DOI: 10.22607/IJACS.2017.503001

Available online at
www.ijacskros.com

Indian
Journal of Advances in
Chemical Science

Indian Journal of Advances in Chemical Science 5(3) (2017) 112-117

Recovery of Total Polyphenols from Pomegranate and *Butia*: A Study of Ultrasound-assisted Extraction and Antioxidant Activity

Naira Carniel[1], Débora Filippi[2], Letícia Mariane Delloss Gullich[2], Denise Bilibio[2], João Paulo Bender[3], Wagner Luiz Priamo[2,4]*

[1]Department of Food Engineering, URI - Campus Erechim, RS, 99709-910, Brazil. [2]Department of Food Technology, IFRS - Campus Sertão, Sertão RS, 99170-000, Brazil. [3]Department of Engineering, UFFS, Chapecó, SC, 99700-000, Brazil. [4]Department of Food Engineering, IFRS - Campus Erechim, Erechim, RS, 99713-028-000, Brazil.

Received: 23rd March 2017; Revised 15th May 2017; Accepted 24th May 2017

ABSTRACT

Ultrasound-assisted extraction technique was developed for the extraction of phenolic acids and flavonoids from pomegranate and Butia. Parameters as ethanol concentration, temperature and solid to liquid ratio were assessed through an experimental design and the antioxidant activity (2,2-diphenyl-1-picrylhydrazyl [DPPH] method) for each assay was evaluated. The maximum amount of total polyphenol content was 6723.42 and 37.99 mg gallic acid equivalent (GAE)/g of extract for pomegranate and Butia, respectively. Individual compounds as phenolic acids and flavonoids ranged depending on experimental conditions but, on the other hand, the antioxidant activity had little influence when these variables were studied. Pomegranate and Butia showed a maximum inhibition of DPPH radicals of 92.35% and 28.50%, respectively.

Key words: *Antioxidant capacity, Arecaceae, Flavonoids, Phenolic acids, Punicaceae.*

1. INTRODUCTION

South America possesses a wide diversity of native fruit bearing species consumed regionally that are slowly gaining popularity for their pleasant sensory attributes such as flavor and color, but also for their proposed nutritional and bioactive potential [1].

Punica granatum L., member of two species comprising the *Punicaceae* family, is a shrub native to occidental Asia and Mediterranean Europe, also grown in warm climate areas of the Americas and other parts of the world, which is popularly referred to as pomegranate [2]. The fruit contains many seeds (arils) separated by a white, membranous peel, individually surrounded by small amounts of tart and red juice. Therapeutic properties have been attributed to all the fruit compartments, and specific studies report that bark, roots, and tree leaves have medicinal benefit as well [3]. Other native fruit to Southern South America and with great potential for expansion is known as *Butia* (*Butia capitata – Arecaceae* family). In Brazil, the State of Rio Grande do Sul (RS) possesses many the existing species and majority of populations. At present, *Butia* fruits are mainly consumed fresh or processed in pulp, juices, alcoholic beverages, jams, jellies, and ice creams [1,4,5].

*Corresponding Author:
E-mail: wagner.priamo@erechim.ifrs.edu.br
Tel.: +555433217500; Fax: +555433217525

Native fruit species have received more attention recently for their potential to improve human health. These benefits are usually associated with specialized metabolites, often referred to as secondary metabolites or natural products, such as carotenoids and phenolic compounds, which have been reported to slow down aging symptoms and to prevent chronic diseases such as cancer, diabetes, and cardiovascular disease [1,6,7].

Different techniques employed in the extraction of polyphenols from solid samples of different plant origins have been critically reviewed [8,9]. Ultrasound-assisted extraction (UAE) is continuously employed to overcome drawbacks, as solvent excess and extraction time [10]. In this approach, the ultrasound waves lead to an intensification of the mass transfer process and increase the solvent penetration into the plant. The cavitation causes collapse that promotes cellular disruption together with penetration of solvent into cells through ultrasonic jet [11,12]. Finally, it is important to check the potential of these extracts, proving the antioxidant efficiency against different free radicals.

