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Ultrasound-assisted extraction using [BMIM][Cl] ionic liquid as an effective method for recovering phenolic compounds from the coproduct of guava processing

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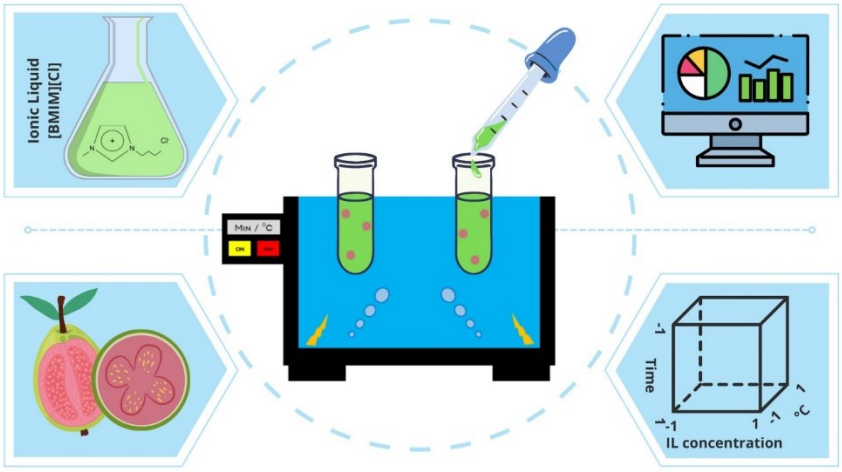
Abstract
Ionic liquids (IL) are an innovative alternative to classic organic solvents for recovering phenolic compounds. In this study, the influence of different parameters for the extraction of phenolic compounds from red guava coproduct using 1-butyl-3-methylimidazolium chloride [BMIM][Cl] IL associated with ultrasound-assisted extraction (ILUAE) was compared to methanolic extraction. The [BMIM][Cl] IL was synthesized and characterized by its melting point and MS/MS fragments. A 2^3 full factorial design was used to evaluate the effects of [BMIM][Cl] concentration, extraction time, and temperature on the total phenolic content (TPC) and antioxidant activity (DPPH) of the obtained extracts. An IL concentration of 2.5 mol L^{-1} and 10 min of extraction at 35 °C showed the highest content of TPC (4.01 mg g^{-1} gallic acid equivalent). On the other hand, the highest AA (8.77 mg g^{-1} ascorbic acid equivalent) was reached using an IL concentration of 2.5 mol L^{-1} and 40 min of extraction at 55 °C. These results were superior to those obtained for methanol extraction (1.58 mg g^{-1} gallic acid equivalent and 3.65 mg g^{-1} ascorbic acid equivalent, respectively). The study indicated that the innovative extraction method using ILUAE was quick, straightforward, and effective for recovering valuable bioactive compounds from red guava coproduct without using organic solvents.

Keywords: Green extraction; 1-butyl-3-methylimidazolium chloride; polyphenolic compounds; guava coproduct; experimental design.

Graphical Abstract



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ROYALDATASET

1. Introduction

The red guava (*Psidium guajava*), which is rich in bioactive compounds, vitamins A, B, and C, and minerals such as iron, calcium, and phosphorous, has been reported as a superfruit (Almulaiky et al., 2018; Campoli et al., 2018; Flores et al., 2015; Koriem et al., 2019; Lima et al., 2019). India, Indonesia, Mexico, China, Pakistan and Brazil are the main producers of guava worldwide (Tridge, 2021). Recent data indicate that guava production reached about 552 thousand tons in 2021 (Brazilian Institute for Geography and Statistics, 2021). It has also been estimated that 53% of the guava produced in Brazil is destinated to the industry (Del'Arco & Sylos, 2018). On the other hand, guava processing generates 30% of coproducts such as peels, seeds, and pulp leftovers (Milani et al., 2018). Although presenting a high concentration of nutrients and phenolic compounds, coproducts are usually discarded as organic residue. The extraction of phenolic compounds from this coproduct may be a strategy for adding value. In addition, the environmental impact caused by its inadequate discard can also be reduced (Lima et al., 2020; Milani et al., 2018; Moon et al., 2018). Reports indicate the application of guava coproducts in feed for chickens (Camelo et al., 2015), soil fertilizer (Souza et al., 2011), and extraction of phenolic compounds (Hassan et al., 2016; Lima et al., 2019).

