Animal 17 (2023) 100879

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

Effect of feeding olive pomace acid oil on pork lipid composition, oxidative stability, colour, and sensory acceptance

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ARTICLE INFO

Article history:
Received 19 December 2022
Revised 1 June 2023
Accepted 5 June 2023
Available online 14 June 2023

Keywords:
Meat quality
Pig feed
Pork loin
Refining by-products
Upcycling

ABSTRACT

One of the targets of the meat industry is to reduce production costs and to increase the sustainability of the food chain, which has driven the attention towards the use of by-products as feed ingredients. Acid oils are fat by-products coming from the chemical refining process of edible oils, with a high energy value and that are approved as feed ingredients in the European Union. However, meat producers are hesitant to utilise them due to their varying composition and the limited understanding of their impact on animal performance and meat quality. The objective of this study was to evaluate the effects of using olive pomace acid oil (**OPAO**) instead of its corresponding crude olive pomace oil (**OPO**) or crude palm oil (**PO**) in pig diets on lipid composition, lipid oxidation and quality of pork loin (*longissimus dorsi*), fresh and after commercial refrigerated storage for 8 days. The experimental design consisted of feeding pigs with four diets supplemented with a 5% of PO, OPO, OPAO or a blend (**B**) of PO and OPAO (50:50, w/w). Fresh and refrigerated pork loin samples were assessed for fatty acid profile; tocopherol (**T**) and tocotrienol (**T3**) composition; lipid oxidative stability with the ferrous oxidation-xylenol orange method; 2-thiobarbituric acid (**TBA**) value; volatile compounds; colour; and sensory acceptance. Results showed that refrigeration reduced the total T + T3 levels and increased the TBA values and the volatile compound concentrations. The refrigerated storage also affected the instrumental colour parameters (L*, a* and b*) but not the overall acceptance of pork. Regarding the diet, pork from OPAO diet showed a higher unsaturated-to-saturated fatty acid ratio than pork from PO and B diets. The lowest T + T3 concentration was found in OPO and OPAO fresh pork and in OPAO refrigerated pork. The oxidative stability of fresh pork was lower for OPAO than for PO diet, but no significant effect of the diet was observed for this parameter in refrigerated pork. The TBA values and volatile compound concentrations of fresh pork were not affected by the diet. After refrigeration, OPAO pork had the highest TBA value and volatile compound concentrations. In any case, colour and consumer acceptance of pork were not affected by diet. In conclusion, in order to upcycle acid oils in pig diets, and considering results on the lipid oxidative stability of pork, it would be preferable to add the OPAO used in this study blended with PO.

Implications

The use of acids oils (fat by-products from edible oil production) as ingredients in pig diets may have a positive impact on the economy of pork production due to their high availability and low price. This study showed that when olive pomace acid oil was added at 5%, pork lipid oxidation increased; although when used at a 2.5% together with a 2.5% of crude palm oil, pork lipid oxidation was not affected. In no case did the use of acid oils affect the colour or the overall acceptance of fresh and commercially refrigerated pork.

Introduction

One of the main targets of meat industry is to reduce the economic costs associated to meat production, which is mostly due to feed formulation. This can be achieved through the replacement of

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https://doi.org/10.1016/j.animal.2023.100879
1751-7311/

some feed ingredients, which are raw materials, by some by-products of food industry. Therefore, the use of food by-products in feeds can contribute to increase the sustainability of the food chain.

The inclusion of fats in animal diets is a common practice in animal production, as they can improve animal growth due to their high energy value. Fats also provide essential fatty acids (**FA**) and lipid-soluble vitamins, such as tocopherols (**T**) and tocotrienols (**T3**). In the last decades, the type of fat added to animal diets has changed to meet consumer demand for leaner and healthier meat (Cardenia et al., 2011). Thus, the use of animal fat sources (rich in saturated FA) in animal nutrition has decreased in favour of crude vegetable oils (generally with a more unsaturated FA profile) (Alonso et al., 2012). Although a more unsaturated meat may be desirable for its high nutritional value, it may be more prone to lipid oxidation, which is the major cause of non-microbial degradation in meat and meat products (Amaral et al., 2018). Lipid oxidation can have a negative impact on meat's nutritional value and on colour, texture and flavour, leading to a loss of sensory acceptability. An option to balance a high concentration of unsaturated FA in meat with its oxidative stability might be the use of dietary fat sources rich in monounsaturated FA, such as oils coming from olives.

Despite crude vegetable oils are widely used in animal feeding, some fat by-products are an interesting cheaper alternative, since they are potential sources of energy (Varona et al., 2021a). One of the main by-products of olive oil industry is olive pomace, which is a mixture of olive pulp and stone, still rich in oil and with a high concentration of phenolic compounds (Difonzo et al., 2021). After removing the water of olive pomace, crude olive pomace oil is extracted and subjected to a chemical refining process to avoid undesirable colours, flavours, and compounds that would affect the stability of the final edible oil. This refining process consists of several steps, one of them being a chemical neutralisation meant to remove free FA from the oil and that produces soap stocks as by-products. The subsequent acidification of these soap stocks generates acid oils, which have a similar FA profile to the crude oil but are richer in free FA (Varona et al., 2021a; 2021b).

Upcycling acid oils as feed fats, as an alternative to crude fats and oils, is a way to increase their value and would contribute to improving the sustainability of the food chain. From an economical point of view, the price of this refining by-product is lower than that of the corresponding crude oil. Therefore, replacing crude oils with acid oils in feeds would reduce the costs associated with animal nutrition, which is the most expensive area within meat production. However, the composition of acid oils is highly variable (Varona et al., 2021a; 2021b), which could compromise animal performance and affect the composition and quality of meat products, decreasing the confidence in them as feeding fats. Despite the potentially significant impact of the use of acid oils as animal feed fats, there is a paucity of information in the literature on this topic, with most studies primarily focused on animal performance. Furthermore, the effect of acid oils on the composition and quality of animal products has been studied even less extensively.

The aim of this trial was to evaluate the effect of using olive pomace acid oil (**OPAO**) as fat source in pig diets, instead of its corresponding crude oil (crude olive pomace oil, **OPO**) or crude palm oil (**PO**), on lipid composition, oxidative stability, colour, and sensory acceptance of fresh and refrigerated pork loin. This study complements the information obtained by Verge-Mèrida et al. (2021) about the impact of using OPAO as fat source in pig diets on animal's digestibility and performance.

Material and methods

Experimental fats

The experimental fat sources used in this study were: PO, supplied by bonÀrea Agrupa (Guissona, Spain); OPO and OPAO, both provided by RIOSA (Refinación Industrial Oleícola S.A., Ibros, Spain). Samples of these fats were collected and were kept in vials at −20 °C under N_2 until the **MIU** value (moisture and volatile matter, insoluble impurities in petroleum ether and unsaponifiable matter), T and T3, FA composition and lipid classes were analysed as detailed by Varona et al. (2021c). The composition of the experimental fat sources is shown in Table 1, and their complete FA profile including minor FA can be found in Supplementary Table S1.

Animals and diets

The animal housing and husbandry was carried out at the animal facilities of bonÀrea Agrupa (Nial farm, Guissona, Spain). A total of 226 boars and gilts [(Landrace × Large White) × Duroc] of approximately 59 kg (103 days of life) were fed up to 103 kg of BW (from 0 to 40 days of the experimental trial) with a grower diet, and from 103 to 130 kg of BW (41 to 62 days of the experimental trial) with a finisher diet. The experimental diets resulted from supplementing the basal diets (grower or finisher) with a

Table 1
Composition of the three fat sources used to formulate the different experimental diets for pigs.

