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Impacts of carob pulp (*Ceratonia siliqua* L.) and vitamin E on pork colour, oxidative stability, lipid composition and microbial growth

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ABSTRACT

This study aimed to evaluate the impact of the dietary by-product rich in polyphenols (Carob pulp, Cp) and supra-nutritional level of vitamin (Vit) E on pork quality and shelf-life of meat stored in modified atmosphere packaging for 15 days. A total of 44 pigs (entire males and gilts, 170 ± 4.5 days of age and 127.8 ± 3.6 kg of body weight) were randomly selected from a larger group (one pig per pen). Pigs were fed ad libitum with one of four diets in a 2 × 2 factorial arrangement, with two feed inclusion levels each for Cp (0 vs. 20 %) and Vit E (30 (Low) vs. 300 IU/kg of feed (High)) for 40 days. No interactions between Cp and Vit E were detected for most variables assessed. Meat colour attributes evolved regardless of diet or sex, although metmyoglobin formation was preserved until 13 days. The Cp diets did not affect malondialdehyde nor α-tocopherol content in meat. High Vit E limited the malondialdehyde production up to 13 days and increased 1.8-fold the muscle α-tocopherol content compared to Low Vit E. The 20 %-Cp group tended to reduce total aerobic microbial count compared to 0 %-Cp group after 15 days of storage. Including Cp slightly affected the meat fatty acid (FA) profile, whereas Vit E did not modify it. Entire males presented higher content of polyunsaturated FA than gilts. Including 20 % Cp into pigs' diets does not impair meat quality, while High Vit E reduces lipid oxidation but not meat discolouration.

1. Introduction

Locally sourced agricultural by-products present a promising alternative for pig feed, aligning with the current consumer demand for natural and low environmental impact ingredients as well as the "clean label" options (Grasso, Estévez, Lorenzo, Pateiro, & Ponnampalam, 2024). Spain is one of the most prominent swine producers (53 million of pigs slaughtered in 2023) and simultaneously leads the production of Carob (*Ceratonia siliqua* L.) with 47,300 t of carob fruit/year (EUROSTAT, 2024; MAPA, 2023). Carob pulp (Cp) is a by-product which constitutes 90 % of the carob fruit by weight and is not readily suitable for human consumption but can be used as a feed source. The Cp is characterised by low crude protein (4.3 %) and energy content (8.0 MJ of metabolizable energy for growing pigs/kg of feed) but high concentration of polyphenols, including gallic acid, flavonol-glycosides, hydrolysable tannins, and mainly condensed tannins (CT) (FEDNA, 2019; Papagiannopoulos, Wollseifen, Mellenthin, Haber, & Galensa, 2004). The importance of CT in terms of human and animal nutrition has partly risen due to their antibacterial and antioxidant properties (Huang, Liu, Zhao, Hu, & Wang, 2018). Indeed, the meat industry has shown a broad interest in exploring these natural compounds to prevent meat deterioration, which represents a challenge during retail display time (Brenes, Viveros, Chamorro, & Arija, 2016).

The reactive oxygen species (ROS) generated during display time may affect the bright red colour and induce the peroxidation of unsaturated fatty acid (FA), developing off-odour and off-flavour and

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decreasing the nutritional value of meat (Pateiro et al., 2018). High-oxygen modified atmosphere packaging (MAP) is commonly used by retailers to preserve the colour of fresh pork for up to 10–14 days of display since it promotes oxymyoglobin formation (Gagaoua, Suman, Purslow, & Lebret, 2023). However, under high-oxygen conditions, the highly peroxidizable (HP) polyunsaturated fatty acids (PUFA) are predisposed to oxidation. Some polyphenols such as CT, might act as scavenging ROS, boosting enzymatic action and protecting other antioxidant agents, such as α-tocopherol (Landete, 2013; Mazur-kuśnirek, Antoszkiewicz, Lipiński, Kotlarczyk, & Żukowski, 2019), which prevent meat spoilage. Dietary Cp inclusion reduced meat lipid oxidation in broilers (Mahmoudi et al., 2022), whereas, in pigs, including Cp in diets increased the malondialdehyde (MDA) meat content (Inserra et al., 2015). Thus, research about the antioxidant effects of Cp in meat from monogastric is limited and controversial.

Vitamin (Vit) E is the most used antioxidant in animal nutrition, and it is commercially supplemented as all-rac-α-tocopheryl acetate. The link between Vit E supplementation, muscle α-tocopherol deposition, and improved meat oxidation stability has been extensively investigated (Buckley, Morrissey, & Gray, 1995; Sales & Koukolová, 2011). Indeed, Trefan et al. (2011) showed that short supplementation periods (< 50 days) can effectively increase muscle α-tocopherol content in pigs. The NRC (2012) recommends 11 IU Vit E/kg feed for finishing pigs, but the optimal level for extending pork shelf-life in oxygen-challenging storing conditions needs research. Onibi, Scaife, Murray, and Fowler (2000) suggested that higher dietary levels of Vit E than 500 IU/kg of feed do not necessarily increase absorption and deposition of α-tocopherol in tissues, while a level of 200 IU/kg of feed prevents pork lipid oxidation under aerobic refrigerated storage. Interestingly, in lambs, high dietary levels of Vit E reduced the microbial count in meat stored 14 days compared to a group fed a polyphenol-rich source (grape pomace) (Guerra-Rivas et al., 2016). However, in pork, few studies have tested antimicrobials effects on meat linked to dietary inclusion of Vit E or polyphenols sources.

Few articles have documented the effects of the dietary inclusion of Cp in pork quality. Indeed, nutritional guidelines and preliminary studies recommend low to moderate levels of Cp in pig's diet ranging from 6 to 15 % (FEDNA, 2019; Inserra et al., 2015). Moreover, none has investigated whether a potential synergistic effect of Cp with Vit E might extend shelf-life. Evidence from monogastric suggests that by-products rich in polyphenols may exhibit similar antioxidant properties on meat quality as Vit E (Brenes et al., 2016). Therefore, the objective of this study was to evaluate the impact of the dietary inclusion of 20 % of Cp and 300 IU Vit E/kg of feed on pork quality and the shelf-life of meat stored refrigerated in MAP for 15 days.

