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Physicochemical, antioxidant, and antibacterial properties of heat-treated edible mushroom extracts

Propiedades fisicoquímicas, antioxidantes y antibacterianas de extractos de hongos comestibles tratados térmicamente

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Abstract

This study describes the thermal effect on physicochemical properties, phenolic content, as well as antioxidant and antibacterial activity of edible mushroom extracts (EME) from *Agaricus brasiliensis*, *Ganoderma lucidum*, and *Pleurotus ostreatus* in aqueous-ethanolic solution. The heating of EME resulted in a reduction of pH and total soluble solids and changes in color values in time dependence ($p < 0.05$). In addition, total phenolic and flavonoid contents, antiradical activity (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ inhibition), and reducing power were enhanced with increasing temperature (90 °C, for 6 h), mainly in the *P. ostreatus* extract ($p < 0.05$). In contrast, the results indicate that high temperatures reduced ($p < 0.05$) the antibacterial effect against all pathogens tested. In conclusion, the thermal treatment affected physicochemical and antibacterial properties of EME and enhanced phenolic composition and antioxidant activity.

Keywords: Mushrooms; phenolic components; biological activity; thermal process.

Resumen

Este estudio describe el efecto térmico sobre las propiedades fisicoquímicas, contenido fenólico, así como actividad antioxidante y antibacteriana de extractos de hongos comestibles (EME) de *Agaricus brasiliensis*, *Ganoderma lucidum* y *Pleurotus ostreatus* en solución acuosa-etanólica. El calentamiento de EME resultó en una reducción del pH y de los sólidos solubles totales, y cambio en los valores de color, en dependencia del tiempo ($p < 0.05$). Además, el contenido total de fenoles y flavonoides, actividad antirradical (inhibición de DPPH$^{\bullet}$ y ABTS$^{\bullet+}$) y el poder reductor se incrementaron con el aumento de temperatura (90 °C, durante 6 h), principalmente en el extracto de *P. ostreatus* ($p < 0.05$). En contraste, los resultados indican que las altas temperaturas redujeron ($p < 0.05$) el efecto antibacteriano contra todos los patógenos probados. En conclusión, el tratamiento térmico afectó las propiedades fisicoquímicas y antibacterianas de EME y aumentó la composición fenólica y la actividad antioxidante.

Palabras clave: Hongos; componentes fenólicos; actividad biológica; proceso térmico.

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Introduction

Edible mushrooms have been considered an important source of nutritional and bioactive compounds like polysaccharides and phenolic compounds (phenolic acids and flavonoids). It has been widely demonstrated that these components are characterized according to their ability to reduce the risk of some chronic diseases (cancer, diabetes, obesity, heart disease, among others); moreover, they act as immunomodulatory, antiinflamatory, antiviral, antioxidant, and antibacterial agents (Mingyi *et al.*, 2019; Pérez-Montes *et al.*, 2021). Hence, investigations about the use of polyphenol extraction methods for different mushrooms have increased in order to use these compounds as functional ingredients for pharmaceutical or food industries (Roselló-Soto *et al.*, 2016).

However, it has been demonstrated that the efficiencies of the methods used for the extraction compounds from natural sources can be influenced by the critical input parameters such as solid-solvent ratio, particle size, extraction time, pressure, temperature, among others (Azmir *et al.*, 2013; Roselló-Soto *et al.*, 2016). In addition, research studies have shown that temperature can affect the phenolic components and the biological properties of natural extracts (Davidov-Pardo *et al.*, 2011; Mokrani & Madani, 2016).

In this scenario, it is necessary to obtain detailed knowledge of the thermal stability of edible mushroom extracts that can be considered as possible functional ingredients for pharmacological or food industries. Therefore, the objective of this work was to determine the thermal effect on the biological properties of three edible mushroom extracts (*Agaricus brasiliensis*, *Ganoderma lucidum*, and *Pleurotus ostreatus*) selected due to their natural antioxidant and antibacterial properties.

Materials and Methods

Extract preparation

The bioactive components to be analyzed were extracted from edible mushroom powder (*A. brasiliensis*, *G. lucidum*, and *P. ostreatus*) with an ethanol:water (1:1) solution by ultrasound-assisted method (42 KHz/30 min/avoiding temperatures above 30 °C), using a 1:10 solid-solvent ratio and an ultrasound bath (Bransonic 3800, Ultrasonics Corp., Jeju, Korea). Afterwards, the extracts were filtered (Whatman No. 4 filter paper) under vacuum (vacuum pump MVP 6, Jeju, Korea), concentrated under reduced pressure at 60 °C (rotary-evaporator Yamato RE301BW, Tokyo, Japan), and dried (freeze-dryer Yamato DC401, Tokyo, Japan). The concentrated-dried edible mushroom extracts (EME) were stored at -20 °C in darkness until analysis (Toma *et al.*, 2001).

