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Antioxidant and Antibacterial Effect of *Agaricus brasiliensis* Extract on Raw and Cooked Pork Patties during Storage

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Abstract: Edible mushrooms have been proposed as a natural ingredient to prevent loss of quality in meat products. This study aimed to compare the antioxidant and antibacterial effects of *Agaricus brasiliensis* aqueous-ethanol extract (ABE, at 0, 0.5, and 1.0%) versus butylated hydroxytoluene (BHT, 0.02% on a fat basis) added to raw and cooked pork patties to prolong shelf-life under chilled storage. All samples were stored at 2 °C for 9 days and subjected to physicochemical (pH, water-holding capacity, and color), chemical (lipid oxidation and antioxidant status), and microbiological evaluation (mesophilic and psychrophilic). Phenolic compounds (TPC) in ABE exert a reducing power ability (Fe^{3+} reduction), free-radical (DPPH), and radical-cation scavenging activity (ABTS), as well as antibacterial activity against Gram-positive bacteria than Gram-negative. Furthermore, incorporating ABE in raw and cooked pork patties reduced ($p < 0.05$) pH and color changes, lipid oxidation, and microbial growth during storage in concentration dependence. No differences ($p > 0.05$) were observed in the WHC and b^* values. In addition, the presence of TPC and the antioxidant status (Fe^{3+} reduction, DPPH, and ABTS activity) of pork patties increased ($p < 0.05$) by the ABE incorporation. ABE can be a natural additive to improve the storage stability of pork patties.

Keywords: mushroom extract; biological properties; meat quality

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1. Introduction

Ground pork meat (a protein and fat source), pork minced back fat, salt, water, spices, and other ingredients are commonly used for the formulation of pork patties [1,2]. They all contribute to technological and sensorial attributes such as firmness, color, smell, flavor, and appearance [1,3,4]. However, it has been demonstrated that salt and water promote pork meat amino and fatty acids (mainly polyunsaturated) oxidation, resulting in meat product quality loss and reduced consumer acceptability [4,5]. Moreover, added water contributes to the growth of spoilage bacteria, which is also associated with losing meat quality and safety [6]. Additionally, the cooking process of meat modifies the quality traits, i.e., physicochemical, technological, and microbiological [3,6].

In this context, synthetic antioxidants (BHT, butylated hydroxytoluene; BHA, butylated hydroxyanisol; TBHQ, tert-butyl hydroquinone; PG, propyl gallate) and antibacterial additives (nitrites and nitrates, among others) are widely used by meat processors to preserve meat quality and safety by preventing oxidation and microbial growth [7]. However, its uncontrolled use in foods has been related to adverse effects on human health [8]. For the

reasons above, using natural antioxidant and antibacterial compounds in processed meat products has been considered a promising strategy to prevent undesirable changes during storage and improve consumer acceptability and safety [6]. Nonetheless, their bioactive effectiveness mainly depends on the chemical composition of the botanical material and its extracts [6,9].

Edible mushrooms have been used for pharmaceutical and food applications. Several studies claim they are an important source of nutrients (protein and amino acids, fat and fatty acids, dietary fiber, and carbohydrates), vitamins (thiamine, riboflavin, niacin, tocopherol, and vitamin D); minerals (Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn), and bioactive components such as polysaccharides and phytochemicals (phenolic acids and flavonoids) [10,11]. *Agaricus brasiliensis* is a mushroom widely produced and consumed in Brazil because of its multiple functional properties, including antidiabetic, anticancer, antihypertensive, antioxidant, and antibacterial, among others [12]. These properties are attributed to polysaccharides and phytochemicals [12,13]. In this context, *A. brasiliensis* has been proposed as a natural feed ingredient to improve broiler chickens' performance and meat quality [14] and as a food ingredient to increase the taste and acceptability of bakery products [15]. However, the use of *A. brasiliensis* as an antioxidant and antibacterial additive for improving meat products' shelf-lives is still limited.

Therefore, this study aims to evaluate the antioxidant and antibacterial activity of *A. brasiliensis* aqueous-ethanol extract (ABE) on the oxidative stability and mitigation of microbial-driven deterioration of raw and cooked pork patties during storage.

2. Materials and Methods

2.1. Chemicasl and Reagents

All reagents used were of analytical grade. Sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), copper sulphate ($Cu(SO_4)_2$), sulfuric acid (H_2SO_4), iron chloride ($FeCl_3$), hydrochloric acid (HCl), acetic anhydride, glacial acetic acid, magnesium ribbon, Folin–Ciocalteu's reagent, ninhydrin reagent, Dragendorff's reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ethanol, gentamicin and butylated hydroxytoluene (BHT) were purchased from Sigma Chemicals (St. Louis, MO, USA). Liquid nutrient broth (BHI) was obtained from Merck (Darmstadt, Germany). Gallic acid, 2-thiobarbituric acid (2-TBA), trichloroacetic acid (TCA), 1,3,3-tetramethoxypropane (TMP) were acquired from J.T. Baker (Baker®, Phillipsburg, NJ, USA).

2.2. Extract Preparation

Bioactive compounds from *A. brasiliensis* powder (donated by ATISA®) were extracted with a solvent mixture (water-ethanol, 1:1) by the ultrasound-assisted method (42 KHz, at 25 °C for 1 h). The resultant mixture was filtered (with Whatman 1 filter paper) under a vacuum (vacuum pump MVP 6, Soosung Vacuum Co., Ltd., Jeju, Korea), concentrated under reduced pressure at 60 °C (rotary evaporator RE301BW, Yamato Scientific Co., Ltd., Tokyo, Japan), and lyophilized (freeze dryer DC401, Yamato Scientific Co., Ltd., Tokyo, Japan). The dried aqueous-ethanol extract (ABE) was stored at −20 °C under dark conditions until further analysis [13].