2. Material and methods

2.1. Animals and dietary treatments

This study was approved by the Ethical Committee for Animal Experimentation of the University of Lleida, Spain (CEEA 02–03/21-procedure 02). At the farm, 220 crossbred fattening pigs [Duroc x (Landrace x Large-White)] were stratified according to sexes (gilts and entire male, EM) and then randomly distributed in 44 partially-slatted pens (5 pigs of the same sex per pen, 22 pens for gilts and 22 for EM). Starting at 130 ± 4.5 days of age and with 78.4 ± 8.93 kg of body-weight (BW), the pigs received ad libitum one of four diets for the last 40 days of the fattening period. The diets were arranged in a 2 × 2 factorial design, with two Cp levels (0 % vs. 20 %) and two Vit E levels (30 vs. 300 IU/kg, Low and High, respectively). The four dietary treatments (0 % Cp-Low Vit E, 0 % Cp-High Vit E, 20 % Cp-Low Vit E and 20 % Cp-High Vit E) were equally distributed across the 44 pens. Thus, there was a total of 11 pens per treatment, 5–6 pens for males and 5–6 pens for females within each treatment. All diets were formulated following the FEDNA (2013) recommendations for finishing pigs to be isoenergetic (12.8 MJ metabolizable energy/kg of feed) and to meet the ideal protein content (15 % crude protein and 0.86 % Lys and balancing the rest of essential amino acids). Diets without Cp were composed of cereals (54.9 % barley, 16.3 % corn and 7.0 % wheat), protein co-products (9.2 % soybean meal and 8.0 % sunflower meal), synthetic amino acids (0.48 % L-Lysine sulphate, 0.02 % Hydroxy analogue of methionine and 0.09 % of L-Threonine), palm oil (1.51 %), and minerals and vitamins (2.50 %). In the diets with 20 % of Cp, cereals were

Table 1
Chemical composition (Mean ± SD), ether extract, fatty acid (FA) profile, tocopherols and polyphenols content of experimental diets including carob pulp (Cp, 0 vs. 20 %) and vitamin E (Vit E, 30 vs. 300 IU/kg of feed).

Item	Dietary treatments			
	0 % Cp		20 % Cp	
	Low Vit E	High Vit E	Low Vit E	High Vit E
Ether Extract, %	4.14 ± 0.16		6.72 ± 0.54	
Fatty acids, g / 100 g of total FA				
C12:0	0.09 ± 0.02		0.14 ± 0.02	
C14:0	0.48 ± 0.02		0.60 ± 0.05	
C16:0	28.2 ± 0.87		33.4 ± 1.23	
C17:0	0.21 ± 0.05		0.17 ± 0.06	
C18:0	7.2 ± 0.18		6.4 ± 0.27	
C16:1 *cis*-9	0.12 ± 0.02		0.12 ± 0.01	
C18:1 *cis*-9	25.3 ± 0.02		32.4 ± 0.01	
C18:1 *cis*-11	0.03 ± 0.47		0.03 ± 0.68	
C20:1	0.02 ± 0.01		0.02 ± 0.01	
C18:2 n-6	36.5 ± 0.73		25.4 ± 1.56	
C18:3 n-3	2.0 ± 0.01		1.4 ± 0.01	
∑ SFA[1]	36.1 ± 0.98		40.7 ± 1.27	
∑ MUFA[2]	25.4 ± 0.45		32.5 ± 0.69	
∑ PUFA[3]	38.5 ± 0.79		26.8 ± 1.87	
Total polyphenols, mg eq. tannic acid /g of dry matter (DM)	5.8 ± 0.4		8.6 ± 0.5	
Total CT[4], g eq. CT carob/kg DM	4.0 ± 1.7		20.9 ± 1.9	
β-carotene, μg/g DM	6.1 ± 0.9	5.5 ± 0.7	12.3 ± 0.8	11.5 ± 0.8
Total carotenoids, μg/g DM	17.2 ± 1.9	15.9 ± 1.4	31.0 ± 1.9	29.4 ± 2.1
α-tocopheryl acetate, μg/g DM	31.4 ± 17.4	299 ± 33.4	30.6 ± 10.1	296 ± 7.6
γ-tocopherol, μg/g DM	5.8 ± 0.6	6.1 ± 0.6	9.2 ± 3.3	7.0 ± 1.1

[1]Sum of saturated fatty acids, [2]Sum of monounsaturated fatty acids, [3]Sum of polyunsaturated fatty acids, [4]Total condensed tannins, expressed in g of the equivalent of condensed tannins (CT) purified from carob pulp/ kg of DM of feed. Polyphenols, carotenoids, and tocopherols are expressed on a dry matter basis.

partially replaced (29.1 % barley, 17.9 % corn and 7.0 % wheat) and they also contained protein co-products (15.8 % soybean meal and 3.9 % sunflower), synthetic amino acids (0.32 % L-Lysine sulphate, 0.05 % Hydroxy methionine and 0.07 % of L-Threonine), minerals and vitamins. Moreover, in the Cp diets palm oil was included in a larger proportion (4.07 %) to comply with the isoenergetic requirement. Vitamin E was supplemented in the form of DL (all-rac)-α-tocopheryl acetate (CUXAVIT E 50, Kaesler Nutrition, Germany, EU Code 3a700) and represented 0.05 % in those diets with the High level (300 IU/kg of feed). The detailed ingredient and proximal chemical composition of diets was reported previously (Bottegal, Latorre, Lobón, Verdú, & Alvarez-Rodriguez, 2024). The FA profile, tocopherols and polyphenols content of the experimental diets are presented in Table 1.

At the age of 170 days and after an 18 h-fasting period, all animals were weighed (124.7 ± 11.30 kg of BW) and transported 3 km to the BonArea Group slaughterhouse (Guissona, Spain). From all the experimental animals, a total of 44 pigs (22 gilts and 22 EM, all belonging to different pens, 11 animals per diet), were randomly selected, considering those animals close to the average BW of the pen, for the study of pork quality and shelf-life. At the slaughter line, the carcass weight was registered, and the dressing percentage calculated. Moreover, backfat thickness (between 12th and 13th ribs) and lean meat percentage were obtained with an AutoFom III ultrasonic system (Frontmatec group; Kolding, Denmark).

2.2. Analysis of feeds

Chemical analyses on feed were run in duplicates. The ether extract was determined with an XT10 Ankom extractor (Ankom Technology Corporation, Fairport, NY, USA) as described by AOAC (2000). The FA of feedstuffs were extracted following the method described by Sukhija and Palmquist (1988) using the C19:0 as internal standard and determined by Bruker Scion 436-GC gas-chromatograph (Bruker, Billerica, MA, USA) with a flame ionization detector equipped with a CP-8400 autosampler and an SP-2560 capillary column (100 m × 0.25 mm ID × 0.20 μm; Sigma-Aldrich, Saint Louis, MO, USA). Total polyphenol compounds (expressed as tannic acid equivalents) and CT content (expressed as Cp CT equivalents) were determined following the procedure described by Pelegrin-Valls et al. (2022). The α- and γ-tocopherol isomers and carotenoids content were determined using ACQUITY UPLC H-Class (Waters, Milford, MA, USA) after extraction with methanol-acetone-petroleum ether, as detailed in the methodology by Bottegal, Lobón, Latorre, Bertolín, and Álvarez-Rodríguez (2023). An all-rac-α-tocopheryl acetate Sigma-Aldrich standard (Munich, Germany) was used to differentiate the α-tocopheryl acetate supplemented from the natural α-tocopherol form of feedstuffs. The α-tocopherol content was expressed as the sum of the natural and the supplemented form.