Items	Fat source[1]		
	PO	OPO	OPAO
MIU (g/100 g of fat)[2]			
Moisture[3]	0.14 ± 0.02	0.28 ± 0.01	1.27 ± 0.03
Insoluble impurities	0.13 ± 0.05	0.79 ± 0.31	7.84 ± 0.42
Unsaponifiable matter	0.22 ± 0.04	3.58 ± 0.02	3.56 ± 0.46
Total	0.50 ± 0.06	4.65 ± 0.31	12.68 ± 0.62
FA (%)[2,4]			
C16:0	42.4 ± 0.02	12.8 ± 0.15	13.5 ± 0.04
C18:0	4.6 ± 0.02	2.6 ± 0.04	3.6 ± 0.01
Saturated FA[5]	48.2 ± 0.02	16.1 ± 0.19	18.6 ± 0.04
C18:1n-9	40.8 ± 0.06	67.0 ± 0.11	62.2 ± 0.23
C18:1n-7	0.8 ± 0.14	2.8 ± 0.07	2.1 ± 0.01
Monounsaturated FA[6]	41.8 ± 0.09	71.1 ± 0.18	65.4 ± 0.20
C18:2n-6	9.7 ± 0.06	12.0 ± 0.01	15.0 ± 0.05
C18:3n-3	0.3 ± 0.01	0.9 ± 0.01	1.0 ± 0.01
Polyunsaturated FA[7]	10.0 ± 0.06	12.8 ± 0.01	15.8 ± 0.04
T and T3 (mg/kg of fat)			
α-T	203.90 ± 9.97	454.03 ± 19.99	383.59 ± 16.35
γ-T	2.15 ± 0.20	10.05 ± 1.29	21.39 ± 0.27
α-T3	219.60 ± 9.34	4.69 ± 0.28	6.02 ± 0.61
γ-T3	275.43 ± 13.14	ND	5.31 ± 0.67
T + T3[8]	740.06 ± 19.09	500.42 ± 20.28	430.38 ± 16.38
Lipid classes (%)[2,9]			
Triacylglycerols	86.7 ± 0.41	82.4 ± 0.05	24.0 ± 0.35
Diacylglycerols	8.4 ± 0.22	8.3 ± 0.02	19.8 ± 0.23
Monoacylglycerols	0.6 ± 0.18	0.6 ± 0.01	2.3 ± 0.03
Free FA	4.3 ± 0.45	8.8 ± 0.08	54.0 ± 0.55
Acid value (mg KOH/g of fat)	10.5 ± 0.06	16.1 ± 0.13	109.5 ± 2.87
Peroxide value (meq O_2/kg of fat)	5.0 ± 0.30	6.35 ± 0.06	2.16 ± 0.16

Abbreviations: PO = crude palm oil; OPO = crude olive pomace oil; OPAO = olive pomace acid oil; MIU = moisture and volatile matter + insoluble impurities + unsaponifiable matter; FA = fatty acids; T = tocopherol; T3 = tocotrienol; ND = not detected.

[1] Mean ± SD (n = 3 determinations).

[2] This information has been previously published by Verge-Mèrida et al. (2021).

[3] It includes moisture and other compounds that volatilise under the conditions of the determination.

[4] The percentage of each FA was obtained by peak area normalisation. See Supplementary Table S1 for the complete FA composition including minor FA results.

[5] Sum of C14:0, C16:0, C18:0, C20:0, C22:0 and C24:0.

[6] Sum of C16:1n-7; C18:1n-9; C18:1n-7 and C20:1n-9.

[7] Sum of C18:2n-6 and C18:3n-3.

[8] Sum of α-T, β-T, γ-T, α-T3, β-T3, γ-T3 and δ-T3.

[9] The percentage of each lipid class was obtained by peak area normalisation.

5% of one of the three experimental fat sources (PO, OPO, OPAO) or a blend of PO and OPAO at 50/50 (w/w) (**B**). All diets were in pelleted form and formulated to cover at least the essential nutritional requirements (FEDNA, 2002). Ingredients, proximate composition and energy of the grower and finisher experimental diets have been previously reported by Verge-Mèrida et al. (2021) and can be found in Supplementary Table S2. The four experimental diets were randomly assigned among the pens, with a total of 12 pens (six for entire males and six for females) per diet. The FA composition, T and T3 content and lipid class composition of the experimental diets are shown in Table 2 (the complete FA profile with minor FA con be found in Supplementary Table S3; analytical methods are also described in Supplementary Material S1). The effects of these diets on animal performance parameters have been published by Verge-Mèrida et al. (2021).

Sampling of pork loin

In March 2019, after 62 days of feeding, the animals were stunned with 85% CO_2 for 120 s and immediately exsanguinated at the commercial slaughterhouse of bonÀrea Agrupa (La Closa, Guissona, Spain). From each dietary treatment, a total of 16 pigs (eight entire males and eight females), closest to the average BW within each treatment (including at least one pig from each pen), were sampled. Then, as Fig. 1 shows, eight replicates per diet were formed, consisting of four pairs of entire males and four pairs of females. From each animal, two portions of loin (one from left and one from right *longissimus dorsi* between L2 and L5) were taken (Fig. 1) and, therefore, each replicate was made up of a total of four portions of pork loin from two different pigs. Each portion was placed in a different EPS tray and stored under commercial conditions (at 3–4 °C, packed in modified atmosphere O_2/CO_2; 70/30, sealed with a PA/EVOH/PE film). From each replicate, two trays (i.e. one loin from each pig) were stored refrigerated until the following morning (fresh pork samples) and the other two trays (i.e. also one loin form each pig) for 8 days (refrigerated pork samples) (four diets × eight replicates × two refrigeration storage times = 64 samples).

For the fresh pork study, in less than 24 h after the slaughter, a portion of the two fresh loins of the replicate was ground and, after colour determination, ground pork was vacuum packed in different high-barrier multilayer bags (Cryovac BB3255; permeability to O_2, 17 cm^3/m^2 per day per bar at 23 °C and 0% relative humidity, ASTMD-3985; Cryovac Europe, Sealed Air S. L., Sant Boi de Llobregat, Spain) and kept at −20 °C until analysis. Some portions of female fresh pork loin from each diet were reserved to perform the sensory acceptance test. For the refrigerated storage study, the sampling process and colour determination were identically performed after 8 days of refrigeration under commercial conditions.

Determination fatty acid composition

The determination of the FA composition was performed only in fresh pork samples according to Albendea et al. (2023). Briefly, after a lipid extraction of 3.5 g of sample with chloroform/methanol (2:1, v/v), a two-step methylation was performed to obtain the FA methyl esters, which were determined by GLC with flame ionisation detector. The percentage of each FA was obtained by peak area normalisation.

Regarding the repeatability of the FA determination, the RSD was below 3%, with the RSD being lower than 1% for FA at a concentration equal to or greater than 4% and between 1 and 3% for FA at a concentration between 0.1 and 3%.

Table 2
Fatty acid, tocopherol and tocotrienol and lipid class compositions of the grower and finisher experimental diets for pigs.

	Grower diets[1]				Finisher diets[1]			
Items	PO	OPO	OPAO	B	PO	OPO	OPAO	B
FA (%)[2,3]								
C16:0	32.9 ± 1.11	13.4 ± 0.03	13.3 ± 0.38	23.7 ± 0.27	31.6 ± 0.13	13.7 ± 0.08	13.5 ± 0.01	23.9 ± 0.27
C18:0	3.8 ± 0.21	2.6 ± 0.02	3.1 ± 0.19	3.5 ± 0.01	3.9 ± 0.17	2.6 ± 0.08	3.0 ± 0.04	3.5 ± 0.06
Saturated FA[4]	37.6 ± 1.13	16.8 ± 0.04	17.2 ± 0.42	28.0 ± 0.27	36.4 ± 0.22	17.1 ± 0.11	17.1 ± 0.04	28.4 ± 0.27
C18:1n-9	35.8 ± 0.46	49.8 ± 0.02	49.4 ± 0.13	41.9 ± 1.26	36.6 ± 0.20	49.8 ± 0.02	48.7 ± 0.03	41.3 ± 0.30
C18:1n-7	0.9 ± 0.06	2.2 ± 0.26	1.6 ± 0.10	0.8 ± 1.07	0.8 ± 0.01	2.0 ± 0.02	1.7 ± 0.06	1.2 ± 0.11
Monounsaturated FA[5]	37.0 ± 0.42	53.0 ± 0.21	51.8 ± 0.05	43.2 ± 0.19	37.7 ± 0.18	52.7 ± 0.02	51.3 ± 0.09	43.1 ± 0.19
C18:2n-6	24.3 ± 0.52	28.8 ± 0.13	29.7 ± 0.90	27.4 ± 0.12	24.7 ± 0.28	28.6 ± 0.08	29.9 ± 0.04	27.1 ± 0.01
C18:3n-3	0.9 ± 0.08	1.4 ± 0.01	0.9 ± 0.82	1.2 ± 0.01	1.0 ± 0.02	1.5 ± 0.02	1.5 ± 0.01	1.3 ± 0.01
Polyunsaturated FA[6]	25.2 ± 0.52	30.2 ± 0.13	30.6 ± 1.22	28.6 ± 0.12	25.7 ± 0.28	30.0 ± 0.08	31.5 ± 0.04	28.4 ± 0.01
T and T3 (mg/kg)[7]								
α-T	43.12 ± 0.90	26.13 ± 1.66	27.12 ± 2.44	30.39 ± 1.01	37.62 ± 0.68	29.91 ± 1.89	26.93 ± 0.52	28.75 ± 0.25
γ-T	15.4 ± 0.31	13.58 ± 0.22	13.96 ± 0.02	14.01 ± 0.47	14.69 ± 0.42	14.07 ± 0.71	14.18 ± 0.35	13.39 ± 0.26
α-T3	13.7 ± 0.30	3.77 ± 0.07	4.00 ± 0.07	7.38 ± 0.46	10.39 ± 0.47	4.02 ± 0.20	4.28 ± 0.09	6.58 ± 0.04
γ-T3	5.17 ± 0.11	2.82 ± 0.01	2.93 ± 0.11	8.03 ± 0.26	13.37 ± 0.26	2.85 ± 0.11	2.97 ± 0.09	7.79 ± 0.12
T + T3[8]	85.81 ± 1.74	53.13 ± 1.58	55.46 ± 2.61	67.95 ± 2.39	83.80 ± 1.86	57.60 ± 3.02	55.97 ± 1.09	64.75 ± 0.69
Lipid classes (%)[2,9]								
Triacylglycerols	83.7 ± 0.46	70.7 ± 0.27	49.3 ± 0.02	68.2 ± 0.42	81.2 ± 0.12	67.1 ± 0.04	44.8 ± 0.04	64.4 ± 0.31
Diacylglycerols	8.9 ± 0.17	11.1 ± 0.06	14.9 ± 0.02	11.8 ± 0.08	9.6 ± 0.04	11.9 ± 0.10	15.1 ± 0.04	12.4 ± 0.04
Monoacylglycerols	0.9 ± 0.01	1.3 ± 0.02	1.7 ± 0.03	1.3 ± 0.01	1.1 ± 0.09	1.4 ± 0.06	1.6 ± 0.03	1.5 ± 0.15
Free FA	6.5 ± 0.29	16.9 ± 0.20	34.2 ± 0.01	18.8 ± 0.33	8.2 ± 0.01	19.6 ± 0.08	38.6 ± 0.10	21.7 ± 0.20

