Effect of Pressure Assisted Thermal Processing on Bioactive Compounds and Antioxidant Activity of Mate and Mate Sweetened with *Stevia rebaudiana*

by

Angelica Maria Chourio Chavez

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Bioresource and Food Engineering

Department of Agricultural, Food and Nutritional Science

University of Alberta

ABSTRACT

Mate *Ilex paraguarensis* is gaining considerable interest worldwide due to its health benefits in the prevention of chronic diseases like cancer, mainly attributed to its content of bioactive compounds. In this study, the effect of Pressure Assisted Thermal Processing (PATP) on total phenolics, total antioxidant activity, and individual bioactive compounds (chlorogenic acid, caffeic acid, quercetin, rutin, theobromine) of mate, mate sweetened with *Stevia rebaudiana* and model systems was investigated. Mate, *Stevia* leaves and mate sweetened with *Stevia* (2.5% w/v) were treated at 10-600 MPa/25-120ºC/1-5 min. The total phenolics (115%) and total antioxidant activity (400%) content of mate significantly increased at 100 MPa/120ºC/1 min and 600 MPa/120ºC/1 min, respectively, compared to untreated mate. Also, temperature and pressure had a significant effect on the increase of quercetin, caffeic acid, and rutin contents on treated mate at 100 MPa/120ºC/1 min. However, chlorogenic acid content significantly decreased up to 91% compared to untreated mate. A significant increase of caffeine from 22.05 ± 2.85 to 35.85 ± 2.59 mg/g mate and theobromine from 15.29± 2.02 to 23.99 ± 0.02 mg/g mate was obtained at 100 MPa/120ºC/1 min compared to untreated mate. Mate sweetened with *Stevia* resulted in an increase up to 88% of total phenolics and up to 74% of total antioxidant activity compared to untreated mate. High correlation (R^2>0.90) between polyphenol and antioxidant activity was observed on PATP treated mate and *Stevia* leaves. Moreover, mate sweetened with *Stevia* leaves resulted in higher release of bioactive compounds compared to mate+commercial *Stevia* powder due to the bioactive compounds and antioxidant properties of *Stevia* leaves. In model systems of pure compounds, the increase of temperature from 25 to 75ºC had no significant effect on quercetin, rutin, caffeic acid and chlorogenic acid contents. However, the increase of pressure from 100 to 300 MPa had a significant impact on the decrease of chlorogenic acid content ($p<0.05$). The effect

of PATP (10-600 MPa/75-120ºC/1-5min) on enzymatic activity of polyphenoloxidase (PPO) and peroxidase (POD) of *Stevia* leaves was also studied. The optimum condition to a complete reduction of PPO and POD enzymatic activity was 600MPa/120ºC/1 min. These results highlight the potential of PATP to release bioactive compounds and enhance antioxidant activity of mate and mate sweetened with *Stevia,* ensuring an effective inactivation of degradative enzymes and promoting the development of ready-to-drink mate-based beverages with health benefits.

Keywords: Emerging technologies, *Ilex paraguariensis,* methylxanthines, enzymes, catechins.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to my supervisor Dr. Marleny D.A. Saldaña for giving me the opportunity to join her research team, for her continuous scientific support, patience, motivation, time and knowledge. I also thank to my committee member Dr. Sergio Martinez Monteagudo for his valuable suggestions, and encouragements throughout my research.

Special thanks to my lab-mates and friends for all their suggestions, help and support, specially to Raquel, Idaresit, Alaleh, Yujia, Carla, Azadeh, Zahid, Eric, Francisca, Renuka and Arantxa.

I want to express my profound gratitude to my family, specially to my beloved husband David for his support and unconditional love, thank you for believing in me and being there for me and my loved parents Nancy and Edgar, my siblings Miguel, Carolina and Juan Pablo and my grandmother Celeste for their prayers and emotional support.

Finally, I thank the University of Alberta and the Natural Sciences and Engineering Research Council of Canada (NSERC) for funding this research project.

TABLE OF CONTENTS

ABSTRACT ii

ACKNOWLEGEMENTS iv

LIST OF TABLES ix

LIST OF FIGURES x

NOMENCLATURE xi

Chapter 1: Introduction 1

1.1 Rationale 1

1.2 Hypothesis 4

1.3 Thesis objectives 5

Chapter 2: Literature review 6

2.1 Mate *IIex paraguariensis* 6

2.2 *Stevia rebaudiana Bertoni* 6

2.2.1 Stability of *Stevia rebaudiana* 15

2.2.2 Effect of *Stevia* addition on color and sensorial properties 18

2.3 Bioactive compounds in model systems 20

2.3.1 Caffeic acid 20

2.3.2 Chlorogenic acid 22

2.3.3 Quercetin 25

2.3.4 Rutin 28

2.4 High Pressure Processing (HPP) 32

2.4.1 Principles of high pressure processing (HPP) 33

2.4.2 Effect of high pressure on bioactive compounds 35

2.4.3 Limitations of high pressure processing 39

2.5 Enzymes 40

2.5.1 Polyphenol oxidase (PPO) 42

2.5.2 Peroxidase (POD) 43

Chapter 3: Effect of Pressure-Assisted Thermal Processing on Bioactive Compounds and Antioxidant Activity of Mate 47

3.1 Introduction 49

3.2 Materials and Methods 50

3.2.1 Sample preparation 50

3.2.2 Chemicals 51

3.2.3 Proximate compositional analysis 51

3.2.3.1 Moisture content 51

3.2.3.2 Ash content 52

3.2.3.3 Protein content 52

3.2.3.4 Total fat content 52

3.2.3.5 Total carbohydrate content 53

3.2.4 Pressure Assisted Thermal Processing (PATP) 53

3.2.4.1 Temperature and pressure profiles of PATP treatment 54

3.2.5 Analysis of treated samples 56

3.2.5.1 Total antioxidant activity 56

3.2.5.2 Total phenolic content 56

3.2.5.3 Individual bioactive compounds 57
3.2.6 Scanning electron microscope (SEM) 57
3.2.7 Statistical analysis 57
3.3 Results and Discussion 58
3.3.1 Proximate compositional analysis of mate 58
3.3.2 Total antioxidant activity and total phenolic content of mate 59
3.3.3 Individual phenolics in mate after PATP treatment 64
3.3.4 Methylxanthines content in mate after PATP treatment 71
3.3.5 Scanning electron microscope (SEM) images of untreated and PATP treated mate. 72
3.4 Conclusions 74
3.5 Recommendation 75
3.6 Acknowledgment 75
Chapter 4: Effect of Pressure Assisted Thermal Processing on Bioactive Compounds and Antioxidant Activity of *Stevia* and Mate Sweetened with *Stevia* 76
4.1 Introduction 77
4.2 Materials and Methods 78
4.2.1 Sample preparation 78
4.2.2 Chemicals 79
4.2.3 Proximate compositional analysis 79
4.2.4 Pressure Assisted Thermal Processing (PATP) 79
4.2.5 Analysis of treated samples 80
4.2.5.1 Total antioxidant activity 80
4.2.5.2 Total phenolic content 80

4.2.5.3 Individual bioactive compounds 81

4.2.5.4 Enzyme activity 81

4.2.5.5 Scanning electron microscope 82

4.2.6 Statistical analysis 82

4.3 Results and Discussion 82

4.3.1 Proximate composition of *Stevia* leaves and commercial *Stevia* powder 82

4.3.2 Total antioxidant activity and total phenolic content of *Stevia* leaves 84

4.3.3 Effect of PATP on bioactive compounds of model systems 87

4.3.4 Enzymatic activity of *Stevia* leaves 91

4.3.5 Total antioxidant activity and total phenolics of PATP treated mate+*Stevia* and mate+commercial *Stevia* powder. 96

4.3.6 Individual phenolics of untreated mate and treated mate sweetened with commercial *Stevia* powder 101

4.4 Conclusions 104

4.5 Recommendations 105

Acknowledgment 105

Chapter 5: Conclusions and recommendations 106

5.1 Conclusions 106

5.2 Recommendations 109

REFERENCES 111

APPENDIX A Effect of pressure-assisted thermal processing on bioactive compounds and antioxidant activity of mate 132

APPENDIX B: Effect of pressure assisted thermal processing on bioactive compounds and antioxidant activity of *stevia* and mate sweetened with *stevia* 148

LIST OF TABLES

Table 2.1 Individual phenolic compounds on mate leaves and commercial beverages. 9

Table 2.2 Effect of treatment and extraction techniques of bioactive compounds from mate and *Stevia*. 10

Table 2.3 Structure and physicochemical properties of bioactive compounds in mate and *Stevia* 12

Table 2.4 Effect of processing on caffeic acid and chlorogenic acid content. 24

Table 2.5 Effect of food processing and storage on chemical stability of quercetin... 26

Table 2.6 Yield of rutin using different extraction methods 30

Table 2.7 Temperature increase during compression heating 34

Table 2.8 Effect of high pressure processing on bioactive compounds. 37

Table 2.9 Effect of pressure-temperature treatment on PPO and POD inactivation from different sources. 45

Table 3.1 Proximate composition of untreated mate. 58

Table 3.2 Individual phenolics of PATP-treated mate at different processing conditions. 66

Table 3.3 Methylxanthines in untreated and treated mate at 120°C 72

Table 4.1 Proximate composition of untreated *Stevia* leaves. 83

Table 4.2 Total antioxidant activity and total phenolic content of mate sweetened with *Stevia* 100

Table 4.3 Individual phenolic compounds (mg/g) of PATP-treated mate+commercial *Stevia* powder. 103

LIST OF FIGURES

Figure 2.1 (a) Flow chart of processing and products from green mate leaves and (b) Steps and processing conditions for roasted mate tea production 8

Figure 2.2 Global trend of HPP treated food market for 2014-2025 33

Figure 2.3 Schematic representation of the elliptic phase pressure-temperature diagram..... 42

Figure 2.4 Catalytical reaction of PPO enzyme 42

Figure 3.1 Pressure assisted thermal processing equipment (Unipress, Warszawa, Poland). 54

Figure 3.2 Pressure and temperature profile of the water surrounding the samples in the 3 mL vial during processing at: (a) 10 MPa, (b)100 MPa, (c) 600 MPa and 90ºC. 56

Figure 3.3 Effect of temperature, pressure, and time on mean values of total antioxidant activity on PATP treated mate. 61

Figure 3.4 (a) Effect of temperature, pressure, and time on mean values of total phenolics on mate treated at 10-600MPa/25-120ºC/1-5 min and (b) Correlation of total phenolics (mg GAE/g) versus total antioxidant activity (mg $FeSO_4.7H_20$/g mate) of PATP treated mate. 64

Figure 3.5 Proposed pathway of caffeic acid degradation in subcritical water: (1) Caffeic acid, (2) 3,4-dihydroxyphenylethanol, (3) 3,4-dihydroxybenzaldehyde, and (4) 2-hydroxy-4-vinylphenol..... 65

Figure 3.6 Chlorogenic acid and caffeic acid (mg/g mate) of untreated mate (UM) and treated mate at 600 MPa 66

Figure 3.7 Scanning electron microscope images of untreated mate (a); and treated mate at 100 MPa/120ºC/5 min (b); and 600 MPa/120ºC/5 min (c)..... 74

Figure 4.1 Influence of main variables on (a) antioxidant activity and (b) total phenolic content. (c) Correlation between total phenolic content and antioxidant activity of *Stevia*. . . 87

Figure 4.2 Influence of variables, temperature, pressure and time on pure model systems: (a) quercetin, (b) rutin, (c) chlorogenic acid and (d) caffeic acid. 90

Figure 4.3 (a) POD, and (b) PPO enzymatic activity of treated *Stevia* leaves..... 94

Figure 4.4 Scanning electron microscope (SEM) images of: untreated *Stevia* (a); and treated *Stevia* at: 100 MPa/120ºC/5 min (b), and 600 MPa/120ºC/5 min (c)..... 95

Figure 4.5 (a) Total antioxidant activity and (b) Total phenolics content of untreated and treated mate+*Stevia* leaves at 25-120ºC/600 MPa/1-5 min. 99

NOMENCLATURE

Symbols and abbreviations

ABTS:	2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
ANOVA:	Analysis of variance
3-CQA:	3-O-Caffeoylquinic acid
4-CQA:	4-O-Caffeoylquinic acid
5-CQA:	5-O-Caffeoylquinic acid
db:	Dry basis
DMSO	Dimethyl sulfoxide
DPPH:	2,2-Diphenyl-1-picrylhydrazyl
EC:	Enzyme Commission
EDTA:	Ethylenediaminetetraacetic acid
FRAP:	Ferric reducing ability of plasma
GAE:	Gallic acid equivalent
HPLC:	High-performance liquid chromatography
HPP:	High pressure processing
ORAC:	Oxygen radical absorbance capacity
PATP:	Pressure assisted thermal processing
PEF:	Pulsed electric field
POD:	Peroxidase
PPO:	Polyphenoloxidase
SEM:	Scanning electron microscope
TEAC:	Trolox equivalent antioxidant capacity
TPTZ:	Tripyridyltriazine

Chapter 1: Introduction

1.1 Rationale

The alarming increase of Canadians with chronic and degenerative diseases, such as diabetes (3 million Canadians in 2014), cardiovascular diseases (2 million Canadians in 2014), obesity (64% of Canadian adults in 2017) and cancer (over 200,000 new cases in Canada in 2017) (Public Health Agency of Canada, 2017), along with an increasingly demanding consumers for healthier and minimally processed foods, have posed significant challenges to the food industry. Plant-based food beverage such as teas can provide health benefits due their high content of polyphenols such as phenolic acids (hydroxybenzene and cinnamic acids) and flavonoids (quercetin, kaempferol, catechins, taxifolin) that scavenge free radicals, inhibiting oxidative reactions. Epidemiological studies have showed evidence that regular consumption of tea and natural sweeteners has the potential of reducing the risk of developing chronic diseases (Gulcinm et al., 2006, Goyal et al., 2010, Gardana et al., 2010).

Mate *Illex paraguarensis* is one of the most consumed teas in the world. It grows mainly in Argentina, Brazil, Uruguay and Paraguay, with remarkable antioxidant properties associated to its high content of polyphenols, particularly chlorogenic acid, caffeic acid, rutin, quercetin, (+)-catechins, and methylxanthines (caffeine and theobromine) (Kungel et al., 2018, Saldaña et al., 1999). In some countries, mate is largely consumed with additives such as sweeteners and milk (Korir et al., 2014). Among natural sweeteners, *Stevia rebaudiana* is a perennial shrub of the Asteraceae family that grows naturally in tropical and subtropical areas of South America, including Paraguay, Brazil and Argentina. Its cultivation has expanded to other countries such as China, Malaysia, Singapore, South Korea, Taiwan, Thailand, England, Canada and the United States (Lemus-Mondaca et al., 2012). *Stevia* is a non-caloric sweetener that contains stevioside (81.2 mg/g), rebaudioside C (3.8 mg/g), rebaudioside A (3.5 mg/g) and dulcoside A (2.8 mg/g)

(Lemus-Mondaca et al., 2012). *Stevia* has gained great interest in the food industry not only as a non-caloric sweetener but also as a sweetener with antimicrobial and antioxidant properties due to its contents of phenolics, and flavonoids, such as quercetin and gallic acid (Barba et al., 2014).

Conventional methods for food preservation and sterilization are based on thermal processing. The application of high temperature is an effective method to achieve microbial safety, inactivation of enzymes and extend shelf-life of products. However, studies have demonstrated that heat treatment compromises the overall quality of food products by causing significant changes in colour, texture, flavour and nutritional value (Terefe et al., 2017).

In the last decade, high pressure processing (HPP) has emerged as a promising alternative to thermal processing, demonstrating high retention of bioactive compounds such as vitamins and polyphenols and retaining organoleptic properties such as flavour, color, and appearance. HPP uses water as a medium to transmit pressure up to 800 MPa. The isostatic pressure is homogenously applied to the product independent of size, shape and composition. Carbonell-Capella et al. (2013) reported high retention of total anthocyanins (>98%) in fruit juices (orange, mango, papaya) sweetened with *Stevia rebaudiana* (0.1-2.5%) after HPP treatment at 500/25ºC/5-15 min. An increase of total phenolics by 30% and 48% in green tea leaves and kiwifruit juice, respectively, was reported after HPP treatment at 500/25ºC/1-5 min (Jun et al., 2009; Xu et al., 2018). Similarly, HPP conditions of 500 MPa/25ºC/5-15 min resulted in an increase of total antioxidant activity up to 273% in papaya seeds (Briones-Labarca et al., 2015). Andres et al. (2016) reported 95% of ascorbic acid was retained in smoothies (orange, papaya, melon, carrot and skimmed milk) treated at 450 MPa/20ºC/3 min after 45 days of storage at 4ºC. Moreover, Santos et al. (2018) reported that the stability of total tannin in treated red wine at 500 MPa/20ºC/5 min was maintained after 5 months of storage at 25ºC. Therefore, HPP is considered a promising alternative to thermal processing. However, it is not possible to achieve inactivation of spores such

Bacillus subtilis and *Clostridium* and complete inactivation of high thermal resistant enzymes such as polyphenoloxidase (PPO) and peroxidase (POD) under HPP conditions (10-1000 MPa, <45ºC, short holding time) (Chourio et al., 2018, Huang et al., 2017, Barba et al., 2014, Garcia-Palazon et al., 2004). Pressure Assisted Thermal Processing (PATP) is an emerging technology with the potential to overcome limitations of HPP. Various studies have demonstrated retention of bioactive compounds after PATP treatment. Recently, Kovacevic et al. (2018) reported high levels of total phenolics of *Stevia rebaudiana* compared to untreated *Stevia* using pressurized hot water extraction at 10 MPa/160ºC/5 min. Increase of total phenolics up to 27% in mango pulp and 65% in pumpkin puree was obtained after PATP at 600 MPa/60ºC/15 min and 600 MPa/70ºC/1 min, respectively (Kaushik et al., 2016; Garcia-Parra et al., 2016). Pressure and thermal processing applied to food products also affect the content of individual phenolics such as chlorogenic acid, caffeic acid, quercetin, rutin, and catechins. Roldan-Marin et al. (2009) studied the effect of HPP and PATP at 400-600MPa/5-50ºC for 5 min on the quercetin content of onion samples, reporting that quercetin-3-glucoside increased by 26% and by 18% at 100MPa/5ºC/5 min and 100MPa/50ºC/5 min respectively, compared to untreated samples. Chen et al. (2015) studied the effect of HPP (200-600MPa/29ºC/10-20min) and thermal treatment (121ºC/3 min) on rutin content of asparagus juice, reporting a retention of rutin by 97% at 200MPa/29ºC/10 min and 86% after thermal treatment. Thus, studying the effects of food processing on the concentration of individual phenolics on food matrices and model system is important to ensure to consumers safety and healthy foods products with antioxidant properties. However, no studies have reported the effect of PATP on model system of chlorogenic acid, caffeic acid, rutin and quercetin.

Moreover, inactivation of spores and inactivation of thermal resistant enzymes including PPO and POD can be achieved under PATP conditions (10-600 MPa/40-120ºC/1-30 min) (Chourio et al., 2018). The PPO enzymatic reaction is associated with the oxidation of phenolic compounds

to quinones, leading to the formation of undesirable brown pigments and off-flavoured products. POD, one of the most thermostable enzymes, is responsible for the oxidation of bioactive compounds in the presence of hydrogen peroxidase generated during PPO enzymatic reactions, resulting in an off-flavor and changes in colour of the fruit, vegetables and beverages. Therefore, the inactivation of PPO and POD enzymes is highly desirable to preserve the quality of food products. Sulaima et al. (2015) reported 98% PPO inactivation in pears at 600 MPa/64ºC/15 min while 96% POD inactivation in blueberries was obtained at 400 MPa/90ºC/15 min (Terefe et al., 2017). Complete inactivation of PPO and POD resulted in treated coconut water at 400-600 MPa/90ºC/2 min (Chourio et al., 2018). Various studies have investigated the effect of HPP and PATP on bioactive compounds and relative activity of PPO and POD enzymes in different food matrices.

No studies have reported the effect of PATP on bioactive compounds of mate and mate sweetened with *Stevia*. Therefore, the aim of this research was to study the effect of PATP processing on phenolic compounds and antioxidant activity of mate, *Stevia*, and mate sweetened with *Stevia* and its effect on relative activity of peroxidase and polyphenol oxidase enzymes of *Stevia* leaves.

1.2 Hypothesis

- PATP treatments of mate, *Stevia* and mate sweetened with *Stevia* will influence the release of bioactive compounds and affect beverage antioxidant activity.
- PATP treatments will inactivate peroxidase (POD) and polyphenol oxidase (PPO) enzymes of *Stevia rebaudiana* leaves.
- Pressure and temperature will affect concentration of individual pure bioactive compounds, such as quercetin, rutin, chlorogenic acid and caffeic acid in model systems.

1.3 Thesis objectives

1.3.1 Main Objective

The main objective of this research was to study the effect of PATP processing conditions on bioactive compounds, and total antioxidant activity of mate, *Stevia rebaudiana* leaves, mate sweetened with *Stevia* leaves and mate sweetened with commercial *Stevia* powder.

1.3.2 Specific objectives

- Study the effect of PATP processing conditions (10-600 MPa/25-120ºC/1-5 min) on bioactive compounds (total phenolics and individual bioactive compounds such as catechins, quercetin, rutin, chlorogenic and caffeic acid), and total antioxidant activity of mate.
- Evaluate the effect of PATP (10-600 MPa/25-120ºC/1-5 min) on bioactive compounds (total phenolics and individual bioactive compounds) of *Stevia* and mate+*Stevia*.
- Study the effect of PATP (100-300MPa/25-75ºC/1-5 min) in model systems of pure quercetin, rutin, chlorogenic acid and caffeic acid in solvents.
- Investigate the effect of PATP (10-600MPa/75-120ºC/1-5 min) on the relative activity of peroxidase and polyphenol oxidase enzymes of *Stevia* leaves.

Chapter 2: Literature review

2.1 Mate *IIex paraguariensis*

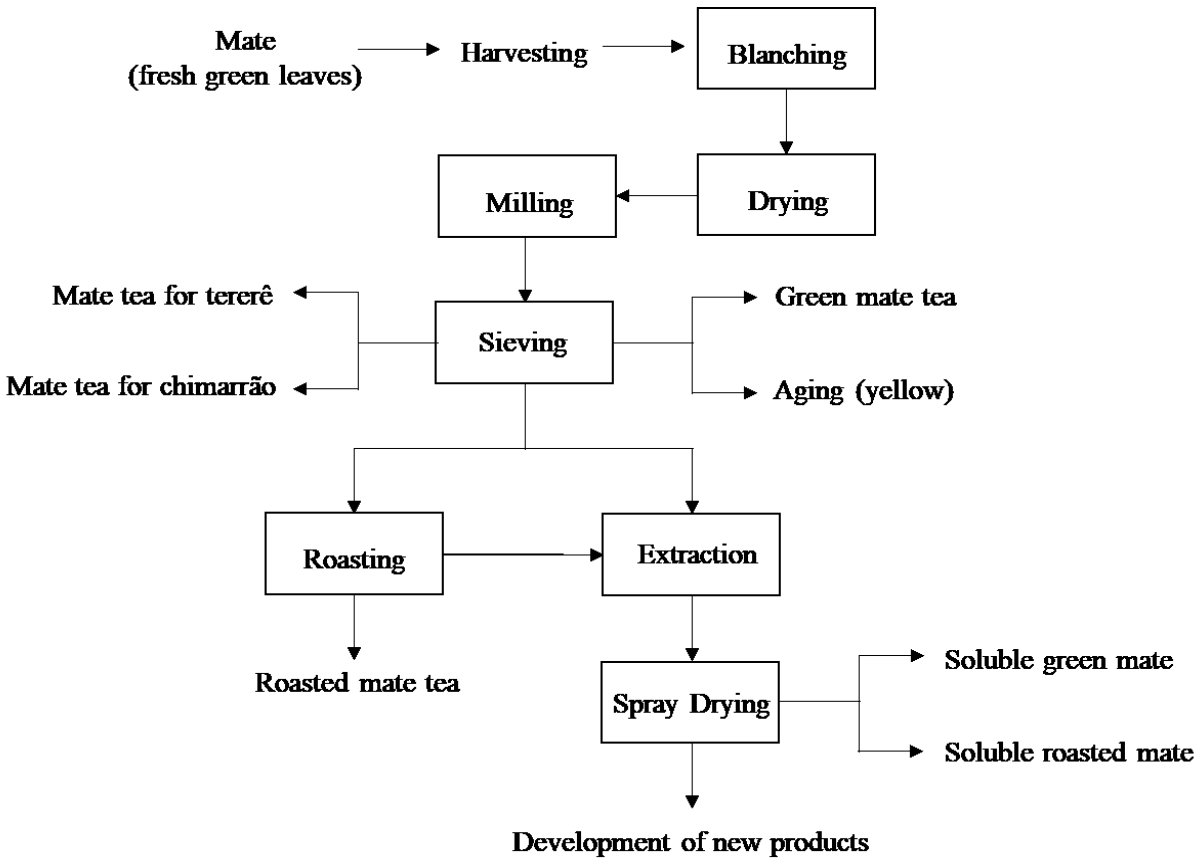
Mate *Ilex paraguarensis* is a plant native from the southern part of South America, specifically Argentina, Brazil, Uruguay and Paraguay. The harvest period of mate is between May and August, where leaves and stems are used in the preparation of commercial beverages, including chimarrão (dried green leaves brewed with hot water); tererê (dried green leaves brewed with cold water); mate tea (roasted leave with hot water); and mate extracts used in multiple applications in functional foods, cosmetics, and pharmaceuticals (Berte et al., 2014). To produce these different products, processing treatments such as blanching, drying, crushing, sieving, extraction and spray drying are used (Fig 2.1a).

The production of mate tea (roasted mate) consists in four main steps illustrated in Fig 2.1b; harvesting of leaves and stems (6 – 12 months), including bagging and transporting to the processing unit; blanching, in a rotating cylinder under the direct fire wood, to inactive oxidative enzymes at 500ºC for 10 s; drying using a rotating cylinder at 80-100ºC for 3 min and roasting at 120ºC for 15 min (Junior et al., 2016).

Dried mate leaves are highly consumed in Uruguay, Argentina, and Brazil with consumption per capita of 8-10 kg/year, 6.5 kg/year, and 3-5 kg/year, respectively (Berte et al., 2014). In the last decade, products based on mate have also been exported to Asia, USA, and Europe and their consumption has increased due to functional properties including antioxidant, stimulant, diuretic, hypocholesterolemic and anti-carcinogenic attributed to the content of caffeine, theobromine, polyphenols, minerals and saponins (Gulcinm et al., 2006). The amount of caffeine in mate leaves depend on raw material, processing, and preparation which might vary between 4.9 to 16.1 mg/g and for commercial products between 5.4 to 36.4 mg/g (Berte et al., 2014). The content of total phenolics of mate also depend on the type of product, varying from 79.1 to 100.3

mg/g (Cardozo et al., 2007; Berte et al., 2014; Da Silveira et al., 2016; Bastos et al., 2006). The predominant individual phenolic compounds on mate leaves and commercial beverages are mono-caffeoylquinic acids (3-O-caffeoylquinic acid, 5-O-caffeoylquinic acid and 4-O-caffeoylquinic acid), di- caffeoylquinic acids (3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid) and tri-caffeoyl quinic isomers, quercetin, rutin and kaempferol (Junior et al., 2016). Mate leaves contain 28.8 mg/mL of chlorogenic acid, 0.6-4.9 mg/g of rutin; 0.07-0.41 mg/mL of caffeic acid and 10.25 μg/mL of quercetin (Junior et al., 2016). Chimarrão infusion contains 5-caffeoylquinic acid (0.25 mg/mL); caffeic acid (0.021 mg/ mL); 3.4-dicaffeoylquinic acid (0.64 mg/mL); 3.5-dicaffeoylquinic acid (0.38 mg/mL); rutin (0.045 mg/mL); and 4.5-dicaffeoylquinic acid (0.18 mg/mL). Tererê infusion contains 5-caffeolqynic acid (0.04 mg/mL); caffeic acid (0.004 mg/mL); 3.4-dicaffeoylquinic acid (0.070 mg/mL); 3.5-dicaffeoylquinic acid (0.72 mg/mL); rutin (0.02 mg/mL); and 4.5-dicaffeoylquinic acid (0.02 mg/mL) (Da Silveira et al., 2016) (Table2.1). Several studies reported the extractability of bioactive compounds from mate leaves using different treatments, summarized in Table 2.2, including solid-liquid extraction, nanofiltration, supercritical CO_2, high pressure CO_2 and high pressure processing.

(a)



(b)

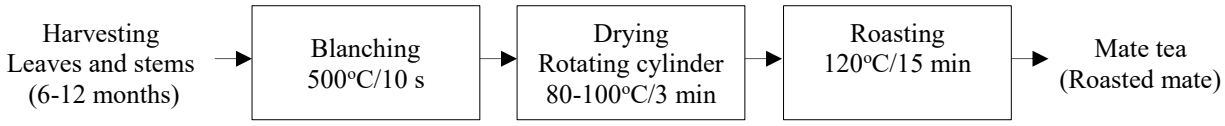


Figure 2.1 (a) Flow chart of processing and products from green mate leaves, and (b) Steps and processing conditions for roasted mate tea production
(Adapted from Berte et al., 2014 and Junior et al., 2016)

Table 2.1 Individual phenolic compounds on mate leaves and commercial beverages.

	Chlorogenic acid	3,4-Dicaffeoylquinic acid	3,5-Dicaffeoylquinic acid	4,5-Dicaffeoylquinic acid	Caffeic acid	Rutin	Quercetin	Reference
	(mg/mL)							
Mate leaves	28.8	NR	NR	NR	0.07-0.41	0.6-4.9	0.10	Junior et al. (2016)
Chimarrão	0.25	0.64	0.38	0.18	0.021	0.045	NR	Da Silveira et al. (2016)
Tererê	0.04	0.07	0.72	0.02	0.004	0.02	NR	

NR: Not reported

Table 2.2 Effect of treatment and extraction techniques of bioactive compounds from mate and *Stevia.*

Treatment/extraction	Results	Reference
***Ilex paraguariensis* leaves**		
Nanofiltration osmosis membrane 150-300 Da 0.3 MPa/24ºC/permeate flux 4.53 L/h.m^2	Increased total phenolic content (338%), chlorogenic acid (483%), theobromine (323%), caffeine (251%), condensed tannins (278%) and saponins (211%) in the concentrated mate extract compared to untreated mate.	Murakami et al. (2013)
Solid-liquid extraction assisted by hydrostatic pressure 0.01 – 0.33 MPa/ 25ºC/100 min	Increased efficiency of soluble matter extraction by hydrostatic pressure pulses at 0.33 MPa for 100 min.	Kotovicz et al. (2013)
High-pressure CO_2 extraction 25 MPa/40 °C/600 min	51 components, mainly esters, fatty acids, hydrocarbons, phytosterols, alcohols, xanthines and vitamin E identified on high-pressure mate tea leaves extracts.	Jacques et al. (2008)
Supercritical CO_2 40 MPa/40-70 °C/400 min 5.7 g min^{-1} of water-saturated supercritical CO_2	96% and 71% of initial caffeine content of mate leaves were extracted at 70 °C and 40 °C, respectively.	Saldaña et al. (2002)
Supercritical CO_2 25.5 MPa/70°C/420 min 0.9-1.2 g/min CO_2, 7 h.	94, 68, and 57% of initial caffeine, theobromine and theophylline extracted.	Saldaña et al. (1999)
***Ilex paraguariensis* fruit**		
Supercritical CO_2 15-25 MPa/35-55 ºC/ /360 min	Extraction of caffeine (163.28 mg/g); theobromine (2.45 mg/g); vitamin E (0.51 mg/g); stigmasterol (9.81 mg/g); total phenolics (9.25 mg GAE/100 g)	Palliga et al. (2016)

Table 2.2 Continued

Treatment/extraction	Results	Reference
Papaya, mango and orange juice mixture sweetened with *Stevia* leaves		
High pressure processing 300–500 MPa/ 25ºC/5–15 min	Increased of total phenolic content up to 22% and 18% on juice mixture with *Stevia* (1.25% w/v) and *Stevia* (2.5% w/v), respectively. ORAC and TEAC increased as a function of *Stevia* concentration, independently of HPP conditions.	Carbonell-Capella et al. (2013)
High pressure processing 300 – 500 MPa/18–22 °C/5-15 min	Significant increase of total phenolics and antioxidant capacity at *Stevia* concentration of 2.5% (w/v) and 453 MPa for 5 min.	Barba et al. (2014)
***Stevia rebaudiana* leaves**		
Pulse electric fields (PEF) 20–40 kV/cm, 100–360 μs	Significant increase of rebaudioside and stevioside contents after treatment at 20 kV/cm for 360 μs compared to untreated sample	Buniowska et al. (2016)
Subcritical water extraction 23 MPa/100-150 °C/30-90 min flow rate (4-6 mL/min)	Increase of stevioside (from 26.94 to 38.67 mg stevioside/g) and rebaudioside A (from 24.65 to 35.68 mg rebaudioside A/g) yields with increase on temperature from 100 °C to 125 °C. The maximum rebaudioside A and stevioside contents were obtained at 125 °C for 45min.	Yildiz-Ozturk et al. (2014)

Table 2.3 Structure and physicochemical properties of bioactive compounds in mate and *Stevia.*

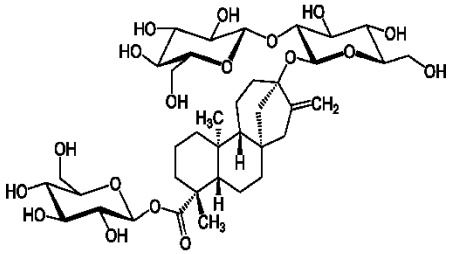
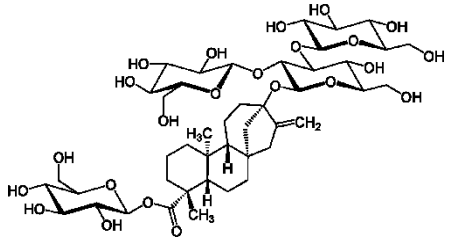
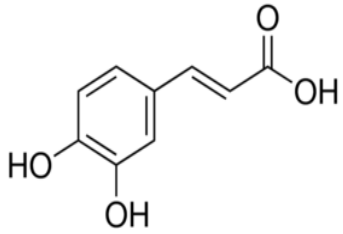
Bioactive compound	Structure	Molecular weight (g/mol)	Melting point (°C)	Solubility	Reference
Stevioside		804.88	198	4.51 mg/mL	Gasmalla et al. (2014)
Rebaudioside A		967.01	210-215	14.4 mg/mL	Gasmalla et al. (2014)
Caffeic acid		180.16	225	Water solubility < 1 mg/mL	Medina et al. (2012)

Table 2.3 Continued.

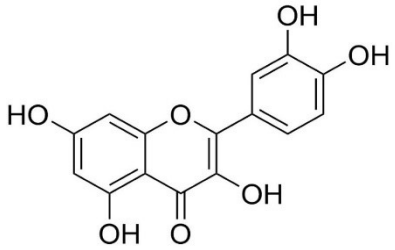
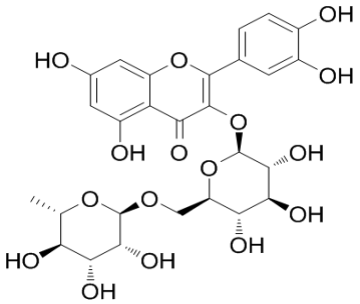
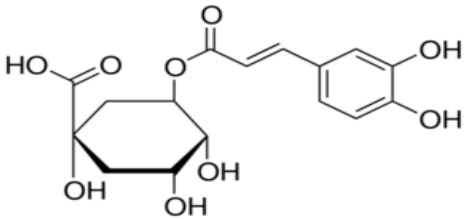
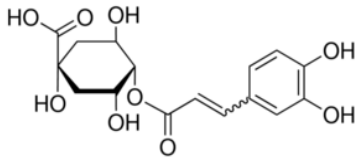
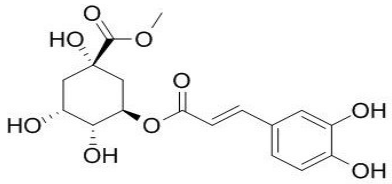
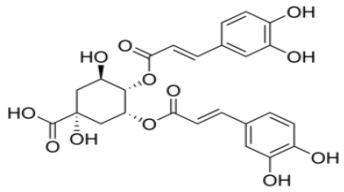
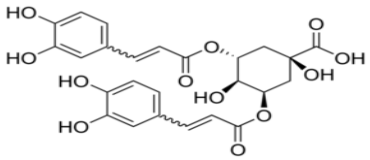
Bioactive compound	Structure	Molecular weight (g/mol)	Melting point (°C)	Solubility	Reference
Quercetin		302.2	316	water solubility 0.024 mg/mL at 25 °C ethanol solubility 4 mg/mL, 37°C	Butiuk et al. (2016) Wang et al. (2016)
Rutin		610.5	242	water solubility 0.125 mg/mL at 25°C	Butiuk et al. (2016)
Chlorogenic acid (5-O-Caffeoylquinic acid)		354.31	207-209	water solubility 25 mg/mL at 25°C	Butiuk et al. (2016)

Table 2.3 Continued.

Bioactive compound	Structure	Molecular weight (g/mol)	Melting point (°C)	Solubility	Reference
4-O-Caffeoylquinic acid		354.31	207-209	water solubility 25 mg/mL at 25°C	Butiuk et al. (2016)
3-O-Caffeoylquinic acid		354.31	207-209	water solubility 25 mg/mL at 25°C	Butiuk et al. (2016)
4,5-Dicaffeoylquinic acid		516.46	234 - 238	-	Da Silveira et al. (2016)
3,5-Dicaffeoylquinic acid		516.46	234 - 238	-	Da Silveira et al. (2016)

2.2 *Stevia rebaudiana Bertoni*

Stevia rebaudiana Bertoni is a plant originally from Paraguay that belongs to the Asteraceae or Chrysanthemum family. *Stevia* is a non-caloric sweetener, 300 times sweeter than sucrose, which main compounds are steviol glycosides, such as stevioside (81.2 mg/g), rebaudioside C (3.8 mg/g), rebaudioside A (3.5 mg/g) and dulcoside A (2.8 mg/g) (Barba et al., 2012; Salazar et al., 2018; Periche et al., 2015). The chemical structure of stevioside and rebaudioside are shown in Table 2.3. *Stevia* has gained interest among consumers and food producers around the world as a healthy, natural, and low-calorie sweetener. Studies have demonstrated the promising potential of *Stevia* on preventing chronic diseases, including anti-hyperglycemic, anti-hypertension, diabetes and anti-cancer, mainly attributed to the presence of antioxidant compounds such as folic acid (52.18 mg 100 g^{-1}), vitamin C (14.98 mg 100 g^{-1}), B-carotene, dulcoside, rabaudioside and riboflavin. *Stevia* is also considered an excellent source of essential minerals (potassium, calcium, magnesium, sodium) and indispensable amino acids (lysine 0.70 g 100 g^{-1} and leucine 0.98 g 100 g^{-1} (Lemus-Mondaca et al., 2012). Varieties of *Stevia rebaudiana* include *Stevia* UEM-320 and *Stevia* UEM-13, which contain rebaudioside A as the main glycose (Dacome et al., 2005). However, *Stevia rebaudiana* Bertoni is still the most used variety due its high content of stevioside and phenolics with health benefits.

2.2.1 Stability of *Stevia rebaudiana*

Food preservation techniques are applied to *Stevia* leaves to prevent microbial growth, to extend shelf-life and facilitate storage and transportation conditions. Drying methods are commonly used to ensure safety and quality requirements of *Stevia* leaves and commercial products. Studies have reported that drying methods can impact the physicochemical and sensorial properties of the natural sweetener. Periche et al. (2015) studied the effect of different drying

methods, hot air drying (100-180 ºC/3 min), freeze drying ($9.5x10^{-1}$ mmHg for 24 h) and shade drying (20ºC for 30 days) on steviol glycosides (stevioside, dulcoside A, rebaudioside A and rebaudioside C) and antioxidant activity of *Stevia rebaudiana*; reporting that all drying methods caused a significant reduction of stevioside (from 81.2±9.3 to 35±8 mg/g)and rebaudioside A (from 3.5±0.3 mg/g to 0.5±0.14 mg/g) compared to fresh *Stevia* leaves. Contrary, the concentration of dulcoside A experimented an increase by 87% and 80% compared to the fresh sample after shade and freeze drying, respectively. The antioxidant activity of treated *Stevia* significantly increased after hot air drying at 180 ºC/3 min that was attributed to the matrix changes occurred during drying, resulting in an extractability of phenolic compounds. Nowicka et al. (2016) investigated the effect of steviol glycosides (0.2% of 98.5% steviol glycosides) and sucrose addition (7%; crystal form) on the stability of phenolic compounds including anthocyanin, flavan-3-ol, flavanol, and phenolic acid of sour cherry puree after processing (100ºC/10 min) and during storage for 6 months at 4ºC and 30ºC. Nowicka et al. (2016) found that the addition of *Stevia*, reduced 6% anthocyanins while the addition of sucrose reduced 34.8% of total anthocyanins content of sour cherry pure due to a possible intermolecular interaction of oligomers to cell wall polysaccharides. After 6 months of storage at 4ºC, the reduction of total anthocyanins of sucrose and *Stevia* addition were 57.4% and 17.2% respectively. After processing at 100ºC for 10 min and storage for 6 months at 4ºC. The addition of *Stevia* in sour cherry puree samples retained up to 96.6% of total flavan-3-ols. Perez-Ramirez et al. (2015) also investigated the effect of *Stevia* and citric acid on the stability of phenolic compounds of a roselle beverage, a plant with antioxidant, ant inflammatory and antidiabetic properties. They reported that roselle beverage sweetened with *Stevia* (14 g/L) and citric acid (0.2 g/L) had a higher concentration of polyphenols (76-80%) compared to the control after storage at 50ºC for 12 days due to bioactive compounds and

antioxidant activity of *Stevia*. The addition of *Stevia*, and *Stevia* + citric acid increased the stability of gallic acid, epigallocatechin gallate, rosmarinic acid, quercetin and anthocyanins of roselle beverage compared to the control after storage at 50ºC for 12 days. It demonstrates that the addition of *Stevia* can provide stability of polyphenols compounds in sour cherry pure.

Korir et al. (2014) also investigated the effect of *Stevia* addition on the antioxidant activity of black and green teas by DPPH radical-scavenging activity method. They found that the addition of 0.1 and 0.3 g *Stevia*/100 mL had no significant influence on the antioxidant activity of teas while sucrose addition (3, and 10 g sugar /100 mL) significantly decreased antioxidant activity of tea beverages compared to the control.