2.3. Meat sample collection and preparation

In the slaughterhouse and after 3 h of chilling, a piece of *Longissimus lumborum* (LL) muscle (1st-4th lumbar *vertebra*) of approximately 15 cm in length and trimmed of subcutaneous fat, was excised from the right half of each carcass. Five slices (3 cm thick) were cut perpendicularly to the muscular fibre's long axis from each sample. Each slice was randomly distributed and assigned to one of five possible display times (0, 9, 11, 13 and 15 days). Before packaging, all slices were weighed (initial storage weight). All slices but the 0-d samples were stored under MAP (80 % O_2 + 20 % CO_2) with an absorbent pad and wrapped with a low gas permeability polyvinyl chloride film (5 cm^3 / m^2 / 24 h O_2 and 25 cm^3 / m^2 / 24 h CO_2 at 23 °C, 50 % relative humidity and 1 atm) and kept in darkness at 4 °C in a vertical fridge. Each tray contained 4 slices of LL, one of each dietary treatment. At 24 h post-mortem and after 15 days of display, pH was measured with pH meter (G-PH7V XS, Italy) equipped with a penetration electrode and calibrated with two buffer solutions pH 7.00 and 4.00.

2.4. Meat colour parameters, purge and thawing losses, and lipid oxidation

At the end of the target display time, 30 min of blooming was allowed before colour measurement. Then, each sample was gently dried with absorbent paper and weighed (final-storage weight) to assess the storage purge losses produced inside the packaging as 100 x (initial – final storage weight) / initial storage weight. Subsequently, meat colour descriptors L^*, a^* and b^* were registered, on two points of the LL muscle slice, in the CIELab space using a portable Minolta CM-700d spectrophotometer (Konica Minolta Sensing Inc., Osaka, Japan) with standard illuminant D65, zero observer angle and an 8-mm diameter viewing area. The reflectance spectra (400–700 nm wavelength) was recorded for calculating the metmyoglobin (MMb) formation, following the AMSA (2012) equation = 100 × {1.395 − [(A572nm − A700nm) / (A525nm − A700nm)]}. In addition, chroma saturation $C^* = (\sqrt{(a^{*2} + b^{*2})})$ and hue angle ($h^\circ = 57.29 \times \tan^{-1}(b^* / a^*)$) were calculated. Finally, samples were weighed again (pre-frozen weight), vacuum-packed, and frozen (−80 °C) until two months for subsequent chemical analysis. The thawed losses were calculated as 100 x (pre-frozen weight − thawed weight) / pre-frozen weight.

After thawing, two sub-samples from each loin were collected, one used for lipid oxidation (10 g) and the other (20 g) was freeze-dried (Freeze-dryer gamma 2–16 LSC- plus, Martin Christ, Osterode am Harz, Germany) and used for the tocopherol, cholesterol and FA analyses. Lipid oxidation was determined on every meat sample (from all days of storage) following an extraction method with ethanol and trichloroacetic acid solution and a derivatization of MDA with a thiobarbituric solution as described by Bertolín, Joy, and Blanco (2019). The concentration of MDA was quantified by UPLC H-Class liquid chromatography.

2.5. Meat tocopherol and cholesterol content

The tocopherols isomers (α- and γ-tocopherols) and the cholesterol content were analysed simultaneously on 200 mg of freeze-dried LL samples collected 24 h hours post-mortem. The methodology performed was described by Bertolín, Joy, Rufino-Moya, Lobón, and Blanco (2018). Briefly, samples were subjected to an overnight saponification process in an orbital shaker at 25 °C with 200 mg of L-ascorbic acid and 3 mL of saponification solution. Then, the extraction was mixed with 5 mL of n-hexane:ethyl acetate (9:1 v:v) and 5 g of BHT, vortexed and shaken for 15 min, and finally centrifuged (2000 *g*, 5 min at 10 °C). The upper layer was recovered and evaporated (30 min at 40 °C). Finally, 1 mL of mobile phase acetonitrile:methanol:dichloromethane (75:15:10, v:v:v) was used to dissolve the evaporated residue, which was then shaken (10 min at 25 °C) and filtered into vial for liquid chromatography. The extracts were injected into an ACQUITY UPLC H-Class liquid chromatograph.

2.6. Meat fatty acid profile

Gas chromatography was used to determine the FA profile of intramuscular fat (IMF) of LL at 0 days of storage. The FA of meat samples were extracted using C23:0 as an internal standard following the methodology described by Argemí-Armengol et al. (2022). A total of 500 mg of freeze-dried sample was minced and processed through a two-step methylation procedure to obtain FA methyl esters (FAME). Identification of FA was performed with the gas chromatograph described in the determination of FA in feed. One μL of the sample with 1/50 split was injected (at 250 °C) and FA identification was based on the retention times as compared with those of the standard FAME mixtures: GLC-532, GLC-401, GLC-463 and GLC-538 (Nu-Check Prep, Elysian, USA). The FAME were quantified following the indications of UNE-EN ISO 12966-4:2015. The IMF content was calculated as the sum of each FA detected, expressed as triglyceride equivalent following the procedure described by Bosch, Tor, Reixach, and Estany (2009). The indices for Δ9-

desaturase and elongase activity were estimated by the ratio of product to precursor FA. The HP-PUFA index indicates the level of FA which are more susceptible to oxidation, and it was calculated as the sum of PUFA with 3 or more double bonds.

2.7. Microbial analysis

Microbiological analyses were performed in male samples on days 0 and 15 (three replicates per diet and storage time). Immediately after opening the tray, 10 g of meat were aseptically removed (inside a laminar flow cabinet) and homogenised during a 1 min with 90 g of saline peptone water in a sterile plastic bag with filter in a Stomacher Masticator (IUL, S.A., Barcelona, Spain) at room temperature. Serial decimal dilutions were made in sterile peptone water and, in duplicate, 1 mL or 0.1 mL samples of appropriate dilutions were spread on plate count agar (Oxoid, Ireland). Incubations of plates were at 30 ± 1 °C for 72 h and at 6.5 ± 1 °C for 10 days for the total count of aerobic mesophilic and psychrotrophic microorganisms, respectively. The aerobics and psychrotrophic microorganisms count were performed according to UNE-EN ISO 4833-1:2013 and the NF-EN ISO 17410:2001 standards, respectively.

