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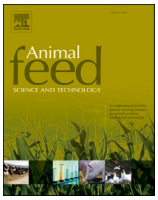

Unravelling the effects of extrusion and drying temperatures on the radical scavenging capacity of aquafeeds supplemented with mango and pineapple by-products

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ABSTRACT

Antioxidant supplementation of aquafeeds with natural sources is a current research trend within the context of a circular economy. However, natural antioxidants are highly vulnerable to thermal conditions during feed manufacturing, particularly during extrusion and drying. This study examines the impact of extrusion and drying temperatures on the antioxidant properties of aquafeeds supplemented with natural antioxidants from mango and pineapple peels. A control dietary mixture and two dietary mixtures with 2 % inclusion of either mango (M) or pineapple (P) peel flour were subjected to different combinations of extrusion temperatures (110 °C, -H vs 25 °C - C) and drying temperatures (60 °C vs 35 °C). Ingredients, manufacturing process intermediate stages, and final diets were analysed for their natural antioxidant composition (vitamins, carotenoids, free and fibre-bound phenolic compounds) and antioxidant capacity via the DPPH^{+}, ABTS$^{\bullet +}$ and ORAC assays, the latter of which is the most representative for biological models. Results show that incorporating 2 % of either pineapple or mango peel flour increases the antioxidant content and capacity of aquafeeds compared to a control diet subjected to the same manufacturing conditions. Phenolic compounds were more abundant and resistant to the feed manufacturing process than vitamins and carotenoids. Specifically, ORAC results for free and fibre-bound extracts in diet PH-60 (1674.3 and 1216.2 mg TE 100 g DM^{-1}, respectively) were significantly higher ($P < 0.001$) than the control CH-60 (694.8 and 422.8, respectively). Moreover, free extracts from diet PC-60 (1312.0 mg TE 100 g DM^{-1}) and fibre-bound extracts from diets MH-60 and PH-35 (719.2 and 871.1 mg TE 100 g DM^{-1}, respectively) were also significantly higher than the control ($P < 0.001$). A PCA analysis showed that pineapple diets with hot extrusion, as well as the mango diet with hot extrusion and hot drying, are more closely

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associated with higher antioxidant capacity in both free and bound extracts. Overall, fruit peel flours show promise as antioxidant supplements for mitigating oxidation in aquafeeds.

1. Introduction

The aquaculture sector currently provides for 53 % of the worldwide production of fish for human consumption (FAO, 2018). This growth has been accompanied by an increasing demand for functional aquafeeds, which are expected to offer additional physiological benefits to the health and growth of farmed fish, going beyond what is provided by their fundamental nutritional content (Bharathi et al., 2019; Dawood et al., 2022).

Aquafeeds contain significant amounts of monounsaturated and polyunsaturated fatty acids (MUFAs and PUFAs, respectively) that are highly sensitive to oxidation via free radical chain reactions initiated by peroxides and hydroperoxides (Colombo et al., 2020). High levels of oxidation can make aquafeeds unsuitable for use due to several factors, such as the depletion of essential nutrients, lower feed palatability, diminished feed intake, and the formation of oxidation by-products that can potentially damage animal health (Gunathilake et al., 2022). Thus, there is a significant demand for diets with strong antioxidant properties, as they help boost the antioxidant defences of fish and prevent feed staling and rancidity (Encarnação, 2016; Olmos-Soto, 2015). This not only helps preserve the nutritional value of the feed but also reduces wastage and the costs associated with handling and storage (Gunathilake et al., 2022; Olmos-Soto, 2015). Moreover, farmed fish are often exposed to stress factors (Reverter et al., 2014) that induce heightened intracellular formation of reactive oxygen species (ROS) that may ultimately lead to reduced growth, higher disease susceptibility, and eventually death (Chowdhury and Saikia, 2020). However, this damage can be mitigated by continuously neutralising ROS with antioxidants, which help maintain healthy metabolic functions (Chowdhury and Saikia, 2020). Therefore, increasing the levels of endogenous and/or exogenous antioxidants can play a crucial role in mitigating oxidative damage by inhibiting the initiation or propagation of oxidative chain reactions, as demonstrated by Baiano and Del Nobile (2016).

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and ethoxyquin (NRC, 2011) are widely used in aquafeeds. However, these compounds have raised certain health concerns. In 2017, the European Union (EU) banned ethoxyquin in feed premixes, additives, and commercialised food (EU 2017/962) due to suspicions of genotoxic and mutagenic properties (European Commission, 2017). After revaluation of potential risks, the authorisation of ethoxyquin (E 324) as an additive in animal nutrition belonging to the additive category 'technological additives' and to the functional group 'antioxidants', was denied by regulation EU 2022/1375 (European Commission, 2022). Furthermore, although the EU still permits using BHA and BHT in animal feeds (European Commission, 2004), BHT has been linked to adverse effects, including immune system suppression and tumour development (Lanigan and Yamarik, 2002). Driven by shifts in global economic interests and consumer preferences, the rising demand for natural ingredients in animal feed and food products (Sanches-Silva et al., 2014) has prompted the feed industry to explore alternative sources of antioxidants, such as fruits and vegetables (Elseady and Zahran, 2013; Bai et al., 2015; Kousoulaki et al., 2015; Encarnação, 2016; Aklakur, 2018; Ehsani et al., 2018; Dawood et al., 2022).

In recent years, the global annual production of fresh vegetables and fruits has increased from 30 to 60 million tons (Shahbandeh, 2019). The processing of these fruits generates millions of tons of antioxidant-rich by-products, such as peels, which are mostly discarded, leading to negative environmental consequences (Kumar et al., 2020; Wu, 2016). However, the antioxidant capacity of these external sources of antioxidants relies on factors such as their chemical composition, feed matrix, and processing technologies. Indeed, the manufacturing processes of feed, including conditioning, extrusion, pelleting, and drying, expose the feed to light, water, heat, pressure, and various forms of mechanical stress. All these factors can affect the stability of natural antioxidants found in aquafeeds (Riaz and Ali, 2009). Particularly, temperatures of over 100 °C are often used (Liu et al., 2021), potentially damaging antioxidant compounds such as vitamins, carotenoids, and polyphenols, which in turn might accelerate feed oxidation (Anderson and Sunderland, 2002; Ortak et al., 2017).

According to Anderson and Sunderland (2002), significant vitamin losses occur in aquafeeds during the extrusion and drying procedures, with extrusion being the most detrimental, causing 54–73 % loss of vitamin E depending on temperature and moisture. Likewise, the high unsaturation of carotenoids makes them susceptible to degradation when exposed to high temperatures, light, and pro-oxidant molecules (Schieber and Weber, 2016). Although phenolic compounds appear to withstand deactivation during the high-temperature extrusion-cooking process to some extent, they remain prone to oxidation (Oniszczuk et al., 2019).

The main objective of this study is to explore the effects of extrusion and drying temperatures on the radical scavenging capacity of aquafeeds supplemented with natural antioxidants from mango and pineapple peels. We will investigate the effects of incorporating 2 % fruit peels into a commercial-like diet for European sea bass, while simultaneously assessing the impact of two different temperatures applied during the extrusion (25 °C vs 110 °C) and drying (35 °C vs 60 °C) processes. The chemical composition and radical scavenging capacity of the fruit peels, as well as the resulting diets, will be evaluated at key steps of their industrial production: homogenisation of fresh fruit, drying fruit biomass, the mixture of feed ingredients, and extrusion and drying of the mixtures.

2. Materials and methods

2.1. Ingredient preparation, dietary formulation and processing

The inclusion of novel ingredients, such as fruit by-products, to produce extruded diets under industrial conditions involves several key phases that may differently impact the nutritional and functional value of the resulting biomass. In this study, five key stages were defined for the feed manufacturing process: Phase 1 – Homogenisation of fresh fruit peels (mango, *Mangifera indica*, and pineapple, *Ananas comosus,* peel biomass); Phase 2 – Drying and grinding of the fruit peel biomass into flour; Phase 3 – Mixing of feed ingredients (Non-extruded dietary mixtures, NE); Phase 4 – Extrusion of the mixtures into pellets; and Phase 5 – Drying of extruded pellets. The technological processes involved in each phase (Fig. 1) are described below:

Phase 1: Fresh mango and pineapple peels were obtained from Nuvi Fruits, S.A., a Portuguese leading company in producing and distributing ready-to-eat fruit and dehydrated fruit products. A small portion of mango and pineapple peels was homogenised using a liquid juicer and analysed for their chemical composition and antioxidant capacity before proceeding to the next step.

Phase 2: Fresh mango and pineapple peels were processed into flour as described previously by Campos et al. (2020b), with adaptations for mango peel. Essentially, while pineapple peels were able to be homogenised in the liquid juicer for conversion into flour as described by Campos et al. (2020b), this was not possible for mango peels due to their physical properties. As such, homogenised pineapple peels and the non-processed mango peels were placed on separate metal trays and dried in an air-circulating oven at 60 °C until the moisture content was reduced to <10 % (72 h for whole mango peels and 48 h for homogenised pineapple peels). The resulting dried biomasses were ground in a granite flour mill and sifted at 0.5 mm to obtain two distinct flours – mango peel flour (M) and pineapple peel flour (P). Both products were stored in separate polyethene bags at - 80 °C until they were chemically analysed.

Phase 3: A control diet was formulated to meet all the nutritional requirements of European sea bass (NRC, 2011), except for the addition of vitamins E and C with antioxidant capacity. A commercial vitamin premix without these vitamins was used to supplement the diet. All ingredients were mixed under industrial conditions by SPAROS Lda. (Portugal) to obtain a non-extruded control mixture (CNE). Two experimental diets were further formulated by including 2 % of either pineapple or mango flour in the CNE at the expense of wheat meal (Table 2). This resulted in two distinct experimental non-extruded mixtures, pineapple (PNE) and mango mixtures (MNE).