Thermal process

Experiments were performed in tubes with screw caps, and all EME (ethanol:water solutions) were thermostated in a water bath (Aquabath, Thermo Fisher Scientific TM, Nueva Jersey, USA) at the desired temperature (60 °C and 90 °C for 2 h, 4 h, and 6 h), except for the control samples, which were maintained at room temperature. Afterwards, test tubes were cooled to halt the thermal degradation and stored (at 4 °C in darkness) until further use (Fernández-López *et al.*, 2013).

pH determination

The pH of EME was measured (50 ml, 5 mg/ml) with a potentiometer (pH211, Hanna Instruments Inc., Woonsocket, Rhode Island, USA) and automatic temperature compensation (Association of Official Analytical Chemists [AOAC], 2020).

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Color measurement

Color changes were monitored using a spectrophotometer (CM 508d, Konica Minolta Inc., Tokyo, Japan) with standard illuminant D_{65} and at an observation angle of 10°. The registered values were lightness (L*, ranging from 0-black to 100-white), redness (a* takes positive values for reddish colors and negative values for greenish colors), and yellowness (b* takes positive values for yellowish colors and negative values for bluish colors). The EME (50 ml, 5 mg/ml) were placed in a quartz cell (3.5 x 4.5 cm), and ten measurements were performed on the surface (Robertson *et al*., 1977).

Total soluble solids

The total soluble solids (TSS) of EME were monitored (AOAC, 2000). A drop of EME (5 mg/ml) was placed on the prism of the refractometer, and the cover slip was closed and positioned against the light, resulting in a percentage value.

Polysaccharide content

Total polysaccharide content (TPC) was performed by the phenol-sulfuric acid method (Albalasmeh *et al*., 2013). An aliquot of EME (0.25 ml, 5 mg/ml) was homogenized in a vortex mixer (Analog vortex mixer, Fisher Scientific TM, Nueva Jersey, USA) with 0.125 ml of aqueous phenol solution (5%, v/v) and 0.625 ml of concentrated H_2SO_4. Subsequently, the reaction mixture (200 µL) was transferred into each well of a flat microplate (96-well) and stored at room temperature (25 °C) for 20 min in darkness. Then, absorbance was measured at 490 nm in a spectrophotometer (Multiskan FC UV-Vis, Thermo Scientific, Tokyo, Japan). TPC was calculated from a standard curve of glucose (62.5 µg/ml–1000 µg/ml; $y = 0.6534x$; $r^2 = 0.9988$) and expressed as mg of glucose equivalent/g of dried extract (mg GE/g).

Total phenolic content

The total phenolic content (TPHC) of EME was measured according to the Folin-Ciocaltecau method (Ainsworth & Gillespie, 2007). An aliquot of the extract (10 µL, 5 mg/ml) was transferred into each well of a flat microplate (96-well) and homogenized with 80 µL of distilled water, 40 µL of Folin–Ciocalteu's reagent (0.25 N), and 60 µL of Na_2CO_3 solution (7%, w/v). The resultant mixture was homogenized with 80 µL of distilled water and incubated at 25 °C for 1 h in darkness. Then, the absorbance was measured at 750 nm. TPHC values were calculated from a standard curve of gallic acid (62.5 µg/ml–1000 µg/ml; $y = 0.4934x$; $r^2 = 0.9996$) and expressed as mg of gallic acid equivalent/g of dried extract (mg GAE/g).

Flavonoids content

Total flavonoids content (TFC) of EME was measured based on the formation of aluminum chloride complexes (Popova *et al*., 2004). An aliquot of EME (10 µL, 5 mg/ml) was mixed with 130 µL of methanol and 10 µL $AlCl_3$ 5% (w/v). The resultant solution was incubated at 25 °C for 30 min in darkness. Then, the absorbance was measured at 412 nm. TFC was calculated from a standard curve of quercetin (62.5 µg/ml–1000 µg/ml; $y = 2.4965x$; $r^2 = 0.9987$) and expressed as mg of quercetin equivalent/g of dried extract (mg QE/g).

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Free-radical scavenging activity

The free-radical scavenging activity (FRSA) of EME was determined by the 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) method (Molyneux, 2004). An aliquot of EME (100 µL, at 100 µg/ml) was mixed with an equal volume of $DPPH^{\bullet}$ solution (300 µmol) and incubated at 25 °C for 30 min in darkness. Ascorbic acid (25 µg/ml) was used as a positive control. The absorbance was measured at 520 nm. The FRSA was calculated by the following equation: FRSA (%) = [1-$Abs_{(S)}$/$Abs_{(0)}$] x 100, where $Abs_{(S)}$ is the absorbance of the antioxidant at t = 30 min, and $Abs_{(0)}$ is the absorbance of the control at t = 0 min.