2.8. Statistical analysis

Statistical analyses were performed using the Infostat 2020 software (Centro de Transferencia InfoStat, UNC, Argentina). The animal was considered the experimental unit for all variables. A mixed linear model with repeated measures was performed for meat colour parameters, purge and thawing loss and MDA content, which were measured during the storage time. The Cp, Vit E, sex, day of display and their interaction were considered fixed effects, whereas the individual was considered random effect. Data of IMF content, FA profile, and meat concentration of tocopherols and cholesterol and microbial counts within each storage day were analysed by a general linear model, considering Cp, Vit E, sex and Cp x Vit E as fixed effects. The same model was used to analyse final BW and carcass traits (weight, dressing, lean percentage and back-fat thickness). Microbial counts were log-transformed (log_{10}) before running the statistical model to approximate the normality of data. Significance was established at $P \leq 0.05$ and differences were considered a trend towards statistical significance when $0.05 < P \leq 0.10$. All interactions were tested but those that showed no effect on variables were removed from the model. Multiple comparisons of the means were performed using Tukey's test.

3. Results

Interactions between Cp, Vit E and sex were not detected in final BW nor carcass traits ($P > 0.10$). Data about carcass quality are shown next in the text. Neither dietary Cp nor Vit E supplementation affected ($P > 0.10$) the final BW (127.8 ± 3.60 kg), carcass weight (93.9 ± 2.51 kg), carcass dressing (73.5 ± 1.00 %), lean percentage (60.9 ± 0.57 %) and back-fat thickness (16.5 ± 0.80 mm). The EM showed higher ($P < 0.05$) final BW, carcass weight and backfat thickness than gilts, 133.3 vs. 122.4 ± 2.23 kg, 96.8 vs. 91.0 ± 1.65 kg and 17.6 vs. 15.4 ± 0.51 mm, respectively. Furthermore, gilts presented greater ($P < 0.05$) carcass dressing and lean percentage than EM, 74.3 vs. 72.7 ± 0.43 % and 61.5 vs. 60.2 ± 0.38 %, respectively.

3.1. Meat colour, purge loss and lipid oxidation

Interactions between factors (Cp, Vit E and sex) and display time were evaluated but no effects were found ($P > 0.10$) on meat colour parameters. Therefore, the effects of Cp, Vit E and sex on colour variables were not dependent on the day of storage. Table 2 summarises the effects of Cp, Vit E, sex, and display time on meat colour, purge losses and pH of LL muscle. As expected, display time affected ($P < 0.001$) all colour parameters (Fig. 1), pH, purge losses except thawing losses ($P > 0.10$). L^* revealed the lowest value on day 0 compared to the rest of the days. The a^* attribute reached a nadir on day 0 and the peak on day 13, which decreased on day 15. Linear effects of time were observed in b^* and C* levels, as well as on MMb formation, which increased during the storage. Meanwhile, h° decreased from day 0 to day 9. The greatest purge losses were observed on day 15 (11.1 ± 0.69, $P < 0.05$), whereas no statistical differences were found in purge losses between days 9, 11 and 13 (8.21 ± 0.69, $P > 0.10$). The pH decreased from day 0 up to day 15, as expected (5.48 vs. 5.43 ± 0.01, $P < 0.001$).

The interaction between Cp and Vit E only affected the b^* ($P = 0.012$) and C* values ($P = 0.031$) (Fig. 2). High Vit E increased b^* and C* values of pork when animals received a 0 % Cp diet whereas no effect was observed when animals fed 20 % Cp. The single effects of Cp or Vit E did not affect ($P > 0.10$) meat colour attributes, purge losses or pH.

The MDA content increased from day 0 to 9 ($P < 0.001$), remaining stable until day 15 ($P > 0.10$). The MDA content ($P > 0.10$) was not influenced by Cp inclusion (Fig. 3 left), while the supplementation of 300 IU Vit E/kg of feed lowered meat MDA content ($P = 0.006$) compared to the 30 IU Vit E group (Fig. 3 right). Regarding sex, the only variable affected was L^* ($P = 0.004$) which was higher in EM than gilts.

3.2. Meat tocopherol and cholesterol content

No interactions between the factors were detected in the tocopherol isomers or cholesterol content ($P > 0.10$). Neither Cp nor sex affected these variables ($P > 0.10$). The supplementation of 300 IU of Vit E/kg of feed increased muscle deposition of α-tocopherol ($P < 0.001$) and cholesterol ($P = 0.019$) and decreased ($P < 0.001$) γ-tocopherol meat concentration compared with the 30 IU of Vit E (Table 3).

Table 2
Effect of dietary inclusion of carob pulp (Cp, 0 vs. 20 %) and vitamin E (Vit E, 30 vs. 300 IU/kg of feed) on pork loin quality (gilts and entire male, EM) stored up to 15 days in modified atmosphere packaging (MAP) at 4 °C in darkness.

	Cp		Vit E		Sex		SEM	*P*-value			
Item	0 %	20 %	Low	High	Gilts	EM		Cp	Vit E	Sex	Day[3]
L^*	53.3	52.9	53.0	53.1	52.5	53.7	0.27	NS	NS	<0.01	<0.001
a^*	3.0	3.2	3.1	3.1	3.2	3.0	0.14	NS	NS	NS	<0.001
b^*	7.2	7.1	7.1	7.2	7.1	7.2	0.13	NS	NS	NS	<0.001
C*[1]	7.9	7.8	7.8	7.9	7.8	7.9	0.15	NS	NS	NS	<0.001
h°[1]	68.2	66.7	67.1	67.8	66.8	68.2	0.83	NS	NS	NS	<0.001
MMb, %[1]	12.2	12.1	12.2	12.1	12.1	12.2	0.61	NS	NS	NS	<0.001
Storage purge losses, %	9.5	8.4	8.8	9.1	8.8	9.1	0.62	NS	NS	NS	<0.01
Thawing losses,%	14.1	13.5	13.6	14.0	14.2	13.4	0.70	NS	NS	NS	NS
pH[2]	5.46	5.45	5.46	5.45	5.45	5.46	0.007	NS	NS	NS	<0.001

[1]C* = chrome; h° = hue angle, MMb (%) = metmyoglobin formation estimated through reflectance. [2]pH of pork loins was measured on day 0 and at the end of display time (day 15). [3]The effect of time on colour parameters is shown in Fig. 1, while significant interactions are shown in Fig. 2. SEM; standard error of the means, NS; $P > 0.10$.