Phenolic compounds are secondary metabolites produced by vegetal tissues in stress conditions such as climate changes, light alterations, extreme temperatures, and during the attack of organisms that cause pathologies (Belwal et al., 2016; Niño-Medina et al., 2017; Shahidi & Ambigaipalan, 2015). The antioxidant properties reported for these compounds are related to their capability to prevent oxidative cell stress, naturally unleashed by free radicals present in the human organism (Shahidi & Ambigaipalan, 2015; Shahidi & Zhong, 2015). Thus, polyphenolic compounds might prevent and/or control diseases related to these mechanisms, such as cardiovascular diseases, neurological and metabolic disorders, and cancer (Martins et al., 2016). Therefore, the consumption of vegetables and fruits, which are rich in phenolic compounds, has been recommended as part of a healthy diet. In addition, phenolic compounds can be used as antioxidants, antimicrobials, and flavoring agents in the food industry (Zeb, 2020).

The conventional methods for extracting phenolic compounds from vegetal sources are usually associated with the use of organic solvents (Alara et al., 2021). However, the continuous exposition of organic solvents might cause damage to the central nervous system, neurotoxic action, respiratory issues, cutaneous irritation, allergies, cancer, and in the worst scenario, death (Claus et al., 2018; Magiera & Sobik, 2017; Tong et al., 2019; Torres et al., 2011).

Considering the harmful effect on human health and the environment, the literature reported alternative methodologies for obtaining phenolic compounds from the vegetal matrices using extractions based on supercritical fluid, pressurized liquid (Teixeira et al., 2021), ultrasound (Lima et al., 2019), ionic liquid (IL) and ultrasound (Santos et al., 2021), and ionic liquid and microwave (Du et al., 2011). ILs are non-molecular and inflammable substances that possess melting point below 100 °C, low vapor pressure, and chemical and thermal stabilities, which have been reported as alternative solvents to replace organic solvents (Faustino et al., 2017; Magiera & Sobik, 2017; Martins et al., 2017; Tuzen et al., 2018). The ILs have a cationic and an anionic fraction, which can be molded according to the specific compound of interest to be obtained and/or the extraction conditions. The ILs have been reported as promising solvents for replacing traditional organic solvents since they can contribute to the reduction of environmental contamination by potentially toxic and pollutants solvents (Faustino et al., 2017; Magiera & Sobik, 2017; Martins et al., 2017; Tuzen et al., 2018).

It has been reported that the extraction using 1-butyl-3-methylimidazolium chloride [BMIM][Cl] for extraction of phenolic compounds from vegetable matrices is effective due to its water miscibility, which allows preparing aqueous solutions, and the property to dissolve vegetal cellulosic fraction (Ma et al., 2010; Martins et al., 2017). In addition, Lou et al. (2012) reported the use of [C_4MIM][Br] for efficient extraction of chlorogenic acid, caffeic acid and quercetin from burdock (*Arctium lappa* L.) leaves. Furthermore, the extraction of phenolic compounds from propolis (Cao et al., 2017), *Myrica rubra* leaves (Du et al., 2011) and seaweed *Saccharina japonica* (Vo Dinh et al., 2018) using [C_{12}MIM][Br], [BMIM][Br] and [BMIM][BF_4], respectively, were also reported.

Combining ILs and ultrasound-assisted extraction for recovering bioactive compounds increases the efficiency and decreases the extraction

time (Murador et al., 2019). The cavitation phenomenon in the extraction process induces the generation of microbubbles, causing precise points of high pressure and temperature, providing the vegetal cells to rupture, broke of solid particles, and consequently, the extraction of target chemical compounds (Ferreira et al., 2014, 2020). Furthermore, using a multivariate approach for the experimental design, such as the full factorial screening, allows the evaluation of the extraction parameters individually and their interactions using a minimum number of experiments (Narenderan et al., 2019). The use of ionic liquids combined with ultrasound-assisted extraction and statistical tools can be an effective alternative for recovering phenolic compounds from vegetal sources.

In this study, a 2^3 full factorial experimental design with center points was combined with the ultrasound-assisted extraction using [BMIM][Cl] to identify the significant parameters in the responses of total phenolic compounds and antioxidant activity in the extracts obtained from red guava coproduct.

