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Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2016.05.002

Influence of peanut skin extract on shelf-life of sheep patties

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ARTICLE INFO

Article history:
Received 4 Nov 2015
Received in revised form 27 Nov 2015
Accepted 20 Dec 2015
Available online 24 May 2016

Keywords:
Peanut skin extract
Phenolic profile
Lipid and protein oxidation
Microbial counts

ABSTRACT

Objective: To evaluate the phenolic profile and antioxidant activity *in vitro* of peanut skin extract (PSE) and effect of PSE on characteristics of sheep patties during storage.
Methods: PSE phenolic profile was evaluated in LC–MS analysis and by total phenolic content, 1,1-diphenyl-2-picrylhydrazyl radical scavenging capacity and ferric reducing/antioxidant power. Patties elaborated with sheep meat were packaged in modified atmosphere and storage at (2 ± 1) °C. The analyses were performed every 5 days for 20 days on microbial counts, physico-chemical properties, lipid oxidation, protein stability and sensory characteristics.
Results: The major group of phenolic compounds in PSE was the proanthocyanidins followed by other flavonoids, which are related to potential phenolic content and antioxidant activity. The addition of PSE and butyl hydroxytoluene (BHT) reduced the microbial counts during the storage time, caused reduction on the loss of redness and sensory properties over time. The lipid and protein oxidation in sheep patties was effectively inhibited by PSE and BHT.
Conclusions: The present results showed the potential application of PSE as a natural alternative to replace synthetic antioxidants (BHT) for increasing the quality and extending the shelf-life of sheep patties.

1. Introduction

Development of society in last decades has changed the diet and food consumption, increasing the demand for food rich in nutrients with good appearance, taste and sold at reasonable price. Thereby, constant changes in food formulation have been done by industries to enhance shelf life, quality and ensure food safety. In this sense, by-products extracts of food processing are potential sources of natural antioxidants and antimicrobials compounds to prevent oxidative reactions on lipids, inhibit undesirable microbial growth and consequently extend shelf life [1].

The chemical changes from lipid oxidation are among the major causes of shelf life reduction and consequent decrease of overall acceptability [2]. Oxidative reactions in lipids and protein generate oxidation products (*e.g.* aldehydes and carbonyl) associated to warmed over flavors [3,4]. Food industries add synthetic antioxidants to retard lipid oxidation and their effects on products characteristics by scavenging free radical, chelating metal ions and decomposing peroxides [5]. On the other hand, the safety concern about synthetic antioxidants and the rising demand for natural products and benefits has increased the search for bioactive compounds. Dietary phenolic have been explored as potential source of natural antioxidants to replace synthetic antioxidant due to the

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Foundation Support: Supported by National Council for Scientific and Technological Development (CNPq No. 248705/2013-0), Foundation for Research Support of São Paulo – FAPESP by PhD scholarship (Case No. 2011/08093-2), Research Internships Abroad (Case n. 517 2013/14120-8).

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

concern of potential toxic effect and consumer increasing consciousness about the safety of food additives [6].

Residues from agro-industry are discarded or sold at low price, wasting natural antioxidants. Processing of peanut seed generates a large amount of skin that has low commercial value. This residue presents a rich diversity of bioactive compounds such as phenolic acids, stilbenes, flavan-3-ols, biflavonoids, isoflavones, flavanols, and flavones [7]. Some studies reported the use of peanut skin extract (PSE) in meat and meat products presenting positive effect on inhibition of lipid oxidation [8,9]. In addition, relative low antimicrobial activity was observed for PSE in ground beef [10].

Thus, the objective of this study was to investigate the effectiveness of natural antioxidant as additive to improve the quality and extend the shelf-life of sheep patties. For this purpose, the ability of PSE to inhibit microbial growth, color and sensory deterioration and lipid and protein oxidation were evaluated in raw sheep patties packaged in modified atmosphere during refrigerated storage.

2. Materials and methods

2.1. PSE

Peanut skin (variety Runner IAC886) was donated by Coplana – Industrial Cooperativa of peanuts. Before the extraction, skins were separated from kernel fragments and kept at −18 °C. The extract was obtained as follows [11]: 30 g of peanut skin were mixed with 80% ethanol (300 mL) and left in water bath (60 °C for 50 min). Then, the mixture was sonicated (15 min at room temperature), centrifugated (6 000 r/min for 15 min) and filtered (Whatman No. 3 paper). Excess solvent was removed by concentration at low pressure (55 °C, −600 kPa) until the final volume of 20 mL. The extract was prepared and used in the same day.

2.2. Phenolic profile by liquid chromatography (LC), diode array detector, electrospray ionization source (ESI), mass spectrometry (MS)

The chromatographic separation was performed on an Agilent 1100 high performance liquid chromatography system equipped with G1379A degasser, G1312B binary gradient pump, G1329A autosampler, G1316A column thermostat and G1315C diode array detector (Agilent Technologies, Waldbronn, Germany). Samples were separated on a Zorbax SB C18 (Agilent Technologies, Santa Clara, CA, USA) (150 × 3.0 mm I.D., 3.5 μm particle size) column, maintained at 25 °C. Gradient elution was performed with acetic acid (2.5%, v/v) (solvent A) and methanol containing 2.5% acetic acid (solvent B). Extract was dissolved in mobile phase to obtain a final concentration of 2 mg/mL. The following gradient program was applied, at a flow rate of 1.0 mL/min: 0 min 95:5 (A:B, v/v), 15 min 85:15 (A:B, v/v), 35 min 70:30 (A:B, v/v), 40 min 60:40 (A:B, v/v), 50 min 40:60 (A:B, v/v), 55 min 10:90 (A:B, v/v), 55.01 min 0:100 (A:B, v/v), 75 min 0:100 (A:B, v/v). Chromatograms were acquired at 240 and 370 nm. Injection volume was 5 μL.

Mass spectrometric analyses were performed with an Agilent 6410B triple quadrupole equipped with an electrospray ionization source (ESI) (Agilent Technologies, Palo Alto, CA, USA). ESI conditions were as follows: temperature: 350 °C, nebulizer pressure: 35 psi, N2 drying gas flow rate: 10 L/min, fragmentor voltage: 135 V, capillary voltage: 4500 V. Full mass scan spectra were recorded in negative ionization mode over the range of *m/z* 100–1 600 Da (scan/s). The Agilent Masshunter Qualitative Analysis B.04.00 software was used for data acquisition and qualitative analysis.

2.3. Estimation of total polyphenol content

The evaluation of PSE phenolic content was estimated using the Folin–Ciocalteau reagent read at 760 nm [12]. The results were expressed as mg gallic acid equivalent (GAE) per g of dry peanut skin.

2.4. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity

The PSE capacity to scavenge radicals was evaluated with 3 150 μL of 72 μmol/L DPPH methanolic solution was mixed with different volumes of PSE (0–150 μL) at 515 nm [13]. PSE concentrations and their respective scavenge activities were used to calculate the concentration required to consume 50% of DPPH amount (EC_{50}).

2.5. Ferric reducing/antioxidant power (FRAP) assay

The FRAP assay was performed according to already establish methodology [14], with FRAP reagent prepared with sodium acetate buffer (pH 3.6), 2,4,6-tris(2-pyridyl)-s-triazine solution (dissolved in HCl) and ferric chlorate. The samples were read at 593 nm and results expressed as μmol trolox equivalents per g of dry peanut skin.

