Beef burgers patties incorporated with *Boletus edulis* extracts: lipid peroxidation inhibition effects

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Running title: Lipid peroxidation inhibition by *B. edulis* extracts

ABSTRACT

The objective of this study was to analyse the lipid peroxidation inhibition in beef burgers after incorporation of *Boletus edulis* extracts in different concentrations. Beef burgers samples were stored for 8 days, and chemical and nutritional parameters, including the profile in free fatty acids, were evaluated. Furthermore, the inhibition of thiobarbituric acid reactive substances (TBARS) formation, free radical scavenging activity and reducing power were determined. Polyunsaturated fatty acids arachidonic (C20:4n6) and *cis*-5,8,11,14,17-eicosapentaenoic (C20:5n3) acids were protected in the presence of mushroom extract. The antioxidant potential increased with the amount of extract added to beef burgers, giving higher radical scavenging properties and TBARS inhibition capacity. These findings indicated that beef burgers patties were protected from lipid peroxidation in the presence of mushroom extract.

Keywords: Beef burgers; *Boletus edulis*; Lipid peroxidation; Antioxidant effects; Fatty acids

1 Introduction

Lipid peroxidation is a major quality deterioration problem in foods. The primary products of the reaction are lipid hydroperoxides, and they are converted to secondary products such as aldehydes. The development of warmed-over flavor, which is caused by aldehydes, can be accelerated by lipid peroxidation in raw meats during storage [1]. Lipid peroxidation is a main problem that reduces meat quality and this is a ubiquitous phenomenon that can lead to rancidic odour and loss of product taste. Moreover, lipid peroxidation products are also considered as compounds harmful to human health. In fact, it has been reported that oral intake of fatty acid autoxidation products stimulated lipid peroxidation in living organs [1]. Furthermore, lipoperoxides and some aldehydes (malonaldehyde and 4-hydroxynonenal) are considered in literature as atherogenic agents and are mutagenic, carcinogenic and cytotoxic [1,2].

Thus, lipid stability is one of the important factors for maintaining meat quality during storage. Several antioxidants are used to prevent lipid oxidations in animal meats, including synthetic compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ) [2]. However, because of toxicological concerns of these compounds, food market is demanding for natural food ingredients free of chemical additives orientated to promote the use of natural products.

Different mushroom species were reported to have antioxidant activity, which was mainly related to their phenolic content [3]. Moreover, our research group reported the antioxidant properties including free radical scavenging and lipid peroxidation inhibition properties of methanolic extracts from commercial [4] and wild [5] samples of *Boletus edulis*. It is a source of important antioxidants such as ascorbic acid (18.71

mg/g dw), tocopherols (18.71 μg/g dw) including α-tocopherol, γ-tocopherol and δ-tocopherol, and phenolic acids (9.74 mg/Kg dw) such as protocatechuic acid, *p*-hydroxibenzoic acid and *p*-coumaric acid [5].

Boletus edulis, king bolete, is a popular edible mushroom in Europe (in Portugal is among the most appreciated), North America, and Asia. Fresh and dried king bolete may be marketed in oriental restaurants and oriental, gourmet, and health food stores. The flavor of this dried king bolete including odour and taste is marvellous-nutty, earthy, and meaty all at once [6,7].

The objective of the present study was to evaluate the antioxidant protective effects, including lipid peroxidation inhibition, during storage, of a *Boletus edulis* hydrophilic extract incorporated in beef burgers patties.

2 Material and Methods

2.1 Materials and chemicals

Beef burgers were prepared from fresh beef meat acquired in a local supermarket in at Bragança, Portugal. *Boletus edulis* Fr. was a commercial dried sample also obtained in a local supermarket in Bragança, Portugal.

The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA). All other chemicals and solvents were of analytical grade, except ethyl ether which was of HPLC grade, and were purchased by Lab-Scan, Lisbon, Portugal. Water was treated in a Milli-Q water purification system (Pure Water Systems, Brea, CA).

2.2 Mushroom extracts preparation and incorporation in beef burgers patties

The samples were prepared following the procedure described by Bao et al. [8]. A 5 g portion of the dried powder of *Boletus edulis* was extracted by stirring with 50 ml of 70% (v/v) aqueous acetone for 1h at 25 ºC at 150 rpm and filtered through Whatman No. 4 paper. The residue was then extracted with an additional 50 ml portion of aqueous acetone. The combined extracts were evaporated at 35 ºC under reduced pressure to remove the acetone, and the water was removed by lyophilisation (Ly-8-FM-ULE, Snijders, Holland).

