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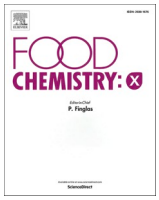

Effects of n-3 polyunsaturated fatty acids and selenomethionine supplementation on physicochemical properties, oxidative stability and endogenous enzyme activities of fresh pork loin

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ABSTRACT

This study evaluated the physicochemical properties, oxidative stability, and endogenous enzyme activities in fresh pork loin from pigs fattened by supplementation of 3 % soybean oil (control), 3 % linseed oil or 3 % linseed oil combined with 0.3 mg/kg selenomethionine (SeMet). Both linseed oil treatments led to higher n-3 polyunsaturated fatty acids (PUFA) levels, lower L^* values, n-6/n-3 ratios, and lipoxygenase activity compared to the control ($P < 0.001$). Supplementation with linseed oil alone reduced a^*, n-6 PUFA, saturated and monounsaturated fatty acids, and GPx activity in pork loin compared to other treatments ($P < 0.05$). Adding SeMet decreased neutral lipase activity but did not affect a^* or GPx activity ($P > 0.05$), likely due to the antioxidant property of SeMet. Overall, linseed oil and SeMet supplementation is a promising strategy to significantly increase n-3 PUFA content in pork without compromising color properties or oxidative stability.

1. Introduction

Pork is a predominant source of animal protein worldwide and is notable for its fatty acid composition. Compared to meat from ruminants, pork generally contains higher levels of polyunsaturated fatty acids (PUFA) and exhibits a greater n-6/n-3 ratio (Nong et al., 2020; Wood et al., 2008). Although PUFA are essential for human health, an excess of n-6 PUFA has been related to the increased production of inflammatory mediators (Simopoulos, 2006; Weaver et al., 2009), and high n-6/n-3 ratios may be associated with an elevated risk of metabolic syndrome (Yi, Huang, Wang, & Shan, 2023; FAO Sources of Meat, 2015). Therefore, the n-6/n-3 ratio in pork is a critical factor that influences consumer purchasing decisions, as it reflects the nutritional quality and health implications of the meat.

Emerging evidence highlights the significant role of n-3 PUFA in reducing the incidence of tumors, obesity, and cardiovascular and cerebrovascular diseases, thereby having a positive impact on human health (Dugan et al., 2015; Shahidi & Ambigaipalan, 2018). Compared to n-6 PUFA, some n-3 PUFA, such as eicosapentaenoic acid (C20:5 n-3, EPA) and docosahexaenoic acid (C22:6 n-3, DHA) are effective in reducing total cholesterol and triglyceride levels in humans (Balk et al., 2006). A low n-6/n-3 ratio is associated with improved vascular endothelial function and lipid metabolism, resulting in multiple benefits in the management and prevention of cardiovascular and cerebrovascular diseases (Li et al., 2021). Various countries recommend n-6/n-3 ratios ranging from 2.3:1 in the United States, 4:1 in Japan, and 4:1 to 6:1 in China (Millward, 2012). Research has shown that dietary intake of n-3 PUFA can be directly absorbed and subsequently deposited into the fat and muscle tissues of pigs (Doran et al., 2006; Raes, De Smet, & Demeyer, 2004; Yi et al., 2023; Woods & Fearon, 2009). Therefore, previous studies have effectively enhanced n-3 PUFA levels in pork products through dietary supplementation with n-3-riched oils, such as linseed oil (Luo et al., 2009), fish oil (Komprda et al., 2020), and microalgae oil (Coelho et al., 2020).

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On the other hand, n-3 PUFA are susceptible to oxidation, leading to deterioration in quality attributes, including color (Juárez et al., 2011), flavor (De Tonnac & Mourot, 2018; Lu, Zhang, Yin, Everts, & Li, 2008), and sensory characteristics (Cannata et al., 2010). It has been reported that loin meat from pigs fed microalgae oil had off-flavor and abnormal odor (De Tonnac & Mourot, 2018). The formation of flavor compounds in meat is closely linked to lipid properties, including lipolysis, lipid oxidation, and antioxidant conditions (Fu, Cao, Yang, & Li, 2022; Shahidi & Hossain, 2022). Lipolysis and lipid oxidation, regulated by muscle lipase and lipoxygenase, respectively, are closely related to the release and oxidation of free fatty acids, both of which are important factors influencing the flavor and sensory attributes of meat products (Fu, Xu, & Wang, 2009; Jin et al., 2010). Furthermore, the antioxidant capacities of meat, regulated by antioxidant enzymes, have been associated with many physicochemical properties of meat (Hernández-García et al., 2024; Pogorzelska-Nowicka, Godziszewska, Horbańczuk, Atanasov, & Wierzbicka, 2018; Saccani et al., 2023). Research has shown that incorporating antioxidants into fattening diets can mitigate quality deterioration in fresh pork. For instance, supplementation with selenomethionine (SeMet) enhanced glutathione peroxidase (GPx) activity and antioxidant capacity in fresh meat (Chen, Wu and Li, 2014), while also improving juiciness and tenderness (Wojtasik-Kalinowska et al., 2016). Additionally, it has been reported that feeding pigs a diet with 3 % linseed oil combined with 1 mg/kg organic selenium altered the volatile compound composition in the *semimembranosus* muscle of these animals (Wojtasik-Kalinowska et al., 2018).

This study aimed to investigate the effects of dietary supplementation of 3 % linseed oil combined with 0.3 mg/kg selenomethionine, in comparison to 3 % soybean oil and 3 % linseed oil, on the physicochemical properties, oxidative stability (antioxidant properties), and enzyme activities (lipases and lipoxygenase) of pork loin. The outcome of this study could offer a viable strategy to enhance the nutritional value of pork through diet management, leading to greater consumer acceptance and healthier meat products.

2. Materials and methods

2.1. Materials and feeding trials

All pig feeding and experimental procedures adhered to the National Research Council's Guide for the Care and Use of Laboratory Animals, Chinese Order No.676 of the State Council (dated March 1, 2017). Thirty-six crossbred (Duroc × Landrace × Large White) female piglets from six sows (six piglets per sow) were transferred to a controlled growing room. After being fed a basal diet (4 % commercial mixed feed, New Hope Liuhe Co., Ltd., Chengdu, China) for 50 days, the pigs were weighed (about 75 kg), and allocated into three groups, each containing twelve pigs (one pig per sow). Each pig was individually housed in a fattening pen within the fattening room and supplemented with one of the three diets: (a) 3 % soybean oil (control, C); (b) 3 % linseed oil (L); (c) 3 % linseed oil combined with 0.3 mg/kg of SeMet (L + SeMet). The formulation and composition of the experiment diets are shown in Table 1. The experimental diets were formulated and prepared by the Teaching and Research Base of Animal Nutrition Institute (Sichuan Agricultural University, Ya'an, China). Linseed oil (cold-pressed linseed oil, DEFU Grease Co., Ltd., Ya'an, China), soybean oil (refined soybean oil, DEFU Grease Co., Ltd., Ya'an, China) and SeMet (SePower® 2000, Sichuan Sinyiml Biotechnology Co., Ltd., Mianyang, China) were purchased for formulating the experimental diets. The fatty acid compositions of the feeding diets are shown in Table 2. The experiment was conducted over two phases based on body weight (Phase 1, 75 kg–100 kg, and Phase 2, 100 kg–140 kg), and lasted for 52 days. At the conclusion of the experiment, six pigs from each group were randomly selected with their average body weights of 140 ± 6.9 kg, being close to the average body weights of each group. The selected pigs were used for processing and analyses described in the following sections.

