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Spray Drying of *Stevia Rebaudiana* Bertoni A*queous* Extract: Effect on Polyphenolic Compounds

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The *Stevia rebaudiana* Bertoni is the raw material of a family of low caloric index sweeteners, the steviol glycosides. *Stevia rebaudiana* also has other compounds with the desired biological effect on human health, the polyphenols. The spray drying process is a physicochemical and microbiological stabilization process widely used for aqueous vegetables extracts. The effect of the spray drying process variables, the inlet air temperature (160-180 °C) and the feed flow rate (2-3 kg h^{-1}); on the total polyphenolic concentration/profile from *Stevia rebaudiana* aqueous extract was evaluated. The polyphenolic profile evaluation was performed by liquid chromatography coupled to time-of-flight mass spectrometer (UHPLC-qTOF-MS); the total polyphenolic concentration, total flavonoids concentration and their antioxidant capacity were evaluated by spectrophotometric method. The concentration of polyphenolic and its associated antioxidant capacity was reduced by all the evaluated spray drying process conditions. At 160 °C - 2 kg h^{-1} the lower phenolic concentration and antioxidant reduction were reached (38.69 % and 3.76 % respectively) referred to the fresh aqueous extract.
The polyphenolic profile change from 14 compounds (phenolic acids, flavonols, flavones, dihydroflavones and isoflavones) in the fresh aqueous extract to the one phenolic acid at 200 °C – 2 kg h^{-1} spray drying condition. The spray drying process reduced the concentration and changed the profile of the phenolic compounds. The lower effect was observed at the lower temperatures (160 °C) and higher feed flows (3 kg h^{-1}).

1. Introduction

Nowadays, the consumption of natural low-calorie sweeteners has increased due to the health benefits they provide. The *Stevia rebaudiana* has been used as a raw material of low caloric sweetener: the steviol glycosides. The steviol glycosides exhibit anti carcinogenic, hypo tensor and anti-diabetic biological activity (Ruiz-Ruiz et al., 2017; Ameer et al., 2020; Martínez-Rojo et al., 2020). The polyphenolic compounds are isolated from *Stevia rebaudiana*, while the steviol glycosides are extracted. The polyphenolic compounds have been catalogued as anti-oxidant (Ameer et al., 2020; Rodríguez-López et al., 2020), anti-inflammatory, hepatoprotective, anti-thrombotic, anti-allergenic, anti-viral, hypo-glycemic, vasodilating, anti-bacterial, and anti-carcinogenic molecules (Sharifi-Rad et al., 2018; Shikalepo et al., 2018; Khan et al., 2020; Li et al., 2020; Salehi et al., 2020). The spray drying is a continuous drying operation process commonly used to obtain high concentrations of soluble compounds with stable physicochemical proprieties (Taylor et al., 2007). For plant extracts, the spray drying has been widely used to obtain products with high physical, chemical and microbiological stability, low storage costs and long shelf life (Anandharamakrishnan and Ishwarya, 2015). The spray drying process has been studied to produce stevia-based sweeteners (Oikonomopoulou et al., 2022; Zorzenon et al., 2020). Several studies have reported the effect of the drying methods on the concentration of phenolic compounds in *Stevia rebaudiana* leaves (Zorzenon et al., 2020; Dong et al., 2021), but so far the authors can investigate, none of them have reported the effect of spray drying without encapsulant material on the polyphenolic compounds profile and concentration. The aim of this research was to evaluate the effect of the spray drying process

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variables (inlet air temperature and flow rate) on the polyphenolic profile /concentration, and their associated in vitro antioxidant capacity, from *Stevia rebaudiana* aqueous extract.

