J Food Sci Technol (September 2023) 60(9):2423–2432
https://doi.org/10.1007/s13197-023-05764-6

ORIGINAL ARTICLE

Antioxidant potential of coffee husks in fresh pork sausage

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Revised: 21 April 2023 / Accepted: 9 May 2023 / Published online: 24 May 2023

Abstract Coffee husks, a by-product of dry coffee processing, present a disposal problem in coffee-producing countries. Valorization of this residue is necessary to reduce its environmental impact and improve benefits to the producer. This study evaluated the antioxidant effect of coffee husks on physicochemical properties and sensory liking of fresh sausages packaged in aerobic (AEP) or modified atmosphere packaging (MAP) (20% CO_2 + 80%N_2). Fresh sausages were prepared with different antioxidants: no addition (control C), sodium nitrite (T2), sodium nitrite + sodium erythorbate + BHA/BHT blend (T3), sodium nitrite + coffee husk 1% (T4), sodium nitrite + coffee husk 2% (T5). Physicochemical properties (TBARs, carbonyl content, pH and instrumental color) were analyzed to evaluate the effect of added synthetic and natural antioxidants on fresh sausages. A sensory test (n = 100) was conducted to assess consumer liking of fresh sausages stored in AEP and MAP. The addition of coffee husks reduced lipid oxidation in fresh sausages, especially under MAP packaging, but did not affect carbonyl content. Consumers reported lower liking scores for products packed in MAP. The addition of coffee husks did not affect the degree of liking. Valorization of coffee husks as an antioxidant in fresh meat products is a viable natural option for the meat industry.

Keywords Coffee by-product · Coffee husk · Antioxidant · Fresh sausage · Meat product

Introduction

Coffee is an agricultural commodity of international importance. The major suppliers are located in developing countries where coffee is grown and produced for domestic supply and export to other countries (Vegro and de Almeida 2020). From June 2020 to May 2021, coffee production was estimated at over 10 million tons of coffee, and total exports worldwide accounted for 7.7 million tones (International Coffee Organization 2021).

Coffee production generates over 20 million tons of liquid and solid waste each year, causing environmental pollution in rural areas with limited possibilities for sustainable treatment of this waste (Arya et al. 2022). The coffee husk is a by-product of dry coffee processing, where the coffee cherries are sun-dried before dehulling. The husk comprises the skin, pulp, mucilage, parchment, and parts of the silverskin (Rebollo-Hernanz et al. 2021). It contains caffeine and tannins and has a low pH, which makes it toxic in nature and poses a problem for disposal (Esquivel et al. 2020). Options for the valorization of this residue are necessary to reduce its environmental impact and improve benefits to the coffee producer.

Coffee husks also contain 1.7–3.9 mg/g of chlorogenic acids, 7–8.47 mgQE/100 g of total flavonoids, and a total phenolic content of 384–455 mg GAE/100 g (Gemechu

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2020), including isomeric caffeolyquinic acids, dicaffeolyquinic acids, feruloylquinic acids, and epicatechin, which are potent antioxidants (Esquivel et al. 2020). Thus, the coffee husk is a great source of bioactive compounds with antioxidant activity, making it an attractive sustainable ingredient in nutraceuticals and foods.

Fresh pork sausages are traditional meat products consumed in many countries in Latin America where coffee is produced, such as Mexico, Brazil, Colombia, and Costa Rica (Becerril Sánchez et al. 2019). Due to their neutral pH, high water activity, high fat content and lack of thermal treatment, fresh pork sausages are susceptible to chemical, enzymatic and microbiological activities that produce a very short shelf life (Becerril Sánchez et al. 2019).

Deterioration of the quality of fresh pork sausages is commonly caused by lipid and protein oxidation (de Florio-Almeida et al. 2017). Lipid oxidation occurs when free-radical chain reactions generate byproducts, such as aldehydes, ketones, and alkanes. This compounds are associated with the spoilage of meat sensory characteristics and the loss of muscle protein stability and functionality (Falowo et al. 2014).

Protein oxidation produces covalent modifications of proteins by reactions with reactive oxygen species (ROS) or indirect reactions with secondary by-products of oxidative stress (Zhang et al. 2013b). ROS can cause protein fragmentation or protein–protein cross-linkages by oxidation of amino acid side chains (cysteine and methionine are the most susceptible). Changes in protein physical and chemical properties, including conformation, structure, solubility, susceptibility to proteolysis, and enzyme activities, may affect the quality of fresh meat and the properties of meat products, and are related to human health implications (Zhang et al. 2013b).

Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are added to meat products to inhibit oxidation. However, growing consumer demand for more natural and healthier products has stimulated the food industry to search for natural options to replace synthetic ingredients in processed foods (Shahidi and Zhong 2010).

Antioxidant compounds in coffee husks, such as phenolic compounds, have been analyzed (Rebollo-Hernanz et al. 2021), but few studies have explored the use of coffee husks as a natural antioxidant in food products. The aim of this study was to evaluate the antioxidant effect of coffee husks on the physicochemical properties and sensory liking of fresh pork sausages stored in aerobic and modified atmosphere packaging.

Materials and methods

Coffee husk preparation

Dehydrated coffee husks from Coffea Arabica variety Caturra and Catuaí were harvested on January–February 2020 and transported from Beneficio Helsar, Zarcero, Alajuela, Costa Rica to the laboratory. Coffee husks were milled into flour (particle size of 1 µm) using an ultracentrifugal mill (ZM 300, Retsch, Germany). The flour was vacuum-packaged and stored at − 60 °C until used as an ingredient.

Sausage production

Formulations for fresh sausages are presented in Table 1. Lean meat and back fat were purchased from a local butcher, cut into approximately 2 cm cubes, and minced using a mincer with a 10 mm plate (Alfa-Laval, Kramer Grebe, Germany). Ingredients for each formulation (Table 1) were weighed and mixed by hand for 6 min to ensure a complete mixture. A manual stuffer was used to stuff the mixtures into 3.2 cm diameter collagen casings (Viscofan, Spain). The sausages were packed in low-density polyethylene (LDPE) sealed bags for aerobic packaging (AEP) or multilayer bags for modified atmosphere packaging (MAP) (80% N_2, 20% CO_2) and stored at 4 ± 1 °C until analysis.