Slaughter and sampling were performed according to the previous study by Hui, Fang, Ma, Hamid, and Li (2023). The selected pigs were shipped to a slaughterhouse (20 min transport time) and slaughtered after a 12 h resting period according to the principles and guidelines of the Sichuan Agricultural University's Animal Care and Use Committee. The *m. longissimus thoracis et lumborum* from 6th to 13th ribs on the left side of the carcass were collected following the Chinese national standard GB/T 9959.3–2019 (Fresh and Frozen Pork and Pig by-Products-Part 3: Pork Cuts) after chilling at 4 °C and 87 % relative humidity for 24 h. The collected muscles were vacuum-packed in nylon/polyethylene bags (Magic Seal®, Chengdu, China) and transported to the lab within 1 h using cold-chain logistics (0–4 °C) for further analysis.

2.2. Proximate content, pH_{24h}, and instrumental color

The moisture, crude protein, and crude fat contents were analyzed following the procedures outlined in the People's Republic of China GB 5009.3–2016, GB 5009.5–2016 and GB 5009.6–2016 (National Health

Table 1
Formulations of three dietary treatment.

Ingredients (%)	C [a]		L		L + SeMet	
	75-100 kg	100-135 kg	75-100 kg	100-135 kg	75-100 kg	100-135 kg
Soybean Oil	3.00	3.00	–	–	–	–
Linseed Oil	–	–	3.00	3.00	3.00	3.00
Selenomethionine	–	–	–	–	0.3	0.3
Corn	72.00	75.12	72.00	75.12	72.00	75.12
Soybean meal	10.38	7.00	10.38	7.00	10.38	7.00
Wheat bran	7.16	7.64	7.16	7.64	7.16	7.64
Linseed cake	5.00	5.00	5.00	5.00	5.00	5.00
Calcium hydrogen phosphate	0.85	0.74	0.85	0.74	0.85	0.74
Calcium phosphate	0.65	0.61	0.65	0.61	0.65	0.61
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30
Choline chloride 50 %	0.10	0.10	0.10	0.10	0.10	0.10
L-Lysine HCl (98.5 %)	0.36	0.31	0.36	0.31	0.36	0.31
DL-Methionine (99 %)	0.01	0.00	0.01	0.00	0.01	0.00
L-Threonine (98.5 %)	0.09	0.08	0.09	0.08	0.09	0.08
L-Tryptophan (98 %)	0.02	0.02	0.02	0.02	0.02	0.02
Trace mineral premix	0.03	0.03	0.03	0.03	0.03	0.03
Vitamin premix	0.03	0.03	0.03	0.03	0.03	0.03
Vitamin E	0.02	0.02	0.02	0.02	0.02	0.02
Total (calculated value)	100.00	100.00	100.00	100.00	100.30	100.30

[a] C, control with 3 % soybean oil supplementation; L, 3 % linseed oil supplementation; L + SeMet, 3 % linseed oil with 0.3 mg/ kg of selenomethionine supplementation.

Table 2
Fatty acid composition of the feeding diets at two phases of the trial.

Fatty acid (mg/100 g feed) [a]		C [b]		L		L + SeMet	
		75–100 kg	100–135 kg	75–100 kg	100–135 kg	75–100 kg	100–135 kg
SFA	C4:0	2.16	2.49	3.01	2.65	2.56	2.34
	C6:0	6.00	2.96	0.52	7.82	0.42	5.04
	C8:0	0.07	0.00	2.90	25.61	20.70	0.00
	C10:0	0.53	0.56	0.47	0.68	0.97	0.59
	C11:0	0.19	0.20	0.14	0.23	0.22	0.19
	C12:0	0.24	0.37	0.36	0.50	0.35	0.28
	C13:0	0.24	0.09	0.22	0.13	0.09	0.09
	C14:0	5.22	7.79	6.31	7.90	7.58	9.14
	C15:0	1.25	1.78	1.53	1.65	1.56	0.82
	C16:0	458.88	614.55	559.85	604.62	442.28	318.13
	C17:0	4.81	5.92	7.03	6.93	5.59	3.70
	C18:0	162.16	210.23	222.30	241.85	182.60	136.32
	C20:0	11.42	14.42	12.99	12.53	9.21	6.04
	C21:0	0.66	1.13	0.00	0.00	0.00	0.00
	C22:0	18.66	23.82	8.50	7.71	6.17	3.52
	C23:0	0.58	1.25	0.59	3.79	0.98	1.80
	C24:0	0.06	0.01	0.02	0.00	0.01	0.04
MUFA	C14:1	0.00	0.21	0.17	0.00	0.07	0.25
	C15:1	0.00	0.08	0.08	0.27	0.24	0.00
	C16:1	5.20	8.59	6.43	11.82	9.09	11.27
	C17:1	2.26	2.43	2.82	2.90	2.49	1.82
	C18:1n9t	0.53	0.86	0.82	1.03	0.67	0.61
	C18:1n9c	1104.31	1466.08	1218.87	1321.96	960.51	680.62
	C20:1n9	0.90	1.23	0.81	0.39	0.62	0.24
	C22:1n9	1.06	1.41	16.50	5.28	10.94	3.77
	C24:1n9	1.14	2.07	1.89	0.78	0.32	0.54
PUFA	C18:2n6t	8.03	0.00	0.00	0.00	0.00	0.00
	C18:2n6c	2498.28	3189.40	1944.05	1933.53	1432.95	871.34
	C18:3n3	186.09	257.21	1706.91	1525.53	1332.28	751.26
	C18:3n6	0.80	1.20	6.16	5.61	4.91	2.79
	C20:2	1.45	2.74	2.71	4.10	2.67	2.08
	C20:3n3	0.44	0.75	0.00	3.84	2.13	3.03
	C20:3n6	0.00	0.21	1.20	1.63	0.78	0.98
	C20:5n3	13.99	18.55	13.79	13.05	12.11	10.31
	C22:2	0.00	0.40	0.30	0.23	0.09	0.21
	C22:6n3	0.85	0.79	2.87	1.32	3.98	4.34
Total	ΣSFA [c]	673.14	887.57	826.75	924.59	681.30	488.05
	ΣMUFA [d]	1115.40	1482.95	1248.39	1344.44	984.95	699.13
	ΣPUFA [e]	2709.94	3471.26	3677.97	3488.85	2791.90	1646.35
	Σn-3 PUFA [f]	201.36	277.30	1723.56	1543.75	1350.49	768.95
	Σn-6 PUFA [g]	2507.12	3190.82	1951.41	1940.77	1438.64	875.11
	n-6/n-3	12.45	11.51	1.13	1.26	1.07	1.14
	PUFA/SFA	4.03	3.91	4.45	3.77	4.10	3.37

[a] Fatty acids content based on raw materials.
[b] C = control with 3 % soybean oil supplementation; L = 3 % linseed oil supplementation; L + SeMet = 3 % linseed oil with 0.3 mg/ kg of selenomethionine supplementation. SFA = saturated FA; MUFA = monounsaturated FA.
[c] Sum of SFA: C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C23:0, and C24:0.
[d] Sum of MUFA: C14:1, C15:1, C16:1, C17:1, C18:1n9t, C18:1n9c, C20:1n9, C22:1n9, and C24:1n9.
[e] Sum of PUFA: C18:2n6t, C18:2n6c, C18:3n6, C18:3n3, C20:2, C20:3n6, C20:3n3, C20:4n6, C20:5n3, C22:2, and C22:6n3.
[f] Sum of n-3 PUFA: C18:3n3, C20:3n3, C20:5n3, and C22:6n3.
[g] Sum of n-6 PUFA: C18:2n6t, C18:2n6c, C18:3n6, and C20:3n6.

Commission of the People's Republic of China, 2016a, 2016b, 2016c), respectively. The pH values at 24 h post-mortem of pork samples were detected using a portable pH meter (Thermo 3star, Thermo Scientific, USA) following the procedure described by Hui, Zhang, Jamali, and Peng (2017a). Instrumental color parameters were assessed using a chroma meter (CR600d1, Konica Minolta, Inc., Tokyo, Japan) as previously described (Hui et al., 2023). The color measurement was performed at three locations, specifically at the 7th, 9th, and 11th ribs perpendicular to the upper surface of pork meat after exposing the meat surface to a temperature of 20 °C for 25 min. Ten observations were obtained from the meat surface at three locations and averaged to calculate the mean values.