2.6. Preparation of sheep patties and package conditions

Three batches of ground sheep patties [control (CON), butyl hydroxytoluene (BHT) and PSE] were manufactured. Sheep patties of 100 g (n = 2 per batch and storage time) were manufactured using the primal cuts of culled sheep meat. These primal cuts were ground using a 6-mm plate in a refrigerated mincer machine (La Minerva, Bologna, Italy). The meat was mixed and compressed by hand with 10 g of NaCl per kg of meat and 50 mg/kg of BHT or 1 000 mg/kg of PSE. Patties were produced in molds of 10-cm diameter and 1-cm height in a burger-maker (Gaser, A-2000, Girona, Spain). Sheep patties were packed in 300 mm thick polystyrene trays, which were sealed with polyethylene film 74-mm thick and permeability by 2 mL/(m^2 bar day) suitable for gas mixtures (Viduca, Alicante, Spain). The packaging was carried out using a heat sealer LARI3/Pn T-VG-R-SKIN (Ca.Ve.Co., Palazzolo, Italy). The composition of the modified atmosphere was 80% O_2 – 20% CO_2. The trays were stored at (2 ± 1) °C with light, to simulate supermarket conditions. The trays were placed over metal shelving and receiving lux values (digital luxometer, HT 306, Italy) in the range of 15–20 depending on the tray position. The light source was conventional, so any wavelength or range (for instance UV) was not filtered. Analyses were carried out at 0, 5, 10, 15 and 20 days of storage. The microbial spoilage, pH values, color parameters, lipid and protein oxidation, and sensory properties were determined in duplicate for every sampling point. The experiment was replicated three times.

2.7. Microbial analysis

Samples of 10 g of sheep patties were aseptically weighed and homogenized with 0.1% sterile peptone water (90 mL) for 2 min at room temperature in a masticator blender (IUL Instruments, Barcelona, Spain). Serial decimal dilutions were prepared for each sample in 0.1% peptone solutions (Merck, Darmstadt, Germany), and 1 mL or 0.1 mL of the appropriate dilutions were poured or spread onto total count and selective agar plates in duplicate, respectively. Enumeration of total viable counts (TVC) were determined in plate count agar (Oxoid, Unipath Ltd., Basingstoke, UK) after incubation at 30 °C for 48 h. Lactic acid bacteria (LAB) were determined on the Man–Rogosa–Sharpe medium Agar (Oxoid, Unipath Ltd., Basingstoke, UK) at pH 5.6, incubated at 30 °C for 5 days. *Pseudomonas* spp. were counted on *Pseudomonas* selective agar (Merck, Darmstadt, Germany) with pseudomonads CFC selective supplement (Merck, Darmstadt, Germany) after incubation at 25 °C for 48 h. Enterobacteriaceae counts were determinate on violet red bile glucose agar (Merck, Darmstadt, Germany) incubated at 37 °C for 24 h. After the incubation period, each plate with 30–300 colonies was counted. Microbiological data were transformed into logarithms of the number of colony forming units (CFU/g).

2.8. Instrumental color and pH value

The pH was determined using a pH-meter equipped with a glass penetration probe (Hanna Instrument HI-9024, Portugal). A portable colorimeter (Konica Minolta CM-600d, Osaka, Japan) was used to estimate instrumental color in the CIELAB space: lightness, (L*); redness, (a*); yellowness, (b*). The colorimeter was equipped with pulsed xenon arc lamp filtered to illuminant D65 lighting conditions, 0° viewing angle geometry and 8 mm aperture size. The color was measured in three different points of each sample. Before each series of measurements, the instrument was adjusted using a white ceramic tile. The samples were allowed to bloom for 1 h before measuring in contact with air.

2.9. Measurement of lipid oxidation

Lipid oxidation was evaluated using the thiobarbituric acid reactive substances (TBARS) index [15], by extraction of secondary oxidation products in trichloroacetic acid. This solution reacted with thiobarbituric acid and was read at 532 nm. A standard curve of malonaldehyde with 1,1,3,3-tetraethoxypropane was used to express the TBARS values as mg MDA/kg sample.

2.10. Measurement of protein oxidation

The total carbonyl content was estimated following an already establish methodology [16]. The 2,4-dinitrophenyl hydrazine reagent was used to estimate carbonyl content in samples as n nmol carbonyls/mg protein read at 370 nm with an adsorption coefficient of 21.0 mM^{-1} cm^{-1} (for protein hydrazones). The protein concentration was quantified using a standard curve with bovine serum albumin read at 280 nm.

2.11. Free fatty acid (FFA) profile

Total intramuscular lipids were extracted from 25 g of sheep patties sample following a establish procedure [17]. FFA were separated in NH_2-aminopropyl mini-columns [18]. Fifty milligrams of the extracted lipids was transesterified with a solution of boron trifluoride (14%) in methanol [19] and the fatty acid methyl ester were stored at −80 °C until chromatographic analysis, followed by separation and quantification of fatty acid methyl ester [20].

2.12. Analysis of volatile compounds

Analysis of volatile compound was performed with 1 g of sheep patties weighed into a 20 mL headspace vial and sealed with a PTFE-faced silicone septum (Supelco, Bellefonte, PA, USA). A solid-phase microextraction device (Supelco, Bellefonte, PA, USA) containing a fused-silica fiber (10-mm length) coated with a 50/30-μm layer of divinylbenzene/carboxen/polydimethylsiloxane was used. The vial was left at 35 °C in a thermo block (Memmert model 100–800, Schwabach, Germany) for 15 min to equilibrate its headspace. Then, a solid-phase microextraction fiber was exposed to the headspace while maintaining the sample at 35 °C for 30 min. The compounds absorbed by the fibers were identified and quantified by gas chromatographic analysis using MS detector [21]. Results for each volatile compound were expressed as arbitrary units (AU) × 10^6/g dry matter.

2.13. Sensory analysis

The sensory evaluation of sheep patties was performed by ten semi-trained panelists, staff of Meat Technology Center. The panellists were trained for 10 h on the scale and attributes (red color, discoloration and off-odor) to be used [22–24]. A descriptive scale of 5-point was used to score the attributes in a paper scoreboard [25]. The evaluation of 'red color' was performed in an area with uniform red color on the patty surface, wherein extremely brilliant fresh-meat red was rated as 1 and extremely faded red was rated as 5. The percentage of patty surface discoloration was used to measure the 'discoloration' attribute: 1 (none), 2 (0%–10%), 3 (11%–20%), 4 (21%–60%), and 5 (61%–100%). The 'off-odour' was associated to the intensity of odors related to meat oxidation, as follows: 1 (none); 2 (slight); 3 (small); 4 (moderate); and 5 (extreme). Any attribute rated as 4 or higher indicated that sheep patties were considered inappropriate for consumption or sale.

2.14. Statistical analysis

A randomized complete block design was adopted and the entire experiment was replicated twice on two different days. ANOVA using the general linear model procedure (SPSS 19.0, Chicago, IL, USA) was performed to analyze the data, which considered the treatments as a fixed effect, and the replications of the experiments as a random term ($n = 3$). When significant treatment effects were found, Duncan tests at 5% significance level ($P < 0.05$) were employed to determine any significant difference between different treatments.

3. Results

3.1. Phenolic profile of PSE

The major group of phenolic compounds in peanut skin was the proanthocyanidins (PACs) followed by other flavonoids and phenolic acids (Table 1). Peaks 1–26 correspond to pentamers (*m/z* 1 439 and 1 437), tetramers (*m/z* 1 151 and 1 149) or trimers (*m/z* 865, 863 and 861) oligomers.