Three replications of each treatment were made using different production batches.

Table 1 Formulations of fresh sausages

Ingredient (%)	C	T2	T3	T4	T5
Lean pork meat	49.9	49.62	49.42	48.62	47.62
Lean beef meat	25	25	25	25	25
Pork fat	14	14	14	14	14
Texturized soy	3	3	3	3	3
Water	6.5	6.5	6.5	6.5	6.5
Salt	0.5	0.5	0.5	0.5	0.5
Spices	1	1	1	1	1
Curing salt*	–	0.28	0.28	0.28	0.28
Sodium trypholiphosphate (TPP)	0.1	0.1	0.1	0.1	0.1
BHA	–	–	0.0015	–	–
BHT	–	–	0.0015	–	–
Sodium erythorbate	–	–	0.2	–	–
Dehydrated coffee husk	–	–	–	1	2

Antioxidant treatments were C, Control; T2, curing salt; T3, curing salt+BHA/BHT blend; T4, curing salt+1% coffee husks; T5, curing salt+2% coffee husks

*6.5% $NaNO_2$. Dosage complies with RTCA 67.04.54:10

Experimental design

A 5 × 4 factorial block design was used with batch as the block (three batches). The two factors were antioxidant (AOx) and time of storage (TS). The first factor had five levels: no antioxidant added (C), curing salt 0.28% (T2), curing salt 0.28% + sodium erythorbate 0.2% + BHA/BHT blend (T3), curing salt 0.28% + coffee husk 1% (T4), and curing salt 0.28% + coffee husk 2%(T5) as described in Table 1. The four levels for the second factor, time of storage, were 1, 5, 8 and 12 days for aerobic packaging and 1, 8, 15, and 23 days for modified atmosphere packaging. This design was used for each type of packaging (AEP and MAP). Times of storage were selected beyond the shelf life of the product to assess if the addition of coffee husk extends the product stability.

One-factor complete randomized design was used to evaluate overall liking on day 3 of storage to assure food safety of the product. The five antioxidant treatments and two packaging types resulted in ten treatments that were evaluated by 100 consumers.

Physicochemical analysis

Samples from each packaging type were removed from storage for physicochemical analysis at the intervals indicated above. The following analyses were performed:

Thiobarbituric acid reactive substances (TBARs)

Thiobarbituric acid reactive substances in fresh sausages were determined according to the method described by Tarladgis et al. (1960) to detect lipid oxidation. Absorbance was measured at 532 nm against a blank, and the results were expressed as mg of malonaldehyde (MDA) per kilogram of meat product. Duplicates were made for each batch.

Carbonyl content

Protein oxidation was measured by estimating carbonyl groups using the method of Oliver et al. (1987) with slight modifications. Carbonyl groups were detected by reactivity with 2,4-dinitrophenylhydrazine (DNPH) to form protein hydrazones. The absorbance of the solution was measured at 370 nm. Protein carbonyl content was determined by a molar extinction coefficient of 21 mM^{-1} cm^{-1}. Results were expressed as nanomoles of DNPH fixed per milligram of protein.

pH

The pH was measured directly in the meat products in duplicate using a pH-meter electrode (Metrohm, 827pH lab, Switzerland) with automatic temperature compensation to 26ºC. The meter was calibrated at room temperature using pH buffers 4.00 and 7.00 (Metrohm, Herisau, Switzerland).

Instrumental color

Instrumental color was measured in duplicate using a Hunterlab colorimeter (ColourFlex, USA). An aperture angle of 10º and illuminant D65 were used for the measurements. The colorimeter was calibrated using a standardized white prior to measurements. Color was expressed as L* (lightness), C* (chroma/saturation), and hº (hue) according to the CIELab system. Chroma (C*), which indicates color intensity or saturation, was calculated as $C^* = (a^{*2} + b^{*2})^{1/2}$. The hue angle (hº) was calculated by $h^\circ = \tan^{-1}$ (b*/a*). The color measurement was performed on the interior of the sausage.

Sensory overall liking test

A total of 100 consumers (70% women, 18–59 years old) were recruited based on their meat products consumption frequency and willingness to participate in the study. Participants signed an informed consent form and received a small gift for their participation.

Samples from the five treatments in AEP and MAP packaging were storage for 3 days of storage before sensory analysis. Preliminary microbiological analyses (psychrotrophic and molds and yeast total counts) showed safe microbial quality on day 3–5 of storage on AEP packaging. Using a safe margin, the consumption of raw sausages to consumers was standardized on the day 3 of storage for both types of packaging.

Sausages were cooked at 72 °C at the cold spot. Cooking procedure was standardized to avoid the effect of the cooking method.

Consumers took the sensory overall liking test in individual cubicles. Samples were coded with random three-digit numbers and presented to the judges in a balanced and randomized order. Each panelist assessed the five treatments for each packaging type in two sets, for a total of ten samples, during the same session. A structured 10-point hedonic scale was used, where 0 meant "dislike extremely" and 10 "extremely like". Participants were asked to rinse their mouths between samples to avoid adaptation and carry-over effects.

Statistical analysis

Statistical analysis was performed using the software XLSTAT (2022.3.2, Adinsoft, USA). The effects of the antioxidant treatments (C, T2, T3, T4 and T5) and time of storage on physicochemical properties (pH, TBARs, protein oxidation and instrumental color), were analyzed

by one-way ANOVA for each packaging type separately. Differences between treatments were tested by the Tukey test at 5% level.

For the overall liking test, cluster analysis was used to identify similarities among the rankings assigned by the panelists. An ANOVA test was applied for each cluster. Differences between samples were tested by the LSD test at 5% level (O`Mahony et al. 1980).