2.3. Metabolites Screening

The metabolites screening of ABE (5 mL, 5 mg/mL) proceeded as follows [16,17]:

Proteins: ABE was homogenized with 100 µL of NaOH (0.1 N) and 100 µL of $Cu(SO_4)_2$ (1%, *w*/*v*). The violet color formation indicated the presence of proteins.

Amino acids: ABE was homogenized with 100 µL of ninhydrin reagent and 100 µL of H_2SO_4. A purple coloration formation indicated the presence of amino acids.

Carbohydrates: ABE was mixed with 1 mL of glacial acetic acid, 100 µL of $FeCl_3$ (1%, *w*/*v*), and 100 µL of H_2SO_4. Brick red precipitate indicated the presence of carbohydrates.

Alkaloids: ABE was homogenized with 2 mL of HCl (2 N) (Analog vortex mixer, Fisher Scientific^{TM}, CA, USA), incubated at 100 °C for 10 min (water bath Aquabath, Thermo Fisher Scientific, OH, USA), cooled at room temperature (25 °C), and filtered (with Whatman 1 filter paper). In total, 100 µL of the filtered solution were mixed with 50 µL of Dragendorff´s reagent. The formation of a reddish-brown precipitate indicated the presence of alkaloids.

Saponins: ABE was incubated at 100 °C for 10 min and frothing persistence indicated the presence of saponins.

Steroids: ABE was mixed with 10 mL of chloroform, 1 mL of acetic anhydride, and 100 µL of H_2SO_4. A green ring formation indicated the presence of steroids.

Terpenoids: ABE was homogenized with 2 mL chloroform and 100 µL of H_2SO_4. The blue/green ring formation indicated the presence of terpenoids.

Tannins and phenols: ABE was homogenized with 100 µL of $FeCl_3$ (0.1%, *w*/*v*) and incubated at 100 °C for 10 min. Blue-black precipitates indicated the presence of these compounds.

Flavonoids: ABE was incubated (100 °C for 10 min) and cooled (25 °C). After that, 0.5 g of magnesium ribbon and 100 µL of HCl were carefully added. A red color formation indicated the presence of flavonoids.

2.4. Total Phenolic Content

The total phenolic content (TPC) of ABE was measured by Folin–Ciocalteu's method [18]. Briefly, 160 µL of distilled water, 40 µL of Folin–Ciocalteu's reagent (2 M), and 60 µL of Na_2CO_3 solution (7%, *w*/*v*) were transferred into each well of a flat 96-well microplate, mixed with 10 µL of ABE (5 mg/mL), and incubated at 25 °C for 1 h, under dark conditions. Therefore, the absorbance was measured at 750 nm in a spectrophotometer (Multiskan FC UV-Vis, Thermo Scientific, Tokyo, Japan). TPC values were calculated from a standard curve (62.5 to 1000 µg/mL; y = 0.4934x; r^2 = 0.9996), and results were expressed as mg of gallic acid equivalent/g of ABE (mg GAE/g).

2.5. Antioxidant Activity

2.5.1. Reducing Power Ability

The reducing power ability (Fe^{3+} reduction) was performed by the ferricyanide/Prussian blue method [19]. An aliquot of ABE (200 µL, 100 µg/mL) was mixed with 500 µL of potassium ferrocyanide (1%, *w*/*v*) and incubated at 50 °C for 20 min in the dark. The resultant mixture was homogenized with 500 µL of TCA (10%, *w*/*v*) and centrifuged at 2300× *g* at 4 °C for 10 min (Sorvall ST18R, Thermo Fisher Scientific, MA, USA). Subsequently, 100 µL of the supernatant was mixed with 100 µL of $FeCl_3$ (0.1%, *w*/*v*). Then, the absorbance was measured at 700 nm. The results were expressed as IC_{50} (i.e., the extract concentration required to reduce Fe^{3+} to Fe^{2+} by 50%).

2.5.2. Free-Radical Scavenging Activity

The free-radical scavenging activity was carried out according to the DPPH method [20]. An aliquot of ABE (100 µL, 100 µg/mL) was mixed with 100 µL of DPPH ethanol solution (300 µM). The reaction mixture was incubated in the dark at 25 °C for 30 min. The absorbance was measured at 517 nm, and the results were expressed as IC_{50} (i.e., the concentration required to inhibit the free radical by 50%).

2.5.3. Radical Cation Scavenging Activity

The radical cation scavenging activity was carried out by the ABTS method [21]. An aliquot of ABE (20 µL, 100 µg/mL) was mixed with 180 µL of ABTS solution (abs 0.7 at 730 nm). In the dark, the resultant solution was incubated at 25 °C for 8 min. The absorbance was measured at 730 nm, and the results were expressed as IC_{50} (i.e., the concentration required to inhibit the cation radical by 50%).

2.6. Antimicrobial Activity

The antimicrobial activity was performed by the liquid nutrient microdilution method against food-borne pathogens [22]. Gram-positive (*Staphylococcus aureus* ATCC 29213B and *Listeria monocytogenes* ATCC 33090) and Gram-negative (*Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14028) bacteria strains were initially reactivated in BHI broth and incubated at 37 °C for 24 h (IC403C, Yamato Scientific Co., Ltd., Tokyo, Japan). An aliquot of ABE (50 µL, 100 µg/mL) was mixed with 50 µL of the cellular suspension (1.5×10^8 CFU/mL, 0.5 McFarland standard) and incubated at 37 °C for 24 h. Gentamicin (25 µg/mL) was used as the standard, and BHI broth solution as the blank. After incubation, the optical density (O.D.) was measured at 630 nm, and the results were expressed as IC_{50} (concentration required to reduce microbial growth by 50%).