The meat was grounded and divided into four batches and the mushroom extract was added to three of the batches to give a final concentration of 5, 3 and 1%. The remaining batch was kept as a control sample (without mushroom extracts; 0%). The ground meat and mushroom extracts were thoroughly mixed and shaped into (100g) patties using an industrial molder, belonging to the local supermarket, packed individually and subsequently stored at 4ºC for 0, 5 and 8 days.

2.3 Chemical constituents

pH Measurement. The pH was measured after homogenization of the samples (~3g) with distilled water, centrifugation and filtration.

Macronutrients. Moisture, protein, fat, carbohydrates, and ash were analysed using the AOAC procedures [9]. Protein content (N × 6.25) of the samples was estimated by the macro-Kjeldahl method; fat was determined by extracting a known weight of powdered sample with petroleum ether (40-60 ºC), using a Soxhlet apparatus; the ash content was

determined by incineration at 600 ± 15 ºC. Carbohydrates were calculated by difference: Carbohydrates = 100 – (g protein + g fat + g ash). Energy was calculated according to the following equation: Energy (kcal) = 4 × (g protein + g carbohydrate) + 9 × (g lipid).

Fatty Acids. Fatty acids were determined by gas chromatography with flame ionization detection (GC-FID) as described previously by the authors [10], and after the following esterification procedure: fatty acids (obtained after Soxhlet extraction) were methylated with 5 mL of methanol:sulphuric acid:toluene 2:1:1 (v/v/v), during at least 12 h in a bath at 50 ºC and 160 rpm; then 3 mL of deionised water were added to obtain phase separation; the FAME were recovered with 3 mL of diethyl ether by shaking in vortex, and the upper phase was passed through a micro-column of sodium sulphate anhydrous, in order to eliminate the water; the sample was recovered in a vial with Teflon, and filtered with 0.2 µm nylon filter from Whatman. The equipment was a DANI model GC 1000 with a split/splitless injector, and a FID. The column used was a 30 m × 0.32 mm i.d., 0.25 µm, 50% cyanopropyl-methyl-50% phenylmethylpolysiloxane (Macherey-Nagel, Düren, Germany). The oven temperature program was as follows: the initial temperature of the column was 50 ºC, held for 2 min, then a 10ºC/min ramp to 240 ºC and held for 11 min. The carrier gas (hydrogen) flow-rate was 4.0 mL/min (0.61 bar), measured at 50 ºC. Split injection (1:40) was carried out at 250 ºC. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using CSW DataApex 1.7 software and expressed in relative percentage of each fatty acid.

2.4 Antioxidant protective effects

In vitro assays already described by the authors in previous studies [5] were applied to evaluate the antioxidant activity of all the samples.

A fine dried powder (20 mesh; ~1.5 g) sample was extracted by stirring with 30 mL of methanol at 25 ºC at 150 rpm for 1 h and filtered through Whatman No. 4 paper. The residue was then extracted with one additional 30 mL portion of methanol. The combined methanolic extracts were evaporated at 35 ºC under reduced pressure, re-dissolved in methanol at a concentration of 40 mg/mL, and stored at 4 ºC for further use.

DPPH radical-scavenging activity. This methodology was performed using an ELX800 Microplate Reader (BioTek Instruments, Inc., Winooski, VT). The reaction mixture in each one of the 96 wells consisted of one of the different concentrations of the extracts (30 µL) and aqueous methanolic solution (80:20 v/v, 270 µL) containing DPPH radicals ($6x10^{-5}$ mol/L). The mixture was kept for 60 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation: % RSA = $[(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50% of radicals scavenging activity (EC_{50}) was calculated from the graph of RSA percentage against extract concentration. Trolox was used as standard.

Reducing power. This methodology was performed using the Microplate Reader described above. The different concentrations of the extracts (0.5 mL) were mixed with sodium phosphate buffer (200 mmol/L, pH 6.6, 0.5 mL) and potassium ferricyanide (1%

w/v, 0.5 mL). The mixture was incubated at 50 ºC for 20 min, and trichloroacetic acid (10% w/v, 0.5 mL) was added. The mixture (0.8 mL) was poured in the 48-wells, as also deionised water (0.8 mL) and ferric chloride (0.1% w/v, 0.16 mL), and the absorbance was measured at 690 nm. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 690 nm against extract concentration. Trolox was used as standard.

Inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS). Brains were obtained from pig (*Sus scrofa*) of body weight ~150 kg, dissected, and homogenized with a Polytron in ice-cold Tris–HCl buffer (20 mM, pH 7.4) to produce a 1:2 (w/v) brain tissue homogenate which was centrifuged at 3000 × *g* for 10 min. An aliquot (0.1 mL) of the supernatant was incubated with the different concentrations of the extracts (0.2 mL) in the presence of $FeSO_4$ (10 μM; 0.1 mL) and ascorbic acid (0.1 mM; 0.1 mL) at 37 ºC for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 0.5 mL), followed by thiobarbituric acid (TBA, 2%, w/v, 0.38 mL), and the mixture was then heated at 80 ºC for 20 min. After centrifugation at 3000 × *g* for 10 min to remove the precipitated protein, the colour intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A – B)/A] x 100%, where A and B were the absorbance of the control and the compound solution, respectively. The extract concentration providing 50% lipid peroxidation inhibition (EC_{50}) was calculated from the graph of TBARS inhibition percentage against extract concentration. Trolox was used as standard.

2.5 Statistical analysis

For each one of the samples, three experiments were performed (n=3). In some analysis the experiments were carried out in triplicate (n=9). The results are expressed as mean values and standard deviation (SD). The results were analysed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test with $\alpha = 0.05$. This treatment was carried out using SPSS v. 16.0 program.

3 Results and Discussion

Synthetic antioxidants are widely used in food industry to avoid lipid peroxidation in meat. A hydrophilic extract of *Boletus edulis* was used as alternative source of natural antioxidants, by incorporation in burgers patties. Chemical and nutritional parameters were evaluated along 8 days of storage and the results are given in **Table 1**.

pH Values decreased along the time of storage, and with the increase of *Boletus edulis* extract concentration, from control to 3% of extract. Nevertheless, there were no significant differences ($P > 0.05$) between 3% and 5% of *B. edulis* extract. In the fresh samples (T0) the pH values were similar in the control and in the burger patties incorporated with mushroom extract. Therefore, the incorporation of *B. edulis* did not change pH values of the burger patties. This was also observed in nutritional parameters, including fat (17.67 g/100 g dw), protein (35.68 g/100 g), ash (3.83 g/100 g), carbohydrates (43.00 g/100g) and energy (472.10 kcal/100 g). Furthermore, these nutrients remained in similar amounts along storage. In fact, in 8 days of storage, is not expectable degradation of total nutrients, but mostly rancidic odour, loss of product

taste, and formation of lipid peroxidation products, according to the following stages [1,2]:

Initiation: generation of reactive radicals after extraction of hydrogen from lipids

$LH + O_2 \rightarrow L^{\bullet} + HO_2^{\bullet}$, where LH is an unsaturated Fatty acid and $L^{\bullet}$ an allyl lipid radical.

Radical Chain Propagation:

$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$, where $LOO^{\bullet}$ is a lipid peroxyl radical

$LH + LOO^{\bullet} \rightarrow LOOH + L^{\bullet}$, where LOOH are hydroperoxides lipids

$LOOH \rightarrow LO^{\bullet} + HO^{\bullet}$

Termination:

$L^{\bullet} + L^{\bullet} \rightarrow L\text{-}L$

$L^{\bullet} + LOO^{\bullet} \rightarrow LOOL$

$LOO^{\bullet} + LOO^{\bullet} \rightarrow LOOL + O_2$

The analysis of the obtained profiles showed that oleic (C18:1n9c), palmitic (C16:0) and stearic (C18:0) acids were the main fatty acids in the beef burgers patties (**Figure 1**). Nineteen fatty acids were identified and quantified. The relative percentage of each fatty acid, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) are shown in **Table 2**. SFA were the main group of fatty acids, followed by MUFA. PUFA contents in burgers patties were very low. Nevertheless, along the storage beef burgers without mushroom extract were subjected to fat oxidation. This can be observed by the decrease in the polyunsaturated fatty acids C20:4n6 (0.21 to 0.16% corresponding to a decrease of 24%) and C20:5n3 (0.18 to 0.09% corresponding to a decrease of 50%) in control samples (**Figure 2**). Particularly, arachidonic acid is the precursor of eicosanoids which are divided in prostanoids

(prostaglandins, prostacyclins and thromboxanes) and leukotrienes, with very important physiological functions such as mediators of inflammatory and anaphylactic reactions [11].

The addition of *Boletus edulis* extract remarkably protected those fatty acids from oxidation after 8 days of storage. Arachidonic acid (C20:4n6) and *cis*-5,8,11,14,17-eicosapentaenoic acid (C20:5n3) contents were maintained in burgers patties with 1%, 3% and 5% of mushroom extract. Apparently, 1% of *B. edulis* extract was enough to protect lipid peroxidation, and no significant differences were observed with the increase of mushroom concentration.