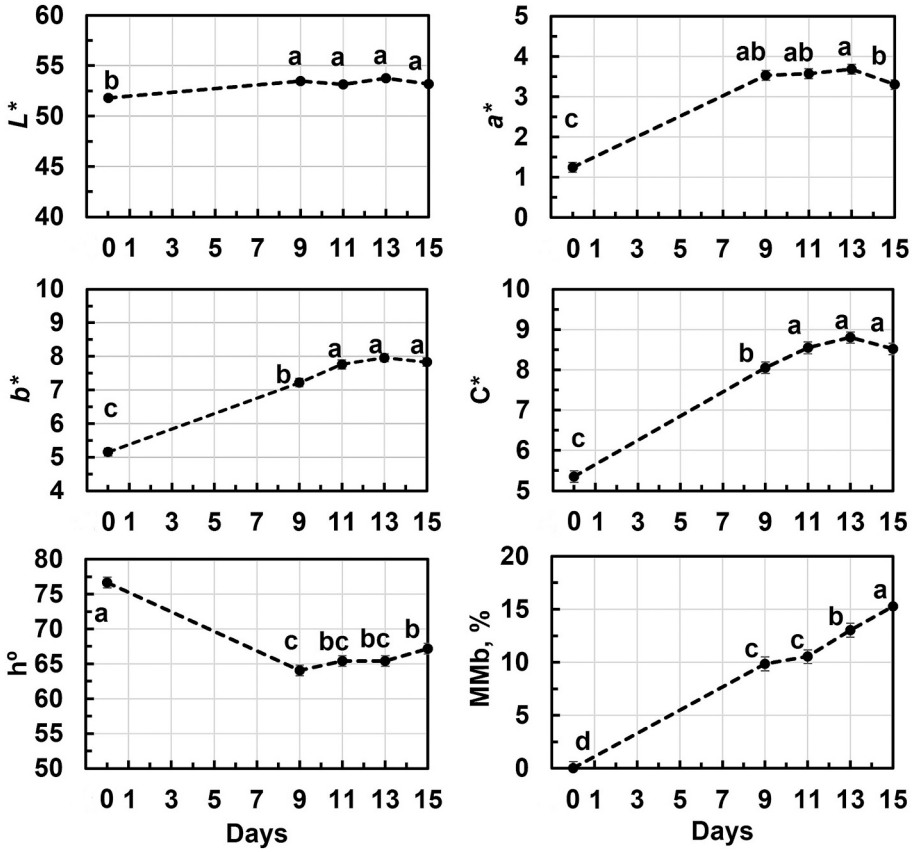

Fig. 1. Pork loin colour evolution (*L**, lightness; *a**, redness; *b**, yellowness; C*, colour saturation; h°, hue angle and MMb, metmyoglobin formation) during display time up to 15 days refrigerated at 4 °C in darkness under modified atmosphere packaging conditions.

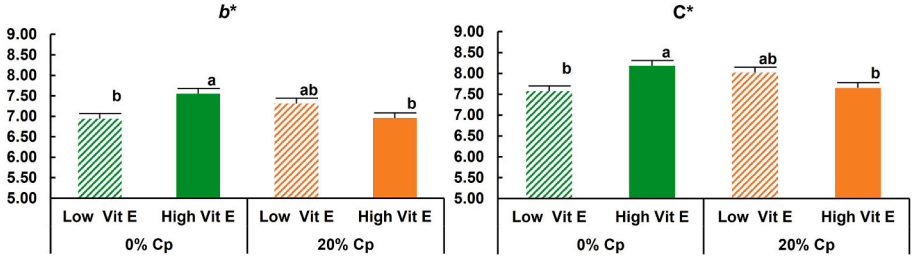

Fig. 2. Interaction between dietary carob pulp inclusion (Cp, 0 vs. 20 %) and vitamin E (Vit E, 30 vs. 300 IU/kg of feed) on the overall *b** index (left figure) and chroma (C*, right figure) of pork stored up to 15 days under modified atmosphere packaging. Values presented are the least-squares means with the standard error bars.

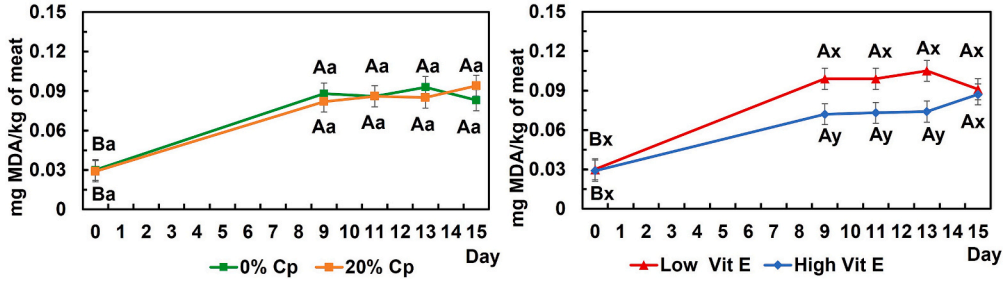

Fig. 3. Effect of dietary inclusion of carob pulp (Cp, 0 vs. 20 %, left) and vitamin E (Vit E, 30 vs. 300 IU/kg of feed, right) on malondialdehyde (MDA) content of pork stored under modified atmosphere packaging for 15 days. ^{A,B} Indicate differences ($P < 0.001$) between days. ^{a,b} Indicate differences between Cp levels within the same day. ^{x,y} Indicate differences ($P < 0.01$) between Vit E levels within the same day. Values presented are the least-squares means with the standard error bars.

Table 3
Effect of dietary inclusion of carob pulp (Cp, 0 vs. 20 %) and Vit E (30 vs. 300 IU/kg of feed) on tocopherols isomers (expressed as μg/g meat) and cholesterol content (mg/g of meat) of pork loin (gilts and entire male, EM).

	Cp		Vit E		Sex			*P*-value		
Item	0 %	20 %	Low	High	Gilts	EM	SEM	Cp	Vit E	Sex
α-tocopherol	4.53	4.27	3.13	5.67	4.43	4.37	0.153	NS	<0.001	NS
γ-tocopherol	0.06	0.06	0.07	0.05	0.06	0.063	0.004	NS	<0.001	NS
Cholesterol	0.58	0.60	0.55	0.63	0.59	0.60	0.023	NS	0.019	NS

SEM; standard error of means, NS; $P > 0.10$.

Table 4
Effect of dietary inclusion of carob pulp (Cp, 0 vs. 20 %) and Vit E (30 vs. 300 IU/kg) on total fatty acid methyl-ester (FAME) content and profile (expressed as mg/100 g of meat) of pork loin from gilts and entire male (EM).