2.7. Patties Elaboration and Storage

Fresh pork meat (*Semimembranosus* muscle, at 48 h post mortem) was purchased from a local processor (Norson®, Hermosillo, Mexico), visible extra muscular fat was trimmed, and the meat was minced through a 4.5 mm-hole plate (meat grinder 4152, 4 Hobart Dayton, OH, USA). The minced pork meat was mixed with salt (1.5%, *w*/*w*) and pork back fat (10% in the final formulation, *w*/*w*). In each of the three replicates, the mass was divided into four different batches (Table 1): Control, without antioxidant; T1, ABE at 0.5% (*w*/*w*); T2, ABE at 1.0% (*w*/*w*); and T3, BHT at 0.02% (fat basis). Raw pork patties were shaped using a manual patty former, and for each batch, 48 raw patties (45 g each) per treatment were used for meat quality measurements. Moreover, 48 patties (45 g each) per treatment were cooked in a preheated grill at 180 °C (George Foreman™, Salton Inc., MI, USA) until reaching an internal temperature of 71 °C and subsequently used for meat quality measurements. Raw and cooked samples were placed on Styrofoam™ trays and wrapped with polyvinyl chloride film (17,400 cm^3 O_2/m^2 at 23 °C for 24 h). Raw and cooked samples were stored in the dark at 2 °C until their evaluation (0, 3, 6, and 9 days).

Table 1. Formulation of pork patties treated with ABE (%).

Item	Treatments			
	Control	**T1**	**T2**	**T3**
Pork meat	83.5	83.0	82.5	83.5
Pork back fat	10.0	10.0	10.0	10.0
Salt (mc®)	1.5	1.5	1.5	1.5
Water	5.0	5.0	5.0	5.0
ABE	0	0.5	1.0	0
Total	100	100	100	100
BHT	0	0	0	0.02

ABE—*A. brasiliensis* extract.

2.8. Meat Quality Measurements

2.8.1. pH Value

Pork patties were homogenized with distilled water (1:10, *w*/*v*) at 6000 rpm in an ice bath at 5 °C for 1 min (Ultraturrax T25, IKA, Braun, Germany). The pH was measured with a potentiometer with automatic temperature control (pH211, Hanna Instruments Inc., Woonsocket, RI, USA) following the 981.12 procedure of AOAC [23].

2.8.2. Water-Holding Capacity

The pork patties' water-holding capacity (WHC) was determined gravimetrically [24]. The samples (5 g each) were placed on fine mesh nylon, inserted into 50 mL tubes with a screwcap, and centrifuged at 4200× *g* at 4 °C for 5 min. The WHC percentage was calculated as [(initial weight − weight after centrifugation)/initial weight] × 100.

2.8.3. Color

The packaging materials of pork patties were removed to stabilize the color surface. The samples were exposed to atmospheric O_2 at 0 °C for 30 min in the dark. After that, 10 measurements on sample surfaces were performed using a spectrophotometer (CM 508d, Konica Minolta Inc., Tokyo, Japan) with a D65 illuminant and a 10° observer calibrated with a white calibration cap (CM-A70). Recorded parameters consisted of lightness (L^*), redness (a^*), and yellowness (b^*) [25].

2.8.4. Lipid Oxidation

Lipid oxidation was determined by the thiobarbituric acid reactive substances (TBARS) method [26]. Pork patties (10 g) were homogenized with 20 mL of TCA (10%, w/v) (4500 rpm at 5 °C for 1 min) and centrifuged (2500× g at 5 °C for 20 min). After that, 2 mL of the filtered supernatant was mixed with 2 mL of 2-thiobarbituric acid solution (20 mM) and incubated at 98 °C for 20 min. The absorbance was measured at 531 nm and the results were expressed as mg of malondialdehyde/kg of pork meat (mg MDA/kg). The TBARS values were calculated from a TMP standard curve (0.001 to 0.01 mmol; y = 95.635× + 0.0134; r^2 = 0.9992)

2.8.5. Phenolic Content and Antioxidant Activity of Meat Samples

Raw and cooked pork patties were homogenized with a solvent mixture of water-ethanol (1:10, w/v) at 4500 rpm for 1 min, and bioactive compounds were extracted by the ultrasound-assisted method (42 kHz, at 25 °C for 1 h). Therefore, the resultant mixture was centrifuged (2300× g at 4 °C for 10 min) and filtered out (Millipore filter 0.2–0.4 µm). As previously described, the filtered solution (meat homogenate) was used to assay the TPC, Fe^{3+} reduction, DPPH, and ABTS inhibition [27]. A higher absorbance of the reaction mixture indicated increased Fe^{3+} reduction, while a lower absorbance indicated higher $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ inhibition.

2.8.6. Microbial Growth

The bacterial growth of psychrophilic and mesophilic bacteria was determined with the pour plate method [28]. Raw and cooked pork patties were aseptically homogenized with peptone water (0.1%, w/v) for 1 min using a stomacher (Seward Stomacher® 400, Norfolk, UK); then, 1 mL of the appropriate dilutions was pour-plated using PCA as the standard. The inoculated plates were incubated at 37 °C for 2 days to assess total viable counts of mesophilic bacteria and incubated at 5 °C (EFR492, Frigidaire Co., Ltd., NC, USA) for 10 days for psychrophilic bacteria. All bacterial counts were expressed as the $\log_{10}$ of colony-forming units/g of meat ($\log_{10}$ CFU/g).

2.9. Statistical Analysis

Three independent experimental trials (with three replicates each) were conducted, and the results were described as mean ± standard deviation (SD). Normal distribution and variance homogeneity were tested (Shapiro–Wilk). Results of the qualitative analysis were expressed as absent or present. TPC, antioxidant, and antimicrobial activity values of ABE in contrast with BHT and gentamicin, respectively, were analyzed by a *t*-test. The data from meat quality measurements were subjected to a 2-way analysis of variance (ANOVA), using the treatments (Control, T1, T2, and T3) and storage time (0, 3, 6, and 9 days) as the fixed effects and their 2-way interaction. TPC and antioxidant activity values of meat samples were subjected to a 1-way ANOVA comparing the treatments on each sampling day (days 0 and 9). A Tukey–Kramer multiple comparison test was performed to determine the significance of mean values for multiple comparisons at $p < 0.05$. All data were analyzed using the National Center for Social Statistics statistical software NCSS version 2007.