2.3. Total and free fatty acid compositions

Lipids in the samples were extracted following the method by Folch, Lees, and Sloane Stanley (1957) with minor modifications as described by Hui, Fang, Li, and Hamid (2022). Extracted lipids were divided into two parts, one for determining the fatty acid composition, and the other half was used for free fatty acid (FFA) analysis. FFAs were separated from lipids using NH2-aminopropyl mini-columns (Sep-Pak Vac 1 cc, 100 mg, Waters, Milford, MA) following the procedures by Lorenzo, Fonseca, Gómez, and Domínguez (2015). Briefly, the extracted lipids (including FFAs) were dissolved in 1 mL of chloroform and loaded onto min-columns. To remove neutral lipids, 3 mL of chloroform-isopropanol (2/1, *v*/v) were added followed by 3 mL of 2 % acetic acid in diethyl

ether to elute the FFAs. The composition of fatty acids and FFA were analyzed following the procedures by Hui, Zhang, Jamali, and Peng (2017b). Fatty acid analysis was conducted using gas chromatography (Trace1310 ISQ, Thermo Scientific, USA) equipped with a TG − FAME column (TG − FAME, 50 m × 0.25 mm × 0.20 μm, Thermo Scientific, USA).

The chromatography conditions were as follows: the injection volume was 1.0 mL with a split ratio was 100:1, and the detector temperature was set at 260 °C. Helium was the carrier gas at a flow rate of 1.0 mL/min. The oven temperature was initially set at 80 °C and maintained for 5 min, then increased to 160 °C at a rate of 20 °C/min and held for 1.5 min. Finally, the temperature was raised to 230 °C at a rate of 5 °C/min and maintained at 230 °C for 6 min. Results were expressed as mg/100 g meat (FA) or mg/100 g dry matter (FFA).

2.4. Lipases activity determination

The extraction and determination of neutral lipase, acid lipase, and phospholipase were performed following the procedures described by Hui et al. (2017b). One unit of activity (U) was defined as the amount of enzyme that hydrolyzes 1 nmol of substrate per hour at 37 °C. The enzyme activities for three lipases were expressed as U/g protein.

2.5. Lipoxygenase activity determination

Lipoxygenase (LOX) activity in pork loin was determined following the method described by Jin, Zhang, Yu, Lei, and Wang (2011). Briefly, the ground sample (5 g) was homogenized with a sodium phosphate buffer (50 mmol/L, pH 7.4) containing dithiothreitol (1 mmol/L) and ethylenediaminetetraacetic acid (1 mmol/L). The homogenate was filtered and centrifuged at 10,000 ×*g* for 30 min at 4 °C. The supernatant was collected to determine protein concentration via the biuret method and LOX activity. A linoleic acid substrate solution was prepared according to Jin et al. (2011). The reaction mixture containing 0.2 mL of linoleic acid solution, 0.1 mL of enzymatic solution, and 2.9 mL of citrate buffer (50 mmol/L, pH 5.5), was analyzed for LOX activity by measuring the increase in absorbance at 234 nm for 1 min with a UV-1800PC spectrophotometer (MAPADA, Shanghai, China). One unit (U) of LOX activity was defined as the amount of enzyme causing an absorption increment of 0.001 per minute and per g protein.

2.6. Antioxidant properties determination

The pork sample (2 g) was mixed with 20 mL of chilled buffer (0.86 % normal saline) and homogenized for 60 s at 10,000 ×*g*. After centrifugation at 8000 ×*g* for 15 min at 4 °C, the supernatant was collected for protein quantification using the biuret method. Commercial kits (Jiancheng Bioengineering Institute in Nanjing, China) were used to assess the activities of glutathione peroxidase (GPx, A005–1), catalase (CAT, A007–1-1), total superoxide dismutase (T-SOD, A001–1), and total antioxidant capacity (T-AOC, A015–1).

2.7. Lipid and protein oxidation

Lipid oxidation level in pork samples was measured following 2-thiobarbituric acid-reactive substances (TBARS) assay using a commercial kit (A003–1-2, Jiancheng Bioengineering Institute, Nanjing, China). The result is expressed as mg of malondialdehyde (MDA) per kg of meat.

Protein oxidation level in pork samples was assessed following the the 2,4-dinitrophenylhydrazine (DNPH) method described by Mercier, Gatellier, Viau, Remignon, and Renerre (1998). The protein carbonyl content in pork loin was expressed as nanomole of carbonyl moieties per milligram of protein (nmol/mg protein). The total sulfhydryl content of pork was analyzed following the method described by Srinivasan and Hultin (1997), and the sulfhydryl contents were expressed as nanomole of sulfhydryl moieties per milligram of protein (nmol/mg protein).

2.8. Statistical analysis

General linear model (GLM) analysis was performed by SPSS 26.0 software (Version 22.0, IBM Co., USA) with one-way analysis of variance (ANOVA), least significant difference, and Tukey multiple comparisons to determine significant differences ($P < 0.05$) in physicochemical parameters, oxidative stability, and enzymatic activities among three dietary treatments (C, L, and L + SeMet). The dietary treatments were considered as a fixed term in the model with animal IDs selected for three dietary treatments and animal IDs selected for slaughtering included as random terms. With 95 % confidence intervals, descriptive statistics, homogeneity tests, and effect size estimations were carried out. Results are presented as means with their standard errors.

The fatty acid composition in pork from different dietary treatments was analyzed by partial least squares regression discrimination analysis (PLS − DA) using the statistical analysis (one factor) function in MetaboAnalyst 5.0. Data normalization was done using a pooled sample from group C (group PQN), mean-centered, and divided by each variable's standard deviation (auto-scaling) after the missing values were handled by excluding features with over 50 % missing values and replacing remaining gaps with 1/5 of each variable's minimum positive value. Key fatty acids with significant changes ($P < 0.05$) among the different dietary treatments were selected based on the variable importance in the projection (VIP) values >1, following methods described previously by Hui et al. (2023).

PLS-DA modelling was employed using XLSTAT software (Version 2019, Microsoft, USA) to assess the effects of dietary treatments on proximate composition, pH_{24h}, instrumental color, FFA content, activities of lipases, lipoxygenases, antioxidant properties and oxidation indicators of lipids and proteins in pork loin, following the procedures described by Hui et al. (2023). All the physicochemical traits were used as the explanatory variables (X), while the dietary treatments (C, L and L + SeMet) were set as the dependent variables (Y). The model calculation included fast algorithm, stop conditions (fixed number = 4), Jackknife (LOO) cross-validation method, variables (center and reduce), and a 95 % confidence interval.

3. Results and discussion

3.1. Proximate content, pH_{24h}, and instrumental color

Visual perception of meat products, especially the meat color, is an important sensory trait affecting consumers' willingness to purchase. The proximate content, pH_{24h}, and instrumental color of pork loin

Table 3
Effects of dietary n-3 polyunsaturated fatty acid (PUFA) treatments on proximate content, instrumental color and pH_{24h} of pork *m. longissimus thoracis et lumborum* ($n = 6$).

Traits [a-b]	Dietary treatments			SEM	*P*-value [c]
	C	L	L + SeMet		
Moisture (%)	72.31	72.44	72.82	0.237	NS
Crude fat (%)	3.78	2.80	3.19	0.249	NS
Crude protein (%)	22.05	23.27	22.74	0.808	NS
*L**	52.40^{a}	45.58^{b}	43.70^{b}	1.125	***
*a**	18.31^{a}	15.46^{b}	17.14^{a}	0.340	***
*b**	9.84	10.04	9.54	0.216	NS
*a**/*b**	1.86^{a}	1.55^{b}	1.81^{a}	0.046	**
pH_{24h}	5.59	5.57	5.57	0.016	NS

Dietary treatments: C = control with 3 % soybean oil supplementation; L = 3 % linseed oil supplementation; L + SeMet = 3 % linseed oil with 0.3 mg/ kg of selenomethionine supplementation. SEM = standard error of means.