Other flavonoids were also identified on PSE: peak 29 presented molecular ion at *m/z* 461 and fragment at *m/z* 299, which correspond to 3′5,7-trihydroxyisoflavone-4′-methoxy-3′-O-β-glucopyranoside. Peak 30 was tentatively identified as luteolin methyl ether presenting [M−H]- at *m/z* 299 and fragment at *m/z* 284. The compound from peak 35 was identified as biflavonoid (eriodictyol → C-methyl robinetinidol) with molecular ion at *m/z* 587 and fragments at *m/z* 551 and 419. The peak 39 was likely another biflavonoid (homoeriodictyol → eriodictyol) giving a molecular ion at *m/z* 587 and fragments at *m/z* 551, 419 and 311. However, some compounds were not identified with fragmentation and absorbance data acquired (peaks 27, 28, 31, 32, 33, 34, 36, 37, 38, 40, 41 and 42).

3.2. Phenolic content and antioxidant activity of PSE

The evaluation of PSE phenolic content analysis by Folin–Ciocalteau reagent indicated (32.1 ± 0.7) mg GAE/g. In FRAP assay PSE displayed (26.6 ± 0.8) μmol Trolox equivalent/g of dry peanut skin. The radical scavenging capacity of showed EC_{50} value of (46.5 ± 0.1) μg/mL (Table 2).

Table 1
Tentative identification of phenolic compounds in PSE.

ID	Identification	RT (min)	$[M-H]^-$ (*m/z*)	UV data (λ, nm)	Product ions (*m/z*)	%	Ref#
1	PAC tetramer 1 A-type linkage	1.60	1 151		863	90.3	[7,26]
2	PAC trimer [(E)C → B → (E)C → B → (E)C]	11.61	865		577*, 575, 289	10.3	[26,27]
3	PAC trimer [(E)C → B → (E)C → B → (E)C]	12.39	865		575*, 289	1.1	[26,27]
4	PAC tetramer 1 A-type linkage	13.84	1 151		863	1.2	[7]
5	PAC tetramer 1 A-type linkage	15.96	1 151	280	863	14.1	[7]
6	PAC pentamer [(E)C → B → (E)C → B → (E)C → A → (E)C → B → (E)C]	19.48	1 439	280	1151, 1149*, 863, 575, 573	3.3	[7]
7	PAC tetramer 1 A-type linkage	20.40	1 151		863*, 711	10.5	[7]
8	PAC tetramer [(E)C → B → (E)C → B → (E)C → A → (E)C]	25.24	1 151	280	863*, 575	8.6	[7]
9	PAC trimer [(E)C → B → (E)C → B → (E)C]	25.70	865	280	577	41.9	[26]
10	PAC tetramer 1 A-type linkage	26.43	1 151	280	573	17.3	[7]
11	PAC tetramer [(E)C → B → (E)C → B → (E)C → A → (E)C]	26.96	1 151	280	575*, 423	74.6	[7]
12	PAC tetramer 1 A-type linkage	27.64	1 151	280	575	30.8	[7]
13	PAC tetramer [(E)C → B → (E)C → A → (E)C → A → (E)C]	28.66	1 149	280	861	6.1	[7,27]
14	PAC tetramer [(E)C → B → (E)C → A → (E)C → B → (E)C]	30.19	1 151		863*, 861	2.8	[7]
15	PAC tetramer [(E)C → B → (E)C → B → (E)C → A → (E)C]	32.06	1 151		861, 573*	1.9	[7,26]
16	PAC tetramer [(E)C → B → (E)C → A → (E)C → A → (E)C]	32.79	1 149		861, 575, 573*	2.9	[26,27]
17	PAC pentamer 2 A-type linkages	33.47	1 437		861, 575*, 573	2.1	[7,26,27]
18	PAC tetramer [(E)C → B → (E)C → B → (E)C → → A → (E)C]	34.37	1 151	280	575	17.7	[7,26]
19	PAC tetramer [(E)C → B → (E)C → B → (E)C → A → (E)C]	34.98	1 151	280	575*, 423	41.4	[7]
20	PAC tetramer [(E)C → B → (E)C → A → (E)C → B → (E)C]	35.78	1 151		863*, 861, 573	4.4	[7]
21	PAC tetramer [(E)C → B → (E)C → B → (E)C → A → (E)C]	37.14	1 151		575*, 285	2.4	[7]
22	PAC tetramer [(E)C → B → (E)C → B → (E)C → A → (E)C]	37.62	1 151		575	6.4	[7]
23	PAC pentamer [(E)C → B → (E)C → A → (E)C → B → (E)C → B → (E)C]	38.45	1 439		1151*, 1149, 861	3.6	[7]
24	PAC pentamer [(E)C → B → (E)C → A → (E)C → B → (E)C → A → (E)C]	39.25	1437		1149, 861, 575*, 573	1.2	[7]
25	PAC trimer [(E)C → B → (E)C → A → (E)C]	40.30	863		575	1.3	[7]
26	PAC trimer [(E)C → A → (E)C → A → (E)C]	42.10	861		575	2.2	[7]
27	Unknown flavonol	42.70	611	367	610, 609*, 589	6.2	[7]
28	Unknown flavonol	46.03	624	352	623*, 593, 477	13.8	[7]
29	3′5,7-trihydroxyisoflavone-4′-methoxy-3′-O-β-glucopyranoside	46.64	461		299	2.8	[7]
30	Luteolin methyl ether	50.52	299		284	2.6	[7]
31	Unknown	54.26	681		351, 329*	7.1	[7]
32	Unknown	54.97	1 121		609*, 391, 193	4.0	
33	Unknown	56.18	1 024	316	941*, 431, 217	6.7	
34	Unknown	56.98	623		541, 250*, 140	18.3	
35	Biflavonoid (Eriodictyol → C-methyl robinetinidol)	57.57	587		551, 419*	4.2	[7]
36	Unknown	58.29	883		663, 280, 279*	42.8	
37	Unknown	58.88	889		363, 281*, 255	100.0	
38	Unknown	59.82	835		447, 365*, 283	17.9	
39	Biflavonoid (Homoeriodictyol → Eriodictyol)	61.31	587		551, 419, 311*	8.1	[7]
40	Unknown	63.49	831		701, 421, 340*	21.1	
41	Unknown	64.73	633		474*, 201, 141	3.5	
42	Unknown	66.75	859		613, 532, 367*	3.2	

RT: Retention time in total ion chromatogram; $[M-H]^-$: Molecular ion; *: The most abundant ions observed in mass spectra; #: Tentatively identified based on the mass spectral data [7,26,27].

Table 2
Phenolic content and antioxidant capacity of PSE.

Method	Peanut skin
Folin–Ciocalteau[a]	32.06 ± 0.70
DPPH[b]	46.50 ± 0.10
FRAP[c]	26.56 ± 0.80

[a]: Results expressed in mg GAE/g; [b]: Results expressed in μg/mL; [c]: Results expressed in μmol Trolox equivalent/g.

3.3. Microbiological evaluation of sheep patties over storage time

The evolution of TVC, Enterobacteriaceae, *Pseudomonas* spp. and LAB counts on sheep patties during storage is summarized in Figure 1a, 1b, 1c and 1d, respectively. Significant differences were not observed ($P > 0.05$) in initial counts of TVC for all treatments displaying values around 5 $\log_{10}$ CFU/g. The TVC showed a significant increase ($P < 0.001$) throughout storage time for all batches, however BHT and PSE batches showed lower TVC than CON samples at the end of storage time.

The Enterobacteriaceae counts did not display significant ($P > 0.05$) differences over storage time for CON and BHT batches, showing values between 3 and 5 $\log_{10}$ CFU/g. However, PSE batch did not display effect on Enterobacteriaceae counts during storage, also showing significant higher ($P < 0.01$) values than CON and BHT batches until day 5.