Phase 4: Extrusion of mixtures was performed by SPAROS Lda. (Portugal) using two extrusion technologies: a) a commonly used hot extrusion employing a pilot-scale twin-screw extruder (CLEXTRAL BC45, France) with a screw diameter of 55.5 mm and a temperature of 110 °C as previously described for aquafeeds (Pereira et al., 2022; Resende et al., 2024); b) a cold extrusion process using a low-shear single screw extruder (ITALPLAST P150, Italy) operated under low-temperature conditions (c.a., 25ºC) at the extruder output to maintain the stability of natural compounds as previously suggested (Hernández et al., 2014). The PNE and MNE mixtures were extruded using either the hot standard temperature (-H, 110 °C) or a colder temperature (-C, <25 °C), generating two extruded pellets from pineapple (PH and PC) and two from mango (MH and MC). The CNE was extruded at 110 °C to simulate current commercial manufacturing processes, resulting in a control hot-extruded (CH) pellet.

Phase 5: The extruded pellets obtained from phase 4 were dried by SPAROS Lda. (Portugal) using two different methods. The first method involved drying at 60 °C, as typically used in aquafeeds (Resende et al., 2024), while the second method involved a colder drying temperature of 35 °C as suggested by Schafberg et al. (2020) until all pellets reached an acceptable dry matter content (>90 %) (Fig. 1). This generated two control hot-extruded dried diets: the CH-60 (hot-extrusion at 110 °C and drying at 60 °C) and the CH-35 (hot-extrusion at 110 °C and drying at 35 °C), as well as four experimental dried diets containing M (MH-60, MH-35, MC-60 and MC-35), and four diets containing P (PH-60, PH-35, PC-60 and PC-35 diets). The resulting biomass from each key processing step was frozen at - 80 °C until analysed for proximate composition and determination of antioxidant properties.

2.2. Proximate composition analysis

Proximate composition of fresh peels, flours, mixtures, and pellets was performed following AOAC methods (AOAC, 2006). Extruded pellets were ground and homogenised before analyses. All samples were analysed in duplicates for dry matter (DM) (105 °C

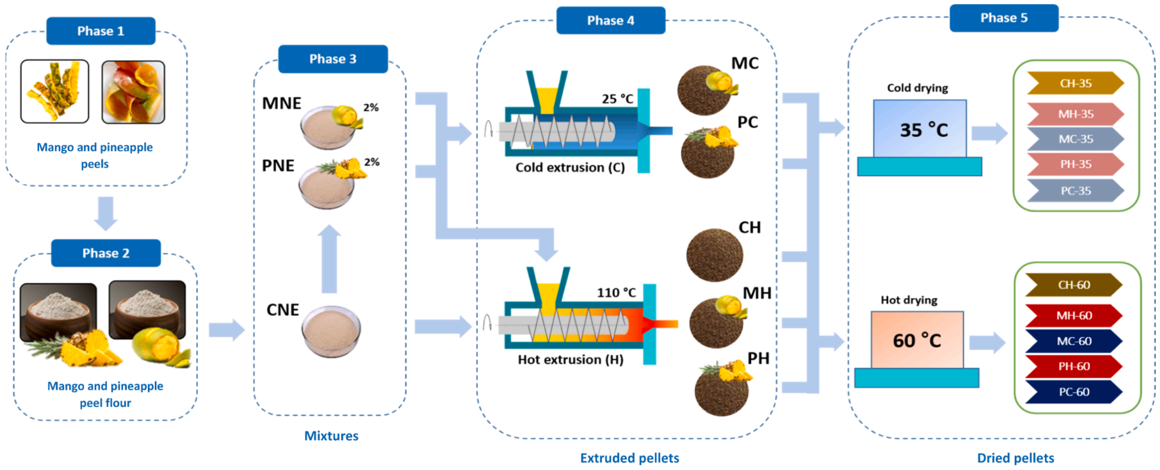


Fig. 1. Illustrative scheme of five key stages for the feed manufacturing process.

for 24 h); ash through muffle furnace combustion at 500 °C for 5 h (Nabertherm L9/11/B170, Bremen, Germany); crude protein (N × 6.25) using a Leco nitrogen analyser (Model FP 528; Leco Corporation, St. Joseph, USA); and crude fat by petroleum ether extraction, using a Soxtec extractor (Model ST 2055 Soxtec™; FOSS, Hillerod, Denmark). Gross energy was determined in an adiabatic bomb calorimeter (Model Werke C2000, IKA, Staufen, Germany). Crude fibre content was analysed according to the intermediate filtration method (ISO 6865:2000); neutral-detergent fibre (NDF) was determined according to ISO 16472:2006 (Robertson and Van Soest, 1981; Van Soest and Robertson, 1985); and starch was determined as described in Thivend et al. (1972).

2.3. Carotenoids and vitamins

In all in fresh peels, flours, mixtures, extruded pellets, and dried pellets (final diets), carotenoids, retinol, and tocopherols were analysed in duplicate using extracts obtained following Slavin and Yu (2012), with slight modifications as described by Gómez-García et al. (2021). The quantification and identification of carotenoids in the resulting extract were also performed according to Gómez-García et al. (2021), through HPLC (Waters Series 600, Mildford MA, USA), using acetonitrile, methanol, dichloromethane, hexane and ammonium acetate (55:22:11.5:11.5:0.02 v/v/v/v/w) under isocratic conditions at 1 mL min^{-1} flow rate during 20 min at 30 °C. Injection volume was 50 μL and detection was performed by a 454 nm diode array detector (Waters, Massachusetts, EUA). α-carotene, β-carotene, lutein, and β-cryptoxanthin were quantified using a pure standard calibration curve and expressed as mg 100 g^{-1} DM. Analysis of retinol and tocopherols were performed via chromatography, using a Beckman System Gold® HPLC system (Beckman Coulter, California, U.S.A.) linked to a Waters™ 474 Scanning Fluorescence Detector (excitation wavelength of 290 nm and emission wavelength of 320 nm) with a VARIAN ProStar Model 410 AutoSampler with a normal-phase silica column (Kromasil 60–5-SIL, 250 mm, 4.6 mm ID, 5 μm particle size). The mobile phase was 1 % (v v^{-1}) isopropanol in n-hexane with a flow rate of 1 mL min^{-1}. The total run time was 20 min and the injection volume was 20 μL. Standard curves of peak area *vs*. concentration were used for each compound quantification.

2.4. Phenolic compounds

Free phenolic compounds were determined in fresh peels, flours, mixtures, extruded pellets, and dried pellets (final diets), in triplicate, according to the method described by Xie et al. (2015) adapted by Coscueta et al. (2018), with some modifications. Briefly, 2.5 g of each sample was mixed with methanol:water (2:8) and homogeneised using an IKA T 25 digital ULTRA-TURRAX® (IKA, Germany) for 1 min, after which they were centrifuged at 15 000 × *g* for 15 min. The resulting supernatant was collected in a light-protected tube and stored at −80 °C for posterior analysis of free phenolic content. The remaining biomass was used for fibre-bound phenolic analysis, hydrolysing the fibre by mixing this pellet with 20 mL of 4 M NaOH in distilled water and placing the resulting mixture in an orbital shaker at 250 rpm for a period of 3 h. After this, acid hydrolysis was performed by adding 8 mL of HCl 32 %, after which sample pH was calibrated using either NaOH or HCl 32 % until the sample reached a pH of 1.5–2. The resulting mixture was washed using 25 mL of ethyl acetate and centrifuged for 5 min at 5000 × g. The obtained ethyl acetate fraction was drained into a new tube, and the process was then repeated, generating about 50 mL of supernatant. The ethyl acetate fraction was placed in a rotary vacuum evaporator at 50 °C (R-210 EBI 136, BÜCHI, Switzerland) until total dryness, after which it was dissolved in 5 mL of pure methanol. The resulting solution was collected in a light-protected tube and stored at - 80 °C.

Identification and quantification of phenolic compounds in both free and fibre-bound phenolic extracts were performed via high-performance liquid chromatography (HPLC, Waters Series 600, Mildford MA, USA). The stationary phase was a C18 Hypersil gold ODS2 analytical column (250 × 4.6 mm i.d.) with a particle size of 5 mm (Thermo Fisher, Waltham, MA, USA) maintained at 30 °C. The flow rate was 1 mL/min, and the absorbance was detected at 280 nm. Mobile phase A was composed of acetic acid/water (2:98, v/v), while mobile phase B was methanol. An elution gradient was implemented as follows: 95 % (A) and 5 % (B) to 80 % (A) and 20 % (B) over 10 min; to 40 % (A) and 60 % (B) over 15 min (25 min, total time); to 30 % (A) and 70 % (B) over 5 min (30 min, total time), and re-equilibration over 10 min (40 min, total time) to the initial composition. The phenolic compounds were quantified by comparing the retention times with those of the corresponding standards using their regression equation and UV spectra obtained with an SPD-20A UV–vis Detector. Measurement of total polyphenols in both free and fibre-bound extracts was performed via spectrophotometry, according to the Folin-Ciocalteu method at 750 nm (Synergy H1 HU126, Vermont, USA), following Singleton and Joseph A. Rossi (1965), and expressed in mg of gallic acid equivalents (GAE) per 100 g of DM.