Based on these premises and aiming to contribute with experimental data for the separation process field, the goals of this work were: (i) To obtain polyphenolic

compounds from pomegranate (*P. granatum* L.) and *Butia* (*B. capitata*) using ultrasound-assisted extraction; (ii) to evaluate the effect of ethanol concentration, temperature and liquid to solid ratio on the total polyphenol content; (iii) to quantify the phenolic acids and flavonoids by high-performance liquid chromatography (HPLC)/diode-array detection (DAD); (iv) to assess the antioxidant activity of the extracts by 2,2-diphenyl-1-picrylhydrazyl (DPPH).

2. EXPERIMENTAL

2.1. Sample Preparation and Materials

Pomegranate and *Butia* were collected and prepared using the same procedure reported by Carniel *et al.,* [13]. Ethanol (95% of purity, Dinamica, Brazil), gallic acid, ellagic acid, caffeic acid, rutin hydrate, quercetin, mangiferin, kaempferol, and Folin–Ciocalteu phenol reagent were purchased from Sigma-Aldrich (St. Louis, MO) and 7.5 wt% Na_2CO_3 solution was prepared by dissolving anhydrous sodium carbonate in deionized water.

2.2. Ultrasound-assisted Extraction (UAE)

All assays were performed using an ultrasonic bath (frequency of 40 kHz and maximum output power of 250 W) [14]. Basically, the solvent (previously prepared with ethanol percentage, Table 1) was added to the test-tubes, and after the solution reaching the temperature desirable, an amount of sample (measured at analytical balance) was left in contact for 60 min. Finally, the solutions were filtered through 0.20 μm PTFE filters to eliminate any suspended solids and analyzed (total phenolic compound [TPC] and individual compounds).

2.3. Experimental Design

Central composite rotatable design (CCRD) was used to investigate the effects of temperature (T, 32-48°C) ethanol concentration (Ec, 14-91%, v/v) and solid-liquid ratio (SLR, 0.01-0.11 $g.mL^{-1}$, w/v) on the TPCs recovery from *Butia* and pomegranate. The CCRD consisted a full 2^3 factorial design (8 experiments), axial points (6 experiments), and replicated central points (2 experiments), totaling 16 experiments [15]. Assays were carried out at maximum ultrasound power and in random order to minimize the effects of unexpected variability in the observed response. The experimental design layout and the observed responses values are presented in Table 1.

2.4. TPC

TPC was determined by Folin–Ciocalteu colorimetric method as previously described by Carniel *et al.,* [13] and the results are expressed as mg of GAE per gram of extract (mg GAE/g extract).

2.5. Chemical Characterization

The analytical conditions reported by Carniel *et al*., [13] and Nayak *et al.* [16] were adopted to identify the individual compounds (phenolic acids and flavonoids) contained in the extracts. Individual compounds were identified by the retention time and quantified from peak area at wavelength using external standards. The standard response curve was a linear regression to the values obtained at each concentration within the range of 1-100 $\mu g.mL^{-1}$ for both compounds.

2.6. Antioxidant Activity

The antioxidant potential of each assay was assessed by DPPH method and expressed in terms of antioxidant activity (AA%, Equation 1) [17-19].

$$\text{DPPH scavenging effect}(\%) = \left(\frac{A_0 - A_1}{A_0} \right) \times 100 \quad (1)$$

Where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample after 30 min. Results are expressed as mean of triplicates.

2.7. Statistical Analysis

The results were statistically evaluated by one-way analysis of variance and significant differences at the level of 5% (α=0.05) were analyzed by the Tukey test using the Software Statistica 5.0 (Statsoft Inc., USA) to detect significant differences on the TPC and individual compounds depending on conditions investigated. Moreover, the effect of each independent variable on the TPC was analyzed and expressed by Pareto chart.

