



## Efficacy of green coffee as an antioxidant in beef meatballs compared with ascorbic acid

Heba Sayed Mostafa<sup>a,\*</sup>, Eman Fawzy El Azab<sup>b,c,2</sup>

<sup>a</sup> Food Science Department, Faculty of Agriculture, Cairo University, Giza 12613, Egypt

<sup>b</sup> Department of Clinical Laboratories Sciences, College of Applied Medical Sciences at Al Qurayyat, Jotif University, Al Qurayyat 77454, Saudi Arabia

<sup>c</sup> Biochemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt

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### ABSTRACT

The effect of green coffee addition on the physicochemical attributes, fatty acid profile, and volatiles of beef meatballs was investigated. Five batches were prepared; no antioxidant (control), ascorbic acid (Asc, 500 ppm), and three concentrations of green coffee (GC) powder (250, 500, and 1000 ppm), then aerobically stored onto polypropylene trays at 4 °C for 14 days. The physicochemical properties (pH, color, metmyoglobin, and antioxidant stability) were assessed at 0, 3, 7, and 14 days. Free fatty acids and hydrocarbons were detected by GC–MS as well. GC addition, compared with control and Asc samples, significantly ( $p < 0.05$ ) improved the oxidative stability and scavenging ability, decreased the microbial count by 30.8 %, and negatively affected the redness and metmyoglobin content. Remarkably, at  $\geq 500$  ppm, GC outperformed ascorbic acid in lowering the aliphatic hydrocarbons (877% reduction). Under the conditions tested, GC at 1000 ppm could be recommended to inhibit meat deterioration, particularly fat oxidation.

### Introduction

Meat is one of the staple foods due to its nutritional value, essential amino acids, iron, and vitamin B content (Mäkinen et al., 2020). Grinding meat to make products like burgers and meatballs exposes the muscle surface to more air, resulting in significant lipid autoxidation. The oxidation of lipids is a complicated chain reaction involving unsaturated fatty acids and oxygen. The first oxidation products of this reaction are hydroperoxides, which contribute to cell cytotoxicity (Domínguez et al., 2019). Because these compounds are highly unstable, they decompose rapidly, producing secondary compounds, mainly hydrocarbons (such as tridecane, hexadecane, and heptadecane), aldehydes such as malonaldehyde and *n*-alkanals (Domínguez et al., 2019), and ketones (Amaral et al., 2018).

The primary consequences of meat oxidation reactions include deterioration of meat flavor, primarily from aldehydes, color, and texture, which reduce the shelf life and induce hazardous compound development (Mäkinen et al., 2020). As a result, adding antioxidants through mixing or spraying is the most common strategy used by meat producers to combat this issue (Parvin et al., 2020). BHA (E320) and

BHT (E321), as synthetic antioxidants, and ascorbic acid (E300), a natural antioxidant, are extensively used in the preparation of meat products to preserve color and flavor (Iset et al., 2009; Parvin et al., 2020). As people become more aware of the potential health risks of synthetic substances, there is a renewed interest in using natural substances. Acerola (Realini et al., 2015), green tea (Amaral et al., 2018), nutmeg seed (Parvin et al., 2020), and many spices and herbs such as oregano, rosemary, and thyme, as well as fruit-derived antioxidants such as strawberries and pomegranates (Manassis et al., 2020), have been evaluated as antioxidants in various meat products to prevent lipid or protein oxidation.

Coffee has been consumed since ancient times for its flavor and positive health benefits. It contains a high concentration of polyphenols, particularly hydroxycinnamic acids, as well as caffeic acid and chlorogenic acid derivatives (Klingel et al., 2020). These polyphenols are 8 % higher than those in roasted coffee and are mostly water-soluble. Therefore, the extraction is both safer and less expensive because no organic or toxic solvents are required. Though green coffee has been shown to have a potential antioxidant effect (Budryn et al., 2017; Klingel et al., 2020), no extensive investigation of its effect on meat has been

\* Corresponding author.

E-mail address: [Hebabitech@agr.cu.edu.eg](mailto:Hebabitech@agr.cu.edu.eg) (H. Sayed Mostafa).

<sup>1</sup> ORCID ID: 0000-0001-6173-3871.

<sup>2</sup> ORCID ID: 0000-0001-9463-5506.

reported. This study was conducted to clarify the effectiveness of green coffee as a natural antioxidant in meatballs during cold aerobic storage.

## Materials and methods

### Reagents and standards

Acetonitrile, methanol, formic acid, chloroform (all HPLC grade), thiobarbituric acid, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ascorbic acid were purchased from Sigma-Aldrich, GmbH (Steinheim am Albuch, Germany). Merck (Darmstadt, Germany) provided the Folin-Ciocalteu reagent, Griess reagent, and MilliQ deionized water. Phenolic compound standards were obtained from Fisher Scientific Co. (Hampton, NH, USA). Standard plate count (SPC) medium was obtained from Merck, Darmstadt, Germany, and solidified with 2 % agar.

### Green coffee extract preparation

A vacuumed bag (100 g) of green coffee powder (Orouba Co., 6th October, Giza, Egypt) was purchased from a coffee store. The aqueous coffee extract was prepared by mixing 100 g of dry coffee powder with 500 ml of distilled water (1:5, w/v) and shaking it for 15 min at 80 °C. The obtained extract was stored at -20 °C until use. Total polyphenols in the filtered extract were determined according to Folin-Ciocalteu's method (Singleton et al., 1999) and compared with ascorbic acid. The results were expressed in mg gallic acid equivalent (GAE) per 100 g of dry sample.

### Estimation of antioxidant activity

It was tested by different assays as follows. The DPPH free radical scavenging capacity was evaluated by the fixed reaction time method of Mishra et al. (2012), while the hydrogen peroxide scavenging assay was performed as described by Ma et al. (2019) and estimated at 230 nm using UV-Spectrophotometer Unico-UV2000, USA. The Boora et al. (2014) technique was used to assess the green coffee extract against nitric oxide radical scavenging (NO) activity. The results of all these assays were calculated using Equation (1) and expressed as percentage scavenging.

$$\% \text{Scavenging} = \frac{A_{\text{control}} - A_{\text{extract}}}{A_{\text{control}}} * 100$$

### HPLC analysis of phenolic compounds

The bioactive polyphenol compounds in aqueous coffee extract were quantified using the El Azab and Mostafa system (2022). The injector was outfitted with a 20-l loop and a PU-1580 pump and UV-157 detector were applied. The sample was injected into a stainless-steel discovery C184 column, which was then run at a flow rate of 1 ml/min at 25 °C. Two solvents were used: A: 0.05 % formic acid and B: acetonitrile/methanol (80:20 v/v). The gradient conditions were as follows: 0–5 min, 10 % B; 5–15 min, 10–18 % B; 15–25 min, 18 % B; 25–30 min, 18–25 % B; 30–35 min, 25 % B; 35–40 min, 25–35 % B; 40–45 min, 35–60 % B; 45–50 min 60–10 % B, and 50–55 min with 10 % B. The existent phenolic compounds were recognized by comparing their retention times (Rt.) with individual standards.

### Meatballs preparation

Fresh beef meat (57 % moisture, 9.66 % protein, and 3 % fat content) and backfat were purchased from a local meat grocery store in Giza, Egypt. The meat and fat were minced separately twice using a 3-grind plate. The meatballs were formulated with the following basic ingredients: 84 % meat, 14 % back fat, and 2 % salt. The mixture was hand-mixed for 5 min before being divided into 5 batches into three

replicates. The first batch was labeled as the control group (without antioxidants). T1, T2, and T3 batches contained 250, 500, and 1000 ppm green coffee powder, respectively. The fifth batch (Asc) contained 500 ppm of ascorbic acid as permitted by the Arab Republic of Egypt Standards (No. 1973/2005). Each meatball (approximately 27 ± 2 mm, 20 g) was hand-shaped before being packed onto polypropylene plastic trays in polyethylene bags and stored at 4 °C. Samples were randomly withdrawn for analysis at zero time and after 3, 7, and 14 days (Oz, 2019).

### Analytical methods

#### pH

For the pH test, 3 g of the meatball was homogenized for 30 sec in 27 ml of purified water. The slurry was set at room temperature for 5 min. A pH meter (301, Orion Research, Boston, USA), calibrated with 4.0 and 9.0 standard buffer solutions, was then used to measure pH (Parvin et al., 2020).