2. Material and Methods

2.1 Materials

The red guava samples were purchased at the local market (Florianópolis, Brazil) in February 2018. This project was registered at National Genetic Heritage Management System (SisGen-Brazil) (register A549A9C). The reagents 1-chlorobutane and 1-methylimidazolium (Sigma Aldrich, St. Louis, USA) were used to synthesize 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]) IL. Gallic acid, ascorbic acid and DPPH were purchased from Sigma-Aldrich (São Paulo, Brazil). Ultrapure water was used to prepare the solutions for all analyses. The other reagents were of analytical grade.

2.2 Sample preparation

Guava fruits were cleaned and sanitized with 100 mg L^{-1} sodium hypochlorite per 15 min, then pulped in an industrial pulp machine (BRAMEITAR, Campinas, Brazil). Next, the coproduct composed of peel, seeds, and pulp leftovers was dried in an air-forced oven (TE 394/2, Tecnal, Piracicaba, Brazil) at 55 °C/12 h. After that, the dry coproduct was grounded in a hammermill (Tecnal, TE 090, Piracicaba, Brazil), and the particle size was adjusted to ≤ 250 mm. Finally, the powder samples were stored under a vacuum at –18 °C until the analysis moment (Lima et al., 2019).

2.3 Synthesis and characterization of [BMIM][Cl]

[BMIM][Cl], a mix of 0.5 mol L^{-1} of 1-methylimidazolium and 1-chlorobutane was kept in shaking and reflux at 80 °C for 72 h until the development of a viscous yellow liquid. Then the IL was dried under reduced pressure using a rotatory evaporator (model 803, Fisatom, São Paulo, Brazil) at 100 °C and crystallized in a freezer (Fang et al., 2007; Martins & de Rosso, 2016). The synthesis of the [BMIM][Cl] IL was confirmed through the melting point (MP) and the correlation of the MS/MS spectrum of the target compound with the literature and with the theoretical software simulation of the given substance.

The MP was determined using a tiny aliquot of the [BMIM][Cl], which was placed in the MP equipment (MQAPF-302, Microquímica Equipamentos, Palhoça, Brazil). First, the temperature was adjusted to increase from room temperature up to 70 °C. Then, MP was registered visually when all the IL was completely molten. Next, the mass spectra were obtained using an ESI high-resolution mass spectrometry (micrOTOF-Q II, Bruker Daltonics, Bremen, Germany) by direct injection of [BMIM][Cl] dissolved in methanol. The following conditions were used: ESI as source type, ionization in positive mode, a capillary voltage of 4500 V, 4.0 L min^{-1} dry gas flow, and nebulizer at 0.4 bar, at 200 °C (Martins & Rosso, 2016). The free software mMass – Open-Source Mass Spectrometry Tool was used for data interpretation. The final IL yield was verified by difference according to the initial mass (**Eq. 1**).

$$IL\ yield = \frac{(final\ mass \times 100)}{initial\ mass} \quad \textbf{(Eq. 1)}.$$

2.4 Experimental design

A 2^3 full factorial experimental design with center points was used to identify the significant parameters ($p < 0.05$) in total phenolic compounds and antioxidant activity responses. The following parameters were considered: [BMIM][Cl] concentration (aqueous solutions at 0.4, 1.45 and 2.5 mol L^{-1}), time (10, 25 and 40 min) and extraction temperature (35, 45 and 55 °C) (Table 1). In addition, an extraction using methanol (MeOH) (25 min, at 45 °C – center point condition of 2^3 design) was

performed for comparison with the results obtained for the IL.

2.5 Phenolic compounds extraction using ionic liquid associated with ultrasound-assisted extraction (ILUAE)

The extractions of phenolic compounds were performed in triplicate in an ultrasonic bath (Ultronique Q5.937A, Eco-Sonics, Indaiatuba, Brazil). An aliquot of 1 mL of the IL solutions was added to 0.06 g of the sample and kept in the ultrasonic bath under the conditions from the experimental design.