Abbreviations: PO = crude palm oil diet; OPO = crude olive pomace oil diet; OPAO = olive pomace acid oil diet; B = diet with a blend of PO and OPAO at 50/50 (w/w); FA = fatty acids; T = tocopherol; T3 = tocotrienol.

[1] Mean ± SD (n = 2 determinations).
[2] This information has been previously published by Verge-Mèrida et al. (2021).
[3] The percentage of each FA was obtained by peak area normalisation. See Supplementary Table S3 for the complete FA composition including minor FA results.
[4] Sum of C12:0, C14:0, C16:0, C18:0, C20:0 and C22:0.
[5] Sum of C16:1n-7, C18:1n-9, C18:1n-7 and C20:1n-9.
[6] Sum of C18:2n-6 and C18:3n-3.
[7] T and T3 results are expressed as fed basis (for DM content of the diets see Table S2).
[8] Sum of α-T, β-T, γ-T, δ-T, α-T3, β-T3, γ-T3 and δ-T3.
[9] The percentage of each lipid class was obtained by peak area normalisation.

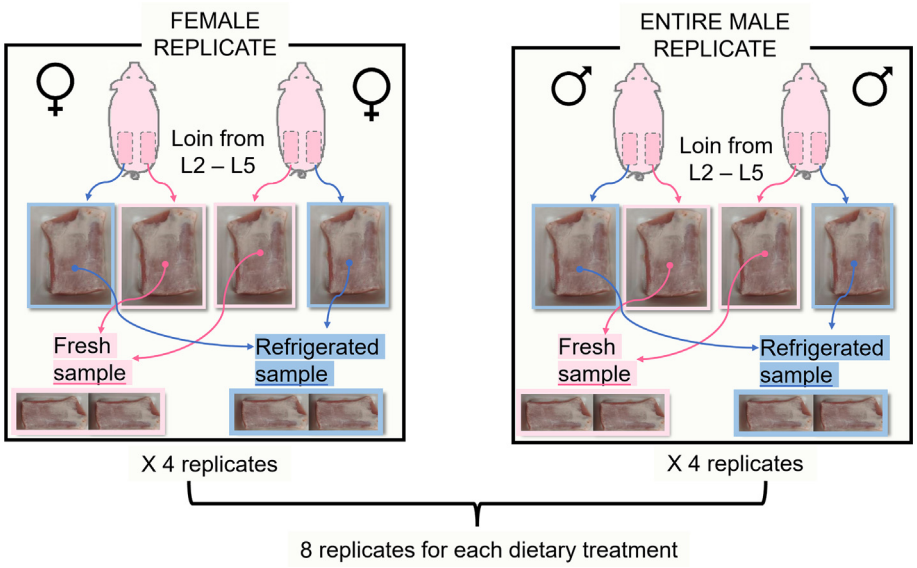

Fig. 1. Pork loin sampling performed to create the different eight replicates (four from female and four from entire male pigs) of fresh and refrigerated samples for each dietary treatment.

Determination of tocopherol and tocotrienol concentrations

The concentrations of T and T3 for fresh and refrigerated pork were determined as detailed by Albendea et al. (2023). It consisted in a saponification with methanolic potassium hydroxide, an extraction of the unsaponifiable fraction with petroleum ether and the determination by HPLC with a fluorescence detector. The repeatability (i.e. RSD of 6% for α-T concentrations of 1.00–2.00 mg/kg; RSD of 16% for around 0.02 mg/kg of β-T; RSD of 12% for γ-T concentrations of 0.07–0.12 mg/kg) and recovery (89, 91, and 90%, respectively) results were in agreement with the requirements stablished by AOAC International (2019).

Lipid oxidation determinations

The primary oxidation and oxidative stability of fresh and refrigerated pork were determined by the ferrous oxidation–xylenol orange assay, expressing the results as mmol of cumene hydroperoxide equivalents/kg of sample (Albendea et al., 2023). In summary, lipid hydroperoxides (**LHPs**) were extracted from 2 g of sample with 15 mL of cold methanol. To perform the colorimetric reaction, for fresh pork analysis, a 940 μL of methanol and 160 μL of the sample extract were used, whereas for refrigerated pork, the volumes were 950 μL of methanol and 150 μL of sample extract. Absorbance at 560 nm after 30 min of incubation revealed the amount of LHP present in the pork sample (named LHP content). Measurements after 74 h of incubation (when the absorbance was stable) evaluated the amount of LHP formed during this reaction time, which is considered a measure of sample's oxidative stability (named final LHP value). The quantification limit of the ferrous oxidation–xylenol orange assay was 0.04 mmol of cumene hydroperoxide equivalents/kg of sample. The repeatability (i.e. RSD of 10% for concentrations of 0.5 mmol of cumene hydroperoxide equivalents/kg of pork loin) fulfilled the requirements stablished by AOAC International (2019).

The determination of 2-thiobarbituric acid (**TBA**) value was carried out to assess the secondary oxidation of fresh and refrigerated pork, expressing the results as μg of malondialdehyde/kg of sample (Albendea et al., 2023). The repeatability of this method (i.e. RSD of 18% for a TBA value of 36 μg of malondialdehyde/kg; RSD of 7% for 74 μg of malondialdehyde/kg; or RSD of 5% for 569 μg of malondialdehyde/kg) was in agreement with the requirements stablished by AOAC International (2019).

The volatile compound content was evaluated in fresh and refrigerated pork by headspace solid–phase microextraction coupled with GLC and mass spectrometry as detailed by Albendea et al. (2023), but using 2 mL of a 20% NaCl water solution instead of 2 mL of double deionised water. Results were expressed as μg of 4-metil-2-pentanol equivalents/kg of sample. The repeatability for the identified volatile compounds (i.e. RSD between 9% and 19% for volatile compounds at concentrations in the range of <1.0 μg/kg and 3.7 μg/kg; between 4% and 12% for concentrations in the range of 9.5 μg/kg and 78.0 μg/kg; or RSD of 3% for 911.4 μg/kg) met the requirements stablished by AOAC International (2019).

Colour determination

Immediately after grinding the pork samples, colour was measured on the CIE L*a*b* colour space (with D-65 illuminant at a 2° observer angle) (Albendea et al., 2023). The repeatability test of the colour determination in pork loin samples showed RSD percentages of 0.3% for L*, 1.3% for a* and 0.8% for b*.

The dimensionless parameter ΔE (Eq. (1)) was calculated to evaluate if the differences between the colour of fresh and refrigerated pork coming from each diet would be appreciable by human eye.

$$\Delta E = \sqrt{(L^*_{0d} - L^*_{8d})^2 + (a^*_{0d} - a^*_{8d})^2 + (b^*_{0d} - b^*_{8d})^2} \quad (1)$$

where L^*_{0d}, a^*_{0d} and b^*_{0d} are the means of colour parameters for fresh pork from one of the diets (n = 8) and L^*_{8d}, a^*_{8d} and b^*_{8d} are the means of refrigerated pork from the same diet (n = 8).