Three different assays were carried out for the *in vitro* evaluation of the antioxidant protective effects of *Boletus edulis* in burgers patties: scavenging activity on DPPH radicals, reducing power, and inhibition of formation of thiobarbituric acid reactive substances (TBARS). The results are shown in **Table 3**. No significant ($P > 0.05$) effects were observed for reducing power. All the samples revealed the same capacity to reduce Fe^{3+}/ferricyanide complex to the ferrous form. Nevertheless, the scavenging effects on DPPH radicals increased (EC_{50} values decreased) with the increase of *Boletus edulis* extract concentration, and increased along the storage time. The addition of mushroom extract increased the radical scavenging capacity of the samples. Furthermore, the addition of mushroom extract inhibited (by decreasing) the formation of TBARS on beef burgers patties. Malonaldehyde (MDA) is one of the products of lipid peroxidation and reacts with thiobarbituric acid (TBA) giving a chromogen MDA-TBA that can be spectrophotometrically measured [12]. A decrease in the chromogen absorbance indicates a decrease in TBARS formation.

Boletus edulis is a source of powerful antioxidants such as ascorbic acid, tocopherols (α-tocopherol, γ-tocopherol and δ-tocopherol), and phenolic acids (protocatechuic, *p*-hydroxibenzoic nd *p*-coumaric acids) [5]. Tocopherols and phenolic acids could act as free radical scavengers, by donating a hydrogen atom or an electron to lipid peroxyl radicals interrupting the chain propagation reactions mentioned above [13,14]. Ascorbic acid neutralizes the tocopherol radical formed in the reaction between tocopherol and lipid peroxyl radicals, producing ascorbate radical. The latter (semidehydroascorbate) is reduced to ascorbate by NADH-dependent semidehydroascorbate reductase [15].

Despite the remarkable increasing antioxidant capacity of the samples with the increase of mushroom extract concentration, the same behaviour was observed in control. The activity of some antioxidant defences (non-enzymatic or enzymatic) present in beef burgers is increasing along the storage time, in response to the oxidative stress inherent to the storage process. Thus, further work is necessary to elucidate the interactions herein reported.

Overall, *Boletus edulis* extract had a protective effect in beef burgers against lipid peroxidation, evident in the protection of arachidonic (C20:4n6) and *cis*-5,8,11,14,17-eicosapentaenoic (C20:5n3) acids. Therefore, the addition of mushroom extract could be used to extend the beef burgers shelf-life during storage. Hydrophilic extract prepared from *Boletus edulis* is a promising source of natural antioxidants for food and food stuffs.

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Conflict of interest statement

The authors have declared no conflict of interest.

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Table 1. Chemical and nutritional parameters in burgers patties incorporated with *Boletus edulis* hydrophilic extracts (control-0%, 1%, 3% and 5%) along storage (T0-fresh, T5-5 days and T8-8 days).