3. Results

3.1. Metabolites and Bioactivity of ABE

The extraction yield of the obtained extract using water and ethanol as solvents was 18.30 ± 1.33. The results of qualitative metabolites screening showed the presence of proteins, amino acids, carbohydrates, phenols, and flavonoids in ABE. Whereas alkaloids, saponins, steroids, and terpenoids were absent. Table 2 presents the results of the phenolic content, as well as the antioxidant and antimicrobial activity of ABE. The phenolic compounds content was greater than 60 mg GAE/g. Respect antioxidant activity, the results showed that ABE exerts high ($p < 0.05$) DPPH and ABTS inhibition (IC_{50}, <200 μg/mL), as well as a weak activity to reduce the Fe^{3+} ion (IC_{50}, >500 μg/mL). Regarding antibacterial activity, the results indicated that ABE showed a higher ($p < 0.05$) inhibition of *S. aureus* and *L. innocua* (IC_{50}, approx. 120 μg/mL) than *E. coli* and *S. typhimurium* (IC_{50}, >500 μg/mL).

Table 2. Phenolic content, antioxidant, and antibacterial activity of ABE.

TPC	Result
mg GAE/g	62.01 ± 0.99
Reducing power activity	(IC_{50}, μg/mL)
Fe^{3+} reduction	800.07 ± 7.70
BHT	35.02 ± 1.01
p-value	
Radical scavenging activity	(IC_{50}, μg/mL)
DPPH inhibition	78.11 ± 2.10
BHT	36.74 ± 1.22
p-value	<0.001
ABTS inhibition	196.08 ± 5.70
BHT	20.60 ± 2.01
p-value	<0.001
Inhibition of microbial growth	(IC_{50}, μg/mL)
S. aureus	120.10 ± 2.51
Gentamicin	12.21 ± 0.20
p-value	<0.001
L. innocua	118.05 ± 1.30
Gentamicin	12.33 ± 0.32
p-value	<0.001
E. coli	550.35 ± 3.54
Gentamicin	12.55 ± 0.10
p-value	<0.001
S. typhimurium	1020.45 ± 17.70
Gentamicin	12.44 ± 0.30
p-value	<0.001

Values expressed as mean ± SD of at least three independent experiments. ABE—*A. brasiliensis* extract; TPC—total phenolic content; Fe^{3+} reduction—reducing power ability; DPPH inhibition—free-radical scavenging activity; ABTS inhibition—radical-cation scavenging activity; BHT—butylated hydroxytoluene; GAE—gallic acid equivalents.

3.2. Physicochemical Analysis

Table 3 presents the results of the physicochemical properties of raw and cooked pork patties. The results showed a significant effect of the treatment x storage time interaction ($p < 0.001$) on pH, L^*, and a^* parameters, without an effect ($p > 0.05$) on the WHC and b^* values. According to the results, at the initial day of storage (day 0), no significant differences ($p > 0.05$) were found in the pH values between treatments of raw and cooked pork patties. During storage time, the pH values decreased in raw and cooked samples ($p < 0.05$). At the end of storage (day 9), raw and cooked pork patties treated with T2 showed the highest ($p < 0.05$) pH values. Concerning color parameters, at day 0, no significant

differences ($p > 0.05$) were found in the L^* values between treatments of raw and cooked pork patties. The L^* values decreased in raw and cooked samples during storage time ($p < 0.05$). On day 9, T1 and T2 showed the lowest L^* values in raw samples, and T1-T3 presented the lowest values in cooked samples ($p < 0.05$). In addition, at day 0, raw patties that were treated with T2 showed the highest ($p < 0.05$) a* values, while no differences ($p > 0.05$) were found in cooked patties between treatments for this parameter. The a^* values decreased in raw and cooked samples during storage time ($p < 0.05$). On day 9, T1 and T2 showed the highest a^* values in raw and cooked samples ($p < 0.05$).

Table 3. Effect of the treatment and storage time on the physicochemical changes of raw and cooked pork patties.