Sensory acceptance test

To evaluate the overall acceptance of fresh and refrigerated pork coming from the different diets, two hedonic tests with a nine-point scale were carried out, one with fresh pork samples and the other one after 8 days of refrigerated storage. The two tests were conducted using pork loin coming from female pigs. It has

been extensively reported in the literature that pork from some entire males can show unpleasant off-odour and off-flavour, commonly known as boar taint (Lundström et al., 2009), which could make the meat unacceptable for consumers. As the main purpose of these sensory acceptance tests was to evaluate the effect of the diet on the overall acceptance of pork loin, the test was focused on female loins to avoid the risk that boar taint from entire male loins masked the diet effect. In all cases, pork steaks of female's pork loin (approximately, 1.5 cm thickness) were grilled for 3 min, keeping the steaks coming from different diets separated during the whole process. A total of 36 regular pork consumers [male (41.7%) and female (58.3%) from different ages (20–60)] participated in each test. In each test, each participant evaluated four steaks (one per dietary treatment), and for each of them, indicated the degree of acceptability (answering the question "How much do you like or dislike the sample overall?") on a 9-point scale (1, "dislike extremely"; 5, "neither like nor dislike"; 9, "like extremely").

Statistical analysis

Multifactor ANOVA (SPSS GLM procedure) was performed to evaluate the impact of the refrigeration time (0 and 8 days) and the interaction of this factor with the experimental diets on the different parameters studied in fresh and refrigerated pork loin (n = 64, two refrigeration times × four diets × eight replicates). One-way ANOVA was carried out to study the influence of the experimental diets (PO; OPO; OPAO and B) on FA profile (only for fresh pork), T and T3 composition, oxidation stability, TBA values, colour, and sensory acceptance of fresh (n = 32, four diets × eight replicates) or refrigerated pork (n = 32, four diets × eight replicates). Significant differences among diets in fresh or refrigerated pork were tested for multiple comparisons with Scheffé's posthoc test. Additionally, the sex effect was evaluated on the different parameters studied in fresh pork loin using multifactor ANOVA tests (n = 32, two sexes × four diets × four replicates). In all cases, differences were considered significant when $P < 0.05$. Statistic tests were carried out using the general linear model procedure of SPSS (version 27.0, IBM Statistics Inc., Chicago, IL, USA).

Results

Fatty acid profile of pork

The FA profile of fresh pork is shown in Table 3 (the complete FA composition including minor FA can be found in Supplementary Table S4). In all cases, pork was rich in monounsaturated FA, with oleic acid being the main FA. Polyunsaturated FA were the FA type found in the lowest proportion in pork, with linoleic acid being the main polyunsaturated FA.

There was a significant effect of the diet on the FA composition of pork, as OPAO pork showed a palmitic acid percentage lower than PO and B pork ($P < 0.001$), a stearic acid percentage lower than B pork ($P = 0.038$) and, consequently, a total saturated FA percentage lower than pork from PO and B diets ($P = 0.002$). Moreover, the unsaturated-to-saturated FA ratio of pork was significantly higher for OPAO pork than for pork from PO and B diets ($P = 0.003$). Overall, the FA profile of pork from OPAO and OPO diets was similar.

The effect of the sex was noticeable in C18:1n-9, C18:2n-6 and C20:2n-6, in total monounsaturated FA, n-6 polyunsaturated FA and total polyunsaturated FA ($P < 0.001$). Pork loin from female pigs showed a lower oleic acid and monounsaturated FA proportions than pork loin from entire male pigs, which was compensated with higher linoleic acid and C20:2n-6, n-6 polyunsaturated FA and total polyunsaturated FA percentages in the former (Table 3).

Tocopherol and tocotrienol composition of pork

The T and T3 composition of fresh and refrigerated pork are presented in Table 4. The main compound in pork loin coming from all diets was α-T, γ-T was quantified in all samples, and α-T3 levels were only quantifiable in fresh and refrigerated pork loin from PO and B diets.

The refrigerated storage of pork loin significantly decreased the α-T ($P < 0.001$) and the T + T3 concentrations ($P = 0.001$) (Table 4). The sex did not affect the T and T3 composition of fresh pork (Supplementary Table S4). The diet significantly affected the α-T and the T + T3 levels of fresh and refrigerated pork ($P < 0.001$, in all cases) (Table 4). In fresh samples, PO pork showed the highest α-T and T + T3 levels, and OPO and OPAO pork the lowest ones. After the refrigerated storage, PO pork remained the meat with the highest α-T and T + T3 amounts, whereas OPAO pork presented the lowest levels. Consequently, OPO and OPAO diets led to similar tocol concentrations in fresh pork, but after the refrigerated storage, OPAO pork showed lower levels. However, no significant effect of the interaction between the two factors (refrigeration × diet) was revealed by multifactor ANOVA (Table 4).

Lipid hydroperoxide concentration and oxidative stability of pork

The primary lipid oxidation (LHP content) of pork was only quantifiable in refrigerated pork coming from OPAO diet, finding levels of 0.07 mmol of cumene hydroperoxide eq/kg.

The oxidative stability of fresh and refrigerated pork, assessed by the final LHP value, is represented in Fig. 2A and B (a higher final LHP value indicates a higher LHP formation during the 74 h incubation of the ferrous oxidation-xylenol orange assay and thus, a lower oxidative stability). The refrigerated storage decreased pork oxidative stability ($P < 0.001$). The oxidative stability in fresh pork was not affected by the sex (Supplementary Table S5) but there was an effect of the diet, as fresh pork from OPAO diet showed a lower oxidative stability than fresh pork from PO diet ($P = 0.004$). However, no significant impact of the diet was found for the oxidative stability of pork after its refrigerated storage (Fig. 2). Despite this different impact of the diet on the oxidative stability of fresh and refrigerated pork, multifactor ANOVA did not reveal a significant interaction between these two factors.

2-Thiobarbituric acid values of pork

Pork TBA values, which measure secondary oxidation, are shown in Fig. 2C and D. There was a significant interaction between the refrigeration and the diet ($P < 0.001$). In fresh pork, TBA values did not depend on the diet or the sex (Supplementary Table S5). After refrigeration, TBA values increased in pork from all diets ($P < 0.001$), being the increase higher in OPAO pork ($P < 0.001$) (Fig. 2).

Composition of volatile compounds of pork

A total of five aldehydes (propanal, pentanal, hexanal, heptanal and octanal), three alcohols (1-pentanol, 1-hexanol and 1-octen-3-ol), four ketones (2-heptanone, 2-octanone, 3-octanone and 1-octen-3-one) and one furan (2-pentylfuran) were identified and quantified in fresh and refrigerated pork (Table 5). There was a significant effect of the interaction between the refrigeration and the diet on propanal ($P = 0.012$), pentanal ($P = 0.019$), all the alcohols

Table 3
Fatty acid profile of fresh pork loin.

	Diet[1]						Sex[2]				
Items	PO	OPO	OPAO	B	SEM	P_{diet}[3]	Female	Entire male	SEM	P_{sex}[4]	$P_{sex\ x\ diet}$[4]
FA (%)											
C14:0	1.1	1.2	1.2	1.1	0.047	0.399	1.2	1.2	0.032	0.740	0.181
C16:0	23.7^{a}	22.7^{bc}	22.2^{c}	23.3^{ab}	0.220	<0.001	22.8	23.1	0.151	0.105	0.438
C18:0	11.1^{ab}	10.9^{ab}	10.4^{b}	11.4^{a}	0.249	0.038	10.8	11.1	0.165	0.343	0.104
Saturated FA[5]	35.9^{a}	34.8^{ab}	33.8^{b}	35.8^{a}	0.409	0.002	34.8	35.4	0.269	0.146	0.131
C16:1n-7	2.4	2.4	2.4	2.3	0.099	0.772	2.3	2.4	0.070	0.137	0.641
C18:1n-9	40.3	42.0	41.7	41.5	0.457	0.065	40.6	42.1	0.260	<0.001	0.799
C18:1n-7	3.1	3.1	3.1	2.8	0.093	0.058	3.0	3.1	0.086	0.052	0.052
Monounsaturated FA[6]	46.4	48.1	47.8	47.2	0.523	0.140	46.5	48.3	0.297	<0.001	0.994
C18:2n-6	14.6	14.1	15.1	14.2	0.591	0.663	15.6	13.4	0.304	<0.001	0.429
C20:4n-6	1.8	1.6	1.7	1.4	0.113	0.058	1.6	1.6	0.079	0.447	0.276
n-6 Polyunsaturated FA[7]	17.1	16.4	17.5	16.3	0.681	0.531	18.0	15.6	0.367	<0.001	0.361
n-3 Polyunsaturated FA[7]	0.5	0.5	0.6	0.5	0.047	0.122	0.6	0.5	0.034	0.200	0.361
Polyunsaturated FA[7]	17.5	16.9	18.2	16.8	0.690	0.484	18.6	16.1	0.371	<0.001	0.411
Unsaturated-to-saturated ratio[8]	1.8^{b}	1.9^{ab}	2.0^{a}	1.8^{b}	0.034	0.003	1.9	1.8	0.022	0.124	0.132