[a-b] Superscript letters indicate the means are significantly different ($P < 0.05$) among dietary treatments (C, L and L + SeMet).

[c] NS, not significantly different ($P > 0.05$); **, significant at 1 % level; ***, significant at 0.1 % level.

muscle following different dietary treatments are shown in Table 3. The yellowness (b^*), moisture, crude fat, crude protein, and pH_{24h} of the pork were not affected by those dietary treatments ($P > 0.05$). Musella et al. (2009) also found that the total lipid content of pork *semimembranosus* muscle remained unchanged following supplementation with 5 % whole extruded linseed in the feed.

Supplementation with L and L + SeMet significantly ($P < 0.01$) reduced lightness (L^*) by 13 % and 17 %, respectively, in pork meat compared to the control samples. Supplementation with 3 % canola oil (Tartrakoon, Tartrakoon, & Kitsupee, 2016) and 5 % flaxseed in the feed (Juárez et al., 2011) were also reported to significantly ($P < 0.01$) decrease the L^* values of pork *longissimus dorsi* and *longissimus thoracis* muscles, respectively. However, other studies did not observe significant changes in L^* values of pork *longissimus lumborum et thoracis* and *semimembranosus* muscles from pigs supplemented with 5 % microalgae (Kalbe, Priepke, Nürnberg, & Dannenberger, 2019) and 1.2 % fish oil (Haak, De Smet, Fremaut, Van Walleghem, & Raes, 2008), respectively. The inclusion of dietary oils in the diet of fattening pigs may cause a slight inhibition of initial post-mortem glycolytic rates and an increased utilization of fatty acids by muscle tissues (Dugan, Aalhus, Robertson, Rolland, & Larsen, 2004). Consequently, the effects of L treatments on the changes of L^* values in pork could be associated with the changes in glycolytic activities.

In terms of redness (a^*), The L treatment resulted in lower ($P < 0.05$) a^* values (by 15 %) than the control, while L + SeMet treatment had no significant effect on the a^* values (Table 3). Liu and Kim (2018) reported that substituting soybean oil with 0.05 %, 0.3 %, and 0.9 % linseed oil in corn-soybean meal-based diets did not affect the a^* values of pork *m. longissimus dorsi*, consistent with findings by Šimkus et al. (2013), who observed no changes in a^* values when pigs were supplemented with 0.2 % blue algae *Spirulina platensis*. The decrease of a^* in meat following L treatment was also reported in *longissimus thoracis and longissimus lumborum* muscles from cull ewes fed with 10 % linseed, where a 13 % reduction in a^* was observed compared to the control (Ben Abdelmalek et al., 2020). Such a decrease in a^* could be linked to the higher levels of n-3 PUFA in pork following L treatment compared to the control (Table 4). Elevated n-3 PUFA levels in meat are more prone to lipid oxidation, which can subsequently lead to myoglobin oxidation and meat discoloration (Qian & Buettner, 1999). However, the addition of 0.3 mg/kg of SeMet in L + SeMet treatment had a protective effect on redness compared to L treatment. Jiang, Tang, Xue, Lin, and Xiong (2017) suggested that the addition of 0.3 ppm Se-Yeast in the feed could enhance glutathione peroxidase activity (GPx) activity in meat, which could prevent PUFA oxidation and retain the integrity of muscle protein structure, thereby reducing myoglobin oxidation and discoloration. The findings from this study indicated that combining SeMet with linseed oil in dietary n-3 PUFA supplementation could better preserve the color properties of pork than using linseed oil alone.

3.2. Fatty acids analysis

3.2.1. Total fatty acids

Thirty-five fatty acids were detected in pork *m. longissimus thoracis et lumborum* in this study (Fig. 1a and Table 4). The heat-map analysis (Fig. 1a) showed clear differences in fatty acid content between treatment groups and control. Compared to the control, the L and L + SeMet treatments resulted in increased n-3 PUFA contents by more than three and five times. As a result, both dietary treatments effectively reduced the ratios of n-6/n-3 from about 17:1 in the control to 2:1–3:1 in L and L + SeMet treatments, which are below the recommended levels of 5:1–10:1 by FAO (2015). Meanwhile, an increased ratio of PUFA/SFA ($P < 0.05$) in pork loin samples following L and L + SeMet treatments may indicate a softer texture in the meat compared to the control samples. The fatty acid results in this study aligned well with findings from Corino, Musella, and Mourot (2008), who reported that n-3 PUFA concentration in *longissimus* muscle of pigs fattened with whole extruded

Table 4
Effects of dietary n-3 polyunsaturated fatty acid (PUFA) treatments on total fatty acid (FA) compositions in the pork *m. longissimus thoracis et lumborum* (n = 6).

FA (mg/100 g meat) [a-c]		Dietary treatments			SEM	*P*-value [d]
		C	L	L + SeMet		
SFA	C4:0	0.10	0.09	0.08	0.012	NS
	C6:0	0.05	0.06	0.04	0.008	NS
	C8:0	0.27	0.17	0.42	0.048	NS
	C10:0	5.30^{a}	2.39^{b}	4.30^{ab}	0.454	*
	C11:0	0.44^{a}	0.21^{b}	0.40^{ab}	0.043	*
	C12:0	3.98^{a}	1.85^{b}	3.24^{ab}	0.330	*
	C13:0	0.18	0.07	0.21	0.026	NS
	C14:0	71.91^{a}	34.11^{b}	58.63^{ab}	5.941	*
	C15:0	0.93^{ab}	0.75^{b}	1.07^{a}	0.056	*
	C16:0	1346.52^{a}	630.96^{b}	1097.50^{ab}	105.613	*
	C17:0	7.98^{a}	4.41^{b}	8.53^{a}	0.698	*
	C18:0	725.45^{a}	300.25^{b}	416.13^{b}	59.875	**
	C20:0	8.99^{a}	4.10^{b}	8.64^{a}	0.822	*
	C21:0	0.92^{a}	0.05^{b}	0.00^{b}	0.145	*
	C22:0	1.62^{a}	0.59^{b}	0.63^{b}	0.142	**
	C23:0	26.52^{a}	17.77^{b}	20.16^{ab}	1.336	*
	C24:0	0.31^{a}	0.05^{b}	0.00^{b}	0.039	**
MUFA	C14:1	0.87	0.66	1.12	0.097	NS
	C16:1	178.55^{a}	80.00^{b}	131.39^{ab}	14.715	*
	C17:1	15.50	12.14	15.00	0.766	NS
	C18:1n9t	2.40^{a}	1.13^{b}	1.92^{ab}	0.183	**
	C18:1n9c	2411.90^{a}	1168.76^{b}	2068.88^{ab}	201.743	*
	C20:1n9	3.55^{a}	1.50^{b}	2.41^{ab}	0.283	**
	C22:1n9	0.42	0.21	0.37	0.045	NS
	C24:1n9	0.63	0.20	0.24	0.100	NS
PUFA	C18:2n6t	0.08	0.00	0.05	0.027	NS
	C18:2n6c	507.23^{a}	306.18^{b}	487.21^{ab}	38.700	*
	C18:3n6	2.84^{ab}	2.05^{b}	3.20^{a}	0.205	*
	C18:3n3	24.07^{c}	94.71^{b}	171.24^{a}	17.458	***
	C20:2	22.08	11.81	19.66	1.886	NS
	C20:3n6	8.06^{a}	5.30^{b}	6.75^{ab}	0.410	*
	C20:3n3	2.70^{c}	12.77^{b}	21.58^{a}	2.282	***
	C20:5n3	0.40^{b}	0.96^{a}	0.48^{b}	0.086	**
	C22:2	2.11^{b}	17.60^{a}	22.27^{a}	2.218	***
	C22:6n3	1.71^{b}	5.43^{a}	7.25^{a}	0.656	***
Total	∑SFA [e]	2201.48^{a}	997.89^{b}	1619.97^{a}	151.559	**
	∑MUFA [f]	2613.83^{a}	1264.59^{b}	2221.31^{ab}	216.870	*
	∑PUFA [g]	571.27	456.82	739.70	52.328	NS
	∑n-3 PUFA [h]	30.58^{c}	130.52^{b}	200.55^{a}	20.047	***
	∑n-6 PUFA [i]	518.21^{a}	313.52^{b}	497.22^{ab}	39.163	*
	n-6/n-3	17.04^{a}	2.44^{b}	2.53^{b}	1.647	***
	PUFA/SFA	0.26	0.47	0.46	0.029	***

