for all applied sonic power levels. For different sonication times, i.e., 6, 12, 18 and 24 min at a fixed power density, their particle size values were determined, for 300 W, 94.18, 89.47, 80.71 and 73.41 μm respectively, for 360 W, 80.10, 78.86, 77.25 and 70.72 μm respectively, for 400 W, 85.42, 80.09, 75.36 and 71.26 μm respectively, for 480 W, 81.34, 81.28, 65.88 and 59.24 μm respectively. This point shows that an increase in the sonication time and sonication power led to a decrease in particle size. It seems that the differences in particle size trends are due to different sonic power. The most important alteration is the ratio of soluble to insoluble components. The ratio of soluble to insoluble components is different in various weights of stevia leaves (Balaghi

Table 3 The effect of sonication on the color of the slurry after ultrasonic treatment

Power [W]	Time [min]	L	a	b	Hue	Chroma	Browning index
300	6	26.44	0.37	0.24	32.96	0.44	1.876989
	12	26.66	0.17	0.57	73.39	0.59	2.54049
	18	26.33	0.71	0.2	15.73	0.74	2.650006
	24	26.27	0.56	0.41	36.21	0.7	3.034751
360	6	26.38	0.4	0.63	57.59	0.75	3.426767
	12	26.29	0.41	0.56	53.79	0.7	3.189301
	18	26.41	0.33	0.68	64.11	0.76	3.40849
	24	26.37	0.46	0.51	47.95	0.69	3.201775
400	6	26.1	0.67	0.46	34.47	0.81	3.541517
	12	26.9	0.41	0.56	53.79	0.69	3.116482
	18	26.76	0.64	1.11	60.05	1.28	5.797488
	24	26.43	0.5	0.81	58.31	0.91	4.353368
480	6	26.34	0.42	0.4	43.6	0.58	2.612485
	12	26.52	0.06	0.17	70.55	0.18	0.784752
	18	26.16	0.47	0.39	39.69	0.61	2.728629
	24	25.48	0.61	0.33	28.41	0.7	2.959861
Control	-	26.95	1.26	1.76	54.4	2.16	9.870125

Table 4 The effect of sonication on the particle size of the slurry after ultrasonic treatment

Power [W]	Time [min]	Mean [μm]	Median [μm]	Mode [μm]	D ₁₀ [μm]	D ₉₀ [μm]
300	6	94.18	87.66	87.99	59.66	136.1
	12	89.47	85.82	87.99	58.24	125.7
	18	80.71	77.98	87.99	54.68	110.6
	24	73.41	73.61	93.53	34.68	111.37
360	6	80.1	81.58	104.6	20.44	128.15
	12	78.86	80.98	104.6	14.59	132
	18	77.25	77.04	87.99	10.07	128.1
	24	70.72	72.87	96.30	13.93	119.2
400	6	85.42	84.91	104.6	42.04	127.6
	12	80.09	82.57	104.6	9.55	135.3
	18	75.36	77.39	104.6	6.90	129.6
	24	71.26	69.09	93.53	22.90	121.13
480	6	81.34	87.18	107.99	37.95	143.67
	12	81.28	83.09	104.6	10.19	131.1
	18	65.88	58.17	87.99	6.41	117.85
	24	59.24	51.72	99.55	12.46	112.8
control	-	93.23	90.92	104.6	57.11	132.5

et al. 2010; Phillips and Williams 2000) as reported that the insoluble part that is less branched produces natural aggregates and the larger the ratio of insoluble components, the larger the resulting aggregates. The soluble component is highly branched and does not normally produce aggregates. It seems that under ultrasonic treatment, soluble and insoluble components change in different ways. The aggregates and some of the chains of the insoluble components break and produce new aggregates on rearrangement. New aggregate formation depends on the chain size of the insoluble component, but the size of these aggregates depends on the total portion of the insoluble component as well.

Results show that ultrasonic treatment at constant power, with increasing time, caused a decrease in particle size distribution in the stevia leaves dispersions. After 24 min of ultrasonic treatment, D₅₀ decreased from 90.92 μm to 73.61, 72.87, 69.09 and 51.72 μm when using ultrasonic power of 300, 360, 400 and 480 W respectively. This shows disruption of large particles, resulting in smaller particles with a narrower particle size distribution. After sonication time of 6 and 12 min and power of 300 W, D₁₀ increased from 57.11 to 59.66 and 58.24 μm . D₉₀ decreased after all treatments, except at a power of 300, 400 and 480 W for 6, 12 and 6 min respectively (Table 4). This indicates that various ultrasound powers lead to different particle size diameters. In the 480 W samples, particle size consistently decreased with increasing ultrasonic treatment duration. D₁₀ decreased from 57.11 to 37.95, 10.19, 6.41 and 12.46 μm and D₉₀ first increased from 132.5 to 143.67 then decreased to 131.1, 117.85 and 112.8 while the