Abbreviations: PO = crude palm oil diet; OPO = crude olive pomace oil diet; OPAO = olive pomace acid oil diet; B = diet with a blend of PO and OPAO at 50/50 (w/w); FA = fatty acids.
[1] Mean of the different experimental replicates from each diet (n = 8). The percentage of each FA was obtained by peak area normalisation. See Supplementary Table S4 for the complete FA composition including minor FA results.
[2] Mean of the different experimental replicates from each sex (n = 16). The percentage of each FA was obtained by peak area normalisation. See Supplementary Table S4 for the complete FA composition including minor FA results.
[3] *P*-values obtained by ANOVA (n = 32). $P < 0.05$ was considered significant. Differences among diets found with Scheffé's posthoc test (n = 32) were noted in the same row as a > b > c.
[4] *P*-values obtained by multifactor ANOVA (n = 32). $P < 0.05$ was considered significant.
[5] Sum of C14:0, C16:0 and C18:0.
[6] Sum of C16:1n-7, C18:1n-9, C18:1n-7 and C20:1n-9.
[7] n-6 Polyunsaturated FA was the sum of C18:2n-6, C20:2n-6, C20:3n-6 and C20:4n-6; n-3 Polyunsaturated FA was equivalent to C18:3n-3; Polyunsaturated FA was the sum of n-3 and n-6 polyunsaturated FA.
[8] Ratio between monounsaturated + polyunsaturated FA and saturated FA.

Table 4
Tocopherol and tocotrienol concentrations of fresh and refrigerated pork loin.

	Diet[1]						Multifactor ANOVA			
Items	PO	OPO	OPAO	B	SEM	P_{diet}[2]	Refrigeration[3]	SEM	$P_{refrigeration}$[4]	$P_{refrigeration\ x\ diet}$[4]
T and T3 (mg/kg)										
α-T										
Fresh pork	2.32^{a}	1.27^{c}	1.15^{c}	1.73^{b}	0.088	<0.001	1.62	0.046	<0.001	0.123
Refrigerated pork	2.20^{a}	1.12^{b}	0.63^{c}	1.42^{b}	0.097	<0.001	1.34			
γ-T										
Fresh pork	0.12	0.10	0.11	0.11	0.009	0.443	0.11	0.004	0.317	0.464
Refrigerated pork	0.11	0.10	0.08	0.11	0.009	0.166	0.10			
T + T3[5]										
Fresh pork	2.45^{a}	1.38^{c}	1.28^{c}	1.87^{b}	0.090	<0.001	1.75	0.047	0.001	0.053
Refrigerated pork	2.46^{a}	1.24^{b}	0.77^{c}	1.62^{b}	0.098	<0.001	1.52			

Abbreviations: PO = crude palm oil diet; OPO = crude olive pomace oil diet; OPAO = olive pomace acid oil diet; B = diet with a blend of PO and OPAO at 50/50 (w/w); T = tocopherol; T3 = tocotrienol.
[1] Mean of the different experimental replicates for each diet (n = 8).
[2] *P*-values obtained from ANOVA (n = 32) of fresh or refrigerated pork. $P < 0.05$ was considered significant. Differences between diets found in fresh or refrigerated pork with Scheffé's posthoc test (n = 32) were noted in the same row as a > b > c.
[3] Pooled means of fresh or refrigerated pork coming from the four dietary treatments (n = 32).
[4] *P*-values obtained for the refrigeration ($P_{refrigeration}$) and the interaction between the refrigeration time and the diet ($P_{refrigeration\ x\ diet}$) from multifactor ANOVA (n = 64). $P < 0.05$ was considered significant.
[5] Sum of α-T, β-T, γ-T and α-T3.

($P < 0.001$), 2-heptanone ($P < 0.001$), 3-octanone ($P < 0.001$), 1-octen-3-one ($P < 0.001$) and 2-pentylfuran ($P < 0.001$). Their concentrations significantly increased after refrigeration ($P \leq 0.003$), but the effect of the diet was significant only for refrigerated pork ($P \leq 0.005$). Refrigeration also increased the concentration of 2-octanone ($P < 0.001$), whereas octanal concentration decreased ($P = 0.002$). After refrigeration, the concentration of all volatile compounds, except 2-octanone, was significantly higher in OPAO pork ($P \leq 0.008$). The sex affected hexanal ($P = 0.035$) and heptanal contents ($P = 0.006$) (Supplementary Table S5). Female fresh pork loin showed higher content of both compounds (50.0 μg/kg and 2.0 μg/kg for hexanal and heptanal, respectively) than entire male fresh pork loin (27.4 μg/kg and 0.9 μg/kg for hexanal and heptanal, respectively).

Colour and sensory acceptance of pork

The colour results obtained for fresh and refrigerated pork are presented in Table 6. The refrigerated storage significantly increased pork lightness (L*) and decreased its redness (a*) and yellowness (b*) ($P < 0.001$). There was no effect of the diet on the colour parameters of fresh or refrigerated pork. Sex also did not affect any colour parameter (Supplementary Table S5). The ΔE parameter that was obtained to study if the differences between

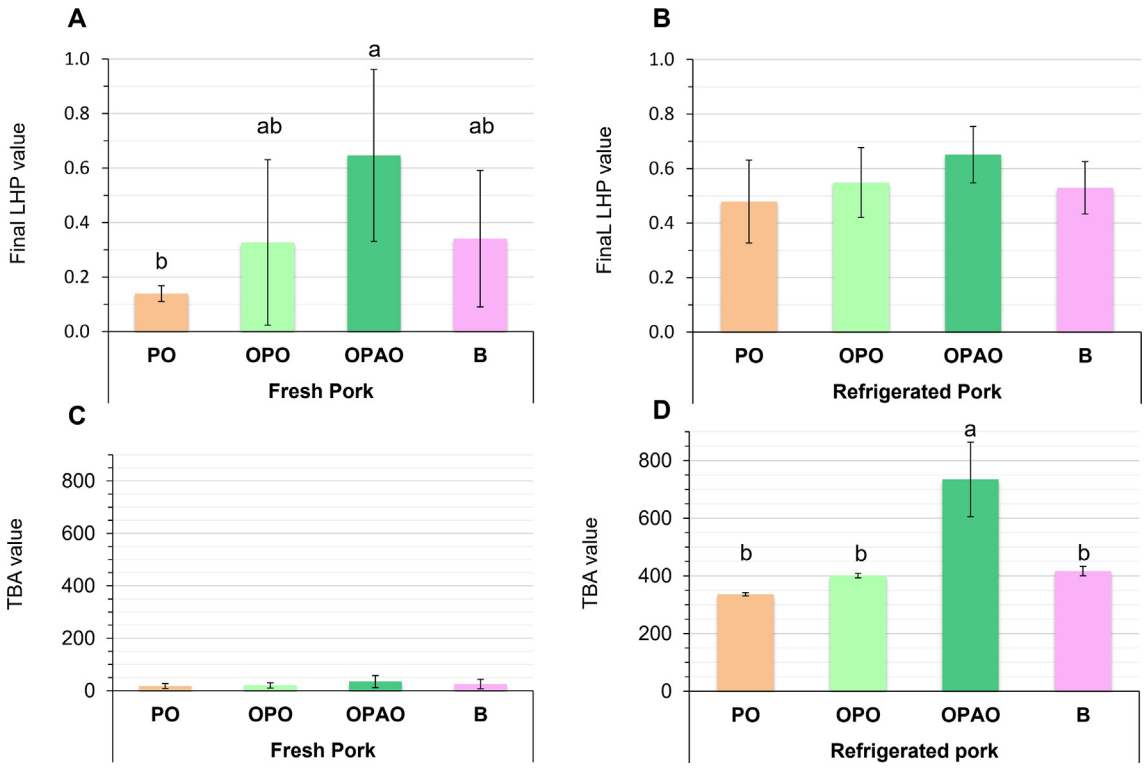

Fig. 2. Oxidative stability (final lipid hydroperoxide (LHP) value after 74 h of incubation, expressed as mmol of cumene hydroperoxide eq/kg) of fresh pork (A) and refrigerated pork (B), and 2-thiobarbituric acid (TBA) values (expressed as μg of malondialdehyde/kg) of fresh pork (C) and refrigerated pork (D) coming from the different diets: PO = crude palm oil diet; OPO = crude olive pomace oil diet; OPAO = olive pomace acid oil diet and B = diet with a blend of PO and OPAO at 50/50 (w/w). The differences between diets found in pork loin with Scheffé's posthoc test (n = 32) were noted as a > b.

the colour of fresh and refrigerated pork coming from each diet would be appreciable by human eye was higher for OPAO pork, followed by OPO pork.

