Frozen storage quality and flavor evaluation of ready to eat steamed meat products treated with antioxidants

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ABSTRACT
The application of synthetic antioxidants to food products has been restricted because of their toxicity. This prompted the use of natural antioxidants to prevent lipid oxidation. Therefore, the objective of this study was to determine the effect of the application of natural antioxidant to beef-pork meat on the lipid oxidation, flavor, color as well as sensory properties during storage (−18 ± 1°C) at 0, 14, 28, 42, 90 days. Three groups of pork and beef meatballs were formulated (control; positive control with an addition of 0.01% of butylhydroxytoluene and experimental group with an addition of 1% of Camellia sinensis L.). The results showed that the addition of 1% of Camellia sinensis L. extract to meatballs is an effective strategy for delaying oxidative changes. After 90 days of frozen storage, the antioxidant activity in group with an addition of 1% of Camellia sinensis L. was twice as high compared to the control group. Also, methanol intensity of steamed frozen meatballs was lower in the experimental group.

Calidad de productos cárnicos al vapor listos para comer tratados con antioxidantes y evaluación de su sabor tras su almacenamiento en congelador

RESUMEN
La aplicación de antioxidantes sintéticos a los productos alimenticios ha sido restringido debido a su toxicidad. Esto impulsó el uso de antioxidantes naturales para prevenir la oxidación de los lípidos. El objetivo de este estudio fue determinar el efecto que conlleva la aplicación de antioxidantes naturales a la carne de cerdo y de res en la oxidación lipídica, el sabor, el color y las propiedades sensoriales tras su almacenamiento (−18 ± 1°C) durante 0, 14, 28, 42 y 90 días. Con este objetivo se elaboraron tres grupos de albóndigas de cerdo y de vaca (control; control positivo adicionado con 0.01% de butilo-hidroxitolueno y grupo experimental adicionado con 1% de Camellia sinensis L.). Los resultados permiten constatar que la adición de 1% de extracto de Camellia sinensis L. a las albóndigas es una estrategia eficaz para retrasar los cambios oxidativos. Después de 90 días de almacenamiento en congelador, la actividad antioxidante del grupo al cual se adicionó 1% de Camellia sinensis L. fue dos veces mayor en comparación con el grupo de control. Además, la intensidad del metanol de las albóndigas cocidas al vapor y luego congeladas fue menor en el grupo experimental.

1. Introduction
Nowadays, people tend to consume ready to eat food because of changes in daily activities. Ready to consume products are a very popular category in the meat industry and demand the development of processing methods that keep their freshness as long as possible (Akcak et al., 2017). Steaming is one of the healthiest cooking techniques (Huang et al., 2013). Meatballs are processed minced meat which can be classified as restructured meat. It can be prepared using pork and beef meat. Meatballs are highly consumed worldwide product (Feiner, 2006; Turgut et al., 2017). However, minced meat has a tendency to become rancid and brown much more rapidly than whole muscle cuts (Ho et al., 1996). Freezing is an effective meat preservation technique (Beltrán & Bellés, 2019). Nonetheless, lipid oxidation can still take place in frozen meat (Karpiszka-Tymoszczyk, 2014). One of the most abundant aldehydes generated during secondary lipid oxidation is malondialdehyde (MDA). This compound is also the most commonly used as a marker of oxidation process (Barriuso et al., 2013). Antioxidants are generally applied to prevent the negative consequences of oxidation process (Estévez & Lorenzo, 2018). The commercial antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butyl hydroquinone (TBHQ) demonstrate many negative health effects (Madsen & Bertelsen, 1995; Półtorak et al., 2018; Saito et al., 2003). Therefore, antioxidants from plant organs such as spices and herbs are being extensively studied. Various researches have obtained the antioxidant characteristics of the Camellia sinensis L., nonfermented products (Jongberg et al., 2015; Rietveld & Wieseman 2003; Tang et al., 2001). Various in vitro researches present that the flavonoids contained in Camellia sinensis L. have antioxidant potential and metal-chelating activities and can protect tissues and cells against free oxygen radicals (Grzesik et al., 2018). Camellia sinensis L. catechins, including epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and also epigallocatechin gallate (EGCG), have a strong antioxidant
potential (Chan et al., 2007). Therefore, *Camellia sinensis* L. extract has been known to have a very good antioxidant activities and have been used in different types of food (Wu et al., 2013).