Several studies have evaluated the impact of processing on *Stevia* leaves and products sweetened with *Stevia*, which are shown in Table 2.2. High Pressure Processing (HPP), a non-thermal technology, has been evaluated as a potential alternative to conventional thermal processing due to remarkable benefits in retaining high levels of bioactive compounds and reducing microorganisms, while extending product shelf-life. Carbonell-Capella et al. (2013) investigated the effect of HPP at 300-500 MPa/25ºC for 5-15 min on physicochemical properties (browning index), and antioxidant activity of a fruit juice mixture (papaya, mango, orange) sweetened with *Stevia.* They showed an increase up to 91% on total antioxidant capacity with the addition of *Stevia* (1.25-2.5%), independently of the treatment conditions tested. Higher values were reported on total antioxidant capacity measured with ORAC (22.2-38.8 mM Trolox) than TEAC (17.9 -26.5 mM Trolox) due to the transfer of hydrogen atoms present in *Stevia* in which ORAC reaction is based.

Barba et al. (2014) evaluated the effects of HPP treatment (300-500 MPa/18-22 ºC/5-15 min) combined with *Stevia* leaves addition (0-0.25% w/v) on total phenolic content, and total

antioxidant capacity of fruit extract, orange, mango, and papaya, reporting significantly increase of total phenolics by 95% and antioxidant activity by 88% at 453 MPa/5 min with *Stevia* addition of 2.5% (w/v) compared to untreated sample due to the presence of phenolics and antioxidant activity of *Stevia*.

Pulsed electric field (PEF) has also emerged as a non-thermal technology with remarkable benefits to retain nutritional and quality characteristics of food product by exposing them to short high-voltage pulses. Buniowska et al. (2016) reported the effects of PEF processing on steviol glycosides, rebaudioside A, stevioside, rebaudioside F, and rebaudioside C of juice beverage sweetened with *Stevia*. They indicated that PEF did not affect the rebaudioside A and stevioside concentration with exception of 20 kV/cm-360 μs and 30 kV/cm-230 μs which resulted in higher concentrations of rebaudioside A and stevioside compared to the control.

2.2.2 Effect of *Stevia* addition on color and sensorial properties

Several studies have shown the effect of sucrose substitution with *Stevia rebaudiana* on the color of the final product. Cadena et al. (2013) reported that lightness values (L^*) increased with the addition of 0.052% of *stevia* in mango nectar. Similarly, Basu et al. (2013) reported that the substitution of sucrose (50%) by *Stevia rebaudiana* increased L^* values in mango jam products. However, Aiddo et al. (2015) indicated that the addition of 0.24 % w/w *Stevia rebaudiana* produced a decrease of lightness in dark chocolate. A decreasing of lightness was also observed in fruit juice mixture and sour cherry pure with the addition of 2.50% w/v and 0.2% w/w *Stevia,* respectively (Carbonell-Capella et al., 2013; Nowicka et al., 2016). The redness values were not affected by *Stevia* addition on mango nectar and mango jam while yellowness significantly increased on *Stevia*-mango jam (Basu et al., 2013; Cadena et al., 2013). Nowicka et al. (2016) reported a decrease in redness values of sour cherry puree sweetened with *Stevia* (0.2% w/w) after

6 months of storage at 25ºC compared to the control. Shah et al. (2010) reported that the addition of *Stevia rebaudiana* with bulking agents (dextrose and inulin) significantly decreased L*, a*, and b* values compared to the sucrose low-fat yogurt samples. Studies demonstrated that the effect of *Stevia rebaudiana* addition on color mainly depends on food matrix, *Stevia* concentration and processing conditions used.

Maintaining acceptable levels of sensorial properties when substituting sucrose with intense sweeteners in food products is one of the major challenges in the development of non-caloric sugar food products. Studies have reported that the addition of *Stevia* can lead to an aftertaste, a bitter taste and to a decrease in overall quality of the final food product. Belščak-Cvitanović et al. (2015) studied different sensorial attributes (color, gloss, surface, breakage, structure, melting, odour, taste, mouthfeel, aftertaste, sweetness, astringency, bitterness, herbal and overall acceptability) of sugar free chocolates using a mixture of fructose, isomalt, *Stevia* leaves, oligofructose, lucuma, agave syrup and peppermint. The results showed that the formulated chocolate containing peppermint and *Stevia* leaves had the highest overall acceptability but also the highest bitterness and astringency compared to chocolate samples without *Stevia*. Cadena et al. (2013) studied the effect of 0.052% of *Stevia* with 97% rebaudioside on sensory profile of mango nectar. Samples formulated with *Stevia* showed great intensity of residual sweetness and bitterness compared to sucrose formulated mango nectar. However, after 120 days of storage at 25ºC, samples sweetened with *Stevia* had the greatest number of attributes including brightness, mango aroma and flavor, sweet aroma and taste compared to the samples sweetened with sucrose. Guggisberg et al. (2011) reported the effects of sucrose substitution with *Stevia* (8%) and Actilight™ (2-6%) plus *Stevia*, on sensorial attributes of low-fat yogurt. This study reported that the addition of *Stevia* showed significantly lower sweetness compared to sucrose samples while bitterness and off-flavor were

significantly higher in the sample containing *Stevia* compared to the control. Therefore, a combination of Antilight^{TM} and *Stevia* was recommended to minimize bitterness and off-flavor in free-sucrose low fat yogurt.

2.3 Bioactive compounds in model systems

Bioactive compounds are chemical compounds present in plant and foods that provide health benefits in the human body to prevent the risk of chronic diseases like cancer (Wang et al., 2015). Bioactive compounds are present especially in functional foods. The definition of functional foods according to Health Canada (2002) states that "A functional food is similar in appearance to, or may be, a conventional food, is consumed as a part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions". Examples of bioactive compounds include polyphenols, carotenoids, dietary fibre, fatty acids, plant sterols, prebiotics/probiotics, soy phytoestrogens, vitamins and minerals (Health Canada, 2002). Caffeic acid, chlorogenic acid, rutin and quercetin are also bioactive compounds found in foods and beverages like mate tea.

2.3.1 Caffeic acid

Caffeic acid ($C_9H_8O_4$), 3,4-hydroxy cinnamic acid, is a phenolic acid with a structure of hydroxy cinnamic and a catechol group, which is widely found in various plants, including *IIex paraguariensis, Stevia rebaudiana, polygonum aviculare,* coffee beans, and potatoes (Saldaña et al., 2002; Ji et al., 2016). It has been extensively studied due to its potent antioxidant capacity and health benefits, such as vasodilatory (Prince et al., 2012), antibacterial (Reinders et al., 2001; Sardi et al., 2016), anti-mutagenesis (Karekar et al., 2000), and anticancer properties (Hudson et al., 2000; Soleas et al., 2002). The catechol group in caffeic acid structure is responsible for the interaction with various types of oxidant radicals (Medina et al., 2012). Therefore, caffeic acid has

exhibited effective *in vitro* antioxidant activity, such as 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 (Gulcin, 2006). In addition, caffeic acid has the strongest and more effective antiradical activity compared with other phenolic acids like ferulic or coumaric acids found in plants (David et al., 2015).

Several studies have shown that the amount of caffeic acid can be affected by processing methods such as pulsed electric field (PEF), thermal pasteurization and high pressure homogenization (Table 2.4). Agcam et al. (2014) investigated the effect of PEF (17.06 kV cm^{-1}, 1206.2 μS) and conventional thermal pasteurisation (90 ºC for 20 s) on caffeic acid of citrus juice, reporting that the concentrations of PEF-caffeic acid and thermal pasteurization- caffeic acid samples were 14% and 20% higher than the untreated samples. Similarly, the concentration of caffeic acid in red beet increased up to 38% during the 5 min-roasting process, up to 30% in tomato sauce after thermal processing (65 ºC, 2 h), up to 25% in tomato sauce after thermal processing (85-95 ºC, 20 min), and up to 360% in non-enzymatic berry juice after pasteurization (95 ºC, 1-10min) (Ravichandran et al., 2012; Re et al., 2002; Kelebek et al., 2017; Makila et al., 2017). However, Ravichandran et al. (2012) observed a decrease up to 40% of caffeic acid on red beet after microwave treatment compared with the control (1800 W, 30 s). Similarly, Yao et al. (2011) found that blanching at 96-98 ºC for 3 min and boiling at 70 ºC for 90 s reduced the levels of phenolic acids up to 35% on Shengjie celery. Friedman et al. (2000) investigated the effect of pH on the stability of caffeic acid, reporting unstable at high pH of 10-11 where the spectrum of caffeic acid changed dramatically under these conditions. However, these changes on the spectrum of

caffeic acid are irreversible when the pH is reduced from 10 to 7. Another phenolic acid widely available in plants is chlorogenic acid.

2.3.2 Chlorogenic acid

Chlorogenic acid ($C_{16}H_{18}O_9$), 5-caffeoylquinic acid (5-CQA), refers to a polyphenol family of esters formed between caffeic acid and the 3-hydroxyl position of L-quinic acid (Clifford et al., 1999). Chlorogenic acid is found in *Stevia rebaudiana Bertoni* leaves, sweet potato and potato, being a major component of coffee (6-10% w/w) and *Ilex paraguariensis* leaves (8-10% w/w) (Narita et al., 2013; Butiuk et al., 2016).

Epidemiological studies have demonstrated that the consumption of chlorogenic acid (100 mg/kg/day) decreases blood pressure, oxidative damage in the brain, and prevents chronic diseases, including, diabetes, bacterial infections, inflammation and cancer (Tajik et al., 2017). However, chlorogenic acid can be affected by processing methods. Butiuk et al. (2016) investigated the content of chlorogenic acid and its isomeric compounds (3-O-caffeoylquinic acid, 3-CQA and 4-O-caffeoylquinic acid, 4-CQA) of *IIex paraguariensis* leaves (mate) along the different processing steps including roasting (250-550 ºC, 2-4 min), and drying (100 ºC/8-24 h). They reported that roasting and drying processes had no significant effect on the content of 3-CQA, 4-CQA and 5-CQA isomers of mate leaves. Also, Isolabella et al. (2010) reported variation of 3-CQA, 4-CQA and 5-CQA during mate processing, with an increase of these compounds during roasting process was obtained followed by a decrease during the drying process. They suggested that temperature conditions (250-550 ºC, 2-4 min) during the roasting process might have released caffeine which combined with caffeoylquinic components induced an increase of caffeoylquinic acid. Narita et al. (2013) studied the degradation of 5-CQA at pH 5-9, reporting degradation of 5-CQA was time and pH dependent. The isomeric compounds of caffeic acid, 4-

CQA and 3-CQA were produced after 5-CQA was incubated at 37°C and pH of 6 - 9. At pH of 5 - 6.5, the amount of 3-CQA and 4-CQA increased from 0 to 0.4 depending on the increase of incubation time from 0 to 200 h.

Table 2.4 Effect of processing on caffeic acid and chlorogenic acid content.

Treatment	Sample	Results	Reference
Caffeic acid			
Pulse electric field 17.06 kV cm^{-1},1206 µS	Citrus juice	Concentration of caffeic acid was significantly higher (5.62 mg/L) than untreated the samples (4.84 mg/L)	Agcam et al. (2014)
Thermal pasteurization 90 ºC/0.33 min	Citrus juice	Caffeic acid content increased up to 25% compared with untreated samples	Agcam et al. (2014)
Thermal processing 65 ºC/120 min	Tomato sauce	Concentration of caffeic acid increased up to 30%	Re et al. (2002)
Pasteurization 95 ºC/1-10 min	Berry juice	Caffeic acid increased up to 360% in non-enzymatic berry juice after pasteurization	Makila et al. (2017)
Chlorogenic acid			
Roasting 250-550 ºC/2-4 min drying 100 ºC/8-24 h	Mate tea	No significant effect on the content of 3-CQA, 4-CQA and 5-CQA isomers	Butiuk et al. (2016)
Incubation 37ºC/pH 6 - 9. 24 h	Chlorogenic acid	4-CQA and 3-CQA were produced after incubation at 37ºC and pH 6 - 9. At pH 5 - 6.5, the amount of 3-CQA and 4-CQA increased from 0 to 0.4.	Narita et al. (2010)

2.3.3 Quercetin

Quercetin (3,5,7-trihydroxy-2-3,4-dihydroxyphenyl) is a natural flavonoid found mainly in vegetables and fruits. Among the vegetables and fruits with high quercetin content are red onion (39 mg/g), cranberry (15 mg/g) and blueberry (8 mg/g) (Lesjak et al., 2018). The daily intake of flavonoids in a typical diet is 23-34 mg/d, being quercetin the major source of these flavonoids (Chen et al., 2015). The amount of quercetin intake in the Western diet is approximately 15 mg per day (Lesjak et al., 2018).

Quercetin has demonstrated potential in the treatment and prevention of different types of cancer cells, including colorectal, prostate, liver, pancreatic and lung cancer as well as obesity, inflammations, viral and bacterial diseases attributed to its strong antioxidant capacity (Lee, et al., 2011; Kim et al., 2013; Kim et al., 2015; Shan et al., 2009). Quercetin is a lipophilic compound with a molecular weight of 448.38 g/mol, melting point of 316 ºC, low water solubility (0.024 mg/mL, 25 ºC), moderate ethanol solubility (4 mg/mL, 37ºC) and high dimethyl sulfoxide solubility (150 mg/mL, 25 ºC) (Wang et al., 2016). Quercetin is bounded to sugars, ethers or phenolic acids (Wang et al., 2016).

Several studies have investigated the stability of quercetin during food processing storage which can be influenced by pH, temperature, metal ions, and food matrix. Wang et al. (2016) indicated that degradation of quercetin could be minimized avoiding high oxygen exposure, high temperature (>121 ºC), and alkaline conditions (pH>8) during processing and storage. The effect of food processing and storage on chemical stability of quercetin are summarized on Table 2.5.

Table 2.5 Effect of food processing and storage on chemical stability of quercetin.

Treatment	Sample	Results	Reference
0.1 MPa/100ºC/60 min	Onions (*Allium cepa*)	43% Degradation of quercetin	Makris and Rossiter (2001)
0.1 MPa/97ºC/240 min pH 8 with and without oxygen		100% Degradation of quercetin (O_2 conditions), 15% degradation of quercetin (no O_2)	Makris and Rossiter (2000)
Storage at 20ºC for 168 days		100% Degradation of quercetin after 168 days storage	Price et al. (1997)
High pressure processing (HPP) 100-400 MPa/5-50ºC/ 5 min		Quercetin-3-glucoside, and quercetin-3,4-diglucoside increased up to 26% at 100 MPa/5ºC/5 min and up to 18% at 100 MPa/50ºC, 5 min	Roldan-Marin et al. (2009)
Thermal treatment 0.1 MPa/100-121ºC/ 119 min	Bean *(Phaseolus vulgaris L.)*	>70% Degradation of quercetin	Ranilla et al. (2009)

Table 2.5 Continued.

Treatment	Sample	Results	Reference
Treatment with different pH values (2.7,7, and 10) for 96 h	Quercetin solution (5μM)	100% Degradation of quercetin after 120 min at pH 10, very stable under acidic condition (pH 2.7)	Moon et al. (2008)
Storage at 20ºC for 180 days	Raspberry (*Rubus idaeus*) jam	40% Degradation of quercetin	Zafrilla et al. (2001)
Thermal pasteurization 80ºC/91 s	Grapefruit juice	17% Degradation of quercetin	Igual et al. (2011)

2.3.4 Rutin

Rutin (quercetin-3-O-rutinoside) is a flavonol glycoside, commonly found in different parts of the plant such as seeds, fruit skin, leaves, flowers, and roots. Rutin is the form of quercetin glycoside with a molecular weight of 610.52 g/mol, melting point of 195ºC and low water solubility (0.13 g/L, 25 ºC) (Gullon et al., 2017). The chemical structure of rutin is shown in Table 2.3. Epidemiological studies have demonstrated the health benefits of rutin including anti-inflammatory effects, prevention of vascular and cardiac diseases and cancer (Gunawardena et al., 2014; Murakami et al., 2014). The content of rutin has been reported in different plant sources, being buckwheat *Fagopyrum sculentum Moench* hulls one of the most important source of rutin (3250 mg/100 g d.b) (Glavac et al., 2017). Rutin can be also found in grapes (*Vitis vinifera L*) (1592 mg/100 g d.b), asparagus (*Asparagus officinalis L*) (2900 mg/100 g d.b), Amaranthus hybrid leaves (*Amaranthus cruentus*), apple peel (*Malus pumila L*.) (800 mg/100 g d.b) and skin onions (*Allium cepa L*) (6 mg/100 g d.b). Rutin content in food product can be affected by processing conditions. Murakami et al. (2004) investigated the effect of heat (100ºC) on rutin samples, founding stability of rutin under these conditions. Makris and Rossiter (2000) also studied the effect of heat-induced on the degradation of quercetin and rutin in aqueous-model, reporting that rutin had more stability compared to quercetin.

Several studies have reported different methods of rutin extraction such as solvent extraction, ultrasound, microwave and infrared assisted solvent extraction, pressurized liquid extraction and supercritical fluid extraction. The table 2.6 summarizes some of studies on the extraction of rutin.

Overall, polyphenols such as quercetin, chlorogenic acid, caffeic acid and rutin are sensitive to temperature (>120ºC), oxygen, changes of pH and light (Grujic et al., 2012; Gullo et

al., 2017; Patras et al., 2009; Santos et al., 2018). Studies have shown that quercetin can be affected by storage time and temperature (Table 2.5). Igual et al. (2011) investigated the changes of quercetin in pasteurized grapefruit juice at 80ºC/11 s during storage in the dark for 25 days, reporting that quercetin concentration decreased by 28% and 38% after refrigeration at 4ºC and frozen conditions at -18ºC, respectively. Also, the stability of tea catechins have been studied. Catechin stability was influenced by oxygen concentration, pH, storage time and temperature (Chen et al., 2001; Wang et al., 2006; Su et al., 2003). Su et al. (2003) reported that green tea catechins decreased by 50% after 1 month of storage at 25ºC. Although polyphenols found in foods and beverages might have health benefits, processing and storage conditions should be considered to minimize their degradation.

Table 2.6 Yield of rutin using different extraction methods.

Treatment	Sample	Yield of rutin	Reference
Supercritical CO_2+ethanol (1-10 g/min) 60 min/50°C/20 MPa	Pecah kaca (*Strobilanthes crispus*)	8.47 mg/g samples, d.b	Liza et al. (2010)
Supercritical CO_2+ethanol 60 min/60°C/20 MPa 70% ethanol	*Mentha spicate* leaves	0.15 mg/g, d.b	Bimakr et al. (2011)
Supercritical CO_2 ethanol:water (1:1 v/v). 10 min/65°C/15 MPa	*Asparagus officinalis*	2.28 mg/g, d.b	Solana et al. (2015)
High pressure extraction, 50% ethanol 10 min/65°C/100 MPa	*Asparagus officinalis*	2.7 mg/g, d.b	Solana et al. (2015)
Solid-liquid extraction (1:10 w/v), 40 min/57°C, 75% ethanol	*Morinda citrifolia* fruit	1.21 mg/g	Thoo et al. (2013)

Table 2.6 Continued.

Treatment	Sample	Yield of rutin	Reference
High pressure extraction, 50 % ethanol 20 min/188ºC/100 MPa	*Amaranthus paniculatus*	14.3 mg/g, d.b	Kraujalis et al. (2015)
Microwave extraction, 40kHz/400 W/70ºC/1min 70% methanol	*Forsythia suspensa*	1.73 mg/g	Fang et al. (2013)
Ultrasound assisted extraction, 60-62 kHz/150 W/20ºC/ 15 min 70% ethanol	*Sophora japonica* olives	182.25 mg/g	Deng et al. (2017)

2.4 High Pressure Processing (HPP)

High pressure processing (HPP), also known as high isostatic or high hydrostatic pressure, is a promising non-thermal technology used to maintain high nutritional food product, extend shelf-life and reduce damaged to heat-sensitive food compounds. HPP operates with pressures up to 1000 MPa and temperatures < 45ºC. The potential of HPP to reduce bacterial growth was reported for the first time in 1895 by Royer. In 1899, HPP was used by Hite and coworkers at the University of West Virginia, USA for milk preservation. In 1914, Percy W. Bridgman reported the coagulation of egg white using HPP. However, it was not until 1992 that the first HPP commercial products, jams, fruit jellies and sauces, were released by Meidiya Food Company in Japan (Tanaka and Kunugi, 1995; Balasubramaniam et al., 2015). In the last decade, HPP has achieved a gradual growth worldwide, mainly in North America, Europe and Asia. The major HPP manufactures in the world are Avure (Middletown, OH, USA), Hiperbaric (Burgos, Spain), Multivac (Germany) and Baotou Kefa High Pressure Technology Co. Ltd. (Baotou, China). Fig. 2.2 shows the global market trend of HPP food products. In 2017, the global market of HPP food products reached approximately US$18.2 billion and it is expected to reach a market value of US$54.8 billion in 2025 (Huang et al., 2017).

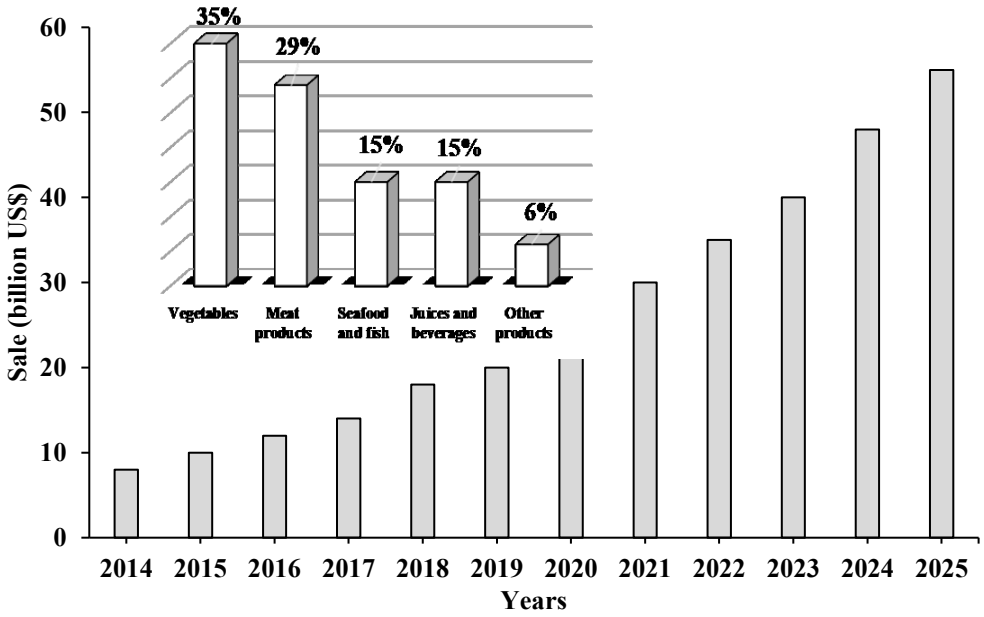


Figure 2.2 Global trend of HPP treated food market for 2014-2025 (Adapted from Campus, 2010 and Huang et al., 2017)

2.4.1 Principles of high pressure processing (HPP)

High pressure processing is mainly based on Le Chatelier's principle, isostatic principle and adiabatic heating. Le Chatelier's principle states that when a system in equilibrium is disturbed by pressure, the system is readjusted, and a new equilibrium is established. Therefore, any phenomenon such as phase transition, change in molecular configuration, or chemical reaction combined to a decrease in volume, is enhanced to counteract the effect of pressure. The increase in pressure and reduction in volume cause an equilibrium change to bond formation and breaking of ionic bonds. Hydrogen bonds and van der Waals forces are distance-dependent and consequently greatly affected by pressure. HPP can also disrupt large molecules and microbial cell structure such as enzymes, cell membranes, proteins and lipids. However, pressure does not affect covalent bonds so small molecules such as vitamin and flavor components are unaffected (Balasubramaniam et al., 2015; Koutchma, 2014). Isostatic principle states that during HPP, packaged food product experiences isostatic compression by the pressure-transmitting fluid. The

pressure is instantaneously and uniformly applied in all directions regardless of structure, shape and size of the foods (Balasubramaniam et al., 2015).

During compression and decompression, food products are exposed to adiabatic heating. The temperature increase of the food components is dictated by the volumetric expansion coefficient, isobaric heat capacity of the materials, and the initial temperature. Temperature change of some foods during HPP per 100 MPa is shown in Table 2.7. Food components with high moisture content (polar components) such as juice, sauce, milk, and fish, experienced temperature increase up to 3ºC while food components with significant content of nonpolar components such as mayonnaise, beef fat, and olive oil experimented temperature increase up to 9.1 ºC (Koutchma, 2014). The combination of pressure-temperature alters interatomic distances, degree of ordering of molecules, changes on reaction rates and bonding interactions of food components. Those interactions result in changes in physical properties including melting points, solubility, density, and viscosity, and affect equilibrium processes such as dissociation of weak acid, acid-base equilibrium and ionization (Balasubramaniam et al., 2015).

Table 2.7 Temperature increase during compression heating (ºC /100 MPa).

Food Material	Temperature Increase (ºC/100 MPa)
Water	2.6-2.8
Orange juice, tomato sauce, skim milk	2.6-3.0
Carbohydrates	2.6-3.6
Proteins	2.7-3.3
Salmon fish	2.8-3.0
Mayonnaise	5.0-7.2
Linolenic acid	5.9-9.0
Olive oil	6.3-8.7
Soybean oil	6.3-8.3
Extracted beef fat	6.2-9.1

(Koutchma et al., 2014; Rasanayagam et al., 2003)

2.4.2 Effect of high pressure processing on bioactive compounds

HPP has been shown to enhance or maintain bioactive compounds of food. Several studies on the effect of HPP on bioactive compounds including total anthocyanins, flavonoids, phenolics, antioxidant activity, tannins, and individual phenolic compounds have been investigated as shown in Table 2.8. Corrales et al. (2008) investigated the effect of HPP-temperature (600 MPa/70ºC/60 min) on grape by-products, reporting that HPP increased total anthocyanins by 41%, due to disruption of hydrophobic bonds that led to a higher permeability and increase of total anthocyanin compared to the control. Carbonell-Capella et al. (2013), investigated the effect of HPP (500MPa/25ºC/5-15 min) on total anthocyanin of fruit juice mixture sweetened with *Stevia rebaudiana* (0-2.5% w/v), reporting the highest anthocyanin content *Stevia rebaudiana* (2.5% w/v). They attributed this behavior to a possible inactivation of enzymes, responsible of anthocyanin degradation, during HPP treatment.

Jun et al. (2009) investigated the effect of high pressure and holding time on the extraction yield of polyphenols of green tea leaves, reporting polyphenols extraction yield of 30% at 500MPa/25ºC/1 min. They indicated that the equilibrium of solvent concentration was achieved at 1 min, due to the uniformly and instantaneously pressure transferred to the food material. Xu et al. (2018) studied the effect of HPP (500 MPa/25ºC/5 min) on total phenolics content of Se-enriched kiwifruit juice. They found that HPP increased total phenolics of the juices by 51.6%. Jez et al. (2018) investigated the effect of HPP (450-550 MPa/25ºC/5-15 min) on total antioxidant activity of tomato *(var.* Maliniak) puree. They reported that total antioxidant activity at 450 MPa/5-15 min was enhanced by 48%. However, a decrease in total antioxidant activity was observed at 550 MPa/15 min due to irregular changes on the concentration of individual phenolics. Briones-Labarca et al. (2015) studied the effect of HPP on total antioxidant activity of papaya seeds

(Vasconcellea pubescens). They found that total antioxidant activity of papaya seeds increased significantly up to 272.8% after HPP (500 MPa/25ºC/15 min). Santos et al. (2018) investigated the effect of HPP (500 MPa/20ºC/5min) on tannins of red wine after 5 months of storage in the dark, at 80% relative humidity and temperatures between 10 and 15ºC. HPP treatment increased tannin content of red wine after storage from 3.38±0.12 to 3.95±0.02 g/L compared to the control (3.38±0.12 g/L). Santos et al. (2018) also reported an increase in the HCl (tannin polymerization) and gelatin (tannin reactivity to proteins) indexes in HPP treated samples compared to the control, obtaining a red wine with a satisfactory astringency for the consumer. The effect of high pressure on individual bioactive compounds has also been studied. Martinez-Monteagudo and Saldaña (2015) investigated the effect of high pressure assisted by temperature on conjugated linoleic acid (CLA) in milk saturated with oxygen. They found that treatment conditions of 600 MPa/120ºC/15 min enhanced CLA retention in milk due to the predominant mechanism of CLA isomerization and free radical polymerization that occurred during high pressure and temperature treatment over the CLA oxidative mechanism. Jun (2009) used HPP to extract caffeine from green tea leaves which was improved by the increase of pressure, from 100 MPa (1.5%) to 600 MPa (4 %), indicating that the higher the hydrostatic pressure, the greater was the amount of solvent entering the cells, leading to an increase of compounds extracted into the solvent. Andres et al. (2016) studied the effect of HPP and thermal treatment on ascorbic acid of smoothies (orange, papaya, melon, carrot, skimmed milk) where higher retention of ascorbic acid was obtained after HPP treatment at 450 MPa (95%), compared to 600 MPa (92%). They indicated that the degradation of ascorbic acid at 600 MPa might be attributed to the activation of enzymes, such as ascorbic acid oxidase and peroxidase, induced by pressure.

Table 2.8 Effect of high pressure processing on bioactive compounds.

Bioactive compound	Food matrix	Treatment	Results	Reference
Total anthocyanins	Grape by-products	600 MPa/70ºC/60 min	Increase of total anthocyanins (41%), anthocyanin monoglucosides (10%) and delphinidin (18%)	Corrales et al. (2008)
	Stevia rebaudiana Bertoni + fruit juice (orange, mango, papaya)	500 MPa/25ºC/5-15 min	Retention of total anthocyanins, between 98-161%	Carbonell-Capella et al. (2013)
Total polyphenols	Green tea leaves	500 MPa/25ºC/1 min	Increase polyphenols extraction yield by 30%	Jun et al. (2009)
	Kiwifruit juice	500 MPa/25 ºC/5 min	Increase in total phenolic content from 451.54±3.59 µg GAE/g to 874.21±9.37 µg GAE/g	Xu et al. (2018)
Total antioxidant activity	Tomato *(var.*Maliniak) puree	450-550 MPa/25 ºC/ 5-15 min	Increase of total antioxidant activity by 48% at 450MPa for 5 and 15 min. Decrease of total antioxidant activity by 35% at 550 MPa for 5 min.	Jez et al. (2018)
	Papaya seeds (*Vasconcellea pubescens*)	500 MPa/25 ºC/ 5-15 min	Increase of total antioxidant activity by 129.3%, 242.7% and 272.8% at 5,10 and 15 min, respectively.	Briones-Labarca et al. (2015)

Table 2.8 Continued.

Bioactive compound	Food matrix	Treatment	Results	Reference
Total tannin	Red wine	500 MPa/20ºC/ 5 min	Higher tannin content (4–7%) compared to the control after 5 months of storage	Santos et al. (2018)
Conjugated linoleic acid	Enriched milk	600 MPa/120ºC/ 15 min	Enhanced the retention of conjugated linoleic acid in enriched milk	Martinez-Monteagudo and Saldaña. (2015)
Caffeine	Green tea leaves	100-600 MPa/25ºC/1-10 min	Yield of caffeine from 1.5±0.3% (100 MPa/25ºC/10 min) to 4.0±0.2% (600 MPa/25ºC/10 min)	Xi. (2009)
Ascorbic acid	Smoothies (orange, papaya, melon, carrot, skimmed milk)	450-600 MPa/20ºC/3 min	High retention of ascorbic acid at 450 MPa (95%) and 600 MPa (92%)	Andres et al. (2016)

2.4.3 Limitation of high pressure processing

High pressure processing has a great potential had preserved food quality, maintained natural flavor and nutritional content of food. However, HPP has some limitations. The investment and operational cost of HPP equipment is high. Depending on the capacity and operating conditions of the equipment, the cost of HPP equipment could reach up to U$2.5 million (Huang et al., 2017). HPP is not applicable to low-moisture content food products, such as flour and powdery flavors due to the use of water as a pressure media, products containing air bubbles can be deformed under pressure. The use of specific packaging materials with a compressibility of at least 15% is required and volume, geometry and composition (polymer type, film thickness, sealing, barrier properties) need to be considered (Balasubramaniam et al., 2015). Finally, pressure treatment alone is not sufficient to inactive bacterial spores of food. *Bacillus subtilis* could survive pressures of 1200 MPa (Huang et al., 2017; Balasubramaniam et al., 2015). HPP at 10-1000 MPa and <45ºC are not enough to inactive spores of harmful pathogens such as *Clostridium botulinum.* The risk of survival of *Clostridium* increases on low-acid HPP products. Therefore, refrigeration at low temperature is required to preserve HPP-food products during transportation and storage (Huang et al., 2017). Pressure assisted thermal processing (PATP) is an emerging sterilization technology with the potential to overcome HPP's disadvantages. PATP operates at pressures of 10-600MPa, temperatures of 40-120ºC and times of 3-10 min. Studies have demonstrated the use of PATP technology not only in the inactivation of spores and preservation of quality and nutritional value of food product, but also in enzyme inactivation for polyphenol oxidase (PPO) and peroxidase (POD).

2.5 Enzymes

Enzymes are a special class of protein present in plants with an active site and three-dimensional configurations (primary, secondary and tertiary structures). The primary structure is formed by amino acid sequence linked via amide (peptide) bonds in a linear chain. The secondary structure refers to the interaction of amino acids in the same chain, forming α-helices or β-sheets by intra- or intermolecular hydrogen bonds, and the tertiary structure is the result of the secondary structure folding into a three-dimensional configuration by amino acid side chain. These structures are maintained not only by the interaction within the polypeptide chain but also by the interactions with the surrounding solvent. Enzymes play an essential role in the metabolism of the plant but after harvesting, the presence of enzymes catalyzes the oxidation of phenolics, resulting in undesirable changes of color, flavor and nutritional value of fruit and vegetables.

Physical and chemical methods have been used to the control enzymatic activity in fruits and vegetables. The conventional chemical methods used to inhibit enzymatic activity are based on the addition of anti-browning agents such as ascorbic acid, citric acid, EDTA, oxalic acid and cyclodextrins. Their action mechanisms are: (i) reduction of o-quinones to diphenols (ii) forming complexes with phenolic substrates, and (iii) forming complexes with copper ion in the structure of the enzyme (Tinello and Lante, 2018). However, it is well known that chemical additives posse low stability in the presence of oxygen and water and result in alteration of organoleptic, bioactive compounds and nutritional properties of the final product. Thermal treatments such as hot drying, high temperature short time and blanching have been used to ensure microbial stability and inactivation of the oxidative enzymes in the food industry. Upon heating, the structure of the protein is unfolded by changes in hydrogen and hydrophobic bonds, electrostatic and van der Waals interactions followed by change in the active site resulting in loss of enzyme activity

(Hendricks et al., 1998). Even though thermal treatment is an effective method used for the food industry to inactivate enzymes, studies have shown that might affect product quality and nutritional value of the final product (Chakraborty et al., 2014). Currently, emerging technologies such as HPP and PATP are alternatives to conventional treatments for enzyme inactivation.

In contrast to temperature, which weakness covalent bonds, pressure destabilizes the enzyme by water penetration into the interior of the protein molecule, destabilizing non-covalent bonds. It is hypothesized that the inactivation of enzyme under high pressure might involve partial or complete unfolding of the native structure of the enzyme, alternating the primary structure of the enzyme at pressure up to 600 MPa while the secondary structure of the enzyme might be affected at pressures above 700 MPa (Hendrick et al., 1998).

The elliptical pressure-temperature diagram shown in Fig. 2.3 describes the relationship between pressure and temperature effects on the activation or inactivation of enzymes such as polyphenoloxidase, pectin methylesterase, myrosinase, naringinase, β-glucanase and α- and β-amylase (Eisenmenger et al., 2009; Smeller, 2002). In point A, at the initial stage of enzymatic inactivation a moderate increase of pressure and temperature might stabilize the enzyme. However, in point D, an increase of pressure and temperature might lead to inactivation or denaturation of the enzyme. In the case of point B, an increase of temperature might stabilize the enzyme while an increase of pressure inactivates the enzyme. In point C, an opposite behavior might occur, an increase of temperature inactivates the enzyme while an increase of pressure might stabilize the enzyme.

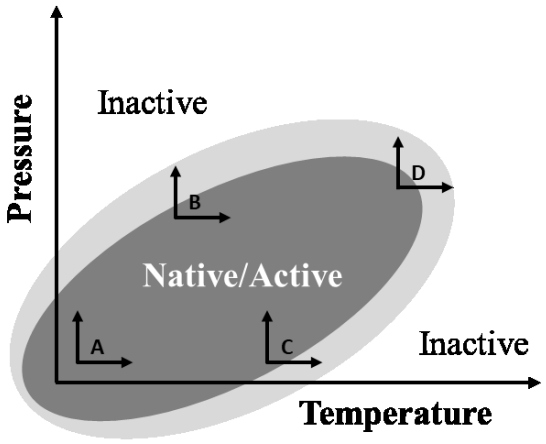


Figure 2.3 Schematic representation of the elliptic phase pressure-temperature diagram of protein activation and inactivation (Adapted from Eisenmenger et al., 2009)

2.5.1 Polyphenol oxidase (PPO)

Polyphenol oxidase (PPO) (EC 1.14.18.1) enzyme is a group of copper proteins that catalyzes the oxidation of phenolics to quinones resulting in undesirable changes of color. Its reaction mechanism is based on two main reactions; the hydroxylation of monophenols to *o*-dephenol, followed by the oxidation of *o*-dephenol to *o*-quinone forming the complex brown polymer (Fig. 2.4) (Nokthai et al., 2010). Because of the negative effect of PPO on appearance, organoleptic properties and nutritional value, its inactivation is desirable in the food industry.

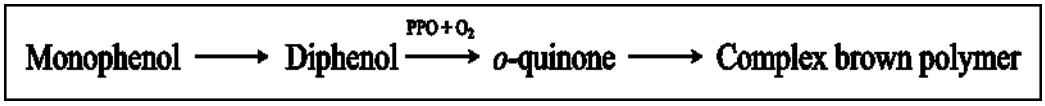


Figure 2.4 Catalytical reaction of PPO enzyme.

Several studies reported in Table 2.9 have investigated the effect of thermal treatment and HPP on PPO enzyme inactivation. Garcia-Palazon et al. (2004) studied the effect of HPP (400-800 MPa/18-22 °C/5-15 min) on PPO enzymes of strawberry puree, reporting that complete inactivation of PPO was achieved at 800 MPa/18-22°C/15 min. Similarly, Yi et al. (2012)

reported 96% of PPO inactivation of mushroom treated at 1600MPa/60ºC/1 min. Terefe et al. (2010) investigated the effect of HPP and thermal treatment on strawberry puree samples at 100-690MPa/24-90ºC/5-15 min and 0.1MPa/40-100ºC/5-15 min, respectively. Maxima inactivation of PPO of 23% and 20% were obtained at 690MPa/90ºC/1-5 min and 100ºC/15 min, respectively. The inactivation of PPO enzyme depends not only on the processing conditions used but also on the fruit type. Sulaiman et al. (2015) reported PPO inactivation by 12%, 41%, 98% of pear, apple, and strawberry purees treated at 600 MPa/64ºC/15 min. The inactivation of PPO was also studied by Barba et al. (2014) in a fruit mixture (orange, mango, papaya) sweetened with *Stevia rebaudiana* (2.5%) at 500 MPa/18-22 ºC/15 min, resulting in 97% of PPO inactivation. Chourio et al. (2018) investigated the inactivation of POD in coconut water treated at 200-600MPa/40-90ºC/1-30 min, reporting complete inactivation of the PPO enzyme at 400-600MPa/90ºC/2 min.

2.5.2 Peroxidase (POD)

Peroxidase (POD) (EC 1.11.1.7) enzyme is a thermal stable enzyme that catalyzes the single electron oxidation of bioactive compounds in the presence of hydrogen peroxidase, resulting in undesirable changes of color (Terefe et al., 2017). It is suggested that PPO promotes POD activity due to the generation of hydrogen peroxidase during PPO catalyzed oxidation of phenolic compounds. The activity of POD is correlated with the degradation of anthocyanin, chlorophyll, ascorbic acid and aromatic amines (Chakraborty et al., 2014). Therefore, POD inactivation is required to preserve food quality, organoleptic and nutritional properties of food products. The effect of HPP and PATP on inactivation of POD enzyme summarized in Table 2.9 has been studied in several fruits, plants and vegetables including green peas (Quaglia et al., 1996), spinach (Jung et al., 2013), strawberry puree (Terefe et al., 2010, Garcia-Palazon et

al., 2004,), mixture of fruit pulp (orange, mango, and papaya) sweetened with *Stevia* (2.5%w/v) (Barba et al., 2014), blueberry (Terefe et al., 2017), and coconut water (Chourio et al., 2018). Antagonism effect between pressure and temperature was reported in blueberry and strawberry puree treated at 100-400 MPa/30-70 ^{o}C/15 min and 400-800 MPa/18-22 ^{o}C/5-15 min, respectively (Terefe et al. 2017; Garcia-Palazon et al. 2004).

Studies have shown that PATP treatments were more effective in the partial or complete inactivation of POD enzyme than HPP treatments. Terefe et al. (2010) investigated the effect of PATP on strawberry puree at 100-690MPa/24-90^{o}C/5-15 min, reporting POD inactivation by 96% at 100-690 MPa/90^{o}C/5 min. Similarly, 96% of POD on blueberry fruit and strawberry pure were inactivated at 400 MPa/90 ^{o}C/15 min and 100-690 MPa/90^{o}C/5 min, respectively (Terefe et al., 2017; Terefe et al., 2010). Chourio et al. (2018) reported complete inactivation of POD on coconut water treated at 90^{o}C/400-600MPa/2 min. Contrary, HPP at room temperature resulted in partial inactivation of POD in a fruit mixture (orange, mango, papaya) with 2.5%w/v *Stevia rebaudiana* (84%) at 500MPa/18-22^{o}C/15 min (Barba et al., 2014) and spinach (83%) at 300MPa/20^{o}C/20 min (Jung et al., 2013). The effect of PATP on bioactive compounds of mate, mate sweetened with *Stevia* and inactivation of PPO and POD enzymes of *Stevia* were studied for the first time (Chapter 3 and 4).

Table 2.9 Effect of pressure-temperature treatment on PPO and POD inactivation from different sources.