2. Materials and methods

2.1 Raw material and aqueous extract

Stevia rebaudiana leaves were collected at Oaxaca, Mexico (15.8327 N and 96.3206 W). The collected material showed uniform color, with no mechanical damage or apparent contamination with fungi or bacteria. The material was disinfected in a solution of 0.05 % sodium hypochlorite (w:v) for 5 minutes and then washed with distilled water. The aqueous extraction process was carried out by maceration (5:1 w:v) at 80 °C for 15 min; followed by an ultrasonic extraction process (40 kHz, 200 W) at 25 °C for 30 min; finally the leaves were stored in dark at 25 °C for 48 h. The liquid phase (named feed flow phase) of the crude extract was recovery by filtration (pore diameter 0.149 mm) and immediately was spray dried. The solid materials were discarded. The feed flow phase aliquot was frozen/stored at -20 °C until they were used for spectrometric determinations.

2.2 Spray drying process conditions

A Mobile Minor concurrent flow spray dryer (Niro Copenhagen, Denmark) equipped with a pneumatic pulse rotary spray dryer (TS-Minor, M02/B) was used. The spray rate was set at 23,000 rpm and the heat drying airflow was 84 ± 2 kg h^{-1}. Distilled water (25 °C) was fed for 5 minutes to stabilize the equipment chamber before each drying process. The spray drying process limits the levels of drying parameters that can be applied, so the inlet air temperature and feed flow rate that were applied were chosen in such a way that no product dripping on the dryer chamber walls would occur. Table 1 shows the spray drying conditions evaluated. The lower drying conditions were set at 160 °C – 2 kg h^{-1}. The higher conditions were set at 200 °C – 3 kg h^{-1}. An intermediate temperature and flow rate was evaluated to improve the drying condition analysis.

Table 1: Spray drying conditions

Treatment	Inlet air temperature (°C)	Feed flow (kg h^{-1})
1	200	2
2	200	3
3	180	2.5
4	160	2
5	160	3

2.3 Determination of total phenolic content (TPC), total flavonoid content (TFC) and antioxidant capacity

TPC was determined by the Folin-Ciocalteu reagent technique according to the method described by Periche et al. (2015) using gallic acid as standard. The concentration of total polyphenolic compounds was expressed as milligrams of gallic acid equivalent per gram of stevia leaves in dry basis ($mg_{GAE}\ g_{dl}^{-1}$).the TFC was determined by the aluminum chloride colorimetric technique according to the methodology described by Kumazawa et al. (2004), using quercetin as standard. The concentration of total flavonoid compounds was expressed as milligrams of quercetin equivalent per gram of stevia leaves in dry basis ($mg_{QE}\ g_{dl}^{-1}$). Antioxidant capacity was determined by the 2,2-diphenyl-1-picrylhydrazyl technique according to the methodology described by Shukla et al. (2012). The antioxidant capacity was expressed as activity antioxidant percentage (Equation 1):

$$AA = \frac{A_0 - A_1}{A_0} \cdot 100 \quad (1)$$

Where: AA is the percentage antioxidant activity of the evaluated phenolic extract aliquots; A_0 is the absorbance of the DPPH* in 80 % (v:v) ethanol:water solution (0.03 mg mL^{-1}); and A_1 is the absorbance of the DPPH* at a concentration of 0.003 mg mL^{-1}

2.4 Characterization of compounds by liquid chromatography coupled with electro-spray ionization quadrupole time-of-flight mass spectrometry (UHPLC-ESI-qTOF-MS)

The characterization of polyphenolic compounds profile was carried out according to the methodology described by Tang et al. (2019) with slight modifications. The liquid chromatographic was carried out by gradient method using water/acetic acid (95:5, v:v) solution (phase A), and acetonitrile/water/acetic acid (100:1:99,v:v:v) solution (phase B) at a flow rate of 0.8 mL min-1. The gradient methodology was: 0–20 min, 10 % B; 20–30 min, 25 % B; 30–40 min, 35 % B; 40–70 min, 40 % B; 70–75 min, 55 % B; 75–77 min, 80 % B; 77–79 min, 100 % B; 79–

82 min, 100 % B; 82–85 min, 10 %. The separation was carried out using a Zorbax Eclipse Plus C18 column (1.8 µm, 150 mm × 4.6 mm). The spectrometric determinations were made using a Bruker Microtus-Q II mass spectrometer equipped with an ESI interface. The electro spray ionization (ESI) interface in negative mode was used, considering a mass range of 50 to 3000 m/z. The mass spectrometry conditions were set as follows: sheath gas temperature at 250 °C with the flow rate 11 L min-1, nitrogen gas temperature at 300 °C with a flow rate 5 L min-1, and a nebulizer gas pressure of 310.2 kPa. The capillary and nozzle voltage were set at 3.5 kV and 500 V, respectively. The identification was carried out comparing the mass spectra pattern and the molecular ion exact mass with the MassBank database.