Meatballs quite popularly consumed, but because of its high lipid content, there is a problem of lipid oxidation during storage, which causes a decrease in shelf-life and quality features (Hong et al., 2009; Ku et al., 2008). Hence, the aim of the research was to determine the effectiveness of extract from *Camellia sinensis* L. on the oxidative stability of steamed meatballs during frozen storage.

2. material and methods

2.1. Experimental design

*Camellia sinensis* L. extract was purchased from a polish local producer. Beef, pork meat and pork jowl were purchased from the local producer. Meat and jowl were minced and then randomly divided into four groups. The first group (control – C1) was mixed with beef (44.2%), pork (27.2%), pork jowl (21%), water (6%), salt (1.2%), black pepper (0.4%). The second group (positive control – C2) contained BHT (0.01%). To the third group (E) *Camellia sinensis* L. extract (1%) was added. Subsequently, the meatballs were formed (20 ± 2 g).

The meatball samples were steam-cooked for 5 minutes. The internal endpoint temperature was 75°C. Thereafter samples were packed in vacuum conditions in polythene bags and stored at −18 ± 1°C for 90 days. The analyses were carried out at days 0, 14, 28, 42 and 90.

2.2. Measurement of pH

Analysis of pH of meatballs was conducted by a potentiometric method and using a hand-held pH meter (Model 205, Testo AG, Lenzkirch, Germany). The pH meter was calibrated by two buffers (pH = 4.01, pH = 7.00). Each sample was measured in triplicate.

2.3. Chemical composition

The chemical components (moisture, protein, fat, ash and connective tissue) of the meatballs were analyzed using a near-infrared spectrometer NIRFlex N-500 (Büchi Labortecnik AG, Flawil, Switzerland). The chemical components were measured in raw meatballs and in the meatballs after heat treatment during freezing storage (0, 14, 28, 42 and 90 days). The meat was homogenized and then a layer of 0.5 cm was placed in a Petri dish. Each sample has been measured in triplicate.

2.4. Water-holding capacity

The measurement of water-holding capacity (WHC) of raw meatballs on 0th day of storage was analyzed according to Grau and Hamm (1953) with a modification (Sz匹cer et al., 2018).

Meatballs samples (300 mg) were transferred on a Whatman no. 1 filter paper between two plates. Then, samples were weighed down for 5 min under 2 kg weight. OImaging MicroPublisher 5.0 RTV (Canada) equipped with a Kaiser system (Germany) to take pictures of the pressed stains was applied. Meat (Am) and liquid (Al) areas were calculated using the Image-Pro Plus (v.7.0) software. WHC was evaluated using formula equation (1).

\[
\text{WHC} = \frac{(1) \times (\text{Am/Al}) \times 100}{100}
\]  

(1)

2.5. Measurement of color

The color measurement on the surface and on the cross-section of meatballs was analyzed in the CIE L*a*b* system. The analysis was performed using a Minolta chromameter (CR-400, Konica Minolta Inc., Tokyo, Japan). The chromometer was calibrated using a white standard calibration plate (L* = 98.45, a* = −0.10, b* = −0.13). The measuring head with a D65 illuminant and a standard 2° observer and diameter of 8 mm was used. Color measurements were carried out on the outside and inside surface on the cooked meatballs. The analysis of color parameters was conducted by the measurement of 10 different parts of the sample.

2.6. Evaluation of antioxidant capacity using DPPH radical-scavenging activity

The antioxidant ability of meatballs from each treatment group was analyzed. For this purpose, the ability or radical scavenging, using the free radical DPPH (1, 1-diphenyl-2-picrylhydrazly) as a reagent according to Mariem et al. (2014) methodology with slight modifications was measured. A 2.5 g of sample from each treatment groups of meatballs was homogenized in 7.5 ml of ethanol. Ultra Turrax (IKA, Germany) for 120 s at 10108 RCF was used. Extraction processes were conducted at room temperature using rotator with shaking (MyLab SLRM-3, NanoEnTek Inc., Korea) and after that samples were centrifuged 15 min and 36288 RCF. Then, the 0.5 ml of supernatant was added with 3.5 ml 0.1 mM DPPH in ethanol and mixed for 30 s and then stored in the darkness at room temperature for 30 min. Then, the absorbance at 517 nm (Tecan Spark TM 10 M, Männedorf, Switzerland) against to ethanol has been measured. For control, all reagents were added except sample solution which was replaced by ethanol (96%). The DPPH has been calculated according to equation (2):

\[
\text{DPPH activity} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100
\]  