Dietary treatments: C = control with 3 % soybean oil supplementation; L = 3 % linseed oil supplementation; L + SeMet = 3 % linseed oil with 0.3 mg/ kg of selenomethionine supplementation. SFA = saturated FA; MUFA = monounsaturated FA; SEM = standard error of means.

[a-c] Superscript letters indicate the means are significantly different ($P < 0.05$) among dietary treatments (C, L and L + SeMet).

[d] NS, not significantly different ($P > 0.05$); *, significant at 5 % level; **, significant at 1 % level; ***, significant at 0.1 % level.

[e] Sum of SFA: C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C23:0, and C24:0.

[f] Sum of MUFA: C14:1, C15:1, C16:1, C17:1, C18:1n9t, C18:1n9c, C20:1n9, C22:1n9, and C24:1n9.

[g] Sum of PUFA: C18:2n6t, C18:2n6c, C18:3n6, C18:3n3, C20:2, C20:3n6, C20:3n3, C20:4n6, C20:5n3, C22:2, and C22:6n3.

[h] Sum of n-3 PUFA: C18:3n3, C20:3n3, C20:5n3, and C22:6n3.

[i] Sum of n-6 PUFA: C18:2n6t, C18:2n6c, C18:3n6, C20:3n6, and C20:4n6.

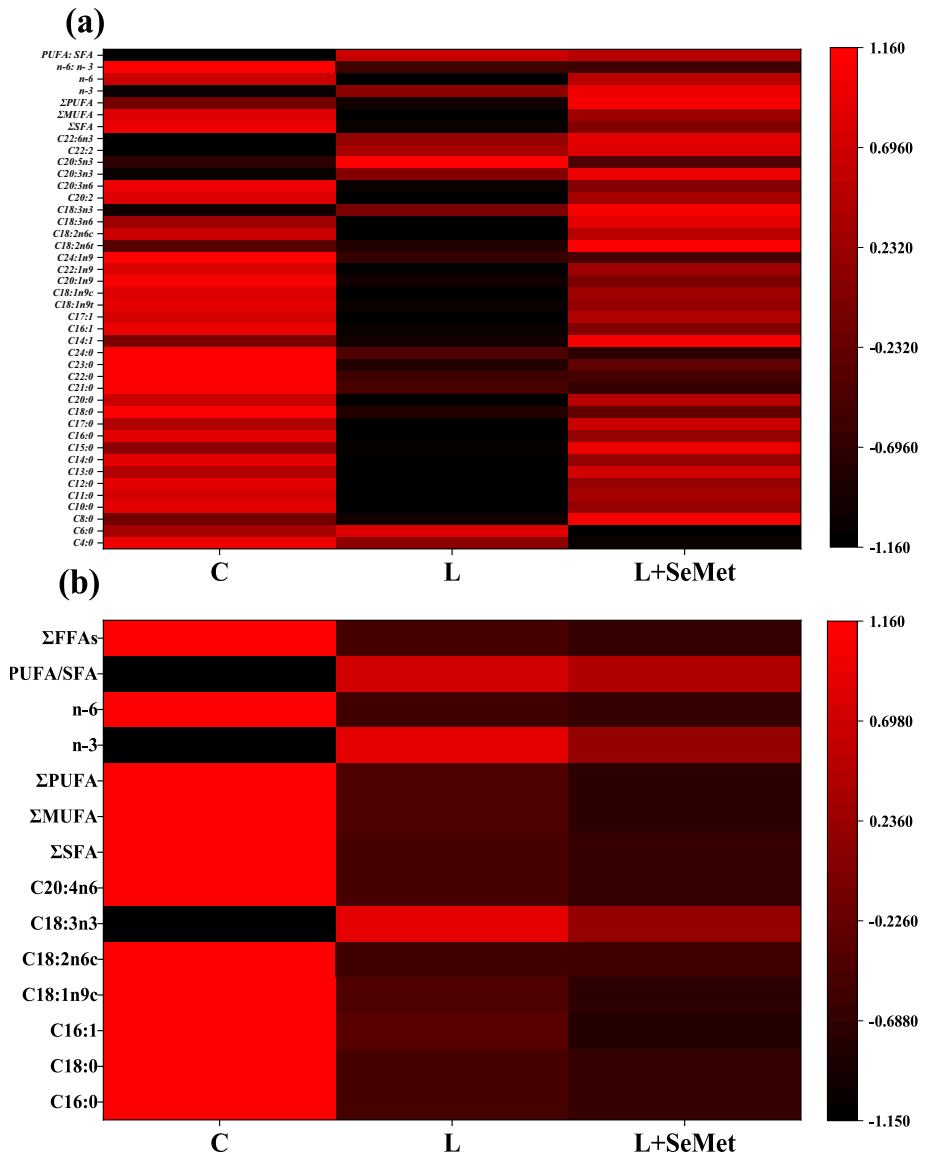

Fig. 1. Effects of dietary supplementation with 3 % soybean oil (control, C), 3 % linseed oil (L) or 3 % linseed oil combined with 0.3 mg/kg of selenomethionine (L + SeMet) on the total and free fatty acid compositions in pork *m. longissimus thoracis et lumborum*. (a) Heatmap analysis of total fatty acid compositions. (b) Heatmap analysis of free fatty acid compositions.

linseed supplements increased by 5 % and n-6/n-3 ratios decreased from 12:1 to 4.5:1. The elevated PUFA/SFA ratios by L and L + SeMet treatments in this study are consistent with the recent study by Czyż, Sokola-Wysoczańska, Wyrostek, and Cholewińska (2021), who found that supplementing the feeds with linseed oil ethyl ester resulted in significant increases in C18:3 n-3 (ALA) and total PUFA levels, and decreases in total SFAs content and n-6/n-3 ratios in pork *m. longissimus dorsi* muscle and *m. biceps femoris*.

In addition, L + SeMet treatment resulted in elevated levels of ALA and total n-3 PUFA in pork loin compared to L treatment, suggesting the enhancing effects of SeMet supplementation on n-3 PUFA composition in pork. To our knowledge, limited information is available on the effects of dietary SeMet supplementation on the fatty acid composition of pork meat. Tian et al. (2022) reported increased levels of ALA and total PUFA in goat *m. longissimus dorsi* following supplementation with 2.4 mg/kg of Se-Yeast. Increased levels of DHA (C22:6n3) and total n-3 PUFA were also observed in rabbit *m. longissimus lumborum* after supplementing with 0.1, 0.5 and 2.5 mg Se-Yeast per kg feed (Papadomichelakis, Zoidis, Pappas, Mountzouris, & Fegeros, 2017). The enhancing effects of SeMet supplementation on n-3 PUFA content in meats could be due to the inhibitory actions of organic selenium on fatty acid peroxidation, especially in long-chain n-3 PUFA such as ALA and DHA (Pappas, Zoidis, Papadomichelakis, & Fegeros, 2011).