The sensory scores obtained for fresh and refrigerated pork did not reveal a significant impact of the refrigeration or the diet on the overall acceptance of pork loin (Table 6).

Discussion

Composition of fat sources and diets

It is known that acid oils, such as OPAO, might present a high variability in their nutritional quality and composition (Varona et al., 2021a). Regarding quality, the MIU percentage represents a fraction of compounds that can dilute the energy value of a fat and, and it has been pointed out as especially relevant for the quality control of acid oils as it can reach high values in them (Varona et al., 2021a). In fact, the OPAO used in this study had the highest total MIU (12.7 g/100 g) and insoluble impurities fraction (7.8 g/100 g) compared to the other experimental fat sources (Table 1). Furthermore, the total MIU and insoluble impurities values in this OPAO were higher than the median values found for acid oils from the Spanish market (7.6 g /100 g and 1.8 g/100 g, respectively) (Varona et al., 2021a). Despite this high content of energy-diluting compounds in OPAO, Verge-Mèrida et al. (2021) did not find significant differences in the productive parameters of pigs when 5% of OPAO was used instead of OPO in diets. Also, OPAO was the richest experimental fat in free FA, as well as in diacylglycerols and monoacylglycerols, these proportions being quite similar to the median values previously reported for acid oils derived from olive origin (60.8%, 17.7% and 4.6%, respectively) (Varona et al., 2021a). Despite a higher free FA content might have conditioned lipid digestibility, no differences were observed in the feed digestible energy and total FA apparent digestibility when using a 5% of OPAO in diets instead of OPO (Verge-Mèrida et al., 2021).

It has also been reported that the FA and tocol composition of acid oils are highly influenced by the botanical origin of the crude oil (Varona et al., 2021a). Accordingly, both the OPAO and the OPO used in this study had a high proportion of oleic acid. On the other hand, OPAO had the lowest T + T3 levels, closely followed by OPO, with α-T being the main tocol compound in both fats, whereas PO presented the highest total T + T3 content, and was richer in T3 than in T. Considering this information, the OPAO used in this study showed a lower quality than that usually found for similar acid oils.

The composition of the diets (Table 2) was clearly influenced by the fat source (Table 1), as the experimental fats were added at 5% and the crude fat (ether extract) was ≈6% (Supplementary Table S2). For instance, the proportion of oleic acid was higher in diets that included oils of olive origin (OPO and OPAO), whereas the concentrations of T3 and T + T3 were higher in diets that included PO. Still, there was a contribution of other ingredients (Supplementary Table S2) to the lipid composition of the diets. The higher proportion of polyunsaturated FA found in the diets compared with the fat sources was due to the contribution of the cereals that were used to formulate the diets, some of which are rich in linoleic acid (Kan, 2015). The T and T3 concentrations of the diets were mainly influenced by the cereals, the premix and the experimental fat source. The contribution of the cereals led to an increment of γ-T, α-T3 and γ-T3 concentrations in the diets compared with the fat sources (Niculita et al., 2007; Nielsen and Hansen, 2008). The supply of 15 mg of α-tocopheryl acetate per kg of feed by the premix (Supplementary Table S2) was clearly noticeable in PO diets (Table 2), as it changed their T and T3 profile

Table 5
Volatile compounds of fresh and refrigerated pork loin.

Items	Diet[1]						Multifactor ANOVA				Previously linked to lipid oxidation
	PO	OPO	OPAO	B	SEM	P_{diet}[2]	Refrigeration[3]	SEM	$P_{refrigeration}$[4]	$P_{refrigeration\ x\ diet}$[4]	
Compound (μg/kg)											
Propanal											
Fresh pork	0.2	0.2	0.2	0.2	0.015	0.299	0.2	0.022	0.002	0.012	Daza et al. (2005)
Refrigerated pork	0.2[b]	0.2[b]	0.5[a]	0.2[b]	0.060	0.005	0.3				
Pentanal											
Fresh pork	1.7	1.6	2.1	0.7	0.546	0.318	1.6	0.408	0.003	0.019	Daza et al. (2005)
Refrigerated pork	2.0[b]	1.9[b]	7.1[a]	2.5[b]	1.016	0.003	3.4				Ross and Smith (2006)
Hexanal[5]											
Fresh pork	36.9	37.7	53.0	15.9	14.731	0.378	35.9	7.995	0.621	0.081	Daza et al. (2005) Del Pulgar et al. (2013)
Refrigerated pork	21.3[b]	19.8[b]	109.5[a]	15.4[b]	17.155	0.001	41.5				Ross and Smith (2006)
Heptanal[5]											
Fresh pork	1.6	1.6	1.9	1.9	0.573	0.982	1.7	0.267	0.126	0.058	Daza et al. (2005)
Refrigerated pork	0.4[b]	0.5[b]	3.1[a]	0.6[b]	0.494	0.001	1.2				Del Pulgar et al. (2013)
Octanal											
Fresh pork	1.7	1.2	1.1	1.5	0.480	0.817	1.4	0.185	0.002	0.195	Daza et al. (2005)
Refrigerated pork	0.2[b]	0.3[b]	1.2[a]	0.4[ab]	0.206	0.008	0.5				Del Pulgar et al. (2013)
1-Pentanol											
Fresh pork	10.2	12.6	11.8	7.5	2.792	0.587	10.5	2.858	<0.001	<0.001	Nieminen et al. (2016)
Refrigerated pork	77.4[b]	88.4[b]	131.4[a]	85.3[b]	7.587	<0.001	95.6				
1-Hexanol											
Fresh pork	13.6	15.2	15.0	13.6	2.381	0.940	14.4	1.675	<0.001	<0.001	Nieminen et al. (2016)
Refrigerated pork	35.9[b]	46.4[b]	85.3[a]	44.2[b]	4.096	<0.001	52.9				
1-Octen-3-ol											
Fresh pork	184.0	194.1	231.0	157.9	29.162	0.374	191.7	17.289	<0.001	<0.001	Nieminen et al. (2016)
Refrigerated pork	329.3[b]	391.1[b]	801.4[a]	363.0[b]	39.254	<0.001	471.2				
2-Heptanone											
Fresh pork	14.0	13.0	15.0	12.5	1.837	0.778	13.6	1.621	<0.001	<0.001	Gardner and Selke (1984)
Refrigerated pork	47.1[b]	51.0[b]	75.5[a]	51.9[b]	4.202	<0.001	56.4				
2-Octanone											
Fresh pork	3.5	3.3	3.9	3.5	0.464	0.787	3.5	0.594	<0.001	0.438	Del Pulgar et al. (2013)
Refrigerated pork	15.9	15.4	19.4	15.6	1.616	0.281	16.6				Takeungwongtrakul et al. (2020)
3-Octanone											
Fresh pork	0.4	0.3	0.4	0.4	0.040	0.511	0.4	0.083	<0.001	<0.001	Kiralan et al. (2018)
Refrigerated pork	0.8[b]	0.8[b]	2.5[a]	0.7[b]	0.231	<0.001	1.2				
1-Octen-3-one											
Fresh pork	6.2	6.5	7.0	4.9	0.726	0.224	6.2	0.487	<0.001	<0.001	Benet et al. (2016)
Refrigerated pork	10.7[b]	12.7[b]	22.6[a]	11.6[b]	1.172	<0.001	14.4				
2-Pentylfuran											
Fresh pork	4.5	5.1	6.2	4.1	0.775	0.271	5.0	0.810	<0.001	<0.001	Del Pulgar et al. (2013)
Refrigerated pork	9.3[b]	12.5[b]	24.9[a]	10.3[b]	2.157	<0.001	14.2				

Abbreviations: PO = crude palm oil diet; OPO = crude olive pomace oil diet; OPAO = olive pomace acid oil diet; B = diet with a blend of PO and OPAO at 50/50 (w/w).
[1] Mean of the different experimental replicates for each diet (n = 8) expressed as μg of 4-metil-2-pentanol equivalents/kg of sample.
[2] *P*-values obtained from ANOVA (n = 32) of fresh or refrigerated pork. $P < 0.05$ was considered significant. The differences among diets found in refrigerated pork with Scheffé's posthoc test were noted in the same row as a > b.
[3] Pooled means of fresh or refrigerated pork coming from the four dietary treatments (n = 32).
[4] *P*-values obtained for the refrigeration ($P_{refrigeration}$) and the interaction between the refrigeration time and the diet ($P_{refrigeration\ x\ diet}$) from multifactor ANOVA (n = 64. $P < 0.05$ was considered significant.
[5] Significant differences ($P < 0.05$) between female and entire male pigs were obtained from multifactor ANOVA (n = 32). See Supplementary Table S5 for the complete information.

Table 6
Colour parameters (L*; a*; b* instrumental parameters and ΔE values) and consumer overall acceptance of fresh and refrigerated pork loin.