(2)

2.7. Total phenolic content

To analyse the total phenolic content (TPC) of the meatballs, the method described by Singleton and Rossi (1965) with modifications was applied. A 2.5 g of meatball from each treatment was homogenised in ethanol (7.5 ml). Then, 0.5 ml of Folin’s reagent and 6.0 ml of distilled water were added to 0.1 ml of ethanolic extract. Then, 1.5 mL of sodium carbonate with the concentration of 200 mg/ml was added to the reactive mixtures. Then, the mixture was filled with water to 10 ml. The solution was incubated for 30 min in the water bath at 40°C. The absorbance was measured using a spectrophotometer at wavelength of 765 nm (SparkTM 10 M -Tecan Group, Männedorf, Switzerland). The content of phenolic compounds was expressed as
a gallic acid equivalent based on calibration curve (the concentrations of gallic acid: 0 mg/ml, 0.1 mg/ml, 0.2 mg/ml, 0.3 mg/ml, 0.4 mg/ml and 0.5 mg/ml and the linear equation was: y = 1.3071x+0.016). Obtained results (from three repetitions) were presented in mg gallic acid equivalent (GAE) per 100 g of the sample.

2.8. Lipid oxidation

The secondary lipid oxidation of meatballs was analysed according to the method of Robles-Martinez et al. (1982) with modification in Brodowska et al. (2016). 2.5 g of ground meat was homogenized with 25 ml of trichloroacetic acid solution and 1.25 ml of antioxidant (0.5% PG and ethylenediaminetetraacetic acid in ethyl alcohol/water 1:1) for 30 s at 1200 rpm (WT 500 homogenizer, Wiggenhauser, Germany). After centrifugation for 10 min at 8000 rpm (centrifuge MPW-251, MPW Med. Instruments, Warsaw, Poland), 5 ml of 2-thiobarbituric acid (0.02 mM/l) was added to 5 ml of supernatant. Then, samples were heated in a water bath (90°C) for 40 min. Samples were cooled and the absorbance was measured at 532 nm, against a blank, using a UV-VIS spectrophotometer (Spark Tecan 10 M, Switzerland). A calibration curve was evaluated with 1.1.3.3-tetramethoxypropane. The results were expressed as mg of MDA/kg of meat. The measurements were performed in three replicates. The obtained results were presented as mg of MDA/kg meat.

2.9. Sensory evaluation of meatballs

The sensory quality of steamed meatballs with an addition of Camellia sinensis L. extract and synthetic antioxidant (BHT) was evaluated according to ISO-13299 2003. Ten females with an age range from 30 to 38 years old, semi-trained panelists recruited from the Department of Technique and Food Development at the University of Life Sciences assessed the sensory properties of the meatballs on 0th, 14th and 28th, 42th and 90th days of frozen storage. Samples were reheated in convection-steamed oven (model CPE 110, Kupperschub, Germany). The consumers’ evaluation session was conducted at 24 ± 1°C in isolated rooms with white light. Samples were served in random order on plastic plates. The taste, texture, flavor, and overall acceptability were assessed using a 10-point hedonic scale (1 = dislike extremely and 10 = like extremely). Data were subjected to Principal Component Analysis (PCA).

2.10. Analysis of volatile compound profile

Volatile compounds in meatballs were obtained using the Heracles II an electronic nose (Alpha M.O.S., Toulouse, France). Incubation of the vials was performed at 55°C for 900 s. The method was presented in the work of Wojtasik-Kalinowska et al. (2017). Five repetitions of the samples were performed on 0th and 90th days of storage. The procedure of PCA was used for data processing using the Alpha Soft (v.8.0) software (Rattray et al., 2014).

2.11. Statistical analysis

To check the distribution of data the Shapiro–Wilk test was carried out. A one-way analysis of variance (ANOVA) was performed using the Fisher’s test – the least significant differences at the level of significance of $P > .05$. The results were analyzed statistically using Statistica 13.1 (StatSoft Inc., Tulsa, USA) program. The sensory and flavor profile data were subjected to principal component analysis using Alphal Soft Version 8.0.