Item	Patties	Treatment	Storage Time (Days)			
			0	3	6	9
pH	Raw	T0	5.95 ± 0.21^{aA}	5.67 ± 0.12^{aB}	5.15 ± 0.01^{cC}	5.17 ± 0.04^{dC}
		T1	5.82 ± 0.10^{aA}	5.70 ± 0.11^{aA}	5.68 ± 0.02^{aA}	5.33 ± 0.01^{cB}
		T2	5.86 ± 0.10^{aA}	5.68 ± 0.01^{aA}	5.48 ± 0.06^{bB}	5.52 ± 0.02^{aB}
		T3	5.90 ± 0.03^{aA}	5.70 ± 0.02^{aB}	5.49 ± 0.01^{bC}	5.36 ± 0.01^{bD}
	Cooked	T0	6.08 ± 0.07^{aA}	5.80 ± 0.01^{aB}	5.64 ± 0.01^{bC}	5.62 ± 0.03^{bC}
		T1	6.15 ± 0.08^{aA}	5.79 ± 0.01^{aB}	5.79 ± 0.06^{aB}	5.63 ± 0.04^{bC}
		T2	6.10 ± 0.05^{aA}	5.85 ± 0.10^{aB}	5.86 ± 0.16^{aB}	5.85 ± 0.12^{aB}
		T3	6.03 ± 0.07^{aA}	5.80 ± 0.01^{aB}	5.80 ± 0.01^{aB}	5.65 ± 0.01^{bC}
WHC (%)	Raw	T0	90.50 ± 3.25	93.09 ± 2.01	93.55 ± 2.59	93.11 ± 2.41
		T1	90.19 ± 2.91	92.86 ± 1.74	92.07 ± 1.80	93.56 ± 1.49
		T2	91.51 ± 2.15	92.22 ± 1.46	92.87 ± 2.44	91.92 ± 2.98
		T3	90.40 ± 1.77	90.94 ± 2.01	90.03 ± 1.67	90.41 ± 1.64
	Cooked	T0	90.33 ± 3.04	92.94 ± 3.03	92.42 ± 2.77	92.37 ± 2.53
		T1	90.74 ± 3.29	91.87 ± 2.07	93.26 ± 2.73	94.25 ± 2.71
		T2	90.26 ± 2.98	91.94 ± 2.21	92.06 ± 2.92	93.12 ± 2.52
		T3	90.09 ± 1.71	90.63 ± 1.77	91.01 ± 2.00	91.13 ± 1.81
L^*	Raw	T0	51.65 ± 1.73^{aB}	53.44 ± 1.86^{aB}	56.44 ± 0.41^{aA}	57.03 ± 1.00^{aA}
		T1	51.78 ± 2.17^{aA}	51.61 ± 1.74^{aA}	54.27 ± 1.69^{bA}	54.41 ± 1.50^{bcA}
		T2	51.91 ± 2.10^{aA}	50.83 ± 2.01^{aA}	49.40 ± 1.43^{bA}	52.11 ± 1.14^{cA}
		T3	51.32 ± 2.11^{aB}	50.98 ± 1.90^{aB}	54.69 ± 1.33^{bAB}	55.02 ± 0.70^{bA}
	Cooked	T0	60.28 ± 1.16^{aB}	60.99 ± 0.62^{aB}	64.07 ± 2.10^{aA}	64.52 ± 1.28^{aA}
		T1	60.63 ± 2.89^{aA}	61.39 ± 1.49^{aA}	61.01 ± 0.70^{bA}	61.90 ± 1.37^{bA}
		T2	59.60 ± 1.04^{aA}	59.98 ± 1.67^{aA}	61.14 ± 1.40^{bA}	60.42 ± 1.58^{bA}
		T3	59.56 ± 1.00^{aA}	59.94 ± 1.15^{aA}	60.07 ± 1.36^{bA}	62.92 ± 1.01^{bA}
a^*	Raw	T0	8.85 ± 1.34^{bA}	5.75 ± 1.29^{bB}	4.34 ± 1.17^{bC}	4.02 ± 0.57^{bC}
		T1	8.50 ± 1.35^{bA}	7.44 ± 0.89^{bA}	6.49 ± 0.92^{abA}	6.66 ± 0.79^{aA}
		T2	11.37 ± 1.59^{aA}	10.03 ± 1.44^{aA}	7.09 ± 1.06^{aB}	7.16 ± 0.64^{aB}
		T3	8.66 ± 1.00^{bA}	7.65 ± 0.69^{bA}	6.43 ± 0.45^{abB}	4.51 ± 0.38^{bC}
	Cooked	T0	5.84 ± 0.68^{aA}	5.44 ± 1.04^{aA}	2.82 ± 1.23^{bB}	2.35 ± 1.07^{bB}
		T1	5.69 ± 0.92^{aA}	5.30 ± 1.15^{aA}	4.61 ± 1.83^{abA}	4.50 ± 0.71^{aA}
		T2	6.13 ± 0.38^{aA}	6.33 ± 0.84^{aAB}	5.36 ± 1.08^{aAB}	5.00 ± 0.37^{aB}
		T3	5.66 ± 0.10^{aA}	5.35 ± 0.50^{aA}	4.52 ± 0.50^{abA}	3.08 ± 0.29^{bB}
b^*	Raw	T0	16.50 ± 1.75	14.17 ± 1.42	14.32 ± 1.01	14.00 ± 1.57
		T1	16.14 ± 1.12	16.33 ± 1.49	15.40 ± 1.09	14.26 ± 1.55
		T2	17.32 ± 1.11	17.50 ± 1.60	16.70 ± 0.89	15.05 ± 1.16
		T3	16.38 ± 0.85	16.57 ± 1.02	15.32 ± 0.47	14.20 ± 1.31
	Cooked	T0	21.69 ± 2.23	19.41 ± 2.29	17.55 ± 1.78	17.41 ± 1.80
		T1	21.35 ± 2.36	19.03 ± 0.49	19.21 ± 0.45	18.63 ± 0.84
		T2	19.79 ± 1.07	19.92 ± 1.74	19.71 ± 2.72	19.63 ± 1.81
		T3	20.84 ± 0.91	19.73 ± 1.41	18.57 ± 0.74	18.44 ± 0.51

Values expressed as mean ± SD of at least three independent experiments. WHC—water-holding capacity; L^*, lightness; a^*, redness; b^*, yellowness; ABE — *A. brasiliensis* extract. Control, without antioxidant; T1, ABE at 0.5%; T2, ABE at 1.0%; and T3, BHT at 0.02%. Lowercase letters indicate differences between treatments on each sampling day; capital letters indicate differences between treatments through the storage period ($p < 0.05$).

3.3. Lipid Oxidation

Figure 1 presents the results of lipid oxidation in raw and cooked pork patties. The results showed a significant effect of the treatment x storage time interaction ($p < 0.001$) on TBARS values. At day 0, T1 and T2 showed the lowest TBARS values in raw and cooked

samples ($p < 0.05$). Lipid oxidation values increased in raw and cooked samples during storage time ($p < 0.05$). At day 9, T1 and T2 showed the lowest TBARS values in raw and cooked samples ($p < 0.05$).