2.5. Antioxidant capacity of samples

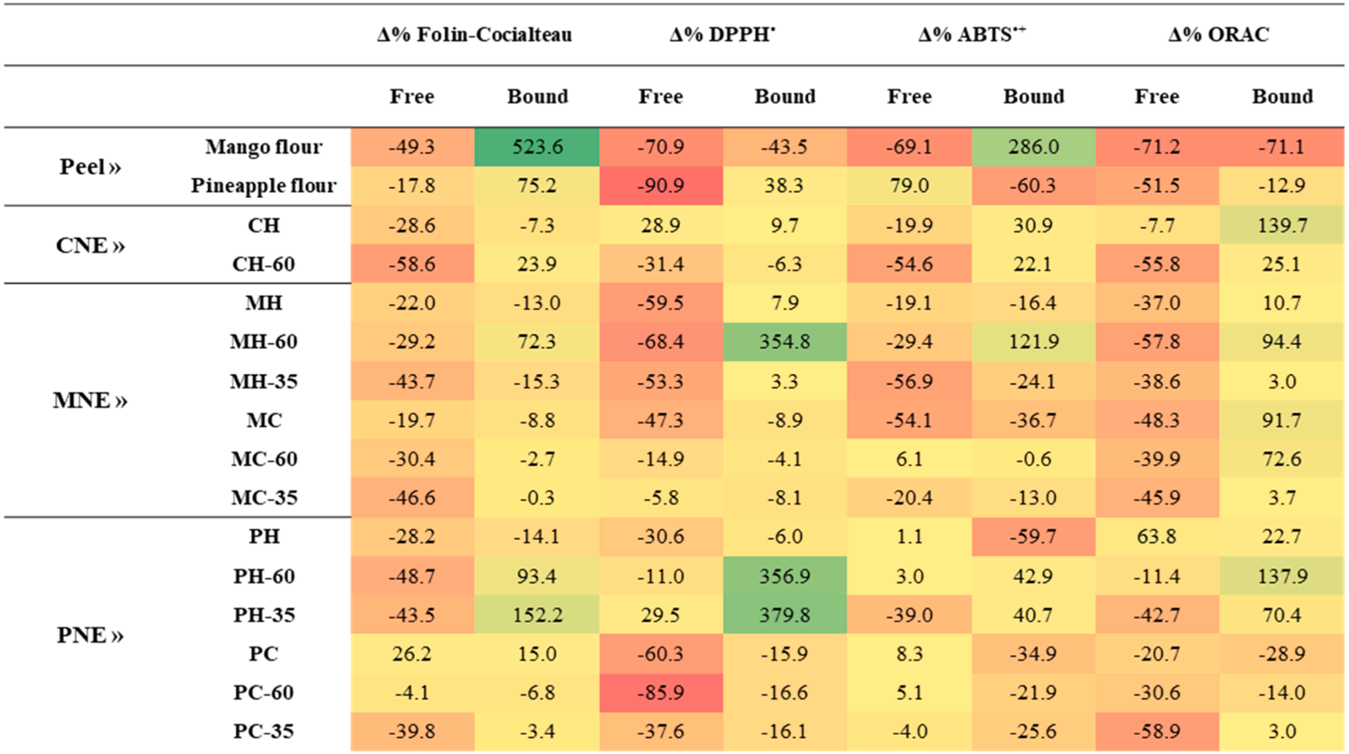
The antioxidant capacity of fresh peels, flours, mixtures, extruded pellets, and dried pellets (final diets) was measured through the assessment of the radical scavenging capacity of ABTS$^{\bullet+}$ and DPPH$^{\bullet}$, as well as ORAC for superoxide anion (O$^{\bullet-}$) radical scavenging capacity. All analyses were done in triplicate using a Multidetector plate reader (Synergy H1 HU126, Vermont, USA), and final results were expressed in mg of Trolox equivalents (TE) per 100 g of DM.

The ABTS$^{\bullet+}$ radical scavenging activity was measured in the methanolic extracts, using the method described by Sánchez-Moreno (2002) and adapted by Gonçalves et al. (2009). Essentially, using a flat-bottom 96 well microplate, 180 μL of ABTS$^{\bullet+}$ working solution was added to 20 μL of sample, in triplicate. The mixture was incubated for 5 min at 30 °C, protected from light, and the absorbance measured at 734 nm. The DPPH$^{\bullet}$ assay was done according to the method of Brand-Williams et al. (1995), with some modifications. The assay was also performed in a flat-bottomed 96-well microplate; 175 μL of DPPH$^{\bullet}$ working solution was added to 25 μL of sample, in triplicate. In both analytical procedures, Trolox was used for the standard curve and methanol 80 %, the solvent used for the analysed extracts, was used for the blanks. The mixture was incubated for 30 min at 25 °C, protected from light, and the absorbance was measured at 515 nm. ORAC was carried out following Dávalos et al. (2004). The reaction was achieved using a phosphate buffer (75 mM, pH 7.4) combined with 20 μL of either the free or the fibre-bound phenolic extracts for each sample, plus 120 μL of fluorescein (116.66 nM) in a black microplate (Nunc, Denmark). The solution was pre-incubated at 37 °C for 15 min, after which, 60 μL of 2, 2′azobis-(2-methylpropionamidine)-dihydrochloride (AAPH) (46,6 mM) was added, followed by a second incubation period at 40 °C for 137 min. A blank was made by using a phosphate buffer instead of a sample.

Table 1
Proximate composition, bioactive compounds and antioxidant capacity of fruit peels from phase 1 and flours from phase 2.

	Mango		Pineapple	
	Peel	**Flour**	**Peel**	**Flour**
Proximate composition (% of DM or kJ g^{-1} DM)[1]				
Dry matter	18.7 ± 0.2	90.2 ± 0.05	13.0 ± 0.4	92.4 ± 0.1
Ash	2.5 ± 0.2	2.6 ± 0.1	3.1 ± 0.2	3.0 ± 0.05
Protein	4.7 ± 0.1	6.2 ± 0.03	7.0 ± 0.1	6.8 ± 0.1
Lipids	1.0 ± 0.2	1.3 ± 0.1	1.8 ± 0.1	0.8 ± 0.1
Carbohydrates	91.9 ± 0.2	89.9 ± 0.1	88.1 ± 0.2	89.3 ± 0.2
Crude fibre	11.3 ± 0.1	13.4 ± 0.1	23.6 ± 0.3	22.5 ± 0.001
Neutral-detergent fibre	15.0 ± 0.02	18.3 ± 0.3	60.4 ± 0.1	56.3 ± 0.01
Starch	1.8 ± 0.03	2.5 ± 0.03	0.1 ± 0.004	0.6 ± 0.03
Energy	17.8 ± 0.1	18.4 ± 0.01	19.1 ± 0.3	18.6 ± 0.1
Vitamins (μg 100 g^{-1} DM)[2]				
Retinol	18.8 ± 1.5	n.a	17.5 ± 0.1	5.0 ± 2.2
α-tocopherol	2732.4 ± 28.8	2580.4 ± 56.0	394.7 ± 25.4	185.0 ± 8.1
β-tocopherol	718.8 ± 59.0	209.8 ± 6.0	1715.5 ± 142.4	834.3 ± 87.8
Sum of identified vitamins	3500.8 ± 2.3	2790.1 ± 54.5	2127.6 ± 117.2	1021.5 ± 80.7
Carotenoids (mg 100 g $^{-1}$ DM)[2]				
Lutein	0.9 ± 0.01	0.7 ± 0.04	1.1 ± 0.1	0.5 ± 0.04
β-cryptoxanthin	0.02 ± 0.004	0.02 ± 0.001	0.02 ± 0.005	0.004 ± 0.001
α-carotene	0.3 ± 0.01	0.3 ± 0.05	0.1 ± 0.005	0.04 ± 0.001
β-carotene	5.2 ± 0.1	4.2 ± 0.1	3.4 ± 0.5	0.9 ± 0.02
Sum of identified carotenoids	6.7 ± 0.5	5.2 ± 0.2	4.6 ± 0.5	1.4 ± 0.2
Total	9.9 ± 1.0	2.5 ± 0.2	11.4 ± 1.0	3.3 ± 0.2
Free phenolic content (mg 100 g $^{-1}$ DM)[2]				
Gallic acid	58.3 ± 2.9	32.7 ± 6.2	2.4 ± 0.1	22.2 ± 5.2
Mangiferin	157.7 ± 35.8	53.8 ± 3.3	n.a	n.a
Rutin	16.5 ± 1.3	8.0 ± 0.3	BQL	n.a
Gentisic acid	n.a	n.a	n.a	51.1 ± 5.7
Isoferulic acid	n.a	n.a	BQL	8.6 ± 3.0
Ferulic acid	n.a	n.a	BQL	115.1 ± 0.4
Sum of identified polyphenols	202.3 ± 2.7	109.3 ± 5.8	7.4 ± 1.4	217.9 ± 12.0
Total (Folin-Ciocalteu)	1155.9 ± 4.2	586.5 ± 36.1	483.5 ± 14.5	397.5 ± 24.7
Bound phenolic content (mg 100 g $^{-1}$ DM)[2]				
Gallic Acid	11.7 ± 0.1	285.9 ± 6.8	n.a.	n.a
Epicatechin gallate	BQL	102.6 ± 3.9	n.a	n.a.
Mangiferin	45.2 ± 7.1	14.3 ± 0.2	n.a	n.a.
Transferulic acid	n.a	n.a	444.5 ± 51.4	141.4 ± 11.8
4-coumaric acid	n.a	n.a	169.5 ± 17.1	63.8 ± 14.8
Sum of identified polyphenols	5.3 ± 1.0	404.2 ± 3.1	614.0± 68.5	225.6 ± 50.8
Total (Folin-Ciocalteu)	54.1 ± 3.3	337.5 ± 18.2	312.2 ± 21.6	547.0 ± 13.3
DPPH$^{\bullet}$ (mg TE 100 g $^{-1}$ DM)[3]				
Free phenolic extracts	2507.6 ± 34.4	729.2 ± 1.8	2600.9 ± 121.4	235.8 ± 16.2
Bound phenolic extracts	162.2 ± 14.1	91.7 ± 13.2	286.0 ± 7.1	395.6 ± 47.7
ABTS$^{\bullet+}$ (mg TE 100 g $^{-1}$ DM)[3]				
Free phenolic extracts	4003.0 ± 275.1	1235.3 ± 149.4	588.0 ± 26.1	963.0 ± 92.4
Bound phenolic extracts	140.1 ± 14.3	540.7 ± 0.2	1289.2 ± 9.2	511.8 ± 2.7
ORAC (mg TE 100 g $^{-1}$ DM)[3]				
Free phenolic extracts	7841.1 ± 53.2	2254.5 ± 114.5	5623.2 ± 47.5	2729.5 ± 59.2
Bound phenolic extracts	5174.3 ± 128.6	1495.4 ± 88.0	4194.9 ± 49.9	3655.4 ± 558.0

Values from analytical samples are presented as mean (n=2) for proximate composition, and mean ± standard deviation (n=3) for remaining variables. “n.a” stands for “non-applicable”, meaning that the chemical compound in question is inexistent in that sample. “BQL” stands for “below quantification limit”, meaning that the chemical compound in question was identified, but was not reported due to being present below the minimal quantities for quantification. 1 Proximate composition is expressed in % of dry matter (DM), except for energy, which is expressed in kJ g-1 DM. Carbohydrates are calculated by difference as 100-(ash+crude protein+crude lipids). DM. 2 Vitamins are expressed in μg of vitamin per 100 g of DM. Total carotenoids were measured spectrophotometrically and are expressed in mg of β-carotene equivalents per 100 g of DM. Specific carotenoids were measured via HPLC are expressed in mg of carotenoid per 100 g of DM. Total phenolic content was measured via the Folin-Ciocalteu method and is expressed in mg of gallic acid equivalents (GAE) per 100 g of DM. Specific phenolic compounds measured via HPLC are expressed in mg of polyphenol per 100 g of DM. 3 DPPH•, ABTS•+ and ORAC, are expressed in mg of Trolox equivalents (TE) per 100 g of DM.