Radical-cation scavenging activity

The radical-cation scavenging activity (RCSA) of EME was determined by the 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid radical cation ($ABTS^{\bullet+}$) method (Re *et al*., 1999). The radical cation was formed by mixing equal parts of ABTS solution (7 mM) and potassium persulfate (2.45 mM) and incubated at 25 °C for 16 h in darkness, and it was diluted with ethanol until an absorbance of 0.8 was obtained. An aliquot of EME was mixed with the formed radical cation (1:99 ratio). Ascorbic acid (25 µg/ml) was used as a positive control. Then, absorbance was measured at 730 nm. The RCSA was calculated by the following equation: RCSA (%) = [1-$Abs_{(S)}$/$Abs_{(0)}$] x 100, where $Abs_{(S)}$ is the absorbance of the antioxidant at t = 30 min, and $Abs_{(0)}$ is the absorbance of the control at t = 0 min.

Reducing power ability

The reducing power ability (RPA) of EME was determined by the Prussian blue method (Berker *et al.*, 2010). An aliquot of EME (200 µL, at 100 µg/ml) was homogenized with 500 µL of phosphate buffer (50 mM, pH 7.0) and 500 µL of potassium ferricyanide (1%, w/v). The resultant solution was incubated at 50 °C for 20 min in darkness. Then, the samples were mixed with 500 µL of trichloroacetic acid (TCA, 10%, w/v) and centrifuged at 3500-x g for 10 min (Sorvall ST18R, Thermo Fisher Scientific, Massachusetts, USA). The supernatant (500 µL) was mixed with 500 µL of Milli-Q distilled water and 100 µL $FeCl_3$ (0.1%, w/v). The obtained mixture (200 µL) was transferred into each well of a flat microplate (96-well). The absorbance was measured at 700 nm, and results were expressed as absorbance increase at the same wavelength.

Antibacterial test

The antibacterial activity of each extract was evaluated according to the broth microdilution method (Jorgensen & Turdnige, 2015), with slight modifications. Four standard bacteria strains obtained from the American Type Culture Collection were used for this phase of the analysis, including two Gram-positive (*Staphylococcus aureus* ATCC 29213B and *Listeria innocua*) and two Gram-negative (*Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14028) strains. The strains were reactivated in liquid nutrient broth (BHI, brain hearth infusion) at 37 °C for 24 h-48 h. Afterwards, the strains' suspension was diluted with saline solution until reaching the turbidity of 0.5 McFarland standard, barium sulfate - $BaSO_4$ (ca. 1.5 x 10^8 CFU/ml). The accuracy of the method was verified by using a spectrophotometer with a 1 cm light path, i.e., for the 0.5 McFarland standard, the absorbance at 620 nm should go from 0.08 to 0.13. Then, the diluted strains' (100 µL) were transferred into each well of a flat microplate (96-well) and homogenized with an aliquot of EME (100 µL, 100 µg/ml). Gentamicin (25 µg/ml) was used as a positive control for bacterial growth inhibition, and BHI was used as bacterial growth blank or negative control. The plates were incubated at 27 °C for 24 h, and the absorbance was read at 620 nm (OD, optical density). The results were expressed as inhibition (%) = (OD_{620} untreated bacteria–OD_{620} nm treated bacteria) / (OD_{620} nm untreated bacteria) × 100.

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Statistical analysis

All variables were conducted in triplicated in at least three independent experiments. The results were expressed as mean ± standard deviation (SD). A two-way analysis of variance (Anova) was conducted, where temperature and time were the fixed effects in the model. A Tukey-Kramer multiple comparison test was performed for mean separation ($p < 0.05$). In addition, a principal component analysis was performed to evaluate the relationships among the analyzed variables (Minitab, version 17).

Results

Thermal x time effect on physicochemical properties

The data of the thermal x time effect on pH, TSS, and color values of edible mushroom extracts (EME) are summarized in Table 1. The results showed that pH and TSS values of control samples were above 5.0% and 16.5%, respectively. At a higher temperature and time (90 °C during 6 h), pH values decreased ($p < 0.05$) by 12.7%, 13.7%, and 14.5% for *A. brasiliensis*, *G. lucidum*, and *P. ostreatus* extracts, in comparison to control samples; while TSS values decreased by 24.7%, 49.4%, and 57.6%, respectively.

Tables and Figures

Table 1. Thermal x time effects on physicochemical properties of EME.