3. RESULTS AND DISCUSSION

3.1. Pomegranate

Table 1 shows the experimental design, total polyphenols content and individual compounds. The maximum content of total polyphenol (6723.42 mg GAE/g of extract) was obtained at 45°C, 0.03 g/mL of ethanol 76% (assay 6) whilst the lowest amount (18.26 mg GAE/g of extract) was also obtained at 40°C, but with a concentration of 0.06 g/mL of ethanol 91% (assay 14). Figure 1 shows the Pareto chart of the independent variables on the TPC. It was observed a linear positive effect of temperature and EWR, i.e., its respective increases may contribute to higher TPC extracted while the increase of SLR was negative decreasing the total phenols extracted.

The observed positive effect of temperature and ethanol percentage could be explained by the higher solubility of polyphenols in the solvent (when a similar amount of ethanol and water was used) and the high diffusivities of mass transfer at higher temperatures. On the other hand, in relation to SLR, the fact that more solvent can enter cells while more polyphenols may permeate into the solvent under higher solid to liquid ratio seems the most plausible to evaluated the opposite behavior [20].

Table 1: Experimental design, total polyphenols content and individual compounds of pomegranate (P) and *Butia* (B).

Assay	Temperature (°C)	SLR (g.mL^{-1})	EWR (% v: v)	TPC mg GAE. (g extract)$^{-1}$		Individual compound (mg.L^{-1})											
						Gallic acid		Ellagic acid		Caffeic acid		Rutin hydrate		Mangiferin		Kaempferol	
				P	B	P	B	P	B	P	B	P	B	P	B	P	B
1	−1 (35)	−1 (0.03)	−1 (30)	82.59^{fg}	6.05^{i}	7.49^{f}	0.41^{g}	4.61^{i}	NI^{e}	NIi	NI^{i}	0.58^{h}	0.23^{f}	0.36^{g}	NI^{h}	0.07^{f}	NI^{a}
2	+1 (45)	−1 (0.03)	−1 (30)	77.85^{fg}	22.33^{c}	5.98^{f}	0.05^{j}	7.45^{ghi}	NI^{e}	0.09^{e}	0.11^{c}	0.46^{h}	0.37^{cd}	0.12^{h}	NI^{h}	NI^{i}	NI^{a}
3	−1 (35)	+1 (0.09)	−1 (30)	297.87^{de}	13.65^{h}	17.92^{d}	0.22^{i}	14.77^{f}	NI^{e}	0.04^{fg}	0.07^{e}	1.90^{f}	0.34^{de}	0.62^{e}	NI^{h}	0.19^{b}	NI^{a}
4	+1 (45)	+1 (0.09)	−1 (30)	317.63^{de}	11.99^{i}	28.21^{a}	0.32^{h}	28.15^{b}	NI^{e}	0.21^{b}	NI^{i}	3.36^{b}	0.09^{h}	3.74^{b}	NI^{h}	NI^{i}	NI^{a}
5	−1 (35)	−1 (0.03)	+1 (76)	1207.03^{b}	17.99^{f}	NI^{h}	0.40^{gh}	NI^{j}	NI^{e}	0.01^{hi}	0.13^{b}	0.45^{h}	0.21^{fg}	NI^{i}	NI^{h}	0.11^{d}	NI^{a}