2.5 Statistical analysis

A factorial design 2^2 with a central point (ANOVA / Tukey significance level of 0.05) was applied to evaluate the effect of inlet air temperature, 200 - 160 °C; and the feed flow rate, 2- 3 kg h^{-1}; on the total phenolic compound concentration and the antioxidant activity. The spray drying treatments were evaluated in triplicate and average data is presented. The analysis was performed with Statistica ver. 7.0.

3. Results and discussion

The total phenolic compounds concentration (TPC) in the fresh materials was 77.00±0.59 mg_{GAE} g_{dl}^{-1}, higher than the concentration reported for *Stevia rebaudiana* fresh leaves by Periche et al. (2015), 44.40±1.04 mg_{GAE} g_{dl}^{-1}; and Lemus-Mondaca et al. (2016), 0.29±0.02 mg_{GAE} g_{dl}^{-1}. The differences can be explained by edaphoclimatic conditions, interspecies genetic variations and postharvest conditions (Wölwer-Rieck, 2012). The temperature of the inlet air has a significant effect on the TPC. At higher air temperature, the TPC decrease (Table 2). The feed flow rate did not have a significant effect on the TPC. This behavior contrast with those reported by Periche et al. (2015) for a convective dried of fresh *Stevia rebaudiana* leaves. They reported an increase of total phenolic concentration when the air temperature increases. The decrease of TCF at the higher evaluated temperatures could be due to the fact that during spray drying, the phenolic compounds in the sprayed aqueous solution were in close contact with hot air, in contrast to the phenolic compounds inside the leaves during the convective drying of fresh stevia leaves where they were protected by layers of different materials such as cellulose or wax and/or cellular organelle cells, e. g. the vacuoles (Buer et al., 2008). The thermal behavior on various polyphenols has been evaluated, especially on molecules of high and medium molecular weight: condensed tannins, flavonoids and flavonols (Ross et al., 2011). Ross et al. (2011) reported that at temperatures below 120 °C, the temperature has a moderate effect on polyphenols degradation, but at higher temperatures the degradation become more intense. Other factors that have been associated with the degradation of phenolic compounds are changes in pH, the presence of oxygen, enzymatic degradation, as well as interactions with other food components as ascorbic acid, metal ions, sugars or pigments (Jackman and Smith, 1996). The relation between the TPC and the evaluated process variables was illustrated the Figure 1. The evaluated spray drying variables had a significant effect on the total flavonoids concentration (TFC). The TFC decreased as the temperature increased (Table 2).

Table 2: Polyphenols concentration and their antioxidant activity

Treatment	Total phenols mg_{GAE} g_{dl}^{-1}	Flavonoids mg_{QE} g_{dl}^{-1}	Antioxidant activity %	Outlet air temperature (°C)
Feed flow phase	$77.01±0.59^{a}$	$170.45±0.61^{a}$	$93.66±0.55^{a}$	
1	$40.90±0.57^{b}$	$110.47±0.48^{b}$	$88.68±0.64^{d}$	102
2	$41.48±0.78^{b}$	$117.26±1.08^{c}$	$76.93±0.30^{c}$	85
3	$41.62±0.59^{b}$	$112.20±0.56^{b}$	$67.97±0.27^{b}$	87
4	$49.93±0.90^{d}$	$117.10±0.24^{c}$	$86.36±0.68^{d}$	83
5	$47.21±0.11^{c}$	$124.68±0.58^{d}$	$90.13±0.78^{e}$	70