3.2.2. *Free fatty acids*

The FFA contents detected in pork *m. longissimus thoracis et lumborum* following different dietary treatments are shown in Fig. 1b and Table 5. FFAs are considered as important flavor precursors contributing to the unique flavor of meat and meat products (Chen et al., 2021). Control samples exhibited significantly higher levels of total and individual free SFA, free MUFA, and free PUFA than other treatments, except for free n-3 PUFA. ALA was the only free n-3 PUFA detected in this study while no free n-3 PUFA was observed in control samples (below detection limit). Supplementation with L and L + SeMet resulted in similar ALA content and n-6/n-3 ratios in pork samples. Furthermore, the total FFA contents in pork loin were significantly ($P < 0.01$) lower in L and L + SeMet treatments compared to the control, which may be linked to alternations in lipase activities influenced by the diets (Table 6), as discussed in section 3.3.

Table 5
Effects of n-3 polyunsaturated fatty acid (PUFA) treatments on free fatty acid (FFA) composition in pork *m. longissimus thoracis et lumborum* (n = 6).

FFA (mg/100 g dry matter) [a-c]		Dietary treatments			SEM	*P*-value [d]
		C	L	L + SeMet		
SFA [e]	C16:0	49.15^{a}	27.77^{b}	25.77^{b}	3.212	***
	C18:0	34.40^{a}	19.97^{b}	19.13^{b}	2.094	***
MUFA	C16:1	3.58^{a}	0.83^{b}	0.00^{b}	0.507	**
	C18:1n9c	61.80^{a}	34.15^{b}	29.52^{b}	5.132	*
PUFA	C18:2n6c	23.91^{a}	17.88^{b}	17.48^{b}	1.145	*
	C18:3n3	0.00^{c}	2.90^{a}	1.85^{ab}	0.508	*
	C20:4n6	8.57^{a}	4.12^{b}	3.78^{b}	0.605	***
Total	∑SFA [ef]	83.55^{a}	47.73^{b}	44.90^{b}	5.290	***
	∑MUFA [g]	65.38^{a}	34.98^{b}	29.52^{b}	5.601	**
	∑PUFA [h]	36.83^{a}	24.90^{b}	23.12^{b}	2.059	**
	∑n-3 [i]	0.00^{c}	2.90^{a}	1.85^{ab}	0.508	*
	∑n-6 [j]	36.83^{a}	22.00^{b}	21.27^{b}	2.118	***
	n-6/n- 3	–	5.74	6.07	–	–
	PUFA/SFA	0.45	0.53	0.52	0.021	NS
	ΣFFA [k]	185.77^{a}	107.62^{b}	97.53^{b}	12.563	**

Dietary treatments: C = control with 3 % soybean oil supplementation; L = 3 % linseed oil supplementation; L + SeMet = 3 % linseed oil with 0.3 mg/ kg of selenomethionine supplementation. SFA = saturated FA; MUFA = mono-unsaturated FA; SEM = standard error of means.

[a-c] Superscript letters indicate the means are significantly different ($P < 0.05$) among dietary treatments (C, L and L + SeMet).

[d] NS, not significantly different ($P > 0.05$); *, significant at the 5 % level; **, significant at the 1 % level; ***, significant at the 0.1 % level.

[e] The superscript letters "a-c" are significantly different ($P < 0.05$ or $P < 0.01$) along with different dietary treatments (C, L and L + SeMet).

[f] Sum of SFA: C16:0 and C18:0.

[g] Sum of MUFA: C16:1 and C18:1n9c.

[h] Sum of PUFA: C18:2n6c, C18:3n3, and C20:4n6.

[i] Sum of n-3 PUFA: C18:3n3.

[j] Sum of n-6 PUFA: C18:2n6c and C20:4n6.

[k] Sum of FFA: C16:0, C18:0, C16:1, C18:1n9c, C18:2n6c, and C18:3n3.

Table 6
Effects of n-3 polyunsaturated fatty acid (PUFA) treatments on activities of lipases and lipoxygenase (LOX) in pork *m. longissimus thoracis et lumborum* (n = 6).

Items [a-b]	Diet treatments			SEM	*P*-value [c]
	C	L	L + SeMet		
Neutral lipase activity (U/mg protein)	3.69^{a}	2.83^{ab}	2.66^{b}	0.186	*
Acid lipase activity (U/mg protein)	1.14	1.04	1.22	0.049	NS
Phospholipase activity (U/mg protein)	0.92	0.89	0.98	0.033	NS
LOX activity (U/mg protein)	4.19^{a}	1.36^{b}	1.08^{b}	0.407	***

Dietary treatments: C = control with 3 % soybean oil supplementation; L = 3 % linseed oil supplementation; L + SeMet = 3 % linseed oil with 0.3 mg/ kg of selenomethionine supplementation. SEM = standard error of means.

[a-b] Superscript letters indicate the means are significantly different ($P < 0.05$) among dietary treatments (C, L, and L + SeMet).

[c] NS, not significantly different ($P > 0.05$); *, significant at the 5 % level; ***, significant at the 0.1 % level.

3.2.3. Key fatty acids

The discriminant analysis of total fatty acid in pork from C, L, and L + SeMet treatments is shown in Fig. 2a. The control group aligned with positive component 1, while L and L + SeMet treatments showed negative loadings, indicating a clear separation. The distinct separation indicated that L and L + SeMet treatments significantly altered the fatty

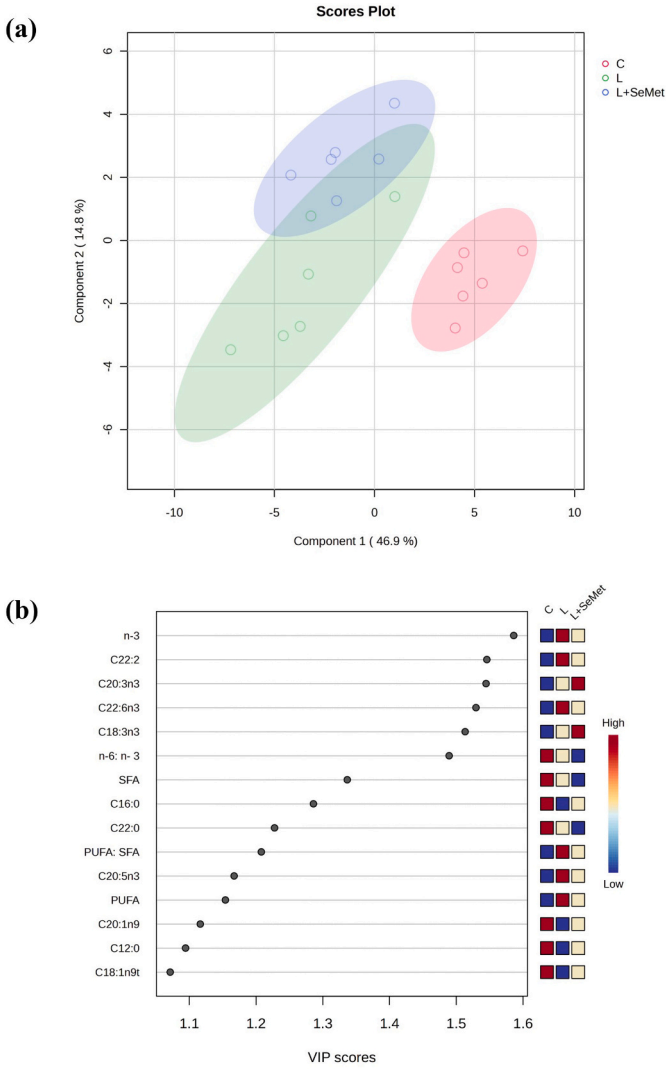

Fig. 2. PLS-DA analysis and VIP values of fatty acid composition in pork *m. longissimus thoracis et lumborum* following dietary supplementation with 3 % soybean oil (control, C), 3 % linseed oil (L) or 3 % linseed oil combined with 0.3 mg/kg of selenomethionine (L + SeMet). (a) The score plot of three dietary treatments in the PLS-DA model. (b) The VIP scores of free fatty acids in the PLS-DA model.