3. Results and discussion

3.1. Physicochemical properties of raw meat balls

The chemical composition of the raw meatballs treated with antioxidants is presented in Table 1. There were no statistically significant differences in fat, protein, L* and b* parameters ($P ≤ 0.05$) **between the treatments. A significant difference in the case of a*color parameter was observed between treatments of raw meatballs. Parameter a* (redness) value of meatballs was affected by Camellia sinensis L. extract addition ($P ≤ 0.05$). In general, the a* value of samples decreased as the extract was added. This implies that the addition of Camellia sinensis L. extract reduced the redness of raw meatballs. Turhan et al. (2005) observed similar results in beef treated with hazelnut pellicle. Statistically significant differences were observed in the moisture content between samples. The lowest values of these parameters were observed in the case of experimental group (E) and control group (C1). The lowest value of ash content was observed in the case of C1 group. However, in the case of connective tissue parameter, the highest value was observed for C2 group.

The pH values analysed on D0 ranged from 5.74 to 5.75. There were no significant differences in pH and WHC values between controls and experimental group.

3.2. Color of steamed frozen meatballs

The effect of the extract obtained from Camellia sinensis L. on color parameters of meatballs is presented in Table 2. There are no statistically significant differences on D0, D21 between C2 and E groups, whereas on D90 there were no differences in the L* parameters between all analyzed groups. This finding suggests that the application of extract obtained from Camellia sinensis L. did not affect L* parameter of the inside surface of meatballs. On D0, D21 and D42 there are statistically significant differences in a* (redness) between C2 and E groups. On D21 the inside surface was redder (higher a* value) in samples with the addition of Camellia sinensis L. extract (E group). Similar results on D21 were also observed on the outside surface where a* parameter was the highest in the case of E group. The red-brownish color of meat after heat treatment is determined by the denatured globin hemichromes appearing as a result of high temperatures (Fox, 1994). The color of raw meat after the addition of extract obtained from Camellia sinensis L. is not attractive (low a* value). The meat after heat treatment characterized an attractive brown color that is fully acceptable by consumers.
Table 1. The effect of *Camellia sinensis* L. extract and synthetic antioxidant (BHT) addition on physicochemical properties of raw meatballs (mean ± SE).

| Group | C1 (%) | C2 (%) | E (%) | P-value |
|-------|--------|--------|-------|---------|
| WHC   | 57.85 ± 4.47 | 71.46 ± 6.08 | 64.64 ± 5.31 | 0.249 |
| L*    | 44.64 ± 1.17 | 44.94 ± 1.32 | 42.68 ± 0.85 | 0.319 |
| a*    | 17.62 ± 0.62 | 18.34 ± 0.60 | 14.80 ± 0.48 | 0.000 |
| b*    | 9.63 ± 0.42 | 9.99 ± 0.42 | 9.77 ± 0.23 | 0.783 |
| Moisture (%) | 63.07 ± 0.18 | 63.69 ± 0.30 | 62.76 ± 0.07 | 0.017 |
| Fat (%) | 17.50 ± 0.17 | 17.60 ± 0.13 | 17.32 ± 0.08 | 0.000 |
| Protein (%) | 16.65 ± 0.06 | 16.80 ± 0.37 | 16.67 ± 0.02 | 0.000 |
| Ash (%) | 1.37 ± 0.09 | 1.97 ± 0.07 | 1.91 ± 0.06 | 0.000 |
| Connective tissue (%) | 1.34 ± 0.08 | 3.29 ± 0.48 | 1.73 ± 0.05 | 0.001 |
| pH    | 5.75 ± 0.01 | 5.74 ± 0.00 | 5.75 ± 0.01 | 0.000 |

Means with a different capital letters within a column indicate a significant effect of treatment group within a row (P ≤ 0.05).

C1 – Control group; C2 – positive control group included 0.01% of BHT; E – experimental group included 1% of *Camellia sinensis* L. extract.

Means with different capital letters in the same column were significantly different (P ≤ 0.05).

