Effect of High Pressure Processing Assisted by Temperature on Bioactive Compounds and Antioxidant Activity of Mate Tea and Mate Tea Sweetened with *Stevia*

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ABSTRACT

Tea is the second most consumed beverage worldwide after water. Mate tea is an infusion from *Ilex paraguariensis*, a native plant of South America, gaining interest for its health benefits in the prevention of chronic diseases. *Stevia Rebaudiana* is a potential sweetener for reduction of sugar consumption. In this study, the effect of pressure and temperature on mate tea leaves and mate tea mixed with *Stevia Rebaudiana* were evaluated with respect to total phenolics (TP), total antioxidant activity (TAA), and individual phenolic compounds, including chlorogenic acid and caffeic acid. Mate and mate sweetened with *Stevia* (2.5% w/v) were treated at 10-600 MPa and 25-120ºC for 1-5 min. The TP and TAA were determined using spectrophotometric methods. Caffeic acid and chlorogenic acid were quantified by high performance liquid chromatography. The TP and TAA contents of mate tea leaves significantly increased after treatment compared to the control. High amounts of TAA (116.33±1.38 $FeSO_4.7H_2O$ (mM) of mate tea leaves were obtained at 120 ºC/100 MPa/1 min while high amounts of TP (137.49±1.29 mg GAE/g) were extracted at 120 ºC/600 MPa/1 min. Also, after treatment at 120 ºC/600 MPa/1 min, *Stevia* addition resulted in an increase of up to 70% of TP and up to 60% of TAA compared to the control. However, the extraction of TP, and TAA of mate sweetened with *Stevia* was lower than mate samples treated without *Stevia* at all conditions investigated. These results highlight the potential use of high pressure processing assisted by temperature to increase antioxidant activity and extraction of total phenolics and caffeic acid of mate and mate sweetened with *Stevia Rebaudiana.*

Keywords: Ilex paraguariensis, Stevia Rebaudiana, high pressure, bioactive compounds.

1. INTRODUCTION

Mate tea, from the leaves of *IIlex paraguariensis*, is originally from the southern part of South America, consumed primarily in Brazil, Paraguay, Uruguay, and Argentina. *IIlex paraguariensis* is used in the preparation of mate tea with hot water (chimarrão) and cold water (tererê). In 2016, the consumption per capita of mate tea in Uruguay was 19 litres, making them the world's leading consumers of mate [1]. Recently, mate tea has gained great interested in Asia, United States, and Europe due to its health benefits and functionality, including antioxidant, stimulant, diuretic, hypocholesterolemic, hepatoprotective and anticarcinogenic effects. These health benefits are associated to the presence of bioactive compounds, including chlorogenic acid (51-388 μg mL^{-1}), caffeic acid (0.66-4.14 μg mL^{-1}), rutin (43.40 μg mL^{-1}), quercetin (10.25 μg mL^{-1}) and theobromine (88.92 μg mL^{-1}) [2]. Studies have demonstrated that the consumption of chlorogenic acid (100 mg/kg/day) might decrease blood pressure, oxidative damage in the brain, and prevent chronic diseases including, diabetes, bacterial infections, inflammation and cancer [3]. Caffeic acid has exhibited effective antioxidant activity *in vitro* by 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging (92.9% ABTS radical decrease), 1,1-diphenyl-2-picryl-

hydrazyl free radical (DPPH) scavenging (93.9% DPPH radical decrease) and ferric thiocyanate method in a linoleic acid system (75.8%) at the concentration of 20 μg/L caffeic acid [4].