		Δ% Folin-Cocialteau		Δ% DPPH$^{\bullet}$		Δ% ABTS$^{\bullet+}$		Δ% ORAC	
		Free	Bound	Free	Bound	Free	Bound	Free	Bound
Peel »	**Mango flour**	-49.3	523.6	-70.9	-43.5	-69.1	286.0	-71.2	-71.1
	Pineapple flour	-17.8	75.2	-90.9	38.3	79.0	-60.3	-51.5	-12.9
CNE »	**CH**	-28.6	-7.3	28.9	9.7	-19.9	30.9	-7.7	139.7
	CH-60	-58.6	23.9	-31.4	-6.3	-54.6	22.1	-55.8	25.1
MNE »	**MH**	-22.0	-13.0	-59.5	7.9	-19.1	-16.4	-37.0	10.7
	MH-60	-29.2	72.3	-68.4	354.8	-29.4	121.9	-57.8	94.4
	MH-35	-43.7	-15.3	-53.3	3.3	-56.9	-24.1	-38.6	3.0
	MC	-19.7	-8.8	-47.3	-8.9	-54.1	-36.7	-48.3	91.7
	MC-60	-30.4	-2.7	-14.9	-4.1	6.1	-0.6	-39.9	72.6
	MC-35	-46.6	-0.3	-5.8	-8.1	-20.4	-13.0	-45.9	3.7
PNE »	**PH**	-28.2	-14.1	-30.6	-6.0	1.1	-59.7	63.8	22.7
	PH-60	-48.7	93.4	-11.0	356.9	3.0	42.9	-11.4	137.9
	PH-35	-43.5	152.2	29.5	379.8	-39.0	40.7	-42.7	70.4
	PC	26.2	15.0	-60.3	-15.9	8.3	-34.9	-20.7	-28.9
	PC-60	-4.1	-6.8	-85.9	-16.6	5.1	-21.9	-30.6	-14.0
	PC-35	-39.8	-3.4	-37.6	-16.1	-4.0	-25.6	-58.9	3.0

Fig. 2. Variation in total free and bound phenolic compounds across key processing steps for the feed production: from peel to flour and from dietary mixtures to extruded pellets (before and after drying). All values were calculated in percentage (Δ%) concerning sample averages. "Free" refers to data from free phenolic extracts while "Bound" refers to data from bound phenolic extracts. Flours were compared to their respective peels. In both extrusions and all final diets, each percentage was calculated concerning its respective mix: CH, CH-60 and CH-35 were calculated in comparison with values from CNE; MH, MH-60, MH-35, MC, MC-60 and MC-35 were calculated in comparison with values from MNE; PH, PH-60, PH-35, PC, PC-60 and PC-35 were calculated in comparison with values from PNE.

2.6. Statistical analysis

All statistical analyses were performed with IBM SPSS STATISTICS, 27.0 package (IBM corporation, New York, NY, USA, 2021). Data were tested for normality and homogeneity of variances by Shapiro-Wilk and Levene's tests, respectively, and transformed whenever required before being submitted to either a two-way ANOVA with extrusion and drying temperatures as main factors (Fig. 3), or a one-way ANOVA with diet as main effect (Table 5). When tests showed significance, individual means were compared using the HSD Tukey post-hoc Test. In all cases, the level of significance was set below 0.05. The principal component analysis (PCA) (Fig. 5) was performed using the XLSTAT (Addinsoft, Inc.) software package to differentiate the samples from the different study groups.

3. Results

3.1. Phases 1 and 2 – characterization of ingredients

The macronutrient composition of mango and pineapple peel and flour is depicted in Table 1. The water content was considerably lower in dried flour (90–92 %) compared to fresh peels (13–19 %), as expected. The protein content of pineapple peel is higher than that of mango peel, but their respective flours have similar protein content (6–7 % DM). The crude lipid content of both pineapple and mango peel is very limited (<2 % DM). Both mango and pineapple peel are very rich sources of carbohydrates (>88 % DM). The content of complex polysaccharides, particularly measured as neutral detergent fibre, *i.e.,* including lignin, hemicellulose, and cellulose, is substantially higher in pineapple peel than in mango peel. This also translates to pineapple peel flour, which is a much richer source of polysaccharides than mango peel flour. Starch content is low in mango peel and flour (1.8–2.5 % DM), and residual in pineapple peel and flour (0.1–0.6 % DM).

Peels from mango and pineapple exhibited different contents of vitamins A (retinol), vitamin E (α and β-tocopherol), carotenoids, and both free and bound and phenolic compounds. When converting peels into flour, heavy losses in vitamins, carotenoids, as well as free phenolic compounds, and antioxidant capacity were perceived in both fruits (Table 1). While levels of retinol were similar in mango and pineapple peel (18.8 and 17.5 μg 100 g $^{-1}$), this compound was completely absent in mango flour, whilst in pineapple flour, 29 % of values from the peel were still retained (5 μg 100 g^{-1} DM). In terms of Vitamin E, mango peel showed higher α-tocopherol quantities (2732.4 vs 394.7 μg 100 g^{-1} DM), but lower β-tocopherol levels than pineapple (718.8 vs 1715.5 g^{-1} DM). Overall, fresh peels consistently showed higher levels of vitamins than the respective corresponding flours. Likewise, total carotenoids were found in higher amounts in mango (9.9 mg 100 g^{-1} DM) and pineapple peels (11.4 mg 100 g^{-1} DM) compared to their respective flours (2.5 and 3.3 mg 100 g^{-1} DM, respectively), possibly due to higher amounts of lutein and β-carotene. Overall, mango flour displayed a better ability to retain carotenoids compared to pineapple flour.

Concerning phenolic compounds, free forms of gallic acid, mangiferin, and rutin were identified in mango peel. However, the content of these phenolic compounds and the total free phenolic content decreased by approximately between −49.3 and −65 % during the conversion process from peel to flour. In contrast, gallic acid emerged as the predominant free phenolic compound in pineapple, with its content in the flour (22.2 mg 100 g^{-1} DM) nearly ten times higher than in the peels (2.4 mg 100 g^{-1} DM). Additionally, pineapple flour contained quantifiable amounts of free gentisic acid, isoferulic acid, and ferulic acid. Overall, the values of total free polyphenols in pineapple peels were 18 % higher than in flour (Fig. 2). In terms of bound phenolic compounds, bound forms of gallic acid, epicatechin gallate, and mangiferin were found in mango peels and flour, while pineapple displayed a presence of transferulic and 4-coumaric acids. The dynamics of fibre-bound phenolic compounds measured via the Folin-Ciocalteu method showed an opposite trend compared to that observed in free phenolic compounds after the conversion from peel to flour. Essentially, in both fruits, the extraction yields of bound phenolic compounds were much higher in mango and pineapple flours (337.5 and 547.0 mg GAE 100 g DM^{-1}, respectively) than in their respective peels (54.1 and 312.2 mg GAE 100 g DM^{-1}, respectively). According to Fig. 2, mango flour exhibited the highest increase, reaching 524 % compared to the peel, whereas pineapple peel displayed a comparatively lower increase of 75 % (Fig. 2).

In terms of the radical scavenging properties (Table 1; Fig. 2), the free polyphenol extracts derived from mango peels showed higher values in the DPPH$^{\bullet}$ (2507.6 mg TE 100 g DM^{-1}), ABTS$^{\bullet+}$ (4003.0 mg TE 100 g DM^{-1}) and ORAC (7841.1 mg TE 100 g DM^{-1}) assays in comparison to mango flour (729.2, 1235.3 and 2254.5 mg TE 100 g DM^{-1}, respectively). The same decreasing trend was observed for the bound phenolic extracts, except for ABTS$^{\bullet+}$, which displayed higher values in mango flour (540.7 mg TE 100 g DM^{-1}) when compared to peel (140.1 mg TE 100 g DM^{-1}). In pineapple peel, the antioxidant capacity of both free and fibre-bound polyphenol extracts was generally higher than in the corresponding flour, except for bound DPPH$^{\bullet}$ and free ABTS$^{\bullet+}$, which exhibited higher antioxidant capacity in the flour (395.6 and 963.0 mg TE 100 g DM^{-1}, respectively) when compared to the peel (286.0 and 588.0 mg TE 100 g DM^{-1}, respectively).

3.2. Phase 3 - characterization of the experimental mixtures prior to extrusion

The proximate composition analysis of the experimental mixtures before extrusion (NE mixtures) revealed that they were isolipidic, isoproteic, and isoenergetic, but they differed in terms of phenolic compounds and antioxidant capacity (Table 2). The quantification of free phenolic compounds using the Folin-Ciocalteu method showed that PNE had the highest levels of these compounds amongst the three mixtures (206.7 vs 163.0–172.3 mg GAE 100 g DM^{-1}). However, MNE had the highest level of free gentisic, sinapic, and isoferulic acids (58.3, 4.5, and 2.8 mg 100 g^{-1} DM, respectively). Moreover, MNE was the only mixture that showed the presence of

Table 2

Ingredients and proximate composition, as well as bioactive compounds and antioxidant capacity of the non-extruded dietary mixtures from phase 3.