Regarding to *Pseudomonas* spp. counts, statistical analysis did not indicate significant ($P > 0.05$) differences over storage time. In addition, significant ($P > 0.05$) differences among treatments were not observed. Finally, LAB counts showed significant ($P < 0.001$) increase over storage time for all treatments, although lower values were observed for BHT and PSE batches compared to CON.

3.4. Evolution of pH values and color parameters during storage of sheep patties

Table 3 shows the pH values and color parameters of sheep patties during the storage for all treatments. The pH values did not display significant differences ($P > 0.05$) over time and ranged between 5.8 and 6.0 (data not shown). Statistical analysis also did not show significant ($P > 0.05$) differences among treatments on pH values, although CON batch presented higher pH values compared to other ones.

Regarding color parameters, L* values significantly ($P < 0.05$) increased over time in CON batch, although the other treatments did not display significant ($P > 0.05$) difference during storage time (Table 3). Samples of the three patties treatments showed significant ($P < 0.001$) decrease in redness (a* values) during refrigeration, although after 10 days, BHT and PSE batches were significant ($P < 0.05$) higher than CON samples. The yellowness showed significant ($P < 0.001$) decrease over storage time for BHT and PSE samples.

3.5. Evolution of lipid and protein oxidation during storage of sheep patties

The effect of PSE addition on lipid stability of sheep patties during chill storage is shown in Figure 2. The PSE and BHT treatments significantly ($P < 0.01$) inhibited oxidative reactions compared to CON samples throughout storage time, when CON batch showed high TBARS values at day 20 [(6.45 ± 0.37) mg MDA/kg]. Such antioxidant effect was in agreement with FRAP and DPPH assays, indicating the activity of phenolic

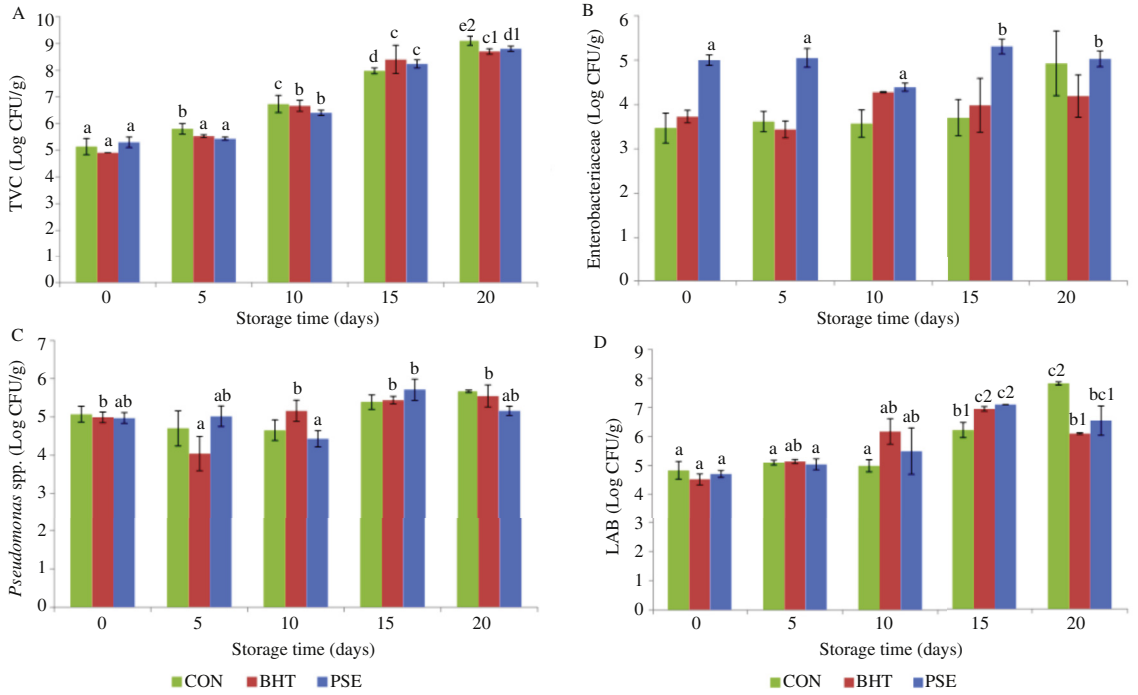

Figure 1. Changes in TVC (A), Enterobacteriaceae (B), *Pseudomonas* spp. (C) and LAB (D) in sheep patties during storage at 2 °C.
[a,b,c,d]: Mean values in the same batch (bars of the same color in different days) not followed by a common letter differ significantly ($P < 0.05$); [1,2]: Mean values in the same day of storage (bars of different color in the same day) not followed by a common number differ significantly ($P < 0.05$).

Table 3
Changes on pH values and color parameters of sheep patties during storage at 2 °C.

Variables	Batches	Days of storage					Significance
		0	5	10	15	20	
pH	CON	5.87 ± 0.03	5.90 ± 0.01	5.90 ± 0.02	5.92 ± 0.03	5.95 ± 0.08	n.s.
	BHT	5.85 ± 0.04	5.87 ± 0.01	5.94 ± 0.05	5.91 ± 0.02	5.84 ± 0.03	n.s.
	PSE	5.84 ± 0.04	5.85 ± 0.01	5.91 ± 0.03	5.91 ± 0.02	5.80 ± 0.05	n.s.
	Significance	n.s.	n.s.	n.s.	n.s.	n.s.	
L^*	CON	39.41 ± 0.67^{a1}	39.43 ± 0.75^{a}	41.25 ± 1.13^{a}	43.28 ± 0.43^{ab}	45.83 ± 2.82^{b}	*
	BHT	42.27 ± 0.69^{2}	40.95 ± 0.41	41.65 ± 0.54	42.18 ± 1.16	40.95 ± 1.07	n.s.
	PSE	$40.35 \pm 0.86^{1,2}$	43.64 ± 1.62	42.33 ± 0.85	42.41 ± 1.64	41.57 ± 0.94	n.s.
	Significance	*	n.s.	n.s.	n.s.	n.s.	
a^*	CON	19.51 ± 0.38^{d}	16.37 ± 0.90^{c}	12.36 ± 0.87^{b1}	10.71 ± 0.12^{b1}	7.50 ± 0.27^{a1}	***
	BHT	21.25 ± 0.49^{d}	17.55 ± 1.07^{c}	14.80 ± 0.25^{b2}	12.94 ± 0.76^{b2}	10.87 ± 0.25^{a3}	***
	PSE	20.43 ± 0.30^{d}	15.92 ± 1.14^{c}	14.93 ± 0.41^{bc2}	13.57 ± 0.41^{b2}	9.56 ± 0.46^{a2}	***
	Significance	n.s.	n.s.	*	**	**	
b^*	CON	17.64 ± 0.56	15.99 ± 0.80	15.61 ± 0.94	16.35 ± 0.29	16.59 ± 0.76	n.s.
	BHT	19.23 ± 0.33^{c}	17.23 ± 0.43^{b}	16.66 ± 0.36^{ab}	16.14 ± 0.24^{ab}	15.44 ± 0.64^{a}	***
	PSE	18.04 ± 0.37^{c}	16.93 ± 0.46^{bc}	16.37 ± 0.23^{b}	15.93 ± 0.56^{b}	14.40 ± 0.47^{a}	***
	Significance	n.s.	n.s.	n.s.	n.s.	n.s.	