Samples	pH	Moisture (g/100 g fw)	Total fat (g/100 g dw)	Crude protein (g/100 g dw)	Ash (g/100 g dw)	Carbohydrates (g/100 g dw)	Energy (kcal/100 g dw)
	n=3	n=3	n=9	n=9	n=9	n=9	n=9
$T0_{Control}$	5.70 ± 0.01 a	72.84 ± 0.32 b	17.67 ± 1.18 a	35.68 ± 1.06 a	3.83 ± 0.13 a	43.00 ± 1.57 a	472.10 ± 5.33 a
$T0_{1\% extract}$	5.79 ± 0.03 a	74.08 ± 0.57 ba	15.00 ± 2.04 a	36.75 ± 1.69 a	3.80 ± 0.20 a	44.15 ± 3.56 a	461.36 ± 10.32 a
$T0_{3\% extract}$	5.71 ± 0.07 a	73.83 ± 0.19 ba	15.15 ± 1.45 a	36.45 ± 1.16 a	4.03 ± 0.17 a	44.36 ± 1.65 a	459.63 ± 6.95 a
$T0_{5\% extract}$	5.70 ± 0.02 a	73.59 ± 0.21 ba	15.76 ± 1.20 a	38.77 ± 0.53 a	4.02 ± 0.06 a	41.45 ± 1.67 a	462.71 ± 6.75 a
$T5_{Control}$	5.37 ± 0.07 b	73.75 ± 0.04 ba	16.06 ± 0.21 a	35.38 ± 0.15 a	3.85 ± 0.05 a	44.70 ± 0.23 a	464.88 ± 0.62 a
$T5_{1\% extract}$	5.29 ± 0.02 cb	74.33 ± 0.39 ba	16.67 ± 1.77 a	35.71 ± 1.04 a	3.92 ± 0.20 a	43.76 ± 2.27 a	467.91 ± 8.06 a
$T5_{3\% extract}$	5.19 ± 0.10 cd	74.27 ± 0.28 ba	15.34 ± 1.29 a	36.91 ± 0.66 a	3.92 ± 0.03 a	43.52 ± 2.87 a	462.59 ± 7.43 a
$T5_{5\% extract}$	5.17 ± 0.04 cd	73.94 ± 0.47 ba	15.69 ± 1.52 a	38.65 ± 0.21 a	3.89 ± 0.23 a	41.77 ± 1.67 a	462.89 ± 8.59 a
$T8_{Control}$	5.28 ± 0.07 cb	73.61 ± 1.29 ba	17.49 ± 1.39 a	35.09 ± 2.70 a	3.88 ± 0.11 a	43.54 ± 2.12 a	471.89 ± 6.50 a
$T8_{1\% extract}$	5.07 ± 0.11 ed	74.50 ± 0.29 a	16.46 ± 2.44 a	35.31 ± 0.80 a	3.77 ± 0.10 a	44.46 ± 3.39 a	467.20 ± 13.06 a
$T8_{3\% extract}$	4.95 ± 0.03 e	74.26 ± 0.79 ba	17.18 ± 2.63 a	36.56 ± 0.86 a	3.83 ± 0.23 a	42.43 ± 2.91 a	470.59 ± 11.80 a
$T8_{5\% extract}$	4.94 ± 0.07 e	73.63 ± 0.42 ba	16.72 ± 1.18 a	36.56 ± 1.00 a	3.69 ± 0.19 a	43.04 ± 0.79 a	468.84 ± 6.81 a

Results are expressed as mean ± SD. In each column different letters mean significant differences ($p<0.05$).
fw- fresh weight; dw- dry weight.

Table 2. Free fatty acids composition of burgers patties incorporated with *Boletus edulis* hydrophilic extracts (control-0%, 1%, 3% and 5%) along storage (T0-fresh, T5-5 days and T8-8 days).