Table 2. The mean and standard error of color components measured on outside surface of steamed meatballs with an addition of *Camellia sinensis* L. extract and synthetic antioxidant (BHT) during the frozen storage (mean ± SE).

| Group | C1 | C2 | E | P-value |
|-------|----|----|----|---------|
| D0 L* (%) | 40.85±a 1.26 | 51.55±a 0.88 | 45.41±b 0.29 | 0.000 |
| a* (°) | 6.56± 0.37 | 6.12±a 0.33 | 5.43±a 0.30 | 0.075 |
| b* (°) | 10.87±a 0.21 | 11.01±a 0.36 | 10.01±a 0.23 | 0.034 |
| D14 L* (%) | 50.07±a 0.59 | 50.79±b 0.85 | 46.47±b 0.60 | 0.000 |
| a* (°) | 6.08±b 0.13 | 5.35±a 0.23 | 5.54±b 0.25 | 0.056 |
| b* (°) | 10.33±b 0.21 | 11.08±a 0.31 | 9.86±a 0.22 | 0.007 |
| D21 L* (%) | 48.71±b 0.93 | 50.83±b 1.17 | 46.49±a 0.53 | 0.009 |
| a* (°) | 6.05±b 0.29 | 5.41±a 0.13 | 6.29±b 0.32 | 0.062 |
| b* (°) | 10.31±b 0.33 | 10.80±a 0.40 | 10.70±b 0.22 | 0.547 |
| D42 L* (%) | 53.21±b 0.68 | 52.33±b 0.52 | 48.89±b 0.74 | 0.000 |
| a* (°) | 6.25±b 0.19 | 5.64±b 0.12 | 6.08±a 0.09 | 0.015 |
| b* (°) | 9.86±ab 0.24 | 9.47±b 0.20 | 10.41±b 0.27 | 0.031 |
| D90 L* (%) | 50.71±a 0.75 | 50.54±a 0.70 | 46.38±b 1.02 | 0.001 |
| a* (°) | 6.26±b 0.28 | 6.47±b 0.29 | 5.61±b 0.19 | 0.063 |
| b* (°) | 10.53±b 0.30 | 10.99±b 0.25 | 10.55±b 0.30 | 0.445 |

Means with different subscript capital letters in the same row were significantly different (P ≤ 0.05).

Means with different subscript small letters in the same column were significantly different (P ≤ 0.05).

C1 – Control group; C2 – positive control group included 0.01% of BHT; E – experimental group included 1% of *Camellia sinensis* L.

Means with different small letters in the same column were significantly different (P ≤ 0.05).

C1 – grupo de control; C2 – grupo de control positivo adicionado con 0.01% de BHT; E – grupo experimental adicionado con 1% de *Camellia sinensis* L.
Table 3. The mean and standard error of a total antioxidant activity (TAA) and a total phenolic content (TPC) of steamed meatballs with an addition of Camellia sinensis L. extract and synthetic antioxidant (BHT) during the frozen storage (mean ± SE).

| Parameters                        | Frozen storage | Group | E | P-value |
|-----------------------------------|----------------|-------|---|---------|
| Total antioxidant activity [% DPPH] | D0             | C1    | C2 | E       | P-value |
|                                   | 14.84 ± 0.81   | 47.85 ± 0.22 | 56.42 ± 1.66 | 0.000005 |
|                                   | 17.71 ± 0.68   | 49.72 ± 0.55 | 47.76 ± 2.57 | 0.000006 |
|                                   | 11.10 ± 1.64   | 44.34 ± 2.01 | 40.31 ± 1.36 | 0.000016 |
|                                   | 10.58 ± 0.86   | 39.35 ± 1.45 | 28.79 ± 1.44 | 0.000012 |
|                                   | 10.0 ± 1.0     | 34.09 ± 2.12 | 21.10 ± 1.65 | 0.000159 |
|                                   | 69.65 ± 1.23   | 182.69 ± 6.32 | 410.32 ± 6.32 | 0.000000 |
|                                   | 65.54 ± 1.09   | 180.78 ± 1.07 | 384.13 ± 1.91 | 0.000000 |
|                                   | 56.72 ± 0.83   | 162.47 ± 3.82 | 307.60 ± 3.01 | 0.000000 |
|                                   | 36.58 ± 2.42   | 153.70 ± 3.10 | 225.51 ± 3.36 | 0.000000 |
| Total phenolic content [mg GAE/100 g F.W.] | D0             | C1    | C2 | E       | P-value |
|                                   | 14.84 ± 0.81   | 47.85 ± 0.22 | 56.42 ± 1.66 | 0.000005 |
|                                   | 17.71 ± 0.68   | 49.72 ± 0.55 | 47.76 ± 2.57 | 0.000006 |
|                                   | 11.10 ± 1.64   | 44.34 ± 2.01 | 40.31 ± 1.36 | 0.000016 |
|                                   | 10.58 ± 0.86   | 39.35 ± 1.45 | 28.79 ± 1.44 | 0.000012 |
|                                   | 10.0 ± 1.0     | 34.09 ± 2.12 | 21.10 ± 1.65 | 0.000159 |
|                                   | 69.65 ± 1.23   | 182.69 ± 6.32 | 410.32 ± 6.32 | 0.000000 |
|                                   | 65.54 ± 1.09   | 180.78 ± 1.07 | 384.13 ± 1.91 | 0.000000 |
|                                   | 56.72 ± 0.83   | 162.47 ± 3.82 | 307.60 ± 3.01 | 0.000000 |
|                                   | 36.58 ± 2.42   | 153.70 ± 3.10 | 225.51 ± 3.36 | 0.000000 |