Values were expressed as mean ± SE of four samples; Mean values in the same row (corresponding to the same batch) not followed by a same superscript letter differ significantly ($P < 0.05$); Mean values in the same column (corresponding to the same days of ripening) not followed by a common superscript number differ significantly ($P < 0.05$); n.s.: Not significant; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

compounds from PSE on prevention of lipids oxidation. Additionally, the phenolic compounds from PSE presents quelating capacity over Fe^{2+} ion, an important pro-oxidant metal ion for lipid peroxidation, and scavenge activity of hydroxyl, superoxide anion and hydrogen peroxide radical, important reactive species related to oxidation process [28]. This result indicated that oxidation of lipids in sheep patties was effectively inhibited by PSE.

The results of protein oxidation of sheep patties over refrigerated storage are presented in Figure 3. CON batch showed significant ($P < 0.001$) increase on amount of protein carbonyls formed by protein oxidation over storage. The same effect was observed on PSE samples, however, after 5 days of storage, PSE treatment displayed significant ($P < 0.05$) lower values of protein oxidation compared to CON samples.

3.6. Evolution of FFA during storage of sheep patties

The effect of PSE on FFA amount of sheep patties at 0 and 20 days of storage is presented in Table 4. The total FFA content did not show significant ($P > 0.05$) differences among treatments at the end of storage showing values in the range between 613 and 773 mg/100 g of fat. The FFA profile of sheep patties in the three studied treatments showed monounsaturated as the predominant FFAs, followed by saturated and polyunsaturated. The main FFAs at the end of storage period by order of amount as follow: oleic (39.9%), palmitic (25.4%) and stearic (17.0%) acids.

3.7. Evolution of volatile compounds of sheep patties

Volatile compounds associated to lipid oxidation from sheep patties storage at 2 °C on days 0 and 20 are displayed in Table 5.

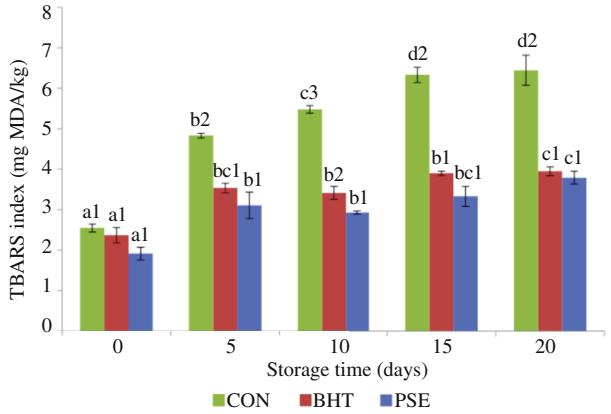

Figure 2. Changes in TBARS values in sheep patties during storage at 2 °C.
[a,b,c,d]: Mean values in the same batch (bars of the same color in different days) not followed by a common letter differ significantly ($P < 0.05$); [1,2]: Mean values in the same day of storage (bars of different color in the same day) not followed by a common number differ significantly ($P < 0.05$).

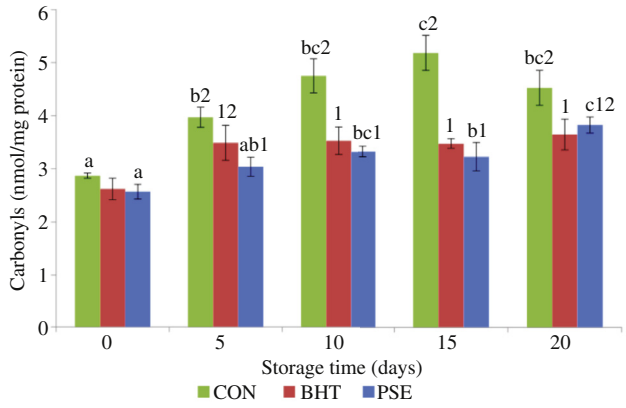

Figure 3. Changes in carbonyls content in sheep patties during storage at 2 °C.
[a,b,c,d]: Mean values in the same batch (bars of the same color in different days) not followed by a common letter differ significantly ($P < 0.05$); [1,2]: Mean values in the same day of storage (bars of different color in the same day) not followed by a common number differ significantly ($P < 0.05$).

Table 4
Changes on FFA of sheep patties during storage at 2 °C.

