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Microwave-Assisted Extraction of Phenolic Compounds from Pineapple Peel Using Deep Eutectic Solvents

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Abstract: Approximately half of the world's pineapple production is marketed as a processed product, leading to the generation of a significant quantity of industrial waste, mainly composed of pineapple peels, cores, and crowns. This study evaluated deep eutectic solvents (DESs) for the assisted microwave extraction of phenolic compounds from pineapple peels and their antioxidant capacity. DESs are considered environmentally friendly solvents characterized by their low toxicity and high capacity for the extraction of bioactive compounds. DESs (choline chloride-glycerol and choline-chloride-malic acid) were used for phenolic compound extraction and compared with traditional solvents such as water, ethanol, methanol, ethanol-water (50%), and methanol-water (50%). A higher concentration of phenolic compounds was achieved using choline chloride-glycerol than traditional solvents as an extraction solvent (7.98 mg eq of gallic acid/g of dry weight). In all the treatments, the antioxidant capacity was higher than 85%. The process variables (drying temperature, extraction time, and solvent/solid ratio) were optimized using choline chloride-glycerol as a solvent. It was found that a drying temperature of 67 °C, an extraction time of 87 s, and a solvent/solid ratio of 60.5 mL/g allow maximizing the content of phenolic compounds and the antioxidant capacity of the extract.

Keywords: polyphenols; antioxidant capacity; waste valorization

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1. Introduction

Pineapple is one of the most commercialized tropical fruits in the world because of its sensory and nutritional properties. In 2018, worldwide pineapple production increased by approximately 8% [1]. Due to the high perishability and the relative difficulty of peeling pineapples, approximately half of the global pineapple production is commercialized as processed fruit (canned fruit or juice), with a high generation (50%) of industrial waste mainly composed of peels, cores, and crowns [2].

Waste disposal can cause environmental damage to soil and water due to the high concentration of nutrients and the low pH of pineapple. Efforts have been concentrated on the valorization of pineapple waste through the extraction of enzymes or fibers for industrial applications [3–5].

However, studies have demonstrated the presence of phenolic compounds such as ferulic acid, syringic acid, tannic acid, and p-coumaric acid in pineapple and its waste. These compounds have a high capacity to inhibit the formation of free radicals with a beneficial effect on health [2]. Different techniques for the extraction of phenolic compounds have been developed. Traditional extraction techniques, such as Soxhlet and maceration, have been gradually replaced by green techniques, such as ultrasound or microwave-assisted extraction, supercritical fluid extraction, and pressurized extraction with solvents or hot water. The microwave technique generates interest due to its simplicity and relatively low processing cost [6].

Microwave irradiation is widely used in the food industry due to the current demand for short processes with low energy demand and to maintain the nutritional quality of the food. The most common applications of microwave irradiation are thermal processing, especially drying, sterilization, and pasteurization [7]. However, the effect of microwave irradiation on the cell membrane allows the possibility of other applications, such as the extraction of essential oils [7], the improvement of the nutritional and organoleptic quality of oils [8,9], the obtaining of antifungal and antibacterial agents for food preservation [10], and the extraction of phenolic compounds, proteins, and others [11–13].

Microwave-assisted extraction is based on the interaction among electromagnetic waves and molecules in the material, causing a change in the electromagnetic and caloric energies. The molecular movement and the structural collapse caused by vapor pressure allow for the entry of the solvent into the matrix, the interaction between the solvent and the substratum, solubilization of the compounds in the solvents, and the mass transfer, achieving a higher extraction yield than that obtained without microwave assistance [14–16].

One of the steps before the extraction is drying. Generally, this process is carried out by traditional drying techniques such as hot air drying or freeze drying [17]. Hydro drying is a relatively new technique in which hot water is in contact with a transparent sheet that allows drying by the radiant heat of the water in thinner materials and all the heat transfer phenomena in thicker materials. It is characterized by a drying technique that requires short process times, low cost, and retention of food quality properties [18]. Although the effect of drying temperature on bioactive compounds of finished products has been briefly studied, as far as we know, the effect of the drying temperature on the extraction process of said compounds has not been reported.