6	+1 (45)	−1 (0.03)	+1 (76)	6723.42^{a}	37.99^{a}	6.68^{f}	2.45^{a}	9.35^{g}	0.92^{a}	0.06^{f}	0.26^{a}	1.13^{g}	0.32^{e}	0.77^{d}	0.52^{a}	0.21^{a}	NI^{a}
7	−1 (35)	+1 (0.09)	+1 (76)	159.53^{efg}	26.76^{b}	28.94^{a}	0.98^{c}	34.15^{a}	0.90^{a}	0.51^{a}	NIi	4.25^{a}	0.11^{h}	4.13^{a}	0.32^{g}	0.09^{e}	NI^{a}
8	+1 (45)	+1 (0.09)	+1 (76)	432.12^{d}	18.56^{ef}	6.89^{f}	1.16^{b}	8.54^{gh}	0.61^{c}	0.03^{gh}	0.004^{g}	0.99^{g}	0.53^{a}	0.33^{g}	0.48^{b}	0.01^{h}	NI^{a}
9	−1.68 (32)	0 (0.06)	0 (53)	123.88^{fg}	16.83^{g}	22.64^{c}	0.55^{e}	23.29^{cd}	NI^{e}	0.18^{c}	0.089^{d}	2.79^{c}	0.36^{cd}	0.93^{c}	0.39^{d}	0.04^{g}	NI^{a}
10	+1.68 (48)	0 (0.06)	0 (53)	387.00^{d}	22.55^{c}	19.3^{d}	0.59^{e}	20.73^{de}	NI^{e}	0.13^{d}	NI^{i}	2.38^{de}	0.35^{cde}	0.81^{d}	0.39^{d}	NI^{i}	NI^{a}
11	0 (40)	−1.68 (0.01)	0 (53)	222.03^{ef}	17.91^{fg}	2.44^{g}	0.22^{i}	3.58^{ij}	NI^{e}	NI^{i}	0.056^{f}	0.37^{h}	0.19^{g}	0.32^{g}	NI^{h}	0.12^{c}	NI^{a}
12	0 (40)	+1.68 (0.11)	0 (53)	769.27^{c}	20.27^{d}	25.25^{b}	0.69^{d}	26.40^{bc}	0.77^{b}	0.23^{b}	NI^{i}	3.14^{b}	0.44^{b}	0.98^{c}	0.42^{c}	NI^{i}	NI^{a}
13	0 (40)	0 (0.06)	−1.68 (14)	19.52^{g}	9.31^{k}	7.25^{f}	0.42^{g}	5.05^{hi}	NI^{e}	NI^{i}	NI^{i}	0.54^{h}	0.42^{b}	0.31^{g}	0.34^{fg}	NI^{i}	NI^{a}
14	0 (40)	0 (0.06)	+1.68 (91)	18.26^{g}	10.58^{j}	12.64^{e}	0.51^{ef}	17.41^{ef}	0.47^{d}	NI^{i}	NI^{i}	2.24^{e}	0.32^{e}	0.51^{f}	0.37^{de}	NI^{i}	NI^{a}
15	0 (40)	0 (0.06)	0 (53)	629.99^{c}	19.53^{de}	23.30^{bc}	0.46^{fg}	22.40^{d}	0.69^{c}	0.19^{cd}	0.03^{h}	2.67^{cd}	0.39^{c}	0.54^{f}	0.37^{ef}	NI^{i}	NI^{a}
16	0 (40)	0 (0.06)	0 (53)	645.40^{c}	19.95^{de}	24.39^{bc}	0.45^{fg}	23.40^{d}	0.61^{c}	0.15^{cd}	0.02^{h}	2.48^{cd}	0.37^{c}	0.49^{f}	0.34^{ef}	NI^{i}	NI^{a}
17	0 (40)	0 (0.06)	0 (53)	662.30^{c}	19.44^{de}	22.98^{bc}	0.47^{fg}	21.53^{d}	0.65^{c}	0.13^{cd}	0.03^{h}	2.62^{cd}	0.38^{c}	0.46^{f}	0.36^{ef}	NI^{i}	NI^{a}

SLR: Solid liquid ratio, TPC: Total phenolic compound, GAE: Gallic acid equivalent, NI: Not identified, HSD: Honest significant difference. Mean of concentration of individual compound in each extraction condition (with standard deviations in parenthesis) within the same compound type followed by the same letter (s) are not significantly different based on Tukey's HSD