2.6 Evaluation of total phenolic content and antioxidant activity from guava coproduct extracts

2.6.1 Total phenolic content (TPC)

The TPC was evaluated using the Prussian Blue method (Margraf et al., 2015) adapted to microplates. An aliquot of 100 μL of iron chloride hexahydrate (0.5 mmol L^{-1}) was added to 100 μL of the extracts in 96 well microplates. After 2 min, 100 μL of potassium ferricyanide (0.5 mmol L^{-1}) was added to the previous solution. The microplate was kept for 15 min in the absence of light, and the absorbance was registered at 725 nm in a microplate reader (SpectraMax®, Paradigm®, Molecular Devices San Jose, CA, USA). The results were expressed as mg g^{-1} of gallic acid equivalent (GAE) using a standard analytical curve (0 to 15 mg L^{-1}).

2.6.2 Antioxidant activity (AA)

The AA of the obtained extracts was evaluated according to the DPPH scavenging method (Brand-Williams et al., 1995), adapted to microplates. First, 260 μL of 0.1 mmol L^{-1} DPPH solution and 40 μL of extract were mixed and kept in the absence of light for 30 min. After that, the absorbance was registered at 517 nm in a microplate reader. The results were expressed as mg g^{-1} of ascorbic acid equivalent (AAE) according to a standard analytical curve (2 to 30 mg L^{-1}).

2.7 Statistical analysis

All the experiments and analyses were performed in triplicate, and the results were expressed as mean ± standard deviation. The 11 experiments from the experimental design with IL and the MeOH control were compared using analysis of variance (ANOVA) followed by Tukey's test to verify statistically significant differences at 5% significance. The lack of fit, coefficient of determination and the adjusted coefficient of determination (R^2 and R^2_{adj}) were evaluated. The significant regression coefficients and estimated effects for the significant parameters were assessed. The normality was evaluated by residual graph plot (predicted values vs. observed values) and Ryan Joiner test (data not shown) to verify the fitted models. Two blank experiments without added samples, one with water and another with IL, were performed for all analyses. The data was analyzed using TIBCO Statistica v.13.5 (TIBCO Statistica Ltd, USA) and SASM-Agri software (Canteri et al., 2001).

3 Results and Discussion

3.1 Characterization of [BMIM][Cl]

The melting point analysis indicated that all the crystalized [BMIM][Cl] was fully melted at 65 °C. This result is in agreement with the temperature reported by Sheldon (2001) for the same IL. The IL melting points are a characteristic of the nature of different anions in their structure (Zhang et al., 2006), and it has been observed that they can be used as a fast tool for the synthesis identification of ILs.

Fig. 1a indicates that the synthesis of [BMIM][Cl] was successful, and the average yield was 98%. The 1-butyl-3-methylimidazolium synthesis was confirmed through the high intensity of the molecular ion mass 139 *m/z* (100%), which confirmed the loss of a chlorine atom [Cl] ($C_8H_{15}N_2$) in the structure of [BMIM][Cl]. Additionally, the MS^2 83 *m/z* (28%) fragment indicates the imidazole ring ($C_4H_7N_2$) (**Fig. 1b**) without the alkyl chain. The MS/MS spectra results agree with those reported in the literature (Martins & Rosso, 2016) and the theoretical simulation made by the mMass software.

3.2 Experimental design for obtaining the extracts using ILUAE, total phenolic content (TPC) and antioxidant activity (AA)

Table 1 shows the full factorial design, total phenolic content and antioxidant activity for the extracts obtained by ILUAE. The total phenolic content in the extracts varied from 0.90 to 4.01 mg g^{-1}

GAE. The highest concentration (4.01 mg g^{-1} GAE) was obtained using 2.5 mol L^{-1} of [BMIM][Cl] during 10 min of extraction at 35 °C (experiment 2). This concentration was able to recover 2.5-fold higher TPC compared to MeOH control experiment, and the extraction was 2.5 times faster ($p < 0.05$) at a lower temperature when compared to the extraction using methanol (1.58 mg g^{-1} GAE, 25 min at 45 °C). Sousa et al. (2011) observed lower concentrations of total phenolic content in a guava coproduct extracted by infusion (1 h at 25 °C) using water and ethanol 80% (between 0.24 and 0.46 mg g^{-1} GAE, respectively). Santos et al. (2017) reported 3.56 mg g^{-1} GAE of TPC for the whole fresh guava.