Food matrix	Treatment	Results	Reference
PPO			
Strawberry puree	400-800 MPa/ 18-22 ºC/5-15 min	Complete inactivation of PPO at 800 MPa/18-22ºC/15 min	Garcia-Palazon et al. (2004)
Strawberry puree	100-690 MPa/ 24-90 ºC/5-15 min 0.1MPa/40-100ºC/5-15 min	Inactivation of PPO by 23% and 20% at 690 MPa/90ºC/1-5 min and 100ºC/15 min, respectively.	Terefe et al. (2010)
Mushroom (*Agaricus bisporus*)	800-1600 MPa/20ºC/1 min	96% of PPO inactivation at 1600 MPa/1 min/20ºC	Yi et al. (2012)
Pear, apple and strawberry puree	600 MPa/64ºC/15 min	PPO inactivation by 12%, 41%, 98% in pear, apples and strawberry, respectively.	Sulaiman et al. (2015)
Fruit mixture (orange, mango, papaya) with *Stevia rebaudiana*	300-500 MPa/18-22 ºC/5-15 min	PPO inactivation by 97% at 500 MPa/18-22ºC/15 min	Barba et al. (2014)
Coconut water	200-600 MPa/40-90ºC/1-30 min	Complete inactivation of PPO at 90ºC/400-600 MPa/2 min	Chourio et al. (2018)

Table 2.9 Continued.

Food matrix	Treatment	Results	Reference
POD			
Green peas	400-900 MPa/60ºC/5-10 min	88% of POD inactivation at 900 MPa/60ºC/5min	Quaglia et al. (1996)
Strawberry puree	100-690 MPa/24-90ºC/5-15 min	96% of POD inactivation at 100-690 MPa/90ºC/5 min	Terefe et al. (2010)
	400-800 MPa/18-22 ºC/5-15 min	Activation of POD by 13% and 1% at 400MPa for 5 and 10 min, respectively.	Garcia-Palazon et al. (2004)
Fruit mixture (orange, mango, papaya) with (2.5% w/v) *Stevia rebaudiana* leaves	300-500 MPa/18-22 ºC/5-15 min	84% of POD inactivation at 500 MPa/18-22 ºC/15 min	Barba et al. (2014)
Blueberry	100-690 MPa/30-90 ºC/15 min	Activation of POD enzyme at 100-400 MPa/30-70 ºC/15 min 96% of POD inactivation at 400 MPa/90 ºC/15 min	Terefe et al. (2017)
Coconut water	200-600 MPa/40-90ºC/1-30 min	Complete inactivation of POD at 90ºC/400-600 MPa/ 2 min	Chourio et al. (2018)
Spinach *Spinacia oleracea L. var. spinoza*	100-500 MPa/20ºC/20 min	83% of POD inactivation at 300 MPa/20ºC/20 min	Jung et al. (2013)

Chapter 3: Effect of Pressure-Assisted Thermal Processing on Bioactive Compounds and Antioxidant Activity of Mate

ABSTRACT

In this study, the effects of Pressure Assisted Thermal Processing (PATP) on mate was evaluated with respect to total phenolics, total antioxidant activity, individual phenolic compounds (quercetin, rutin, chlorogenic acid, caffeic acid) and methylxanthines (caffeine and theobromine) and catechins (catechin, gallocatechin, epigallocatechin and gallocatechin gallate). Mate was treated at 10-600 MPa and 25-120ºC for 1-5 min. The total phenolics and total antioxidant activity were determined using spectrophotometric methods. Individuals phenolics were quantified by high performance liquid chromatography. The total phenolics and total antioxidant activity contents of mate significantly increased after PATP treatment compared to untreated mate. High amounts of total antioxidant activity (698±10 $FeSO_4.7H_2O$ mg/g) of mate were obtained at 100 MPa/120 ºC/1 min while high amounts of total phenolics (148±7 mg GAE/g) were extracted at 600 MPa/120ºC/1 min, probably due to the formation of compounds with low antioxidant activity. Chlorogenic acid content in mate decreased after PATP treatment due to its conversion to caffeic acid. The highest amount of caffeic acid (28.94±1.45 mg/g mate) was obtained at 120ºC/600MPa/1 min while the highest concentrations of caffeine (35.90±2.6 mg/g mate), theobromine (23.99±0.02 mg/g mate) and quercetin (5.36±0.27 mg/g mate) were obtained on treated mate extracts at 100MPa/120ºC/1 min. Also, catechin (0.36±0.02 mg/g mate), gallocatechin gallate (0.59±0.03) mg/g mate), and epigallocatechin (0.15±0.01 mg/g mate) were quantified on untreated mate. Catechin content increased up to 64% at 600MPa/75ºC/1 min compared to untreated mate. However, only catechin traces were detected with the mate extracts after PATP treatment at 120ºC. The maximum

gallocatechin gallate content (0.94±0.05 mg/g mate) and epigallocatechin (0.43±0.02 mg/g mate) on treated mate was obtained at 600 MPa/120ºC/1 min.

Moreover, the analysis of the influence of parameters (ANOVA, α =0.05), showed that temperature (p-value <0.001), pressure (p-value <0.001) and holding time (p-value ≤0.006) significantly influenced total phenolic and total antioxidant contents of treated mate, obtaining the greatest release of total phenolics and total antioxidant activity with the increase of temperature. These results highlight the potential use of PATP to enhance release of phenolics compounds, methylxanthines and catechins, and antioxidant activity of treated mate.

Keywords: *Ilex paraguariensis;* emerging technology; methylxanthines; catechins, rutin, quercetin.

3.1 INTRODUCTION

Mate, from the leaves of *Illex paraguariensis*, is originally from the southern part of South America, consumed primarily in Brazil, Paraguay, Uruguay, and Argentina. *Illex 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 (Euromonitor International, 2016). Recently, mate tea has gained great interest 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}) (Berte et al., 2014). Studies have demonstrated that the consumption of chlorogenic acid (100 mg/kg/day) decreases blood pressure, oxidative damage in the brain, and prevent chronic diseases, including, diabetes, bacterial infections, inflammation and cancer (Gulcinm et al., 2006). Caffeic acid also 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 a concentration of 20 μg/L caffeic acid (Wu et al., 2006). Quercetin has also potential for the treatment and prevention of different types of cancer cells, including colorectal, prostate, liver, pancreatic and lung cancer as well as obesity, inflammations, viral and bacterial diseases due to its strong antioxidant capacity (Goyal et al., 2010; Gardana et al., 2010).

Various processing techniques have been used to extract bioactive compounds from *mate Ilex paraguariensis.* Supercritical CO_2 (25.5 MPa/70°C/0.9-1.2 g CO_2/min/7h) extracted 94, 68, and

57% of initial caffeine, theobromine, and theophylline contents from mate (Saldaña et al., 1999). Also, 51 compounds, mainly esters, fatty acids, hydrocarbons, phytosterols, alcohols, xanthines and vitamin E were identified after pressurized liquid extraction of mate tea leaves (10 MPa/50-100ºC/10-30 min) using methanol and hexane as solvents (Jacques et al., 2008). High pressure processing at 500 MPa/25ºC/1 min was used to extract caffeine from green tea leaves (4.0 ± 0.22% yield of caffeine) with same extraction yield obtained at room temperature for 20 h (Xi et al., 2009). Pressure assisted thermal processing (PATP) 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 PATP on increasing total anthocyanin, total carotenoids, flavonoids, total phenolic contents, antioxidant activity of grape by products, green tea, and *Stevia rebaudiana* leaves (Corrales et al., 2008 and Carbonell-Capella et al., 2013) and inactivation of enzymes PPO and POD in blueberries (Terefe et al., 2010), strawberry (Terefe et al., 2013), fruit extract sweetened with *Stevia* (Barba et al., 2014), raspberry (Garcia-Palazon et a., 2004), coconut water (Chourio et al., 2018). However, no data is available on the effect of PATP on mate. Therefore, the objective of this study was to investigate the effect of PATP on total antioxidant activity, total phenolic content, and specific bioactive compounds of mate.

3.2 MATERIALS AND METHODS

3.2.1 Sample preparation

Mate was purchased at the "Paraiso Tropical" latin food market (Edmonton, AB, Canada), "Guayaki" brand from Sao Mateus, Espiritu Santo, Brazil. Mate was ground to a mean particle size of 500 µm, sieved and stored at 4ºC for further use.

3.2.2 Chemicals

Chemicals such as (−) epigallocatechin (≥95% purity), (−)epicatechin gallate (≥98% purity), catechin (≥98%) chlorogenic acid (≥95% purity), caffeic acid (≥98% purity), rutin hydrate (≥98% purity), ABTS (2.2 azino-bis 3–ethylbenzothiazoline-6-sulfonic acid) (≥98% purity), pyrocatechol (≥99% purity), caffeine (≥99% purity), theobromine (≥98% purity), theophylline (≥98% purity), Fe_2SO_4 (≥98% purity), tripyridyltriazine, and quercetin (≥95% purity) were purchased from Sigma-Aldrich (Oakville, ON, Canada).

Chemical reagents, such as sodium hydroxide pellets (≥97%, ACS grade), Folin-Ciocalteu phenol reagent (2M) and petroleum ether were purchased from Fisher Scientific Co (Toronto, ON, Canada).

3.2.3 Proximate compositional analysis

3.2.3.1 Moisture content

Moisture content of samples was determined using a gravimetric method (AOAC, 2000). Approximately 2 g of sample was weighed using an analytical balance (Mettler Toledo, Mississauga, ON, Canada). The samples were uniformly spread into a pre-weighed aluminium dish of 50 mm diameter x 23 mm deep. The aluminium dishes were placed into a hot air oven (Model 655G, Fisher Scientific Isotherm oven, Toronto, ON, Canada) maintained at 105ºC for 3 h. After drying, the aluminium dishes were placed in a desiccator for cooling for 1 h. The weight of the aluminium dishes with the samples were recorded. The moisture content (%) was calculated using the following equation:

$$\text{Moisture content (\%)} = \frac{m_1 - m_2}{m_2}\ x\ 100 \qquad (3.1)$$

where, m_1 = weight (g) of the sample before drying, and m_2= weight (g) of the sample after drying.

3.2.3.2 Ash content

Ash content of the samples was determined by incineration (AOAC, 2000). One gram of sample was weighed in pre-weighed porcelain crucibles. The samples were incinerated overnight in a muffle furnace (Model F-A1730, Thermolyne Corporation, Dubuque, IA, USA) at 550°C. The crucibles were removed, cooled in a desiccator for 1 h, and weighed. Ash content was calculated using the following equation:

$$\text{Ash content (\%)} = \frac{m2}{m_1}\ x\ 100 \tag{3.2}$$

where, m_1 = weight (g) of the sample before incineration, and m_2 = weight (g) of the sample after incineration.

3.2.3.3 Protein content

Protein content was calculated by determining the nitrogen content of the sample using the LecoTruSpec nitrogen analyser (Leco instruments Ltd., Mississauga, ON, Canada). Approximately 0.16 g of sample was weighed into an aluminum foil cone, which was pressed to form pellets. The aluminum foil conned samples were placed into a loading head. The combustion of the sample was performed in a furnace at 950°C using oxygen. The sample was loaded in a thermal conductivity cell for nitrogen content determination (%). The calibration of the apparatus was performed using corn starch standard.

$$\text{Protein content (\%)} = 6.25 \text{ x Nitrogen content} \tag{3.3}$$

3.2.3.4 Total fat content

Total fat content was determined following the procedure described in ISO 17189 (2003), with slight modifications. First, the sample was weighed (4.0±0.1 g) into a centrifuge tube. Then, 20 mL of petroleum ether was added to the tube, which was closed, and vigorously mixed for 1 min using a vortex mixer. The solution was centrifuged using radical acceleration of 600 *x g* for 5 min.

Then, the supernatant was transferred into a fat-collecting Pyrex tube previously weighed and placed in a hot drying oven preheated at 102±2ºC for 30 min, cooled to room temperature in a desiccator for 1 h and weighed. The total fat content of the sample was calculated and expressed as a mass fraction in percent using the following equation:

$$Total\ Fat\ (\%) = \frac{(m1-m2)}{m_0}\ x\ 100 \quad (3.4)$$

where, m_o = initial mass of the sample in grams, m_1 = mass of the collecting Pyrex tube and extracted matter, and m_2 = mass of the collecting Pyrex tube.

3.2.3.5 Total carbohydrate content

Total carbohydrate content was calculated by difference using the following equation:

Total carbohydrate (%) = 100% - (moisture (%) - ash (%) - protein (%) - total fat (%)) (3.5)

All proximate compositional analyzes were performed at least in duplicate.

3.2.4 Pressure Assisted Thermal Processing (PATP)

The effects of pressure (MPa), temperature (ºC) and process time (min) on total antioxidant activity, total phenolic content, and specific bioactive compounds was investigated using a full factorial experimental design (3 × 3 × 2) with pressures of 10, 100 and 600 MPa, temperatures of 25, 75 and 120 ºC and holding times of 1 and 5 min. These processing conditions were selected based on literature and previous study on coconut water (Chourio et al., 2018) and typical pressure and temperature conditions used in the food industry.

A four-vessel system (Apparatus U111 Unipress, Warszawa, Poland) was used (Fig. 3.1). 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 according to the experimental design

performed in this study. 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.

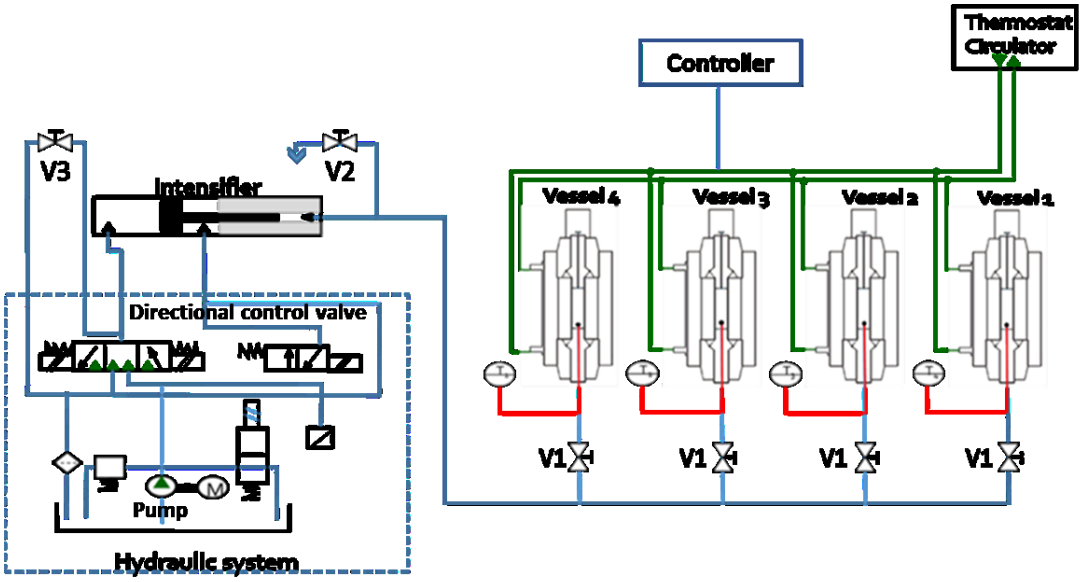
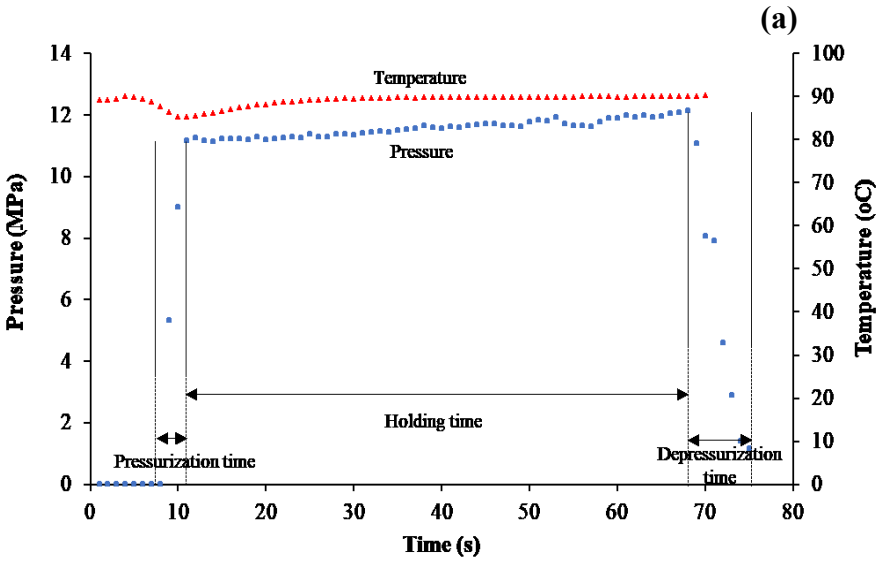


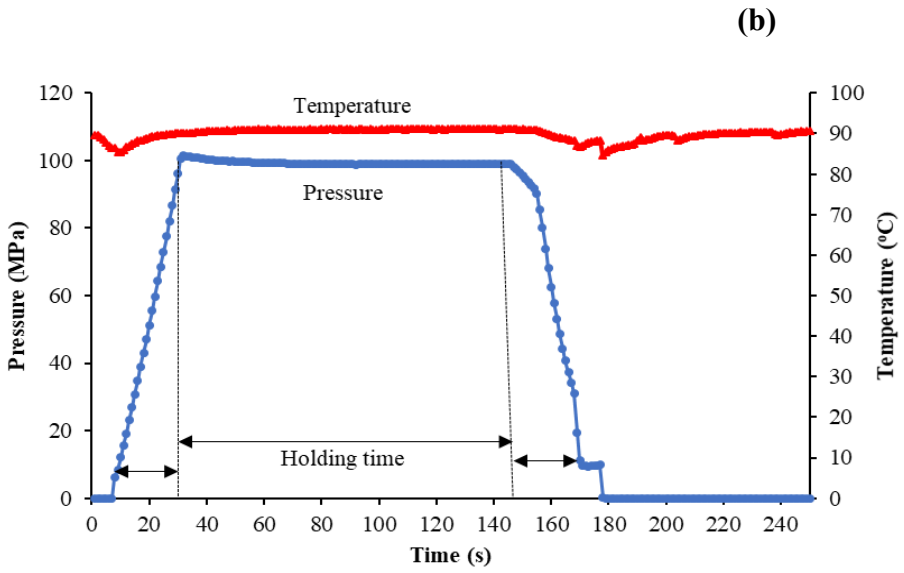
Figure 3.1 Pressure assisted thermal processing equipment (Unipress, Warszawa, Poland). T: thermocouple, V1: valve, V2: release valve, V3: filling valve, M: electric motor.

3.2.4.1 Temperature and pressure profiles of PATP treatment

Fig. 3.2a-c shows the pressure and temperature profiles of the water surrounding the samples inside a 3 mL vial at 10, 100, and 600 MPa and 90ºC. First, the high-pressure system is programmed to the desired temperature, in this case 90ºC. Then, samples are pressurized from atmospheric pressure of 0.1 MPa to the target pressure. The pressurization rates were approximately 3.3, 4.2 and 6.25 MPa/s for 10, 100 and 600 MPa, respectively. Once these pressure and temperature conditions are reached, samples are processed under isothermal and isobaric conditions for the target holding times (60-300 s). After processing at this holding time, the samples are depressurised to atmospheric pressure. The depressurization rates were approximately 1.4, 4.5 and 8.7 MPa/s for 10, 100 and 600 MPa, respectively. The system was manually

depressurized where the release valve was slowly opened. It is observed that pressure of 100 and 600 MPa (Fig. 3.2c-b) were more stable than pressure of 10 MPa (Fig. 3.2a) while that targeted temperature was well maintained at all pressures evaluated.





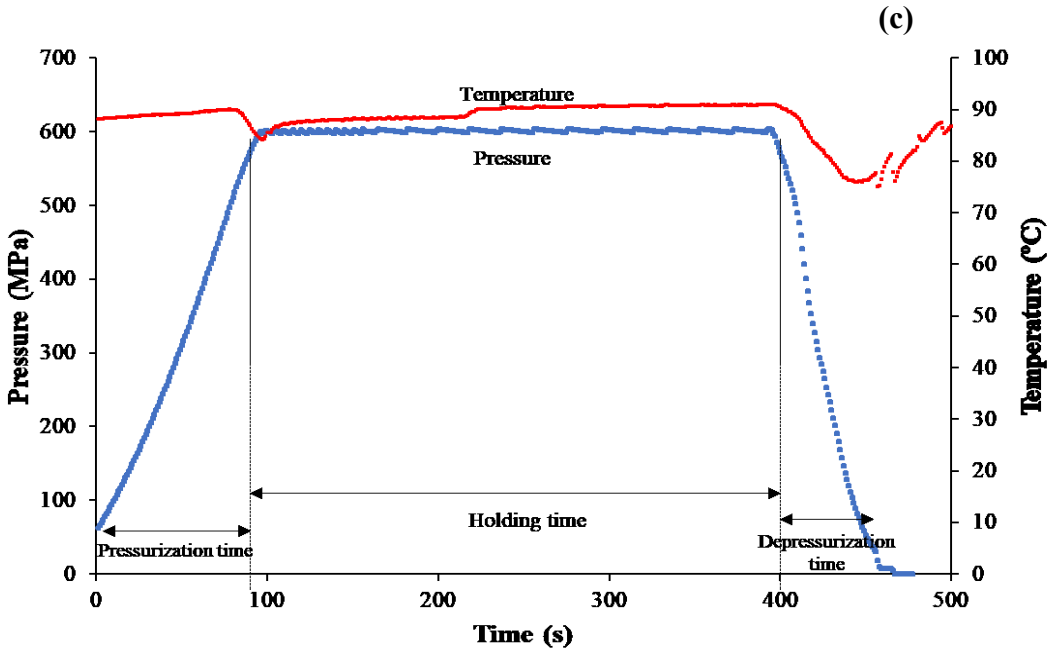


Figure 3.2 Pressure and temperature profile of the water surrounding the samples in the 3 mL vial during processing at 90°C: (a) 10 MPa, (b)100 MPa and (c) 600 MPa.

3.2.5 Analysis of treated samples

3.2.5.1 Total antioxidant activity

The total antioxidant activity was determined using the FRAP assay with slight modifications (Sarkar et al., 2014). A buffer acetate of pH 3.6, 10 mM *tripyridyltriazine* (*TPTZ*) and 20 mM ferric chloride solution were mixed at a ratio of 10:1:1 (v/v/v) to prepare the FRAP solution. A mixture of FRAP solution (3 mL), distilled water (0.3 mL), and extract solution (0.1 mL) was vortexed for 10 s and incubated at 37°C in a water bath for 30 min. The absorbance was read at 593 nm using a UV-VIS spectrophotometer (Jenway 6320D, Standford, United Kingdom).

3.2.5.2 Total phenolic content

The Folin-Ciocalteu method was used to determine total phenolic content (Sarkar et al., 2014). 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 °C). The absorbance

was measured at 765 nm using a UV-VIS spectrophotometer. The results were expressed as milligrams of gallic acid equivalent per gram of sample (mg GAE g^{-1}).

3.2.5.3 Individual bioactive compounds

Individual phenolic compounds, such as chlorogenic acid, quercetin, caffeic acid and rutin were analyzed with a Shimadzu 20 HPLC (Shimadzu, Kyoto, Japan) equipped with a Shimadzu SPD-M10A diode array detector. The column used was a ZORBAX Eclipse XDB 80 Å C18 column (150x4.6 mm, 5μm Nomura Chemical Co., Seto, Japan) with a guard column (10x4 mm, Nomura Chemical Co., Seto, Japan). The flow rate of the mobile phase was 1 mL/min and the injection volume was 20 μL. 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% B for 36-41 min and finally back to the initial concentration of 8% B for another 47 min. The wavelengths were scanned from 190 nm to 500 nm, and data was quantified at 268 nm. Extracts were filtered using a 0.22 μm EMD Millipore MillexTM Sterile Syringe Filter purchased from Sigma-Aldrich (Oakville, ON, Canada).

3.2.6 Scanning electron microscope (SEM)

A scanning electron microscope (SEM) (Zeiss Sigma 300 VP-FESEM, Oberkochen, Germany) was used to visualize the structure of untreated and PATP treated residue mate and *Stevia*. The PATP residues were dried using freeze drying and mounted on SEM specimen stubs with double-size conductive carbon tape and sputter-coated with carbon using a Nanotek SEM prep 2 sputter coater (Nanotech, Manchester, England)

3.2.7 Statistical analysis

The influence of parameters, temperature, pressure and time, on total phenolics and total antioxidant activity were evaluated by analysis of variance (ANOVA) using Minitab 16 ® software

(Minitab Inc, State Collage, Pennsylvania, USA) with a 95% confidence level (p-value ≤ 0.05). The parameters were evaluated using a full factorial design (3x3x2). Individual phenolics results were analyzed using R studio software (Minitab Inc, State Collage, PA, USA). R studio software was used to examine differences in mean values by Tukey's test of means with a 95% confidence level (p-value ≤ 0.05).

3.3 RESULTS AND DISCUSSION

3.3.1 Proximate compositional analysis of mate

Table 3.1 and Table A.1, Appendix A show the proximate compositional analysis of mate. The mate used in this study has a pH of 5.8 and 76.41% of total carbohydrates. Similarly, Berte et al. (2014) reported that instant mate tea (unroasted) had 80.7 – 81.5% total carbohydrates while mate tea (chimarrão) had 72.3-79.6%. The other major component of mate, after total carbohydrates, was protein with 12.60 %. Berte et al. (2014) reported 7.9 – 9.8% and 3.7-4.1% protein content in mate tea (chimarrão) and instant mate tea, respectively. The amounts of lipids, moisture, and ash of mate used in this study were 0.5%, 5.2% and 5.3%, respectively.

Table 3.1 Proximate composition of untreated mate.

Component (g/100 g)	Mate (This study)	Mate tea (chimarrão)[1]	Instant mate tea (unroasted)[1]
Total carbohydrates (%)	76.4 ± 1.1	72.3 - 79.6	80.1- 82.2
Protein (%)	12.6 ± 0.5	7.9 - 9.8	3.7 - 4.1
Lipids (%)	0.5 ± 0.0	3.8 - 4.2	0.7 - 0.9
Moisture (%)	5.2 ± 0.5	3.6 - 8.0	3.9 - 4.8
Ash (%)	5.3 ± 0.1	5.1- 5.7	9.5 - 10.1

[1]Berte et al. (2014). Data reported as mean ± standard deviation between duplicates.

3.3.2 Total antioxidant activity and total phenolics content of mate

The total antioxidant activity values for untreated and treated mate were 174±6 and 494±4–698±10 mg $FeSO_4.7H_20$/g of mate, respectively (Fig A.1a, Appendix A). An increase of temperature from 25ºC to 120ºC resulted in a gradual increase of total antioxidant activity with more than 300% at conditions of 10 MPa/120 ºC/1 min compared with untreated mate. The highest value of total antioxidant activity was achieved at 100 MPa/120ºC/1 min (698±10 mg $FeSO_4.7H_20$/g of mate) (Table A.2, Appendix A).

In addition, analysis of the influence of parameters by ANOVA at α =0.05 showed that the temperature (p-value<0.001), pressure (p-value<0.001) and holding time (p-value=0.003) significantly influenced total antioxidant activity of treated mate (Fig. 3.3). The values of total antioxidant activity presented in Fig 3.3 for pressure, temperature and holding time corresponded the to overall means of total antioxidant activity at 10-600MPa, 25-120ºC and 1-5 min. For example, for the effect of pressure at 10 MPa values obtained at 25-120ºC and 1-5 min were averaged. Also, for the effect of temperature at 120ºC values obtained at 10-600MPa and 1-5 min were averaged.

An increase of pressure from 10 to 100 MPa resulted in an increase of total antioxidant activity (p-values<0.001) while no significant difference was observed with the increase of pressure from 100 to 600 MPa (p-values<0.001). Also, the increase of temperature from 25 to 120ºC resulted in a significant increase of total antioxidant activity values (p-values<0.001), with the highest total antioxidant activity at 120ºC. Contrary, the increase of holding time from 1 to 5 min resulted in a significant slight decrease of total antioxidant activity mean values. Therefore, holding time of 1 min resulted to be enough to obtain PATP treated mate with the highest total antioxidant activity, probably due to the saturation of the solvent with the bioactive and attainment of the extraction

equilibrium. Thus, a longer time is not recommended. In general, temperature was the parameter with the highest influence on total antioxidant activity compared to pressure and holding time.

The increase of total antioxidant activity at high temperature and pressure of 100 MPa was attributed to the disruption of cell membranes that occurred during PATP, resulting in an enhancement of antioxidant compound extractability. It was also hypothesized that PATP induced water ionization resulting in a pH decrease and a disruption of hydrophobic bonds like non-covalent bonds involved in the stability of proteins, micelles, and lipids, and inactivation of peroxidase and polyphenoloxidase enzymes, resulting in high antioxidant activity (Hendrickx et al., 199).

No study reported the effect of PATP on antioxidant activity of mate. However, various studies reported the effect of pressure and/or temperature in other food matrices. An increase of total antioxidant activity by 12% was reported in Aronia berry puree after high pressure treatment at 400 MPa/25^{o}C for 5 min compared to untreated sample. However, no significant difference was reported on treated aronia berry puree at 200 MPa/2.5-5 min and 600 MPa/2.5-5 min compared to untreated sample (Yuan et al., 2018). After high pressure processing at room temperature, an increase of total antioxidant activity up to 67% and 46% were reported in treated blueberry puree at 600 MPa/15 min and treated jabuticaba *Myrciaria jaboticaba* juice at 500 MPa/10 min, respectively (Patras et al., 2009; Inada et al., 2018).

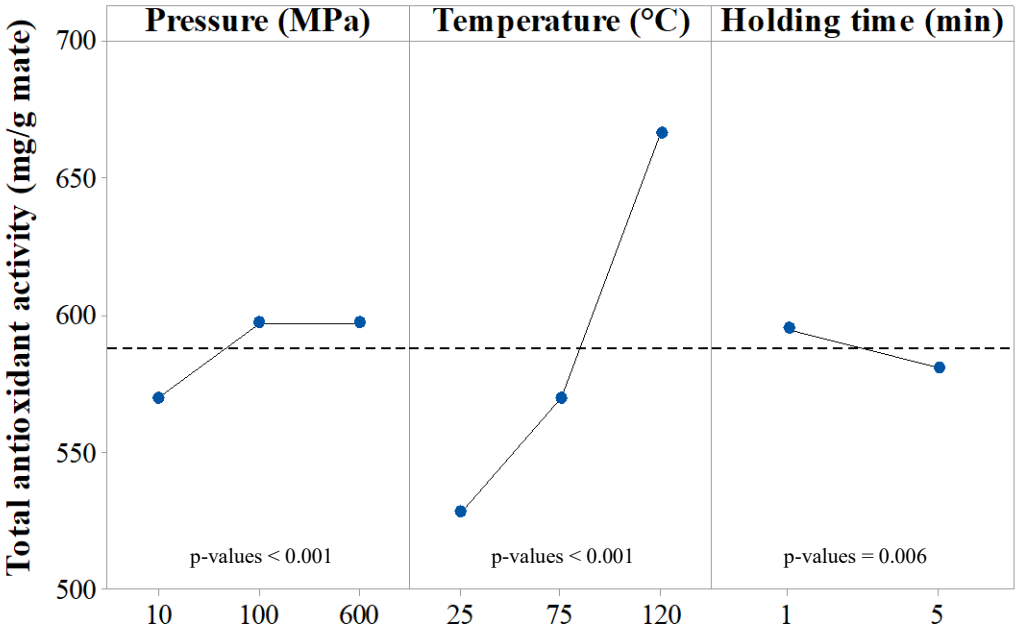


Figure 3.3 Effect of temperature, pressure, and time on mean values of total antioxidant activity on PATP treated mate.

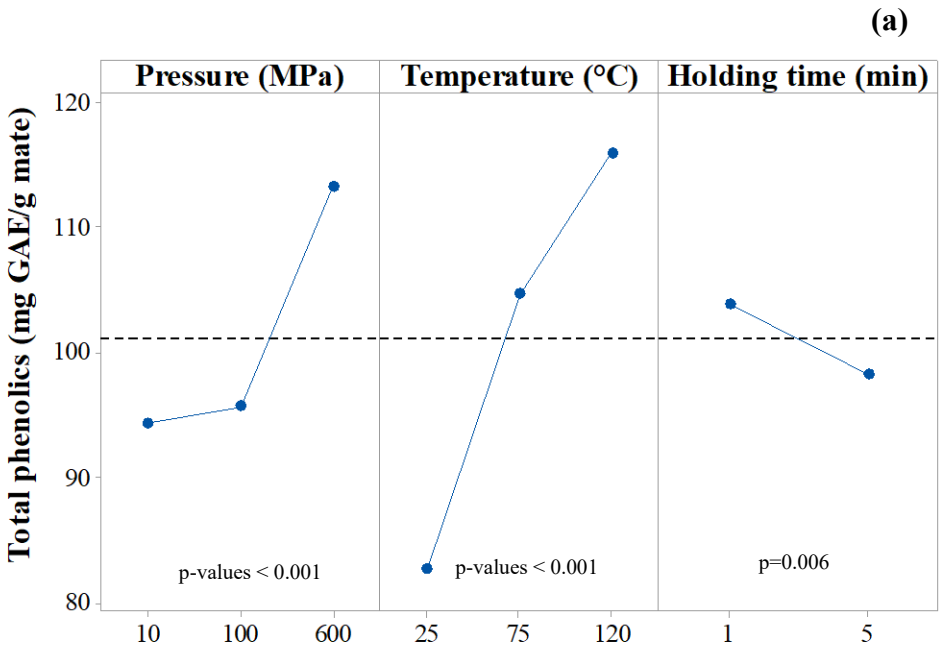
Total phenolic content of mate after PATP treatment is shown in Fig A.1b, Appendix A. Overall, TP values of treated mate were higher compared to untreated mate at all conditions investigated. High values of total phenolics of treated mate were achieved at 600 MPa/120ºC/1 min (148 ± 7 mg GAE/g) and 600 MPa/120ºC/5 min (140± 7 mg GAE/g) (Fig A.1b, Appendix A and Table A.2, Appendix A). This increase of total phenolic content of treated mate can be related to the enhancement of cell permeability (Yuan et al., 2018) and liberation of matrix-bound phenolic compounds promoted by PATP treatment.

Moreover, the analysis of influence of parameters (ANOVA, $\alpha = 0.05$) showed that temperature (p-value<0.001), pressure (p-value<0.001) and holding time (p-value=0.006) significantly influenced total phenolic content of treated mate. An increase of temperature from 25 to 120 ºC MPa resulted in an increase of total phenolic content (p-values<0.001) (Fig. 3.4a). Contrary to total antioxidant activity, total phenolic content increased with the increase of pressure

from 100 to 600 MPa, while holding time caused a significant slight decreased of total phenolic content from 1 to 5 min (Fig 3.4a).

No studies have reported the effect of PATP on total phenolic content of mate. However, several studies have investigated the effect of temperature, and solvents on the extractability of total phenolic compounds from mate. Turkmen et al. (2006) investigated the extraction of total phenolics from mate using different solvents (water, 50-100% acetone, 50-100% dimethylformamide, 50-100% ethanol and 50-100% methanol) at 100ºC for 10 min. They reported that the highest total phenolic value (120±1 mg/g) was obtained using 50% acetone while total phenolic values of 64±1mg/g was obtained using water as a solvent, demonstrating that the high content of polyphenols was obtained with an increase in polarity of the solvent used. Also, Grujic et al. (2012) studied the effect of different ethanol concentrations (40-60% v/v) and liquid CO_2 extraction (5-10 MPa/27ºC) on the total phenolic content of mate extracts and the highest values of total phenolics (115.0 ± 0.3 mg/g) were obtained using 40% ethanol as a co-solvent and a pressure of 10 MPa due to the increase of polarity favouring polyphenols extraction. Chandra and Mejia (2004) determined total phenolic content of aqueous mate extraction at 98ºC for 10 min obtaining 96 ± 4 mg GAE/g mate. Kaushik et al. (2016) reported an increase of total phenolic on treated mango pulp at 400-600 MPa/40-60ºC/5-15 min by 7-27% compared to the untreated sample due to the interactive effect of pressure and temperature to enhance or retard enzymatic and chemical reactions and disrupt food matrix to an increase phenolics in the extract. Garcia-Parra et al. (2016) investigated the effect of PATP on total phenolic content of pumpkin puree, where 600 MPa/70ºC/1 min increased total phenolic content by 65% of pumpkin puree compared with the untreated sample, suggesting that PATP might produce a change in chemical and biological structure of the matrix, converting insoluble phenolics to more soluble phenolics.

The correlation between total phenolics and total antioxidant activity is shown in Fig 3.4b. It can be observed that total phenolic content had a good correlation of $R^2 = 0.923$ with their total antioxidant activity, confirming that total phenolics contributed to the antioxidant activity of mate. This result agrees with those reported by Turkmen et al. (2006), who investigated the effect of solvents with different polarities (methanol, ethanol, acetone and dimethylformamide) on the extraction of total phenolics and on the total antioxidant activity of mate and black tea at 0.1 MPa/100ºC/60 min, reporting a good correlation of $R^2 = 0.980$ between total phenolic content and antioxidant activity. Similarly, Mello et al. (2004) investigated the correlation between total phenolic content and total antioxidant activity in black and mate tea infusions at 0.1 MPa/100ºC, obtaining a good correlation of $R^2 = 0.986$ between total phenolic content and total antioxidant activity of mate and black tea.



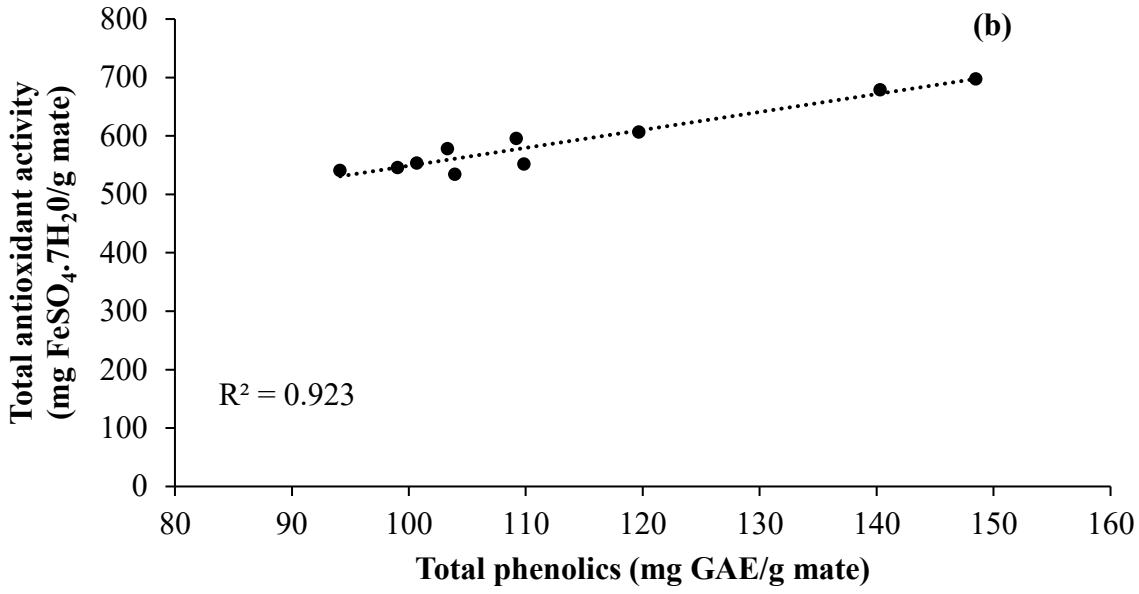


Figure 3.4 (a) Effect of temperature, pressure, and holding time on mean values of total phenolics on mate treated at 10-600 MPa/25-120ºC/1-5 min and **(b)** Correlation of total phenolics (mg GAE/g) versus total antioxidant activity (mg $FeSO_4.7H_20$/g mate) of PATP treated mate.

3.3.3 Individual phenolics in mate after PATP treatment

Fig. 3.6 and Table A.5, Appendix A show chlorogenic acid and caffeic acid contents of untreated and PATP treated mate identified by HPLC. The concentrations of chlorogenic acid and caffeic acid in untreated mate were 8.3±0.4 and 6.5±0.3 mg/g, respectively. The amounts of caffeic acid on treated mate gradually increased with the increase of temperature from 25ºC to 120ºC at 600 MPa. The highest amount of caffeic acid was obtained at 600 MPa/120ºC/1 min. However, a slight decrease was observed at 600MPa/120ºC/5min, probably due to decarboxilation of caffeic acid to hydroxytyrosol (3,4-dihydroxyphenylethanol), protocatechuic aldehyde (3,4-dihydroxybenzaldehyde), and 4-vinylcatechol (2-hydroxy-4-vinylphenol) formed at the early stage of caffeic degradation (Khuwijitjaru et al., 2014). These compounds are associated with low antioxidant capacity and pro-oxidant activity (Andueza et al., 2009). The pathway of caffeic acid

decomposition at subcritical water conditions at 5 MPa/160-240ºC/20-1080 s, shown in Fig. 3.5, is a reaction via decarboxylation and cyclisation of caffeic acid into hydroxytyrosol, protocatechuic aldehyde, and 4-vinylcatechol as described by Khuwijitjaru et al., (2014). Chlorogenic acid (5-CQA) contents decreased about 41% and 91% at 600MPa/25ºC/1 min and 600MPa/120ºC/5 min, respectively, probably due to chlorogenic acid isomerization, transforming 5-O-caffeoylquinic acid into caffeic acid (Dawidowicz et al., 2010).

Earlier, De Maria et al. (1995) reported a decrease of 90% of chlorogenic acid 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.

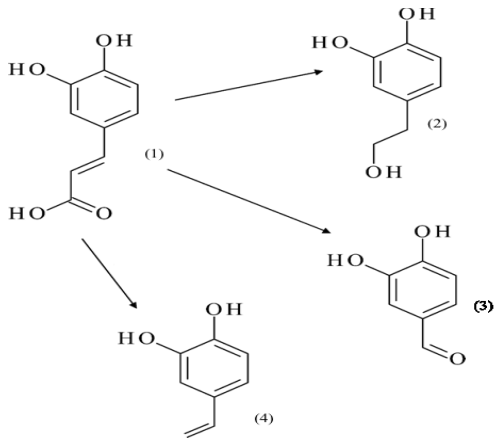


Figure 3.5 Proposed pathway of caffeic acid degradation in subcritical water: (1) Caffeic acid, (2) 3,4-dihydroxyphenylethanol, (3) 3,4-dihydroxybenzaldehyde, and (4) 2-hydroxy-4-vinylphenol. (Adapted from Khuwijitjaru et al., 2014).