Mate tea is currently consumed with additives like sucralose. However, high intake of sucralose can lead to chronic and degenerative diseases, including type II diabetes, obesity, and cardiovascular diseases [5]. *Stevia Rebaudiana,* a non-caloric sweetener, is 100-300 times sweeter than sucrose, being an alternative to reduce sugar consumption [6]. Epidemiological studies have reported that a product sweetened with *Stevia* has the potential of managing type-2 diabetes [7]. Glycosides of *Stevia* leaves include stevioside (5.8±1.3% dry leaves), rebaudioside A (1.8±1.2% dry leaves) and rebaudioside C (1.3±1.2% dry leaves). Some studies have evaluated the effect of *Stevia* addition on phenolic compounds of fruit and tea beverages. Stevia addition on Roselle (*Hibiscus sabdariffa L)* beverage increased quercetin, gallic acid and rosmarinic acid during storage [8]. The addition of *Stevia* rebaudiana (1.25-2.5%w/w) in fruit-orange-oat beverage increased concentration and bioaccesibility of total phenolic content, total antioxidant capacity, total carotenoids and total anthocyanins [9].

Various processing techniques have been used to extract bioactive compounds from *Mate Ilex paraguariensis* and *Stevia rebaudiana*. Supercritical CO_2 (25.5 MPa/70 ^oC/0.9-1.2 g CO_2/min /7h) extracted 94, 68, and 57% of initial caffeine, theobromine, and theophylline contents from mate leaves [10]. Also, 51 compounds, mainly esters, fatty acids, hydrocarbons, phytosterols, alcohols, xanthines and vitamin E were only identified on mate tea leaves extracts (25.5 MPa/40 ^oC/10 h) using methanol and hexane as solvents [11]. High pressure processing at 500 MPa/25^oC/1 min was used to extract caffeine from green tea leaves (4.0±0.22% yield of caffeine). The same extraction yield was obtained at room temperature for 20 h [12]. High pressure processing assisted by temperature is an emerging technology that consists in applying high hydrostatic pressure (10-800 MPa) at uniform heating (25-120 ^oC) for short period of time (3-60 min). Various studies have reported the effect of high pressure processing and high pressure assisted by thermal processing on increasing total anthocyanin, total carotenoids, flavonoids, total phenolic content and antioxidant activity of plant materials and fruits, including grape by products, green tea, and *Stevia rebaudiana* leaves [13-14]. However, no data is available on the effect of high pressure processing assisted by temperature on mate tea and mate tea sweetened with *Stevia.* Therefore, the objective of this study was to investigate the effect of high pressure assisted thermal processing on total antioxidant activity, total phenolic content, and specific bioactive compounds of mate tea and mate tea sweetened with *Stevia*.

2. MATERIALS AND METHODS

2.1. Materials

Yerba mate was purchased at a local market in Edmonton, AB with a proximate composition of 76.4% carbohydrates, 12.6% protein, 0.5% lipids, 5.3% moisture, and 5.3% ash. Yerba mate was ground, sieved (mean particle size of 500 μm) and stored at 4^oC for further analysis. *Stevia Rebaudiana* powder, SweetLeaf Stevia®, was obtained in a local market in Edmonton, AB and stored at 4^oC for further analysis.

Chemicals such as (−) epigallocatechin (EGC, ≥95%), (−) epicatechin (EC, ≥90%), (−)epigallocatechin gallate (EGCG, ≥ 95%), (−)epicatechin gallate (ECG, ≥98%), chlorogenic acid (CA, ≥95%), caffeic acid (CF, ≥98%), rutin hydrate (RT, ≥98%) and quercetin (QC, ≥95%) were purchased from Sigma-Aldrich (Oakville, ON, Canada).

2.2. Methods

High Pressure Processing assisted by Temperature (HPPT)

A four-vessel system (Apparatus U111 Unipress, Warszawa, Poland) was used. Each vessel has a capacity of 8 mL. The vessels were heated with a circulator thermostat (Lauda Proline RP 855 Low Temperature, Lauda-Konigshofen, Germany) using propylene glycol as the pressure transmission fluid. Polypropylene tubes (Cryogenic vial, Fisher Scientific, Pittsburgh, PA) of 3 mL were filled with samples that were pressurized to 10, 100, and 600 MPa at temperatures of 25, 75, and 120°C with holding times of 1 and 5 min. The samples were pressurized at a rate of 10 MPa s^{-1}. At the end of the holding time, the vessels were decompressed, and the samples were removed immediately from the high-pressure vessels, cooled down with ice and stored at -18 ˚C for further analysis.