The extract obtained with 2.5 mol L^{-1} [BMIM][Cl] and 40 min at 55 °C (experiment 8) showed the highest antioxidant activity (8.77 mg g^{-1} AAE). This result was significantly ($p < 0.05$) higher and 2.4-fold superior when compared to the control extraction using methanol as solvent (3.65 mg g^{-1} AAE) (**Table 1**). On the other hand, using IL the extraction time and temperature were slightly superior when compared to the control.

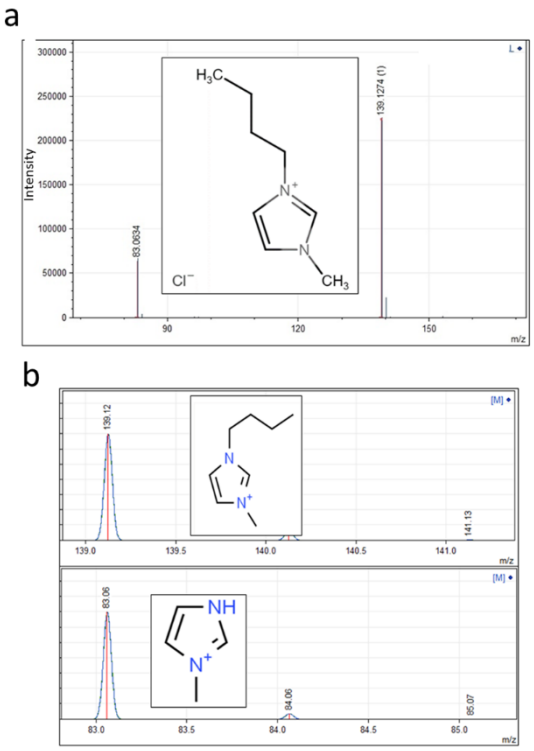

Fig. 1 Mass spectra of synthesized [BMIM][Cl] (a) and theoretical mass spectra according to *m/z* 139 and 83 (b).

Table 1 Theoretical and actual values from a 2^3 full factorial design for total phenolic compounds and antioxidant activity of extracts obtained from guava coproduct

Run	[BMIM][Cl] concentration (mol L^{-1})		Time (min)		Temperature (°C)		Total phenolic compounds (mg g^{-1} GAE)	Antioxidant activity (mg g^{-1} AAE)
1	−1	0.40	−1	10	-1	35	1.68 ± 0.18^{e}	3.18 ± 0.22^{cd}
2	+1	2.50	−1	10	-1	35	4.01 ± 0.08^{a}	8.13 ± 0.27^{a}
3	−1	0.40	+1	40	-1	35	1.07 ± 0.07^{gh}	2.72 ± 0.19^{de}
4	+1	2.50	+1	40	-1	35	3.80 ± 0.08^{ab}	8.04 ± 0.46^{a}
5	−1	0.40	−1	10	+1	55	0.90 ± 0.04^{h}	2.17 ± 0.19^{e}
6	+1	2.50	−1	10	+1	55	3.36 ± 0.05^{c}	8.12 ± 0.54^{a}
7	−1	0.40	+1	40	+1	55	1.30 ± 0.05^{fg}	3.61 ± 0.19^{c}
8	+1	2.50	+1	40	+1	55	3.47 ± 0.09^{bc}	8.77 ± 0.36^{a}
9	0	1.45	0	25	0	45	2.58 ± 027^{d}	5.83 ± 0.28^{b}
10	0	1.45	0	25	0	45	2.45 ± 0.12^{d}	6.08 ± 0.11^{b}
11	0	1.45	0	25	0	45	2.53 ± 0.24^{d}	6.05 ± 0.42^{b}
MeOH control			25		45		1.58 ± 0.40^{ef}	3.65 ± 0.41^{c}

n = 3. −1, 0 and +1 represent the coded parameters. Different letters in the same column indicate statistical differences (Tukey's test at 5 % significance). GAE, gallic acid equivalents; AAE, ascorbic acid equivalents

According to Tukey's test, the control extract using MeOH did not show a statistical difference ($p > 0.05$) with runs 1 and 7 from the experimental design for TPC and AA responses (**Table 1**). Both experiments used the lowest [BMIM][Cl] concentration (0.4 mol L^{-1}, level −1) and showed 1.68 and 1.30 mg g^{-1} GAE for TPC and 3.18 and 3.61 mg g^{-1} AAE for the AA, respectively.