Antioxidant capacity and content of phenolic compounds in the extract are strongly related to the solvent used due to the diversity of compounds in the tissue and their polarity and solubility [19].

The polar solvents most commonly used in polyphenol extraction are ethanol and methanol. Since 2015, an increase in the study of solvents that are considered environmentally friendly has been observed, including deep eutectic solvents (DESs), which are characterized by their low toxicity and high capacity for extraction of bioactive compounds [20,21].

The general principle of DES is its ability to donate and absorb hydrogen. This ability allows the formation of hydrogen bonds with the components to be extracted and facilitates the extraction. DESs are a mixture of a quaternary ammonium salt, such as choline chloride, and a compound that can act as a hydrogen donor, such as sugars, vitamins, amines, alcohols, and carboxylic acids. The mixture of these compounds results in a solution with optimal properties for the extraction of natural products such as phenolic compounds [22] DESs based on the use of choline chloride, known as CH-DESs, are characterized by significant retention of bioactive target compounds, better biodegradability compared with other DESs, low cost, low toxicity, and environmental compatibility. These properties make them a promising alternative for food, pharmaceutical, and cosmetic applications [23].

The extraction of phenolic compounds from pineapple waste has been carried out by autohydrolysis [2] and microwave-assisted extraction using traditional solvents (water, ethanol, and methanol) [24]. Meanwhile, DESs have been used for the extraction of bioactive compounds of different matrices, such as olive pomace [25], orange peels [26], and grape skins [22]. Additionally, the interaction between DESs and green extraction techniques, such as ultrasound and microwave-assisted extraction in olive pomace [25] and grape skins [22] has been studied. However, the combined effect of microwave-assisted extraction and DESs for the extraction of phenolic compounds from pineapple peels has not been studied. The objective of this work was to evaluate DESs as alternatives to conventional solvents for the microwave-assisted extraction of total phenolic compounds from pineapple peel.

2. Materials and Methods

2.1. Raw Material

Pineapples (variety MD2) with 13.65 ± 0.06 °Brix and green peels were used. Fruits were obtained from the Zambrano S.A.S. Farm (Valle del Cauca, Colombia). The peels were cut and dried in refractance window (RW®) equipment at 80 °C for 4 h, ground for 1 min in a knife mill, and sieved to obtain a uniform particle size.

2.2. Extraction Process

Phenolic compounds were extracted using a microwave oven with a 2.55 MHz frequency (LG brand reference MD-0746T) that was operated at 60% of its maximum power (420 W) for 1 min. These conditions were determined in preliminary tests. Samples of 1 g mixed with 15 mL of solvent were used for the extractions. The supernatant was separated, and the extracts were stored in the dark until analysis.

The evaluated DESs were choline chloride-glycerol (CC-glycerol) and choline chloride-malic acid (CC-malic acid). Additionally, extraction with conventional solvents, water-ethanol 96% (*v*/*v*) (Conquímica brand), ethanol-water 50% (*v*/*v*), methanol (Honeywell brand), and methanol-water 50% (*v*/*v*), were performed.

2.3. DESs Preparation

Choline chloride (Acros Organics (Geel, Belgium)) was dried in a vacuum oven at 50 °C for 24 h and mixed with glycerol (Loba Chemie (Maharashtra, India)) at a molar ratio of 1:2. The mixture was stirred for 2 h at 80 °C, dried in a vacuum oven for 18 h at 50 °C, and weighed. Distilled water at 10% in weight [25] was added [26].

Similarly, the choline chloride-malic acid solvent (Alfa Aesar (Ward Hill, MA, USA)) was prepared at a molar ratio of 1:1.5, and the mixture was heated for 6 h. The solution was rehydrated with water in a relation 50% *w*/*w* [22].

2.4. Total Polyphenol Content

The Folin-Ciocalteu colorimetric method was used [26,27]. For this purpose, 1560 µL of distilled water was mixed with 40 µL of extract, 100 µL of Folin-Ciocalteu reagent (Panreac applichem (Chicago, IL, USA)), and 300 µL of a filtered solution of 20% sodium carbonate (Loba Chemmie PVT, Maharashtra, India). The mixture was stirred manually for 1 min and stored in the dark for 1 h. Absorbance was read in a spectrophotometer (Genesys™ 20 de Thermo Scientific, Waltham, MA, USA) at 755 nm, and ethanol was used as a blank. A calibration curve was made with gallic acid following the above procedure with concentrations of 0.04, 0.1, 0.16, 0.2, and 0.3 mg of gallic acid/mL of solution. The results are expressed as mg eq of gallic acid/g of dry weight (mg eq of GA/g DW).