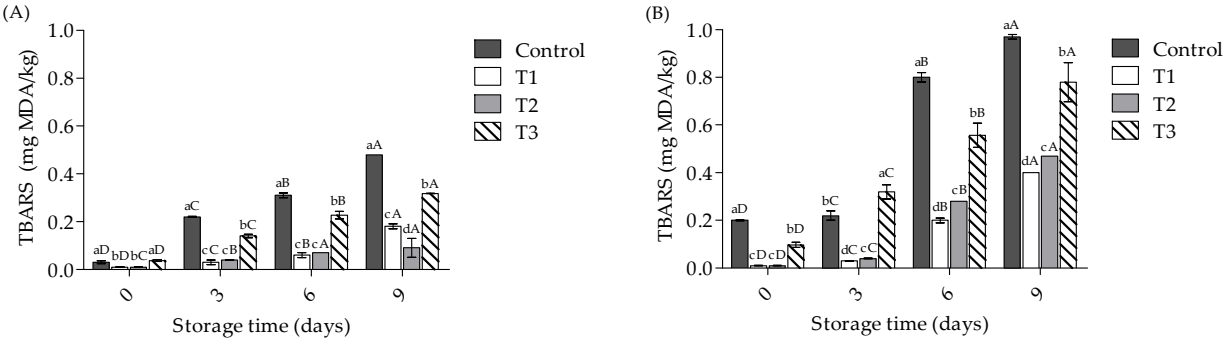

Figure 1. Effect of the treatment and storage time on lipid oxidation levels of raw (**A**) and cooked (**B**) pork patties. ABE—*A. brasiliensis* extract. Control, without antioxidant; T1, ABE at 0.5%; T2, ABE at 1.0%; and T3, BHT at 0.02%. Lowercase letters indicate differences between treatments on each sampling day; capital letters indicate differences between treatments through the storage period ($p < 0.05$).

3.4. Phenolic Content and Antioxidant Activity of Meat Samples

Table 4 presents the results of raw and cooked pork patties' phenolic content and antioxidant activity. At day 0, T1 and T2 showed the highest TPC and Fe^{3+} reduction values in raw and cooked samples ($p < 0.05$) and the lowest DPPH and ABTS absorbance values in raw samples, i.e., high antioxidant activity. On the same day, T2 showed the lowest DPPH and ABTS absorbance values in cooked samples ($p < 0.05$). Furthermore, on day 9, T2 showed the highest TPC and Fe^{3+} reduction values in raw and cooked samples ($p < 0.05$). In addition, T2 showed the lowest DPPH and ABTS absorbance values in raw samples, while T1 and T2 showed the lowest values for these parameters in cooked samples ($p < 0.05$).

Table 4. Effect of the treatment on the phenolic content and the antioxidant status of raw and cooked pork patties.

Item	Patties	Days	Treatments			
			T0	T1	T2	T3
TPC (mg GAE/g)	Raw	0	14.90 ± 1.10^{b}	27.40 ± 1.40^{a}	28.40 ± 1.50^{a}	15.22 ± 0.50^{b}
		9	3.60 ± 0.50^{c}	13.60 ± 2.50^{b}	20.90 ± 0.10^{a}	3.67 ± 0.10^{c}
	Cooked	0	3.20 ± 0.20^{c}	7.90 ± 0.40^{b}	10.10 ± 1.20^{a}	3.52 ± 0.43^{c}
		9	3.40 ± 0.10^{b}	3.10 ± 0.70^{b}	6.80 ± 0.80^{a}	3.49 ± 0.04^{b}
Fe^{3+} reduction (Abs 700 nm)	Raw	0	0.04 ± 0.01^{c}	0.07 ± 0.01^{b}	0.11 ± 0.01^{a}	0.07 ± 0.01^{b}
		9	0.15 ± 0.01^{d}	0.25 ± 0.01^{b}	0.28 ± 0.01^{a}	0.20 ± 0.01^{c}
	Cooked	0	0.07 ± 0.01^{b}	0.11 ± 0.01^{a}	0.13 ± 0.01^{a}	0.07 ± 0.01^{b}
		9	0.10 ± 0.01^{b}	0.09 ± 0.01^{b}	0.13 ± 0.01^{a}	0.10 ± 0.01^{b}
DPPH (Abs 517 nm)	Raw	0	0.42 ± 0.04^{a}	0.15 ± 0.01^{c}	0.14 ± 0.01^{c}	0.36 ± 0.02^{b}
		9	0.41 ± 0.01^{a}	0.34 ± 0.02^{c}	0.19 ± 0.01^{d}	0.36 ± 0.01^{b}
	Cooked	0	0.38 ± 0.03^{a}	0.30 ± 0.02^{b}	0.26 ± 0.01^{c}	0.37 ± 0.01^{a}
		9	0.79 ± 0.05^{a}	0.36 ± 0.04^{b}	0.33 ± 0.01^{b}	0.78 ± 0.01^{a}
ABTS (Abs 730 nm)	Raw	0	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}
		9	0.12 ± 0.01^{a}	0.10 ± 0.01^{b}	0.07 ± 0.01^{c}	0.12 ± 0.01^{a}
	Cooked	0	0.31 ± 0.01^{a}	0.31 ± 0.02^{a}	0.20 ± 0.01^{b}	0.30 ± 0.01^{a}
		9	0.78 ± 0.01^{a}	0.67 ± 0.01^{b}	0.65 ± 0.01^{b}	0.78 ± 0.01^{a}

Values expressed as mean ± SD of at least three independent experiments. ABE—*A. brasiliensis* extract; TPC—total phenolic content; Fe^{3+} reduction—reducing power ability; DPPH inhibition—free-radical scavenging activity; ABTS inhibition—radical-cation scavenging activity; BHT—butylated hydroxytoluene; GAE—gallic acid equivalents. Control, without antioxidant; T1, ABE at 0.5%; T2, ABE at 1.0%; and T3, BHT at 0.02%. Lowercase letters indicate differences between treatments on each sampling day ($p < 0.05$).

3.5. Microbial Growth

Figure 2 presents the results of the microbial growth of raw and cooked pork patties. The results showed a significant effect of the treatment x storage time interaction ($p < 0.001$) on microbial growth values. At day 0, no significant differences ($p > 0.05$) were found in mesophilic psychrophilic counts between raw and cooked pork patties treatments. Both microbial values increased in raw and cooked samples during storage time ($p < 0.05$). At day 9, T2 showed the lowest mesophilic and psychrophilic counts in raw and cooked samples ($p < 0.05$).