Fig. 2a and **Table 2** show that the IL concentration and the interaction between time and temperature had a positive and significant effect on the extraction of the phenolic compounds.

Fig. 3a shows that an increase in the IL concentration from 0.4 to 2.5 mol L^{-1} (−1 to +1) increased the recovery of TPC in the extracts. On the other hand, the effect of temperature was significant and negative (**Fig. 2a**), indicating that increasing the extraction temperature from 35 to 55 ºC causes a reduction in the extraction of TPC.

The response surface (**Fig. 3a**) suggests that the extraction at low temperatures is effective for the extraction of phenolic compounds. However, this result indicates that the increase in temperature might destroy the thermolabile compounds.

Silva et al. (2019) verified that the temperature was a significant parameter in the extraction of total phenolic compounds from jatobá fruit (*Hymenaea courbaril* L.) using a water bath and

ethanol 60.6% for 71.93 min. Furthermore, the authors showed a direct relationship between the increase in temperature (from 25 to 65 °C) and the recovery of total phenolic compounds.

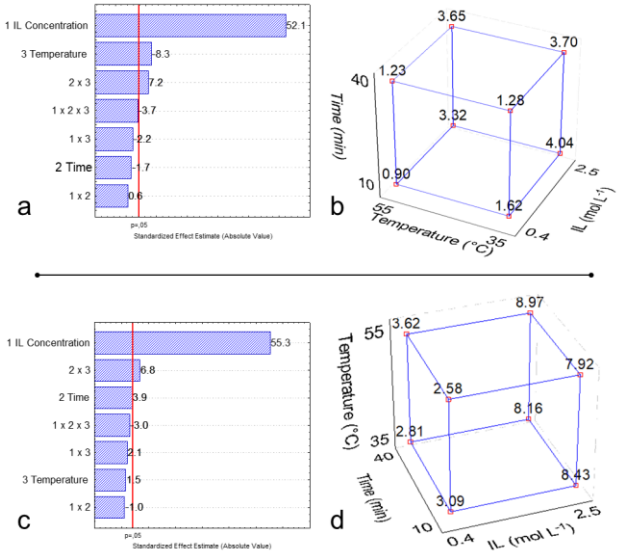

Fig. 2 Pareto's chart (left) and cube plot chart for predicted values (right) obtained for total phenolic compounds (a, b) and antioxidant activity (c, d) in guava coproduct extracts obtained with [BMIM][Cl] ionic liquid assisted by ultrasound.

The extraction of phenolic compounds was not significantly ($p < 0.05$) affected by the extraction time (10, 25, and 40 min). Similarly, Silva et al. (2016) reported that the extraction time (30, 45, and 60 min) was not a significant parameter for extracting phenolic compounds using ultrasound-assisted extraction with ethanol 70% from lychee (*Litchi chinensis* Sonn.) peel.

Table 2 Significant regression coefficients (estimated effects), R^2 and $R^2_{adjusted}$ coefficients, and lack of fit of 2^3 full factorial design for total phenolic compounds and antioxidant activity of guava coproduct extracts.

	Total phenolic content	Antioxidant activity (DPPH)
Mean	2.47	5.70
IL concentration	1.21 (2.42)	2.67 (5.34)
Temperature	–0.19 (–0.38)	NS
Time × Temperature	1.17 (0.33)	0.33 (0.66)
R^2	0.9905	0.9889
$R^2_{adjusted}$	0.9864	0.9815
Lack of fit (α = 0.05)	0.17	0.11

NS: not significant ($p > 0.05$).

The lowest [BMIM][Cl] concentration (0.40 mol L^{-1}) and extraction time (10 min), and highest temperature (55 °C) were the conditions that promoted the lowest extraction of total phenolic compounds (0.90 mg g^{-1} GAE) (**Fig. 2b**). On the other hand, the IL concentration and the interaction between extraction time and temperature showed a significant and positive effect in the extraction (**Fig. 22c**), as confirmed by the results for DPPH, which indicate that the antioxidant activity increases with extraction time and temperature (8.97 mg g^{-1} AAE, **Fig. 2d**).

Fig. 3b indicates that increasing IL concentration significantly improved the antioxidant activity of the extracts (**Fig. 2d**). On the other hand, the extraction time and temperature (**Fig. 2d**) did not significantly change the AA (**Table 2**).