	CNE	MNE	PNE
Ingredients (% of feed)			
Fishmeal Super Prime	10.0	10.0	10.0
Porcine blood meal	2.5	2.5	2.5
Poultry meal	5.0	5.0	5.0
Soy protein concentrate	16.0	16.0	16.0
Wheat gluten	9.5	9.5	9.5
Corn gluten	7.0	7.0	7.0
Soybean meal 48	10.0	10.0	10.0
Rapeseed meal	5.0	5.0	5.0
Wheat meal	16.5	14.5	14.5
Fish oil	5.1	5.1	5.1
Rapeseed oil	9.4	9.4	9.4
Soy lecithin	0.2	0.2	0.2
Vitamin Premix PV02[1]	0.2	0.2	0.2
Brewer's yeast	2.5	2.5	2.5
MAP	0.9	0.9	0.9
DL-Methionine	0.2	0.2	0.2
Mango flour	-	2.0	-
Pineapple flour	-	-	2.0
Proximate composition (% of DM or kJ g^{-1} DM)[2]			
Dry Matter	93.0 ± 0.1	93.1 ± 0.1	92.2 ± 0.1
Ash	6.5 ± 0.3	6.5 ± 0.3	6.7 ± 0.002
Protein	57.7 ± 0.3	57.1 ± 0.4	57.4 ± 0.2
Lipids	3.0 ± 0.03	3.2 ± 0.1	2.8 ± 0.05
Carbohydrates	32.8 ± 0.2	33.3 ± 0.1	33.1 ± 0.2
Crude fibre	3.4 ± 0.1	3.7 ± 0.0003	3.8 ± 0.05
Starch	16.1 ± 0.6	14.7 ± 0.5	15.0 ± 0.6
Energy	20.5 ± 0.2	20.6 ± 0.02	20.9 ± 0.04
Free phenolic content (mg 100 g^{-1} DM)[3]			
Gentisic acid	55.2 ± 0.4	58.3 ± 0.3	49.8 ± 0.9
Mangiferin	n.a	0.2 ± 0.02	n.a
Sinapic acid	3.6 ± 0.1	4.5 ± 0.1	3.9 ± 0.2
Isoferulic acid	1.7 ± 0.04	2.8 ± 0.1	0.5 ± 0.03
Sum of identified polyphenols	57.6 ± 0.5	65.8 ± 0.2	50.2 ± 1.0
Total (Folin-Ciocalteu)	163.0 ± 4.2	172.3 ± 6.4	206.7 ± 13.3
Bound phenolic content (mg 100 g^{-1} DM)[3]			
Gallic acid	n.a	4.5 ± 0.7	n.a
Caffeic acid	BQL	BQL	0.2 ± 0.01
Syringic acid	2.3 ± 0.1	1.1 ± 1.5	2.8 ± 0.03
Neochlorogenic acid	2.0 ± 0.1	1.5 ± 0.1	3.1 ± 0.2
4-coumaric acid	BQL	BQL	5.7 ± 0.02
Transferulic acid	5.5 ± 1.2	5.3 ± 0.4	13.8 ± 0.5
Sinapic acid	21.7 ± 1.9	18.3 ± 0.1	25.9 ± 0.4
Sum of identified polyphenols	31.5 ± 0.7	30.4 ± 0.2	50.5 ± 2.7
Total (Folin-Ciocalteu)	27.5 ± 0.1	35.3 ± 3.5	35.4 ± 0.3
DPPH$^{\bullet}$ (mg TE 100 g^{-1} DM)[4]			
Free phenolic extracts	107.7 ± 16.2	154.0 ± 4.5	154.1 ± 7.7
Bound phenolic extracts	24.7 ± 0.002	27.5 ± 0.5	25.6 ± 2.0
ABTS$^{\bullet+}$ (mg TE 100 g^{-1} DM)[4]			
Free phenolic extracts	183.4 ± 2.8	181.4 ± 5.4	154.1 ± 7.7
Bound phenolic extracts	66.3 ± 10.8	91.6 ± 4.5	101.8 ± 9.2
ORAC (mg TE 100 g^{-1} DM)[4]			
Free phenolic extracts	1572.1 ± 40.5	2043.8 ± 47.8	1889.4 ± 26.8
Bound phenolic extracts	338.1 ± 98.6	370.0 ± 74.2	511.2 ± 29.7

Values from analytical samples are presented as mean (n=2) for proximate composition, and mean ± standard deviation (n=3) for remaining variables. "n.a" stands for "non-applicable", meaning that the chemical compound in question is inexistent in that sample. "BQL" stands for "below quantification limit", meaning that the chemical compound in question was identified, but was not reported due to being present below the minimal quantities for quantification. To the control mix with no extrusion (CNE) 2 % of either mango peel flour (MNE) or pineapple peel flour (PNE) were added. 1Vitamin premix (mg or IU/kg mix): vitamin D3 (DL-cholecalciferol), 2000 IU; vitamin K3 (menadione sodium bisulfite), 25 mg; vitamin B1 (thiamine hydrochloride), 30 mg; vitamin B2 (riboflavin), 30 mg; vitamin B6 (pyridoxine hydrochloride), 20 mg; vitamin B12 (cyanocobalamin), 0.1 mg; vitamin B5 (pantothenic acid), 100 mg; vitamin B3 (nicotinic acid), 200 mg; vitamin B9 (folic acid), 15 mg; vitamin H (biotin), 3 mg; betaine, 500 mg; inositol, 500 mg; choline chloride, 1000 mg; vitamin C (stay C).2Proximate composition is expressed in % of dry matter (DM), except for energy, which is expressed in kJ g-1 DM. 3Total polyphenols measured via the Folin-Ciocalteu method are expressed in mg of gallic acid equivalents (GAE) per 100 g of DM. Specific phenolic compounds measured via HPLC are expressed in mg of polyphenol per 100 g of DM.4 DPPH● ABTS●+ and ORAC, are expressed in mg of Trolox equivalents (TE) per 100 g DM.

mangiferin, distinguishing it from the other two mixtures. In terms of bound polyphenolic extracts, gallic acid was only detected in MNE in quantities consistent with the expected 2 % derived from mango peel flour (4.5 mg 100 g^{-1} DM). Additionally, the higher amounts of 4-coumaric (5.7 mg 100 g^{-1} DM) and transferulic (13.8 mg 100 g^{-1} DM) acids found in PNE corresponded to the addition of 2 % pineapple peel flour. Overall, higher levels of free and bound polyphenols were identified and quantified by Folin-Ciocalteu method in both MNE (65.8 and 172.3 mg GAE 100 g DM^{-1}, respectively) and PNE (50.2 and 206.7 mg GAE 100 g DM^{-1}, respectively) when compared to CNE (57.6 and 163.0 mg GAE 100 g DM^{-1}, respectively), although the increase was more evident on bound polyphenols.

The analysis of antioxidant capacity revealed that free phenolic extracts of both MNE (154.0 mg TE 100 g^{-1} DM) and PNE (154.1 mg TE 100 g^{-1} DM), had higher $DPPH^{\bullet}$ compared to those from CNE (107.7 mg TE 100 g^{-1} DM). In terms of free $ABTS^{\bullet +}$, PNE showed the lowest radical scavenging activity of all mixtures (154.1 vs 181.4–183.4 mg TE 100 g^{-1} DM), contrarily to bound extracts that had higher bound $ABTS^{\bullet +}$ in MNE and PNE (91.6 and 101.8 mg TE 100 g^{-1} DM, respectively) compared to CNE (66.3 mg TE 100 g^{-1} DM). Concerning ORAC, values were lowest in CNE for both free and bound phenolic extracts (1572.1 and 338.1 mg TE 100 g^{-1} DM, respectively). MNE displayed the highest superoxide anion ($O^{\bullet -}$) radical scavenging capacity in free phenolic extracts (2043.8 mg TE 100 g^{-1} DM), whereas PNE exhibited the highest capacity in bound phenolic extracts (511.2 mg TE 100 g^{-1} DM).

3.3. Phase 4 - characterization of the extruded pellets

The impact of extrusion on the antioxidant capacity of the resulting pellets, before drying, was evaluated (Table 3). Concerning free phenolic content, the sinapic acid was exclusively detected in the cold extruded pellets MC and PC. Moreover, only the MH and MC pellets, showed the presence of mangiferin. This phenolic compound was exclusively detected in the MNE mixture, but its amount decreased greatly after extrusion. Among all extruded pellets, the PC displayed the highest level of total free phenolics, having experienced a reduction of −9 % compared to PNE, while the remaining extruded pellets displayed a decrease between −20 % and −29 % in free phenolic compounds compared to their corresponding mixtures (Fig. 2). In the bound phenolic extracts, 4-coumaric acid had the highest amounts in both PH and PC pellets (4.8 and 3.4 mg 100 g^{-1} DM, respectively) (Table 3). This acid was already present in much higher amounts in PNE (5.7 mg 100 g^{-1} DM) compared to MNE and CNE mixtures where it was below the quantification limit (Table 2), but after extrusion, a lower level was detected in both PH and PC, yet mainly in the PC pellets. Moreover, despite a general decrease in bound transferulic acid content observed in all extruded pellets, compared to their respective dietary mixtures, higher levels of this polyphenol were still found in both PH and PC (8.7 and 7.7 mg 100 g^{-1} DM, respectively) (Table 3). This is consistent with the fact that PNE was the dietary mixture with higher amounts of bound transferulic acid (13.8 mg 100 g^{-1} DM) (Table 2). Finally,

Table 3
Phenolic profile and antioxidant capacity of the extruded pellets from phase 4, prior to drying.