Results

Physicochemical analysis

AOx and TS ($P < 0.05$) had significant effects on lipid oxidation in sausages in both types of packaging, but the interaction was not significant. For sausages in aerobic packaging (Fig. 1a), significant differences ($P < 0.003$) were observed between the treatments with added antioxidants and the control (C) on day 1 of storage. At this time only T5, with 2% of coffee husk added, presented a value below 2,5 mg MDA/

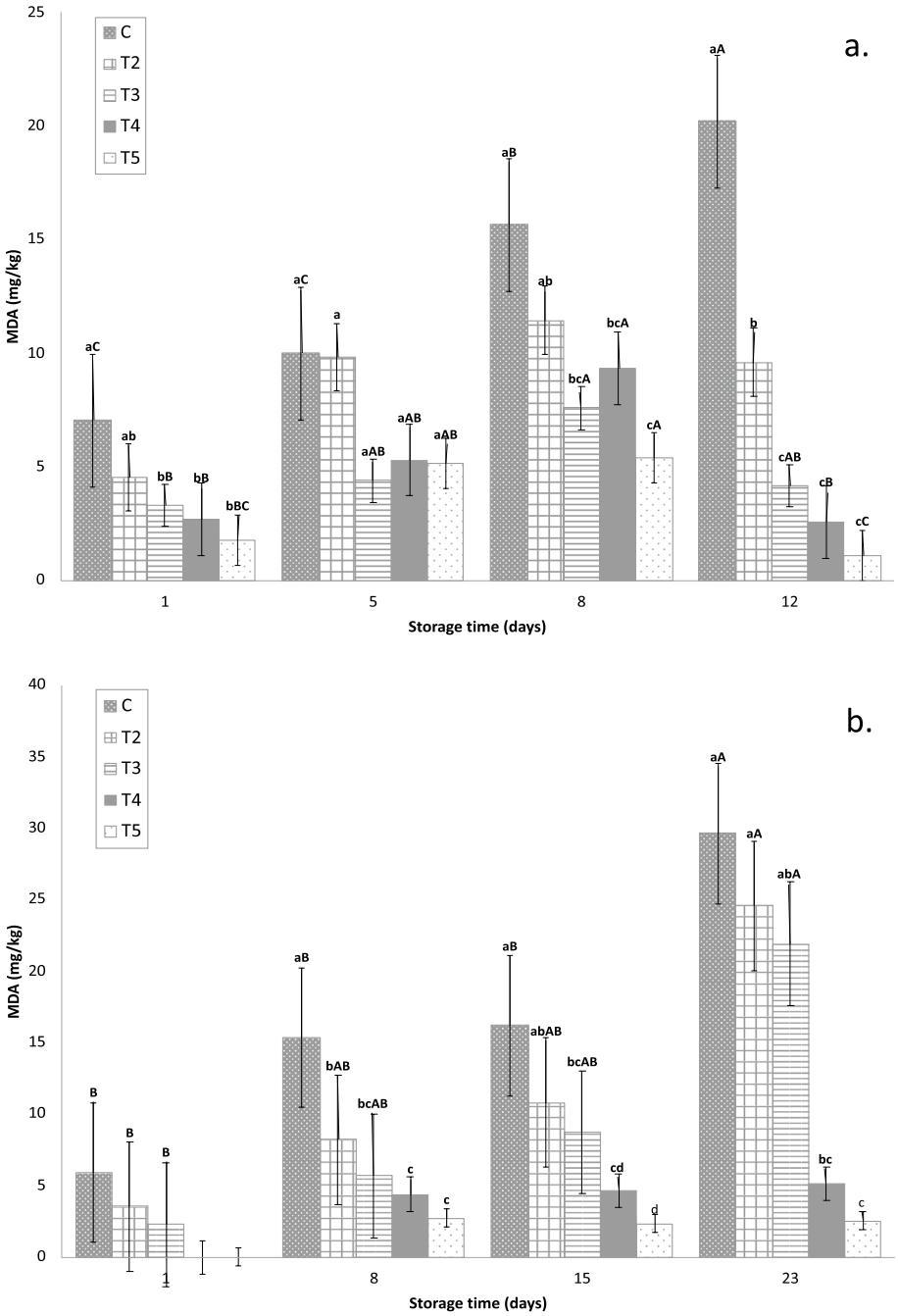

Fig. 1 Effect of antioxidants on lipid oxidation TBARs (mg MDA/kg) in fresh pork sausage during storage in **a** aerobic packaging (AEP) and **b** modified atmosphere packaging (MAP). Antioxidant treatments: C, Control; T2, curing salt; T3, curing salt+BHA/BHT blend; T4, curing salt + 1% coffee husks; T5, curing salt + 2% coffee husks. Different letters (a–c) within the same day of storage indicate significant differences between treatments ($P < 0.05$). Different letters (A–B) indicate significant differences within a treatment on different days of storage ($P < 0.05$)

kg. On day 5 of storage, MDA was greater than 5 mg/kg in all treatments, with no significant differences ($P = 0.183$) among treatments. In treatments with added antioxidants, MDA reached a maximum level on day 8 of storage and decreased on day 12. Oxidation continued to increase in the control for the duration of the storage period. Lipid oxidation values for sausages with added coffee husks (T4 and T5) did not differ significantly from those of sausages with synthetic antioxidants (T3).

In sausages packed under MAP, lipid oxidation was not significantly different among AOx treatments on day 1. MDA was not detectable in T4 and T5 at the beginning of the storage (Fig. 1b). On day 8, C presented the highest value. It is noticeable that only T4 and T5 had significantly lower lipid oxidation values than C and T2, and the values did not increase significantly during the storage period. T5 did not surpass the 2.5 MDA mg/kg limit in the 23 days of storage.

Carbonyl content is shown in Table 2. The antioxidant treatments did not affect protein oxidation significantly, but the effect of time of storage was significant in both AEP ($P < 0.0001$) and MAP ($P = 0.019$). The interaction was not significant ($P > 0.05$). Relatively high protein oxidation values were observed in the two types of packaging. In AEP packaging, carbonyl content was highest on day 1 of storage and decreased over time. Average carbonyl values for AEP were between 27.7 and 1.5 nmol DNPH/mg protein. Carbonyl content in the MAP samples was highest on days 8 ($\bar{x} = 8.7$ nmol DNPH/mg protein) and 15 ($\bar{x} = 14.1$ nmol DNPH/mg protein) but was not detectable on day 1.