Determination of total antioxidant activity (TAA)

The TAA was determined using the FRAP assay with slight modifications [15]. A buffer acetate of pH 3.6, 10Mm tripyridyltriazine (TPTZ) and 20mM ferric chloride solution were mixed in a ratio of 10:1:1 (v/v/v) to prepare the FRAP solution. The FRAP solution (3 mL) reacted with the extract solution (0.1 mL) and distilled water (0.3 mL). Then, the mixture was vortexed for 10 s and incubated at 37 ºC in a water bath for 30 min. The absorbance was read at 593 nm using a UV-VIS spectrophotometer (Jenway 6320D, Standford, United Kingdom).

Determination of total phenolic content

The Folin-Ciocalteu method was used to determine total phenolic content [15]. Briefly, a sample aliquot (0.04 mL), distilled water (3.10 mL) with Folin-Ciocalteu reagent (0.20 mL) was vortexed for 10 s. Then, sodium carbonate (20% w/v; 0.60 mL) was added and vortexed for 10 s. The mixture was incubated for 2 h in dark at room temperature (23±2 ºC). The absorbance was measured at 765 nm using a UV-VIS spectrophotometer. The result was expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE g^{-1}).

Determination of individuals bioactive compounds

Individual bioactive compounds were analyzed with an HPLC system (Shimadzu 20, Kyoto, Japan) equipped with a Phenomenex Luna column (5 mm, 4.6 x 150 mm, 35 °C) at 268 nm and a flow rate of 1 mL/min. Solvents A (0.5% formic acid in water) and B (0.5% formic acid in methanol) were run with 8% B, 27% B for 25 min, 30% B for 32 min, 100% for 36-41 min and finally back to initial concentration (8%) for another 42 and 47 min.

Statistical analysis

Results were expressed as mean ± standard deviation of at least three replications and analyzed using the R Studio software. One-way ANOVA was used to analyse the data, $p < 0.05$.

3. RESULTS AND DISCUSSIONS

3.1. Total antioxidant activity of mate and mate + stevia

Total antioxidant activity (TAA) of mate and mate + stevia after treatment at 10–600 MPa/25-120 ˚C/1-5 min is shown in Fig. 1. The TAA values for treated and untreated mate were 88-116 and 28.98±1.03 $FeSO_4.7H_20$ (mM), respectively. An increase in temperature from 25 to 120 ˚C resulted in a significant increase of mate tea TAA. The TAA values of mate tea increased more than 300% at conditions of 10 MPa/120 ºC/1 min compared with untreated mate. The highest value of TAA was achieved at 100 MPa/120 ºC/1 min (116.33±3.46 $FeSO_4.7H_20$ (mM)). This increase of TAA is attributed to the disruption of cell membranes, improving the extractability of antioxidant compounds after HPPT. It is also suggested that high pressure and temperature could

deprotonate charged groups, disrupt hydrophobic bonds in cell membranes, and inactivate peroxidase and polyphenoloxidase enzymes, resulting in high antioxidant activity.
No study reported the effect of HPPT on antioxidant activity of mate tea. However, various studies reported the effect of pressure and/or temperature in other food matrices. Increases of TAA contents by 68% and 19% were observed in asparagus juice (121 ºC/3 min) and carrot purees after thermal processing (70ºC/2 min), respectively [16].

The TAA values for mate tea sweetened with *Stevia Rebaudiana* were 49-71 $FeSO_4.7H_20$ (mM) (Fig 1b). The highest value of TAA was achieved at 600 MPa/120 ºC/1 min (71.15±0.34 $FeSO_4.7H_20$ (mM)) while the TAA lowest value was obtained at 600 MPa/120 ºC/5 min (49.09±2.56 $FeSO_4.7H_20$ (mM)). At conditions of 600 MPa/120 ºC/1 min and 600 MPa/120 ºC/5 min, the TAA values of mate tea sweetened with stevia increased by 146% and by 69.5%, respectively, compared with untreated mate samples. This increase in TAA can be attributed to the superoxide radical scavenging activity of *Stevia* leaf extract [17]. An increase in TAA (393-762%) was reported for untreated and treated fruit juice mixture (papaya 32.5% v/v, mango 10% v/v, and orange 7.5% v/v) sweetened with *Stevia rebaudiana* (1.25-2.5% w/v) at 300-500 MPa, 5-15 min, and 15-32ºC.