Means with different superscript capital letters in the same row were significantly different (P < 0.05).

Means with different superscript small letters in the same column were significantly different (P < 0.05).

C1 – Control group; C2 – positive control group included 0.01% of BHT; E – experimental group included 1% of Camellia sinensis L.

Las medias con distintas letras mayúsculas de superíndice en la misma fila son significativamente diferentes (P < 0.05).

Las medias con distintas letras minúsculas de superíndices en la misma columna son significativamente diferentes (P < 0.05).

C1 – grupo de control; C2 – grupo de control positivo adicionado con 0.01% de BHT; E – grupo experimental adicionado con 1% de Camellia sinensis L.

3.3. Antioxidant activity [% DPPH]

Antioxidant activity of frozen meatballs treated with different antioxidants is presented in Table 3. The antioxidant activity of plant extracts is affected by the content of polyphenolic compounds (Lee et al., 2015). The highest value of antioxidant activity (DPPH) was observed in the group with an addition of extract from Camellia sinensis L. on D0 (P < 0.05). However, during the storage, this value decreased. On D90, the value of DPPH in the E group was significantly higher P < 0.05 (21.10 ± 1.65) compared to C1 group (10.0 ± 1.01). The Camellia sinensis L. extract contains high content of flavonoids, catechins and gallic acid. The antioxidant mechanisms are single-electron transfer and hydrogen atom transfer. These mechanisms are related to the position of phenolic hydroxyl groups. The higher antioxidant capacity of meatballs with extract is related to the high content of catechins (Carrizo et al., 2014; Quideau et al., 2011; Song et al., 2020). The results of antioxidant capacity showed that the Camellia sinensis L. extract have the potential to extend the shelf life of frozen meatballs.

3.4. The content of total phenolic

Values of total phenolic content are presented in Table 3. On D0 the phenolic content in the control group (C1) was 69.65 ± 1.23%. After 90 days of frozen storage, this value decreased by 61.52%. In the group where Camellia sinensis L. extract was added, the value of total phenolic content decreased by 57.77%. However, on the last day of frozen storage, the value of analysed parameter was still on the high level (173.28 ± 2.98%). In all analysed days of frozen storage, the values of TPC was significantly higher (P < 0.05) compared to both C1 and C2 groups. The antioxidant activity of phenolic compounds in spices and...
herbs results from redox and chemical properties, which can function as reducing agents, Fe2+ chelators, free radical scavengers or quenchers of the formation of singleton oxygen (Pizzale et al., 2002).

3.5. The TBARS analysis

Table 4 presents the results of lipid oxidation in frozen meatballs treated with different antioxidants. The values of the TBARS of all samples increased during the storage. The extract obtained from Camellia sinensis L. lowered oxidation of lipids during the frozen storage period. Chouliara et al. (2009) reported that when the value of TBARS is on the level of 3 mg MDA·kg\(^{-1}\) in meat, it can be regarded as well preserved. The results showed that the addition of BHT was the most effective against malondialdehyde formation during 90 days of frozen storage of meatballs.