The pH values are shown in Table 2. The pH did not differ significantly among the AOx treatments ($P > 0.05$), and the AOx x TS interaction was not significant. TS had a significant effect on pH in both AEP ($P = 0.01$) and MAP ($P < 0.0001$) samples. The average pH value was 5.6 in both types of packaging at the beginning of the storage and decreased during storage.

Luminosity (L*) of the AEP samples (Table 2) differed significantly among AOx treatments and storage times (TS) ($P < 0.0001$), but the interaction was not significant. The L* value ($P = 0.006$) was significantly lower for T5 than for the control on days 1 and 5 of storage. L* values increased during storage in all treatments but were lower for T5 than for the other treatments throughout the storage period. A similar trend in L* values was observed for MAP samples; differences were significant ($P < 0.0001$) among AOx and TS and non-significant for the interaction. On days 1 and 23, L* values were significantly lower for T5 than for C. Treatments T2, T3, and T4 did not differ significantly from the control or T5 during storage in either type of packaging.

Chroma (C*) was not significantly different among treatments (AOx) or time of storage for AEP and MAP samples, and the interaction was not significant ($P < 0.05$). Values were slightly lower in the control than in the other treatments although non-significant.

Hue (h*) of the AEP samples differed significantly among AOx ($P < 0.0001$) within days of storage. The hue was significantly higher in the control than in the other treatments but was not significantly different from T5 on days 1 and 5 of storage. The hue of sausages in MAP packaging did not differ significantly among treatments ($P < 0.05$) until days 15 and 23 of storage. On day 23, the hº values were higher for C than for the other treatments except T5.

Sensory overall liking test

Three clusters of sensory liking were obtained from the panelists' overall liking responses using the hedonic scale. In the first cluster, representing 43% of the sampled population, liking scores were significantly ($P = 0.04$) higher for the samples packed in aerobic conditions (AEP) (Fig. 2a). Liking values were higher for T4 and T5 than for T2. Liking rankings for the MAP samples were around 5 ("do not like or dislike"). T4 was the least liked, but the difference was non-significant compared to C and T5.

Cluster 2 represented 31% of the consumers (Fig. 2b). In this cluster, all the panelists ranked the samples with a liking score higher than 6.5, which means they liked all the samples. The control had a slightly lower liking score than the other treatments ($P = 0.0007$).

Cluster 3 consisted of 26% of the panelists (Fig. 2c). These participants ranked all samples around the middle point of liking (5), except for T5 packaged in MAP conditions, which was ranked significantly ($P < 0.0001$) lower than the other treatments.

Discussion

Physicochemical analysis

Aldehydes, ketones and other carbonyl compounds are secondary products of lipid oxidation of meat and are associated with rancid, pungent and other off-flavors during meat storage (Falowo et al. 2014). Studies have established a limit of 2–2.5 mg MDA/kg, at which there is no rancidity in meat products (Zhang et al. 2013a). Fresh pork sausages are made from by-products of meat cuts that are minced and mixed with pork fat favoring the oxidation processes.

The two sausages with added coffee husks presented the lowest TBARs values compared to the control in both types of packaging used in this study. Coffee husks contain phenolic compounds responsible for the high ORAC values of coffee husks (~60,000 µmol TE/100 g). These antioxidant compounds include flavonoids, such as anthocyanins (cyanidin-3-glucoside, cyanidin 3-o-ruthinoside) and phenolic

Table 2 Physicochemical parameters of fresh sausages during storage in aerobic (AEP) or modified atmosphere (MAP) packaging

Type of packaging	AEP				MAP			
Time of storage (days)	1	5	8	12	1	8	15	23
pH								
C	5.6	5.5	5.5	5.4	5.5	5.5	5.3	5.3
T2	5.6	5.4	5.2	5.2	5.8	5.1	5.1	5.1
T3	5.7	5.4	5.2	5.2	5.6	5.2	5.1	5.2
T4	5.6	5.1	5.0	5.0	5.5	5.0	5.0	5.0
T5	5.8	5.3	5.0	5.2	5.8^{A}	4.9^{AB}	4.8^{B}	4.9^{AB}
SEM	0.1	0.1	0.07	0.1	0.1	0.09	0.06	0.06
Carbonyl content (nmol DNPH/mg protein)								
C	20.4	12.1	0.0	6.8	0.0^{C}	8.8^{AB}	12.9^{A}	0.7^{BC}
T2	43.3^{A}	7.6^{AB}	12.7^{AB}	0.0^{B}	0.0	21.1	11.1	3.7
T3	20.6^{A}	21.1^{A}	3.7^{B}	0.0^{B}	0.0	6.1	9.4	13.8
T4	40.3^{A}	13.2^{AB}	21.0^{AB}	0.7^{B}	0.0^{B}	7.7^{AB}	25.4^{A}	4.4^{AB}
T5	14.0	10.5	14.0	0.0	0.0	0.0	11.8	0.4
SEM	4.0	1.7	4.3	1.4	3.3	2.0	2.9	2.6
Luminosity (L)*								
C	50.3^{aB}	51.8^{aAB}	53.3^{A}	51.5^{abAB}	50.6^{aB}	51.6^{AB}	50.9^{B}	54.0^{aA}
T2	48.6^{ab}	49.0^{ab}	52.0	50.8^{ab}	48.0^{abB}	50.6^{AB}	50.3^{AB}	52.5^{abA}
T3	49.4^{abc}	50.0^{ab}	51.6	52.3^{a}	48.9^{ab}	50.8	51.2	52.3^{ab}
T4	44.9^{bcB}	47.9^{abAB}	50.4^{A}	50.8^{abA}	45.3^{abB}	51.6^{A}	50.5^{AB}	51.4^{abA}
T5	44.1^{cB}	45.5^{bAB}	48.5^{A}	47.4^{bAB}	44.0^{b}	47.3	49.0	49.4^{b}
SEM	0.9	0.7	0.7	0.5	1.0	0.8	0.5	0.6
Chroma (C)*								
C	19.1	18.5	17.2	17.7	18.6	18.8	16.8	17.6
T2	21.7	21.1	18.3	19.5	21.7	19.4	19.8	18.7
T3	21.7	20.2	19.5	19.5	21.8	20.4	19.9	18.8
T4	20.1	20.5	19.3	19.3	20.1	19.9	19.0	19.3
T5	19.8	19.5	19.9	18.5	19.7	20.1	20.0	19.3
SEM	0.5	0.6	0.4	0.3	0.5	0.3	0.5	0.4
Hue (h°)								
C	67.7^{a}	71.1^{a}	75.0^{a}	75.5^{a}	63.9	64.3	69.4^{a}	69.0^{a}
T2	56.0^{b}	54.4^{b}	58.1^{b}	54.8^{b}	55.1	56.0	51.4^{c}	53.4^{b}
T3	54.5^{b}	55.3^{b}	58.2^{b}	60.7^{b}	56.2^{A}	54.7^{AB}	50.3^{cB}	52.4^{bAB}
T4	57.7^{b}	54.4^{b}	58.2^{b}	59.4^{b}	58.3	58.4	56.2^{bc}	55.4^{b}
T5	60.8^{ab}	60.7^{ab}	62.2^{b}	62.1^{b}	61.1	63.4	60.5^{b}	60.8^{ab}
SEM	1.4	1.9	1.9	2.0	1.2	1.3	2.2	1.9