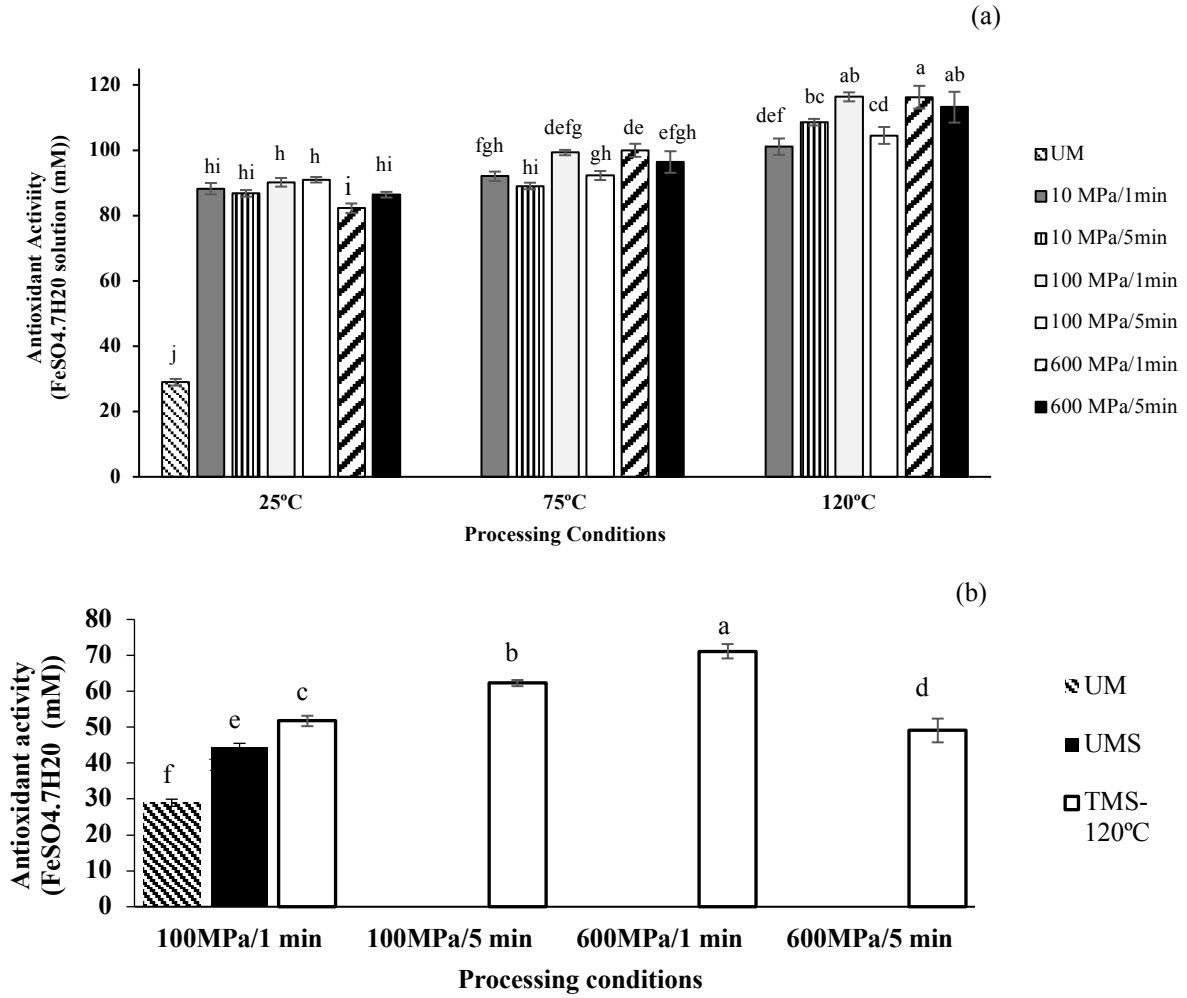

Figure 1. Total antioxidant activity of untreated and: (a) treated mate and (b) treated mate + stevia. (UM: untreated mate, UMS: untreated mate + stevia, TMS: treated mate + stevia)