#### Color and metmyoglobin determination

A colorimeter (CR-300, Konica Minolta, Tokyo, Japan) was used to measure the color characteristics of the exterior surface of the raw meatballs. The color values, i.e., a\* (redness), b\* (yellowness), and L\* (lightness) were assessed in triplicate (Mashau et al., 2021). Metmyoglobin percentage was calculated using Equation (2) by measuring the absorbance of the meat supernatant at 525, 545, 565, and 575 nm, as described by Ouerfelli et al. (2019).

$$\text{Metmyoglobin}(\%) = \left[ -2.51 \times \left( \frac{A_{572}}{A_{525}} \right) + 0.777 \times \left( \frac{A_{565}}{A_{525}} \right) + 0.8 \right. \\ \left. \times \left( \frac{A_{545}}{A_{525}} \right) + 1.098 \right] * 100$$

#### TBARs determination

The lipid oxidation of raw meatballs was assessed by thiobarbituric acid reactive substances (TBARs) assay as described by Fan et al. (2019). Briefly, 5 g of sample + 15-ml TCA containing 0.1 % EDTA were mixed for 30 sec before filtering. Three milliliters of the filtrate were mixed with 3-ml TBA and heated in closed tubes in a boiling water bath for 40 min. The absorbance of the cooled mixture was measured by UV-Spectrophotometer (Unico-UV2000, USA) set at a wavelength of 532 nm.

#### Antioxidant capacity and maintenance

The antioxidant activity and maintenance of the raw meatballs during cold storage were assessed by determining lipophilic and hydrophilic DPPH (L-DPPH and H-DPPH) with 0.04 mM of methanolic DPPH (Gallego et al., 2015). L-DPPH was measured in the extract of acetone: ethanol: MilliQ-water (5:4:1, v/v), whereas MilliQ-water was the extraction solvent for the H-DPPH assay. Equation (1) was used to calculate the results, which were represented as scavenging %. This test gives an indication of the antioxidant capacity and stability of the added green coffee compared to Asc and control samples.

#### Microbiological examination

The microbial analysis of meatball samples was carried out on days 0, 3, 7, and 14. Samples of 10-g meatballs were diluted with 90 ml of 0.85 % sterile saline to create a 10<sup>-1</sup> dilution for analysis. Different dilutions were prepared and plated with SCP medium before being incubated for 72 h at 30 ± 1 °C for total viable counts (TVC) and 7 days at 4–5 ± 1 °C for psychophilic. The results were expressed as log<sub>10</sub> CFU (colony forming units) per g of ground meat (Realini et al., 2015).

### Fatty acid profile

The total lipids were extracted with the solvent chloroform: methanol (2:1, v/v), then fatty acid methyl esters (FAMES) were synthesized by the sulfuric acid derivatization method described by Hewavitharana et al. (2020). Gas-chromatography-mass spectrometry (GC-MS) analysis using a Trace GC 1310-ISQ Mass Spectrometer (Thermo Fisher Scientific, Austin, TX, USA) was utilized to estimate the fatty acids and volatile compounds of raw meatballs. A TG-5MS direct capillary column (length of 30 m, ID 0.25 mm, and film thickness of 0.25  $\mu\text{m}$ ) was used. The column oven's temperature was initially held at 50  $^{\circ}\text{C}$ , then raised by 5  $^{\circ}\text{C}/\text{min}$  to 230  $^{\circ}\text{C}$  and held for an additional 2 min. The temperature was then increased at a rate of 30  $^{\circ}\text{C}/\text{min}$  to 290  $^{\circ}\text{C}$  and held for 2 min. The MS transfer line, injector, and ion source, on the other side, were kept at 260, 250, and 200  $^{\circ}\text{C}$ , respectively. Helium gas was the carrier gas that was kept at a flow rate of 1 ml/min. The diluted sample of 1  $\mu\text{l}$  was injected automatically using the Autosampler S1300 coupled with the GC in the split mode. EI mass spectra were collected in a full scan mode at 70 eV. The mass spectral databases WILEY 09 and NIST 11 were used to identify the components and their mass spectra (Zhao et al., 2018). The lipid quality indices, nutritive value index (NVI), hypocholesterolemic/hypercholesterolemic ratio (HH), index of atherogenicity (IA), and health-promoting index (HPI) were calculated based on the fatty acid profile (Chen & Liu, 2020) by the following Equations.

$$\text{NVI} = \frac{\text{C18:0} + \text{C18:1}}{\text{C16:0}}$$

$$\text{HH} = \frac{\text{C18:1 cis} + \text{PUFA}}{\text{C12:0} + \text{C14:0} + \text{C16:0}}$$

$$\text{IA} = \frac{\text{C12:0} + (4 * \text{C14:0}) + \text{C16:0}}{\Sigma\text{UFA}}$$

$$\text{HPI} = \frac{\Sigma\text{UFA}}{\text{C12:0} + (4 * \text{C14:0}) + \text{C16:0}}$$

### Statistical analysis

All results were evaluated using a one-way analysis of variance (ANOVA). A significance level of  $p < 0.05$  was used to assess the significant differences between the treatment means, storage period, and interaction. Duncan's test was used to calculate and statistically compare all data with three replicated mean values using the Costat software.

## Results and discussion

### Antioxidant activity of green coffee

**Supplementary 1** contains information on the polyphenol content and antioxidant capacity of green coffee aqueous extract as determined by various tests (e.g., DPPH,  $\text{H}_2\text{O}_2$ , and NO). The coffee extract contained polyphenols of 391.6 mg GAE/100 g dry weight and outperformed ascorbic acid in all three tests, despite the fact that ascorbic acid is a pure compound and the coffee extract contains a percentage of antioxidant compounds. That indicates that green coffee is a powerful antioxidant, as proved by many studies. Masek et al. (2020) evaluated green coffee's antioxidant capability using DPPH, FRAP, and ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assays. They reported a value of 80 % DPPH inhibition by 4 mg/ml of green coffee, which is lower than the value given in this study.

The main polyphenol compounds identified in the aqueous green coffee extract are listed in **Supplementary 2**. In descending order, the most important antioxidant and phytochemical substances were chlorogenic acid, cinnamic acid, caffeic acid, and *p*-coumaric acid. Benzoic acid and syringic acid also existed in the obtained extract, but at

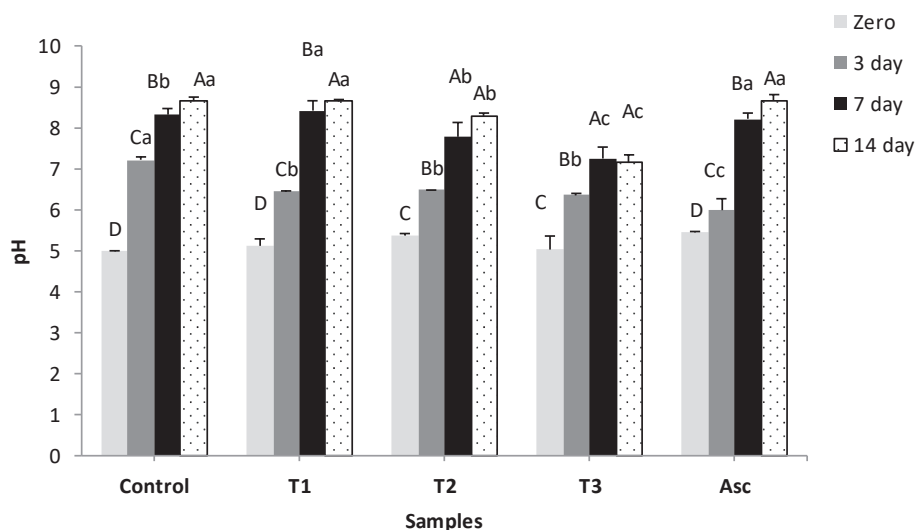
too low concentrations to contribute effectively to its antioxidant capacity. Similarly, Ramalakshmi et al. (2007) observed a significant quantity of chlorogenic acid in green coffee beans (6.35–8.8 %). To reap the full benefits of the bioactive compounds in green coffee, the powder rather than the aqueous extract was applied directly to ground meatballs. Because the meat contains > 50 % moisture, that means all water-soluble and non-soluble compounds may have an effect.