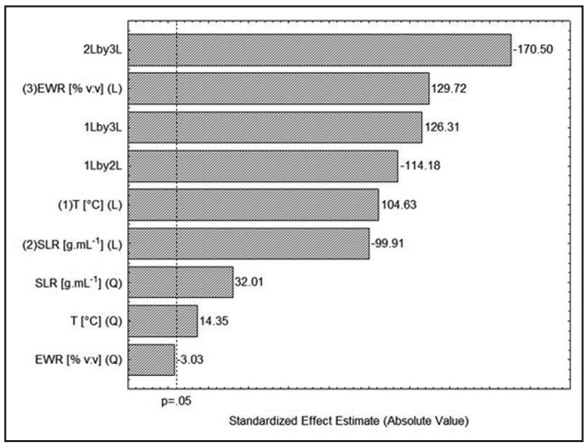


Figure 1: Pareto chart of the standardized effects of independent variables on total phenolic compound extracted from pomegranate.

Gallic and ellagic acid were the two most abundant compounds. Indeed, gallic acid ranged from 5.98 mg.L^{-1} (assay 2) to 28.94 mg.L^{-1} (assay 7), and ellagic acid from 3.58 mg.L^{-1} (assay 11) to 34.15 mg.L^{-1} (assay 7). Caffeic acid also was analyzed and its content ranged from 0.00 mg.L^{-1} to 0.51 mg.L^{-1} (assay 7). The minimum content of rutin, mangiferin, and kaempferol was 0.45 mg.L^{-1}, 0.12 mg.L^{-1}, and 0.00 mg.L^{-1} while the maximum amount was 4.25 mg.L^{-1}, 4.13 mg.L^{-1}, and 0.21 mg.L^{-1}, respectively. Curiously, the experimental condition that promoted the highest content of TPC was not the same that gallic and ellagic acid were obtained. This is justified because the TPC analysis considers all polyphenols compound presents in the extract and from HPLC-DAD analysis, only those obtained in the calibration curve using external standards.

According to statistical analysis, there are significant differences ($p<0.05$) between the extractions conditions in relation to TPC and each compound. For example, TPC obtained from assay 6 (6723.42 mg GAE/g of extract) is completely different of others assays. Ellagic acid, caffeic acid, rutin hydrate, mangiferin, and kaempferol also presented a specific experimental condition with the highest amount extracted. However, the concentration of gallic acid found in the assay 4 (28.21 mg.L^{-1}) and 7 (28.94 mg.L^{-1}) is statistically equals although experimental conditions are different.

3.2. Butia

Equally assessed, the results of TPC and individual compounds present in *Butia* are shown in Table 1 together with the experimental design. The maximum content of total polyphenol (37.99 mg GAE/g of extract) was obtained at 45°C, 0.03 g/mL of ethanol 76% (assay 6) whilst the lowest amount (6.05 mg GAE/g of extract) was also obtained at 35°C, but with a concentration of 0.03 g/mL of ethanol 30% (assay 1). Figure 2 shows the Pareto chart of the independent variables on the TPC and the same behavior it was observed, i.e., a linear positive effect of temperature and EWR and a linear negative effect of SLR.

For this vegetable, gallic acid and rutin hydrate were the two most abundant compounds. Indeed, gallic acid ranged from 0.05 mg.L^{-1} (assay 2) to 2.45 mg.L^{-1} (assay 6) and rutin hydrate from 0.09 mg.L^{-1} (assay 4) to 0.53 mg.L^{-1} (assay 8). Ellagic acid, caffeic acid, and mangiferin also were analyzed and its content ranged from 0.00 mg.L^{-1} to 0.92 mg.L^{-1} (assay 6) and 0.00 mg.L^{-1} to 0.26 mg.L^{-1} (assay 6), and 0.00 mg.L^{-1} to 0.52 mg.L^{-1} (assay 6), respectively. The presence of kaempferol was not detected in the extracts from *Butia*. Likewise, it should be mentioned that quercetin also was not detected in both fruits, but we may not infer the total absence of this compound because the contents may vary according to experimental conditions and intrinsic properties.

In terms of TPC and individual compounds also were found significant differences ($p<0.05$) between the extractions conditions. TPC, gallic acid, caffeic acid, rutin, and mangiferin presented a specific experimental condition with the highest amount extracted. However, the concentration of ellagic acid found in the assay 6 (0.92 mg.L^{-1}) and 7 (0.90 mg.L^{-1}) is statistically equals although experimental conditions are different.