	CH	MH	MC	PH	PC
Free phenolic content (mg 100 g^{-1} DM)[1]					
Gentisic acid	28.9 ± 11.9	14.0 ± 0.2	14.7 ± 0.8	14.3 ± 0.4	14.8 ± 0.2
Mangiferin	n.a	BQL	BQL	n.a.	n.a.
Sinapic acid	n.a	n.a	7.1 ± 1.9	n.a	6.2 ± 0.6
Isoferulic acid	BQL	BQL	BQL	BQL	BQL
Sum of identified polyphenols	28.9 ± 11.9	14.0 ± 0.2	21.5 ± 0.7	14.3 ± 0.4	22.1 ± 0.2
Total (Folin-Ciocalteu)	123.5 ± 0.7	134.5 ± 2.1	138.3 ± 8.5	147.3 ± 6.8	186.0 ± 5.7
Bound phenolic content (mg 100 g^{-1} DM)[1]					
Gallic acid	n.a	3.4 ± 0.1	2.1 ± 0.4	n.a	n.a.
Caffeic acid	BQL	BQL	BQL	BQL	BQL
Syringic acid	2.1 ± 0.4	1.1 ± 0.1	0.7 ± 0.01	1.4 ± 0.1	0.9 ± 0.1
Neochlorogenic acid	1.1 ± 1.2	BQL	BQL	BQL	BQL
4-coumaric acid	BQL	0.1 ± 0.1	BQL	4.8 ± 0.1	3.4 ± 0.2
Transferulic acid	6.2 ± 0.1	3.8 ± 0.3	3.6 ± 0.3	8.7 ± 0.7	7.7 ± 0.9
Sinapic acid	21.2 ± 1.0	27.9 ± 1.7	7.6 ± 0.6	28.8 ± 0.6	9.2 ± 0.04
Sum of identified polyphenols	29.5 ± 0.3	33.0 ± 1.1	14.3 ± 1.5	43.6 ± 1.6	21.1 ± 1.2
Total (Folin-Ciocalteu)	25.5 ± 6.2	30.7± 4.7	32.2 ± 0.2	30.4 ± 1.1	40.5 ± 6.5
$DPPH^{\bullet}$ (mg TE 100 g^{-1} DM)[2]					
Free phenolic extracts	138.9 ± 9.6	62.6 ± 12.3	81.5 ± 23.1	78.1 ± 18.9	62.4 ± 10.7
Bound phenolic extracts	27.1 ± 3.6	29.7 ± 0.1	25.1 ± 3.9	24.1 ± 0.004	21.5 ± 3.8
$ABTS^{\bullet +}$ (mg TE 100 g^{-1} DM)[2]					
Free phenolic extracts	146.9 ± 24.9	146.8 ± 14.9	83.3 ± 9.3	155.8 ± 14.9	166.9 ± 15.1
Bound phenolic extracts	78.4 ± 10.9	76.6 ± 18.1	58.0 ± 4.9	40.7 ± 0.8	65.9 ± 9.7
ORAC (mg TE 100 g^{-1} DM)[2]					
Free phenolic extracts	1450.8 ± 585.4	1286.9 ± 8.9	1056.4 ± 41.3	3093.9 ± 313.4	1498.5 ± 77.7
Bound phenolic extracts	810.3 ± 138.9	409.4 ± 75.1	709.2 ± 91.8	627.2 ± 41.1	363.6 ± 75.8

Values are presented as mean ± standard deviation (n=3). “n.a” stands for “non-applicable”, meaning that the chemical compound in question is inexistent in that sample. “BQL” stands for “below quantification limit”, meaning that the chemical compound in question was identified, but was not reported due to being present below the minimal quantities for quantification. 1Total phenolic content was measured via the Folin-Ciocalteu method and is expressed in mg of gallic acid equivalents (GAE) per 100 g of DM. Specific phenolic compounds measured via HPLC are expressed in mg of polyphenol per 100 g of DM. 2DPPH•, ABTS•+ and ORAC, are expressed in mg of Trolox equivalents (TE) per 100 g of DM.

Table 4
Proximate composition, phenolic profile and antioxidant capacity of all experimental diets from phase 5 (pellets after extrusion and drying processes).

	Control diets			Mango diets			Pineapple diets			
	CH-60	**CH-35**	**MH-60**	**MH-35**	**MC-60**	**MC-35**	**PH-60**	**PH-35**	**PC-60**	**PC-35**
Proximate composition (% of DM or kJ g^{-1} DM)[1]										
Dry matter	95.4 ± 0.1	93.8 ± 0.1	94.4 ± 0.1	94.3 ± 0.1	98.8 ± 0.1	92.3 ± 0.1	94.5 ± 0.1	93.1 ± 0.2	97.8 ± 0.04	92.2 ± 0.03
Ash	5.3 ± 0.03	5.5 ± 0.1	5.3 ± 0.4	5.3 ± 0.3	5.6 ± 0.03	5.5 ± 0.1	5.5 ± 0.04	5.6 ± 0.2	5.6 ± 0.1	5.7 ± 0.02
Protein	48.9 ± 0.8	48.5 ± 0.6	45.4 ± 0.2	47.5 ± 0.7	48.1 ± 0.04	48.5 ± 0.3	48.0 ± 0.7	49.8 ± 0.01	48.6 ± 0.2	49.0 ± 0.03
Energy	22.4 ± 0.05	22.3 ± 0.02	23.2 ± 0.2	23.7 ± 0.1	23.8 ± 0.7	24.0 ± 0.2	23.4 ± 0.05	23.8 ± 0.4	23.5 ± 0.1	23.6 ± 0.1
Lipids	16.0 ± 0.6	16.3 ± 0.1	18.3 ± 0.04	18.6 ± 0.7	18.2 ± 0.1	18.3 ± 0.2	16.8 ± 0.4	15.9 ± 0.004	18.0 ± 0.6	17.6 ± 0.2
Carbohydrates	29.8 ± 1.3	29.7 ± 0.4	31.0 ± 0.7	28.7 ± 0.3	28.1 ± 0.04	27.7 ± 0.3	29.7 ± 1.0	28.7 ± 0.15	27.8 ± 0.9	27.8 ± 0.1
Crude fibre	3.6 ± 0.2	3.2 ± 0.2	3.7 ± 0.2	3.7 ± 0.2	4.5 ± 0.02	4.0 ± 0.2	3.9 ± 0.1	3.1 ± 0.1	4.7 ± 0.1	3.8 ± 0.001
Starch	14.7 ± 0.5	14.8 ± 0.6	12.7 ± 0.1	12.7 ± 0.4	12.6 ± 0.4	13.1 ± 0.4	12.9 ± 0.4	13.6 ± 0.4	13.1 ± 0.3	13.5 ± 0.4
Free phenolic extracts (mg 100 g^{-1} DM)[2]										
Gentisic Acid	23.7 ± 2.7	18.4 ± 0.7	26.5 ± 5.7	20.7 ± 1.3	40.2 ± 1.2	36.5 ± 1.9	40.3 ± 1.6	29.1 ± 0.1	21.0 ± 0.2	38.0 ± 1.0
Mangiferin	n.a	n.a	n.a	BQL	n.a	BQL	n.a	n.a	n.a	n.a
Sinapic acid	n.a	n.a	4.1 ± 0.1	2.0 ± 0.7	7.3 ± 0.6	2.2 ± 0.1	4.5 ± 0.1	1.8 ± 0.01	1.1 ± 0.05	5.7 ± 1.5
Isoferulic acid	BQL	BQL	BQL	BQL	0.4 ± 0.1	BQL	0.5 ± 0.1	BQL	BQL	0.2 ± 0.02
Sum of identified polyphenols	23.7 ± 2.7	18.4 ± 0.7	30.0 ± 6.7	22.7 ± 0.5	47.5 ± 0.5	39.2 ± 1.7	45.8 ± 0.8	30.8 ± 0.05	22.1 ± 0.2	43.7 ± 1.6
Bound phenolic extracts (mg 100 g^{-1} DM)[2]										
Caffeic acid	BQL	BQL	n.a.	n.a.	BQL	BQL	BQL	0.1 ± 0.0002	BQL	BQL
Gallic acid	n.a	n.a	3.9 ± 0.5	2.1 ± 0.9	4.2 ± 0.5	3.2 ± 0.2	n.a.	n.a.	n.a.	n.a.
Syringic acid	2.1 ± 0.1	2.0 ± 0.1	3.7 ± 0.3	1.3 ± 0.7	1.7 ± 0.04	1.5 ± 0.1	3.5 ± 0.6	4.1 ± 0.5	2.0 ± 0.1	1.5 ± 0.1
Neochlorogenic acid	1.8 ± 0.1	2.3 ± 0.1	4.1 ± 0.8	n.a	1.0 ± 0.1	0.9 ± 0.2	2.5 ± 0.4	2.6 ± 0.5	1.1 ± 0.4	0.6 ± 0.1
4-coumaric acid	BQL	0.1 ± 0.01	2.8 ± 0.2	BQL	BQL	BQL	11.8 ± 1.2	12.9 ± 0.3	4.1 ± 0.005	3.4 ± 0.4
Transferulic acid	4.7 ± 0.5	5.1 ± 0.4	13.5 ± 0.6	3.6 ± 1.2	4.4 ± 0.1	4.3 ± 0.3	25.0 ± 3.1	24.1 ± 0.4	10.5 ± 0.01	9.0 ± 1.1
Sinapic acid	28.5 ± 0.4	31.0 ± 1.2	57.0 ± 9.6	19.7 ± 9.8	20.0 ± 0.2	19.6 ± 1.1	55.5 ± 2.7	65.6 ± 6.4	19.6 ± 1.3	19.2 ± 1.0
Sum of identified polyphenols	37.1 ± 1.2	40.3 ± 1.3	97.0 ± 3.3	26.8 ± 12.6	31.5 ± 0.5	29.6 ± 1.9	98.4 ± 5.0	109.3 ± 8.0	37.4 ± 1.8	32.4 ± 2.7

Values are presented as mean ± standard deviation (n=2) for proximate composition, and mean ± standard deviation (n=3) for remaining variables. “n.a” stands for “non-applicable”, meaning that the chemical compound in question is inexistent in that sample. “BQL” stands for “below quantification limit”, meaning that the chemical compound in question was identified, but was not reported due to being present below the minimal quantities for quantification. The feed mix with 2 % of mango peel flour inclusion was subjected to different extrusion and drying temperatures, thus originating four experimental diets: i) MH-60 (110 °C extrusion; 60 °C drying - conventional manufacturing temperatures) ii) MH-35 (110 °C; 35 °C) iii) MC-60 (25 °C; 60 °C) and iv) MC-35 (25 °C; 35 °C). 1Proximate composition, fibre and starch are expressed in % of dry matter (DM), with the exception of energy, which is expressed in kJ g-1 DM. Carbohydrates are calculated by difference as % of DM by adding values from protein, ash and lipids and subtracting that value from DM. Specific phenolic compounds measured via HPLC are expressed in mg of polyphenol per 100 g of DM.