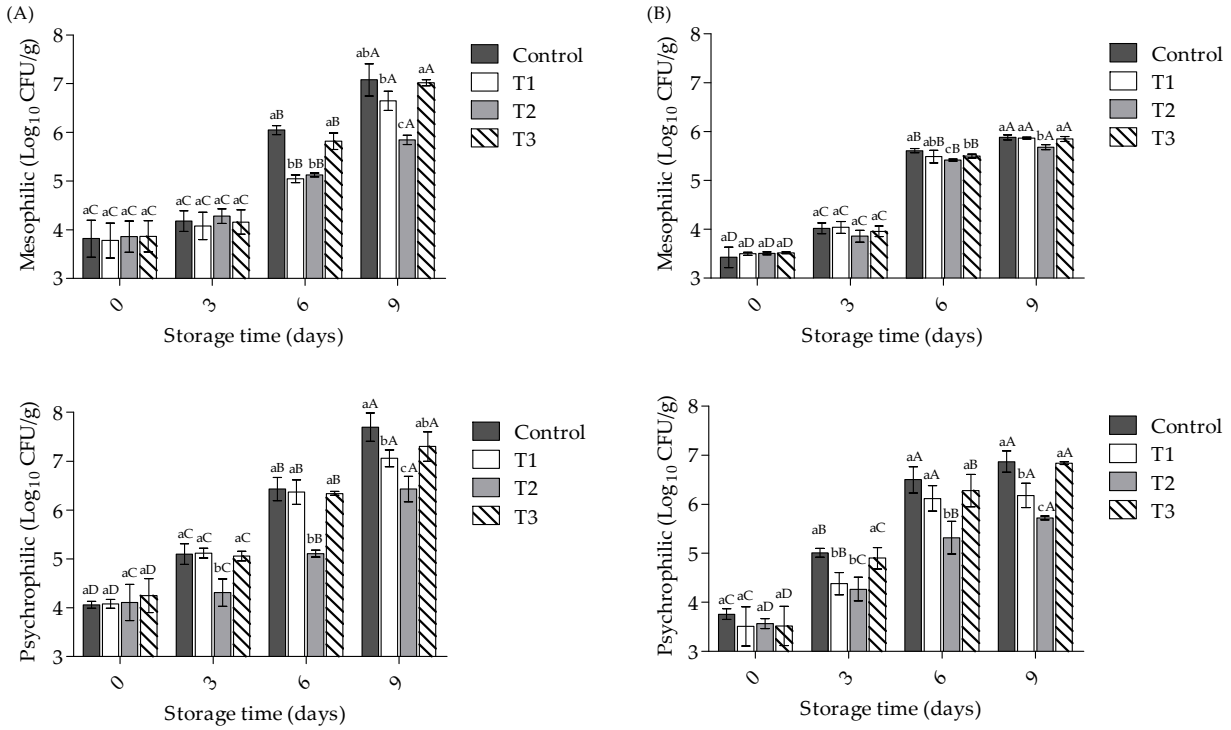

Figure 2. Effect of the treatment and storage time on the microbial growth of raw (**A**) and cooked (**B**) pork patties. ABE—*A. brasiliensis* extract. Control, without antioxidant; T1, ABE at 0.5%; T2, ABE at 1.0%; and T3, BHT at 0.02%. Lowercase letters indicate differences between treatments on each sampling day; capital letters indicate differences between treatments through the storage period ($p < 0.05$).

4. Discussion

The addition of food ingredients of plant origin should be cautiously evaluated because some antinutritional factors have been reported (alkaloids, phlobatannins, tannins, saponins, steroids, and terpenoids) in plant powders and extracts [16,17]. Our results demonstrated that ABE is an important source of nutritional components, including proteins, amino acids, and carbohydrates, as well as bioactive compounds such as phenolic acids and flavonoids (Ax-OH), without detecting any antinutritional factors.

In previous reports, phenolic compounds found in edible mushrooms have shown antioxidant and antibacterial activity [10]. Our results agree with other studies indicating that ABE exerts Fe^{3+} reduction and $ABTS^{\bullet+}$ inhibition, dominated by the electron transfer mechanism (Fe^{3+} + Ax-OH or antioxidant $\rightarrow$ Fe^{2+} + Ax-$OH^{\bullet+}$; $ABTS^{\bullet+}$ + Ax-OH $\rightarrow$ ABTS + Ax-$O^{\bullet}$ + H^{+}, respectively) [19,21]. $DPPH^{\bullet}$ inhibition elicited by ABE could be associated with the H-atom transfer mechanism of the phenolic compound ($DPPH^{\bullet}$ + Ax-OH $\rightarrow$ DPPH + Ax-$O^{\bullet}$) [20]. Concerning antibacterial activity, the reduction or inhibition of microbial growth with natural extracts could be associated with the OH-group site in the phenolic compound structure. For example, 5,7-dihydroxil substitution for flavone and flavanone, 2´ or 4´ hydroxylation of chalcones, hydroxylation group at 3-position on the C

ring of flavone, lipophobicity of A ring of chalcones, and hydrophobic substituents (alkyl chains, alkylamino chains, prenyl groups, among others) [29]. Our results indicated that ABE exerts a higher inhibition against Gram-positive bacteria than Gram-negative bacteria, and this selective effect is most likely associated with structural differences in cell walls between these bacteria [30].