The ability of both fruits against the scavenging of DPPH radicals showed very distinct (Figure 3) behaviors. Indeed, pomegranate showed an inhibition ranged from 88.00% (assay 5) to 92.35% (assay 12). On the other hand, *Butia* showed the lowest potential of scavenging, ranging from 7.13% (assay 11) to 28.50% (assay 8). It was observed a low dependence of the experimental conditions in relation to an antioxidant activity for each fruit, i.e., any experimental condition, in the ranges evaluated, promoted a similar potential.

Likewise, the results may be compared to the scientific literature. Msaada *et al.* [19] studied the methanolic extract of three coriander varieties and obtained results of TPC and total flavonoids are ranging from 0.94 to 1.09 mg GAE/g DW and 2.03-2.51 mg EC/g DW, respectively. The main compounds found were the chlorogenic and gallic acids, and finally, IC_{50} values ranged from 27.00 to 36.00 µg/mL. Hmid *et al.* [21] investigated TPC, individual phenolic compounds and antioxidant capacity of pomegranate juices from local and foreign cultivars. The main compounds found were the ellagic and gallic acids and the values of TPC and antioxidant activity ranging from 1284 to 9476 mg GAE/L and 31.16% to 76.3%, respectively. Tezcan *et al.* [22] found values of TPC ranging from 2602 to 10086 mg/L and antioxidant activity around of 70%. Denardin *et al.* [23] showed 359.50 mg GAE/g of *Butia* (TPC) and antioxidant activity of 253.80 mg/L and finally when compared to other studies [18,24-26]

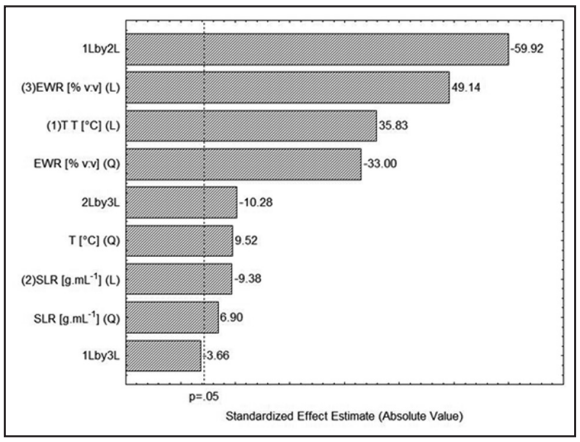


Figure 2: Pareto chart of the standardized effects of independent variables on total phenolic compound extracted from Butia.

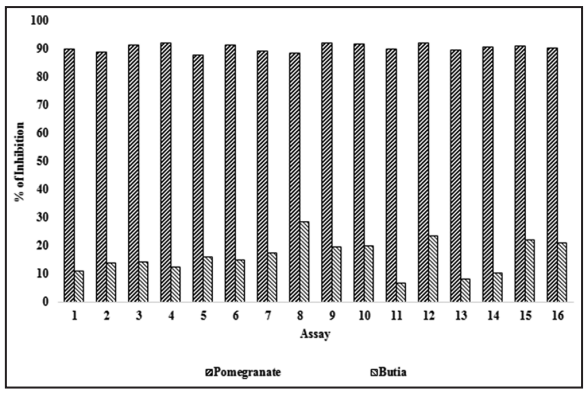


Figure 3: Antioxidant activity assessed by 2,2-diphenyl-1-picrylhydrazyl method.

pomegranate extract may be highlighted as a promising natural source as benefits on several purposes.