Different letters (a–c) in the same column indicates significant differences between treatments ($P < 0.05$) within the day of storage. Different letters (A–B) in the same row indicates significant differences between days of starge ($P < 0.05$) within treatments

acids, such as chlorogenic acid (Esquivel et al. 2020). Several studies have shown the effectiveness of plant-derived antioxidants for delaying lipid oxidation in meat products. de Florio-Almeida et al. (2017) found that lyophilized bee pollen extract (ORAC ~ 12,000 µmol TE/100 g) controlled lipid oxidation of fresh sausages during storage. The TBARs values (1.29–4.22 mg malonaldehyde/kg) were similar to those obtained in this study in treatments with added coffee husks (T4 and T5). Comparable results were seen for Chinese fresh sausages with added sage (ORAC ~ 32,000 µmol TE/100 g), with TBARs values between 1.5 and 3.4 MDA/kg for sausages with the natural antioxidant (Zhang et al. 2013a).

In MAP packaging, the conditions of low oxygen and limited light exposure during storage were not favorable for lipid oxidation, as reported in several studies (Redondo-Solano et al. 2021). In contrast, the level of MDA in AEP samples exceeded the 2.5 mg/kg limit after 5 days of storage, which indicates an important degree of lipid oxidation that

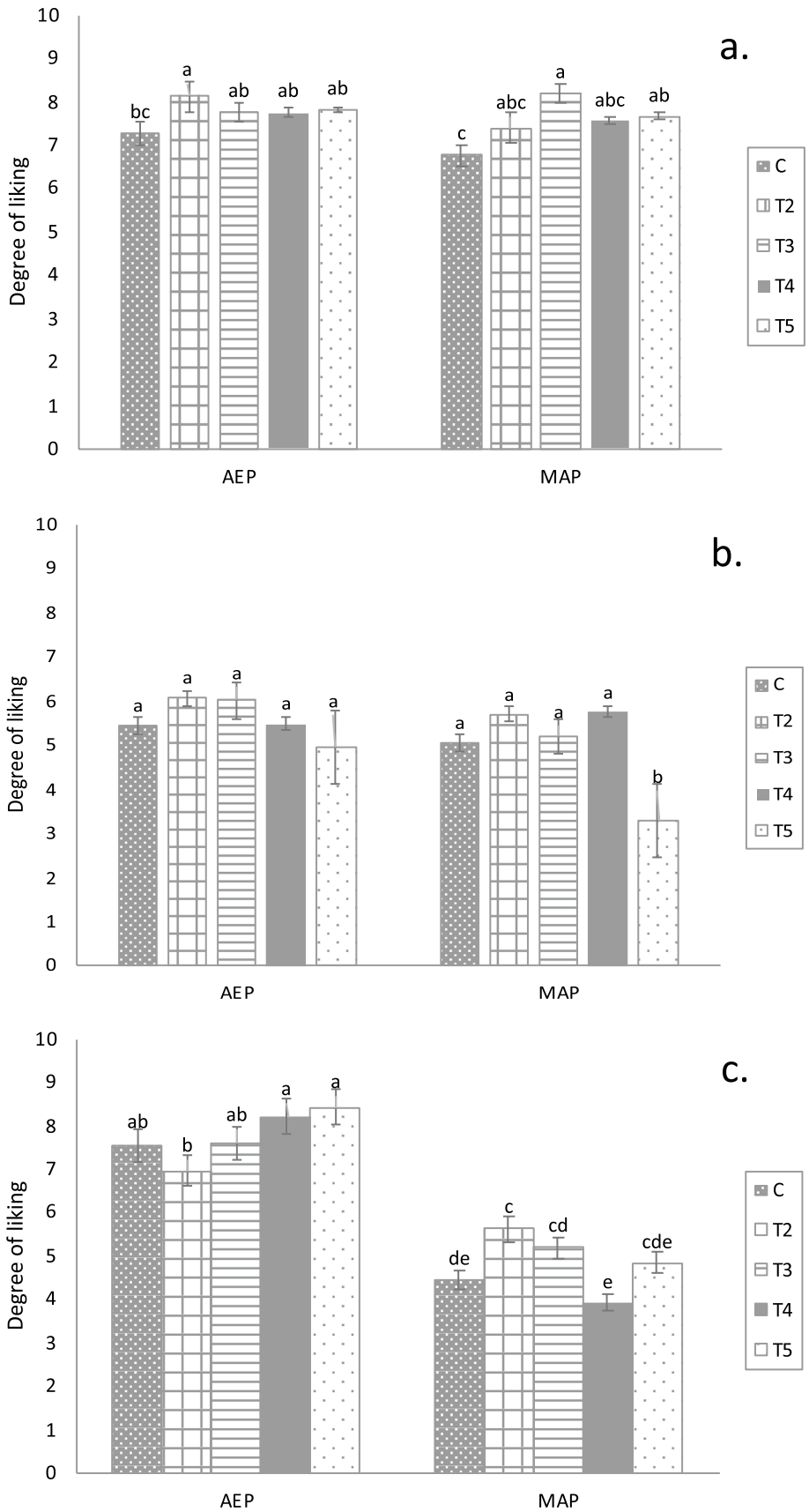

Fig. 2 Consumer liking of fresh pork sausage with added antioxidants and stored in aerobic (AEP) or modified atmosphere packaging (MAP). Clusters represent responses of a) 43%, b) 31% and c) 26% of the panelists. Antioxidant treatments: C, Control; T2, curing salt; T3, curing salt+BHA/BHT blend; T4, curing salt + 1% coffee husks; T5, curing salt + 2% coffee husks. Different letters (a–c) within the same type of packaging indicate significant differences between treatments ($P < 0.05$)

was probably due to the high oxygen permeability of the LDPE used. Similar results were reported for beef patties (Lund et al. 2007) and fresh sausages (de Florio-Almeida et al. 2017) packed for aerobic storage conditions, but the TBARs values in those studies were lower. These differences may be due to the lipogenesis process in the meat, which is affected by the animal diet, the muscles used to prepare the samples, and the handling and processing steps (Domínguez et al. 2019).

In sausages in MAP packaging, lipid oxidation was inhibited in T5 (2% coffee husks) throughout the entire storage period, and MDA did not exceed the 2.5 mg/kg limit. These results demonstrate the efficacy of coffee husks as an antioxidant in fresh sausages. Although ORAC values have been used as a measure of antioxidant activity, they can be reduced significantly during heat treatment (Esquivel et al. 2020). Thus, the addition of coffee husks to cooked meat products should be further analyzed to evaluate whether there is a significant reduction in total polyphenol content, and consequently, the antioxidant properties.

Interest in quantifying protein oxidation in meat products has grown as a novel research field because of the impact of oxidized protein on meat quality. In this study, added antioxidants did not affect protein oxidation in either type of packaging ($P > 0.05$) (Table 2). This finding is not consistent with the solid inhibitory effect on lipid oxidation (Fig. 1). While many studies have shown antioxidant effects of phenolic-rich plant extracts in meat applications (Jia et al. 2012), others have reported no effect or even prooxidative effects on the formation of protein carbonyls in meat products (Lund et al. 2007; Estévez 2011).