Mushrooms	T / t	pH	TSS	L*	a*	b*
A. brasiliensis	Control	5.5 ± 0.1^{b}	17.0 ± 0.1^{b}	30.2 ± 0.3^{b}	-0.3 ± 0.1^{a}	3.6 ± 0.2^{a}
	60 °C/ 2 h	5.4 ± 0.1^{b}	17.0 ± 0.1^{b}	30.3 ± 0.3^{b}	-0.4 ± 0.2^{a}	3.7 ± 0.1^{a}
	60 °C/ 4 h	5.5 ± 0.1^{b}	17.2 ± 0.1^{b}	30.1 ± 0.3^{b}	-0.4 ± 0.3^{a}	3.6 ± 0.5^{a}
	60 °C/ 6 h	5.5 ± 0.1^{b}	16.9 ± 0.1^{b}	30.3 ± 0.2^{b}	-0.4 ± 0.1^{a}	3.8 ± 0.2^{a}
	90 °C/ 2 h	5.3 ± 0.2^{b}	17.1 ± 0.1^{b}	28.7 ± 0.4^{a}	-0.4 ± 0.2^{a}	3.9 ± 0.3^{a}
	90 °C/ 4 h	5.0 ± 0.1^{a}	16.7 ± 0.1^{b}	28.3 ± 0.7^{a}	0.3 ± 0.0^{b}	4.8 ± 0.3^{b}
	90 °C/ 6 h	4.8 ± 0.2^{a}	12.8 ± 0.1^{a}	28.2 ± 0.1^{a}	0.7 ± 0.1^{c}	4.9 ± 0.1^{b}
G. lucidum	Control	5.1 ± 0.1^{c}	17.0 ± 0.1^{c}	56.2 ± 0.1^{c}	-0.4 ± 0.2^{a}	1.1 ± 0.1^{a}
	60 °C/ 2 h	5.1 ± 0.1^{c}	17.0 ± 0.1^{c}	29.7 ± 0.2^{b}	-0.2 ± 0.1^{a}	3.5 ± 0.2^{b}
	60 °C/ 4 h	5.0 ± 0.1^{c}	17.0 ± 0.1^{c}	29.7 ± 0.2^{b}	-0.2 ± 0.0^{a}	3.4 ± 0.1^{b}
	60 °C/ 6 h	5.0 ± 0.1^{c}	17.0 ± 0.1^{c}	29.7 ± 0.2^{b}	-0.3 ± 0.1^{a}	3.3 ± 0.1^{b}
	90 °C/ 2 h	4.7 ± 0.1^{b}	12.8 ± 0.1^{b}	28.6 ± 0.1^{a}	0.1 ± 0.1^{b}	4.0 ± 0.1^{c}
	90 °C/ 4 h	4.5 ± 0.1^{a}	8.7 ± 0.1^{a}	28.2 ± 0.2^{a}	0.4 ± 0.1^{c}	4.4 ± 0.2^{c}
	90 °C/ 6 h	4.4 ± 0.1^{a}	8.6 ± 0.2^{a}	28.3 ± 0.2^{a}	0.5 ± 0.1^{d}	4.4 ± 0.1^{c}

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P. ostreatus	Control	5.5 ± 0.1^{b}	17.0 ± 0.1^{d}	27.6 ± 0.3^{c}	1.7 ± 0.2^{a}	6.0 ± 0.3^{d}
	60 °C/ 2 h	5.4 ± 0.1^{b}	17.2 ± 0.1^{d}	26.4 ± 0.1^{c}	2.0 ± 0.2^{a}	6.3 ± 0.3^{d}
	60 °C/ 4 h	5.5 ± 0.1^{b}	17.3 ± 0.1^{d}	27.3 ± 0.1^{c}	2.0 ± 0.2^{a}	5.6 ± 0.2^{c}
	60 °C/ 6 h	5.4 ± 0.1^{b}	17.1 ± 0.1^{d}	27.5 ± 0.2^{c}	1.8 ± 0.1^{a}	5.2 ± 0.2^{c}
	90 °C/ 2 h	5.2 ± 0.2^{b}	13.3 ± 0.1^{c}	26.0 ± 0.1^{b}	2.4 ± 0.2^{b}	3.9 ± 0.2^{b}
	90 °C/ 4 h	4.9 ± 0.1^{a}	9.4 ± 0.1^{b}	25.2 ± 0.1^{a}	2.4 ± 0.1^{b}	2.5 ± 0.1^{a}
	90 °C/ 6 h	4.7 ± 0.2^{a}	7.2 ± 0.1^{a}	25.1 ± 0.2^{a}	2.3 ± 0.1^{b}	2.3 ± 0.2^{a}

Results expressed as mean ± SD (n = 9). T = temperature; t = heating time; TSS = total soluble solids; L* = lightness; a* = redness; b* = yellowness. Different superscripts (a-e) indicate significant differences among thermal x time effect (p < 0.05).
Source: Authors' own elaboration.

Furthermore, with regards to control samples, *G. lucidum* extract presented the highest (p < 0.05) lightness (*L), and *P. ostreatus* extract control showed the highest (p < 0.05) redness (a*) and yellowness (b*). At a higher temperature and time (90 °C during 6 h), L* values decreased by 6.6%, 49.6%, and 9.1% for *A. brasiliensis*, *G. lucidum*, and *P. ostreatus* extracts, respectively, in comparison to control samples. In contrast, a* values increased by >50% for *A. brasiliensis* and *G. lucidum* extracts, and 26.1% for *P. ostreatus* extract. Also, b* values increased 26.5% and 75% for *A. brasiliensis* and *G. lucidum* extracts, except for b* values of *P. ostreatus*, which was reduced 61.7% (p < 0.05).