	Diet						Multifactor ANOVA			
Items	PO	OPO	OPAO	B	SEM	P_{diet}[1]	Refrigeration[2]	SEM	$P_{refrigeration}$[3]	$P_{refrigeration\ x\ diet}$[3]
Colour parameters[4,5]										
L*										
Fresh pork	60.97	60.77	61.64	62.25	0.622	0.338	61.41	0.270	<0.001	0.765
Refrigerated pork	64.56	64.54	65.69	65.18	0.445	0.229	64.99			
a*										
Fresh pork	21.11	21.67	21.24	20.92	0.365	0.525	21.24	0.198	<0.001	0.238
Refrigerated pork	15.51	15.18	14.21	15.20	0.424	0.178	15.03			
b*										
Fresh pork	12.14	12.42	12.32	12.62	0.125	0.079	12.38	0.061	<0.001	0.447
Refrigerated pork	11.80	11.89	11.75	11.87	0.119	0.829	11.82			
ΔE	6.66	7.53	8.13	6.47						
Overall acceptance[6]										
Sensory scores										
Fresh pork	6.64	5.89	6.19	6.28	0.345	0.495	6.25	0.160	0.160	0.770
Refrigerated pork	5.92	5.67	6.11	6.03	0.295	0.732	5.93			

Abbreviations: PO = crude palm oil diet; OPO = crude olive pomace oil diet; OPAO = olive pomace acid oil diet; B = diet with a blend of PO and OPAO at 50/50 (w/w); L* = lightness; a* = redness; b* = yellowness.
[1] *P*-values obtained from ANOVA (n = 32 for colour parameters and n = 144 for sensory scores) of fresh or refrigerated pork. $P < 0.05$ was considered significant.
[2] Pooled means of fresh or refrigerated pork coming from the four dietary treatments (n = 32 for colour parameters and n = 144 for sensory scores).
[3] *P*-values obtained for the refrigeration ($P_{refrigeration}$) and the interaction between the refrigeration time and the diet ($P_{refrigeration\ x\ diet}$) from multifactor ANOVA (n = 64 for colour parameters and n = 288 for sensory scores). $P < 0.05$ was considered significant.
[4] For L*, a* and b* mean of the different experimental replicates for each dietary treatment (n = 8).
[5] ΔE values were calculated by Eq. (1) to compare the colour parameters between fresh and refrigerated pork from the same diet.
[6] Data were expressed as the mean of the different sensory scores obtained for each dietary treatment (n = 36).

(richer in T than in T3) with respect to the PO fat source (richer in T3 than in T) (Table 1). Regarding the lipid classes of the diets, lipids supplied by cereals were mainly triacylglycerols, which caused the increment of this fraction in OPAO diets (45–49%) with respect to the fat source (24%) and, consequently, diluted the final free FA percentage in OPAO diets (34–39%) (Table 2) compared to the fat source (54%) (Table 1).

Lipid composition of pork

It has been demonstrated that the FA composition of the diet can be mirrored in the FA profile of meat from monogastric animals. In this study, oleic acid was the main FA found in pork (≈41%) and in all diets, but even if OPO and OPAO diets presented a higher monounsaturated FA proportion (≈52%) than PO diets (≈37%), the effect of the diet on pork monounsaturated FA was not significant. Regarding saturated FA, the diet significantly affected some saturated FA ($P \leq 0.038$) and total saturated FA ($P = 0.002$) of pork, but the numerical difference between treatments was approximately of 2%, even though the PO diet had a higher total saturated FA content (≈37%) than OPO and OPAO diets (≈17%). In fact, there are several factors that can affect the FA profile of the meat besides the diet, as not all absorbed FA are deposited. Some studies have suggested that the FA metabolism of pigs can be adapted to the FA profile of the diet, as for instance reflected by the activity of some desaturases. Particularly, Δ-9 desaturase activity increases when the diet is rich in saturated FA and decreases when the diet is rich in oleic acid (Vehovský et al., 2018). Thus, this adaptability of the FA metabolism in pigs depending on their diet might explain why the differences in the FA profiles between the experimental diets were attenuated in pork in this study. The same attenuation was observed by Verge-Mèrida et al. (2021), who evaluated the effect of the same dietary treatments (PO, OPO, OPAO or B) on pork loin taken from a different location (between the last rib and the first lumbar). In general, the use of OPAO diet had the same effect as OPO diet, but the differences observed in the pork FA profile between diets were slightly different to the ones obtained in our study. This could be due to the fact that Verge-Mèrida et al. (2021) only studied pork loin coming from female pigs, and our results suggested that loins from entire male pigs had a higher proportion of oleic acid, and a lower C18:2n-6 percentage (Table 3). Generally, the studies in the literature about the differences in the FA composition of pork loin coming from entire males and females show the opposite behaviour when entire males and females of similar BW are compared (Hallenstvedt et al., 2010; Grela et al., 2013). However, it has also been reported that the proportion of oleic acid increases with the BW of the pigs (both in entire males and in females), whereas that of linoleic and linolenic decreases (Kouba et al., 2003; Zomeño et al., 2023). Thus, these differences might be attributed to the different composition of the diets, the breed of the pigs, or the anatomical location from where the loin sample was taken in each study, but also to the fact that in our study, females reached a significantly lower final BW than entire males (Verge-Mèrida et al., 2021).

Regarding T and T3 levels, animals are unable to synthetise them, so the concentrations found in pork came entirely from the diet. The highest T + T3 concentration was found in fresh and refrigerated pork from the PO diet, which had the highest T + T3 concentration. Thus, the use of a 5% OPAO in diets, or its blend with PO, led to lower T + T3 concentrations than the use of 5% PO, but similar to those obtained when using 5% OPO, except for α-T in refrigerated pork, for which, 5% OPAO resulted in lower levels (Table 4). The α-T levels in fresh and refrigerated pork loin (0.63–2.32 mg/kg) were similar to those observed by Nuernberg et al. (2005) for fresh pork loin (0.8–1.2 mg/kg) when 5% of linseed oil or olive oil was added to a basal diet containing 12 mg α-T/kg. In the same line, a study performed with pig diets supplemented with 40 mg/kg of α-tocopheryl acetate and containing different blends of olive acid oil, sunflower acid oil and Iberian pig lard reported levels between 2 and 3 mg of α-T/kg and between 0.1 and 0.2 mg of γ-T/kg in fresh loin (Daza et al., 2005). These concentrations are similar to the ones found in this study for fresh pork, α-T (1.2–2.3 mg/kg) and γ-T (0.1 mg/kg), even though the vitamin premix of our diets provided a much lower amount of α-tocopheryl acetate (15 mg/kg).

Lipid oxidation in pork

The use of OPAO in pig diets did not result in a higher lipid oxidation in fresh pork, but after the refrigerated storage, OPAO pork showed a higher increase in TBA values and in the content of most volatile compounds. The volatile compound composition of meat has been widely studied, as it can affect the aroma of the meat and, therefore, its sensory acceptance. The presence of certain volatile compounds in meat, such as those identified in pork in this study, has been linked to lipid oxidation reactions (Table 5). Aldehydes are typically the main secondary products of meat lipid oxidation, with hexanal being the most predominant and prone to a higher increase (Amaral et al., 2018). However, in this study, hexanal was not the predominant volatile compound in pork and, furthermore, it was not affected by refrigeration. Instead, the main volatile compound was 1-octen-3-ol (Table 5), which suffered the highest increment after the refrigerated storage. Both may arise from the oxidation of n-6 polyunsaturated FA, such as linoleic and arachidonic acids, although the chemical pathways of production differ. Hexanal can be produced by the homolytic cleavage of five different hydroperoxides derived from linoleic and arachidonic acids, whereas 1-octen-3-ol may be formed after a multistep decomposition of two different hydroperoxides, involving intermediate reactants (Meynier et al., 1998). It is important to notice that, besides lipid oxidation, there are other chemical or microbiological process that can be involved in the production of volatile compounds. For instance, Park et al. (2009) revealed that heat and refrigeration had a different impact on the volatile compound composition of pork loin. During heating, they found a greater increase in aldehydes, which suggested that lipid oxidation was the main pathway for the formation of volatile compounds. However, during pork refrigeration, they observed an increase in methyl alcohols and ketones (overall 1-methoxy-2-propanol and 3-hydroxy-2-butanone, both not detected in our study), suggesting that, contrarily to our outcomes, branched-chain amino acids and pyruvate catabolism had a greater impact than lipid oxidation on the production of volatile compounds during refrigeration (Park et al., 2009). In our study, the increase in TBA values (Fig. 2C and D) and volatile compound concentrations (Table 5) plus the decrease in oxidative stability (Fig. 2A and B) and α-T levels (Table 4) after refrigerated storage, revealed a clear development of lipid oxidation reactions in pork during refrigeration. This is consistent with the results obtained by Fan et al. (2019), who found an increase in TBA values from ≈200 μg malondialdehyde/kg to ≈1 000 μg malondialdehyde/kg after seven days of refrigeration (at 4 ± 1 °C) of pork tenderloin placed in enamel trays and sealed with plastic wrap with bleeder vents. However, their TBA values were higher than the ones obtained in the present work (Fig. 2C and D), which might be attributed to the modified atmosphere used in this study.