4. CONCLUSIONS

Total polyphenols, especially phenolic acids and flavonoids from pomegranate and *Butia*, were extracted using UAE. The parameters investigated had a significant impact on the phenols extracted and may be modulated to obtain a desirable amount of target compounds. Total polyphenols, especially phenolic acids and flavonoids from pomegranate and *Butia*, were extracted using UAE. The parameters investigated had a significant impact on the phenols extracted and may be modulated to obtain a desirable amount of target compounds. Different antioxidant activities were reached depending on the fruit; however, both are natural sources and may be used to promote benefits to human health as well as to enrich food and products.

5. ACKNOWLEDGMENTS

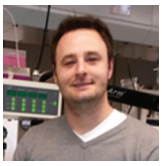
This work was supported by the CAPES and Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Sul (IFRS).

6. REFERENCES

1. J. F. Hoffmann, R. L. Barbieri, C. V. Rombaldi, F. Chaves, (2014) *Butia* spp. (*Arecaceae*): An overview, ***Scientia Horticulturae***, **179:** 122-131.
2. A. Vidal, A. Fallarero, B. R. Pena, (2003) Studies on the toxicity of *Punica granatum* L. (*Punicaceae*) whole fruit extracts, ***Journal of Ethnopharmacology***, **89(2-3):** 295-300.
3. C. Pagliarulo, V. de Vito, G. Picariello, (2016) Inhibitory effect of pomegranate (*Punica granatum* L.) polyphenol extracts on the bacterial growth and survival of clinical isolates of pathogenic *Staphylococcus aureus* and *Escherichia coli*, ***Food Chemistry,*** **190:** 824-831.
4. E. Schwartz, J. C. Fachinello, R. L. Barbieri, J. B. da Silva, (2010) Evaluation of populations of Butia capitata from Santa Vitória do Palmar, ***Brazilian Journal of Fruticulture***, **32:** 736-745.
5. M. Sganzerla, (2010) ***Caracterização Físico-Química e Capacidade Antioxidante do Butiá***, Pelotas: Universidade Federal de Pelotas. p104.
6. N. Martínez-Navarrete, M. del Mar Camacho Vidal, J. J. M. Lahuerta, (2008) Bioactive compounds of fruits and their effects on health, ***Dietetic Activity***, **12(2):** 64-68.
7. M. A. S. van Duyn, E. Pivonka, (2000) Overview of the health benefits of fruit and vegetable consumption for the dietetics professional: Selected literature, ***Journal of the American Dietetic Association,*** **100(12):** 1511-1521.
8. R. E. Ghitescu, I. Volf, C. Carausu, A. M. Bühlmann, I. A. Gilca, V. I. Popa, (2015) Optimization of ultrasound-assisted extraction of polyphenols from spruce wood bark, ***Ultrasonics Sonochemistry***, **22:** 535-541.
9. I. Ignat, I. Volf, V. I. Popa, (2011) A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables, ***Food Chemistry,*** **126(4):** 1821-1835.
10. K. Vilkhu, R. Mawson, L. Simons, D. Bates, (2008) Applications and opportunities for ultrasound assisted extraction in the food industry - A review, ***Innovative Food Science and Emerging Technologies,*** **9(2):** 161-169.
11. C. da Porto, E. Porretto, E. Decorti, (2013) Comparison of ultrasound-assisted extraction with conventional extraction methods of oil and polyphenols from grape (*Vitis vinifera* L.) seeds, ***Ultrasound Sonochemistry***, **20:** 1076-1080.
12. M. Toma, M. Vinatoru, L. Paniwynk, T. J. Masson, (2001) Investigation of the effects of ultrasound on vegetal tissues during solvent extraction, ***Ultrasound Sonochemistry,*** **8:** 137-142.
13. N. Carniel, R. M. Dallago, C. Dariva, J. P. Bender, A. L. Nunes, O. Anella, W. L. Priamo, (2016) Microwave-assisted extraction of phenolic acids and flavonoids from physalis angulata, ***Journal of Food Process Engineering***, **1:** 1-16.