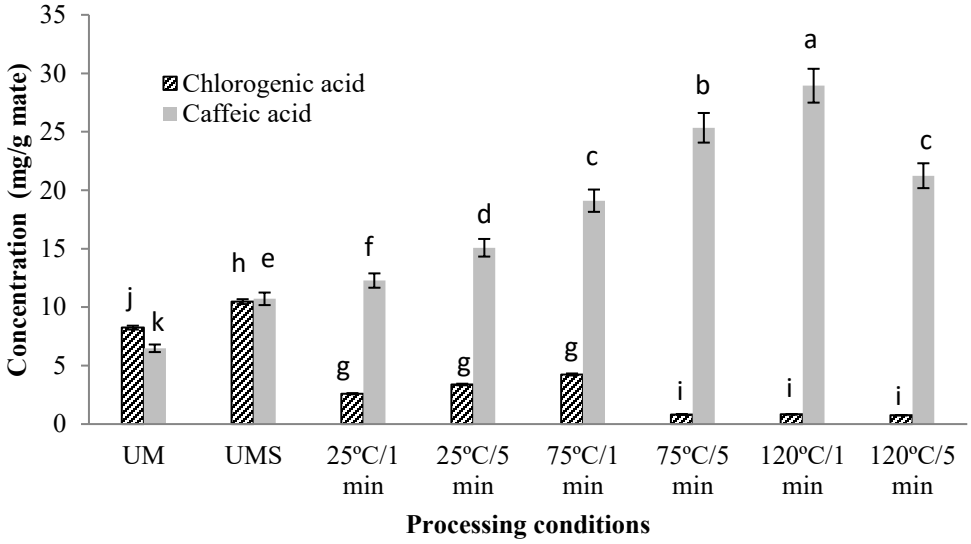


Figure 3.6 Chlorogenic acid and caffeic acid (mg/g mate) of untreated mate (UM) and treated mate at 600 MPa.

Table 3.2 show chlorogenic acid, caffeic acid, quercetin, rutin, catechin, gallocatechin gallate, and epigallocatechin concentrations of untreated and treated mate extract at 10-600 MPa/25-120ºC/1-5 min. The quercetin content on untreated sample (1.25±0.06 mg/g mate) at 10 MPa/25ºC/1 min increased by 42% (2.18±0.11 mg/g) while the highest concentration of quercetin (5.36±0.27 mg/g) was achieved at 100 MPa/120ºC/1 min (Table A.5, Appendix A). These results are in agreement with total antioxidant activity values of treated mate reported in this study (Fig A.1a, Appendix A), where the maximum value of total antioxidant activity (400% increase) was achived at 100 MPa/120 ºC/1 min. A significant slighly decrease of quercetin concentration was found on treated mate at 100 MPa/120ºC/5 min (2.596±0.130 mg/g), which is in agreement with a decrease of total antioxidant activity on treated mate samples at 100 MPa/120ºC/5 min. Similarly, a slightly decrease of chlorogenic acid was observed at 100 MPa/120 ºC/5 min while only traces of gallocatechin gallate and catechin were obtained at 100 MPa/120 ºC/5 min (Table 3.2). This

behaviour can be attributed to the formation of decomposition products with lower antioxidant capacity compared to the original sample, exerting pro-oxidant activity (Andueza et al., 2009).

Studies reported the effect of PATP on quercetin in different food matrices. Roldan-Marin et al. (2009) investigated the effect of 100 MPa/50ºC/5 min on onion, reporting an increase of 18% of quercetin on treated onion compared to untreated samples. The increase of quercetin after PATP treatment could be associated to the disruption of the vegetative vacuoles and cell wall membrane, where quercetin is confined.

Rutin content of untreated and treated mate (Table 3.2 and Table A.5, Appendix A) indicated that rutin remained the same during PATP treatment at 10 MPa/25ºC/1-5 min and 120ºC/10 MPa/1-5 min compared to untreated mate (0.28±0.01 mg/g). The melting point of rutin is 242ºC. However, the highest increase of rutin concentration was observed at 100 MPa/120ºC/1-5 min (0.36 ± 0.02-0.38 ± 0.02 mg/g) and 600 MPa/120ºC/1 min (0.38 ± 0.02 mg/g). Similarly, Isolabella et al. (2010) studied the variation of rutin during mate *Ilex paraguarensis* processing, reporting an increase in rutin content in the roasting stage at 250ºC for 2-4 min, while no significant difference on rutin content was observed in the drying process (90-110ºC/3-6 h) compared to untreated mate.

Catechin, gallocatechin gallate and epigallocatechin were identified on untreated and treated mate samples (Table 3.2 and Table A.6, Appendix A). The concentrations of catechin, gallocatechin gallate, and epigallocatechin on untreated mate were 0.35 ± 0.02 mg/g, 0.59 ±0.03 mg/g and 0.15 ± 0.01 mg/g, respectively. It can be observed that the concentration of catechin significantly increased after 10-600 MPa/25ºC/1-5 min compared to untreated mate (pH of 5.8). However, catechin was not detected at 100 MPa/75ºC/1-5 min and 10-600 MPa/120ºC/1-5 min. Thermal stability of catechin was studied on Longjing green tea and Nestle green tea powder, reporting that catechin is heat and pH sensitive (Li et al., 2012; Chen et al., 2001). Catechin on

Nestle green tea powder was more stable at pH levels from 4.0 to 5.2 at 120ºC, while degradation was accelerated at pH above 5.2 and pH levels below 4.0. No significant difference was observed on catechin concentration of Longjing green tea at 37ºC for 7 h. However, Longjing green tea-catechin was degraded about 20% and 24% after thermal treatment at 98ºC/pH 3-4 for 7 h and 120ºC pH 3-4 for 20 min, respectively, while 80% of catechin was lost after thermal treatment at 120ºC for 20 min at pH 6.0 (Chen et al., 2001).

No significant difference was found on gallocatechin gallate concentrations of treated mate at 10-600 MPa/25ºC/1-5 min compared to untreated mate (0.59±0.03 mg/g) and only traces were observed for treated mate at 10 MPa/120ºC/5min and 100 MPa/120ºC/1-5min. However, the concentration of gallocatechin gallate significanly increased on treated mate at 600 MPa/75ºC/5 min and at 600 MPa/120ºC/1-5min. This result can be attributed to the epimerization of epigallocatechin gallate into gallocatechin gallate as demostrated by Chen et al. (2001). They investigated the degradation of green tea catechins in tea drinks, reporting that when Longjing green tea was thermally treated at 80ºC, 90ºC and 120ºC in aqueous solution, gallocatechin gallate (GCG) concentration was gradually increasing with heating where epigallocatechin gallate is epimerizated to gallocatechin gallate at high temperatures of 80-120ºC. However, gallocatechin gallate formation was more efficient at pH 5 when Longjing green tea was autoclaved at 120ºC for 20 min.

Epigallocatechin concentration significantly increased after PATP treatment at all conditions evaluated, compared to the untreated mate, with the exception of treated mate at 600 MPa/25ºC/5 min and 10-100MPa/120ºC/1-5 min in which no significant difference was observed compared to untreated mate (Table 3.2 and Table A.6, Appendix A). Pressure, temperature, and holding time had a significant effect (p-value<0.001) on the epigallocatechin concentration of treated mate. The

increase of holding time from 1 to 5 min resulted in significant increase (11%) (p-value<0.001) of epigallocatechin on treated mate samples (Fig A.6, Appendix A). These results are in agreement with those reported by Labbe et al. (2006) who investigated the effect of temperature and time on epigallocatechin on green tea. They observed that epigallocatechin increased by 29% with the increase of time from 5 to 20 min at 70ºC. Sharma et al. (2005) reported that green tea catechins, especially epicatechin gallate showed higher amounts at 100ºC than the infusions at 80ºC. These results suggested that catechins on treated mate had different behavior. Thus, it is possible to target specific catechin types in the extracts by adjusting pressure, temperature and holding time.

Table 3.2 Individual phenolics of PATP-treated mate at different processing conditions.

Processing conditions		Caffeic acid (mg/g mate)	Chlorogenic acid (mg/g mate)	Quercetin (mg/g mate)	Rutin (mg/g mate)	Catechin (mg/g mate)	Gallocatechin gallate (mg/g mate)	Epigallocatechin (mg/g mate)
25ºC	Untreated mate	6.48 ± 0.32^{f}	8.25 ± 0.41^{a}	1.25 ± 0.06^{a}	0.28 ± 0.01^{a}	0.36 ± 0.02^{a}	0.59 ± 0.02^{a}	0.15 ± 0.01^{a}
	10 MPa/1 min	17.80 ± 0.89^{d}	0.72 ± 0.52^{b}	2.18 ± 0.11^{b}	0.30 ± 0.02^{a}	0.73 ± 0.04^{b}	0.60 ± 0.03^{a}	0.17 ± 0.01^{b}
	10 MPa/5 min	23.45 ± 1.17^{d}	0.80 ± 0.04^{b}	3.41 ± 0.17^{c}	0.31 ± 0.06^{a}	0.91 ± 0.05^{c}	0.62 ± 0.03^{a}	0.22 ± 0.02^{c}
	100 MPa/1 min	17.93 ± 0.90^{d}	3.88 ± 0.19^{c}	3.41 ± 0.17^{c}	0.32 ± 0.06^{b}	0.94 ± 0.05^{c}	0.62 ± 0.03^{a}	0.37 ± 0.03^{d}
	100 MPa/5 min	18.47 ± 0.92^{c}	3.98 ± 0.20^{c}	3.11 ± 0.16^{c}	0.32 ± 0.02^{b}	0.97 ± 0.05^{c}	0.64 ± 0.03^{a}	0.38 ± 0.03^{d}
	600 MPa/1 min	12.28 ± 0.61^{e}	2.60 ± 0.13^{d}	2.57 ± 0.13^{b}	0.34 ± 0.02^{b}	0.62 ± 0.03^{d}	0.59 ± 0.03^{a}	0.15 ± 0.01^{a}
	600 MPa/5 min	15.08 ± 0.75^{e}	3.39 ± 0.17^{e}	3.11 ± 0.16^{c}	0.33 ± 0.02^{b}	0.76 ± 0.04b	0.60 ± 0.03^{a}	0.19 ± 0.02^{b}
75ºC	10 MPa/1 min	16.40 ± 0.82e^{d}	3.78 ± 0.19^{c}	3.31 ± 0.17^{c}	0.32 ± 0.02^{b}	0.16 ± 0.01^{e}	0.62 ± 0.03^{a}	0.34 ± 0.03^{d}
	10 MPa/5 min	17.79 ± 0.89^{d}	4.09 ± 0.20^{c}	3.50 ± 0.17^{c}	0.33 ± 0.02^{b}	0.99 ± 0.05^{c}	0.63 ± 0.03^{a}	0.39 ± 0.02^{d}
	100 MPa/1 min	17.76 ± 0.89^{d}	3.95 ± 0.20^{c}	3.53 ± 0.18^{c}	0.33 ± 0.02^{b}	traces	0.62 ± 0.03^{a}	0.31 ± 0.03^{d}
	100 MPa/5 min	17.57 ± 0.88^{d}	3.78 ± 0.19^{c}	3.56 ± 0.18^{c}	0.32 ± 0.02^{b}	0.88 ± 0.04^{c}	0.62 ± 0.03^{a}	0.34 ± 0.02^{d}
	600 MPa/1 min	19.11 ± 0.95^{c}	4.24 ± 0.21^{c}	3.55 ± 0.18^{c}	0.32 ± 0.02^{b}	1.00 ± 0.05^{c}	0.63 ± 0.03^{a}	0.26 ± 0.01^{c}
	600 MPa/5 min	25.34 ± 1.27^{b}	0.82 ± 0.04^{b}	3.55 ± 0.18^{c}	0.30 ± 0.02^{b}	traces	0.81 ± 0.04^{b}	0.34 ± 0.01^{d}
120ºC	10 MPa/1 min	15.12 ± 0.76^{e}	0.68± 0.03^{f}	1.70 ± 0.09^{a}	0.29 ± 0.01^{ab}	traces	0.67 ± 0.033^{a}	0.21 ± 0.03^{b}
	10 MPa/5 min	17.46 ± 0.87^{d}	3.90 ± 0.20^{c}	4.16 ± 0.21^{d}	0.28 ± 0.01^{ab}	traces	traces	0.18 ± 0.03^{ab}
	100 MPa/1 min	18.96 ± 0.95^{c}	4.07 ± 0.20^{c}	5.36 ± 0.27^{e}	0.38 ± 0.02^{c}	traces	traces	0.18 ± 0.02^{ab}
	100 MPa/5 min	25.05 ± 1.25^{b}	2.60 ± 0.13^{d}	2.45 ± 0.12^{b}	0.36 ± 0.02^{c}	traces	traces	0.36 ± 0.02^{d}
	600 MPa/1 min	28.94 ± 1.45^{a}	0.83 ± 0.04^{b}	3.08 ± 0.154^{c}	0.38 ± 0.02^{c}	traces	0.94 ± 0.05^{c}	0.43 ± 0.02^{e}
	600 MPa/5 min	21.24 ± 1.06^{c}	0.76 ± 0.04^{b}	2.00 ± 0.100^{b}	0.34 ± 0.02^{bc}	traces	0.83 ± 0.04^{b}	0.30 ± 0.03^{bd}

$^{a-f}$ Different lowercase letters in the same column indicate significant differences ($p < 0.05$).

3.3.4 Methylxanthines content in mate after PATP treatment

Table 3.3 and Table A.7, Appendix A show the content of caffeine and theobromine of untreated and treated mate at 10-600 MPa/120ºC/1 min. The effect of PATP on caffeine and theobromine of mate was studied at holding time of 1 min based on a previous study, in which a holding time of 5 min had not significant effect on caffeine and theobromine of PATP treated mate (Saldaña et al., 2015). Theophylline concentration was not detected by HPLC on untreated and treated mate. The concentrations of caffeine and theobromine of untreated mate were 22.05±2.85mg/g and 15.29±2.02 mg/g, respectively. No significant difference was observed on caffeine content of treated mate at 600 MPa/120ºC/1 min compared to caffeine content of untreated mate. However, a significant increase of caffeine concentration was observed at 10-100 MPa/120ºC/1 min on treated mate extract. The content of theobromine on treated mate extract increased significantly at 100 MPa/120ºC/1 min (17.9±3.3 mg/g) compared to untreated mate (15.29±2.02 mg/g). However, no significant difference was observed on treated mate at 10 MPa/120ºC/1 min and 600 MPa/120ºC/1 min compared to untreated mate.

Various studies have investigated the extraction of methylxanthines from mate leaves using different extraction techniques. Saldaña et al. (2002) used supercritical CO_2 (40-70°C/40 MPa/5.7 g min^{-1} of water-saturated supercritical CO_2/400 min) to extract 96% and 71% of caffeine at 40ºC and 70ºC, respectively. Fernandes et al. (2017) investigated the extraction of caffeine and theobromine from mate *Ilex paraguariensis* using supercritical CO_2 at 20 MPa/45ºC/6 h obtaining 163.28 mg/g of caffeine and 2.45 mg/g of theobromine. Riachi et al. (2018) studied the effect of roasting temperature and time on caffeine content of mate, reporting no significant difference on caffeine concentration at 180ºC/10 min compared to caffeine concentration on untreated samples (13.4 mg/g). However, a significant decrease on caffeine content (74%) was obtained on treated

mate at 240ºC/10-40 min, the authors explained that partial sublimation of the caffeine might occur when the degree of roasting is intense (≥240ºC), suggesting that caffeine degradation on mate could be related to this phase change. Jun et al. (2009) studied the effect of high-pressure processing on the extraction of caffeine from green tea leaves at 100-600 MPa/25ºC/1-10 min using different solvents (acetone, methanol, ethanol and water). They found that the increase of pressure from 100 MPa/1 min to 600 MPa/1 min caused an increase of caffeine yield extraction by 4.2±0.2% because high pressure processing induces the reduction of volume causing an increase of solvent into the cells and releasing caffeine into the solvent.

Table 3.3 Methylxanthines in untreated and treated mate at 120ºC.

Processing conditions	Caffeine (mg/g mate)	Theobromine (mg/g mate)
Untreated mate	22.05 ± 2.85^{c}	15.29± 2.02^{b}
10 MPa/1 min	24.64 ± 0.99^{cb}	14.96 ± 0.41^{b}
100 MPa/1 min	35.85 ± 2.59^{a}	23.99 ± 0.02^{a}
600 MPa/1 min	21.15 ± 1.70^{c}	17.90 ± 3.30^{b}
Melting point (ºC)	223-225	375
Molecular weight (g/mol)	180.16	180.17
Boiling point (ºC)	178	290-295

$^{a-c}$Different lowercase letters in the same column indicate significant differences ($p < 0.05$)

3.3.5 Scanning electron microscope (SEM) images of untreated and PATP treated mate

Fig. 3.7 shows SEM images of untreated and treated mate. A compact and dense structure was observed for untreated mate (Fig. 3.7a). When mate samples were treated at 100 MPa/120ºC and 600 MPa/120ºC for 5 min, a change in the granule structure was observed compared to untreated mate (Fig. 3.7b,c). A clear damage, deformation and disintegration of the granule structure caused by PATP treatment was observed in both processing conditions (Fig. 3.7b,c). These changes in the structure of the cells can be related to the movement of water

from inside to outside the cell due to the changes on cell permeability during processing. Prestamo and Arroyo (1998) reported the structural changes occurred on cauliflower and spinach at 400MPa/5ºC for 30 min using SEM. They observed deformation and cavities of the treated samples compared to a rigid and well-maintained cell structure of untreated sample. These changes were attributed to a mass-transfer of molecules such as ions, sugar, amino acids, causing a collapse of the cell membrane.

The cell structure of treated mate at 600 MPa had various cavities, big deformation, and cell damage compared to treated mate at 100 MPa. This behaviour can be attributed to different depressurization rates after PATP treatment (600 MPa with 8.7 MPa/s versus 100 MPa with 4.5 MPa/s, Fig 3.2, Chapter 3). Earlier Goñi et al. (2016) investigated the effect of pressures of 10, 12 and 15 MPa and depressurization rates of 0.5, 1 and 5 MPa/min on impregnation yield of eugenol on low density polyethylene films using supercritical CO_2, where a fast depressurization of 5 MPa/min produced mechanical damages to the films.

These structural changes observed on treated mate can explain the increase of total antioxidant activity and total phenolics at high pressure and temperature conditions, associated to the release of intracellular compounds to the solvent and inactivation of enzymes (Chourio et al., 2018).

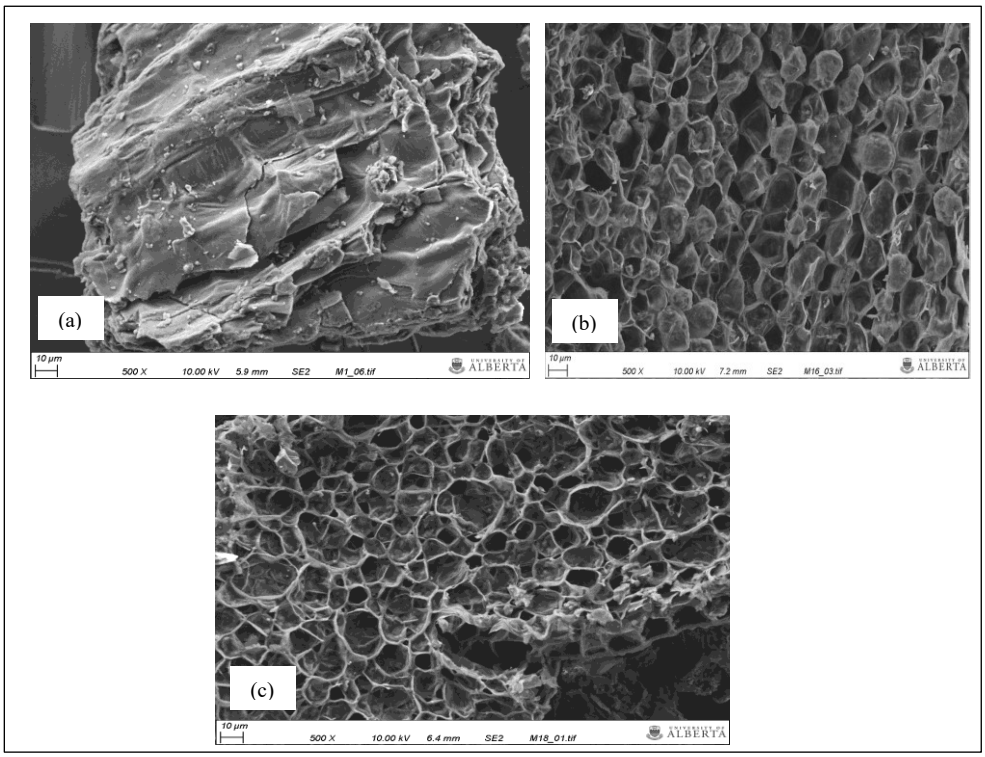


Figure 3.7 Scanning electron microscope images of (a) untreated mate; and treated mate at (b) 100 MPa/120ºC/5 min; and (c) 600 MPa/120ºC/5 min.

3.4 CONCLUSIONS

The effect of PATP on the extraction of total phenolics, individual phenolics, methylxanthines, catechins and antioxidant activity of mate was investigated for the first time. Total phenolic and total antioxidant activity content on treated mate were significantly influenced by temperature (p-value ≤0.001), pressure (p-value ≤0.001) and holding time (p-value ≤0.006). However, temperature had the most influence on the increase of antioxidant activity, total phenolic content while increase of holding time affected negatively the release of total phenolic content. The best PATP condition to obtain the highest total phenolic content (148±7 mg GAE/g) was 600 MPa/120 ºC/1 min. Also, the highest content of caffeic acid (28.94±1.45 mg/g mate) was obtained at this condition. The highest concentrations of caffeine (35.9±2.6 mg/g mate), theobromine (23.99±0.02 mg/g mate) and quercetin (5.36±0.27 mg/g mate) were obtained on treated mate

extracts at 100MPa/120ºC/1 min. Catechin (0.36±0.02 mg/g mate), gallocatechin gallate (0.59±0.03) mg/g mate), and epigallocatechin (0.15±0.01 mg/g mate) were the main catechins identified in mate extracts. The highest concentration of epigallocatechin (0.43 ± 0.02 mg/g) on the treated extract was obtained at 600 MPa/120ºC/1 min. The SEM images showed the effect of pressure (100-600MPa) and temperature(120ºC) on the cell structure of mate, PATP-treated mate residue at 600MPa/120ºC/1 min had bigger cavities than treated mate at 600MPa/100ºC/1 min. This study showed that PATP has the potential to be a promising alternative to conventional treatment to enhance the release of bioactive compounds and the development of ready-to drink beverages with health properties.

3.5 RECOMMENDATION

- Study of pH change on mate sample is recommended for a better understanding of stability of phenolic compounds of mate during PATP treatment.

ACKNOWLEDGMENT

The authors thank to Natural Sciences and Engineering Research Council of Canada (NSERC, #05356-2014) for the financial support of this research.

Chapter 4: Effect of Pressure Assisted Thermal Processing on Bioactive Compounds and Antioxidant Activity of *Stevia* and Mate Sweetened with *Stevia.*

ABSTRACT

The effect of PATP was studied on bioactive compounds and antioxidant activity of *Stevia* leaves and mate sweetened with *Stevia* leaves and powder (2.5%w/v). The effect of temperature (25-120ºC), pressure (10-600 MPa), and holding time (1-5 min) was evaluated on total antioxidant activity and total phenolics of *Stevia*. The highest total antioxidant activity value was obtained at 600MPa/120ºC/1 min (429.06 mg $FeSO_4.7H_20$/g of *Stevia*). Temperature significantly favoured the extraction of phenolics compounds and total antioxidant activity of PATP treated *Stevia* samples. Then, a positive correlation was found between total phenolics and antioxidant activity of PATP treated Stevia (R>0.90). Also, PATP conditions of 10-600MPa/120ºC/1-5min resulted in a complete inactivation of PPO and POD enzymes of *Stevia* leaves. The POD enzyme was more resistant to PATP treatment than PPO enzyme. Moreover, the addition of Stevia to mate samples resulted in an increase up to 88% of total phenolics and up to 74% of total antioxidant activity. However, treated mate sweetened with stevia had lower total antioxidant activity and total phenolics than treated mate at all conditions evaluated. Pure compounds of model systems were also studied under PATP conditions of 10-300MPa/25-75ºC/1-5 min. However, the variables evaluated, temperature, pressure and holding time did not cause a significant change on concentration of treated rutin, quercetin, chlorogenic acid and caffeic acid at 10-300MPa/25-75ºC/1-5 min. Overall, it suggested a combination of PATP conditions of 120ºC/100 MPa/5 min to obtain mate+*Stevia* beverage with high content of total phenolics and total antioxidant activity, which ensures complete inactivation of PPO and POD enzymes.

Keywords: Emerging technology, enzymes, peroxidase, polyphenoloxidase, phenolics, *Stevia rebaudiana*.

4.1 INTRODUCTION

Mate tea is sometimes consumed with additives like sucralose. However, it has been demonstrated that high intake of sucralose can lead to chronic diseases, including type-2 diabetes and obesity (Perez-Ramirez et al., 2015). *Stevia rebaudiana,* a non-caloric sweetener, approximately 100-300 times sweeter than sucrose, is an alternative to reduce sugar consumption (Carbonell-Capella et al., 2015). Epidemiological studies have reported that products sweetened with *Stevia* have the potential of managing type-2 diabetes (Gardana et al., 2010). Glycosides of *Stevia* leaves include stevioside (81.2±9.3 mg/g), rebaudioside A (3.5±0.3 mg/g) and rebaudioside C (3.8±0.3 mg/g) (Periche et al., 2015). The health benefits of *Stevia* are mainly attributed to its carbohydrates (52% d.b), protein (10% d.b), and crude fibre (18% d.b) contents (Jacques et al., 2008). Some studies have evaluated the effect of *Stevia* addition on fruit and tea beverages. *Stevia* addition on Roselle *Hibiscus sabdariffa L* beverage increased the stability of quercetin, gallic acid and rosmarinic acid during storage for 70 days at 20ºC (Perez-Ramirez et al., 2015). But, further research is needed to understand the stabilizing effect of *Stevia* on individual phenolic compounds during storage The addition of *Stevia rebaudiana* (1.3-2.5% w/w) in fruit-orange-oat beverage increased the release of total phenolic content, total antioxidant capacity, total carotenoids and total anthocyanins (Corrales et al., 2008) while the use of milk, sugar or honey significantly decreased antioxidant activity of Kenyan teas (Korir et al., 2014). *Stevia* leaves have enzymes such peroxidase (POD) and polyphenoloxidase (PPO), which can degrade polyphenols and affect antioxidant activity and organoleptic properties of fruit and vegetables, therefore the inactivation of POD and PPO is required (Hendrickx et al., 1998). Conventional processes to inactive POD and PPO are based on thermal and chemical treatment. However, the use of high temperatures and chemical additives might affect product quality and nutritional value of the final product. Pressure

assisted thermal processing (PATP) 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 PATP treatment on the relative activity of POD and PPO enzymes in different food matrices such as strawberry treated at 100-690MPa/24-90ºC/5-15 min (Terefe et al., 2010), mushroom treated at 800-1600MPa/20ºC/1 min (Yi et al., 2012), pear treated at 600MPa/64ºC/15 min (Sulaiman et al., 2015), fruit mixture sweetened with *Stevia* treated at 300-500MPa/18-22ºC/5-15 min (Barba et al., 2014), coconut water treated at 200-600MPa/40-90ºC/1-30 min (Chourio et al., 2018), blueberry treated at 100-690MPa/30-90ºC/15 min (Terefe at al., 2017) and spinach treated at 100-500 MPa/20ºC/20 min (Jung et al., 2013). Overall, PATP treatment demonstrated a higher effectiveness in reducing the relative activity of PPO and POD enzymes compared to high pressure processing. In addition, total anthocyanin, total carotenoid, flavonoid, total phenolic contents and antioxidant activity of grape by-products, green tea, and *Stevia rebaudiana* leaves were maintained or increased after PATP compared to untreated samples (Corrales et al., 2008 and Carbonell-Capella et al., 2013). However, no data is available on the effect of PATP on *Stevia* leaves and mate sweetened with *Stevia.* Therefore, the objective of this study was to investigate the effect of PATP on total antioxidant activity, total phenolic content, and specific bioactive compounds of *Stevia* leaves and mate sweetened with *Stevia* leaves. Inactivation of PPO and POD enzymes of *Stevia* leaves treated by PATP was also studied.

4.2 MATERIALS AND METHODS

4.2.1 Sample preparation

Commercial *Stevia* powder, SweetLeaf Stevia®, was obtained at "Paraiso Tropical" Latin food market (Edmonton, AB, Canada) and stored at 4ºC for further use. *Stevia rebaudiana* plant was

purchased at a local garden store center, Creekside Home and Garden (Edmonton, AB, Canada). Fresh *Stevia* leaves were dried with a hot air oven (Model 655G, Fisher Scientific Isotherm oven, Toronto, ON, Canada) pre-heated at 40ºC for 24 h. Dried leaves were then ground and sieved to a mean particle size of 500 µm and stored at 4ºC for further use.

4.2.2 Chemicals

Chemicals such as (−) epigallocatechin (≥95% purity), (−), (−)epicatechin gallate (≥98% purity), catechin (≥98% purity) chlorogenic acid (≥95% purity), caffeic acid (≥98% purity), rutin hydrate (≥98% purity), ABTS (2,2 azino-bis 3–ethylbenzothiazoline-6-sulfonic acid) (≥98% purity), pyrocatechol (≥99% purity), caffeine (≥99% purity), theobromine (≥98% purity), theophylline (≥98% purity), Fe_2SO_4 (≥98% purity), tripyridyltriazine, and quercetin (≥95% purity) were purchased from Sigma-Aldrich (Oakville, ON, Canada).

Chemical reagents, such as sodium hydroxide pellets (≥97%, ACS grade), Folin-Ciocalteu phenol reagent (2M) and petroleum ether were purchased from Fisher Scientific Co (Toronto, ON, Canada).

4.2.3 Proximate compositional analysis

Moisture, ash, protein, total fat and total carbohydrates content of *Stevia* were determined using the same methods described in Chapter 3, Section 3.2.3.

4.2.4 Pressure Assisted Thermal Processing (PATP)

The high pressure processing (HPP) used to treat mate sweetened with *Stevia* and *Stevia* samples was the same as described in Section 3.4.1 in Chapter 3. Mate samples (1:20 w/v) were sweetened with 2.5% (w/v) *Stevia* and treated at 10, 100 and 600 MPa and 25, 75, and 120ºC for 1 and 5 min. The *Stevia* concentration (2.5% w/v) was selected based on the literature in which sweetness of *Stevia* (300 times sweeter than sucrose) was equivalent to sucrose concentration of

commercial beverages (7-26 ºBrix) (Barba et al., 2014., Carbonell-Capella et al., 2013., Criado et al., 2014).

Stevia samples (2.5% w/v) were treated at 10, 100 and 600 MPa, and 75, and 120ºC for 1 and 5 min. For the pure compounds, solutions of quercetin, rutin, chlorogenic acid and caffeic acid in solvents were individually prepared. Briefly, quercetin or rutin solution (0.30 mg/mL) was prepared using 15.1±0.4 mg of quercetin dissolved in 50 mL of methanol. A chlorogenic acid solution (0.30 mg/mL) was prepared using 15.1±0.4 mg of chlorogenic acid dissolved in distilled water. A caffeic acid solution (0.30 mg/mL) was prepared using 15.1±0.4 mg of caffeic acid in 50 mL of 40% methanol-water. Then, polypropylene tubes (Cryogenic vial, Fisher Scientific, Pittsburgh, PA) of 3 mL were filled with the solution. Samples were pressurized to 100, 300 and 600 MPa at temperatures of 25, 75 and 120ºC with holding times of 1 and 5 min. All 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 -18ºC for further analysis.

4.2.5 Analysis of treated samples

4.2.5.1 Total antioxidant activity

The total antioxidant activity was determined using the FRAP assay described in Section 3.2.5, Chapter 3.

4.2.5.2 Total phenolic content

The Folin-Ciocalteu method was used to determine total phenolic content as described in Section 3.2.5, Chapter 3.

4.2.5.3 Individual bioactive compounds

Individual phenolic compounds such as chlorogenic acid, quercetin, caffeic acid and rutin were analyzed using a Shimadzu 20 HPLC (Shimadzu, Kyoto, Japan) as described in Section 3.3.3, Chapter 3.

4.2.5.4 Enzyme activity

The peroxidase (POD) activity was determined using a UV-VIS spectrophotometer (Jenway 6320D, Stanford, United Kingdom) according to the method described by Matsui et al. (2008), with slight modifications. A test tube containing 3.5 mL of buffer ($Na_2HPO_4 \cdot 2H_2O + KH_2PO_4$, pH 6), 0.4 mL of ABTS (2.2 azino-bis 3–ethylbenzothiazoline-6-sulfonic acid) solution (0.02 mol/L) and 0.4 mL of hydrogen peroxide (0.1% v/v) was placed in a water bath at 25 °C for 5 min. Then, 1 mL of *Stevia* extract was added to this solution and transferred to a cuvette where the absorbance of the solution was measured at 405 nm and recorded every 20 s for 5 min. The reference value of the POD was determined using a blank solution containing 3.5 mL of buffer ($Na_2HPO_4 \cdot 2H_2O + KH_2PO_4$, pH 6.0), 0.4 mL of ABTS solution (0.02 mol/L), 0.4 mL of hydrogen peroxide (0.1% v/v) and 1 mL of distilled water.

The polyphenol oxidase (PPO) activity was measured using the same UV-VIS spectrophotometer according to the method described by Matsui et al. (2008), with slight modifications. A test tube containing 2.25 mL of sodium phosphate buffer (0.2 mol/L, pH 6.0) and 0.75 mL of 0.2 mol/L pyrocatechol solution was placed in a water bath at 25 °C for 5 min. *Stevia* extract (1 mL) was added to this solution and the absorbance was measured at 425 nm and recorded every 10 s for 5 min. The reference value of PPO was determined using a blank solution containing 0.75 mL of pyrocatechol, 2.25 mL of sodium phosphate buffer (0.2 mol/L, pH 6) and 1 mL of distilled water. All POD and PPO analyses were carried out at least in triplicate.

For both enzymes, absorbance was plotted against time and the values of enzymatic activity were calculated from the slope of the initial linear part of the curves following the method reported by Matsui et al. (2008).

The relative activity in *Stevia* leaves extract was determined using equation (4.1):

$$\text{Relative activity of enzyme} = (A_t/A_o) \times 100\ \% \quad (4.1)$$

where, A_t is the mean of the enzyme activity after PATP treatment at specific processing conditions, and A_o is the mean of the initial enzyme activity before the PATP treatment.

All analysis were performed at least in duplicate.

4.2.5.5 Scanning electron microscope

A scanning electron microscope (SEM) (Zeiss Sigma 300 VP-FESEM, Oberkochen, Germany) was used to visualize the structure of untreated *Stevia* extract and PATP treated *Stevia* residue as described in Section 3.5.5, Chapter 3.

4.2.6 Statistical analysis

The influence of parameters, temperature, pressure and time, on total phenolics, total antioxidant activity, pure compounds of model systems and relative activity of enzymes were evaluated by analysis of variance (ANOVA) using Minitab 16® software (Minitab Inc, State Collage, Pennsylvania, USA) with a 95% confidence level ($p \leq 0.05$).

4.3 RESULTS AND DISCUSSION

4.3.1 Proximate composition of *Stevia* leaves and commercial *Stevia* powder

Table 4.1, Table B1 and Table B.2, Appendix B show the proximate compositional analysis and pH of untreated *Stevia rebaudiana* leaves (pH 6.50 ± 0.20) and commercial *Stevia* powder (pH 5.60±0.02). *Stevia rebaudiana* leaves area good source of carbohydrates (80.57 ± 0.16%), protein (6.7 ± 0.01%) and ash (10.90 ± 0.02%). Mishra et al. (2010) reported that *Stevia rebaudiana* leaves

had 52% of carbohydrates, 10% of protein and 11% of minerals. Savita et al. (2004) reported that *Stevia rebaudiana* leaves had 52% of carbohydrates, 11.40% of protein and 10.5% of minerals. The carbohydrate content on the Stevia leaves used in this study were higher than those values reported in the literature due to the analytical method used. Abou-Arab et al. (2010) identified essential amino acids on *Stevia* leaves, including arginine (0.45 g/100 g d.b), lysine (0.70 g/100 g d.b), histidine (1.13 g/100 g d.b), phenylalanine (0.77 g/100 g d.b), leucine (0.98 g/100 g d.b), methionine (1.45 g/100 g d.b), valine (0.64 g/100 g d.b), threonine (1.13 g/100 g d.b) and isoleucine (0.42 g/100 g d.b). The minerals identified on *Stevia rebaudiana* include calcium (464-1550 mg/100 g), phosphorous (11-350 mg/100 g), sodium (89-190 mg/100 g), iron (4-55 mg/100 g), magnesium (349-500 mg/100 g) and zinc (1.5-6.39 mg/100 g) (Mishra et al., 2010; Serio, 2010; Tadhani and Subhash, 2006). *Stevia* used in this study had 1.5±0.01% of lipids. Similarly, the content of lipids on *Stevia* leaves was reported between 1.9 – 5.6% (Mishra et al., 2010; Abou-Arab et al., 2010; Savita et al., 2004). Tadhani and Subhash (2006) identified six types of fatty acids on *Stevia* leaves, including palmitic acid (27.5%), palmitoleic acid (1.3%), stearic acid (1.2%), oleic acid (4.4%), linoleic acid (12.4%), and linolenic acid (21.6%).

Table 4.1. Proximate composition of untreated *Stevia* leaves.

Component	***Stevia rebaudiana* leaves (This study)**	**Commercial *Stevia* powder (This study)**	***Stevia rebaudiana* leaves [(1)]**	***Stevia rebaudiana* leaves [(2)]**	***Stevia rebaudiana* leaves [(3)]**
Total carbohydrates (%)	80.57 ± 0.16	95.51 ± 0.01	52.0	61.9	52.0
Protein (%)	6.70 ± 0.01	0.40 ± 0.01	10.0	11.40	9.80
Lipids (%)	0.20 ± 0.01	0.10 ± 0.01	1.90	3.73	2.50
Moisture (%)	1.67 ± 0.01	3.98 ± 0.05	7.0	5.37	7.0
Ash (%)	10.90 ± 0.02	0.01 ± 0.02	11.0	7.41	10.50
pH	6.50 ± 0.02	5.60 ± 0.02	NR	NR	NR

NR: not reported, [(1)]Mishra et al. (2010), [(2)]Abou-Arab et al. (2010), [(3)]Savita et al. (2004)

4.3.2 Total antioxidant activity and total phenolic content of *Stevia* leaves

Fig. 4.1a-b shows the total antioxidant activity and total phenolic values of untreated and treated *Stevia* leaves at 75-120ºC/100-600MPa for 1-10 min. The total antioxidant activity of treated *Stevia* leaves (198.66- 429.06 mg $FeSO_4.7H_20$/g of *Stevia*) at all conditions evaluated were higher than untreated *Stevia* leaves (110.95 mg $FeSO_4.7H_20$/g of *Stevia*) (Fig B.1 and Table B.3, Appendix B). Temperature had a major effect on total antioxidant activity compared to pressure and holding time. An increase of temperature from 75ºC to 120ºC for 5 min enhanced total antioxidant activity of *Stevia* leaves extract by 14%, 31% and 20% at 10, 100 and 600 MPa, respectively (Fig B.1a, Appendix B). These results are in agreement with the analysis of variance shown in Fig. 4.1a, where the increase of temperature (p-values<0.001) significantly increased mean values of total antioxidant activity. This increase of total antioxidant activity can be associated to the inactivation of enzymes such as peroxidase (POD) and polyphenoloxidase (PPO) responsible for the total antioxidant activity degradation on food products (Chourio et al., 2018).

The total phenolic content of treated and untreated *Stevia* leaves is shown in Fig. 4.1b and Table B.3, Appendix B. The in total phenolic content of treated *Stevia* extract significantly increased (33.82 – 110.35 mg GAE/g) at all conditions evaluated compared to untreated *Stevia* (25.41 mg GAE/g) with the exception of 75ºC/10MPa/1 min in which no significant difference of in total phenolics was found compared to in total phenolics of untreated *Stevia* leaves. The highest total phenolics increase of 77% was obtained at 120ºC/600MPa/5 min (110.35 mg GAE/g) compared to untreated *Stevia* leaves. Similarly, the analysis of variance on total phenolics mean values shown in Fig 4.1b indicated that the increase of temperature (p-values<0.001), pressure (p-values<0.001) and time (p-values<0.001) resulted in a significantly increase of in total phenolics of *Stevia* leaves. Studies have attributed the increase in total phenolics at high pressure and

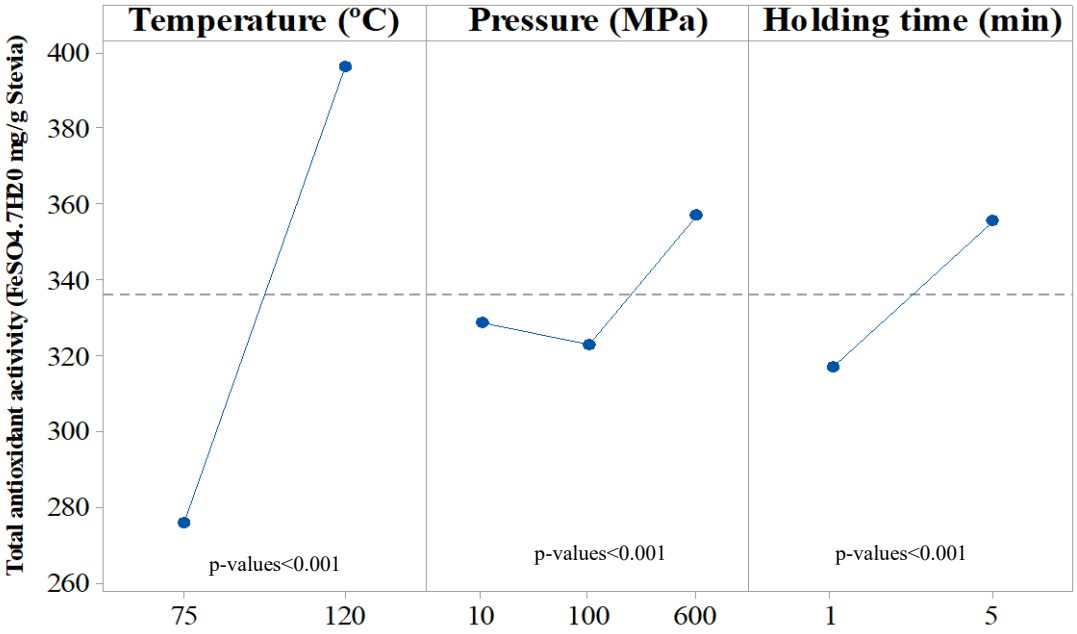
temperature to the decrease in water polarity (low dielectric constant) favouring the solubility of total phenolics at high temperatures.