2.5. Determination of the Antioxidant Capacity

Free radical scavenging activity (2,2-diphenyl-1-picryl-hydrazyl, DPPH) of the extracts was determined using 300 µL of extract and 3700 µL of a 100 mM DPPH (Sigma-Aldrich (Burlington, MA, USA)) solution in methanol. The mixture was stored in the dark for 30 min. The absorbance of the solution was measured at 515 nm in a spectrophotometer (Genesys™ 20 de Thermo Scientific) using methanol as a control solution. The radical scavenging activity (*RSA*) was calculated according to Equation (1) [28]:

$$RSA = (1 - As/Ac) \times 100 \quad (1)$$

where *As* is the absorbance of the sample and *Ac* is the absorbance of the control solution [28].

The oxygen radical absorbance capacity (ORAC) analyses were carried out as described by Ganske et al. [29] with some modifications. Aliquots of 150 µL of fluorescein (5 µM) and 25 µL of extract were placed in a black 96-well polystyrene microplate. The mixtures were incubated for 30 min at 37 °C before adding 25 µL of APPH solution (240 mM). The reactants were prepared using phosphate buffer (10 mM pH 7.4). Fluorescence measurements (ex

485 nm, em 520 nm) were taken every 90 s during 80 cycles. A calibration curve was made (10, 300, 500, 700, 900, and 1000 μM) using Trolox as a standard.

2.6. Statistical Analysis

The results of three replicates for each experiment were averaged. Data in the text are expressed as the mean ± standard deviation (±SD), and error bars in the figures indicate the standard deviation. Differences between means were analyzed by ANOVA and post hoc Tukey's test using Minitab ® 20.3, Minitab LLC (State College, PA, USA) software. A confidence level of 95% was utilized. The normality of the distribution was tested by the Anderson-Darling test, and the homogeneity of variance was tested by the Bonferroni test.

2.7. Optimization

A rotatory central response surface design for the selected DES was carried out with 17 experiments and three replicates in the central point. The best variables to increase total phenolic content and antioxidant capacity were determined. The process variables were drying temperature, extraction time, and liquid/solid ratio. Each variable was coded in levels, which is shown in Table 1. The assumption of normality was evaluated, and Minitab® 20 software was used.

Table 1. Actual and coded levels of the process variables.

Process Variables	Variable Levels			Unit
	−1	0	1	
Drying temperature	70	77.5	85	°C
Extraction time	50	70	90	s
Liquid/solid ratio	15	32.5	50	mL/g

3. Results and Discussion

3.1. Total Phenolic Content

The total phenolic content expressed as mg eq of GA/g DW is shown in Figure 1. The determined values varied between 1.84 and 7.98 mg eq of GA/g DW.