3.2. Total phenolic content (TP) of mate and mate + stevia

The effect of high pressure assisted by temperature (10-600MPa/1-5min/25-120ºC) of mate and mate sweetened with *Stevia* for total phenolics before and after treatment is presented in Fig. 2. In general, treated mate had higher TP compared to untreated mate tea. The process condition of 100 MPa/120 ºC/5 min showed significant increase in TP content (187.23±10.29 mg GAE/g) compared to untreated mate (11.94±0.46 mg GAE/g). The increase of TP content can be related to an increase of individual phenolic components extractability after HPPT.

In this study, higher TP content was obtained (41% more) using high pressure processing assisted by temperature (100 MPa/120 ºC/5 min) compared to 50% acetone extraction [18]. Earlier, the TP content of mate after extraction using different solvents (water, 50-100% acetone, 50-10% dimethylformamide, 50-100% ethanol, and 50-100% methanol) was reported. The highest TP content was obtained using 50% acetone (120.4±1.49 mg/g) at 100 ºC for 10 min.

The TP contents for lime, mandarin and lemon citrus peels were reported, where an increment of 9%, 12%, and 20%, respectively, were observed at 300 MPa/10 ºC/3 min [19]. Similarly, an increase of TP content of mango pulp by 7-27% relative to the untreated sample after treatment at 400-600 MPa/40-60 ºC/ 5-15 min was reported [20].

The *Stevia* addition resulted in a significantly increase of TP content compared to untreated mate tea at all conditions investigated (10-100MPa, 120ºC, 1-5 min). The highest TP content was achieved at 600 MPa/120ºC/1 min (69.87 mg GAE/g) (Fig 2b). These results can be attributed to the presence of phenolic and flavonoid components on *Stevia rebaudiana*, and its capacity of acting as an antibrowning agent, minimizing oxidation [21]. Increases of TP contents of 22% and 18% were reported for a juice mixture of papaya (32.5%, v/v), mango (10%, v/v) and orange (7.5%, v/v) with *Stevia* (1.25% w/v), and with *Stevia* (2.5% w/v), respectively, after treatments at 500 MPa/25ºC/15 min [14].