No studies have investigated the effect of PATP on total antioxidant activity and total phenolics of *Stevia rebaudiana*. However, some studies reported the effect of temperature and pressure on antioxidant activity and total phenolic content of *Stevia* leaves. Kovacevic et al. (2018) investigated the effect of pressurized hot water extraction on in total phenolics and total antioxidant activity of *Stevia rebaudiana* leaves at 100-160ºC/10 MPa/5-10 min, reporting that the highest in total phenolics yield was observed at 160ºC/10MPa/5 min (8.96 mg GAE/g) as compared to 100ºC/10MPa/10 min (5.22 mg GAE/g). Periche et al. (2015) investigated the effect of hot air drying at 100 °C and 180 °C for 3 min on *Stevia* leaves, reporting an increase of total antioxidant activity from 52.92±0.84 to 129 mg equivalent/g and total phenolics from 44.40±1.04 mg GAE/g *Stevia* to 76.8 mg GAE/g *Stevia* using hot air-drying at 180ºC for 3 min. Similarly, Lemus-Mondaca et al. (2012) investigated the effect of drying processes using convective dryer at 30-80ºC, reporting a significant increase in total phenolic content by 91% and total antioxidant activity by 57% at 40ºC compared to the untreated *Stevia* due to the matrix changes occurred during drying, resulting in an increase of phenolic concentration. However, a slight decrease in total phenolics and total antioxidant activity was obtained at temperatures higher or equal to 60ºC that can be associated to the oxidation of phenolic compounds due to active enzymes (Mrad et al., 2012).

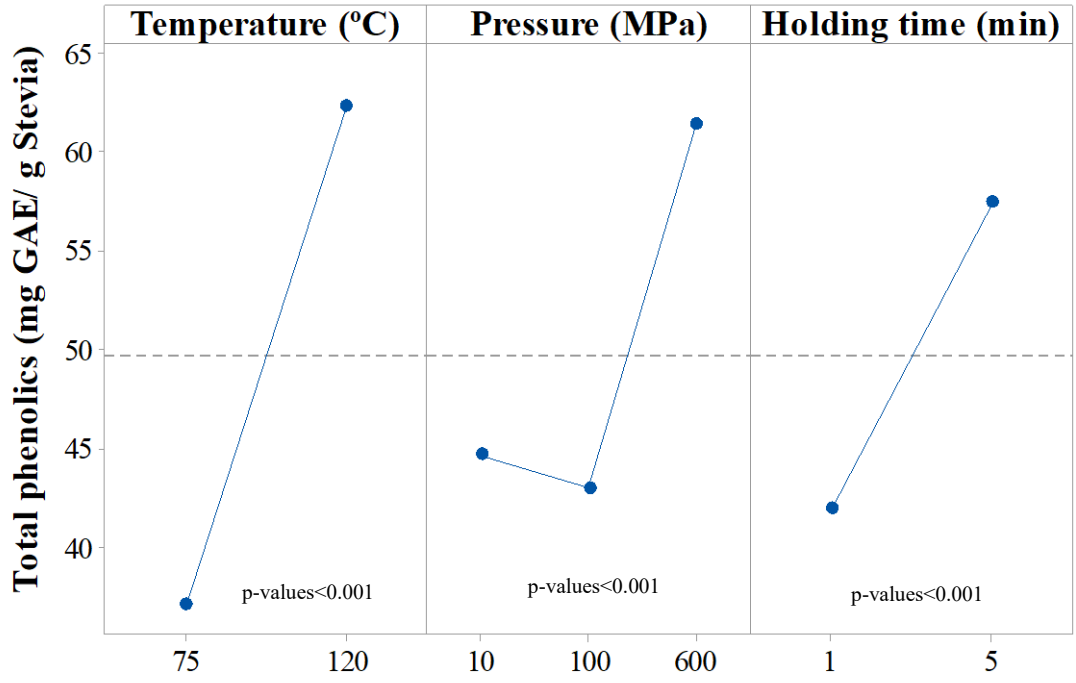
Correlations between total phenolics and total antioxidant activity of treated PATP *Stevia* leaves are shown in Fig. 4.1c. A high positive correlation was R^2=0.92 was found for total phenolic content versus antioxidant activity. Correlations between total phenolics and antioxidant activity have been reported in the literature. Romero-Diaz et al. (2018) reported a good correlation (R>0.90) between total phenolics and total antioxidant activity extracted from aging wine obtained

using ultrasound assisted solid-liquid extraction with different solvents: distilled water, ethanol, acetone and methanol. Doshi et al. (2015) also reported high correlation between total phenolics and total antioxidant activity (R^2=0.915) from grapes *Vitis vinifera L* extracts obtained using 80% methanol-water at 25°C for 12 h.

(a)



(b)



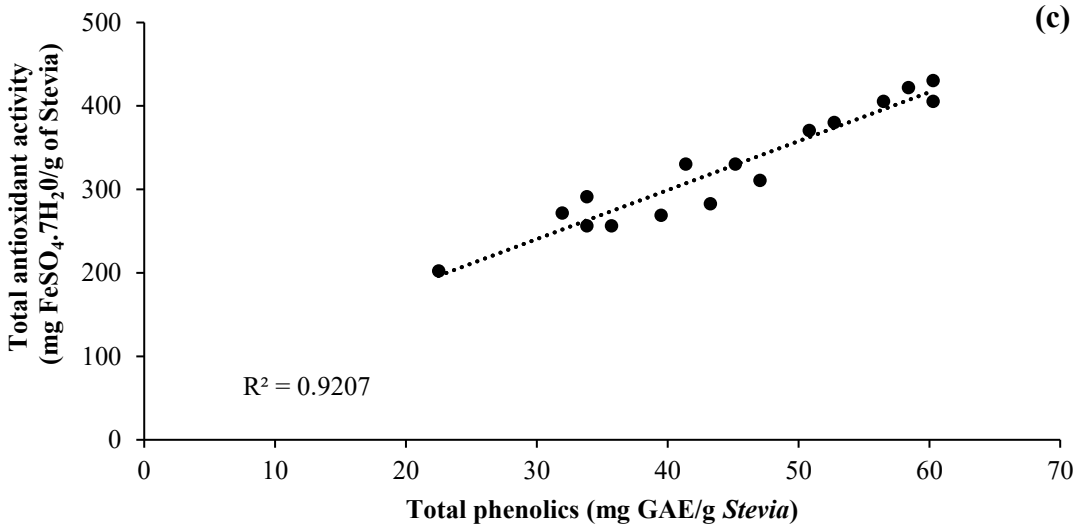


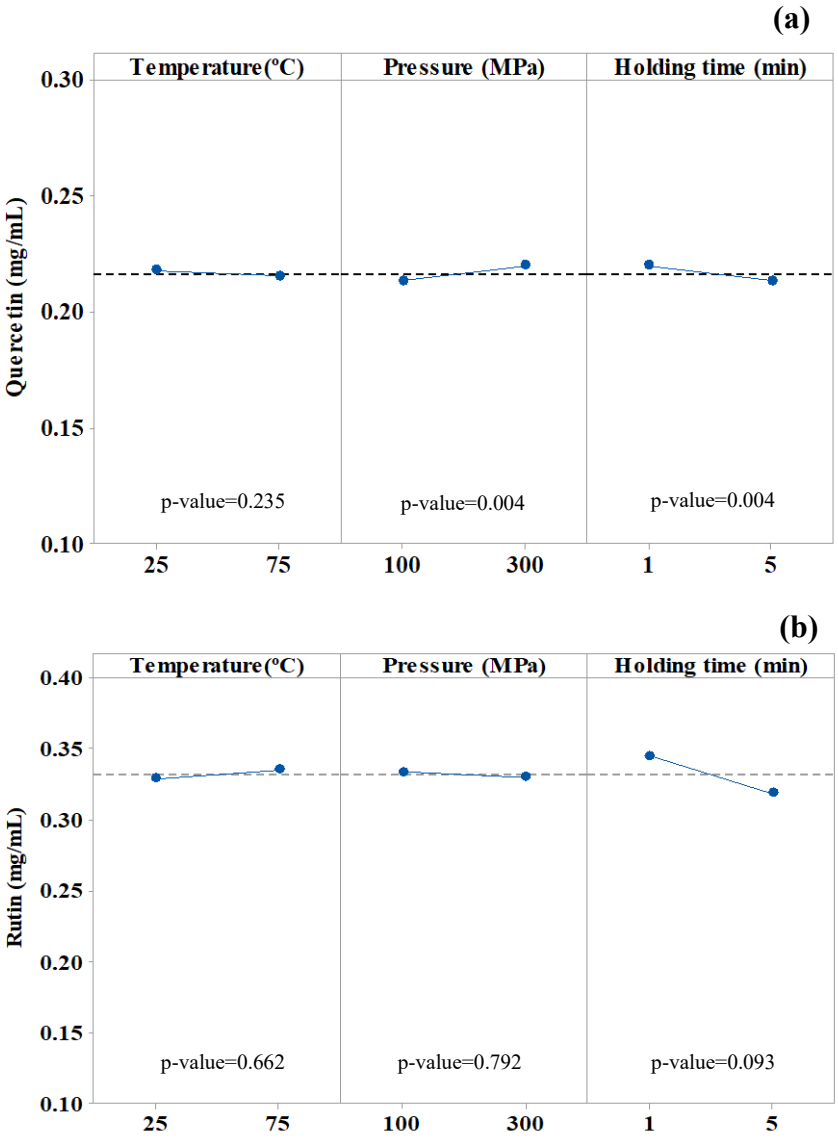
Figure 4.1 Influence of main variables on (a) antioxidant activity and (b) total phenolic content. (c) Correlation between total phenolic content and antioxidant activity of *Stevia*.

4.3.3 Effect of PATP on bioactive compounds of model systems

Pure compounds such as quercetin, rutin, chlorogenic acid and caffeic acid were treated at 100-300 MPa/25-75ºC/1-5 min. The influence of temperature, pressure, and holding time on concentration of quercetin, rutin, chlorogenic acid and caffeic acid are shown in Fig. 4.2a-d and Table B.4, Appendix B. For quercetin, the increase of pressure from 100 to 300 MPa at 75ºC resulted in a significant increase of quercetin concentration while holding time from 1 to 5 min significantly decreased quercetin concentration. Temperature (p-value=0.235) had no significant difference on quercetin (Fig 4.2a). Earlier, Buchner et al. (2006) reported 20% degradation of pure quercetin (1,2 and 5 mM) at pH 5, and complete degradation at pH 8 after heating at 0.1 MPa/100ºC/60 min in aqueous solution. The chemical stability of quercetin can be influenced by pH, temperature, treatment, concentration of other antioxidants compounds and oxygen

concentration (Wang et al., 2016). Sharma et al. (2015) who found no significant difference on quercetin content of different onion varieties (Colossal and Chairman) treated at 80ºC for 30 min compared to untreated samples. Ko et al. (2011) investigated the effectiveness of solid-liquid extraction and subcritical water extraction of quercetin from onion skins. They reported that solid-liquid extraction using methanol and ethanol at 60ºC/2 h showed no significantly difference for quercetin yield while quercetin extracted by subcritical water at 9-13 MPa/100-190ºC/5-30 min resulted in over fourfold higher yield than the solid-liquid extraction method. For rutin, temperature, pressure and holding time had no significant impact on the concentration of rutin (Fig 4.2b). Overall, the treatment conditions evaluated (100-300 MPa/25-75ºC/1-5 min) had no influence on the hydrolysis of rutin into quercetin. No studies have reported the effect of PATP on rutin model solutions. However, various studies investigated its stability of rutin in model solutions and food matrices. Makris and Rossiter (2000) investigated the degradation of rutin (1 mM) and quercetin (1 mM) in phosphate buffer solution, pH 8.0, at 97ºC under oxidative conditions (Fe^{2+} and Cu^{2+}), reporting higher stability of rutin compared to quercetin where the presence of oxygen accelerated the degradation of both flavonols. Ravber et al. (2015) reported the conditions to hydrolyze rutin into quercetin using subcritical water at 0.5-30 MPa/160-205ºC/30 min. They found that rutin (1.08 mg/mL) was hydrolyzed at 21.5MPa/196ºC/12.35 min with the addition of CO_2 (21.5 MPa) to water, obtaining quercetin maximum yield of 89.11±0.55%. Ravber et al. (2015) demonstrated that temperature was the most influential factor on the hydrolysis of rutin into quercetin. For chlorogenic acid, the increase of temperature and time had no significant difference while the increase of pressure from 100 to 300 MPa at 75ºC resulted in a significant decrease (12%) of chlorogenic acid concentration compared to the control (Fig 4.2c). Moreover, the effect of PATP on chlorogenic acid was also studied at 600MPa/120ºC/1-5 min where a

decrease up to 65% was observed compared to untreated sample. Similarly, the effect of temperature, pressure, and time (Fig. 4.2d) had no significant impact on the concentration of caffeic acid. The degradation of chlorogenic acid was greater than caffeic acid, probably because the melting point of chlorogenic acid (207-209^{o}C) is lower than caffeic acid (225^{o}C) (Table 2.3, Chapter 2). Daraee et al. (2019) studied the extraction of chlorogenic acid from sunflower seed kernels using supercritical CO_2 extraction and ethanol as a co-solvent, reporting that the optimum operating conditions to obtain the maximum chlorogenic acid recovery (52.08%) was 40^{o}C/16.9 MPa/104.6 min and CO_2 flow rate of 1.6 mL/min. Overall, chlorogenic acid is the compound less stable based on melting point (Table 2.3, Chapter 2)compared to rutin, quercetin and caffeic acid.



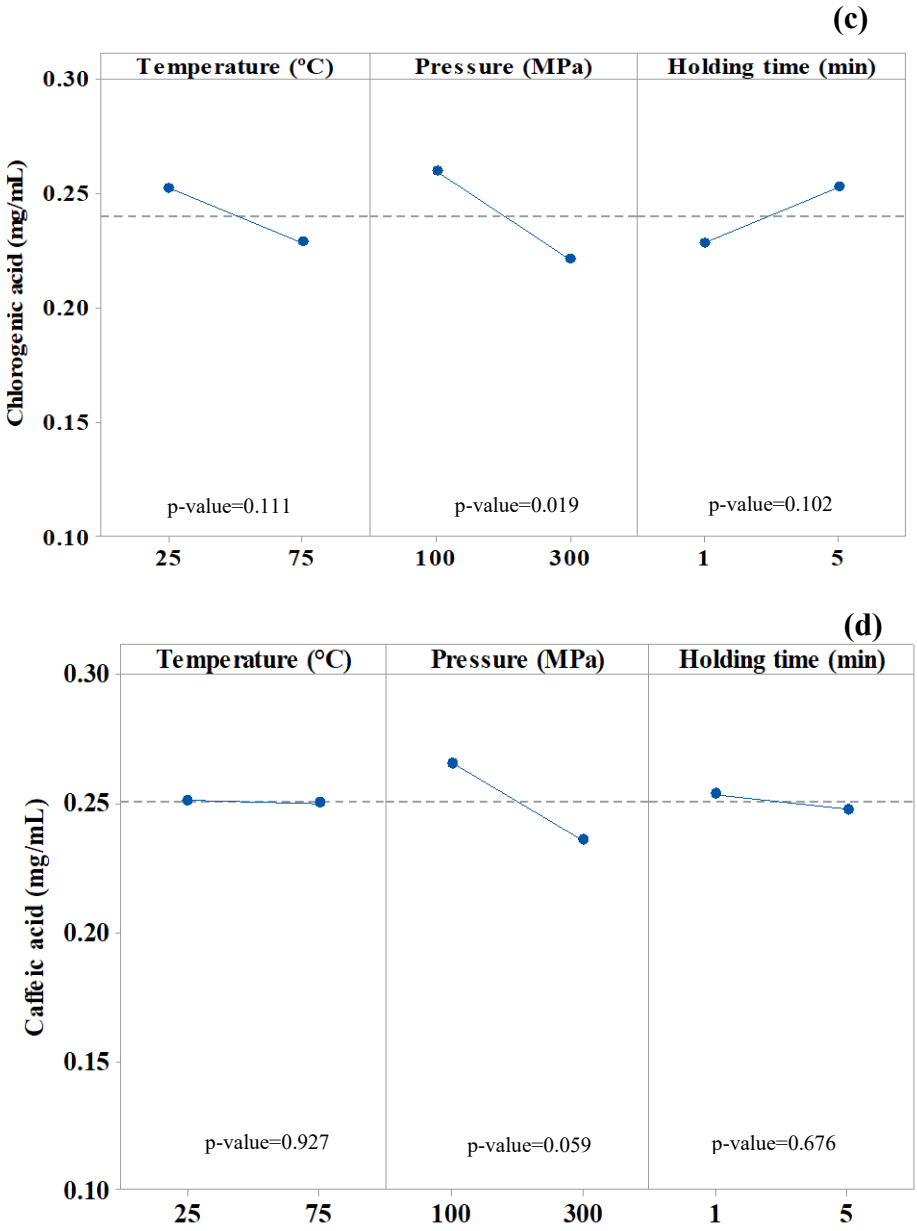


Figure 4.2 Influence of variables, temperature, pressure and time on pure model systems: (a) quercetin, (b) rutin, (c) chlorogenic acid and (d) caffeic acid.

4.3.4 Enzymatic activity of *Stevia* leaves

The effect of PATP on peroxidase (POD) activity of treated *Stevia* samples is shown in Fig. 4.3a and Table B.5, Appendix B. The POD of treated *Stevia* at 75ºC/10-600MPa/1-5 min had a higher enzymatic activity (0.36-0.75) than the treated *Stevia* at 90ºC/10-600MPa/1-5 min (0.03-0.17) (Figure B.2a and Table B.5, Appendix B). The POD activity was reduced up to 99% at 90ºC/10-600MPa/5 min while no POD enzymatic activity was observed at 120ºC/10-600MPa/1-5 min. The increase of temperature from 75 to 120ºC significantly decreased (p-values<0.001) the POD activity (Fig 4.3a). No studies have reported the effect of PATP on the enzymatic activity of *Stevia* POD enzymes, but some studies have evaluated the effect of high pressure and temperature on the activity of POD enzymes in other matrices. Chourio et al. (2018) studied the effect of PATP treatment at 40-120ºC/200-600MPa/1-30 min on enzymatic activity of green coconut water where the activity of POD decreased as a function of increasing temperature from 40 to 90ºC, achieving complete inactivation at 90ºC/400-600MPa/2 min. Similarly, Chisari et al. (2007) studied the effect of thermal treatment on POD activity on strawberry fruit, where temperatures above 30ºC/1 h caused a progressive decrease of POD activity until 10% of enzymatic activity at 70ºC/1 h. Terefe et al. (2017) investigated the thermal effect on blueberry POD, reporting no relative activity after treatment at 90ºC/690MPa/15 min. In this study, complete inactivation of *Stevia* POD required a higher temperature of 120ºC/10MPa/1 min compared to data reported in the literature (Chourio et al., 2018; Terefe et al., 2017; Chisari et al., 2007). These differences could be attributed to the fact that the inactivation of the POD enzyme was influenced by different factors, such as pH, treatment conditions, composition, and thermostability of the POD isoenzyme present in the food matrix (Terefe et al., 2017; Barba et al., 2014).

The enzymatic activity of *Stevia* POD at 75ºC, increased by 50% with the increase of pressure from 10MPa/1 min to 100MPa/1 min and by 42% with the increase of pressure from 10MPa/5 min to 100MPa/5 min. Similarly, the analysis of variables shown in Fig.4.3a indicated that the increase of pressure from 10 to 600 MPa significantly increased (p-values<0.001) POD activity while the holding time (1-5 min) had no significant impact on the POD activity.

This effect of pressure-induced activation of POD enzyme at constant temperature has also been reported in the literature for other matrices. Terefe et al. (2017) reported an increase in the activity of blueberry POD at 70 ºC with increase of pressure from 0.1 MPa to 395 MPa. Similarly, Anese et al. (1995) reported an increase by 70% on carrot POD activity with increase of pressure from 100 MPa to 500 MPa at 20ºC/1 min. Terefe et al. (2010) also studied the effect of PATP treatment at 100-690MPa/24-90ºC/5-15 min on enzymatic activity of POD enzyme in strawberry puree, reporting an antagonist effect when pressure increases at constant temperature in the range of 100-400 MPa and 24-75ºC, resulting in activation of POD enzyme. One possible explanation of this behavior is the antagonist effect of pressure and temperature at the initial stage of inactivation of the POD enzyme. Thermal treatment promotes a rearrangement of the protein caused by the dehydration of the protein while high-pressure favours the hydration of charged and non-polar groups, inhibiting the thermal inactivation of the enzyme (Eisenmenger et al., 2009; Mozhaev et al., 1996). Another possible explanation is that high pressure enhances the extraction of membrane bound enzymes, resulting in enzyme-substrate contact. The catalytic activity of the enzyme can be accelerated or decelerated by pressure, depending on pH, medium composition and temperature (Terefe et al., 2017; Hendrickx et al., 1998).

The residual activity of *Stevia* PPO was significantly affected by temperature (p-values<0.001) (Fig 4.3b, Fig B.2b and Table B.5, Appendix B). The activity of PPO was 37% at

75ºC/10MPa/1 min while 2% of PPO relative activity was obtained at 10 MPa/90ºC/1 min. Complete inactivation of *Stevia* PPO was observed at 10-600 MPa/120ºC/1-5 min. Earlier, Terefe et al. (2016) reported that the activity of pear PPO was 64% at 0.1 MPa/60ºC/3 min while 10% of pear PPO was obtained after thermal treatment at 0.1 MPa/100ºC/3-5 min. Similarly, a gradual decrease of coconut water PPO activity was observed with the increase on temperature from 60ºC (33%) to 80ºC (13%) at 600 MPa at 1 min (Chourio et al., 2018).

The residual activity of *Stevia* PPO was significantly affected by pressure at 75ºC. The PPO activity decreased from 40% at 10 MPa/5 min to 3% at 600MPa/5 min. Also, the analysis of variables shown in Fig. 4.3b indicated that the increase of pressure from 10 to 600 MPa significantly decreased PPO activity while holding time (1-5 min) had no significant impact (p-values of 0.117) on the PPO enzymatic activity. This result can be attributed to the rupture of ionic, hydrophobic and hydrogen bonds by pressure increament, causing a volume reduction that inactivates the enzyme (Hendrickx et al., 1998). Garcia-Palazon et al. (2004) and Chakraborty et al. (2014) found that complete inactivation of PPO on strawberry was achieved at 25ºC/800MPa/15 min mainly due to the irreversible unfolding of the secondary structure of the enzyme at very high pressure (800 MPa) treatment.

Although processing time had no significant effect on mean relative activity values of PPO, a significant effect of holding time was observed at 10 MPa/75ºC (Fig. 4.3b). However, no further decrease of *Stevia* PPO was observed with increasing holding time. Similar results were reported by Terefe et al. (2016) on pear PPO where the residual activity decreased with increasing the holding time from 3 min to 5 min at temperatures between 20 and 60ºC/0.1 MPa while no further decrease of residual pears PPO activity was observed after 100ºC/0.1MPa with increasing the holding time from 3 to 5 min. Terefe et al. (2016) explained that processing time might have a

significant effect in the relative activity of PPO enzymes, depending on pH, processing conditions and level of tissue disruption.

The POD enzyme was more PATP resistant than the PPO enzyme. POD activity was 0.75 at 600MPa/75°C/5 min while the PPO activity was <0.03 at the same processing conditions (Table B.5, Appendix B). Similarly, POD was more resistant to pressure and temperature than PPO on coconut water, banana and a fruit extract of orange, mango, and papaya (Chourio et al., 2018; Barba et al., 2014; Terefe et al., 2016; MacDonald and Schaschke, 2000).

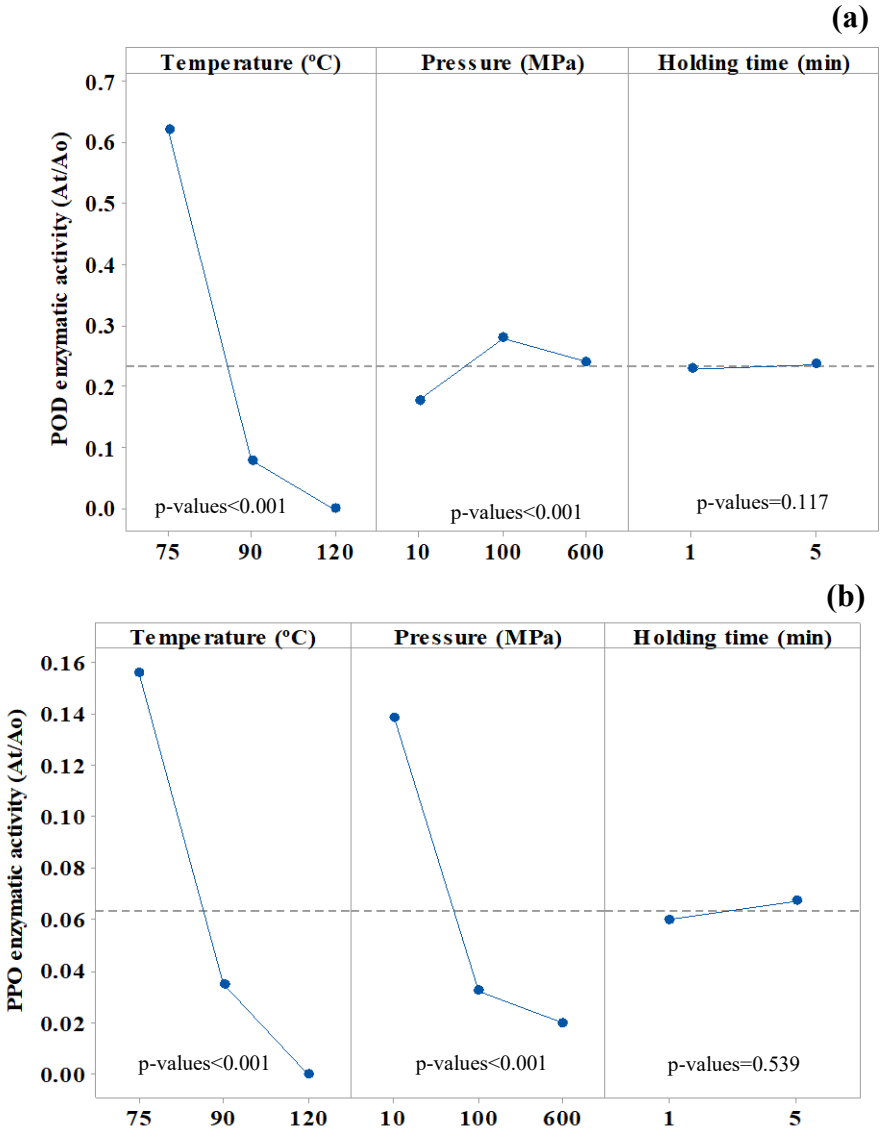


Figure 4.3 (a) POD, and (b) PPO enzymatic activity of treated *Stevia* leaves.

Scanning electron microscope (SEM) images of untreated *Stevia* and treated *Stevia* at 100-600 MPa/120ºC/5 min are shown in Fig. 4.4. The images showed the structural changes in the tissue of *Stevia* after PATP treatments where the structure of treated *Stevia* at 100 MPa/120ºC/5 min had a ruptured and perforated appearance (Fig. 4.4b) compared to the untreated *Stevia* where the structure was intact (Fig. 4.4a). More deformation and agglomeration of the guard cell is observed on *Stevia* treated at 600 MPa/120ºC/5 min (Fig 4.4c). Similarly, Jung et al. (2013) observed by SEM images of carrot and spinach treated at 500MPa/25ºC/20 min deformation of the tissue and agglomeration of the structure at high pressure, causing the leakage of intracellular compounds in the extract.

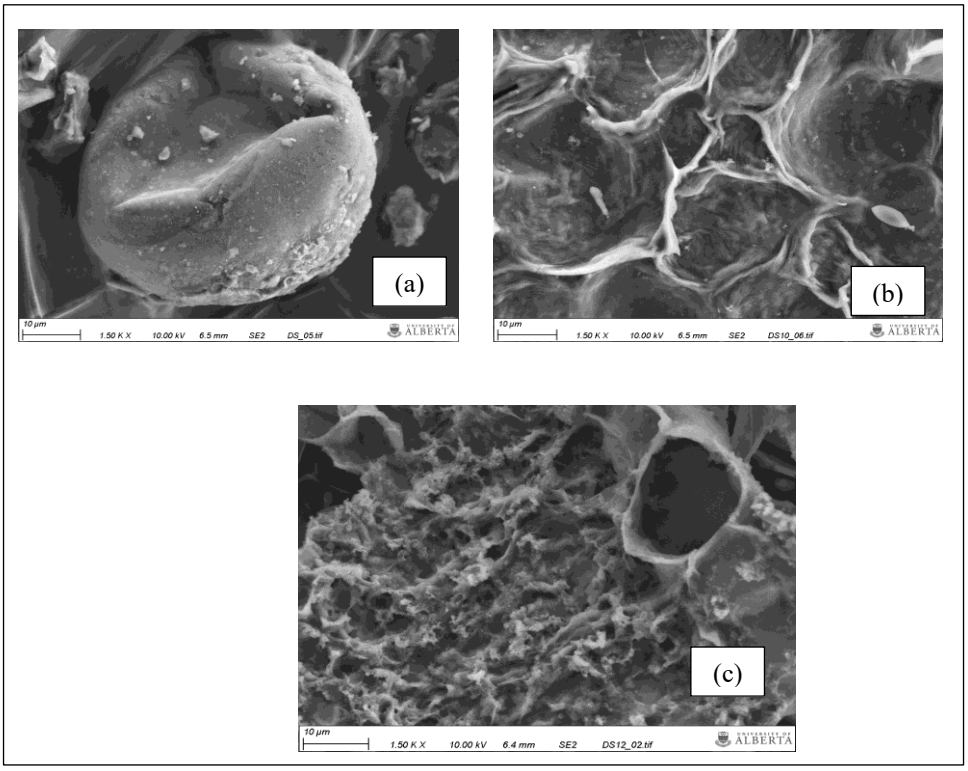


Figure 4.4 Scanning electron microscope (SEM) images of: (a) untreated *Stevia*; and treated *Stevia* at: (b) 100 MPa/120ºC/5 min, and (c) 600 MPa/120ºC/5 min.

4.3.5 Total antioxidant activity and total phenolics of PATP treated mate + *Stevia* and mate + commercial *Stevia* powder

The total antioxidant activity values of treated mate+*Stevia* leaves significantly increased with increasing temperature from 25ºC (233.8±22.5 mg $FeSO_4.H_20$/g mate) to 120ºC (661.6 ± 12.8 $FeSO_4.H_20$ mg/g mate) at 600 MPa/5 min (Fig. 4.5a and Table B.9, Appendix B) and were significantly higher than untreated mate (173.74±6.14 $FeSO_4.H_20$ mg/g mate), with the exception of treated samples at 25ºC/600MPa/1 min. The increase of total antioxidant activity of treated mate+*Stevia* leaves might be related to the cell lysis occurred at combined high pressure-temperature treatment causing a release of compounds into the solvent (Eylen et al., 2008).

The addition of *Stevia* leaves resulted in a decrease of total antioxidant activity values by 41% on treated mate+*Stevia* leave (412.44±14.32 mg/g mate) at 120ºC/100MPa/1 min compared to treated mate without *Stevia* (697.98±9.79 $FeSO_4.H_20$ mg/g mate, Fig A.1a, Appendix A) at the same conditions. Korir et al. (2014) studied the effect of additives such as *Stevia rebaudiana* leaves, milk, sugar and honey on antioxidant activity of green and black tea. They reported that the addition of 2.5% *Stevia* showed no significant influence on the antioxidant activity of green and black tea. However, the addition of milk, sugar and honey significantly decreased the antioxidant activity of green and black tea. Similarly, Barba et al. (2014) studied the effect of 2.5% of *Stevia rebaudiana* leave addition on the antioxidant activity of fruit extract (orange, mango, and papaya) treated at 22ºC/300-500MPa/5-15 min. They reported a significant increase of the total antioxidant activity of the fruit extract (acid pH of < 5) sweetened with *Stevia*. The difference of the results can be related to treatment conditions and pH of the beverage due to the oxidation of polyphenols at pH of 5 to 8 (Barba et al., 2014).

Similarly, the concentration of total antioxidant activity on treated mate+commercial *Stevia* powder (Table 4.2 and Table B.10, Appendix B) significantly increased at all conditions evaluated (120ºC/100-600MPa/1-5 min) compared to untreated mate (174.74±19.95 mg $FeSO_4.H_20$/g mate). However, lower total antioxidant activity values of treated mate+commercial *Stevia* powder were observed compared to treated mate without *Stevia* at all conditions evaluated (Fig A.1a, Appendix A). For instance, the total antioxidant activity of treated mate+commercial *Stevia* powder at 120ºC/600MPa/5 min (294.54±15.34 mg $FeSO_4.H_20$ /g mate) decreased by 58% compared to treated mate without *Stevia* (697.44±15.82 mg $FeSO_4.H_20$ /g mate) at 120ºC/600MPa/5 min (Fig A.1a, Appendix A).

Higher total antioxidant activity values were obtained on treated mate+*Stevia* leaves (412.44 –661.63 mg $FeSO_4.H_20$/g mate) (Fig. 4.5a) than treated mate+commercial *Stevia* powder (294.54–426.91 mg $FeSO_4.H_20$/g mate) at 100-600MPa/120ºC/1-5 min (Table. 4.3). These results can be attributed to the presence of hydroxybenzoic acids (gallic acid, protocatechuic acid), hydroxycinnamic acids (chlorogenic acid, caffeic acid, cinnamic acid, coumaric acid) and flavonoids (catechin, epicatechin, rutin and quercetin) on *Stevia rebaudiana* leaves (Kovacevic et al., 2018). The PATP treatment at 10-600MPa/25-120ºC/1-5 min significantly increased the total phenolic content of mate+*Stevia* leaves compared to untreated mate (Fig. 4.5b). Different PATP conditions were needed to extract maximum total phenolics content from mate compared to mate+ *Stevia* leaves. The highest total phenolics values obtained on treated mate+*Stevia* leaves was 113.88±2.78 mg GAE/g mate at 75ºC and 100MPa/120ºC/5 min while the highest total phenolic values of treated mate without *Stevia* was 148.49±6.87 mgGAE/g mate at 600MPa/120ºC/1-5 min. These results are probably due to the high degree

of cell damage during PATP treatment and extraction equilibrium achieved that further treatment does not cause increase in total phenolic content.

The amount of total phenolics of untreated and treated mate+commercial *Stevia* powder at 120ºC/100-600MPa/1-5 are shown in Table 4.2 and Table B.10, Appendix B. Total phenolics of treated mate+commercial *Stevia* significantly increased at all conditions evaluated (120ºC/100-600MPa/1-5 min) compared to untreated mate without *Stevia* (12.90±0.01 mg GAE/g mate) (Table 4.2). However, the extraction of total antioxidant activity of treated mate+commercial *Stevia* powder decreased compared to treated mate without *Stevia* at all conditions evaluated (Fig A.1a, Appendix A). The total phenolic content of treated mate+commercial *Stevia* powder at 600MPa/120ºC/5 min was 63.50±0.01 mg GAE/g mate, decreasing by 55% compared to treated mate without *Stevia* (140.31±6.50 mg GAE/g mate) at the same PATP conditions, 600MPa/120ºC/5 min (Table A.4, Appendix A). This result can be related to a possible interaction between the steviol glycosides (stevioside, rebaudioside, dulcoside) and antioxidant compounds in mate. However, the mechanism in which steviol glycosides might inhibit the antioxidant activity of mate is still not fully understood.

Also, higher total phenolic contents were obtained for treated mate+*Stevia* leaves (63.77±11.12–113.88±2.78 mg GAE/g) (Fig. 4.5b and Table B.9, Appendix B) than treated mate+commercial *Stevia* powder (46.52±4.58–69.87±6.43 mg GAE/g) at the same PATP conditions of 120ºC/100-600 MPa/1-5 min (Table 4.2 and Table B.10, Appendix B) due to the presence of phenolic, glycosides and flavonoids compounds in *Stevia* leaves such as chlorogenic acid, gallic acid, quercetin-3-O-*β*-d-arabinoside, quercetin-3-O-*β*-d-rhamnoside, apigenin, apigenin-4-O-*β*-d-glycoside, luteolin, quercetin-3-O-glucoside, quercetin-3-O-rutinoside,

apegenin-7-O-*β*-d-glycoside, luteolin-7-O-*β*-d- glycoside, contributing to the increase of total phenolics in the extracts (Ghanta et al., 2007; Cacciola et al., 2011; Karakose et al., 2015).

Increases of total phenolic 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 (Carbonell-Capella et al., 2013).

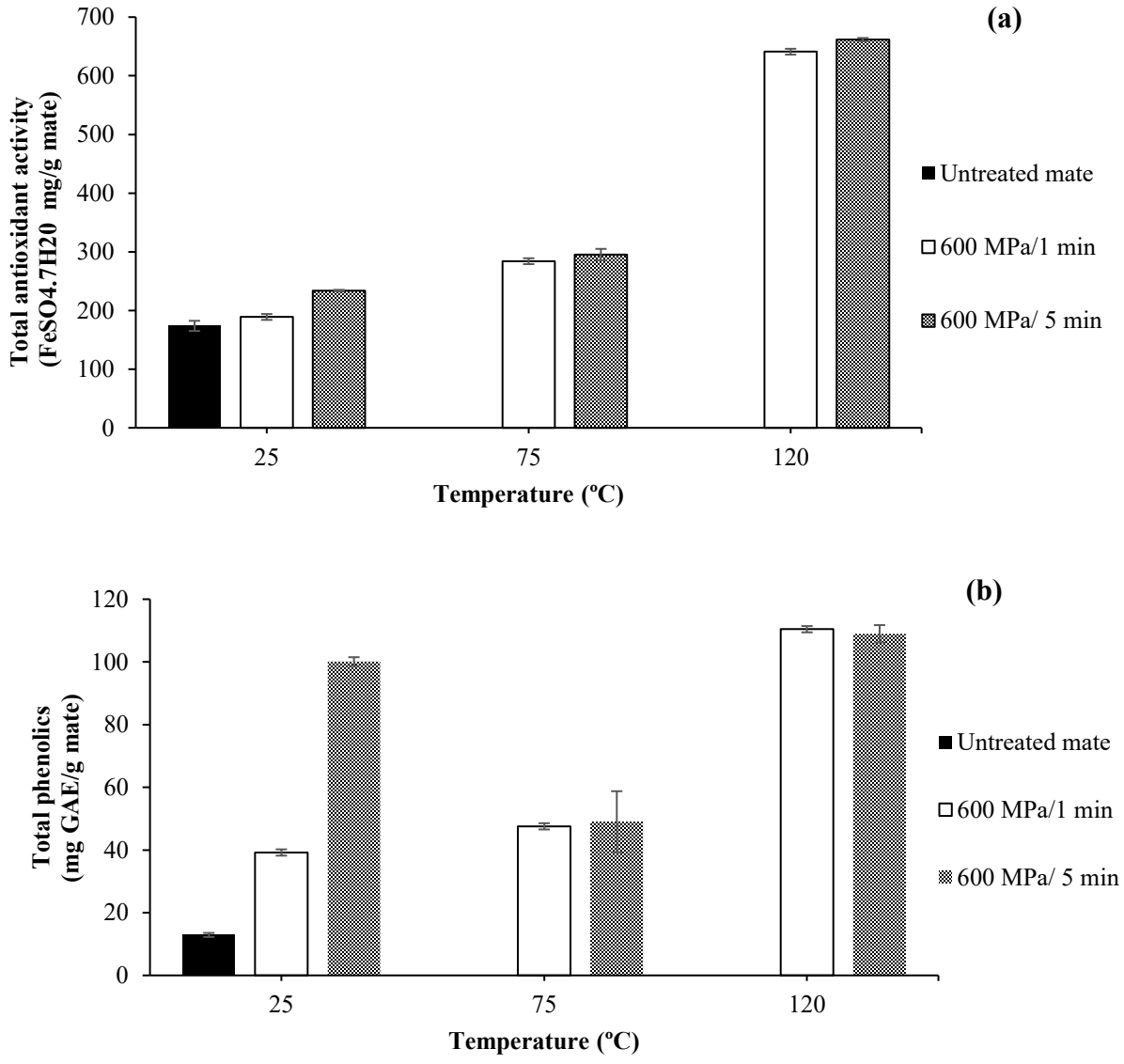


Figure 4.5 (a) Total antioxidant activity and (b) Total phenolics content of untreated and treated mate+*Stevia* leaves at 25-120ºC/600 MPa/1-5 min.

Table 4.2 Total antioxidant activity and total phenolic content of mate sweetened with *Stevia*.

Processing condition		Mate + *Stevia* leaves Total phenolics (mg GAE/g)	Mate + *Stevia* leaves Total antioxidant activity (mg $FeSO_4.7H_20$ /g mate)
25ºC	Untreated mate	12.90 ± 0.01[a]	174.74 ± 19.95[a]
	10 MPa/1 min	47.06 ± 0.01[b]	320.94 ± 38.39[b]
	10 MPa/5 min	67.21 ± 3.47[c]	227.63 ± 11.76[c]
	100 MPa/1 min	98.16 ± 1.39[d]	258.37 ± 2.05[c]
	100 MPa/5 min	90.29 ± 0.01[e]	525.64 ± 8.70[d]
	600 MPa/1 min	39.20 ± 9.73[b]	188.93 ± 11.25[a]
	600 MPa/ 5 min	100.12 ± 1.39	233.78 ± 22.50[c]
75ºC	10 MPa/1 min	83.91 ± 2.08[e]	518.05 ± 7.16[d]
	10 MPa/5 min	61.31 ± 0.69[c]	307.56 ± 14.32[b]
	100 MPa/1 min	83.91 ± 3.47[e]	459.82 ± 1.53[e]
	100 MPa/5 min	113.38 ± 4.86[f]	669.95 ± 19.44[f]
	600 MPa/1 min	47.55 ± 11.81[b]	284.05 ± 2.56[c]
	600 MPa/ 5 min	49.03 ± 9.73[b]	295.26 ± 11.25[b]
120ºC	10 MPa/1 min	63.77 ± 11.12[c]	316.96 ± 14.32[b]
	10 MPa/5 min	77.03 ± 2.08[c]	414.25 ± 1.53b[e]
	100 MPa/1 min	81.45 ± 2.78[e]	412.44 ± 14.32b[e]
	100 MPa/5 min	113.88 ± 2.78[f]	419.68 ± 13.30b[e]
	600 MPa/1 min	110.44 ± 0.69[f]	641.02 ± 42.96[f]
	600 MPa/ 5 min	108.96 ± 2.78[f]	661.63 ± 12.79[f]
Processing condition		**Commercial *Stevia* Total phenolics (mg GAE/g)**	**Commercial *Stevia* Total antioxidant activity (mg $FeSO_4.7H_20$ /g mate)**
120 ºC	Untreated mate	12.90 ± 0.01[a]	174.74 ± 19.95[a]
	100 MPa/1 min	50.76 ± 5.15[b]	310.45 ± 7.16[b]
	100 MPa/5 min	46.52 ± 4.58[b]	373.74 ± 4.63[b]
	600 MPa/1 min	69.87 ± 6.43[c]	426.91 ± 2.05[e]
	600 MPa/5 min	63.50 ± 0.01[c]	294.54 ± 15.34[b]

[a-f] Different lowercase letters in the same column indicate significant differences ($p < 0.05$).