Thermal x time effect on chemical composition

The data of the thermal x time effect on the chemical composition of EME are summarized in Table 2. Regarding the control samples, the results showed that the highest TPC, TPHC, and TFC values were found in *P. ostreatus* extract (p < 0.05). At a higher temperature and time (90 °C during 6 h), TPC values increased (p < 0.05) 47.9%, 61.6%, and 45.2% for *A. brasiliensis*, *G. lucidum*, and *P. ostreatus* extracts, in comparison to control samples. Also, TPHC values increased (p < 0.05) by 47.9%, 46.3%, and 45.2%; and TFC values increased by 57.6%, 49.4%, and 57.4%.

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Table 2. Thermal x time effects on chemical composition and antioxidant activity of EME.

Mushrooms	T / t	TPC	TPHC	TFC	FRSA	RCSA	RPA
A. brasiliensis	Control	86.2 ± 5.3^{a}	16.8 ± 0.5^{a}	15.3 ± 0.4^{a}	48.7 ± 1.0^{a}	15.6 ± 1.6^{a}	0.38 ± 0.02^{a}
	60 °C/ 2 h	104.2 ± 1.6^{b}	17.1 ± 1.0^{a}	15.8 ± 0.4^{a}	57.4 ± 0.8^{b}	14.8 ± 1.0^{a}	0.39 ± 0.01^{a}
	60 °C/ 4 h	104.2 ± 6.8^{b}	19.3 ± 0.7^{b}	15.4 ± 0.2^{a}	58.2 ± 0.9^{b}	14.8 ± 1.2^{a}	0.39 ± 0.01^{a}
	60 °C/ 6 h	109.2 ± 2.3^{b}	18.1 ± 0.6^{b}	16.0 ± 0.2^{b}	59.2 ± 2.3^{b}	15.8 ± 1.7^{a}	0.38 ± 0.01^{a}
	90 °C/ 2 h	120.9 ± 5.9^{c}	28.6 ± 1.1^{c}	17.3 ± 0.7^{c}	57.4 ± 0.1^{b}	25.7 ± 2.6^{b}	0.51 ± 0.01^{b}
	90 °C/ 4 h	156.0 ± 3.0^{d}	39.5 ± 1.8^{d}	19.8 ± 0.6^{d}	57.4 ± 0.6^{b}	31.4 ± 2.1^{c}	0.62 ± 0.02^{c}
	90 °C/ 6 h	165.3 ± 4.7^{e}	39.6 ± 0.6^{d}	20.4 ± 0.1^{d}	66.9 ± 2.2^{c}	36.2 ± 0.8^{d}	0.62 ± 0.03^{c}
G. lucidum	Control	85.0 ± 5.0^{a}	16.5 ± 1.1^{a}	27.2 ± 2.3^{a}	48.1 ± 1.2^{a}	8.7 ± 2.0^{a}	0.23 ± 0.02^{a}
	60 °C/ 2 h	82.8 ± 2.3^{a}	15.8 ± 0.7^{a}	30.5 ± 2.5^{a}	57.2 ± 0.1^{b}	9.4 ± 0.5^{a}	0.23 ± 0.01^{a}
	60 °C/ 4 h	78.2 ± 4.4^{a}	14.7 ± 0.7^{a}	29.1 ± 2.2^{a}	57.4 ± 1.3^{b}	9.2 ± 2.5^{a}	0.25 ± 0.01^{a}
	60 °C/ 6 h	186.1 ± 7.3^{d}	14.2 ± 0.2^{a}	29.9 ± 1.2^{a}	55.3 ± 1.6^{b}	10.6 ± 3.0^{a}	0.25 ± 0.01^{a}
	90 °C/ 2 h	103.6 ± 10.5^{b}	22.9 ± 1.2^{b}	29.8 ± 1.4^{a}	56.9 ± 0.1^{b}	13.9 ± 2.1^{a}	0.30 ± 0.01^{b}
	90 °C/ 4 h	157.9 ± 9.1^{c}	28.5 ± 1.8^{c}	28.5 ± 2.8^{a}	57.1 ± 0.3^{b}	18.1 ± 0.7^{b}	0.34 ± 0.02^{c}
	90 °C/ 6 h	221.5 ± 3.6^{e}	30.7 ± 1.1^{c}	36.3 ± 1.0^{b}	59.0 ± 0.1^{c}	21.3 ± 1.1^{c}	0.35 ± 0.02^{c}
P. ostreatus	Control	125.2 ± 3.1^{a}	34.0 ± 0.2^{a}	24.9 ± 1.1^{a}	52.4 ± 1.1^{a}	31.0 ± 1.4^{a}	0.47 ± 0.01^{a}
	60 °C/ 2 h	122.3 ± 2.4^{a}	35.8 ± 0.3^{b}	24.7 ± 0.1^{a}	58.0 ± 0.6^{b}	32.3 ± 1.4^{a}	0.47 ± 0.01^{a}
	60 °C/ 4 h	121.0 ± 4.5^{a}	36.9 ± 0.8^{c}	25.5 ± 1.3^{a}	58.5 ± 0.2^{b}	31.4 ± 0.8^{a}	0.48 ± 0.01^{a}
	60 °C/ 6 h	207.5 ± 9.9^{d}	37.4 ± 0.8^{c}	25.1 ± 0.7^{a}	67.5 ± 0.8^{c}	36.6 ± 0.8^{b}	0.48 ± 0.02^{a}
	90 °C/ 2 h	138.6 ± 0.8^{b}	54.8 ± 0.2^{d}	29.5 ± 1.1^{b}	60.2 ± 1.0^{b}	51.9 ± 2.3^{c}	0.66 ± 0.01^{b}
	90 °C/ 4 h	195.1 ± 6.5^{c}	73.7 ± 2.9^{e}	34.6 ± 1.3^{c}	60.1 ± 0.9^{b}	66.4 ± 2.9^{d}	0.83 ± 0.01^{c}
	90 °C/ 6 h	228.3 ± 5.0^{e}	79.8 ± 0.8^{f}	39.5 ± 0.6^{d}	72.1 ± 1.6^{d}	72.3 ± 2.3^{e}	0.88 ± 0.03^{d}