	Carob pulp		Vit E		Sex		SEM	*P*-value		
	0 %	20 %	Low	High	Gilts	EM		Cp	Vit E	Sex
IMF, g FAME/100 g meat	1.46	1.28	1.36	1.39	1.24	1.50	0.10	NS	NS	0.070
C10:0	1.42	1.12	1.17	1.37	1.20	1.34	0.133	NS	NS	NS
C12:0	1.10	0.96	1.00	1.06	0.92	1.14	0.098	NS	NS	NS
C14:0	14.2	12.0	12.8	13.5	11.7	14.5	1.41	NS	NS	NS
C15:0	0.85	0.55	0.71	0.69	0.62	0.79	0.064	0.002	NS	0.068
C16:0	353	305	327	331	300	359	24.6	NS	NS	0.097
C17:0	4.83	3.76	4.21	4.38	4.10	4.48	0.584	NS	NS	NS
C18:0	214	187	199	202	182	219	11.6	NS	NS	0.030
C19:0	0.19	0.15	0.16	0.17	0.14	0.20	0.030	NS	NS	NS
C20:0	1.13	1.07	1.06	1.14	0.97	1.23	0.180	NS	NS	NS
C22:0	0.32	0.25	0.27	0.31	0.27	0.30	0.066	NS	NS	NS
C24:0	0.06	0.06	0.06	0.06	0.06	0.06	0.006	NS	NS	NS
C12:1	0.10	0.11	0.10	0.11	0.09	0.12	0.037	NS	NS	NS
C14:1	0.29	0.28	0.29	0.28	0.30	0.27	0.040	NS	NS	NS
C16:1 *cis*-9	40.0	30.2	34.3	35.9	33.4	36.8	3.81	0.076	NS	NS
C18:1 *cis*-9	471	414	426	458	397	488	40.4	NS	NS	NS
C18:1 *cis*-11	49.7	38.4	42.7	45.4	41.4	46.7	4.19	0.062	NS	NS
C20:1	5.14	4.96	4.97	5.13	4.30	5.80	0.600	NS	NS	0.089
C22:1	0.17	0.15	0.16	0.16	0.14	0.18	0.020	NS	NS	NS
C24:1	0.04	0.03	0.03	0.04	0.03	0.04	0.005	0.088	NS	NS
C18:2 n-6	206	188	201	192	175	218	9.9	NS	NS	0.004
C18:3 n-6	1.97	1.72	1.92	1.78	1.72	1.98	0.098	0.080	NS	0.069
C18:3 n-3	5.41	5.25	5.33	5.32	4.27	6.39	0.497	NS	NS	0.004
C19:2 n-6	0.32	0.31	0.33	0.30	0.31	0.32	0.064	NS	NS	NS
C20:2 n-6	5.11	4.46	4.74	4.83	3.99	5.58	0.390	NS	NS	0.007
C20:3 n-6	8.58	7.79	8.46	7.91	7.44	8.94	0.431	NS	NS	0.019
C20:3 n-3	0.20	0.14	0.15	0.19	0.17	0.17	0.032	NS	NS	NS
C20:4 n-6 ARA[1]	60.0	54.6	59.7	54.9	53.9	60.7	2.69	NS	NS	0.080
C20:5 n-3 EPA[2]	1.85	1.60	1.81	1.64	1.57	1.88	0.147	NS	NS	NS
C22:4 n-6	7.87	6.88	7.40	7.36	6.73	8.03	0.315	0.031	NS	0.006
C22:5 n-6	1.15	0.95	1.08	1.03	1.02	1.09	0.058	0.020	NS	NS
C22:5 n-3 DPA[3]	6.30	6.12	6.50	5.92	5.81	6.61	0.262	0.620	NS	0.038
C22:6 n-3 DHA[4]	2.67	2.11	2.51	2.27	2.24	2.54	0.195	0.048	NS	NS
∑SFA	591	513	547	556	501	602	37.9	NS	NS	0.069
∑MUFA	567	488	509	545	476	578	48.7	NS	NS	NS
∑PUFA	307	280	301	286	265	322	13.7	NS	NS	0.005
∑n-6	291	265	285	270	250	305	13.1	NS	NS	0.006
∑n-3	16.4	15.2	16.3	15.3	14.0	17.6	0.76	NS	NS	0.002
n-6/n-3	17.9	17.4	17.5	17.6	17.8	17.3	0.46	NS	NS	NS
PUFA/SFA	0.52	0.56	0.55	0.51	0.53	0.54	0.018	NS	NS	NS
HP-PUFA[5], mg/g muscle	0.95	0.87	0.95	0.87	0.84	0.99	0.040	NS	NS	0.007
Δ9-desaturase C16:1 *cis*-9/C16:0	0.11	0.09	0.10	0.10	0.11	0.10	0.004	0.015	NS	NS
Δ9-desaturase C18:1 *cis*-9/C18:0	2.14	2.12	2.07	2.2	2.12	2.15	0.08	NS	NS	NS
Elongase C18:1 *cis*-11/C16:1 *cis*-9	1.28	1.32	1.31	1.29	1.29	1.31	0.04	NS	NS	NS

[1]Arachidonic acid, [2]Eicosapentaenoic acid, [3]Docosapentaenoic acid, [4]Docosahexaenoic acid, [5]Highly peroxidizable (HP)–Polyunsaturated fatty acids (PUFA) calculated as the sum of PUFA with more than 3 double bonds. SEM; standard error of means, NS; $P > 0.10$.

3.3. Intramuscular fat content and fatty acids profile

No effect of the interaction between the Cp and Vit E dietary inclusion and sex was found ($P > 0.10$) in the IMF content or FA profile of pork (Table 4). The IMF was not affected by the Cp inclusion nor the Vit E supplementation, although EM tended ($P = 0.07$) to have a higher IMF than gilts.

The sum of SFA was not affected by the diets, however, the 20 %-Cp diet decreased ($P = 0.002$) the C15:0 meat content compared to the 0 %-Cp diet. The Vit E did not affect the content of any FA ($P > 0.10$). Regarding sex, EM tended ($P < 0.10$) to show a higher amount of C15:0, C16:0 and total SFA than gilts, meanwhile, the C18:0 was also greater ($P = 0.030$) in EM.

The sum of MUFA was not affected by any factor studied, although the content of C16:1 *cis*-9, C18:1 *cis*-11 and C24:1 tended ($P < 0.10$) to be lower in meat from the 20 %-Cp group compared with the 0 %-Cp group. The EM meat tended ($P = 0.089$) to have a higher content of C20:1 than gilts.

Diets did not affect the sum of PUFA ($P > 0.10$), but sex did since EM had greater content than gilts ($P < 0.01$). The dietary inclusion of 20 % Cp tended ($P = 0.080$) to decrease the C18:3 n-6 γ-Linolenic and reduced ($P < 0.05$) the meat content of C22:4 n-6, C22:5 n-6 and C22:6 n-3 DHA compared with 0 %-Cp. The content of linoleic acid (C18:2 n-6), linolenic acid (C18:3 n-3), eicosadienoic acid (C20:2 n-6), dihomo-gamma-linolenic acid (C20:3 n-6), adrenic acid (C22:4 n-6), docosapentaenoic acid (C22:5 n-3) and HP-PUFA were greater in EM meat than gilts ($P < 0.05$). The γ-linolenic acid (C18:3 n-6) and arachidonic acid (C20:4 n-6) tended to be higher in EM meat ($P < 0.10$) compared to gilts.

The activity Δ9-desaturase (C16:1 *cis*-9/C16:0) decreased in the Cp animals ($P < 0.015$), though the Vit E or sex did not affect the estimated activity of this enzyme. Neither the Δ9-desaturase (C18:1 *cis*-9/C18:0) nor elongase activity were influenced by any of the studied factors ($P > 0.10$).