However, on D28 and D42, there were no significant differences between BHT and the extract obtained from Camellia sinensis L. addition. Lorenzo et al. (2014) examined the antioxidant potential of different plant extracts...
added to pork patties and demonstrated that extract obtained from *Camellia sinensis* L. was one of the most effective antioxidants against lipid oxidation. After 90 days of frozen storage, an addition of *Camellia sinensis* L. extract decreased the value of TBARS (1.75 ± 0.08 mg MDA·kg⁻¹). The value of TBARS in E group was significantly lower compared to C1 group (2.01 ± 0.02 mg MDA·kg⁻¹). 2 mg MDA/kg is considered as the point where rancid flavor is perceptible (Greene & Cumuze, 1981). Different studies (Campo et al., 2006) set this limit as 2.28 mg MDA/kg of meat and reported that in samples with TBARS above this value beef flavor was changed by rancidity.

### 3.5.1. Volatile compounds in meatballs

Figures 1–3 show the 24 characteristic volatile compounds in all the groups. Based on the chromatographic diagrams (D0), 13 compounds in C1 group, 13 in C2 group, 17 in E group were identified. After 90 days of frozen storage 15 characteristic compounds were identified in the case of C1 and C2.

![Diagram of volatile compounds in meatballs](image-url)
groups, and 16 compounds in the case of E group. In groups with an addition of 1% of *Camellia sinensis* L. extract, methanol intensity was significantly lower compared to control groups both on D0 and D90 days of storage. Alcohols (Figure 2) are considered as a characteristic product of microorganism activity in meat. Therefore, the significantly lower value of methanol in samples with *Camellia sinensis* L. extract addition could suggest that microbiological growth was inhibited during frozen storage of meatballs (Casaburi et al., 2015; Gantner et al., 2017). The 0.01% BHT addition to meatballs caused decrease an intensity of a specific for heated meat volatile compounds such as: butane-2,3-dione, butanal, 1 R(+) -alpha-pinene, 1S-(a) -pinene, pentane.

### 3.6. Sensory evaluation of meatballs

PCA on sensory data for the 15 samples resulted in two principal factors that accounted for 91.66% of the variance (Figure 4). Factor 1 (69.78%) was associated with taste, flavor and overall acceptance. Factor 2 (21.88%) was associated with color and texture. Consumers were able to detect differences between samples, grouping them into distinctive clusters. This is associated with changes in sensory attributes during the storage of samples. The dominating descriptors responsible for samples discrimination were texture and color. The highest values of the evaluation characterized the samples with an addition of *Camellia sinensis* L. extract on all storage days. Gantner et al. (2017) and Contini et al. (2014) reported that the addition of natural antioxidant extracts increase the overall acceptability of meat.

### 3.7. Principal components analysis of meatballs during the frozen storage

PCA was performed to indicate the differences in the volatile compounds of different groups during frozen storage. The results are shown in Figure 5. In C group after 90 days of frozen storage, the flavor was changed.
compared to D0. Due to the addition of BHT and *Camellia sinensis* L. extract, the volatile compounds profile did not change significantly. This fact was also confirmed in consumers’ panel. However, the samples with the addition of BHT were disliked by the panelists.

The electronic nose is a rapid and feasible method in the detection and monitoring of off-flavors in foods and can be successfully used to replace chemical and traditional sensory methods (Haugen et al., 2006; Tikk et al., 2008).

Figure 4. PCA analysis of steamed meatballs with an addition of *Camellia sinensis* L. extract and synthetic antioxidant (BHT) on 0th, 14th, 28th, 42th and 90th days of frozen storage.

**Figura 4.** Análisis de PCA de las albóndigas al vapor a las que se adicionó con extracto de *Camellia sinensis* L. y antioxidante sintético (BHT) en los días 0, 14, 28, 42 y 90 de almacenamiento en congelador.
4. Conclusions

The addition of *Camellia sinensis* L. extract has a preservative result in beef-pork meatballs during frozen storage. A beneficial effect on lipid oxidation inhibition by reducing TBARS value compared to the control during frozen storage at -18°C for 90 days of meatballs was observed. Therefore, *Camellia sinensis* L. extract is an effective natural compound towards replacing the use of commercial antioxidants for extending the shelf-life of ready to eat pork and beef meatballs during frozen storage. Meatballs with an addition of plant extracts were also more acceptable by consumers than meatballs with synthetic antioxidants.

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Disclosure statement

The authors declare no conflict of interest.

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