Moreover, the incorporation of ABE positively affected the pH stability of raw and cooked pork patties. The initial pH values of pork patties remained in the 5.75–6.04 range, which is considered high/normal for fresh meat [31]. In agreement with our work, it has been demonstrated that the initial pH values were not affected by incorporating the *A. brasiliensis* aqueous-ethanolic extract (0.5 and 1.0%) in uncooked pork sausages [32]. No significant effect on the pH values of fresh beef patties treated with *Boletus edulis* aqueous extract (1, 3, and 5%) was also reported [33]. However, in our study, the pH values decreased during the storage of control (no antioxidants added) samples, which can be associated with post mortem muscle energy metabolism, i.e., the accumulation of H+ ions [34]. At the same time, an increase in pH values was reported in uncooked beef patties treated with 2.5 and 5% of *Agaricus bisporus* powder during storage at 4 °C for 6 days [35]. A decrease in the pH values of uncooked pork sausages was observed during storage at 4 °C for 7 days by adding *A. brasiliensis* aqueous-ethanolic extract (0.5 and 1.0%) [32]. A high correlation has been found between the pH values of pork meat and its quality traits. For example, a decrease in pH values can affect the functional (WHC and cooking loss), physicochemical (color, texture, lipid, and protein oxidation, among others), and chemical properties (lipid and protein oxidation) of pork meat during storage [31,34].

The color of minced meat patties is one of the most important quality parameters for consumer selection [36]. Our results showed that ABE incorporation positively affected the color stability of raw and cooked pork patties. In agreement with our work, adding 2.5% of *A. bisporus* avoided increasing the L^* values of raw beef patties stored at 4 °C for 13 days and protected the meat samples against decreasing a^* values [35]. Additionally, incorporating *A. bisporus* (5, 10, and 15%) into raw beef patties avoided an increase in L^* values and increased the a^* values during storage without significant effects on the b^* values [37]. In contrast with our study, no significant differences were found in the a^* values of raw pork sausages stored at 4 °C for 7 days and treated with 1.0% of *A. brasiliensis* aqueous-ethanolic extract [32].

The quality of meat products is impacted by lipid oxidation during processing and storage, and it is associated with the formation of aldehydes and other peroxidation products capable of posing a risk to human health [2,26]. Our study indicates that incorporating ABE into raw and cooked pork patties reduces lipid oxidation values. In agreement, it was demonstrated that the incorporation of *A. bisporus* (2.5%) in raw beef patties reduced the MDA formation (8.2% of inhibition) during storage at 4 °C for 13 days in the concerning control samples [35]. Additionally, adding *A. bisporus* (5, 10, and 15%) to raw beef patties reduced MDA formation during storage at 4 °C for 16 days in concentration dependence [37]. Furthermore, the addition of *B. edulis* aqueous-acetone extract (1.0%) to raw beef patties reduced (ca. 70%) MDA formation [33]. The incorporation of *A. bisporus* (1.0%) into cooked ground beef reduced MDA formation (>80% of inhibition) during storage at 4 °C for 16 days in comparison to the control samples [5]. In contrast, adding *A. brasiliensis* aqueous-ethanolic extract (0.5 and 1.0%) to raw pork patties increased MDA formation during storage at 4 °C for 7 days [32]. Phenolic compounds found in natural extracts have shown lipid oxidation, dominated by an electron transfer mechanism ($RO_2^{\bullet}$ + Ax-OH → RO_2H + Ax-$OH^{\bullet+}$). Moreover, the effectiveness of an antioxidant could be related to the hydroxyl group position (ortho and para) due to their ability to donate hydrogen atoms, antioxidant concentrations, and food processing, among others. Although, under certain conditions, the antioxidant activity of synthetic compounds is weak due to a reduction in resonance stability and the tendency to act as chain-carriers by generating radicals and becoming pro-oxidants [38]. In accordance with our study, it is reported that the BHT has greater in vitro activity against the radical DPPH, as well as high capacity to reduce

the FE^{3+} ion, compared with the lotus root and leaf extracts (*Nelumbo nucifera*). However, when BHT was incorporated into pork patties and stored at 4 °C for 10 days, it was less effective than the tested extracts [39]. This behavior could be attributed to the fact that these extracts presented a high content of phenolic and flavonoid compounds, as well as the characteristics of these acting as amphiphilic molecules (lipophilic and hydrophilic) compared to the BHT, which is currently only lipophilic [39,40].

The presence or bioavailability of phenolic compounds in meat products formulated with natural powders and extracts improves oxidative stability during storage [6,9]. In our results, incorporating ABE enhanced the bioavailability of phenolic compounds and improved the antioxidant status of raw and cooked pork patties. In agreement with our study, the incorporation of *A. bisporus* (1, 2, and 4%) enhanced the presence of phenolic compounds (gallic, *p*-coumaric, and caffeic acids) in cooked ground beef during storage [5]. Although adding *B. edulis* aqueous-acetone extract (1.0%) to raw beef patties improved anti-radical and reduced power ability during storage at 4 °C for 8 days, the antioxidant status was reduced during the storage period [33].

Microbial growth negatively impacts the quality of meat products [6]. In our study, incorporating ABE proved to have antimicrobial effects in raw and cooked pork patties. In agreement with our work, the incorporation of *Pleurotus ostreatus* aqueous-methanol extract (3, 6, and 9 mL/kg) to raw beef balls reduced the mesophilic and psychrophilic values during storage at 4 °C for 9 days [41]. However, no significant effect was observed on mesophilic and psychrophilic counts of raw pork sausages treated with 0.5 and 1.0% of *A. brasiliensis* aqueous-ethanolic extract during storage at 4 °C for 7 days [32]. Likewise, no significant effect was observed on mesophilic and psychrophilic counts of raw pork patties treated with *A. bisporus* during storage at 4 °C for 13 days [35].

5. Conclusions

ABE incorporation into pork patties brought about beneficial effects on physicochemical properties and improved antioxidant status, protecting these products from lipid oxidation and microbial deterioration. Therefore, our findings justify using ABE as a natural additive for extending the shelf life of raw and cooked pork patties. Further studies are necessary to know the health benefits of meat products incorporated with edible mushroom extracts.

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