Results expressed as mean ± SD (n = 9). T = temperature; t = heating time; TPC = total polysaccharide content (mg GE/g); TPHC = total phenolic content (mg GAE/g); TFC = total flavonoids content (mg QE/g); FRSA = free-radical scavenging activity (%); RCSA = radical-cation scavenging activity (%); RPA = reducing power ability (abs). Different superscripts (a-f) indicate significant differences among thermal x time effect (p < 0.05).
Source: Authors' own elaboration.

Thermal x time effect on antioxidant activity

The data of the thermal x time effect on antioxidant activity of EME are also summarized in Table 2. In relation to the control samples, the results showed that the highest FRSA, RCSA, and RPA values were found in *P. ostreatus* extract (p < 0.05). At a higher temperature and time (90 °C during 6 h), FRSA values increased (p < 0.05) by 27.2%, 18.5%, and 27.3% for *A. brasiliensis*, *G. lucidum*, and *P. ostreatus* extracts, in comparison to control samples. Also, RCSA values increased (p < 0.05) by 58.9%, 51.9%, and 57.1%; and RPA values increased 38.7%, 34.3%, and 46.6%.

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Thermal x time effect on antibacterial activity

The data of the thermal x time effect on antibacterial activity of EME are summarized in Table 3. With regards to the control samples, the results showed that the highest bacterial inhibition values were found in *G. lucidum* extract ($p < 0.05$). At a higher temperature and time (90 °C during 6 h), *S. aureus* inhibition decreased ($p < 0.05$) 3.8%, 6.4%, and 4.8% for *A. brasiliensis*, *G. lucidum*, and *P. ostreatus* extracts, in comparison to control samples. Also, *L. innocua* inhibition decreased ($p < 0.05$) by 3.6%, 4.9%, and 6.1%; and *E. coli* inhibition decreased by 15.9%, 26.1%, and 14.0%. Similarly, *S. typhi* inhibition decreased by 6.3%, 6.4%, and 8.7%.

Table 3. Thermal x time effects on antibacterial activity of EME.