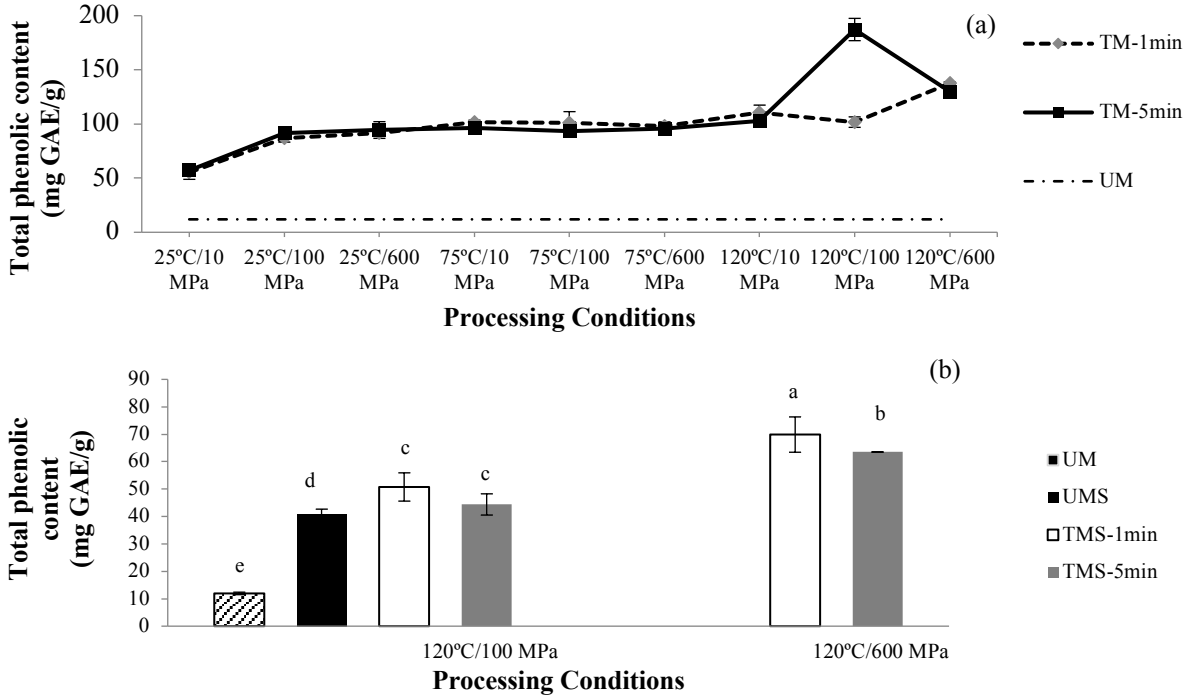

Figure 2. Total phenolic content of untreated and: (a) treated mate, (b) treated mate + stevia.
(UM: untreated mate, UMS: untreated mate + stevia, TM: treated mate, TMS: treated mate + stevia)

Fig. 3 shows chlorogenic and caffeic acid contents of untreated and treated mate at 25-120 ºC/600 MPa/1-5 min. The concentrations of chlorogenic acid and caffeic acid determined by HPLC of untreated mate were 8.25 mg/g mate and 6.48 mg/g mate, respectively. The amount of caffeic acid on mate samples gradually increased with the increase of temperature from 25 ºC to 120ºC at 600 MPa. The highest amount of caffeic acid was achieved at 120ºC/600MPa/1min. However, a slight decrease was observed at 120 ºC/600MPa/5 min, probably due to degradation of caffeic acid to hydroxytyrosol (3,4-dihydroxyphenylethanol), protocatechuic aldehyde (3,4-dihydroxybenzaldehyde), and 4-vinylcatechol (2-hydroxy-4-vinylphenol) [22].
Chlorogenic acid contents decreased about 41% and 91% at 25ºC/600MPa/1min and 120ºC/600MPa/5 min, respectively, probably due to chlorogenic acid isomerization, transforming 5-O-caffeolquinic acid into caffeic acid [23]. It was reported a decrease of 90% of 5-CQA content of green coffee samples after aqueous extraction at 80ºC/15 min due to chlorogenic acid isomerization and transformation to 3-O-caffeoylquinic acid, 4-O-caffeoylquinic acid, quinic acid, and caffeic acid [24].