4.3.6 Individual phenolics of untreated mate and treated mate sweetened with commercial *Stevia* powder

Table 4.3 shows main individual phenolics, such as caffeic acid, chlorogenic acid, quercetin, rutin, catechin, gallocatechin gallate and epigallocatechin, of untreated mate and treated mate+commercial *Stevia* powder at 120ºC/100-600MPa/1-5 min. The amounts of caffeic acid on treated mate+commercial *Stevia* powder at 120ºC/100-600MPa/1-5 min significantly increased compared to untreated mate (Table 4.3 and Table B.12, Appendix B). However, no significant difference was found with the increase of pressure and holding time from 100MPa/1 min to 600MPa/5 min. Chlorogenic acid concentration of treated mate+commercial *Stevia* powder was significantly reduced by 90% at all conditions evaluated compared to untreated mate while no significant difference was observed with the increase of pressure and holding time from 100MPa/1 min to 600MPa/5 min. The concentration of quercetin of treated mate+commercial *Stevia* powder significantly increased by 61% and 46% at 120ºC/100MPa/1 min and 120ºC/600MPa/1 min, respectively compared to untreated mate (Table 4.3 and Table B.12, Appendix B). No significant difference was observed at 120ºC/100-600MPa/5 min compared to untreated mate. The rutin concentration of mate+commercial *Stevia* significantly increased by 20% at all conditions evaluated. Catechin content of treated mate+commercial *Stevia* powder increased by 38% and 20% at 120ºC/100MPa/1 min and 120ºC/100MPa/5 min compared to untreated mate (Table 4.3). However, no significant difference was observed on treated mate+commercial *Stevia* powder at 120ºC/600MPa/5 min compared to untreated mate while only catechin traces was obtained at 120ºC/600MPa/1 min. The concentration of gallocatechin gallate of treated mate+commercial *Stevia* increased by 17% after PATP treatment at 120ºC/100-600MPa/1-5 min while the concentration of epigallocatechin increased by 40% after PATP treatment at 120ºC/100-

600MPa/1-5 min (Table 4.3 and Table B.11). It was reported that Stevia addition (14 g/L) on Roselle *Hibiscus sabdariffa L* beverage increased quercetin, gallic acid and rosmarinic acid during storage (Perez-Ramirez et al., 2015).

Table 4.3 Individual phenolic compounds (mg/g) of PATP-treated mate+commercial *Stevia* powder at 120°C.

Processing conditions	Caffeic acid	Chlorogenic acid	Quercetin	Rutin	Catechin	Gallocatechin gallate	Epigallocatechin
Untreated mate	6.48 ± 0.32[a]	8.25 ± 0.41[a]	1.25 ± 0.06[a]	0.28 ± 0.01[a]	0.36 ± 0.02[a]	0.59 ± 0.02[a]	0.15 ± 0.01[a]
100 MPa/1 min	24.18 ± 1.21[b]	0.81 ± 0.04[b]	3.20 ± 0.16[b]	0.36 ± 0.02[b]	0.59 ± 0.03[b]	0.71 ± 0.04[b]	0.33 ± 0.03[b]
100 MPa/5 min	17.20 ± 0.86[b]	0.71 ± 0.04[b]	1.57 ± 0.08[a]	0.31 ± 0.02[b]	0.46 ± 0.02[c]	0.78 ± 0.04[b]	0.24 ± 0.02[b]
600 MPa/1 min	21.90 ± 1.01[b]	0.77 ± 0.04[b]	2.34 ± 0.12[c]	0.36 ± 0.02[b]	traces	0.83 ± 0.04[b]	0.31 ± 0.03[b]
600 MPa/5 min	17.53 ± 0.88[b]	0.71 ± 0.03[b]	1.15 ± 0.06[a]	0.31 ± 0.02[b]	0.38 ± 0.03[a]	0.74 ± 0.04[b]	0.25 ± 0.02[b]

[a-c] Different lowercase letters in the same column indicate significant differences ($p < 0.05$).

4.4 CONCLUSIONS

The effect of PATP on total phenolics and individual phenolics such as caffeic acid, chlorogenic acid, quercetin, rutin, and antioxidant activity of *Stevia* and mate sweetened with *Stevia* leaves was reported for the first time. In addition, the inactivation of PPO and POD of *Stevia* leaves using PATP was reported for the first time.

The enzymatic activity of PPO and POD enzymes of *Stevia* leaves was reduced up to 2% at 90ºC/600MPa/1 min. Both temperature and pressure had a significant effect (p-values<0.001) on the relative activity of PPO and POD while the holding time (1-5 min) had no significant effect. An increase of POD enzymatic activity by 50% was observed at 75ºC/100MPa/1-5 min due to the antagonist effect between temperature and pressure and the rearrangement of the enzyme at the initial stage of POD inactivation. No POD and PPO enzymatic activity was observed at 120ºC/10-600MPa/1-5 min. The POD was more PATP-resistant than the PPO. The addition of *Stevia* (2.5%w/v) to mate samples resulted in an increase up to 88% of total phenolics and up to 74% of total antioxidant activity compared to untreated mate. However, the *Stevia* addition reduced up to 40% and 55% the extraction of total phenolics and total antioxidant activity, respectively compared to treated mate without *Stevia* at all conditions investigated (25-120ºC/10-600MPa/1-5 min). Moreover, higher total phenolics (108.96±2.78 mg GAE/g mate) and total antioxidant activity (661.63±12.79 mg $FeSO_4.7H_20$/g mate) were obtained on mate+*Stevia* leaves compared to mate+commercial *Stevia* powder at 120ºC/600MPa/5 min (63.50 ± 0.01 mgGAE/ g mate and 294.54±15.34 mg $FeSO_4.7H_20$/g mate of total phenolics and total antioxidant activity, respectively).

The effect of PATP on pure compounds (quercetin, rutin, caffeic acid, chlorogenic acid and caffeic acid) was studied for the first time. Also, no hydrolysis of rutin into quercetin was observed

at the conditions evaluated (25-75ºC/100-300MPa/1-5 min) while that quercetin was not affected by PATP conditions. A slight degradation of chlorogenic acid was observed with the increase of temperature (p-value<0.001) while caffeic acid concentration was not significantly affected by temperature. This study showed that PATP is a promising technology to increase extractability of bioactive compounds of *Stevia* leaves and to inhibit degradative enzymes such as PPO and POD, improving nutritional and functionality value of beverages including mate and mate sweetened with *Stevia*.

4.5 RECOMMENDATIONS

- Study the effect PATP on pure compounds quercetin, rutin, chlorogenic acid, and caffeic at temperatures at 120ºC/600 MPa at different pH for a better understanding of pure compound stability under PATP conditions.
- Study the effect of PATP on enzymatic activity of PPO and POD on mate is recommended using fresh mate leaves before thermal treatments.
- Study the consumer acceptance of *Stevia* as a sweetener on mate beverages to complement this study.

ACKNOWLEDGMENT

The authors thank to Natural Sciences and Engineering Research Council of Canada (NSERC, #05356-2014) for the financial support of this research.

CHAPTER 5: Conclusions and recommendations

5.1 Conclusions

- The effect of PATP treatment was investigated for the first time on mate *Illex paraguarensis* samples. The increase of temperature from 25ºC to 120ºC and pressure from 10MPa to 600MPa had a significant impact ($p<0.001$) on the increase of total phenolic content and total antioxidant activity. The maximum increase of total antioxidant activity (400%) and total phenolics (115%) was obtained on treated mate at 100 MPa/120ºC/1 min and 600 MPa/120ºC/1 min, respectively. These results are attributed to the enhanced disruption of cell membranes during PATP treatment, resulting in the increase of bioactive compounds and antioxidant activity of treated mate.
- A good correlation of R^2=0.92 was obtained between total phenolic content and antioxidant activity of PATP treated mate, indicating that phenolics contributed to the antioxidant activity of mate.
- The main phenolic compounds found in untreated mate were chlorogenic acid (8.25±0.41 mg/g mate) caffeic acid (6.48±0.32 mg/g mate), quercetin (1.25±0.06 mg/g mate), rutin (0.28±0.01 mg/g mate), gallocatechin gallate (0.59±0.02 mg/g mate), catechin (0.36±0.02 mg/g mate) and epigallocatechin (0.15±0.01 mg/g mate). The increase of temperature from 25 to 120ºC and pressure from 10 to 600 MPa significantly decreased the content of chlorogenic acid up to 91% while caffeic acid content significantly increased up to 78% compared to untreated mate due to a possible conversion of chlorogenic acid into caffeic acid.
- The effect of PATP on quercetin, rutin, and catechins was also studied. Temperature and pressure had a significant effect on the increase of quercetin content on treated mate

extracts compared to untreated mate. Thus, maximum quercetin values (5.36±0.27 mg/g mate) were obtained on mate treated at 100 MPa/120ºC/1 min. These PATP conditions agreed with the optimum conditions required to obtain maximum total antioxidant activity on treated mate samples. Similarly, rutin increased up to 10% on treated mate extracts at 100 MPa/120ºC/1 min, compared to untreated mate but the effect of PATP on catechins of treated mate had a different behaviour. Catechin content significantly decreased with the increase of temperature from 75 to 120ºC, pressure and holding time compared to untreated mate. The pH of mate 5.8 and high temperature (120ºC) accelerated the degradation of catechin. Similarly, the content of gallocatechin gallate significantly decreased on treated mate at 10-100MPa/120ºC/1-5 min with while gallocatechin gallate content increased up to 37% at 600MPa/120ºC/1 min compared to untreated mate. These results might be attributed to epimerization of epigallocatechin gallate into gallocatechin gallate. Moreover, maximum caffeine (35.85±2.59 mg/g mate) and theobromine contents (23.99±0.02 mg/g mate) of treated mate were obtained at 100MPa/120ºC/1 min.

- The effect of PATP (10-600MPa/75-120ºC/1-5 min) was also studied on total phenolics, total antioxidant activity and relative activity of peroxidase and polyphenoloxidase enzymes of *Stevia* leaves. The analysis of variance showed that the increase of temperature from 75 to 120ºC, pressure from 10 to 600MPa, and holding time from 1 to 5 min significantly increased total antioxidant activity and total phenolic content of treated *Stevia* leaves. The highest values of total phenolics (110.35 mg GAE/g *Stevia*) and total antioxidant activity (110.95 mg $FeSO_4.7H_20$/g of *Stevia*) were obtained on treated *Stevia* at 600MPa/120ºC/5 min with a correlation of R^2=0.92. These results could be related to the complete inactivation of PPO and POD enzymes at high temperature (120ºC) favouring

the increase of total phenolics and total antioxidant activity levels. The activity of PPO and POD enzymes were significantly affected by temperature and pressure ($p<0.001$). The optimum conditions to achieve complete reduction of both PPO and POD enzymatic activities was 600 MPa/120ºC/1 min. In addition, activation of POD was observed on treated *Stevia* at 100 MPa/90ºC/1 min due to an antagonistic effect between pressure and temperature. Thus, POD enzyme was more resistant to PATP treatment than PPO enzyme.

- The effect of PATP (100-300 MPa/25-75ºC/1-5 min) were investigated on model systems of pure quercetin, rutin, chlorogenic acid and caffeic acid. The analysis of variance showed that the increase of temperature from 25 to 75ºC had no significant different on the model systems evaluated. However, the increase of pressure from 100 to 300 MPa had a significant impact on the decrease of chlorogenic acid (p-value=0.02) and the increase of quercetin content while holding time had no significant difference on the contents of rutin, chlorogenic acid, and caffeic acid.
- The scanning electron microscope images of treated *Stevia* leaves and mate at 100MPa-600MPa/120ºC/5 min illustrated the changes of cell structure occurred after PATP treatment, demonstrating clear deformation and some disintegration of the cell structure caused by the migration of molecules inside and outside the cell. Greater damage of the structure was observed at 600MPa/120ºC/5 min compared to 100MPa/120ºC/5 min.
- The effect of PATP treatment (10-600 MPa/25-120ºC/1-5 min) on bioactive compounds and antioxidant activity of mate sweetened with *Stevia* leaves and commercial *Stevia* powder were investigated for the first time. Temperature and pressure had a major impact on the release of total phenolic content and total antioxidant activity of both matrices. The addition of *Stevia* leaves to mate resulted in better release of bioactive compounds

compared to mate+commercial *Stevia* powder probably due to the bioactive compounds and antioxidant properties of *Stevia* leaves. These results suggested that the combination of 600MPa/120ºC/1 min and the addition of *Stevia* leaves (2.5% w/v) to mate enhanced bioactive compounds and antioxidant activity of the final product.

5.2 Recommendations

- No studies have reported the effect of PATP on inactivation of PPO and POD enzymes of mate tea. Thus, to study the effect of this promising technology on PPO and POD enzymatic activity, the use of fresh *Ilex paraguarensis* leaves is suggested.
- As the effect of PATP (100-300 MPa/25-75ºC/1-5 min) on model systems of quercetin, rutin, chlorogenic acid and caffeic acid was studied, temperatures of 120ºC, holding time 5-15 min, and change in pH from 2-10 should be included for a better understanding of degradation of pure compounds in PATP treatment.
- As the phytochemical profile of *Stevia rebaudiana* leaves can be affected by harvest factors such as harvest time, weather conditions and crop age. It is suggested to study the impact of *Stevia rebaudiana* leaves at different growth stages and harvest conditions, mainly to quantify phenolic compounds and steviol glycosides.
- The quality parameters such as color and overall consumer acceptance should be included to obtain a complete evaluation of the development of a ready to drink beverage. Moreover, the effect of *Stevia* leaves addition on the stability of phenolics compounds such as chlorogenic acid, caffeic acid, quercetin and rutin on treated PATP mate during storage conditions should be included. The effect of PATP on steviol glycosides of *Stevia* such as stevioside, dulcoside A, rebaudioside A and

rebaudioside C by HPLC should be evaluated and sensorial properties of the natural sweeteners should be included.

REFERENCES

Abou-Arab, A., Abu-Salem M.F. (2010) Physico-chemical assessment of natural sweeteners steviosides produced from *Stevia rebaudiana* Bertoni plant. *African Journal of Food Science*, 4, 269-281.

Agcam E., Akyildiz, A., Evrendilek, G.A. (2014) Comparison of phenolic compounds of orange juice processed by pulsed electric fields (PEF) and conventional thermal pasteurisation. *Food Chemistry,* 143, 354-361.

Aiddo, R., Afoakwa, E., Dewettinck, K. (2015) Rheological properties, melting behaviours and physical quality characteristics of sugar-free chocolates processed using inulin/polydextrose bulking mixtures sweetened with *Stevia* and traumatic extracts. *LWT-Food Science and Technology,* 62, 592-597.

Andres, V., Villanueva, M.J., Tenorio, M. (2016) The effect of high-pressure processing on colour, bioactive compounds, and antioxidant activity in smoothies during refrigerated storage. *Food Chemistry,* 192, 328-335.

Andueza, S., Manzoco, L., Peña, M.P., Cid, C., Nicoli, C. (2009) Caffeic acid decomposition products: Antioxidants or pro-oxidants?. *Food Research International*, 42, 51-55.

Anese, M., Nicole, M.C., Dallaglio, G., Lerici, C.R. (1995) Effects of high pressure treatments on peroxidase and polyphenol oxidase activities. *Journal of Food Biochemistry*, 18, 285-293.

Balasubramaniam, V.B., Martinez-Monteagudo, S.I., Gupta, R. (2015) Principles and application of high pressure-based technologies in the food industry. *Annual Review of Food Science and Technology*, 6, 435-62.

Barba et al., F.J., Criado, M.N., Belda-Galbis, C.M., Esteve, M.J. (2014) *Stevia rebaudiana* Bertoni as a natural antioxidant/antimicrobial for high pressure processed fruit extract: Processing parameter optimization. *Food Chemistry,* 148, 261-267.

Bastos, D.H.M., Ishimoto, E.Y., Marques, M.O.M., Ferri, A.F., Torres, E.A.F.S. (2006) Essential oil and antioxidant activity of green maté and maté tea (*Ilex paraguariensis*) infusions. *Journal of Food Composition and Analysis,* 19, 538–543.

Basu, S., Shivhare, U.S., Singh, T.V. (2013) Effect of substitution of stevioside and sucralose on rheological, spectral, color, and microstructural characteristics of mango jam. *Journal of Food Engineering,* 114, 465-467.

Berte, K., Rodriguez-Amaya, D.B., Hoffman-Ribani, R., Maccari. (2014) Antioxidant activity of mate tea and effects of processing. In: Preedy, V (Ed.), Processing and Impact on Antioxidants in Beverages, vol. 1, Chapter 15, Academic Press, London, UK. pp. 145-153.

Bimakr, M., Rahman, R.A., Taip, F.S., Ganjloo, A., Salleh, L.M.D., Selamat, J. (2011) Comparison of different extraction methods for the extraction of major bioactive flavonoid compouds from spearmint (*Mentha spicate L*) leaves. *Food and Bioproducts Processing,* 89, 67-72.

Briones-Labarca, V., Jamett, F. (2015) High hydrostatic pressure and ultrasound extractions of antioxidant compounds, sulforaphane and fatty acids from Chilean papaya (*Vasconcellea pubescens*) seeds: Effects of extraction conditions and methods. *LWT-Food Science and Technology*, 60, 525-534.

Buchner, N., Krumbein, A., Rohn, S., Kroh, L.W. (2006) Effect of thermal processing on the flavonols rutin and quercetin. *Rapid Communications in Mass Spectrometry*, 20, 3229-3235.

Buniowska, M., Carbonell-Capella, J.M., Frígola A., Esteve M.J. (2016) Steviol glycosides stability after pulsed electric technologies and ultrasounds treatments in fruit juice blend sweetened with *Stevia rebaudiana*. In: Jarm T., Kramar P. (Eds.), 1st World Congress on Electroporation and Pulsed Electric Fields in Biology, Medicine and Food and Environmental Technologies. IFMBE Proceedings, vol. 53. Springer, Singapore. pp. 136-139.

Butiuk, A., Martos, M., Adachi, O., Hours, R. (2016) Study of the chlorogenic acid content in yerba mate *(Ilex paraguariensis St. Hil)*: Effect of plant fraction, processing step and harvesting season. *Journal of Applied Research on Medicinal and Aromatic Plants,* 3, 27-33.

Cacciola, F., Delmonte, P., Jaworska, K., Dugo, P., Mondello, L., Rader, J. (2011) Employing ultra-high pressure liquid chromatography as the second dimension in a comprehensive two-dimensional system for analysis of *Stevia rebaudiana* extracts. *Journal of Chromatography*, 1218, 2012-2018.

Cadena, R.S., Cruz, A.G., Netto, R.R., Castro, W.F., Faria, J.A.F., Bolini, H.M.A. (2013) Sensory profile and physicochemical characteristics of mango nectar sweetened with high intensity sweeteners throughout storage time. *Food Research International,* 54, 1670-1679.

Campus, M. (2010) High pressure processing of meat, meat products and seafood. *Food Engineering Reviews*, 2, 256-273.

Carbonell-Capella, J.M., Bunioswska, M., Esteve, M.J., Frigola, A. (2015) 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, 122-130.

Carbonell-Capella, J.M., Frigola, A. (2013) 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, 48-56.

Cardozo, E.L., Ferrarese Filho, O., Cardozo Filho, L., Ferrarese, M.L.L., Donaduzzi, C.M., Sturion, J.A. (2007) Methylxanthines and phenolic compounds in mate (*Ilex paraguarensis* St. Hill.) progenies grown in Brazil. *Journal of Food Composition and Analysis,* 20, 378-384.

Chakraborty, S., Kaushik, N., Rao, P.S., Mishra, H.N. (2014) High-pressure inactivation of enzymes: a review on its recent applications on fruit purees and juices. *Comprehensive reviews in Food Science and Food Safety*, 13, 578-596.

Chen, X., Qin, W., Ma, L., Xu, F., Jin, P., Zheng, Y. (2015) Effect of high pressure processing and thermal treatment on physicochemical parameters, antioxidant activity and volatile compounds of green asparagus juice. *LWT- Food Science and Technology,* 62, 927-933.

Chen, Z.Y., Zhu, Q.Y., Tsang, D., Huang, Y (2001) Degradation of green tea catechin in tea drinks. *Journal of Agricultural and Food Chemistry*, 49, 477-482.

Chisari, M., Barbagallo, R.N., Spagna, G. (2007) Characterization of polyphenol oxidase and peroxidase and influence on browning of cold stored strawberry fruit. *Journal of Agricultural and Food Chemistry*, 55, 3469-3476.

Chourio, A.M., Salais-Ferro, F., Mehmood, Z., Martinez-Monteagudo, S.I., Saldaña, M.D.A. (2018) Inactivation of peroxidase and polyphenoloxidase in coconut water using pressure-assisted thermal processing. *Innovative Food Science and Emerging Technologies,* 49, 41-50.

Clifford, H.M. (1999) Review: Chlorogenic acids and other cinnamates-nature, occurrence and dietary burden. *Journal of the Science of Food and Agriculture*, 79, 362-372.

Corrales, M., Tauscher, B. (2008) 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, 85-91.

Da Silveira, T.F.F; Meinhart, A.D., De Souza, T.C.L; Filho, J.T., Godoy, H.T. (2016) Phenolic compounds from yerba mate based beverages- A multivariate optimisation. *Food Chemistry* 190, 1159-1167.

Dacome, A.S., Silva, C.C., Costa, C.E.M., Fontana, J.D., Adelmann, J.A., Costa, S.C. (2005) Sweet diterpenic glycosides balance of a new cultivar of *Stevia rebaudiana* (Bert.) Bertoni: Isolation and quantitative distribution by chromatographic, spectroscopic, and electrophoretic methods. *Process Biochemistry*, 40, 3587-3594.

David, L.G., Bizgan, A.M.C., Popa, D.E., Buleandra, M., Moldovan, Z., Badea, I.A., Tekiner, A., Basaga, H., Ciucu, A. (2015) Rapid determination of total polyphenolic content in tea samples based on caffeic acid voltammetric behaviour on a disposable graphite electrode. *Food Chemistry*, 173, 1059-1065.

Dawidowicz, A.L., Typek, R. (2010) Thermal stability of 5-O-caffeoylquinic acid in aqueous solutions at different heating conditions. *Journal of Agricultural and Food Chemistry*, 58, 12578-12584.

De Maria, C.A.B., Trugo, L.C., Moreira, R.F.A., Petraco, M. (1995) Simultaneous determination of total chlorogenic acid, trigonelline and caffeic in green coffee samples by high performance gel filtration chromatography. *Food Chemistry,* 52, 447-449.

Deng, J., Xu, Z., Xiang, C., Liu, J., Zhou, L., Li, T. (2017) Comparative evaluation of maceration and ultrasonic-assisted extraction of phenolic compounds from fresh olives. *Ultrasonics Sonochemistry*, 37, 328-334.

Doshi, P., Adsule, P., Banerjee, K., Oulkar, D (2015). Phenolic compounds, antioxidant activity and insulinotropic effect of extracts prepared from grape (*Vitis vinifera L*) byproduts. *Journal of Food Science and Technology*, 52, 181-190.

Eisenmenger, M.J., Reyes-De-Corcuera, J.I. (2009) High pressure enhancement of enzymes: A review. *Enzyme and Microbial Technology*, 45, 331-347.

Euromonitor International. (2016) As consumption stagnates in South America, will yerba Mate move north? Accessed on November 10, 2018. https://blog.euromonitor.com/as-consumption-stagnates-in-south-america-will-yerba-mate-move-north/

Eylen, D.V., Oey, I., Hendrickx, M., Loey, A.V. (2008) Effects of pressure/temperature treatments on stability and activity of endogenous broccoli (*Brassica oleracea L. cv. Italica*) myrosinase and on cell permeability. *Journal of Food Engineering*, 89, 178-186.

Fang, X., Wang, Y., Wang, J., Zhang, J., Wang, X. (2013) Microwave-assisted extraction followed by RP-HPLC for the simultaneous extraction and determination of forsythiaside A, rutin, and phillyrin in the fruits of Forsythia suspense. *Journal of Separation Science*, 36, 2672-2679.

Fernandes, C.E.F., Scapinello, J., Bohn, A., Boligon, A., athayde, M., Magro, J.D., Palliga, M., Olivieira, M.V.T. (2017) Phytochemical profile, antioxidant and antimicrobial activity of extracts obtained from erva-mate (*Ilex paraguariensis*) fruit using compressed propane and supercritical CO_2. *Journal of Food Science and Technology*, 54, 98-104.

Friedman, M., Jurgens, H.S. (2000) Effect of pH on the stability of plant phenolic compounds. *Journal of Agricultural and Food Chemistry,* 48, 2101-2110.

Garcia-Palazon, A., Suthamthamgjai, W., Kajda, P., Zabetakis, I. (2004) The effects of high hydrostatic pressure on β-glucosidase, peroxidase and polyphenoloxidase in red raspberry (*Rubus idaeus*) and strawberry (*Fragaria ananassa*). *Food Chemistry*, 88, 7-10.

Garcia-Parra J., Gonzalez-Cebrino, F., Delgado, J. (2016) High pressure assisted thermal processing of pumpkin puree: Effect on microbial counts, color, bioactive compounds and polyphenoloxidase enzyme. *Food and Bioproducts Processing*, 98, 124-132.

Gardana, C., Scaglianti, M., Simonetti, P. (2010) 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, 1463-1470.

Gasmalla, M.A., Yang, R., Hua, X. (2014*) Stevia rebaudiana* Bertoni: An alternative sugar replacer and its application in food industry. *Food Engineering,* 6, 150-162.

Ghanta, S., Banerjee, A., Poddar, A., Chattopadhyay, S. (2007) Oxidative DNA damage preventive activity and antioxidant potential of *Stevia rebaudiana* (Bertoni) Bertoni, a natural sweetener. *Journal of Agricultural and Food Chemistry*, 55, 10962-10967.

Glavac, K., Stojilkovski, K., Kreft, L. (2017) Determination of fagopyins, rutin and quercetin in Tartary buckwheat products. *LWT Food Science and Technology*, 79, 423-427.

Goñi, M.L., Ganan, N., Strumia, M.C., Martini, R.E. (2016) Eugenol-loaded LLDPE films with antioxidant activity by supercritical carbon dioxide impregnation. *The Journal of Supercritical Fluids*, 111, 28-35.

Grujic, N., Lepojevic, Z., Srdjenovic, B., Vladic, J., Sudji, J. (2012) Effect of different extraction methods and conditions on the phenolic composition of mate tea extracts. *Molecules*, 17,

2518-2528.

Guggisberg D., Piccinali P., Schreier K. (2011) Effects of sugar substitution with *Stevia*, Actilight™ and *Stevia* combinations or Palatinose™ on rheological and sensory characteristics of low-fat and whole milk set yoghurt. *International Dairy Journal*, 9, 636-644.

Gulcin, I. (2006) Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid). *Toxicology*, 217, 213-220.

Gullo, B., Lu-Chau, T., Moreira, M.T., Lema, J.M., Eibes, G. (2017) Rutin: A review on extraction, identification and purification methods, biological activities and approaches to enhance its bioavailability. *Trends in Food Science and Technology,* 67, 220-235.

Gunawardena, D., Govindaraghavan, S., Munch, G (2014). Anti-inflammatory properties of cinnamon polyphenols and their monomeric precursors. In: Watson, R and Zibadi, S (Eds.), Polyphenols in Human Health and Disease, vol. 1, Chapter 30, Academic Press, London, UK. pp. 409-425.

He, Z., Tao, Y., Zeng, M., Zhang, S., Tao, G., Qin, F., Chen, J. (2016) High pressure homogenization processing, thermal treatment and milk matrix effect in vitro bioaccessibility of phenolics in apple, grape and orange juice to different extents. *Food Chemistry,* 200, 107-116.

Hendrickx, M., Ludikhuyze, L., Van den Broeck, I., Weemaes, C. (1998) Effects of high pressure on enzyme related to food quality. *Trends in Food Science and Technology*, 9, 197-203.

Huang, H., Wu, S., Lu, J., Shyu, Y., Wang, C. (2017) Current status and future trends of high pressure processing in food industry. *Food Control*, 72, 1-8.

Hudson, E.A., Dinh, P.A., Kokubun, T., Simmonds, M.S. (2000) Characterization of potentially chemopreventive phenols in extracts of brown rice that inhibit the growth of human breast and colon cancer cells. *Cancer Epidemiology, Biomarkers and Prevention,* 9, 1163-1170.

Igual, M., Garcia-Martinez, E., Camacho, M, Martinez-Navarrete, N. (2011) Changes in flavonoid content of grapefruit juice caused by thermal treatment and storage. *Innovative Food Science and Emerging Technologies*, 12, 153-162.

Inada, K.O.P., Torres, A.G., Perrone, D., Monteiro, M. (2018) High hydrostatic pressure processing affects the phenolic profile, preserves sensory attributes and ensures microbial quality of jabuticaba (*Myrciaria jaboticaba*) juice. *Journal of the Science of Food and Agriculture*, 98, 231-239.

Isolabella, S., Cogoi, L., Lopez, P., Anesini, C., Ferraro, G., Filip. R. (2010) Study of the bioactive compound variation during yerba mate (*llex paraguariensis)* processing. *Food Chemistry,* 122, 695-699.

Jacques, R.A., Dariva, C., De Oliveira, J.V., Caramao, E.B. (2008) Pressurized liquid extraction of mate tea leaves. *Analytica Chimica Acta*, 5, 70-76.

Jez, M., Wiczkowski, W., Zielinska, D., Bialobrzewski, Blaszczak, W. (2018) The impact of high pressure processing on the phenolic profile, hydrophilic antioxidant and reducing capacity of puree obtained from commercial tomato varieties. *Food Chemistry,* 261, 201-209.

Ji, W., Meng, Q., Ding, L., Wang, F., Dong, J., Zhou, G., Wang, B. (2016) Measurement and correlation of the solubility of caffeic acid in eight mono and water + ethanol mixed solvents at temperatures from (293.15 to 333.15) K. *Journal of Molecular Liquids,* 224, 1275-1281.

Jun, X. (2009) Caffeine extraction from green tea leaves assisted by high pressure processing. *Journal of Food Engineering*, 94, 105-109.

Jun, X., Shen, Deji, Zhao, Shou, Lu, Bingbing, Li, Ye, Zhanf, Rui. (2009) Characterization of polyphenols from green tea leaves using a high hydrostatic pressure extraction. *International Journal of Pharmaceutics,* 382, 139-143.

Jung, L.S., Lee., S.H., Kim, S., Ahn, J. (2013) Effect of high hydrostatic pressure on the quality-related properties of carrot and spinach. *Food Science and Biotechnology*, 22, 189-195.

Junior, E.L.C., Morand, C. (2016) Interest of mate (*Ilex paraguariensis* A. St.-Hill.) as a new natural functional food to preserve human cardiovascular health-A review. *Journal of Functional Foods,* 21, 440-454.

Karadag, A., Ozcelik, B., Huang, Q. (2014) Quercetin nanosuspensions produced by high-pressure homogenization. *Journal of Agricultural and Food Chemistry*, 62, 1852-1859.

Karakose, H., Muller, A., Kuhnert, N. (2015) Profiling and quantification of phenolics in *Stevia rebaudiana* leaves. *Journal of Agricultural and Food Chemistry*, 63, 9188-9198.

Karekar, V., Joshi, S., Shinde, S.L. (2000) Antimutagenic profile of three antioxidants in the Amess assay and the Drosophila wing spot test. *Mutation Research,* 468, 183-194.

Kaushik, N., Rao, P.S., Mishra, H.N. (2016) Process optimization for thermal-assisted high pressure-processing of mango (*Mangifera indica L*) pulp using response surface methodology. *LWT-Food Science and Technology*, 69, 372-381.

Kelebek, H., Selli, S., Kadiroglu, P., Kola, O., Kesen, S., Ucar, B., Cetiner, B. (2017) Bioactive compounds and antioxidant potential in tomato pastes as affected by hot and cold break process. *Food Chemistry*, 220, 31-41.

Khuwijitjaru, P., Suaylam, B., Adachi, S. (2014) Degradation of caffeic acid in subcritical water and online HPLC-DPPH assay of degradation products. *Journal of Agricultural and Food Chemistry*, 62, 1945-1949.

Kim, H., Seo, E.M., Sharma, A.R., Ganbold, B., Park, J., Sharma, G. (2013) Regulation of Wnt signaling activity for growth suppression induced by quercetin in 4T1 murine mammary cancer cells. *International Journal of Oncology*, 43, 1319-1325.

Kim, M.K., Park, K.S., Choo, H., Chong, Y. (2015) Quercetin-POM (pivaloxymethyl) conjugates: Modulatory activity for P-glycoprotein-based multidrug resistance. *Phytomedicine,* 22, 778-785.

Korir, M. W., Wachira, F. N., Wanyoko, J. K., Ngure, R. M., and Khalid, R. (2014) The fortification of tea with sweeteners and milk and its effect on in vitro antioxidant potential of tea product and glutathione levels in an animal model. *Food Chemistry,* 145, 145–153.

Kotovicz, V., Zanoelo, E.F. (2013) Hydrostatic pressure cycling extraction of soluble matter from mate leaves. *Journal of Food Engineering*, 116, 656-665.

Koutchma, T. (2014) Fundamentals of HPP technology. In: Koutchma, T (Ed.), Adapting High Hydrostatic Pressure for Food Processing Operations, vol. 1, Chapter 2, Academic Press, London, UK. pp. 5-10.

Kovacevic, D.B., Barba, F.J., Granato, D., Galanakis, C.M., Herceg, Z., Dragovic-Uzelac, V., Putnik, P. (2018) Pressurized hot water extraction (PHWE) for the green recovery of bioactive compounds and steviol glycosides from *Stevia rebaudiana* Bertoni leaves. *Food Chemistry*, 15, 150-157.

Kraujalis, P., Venskutonisa, P.R., Ibanez, E, Herrere, M. (2015) Optimization of rutin isolation from *Amaranthus paniculatus* leaves by high pressure extraction and fractionation techniques. *The Journal of Supercritical Fluids*, 104, 234-242.

Kungel, P., Correa, V.G., Correa, R.C.G., Peralta, R.A., Sokovic, M., Calhelha, R., Bracht, A., Ferreira, I, Peralta, R. (2018) Antioxidant and antimicrobial activities of a purified

polysaccharide from yerba mate (*Ilex paraguariensis*). *International Journal of Biological Macromolecules*, 114, 1161-1167.

Labbe, D., Tremblay, A., Bazinet, L. (2006) Effect of brewing temperature and duration on green tea catechin solubilization: Basis for production of EGC and EGCG-enriched fractions. *Separation and Purification Technology*, 49, 1-9.

Lee, K.W., Bode, A.M., Dong, Z. (2011) Molecular targets of phytochemicals for cancer prevention. *Nature Reviews Cancer,* 11, 211-218.

Lemus-Mondaca, R., Vega-Galvez, A., Zura-Bravo, L., Ah-Hen, K. (2012) *Stevia rebaudiana Bertoni*, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. *Food Chemistry*, 132, 1121-1132.

Lesjak, M., Beara., I; Simin, N., Pintac, D., Majkic, T., Bekvalac, K., Orcic, D., Mimica-Dukic, N. (2018) Antioxidant and anti-inflammatory activities of quercetin and its derivatives. *Journal of Functional Foods*, 40, 68-75.

Li, N., Taylor, L.S., Ferruzzi, M.G., Mauer, L. (2012) Kinetic study of catechin stability: Effects of pH, concentration, and temperature. *Journal of Food Agricultural and Food Chemistry*, 60, 12531-12539.

Liza, M.S., Rahman, R.A., Mandanam B., Jinap, S., Rahmat, A., Zidul, I.S.M. (2010) Supercritical carbon dioxide extraction of bioactive flavonoid from Strobilanthes crispus (*Pecah kaca*). *Food and Bioproducts Processing,* 88, 319-326.

MacDonald, L., Schaschke, C.J. (2000) Combined effect of high pressure, temperature, and holding time on polyphenoloxidase and peroxidase activity in banana (*Musa acuminata*). *Journal of the Science of Food and Agriculture*, 80, 719-724.

Makila, L., Laaksonen, O., Kallio, H., Yang, B. (2017) Effect of processing technologies and storage conditions on stability of blackcurrant juices with special focus on phenolic compounds and sensory properties. *Food Chemistry,* 221, 422-430.

Makris, D.P., and Rossiter, J. T (2000). Heat-induced, metal-catalyzed oxidative degradation of quercetin and rutin (quercetin 3-O-rhamnosylglucoside) in aqueous model systems. *Journal of Agricultural and Food Chemistry*, 48, 3830-3838.

Martinez-Monteagudo, S.I., Saldaña, M.D.A. (2015) Combined effect of pressure-assisted thermal processing and antioxidants on the retention of conjugated linoleic acid in milk. *Foods*, 4*,* 65-79.

Matsui, K.T., Gut, J.A.W., Oliveira, P.V., Tadini, C.C. (2008) Inactivation kinetics of polyphenol oxidase and peroxidase in green coconut water by microwave processing. *Journal of Food Engineering*, 88, 169-176.

Medina, I., Undeland, I., Larsson, K., Storro, I., Rustad, T., Jacobsen, C., Kristinova, V., Gallardo, J.M. (2012) Activity of caffeic acid in different fish lipid matrices: A review. *Food Chemistry,* 131, 730-740.

Mello, L.D., Alves, A.A., Macedo, D.V., Kubota, L.T. (2004) peroxidase-based biosensor as a tool for a fast evaluation of antioxidant capacity of teas. *Food Chemistry*, 92, 515-519.

Moon, Y.J., Wang, L., DiCenzo, R., Morris, M.E. (2008) Quercetin pharmacokinetics in humans. *Biopharmaceutics and Drugs Disposition*, 29, 205-217.

Mozhaev,V.V., Lange, R., Kudryashova, E.V., Balny, C. (1996) Application of high hydrostatic pressure for increasing activity and stability of enzymes. *Biotechnology Bioengineering*, 52, 320-331.

Mrad, N.D., Boudhrioua, N., Courtois, F., Bonazzi, C. (2012) Influence of air drying temperature on kinetics, physicochemical properties, total phenolic content and ascorbic acid of pears. *Food and Bioproducts Processing*, 3, 433-441.

Murakami, A.N.N., Amboni, R.D., Prudêncio, E.S., Amante, E.R., Fritzen-Freire, C.B., Boaventura, B.C., Muñoz, I.B., Branco, C.D., Marashin, M. (2013) Concentration of biologically active compounds extracted from *Ilex paraguariensis* St. Hil. by nanofiltration. *Food Chemistry,* 141, 60-65.

Murakami, M., Yamaguchi, T., Takamura, H., Matoba, T. (2004) Effects of thermal treatment on radical-scavenging activity of single and mixed polyphenolic compounds. *Journal of Food Science: Food Chemistry and Toxicology*, 69, 7–10.

Narita, Y., Inouye, K. (2013) Degradation kinetics of chlorogenic acid at various pH values and effects of ascorbic acid and epigallocatechin gallate and its stability under alkaline conditions. *Journal of Agricultural and Food Chemistry,* 61, 966–972

Nokthai, P., Lee, V.S., Shank, L. (2010) Molecular modeling of peroxidase and polyphenol oxidase: substrate specificity and active site comparison. *International Journal of Molecular Science,*11, 3266-3276.

Nowicka, P., Wojdylo, A. (2016) Stability of phenolic compounds, antioxidant activity and colour through natural sweeteners addition during storage of sour cherry puree. *Food Chemistry* 196, 925-934.

ñyal, S.K., Goyal, R.K., Goyal, S. (2010) Stevia (*Stevia rebaudiana*) a bio-sweetener: a review, *International Journal of Food Sciences and Nutrition*, 61, 1-10.

Palliga, M., Oliveira, J.V., Tres, M.V. (2016) Phytochemical profile, antioxidant and antimicrobial activity of extracts obtained from erva-mate (*Ilex paraguariensis*) fruit using compressed propane and supercritical CO_2. *Journal of Food Science and Technology,* 54, 98-104.

Patras, A., Brunton N.P., Da Pieve S., Butler F. (2009) Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. *Innovative Food Science and Emerging Technologies*, 10, 308-313.

Perez-Ramirez, I.F., Castano-Tostado, E., Ramirez-De Leon, J.A., Rocha-Guzman, N.E., Reynoso-Camacho, R. (2015) 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, 885-892.

Periche, A., Castello, M.L., Heredia, A., Escriche, I. (2015) Influence of drying method on steviol glycosides and antioxidant in *Stevia rebaudiana* leaves. *Food Chemistry* 172, 1-6.

Prestamo, G., Arroyo, G. (1998) High hydrostatic pressure effects on vegetables structure. *Journal of Food Science*, 63, 878-881.

Prince, P.S.M., Kumara, K.S. (2012) Preventive effects of caffeic acid on lipids, lipoproteins and glycoproteins in isoproterenol induced myocardial infarcted rats. *Food Research International,* 45, 155-160.

Quaglia, G.B., Gravina, R., Paperi, R., Paoletti, F. (1996) Effect of high pressure treatments on peroxidase activity ascorbic acid content and texture in green peas. *LWT-Food Science and Technology*, 29, 552-555.

Ranilla, L.G., Genovese, M.L., Lajolo, F.M. (2009) Effect of different cooking conditions on phenolics compounds and antioxidant capacity of some selected Brazilian bean (*Phaseolus vulgaris L.*) cultivars. *Journal of Agricultural and Food Chemistry*, 57, 5734-5742.

Rasanayagam, V., Balasubramaniam, V.M., Ting, E., Sizer, C.E., Bush, C., Anderson, C. (2003) Compression heating of selected fatty food materials during high-pressure processing. *Journal of Science,* 68, 251-259.

Ravber, M., Knez, Z., Skerget, M. (2015) Optimization of hydrolysis of rutin in subcritical water using response surface methodology. *The Journal of Supercritical Fluids*, 104, 145-152.

