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Microwave extraction of champa (*Campomanesia lineatifolia* Ruiz & Pav.) fruit: alternative to obtain natural antioxidants

Extracción por microondas de la champa (*Campomanesia lineatifolia* Ruiz & Pav.): alternativa para la obtención de antioxidantes naturales

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Abstract

Champa (*Campomanesia lineatifolia* Ruiz & Pav.) is a fruit tree that belongs to Myrtaceae family and is native from the Amazon. Champa fruit has special organoleptic characteristics like its flavor and is an important source of phenolic compounds useful in food industry. Currently, the postharvest process of champa fruit needs to be improved due to fruit losses, which had achieved a 97%. In this sense, the aim of this research was to evaluate microwave extraction to obtain extracts enriched in phenolic compounds from champa fruit. The evaluated variables were as follows: microwave power (100-200W), extraction time (1-2min) and solvent (water, ethanol-water 70:30 and ethanol-water 96:4), respectively. Results showed that the best condition to obtain phenolic extracts involved the use of water as solvent, 100W of power and 2min of extraction time. Given these concerns, was possible to obtain a yield higher than 60%. The obtained extracts had achieved a maximum antioxidant activity of 11.36 and 31.44 mg of Trolox.g^{-1} of dry pulp through DPPH and FRAP methods. In fact, the higher phenolic concentration (3450.73 µg Gallic acid. g^{-1} of dry pulp) was obtained with a 200 W of microwave power, water as solvent and 1 min of extraction time. These results suggest that champa fruit is a source of phenolic extracts with antioxidant activity, which are useful for agroindustry sector, especially as natural additives to extend the shelf life of different products.

Key words: Agroindustrial biotechnology, food processing, fruit extracts, natural additives, phenolic compounds, fruit postharvest.

Resumen

La Champa (*Campomanesia lineatifolia* Ruiz & Pav.) es un árbol frutal nativo del amazonas perteneciente a la familia Myrtaceae. La fruta de la champa tiene unas características organolépticas especiales como su sabor y es una importante fuente de compuestos fenólicos útiles para la industria de los alimentos. Actualmente los procesos poscosecha de la fruta requieren mejoras pues las pérdidas alcanzan el 97%. En ese sentido, el objetivo de esta investigación fue evaluar la extracción por microondas de la champa para la obtención de extractos enriquecidos en compuestos fenólicos. Las variables estudiadas fueron: potencia del microondas (100-200W), tiempo de extracción (1-2min) y disolvente (agua, mezcla etanol: agua 70:30 y mezcla etanol: agua 96:4). Los resultados mostraron que la mejor condición para la obtención de extractos fenólicos fue empleando agua como disolvente, una potencia de 100W y 2 min de tiempo de extracción. A esta condición, se alcanzó un rendimiento de extracción superior al 60%. La máxima actividad antioxidante en los extractos fue de 11.36 y 31.44 mg eq de Trolox.g^{-1} de pulpa seca, evaluada por los métodos del DPPH y FRAP respectivamente. La mayor concentración de contenido fenólico fue obtenida (3450.73 µg de ácido gálico.g^{-1} de pulpa seca) utilizando como condiciones de proceso una potencia del microondas de 200W, agua como disolvente y un tiempo de extracción de 1min. Estos resultados sugieren que la champa es una fuente de extractos de compuestos fenólicos con actividad antioxidante útiles para el sector agroalimentario, especialmente como aditivos naturales para extender la vida útil de diferentes productos.

Palabras clave: Aditivos naturales, biotecnología agroindustrial, compuestos fenólicos, extractos de frutas, poscosecha de frutas, procesamiento de alimentos.

Introduction

A healthy lifestyle is a strategy to reduce the incidence of chronic diseases. Among other things, comprises a diet rich in fruits and vegetables and low in refined sugars and saturated fats. The World Health Organization (WHO) has indicated that 85% of cardiovascular diseases and a 15% of some cancers cases are associated with a low fruit diet (Wang, Li, Wang, Qiu, Shen & Wang, 2015), which is consistent with the Food and Agriculture Organization (FAO) statistics.

The world production of vegetable and fruits had achieved 1740 million tons for 2013 (950 million tons for vegetables and 760 million tons for fruits) (FAO, 2013). Colombian fruit production rose from 3.1 million tons in 2010 to 3.8 tons in 2014, which represents an increasing in annual rate of 5.0%. Likewise, fruit and vegetables production in Colombia reached 46.2% from total agricultural sector production in 2013 (Asohofrucol, 2014).

In this sense, is clear that fruit and vegetables subsector is strategic for Colombian development. This is an opportunity to generate knowledge addressed to enables producers to acquire new tools to face the market challenges. Conversely, is important to study harvest and post-harvest losses, which are the most important limitation in fruits and vegetables industrialization. In fruits case, losses are related to high water content, ethylene production, breathing processes and maturity effect among them (FAO, 2013).