### The effect of green coffee addition on beef meatballs' quality

#### Physicochemical properties.

The influence of cold storage and green coffee powder concentration on the pH levels of meatballs is presented in Fig. 1. At the beginning of the trial, there was no significant ( $p \geq 0.05$ ) difference between the pH values of all treatments and control samples. The pH was significantly increased from acidic values by increasing the storage time in all treatments, reaching the highest values (ranging from 8.63 to 8.66) in the control, T1, and ascorbic acid samples after 2 weeks of storage. After one week of cold storage, the coffee powder addition had a substantial effect on pH values that decreased with increasing coffee concentration. That is due to green coffee's low pH of 5.7–6.4 and the presence of phenolic compounds, especially acids such as caffeic acid, cinnamic acid, and *p*-coumaric acid (Ramalakshmi et al., 2007; Espíndola et al., 2019). Two weeks after storage, T3 treatment showed the lowest pH (7.14) value. That means it kept its pH neutral throughout the cold storage period. That suggests a possible inactivation of lactic acid bacteria and inhibition of substrate decomposition. According to Ouerfelli et al. (2019), the increase in pH is caused by microbes and enzymes that degrade meat proteins and yield basic compounds such as amines and ammonia. Cantú-Valdéz et al. (2020) likewise noticed a rise in pH during the storage of oregano oil-added meat.

**Table 1** illustrates the color changes of the various meatball samples during storage based on the perception of the apparel meat color. Generally, at zero time, the color parameters  $a^*$  and  $b^*$  of raw samples were negligible across samples, except between the control and T1 and Asc samples. After 3 days of storage, the color redness values were significantly affected ( $p < 0.05$ ) by the green coffee percentage and the storage period. Increased storage time reduced the redness of both the control and ascorbic-added samples, but ascorbic acid, followed by the free sample, exhibited higher red  $a^*$  values than green coffee samples (T1, T2, and T3). The control sample discolored after 3 days of cold storage, which was also observed by Realini et al. (2015), who observed the discoloration of beef patties between 3 and 6 days of cold storage. Myoglobin oxidation and the beginning development of metmyoglobin, a brownish hue, might explain the fading of the most crucial color parameter,  $a^*$ , in all samples (Ouerfelli et al., 2019). Increasing the percentage of green coffee powder from 250 to 1000 ppm lowered  $a^*$  values considerably (from 9.3 to 5.32 after 3 days of storage), with T3 having the lowest  $a^*$  value (61 % reduction). There were no significant variations in  $a^*$  values between the control and green coffee-added sample at a concentration of 250 ppm during the cold storage, indicating that this concentration had no impact. Green coffee at 500 and 1000 ppm, on the other hand, considerably affected the raw red color stability compared with ascorbic acid and free meatballs. This may be attributed to the existence of chlorogenic acid, which becomes green when oxidized (Wildermuth et al., 2016). The resulting color may have obscured and darkened the appearance of the meatballs. Some natural antioxidants such as taurine and carnosine have also been shown to reduce  $a^*$  values in beef patties during cold storage compared with ascorbic acid alone (Sa'nchez-Escalante et al., 2001) and in deer burgers containing healthier oils (Vargas-Ramella et al., 2020). Additionally, the maintenance of redness by ascorbic acid was repeatedly reported in meat (Is et al., 2009), whereas improved  $a^*$  values were achieved by ascorbic acid + rosemary (Sa'nchez-Escalante et al., 2001), acerola (Realini et al., 2015), and *Moringa olivera* leaf powder (Mashau et al., 2021). Regarding the yellowness value,  $b^*$ , a similar trend was observed.



**Fig. 1.** pH values of raw meatballs during cold storage for 14 days.  $\pm$  T1, T2, and T3 meatballs contain 250, 500, and 1000 ppm of green coffee powder, respectively. Asc sample is the meatballs contain 500 ppm of ascorbic acid. \* Capital letters denotes significance difference ( $p < 0.05$ ) in the same sample between days of storage. Different small letters denote significant differences ( $p < 0.05$ ) between samples.

**Table 1**

Effects of time (0, 3, 7, and 14 days of storage), and green coffee addition on color parameters ( $a^*$ ,  $b^*$  and  $L^*$ ) of raw meatballs during cold storage for two weeks (mean  $\pm$  SD).

$\pm$ Sample	$a^*$				$b^*$				$L^*$			
	0	3	7	14	0	3	7	14	0	3	7	14
Control	12.12 <sup>A</sup> $\pm$ 2.17	6.04 <sup>Bc</sup> $\pm$ 0.01	9.38 <sup>Aa</sup> $\pm$ 0.17	9.13 <sup>Ac</sup> $\pm$ 0.02	3.81 <sup>ABab</sup> $\pm$ 0.90	3.05 <sup>Bb</sup> $\pm$ 0.07	3.72 <sup>Bb</sup> $\pm$ 0.11	5.05 <sup>Aa</sup> $\pm$ 0.07	35.48 <sup>C</sup> $\pm$ 2.17	37.26 <sup>Bcd</sup> $\pm$ 0.05	39.35 <sup>Bd</sup> $\pm$ 0.21	42.48 <sup>Aa</sup> $\pm$ 3.91
T1	13.18 <sup>A</sup> $\pm$ 0.67	7.04 <sup>Ba</sup> $\pm$ 0.00	7.78 <sup>Bb</sup> $\pm$ 0.00	12.30 <sup>Aa</sup> $\pm$ 1.14	3.78 <sup>ABab</sup> $\pm$ 0.79	1.44 <sup>Ce</sup> $\pm$ 0.22	3.48 <sup>Bc</sup> $\pm$ 0.05	4.76 <sup>Ab</sup> $\pm$ 0.03	37.91 <sup>B</sup> $\pm$ 1.18	35.27 <sup>Ce</sup> $\pm$ 0.02	40.74 <sup>Ab</sup> $\pm$ 0.02	38.86 <sup>Bb</sup> $\pm$ 0.10
T2	13.18 <sup>A</sup> $\pm$ 0.01	6.31 <sup>Cb</sup> $\pm$ 0.32	5.60 <sup>Dd</sup> $\pm$ 0.07	8.20 <sup>Bd</sup> $\pm$ 2.02	4.35 <sup>Aab</sup> $\pm$ 0.12	2.67 <sup>Dc</sup> $\pm$ 0.32	4.07 <sup>Ba</sup> $\pm$ 0.02	3.20 <sup>Cd</sup> $\pm$ 0.42	37.01 <sup>C</sup> $\pm$ 0.42	38.37 <sup>Bb</sup> $\pm$ 2.00	39.72 <sup>Ac</sup> $\pm$ 2.15	37.77 <sup>Bd</sup> $\pm$ 0.17
T3	11.11 <sup>A</sup> $\pm$ 0.54	5.30 <sup>Bd</sup> $\pm$ 0.14	5.38 <sup>Be</sup> $\pm$ 0.12	4.32 <sup>Ce</sup> $\pm$ 0.00	2.96 <sup>Bb</sup> $\pm$ 0.41	3.16 <sup>Ba</sup> $\pm$ 0.01	4.17 <sup>Aa</sup> $\pm$ 0.16	2.73 <sup>Be</sup> $\pm$ 0.10	38.46 <sup>A</sup> $\pm$ 3.00	37.36 <sup>Ac</sup> $\pm$ 1.51	41.44 <sup>Aa</sup> $\pm$ 0.02	38.37 <sup>Ac</sup> $\pm$ 3.33
Asc	13.22 <sup>A</sup> $\pm$ 0.64	6.92 <sup>Ca</sup> $\pm$ 0.08	7.09 <sup>Cc</sup> $\pm$ 1.01	10.62 <sup>Bb</sup> $\pm$ 0.03	4.93 <sup>Aa</sup> $\pm$ 1.17	2.21 <sup>Dd</sup> $\pm$ 0.03	2.88 <sup>Cd</sup> $\pm$ 0.72	3.47 <sup>Bc</sup> $\pm$ 0.02	41.16 <sup>A</sup> $\pm$ 2.97	41.13 <sup>Aa</sup> $\pm$ 3.03	39.33 <sup>Ad</sup> $\pm$ 0.22	37.88 <sup>Ad</sup> $\pm$ 0.11

$\pm$ T1, T2 and T3 meatballs contain 250, 500 and 1000 ppm of green coffee powder, respectively. Asc sample is the meatballs contain 500 ppm of ascorbic acid.