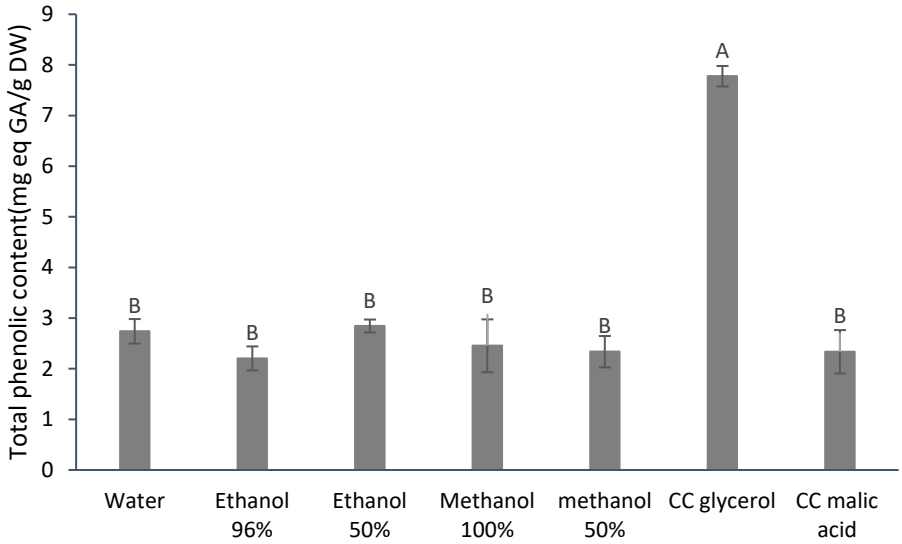

Figure 1. Effect of the extraction solvent on the total polyphenolic content of pineapple peel extracts.

The extraction with CC-glycerol showed a higher extraction yield than the other treatments, with a value of 7.78 ± 0.2 mg eq GA/g DW and no significant differences between the extraction yields of the conventional solvents (water, ethanol, and methanol); assumptions of normality and equality of variance (p-value > 0.05) were validated. The

mixture of CC-malic acid as a DES showed similar results to conventional solvents. These results were due to the high interaction among the phenolic compounds and the hydrogen bonds generated by DES and the higher polarity of the DES compared to that of the water and alcohol-based solvents [30].

These results are similar to those of Ozturk et al. [26]. They found that higher contents of total phenolic compounds were achieved using choline chloride-glycerol than other extraction solvents. Similarly, Rajha et al. [31] reported that the extraction of polyphenols from pomegranate was better when using a DES combined with green extraction techniques, such as ultrasound and infrared extraction, than when using other solvents, such as water and ethanol-water [30].

Figure 2 shows the results of the extraction yield of polyphenols from pineapple peels. As expected, this response was similar to the total phenolic compound content, indicating that the solvent with the highest extraction yield (0.71%) was the mixture of choline chloride-glycerol.

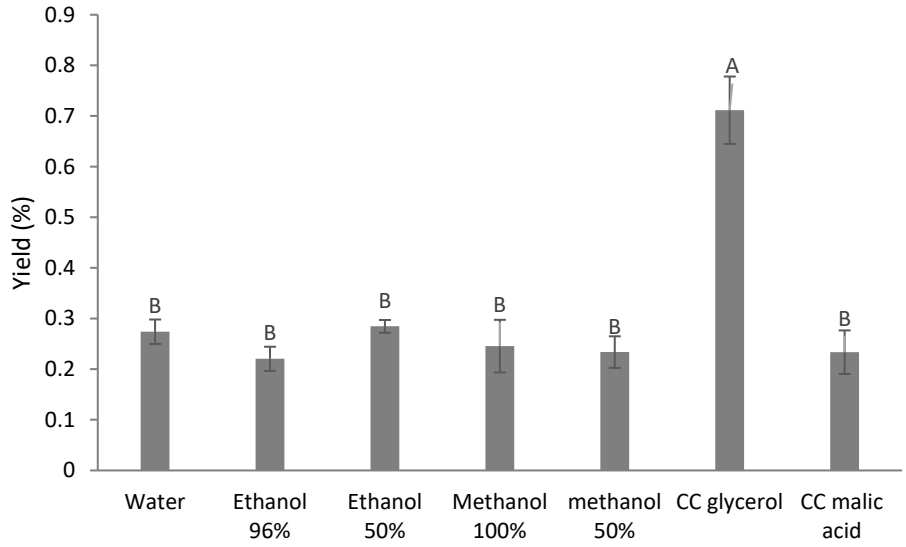

Figure 2. Extraction yield of phenolic compounds of pineapple peel.

3.2. Antioxidant Activity

All the extracts exhibited an RSA higher than 80% (Figure 3) without statistically significant differences between the water, methanol (50%), and DES treatments; assumptions of normality and equal variances showed p-value > 0.005. This behavior (opposite to that found for the total phenolic content, not flavonoids) could be due to other compounds, such as sugars, flavonoids, and others, that also have a high antioxidant capacity. Moreover, antagonistic interactions between the antioxidant compounds in the extract can exist [2].

According to Radosevic et al. [30], a consistent relationship between the total phenolic content and the antioxidant capacity has not been found. Ozturk et al. [26] found a lack of correlation between the total phenolic content and the antioxidant capacity of the extract of orange peels. This was due to the interactions generated between the solvent and radicals, which decreased the RSA.