3.4. Shelf-life: Microbial count

At 24 h postmortem, there were no differences ($P > 0.10$) in the total count of aerobics measured on LL of EM due to the dietary inclusion of Cp or Vit E (Table 5). However, after 15 days of storage, those samples obtained from Cp-pigs tended ($P = 0.055$) to show a lower total aerobic count, whereas Vit E addition did not affect this variable ($P > 0.10$). The total psychrotrophic count was not affected ($P > 0.10$) by any dietary factors.

4. Discussion

To the best of our knowledge, there is no evidence of including a high Cp level (200 g/kg of feed) and a supra-nutritional vitamin E level (300 IU/kg of feed) in the fast-growing fattening pig diets to improve the chemical composition and shelf-life of pork under MAP. Although a synergistic effect was hypothesised between Cp polyphenols and Vit E on pork quality, the current results suggest that this combination produced neither harmful nor beneficial effects on carcass traits or meat quality parameters.

Including 20 % of Cp in pig diets fed ad libitum did not affect animal performance (Bottegal et al., 2024) and carcass traits. This suggests no negative effects of Cp CT and that an adequate dietary balance was maintained, as a deficiency of crude protein and amino acids would have impaired muscle growth and increased fat deposition. Similar findings were reported in pigs supplemented with moderate levels of carob (12.5 - 15 %) (Inserra et al., 2015; Kotrotsios, Christaki, Bonos, & Florou-Paneri, 2012). Based on Spain's annual carob fruit production with a seed yield of 10 %, Cp production is estimated to be approximately 42,570 t/year. Dietary inclusion of Cp (200 g/kg of feed) during the last 40 days of the fattening period of pigs could theoretically feed approximately 1,715,000 pigs, assuming an average feed daily feed intake of 3.1 kg of feed/pig (Bottegal et al., 2024).

In heavy pigs, Corino, Oriani, Pantaleo, Pastorelli, and Salvatori (1999) observed that supra-nutritional levels (200–300 IU) of Vit E improved carcass weight and dressing. However, in the current trial, the supplementation of 300 IU of Vit E did not influence the final BW or carcass traits, which was in agreement with other studies supplementing up to 200 IU of Vit E in fattening pigs (Boler et al., 2009; Wang, Jang, Rentfrow, Azain, & Lindemann, 2022).

Despite similar slaughter age, EM reached greater BW and produced heavier carcasses than gilts, linked to greater backfat thickness and higher IMF. Xie et al. (2023) suggested that the higher the carcass weight the greater the backfat thickness and the fat deposition. In contrast, other studies observed similar carcass traits between sexes (Vanheukelom, Van Beirendonck, Van Thielen, & Driessen, 2012) which is likely expected in animals slaughtered at the same BW. The lower lean content in the EM than in gilts could be attributed to the genetic line used which is intended for increasing the marbling in pork as an alternative to the business-as-usual Pietrain paternal lines reared in Spain. Moreover, the current nutritional recommendations are likely not in line with the digestible amino acid requirements of the EM of this fast-growing line, which predispose them to precocious fat accretion.

Meat colour as well as water holding capacity were affected by the display time. The MMb (oxidized form of myoglobin) reached the highest value on day 15, indicating the highest myoglobin oxidation. During the display time, protein and lipid oxidation produced in high-oxygen MAP systems can lead to reduce water-holding capacity and increase the formation of MDA (Wang, Wang, Li, & Zhang, 2019). However, the MMb percentages kept always below 20 % considered the threshold for slight discolouration (AMSA, 2012). Additionally, MDA values were kept steady from day 9 onwards, reflecting minimal lipid oxidation. It is worth noting that meat samples were kept in darkness inside a vertical fridge to stabilize the storage conditions across trays. However, these conditions may slightly differ to those available at the purchase points.

The interaction between Cp and Vit E was unobserved in almost all variables evaluated during the display time (except b^* and C*) indicating the lack of synergistic effects on colour parameters. The high C* value observed in 0 %Cp-High Vit E may indicate the preservation of the intensity of the red colour (Álvarez-Rodríguez et al., 2015), while 20 % Cp-Low Vit E produced it partially. Likewise, the high level of yellowness (b^*) was previously observed in pork from pigs fed High Vit E levels (Hasty, Van Heugten, See, & Larick, 2002; Zhu et al., 2022), and it would be linked to the Vit E fat-soluble properties. However, including at the same time Cp in diets counterbalanced the High Vit E effects on b^*.

Interestingly, a level of 300 IU of Vit E did not prevent meat discolouration. Dunshea, D'Souza, Pethick, Harper, and Warner (2005) reported that long-term and high supplementation of Vit E (200 IU/kg of feed) might benefit pork colour and reduce purge loss. However, several studies in line with the current findings (Boler et al., 2009) suggest that the impact of Vit E on pork discolouration appears not detectable compared to ruminant meat (Suman, Faustman, Stamer, & Liebler, 2006).

The concentration of MDA in meat is a reliable marker of lipid oxidation. Herein, regardless of the diet or sex, MDA was always below the borderline (0.5 mg MDA/kg) at which lipid oxidation and the off-flavours (rancidity) may be detectable (Dunshea et al., 2005). Our results showed no effects of Cp inclusion on MDA content, likely due to the low bioavailability of Cp's polyphenols with large molecular weight (Landete, 2013). Nevertheless, the High Vit E prevented the MDA production in pork compared to the Low Vit E up to 13 days of storage. The

Table 5
Effect of dietary inclusion of carob pulp (Cp, 0 vs. 20 %) and vitamin E (Vit E, 30 vs. 300 IU/kg) on total aerobic and psychrotrophic microbial counts (log_{10} CFU/g meat) of pork loins from entire males at the day 0 and 15 of storage.

		Cp		Vit E		SEM	*P*-value	
	Day	0 %	20 %	Low	High		Cp	Vit E
Total aerobic count	0	1.9	2.2	2.1	2.0	0.24	NS	NS
	15	2.6	2.1	2.3	2.4	0.17	0.055	NS
Total psychrotrophic count	0	0.50	nd	nd	0.50	0.354	NS	NS
	15	0.58	0.55	0.55	0.58	0.565	NS	NS

SEM; standard error of means, nd; levels not detected, NS; $P > 0.10$.

current results support prior findings that Vit E supplementation between 200 and 400 IU effectively reduces meat lipid oxidation compared to lower levels (Boler et al., 2009; Popova, Marinova, & Ignatova, 2014). Similarly, Kim et al. (2015) supplementing 300 IU of Vit E for 42 days observed a lower MDA after 24 h post-slaughter compared to the control group (35 IU).