Fatty acid	$T0_{Control}$	$T0_{1\%\ extract}$	$T0_{3\%\ extract}$	$T0_{5\%\ extract}$	$T5_{Control}$	$T5_{1\%\ extract}$	$T5_{3\%\ extract}$	$T5_{5\%\ extract}$	$T8_{Control}$	$T8_{1\%\ extract}$	$T8_{3\%\ extract}$	$T8_{5\%\ extract}$
C10:0	0.11 ± 0.00	0.14 ± 0.02	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.13 ± 0.00	0.14 ± 0.02	0.13 ± 0.01	0.12 ± 0.01	0.19 ± 0.01	0.11 ± 0.01
C12:0	0.13 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.14 ± 0.00	0.15 ± 0.01	0.14 ± 0.06	0.15 ± 0.00	0.14 ± 0.00	0.14 ± 0.01	0.14 ± 0.01
C14:0	3.66 ± 0.01	3.56 ± 0.28	3.52 ± 0.17	3.74 ± 0.02	3.60 ± 0.04	3.36 ± 0.14	3.64 ± 0.09	3.64 ± 0.05	3.66 ± 0.10	3.59 ± 0.08	3.58 ± 0.12	3.51 ± 0.15
C14:1	0.54 ± 0.03	0.53 ± 0.05	0.51 ± 0.02	0.49 ± 0.01	0.49 ± 0.01	0.49 ± 0.08	0.50 ± 0.02	0.52 ± 0.06	0.51 ± 0.01	0.49 ± 0.01	0.45 ± 0.04	0.48 ± 0.03
C15:0	0.91 ± 0.03	0.90 ± 0.01	0.88 ± 0.06	0.95 ± 0.04	0.90 ± 0.00	0.86 ± 0.07	0.89 ± 0.04	0.92 ± 0.01	0.91 ± 0.02	0.89 ± 0.03	1.00 ± 0.05	0.95 ± 0.03
C16:0	29.85 ± 0.04	29.83 ± 0.19	29.08 ± 0.50	29.64 ± 0.26	29.45 ± 0.13	28.80 ± 0.14	29.38 ± 0.22	29.40 ± 0.22	29.89 ± 0.28	29.21 ± 0.27	29.52 ± 0.16	29.37 ± 0.16
C16:1	2.57 ± 0.24	2.79 ± 0.11	2.85 ± 0.11	2.60 ± 0.13	2.77 ± 0.04	2.60 ± 0.38	2.78 ± 0.14	2.60 ± 0.08	2.77 ± 0.07	2.72 ± 0.05	2.71 ± 0.10	2.64 ± 0.01
C17:0	2.22 ± 0.14	2.11 ± 0.08	2.12 ± 0.04	2.28 ± 0.11	2.14 ± 0.02	2.22 ± 0.15	2.10 ± 0.05	2.22 ± 0.14	2.11 ± 0.02	2.20 ± 0.09	2.14 ± 0.06	2.13 ± 0.05
C17:1	0.26 ± 0.03	0.33 ± 0.01	0.68 ± 0.04	0.69 ± 0.04	0.26 ± 0.02	0.62 ± 0.06	0.67 ± 0.03	0.63 ± 0.06	0.41 ± 0.04	0.64 ± 0.06	0.53 ± 0.01	0.70 ± 0.08
C18:0	20.52 ± 0.09	21.07 ± 0.26	20.91 ± 0.36	20.78 ± 0.19	21.03 ± 0.16	20.73 ± 0.15	20.98 ± 0.31	20.98 ± 0.20	21.28 ± 0.14	21.04 ± 0.20	21.53 ± 0.16	21.30 ± 0.25
C18:1n9c	36.46 ± 0.04	35.75 ± 0.44	36.45 ± 0.95	35.82 ± 0.13	36.26 ± 0.10	36.85 ± 0.41	36.03 ± 0.47	35.97 ± 0.37	36.67 ± 0.08	35.86 ± 0.33	35.45 ± 0.17	35.60 ± 0.20
C18:2n6c	1.14 ± 0.01	1.24 ± 0.16	1.21 ± 0.05	1.08 ± 0.18	1.26 ± 0.08	1.50 ± 0.07	1.25 ± 0.11	1.23 ± 0.03	1.22 ± 0.02	1.43 ± 0.02	1.25 ± 0.00	1.52 ± 0.08
C18:3n3	0.54 ± 0.03	0.63 ± 0.01	0.62 ± 0.02	0.74 ± 0.06	0.68 ± 0.01	0.76 ± 0.02	0.62 ± 0.01	0.65 ± 0.02	0.61 ± 0.01	0.67 ± 0.02	0.65 ± 0.01	0.68 ± 0.03
C20:0	0.30 ± 0.01	0.33 ± 0.06	0.30 ± 0.00	0.29 ± 0.01	0.29 ± 0.01	0.32 ±0.04	0.29 ± 0.01	0.34 ± 0.09	0.20 ± 0.02	0.28 ± 0.00	0.26 ± 0.03	0.25 ± 0.01
C20:1	0.07 ± 0.00	0.06 ± 0.01	0.06 ± 0.00	0.06 ±0.00	0.05 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.07 ± 0.01	0.05 ± 0.00	0.15 ± 0.00	0.06 ± 0.01	0.05 ± 0.01
C20:3n6	0.13 ± 0.02	0.14 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.05	0.14 ± 0.00	0.13 ± 0.03	0.12 ± 0.01	0.14 ± 0.02	0.13 ± 0.01
C20:4n6	**0.21 ± 0.00**	**0.22 ± 0.01**	**0.22 ± 0.00**	**0.23 ± 0.01**	**0.22 ± 0.02**	**0.23 ± 0.00**	**0.22 ± 0.01**	**0.21 ± 0.03**	**0.16 ± 0.00**	**0.22 ± 0.00**	**0.23 ± 0.00**	**0.22 ± 0.00**
C20:3n3+C21:0	0.08 ± 0.00	0.06 ± 0.00	0.07 ± 0.01	0.07 ± 0.00	0.05 ± 0.00	0.05 ± 0.01	0.06 ± 0.00	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
C20:5n3	**0.18 ± 0.02**	**0.16 ± 0.01**	**0.12 ± 0.03**	**0.16 ± 0.03**	**0.15 ± 0.01**	**0.16 ± 0.03**	**0.13 ± 0.002**	**0.14 ± 0.02**	**0.09 ± 0.01**	**0.16 ± 0.01**	**0.13 ± 0.01**	**0.16 ± 0.01**
SFA	57.70 ± 0.09	58.08 ± 0.34	57.07 ± 1.09	57.94 ± 0.34	57.67 ± 0.16	56.54 ± 0.57	57.55 ± 0.58	57.78 ± 0.41	58.33 ± 0.28	57.48 ± 0.39	58.35 ± 0.16	57.77 ± 0.17
MUFA	39.90 ± 0.15	39.46 ± 0.45	40.54 ±1.04	39.66 ± 0.28	39.84 ± 0.11	40.63 ± 0.78	40.03 ± 0.62	39.78 ± 0.35	39.40 ± 0.24	39.87 ± 0.39	39.19 ± 0.15	39.47 ± 0.28
PUFA	2.40 ± 0.06	2.46 ±0.17	2.39 ± 0.07	2.40 ± 0.10	2.49 ± 0.10	2.83 ± 0.80	2.42 ± 0.07	2.43 ± 0.06	2.27 ± 0.06	2.65 ± 0.02	2.46 ± 0.01	2.76 ± 0.11