Ravichandra, K., Ahmed, A., Knorr, D., Smetanska, I. (2012) The effect of different processing methods on phenolic acid content and antioxidant activity of red beet. *Food Research International,* 48, 16-20.

Re, R., Bramley, P.M., Rice-Evans, C. (2002) Effects of food processing on flavonoids and lycopene status in a Mediterranean tomato variety. *Free Radical Research,* 36,803-810.

Reinders, R.D., Biesterveld, S., Bijker, P.G. (2001) Survival of Escherichia coli O157:H7 AT CC 43895 in a model apple juice medium with different concentrations of proline and caffeic acid. *Applied and Environmental Microbiology,* 67, 2863-2866.

Riachi, L.G., Simas, D.L.R., Coelho, G.C., Marcellini, P.S., Da Silva, A.J.R., De Maria, C.A.B. (2018) Effect of light intensity and processing conditions on bioactive compounds in mate extracted from yerba mate (*IIex paraguariensis* a. St.-Hil). *Food Chemistry*, 266, 317-322.

Roldan-Marin E., Sanchez-Moreno, C., Lloria, R., Ancos, B. (2009) Onion high-pressure processing: Flavanol content and antioxidant activity. *LWT-Food Science and Technology*, 42, 835-841.

Romero-Diez, R., Rojo-Rodriguez, S., Cocero, M.J., Duarte, C.M.M., Matias, A.A., Bronze, M.R. (2018) Phenolic characterization of aging wine less: Correlation with antioxidant activities. *Food Chemistry,* 259, 188-195.

Salazar, V.A.G; Encalada, S.V., Cruz, A.C., Campos, M.R.S. (2018) *Stevia rebaudiana*: A sweetener and potential bioactive ingredient in the development of functional cookies. *Journal of Functional Foods,* 44, 183-190.

Saldaña M.D.A., Zetzl, C., Mohamed R.S., Brunner, G. (2002) Extraction of methylxanthines from guarana seeds, mate, and cocoa beans using supercritical carbon dioxide and ethanol. *Journal of Agricultural and Food Chemistry,* 50, 4820-4826.

Saldaña, M.D.A., Espirito Santo, A.T., Feihrmann, A.C., Du, J., Cassel, E. (2015). High pressure processing of beverages: Mate/green tea+milk. In proceedings of the 2015 International Nonthermal Processing Workshop, November 12-13, 2015, Athens, Greece, 5 pp.

Saldaña, M.D.A., Mohamed, R.S., Baer, M.G. And Mazzafera, P. (1999) Extraction of purine alkaloids from maté (*Ilex paraguariensis*) using supercritical CO_2. *Journal of Agriculture and Food Chemistry*, 47, 3804-3808.

Santos, M.C., Nunes, C., Ferreira, A., Jourdes, M., Teissedre, P., Rodrigues, A., Amado, O., Saraiva, J., Coimbra, M. (2018) Comparison of high pressure treatment with conventional red wine aging processes: impact on phenolic composition. *Food Research International*, In Press. https://doi.org/10.1016/j.foodres.2018.08.018.

Sardi J.C.O., Gullo, F.P., Freires, I.A., Pitangui, N.S., Segalla, M.P., Fusco-Almeida, P., Regasini, L.O., Mendes-Giannini. (2016) Synthesis, antifungal activity of caffeic acid derivative esters, and their synergism with fluconazole and nystatin against *Candida* spp. *Diagnostic Microbiology and Infectious Disease,* 86, 387-391.

Savita, S., Sheela, K., Sunanda, S., Shankar, A., Ramakrishna, P. (2004) *Stevia rebaudiana* – A functional component for food industry. *Journal of Human Ecology*, 15, 261-264.

Serio, L. (2010) La *Stevia rebaudiana***,** une alternative au sucre. *Phytothérapie*, 8, 26-32.

Shah, A., Jones, G., Vasiljevic, T., Vasiljevic, J. (2010) Sucrose-free chocolate sweetened with *Stevia rebaudiana* extract and containing different bulking agents – effects on physicochemical and sensory properties. *International Journal of Food Science and Technology,* 45, 1426-1435.

Shan, B.E., Wang, M.X., Li, R.Q. (2009) Quercetin inhibit human SW480 colon cancer growth in association with inhibition of cyclin D1 and survivin expression through Wnt/beta-catenin signaling pathway. *Cancer Investigation*, 27, 604-612.

Sharma, K., Ko, E.Y., Assefa, A.D., Ha, S., Nile, S.H., Lee, E.T., Park, S.W. (2015) Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onions varieties. *Journal of Food and Drug Analysis*, 23, 243-252.

Sharma, V., Gulati, A., Ravindranath, S.D. (2005) Extractability of tea catechins as a function of manufacture procedure and temperature of infusion. *Food Chemistry*, 93, 141-148.

Smeller, L. (2002) Pressure-temperature phase diagram of biomolecules. *Biochimica et Biophysica Acta,* 1595, 11-29.

Solana, L. M., Boschiero, I., Dall'Acqua, S., and Bertucco, A. (2015) A comparison between supercritical fluid and pressurized liquid extraction methods for obtaining phenolic compounds from Asparagus officinalis L. *The Journal of Supercritical Fluids*, 100, 201-208.

Soleas, G.J., Grass, L., Josphy, P.D., Goldberg, D.M., Diamandis, E.P. (2002) A comparison of the anticarcinogenic properties of four red wine polyphenols. *Clinical Biochemistry,* 35, 119-124.

Su, Y.L., Leung, L.K., Huang, Y., Chen, Z.Y. (2003) Stability of tea theaflavins and catechins. *Food Chemistry*, 83, 189-195

Sulaima, A., Soo, M.J., Yoon, M.M., Farid, M., Silva, F.V. (2015) Modeling the polyohenoloxidase inactivation kinetics in pear, apple and strawberry purees after high pressure processing. *Journal of Food Engineering*, 147, 89-94.

Tadhani, M., Subhash, R. (2006) Preliminary studies on *Stevia rebaudiana* leaves: Proximal composition, mineral analysis and phytochemical screening. *Journal of Medical Sciences*, 6, 321-326.

Tajik, N., Tajik, M., Mack, I., Enck, P. (2017) The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: a comprehensive review of the literature. *European Journal of Nutrition*, 56, 2215-2244.

Tanaka, N., Kunugi, S (1995) Structure of pressure-induced "denatured" state of proteins. In: Hayashi, R and Balny, C (Eds.), High Pressure Bioscience and Biotechnology, vol. 13, Chapter 2, Elsevier Science B.V, Amsterdam, The Netherlands. pp. 153-156.

Terefe, N.S., Delon, A., Versteeg, C. (2017) Thermal and high pressure inactivation kinetics of blueberry peroxidase. *Food Chemistry*, 232, 820-826.

Terefe, N.S., Tepper, P., Ullman, A., Knoerzer, K., Juliano, P. (2016) High pressure thermal processing of pears: Effect on endogenous enzyme activity and related quality attributes. *Innovative Food Science and Emerging Technologies*, 33, 56-66.

Terefe, N.S., Yang, Y.H., Knoerzer, K., Buckow, R., Versteeg, C. (2010) High pressure and thermal inactivation kinetics of polyphenol oxidase and peroxidase in strawberry puree. *Innovative Food Science and Emerging Technologies*, 11, 52-60.

Thoo, Y. Y., Ho, S. K., Abas, F., Lai, O. M., Ho, C. W., and Tan, C. P. (2013) Optimal binary solvent extraction system for phenolic antioxidants from Mengkudu (*Morinda citrifolia*) fruit. *Molecules*, 18, 7004-7022.

Tinello, F., Lante, A. (2018) Recent advances in controlling polyphenol oxidase activity of fruit and vegetable products. *Innovative Food Science and Emerging Technologies*, 50, 73-83.

Turkmen, N., Sari, F., Velioglu, Y.S. (2006) 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, 835-841.

Wang, R., Zhou, W., Wen, R.A.H. (2006) Kinetic study of the thermal stability of tea catechins in aqueous systems using a microwave reactor. *Journal of Agricultural and Food Chemistry*, 54, 5924-5932.

Wang, W., Sun, C., Mao, L., Liu, Fuguo., Yang, J., Gao, Y. (2016) The biological activities, chemical stability, metabolism and delivery systems of quercetin: A review. *Trends in Food Science and Technology*, 56, 21-38.

Wang, Y., Miao, X, Sun, J., Cai, L. (2015) Oxidative stress in diabetes: Molecular basis for diet supplementation. In: Didac, M. (Ed.), Molecular Nutrition and Diabetes, vol. 1, Chapter 6, Academic Press, London, UK. pp. 65-72.

Wu, L., Shi, A., Zhu, D., Bo, L., Zhong, Y., Wang, J., Xu, Z. (2006) High sucrose intake during gestation increases angiotensin II type 1 receptor-mediated vascular contractility associated with epigenetic alterations in aged offspring rats. *Peptides,* 86, 133-144.

Xi, Jun. (2009) Caffeine extraction from green tea leaves assisted by high pressure processing. *Journal of Food Engineering*, 94, 105-109.

Xu, X., Deng, J., Luo, D., Bao, Y., Liao, X., Gao, H., Wu, J. (2018) Comparative study of high hydrostatic pressure and high temperature short time processing on quality of clear and cloudy Se-enriched kiwifruit juices. *Innovative Food Science and Emerging Technologies*, 49, 1-12.

Yao, Y., Ren, G. (2011) Effect of thermal treatment on phenolic composition and antioxidant activities of two celery cultivars. *LWT-Food Science and Technology,* 44, 181-185.

Yi, J., Jiang, B, Zhang, Z., Liao, X., Hu, X. (2012) Effect of ultrahigh hydrostatic pressure on the activity and structure of mushroom (*Agaricus bisporus*) polyphenoloxidase. *Journal of Agricultural and Food Chemistry*, 60, 593-599.

Yildiz-Ozturk, E., Tag, O., Yesil-Celiktas, O. (2014) Subcritical water extraction of steviol glycosides from Stevia rebaudiana leaves and characterization of the raffinate phase. *The Journal of Supercritical Fluids*, 95, 422-430.

Yuan, B., Danao, M.G.C., Stratton, J.E., Weier, S.A., Weller, C.L., Lu, M. (2018) High pressure processing (HPP) of aronia berry puree: Effects on physicochemical properties, microbial counts, bioactive compounds, and antioxidant capacity. *Innovative Food Science and Emerging Technologies,* 47, 249-255.

Zafrilla, P., Ferreres, F., Tomas-Barberan, F.A. (2001) Effect of processing and storage on the antioxidant ellagic acid derivatives and flavonoids of red raspberry (*Rubus idaeus*) jams. *Journal of Agricultural and Food Chemistry*, 49, 3651-3655.

APPENDIX A
Effect of pressure-assisted thermal processing on bioactive compounds and antioxidant activity of mate

Table A.1 Proximate composition and pH of untreated mate.

Component (g/100 g)	Sample mate A	Sample mate B	Average Mate
Total carbohydrates (%)	77.0	75.8	76.4±1.1
Lipids (%)	0.5	0.5	0.5±0.0
Moisture (%)	5.2	5.3	5.2±0.5
Ash (%)	5.1	5.5	5.3±0.1
Protein (%)	12.2	12.9	12.6±0.5
pH	5.8	5.7	5.8±0.0

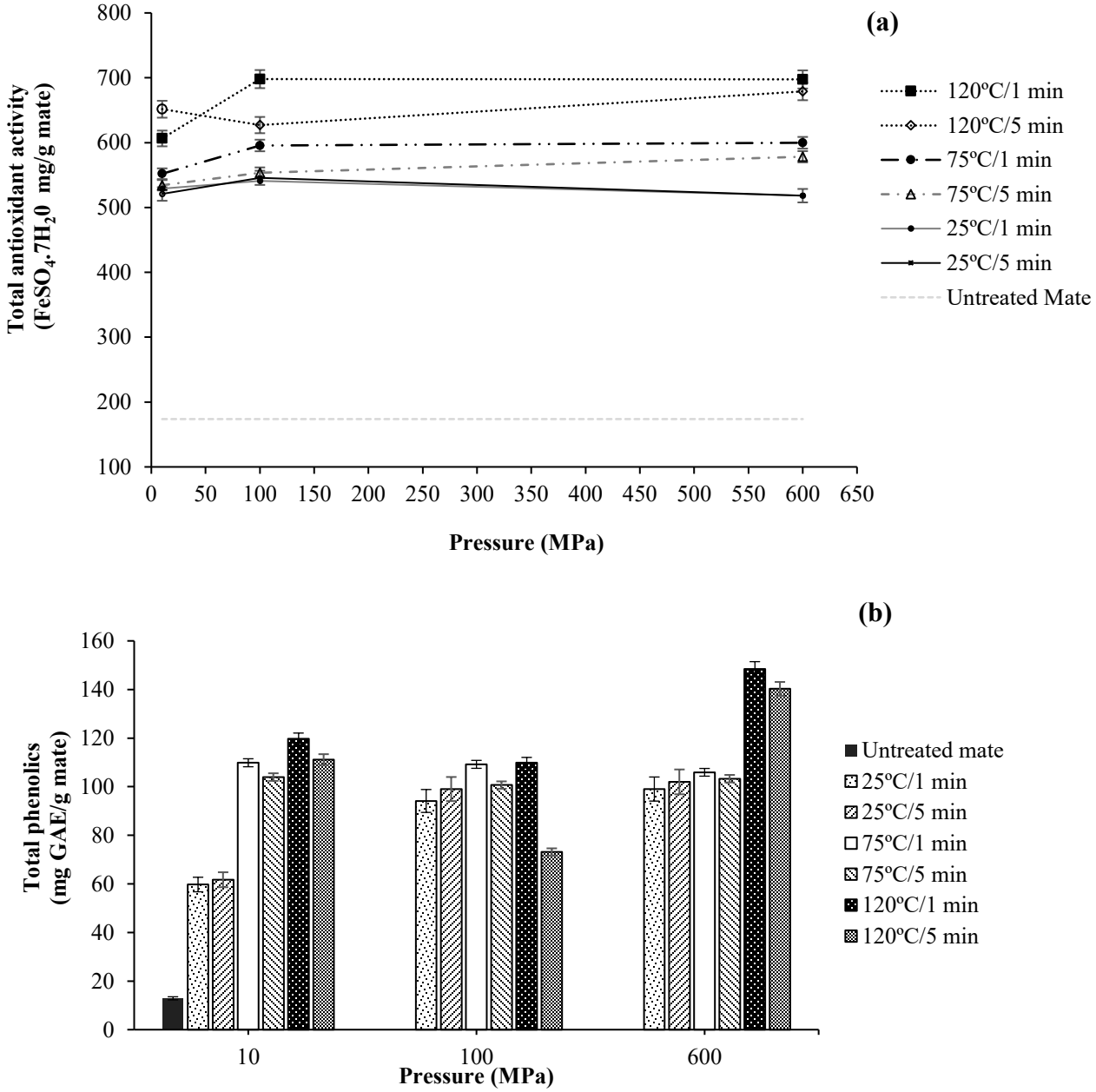


Figure A.1 (a) Total antioxidant activity and (b) Total phenolics of untreated and PATP treated mate.

Table A.2 Total antioxidant activity and total phenolics of PATP treated mate

Temperature	Pressure	Time	Total antioxidant activity (mg $FeSO_4.7H_20$/g mate)		Total phenolics (mg GAE/g mate)	
(ºC)	(MPa)	(min)	Mate	Average	Mate	Average
25	10	1	544.09	536.13±11.25	64.65	59.74±6.43
	10	1	528.18		54.83	
	10	5	533.96	528.18±8.18	54.83	58.76±4.20
	10	5	522.39		62.69	
	100	1	550.60	546.98±5.11	80.37	83.32±3.86
	100	1	543.37		86.27	
	100	5	551.32	545.90±7.67	101.99	101.00±1.82
	100	5	540.47		100.02	
	600	1	499.24	496.35±4.09	96.25	97.15±3.15
	600	1	493.46		98.06	
	600	5	512.26	514.43±3.07	90.20	96.09±7.72
	600	5	516.60		101.99	
75	10	1	560.73	559.28±2.05	109.85	108.86±1.82
	10	1	557.83		107.88	
	10	5	517.33	527.45±14.32	107.88	106.90±4.81
	10	5	537.58		105.92	
	100	1	588.94	594.72±8.18	111.81	119.67±10.29
	100	1	600.51		127.53	
	100	5	554.22	558.56±6.14	90.20	93.14±3.86
	100	5	562.90		96.09	
	600	1	607.02	608.47±2.05	92.61	99.26±3.86
	600	1	609.91		105.92	
	600	5	559.28	570.13±15.34	100.02	100.02±0.00
	600	5	580.98		100.02	
120	10	1	626.55	616.42±14.34	115.74	116.91±2.57
	10	1	606.30		118.09	
	10	5	659.82	651.87±11.25	114.45	115.09±5.15
	10	5	643.91		115.74	
	100	1	706.12	699.24±9.72	105.35	104.65±4.81
	100	1	692.37		103.95	
	100	5	647.53	637.04±14.83	79.88	72.26±14.15
	100	5	626.55		64.65	
	600	1	709.73	698.42±10.00	152.01	148.49±6.9
	600	1	687.31		145.22	
	600	5	719.14	693.46±36.31	138.20	140.31±6.50
	600	5	667.78		143.25	

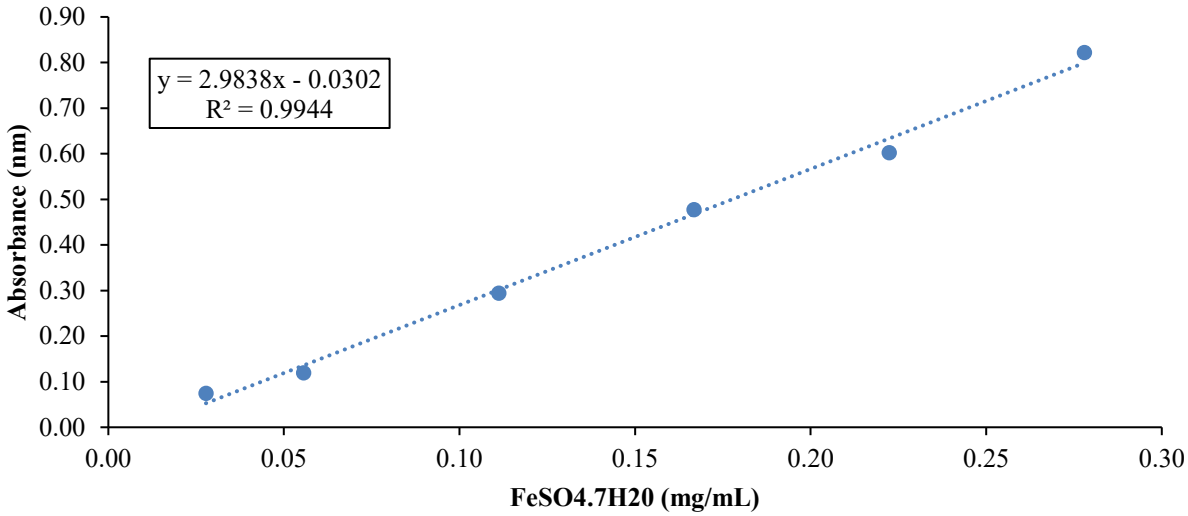


Figure A.2 Calibration curve of antioxidant activity

Table A.3 Analysis of variance of total antioxidant activity of PATP treated mate.

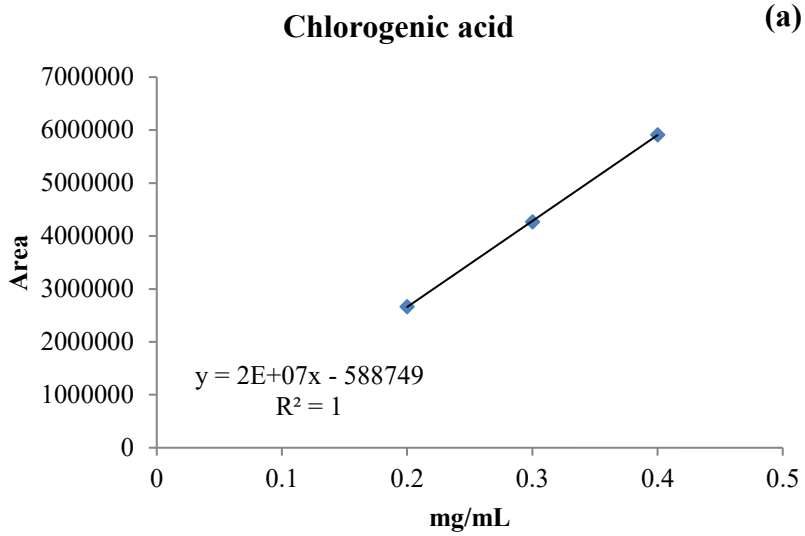
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	18	145825	8101.4	52.79	0.000
Blocks	1	466	465.7	3.03	0.100
Linear	5	128100	25620.0	166.94	0.000
Pressure (MPa)	2	5873	2936.6	19.13	0.000
Temperature (°C)	2	120374	60187.2	392.18	0.000
Holding time (min)	1	1852	1852.3	12.07	0.003
2-Way Interactions	8	13724	1715.5	11.18	0.000
Pressure (MPa)*Temperature (°C)	4	9778	2444.5	15.93	0.000
Pressure (MPa)*Holding time (min)	2	1665	832.5	5.42	0.015
Temperature (°C)*Holding time (min)	2	2281	1140.6	7.43	0.005
3-Way Interactions	4	3535	883.8	5.76	0.004
Pressure (MPa)*Temperature (°C)*Holding time (min)	4	3535	883.8	5.76	0.004

S	R-sq	R-sq(adj)	R-sq(pred)
12.3882	98.24%	96.38%	92.12%

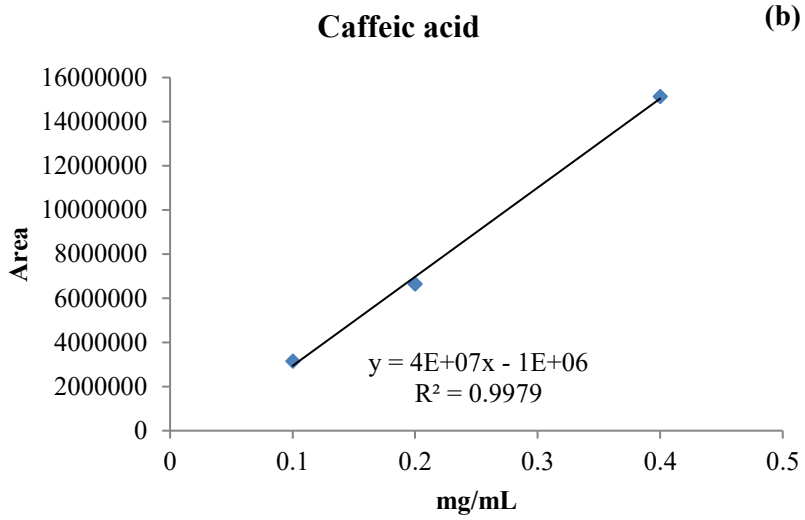
Table A.4 Analysis of variance of total phenolics of PATP treated mate

Source	p-value
Model	0.000
Blocks	0.315
Linear	0.000
Pressure (MPa)	0.000
Temperature (°C)	0.000
Holding time (min)	0.006
2-Way Interactions	0.000
Pressure (MPa)*Temperature (°C)	0.000
Pressure (MPa)*Holding time (min)	0.018
Temperature (°C)*Holding time (min)	0.002
3-Way Interactions	0.001
Pressure (MPa)*Temperature (°C)*Holding time (min)	0.001

(a)


Chlorogenic acid
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
Area
y = 2E+07x - 588749
R² = 1
0
0.1
0.2
0.3
0.4
0.5
mg/mL

(b)


Caffeic acid
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
Area
y = 4E+07x - 1E+06
R² = 0.9979
0
0.1
0.2
0.3
0.4
0.5
mg/mL

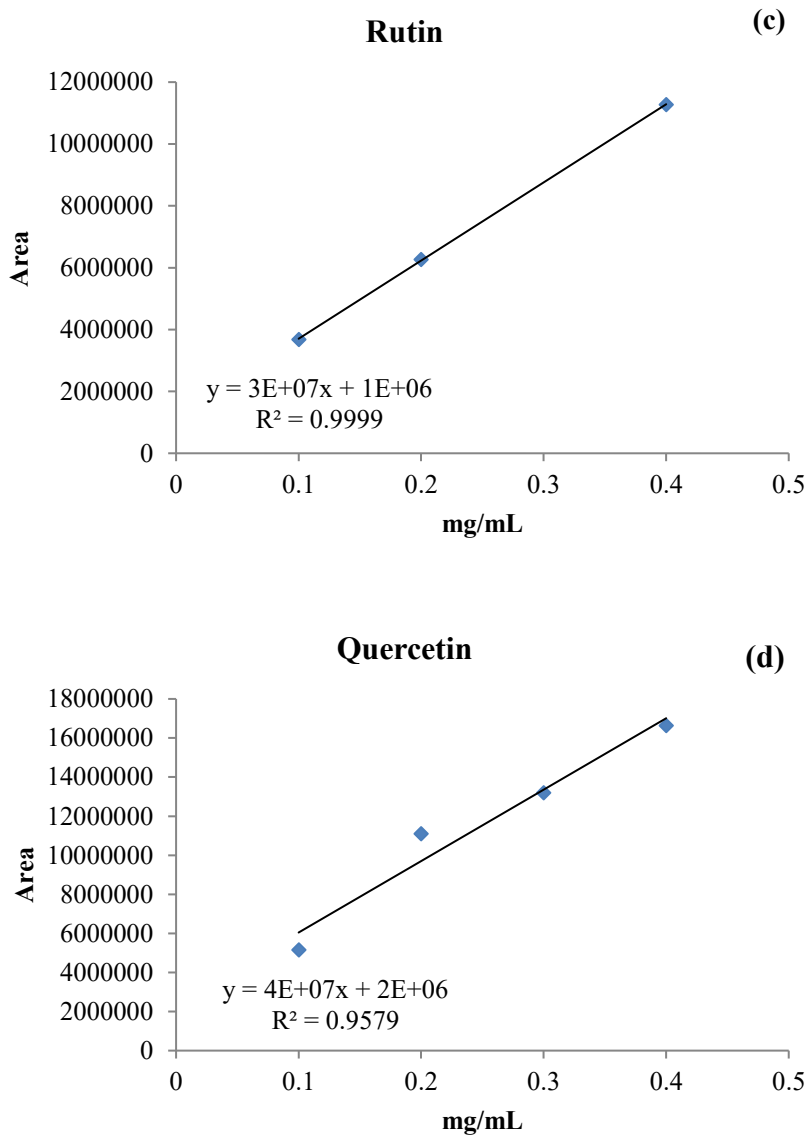


Figure A.3 HPLC calibration curve of individual phenolics (a) chlorogenic acid, (b) caffeic acid, (c) rutin and (d) quercetin.

Table A.5 Individual phenolic compounds of mate.

Temperature (ºC)	Pressure (MPa)	Time (min)	Caffeic acid (mg/g mate)		Chlorogenic acid (mg/g mate)		Rutin (mg/g mate)		Quercetin (mg/g mate)	
Untreated mate (25ºC/0.1 MPa)			6.71	6.48±0.32^{f}	8.539	8.25±0.41^{a}	0.294	0.28±0.01^{a}	1.297	1.25±0.06^{a}
			6.25		7.962		0.275		1.209	
25ºC	10	1	18.42	17.80±0.89^{d}	0.744	0.72±0.52^{b}	0.313	0.30±0.02^{a}	2.251	2.18±0.11^{b}
	10	1	17.18		0.693		0.292		2.099	
	10	5	24.27	23.45±1.17^{d}	0.831	0.80±0.04^{b}	0.317	0.31±0.06^{a}	3.531	3.41±0.17^{c}
	10	5	22.83		0.775		0.295		3.292	
	100	1	18.55	17.93±0.90^{d}	4.019	3.88±0.19^{c}	0.333	0.32±0.06^{b}	3.528	3.41±0.17^{c}
	100	1	17.30		3.747		0.310		3.289	
	100	5	19.12	18.47±0.92^{c}	4.123	3.98±0.20^{c}	0.330	0.32±0.02^{b}	3.218	3.11±0.16^{c}
	100	5	17.83		3.844		0.307		3.000	
	600	1	12.71	12.28±0.61^{e}	2.687	2.60±0.13^{d}	0.350	0.34±0.02^{b}	2.660	2.57±0.13^{b}
	600	1	11.85		2.505		0.327		2.480	
	600	5	15.61	15.08±0.75^{e}	3.504	3.39±0.17^{e}	0.345	0.33±0.02^{b}	3.220	3.11±0.16^{c}
	600	5	14.55		3.267		0.322		3.003	
75ºC	10	1	16.98	16.40±0.82e^{d}	3.909	3.78±0.19^{c}	0.334	0.32±0.02^{b}	3.420	3.31±0.17^{c}
	10	1	15.83		3.645		0.311		3.189	
	10	5	18.41	17.79±0.89^{d}	4.230	4.09±0.20^{c}	0.345	0.33±0.02^{b}	3.611	3.50±0.17^{c}
	10	5	17.17		3.944		0.322		3.367	
	100	1	18.38	17.76±0.89^{d}	4.086	3.95±0.20^{c}	0.338	0.33±0.02^{b}	3.653	3.53±0.18^{c}
	100	1	17.13		3.809		0.315		3.406	
	100	5	16.95	17.57±0.88^{d}	3.911	3.78±0.19^{c}	0.329	0.32±0.02^{b}	3.680	3.56±0.18^{c}
	100	5	18.18		3.646		0.307		3.431	
	600	1	18.44	19.11±0.95^{c}	4.392	4.24±0.21^{c}	0.336	0.32±0.02^{b}	3.670	3.55±0.18^{c}
	600	1	19.77		4.095		0.313		3.422	
	600	5	26.23	25.34±1.27^{b}	0.850	0.82±0.04^{b}	0.314	0.30±0.02^{b}	3.670	3.55±0.18^{c}
	600	5	24.45		0.792		0.292		3.422	
120ºC	10	1	15.65	15.12 ±0.76^{e}	0.704	0.68±0.03^{f}	0.296	0.29±0.01^{ab}	1.755	1.70±0.09^{a}
	10	1	14.59		0.657		0.276		1.637	

Table A.5 Continued.

Temperature (ºC)	Pressure (MPa)	Time (min)	Caffeic acid (mg/g mate)		Chlorogenic acid (mg/g mate)		Rutin (mg/g mate)		Quercetin (mg/g mate)	
120ºC	10	5	18.07	17.46±0.87^{d}	4.03	3.90±0.20^{c}	0.28	0.28±0.01^{ab}	4.31	4.16±0.21^{d}
	10	5	16.85		3.76		0.27		4.02	
	100	1	19.62	18.96±0.95^{c}	4.22	4.07±0.20^{c}	0.39	0.38±0.02^{c}	5.56	5.36±0.27^{e}
	100	1	18.29		3.93		0.37		5.18	
	100	5	25.91	25.05±1.25^{b}	2.67	2.60±0.13^{d}	0.37	0.36±0.02^{c}	2.54	2.45±0.12^{b}
	100	5	24.16		2.51		0.35		2.36	
	600	1	29.95	28.94±1.45^{a}	0.86	0.83±0.04^{b}	0.39	0.38±0.02^{c}	3.18	3.08±0.154^{c}
	600	1	27.93		0.81		0.37		2.97	
	600	5	21.99	21.24±1.06^{c}	0.77	0.76±0.04^{b}	0.35	0.34±0.02^{bc}	2.07	2.00±0.100^{b}
	600	5	20.50		0.73		0.32		1.93	

Table A.6 Individual catechins of mate.

Temperature	Pressure	Time	Catechin		Gallocatechin gallate		Epigallocatechin	
(ºC)	(MPa)	(min)	(mg/g mate)		(mg/g mate)		(mg/g mate)	
Untreated mate (25ºC/0.1 MPa)			0.38	0.36±0.02[a]	0.61	0.59±0.02[a]	0.15	0.15±0.01[a]
			0.35		0.57		0.14	
25ºC	10	1	0.75	0.73±0.04[b]	0.62	0.60±0.03[a]	0.17	0.17±0.01[b]
	10	1	0.70		0.58		0.17	
	10	5	0.94	0.91±0.05[c]	0.64	0.62±0.03[a]	0.23	0.22±0.02[c]
	10	5	0.88		0.60		0.22	
	100	1	0.97	0.94±0.05[c]	0.64	0.62±0.03[a]	0.38	0.37±0.03[d]
	100	1	0.91		0.60		0.36	
	100	5	1.00	0.97±0.05[c]	0.66	0.64±0.03[a]	0.40	0.38±0.03[d]
	100	5	0.94		0.61		0.37	
	600	1	0.64	0.62±0.03[d]	0.61	0.59±0.03[a]	0.16	0.15±0.01[a]
	600	1	0.60		0.57		0.14	
	600	5	0.78	0.76±0.04[b]	0.62	0.60±0.03[a]	0.20	0.19±0.02[b]
	600	5	0.73		0.58		0.18	
75ºC	10	1	0.17	0.16±0.01[e]	0.64	0.62±0.03[a]	0.35	0.34±0.03[d]
	10	1	0.16		0.59		0.33	
	10	5	1.02	0.99±0.05[c]	0.65	0.63±0.03[a]	0.39	0.39±0.02[d]
	10	5	0.95		0.62		0.37	
	100	1	traces	traces	0.65	0.62±0.03[a]	0.32	0.31±0.03[d]
	100	1	traces		0.60		0.30	
	100	5	0.91	0.88±0.04[c]	0.64	0.62±0.03[a]	0.35	0.34±0.02[d]
	100	5	0.85		0.60		0.33	
	600	1	1.03	1.00±0.05[c]	0.65	0.63±0.03[a]	0.27	0.26±0.01[c]
	600	1	0.97		0.61		0.25	
	600	5	traces	traces	0.84	0.81±0.040[b]	0.35	0.34±0.01[d]
	600	5	traces		0.78		0.33	

Table A.6 Continued.

Temperature (ºC)	Pressure (MPa)	Time (min)	Catechin (mg/g mate)		Gallocatechin gallate (mg/g mate)		Epigallocatechin (mg/g mate)	
120ºC	10	1	traces	traces	0.67	0.67±0.033[a]	0.22	0.21±0.03[b]
	10	1	traces		0.64		0.20	
	10	5	traces	traces	traces	traces	0.17	0.18±0.03[ab]
	10	5	traces		traces		0.17	
	100	1	traces	traces	traces	traces	0.18	0.18±0.02[ab]
	100	1	traces		traces		0.17	
	100	5	traces	traces	traces	traces	0.37	0.36±0.02[d]
	100	5	traces		traces		0.35	
	600	1	traces	traces	0.977	0.94±0.05[c]	0.44	0.43±0.02[e]
	600	1	traces		0.911		0.41	
	600	5	traces	traces	0.856	0.83±0.04[b]	0.31	0.30±0.03[bd]
	600	5	traces		0.798		0.29	

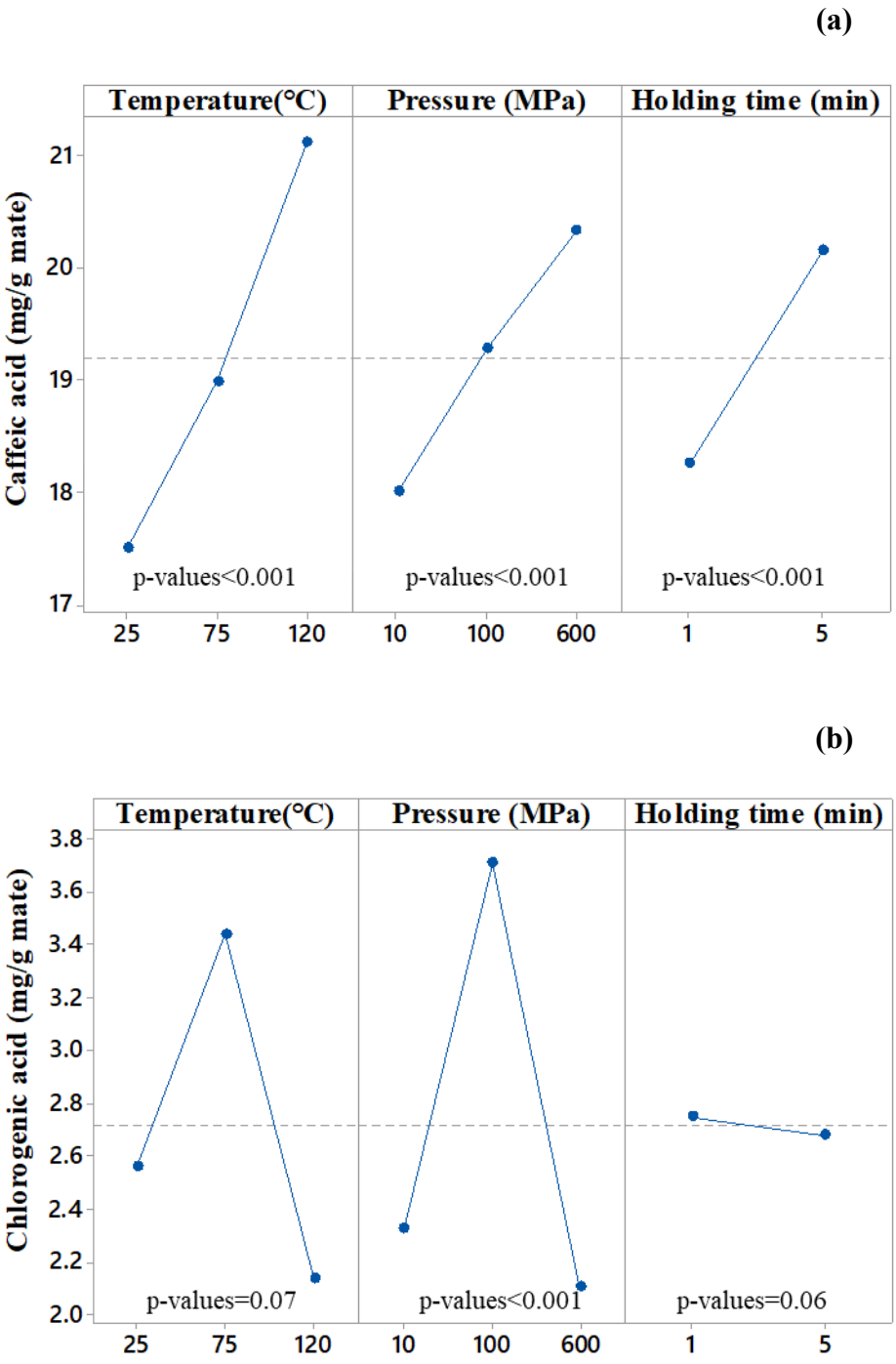


Figure A.4 Influence of variance, temperature, pressure, and holding time on (a) the caffeic and (b) chlorogenic acid content of PATP-treated mate.

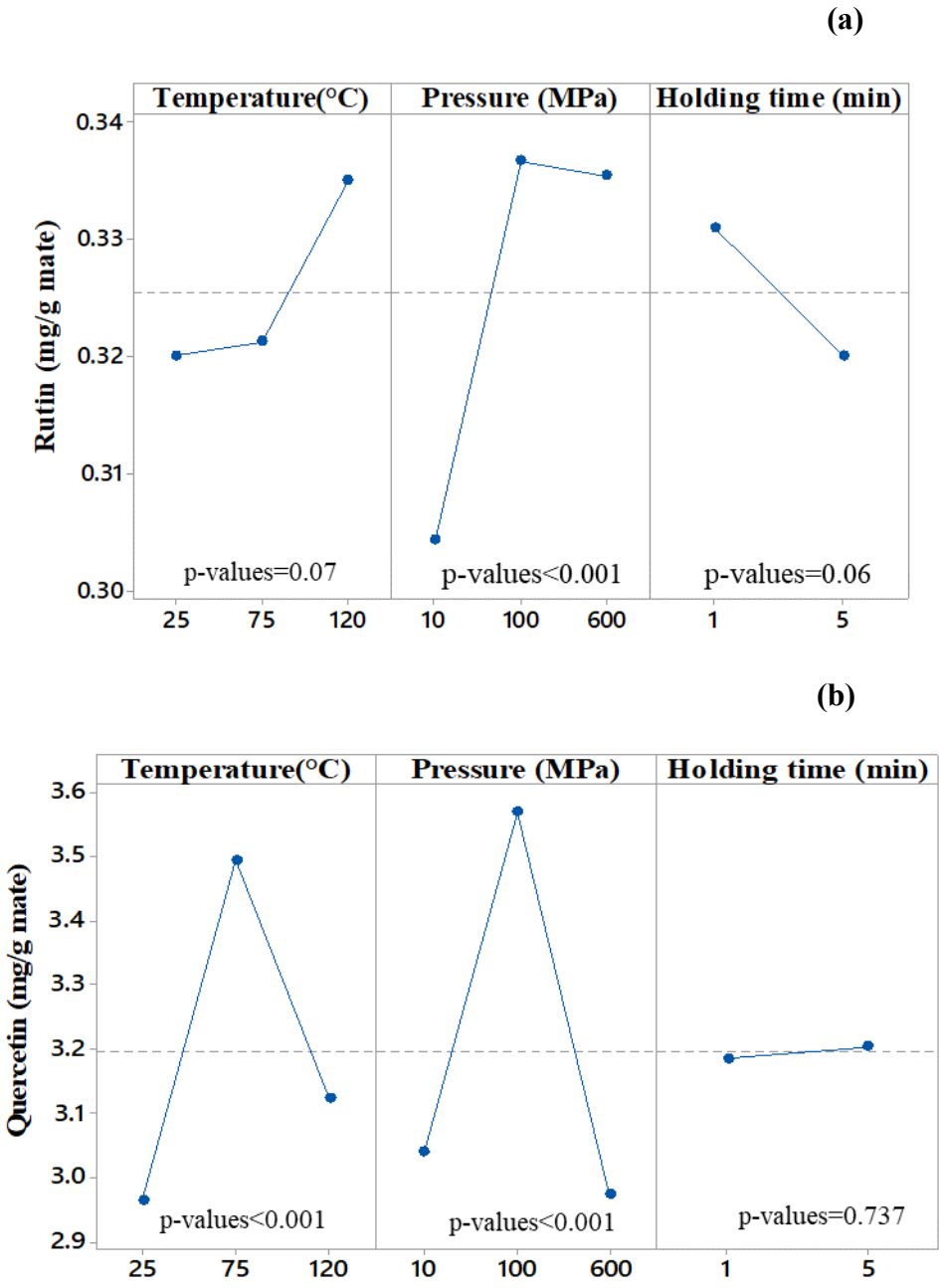


Figure A.5 Influence of variance, temperature, pressure, and holding time on (a) rutin and (b) quercetin content of PATP-treated mate.