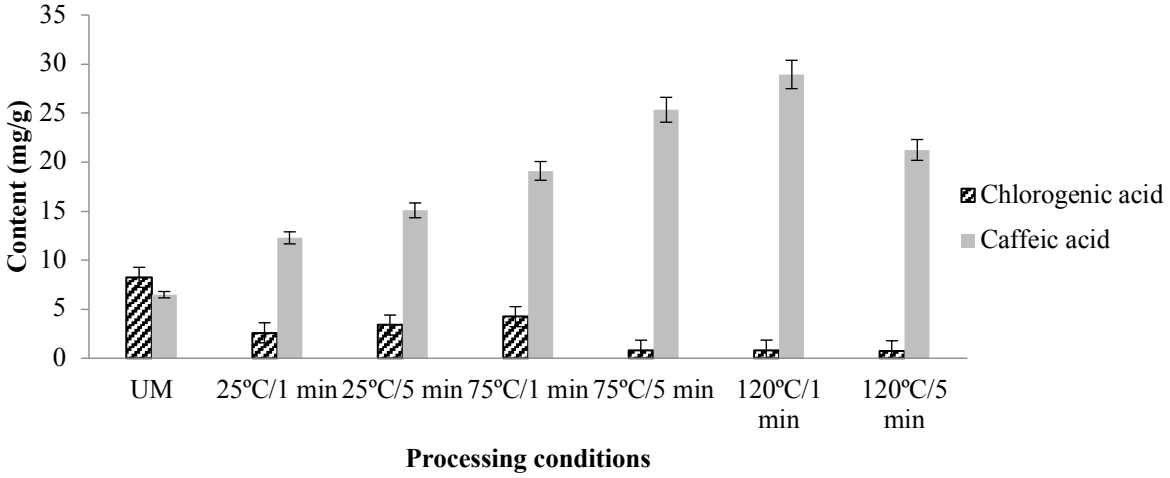

Figure 3. Chlorogenic acid and caffeic acid (mg/g) of untreated and treated mate at 600 MPa. (UM: untreated mate)

4. CONCLUSIONS

Antioxidant activity and bioactive compounds, including total phenolics, chlorogenic acid and caffeic acid of mate and mate sweetened with *Stevia* were affected by HPPT. The TAA increase of mate tea after HPPT compared to untreated mate tea was observed at all conditions evaluated (25-120 ºC/10-600MPa/1-5 min). The highest TAA value was obtained at 120ºC/100 MPa/1 min. HPPT applied to mate resulted in an increase of total phenolic content at all conditions investigated, obtaining the highest value (187.23 mgGAE/g) at 120ºC/100 MPa/5 min. The chlorogenic acid content of mate and mate sweetened with stevia were negatively affected by HPPT, however a conversion of this compound to caffeic acid was observed at all conditions evaluated. The *Stevia* addition reduced the extraction of TP and TAA compared to mate without *Stevia* at all conditions investigated. This study showed that high pressure processing assisted by temperature is an alternative treatment to increase bioactive compounds extractability (except for chlorogenic acid), improving nutritional and functionality of mate and mate sweetened with *Stevia*.

Acknowledgments
The authors acknowledge the financial support of the Natural Sciences and Engineering Research Council of Canada (NSERC, #05356-2014).

References

[1] Euromonitor International (2016, October), As consumption stagnates in South America, will yerba Mate move north?
[2] BERTE, K., RODRIGUEZ-AMAYA, D.B., HOFFMAN-RIBANI, R., MACCARI. Antioxidant activity of mate tea and effects of processing, Processing and Impact on Antioxidants in Beverages, 2014, 145-153.
[3] TAJIK, N., TAJIK, M., MACK, I., ENCK, P., The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: a comprehensive review of the literature, Europen Journal of Nutrition, 56, 2017, 2215-2244.
[4] GULCINM, I. Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid), Toxicology, 217, 2006, 213-220.
[5] WU, L., SHI, A., ZHU, D., BO, L., ZHONG, Y., WANG, J., XU, Z. High sucrose intake during gestation increases angiotensin II type 1 receptor-mediated vascular contractility associated with epigenetic alterations in aged offspring rats, Peptides, 2016, 133-144.
[6] GOYAL, S.K., GOYAL, R.K., GOYAL, S. Stevia (*Stevia rebaudiana*) a bio-sweetener: a review, International Journal of Food Sciences and Nutrition, 61, 2010, 1-10.
[7] GARDANA, C., SCAGLIANTI, M., SIMONETTI, P. Evaluation of steviol and its glycosides in *Stevia rebaudiana* leaves and commercial sweetener by ultra-high-performance liquid chromatography-mass spectrometry, Journal of Chromatography A, 1217, 2010, 1463-1470.
[8] PEREZ-RAMIREZ, I.F., CASTANO-TOSTADO, E., RAMIREZ-DE LEON, J.A., ROCHA-GUZMAN, N.E., REYNOSO-CAMACHO, R. Effect of stevia and citric acid on the stability of phenolic compounds and *in vitro* antioxidant and antidiabetic capacity of a roselle (*Hibiscus sabdariffa L.)* beverage, Food Chemistry, 172, 2015, 885-892.
[9] CARBONELL-CAPELLA, J.M., BUNIOSWSKA, M., ESTEVE, M.J., FRIGOLA, A. Effect of *Stevia rebaudiana* addition on bioaccessibility of bioactive compounds and antioxidant activity of beverages based on exotic fruits mixed with oat following simulated human digestion, Food Chemistry, 184, 2015, 122-130.
[10] SALDAÑA, M.D.A., MOHAMED, R.S., BAER, M.G. AND MAZZAFERA, P. Extraction of purine alkaloids from maté (*Ilex paraguariensis*) using supercritical CO_2, Journal of Agriculture and Food Chemistry, 47 (9), 1999, 3804-3808.
[11] JACQUES, R.A., DARIVA, C., DE OLIVEIRA, J.V., CARAMAO, E.B. Pressurized liquid extraction of mate tea leaves, Analytica Chimica Acta, 5, 2008, 70-76.
[12] XI, JUN. Caffeine extraction from green tea leaves assisted by high pressure processing, Journal of Food Engineering, 94, 2009, 105-109.
[13] CORRALES, M., TAUSCHER, B. Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: A comparison, Innovative Food Science and Emerging Technologies, 9, 2008, 85-91.
[14] CARBONELL-CAPELLA, J.M., FRIGOLA, A. High pressure processing of fruit juice mixture sweetened with *Stevia rebaudiana* Bertoni: Optimal retention of physical and nutritional quality, Innovative Food Science and Emerging Technologies, 18, 2013, 48-56.
[15] SARKAR, S., ALVAREZ, V.H. AND SALDAÑA, M.D.A. Relevance of ions in pressurized fluid extraction of carbohydrates and phenolics from barley hull. The Journal of Supercritical Fluids, 93, 2014, 27-37.
[16] CHEN, X., QIN, W., MA, L., XU, F., JIN, P., ZHENG, Y. Effect of high pressure processing and thermal treatment on physicochemical parameters, antioxidant activity and volatile compounds of green asparagus juice, LWT- Foof Science and Technology, 62, 2015, 927-933.