Fatty acid (mg/100 g fat)	Batches	Days of storage		Significance
		0	20	
Myristic acid (C14:0)	CON	14.78 ± 0.83	14.15 ± 0.60	n.s.
	BHT	11.47 ± 1.12	15.87 ± 3.54	n.s.
	PSE	12.97 ± 1.30	17.55 ± 2.64	n.s.
	Significance	n.s.	n.s.	
Myristoleic acid (C14:1n5)	CON	3.27 ± 0.42	4.98 ± 1.39	n.s.
	BHT	3.89 ± 0.80	5.38 ± 0.32	n.s.
	PSE	2.52 ± 0.64	4.88 ± 1.25	n.s.
	Significance	n.s.	n.s.	
Pentadecanoic acid (C15:0)	CON	3.02 ± 0.02	3.25 ± 0.32	n.s.
	BHT	2.20 ± 0.50	1.88 ± 0.12	n.s.
	PSE	2.20 ± 0.14	3.53 ± 0.61	n.s.
	Significance	n.s.	n.s.	
Palmitic acid (C16:0)	CON	151.00 ± 5.13	151.66 ± 18.33	n.s.
	BHT	137.76 ± 14.88	183.11 ± 9.69	n.s.
	PSE	158.23 ± 27.21	164.39 ± 18.99	n.s.
	Significance	n.s.	n.s.	
Palmitoleic acid (C16:1n7)	CON	18.45 ± 1.88	18.44 ± 1.38	n.s.
	BHT	14.66 ± 0.92[a]	22.55 ± 2.04[b]	*
	PSE	16.63 ± 1.85	19.33 ± 2.59	n.s.
	Significance	n.s.	n.s.	
Heptadecanoic acid (C17:0)	CON	6.86 ± 0.60	6.23 ± 0.35	n.s.
	BHT	6.17 ± 1.18	10.73 ± 0.68	n.s.
	PSE	5.11 ± 0.79	8.32 ± 1.26	n.s.
	Significance	n.s.	n.s.	
Cis-10-heptadecanoic (C17:1n7)	CON	4.23 ± 0.64	6.36 ± 2.41	n.s.
	BHT	4.79 ± 1.21	7.07 ± 0.57	n.s.
	PSE	3.01 ± 0.91	6.20 ± 1.47	n.s.
	Significance	n.s.	n.s.	
Stearic acid (C18:0)	CON	108.68 ± 7.45	114.15 ± 23.48	n.s.
	BHT	88.94 ± 11.05	107.06 ± 17.20	n.s.
	PSE	102.18 ± 14.84	115.17 ± 13.82	n.s.
	Significance	n.s.	n.s.	
Elaidic acid (C18:1n9t)	CON	4.45 ± 0.78	3.82 ± 0.36	n.s.
	BHT	3.43 ± 0.71	2.57 ± 0.46	n.s.
	PSE	2.92 ± 0.45	3.00 ± 0.40	n.s.
	Significance	n.s.	n.s.	
Trans-vaccenic acid (C18:1n11t)	CON	15.03 ± 2.34	14.66 ± 1.49	n.s.
	BHT	16.94 ± 4.20	21.58 ± 7.30	n.s.
	PSE	10.43 ± 1.94	17.04 ± 2.67	n.s.
	Significance	n.s.	n.s.	
Oleic acid (C18:1n9c)	CON	253.89 ± 25.41	226.96 ± 26.63	n.s.
	BHT	219.36 ± 31.75	318.04 ± 38.69	n.s.
	PSE	170.67 ± 33.98	301.01 ± 44.88	n.s.
	Significance	n.s.	n.s.	
Cis-vaccenic acid (C18:1n7c)	CON	12.55 ± 1.09[2]	12.04 ± 1.69	n.s.
	BHT	8.47 ± 1.12[1]	12.13 ± 0.94	n.s.
	PSE	7.68 ± 1.20[1]	9.17 ± 0.95	n.s.
	Significance	*	n.s.	
Linolelaidic acid (C18:2n6t)	CON	3.19 ± 0.24	3.46 ± 0.83	n.s.
	BHT	3.12 ± 0.61	5.31 ± 0.37	n.s.
	PSE	2.14 ± 0.33[a]	4.28 ± 0.71[b]	*
	Significance	n.s.	n.s.	
Linoleic acid (C18:2n6c)	CON	38.74 ± 7.16	44.34 ± 8.22	n.s.
	BHT	25.79 ± 4.37	31.37 ± 1.60	n.s.
	PSE	34.90 ± 9.84	40.91 ± 1.93	n.s.
	Significance	n.s.	n.s.	
Arachidic acid (C20:0)	CON	1.70 ± 0.31	1.86 ± 0.37	n.s.
	BHT	0.63 ± 0.07	1.42 ± 0.46	n.s.
	PSE	1.54 ± 0.28	1.51 ± 0.35	n.s.
	Significance	n.s.	n.s.	
Eicosenoic acid (C20:1n9)	CON	5.54 ± 1.49	9.62 ± 2.92	n.s.
	BHT	5.35 ± 1.93	10.89 ± 0.86	n.s.
	PSE	5.01 ± 0.65	10.65 ± 3.61	n.s.
	Significance	n.s.	n.s.	
Linolenic acid (C18:3n3)	CON	5.58 ± 0.33	4.56 ± 0.21	n.s.
	BHT	5.78 ± 0.57[a]	9.67 ± 0.93[b]	*
	PSE	4.36 ± 1.07	8.11 ± 1.26	n.s.
	Significance	n.s.	n.s.	
Cis-11,14-eicosadienoic acid (C20:2n6)	CON	1.46 ± 0.08	1.46 ± 0.43	n.s.
	BHT	1.12 ± 0.26	1.22 ± 0.22	n.s.
	PSE	2.21 ± 0.78	1.09 ± 0.07	n.s.
	Significance	n.s.	n.s.	
Cis-8, 11, 14-eicosatrienoic acid (C20:3n6)	CON	1.26 ± 0.08	1.45 ± 0.13	n.s.
	BHT	1.22 ± 0.29	0.91 ± 0.37	n.s.
	PSE	1.30 ± 0.05	1.79 ± 0.28	n.s.
	Significance	n.s.	n.s.	
Cis-11,14, 17-eicosatrienoic acid (C20:3n3)	CON	4.02 ± 0.002	3.76 ± 0.44	n.s.
	BHT	2.92 ± 0.231	5.40 ± 0.69	n.s.
	PSE	2.17 ± 0.691	5.40 ± 1.71	n.s.
	Significance	*	n.s.	
Arachidonic acid (C20:4n6)	CON	2.58 ± 1.60	1.59 ± 1.48	n.s.
	BHT	2.98 ± 2.11	2.78 ± 1.97	n.s.
	PSE	1.93 ± 1.12	2.83 ± 1.39	n.s.
	Significance	n.s.	n.s.	
Tricosanoic acid (C23:0)	CON	0.65 ± 0.01a	11.40 ± 0.82b2	**
	BHT	0.60 ± 0.03	0.46 ± 0.061	n.s.
	PSE	0.66 ± 0.02a	0.90 ± 0.04b1	***
	Significance	n.s.	***	
Cis-5, 8, 11, 14, 17-eicosapentaenoic acid (C20:5n3)	CON	1.55 ± 0.16	1.63 ± 0.08	n.s.
	BHT	1.55 ± 0.28	1.95 ± 0.50	n.s.
	PSE	1.36 ± 0.24	2.46 ± 0.61	n.s.
	Significance	n.s.	n.s.	
Docosapentaenoic acid (C22:5n3)	CON	2.25 ± 0.06	1.92 ± 0.62	n.s.
	BHT	2.32 ± 0.21	3.07 ± 0.60	n.s.
	PSE	2.04 ± 0.10	3.67 ± 0.89	n.s.
	Significance	n.s.	n.s.	
Cis-4, 7, 10, 13, 16, 19-docosahexaenoic acid (C22:6n3)	CON	1.73 ± 0.96	2.49 ± 0.75	n.s.
	BHT	1.88 ± 0.75	2.24 ± 0.43	n.s.
	PSE	1.65 ± 0.59	1.77 ± 0.30	n.s.
	Significance	n.s.	n.s.	
Saturated fatty acid	CON	246.67 ± 30.31	251.89 ± 33.84	n.s.
	BHT	243.18 ± 23.19	330.00 ± 33.32	n.s.
	PSE	282.61 ± 43.95	309.16 ± 36.50	n.s.
	Significance	n.s.	n.s.	
Monounsaturated fatty acid	CON	309.14 ± 31.99	279.13 ± 26.09	n.s.
	BHT	263.30 ± 36.65	396.54 ± 55.75	n.s.
	PSE	208.03 ± 41.93	362.40 ± 46.20	n.s.
	Significance	n.s.	n.s.	
Polyunsaturated fatty acid	CON	50.53 ± 4.472,3	60.01 ± 6.58	n.s.
	BHT	37.76 ± 6.662	54.15 ± 8.72	n.s.
	PSE	13.49 ± 0.73a1	66.28 ± 6.14b	**
	Significance	**	n.s.	
Total FFA	CON	614.67 ± 29.66	613.21 ± 67.70	n.s.
	BHT	482.45 ± 25.53	773.92 ± 104.03	n.s.
	PSE	501.44 ± 41.31	744.09 ± 106.19	n.s.
	Significance	n.s.	n.s.	

Values were expressed as mean ± SE of four samples; Mean values in the same row (corresponding to the same batch) not followed by a same superscript letter differ significantly ($P < 0.05$); Mean values in the same column (corresponding to the same days of ripening) not followed by a common superscript number differ significantly ($P < 0.05$); n.s.: Not significant; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Eight volatile compounds were identified on sheep patties. The main volatile compound detected after 20 days of storage was 3-hydroxy-2-butanone followed by octane and 1-pentanol. The analysis of the volatile compounds gives an indication of the chemical and metabolic processes that occur during storage period. The total amount of volatile compounds increased over time ($P < 0.01$), which is in accordance with TBARS values, that also presented an increase during storage period. At the end of storage time, statistical analysis did not show significant

Table 5
Changes on volatile compounds (AU × 10^6/g dry matter) of sheep patties during storage at 2 °C (mean ± SE of four samples).