mode increased from 104.6 to 107.99. During sonoprocessing of the soluble component, the branches are the first to be influenced and broken up. This probable phenomenon can result in the formation of unbranched backbones, which in turn can form new aggregates. Although these aggregates can increase the particle size distribution to some extent, they are smaller than insoluble component aggregates. In the insoluble particle size of stevia leaves, with sonoprocessing exposure, the primary aggregates and some of the insoluble component chains break down and produce new larger aggregates through rearrangement. The phenomena described above were observed in this study in the particle size distribution analysis of stevia leaves. Our results indicated that decrease of the particle size with the increase of total energy dissipated into the system was the function of time at a constant power density. In other words, increasing sonication power significantly reduces the sonication time until it reaches to the point where no further effect is noted (Delmas et al. 2011).

The increase and corresponding decrease in particle size distribution is clear, as shown in Table 4.

Effect of addition of polymers on the reducing sugar

In order to evaluate the effect of polymer treatment on reducing sugar yield, stevia solution samples were treated under sonication conditions. The reducing sugar release rates of sonicated samples with A, B and C were compared with unsonicated samples (control). The reducing sugar yields for each treatment are presented in Table 5. The reducing sugar yields at the completion of the reaction between samples and polymers were 2.96, 2.04, and 0.76 mg/ml for sample A, B and C, respectively. For untreated sample, the value was 3.9 mg/ml. It is obvious that treated samples require more time for the completion of the reaction. However, reducing sugar values of treated samples were much lower than that of the control. The highest content was found in sample A. Both values for B and C decreased with increase in TSS levels. At high TSS levels, there were more particles (starch granules) available for ultrasound to react with, which resulted in both lower reaction rate coefficients and overall reducing sugar yields at completion of reaction (Table 5).

Effect of addition of polymers on the color removal

First, a series of experiments feeding the thin juice through the polymers were carried out, analyzing the influence of polymers on decolorization efficiencies. The volumes of treated stevia extract were 25 ml. The highest color removal percentage was 65.45 %, corresponding to Separan AP30 plus CaO whereas the lowest percentage, corresponding to Separan AP30 only, was 29.61 %. From these results, a separan AP30 plus CaO of 1 ml/25 ml may be considered suitable for decolorizing thin stevia solution, with a percentage of color removal of about

Fig. 3 Particle-size distribution **a** Particle-size distribution at different sonication times 6, 12, 18 and 24 min at fixed power density 300 W, **b** Particle-size distribution at different sonication times 6, 12, 18 and 24 min at fixed power density 360 W, **c** Particle-size distribution at different

sonication times 6, 12, 18 and 24 min at fixed power density 400 W, **d** Particle-size distribution at different sonication times 6, 12, 18 and 24 min at fixed power density 480 W

65.45 %, after 18 min sonication as presented in Table 5. These results show that removal of colorants depends on the chemical treatment. This fact may be due to the different conditions of polymers and reactants between the polymers and stevia extract. The total soluble substance content decreased from 17.03 to 13.83 %. Total soluble solids and pH values were about 13–21 % and 5–10, respectively.

Conclusion

In the current study the effects of ultrasound conditions on the extraction efficiency and total rebaudioside A yield were investigated. We found using isopropanol was economically which have higher value (3561 mg/g) when we use power 360 W with sonication for 18 min. This study revealed the

Table 5 Effect of different polymer on color and sugar removal

Polymer	T.S.S. [%]	Initial pH	R.S. [g/ml]	Color Removal [%]
Separan AP30	14.17±0.764 ^b	5.74±0.045 ^c	2.96±0.073 ^b	29.61±1.57 ^c
Separan AP30+CaO	13.83±4.072 ^b	9.5±0.226 ^a	2.04±0.03 ^c	65.45±5.789 ^a
Resin ADS-7	20.33±0.577 ^a	5.17±0.128 ^d	0.76±0.005 ^c	43.51±1.43 ^b
Control	17.03±0.97 ^{ba}	7.00±0.00 ^b	3.9±0.012 ^a	–

Mean values in the same column with different letters are significantly different ($p \leq 0.05$)

benefit of ultrasonic treatment on the particle size distribution and physicochemical properties of *stevia rebaudiana* bertonii leaves dispersions. It is that foods containing stevia extract could be treated with separan AP30 without any worry about color. Although, there is still a need for more research to understand the exact reasons for the different behaviours of color removal when considering the polymers differences.

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