Alothman et al. [32] reported RSA values for pineapple between 67 and 78% using pure methanol and ethanol and their mixtures with water, while Sepúlveda et al. [2] reported an RSA lower than 85% for the extract of pineapple residues using water as the solvent. The RSA values in this research are higher than those reported in other studies. Higher antioxidant capacity may be due to the fact that the processes used in this work decrease the degradation of antioxidant compounds and their biological activity.

On the other hand, the ORAC assay has been associated with the simulation of the oxidation process that occurs during cell degeneration in an organism, hence its importance in studying antioxidant compounds [33].

Figure 4 shows the effect of the extraction solvent on the oxygen radical absorbance capacity. The results obtained with ethanol at 50%, methanol at 50 and 100%, and choline-chloride-glycerol are statistically similar; data showed normal distribution and equality of variances (p-value > 0.05).

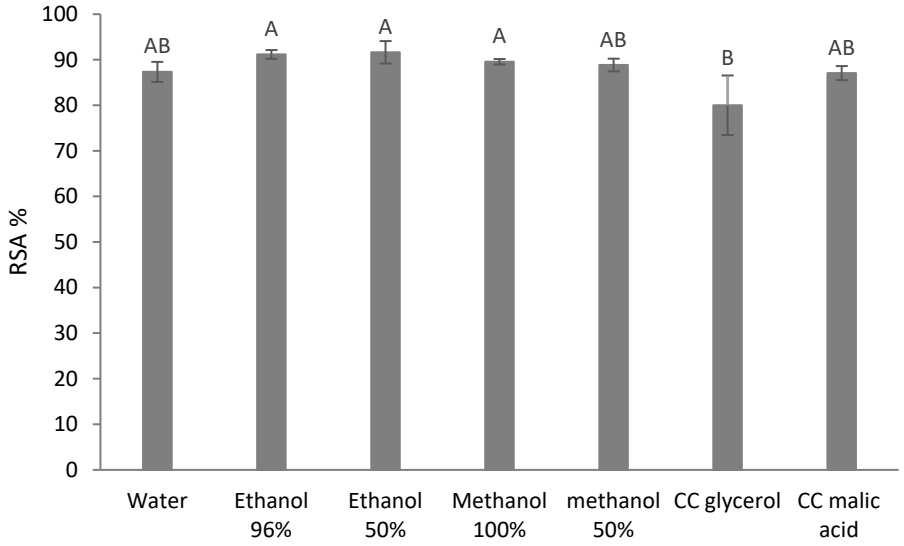

Figure 3. Effect of the extraction solvent on the Radical Scavenging Activity (RSA).

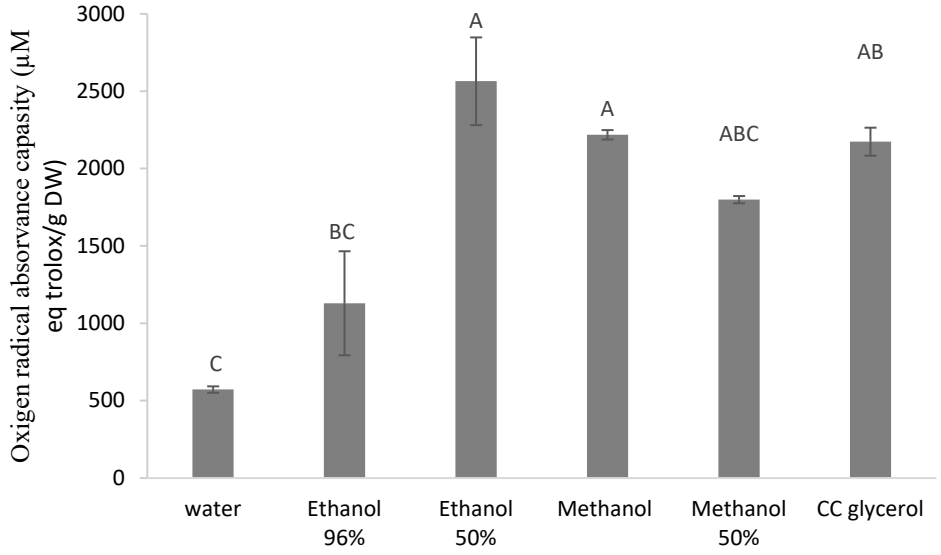

Figure 4. Effect of extraction solvents on the oxygen radical absorbance capacity (ORAC).