Protein oxidation values reported in this study showed high levels of carbonyl content. In general, the carbonyl content in non-oxidized muscle tissue is 1 nmol/mg protein, whereas in oxidized tissue, it can vary from 2 to 14 nmol/mg protein depending on the initiator of oxidation, the level of oxidation, the muscle type, the protein solubility (Estévez et al. 2008) and the type of packaging (Lund et al. 2007). With MAP packaging, the carbonyl content was below a detectable level in all treatments on day 1 of storage but was higher on days 8 and 15 (Table 2). Other studies have shown no sign of protein oxidation at the beginning of storage, even when lipid oxidation was quantified (Lund et al. 2007). These results indicate that the reaction rate of lipid oxidation was faster than protein oxidation on the quality of fresh pork sausage packed in MAP.

Fresh pork sausage in AEP packaging was oxidized on day 1 of storage (Table 2), as seen by the high TBARs values. However, the total amount of protein carbonyls may have been overestimated by accounting lipid-derived carbonyls from lipid oxidation (Estévez et al. 2008). Although the DNPH method is used routinely to quantify carbonyls due to protein oxidation, meat researchers often find it difficult to obtain consistent and reliable results (Estévez 2011). With a complex sample such as fresh pork sausage, in which the state of oxidation is presumably high, the method could produce many artifacts that affect the results (Cao and Cutler 1995).

Some phenolic compounds with antioxidant activity, such as cyanidin-3-glucoside, also found in coffee husks, are more protective against lipid oxidation than protein oxidation (Viljanen et al. 2004). The protective effect is dependent on the composition and chemical structure of the phenolic compounds and the nature and conformation of the meat proteins, the antioxidant concentration, and production technologies (Estévez et al. 2008). It is also important to mention that the antioxidant compounds content might change within different coffee varieties.

Antioxidants added to fresh sausages had no effect on pH; however, in MAP packaging, pH values were lower after one day of storage. The modified atmosphere used (80% N_2, 20% CO_2) has been associated with a lower pH in food products due to the dissolution of CO_2 into H_2CO_3 in the product and the activity of Gram+bacteria, such as lactic acid bacteria (LAB) (Kandeepan and Tahseen 2022).

The addition of coffee husks did not modify the pH of the product. It has been shown that the effect of additives of natural origin on pH is very low due to the buffering effect of muscle tissues (Riel et al. 2017). Studies on pork-based meat products with added natural antioxidants such as cranberry pomace extract (Tamkutė et al. 2019) and grape seed extract (Garrido et al. 2011) showed no significant variation in pH during prolonged refrigerated storage (4 °C).

Luminosity is associated with the physical state of the meat, pH, and water holding capacity (WHC) and is used as a measure of brightness (Limbo et al. 2010). The decrease in pH during storage may be associated with lower WHC, which results in higher light reflectance on the surface of the product. This effect generates a bright, wet surface that gives higher L* values, as observed during storage of fresh sausages in both types of packaging (Limbo et al. 2010). Accordingly, lower L* values were lower for the treatment with 2% coffee husks (T5) than for the control. This could be due to high water retention by the coffee husks and, thus, a drier surface of the meat product. Similarly, Tamkutė et al. (2019) showed that adding cranberry pomace extract to pork products significantly reduced the L* value due to the added fiber in the product.