\* Capital letters within columns denotes significance difference ( $p < 0.05$ ) in the same color parameter between days of storage. Different small letters within rows denote significant differences ( $p < 0.05$ ) between samples.

The green coffee-added samples had lower  $b^*$  values during storage compared to the control and Asc samples. Ouerfelli and colleagues (2019) also stated a reduction in the yellowness of beef patties containing neem (*Azadirachta indica* L.) during refrigerated storage from 15.25 to 10.27 after 11 days.

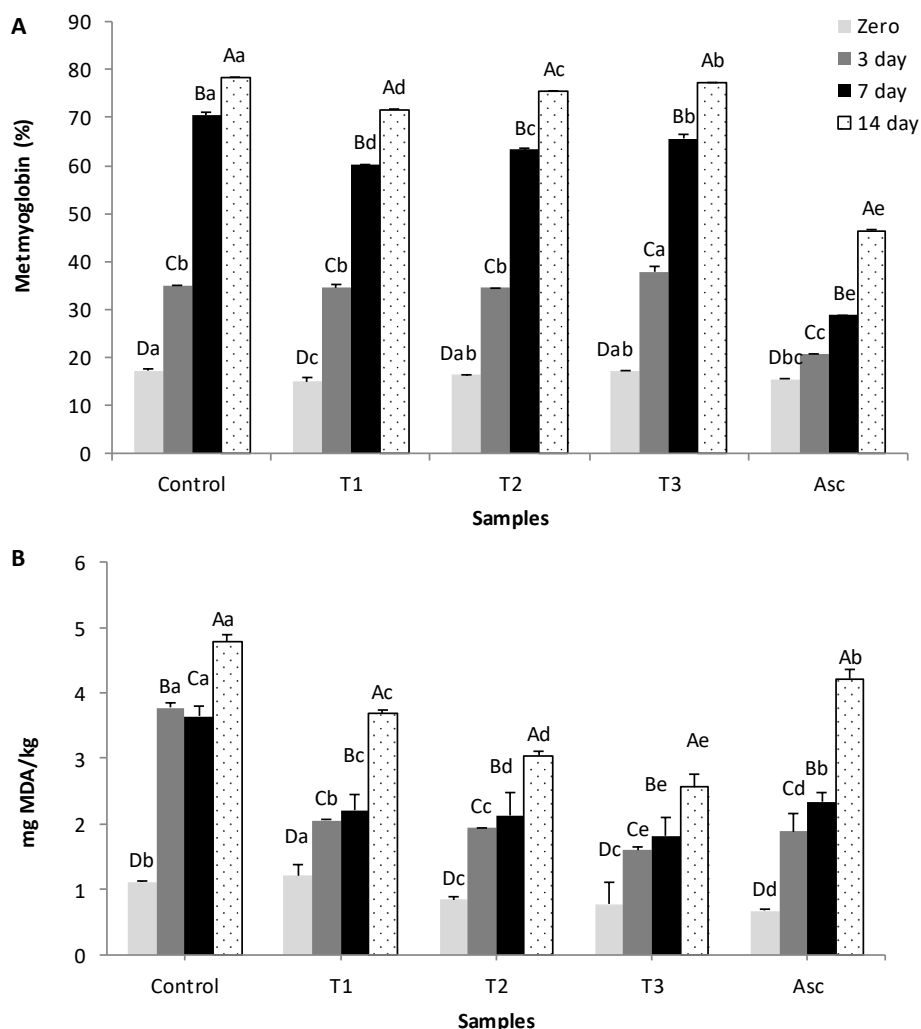
The lightness of all meatball samples progressively decreased during storage, although it remained stable in T1 and T2 for 1 week ( $p \geq 0.05$ ). From the beginning through the completion of the trial, the lightness of the green coffee-added samples was greater than the control and lower than the Asc samples, and it rose as the green coffee amount increased. This might be linked to the antioxidant effects of green coffee. Other studies also reported a reduction in the  $L^*$  value of ground beef samples by the addition of *Moringa olivera* leaf powder during cold storage for 15 days (Mashau et al., 2021). Instrumental assessment of meat color indicates that the addition of green coffee to meatballs has a detrimental influence on their color. It also appears that ascorbic acid at 500 ppm, followed by green coffee at 1000 ppm, can preserve the lightness of the prepared samples.

#### Metmyoglobin content

After slaughter and in well-bled carcasses, myoglobin accounts for 95 % of the total iron in the meat. Myoglobin is a circular protein that comprises a heme group surrounded by a globin moiety. The primary

factors responsible for myoglobin's color and, accordingly, the color of meat are the state of the iron atom and the ligand bond to the free binding site of the heme. When meat muscle is exposed to oxygen, oxygen binds to the free binding site of reduced myoglobin, resulting in the desirable red meat. Brown metmyoglobin, on the other hand, is caused by the oxidation of iron in heme by losing an electron, which changes the iron molecule from  $Fe^{++}$  to  $Fe^{+++}$  (Seideman et al., 1984).

The metmyoglobin percentage of the meatballs over 14 days of refrigerated storage is shown in Fig. 2 A. Metmyoglobin levels in all meatball samples increased progressively ( $p < 0.05$ ) as the experiment progressed. This validated the redness  $a^*$  values of these samples (Table 1). Starting from the third day of storage, the metmyoglobin significantly differed between samples substantially, in which the greatest percentage was found in the T3 sample, followed by the control. The green coffee percentage significantly increased the metmyoglobin formation ( $p < 0.05$ ). The lowest relative amount of metmyoglobin ( $p < 0.05$ ) was formed in ascorbic acid meatballs (15.5–46.4 %). For 3 days of storage, all samples maintained the critical level of metmyoglobin (30–40 %), and ascorbic acid preserved this percentage till the end of the experiment. Many authors (Sa'nchez-Escalante et al., 2001; Iset al., 2009) have agreed on the effectiveness of ascorbic acid in delaying the oxidation of meat pigments. Ascorbic acid can scavenge free radicals and oxygen and acts as a reducing agent by keeping the heme-protein in a



**Fig. 2.** (A) Metmyoglobin (%) and (B) TBARS (mg/kg) values of raw meatballs during cold storage for 14 days.  $\pm$  T1, T2, and T3 meatballs contain 250, 500, and 1000 ppm of green coffee powder, respectively. Asc sample is the meatballs contain 500 ppm of ascorbic acid. \* Capital letters denotes significance difference ( $p < 0.05$ ) in the same sample between days of storage. Different small letters denote significant differences ( $p < 0.05$ ) between samples.

reduced non-catalytic form (Amaral et al., 2018). All green coffee-added samples, on the other hand, surpassed the metmyoglobin acceptance limit after a week of storage, as previously observed by the low redness  $a^*$  values. That means that green coffee had no inhibitory effect on myoglobin oxidation after one week of storage. pH could be considered a factor affecting metmyoglobin formation, as low pH can cause the myoglobin fraction to be readily oxidized to metmyoglobin (Seideman et al., 1984). That could explain this phenomenon, as green coffee reduces pH, especially after a week.

#### Oxidative stability

The direct addition of green coffee powder to meatballs was evaluated against the formation of malonaldehyde, aldehyde compounds, and ketones resulting from lipid oxidation (Fig. 2 B). Storage and the added antioxidant influenced TBARS values of the meatballs. The control sample had the greatest TBARS values, which gradually increased with storage time. Compared to the ascorbic-added sample, green coffee addition (from 250 to 1000 ppm) was more effective as an antioxidant during storage for 14 days. T1, T2, and T3 meatball batches were kept below the oxidation acceptability limit of 2.5 mg MDA/kg for 7 days (Zhang et al., 2019), but only the T3 meatball sample was kept below this limit for even two weeks. Starting at 7 days and continuing until the end of the experiment, T1 and T2 samples had lower MDA/kg (2.21–3.69 and 2.13–3.04, respectively) than both ascorbic acid and

control samples. The effectiveness of the hydrophilic antioxidant, ascorbic acid, in reducing TBARS level was observed at zero-day, but it declined after 3 days as the storage duration under cooling increased. Compared to green coffee powder, ascorbic acid addition to meatballs only had an antioxidant impact for just 3 days under aerobic cold storage. Iset et al. (2009) also observed increased TBARS values in ascorbic-containing beef patties after 3 days of refrigerated storage.