[17] SHUKLA, S., EHTA, A., BAJPAI, V.K., SHUKLA, S. *In vitro* antioxidant activity and total phenolic content of ethanolic leaf extract of S*tevia rebaudiana* Bert, Food and Chemical Toxicology, 47, 2009, 2338-2343.
[18] TURKEMEN, N., SARI, F., VELIOGLU, Y.S. Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods, Food Chemistry, 99, 2006, 835-841.
[19] CASQUETE, R., CASTRO, S.M., MARTIN, A., RUIZ-MOYANO, S., SARAIVA, J.A., CORDOBA, M.G., TEIXEIRA, P. Evaluation of the effect of high pressure on total phenolic content, antioxidant and antimicrobial activity of citrus peels, Innovative Food Science and Emerging Technologies, 31, 2015, 37-44.
[20] KAUSHIK, N., RAO, P.S., MISHRA, H.N. Process optimization for thermal-assisted high pressure-processing of mango (*Mangifera indica L*) pulp using response surface methodology, LWT-Food Science and Technology, 69, 2016, 372-381.
[21] BARBA, F.J., CRIADO, M.N., BELDA-GALBIS, C.M., ESTEVE, M.J., RODRIGO, G. *Stevia rebaudiana Bertoni* as a natural antioxidant/antimicrobial for high pressure processed fruit extract: Processing parameter optimization. Food Chemistry, 148, 2014, 261-267.
[22] KHUWIJITJARU, P., SUAYLAM, B., ADACHI, S. Degradation of caffeic acid in Subcritical Water and online HPLC-DPPH assay of degradation products. Journal of Agricultural and Food Chemistry, 62, 2014, 1945-1949.
[23] DAWIDOWICZ, A.L., TYPEK, R. Thermal stability of 5-o-caffeoylquinic acid in aqueous solutions at different heating conditions, J. Agric. Food Chemistry, 58, 2010, 12578-12584.
[24] DE MARIA, C.A.B., TRUGO, L.C., MOREIRA, R.F.A., PETRACO, M. Simultaneous determination of total chlorogenic acid, trigonelline and caffeic in green coffee samples by high performance gel filtration chromatography, Food Chemistry, 52, 1995, 447-449.