14. R. Conte, L. M. D. Gullich, D. Filippi, C. A. Pazinatto, D. Bilibio, N. Carniel, M. A. Mazutti, W. L. Priamo, J. P. Benderet, (2016) Ultrasound-assisted extraction of total polyphenols from black poplar (*Populus nigra*) and evaluation of antioxidant potential, ***Indian Journal of Advances in Chemical Science***, **4(1):** 25-30.
15. M. I. Rodrigues, A. F. Iemma, (2005) ***Planejamento de Experimentos e Otimização de Processos: Uma Estratégia Seqüêncial de Planejamentos***. Campinas-SP: Casa do Pão.
16. B. Nayak, F. Dahmoune, K. Moussi, H. Remini, S. Dairi, O. Aoun, M. Khodir, (2015) Comparison of microwave, ultrasound and accelerated-assisted solvent extraction for recovery of polyphenols from *Citrus sinensis* peels, ***Food Chemistry,*** **187:** 507-516.
17. W. Brand-Williams, M. E. Cuvelier, C. Berset, (1995) Use of a free radical method to evaluate antioxidant activity, ***Food Sciences Technology***, **28:** 25-30.
18. D. Filippi, D. Bilibio, J. P. Bender, N. Carniel, (2015) Kinetic extraction of total polyphenols from pitanga (*Eugenia Uniflora* L.): Effect of ultrasonic treatment, modeling and antioxidant potential, ***Journal of Food Process Engineering,*** **38(4):** 320-328.
19. K. Msaada, M. B. Jemia, (2013) Antioxidant activity of methanolic extracts from three coriander (*Coriandrum sativum* L.) fruit varieties, ***Arabian Journal of Chemistry***, 10: S3176-S3183.
20. C. M. Galanakis, V. Goulas, V. Gekas, (2011) Predicting the solubilization preference of natural phenols to different solvents. In: *11th International Congress on Engineering and Food (iCEF11)*, Athens, Greece.
21. I. Hmid, D. Elothmani, H. Hanine, (2013) Comparative study of phenolic compounds and their antioxidant attributes of eighteen pomegranate (*Punica granatum* L.) cultivars grown in Morocco, ***Arabian Journal of Chemistry***, **10**: S2675-S2684.
22. F. Tezcan, M. Gultekin-Ozguven, T. Diken, B. Ozcelik, F. B. Erim, (2009) Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices, ***Food Chemistry,*** **115(3):** 873-877.
23. C. C. Denardin, G. E. Hirsch, R. F. da Rocha, M. Vizzotto, A. T. Henriques, (2015) Antioxidant capacity and bioactive compounds of four Brazilian native fruits, ***Journal of Food and Drug Analysis,*** **23(3):** 387-398.
24. Y. Shen, J. He, B. Huang, X. Ban, J. Tian, L. Zhu, Y. Wang, (2016) *In vitro* and *in vivo* antioxidant activity of polyphenols extracted from black highland barley, ***Food Chemistry,*** **194:** 1003-1012.
25. J. H. Xie, J. Y. Peng, G. R. Fan, (2015) Extraction, chemical composition and antioxidant activity of flavonoids from *Cyclocarya paliurus* (Batal.) Iljinskaja leaves, ***Food Chemistry***, **186:** 97-105.
26. H. F. Zhang, X. H. Yang, N. X. Qiu, Y. Wang, Z. Z. Wang, (2013) Microwave assisted extraction of flavonoids from cultivated *Epimedium sagittatum*: Extraction yield and mechanism, antioxidant activity and chemical composition, ***Industrial Crops and Products,*** **50:** 857-865.

****Bibliographical Sketch***



Professor Wagner Luiz Priamo has graduated from Food Engineering (URI-Erechim), M.Sc from Food Engineering (UNICAMP) and Ph.D degree from Food Engineering (UFSC) both Institutions in Brazil. His research areas include: Thermodynamic, with emphasys in separation process, supercritical technology, nanotechnology, and physical properties. He has published 22 research papers in International Journals, 21 papers in National and International conferences/seminars. Dr. Priamo is a professor at Federal Institute of Education, Science and Technology of Rio Grande do Sul (IFRS) - Erechim