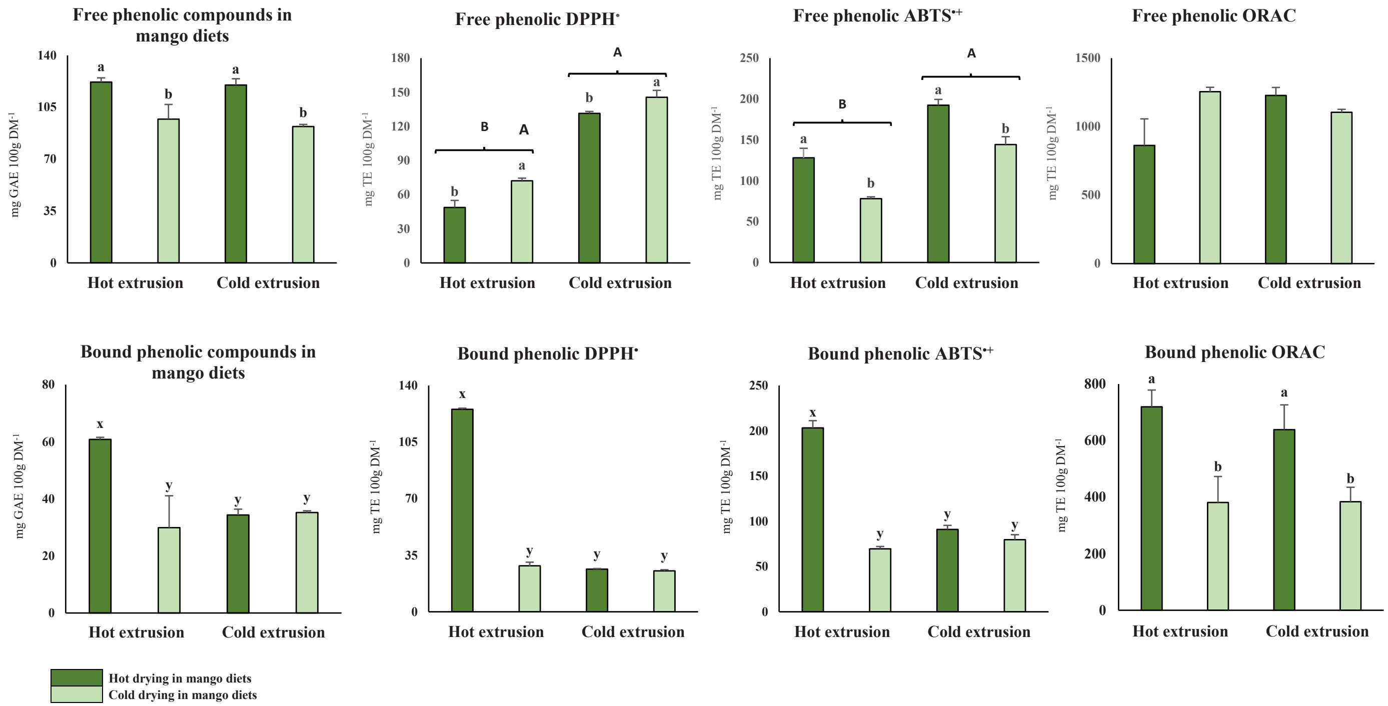


Fig. 3. Histograms concern phenolic compound quantification and antioxidant capacity of dried pellets from diets with 2 % mango inclusion. The mixture with 2 % of mango peel flour inclusion was subjected to different extrusion and drying temperatures, thus originating four experimental extruded diets: i) MH-60 (110 °C extrusion; 60 °C drying - conventional manufacturing temperatures) ii) MH-35 (110 °C; 35 °C) iii) MC-60 (25 °C; 60 °C) and iv) MC-35 (25 °C; 35 °C). Values are presented as mean ± standard deviation (n=3). A 2-way ANOVA was performed. Different superscript uppercase letters (^{A,B}) indicate significant differences between different extrusions while different superscript lowercase letters indicate significant differences between different drying temperatures(^{a,b}) or in the intersection between both variables “Extrusion” and “Drying” (^{x,y}) ($P < 0.05$).

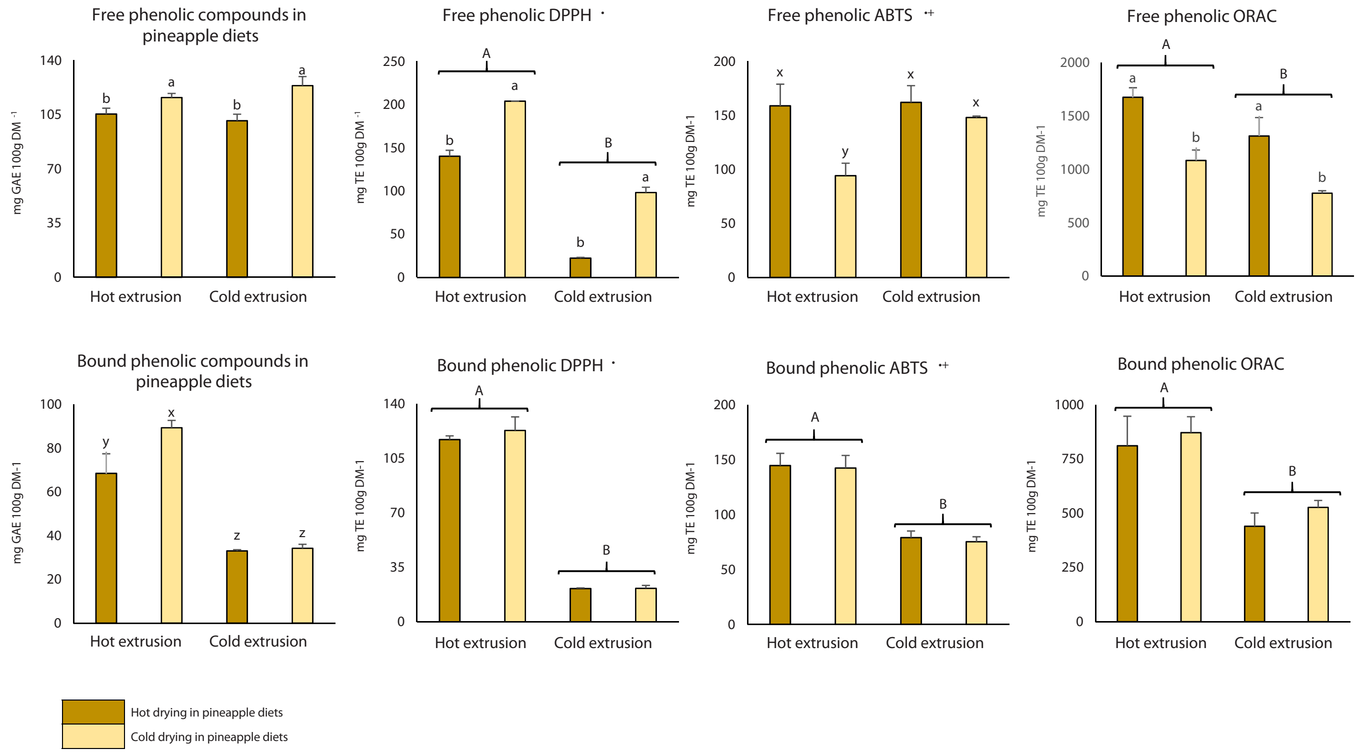


Fig. 4. Histograms concern phenolic compound quantification and antioxidant capacity of dried pellets from diets with 2 % pineapple peel flour inclusion. The mixture with 2 % of pineapple peel flour inclusion was subjected to different extrusion and drying temperatures, thus originating four experimental extruded diets: i) PH-60 (110 °C extrusion; 60 °C drying - conventional manufacturing temperatures) ii) PH-35 (110 °C; 35 °C) iii) PC-60 (25 °C; 60 °C) and iv) PC-35 (25 °C; 35 °C). Values are presented as mean ± standard deviation (n=3). A 2-way ANOVA was performed. Different superscript uppercase letters (^{A,B}) indicate significant differences between different extrusions while different superscript lowercase letters indicate significant differences between different drying temperatures(^{a,b}) or in the intersection between both variables "Extrusion" and "Drying" (^{x,y}) ($P < 0.05$).

bound sinapic acid was found in higher quantities in hot extruded pellets compared to those extruded at a lower temperature, regardless of the diet. Concerning bound phenolics, quantified by the Folin-Ciocalteu method, PC displayed higher amounts than all other extruded pellets (40.5 mg GAE 100 g^{-1} DM), even showing a 15 % increase in these compounds compared to its initial dietary mixture (Fig. 2). Comparatively, mango extruded pellets, MH and MC, lost −13 % and −9 % of bound phenolics, respectively, when compared to the MNE mix.