Treatment with antioxidant substances had achieved a preservation technique to diminish the effects of senescence processes caused by oxidation and subsequently, to control fruit post-harvest losses. There are several synthetic antioxidants used in foods and drugs in the market, including butylhydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ) and propyl gallate, respectively. However, BHA and BHT use is associated with toxic and carcinogenic effects. The new generations of antioxidants are focus on natural additives, as plant extracts (rich in vitamin C, tocopherols, carotenoids and flavonoids) useful to protect products against oxidation (Barbosa, Bilbao, Vilches, Angulo, Fité, Paseiro & Cruz, 2014; Martín & Soliva, 2010; Tuesta, Orbe, Merino, Rengifo & Cabanillas, 2014).

Natural antioxidants are a topic of much scientific research due to its chemical properties and its food interaction. For instance, they can help in food conservation while reducing the risk of some diseases (cancer, heart problems and neurological disorders). In other words, they have a protective effect on health (Cerón, Higuita & Cardona, 2010; Freid, 2012). This protective effect is attributed to various metabolites including the group of polyphenols (flavonoids, anthocyanins, lignans, among others) (Muñoz, Ramos, Alvarado & Castañeda, 2007).

Colombian native fruits are a source of bioactive compounds with antioxidant activity, which can be used as natural additives (Zapata, Piedrahita & Rojano, 2014). An example of them, is the champa (*Campomanesia lineatifolia* Ruiz & Pav.), a fruit tree of the Myrtaceae family native from the Amazon. In Colombia, grows in Choco, Amazonas, Caquetá, Casanare, Cundinamarca and Boyacá departments. This fruit has a high concentration of acids (citric acid predominating) and sugars (mostly sucrose). Therefore, its total titratable acidity is close to 3% and total soluble solids (TSS) are between 11.2 and 13.4% (Álvarez, Galvis & Balaguera, 2009; Balaguera, Álvarez & Bonilla, 2009).

Even though there are a small number of studies related to champa phytochemicals, the presence of three triketones in seeds and nine volatile compounds isolated from pulp, peel, leaves and seeds is demonstrated. In fact, these compounds show antimicrobial activity and potential use as colorants or additives in food matrices (to improve functional characteristics or shelf life extension) (Bonilla, Duque, Garzón, Takaishi, Yamaguchi, Hara & Fujimoto, 2005).

In fact, in a previous work carried out by Balaguera, Álvarez & Bonilla (2009), harvest and post-harvest losses of champa had achieved 97% in the Lengupá Province of Boyacá, Colombia. In this context, the development of new products obtained from champa is a strategy to improve the benefits to producers, despite having enriched phenolic extracts, which were obtained through solid liquid extraction process of champa (Muñoz, Chávez, Pabón, Rendón, Chaparro & Otálvaro, 2015). The results confirmed antioxidant activity and phenolic compounds presence on the extracts. To continue with this research, this work aimed to evaluate microwave extraction with different conditions as an alternative extraction method. In this research, different conditions of extraction time, microwave power and solvent looking for cheaper, easily, safe and environmental friendly extraction conditions to carry out this process, were considered. The results will contribute to the species knowledge and as source of phenolic compounds useful for food industries.

Materials and methods

The fruits of *C. lineatifolia* came from the municipality of Miraflores (Boyacá, Colombia 5°11′47″N 73°08′40″O). The mean fruit weight ranged from 25-30 g and their ripening degree ranged from 3 to 6, equivalent conditions to green, ripe and overripe, respectively. Champa fruits were selected by their physics attributes (without wounds, fermentation, fungal or insect damage). After selection, fruits were scalded to inactivate enzymes prior to pulp production (Muñoz *et al.*, 2015). Pulp was obtained and subsequently, was dehydrated into a freeze-dryer (FreeZone Plus 12 Liter, Labconco ®) until 6% of moisture (experimental conditions: 24 h, 0.12 mBar and -89 °C). Dehydrated pulp was stored at -20°C in airtight containers protected from light until microwave extractions.

Evaluation of different extraction conditions

Extractions took place in a conventional microwave oven. Extraction conditions were as follows: power ranged from 100 and 200W, extraction time: 1-2min. Distilled water and ethanol mixes (J.T. Baker absolute)-water 70:30 and ethanol-water 96:4, were used as extraction solvents. The solute-solvent ratio was constant (1:10) and all extracts were concentrated though distillation under reduced pressure. Each treatment was evaluated in triplicate.