Chroma C* describes the vividness or dullness of a color. The antioxidants added to fresh sausages did not affect this parameter during storage. The h° values showed that the hue angles for C and T5 in the spectrum shifted from red to yellow in both types of packaging. This was expected for the control since it had no added sodium nitrite, an ingredient related to the pink color in meat products, but the color change in T5 was likely due to the coffee husks. The

synthetic antioxidants seemed to improve the redness of the sausage during storage in MAP packaging for T3, but not for T4 and T5, probably due to the brown color provided by the coffee husks.

Sensory overall liking test

The sensory overall liking test was conducted on day 3 of storage for both types of packaging to ensure the quality and safety of the fresh sausages. Packaging conditions affected the liking of the product for 42% of the consumers, indicating that changes occurred in the sausage in a 20%CO_2/80%N_2 atmosphere. Sausages in oxygen-free modified packaging have been shown to have increased sour odors and flavors during storage (Santos et al. 2005). This effect could be due to dissolution of CO_2 in the product and the growth of LAB (Kandeepan and Tahseen 2022). Typical spoilage microorganisms, predominantly pseudomonads, are replaced by LAB when meat products are packaged under vacuum or in modified atmospheres. LAB decrease the pH and cause sensory spoilage of meat products (Santos et al. 2005). These results appeared to be in close agreement with the lower pH values obtained for MAP fresh sausages during storage.

Studies have shown that the use of high CO_2 concentrations (50–80%) or high O_2 (80%) in packaging delays the growth of LAB and improves the shelf life of the product (Santos et al. 2005). However, microbiological quality can also be affected by the composition of the spoilage population in the product. For instance, *Lactobacillus* and *Leuconostoc* are more resistant to CO_2 concentrations (Pexara et al. 2002). In this study, although oxidation of lipids and proteins was lower in MAP, this type of packaging could affect the overall consumer liking of the product. Further studies are needed to find the best gas mixture for modified packaging of this type of product.

For 74% of consumers, sensory acceptance of the fresh sausage was not affected by the added coffee husks (T4 and T5). Previous studies have shown that coffee husk has a low taste profile (Neves et al. 2019). Results in this study confirms these statements. Several reports have shown the sensory acceptance of fresh meat products with plant-derived antioxidants at concentrations from 0.02 to 2%. These natural antioxidants include turmeric extract (de Carvalho et al. 2020), rosemary and green tea extracts (Schilling et al. 2018), and green coffee extract (Valencia et al. 2008).

Conclusion

The addition of coffee husks from Coffea Arabica varieties Caturra and Catuaí to fresh pork sausages reduced lipid oxidation. Although the use of coffee husks as a natural antioxidant affected color parameters of the sausage, sensory overall liking remained unaffected for most of the consumers. Thus, valorization of coffee husks as a food ingredient is an approach for reducing the environmental impact of this industrial waste.

Packaging fresh pork sausages in an oxygen-free modified atmosphere extended the shelf-life in terms of lower oxidation rates, but sensory acceptance of the product was affected. Further research is necessary to ensure oxidative stability and consumer acceptance of fresh sausages with added coffee husks packaged in a modified atmosphere.

Acknowledgements The authors would like to thank the consumers that participated in the sensory panels.

Author contributions AAM Conceptualization, validation, formal analysis, investigation, data curation, writing- original MS, funding project administration and supervision. YAQ Conceptualization, supervised the work, validation, formal analysis, writing-reviewing, and editing. NC Conceptualization, carring out the work, writing-reviewing, and editing. JC Conceptualization, carring out the work, writing-reviewing, and editing. LA Setting of methods, validation of methods, formal analysis, writing-reviewing, and editing.

Funding This research was financed by University of Costa Rica [735-B9-037].

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The consent form and sensory protocols were approved by the Scientific Ethics Committee of the University of Costa Rica (nº VI-4141–2014).

References

Arya SS, Venkatram R, More PR, Vijayan P (2022) The wastes of coffee bean processing for utilization in food: a review. J Food Sci Technol 59:429–444. https://doi.org/10.1007/s13197-021-05032-5

Becerril Sánchez AL, DublánGarcía O, Domínguez-López A et al (2019) La calidad sanitaria del chorizo rojo tradicional que se comercializa en la ciudad de Toluca Estado de México. Rev Mex Ciencias Pecu 10:172–185. https://doi.org/10.22319/rmcp.v10i1.4344

Cao GH, Cutler RG (1995) Protein oxidation and aging 1. Difficulties in measuring reactive protein carbonyls in tissues using 2,4-dinitrophenylhydrazine. Arch Biochem Biophys 320:106–114. https://doi.org/10.1006/abbi.1995.1347

de Carvalho FAL, Munekata PES, Lopes de Oliveira A et al (2020) Turmeric (*Curcuma longa* L.) extract on oxidative stability, physicochemical and sensory properties of fresh lamb sausage with fat