Compounds (AU)	Batches	Days of storage		Significance
		0	20	
1-Pentanol	CON	55.80 ± 7.97	51.61 ± 13.20	n.s.
	BHT	39.19 ± 8.46	43.97 ± 9.74	n.s.
	PSE	41.69 ± 3.25	47.27 ± 8.90	n.s.
	Significance	n.s.	n.s.	
3-hydroxy-2-Butanone	CON	0.00 ± 0.00^{a}	1 073.45 ± 100.27^{b2}	***
	BHT	0.00 ± 0.00^{a}	763.63 ± 22.45^{b1}	***
	PSE	0.00 ± 0.00^{a}	1 281.67 ± 85.58^{b2}	***
	Significance	n.s.	**	
Decane	CON	12.74 ± 2.71	8.33 ± 1.16	n.s.
	BHT	5.35 ± 0.05^{a}	11.58 ± 1.05^{b}	*
	PSE	8.95 ± 1.26	11.40 ± 1.93	n.s.
	Significance	n.s.	n.s.	
Dodecane	CON	10.68 ± 0.22^{2}	9.39 ± 2.53	n.s.
	BHT	12.93 ± 1.64^{a2}	7.82 ± 0.68^{b}	*
	PSE	6.48 ± 0.15^{1}	7.48 ± 1.53	n.s.
	Significance	*	n.s.	
Heptanal	CON	17.84 ± 1.71^{2}	13.58 ± 1.76^{1}	n.s.
	BHT	8.10 ± 0.77^{1}	14.27 ± 2.91^{1}	n.s.
	PSE	8.07 ± 0.49^{a1}	35.48 ± 4.55^{b2}	*
	Significance	**	**	
3-ethyl-Heptane	CON	10.70 ± 1.58^{a}	4.43 ± 0.62^{b}	*
	BHT	15.06 ± 0.48^{a}	4.72 ± 0.31^{b}	**
	PSE	7.16 ± 1.56	6.30 ± 0.96	n.s.
	Significance	n.s.	n.s.	
3-methyl-nonane	CON	9.49 ± 1.71	7.47 ± 1.00	n.s.
	BHT	9.25 ± 0.57^{a}	6.45 ± 0.79^{b}	*
	PSE	6.34 ± 1.39	6.14 ± 0.36	n.s.
	Significance	n.s.	n.s.	
Octane	CON	48.10 ± 5.77^{a2}	113.86 ± 2.48^{b2}	**
	BHT	40.77 ± 7.86^{2}	26.78 ± 3.91^{1}	n.s.
	PSE	24.07 ± 2.10^{1}	41.34 ± 7.83^{1}	n.s.
	Significance	*	***	
Total volatile compounds	CON	167.70 ± 28.01^{a2}	1 393.59 ± 119.42^{b2}	**
	BHT	115.60 ± 22.26^{a1,2}	862.30 ± 33.90^{b1}	***
	PSE	77.46 ± 12.00^{a1}	1 325.14 ± 101.66^{b2}	***
	Significance	*	**	

Mean values in the same row (corresponding to the same batch) not followed by a same superscript letter differ significantly ($P < 0.05$); Mean values in the same column (corresponding to the same days of ripening) not followed by a common superscript number differ significantly ($P < 0.05$); n.s.: Not significant; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

($P > 0.05$) differences between CON and PSE batches on total volatile compounds. Only, octane presented lower amounts in sheep patties with PSE compared to CON sample (111.9 *vs.* 41.3 × 10^6 AU/g DM for CON and PSE treatment, respectively).

3.8. Evolution of sensory properties during storage of sheep patties

The sensory evaluation of red color, surface discoloration and off-odor of sheep patties during storage is show in Figure 4. The addition of PSE did not promote significant ($P > 0.05$) changes for any attribute at day 0. Regarding to red color of patties (Figure 4a), all treatments showed significant ($P < 0.001$) increase over time, indicating the loss of redness. However, PSE and BHT batches showed significant ($P < 0.001$) lower value than CON samples at 15 days, indicating better sensory perception of redness in antioxidant treatments than observed for control batch.

Surface discoloration of sheep patties also presented significant ($P < 0.001$) increase over storage (Figure 4b). CON samples were scored with values around 3 at 15 days of storage, whereas discoloration on BHT and PSE samples were scored as below 10% at the same period.

The off-odor attribute displayed significant ($P < 0.001$) increase for all treatments over time, indicating the development and perception of undesirable compounds (Figure 4c). The sheep patties of CON batch were score as 4 at day 15, which could be explained by the value above 6 on TBARS index (Figure 2). On the other hand, PSE samples were scored as excellent or good until day 20 of storage.

4. Discussion

Phenolic acids and flavonoids show characteristic UV-range absorbance patterns from 190 to 380 nm [7]. Four groups of phenolic compounds were distinguished by UV–vis diode array detector, namely hydroxybenzoic acids (255 nm), flavan-3-ols and polymers (280 nm), trans-cinnamic acids (320 nm) and other flavonoids (360 nm). These compounds were subsequently introduced into the ESI mass spectrometer and analyzed based on their *m/z* charge. The identification was performed by matching spectral data with those published in literature or tentative identification was based on mass spectra and/or UV data.

PACs are the main group of compounds usually observed for PSE, which are polymers of flavan-3-ols composed by units of catechin and epicatechin. PACs fragmentation can occur by different mechanism: direct cleavage of interflavan linkage, quinone methide fission, reto-Denis-Alder fission and

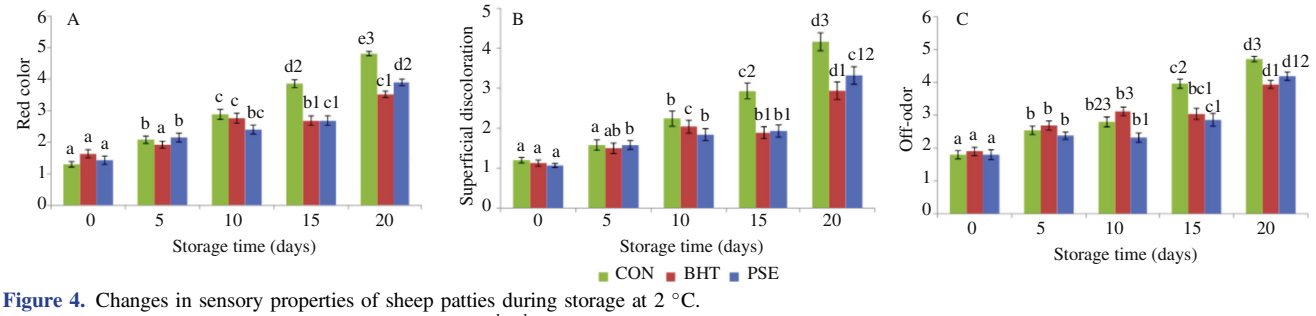

Figure 4. Changes in sensory properties of sheep patties during storage at 2 °C.
A: Red color; B: Superficial discoloration; C: Off-odor; [a,b,c,d]: Mean values in the same batch (bars of the same color in different days) not followed by a common letter differ significantly ($P < 0.05$); [1,2]: Mean values in the same day of storage (bars of different color in the same day) not followed by a common number differ significantly ($P < 0.05$).

heterocyclic ring fission. In the present study, direct cleavage was the main fragmentation mechanism observed and maximum absorbance at 280 nm was also detected to indicate the corr1e-sponding peak as a PAC. Particularly for PACs, the difference observed in *m/z* of molecular ion for similar structures, as observed for both peaks 15 and 16 (*m/z* 1 151 and 1 149, respectively) is attributed to intermolecular linkage between 2 anthocyanidins. In the condition of two adjacent anthocyanidins are linked by a A-type linkage, the *m/z* of molecular ion will be 2 units lower than B-type linkage, indicating at least 1 A-type intermolecular linkage in the compounds of peak 16 [7,26].

Besides the presence of PACs, other flavonoids (*e.g.* luteolin and eriodictyol derivatives) were also present in PSE. Is worth noting, peaks 27 and 28 displayed maximum absorbance at 365 and 352 nm, respectively, which can indicate the presence of flavonoids [7], which suggest flavonoid as the main group of phenolic compounds. Finally, the presence of PACs and other flavonoids are in agreement with [26] who noticed elevated PACs content among all phenolic compounds and at different polymerization degrees, from PACs dimers to octamers.