Because of its high caffeic acid concentration, green coffee has been found to be an efficient inhibitor of lipid peroxidation. Caffeic acid acts as a metal chelator (mainly iron and copper) as well as a hydrogen donor, and it could prevent the harmful action promoted by lipid-derived peroxy and alkoxy radicals. Caffeic acid may also be responsible for controlling oxidation by reducing metals. The catechol group with a chain of  $\alpha$ ,  $\beta$ -unsaturated carboxylic acid in its structure is accountable for its interactions with different kinds of oxidizing radicals (Espíndola et al., 2019). Because of the presence of hydrophobic benzenoid rings, and the capacity of phenolic hydroxyl groups to form hydrogen-bonding connections, ellagic acid also has a positive effect. It might interact with enzymes that produce radicals, such as lipoxygenases, preventing oxidation (Masek et al., 2020). Previously, Gawlik-Dziki et al. (2014) proved the lipoxygenase inhibition activity of green coffee. Lipoxygenase can oxygenate polyunsaturated fatty acids (PUFAs) forming fat hydroperoxides. Lipoxygenase may thus play a role in the onset of lipid peroxidation in this type of meat (Domínguez et al.,

2019); and inactivating it may help break the cycle. Many researchers observed a decline in TBARs when applying antioxidants such as black cumin in meatballs (Oz, 2019) and berry leaf extract in pork sausage (Mäkinen et al., 2020) compared with ascorbic acid. However, during cold preservation for 18 days, the TBARs values of deer burgers prepared with linseed oil and chia were higher than control (Vargas-Ramella et al., 2020).

#### Antioxidant capacity and maintenance

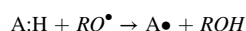
The antiradical ability of green coffee assessed by L-DPPH and H-DPPH over 2 weeks of refrigerated storage is illustrated in Fig. 3 A and B of this research. The L-DPPH assay (Fig. 3 A) revealed reduced antioxidant activity compared to the H-DPPH assay (Fig. 3 B). Similar results were obtained by Gallego et al. (2015), where *Casalpinia decapetala* extract had hydrophilic FRAP assay values higher than those of lipophilic ones in beef meat patties. Contrarily, Ouerfelli et al. (2019) observed H-DPPH values lower than L-DPPH in chilled meat patties containing neem (*Azadirachta indica* L.), a natural antioxidant herbal plant.

Fig. 3 A also shows substantial ( $p < 0.05$ ) variations in L-DPPH scavenging between samples and the time of analysis, all of which are decreased by extending the cold storage period. Green coffee and ascorbic acid improved the hydrophilic antioxidant capacity of the free-antioxidant sample (control). The meatballs formulated with 1000 ppm green coffee powder had the maximum-scavenging value at zero and the third day of storage (ranging from 2.74 to 2.4 % scavenging), but after a week, the value dropped drastically. Although the T2 sample (500 ppm) has a higher scavenging capacity than the control, T1, and Asc samples

at zero-day, this capacity drops lower than the Asc and T1 samples after 3 days of storage. After one week, L-DPPH scavenging percentages were greater in T1, T2, as well as, T3 compared with the ascorbic-containing and control formulas. This demonstrates the antioxidative effect of this type of coffee, which was maintained for 2 weeks compared with ascorbic acid, which lost 25.94 % and 48.33 % of its efficacy after three days and one week of cold storage, respectively.

Regarding the H-DPPH values (Fig. 3 B), they also exhibited a similar trend, where there are significant differences between all samples and between the storage intervals. Compared with the control and ascorbic acid-containing samples, the formulas including green coffee powder (500 and 1000 ppm) had significantly higher H-DPPH values (3–3.5 % at zero time) until the second week of storage (2–2.2 %). These findings support the hydrophilic antioxidative action of green coffee.

As demonstrated in the following equation, the capacity of an antioxidant is affected by its ability to donate hydrogen radicals to free radicals to avoid oxidative harm.



Since green coffee is rich in phenolic compounds (Supplementary 2), this may explain the mode of its action on the physicochemical properties of preserved meatballs. Antioxidants are classified based on their actions into categories. Green coffee can be classified as one of the primary antioxidants, which stop free-radical chain reactions by donating electrons or hydrogen. This possible effect might be due to its *p*-coumaric acid and cinnamic acid content. Both acids include phenolic hydroxyl, which may directly scavenge reactive oxygen species, for

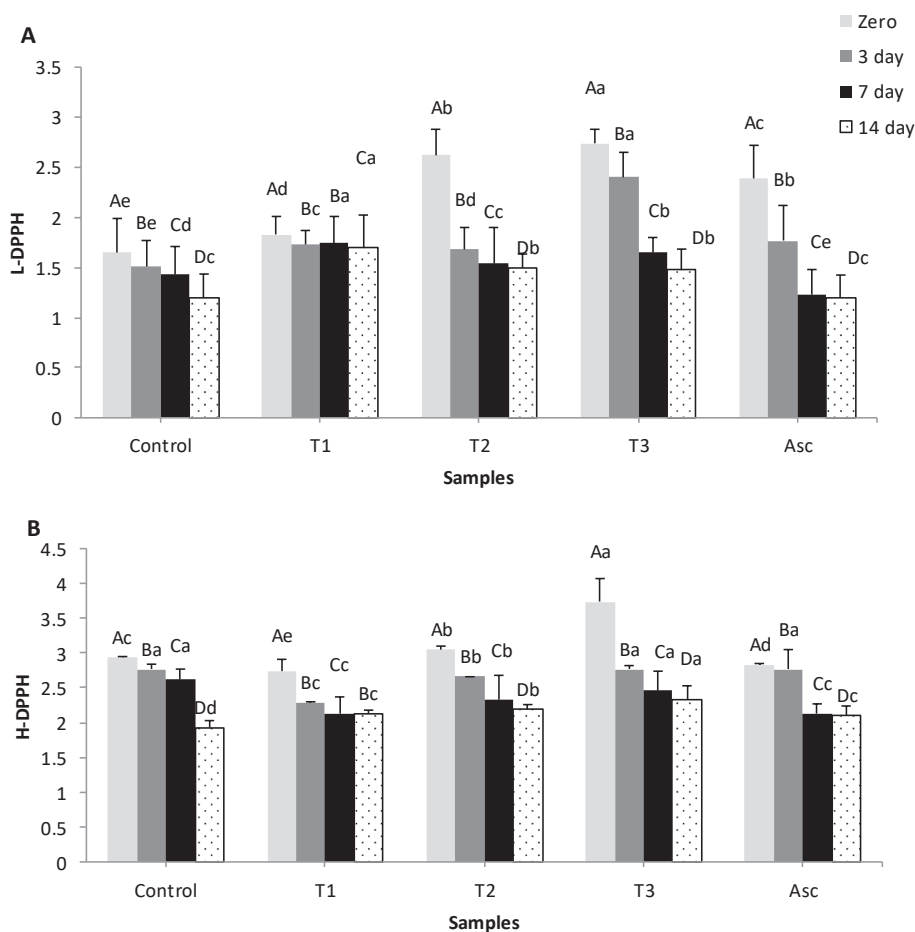


Fig. 3. Antioxidant capacity as measured by L-DPPH (A) and H-DPPH (B) of raw meatballs during cold storage for 14 days.  $\pm$  T1, T2, and T3 meatballs contain 250, 500, and 1000 ppm of green coffee powder, respectively. Asc sample is the meatballs contain 500 ppm of ascorbic acid. \* Capital letters denotes significance difference ( $p < 0.05$ ) in the same sample between days of storage. Different small letters denote significant differences ( $p < 0.05$ ) between samples.

instance, hydroxyl radical, superoxide anion, and hydrogen peroxide, which may attack biological molecules, i.e., fats, proteins, and enzymes (Shen et al., 2019). Because they include phenols with 2-OH groups, chlorogenic acid, the major phenolic ingredient in green coffee, and caffeic acid have a strong antioxidant impact by donating hydrogen atoms to reduce free radicals and limit oxidation processes (Liang & Kitts, 2015). Many plant extracts with high levels of phenolic compounds demonstrate a protective effect in decreasing lipid oxidation in meat due to the OH groups bonded to the phenolic ring. Green tea, which contains catechins and polyphenolic flavonoids, and grape seed extract, which contains caffeic acid, proanthocyanidins, resveratrol, gallic acid, and epigallocatechin, are two examples (Amaral et al., 2018).