replacement by tiger nut (*Cyperusesculentus* L.) oil. Food Res Int 136:109487. https://doi.org/10.1016/j.foodres.2020.109487
de Florio-Almeida J, Soares-dosReis A, Serafini-Heldt LF et al (2017) Lyophilized bee pollen extract: a natural antioxidant source to prevent lipid oxidation in refrigerated sausages. LWT–Food Sci Technol 76:299–305. https://doi.org/10.1016/j.lwt.2016.06.017
Domínguez R, Pateiro M, Gagaoua M et al (2019) A comprehensive review on lipid oxidation in meat and meat products. Antioxidants 8(10):429
Esquivel P, Viñas M, Steingass CB et al (2020) Coffee (*Coffeaarabica* L.) by-products as a source of carotenoids and phenolic compounds—evaluation of varieties with different peel color. Front Sustain Food Syst. https://doi.org/10.3389/fsufs.2020.590597
Estévez M (2011) Protein carbonyls in meat systems: a review. Meat Sci 89:259–279. https://doi.org/10.1016/j.meatsci.2011.04.025
Estévez M, Morcuende D, Ventanas S (2008) Determination of oxidation. In: Mollet LM, Toldrá F (eds) Handbook of processed meat and poultry analysis. CRC Press, Boca Raton, pp 141–162
Falowo AB, Fayemi PO, Muchenje V (2014) Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: a review. Food Res Int 64:171–181. https://doi.org/10.1016/j.foodres.2014.06.022
Garrido MD, Auqui M, Martí N, Linares MB (2011) Effect of two different red grape pomace extracts obtained under different extraction systems on meat quality of pork burgers. LWT–Food Sci Technol 44:2238–2243. https://doi.org/10.1016/j.lwt.2011.07.003
Gemechu FG (2020) Embracing nutritional qualities, biological activities and technological properties of coffee byproducts in functional food formulation. Trends Food Sci Technol. 104:235–261. https://doi.org/10.1016/j.tifs.2020.08.005
International Coffee Organization (2021) Monthly Coffee Market Report (2021). https: //www.ico.org/documents/cy2020-21/cmr-0621-e.pdf (accessed on 12 August 2021)
Jia N, Kong B, Liu Q et al (2012) Antioxidant activity of black currant (Ribes nigrum L.) extract and its inhibitory effect on lipid and protein oxidation of pork patties during chilled storage. Meat Sci 91:533–539. https://doi.org/10.1016/j.meatsci.2012.03.010
Kandeepan G, Tahseen A (2022) Modified atmosphere packaging (MAP) of meat and meat products: a review. J Packag Technol Res 6:137–148. https://doi.org/10.1007/s41783-022-00139-2
Limbo S, Torri L, Sinelli N et al (2010) Evaluation and predictive modeling of shelf life of minced beef stored in high-oxygen modified atmosphere packaging at different temperatures. Meat Sci 84:129–136. https://doi.org/10.1016/J.MEATSCI.2009.08.035
Lund MN, Hviid MS, Skibsted LH (2007) The combined effect of antioxidants and modified atmosphere packaging on protein and lipid oxidation in beef patties during chill storage. Meat Sci 76:226–233. https://doi.org/10.1016/j.meatsci.2006.11.003
Neves JVD, Borges MV, de Silva DM et al (2019) Total phenolic content and primary antioxidant capacity of aqueous extracts of coffee husk: chemical evaluation and beverage development. Food Sci Technol 39:348–353
Oliver CN, Ahn BW, Moerman EJ et al (1987) Age-related changes in oxidized proteins. J Biol Chem 262:5488–5491
Omahony M, Garske S, Klapman K (1980) Rating and ranking procedures for short cut signal detection multiple difference tests. J Food Sci 45:392–393
Pexara ES, Metaxopoulos J, Drosinos EH (2002) Evaluation of shelf life of cured, cooked, sliced turkey fillets and cooked pork sausages—'piroski'—stored under vacuum and modified atmospheres at +4 and +10 °C. Meat Sci 62:33–43. https://doi.org/10.1016/S0309-1740(01)00224-8
Rebollo-Hernanz M, Cañas S, Taladrid D et al (2021) Revalorization of coffee husk: modeling and optimizing the green sustainable extraction of phenolic compounds. Foods 10(3):653
Redondo-Solano M, Guzmán-Saborío P, Ramírez-Chavarría F et al (2021) Effect of the type of packaging on the shelf life of ground rabbit meat. Food Sci Technol Int. https://doi.org/10.1177/1082013221100370 5
Riel G, Boulaaba A, Popp J, Klein G (2017) Effects of parsley extract powder as an alternative for the direct addition of sodium nitrite in the production of mortadella-type sausages – Impact on microbiological, physicochemical and sensory aspects. Meat Sci 131:166–175. https://doi.org/10.1016/j.meatsci.2017.05.007
Santos EM, Diez AM, González-Fernández C et al (2005) Microbiological and sensory changes in "Morcilla de Burgos" preserved in air, vacuum and modified atmosphere packaging. Meat Sci 71:249–255. https://doi.org/10.1016/j.meatsci.2005.03.028
Schilling MW, Pham AJ, Williams JB et al (2018) Changes in the physiochemical, microbial, and sensory characteristics of fresh pork sausage containing rosemary and green tea extracts during retail display. Meat Sci 143:199–209. https://doi.org/10.1016/J.MEATSCI.2018.05.009
Shahidi F, Zhong Y (2010) Novel antioxidants in food quality preservation and health promotion. Eur J Lipid Sci Technol 112:930–940. https://doi.org/10.1002/ejlt.201000044
Tamkutė L, Gil BM, Carballido JR et al (2019) Effect of cranberry pomace extracts isolated by pressurized ethanol and water on the inhibition of food pathogenic/spoilage bacteria and the quality of pork products. Food Res Int 120:38–51. https://doi.org/10.1016/j.foodres.2019.02.025
Tarladgis BG, Watts BM, Younathan MT, Dugan L Jr (1960) A distillation method for the quantitative determination of malonaldehyde in rancid foods. J Am Oil Chem Soc 37:44–48. https://doi.org/10.1007/BF02630824
Valencia I, O'Grady MN, Ansorena D et al (2008) Enhancement of the nutritional status and quality of fresh pork sausages following the addition of linseed oil, fish oil and natural antioxidants. Meat Sci 80:1046–1054. https://doi.org/10.1016/j.meatsci.2008.04.024
Vegro CLR, Almeida de LF (2020) Chapter 1 - Global coffee market: Socio-economic and cultural dynamics. In: de Almeida LF, Spers EEBTCC (eds), Woodhead Publishing Series in Consumer Sci & Strat Market. Woodhead Publishing, Sawston, pp 3–19
Viljanen K, Kivikari R, Heinonen M (2004) Protein−Lipid interactions during liposome oxidation with added anthocyanin and other phenolic compounds. J Agric Food Chem 52:1104–1111. https://doi.org/10.1021/jf034785e
Zhang L, Lin YH, Leng XJ et al (2013a) Effect of sage (Salvia officinalis) on the oxidative stability of Chinese-style sausage during refrigerated storage. Meat Sci 95:145–150. https://doi.org/10.1016/j.meatsci.2013.05.005
Zhang W, Xiao S, Ahn DU (2013b) Protein oxidation: basic principles and implications for meat quality. Crit Rev Food Sci Nutr 53:1191–1201. https://doi.org/10.1080/10408398.2011.577540