In terms of the effect of the diet, fresh OPAO pork exhibited a higher tendency to form LHP than pork from other diets (Fig. 2A), which is consistent with its greater increase in TBA values (Fig. 2D) and volatile compound concentrations (Table 5) after refrigeration. Moreover, this agreed with the greater reduction in α-T in OPAO pork during the refrigerated storage (Table 4). This different behaviour of OPAO pork might be due to its higher unsaturated-to-saturated FA ratio (Table 3) and lower α-T concentration (Table 4) compared with PO and B pork. However, the lower quality of the OPAO fat source used in this study compared with other OPAO from the Spanish market (Varona et al., 2021a) could also have had some negative impact on the lipid quality of OPAO pork.

The relationship between pork unsaturated-to-saturated FA ratio and the lipid oxidation has been previously reported by Nuernberg et al. (2005), who observed a significantly lower oxidative stability in pork coming from a diet rich in polyunsaturated FA (5% of added linseed oil) compared with pork from a diet rich in monounsaturated FA (5% of added olive oil). In addition, Rey et al. (2001) found that the TBA values in pork loin kept in PVC stretch overwrap after three days of refrigerated storage, also depended on the FA profile of the diet (no added fat or 20% of sunflower, olive oil or a blend of sunflower and linseed oils), whereas they observed similar α-T levels in fresh pork coming from the four diets used (≈1.0 mg/kg). In our study, fresh PO pork showed the highest α-T level (2.32 mg/kg); however, this did not lead to a significant positive effect on the oxidative stability (Fig. 2A and B), the TBA values (Fig. 2C and D) or the volatile compound concentrations (Table 5) compared with OPO and B pork, as they showed similar unsaturated-to-saturated ratio. Likewise, Daza et al. (2005) observed no clear influence of the dietary α-tocopheryl acetate levels on TBA values of pork loin placed into polystyrene trays and wrapped in an oxygen-permeable PVC stretch wrap after 9 days of refrigerated storage, but TBA values depended on the FA profile of the diet used. Moreover, although *longissimus muscle* has a lower capacity to accumulate T and T3 compared to other pig muscles richer in α-T, such as *psoas major*, *tibialis*, or *biceps femoris*, it has been suggested that it is a muscle more stable to oxidation (O'Sullivan et al., 1997; Mason et al., 2005).

Although female pork loin had a higher polyunsaturated FA proportion than entire male pork loin (Table 3), the oxidative stability, the TBA values and the concentration of most of the volatile compound observed in fresh pork loin were not affected by the sex. This is in agreement with the results obtained by Grela et al. (2013), who found similar TBA values in pork loin from entire males and females (370 vs 360 μg malondialdehyde/kg), despite the pork loin from entire males showed a higher percentage of polyunsaturated FA. This means that the differences in the FA profile of pork loin due to the sex of the pigs were negligible in terms of lipid oxidation in any of the two studies.

Colour and sensory acceptance of pork

The addition of OPAO to pig diets did not let to colour differences in fresh or refrigerated pork compared to the other fat sources assayed. However, after the storage of the loin portions for 8 days under commercial conditions (at 3–4 °C, packed in modified atmosphere O_2/CO_2; 70/30), the colour parameters of pork measured after grinding were modified: L* increased, and a* and b* decreased. It is well known that meat colour depends on the myoglobin concentration, its oxidation state and the meat structure. Concretely, the increase in pork lightness (L*) after refrigeration (Table 6) might be linked to a higher oxymyoglobin fraction (Lindahl et al., 2001), which is in concordance with the promotion of myoglobin oxygenation in meat packed in high-oxygen atmospheres (Mancini and Hunt, 2005), such as the one used in this study. Similarly, Jongberg et al. (2018) reported an increase in L* on the surface of pork chops after their refrigerated storage in a high-oxygen atmosphere (80% O_2 and 20% CO_2). According to Lindahl et al. (2001), the decrease in pork redness (a*) after the refrigerated storage (Table 6) may be related to a lower myoglobin oxidation (lower proportion of metmyoglobin) and to a higher deoxymyoglobin (Lindahl et al., 2001), whereas the reduction in pork yellowness (b*) may be related with a lower oxymyoglobin/deoxymyoglobin proportion and to a lower internal reflectance (Lindahl et al., 2001). In some cases, decreases in a* (Table 6) have also been observed together with the development of meat lipid oxidation during refrigerated storage. In our study, this agreed with the increase in the TBA values (Fig. 2C and D) and the volatile compound concentrations (Table 6).

According to the National Bureau of Standards Unit (NBS unit), the following scale for ΔE reveals if colour differences could be

perceived by humans: 0–0.5, *trace*; 0.5–1.5, *slight*; 1.5–3.0, *noticeable*; 3.0–6.0, *appreciable*; 6.0–12.0, *much*; and > 12.0, *very much* (Pogorzelska et al., 2018). This means that the colour differences between fresh and refrigerated pork from all diets studied in this trial, which led to ΔE values between 6.5 and 8.1, would be highly (*much*) appreciable by the human eye (Table 6).

Regarding sensory acceptance, the use of a 5% of OPAO as feed fat led to the highest TBA value in refrigerated pork (734 μg malondialdehyde/kg), but it did not result in a loss of sensory acceptance. Several authors in the literature have studied the correlation between TBA levels and the sensory acceptance of different food products, but the TBA value threshold that can be linked to an acceptance loss is difficult to stablish, since there are several analytical methods to evaluate the TBA values in meat and different types of sensory acceptance tests. For example, Gray et al. (1996) reported different ranges of TBA values associated with the detection of rancid odours and flavours in cooked meat by trained taste panelists (0.5–1.0 mg malondialdehyde/kg) or by inexperienced taste panelists (0.6–2.0 mg malondialdehyde/kg). Even if the sensory acceptance results should be corroborated in loins from male pigs, which are also commercialised, it is highly probable that there would also be an absence of diet effect, as the FA composition revealed that male loins were less rich in PUFA, no sex differences were observed for TBA values, and the only volatile compounds for which a sex difference was observed (hexanal and heptanal) were lower in male loins.

Conclusion

The addition of a 5% of OPAO to pig diets had a negative impact on pork's oxidative parameters which might be due to the higher unsaturated-to-saturated FA ratio and the lower α-T levels of OPAO pork but might also be related to the lower quality of the acid oil used in this study. This effect on the oxidative parameters was not observed when OPAO was added to the diet as a blend with PO (B diet) or when using OPO diet. In addition, the colour and the overall acceptance of pork were not significantly affected by any of the diets. Thus, in order to upcycle acid oils, the use of 5% of OPAO in pig diets would not be noticed by pork consumers, but considering the results obtained for the lipid oxidative stability of pork, it would be preferable the addition of the OPAO source used in this study as a blend with PO.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2023.100879.

Ethics approval

The Ethics Committee on Animal and Human Research (CEEAH) of the Universitat Autònoma de Barcelona (code 4006), on the 19/04/2021, confirmed that this experiment did not require their approval since all the procedures were performed under commercial conditions following the European Regulations for pork meat production.

Data and model availability statement

None of the data were deposited in an official repository. Available upon request.

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Declaration of interest

The authors declare that bonÀrea Agrupa provided technical support, staff and facilities free of charge. M. Verdú works for bonÀrea Agrupa. All authors contributed to analysing and interpreting the data and declare no conflict of interest.

Acknowledgements

The authors acknowledge bonÀrea Agrupa for the support during pig production, slaughtering and meat processing and to our colleagues from Universitat Autònoma de Barcelona for their collaboration in this study. The authors are also grateful to RIOSA S. A. for providing two experimental fats. Also, the authors would like to thank all the participants in the sensory acceptance test. The *Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB)* is recognised as a Maria de Maeztu Unit of Excellence (grant CEX2021-001234-M) funded by MICIN/AEI/FEDER, EU.

Financial support statement

This research has been supported by MCIN/AEI/10.13039/5011 00011033 and by "ERDF A way of making Europe" through the project AGL2015-64431-C2-2-R; by MCIN/AEI/10.13039/5011000110 33 and by "ESF Investing in your future" through the grant RYC-2017-23601; by Spanish Ministry of Universities through the FPU program (FPU18/01010), the University of Barcelona through the predoctoral program APIF (APIF_18_19_10) and the Universitat Autònoma de Barcelona -Banco de Santander through the Talent Program.

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