The antioxidant capacity measured by the ORAC method for pineapple peel is in the range that Campos et al. [5] reported. The results obtained in this study with DES is higher than those obtained by Radošević et al. [30], who report values for antioxidant capacity for grape skin extracts with choline chloride-glycerol of 111 µM/gdm, and those obtained with traditional solvents in litchi pulp reported by Su et al. [34].

The differences observed in the antioxidant capacity determined by RSA or ORAC may be due to the nature of the phenolic compounds in the extract. It has been shown that extracts with a majority composition of some compounds such as epicatechin show better results with the ORAC antioxidant capacity method than other methods [30].

3.3. Optimization Process

Once CC-glycerol was determined as the most efficient solvent, the extraction process was optimized by evaluating the drying temperature, microwave irradiation time, and

solvent/solid radio. In Figure 5, the behavior of the total content of phenolic compounds extracted from pineapple peel is observed.

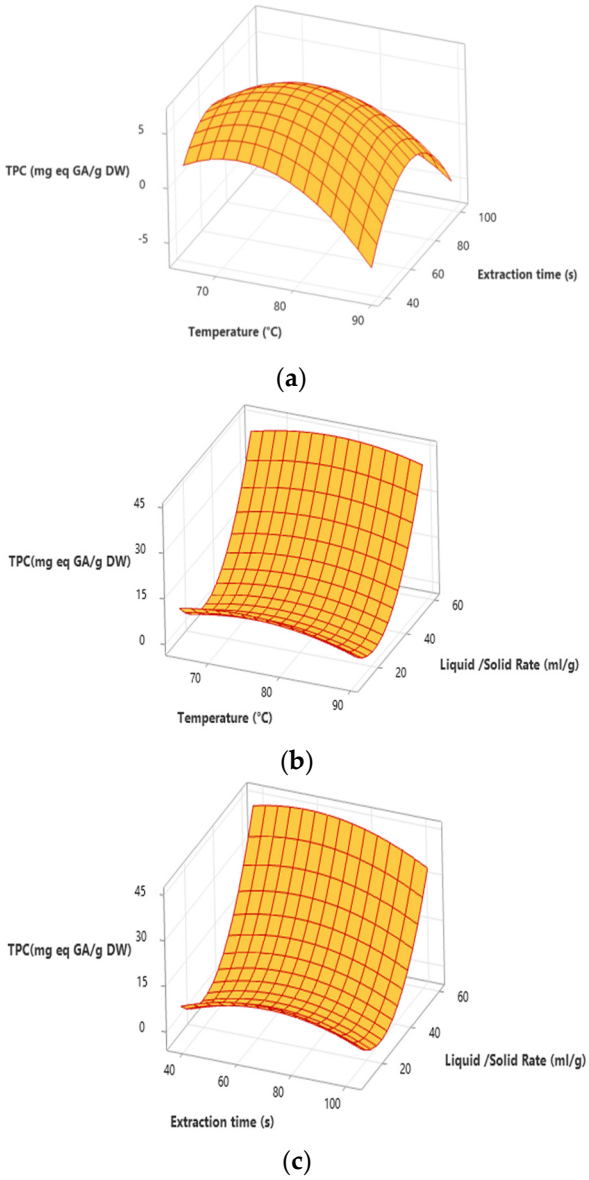

Figure 5. Response surface of the effect of (**a**) drying temperature and extraction time, (**b**) drying temperature and solid/liquid ratio, and (**c**) extraction time and solid liquid ratios on total phenolic content of pineapple peel extracts.