Different super index means significate difference ($p<0.05$)

The flavonoids compounds are sensitive to oxidation and thermal degradation during spray drying (Anandharamakrishnan and Ishwarya, 2015), when the flavonoid molecule exhibit great number of hydroxyl attached to the aglicon molecule, they are more susceptible to the thermal degradation (Buchner et al., 2006). The quercetin, one of the flavonoids reported with more frequency for the *Stevia rebaudiana* leafs, has four hydroxyl groups and is reported as a flavonoid susceptible to degradation at temperatures above 100 °C. Some investigations have proposed that the breaking of the glycoside part of high molecular weight polyphenols modifies the structural properties, leading to degradation towards simpler structures (e.g., the degradation to monomers in tannins) derived from the increase of free energy in the environment caused by the increase in

temperature (Stamatopoulos et al., 2014). In the treatments with higher feed flows, the lowest outlet temperatures were observed (Table 2), so this also helps the flavonoids suffer less degradation. Also, as it is a concurrent flow dryer, despite the fact that inlet air temperatures of up to 220 °C are applied, water evaporation occurs instantly, so the dried product is exposed to moderate temperatures (50 – 100 °C), which limits thermal degradation of heat-sensitive compounds (Anandharamakrishnan and Ishwarya, 2015). The air temperature and the feed flow rate have no significant effect in antioxidant capacity. The highest antioxidant capacity was observed at the lowest temperature and the highest feed flow, 160 °C 3 kg h^{-1} (Table 2).

The fact that the inlet temperature and the feed flow did not have a significant effect on the antioxidant activity could be because, in addition to flavonoids and phenolic compounds, the aqueous extract of stevia contains other antioxidant compounds that are not sensitive to high temperatures as reported by Georgetti et al. (2008) in the spray drying of soy extract with/without encapsulant, and by Krishnaiah et al. (2012) in the spray drying of *Morinda citrifolia* extracts.The spectrometry data obtained by UHPLC-ESI-qTOF-MS inferred the presence of several phenolic acids, flavonols, flavones and isoflavones (Table 3). At a higher temperature and lower feed flow (200 °C – 2 kg h^{-1}), the profile of phenolic compounds consists only of chlorogenic acid. At lower temperature and higher feed flow (160 °C – 3 kg h^{-1}), the phenolic profile is constituted by 8 compounds.

Table 3 Polyphenolic compounds profile

Treatment	Phenolic compound	Molecular formula	Observed $[M-H]^-$ m/z
Feed flow phase	Chlorogenic acid	$C_{16}H_{18}O_9$	353.0876
	3-Feruloylquinic acid	$C_{17}H_{20}O_9$	367.1045
	3,4-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	515.1194
	3,5-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	515.1194
	Diosmin	$C_{28}H_{32}O_{15}$	607.1741
	Rutin	$C_{27}H_{30}O_{16}$	609.1416
	Apigenin 6,8-di-c-glucoside	$C_{27}H_{30}O_{15}$	593.1536
	Quercetin-rhamnoside	$C_{21}H_{20}O_{11}$	447.0942
	Quercetin	$C_{15}H_{10}O_7$	301.0354
	Quercetin derivate	$C_{15}H_{10}O_7$	301.0354
	Naringenin	$C_{15}H_{12}O_5$	271.0612
	3'-hydroxygenistein	$C_{15}H_{10}O_6$	285.0413
	Rosmarinic acid	$C_{18}H_{16}O_8$	359.0785
1	Chlorogenic acid	$C_{16}H_{18}O_9$	353.0876
2	Chlorogenic acid	$C_{16}H_{18}O_9$	353.0876
	5-Caffeoylquinic acid	$C_{16}H_{18}O_9$	353.0876
	Caffeic acid	$C_9H_8O_4$	179.0350
3	Chlorogenic acid	$C_{16}H_{18}O_9$	353.0876
	3,4-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	515.1194
	Rutin	$C_{27}H_{30}O_{16}$	609.1416
	Rutin derivate	$C_{27}H_{30}O_{16}$	609.1416
	Quercetin derivate	$C_{15}H_{10}O_7$	301.0354
4	Chlorogenic acid	$C_{16}H_{18}O_9$	353.0876
	4-Caffeoylquinic acid	$C_{16}H_{18}O_9$	353.0876
	3,4-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	515.1194
	Quercetin	$C_{15}H_{10}O_7$	301.0345
5	Chlorogenic acid	$C_{16}H_{18}O_9$	353.0876
	3-Feruloylquinic acid	$C_{17}H_{20}O_9$	367.1045
	3,4-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	515.1194
	3,5-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	515.1194
	Rutin	$C_{27}H_{30}O_{16}$	609.1416
	Apigenin 6,8-di-c-glucoside	$C_{27}H_{30}O_{15}$	593.1536
	Quercetin-rhamnoside	$C_{21}H_{20}O_{11}$	447.0942
	Quercetin	$C_{15}H_{10}O_7$	301.0354