#### Microbial count

There was a significant effect ( $p < 0.05$ ) of green coffee addition to meatballs on the microbial counts, either total counts or psychrophilic bacteria, throughout the experiment (Supplementary 3). Increasing the content of green coffee in meatballs altered the microbial status of the samples, showing that green coffee had an antimicrobial effect compared with the control and ascorbic acid. That indicates that green

coffee is more efficient than other commonly used meat antioxidants such as acerola, which had no antimicrobial effect (Realini et al., 2015). Different green coffee extracts were found to be antibacterial (MIC = 15.62–31.25 mg/ml), owing to the high content of caffeic acid and chlorogenic acid (Tasew et al., 2020).

#### Free fatty acid profile

The fatty acids of the different meatball formulations are listed in Table 2. The major fatty acids identified in all samples were SFA followed by MUFA, and then PUFA. Palmitic acid (C16:0; 32.51–36.02 g/100 g fatty acids), oleic acid (C18:1n-9; 18.34–24.14 g/100 g fatty acids), followed by stearic acid (C18:0; 14.83–18.77 g/100 g of fatty acids) were the most abundant fatty acids. A similar profile was detected in beef steers fed soybean oil-containing diets (Castro et al., 2016).

At zero time, the addition of green coffee substantially reduced SFA and PUFAs and increased MUFAs. The cooling storage for two weeks resulted in a significant drop in MUFAs in both the control and Asc samples. SAFs, on the other hand, were gradually decreased by increasing the green coffee percentage in meatballs (from 69.19 to 64.16 g/100 g fatty acids). It is well known that the various mechanisms that occur during cold storage, such as lipid oxidation and water loss,

**Table 2**

Fatty acid composition of raw meatballs at zero time and after two weeks of cold storage (g/100 g fatty acid), mean  $\pm$  SD.