The drying causes damage to the cellular structure, improving the interaction of the matrix with the solvent in the extraction process [17]. Figure 5a shows a maximum in the total phenolic content between 70 and 80 °C. However, higher temperatures decrease the total phenolic content because these compounds are highly temperature sensitive. On the other hand, it is observed that the process is favored by extraction times between 60 and 80 s. Increasing the irradiation time increases cell membrane breakdown [35] and decreases DESs' viscosity, enhancing the interaction between the solvent and the phenolic compounds and improving the extraction process. This phenomenon is observed up to a specific irradiation time. When the irradiation is further prolonged, DESs overheat and possibly cause the degradation of phenolic compounds.

Figure 5b,c show that the higher liquid/solid ratio, the higher the total phenolic content. A higher amount of solvent in the system avoids solvent saturation and improves

the mass transfer and irradiation process [35]. Similar behavior was found for the yield (data not shown).

Figure 6 shows the behavior of the antioxidant capacity measured by DPPH; it is observed that higher antioxidant capacity does not coincide with higher TPC, showing that high concentrations of phenolic compounds do not imply high biological activity or that the predominant antioxidant compounds in the extracts are not phenolic compounds [13].

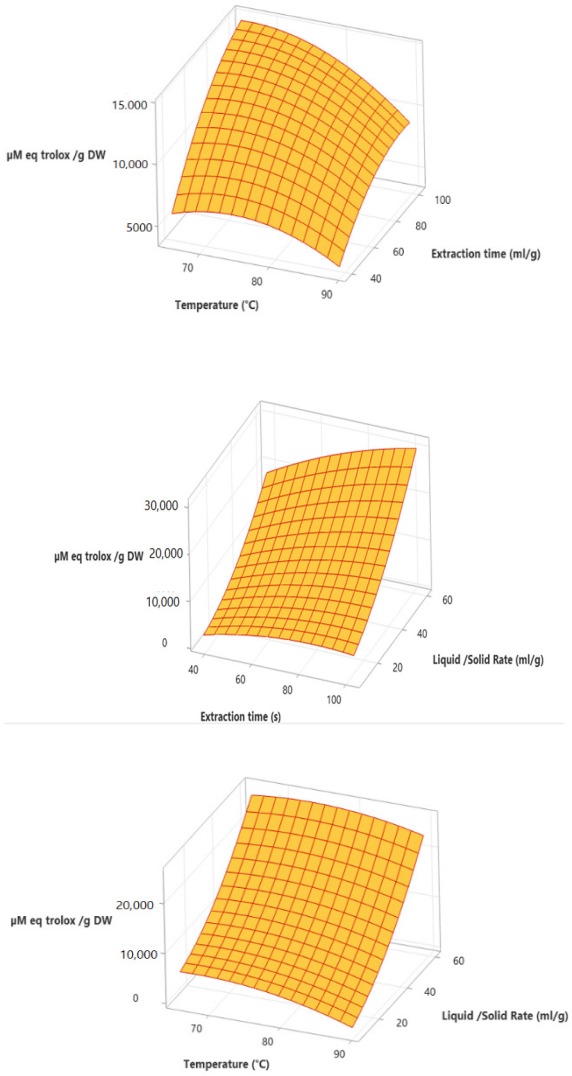

Figure 6. Response surface of the effect of drying temperature, extraction time, and liquid-solid ratio on antioxidant capacity measured by the DPPH method of pineapple peel extracts.

Lower temperatures and longer extraction times favored the antioxidant capacity measured by the DPPH method. Prolonged exposure to high temperatures degrades bioactive compounds and their biological activity. In addition, longer microwave irradiation times allow higher membrane breakdown facilitating the solvent input and the extraction of compounds with an antioxidant capacity [36].

The antioxidant capacity measured by the ORAC assay is shown in Figure 7. The effect of the process variables on antioxidant capacities measured by DPPH and ORAC are similar. However, the ORAC values are lower than those of DPPH. Although both methods are valid, the ORAC method provides the best approximation to the in vitro antioxidant capacity since this technique allows simulating the antioxidant capacity of an extract against peroxyl radicals, which are the primary free radicals produced in lipid oxidation in food and other biological systems [36].