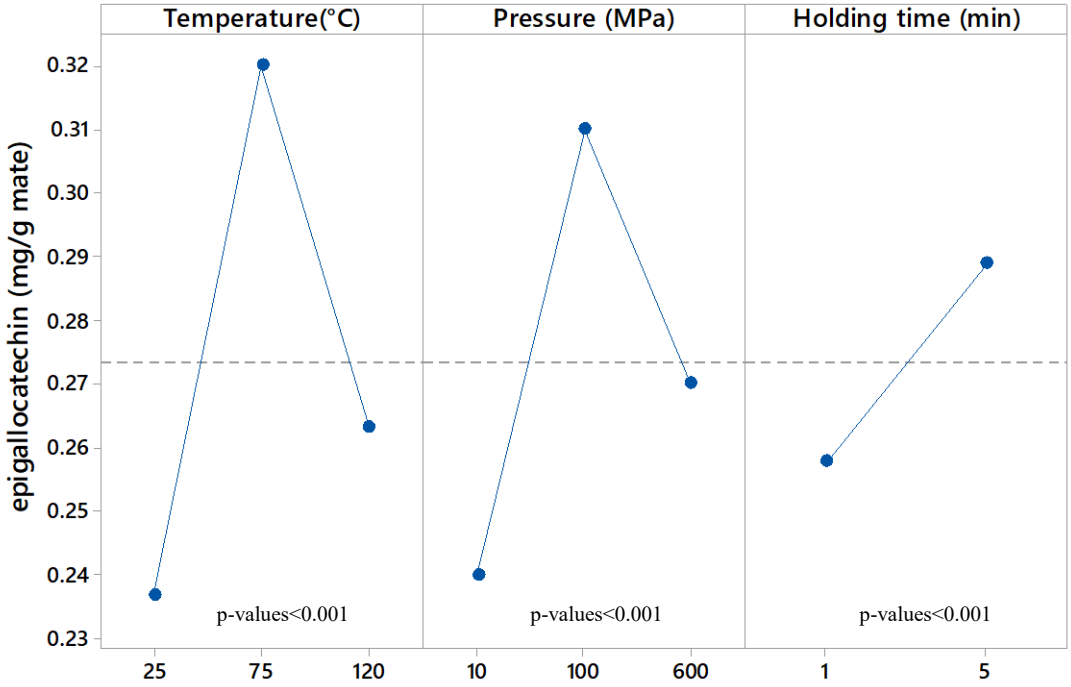
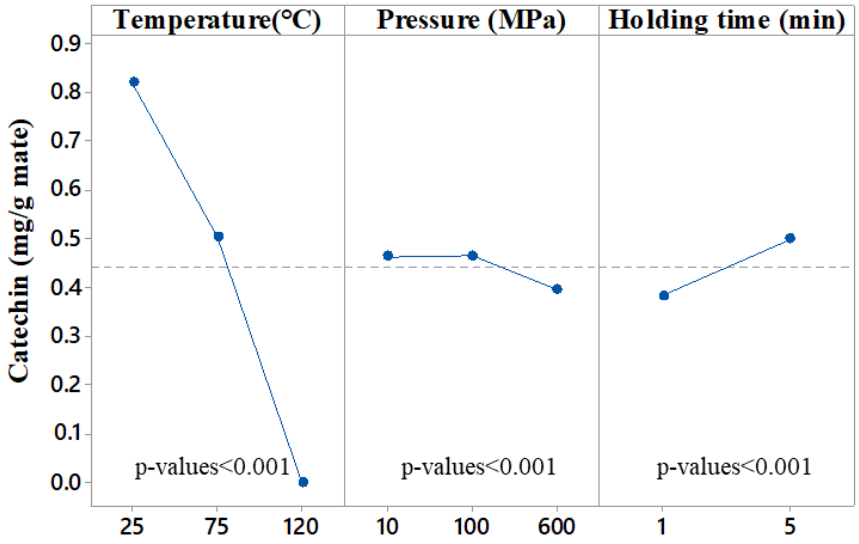


Figure A.6 Influence of variance, temperature, pressure, and holding time on the epigallocatechin concentration of treated mate.

(a)



(b)

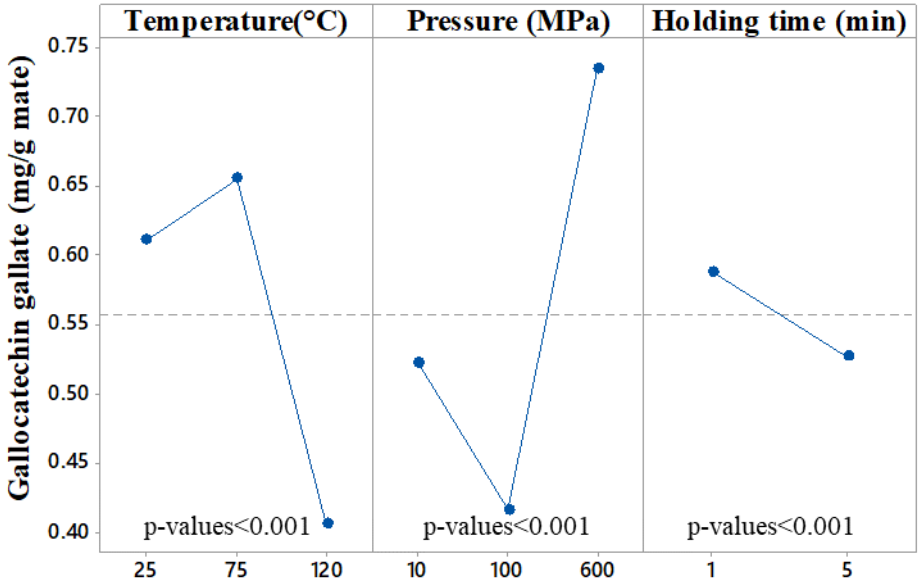


Figure A.7 Influence of variance, temperature, pressure, and holding time on the epigallocatechin concentration of treated mate.

Table A.7 Methylxanthines of untreated and treated mate at 120ºC.

Processing conditions	Caffeine (mg/g)		Theobromine (mg/g)	
Untreated mate A	20.03	22.05±2.85	16.71	15.29±2.02
Untreated mate B	24.07		13.86	
10 MPa/1 min	25.34	24.64±0.99	14.67	14.96±0.41
10 MPa/1 min	23.94		15.26	
100 MPa/1 min	34.02	35.88±2.59	23.99	23.99±0.02
100 MPa/1 min	37.68		24.00	
600 MPa/1 min	22.35	21.15±1.70	20.23	17.90±3.30
600 MPa/1 min	19.95		15.57	

APPENDIX B
Effect of pressure assisted thermal processing on bioactive compounds and antioxidant activity of *Stevia* and mate sweetened with *Stevia*

Table B.1 Proximate composition and pH of untreated commercial *Stevia* powder.

Component (g/100 g)	**Sample *Stevia* powder A**	**Sample *Stevia* powder B**	**Average *Stevia* powder**
Total carbohydrates (%)	95.5	95.5	95.5±0.01
Lipids (%)	0.1	0.1	0.1±0.01
Moisture (%)	4.01	3.95	3.98±0.05
Ash (%)	0.01	0.01	0.01±0.02
Protein (%)	0.40	0.40	0.40±0.01
pH	5.60	5.50	5.60±0.02

Table B.2 Proximate composition and pH of untreated *Stevia* leaves.

Component (g/100 g)	**Sample *Stevia* leaves A**	**Sample *Stevia* leaves B**	**Average *Stevia* leaves**
Total carbohydrates (%)	80.68	80.46	80.57±0.16
Lipids (%)	0.20	0.21	0.20±0.01
Moisture (%)	1.62	1.73	1.67±0.01
Ash (%)	10.80	10.90	10.9±0.02
Protein (%)	6.70	6.70	6.7±0.01
pH	6.40	6.60	6.50±0.2

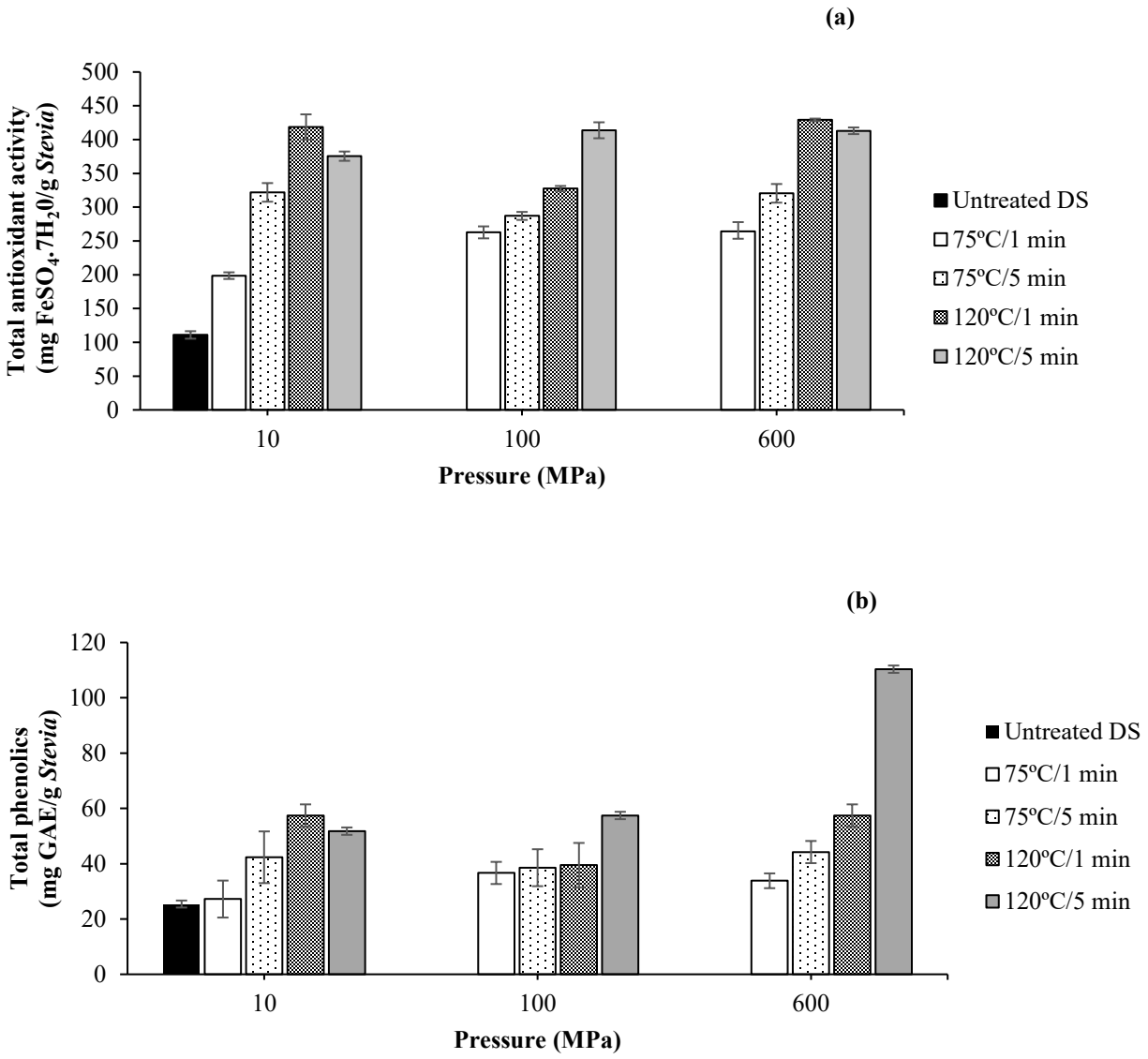


Figure B.1 (a) Total antioxidant activity and (b) Total phenolics of untreated and PATP treated *Stevia*.

Table B.3 Total antioxidant activity and total phenolics of PATP *Stevia.*

Temperature	Pressure	Time	Total antioxidant activity (mg $FeSO_4.7H_20$/g *Stevia*)		Total phenolics (mg GAE/g *Stevia*)	
(ºC)	(MPa)	(min)	*Stevia*	Average	*Stevia*	Average
75	10	1	202.1	198.7±4.9	22.5	27.2±0.7
	10	1	195.2		31.9	
	10	5	312.1	321.9±13.8	48.9	42.3±1.0
	10	5	331.6		35.7	
	100	1	256.4	262.7±8.9	33.8	36.7±0.9
	100	1	269.0		39.5	
	100	5	282.9	287.1±5.9	43.3	38.5±0.9
	100	5	291.2		33.8	
	600	1	256.4	264.1±10.8	35.7	33.8±1.2
	600	1	271.7		31.9	
	600	5	310.7	320.5±13.8	47.0	44.2±1.1
	600	5	330.2		41.4	
120	10	1	405.4	418.6±18.7	60.3	57.4±1.4
	10	1	431.8		54.6	
	10	5	370.6	375.5±6.9	50.8	51.8±1.2
	10	5	380.3		52.7	
	100	1	324.6	327.4±3.9	33.8	39.5±1.0
	100	1	330.2		45.2	
	100	5	422.1	413.7±11.8	58.4	57.4±1.4
	100	5	405.4		56.5	
	600	1	427.7	429.1±2.0	54.6	57.4±1.4
	600	1	430.4		60.3	
	600	5	416.5	413.0±4.9	111.3	110.3±2.2
	600	5	409.6		109.4	

Table B.4 Pure compounds in model systems.

Processing conditions	Quercetin (mg/mL)		Rutin (mg/mL)		Chlorogenic acid (mg/mL)		Caffeic acid (mg/mL)	
Control A	0.21	0.21±0.01	0.33	0.32±0.01	0.25	0.25±0.00	0.27	0.27±0.00
Control B	0.20		0.31		0.25		0.27	
25ºC/100 MPa/1 min	0.21	0.21±0.00	0.36	0.35±0.01	0.28	0.27±0.00	0.29	0.27±0.03
25ºC/100 MPa/1 min	0.21		0.34		0.25		0.25	
25ºC/100 MPa/5 min	0.21	0.21±0.00	0.28	0.30±0.02	0.23	0.22±0.01	0.22	0.26±0.06
25ºC/100 MPa/5 min	0.21		0.33		0.21		0.30	
25ºC/300 MPa/1 min	0.24	0.24±0.00	0.04	0.34±0.00	0.26	0.26±0.00	0.25	0.23±0.02
25ºC/300 MPa/1 min	0.24		0.04		0.26		0.22	
25ºC/300 MPa/5 min	0.21	0.21±0.00	0.32	0.32±0.00	0.26	0.26±0.01	0.24	0.24±0.01
25ºC/300 MPa/5 min	0.21		0.32		0.27		0.23	
75ºC/100 MPa/1 min	0.22	0.22±0.01	0.31	0.35±0.03	0.20	0.16±0.04	0.26	0.26±0.01
75ºC/100 MPa/1 min	0.21		0.38		0.11		0.27	
75ºC/100 MPa/5 min	0.22	0.22±0.01	0.35	0.33±0.02	0.24	0.25±0.01	0.27	0.27±0.01
75ºC/100 MPa/5 min	0.21		0.32		0.25		0.26	
75ºC/300 MPa/1 min	0.22	0.21±0.01	0.32	0.35±0.02	0.25	0.24±0.01	0.25	0.24±0.01
75ºC/300 MPa/1 min	0.21		0.37		0.22		0.24	
75ºC/300 MPa/5 min	0.21	0.21±0.00	0.30	0.32±0.02	0.29	0.28±0.01	0.22	0.23±0.01
75ºC/300 MPa/5 min	0.21		0.33		0.27		0.23	
120ºC/600 MPa/1 min	Nd	Nd	Nd	Nd	0.18	0.18±0.00	0.19	0.20±0.01
120ºC/600 MPa/1 min	Nd	Nd	Nd	Nd	0.19		0.20	
120ºC/600 MPa/5 min	Nd	Nd	Nd	Nd	0.18	0.17±0.01	Nd	Nd
120ºC/600 MPa/5 min	Nd	Nd	Nd	Nd	0.15		Nd	

Nd: not detected

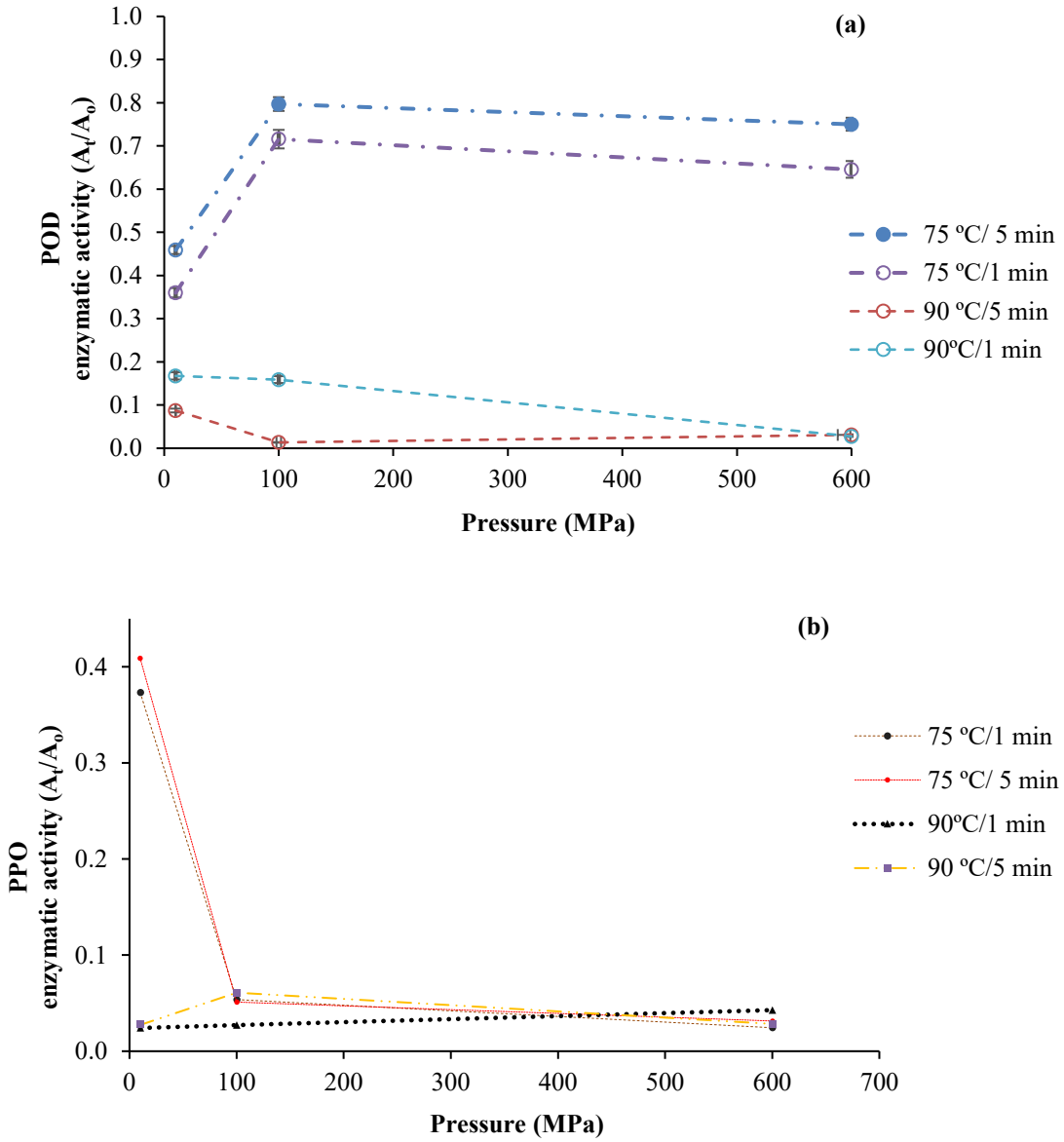


Figure B.2 (a) POD and (b) PPO enzymatic activity of PATP-treated *Stevia* leaves.

Table B.5 Peroxidase activity of treated *Stevia* leaves.

Peroxidase (POD)	Slope (nm)	Absorbance (Average) (nm)	Relative Activity POD (Average)	Relative Activity POD (Average)	Inactivation	Inactivation (%)
Control A	9.6750E-04	1.0000E+00	1.00	1.00	0.00	0.0
Control B	1.2298E-03		1.00			
75°C/10 MPa/1 min	3.5179E-04	3.6010E-01	0.32	0.36	0.64	64
75°C/10 MPa/1 min	4.3946E-04		0.40			
75°C/10 MPa/5 min	5.1446E-04	4.5900E-01	0.47	0.46	0.54	54
75°C/10 MPa/5 min	4.9411E-04		0.45			
75°C/100 MPa/1 min	8.2071E-04	7.1581E-01	0.75	0.72	0.28	28
75°C/100 MPa/1 min	7.5214E-04		0.68			
75°C/100 MPa/5 min	8.5089E-04	7.9707E-01	0.77	0.80	0.20	20
75°C/100 MPa/5 min	9.0054E-04		0.82			
75°C/600 MPa/1 min	7.0286E-04	6.4575E-01	0.64	0.65	0.35	35
75°C/600 MPa/1 min	7.1607E-04		0.65			
75°C/600 MPa/5 min	7.8339E-04	7.5035E-01	0.71	0.75	0.25	25
75°C/600 MPa/5 min	8.6536E-04		0.79			
90°C/10 MPa/1 min	1.7929E-04	1.6757E-01	0.16	0.17	0.83	83
90°C/10 MPa/1 min	1.8893E-04		0.17			
90°C/10 MPa/5 min	1.0071E-04	8.7444E-02	0.09	0.09	0.91	91
90°C/10 MPa/5 min	9.1429E-05		0.08			
90°C/100 MPa/1 min	2.4786E-04	1.5904E-01	0.23	0.16	0.84	84
90°C/100 MPa/1 min	1.0161E-04		0.09			
90°C/100 MPa/5 min	8.0357E-06	1.3572E-02	0.01	0.01	0.99	99
90°C/100 MPa/5 min	2.1786E-05		0.02			

Table B.5 Continued.

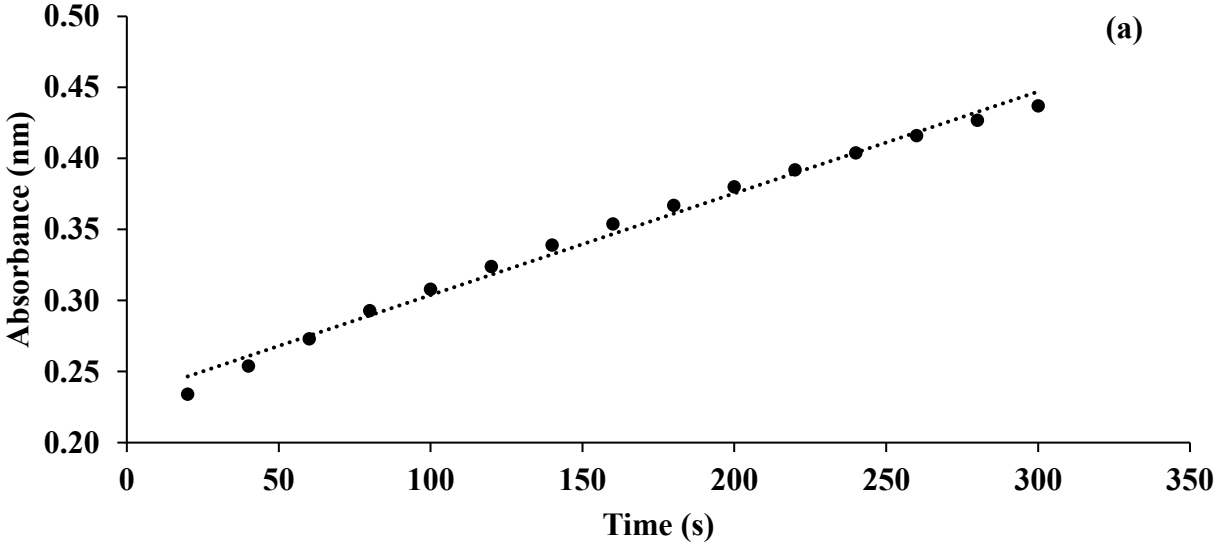
Peroxidase (POD)	Slope (nm)	Absorbance (Average) (nm)	Relative Activity POD (Average)	Relative Activity POD (Average)	Inactivation	Inactivation (%)
90°C/600 MPa/1 min	3.0557E-02	2.7712E-02	0.03	0.03	0.97	97
90°C/600 MPa/1 min	2.4868E-02		0.02			
90°C/600 MPa/5 min	3.0557E-02	3.0882E-02	0.03	0.03	0.97	97
90°C/600 MPa/5 min	3.1207E-02		0.03			
120°C/10 MPa/1 min	0.0000	0.0000	0.00	0.00	1.00	100
120°C/10 MPa/1 min	0.0000		0.00			
120°C/10 MPa/5 min	0.0000	0.0000	0.00	0.00	1.00	100
120°C/10 MPa/5 min	0.0000		0.00			
120°C/100 MPa/1 min	0.0000	0.0000	0.00	0.00	1.00	100
120°C/100 MPa/1 min	0.0000		0.00			
120°C/100 MPa/5 min	0.0000	0.0000	0.00	0.00	1.00	100
120°C/100 MPa/5 min	0.0000		0.00			
120°C/600 MPa/1 min	0.0000	0.0000	0.00	0.00	1.00	100
120°C/600 MPa/1 min	0.0000		0.00			
120°C/600 MPa/5 min	0.0000	0.0000	0.00	0.00	1.00	100
120°C/600 MPa/5 min	0.0000		0.00			

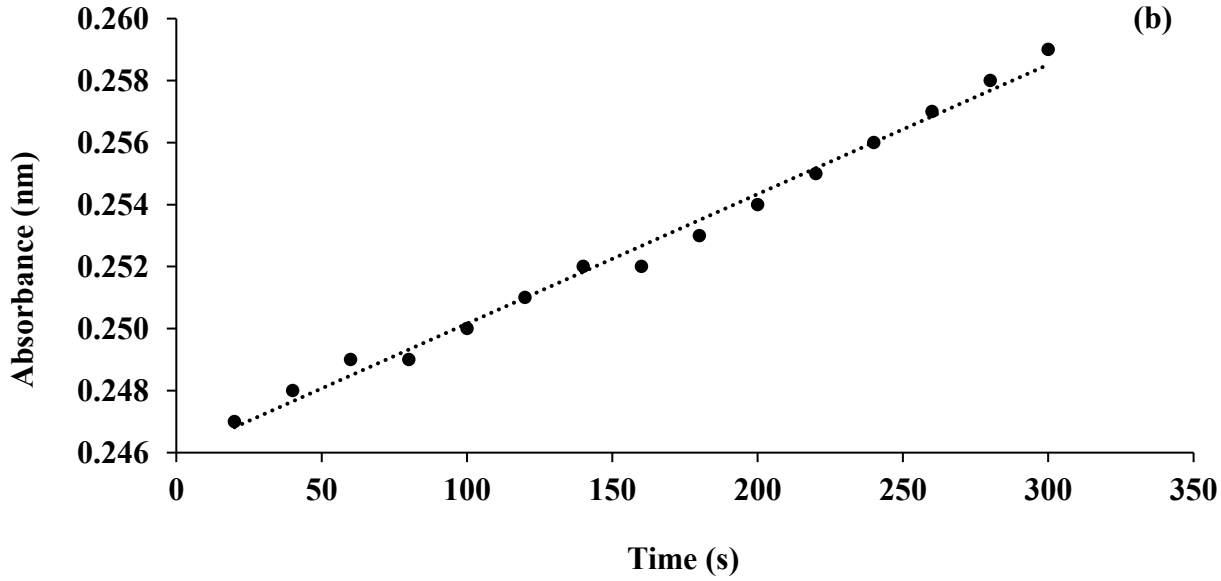
Table B.6 Polyphenoloxidase activity of treated *Stevia* leaves.

Polyphenoloxidase (PPO)	Slope (nm)	Absorbance (Average) (nm)	Relative Activity PPO (Average)	Relative Activity PPO (Average)	Inactivation	Inactivation (%)
Control A	1.2945E-03	1.2945E-03	1.00	1.00	0.00	0.0
Control B	1.2945E-03		1.00			
75°C/10 MPa/1 min	4.5661E-04	4.8304E-04	0.35	0.37	0.63	63
75°C/10 MPa/1 min	5.0946E-04		0.39			
75°C/10 MPa/5 min	5.7071E-04	5.2902E-04	0.44	0.41	0.59	59
75°C/10 MPa/5 min	4.8732E-04		0.38			
75°C/100 MPa/1 min	6.6964E-05	6.9375E-05	0.05	0.05	0.95	95
75°C/100 MPa/1 min	7.1786E-05		0.06			
75°C/100 MPa/5 min	6.4643E-05	6.6161E-05	0.05	0.05	0.95	95
75°C/100 MPa/5 min	6.7679E-05		0.05			
75°C/600 MPa/1 min	3.1786E-05	3.1607E-05	0.02	0.02	0.98	98
75°C/600 MPa/1 min	3.1429E-05		0.02			
75°C/600 MPa/5 min	3.9643E-05	4.0714E-05	0.03	0.03	0.97	97
75°C/600 MPa/5 min	4.1786E-05		0.03			
90°C/10 MPa/1 min	2.8750E-05	3.1250E-05	0.02	0.02	0.98	98
90°C/10 MPa/1 min	3.3750E-05		0.03			
90°C/10 MPa/5 min	2.8929E-05	3.5357E-05	0.02	0.03	0.97	97
90°C/10 MPa/5 min	4.1786E-05		0.03			
90°C/100 MPa/1 min	3.7679E-05	3.5179E-05	0.03	0.03	0.97	97
90°C/100 MPa/1 min	3.2679E-05		0.03			
90°C/100 MPa/5 min	7.0357E-05	7.8839E-05	0.05	0.06	0.94	94
90°C/100 MPa/5 min	8.7321E-05		0.07			

Table B.6 Continued.

Polyphenoloxidase (PPO)	Slope (nm)	Absorbance (Average) (nm)	Relative Activity PPO (Average)	Relative Activity PPO (Average)	Inactivation	Inactivation (%)
90°C/600 MPa/1 min	5.5357E-05	0.0001	0.04	0.04	0.96	96
90°C/600 MPa/1 min	5.5357E-05		0.04			
90°C/600 MPa/5 min	3.6786E-05	0.0000	0.03	0.03	0.97	97
90°C/600 MPa/5 min	3.6429E-05		0.03			
120°C/10 MPa/1 min	0.0000	0.0000	0.00	0.00	1.00	100
120°C/10 MPa/1 min	0.0000		0.00			
120°C/10 MPa/5 min	0.0000	0.0000	0.00	0.00	1.00	100
120°C/10 MPa/5 min	0.0000		0.00			
120°C/100 MPa/1 min	0.0000	0.0000	0.00	0.00	1.00	100
120°C/100 MPa/1 min	0.0000		0.00			
120°C/100 MPa/5 min	0.0000	0.0000	0.00	0.00	1.00	100
120°C/100 MPa/5 min	0.0000		0.00			
120°C/600 MPa/1 min	0.0000	0.0000	0.00	0.00	1.00	100
120°C/600 MPa/1 min	0.0000		0.00			
120°C/600 MPa/5 min	0.0000	0.0000	0.00	0.00	1.00	100
120°C/600 MPa/5 min	0.0000		0.00			


(a)
Absorbance (nm)
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0
50
100
150
200
250
300
350
Time (s)


(b)
Absorbance (nm)
0.260
0.258
0.256
0.254
0.252
0.250
0.248
0.246
0
50
100
150
200
250
300
350
Time (s)

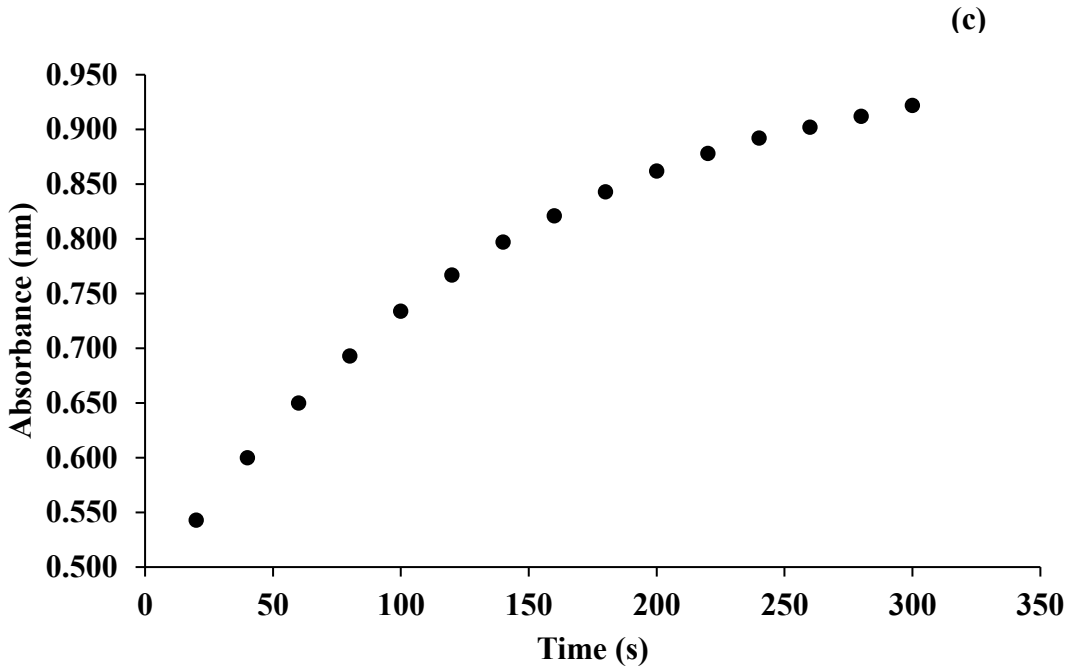


Figure B.3 Slope of (a) POD at 75ºC/600MPa/1 min and (b) PPO at 90ºC/10MPa/5 min of *Stevia* leaves (c) untreated *Stevia*.

The slope of the PPO curve, absorbance versus time, was calculated using Microsoft Excel slope function where the slope (A_t)=slope({0.247, 0.248, 0.249…0.259},{20, 40, 60…300})=3.5E-05 nm/s. The slope of the initial PPO enzyme activity before PATP treatment (A_o) was also calculated using Microsoft Excel where the slope (A_o)=slope({0.543,0.600, 0650…0.922},{20,40, 60…300})=1.3E-05 nm/s. Relative activity of the enzyme was calculated using the equation 4.1, Chapter 4.

Table B.7 Analysis of variance of relative activity of POD.

Source	p-value
Model	0.000
Blocks	0.962
Linear	0.000
Temperature (ºC)	0.000
Pressure (MPa)	0.000
Holding time (min)	0.539
2-Way Interactions	0.000
Temperature (ºC)*Pressure (MPa)	0.000
Temperature (ºC)*Holding time (min)	0.000
Pressure (MPa)*Holding time (min)	0.149
3-Way Interactions	0.286
Temperature (ºC)*Pressure (MPa)*Holding time (min)	0.286

S	R-sq	R-sq(adj)	R-sq(pred)
0.035	99.34%	98.65%	97.06%

Table B.8 Analysis of variance of relative activity of PPO.

Source	p-value
Model	0.000
Blocks	0.708
Linear	0.000
Temperature (ºC)	0.000
Pressure (MPa)	0.000
Holding time (min)	0.117
2-Way Interactions	0.000
Temperature (ºC)*Pressure (MPa)	0.000
Temperature (ºC)*Holding time (min)	0.394
Pressure (MPa)*Holding time (min)	0.469
3-Way Interactions	0.092
Temperature (ºC)*Pressure (MPa)*Holding time (min)	0.092

S	R-sq	R-sq(adj)	R-sq(pred)
0.013	99.41%	98.79%	97.36%

Table B.9 Total antioxidant activity and total phenolics of PATP treated mate+*Stevia* leaves

Temperature	Pressure	Time	Total antioxidant activity (mg $FeSO_4.7H_20$ /g mate)		Total phenolics (mg GAE/g mate)	
(ºC)	(MPa)	(min)	mate+*Stevia* leaves	Average	mate+*Stevia* leaves	Average
25	10	1	293.82	320.94±38.39	47.06	47.06±0.01
	10	1	348.07		47.06	
	10	5	219.31	227.63±11.76	69.66	67.21±0.01
	10	5	235.95		64.75	
	100	1	256.93	258.37±2.05	97.17	98.16±3.47
	100	1	259.82		99.14	
	100	5	519.50	525.64±8.70	90.29	90.29±1.39
	100	5	531.79		90.29	
	600	1	180.98	188.93±11.25	32.33	39.20±0.01
	600	1	196.89		46.08	
	600	5	217.87	233.78±22.50	99.14	100.12±1.39
	600	5	249.69		101.10	
75	10	1	512.99	518.05±7.16	82.43	83.91±2.08
	10	1	523.11		85.38	
	10	5	317.69	307.56±14.32	60.82	61.31±0.69
	10	5	297.43		61.80	
	100	1	458.74	459.82±1.53	81.45	83.91±3.47
	100	1	460.91		86.36	
	100	5	656.21	669.95±19.44	109.95	113.38±4.86
	100	5	683.69		116.82	
	600	1	285.86	284.05±2.56	55.91	47.55±11.81
	600	1	282.24		39.20	
	600	5	303.22	295.26±11.25	55.91	49.03±9.73
	600	5	287.31		42.15	
120	10	1	306.84	316.96±14.32	71.63	63.77±11.12
	10	1	327.09		55.91	
	10	5	413.17	414.25±1.53	75.56	77.03±2.08
	10	5	415.34		78.50	
	100	1	402.32	412.44±14.32	83.42	81.45±2.78
	100	1	422.57		79.49	
	100	5	410.27	419.68±13.30	111.91	113.88±2.78
	100	5	429.08		115.84	
	600	1	610.64	641.02±42.96	110.93	110.44±0.69
	600	1	671.40		109.95	
	600	5	670.67	661.63±12.79	110.93	108.96±2.78
	600	5	652.59		107.00	

Table B.10 Total antioxidant activity and total phenolics of PATP treated mate+commercial *Stevia* powder.

Temperature	**Pressure**	**Time**	**Total antioxidant activity (mg $FeSO_4.7H_20$ /g mate)**		**Total phenolics (mg GAE/g mate)**	
(ºC)	**(MPa)**	**(min)**	**mate+commercial *Stevia* powder**	**Average**	**mate+commercial *Stevia* powder**	**Average**
120	100	1	315.5	310.4±7.2	50.76	50.76±5.15
	100	1	305.4		58.04	
	100	5	377.0	373.7±4.6	41.67	46.52±4.58
	100	5	370.5		47.13	
	600	1	428.4	426.9±2.0	74.42	69.87±6.43
	600	1	425.5		65.32	
	600	5	305.4	294.5±15.3	63.50	63.50±0.01
	600	5	283.7		63.50	

Table B.11 Individual catechins of mate+commercial *Stevia* powder at 120ºC.

Pressure	Time	Catechin		Gallocatechin gallate		Epigallocatechin	
(MPa)	(min)	(mg/g mate)		(mg/g mate)		(mg/g mate)	
100	1	0.59	0.59±0.03	0.73	0.71±0.04	0.34	0.33±0.03
100	1	0.61		0.68		0.32	
100	5	0.46	0.46±0.02	0.80	0.77±0.04	0.25	0.24±0.02
100	5	0.47		0.74		0.24	
600	1	traces	traces	0.86	0.83±0.04	0.32	0.31±0.03
600	1	traces		0.80		0.30	
600	5	0.4	0.38±0.02	0.77	0.74±0.04	0.26	0.25±0.02
600	5	0.4		0.72		0.24	

Table B.12 Individual phenolics of mate+commercial *Stevia* powder at 120ºC.

Pressure	Time	Caffeic acid		Chlorogenic acid		Quercetin		Rutin	
(MPa)	(min)	(mg/g mate)		(mg/g mate)		(mg/g mate)		(mg/g mate)	
100	1	25.02	28.18±1.21	0.83	0.81±0.04	3.32	3.2±0.16	0.37	0.36±0.02
100	1	23.33		0.78		3.09		0.35	
100	5	17.81	17.20±0.86	0.73	0.71±0.04	1.62	1.56±0.01	0.32	0.31±0.02
100	5	16.60		0.68		1.51		0.30	
600	1	22.67	21.90±1.10	0.80	0.77±0.04	2.42	2.34±0.12	0.37	0.36±0.02
600	1	21.14		0.74		2.26		0.35	
600	5	18.15	17.53±0.86	0.73	0.71±0.04	1.19	1.15±0.06	0.32	0.31±0.02
600	5	16.92		0.68		1.11		0.30	

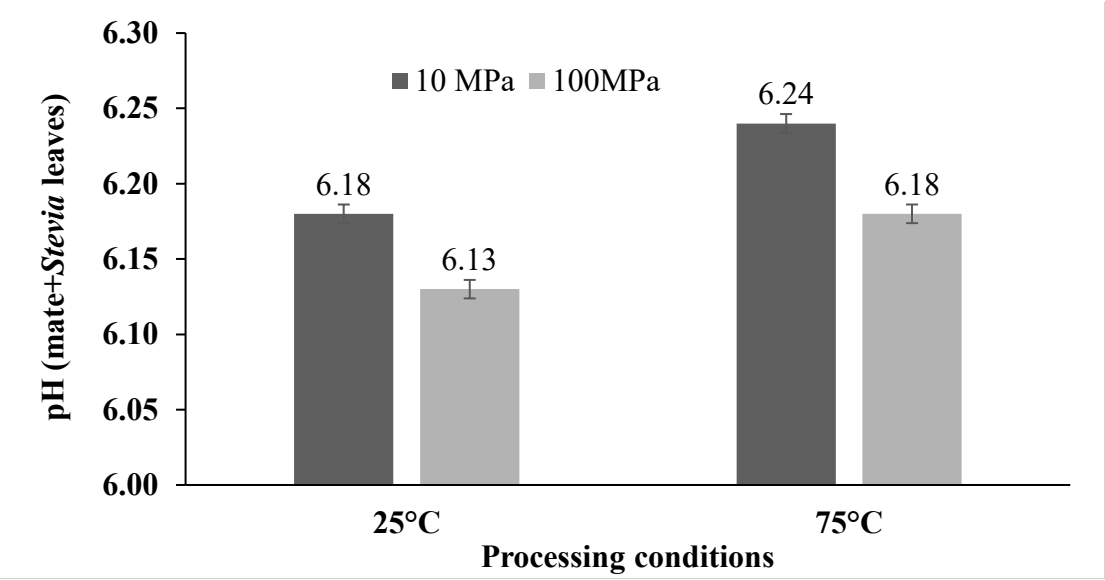


Figure B.4 pH of PATP-treated mate+*Stevia* leaves