Fatty acid	$\pm$ Control	T1	T2	T3	Asc	Control	T1	T2	T3	Asc
	Zero time					After 2 weeks				
C10:0	0.18 <sup>c</sup> $\pm$ 0.05	0.41 <sup>a</sup> $\pm$ 0.14	0.34 <sup>b</sup> $\pm$ 0.02	0.33 <sup>b</sup> $\pm$ 0.03	0.17 <sup>d</sup> $\pm$ 0.00	0.00 <sup>e</sup> $\pm$ 0.00	0.45 <sup>a</sup> $\pm$ 0.34	0.25 <sup>c</sup> $\pm$ 0.04	0.11 <sup>d</sup> $\pm$ 0.25	0.31 <sup>b</sup> $\pm$ 0.16
C12:0	1.19 <sup>b</sup> $\pm$ 0.04	2.53 <sup>a</sup> $\pm$ 0.07	1.16 <sup>c</sup> $\pm$ 0.06	0.90 <sup>d</sup> $\pm$ 0.04	1.20 <sup>b</sup> $\pm$ 0.02	2.51 <sup>a</sup> $\pm$ 0.41	0.90 <sup>b</sup> $\pm$ 0.27	0.75 <sup>d</sup> $\pm$ 0.06	0.59 <sup>e</sup> $\pm$ 0.03	0.78 <sup>c</sup> $\pm$ 0.42
C14:0	12.66 <sup>b</sup> $\pm$ 0.02	13.20 <sup>a</sup> $\pm$ 0.05	11.86 <sup>c</sup> $\pm$ 0.15	11.65 <sup>e</sup> $\pm$ 0.14	12.67 <sup>d</sup> $\pm$ 0.01	12.77 <sup>b</sup> $\pm$ 0.35	12.05 <sup>c</sup> $\pm$ 0.61	11.55 <sup>d</sup> $\pm$ 0.15	11.18 <sup>e</sup> $\pm$ 0.00	13.83 <sup>a</sup> $\pm$ 0.31
C15:0	1.77 <sup>b</sup> $\pm$ 0.03	1.91 <sup>a</sup> $\pm$ 0.12	1.77 <sup>b</sup> $\pm$ 0.24	1.77 <sup>b</sup> $\pm$ 0.12	1.63 <sup>c</sup> $\pm$ 0.04	2.10 <sup>b</sup> $\pm$ 0.03	1.97 <sup>d</sup> $\pm$ 0.31	2.00 <sup>cd</sup> $\pm$ 0.12	2.20 <sup>a</sup> $\pm$ 0.12	2.04 <sup>c</sup> $\pm$ 0.04
C16:0	35.40 <sup>c</sup> $\pm$ 0.06	35.21 <sup>d</sup> $\pm$ 0.14	35.80 <sup>b</sup> $\pm$ 0.23	36.02 <sup>a</sup> $\pm$ 0.17	34.79 <sup>e</sup> $\pm$ 0.12	33.26 <sup>d</sup> $\pm$ 0.04	35.22 <sup>b</sup> $\pm$ 0.21	34.92 <sup>c</sup> $\pm$ 0.25	32.51 <sup>e</sup> $\pm$ 0.01	35.26 <sup>a</sup> $\pm$ 0.05
C17:0	3.26 <sup>a</sup> $\pm$ 0.01	2.00 <sup>b</sup> $\pm$ 0.21	1.77 <sup>c</sup> $\pm$ 0.14	1.52 <sup>d</sup> $\pm$ 0.08	1.43 <sup>e</sup> $\pm$ 0.02	1.36 <sup>e</sup> $\pm$ 0.13	2.06 <sup>c</sup> $\pm$ 0.26	2.18 <sup>b</sup> $\pm$ 0.26	2.52 <sup>a</sup> $\pm$ 0.00	1.82 <sup>d</sup> $\pm$ 0.07
C18:0	16.56 <sup>c</sup> $\pm$ 0.33	18.77 <sup>a</sup> $\pm$ 0.03	18.49 <sup>b</sup> $\pm$ 0.06	18.77 <sup>a</sup> $\pm$ 0.91	16.12 <sup>d</sup> $\pm$ 0.03	16.94 <sup>b</sup> $\pm$ 0.03	16.23 <sup>d</sup> $\pm$ 0.15	16.51 <sup>c</sup> $\pm$ 0.31	14.83 <sup>e</sup> $\pm$ 0.23	16.96 <sup>a</sup> $\pm$ 0.91
Other SFU	0.28 $\pm$ 0.12	–	0.26 $\pm$ 0.05	–	1.91 $\pm$ 0.03	–	0.31 <sup>b</sup> $\pm$ 0.21	0.30 <sup>b</sup> $\pm$ 0.11	0.30 <sup>b</sup> $\pm$ 0.02	–
<b>SFAs</b>	<b>71.30<sup>b</sup> <math>\pm</math> 0.08</b>	<b>73.03<sup>a</sup> <math>\pm</math> 0.22</b>	<b>71.45<sup>b</sup> <math>\pm</math> 0.17</b>	<b>70.96<sup>c</sup> <math>\pm</math> 0.13</b>	<b>72.92<sup>a</sup> <math>\pm</math> 0.13</b>	<b>68.94<sup>c</sup> <math>\pm</math> 0.16</b>	<b>69.19<sup>b</sup> <math>\pm</math> 0.16</b>	<b>68.46<sup>d</sup> <math>\pm</math> 0.13</b>	<b>64.16<sup>e</sup> <math>\pm</math> 0.07</b>	<b>71.00<sup>a</sup> <math>\pm</math> 0.04</b>
C14:1	2.20 <sup>b</sup> $\pm$ 0.25	2.42 <sup>a</sup> $\pm$ 0.06	2.42 <sup>a</sup> $\pm$ 0.17	1.98 <sup>d</sup> $\pm$ 0.23	2.11 <sup>c</sup> $\pm$ 0.24	2.18 <sup>d</sup> $\pm$ 0.05	2.87 <sup>a</sup> $\pm$ 0.08	2.65 <sup>c</sup> $\pm$ 0.81	2.64 <sup>c</sup> $\pm$ 0.48	2.77 <sup>b</sup> $\pm$ 0.04
C16:1	4.09 <sup>c</sup> $\pm$ 0.11	3.96 <sup>d</sup> $\pm$ 0.12	4.37 <sup>b</sup> $\pm$ 0.31	4.09 <sup>c</sup> $\pm$ 0.06	4.76 <sup>a</sup> $\pm$ 0.21	3.64 <sup>e</sup> $\pm$ 0.51	7.04 <sup>a</sup> $\pm$ 0.23	6.64 <sup>b</sup> $\pm$ 0.46	5.67 <sup>c</sup> $\pm$ 0.24	4.29 <sup>d</sup> $\pm$ 0.03
C18:1n-9 cis	20.78 <sup>b</sup> $\pm$ 0.08	18.72 <sup>d</sup> $\pm$ 0.18	19.93 <sup>c</sup> $\pm$ 0.21	21.28 <sup>a</sup> $\pm$ 0.04	20.11 <sup>e</sup> $\pm$ 0.02	18.34 <sup>e</sup> $\pm$ 0.26	18.99 <sup>c</sup> $\pm$ 0.09	19.87 <sup>b</sup> $\pm$ 0.02	24.14 <sup>a</sup> $\pm$ 0.04	18.67 <sup>d</sup> $\pm$ 0.15
<b>MUFAs</b>	<b>27.07<sup>c</sup> <math>\pm</math> 0.07</b>	<b>25.10<sup>e</sup> <math>\pm</math> 0.16</b>	<b>26.72<sup>d</sup> <math>\pm</math> 0.06</b>	<b>27.35<sup>b</sup> <math>\pm</math> 0.61</b>	<b>27.97<sup>a</sup> <math>\pm</math> 0.14</b>	<b>22.16<sup>e</sup> <math>\pm</math> 0.22</b>	<b>28.90<sup>c</sup> <math>\pm</math> 0.12</b>	<b>29.16<sup>b</sup> <math>\pm</math> 0.18</b>	<b>32.45<sup>a</sup> <math>\pm</math> 0.17</b>	<b>25.87<sup>d</sup> <math>\pm</math> 0.14</b>
C18:2n-6	0.27 <sup>d</sup> $\pm$ 0.09	0.82 <sup>b</sup> $\pm$ 0.04	0.43 <sup>a</sup> $\pm$ 0.05	0.18 <sup>e</sup> $\pm$ 0.02	0.29 <sup>c</sup> $\pm$ 0.02	0.41 <sup>e</sup> $\pm$ 0.11	0.41 <sup>d</sup> $\pm$ 0.20	1.20 <sup>c</sup> $\pm$ 0.23	1.30 <sup>b</sup> $\pm$ 0.13	1.91 <sup>a</sup> $\pm$ 0.14
C20:4n-6	1.06 <sup>b</sup> $\pm$ 0.05	2.05 <sup>c</sup> $\pm$ 0.10	0.95 <sup>d</sup> $\pm$ 0.24	1.05 <sup>c</sup> $\pm$ 0.32	2.31 <sup>a</sup> $\pm$ 0.03	1.16 <sup>a</sup> $\pm$ 0.14	0.31 <sup>d</sup> $\pm$ 0.31	0.31 <sup>d</sup> $\pm$ 0.21	0.87 <sup>b</sup> $\pm$ 0.14	0.49 <sup>c</sup> $\pm$ 0.16
Other PUFA	–	–	–	–	–	–	0.53 $\pm$ 0.36	0.32 $\pm$ 0.26	0.18 $\pm$ 0.17	–
<b>PUFAs</b>	<b>1.42<sup>c</sup> <math>\pm</math> 0.26</b>	<b>1.87<sup>b</sup> <math>\pm</math> 0.13</b>	<b>1.38<sup>d</sup> <math>\pm</math> 0.17</b>	<b>1.23<sup>e</sup> <math>\pm</math> 0.07</b>	<b>2.60<sup>a</sup> <math>\pm</math> 0.29</b>	<b>1.56<sup>d</sup> <math>\pm</math> 0.14</b>	<b>1.25<sup>e</sup> <math>\pm</math> 0.23</b>	<b>1.83<sup>c</sup> <math>\pm</math> 0.17</b>	<b>2.37<sup>b</sup> <math>\pm</math> 0.19</b>	<b>2.40<sup>a</sup> <math>\pm</math> 0.18</b>
Unknown FAs	0.20 <sup>d</sup> $\pm$ 0.20	0.28 <sup>c</sup> $\pm$ 0.18	0.55 <sup>b</sup> $\pm$ 0.22	0.74 <sup>a</sup> $\pm$ 0.19	–	0.37 <sup>d</sup> $\pm$ 0.17	1.00 <sup>a</sup> $\pm$ 0.09	0.55 <sup>c</sup> $\pm$ 0.20	0.54 <sup>c</sup> $\pm$ 0.23	0.70 <sup>b</sup> $\pm$ 0.03
<b>NVI</b>	<b>1.052<sup>d</sup> <math>\pm</math> 0.13</b>	<b>1.060<sup>c</sup> <math>\pm</math> 0.17</b>	<b>1.071<sup>b</sup> <math>\pm</math> 0.05</b>	<b>1.110<sup>a</sup> <math>\pm</math> 0.21</b>	<b>1.040<sup>e</sup> <math>\pm</math> 0.10</b>	<b>1.060<sup>b</sup> <math>\pm</math> 0.22</b>	<b>1.005<sup>d</sup> <math>\pm</math> 0.30</b>	<b>1.041<sup>c</sup> <math>\pm</math> 0.18</b>	<b>1.194<sup>a</sup> <math>\pm</math> 0.19</b>	<b>1.011<sup>d</sup> <math>\pm</math> 0.26</b>
<b>HH</b>	<b>0.450<sup>c</sup> <math>\pm</math> 0.07</b>	<b>0.423<sup>e</sup> <math>\pm</math> 0.06</b>	<b>0.436<sup>d</sup> <math>\pm</math> 0.09</b>	<b>0.463<sup>b</sup> <math>\pm</math> 0.13</b>	<b>0.466<sup>a</sup> <math>\pm</math> 0.02</b>	<b>0.409<sup>c</sup> <math>\pm</math> 0.18</b>	<b>0.420<sup>d</sup> <math>\pm</math> 0.14</b>	<b>0.459<sup>b</sup> <math>\pm</math> 0.06</b>	<b>0.598<sup>a</sup> <math>\pm</math> 0.16</b>	<b>0.422<sup>c</sup> <math>\pm</math> 0.24</b>
<b>IA</b>	<b>3.060<sup>b</sup> <math>\pm</math> 0.12</b>	<b>3.357<sup>a</sup> <math>\pm</math> 0.18</b>	<b>3.005<sup>c</sup> <math>\pm</math> 0.20</b>	<b>2.921<sup>d</sup> <math>\pm</math> 0.24</b>	<b>2.832<sup>e</sup> <math>\pm</math> 0.21</b>	<b>3.661<sup>a</sup> <math>\pm</math> 0.17</b>	<b>2.793<sup>c</sup> <math>\pm</math> 0.14</b>	<b>2.640<sup>d</sup> <math>\pm</math> 0.10</b>	<b>2.232<sup>e</sup> <math>\pm</math> 0.18</b>	<b>3.230<sup>b</sup> <math>\pm</math> 0.22</b>
<b>HPI</b>	<b>0.326<sup>d</sup> <math>\pm</math> 0.18</b>	<b>0.297<sup>e</sup> <math>\pm</math> 0.11</b>	<b>0.332<sup>c</sup> <math>\pm</math> 0.07</b>	<b>0.342<sup>b</sup> <math>\pm</math> 0.08</b>	<b>0.351<sup>a</sup> <math>\pm</math> 0.06</b>	<b>0.273<sup>e</sup> <math>\pm</math> 0.09</b>	<b>0.357<sup>c</sup> <math>\pm</math> 0.06</b>	<b>0.378<sup>b</sup> <math>\pm</math> 0.07</b>	<b>0.447<sup>a</sup> <math>\pm</math> 0.03</b>	<b>0.307<sup>d</sup> <math>\pm</math> 0.09</b>

$\pm$ T1, T2 and T3 meatballs contain 250, 500 and 1000 ppm of green coffee powder, respectively. Asc sample is the meatballs contain 500 ppm of ascorbic acid.