Fruit extracts characterization

Total phenolic compounds in fruit extracts were determined through Folin Ciocalteu method while antioxidant activity was determined by DPPH method, both previously described by Muñoz *et al.* (2015). The results of phenolic compounds were expressed in μg of gallic acid.g^{-1} dry pulp and antioxidant activity results, were expressed in mg of Trolox.g^{-1} dry pulp, according to calibration curves.

The antioxidant activity was also determined through FRAP methodology. FRAP reagent was obtained mixing 25mL of acetate buffer solution (300mM, pH 3,6), 2.5mL of TPTZ (Sigma-Aldrich) solution (10mM) and 2.5mL of $FeCl_3.6H_2O$ solution (20mM). To quantify antioxidant activity in the fruit extracts, 50μL of fruit extract solution (20 mg of extract.mL^{-1}) was mixed with 1mL of FRAP solution. In addition, the mix was heated in a water bath at 37°C for half an hour and then the absorbance was determined at 593nm. The results were expressed in mg of Trolox.g^{-1} dry pulp. Antioxidant activity and phenolic content data were analyzed using ANOVA, to identify significant difference among treatments and statistical significance for all comparisons was made at $p<0.05$.

Results

Microwave extraction

According to the results presented in Table 1, corresponding to the microwave extraction, it was observed that the solvent with higher extraction yields obtained was water, followed by the mix ethanol-water (70:30), which is an advantage considering that extraction costs are in decreasing when water is used as solvent.

Table 1. Microwave extraction yields

Time (min)	Solvent	Power (W)	Extraction Yield
1	Ethanol- water (96:4)	100	21.61 ± 1.68
2	Ethanol- water (96:4)	100	26.56 ± 0.75
1	Ethanol- water (96:4)	200	25.24 ± 3.56
1	Ethanol-water (70:30)	100	42.08 ± 5.92
2	Ethanol-water (70:30)	100	59.51 ± 3.03
1	Ethanol-water (70:30)	200	48.46 ± 8.39
1	Water	100	40.59 ± 2.43
2	Water	100	67.68 ± 18.20
1	Water	200	65.32 ± 11.30

Quantification of phenolic content and antioxidant activity

The highest content of phenolic compounds (3450.73 ± 368.26 μg of gallic acid.g^{-1} of dry pulp) was obtained using water as solvent for 1min at 200W. The highest antioxidant activity (DPPH 11.36 ± 4.65 and FRAP 31.44 ± 20.18 mg of Trolox/g dry pulp) had achieved using water as solvent, 2min of extraction time and 100W of power, respectively (Table 2).

Table 2. Champa fruit extracts characterization

Time (min)	Solvent	Power (W)	Folin Ciocalteu (mg of gallic acid. g^{-1} dry pulp)	DPPH (mg of Trolox. g^{-1} dry pulp)	FRAP (mg of Trolox. g^{-1} dry pulp)
1	Ethanol- water (96:4)	100	1208.38 ± 184.27	3.09 ± 0.71	6.16 ± 1.07
2	Ethanol- water (96:4)	100	1649.63 ± 83.03	3.43 ± 0.36	7.66 ± 0.85
1	Ethanol- water (96:4)	200	1893.60 ± 397.58	2.68 ± 0.10	5.91 ± 0.45
1	Ethanol-water (70:30)	100	1693.85 ± 347.66	7.34 ± 0.83	16.02 ± 2.00
2	Ethanol-water (70:30)	100	2222.01 ± 634.77	10.61 ± 1.31	23.24 ± 4.38
1	Ethanol-water (70:30)	200	1932.22 ± 713.09	8.36 ± 0.88	19.83 ± 4.39
1	Water	100	3432.66 ± 722.67	5.86 ± 0.73	15.63 ± 0.81
2	Water	100	2916.71 ± 396.70	11.36 ± 4.65	31.44 ± 20.18
1	Water	200	3450.73 ± 368.26	9.61 ± 1.99	23.93 ± 2.62

Discussion

Microwave extraction yields have had achieved a similar behavior of the results obtained in solid-liquid extractions of champa fruits, where aqueous extracts present the highest yields (reaching efficiencies from above 45%) (Muñoz *et al.*, 2015). Although, statistically significant differences were observed in the extraction yields respect to power and extraction time (95% confidence interval) for all evaluated variables (solvent, time and power). The highest yield extraction was 67.68 ± 18.20%, which was higher than the results obtained with solid-liquid extraction. The extraction conditions from which this yield had achieved were 100W, 2min and water as solvent. These results are comparable in variability to the report by Martínez, Contreras & Belares (2010); Muñoz *et al.* (2015); Alonso, Aguilar, Vernon, Jiménez, Cruz & Román (2017), where the application of ultrasonic or microwave extraction have allowed an increasing in the rate of mass transfer and thus the extraction yield of antioxidant compounds from plant material.