This could be because in the drying treatments in which the feed flow was higher, the temperature of the drying chamber and the outlet temperature were lower; for this reason, there was a greater number of phenolic compounds in the treatments with lower temperature and higher feed flow.

Chlorogenic acid was the compound that presented the greatest stability at high temperatures, this molecule requires up to 200 °C for its degradation (Dawidowicz and Typek, 2017).

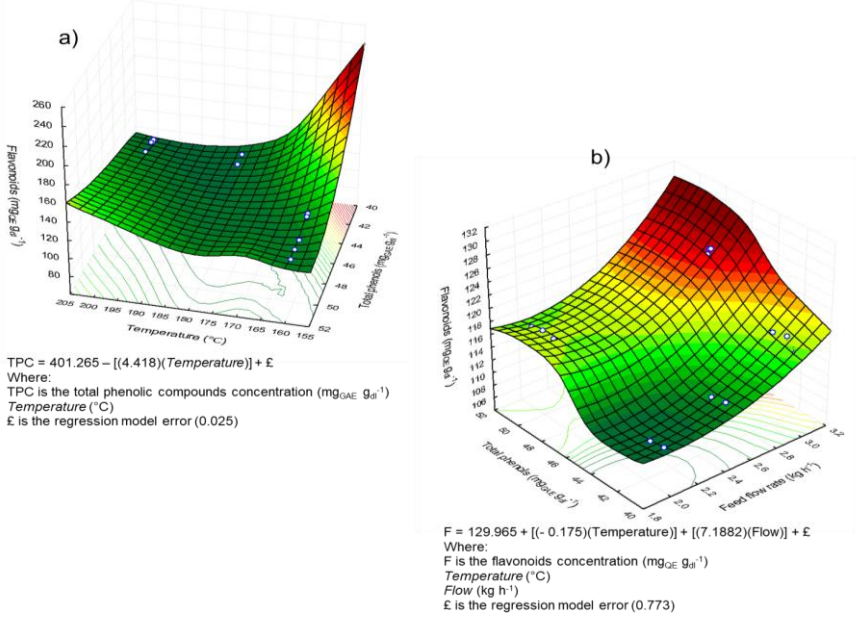

Figure 1: Response Surface Design. (a) TPC multiple regression model. (b) flavonoids multiple regression model

4. Conclusions

Phytochemical changes in phenol content, flavonoid content and antioxidant activity occurred during the spray drying process of stevia leaves aqueous extract. The inlet air temperature and feed flow rate had a significant effect ($p<0.05$) on the total flavonoid concentration. The inlet air temperature had a significant effect ($p<0.05$) on the total phenol. When the inlet air temperature increased, the total phenolic compound concentration, and the total flavonoid concentration decreased. The inlet air temperature and feed flow rate did not have a significant effect on antioxidant activity. The application of high feed flow rates in spray drying preserves the concentration of flavonoids in the extracts, since it decreases the air temperature at which the compounds are exposed. The final spray dried material could be considered, more than a low caloric index sweetener, a source of polyphenolic compounds.

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