acid compositions in pork loin compared to the control. There were some overlaps in the score plot between L and L + SeMet treatments, suggesting some similarity in fatty acid profiles between these two treatments. The results of VIP analysis based on PLS-DA are shown in Fig. 2b. The VIP chat revealed that the changes in total and some n-3 PUFA (including C20:3 n-3, DHA, and ALA), C22:2, and n-6/n-3 ratios were the key variables driving the differences among dietary treatments. The increases of ALA content in pork samples of L and L + SeMet may be derived from linseed oil which is rich in ALA (Bertol et al., 2013). Matthews, Homer, Thies, and Calder (2000) also observed the increases of ALA concentration in pork *m. longissimus thoracis* as the result of supplementation with 50 g/kg to 100 g/kg of flaxseed in the feed. Both L and L + SeMet treatments increased the concentrations of DHA ($P < 0.001$, Table 4), while only L resulted in significantly improvement in EPA content ($P < 0.01$, Table 4). A similar result was found by Enser, Richardson, Wood, Gill, and Sheard (2000), who reported that adding 2 % flaxseed to pig fattening diet resulted in over a 70 % increase in ALA content and a 20 % increase in DHA level in *m. longissimus lumborum*. However, there was no significant change ($P > 0.05$) in DHA content in pork *m. longissimus dorsi* after feeding with 7 % to 10 % linseed oil

supplementation (Martínez-Ramírez, Cant, Shoveller, Atkinson, & de Lange, 2014). Dugan et al. (2015) and Cameron et al. (2000) suggested that high ALA levels in pig fattening diet could promote its transformation to C18:4 n-3 through the catalysis of Δ6 desaturase, thereby reducing the availability of Δ6 desaturase for the conversion of C24:6 n-3 into DHA. Consequently, lower dietary ALA levels could lead to increased DHA content in meat compared to higher ALA supplementation.

3.3. Analysis of lipase and lipoxygenase activities

The activities of lipases and LOX in pork loin following different dietary treatments are shown in Table 6. Dietary treatments did not alter the activities of acid lipase and phospholipase in this study ($P > 0.05$). The activities of neutral lipase and LOX decreased due to L and L + SeMet treatments compared to the control ($P<0.05$). The lipases play a crucial role for the release of FFAs that are the precursors of the generation of volatiles or aroma compounds in meat products, especially the long-term cured meats (Gandemer, 2002). However, the effects of nutrient supplementation in pig fattening diets on the activities of these lipases in pork are less investigated. Hui et al. (2017a) observed increased neutral lipase activity in restructured dry-cured ham by adding 2–4 % pork back fat. The activities of acid lipase and phospholipase remained unchanged in their study. Neutral lipids constitute the majority of total lipids in smoke-cured bacon and ham, which can be hydrolyzed by acid and neutral lipases in muscles (Jin et al., 2010; Zhou & Zhao, 2007). Thus, the changes in neutral lipase activities could alter the FFA resulting in modification of the flavor profile in meat products. The decreases in neutral lipase activities due to L and L + SeMet treatments supported the observations in FFA (Table 5), where lower levels of FFAs were found compared to the control ($P<0.01$).

LOX plays an important role in lipid oxidation during meat processing, contributing to the formation and regulation of qualities in cured meat products, especially dry-cured ham and sausages (Casaburi et al., 2008). LOX activities were reduced ($P < 0.05$) by 67 % and 74 % following L and L + SeMet treatments, respectively compared to the control, which suggests improved oxidative stability in meat following these dietary treatments. However, in previous studies, dietary n-3 PUFA treatments usually increased lipid oxidation in meat products (Bryhni, Kjos, Ofstad, & Hunt, 2002; Domínguez et al., 2019; Jon Meadus et al., 2011), because the double bonds of increasing n-3 PUFA can lead to rapid lipid oxidation (Wood et al., 2004). The present results suggested that the change of LOX activity was also a crucial factor influencing the oxidative stability of pork meat treated with dietary n-3 PUFA supplementation, besides of increasing n-3 PUFA content.

3.4. Oxidative stability

Oxidative stability was determined based on antioxidant properties and levels of lipid and protein oxidation, the results are shown in Table 7. Most antioxidant properties were not affected by the L and L + SeMet treatments except for GPx, which was significantly reduced by 63 % ($P < 0.05$) and 37 % ($P > 0.05$), respectively, compared to control. Given the ability of n-3 PUFA to elevate lipid peroxide levels in muscle tissues, the observed inhibition of GPx activities in L and L + SeMet treatments may be attributed to the reactive aldehydes derived from lipid peroxidation that modified the GSH-binding sites (Lee, Takahashi, Hatakawa, & Oe, 2023). However, Jiang et al. (2017) reported that the supplementation of 1.5 % linseed oil in the pig fattening diet increased the GPx activity in pork loins by 18 %–22 % compared to those from 1.5 % soybean oil treatment. Their results suggested that the animals may have greater demands for antioxidants for cellular protection due to higher PUFA intake, necessitating increased selenium to enhance the redox system. It has been reported that SeMet was more effective than inorganic selenium for depositing selenium in muscle tissues (Wang, Zhan, Yuan, Zhang, & Wu, 2011), suggesting that the levels of linseed oil and the type of selenium source could significantly influence the results.

Table 7
Effects of n-3 polyunsaturated fatty acid (PUFA) treatments on the antioxidant properties of pork *m. longissimus thoracis et lumborum* (n = 6).

Traits [a-b]	Dietary treatments			SEM	*P*-value [c]
	C	L	L + SeMet		
TBARS (mg/kg meat)	0.34	0.27	0.17	0.039	NS
T-AOC (U/mg protein)	0.09	0.07	0.08	0.006	NS
T-SOD (U/mg protein)	59.60	61.28	57.59	1.031	NS
CAT (U/mg protein)	2.44	3.25	3.33	0.294	NS
GPx (U/mg protein)	1.37[a]	0.51[b]	0.86[ab]	0.150	*
Carbonyl (nmol/mg protein)	6.56	7.07	7.17	0.166	NS
Sulfhydryl (nmol/mg protein)	59.71	66.36	63.26	2.266	NS

Dietary treatments: C = control with 3 % soybean oil supplementation; L = 3 % linseed oil supplementation; L + SeMet = 3 % linseed oil with 0.3 mg/ kg of selenomethionine supplementation. TBARS: thiobarbituric acid reactive substances; T-AOC = total antioxidant capacity; T-SOD = total superoxide dismutase; CAT = catalase; GPx = glutathione peroxidase. SEM = standard error of means.

[a-b] Superscript letters indicate indicate the means are significantly different ($P < 0.05$) among dietary treatments (C, L and L + SeMet).

[c] NS, not significantly different ($P > 0.05$); *, significant at the 5 % level.

GPx is an important peroxy-decomposing enzyme, whose activity reflects the level of selenium in tissues (Arthur, 2001), thus the decrease of GPx activity in the pork from L treatment might lead to lipid oxidation and the accumulation of oxidation primary products. However, no significant difference was observed among dietary treatments for all the indicators lipid and protein oxidation (Table 7). This aligns with findings by Hallenstvedt, Kjos, Rehnberg, Overland, and Thomassen (2010), who reported that adding 0.5 % fish oil to the fattening diet had no effect on TBARS values in pork *m. longissimus dorsi*. However, dietary n-3 PUFA supplementation have been reported to increase lipid oxidation in processing meat products (Bryhni et al., 2002; Domínguez et al., 2019; Jon Meadus et al., 2011). Bartkovský et al. (2022) observed increased levels of MDA in pork *m. gluteobiceps* and *m. longissimus dorsi* from pigs supplemented with 10 % flaxseed for 6 weeks. The TBARS values were approximately 1.2 times higher in dry-cured hams from the pigs supplemented with 0.3, 0.6 or 1.2 g algae/100 g diet compared to those in the control group (Vossen, Raes, Van Mullem, & De Smet, 2017). A similar trend was observed in pork sausages processed from pigs supplemented with 0.4 % capelin oil in the fattening diet, where the TBARS values were 46 % higher than those in the control group (Bryhni et al., 2002).