Results are expressed as mean ± SD (n=9). In each column different letters mean significant differences ($p<0.05$).
Capric acid (C10:0); Lauric acid (C12:0); Myristic acid (C14:0); Myristoleic Acid (C14:1); Pentadecanoic acid (C15:0); Palmitic acid (C16:0); Palmitoleic acid (C16:1); Heptadecanoic acid (C17:0); *cis*-10-Heptadecenoic acid (C17:1); Stearic acid (C18:0); Oleic acid (C18:1n9c); Linoleic acid (C18:2n6c); α-Linolenic acid (C18:3n3); Arachidic acid (C20:0); *cis*-11-Eicosenoic acid (C20:1); *cis*-8,11,14-Eicosatrienoic acid (C20:3n6); Arachidonic acid (C20:4n6); *cis*-11,14,17-Eicosatrienoic acid + Heneicosanoic acid (C20:3n3+C21:0); *cis*-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3). SFA (Saturated Fatty Acids); MUFA (Monounsaturated Fatty Acids); PUFA (Polyunsaturated Fatty Acids).

Table 3. Antioxidant activity EC_{50} values (mg/mL) of burgers patties incorporated with *Boletus edulis* hydrophilic extracts (control-0%, 1%, 3% and 5%) along storage (T0-fresh, T5-5 days and T8-8 days). The results are expressed in mean ± SD. In each column, different letters mean significant differences ($p<0.05$).

Samples	DPPH Scavenging activity	Reducing power	TBARS inhibition
$T0_{Control}$	244.77 ± 45.51 a	5.09 ± 1.39 a	43.21 ± 2.94 a
$T0_{1\%\ extract}$	47.48 ± 3.03 de	3.78 ± 1.10 ba	13.75 ± 0.96 c
$T0_{3\%\ extract}$	44.28 ± 3.24 def	2.98 ± 0.80 ba	6.26 ± 0.73 d
$T0_{5\%\ extract}$	42.09 ± 3.49 def	2.43 ± 0.48 ba	6.93 ± 0.74 d
$T5_{Control}$	91.89 ± 15.41 b	4.78 ± 1.33 ba	25.91 ± 3.27 b
$T5_{1\%\ extract}$	61.57 ± 8.32 dc	3.32 ± 1.17 ba	12.29 ± 5.51 d
$T5_{3\%\ extract}$	41.79 ± 10.92 def	3.64 ± 0.29 ba	4.71 ± 0.15 d
$T5_{5\%\ extract}$	24.42 ± 2.78 f	2.78 ± 0.44 ba	4.26 ± 0.14 d
$T8_{Control}$	70.96 ± 9.58 c	4.90 ± 0.73 ba	27.51 ± 3.49 b
$T8_{1\%\ extract}$	40.66 ± 0.63 ef	2.73 ± 0.30 ba	7.08 ± 1.48 d
$T8_{3\%\ extract}$	32.16 ± 3.79 ef	3.75 ± 0.71 ba	4.57 ± 0.09 d
$T8_{5\%\ extract}$	30.11 ± 3.71 ef	2.35 ± 0.24 b	4.60 ± 0.10 d