The phenolic compounds extraction are thought was independent of power and time and only the solvent, presented a statistically significant effect (confidence interval 95%) at the evaluated conditions. In this sense, at higher solvent polarity an increasing in the extraction feasibility of phenolic compounds was observed (Martínez, Contreras & Belares, 2010; Alonso *et al.*, 2017). On the other hand, the solubility in water and alcohol of phenolic compounds is higher for diphenols and polyphenols such as flavonoids, suggesting their presence in the studied matrix (Cerón, Higuita & Cardona, 2010).

The phenolic compounds concentration obtained from champa fruits was lower than the value established for Guabiroba Brazilian (*C. adamantium*) (7240 - 21190 µg of gallic acid.g^{-1} of dry sample). However, was comparable to other fruits and tubers, for example, carambola (*Averrhoa carambola* L.) (13.10 µg of gallic acid.g^{-1} of dry sample) and sapodilla (*Manilkara zapota* L. P. Royen), (1.421 µg of gallic acid.g^{-1} of dry sample) (Rodríguez, López & García, 2010).

Bearing this in mind, the present work is intended to present phenolic compounds extracted by solid-liquid extraction from champa fruits, it was observed that using this technique after 4 h of extraction with a mixture of ethanol-water (70:30) as solvent at 70°C, the amount of those compounds was 5272.51 ± 424.89 µg of gallic acid.g^{-1} of dry pulp. In this sense, the results obtained by microwave extraction were lower than solid extraction results (Muñoz *et al.*, 2015). It was also noted in Table 2, in most treatments FRAP values were approximately twice as those obtained through DPPH. Nevertheless, is possible that some antioxidant compounds, such as carotenoids and some sugars can interfere in the measurement of DPPH methodology (Pérez, Lugo, Gutiérrez & Del-Toro, 2013).

It was observed that antioxidant activity measured through DPPH method was affected by time, solvent and power ($P<0.05$) while by FRAP method was only affected by solvent and power ($P<0.05$). In both cases, this parameter had achieved an increasing effect with time and power used in extraction process.

When the results were compared against the antioxidant activity obtained by solid-liquid extraction using water as solvent at 50°C (6.31±0.61 and 7.90±1.05 mg of Trolox.g^{-1} of dry pulp for DPPH and FRAP methods, respectively), is clear that microwave extraction have allowed to achieve better results (Muñoz *et al.*, 2015).

On the other hand, in both extractions, fruit extracts with higher phenolic content had the higher antioxidant activity. It shows that biological activity is attributed exclusively to the phenolic compounds. Although in previous studies have determined a direct relationship between the phenolic compounds concentration in different fruit extracts and their antioxidant activity, is possible that in extractions with solvents such as water, these compounds had formed macromolecules and then, extracts, which had a lower antioxidant activity than it was expected.

When comparing the results of antioxidant activity obtained in this study with previous reports, we found that for different species, including carambola, the antioxidant activity was 8.00 mM TEAC.g^{-1} from wet pulp, which is very close to the maximum obtained for champa (*C. lineatifolia*) fruit. However, fruits such as yacon (*Smallanthus sonchifolius* (Poepp.) H.Rob.), noni (*Morinda citrifolia* L.), cherry (*Prunus avium* (L.) L.), banana (*Musa acuminata* Colla.), passionfruit (*Passiflora edulis* Sims.) and camu-camu (*Myrciaria dubia* (Kunth) McVaugh.), had achieved higher activities 22.20 mM TEAC. g^{1} of pulp (Morillas & Delgado, 2012).

Considering results related to antioxidant activity, this paper has shown that the extracts obtained had potential in agroindustrial processes. Conversely,in meat products, they can be used to control lipid oxidation. Alternatively, in baking, they can increase the functional properties as well as to enrich products with antioxidants. In dairy industry, are used to increase the concentration of biologically active compounds in these products and finally, in fruits and vegetables industry, they can help to control the oxidation and can be used to extend the shelf life of products. In addition, natural antioxidants are also employed as raw materials for the pharmaceutical and cosmetic industries due to its antioxidant activity, moisturizing and emollient activity (Rodríguez, López & García, 2010).

Conclusion

This study evaluated the effects of fruit the extracts obtained by microwave extraction, which have demonstrated that had potential in agroindustrial processes due to their antioxidant activity. Given these concerns, the fruit extracts offers a new way to take advantage of this native amazon fruit as a source of extracts, which can be used as functional additives in other food matrices, adding value to this species.

The best results for extraction had achieved using water as solvent; this provides more accurate and reliable estimates of an advantage for food industry because water is a green and nontoxic solvent. On the other hand, short times involved in microwave phenolic compounds extraction compare with other extraction methods; helps ameliorate process cost in the industrialization of this process.

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