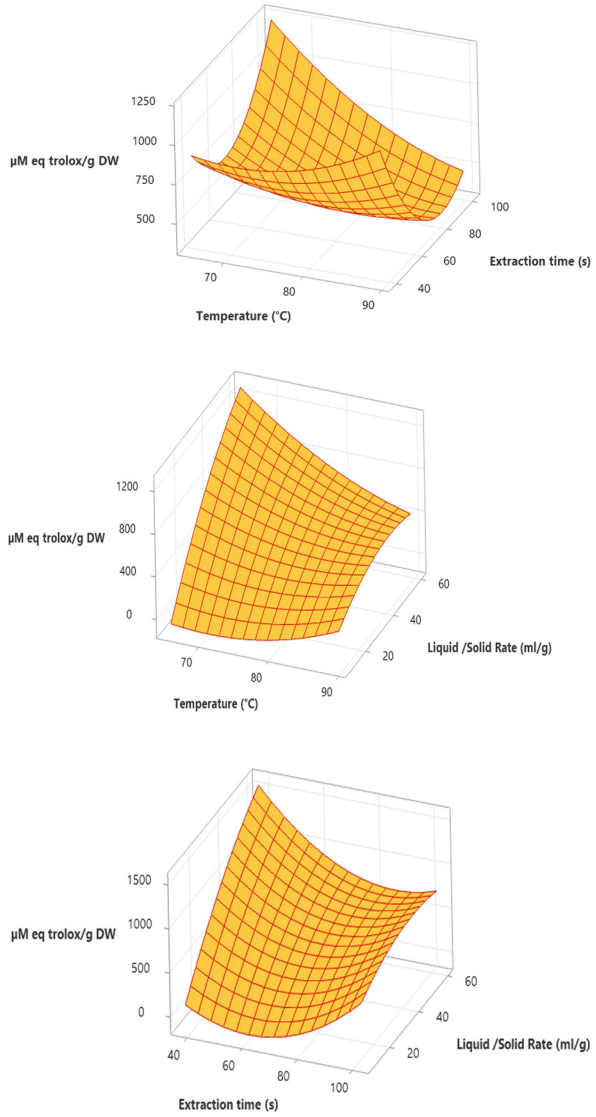

Figure 7. Response surface of the effect of drying temperature, extraction time, and liquid-solid ratio on antioxidant capacity measured by ORAC method of pineapple peel extracts.

TPC, antioxidant capacity (DPPH, ORAC), yield, and RSA (%) were maximized to obtain the optimal conditions. The composite desirability of the optimization process was 0.79, and the optimal conditions were temperature of 67 °C, liquid/solid ratio of 60.5 mL/g, and extraction time of 87 s. The results for each response variable are shown in Table 2.

Table 2. Optimization results.

	TPC	DPPH	ORAC	Yield	RSA
Predicted value	35.95	28630	1173.3	3.16	93.97
Desirability	0.57	0.94	0.99	0.59	0.99

TPC (mg eg GA/g DW), DPPH (µM eq Trolox/g DW), ORAC (µM eq Trolox/g DW), Yiel (%), RSA (%).

For an efficient extraction, it is necessary to reduce the thermal damage of the antioxidant compounds, which is achieved using low drying temperatures such as those obtained in the optimization. Low temperatures also have the advantage of reducing the energy cost of the process. The value of the solvent/solid ratio obtained indicates that in the optimal process, the saturation of the solvent with the solute was avoided. In addition, the solvent/solid ratio increase is associated with less overheating of the medium and

less thermal damage to the compounds of interest. The microwave irradiation time was sufficient to allow better solvent interaction with the solute, increasing the extraction yield. This behavior could be because the cell membrane ruptures, increasing the mass transfer. This effect was not observed in shorter irradiation times.

The desirability results of the response variables studied show that the antioxidant capacity measured by both ORAC and DPPH were the best modeled parameters. Antioxidant capacity is the most important indicator of the bioactivity of the extract.

4. Conclusions

DES choline chloride-glycerol and microwave-assisted extraction are an alternative to a green process for extracting phenolic compounds from pineapple peel. The drying temperature of 67 °C, liquid/solid ratio of 60.5 mL/g, and extraction time of 87 s allowed us to obtain extracts with a high concentration of phenolic compounds and high antioxidant capacity. To the best of our knowledge, for the first time, convective hydro-drying and microwave extraction conditions are optimized to obtain phenolic compounds from pineapple waste. This is a significant basis for the valorization of by-products using combinations of eco-friendly technologies.

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