Musrooms	T / t	Gram positive bacteria		Gram negative bacteria	
		S. aureus	*L. innocua*	*E. coli*	*S. typhi*
A. brasiliensis	Control	73.9 ± 1.1^{b}	80.5 ± 0.8^{b}	21.4 ± 0.8^{c}	28.7 ± 0.1^{b}
	60 °C/ 2 h	73.7 ± 0.5^{b}	80.4 ± 0.6^{b}	21.8 ± 0.1^{c}	28.5 ± 0.1^{b}
	60 °C/ 4 h	73.1 ± 0.7^{b}	70.9 ± 0.9^{b}	21.2 ± 0.8^{c}	28.4 ± 0.4^{b}
	60 °C/ 6 h	73.0 ± 0.9^{b}	80.1 ± 0.4^{b}	21.1 ± 0.6^{c}	28.4 ± 0.7^{b}
	90 °C/ 2 h	72.7 ± 0.6^{b}	79.2 ± 1.5^{b}	21.5 ± 0.4^{c}	28.2 ± 0.4^{b}
	90 °C/ 4 h	72.9 ± 0.2^{b}	80.2 ± 0.6^{b}	19.6 ± 0.2^{b}	28.2 ± 0.2^{b}
	90 °C/ 6 h	71.1 ± 0.9^{a}	77.6 ± 0.5^{a}	18.0 ± 0.2^{a}	26.9 ± 0.8^{a}
G. lucidum	Control	77.6 ± 2.2^{b}	82.9 ± 1.0^{b}	26.8 ± 1.1^{c}	29.5 ± 0.2^{c}
	60 °C/ 2 h	75.3 ± 0.9^{b}	81.5 ± 1.4^{b}	22.3 ± 0.2^{b}	29.8 ± 0.2^{c}
	60 °C/ 4 h	75.6 ± 0.5^{b}	80.8 ± 1.4^{b}	22.7 ± 1.0^{b}	29.2 ± 0.2^{c}
	60 °C/ 6 h	74.0 ± 1.1^{b}	80.1 ± 0.1^{b}	21.2 ± 0.1^{b}	29.0 ± 0.2^{c}
	90 °C/ 2 h	74.4 ± 0.7^{b}	81.0 ± 0.6^{b}	21.3 ± 0.7^{b}	29.0 ± 0.1^{c}
	90 °C/ 4 h	72.5 ± 0.7^{a}	81.1 ± 0.5^{b}	19.6 ± 0.2^{a}	28.7 ± 0.2^{b}
	90 °C/ 6 h	72.6 ± 1.2^{a}	78.8 ± 0.6^{a}	19.8 ± 0.7^{a}	27.6 ± 0.4^{a}
P. ostreatus	Control	72.3 ± 0.9^{c}	79.0 ± 1.0^{b}	21.4 ± 0.4^{c}	28.9 ± 0.2^{c}
	60 °C/ 2 h	72.9 ± 0.8^{c}	79.1 ± 0.4^{b}	21.7 ± 0.1^{c}	28.2 ± 0.1^{c}
	60 °C/ 4 h	71.5 ± 0.4^{b}	79.1 ± 0.4^{b}	21.8 ± 0.2^{c}	28.9 ± 0.2^{c}
	60 °C/ 6 h	71.5 ± 0.5^{b}	77.9 ± 1.0^{b}	21.0 ± 0.1^{c}	28.0 ± 0.2^{c}
	90 °C/ 2 h	71.1 ± 0.4^{b}	77.8 ± 1.5^{b}	21.6 ± 0.1^{c}	27.8 ± 0.2^{b}
	90 °C/ 4 h	71.9 ± 0.4^{b}	77.6 ± 1.6^{b}	20.0 ± 0.3^{b}	27.2 ± 0.2^{b}
	90 °C/ 6 h	68.8 ± 0.7^{a}	74.2 ± 1.7^{a}	18.4 ± 1.0^{a}	26.4 ± 0.3^{a}

Results expressed as mean ± SD ($n = 9$). T = temperature; t = heating time. Different superscripts (a-c) indicate significant differences among thermal x time effect ($p < 0.05$).
Source: Authors' own elaboration.

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Physicochemical, antioxidant, and antibacterial properties of heat-treated edible mushroom extracts | 1-12

Multivariate analysis

A principal component analysis was carried out (Figure 1) to evaluate the differences between treatments and evaluated parameters. The first and second components showed a variance of 62.9% and 17.6%, respectively; thus, an accumulative 80.5% of the total variation was explained by the two components. In addition, the results showed a separation of analyzed treatment with respect to physicochemical, chemical components, and biological activity ($p < 0.05$). For example, EME extracts subjected to thermal x time treatment (90 °C during 6 h) were associated with an increase in some physicochemical parameters like a* value, chemical composition (TPC, TPHC, and TFC), and antioxidant activity (FRSA, RCSA, and RPA).