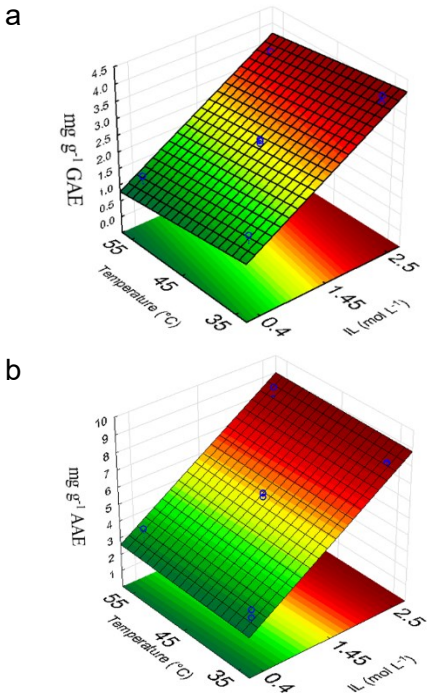

Fig. 3 Response surface for total phenolic compounds (a) and antioxidant activity (b) for guava coproduct extracts obtained with [BMIM][Cl] ionic liquid assisted by ultrasound.

Gruz et al. (2013) found that the extraction temperature (using ethanol 30%, pH 4, and 1:3 sample/solvent ratio) had a significant and positive effect on the antioxidant activity of grape marc, a coproduct from grape juice processing. The authors also indicated that the highest antioxidant activity was obtained using 50 °C, which was close to the temperature for the highest AA observed in this work (55 °C).

The statistical model proposed for TPC and AA did not show a lack of fit ($p > 0.05$). The coefficient of determination (R^2) of 0.9905 and 0.9889 indicates

the model's accuracy to the experimental data at 99% for TPC and AA (**Table 2**).

Fig. 2a, 2c and **Fig. 3** show that the [BMIM][Cl] concentration is a significant parameter for the effective recovery of phenolic compounds from guava coproduct. Experiments 2, 4, 6 and 8 using 2.5 mol L^{-1} of [BMIM][Cl] showed 3.36-4.01 mg g^{-1} GAE (**Table 1**).

Kou et al. (2018) evaluated the effect of 0.2 to 2 mol L^{-1} aqueous solutions of 1-butyl-3-methylimidazolium tetrafluoroborate [BMIM][BF_4] on the extraction of gingerols from ginger using ultrasound-assisted extraction (25 °C, 10 min). The authors reported that the recovery of the bioactive compound was higher when the IL solutions were more concentrated.

Du et al. (2007) assessed the effect of 0.5 to 3 mol L^{-1} aqueous solutions of [BMIM][BF_4] and 1-butyl-3-methylimidazolium bromide [BMIM][Br] on the extraction of *trans*-resveratrol from the rhizome of *Polygonum cuspidatum*, using microwave-assisted extraction (MAE). The authors described that [BMIM][Br] at the concentration of 2.5 mol L^{-1} was more effective for recovering *trans*-resveratrol. Du et al. (2011) reported that the use of 2.5 mol L^{-1} of [BMIM][Br] with 0.8 mol L^{-1} HCl was efficient for the recovery of myricetin and quercetin from *Myrica rubra* leaves using MAE.

4 Conclusion

The ILUAE approach combined with the 2^3 full factorial design was an efficient method for recovering phenolic compounds with potential antioxidant activity from red guava agro-industrial coproduct. The total phenolic coontent and antioxidant activity obtained for the extracts indicated that 2.5 mol L^{-1} [BMIM][Cl] IL was a potential alternative in replacing the traditional extraction method with methanol. This innovative method can be used for obtaining bioactive compounds and adding value to this important agro-industrial coproduct.

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Authors' Contributions

BLF: Conceptualization, Methodology, Formal analysis, Writing - Original Draft, Writing - Review & Editing. VFB: Investigation, Formal analysis, Writing - Original Draft. LW: Investigation. JMB: Resources, Writing - Review & Editing. DG: Writing - Review & Editing. ILN: Conceptualization, Methodology, Resources, Writing - Review & Editing. All authors read and approved the final manuscript.

Availability of data and materials

Data are available under request from the corresponding author.

Ethics approval and consent to participate.

Not applicable.

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Informed Consent Statement

Not applicable.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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