Since PSE has elevated proportion of PACs, these compounds may be exert other properties as antioxidant activity. *In vitro* tests, phenolic content of PSE was lower than presented by other authors as [29], who observed values in the range of 101.43–280.42 mg/g for the Runner variety, between 106.60 and 148.84 mg/g for Virginia and between 95.56 and 136.80 mg/g for Spanish variety. However, the phenolic content in the present study is in the same range as grape pomace (20.17–66.69 mg GAE/g) [30] and leafy green vegetables (4.53–23.87 mg GAE/g) [31].

The antioxidant activity evaluation requires more than one methodology [32]. In this sense, in the present study, the DPPH and FRAP tests were performed. In FRAP assay, the capacity of PSE to reduce ferric ion to ferrous form in low pH buffer was tested and showed values superior to mango, passion fruit, pineapple and guava co-products (between 2.5 and 19.1 μmol/L Trolox equivalents/g) [33]. Additionally, DPPH assay measure the capacity to scavenge the DPPH radical in methanolic solution and indicated similar capacity reported for *Rubus geoides* fruits (wild raspberry) collected at different regions of Patagonia (between 7.20 and 64.75 μg/mL) [34].

Meat and meat products are highly susceptible to microbial spoilage due to the composition and physic-chemical characteristics. The use of modified atmosphere is an efficient technology to prevent the development of undesirable microorganisms in food, especially in meat. In the present study, the microenvironment inside the package was modified to contain a mixture to 80% O_2–20% CO_2, which is associated to reduction of microbiologic development in meat [35]. In this condition, the results of PSE and BHT treatments in sheep patties are in agreement with previous study that observed that TVC were significantly reduced ($P < 0.05$) in the presence of PSE, particularly, at the last days of storage while CON samples showed steady increase during storage [10]. Additionally, in this study [10], it was observed that TVC counts for PSE samples were 1.0 $\log_{10}$ CFU/g lower than CON batch at 6 days of storage and 2.0 $\log_{10}$ CFU/g lower at 12 days. However, PSE at levels of 0.1% and 0.2%, showed no significant effect on TVC before 12 days.

Enterobacteriaceae counts were not positively influenced by PSE and BHT treatment. However, *Rosmarinus officinalis* at 250 mg/L and *Mentha longifolia* (L.) at 62 mg/L presented potential to inhibit Enterobacteriaceae development in beef sausages (counts around 4 $\log_{10}$ CFU/g *vs* 5.5 $\log_{10}$ CFU/g after 25 days, respectively) [36]. In the same way, *Pseudomonas* spp. counts in foal meat protected by antioxidant film with 1% of green tea inhibited the growth of pseudomonas (1.41 $\log_{10}$ CFU/g for green treatment and 3.36 $\log_{10}$ CFU/g for control after 14 days) [37]. On the other and, like PSE and BHT batches in the present study, the presence of tea and grape extracts in pork patties also inhibited LAB development (around 7 $\log_{10}$ CFU/g for both extracts *vs* above 8.5 $\log_{10}$ CFU/g in control treatment) [1]. These results indicated a general positive effect of PSE on microbial inhibition similar to BHT effect.

The addition of PSE also caused changes in some physical–chemical parameters. In pH values a similar effect was observed for pork patties elaborated with extracts of other natural extracts over storage (pH values around 6.10) [1]. However, for color parameters, the positive effect on reduction of redness loss can be attributed to the antioxidant activity of phenolic compounds to retard metmyoglobin formation during chilled storage [5]. These findings are in agreement with those reported by previous studies [9,10] that observed the effect of PSE on the reduction of a* value on meat products. According these authors, the preservation of redness or at least slowing the loss of redness is positively related to the visual perception of quality.

The addition of PSE in sheep patties inhibited both lipid and protein oxidative reactions that may be attributed to phenolic compounds activity. In lipid oxidation, the deterioration is usually measured by TBARS assay in meat and meat products, as observed for peach seeds (*Prunus persia*) in raw chicken ground meat after 5 days reached TBARS values around 1 mg MDA/kg, whereas control sample displayed 1.5 mg MDA/kg [6]. In addition, previous studies [9,10] also observed that lipid oxidation was significantly reduced by addition of PSE throughout the storage period. The aforementioned color degradation may have been indirectly reduced by the antioxidant effect of PSE. Many authors have correlated the lower redness loss to the antioxidant activity from natural extracts over primary and secondary products from lipid oxidation reactions [1,5,38]. In the same way, protein oxidation was also reduced in PSE and BHT batches. Similar carbonyl content was observed for 500 mg/L of white grape extract in refrigerated beef patties after 9 days (3.5 nmol/mg protein) [39], but superior to chilled porcine patties elaborated with avocado by-products extract (between 1 and 1.5 nmol/mg protein) [5].

The increase in carbonyl content is observed in many muscle products and may be affected by natural extracts, rich in phenolic compounds. This hypothesis is related to the positive effects of phenolic compounds can exert in main known initiators of protein oxidation (transition metals, myoglobin and oxidizing lipids), wherein phenolic compounds act as metal chelators, free radical scavengers and present covalente/non-covalent interactions with proteins. However, the mechanism related to phenolic compounds activity over protein oxidation still unclear [40].

Besides the secondary compounds of lipid oxidation, lipolysis is present in processing and storage in meat products. The events related to lipolysis are associated to release of fatty acids from triglycerides and phospholipids [41]. However, presence of phenolic compounds has little impact in this aspect, since minor effects were observed for all treatments. Increase of total FFA was observed for salami by enzymatic activity during ripening, but PSE did not prevent lipolysis [8].

The processes of volatile compounds formation is related to chemical and enzymatic reactions over unsaturated fatty acids that consequently can interact with proteins, peptides and free amino acids. Other pathways include Maillard reaction and Strecker degradation of free amino acids [18,42]. The results in the present study are in disagreement with previous study about the addition Mediterranean berries extracts in cooked and chilled porcine patties that noticed reduction of volatile compounds, including lipid-derived volatiles [43].

Sensory analysis revealed changes in perception of patties quality over storage. Among the main changes, the loss of redness was also observed in instrumental evaluation and corroborate with sensory perception of red color. Concerning to surface discoloration, the score of 3 was used as the limit of acceptance [25], wherein the discoloration area of samples was perceived as 10%–20% of whole patty. The increase of off-odor parameter during storage is likely associated to lipid oxidation, which is in accordance with higher TBARS values of CON batch compared to PSE and BHT treatments. Similar positive effects of reduced oxidized flavor were observed for ground beef treated with marjoram, rosemary and sage extracts [44] and for cooked, frozen, reheated ground beef patties with grape seed extract [45].

Peanut skin is an interesting source of phenolic compounds, presenting PAC, isoflavone and other flavonoids. Their antioxidant activity *in vitro* showed potential application in meat and meat products. The addition of PSE in sheep patties presented inhibitory effect on oxidative reactions (for proteins and lipids) and reduced the loss of red and changes on sensory attributes during storage. However, microbial counts, FFAs and volatile compounds related to lipid oxidation were not affected by the addition of PSE. Due to the influence on preservation of sheep patties, PSE could be a natural source of antioxidants and replace butyl hydroxytoluene to extend shelf-life.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors would like to thank National Council for Scientific and Technological Development (CNPq No. 248705/2013-0), Foundation for Research Support of São Paulo – FAPESP by PhD scholarship (Case No. 2011/08093-2), Research Internships Abroad (Case No. 517 2013/14120-8), COPLANA for donation of peanut skin and Esteban Guitián from the University of Santiago de Compostela Mass Spectrometry Core Facility for technical support in mass spectrometric analysis.

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