\* Different small letters within rows denote significant differences ( $p < 0.05$ ) between samples at the same time of analysis.

may encourage certain changes in the fatty acid profile, leading to an increase in the SFA content (Muzolf-Panek & Kaczmarek, 2021). Several international authorities, like FAO and USDA, advise low SFA intake as much as possible, thus addition of more than 500 ppm of green coffee to meatballs may be better than ascorbic acid. Contrarily, increasing the proportion of green coffee increased the MUFAs and PUFAs compared with the control and Asc samples after 2 weeks of storage. The dominant MUFAs and PUFAs in the green coffee-containing samples were 18:1n-9 cis and 18:2n-6, respectively. It is ascribed to the fatty acid composition of green coffee, which is high in oleic acid (C18:1) and linoleic acid (C18:2n-6) (Mehari et al., 2019). MUFAs are hypocholesterolemic, and among the unsaturated fatty acids, they are more helpful than PUFAs in terms of raising HDL-cholesterol, which is an essential factor in preventing cardiovascular diseases (Barros et al., 2020).

Regarding the nutritional value of the preserved meatballs, because the PUFA/SFA ratio (P/S) was so low, other indices were evaluated (i.e., NVI, HH, IA, and HPI). These indices, particularly the HH index, appropriately indicate the impact of fatty acid composition on cardiovascular disease compared with the P/S ratio. The HH index, which should be high, characterizes the association between hypocholesterolemic fatty acids (C18:1-cis and PUFA) and hypercholesterolemic fatty acids (palmitic, myristic, and lauric acids) (Barros et al., 2020). Consuming fats or lipid-containing foods with a low IA value can lower LDL-cholesterol and total cholesterol levels in the blood plasma. The HPI index, which is the inverse of the IA index, reflects the effect of fatty acid content on cardiovascular disease (Chen & Liu, 2020).

From that nutritional standpoint, it is important to emphasize the gradual increase in NVI, HH, and HPI indices as well as a reduction in the IA index of green coffee-formulated meat samples by increasing the added coffee, at the beginning and end of storage. Ascorbic acid-containing meatballs had greater HH and HPI than the control sample and all green coffee-containing samples at zero-day, but this was reversed after 2 weeks. After 14 days of storage, green coffee  $\geq 500$  ppm was more effective than the control and Asc samples, regarding all these indices, which could be related to its inhibitory influence on lipooxygenase activity, as previously mentioned. The most significant effect ( $p < 0.05$ ) was recorded in meatballs containing 1000 ppm green coffee (T3). These changes are directly connected to the fatty acid composition of green coffee powder. These findings are consistent with those of Barros et al. (2020), who reduced the AI index in burgers by substituting fats with tiger nut oil.

### Hydrocarbons content

Aliphatic hydrocarbons are straight and saturated carbon chain molecules with carbon atom numbers ranging from C6–C40. It has been gaining increasing concern due to its toxic, mutagenic, and carcinogenic

effects (Aly Salem et al., 2014). Table 3 displays the total and individual hydrocarbon contents of different meatball samples. At zero time, the total hydrocarbon content in the control sample was the highest and the lowest in ascorbic-containing samples. Octadecane was the prevalent hydrocarbon in the control and green coffee meatballs, but it vanished in the Asc sample. Similarly, the lowest numbers of hydrocarbons were found in ascorbic non-irradiated ground beef (Iset et al., 2009). These identified hydrocarbon compounds were reduced in this study by increasing the green-coffee proportion (from 0.55 to 0.28 %). After the cold storage period ended, the total hydrocarbons increased approximately-three times in the Asc sample and twice in the control and T1 samples. Three new hydrocarbons, tridecane, tetradecane, and pentadecane, appeared in the control sample. Interestingly, after 2 weeks of cold storage, green coffee addition was more efficient than ascorbic acid in decreasing the levels of these carcinogenic chemicals, namely, T2 and T3 samples, by 87.4 % and 87.6 %, respectively, compared to 84.0 % for ascorbic acid. Iset et al. (2009) also stated that adding  $\alpha$ -tocopherol and sesamol to ground beef, cooled for 7 days, successfully reduced the hydrocarbons. Likewise, Wang et al. (2019) reduced the total of four polycyclic aromatic hydrocarbons (PAHs) content: benzo (a) pyrene, chrysene, benzo (a) anthracene, and benzo (b) fluoranthene, as indicators of PAHs, in chicken wings before grilling, by marinating them in green tea. The greatest percentage drop recorded ranged between 15.1 and 61.7 %. This finding proposes a possible inhibition of the phenolic compounds found in green coffee by scavenging the free radicals or by combining them with hydrocarbon intermediates to inhibit the fat oxidation reaction.

### Conclusions

Many herbs and spices have been applied to preserve meat quality during storage. Our results demonstrated, for the first time, the antioxidant effect of green coffee added directly to beef meatballs during cold storage. The addition substantially improved the luminosity and the oxidative stability of the formulated meatballs, as well as decreased the visible microbial count. Green coffee maintained its antioxidant capacity for two weeks compared with the ascorbic acid-containing meatballs. Furthermore, from the nutritional viewpoint, it is important to highlight the impact of green coffee on lipid indices and their inhibitory influence on aliphatic hydrocarbons formation during cold storage. Thus, with all these findings in mind, we can conclude that green coffee may substitute ascorbic acid or maybe be tested along with it or other antioxidants since it demonstrated a far greater protective impact against fat breakdown in chilled meatballs.

**Table 3**

Hydrocarbons content in raw meatballs at zero time and after two weeks of cold storage (%), mean  $\pm$  SD.

Hydrocarbon	$\pm$ Control					Asc				
	Control	T1	T2	T3	Asc	Control	T1	T2	T3	Asc
	Zero time					After 2 weeks				
Tridecane	–	–	–	–	–	0.46 $\pm$ 0.24	–	–	–	0.14 $\pm$ 0.02
Tetradecane	–	–	–	–	–	1.65 $\pm$ 0.27	–	–	–	–
Pentadecane	–	–	–	–	–	0.66 $\pm$ 0.30	–	–	–	–
Hexadecane	0.31 $\pm$ 0.02	–	–	0.06 $\pm$ 0.14	0.20 $\pm$ 0.36	0.63 $\pm$ 0.05	–	–	–	–
Heptadecane	0.65 <sup>a</sup> $\pm$ 0.01	0.30 <sup>b</sup> $\pm$ 0.41	0.20 <sup>c</sup> $\pm$ 0.10	0.06 <sup>d</sup> $\pm$ 0.13	0.20 <sup>c</sup> $\pm$ 0.22	0.44 <sup>a</sup> $\pm$ 0.06	0.17 <sup>c</sup> $\pm$ 0.12	0.32 <sup>b</sup> $\pm$ 0.24	0.15 <sup>d</sup> $\pm$ 0.34	0.00 <sup>e</sup> $\pm$ 0.00
Octadecane	0.96 <sup>a</sup> $\pm$ 0.03	0.25 <sup>d</sup> $\pm$ 0.23	0.26 <sup>c</sup> $\pm$ 0.08	0.42 <sup>b</sup> $\pm$ 0.17	0.00 <sup>e</sup> $\pm$ 0.00	0.53 <sup>c</sup> $\pm$ 0.15	0.85 <sup>a</sup> $\pm$ 0.06	0.23 <sup>e</sup> $\pm$ 0.17	0.39 <sup>d</sup> $\pm$ 0.12	0.56 <sup>b</sup> $\pm$ 0.06
<b>Total</b>	<b>1.92<sup>a</sup> <math>\pm</math> 0.03</b>	<b>0.55<sup>b</sup> <math>\pm</math> 0.32</b>	<b>0.46<sup>c</sup> <math>\pm</math> 0.09</b>	<b>0.48<sup>c</sup> <math>\pm</math> 0.17</b>	<b>0.20<sup>d</sup> <math>\pm</math> 0.22</b>	<b>4.37<sup>a</sup> <math>\pm</math> 0.17</b>	<b>1.02<sup>b</sup> <math>\pm</math> 0.09</b>	<b>0.55<sup>d</sup> <math>\pm</math> 0.20</b>	<b>0.54<sup>d</sup> <math>\pm</math> 0.23</b>	<b>0.70<sup>c</sup> <math>\pm</math> 0.03</b>
<b>Reduction percent</b>	–	<b>71.4</b>	<b>76.0</b>	<b>75.0</b>	<b>89.6</b>	–	<b>76.7</b>	<b>87.4</b>	<b>87.6</b>	<b>84.0</b>

$\pm$ T1, T2 and T3 meatballs contain 250, 500 and 1000 ppm of green coffee powder, respectively. Asc sample is the meatballs contain 500 ppm of ascorbic acid.

\* Different small letters within rows denote significant differences ( $p < 0.05$ ) between samples at the same time of analysis.



## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2022.100336>.

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