In broilers, some studies reported a protective effect of polyphenols on Vit E (Brenes et al., 2016; Goñi et al., 2007). Nevertheless, in pork, the consumption of Cp did not affect the tocopherols deposition, failing to demonstrate the protective or regenerating effect mentioned on Vit E. Additionally, the Vit E muscle deposition as well as its antioxidants effects highly depend on the length and the level supplemented (Phillips et al., 2001; Sales & Koukolová, 2011; Trefan et al., 2011). The α-tocopherol muscle content in the High Vit E group increased 1.80-fold compared to the Low Vit E group. Likewise, the decrease of γ-tocopherol muscle in the High Vit E compared to the Low Vit E group was likely due to an interaction between Vit E isomers (α-, γ-, δ-) which influences their deposition in the muscle. Overall, Vit E enrichment is associated with elevated muscle tocopherol content, potentially augmenting the nutritional value of pork for human consumers (Chu, Chew, Liew, & Nyam, 2023).

Cholesterol is an integral part of cell membranes, and it is simultaneously vulnerable to oxidation which occurs during the formation of modified low-density lipoprotein, enhancing the potential for cytotoxicity and atherogenicity (Buckley et al., 1995). Herein, the High Vit E supplementation increased the amount of cholesterol in pork. Although the causes of this increase are difficult to demonstrate with the present trial, both the higher muscle cholesterol content and the lower MDA concentration observed in the High Vit E group might be a consequence of its lipidic antioxidant effect (de Oliveira et al., 2018).

The inclusion of 20 % of Cp in diets slightly altered the feed ether extract content and the FA profile. However, the use of palm oil as a source of energy increased the saturation level of the Cp diet, unlike other studies with carob (Inserra et al., 2015; Kotrotsios et al., 2012). Despite the different profile of FA in the diets due to the inclusion of Cp, no differences were observed in the main ratios on meat. Conversely, Kotrotsios et al. (2012) found a tendency to increase the PUFA content in pork when carob pods were included in diets, although dietary FA composition was not informed. Inserra et al. (2015) including Cp in pigs' diets rich in PUFA (around 68 % PUFA) found a decrease in SFA and an increase in the MUFA content of pork with Cp. Furthermore, these authors included lower levels of C18:2 and higher levels of C18:3 n-3 in the diets with Cp compared to the 0 %Cp-diet, and consequently, those differences reflected in the FA composition of meat.

Herein, the Cp-group displayed a lower meat content of some minor SFA, such as C15:0, than the control group. Also, Cp meat tended to decrease the content of some MUFA (C16:1 *cis*-9, C18:1 *cis*-11 and C24:1) since Cp diet contained 7 % more oleic acid (C18:1 n-9) which inhibited the Δ9-desaturase activity in the adipose tissue, responsible for catalysing the conversion of SFA to MUFA. Likewise, in the meat from the Cp-group, the lower content of some long chain n-6 and n-3 FA, particularly DHA (C22:6 n-3), was likely due to the lower C18:2 n-6 and C18:3 n-3 level in the Cp diets, since these FA serve as precursors for the synthesis of essential long-chain FA (Kloareg, Le Bellego, Mourot, Noblet, & van Milgen, 2005). Therefore, the changes observed in the FA profile of meat, both here and in the aforementioned literature, would be related to the rest of the lipid sources used in the diets rather than to Cp lipid composition, which has only 0.6 % of ether extract.

Pigs with higher growth performance, body weight, and larger fat deposits (measured by backfat thickness and IMF) increase de novo synthesis acids, i.e., SFA and MUFA (Argemí-Armengol et al., 2023). This may explain the tendency in EM to present higher SFA and C18:0 content than gilts. However, in the current study, no differences were observed in the MUFA content between the sexes. In agreement with other studies (Argemí-Armengol et al., 2022), males showed higher PUFA content (C18:2 n-6, C18:3 n-3, C20:2 n-6, C20:3 n-6, C22:4 n-6 and C22:5 n-3) than gilts. Although both sexes ate the same diets, EM had higher daily feed intake (Bottegal et al., 2024) hence higher consumption of essential FA, increasing their muscle deposition, especially PUFA since it is related to their dietary inclusion. In addition, the tendency to higher MDA formation in EM than in gilts was likely associated with the higher deposition of HP-PUFA.

Finally, after 15 days of refrigerated storage, all samples were far below the limit of 7 $\log_{10}$ CFU/g established in the microbiological criteria for foods of the European Union (No. 2073/2005) above which meat is unacceptable for human consumption. Thus, MAP condition could preserve pork shelf-life for up to 15 days since CO_2 concentrations of 10–20 % inhibit most aerobic bacteria (Livingston, Brewer, Killifer, Bidner, & McKeith, 2004). All treatments had similar initial microbial counts, however, the dietary inclusion of 20 %-Cp tended to have less aerobic count than 0 %-Cp after 15 days of storage. Similar results were obtained in pork including rosemary (feedstuff rich in polyphenols) in the diets (Peñaranda, Auqui, Egea, Linares, & Garrido, 2021). Recently, it was highlighted an antimicrobial activity of Cp extract against a wide range of strains, including *Escherichia coli* (Djebari et al., 2024). To the best of our knowledge there are not studies evaluating the impact of Vit E on microbial growth in pork, but it has been evaluated in ruminant meat. For example, Vieira, Guerra-Rivas, Martínez, Rubio, & Manso (2022) found no effects on the total viable microbial count in suckling lamb meat when Vit E or polyphenols sources were included in the ewe diets.

5. Conclusions

Incorporation of Cp in fast-growing pig diets was feasible without compromising carcass characteristics, pork colour, drip losses or lipid oxidation. Unlike other by-products, Cp failed to elevate the intramuscular α-tocopherol concentration or enhance the antioxidant capacity of pork, rejecting the hypothesis of synergistic effects between Cp and Vit E on key variables linked to lipid oxidation and meat shelf-life. Minimal alterations to the overall FA composition were detected upon Cp inclusion, although could exert antimicrobial activity, potentially extending the refrigerated shelf-life of pork. Dietary Vit E supplementation of 300 IU/kg of feed for 40 days increased muscle α-tocopherol deposition and limited lipid oxidation of pork under MAP conditions without limiting meat discolouration or microbial growth. Meat quality differed between sexes, since EM were heavier and fatter and had lighter meat and deposited more PUFA than gilts at the same age, which made them more prone to fat oxidation.

Ethics statement

The Ethical Committee for Animal Experimentation of the University of Lleida approved the protocol used in this study (CEEA 02-03/21-procedure 02). All animals were raised, managed and slaughtered under the Spanish Animal Protection Regulations RD 53/2013 and EU Directive 2010/63 for animal experiments.

CRediT authorship contribution statement

Diego Nicolas Bottegal: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **María Ángeles Latorre:** Writing – review & editing, Visualization, Supervision, Methodology, Investigation, Conceptualization. **Sandra Lobón:** Writing – original draft, Visualization, Supervision, Methodology, Investigation, Conceptualization. **Immaculada Argemí-Armengol:** Writing – review & editing, Visualization, Methodology, Conceptualization. **Javier Álvarez-Rodríguez:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that no conflict of interest is involved in this work.

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Data availability

Data will be made available on request.

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