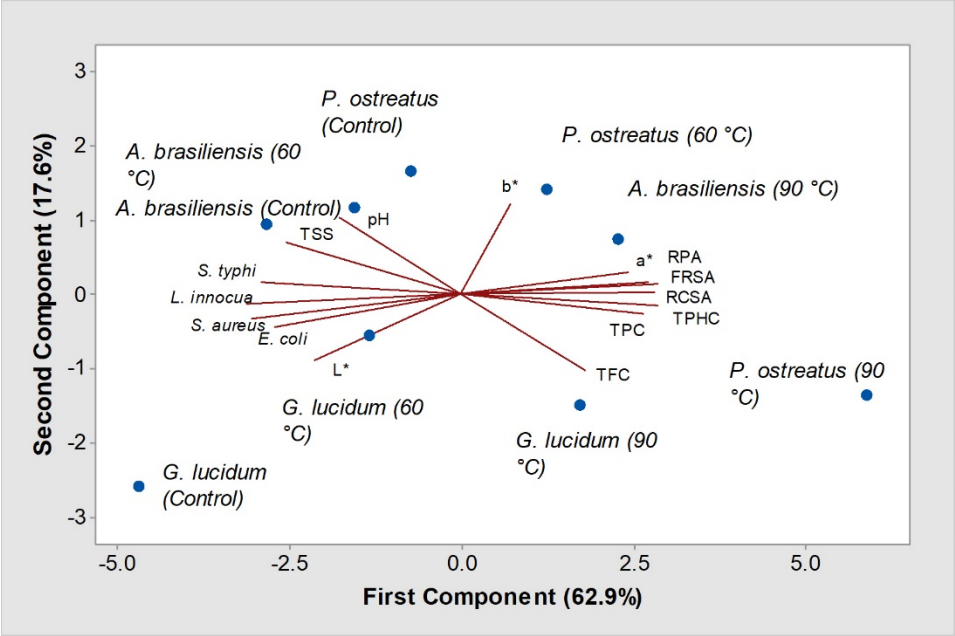

Figure 1. Principal components analysis of the thermal x time effects on the evaluated parameters.
Source: Authors' own elaboration.

Discussion

It has been demonstrated that temperature and time may affect physicochemical properties like pH, TSS, and color of foods matrices and natural extracts (Fernández-López *et al.*, 2013). A reduction in pH values of heat-treated natural extracts may be related to the degradation of labile acid compounds (Olvera-García *et al.*, 2015). In addition, it has been reported that temperature can increase TSS by release of solids bound to plant tissues, in time dependence (Park *et al.*, 2019). In contrast, an increase of temperature and time exposure can be related with a TSS decrease (Nguyen & Chuyen, 2020). In agreement, it has been reported a reduction on pH value and TSS of aqueous extracts from heat-treated pomegranate marc peel, in time dependence (Qu *et al.*, 2013).

Regarding color parameters, L* value is commonly associated with darkening of foods due to enzymatic and non-enzymatic browning, and when heat-treatment is employed during processing, browning is related to Maillard reaction, a non-enzymatic reaction that involves carbonyl and amino compounds that leads to brown pigments formation (Kurozawa *et al.*, 2012). Additionally, phenolic components of mushroom in presence of oxygen and polyphenol oxidase are oxidized to the

corresponding o-quinones, which subsequently polymerize non-enzymatically to brown pigments (Golan-Goldhirsh & Whitaker, 1984; Weemaes *et al.*, 2006). Thus, a greater browning formation results in a decrease of L* values (Kurozawa *et al.*, 2012). In accordance with the above, a reduction of L* values and an increase of a* values were reported of heat-treated radish ethanol extracts (Lee *et al.*, 2009).

Moreover, it has been demonstrated that edible mushroom extracts contain bioactive compounds, including polysaccharides and phenolic compounds, which are characterized by possessing antioxidant and antibacterial properties (Pérez-Montes *et al.*, 2021). However, previous studies have also demonstrated that temperature and time may affect chemical composition and biological properties of the natural extracts (Lee *et al.*, 2009; Park *et al.*, 2019). In accordance with the above, a previous work reported that total phenolic composition and antioxidant activity of *Lentinus edodes* extract (80% ethanol) was increased two-fold by heat x time-treated effect (Choi *et al.*, 2006). An increase in total phenolic components during the heat-treatment may be related to the decrease/inhibition of enzymatic oxidation involving antioxidant compounds of mushrooms (Devece *et al.*, 1999).

It is recognized that many antioxidant compounds present in mushroom extracts may also possess different effects on foodborne pathogens, mainly against Gram-positive (Ren *et al.*, 2014). Gram-positive and Gram-negative bacteria have a peptidoglycan cover located outside the cytoplasmic membrane, Gram-negative bacteria have a thinner cover, and the composition of their outer membranes is also asymmetric. The inner sheet of this membrane consists of phospholipids, while the external part contains a glycosylated lipid, referred to as an endotoxin or lipopolysaccharide (Calvo & Martínez-Martínez, 2009). In this context, phenolic compounds can get inserted in these membranes, in addition to several other natural compounds, which are characterized by having a hydrophilic region and thus exert an antibacterial activity (Xu & Lee, 2001). In a previous work, it has been reported that bacterial inhibition of natural extract against *S. aureus*, *E. coli*, and *S. typhi* was decreased by temperature (28 °C > 4 °C > -20 °C) x time effect (Durairaj *et al.*, 2009).

Conclusions

The present study showed that thermal x time treatment has a significant effect on physicochemical properties (pH, TSS and color) of edible mushrooms extracts. In addition, thermal x time treatment (90 °C during 6 h) significantly increase chemical compounds (TPC, TPHC, and TFC) and antioxidant activity of mushroom extracts (*P. ostreatus* > *A. brasiliensis* > *G. lucidum*). In contrast, thermal x time treatment reduced antibacterial activity against all pathogens tested. In this regard, heat-treatment x time could improve or reduce the physical-chemical and functional properties of ingredients of natural origin with potential use for the pharmaceutical or food industry.

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Conflicts of interest

The authors declare that there is not conflict of interest.

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