3.5. Correlation analysis between dietary treatments and selected physicochemical traits

The correlation between dietary treatments and selected physicochemical traits is shown in Fig. 3, with Fig. 3a showing the projection of the explanatory variables and Fig. 3b showing the correlations between fatten diet treatments and pork meat qualities. The Q^2 in PLS-DA was 0.63, indicating that this model has a goodness of fit and predictive quality. The cumulative R^2Y and R^2X cum represent the correlations between the explanatory (X) and dependent (Y) variables are more than 50 %, suggesting that both Xs and Ys are well-summarized by two components.

The global correlation between the variables is illustrated in Fig. 3a, with dependent variables on the c vectors and explanatory variables on the w* vectors. The dietary treatments, marked in blue, were situated in different quadrants. The clear separation among treatments indicates significant differences in those variables across pork from C, L, and L + SeMet treatments. Key variables correlated with the dietary treatments are shown in Fig. 3b, where the explained variance is depicted by two ellipses: a large one at 100 % and a small one at 50 %. Projections within

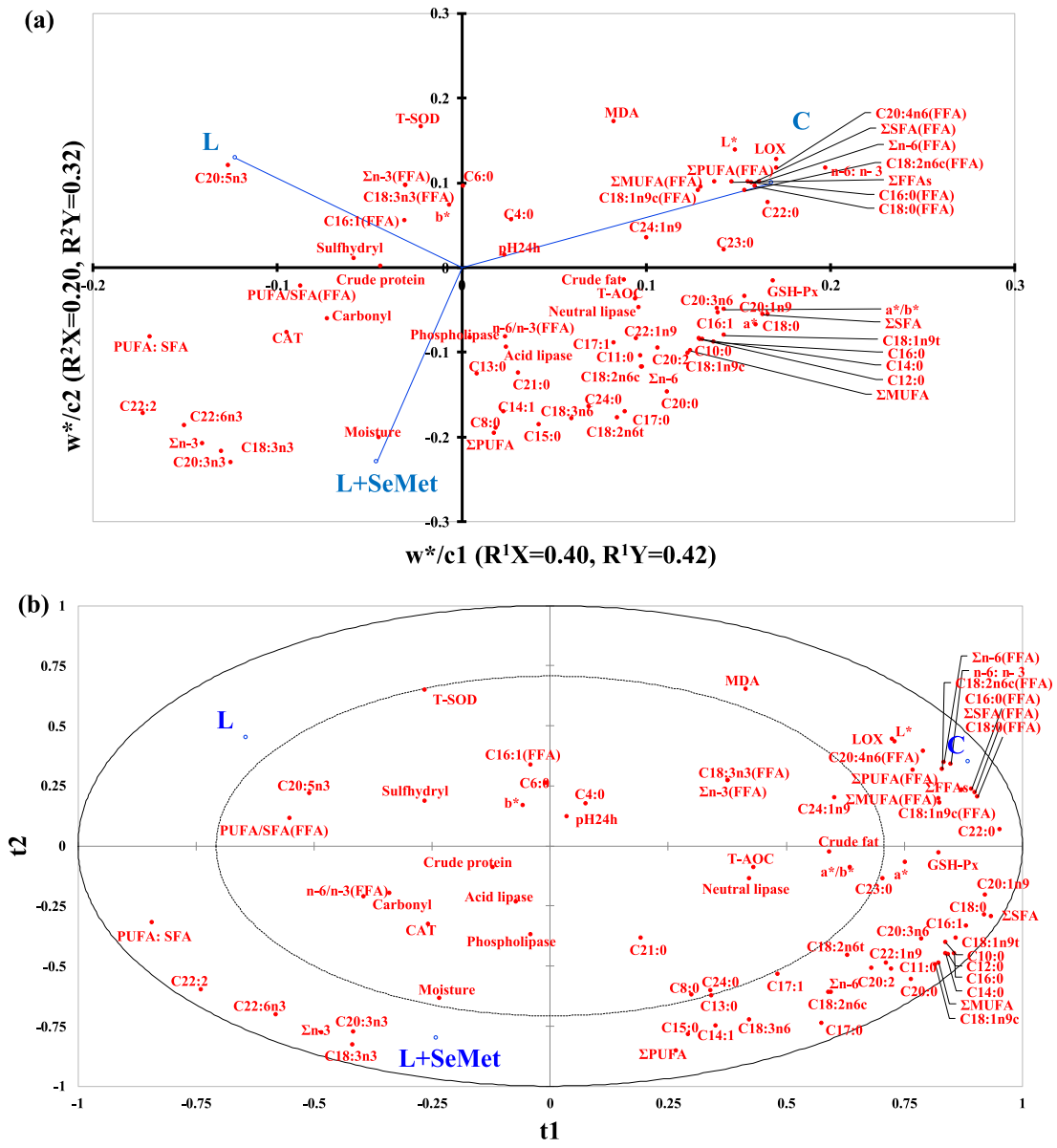

Fig. 3. The PLS-DA analysis of the selected physicochemical traits of pork *m. longissimus thoracis et lumborum* following dietary supplementation with 3 % soybean oil (control, C), 3 % linseed oil (L) or 3 % linseed oil combined with 0.3 mg/kg of selenomethionine (L + SeMet). (a) Projections of physicochemical traits and dietary treatments in the PLS-DA model. (b) Correlations between the dietary treatments and physicochemical traits in the PLS-DA model.

the small ellipse indicate weak association, while those located between two ellipses were considered highly associated. Compared to the control, both the L and L + SeMet treatments reduced the n-6/n-3 ratios and increased the levels of key n-3 PUFA (C20:3 n-3, DHA, and ALA) and total n-3 PUFA. The addition of 3 % linseed oil in the fattening diet significantly reduced L^*, LOX activity, and total FFA content, while increasing PUFA/SFA ratios, particularly in the L + SeMet treatment. Overall, dietary supplementation with both linseed oil and SeMet could be a viable strategy over using linseed oil alone in improving the n-3 PUFA level in pork while maintaining acceptable color properties, oxidative stability and lipase activities.

4. Conclusion

This study demonstrated that adding 3 % linseed oil alone or combined with 0.3 mg/kg SeMet to pig fattening diets effectively enhanced the levels of n-3 PUFA in pork loin and reduced n-6/n-3 ratios to below the recommended ranges in both the US and China. However, supplementation with 3 % linseed oil compromised the lightness and redness, and reduced the activities of LOX and GPx, which are closely correlated with the processing qualities. The inclusion of SeMet (L + SeMet) preserved the redness and GPx activity, while reducing the neutral lipase activity. Collectively, the combination of 3 % linseed oil and 0.3 mg/kg of SeMet supplementation proved beneficial for nutritional quality, visual acceptability and oxidative stability of fresh pork products. Further research is warranted to explore the interactions between linseed oil and SeMet, particularly in relation to lipase, LOX and antioxidant properties, to optimize processibility and eating qualities of fresh pork.

CRediT authorship contribution statement

Yiming Sun: Formal analysis, Conceptualization, Writing – original draft. **Hu Zhang:** Writing – review & editing, Formal analysis, Conceptualization. **Renyu Zhang:** Writing – review & editing. **Yong Yang:** Formal analysis. **Teng Hui:** Supervision, Methodology,

Investigation. **Zhengfeng Fang:** Supervision, Methodology, Investigation.

Declaration of competing interest

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

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Data availability

Data will be made available on request.

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