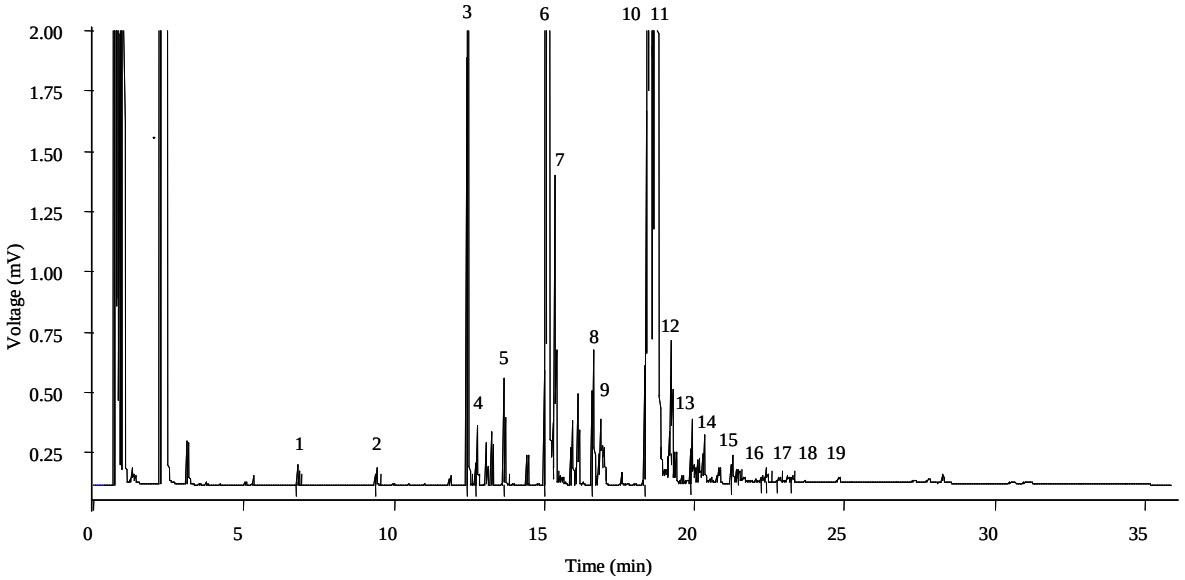

Figure 1. GC-FID profile of methyl esters of free fatty acids of fresh (T0) burgers patties without *Boletus edulis* hydrophilic extracts (control). 1. Capric acid (C10:0); 2. Lauric acid (C12:0); 3. Myristic acid (C14:0); 4. Myristoleic Acid (C14:1); 5. Pentadecanoic acid (C15:0); 6. Palmitic acid (C16:0); 7. Palmitoleic acid (C16:1); 8. Heptadecanoic acid (C17:0); 9. *cis*-10-Heptadecenoic acid (C17:1); 10. Stearic acid (C18:0); 11. Oleic acid (C18:1n9c); 12. Linoleic acid (C18:2n6c); 13. α-Linolenic acid (C18:3n3); 14. Arachidic acid (C20:0); 15. *cis*-11-Eicosenoic acid (C20:1); 16. *cis*-8,11,14-Eicosatrienoic acid (C20:3n6); 17. Arachidonic acid (C20:4n6); 18. *cis*-11,14,17-Eicosatrienoic acid + Heneicosanoic acid (C20:3n3+C21:0); 19. *cis*-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3).

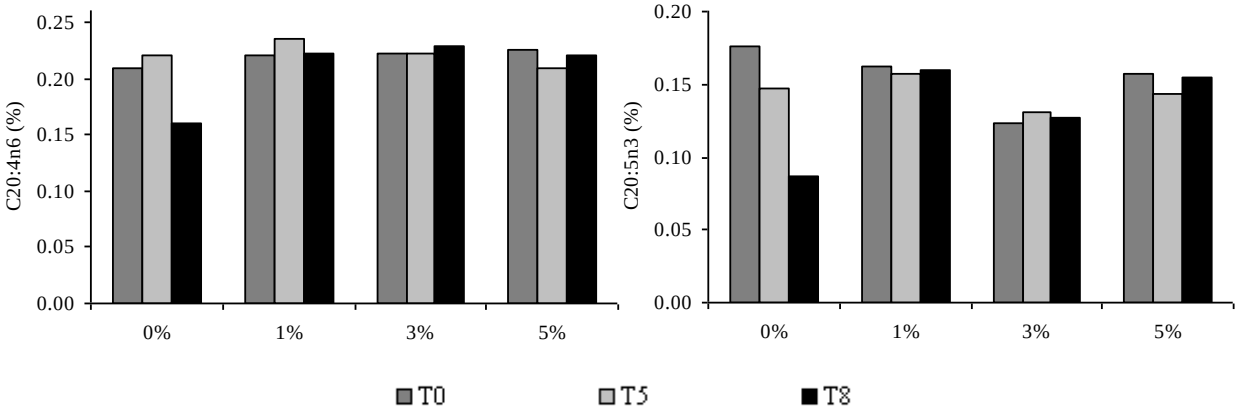

Figure 2. Arachidonic acid (C20:4n6) and *cis*-5,8,11,14,17-eicosapentaenoic acid (C20:5n3) contents in burgers patties incorporated with *Boletus edulis* hydrophilic extracts (control-0%, 1%, 3% and 5%) along storage (T0-fresh, T5-5 days and T8-8 days).