In terms of the antioxidant capacity of free phenolic extracts, $DPPH^{\bullet}$ values were lower in all extruded pellets (62.4–81.5 mg TE 100 g^{-1} DM) when compared to the CH (138.9 mg TE 100 g^{-1} DM) (Table 3). Mango and pineapple extruded pellets exhibited a −60 % and −31 % reduction in their $DPPH^{\bullet}$ values compared to their corresponding mixtures, MNE and PNE, whilst $DPPH^{\bullet}$ values increased by 29 % in CH pellets compared to the CNE mixture (Fig. 2). In bond phenolic extracts, $DPPH^{\bullet}$ is quite similar among extruded pellets (21.5–29.7 mg TE 100 g^{-1} DM) (Table 3). $ABTS^{\bullet+}$ radical scavenging capacity of free extracts lowered by −20 % in the CH pellets compared to CNE. The very same trend was observed in both mango (MH and MC) extruded pellets, with an $ABTS^{\bullet+}$ reduction of −19 and −54 % respectively, in relation to MNE. Contrarily, pineapple (PH and PC) free extracts kept their $ABTS^{\bullet+}$ values after extrusion (Fig. 2). In the bound extracts, the CH increased $ABTS^{\bullet+}$ scavenging capacity by 31 % in relation to the respective mixture, while all remaining extruded pellets showed a decrease, particularly pronounced in MC, PH and PC pellets (reduction from −16 % to −60 %). Finally, the free extracts of the PH pellets showed much higher ORAC values (3093.9 mg TE 100 g^{-1} DM) than the remaining extruded pellets (1056.4–1498.5 mg TE 100 g^{-1} DM), being the only pellets that increased ORAC values after extrusion (Table 2). Moreover, hot extrusion resulted in higher ORAC values for each fruit (Fig. 2). ORAC in bound phenolic extracts generally increased after extrusion, with the exception of PC.

3.4. *Phase 5 – characterization of the dried extruded pellets*

The final chemical composition of dried extruded pellets was evaluated for each diet (Table 4). The extrusion and drying processes did not alter the macronutrient composition of all diets.

In free phenolic extracts, the highest amounts of gentisic acid were found in diets MC-60, MC-35, PH-60, and PC-35 (40.2, 36.5, 40.3, and 38.0 mg 100 g^{-1} DM, respectively) (Table 4). Moreover, sinapic acid was only found in the free phenolic extracts of diets with fruit flour inclusion. Additionally, mangiferin was only identified in mango diets with cold drying, namely MH-35 e MC-35, albeit in trace amounts below the quantification limit, while disappearing completely in mango diets after hot extrusion. The drying stage had a significant impact on total free phenolic compounds: hot drying led to higher levels in mango diets MH-60 and MC-60 compared to cold drying MH-35 and MC-35, but the same trend was not observed in both the controls and the pineapple diets (Fig. 4). Moreover, pineapple diets with cold drying PH-35 and PC-35 showed the highest total free phenolic compounds (Fig. 3). Overall, the drying process resulted in a −59 % and −41 % free phenolic compound decrease, in CH-60 and CH-35, respectively, when compared to the CNE mixture. Mango diets showed a decrease between −29 % and −30 %, and between −44 % and −47 % in free phenolic compounds, after hot and cold drying, respectively, when compared to the MNE mixture (Fig. 2). In the pineapple diets, drying led to a −4 % decrease in free phenolic compounds for the PC-60 diet, while all other diets exhibited reductions of between −40 % and −49 % compared to the PNE mixtures (Fig. 2).

In extracts made from the antioxidant fibre, gallic acid was only found in diets with mango peel flour inclusion (Table 4), which is consistent with the high amounts of gallic acid found in mango peel flour (Table 1). With the sole exception of MH-35, all mango diets demonstrated a rise in bound gallic acid concentrations following the drying process in comparison to their respective extruded mixtures (Table 4). Fibre-bound 4-coumaric and transferulic acids were found in much higher quantities in pineapple diets compared to mango diets, with the highest values observed in diets that underwent hot extrusion (PH-60 and PH-35), regardless of the drying temperature (Table 4). Total bound phenolic compounds were significantly affected by both the extrusion process and drying temperature, with a significant interaction observed between these two factors (Fig. 3). The PH-35 diet exhibited the highest final amounts of bound polyphenols, followed by PH-60 and MH-60 (Table 4). These diets represent an increase of 152 %, 93 %, and 72 %, respectively, in comparison to their corresponding initial mixtures (Fig. 2).

Fig. 3 presents a 2-way ANOVA analysis comparing phenolic compounds and antioxidant capacity in mango and pineapple diets. Although the “Extrusion” process did not seem to have a significant impact on free phenolic extracts of the different diets, the antioxidant capacity varied significantly among diets. In mango diets, analysis of free phenolic extracts showed that cold extrusion (MC-60 and MC-35) induced increased levels $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical scavenging capacity when compared to mango diets with hot extrusion (MH-60 and MH-35). In pineapple diets, hot extrusion induced higher $DPPH^{\bullet}$ radical scavenging capacity and ORAC in both free and bound phenolic extracts, as well as higher $ABTS^{\bullet+}$ radical scavenging capacity in bound phenolic extracts (Fig. 3). Moreover, when compared to the PNE mixture, diet PH-35 increased free phenolic $DPPH^{\bullet}$ radical scavenging capacity by 29 % when compared to the PNE mixture. Additionally, compared to PNE, PH-60 and PH-35 increased bound phenolic $DPPH^{\bullet}$ radical scavenging capacity by 357 % and 380 %, while also increasing bound phenolic $ABTS^{\bullet+}$ radical scavenging capacity by 43 % and 41 %, and bound phenolic ORAC by 138 % and 70 %, respectively (Fig. 2).

Significant differences associated with the drying stage were observed among the various experimental diets. Results from mango diets show that higher drying temperatures increase total free phenolic compounds, free phenolic $ABTS^{\bullet+}$ radical scavenging capacity, and bound phenolic ORAC when compared to lower drying temperatures (Fig. 3). Indeed, hot drying increased bound ORAC by 94.4 and 72 %, respectively, when compared to the MNE mixture (Fig. 2). However, in pineapple diets, data shows that lower drying temperatures lead to higher total free phenolic values (Fig. 3). This was accompanied by higher $DPPH^{\bullet}$ radical scavenging capacity in free phenolic extracts when compared to pineapple diets dried with hotter temperatures (Fig. 3). Specifically, diet PH-35 was able to increase free phenolic $DPPH^{\bullet}$ radical scavenging capacity by 29 % when compared to the PNE mixture (Fig. 2).

Table 5

Comparison of antioxidant capacity between all diets from phase 5 (pellets after extrusion and drying processes).

	Control diets			Mango diets			Pineapple diets				*p-value*
	CH−60	CH−35	MH−60	MH−35	MC−60	MC−35	PH−60	PH−35	PC−60	PC−35	
DPPH$^{\bullet+}$ (mg TE 100 g^{-1} DM)^{1}											
Free phenolic extracts	73.9 ± 2.1^{d}	28.2 ± 2.0^{e}	48.8 ± 6.2^{de}	72.2 ± 2.4^{d}	131.4 ± 1.7^{bc}	145.6 ± 6.1^{b}	140.1 ± 6.9^{b}	203.8 ± 0.2^{a}	62.4 ± 10.7^{e}	98.1 ± 6.1^{c}	**<0.001**
Bound phenolic extracts	23.1 ± 0.2^{b}	27.9 ± 0.4^{b}	125.3 ± 0.7^{a}	28.4 ± 2.3^{b}	26.4 ± 0.4^{b}	25.3 ± 0.7^{b}	117.1 ± 2.4^{a}	123.0 ± 8.7^{a}	21.4 ± 0.4^{b}	21.5 ± 1.9^{b}	**<0.001**
ABTS$^{\bullet+}$ (mg TE 100 g^{-1} DM)^{1}											
Free phenolic extracts	83.3 ± 6.3^{d}	69.9 ± 5.5^{d}	128.0 ± 11.7^{bc}	78.1 ± 2.2^{d}	192.4 ± 7.1^{a}	144.3 ± 9.6^{b}	158.7 ± 20.1^{b}	$94.1 \pm 11.6\ ^{cd}$	161.9 ± 15.5^{b}	147.9 ± 1.3^{b}	**<0.001**
Bound phenolic extracts	81.0 ± 8.8^{d}	75.8 ± 1.4^{d}	203.2 ± 8.0^{a}	69.5 ± 2.6^{d}	$91.0 \pm 4.5\ ^{cd}$	79.7 ± 5.5^{d}	144.6 ± 11.1^{b}	142.3 ± 11.5^{bc}	79.0 ± 6.0^{d}	75.2 ± 4.6^{d}	**<0.001**
ORAC (mg TE 100 g^{-1} DM)^{1}											
Free phenolic extracts	694.8 ± 274.4^{c}	1036.6 ± 87.0^{bc}	862.3 ± 194.2^{abc}	1255.1 ± 31.9^{ab}	1227.3 ± 58.7^{abc}	1104.7 ± 21.6^{abc}	1674.3 ± 89.3^{a}	1082.9 ± 97.8^{bc}	1312.0 ± 172.8^{ab}	776.1 ± 22.9^{bc}	**<0.001**
Bound phenolic extracts	422.8 ± 26.0^{de}	428.7 ± 13.0^{de}	719.2 ± 59.4^{bc}	381.1 ± 91.8^{e}	638.7 ± 87.5^{bcd}	383.7 ± 51.2^{e}	1216.2 ± 136.1^{a}	$871.1 \pm 187.5a^{b}$	439.5 ± 61.3^{de}	526.6 ± 31.9^{cde}	**<0.001**

Values are presented as mean ± standard deviation (n=3). A 1-way ANOVA was performed. Different superscript uppercase letters (a,b,c) indicate significant differences ($P < 0.05$). A control mix (CNE) was subjected to a conventional hot extrusion process (110 °C), after which it was subjected to two distinct drying temperatures (60 and °°C), generating control diets CH-60 and CH°, respectively. The feed mix with 2 % of mango peel flour inclusion was subjected to different extrusion and drying temperatures, thus originating four experimental diets: i) MH-60 (110 °C extrusion; 60 °C drying - conventional manufacturing temperatures) ii) MH-35 (110 °C; 35 °C) iii) MC-60 (25 °C; 60 °C) and iv) MC-35 (25 °C; 35 °C). 1DPPH•, ABTS•+ and ORAC are expressed in mg of Trolox equivalents (TE) per 100 g DM.

Some interaction effects between variables “Extrusion” and “Drying” were observed (Fig. 3). In mango diets, the interaction between hot extrusion and hot drying (MH-60) strongly benefited both bound phenolic content and antioxidant capacity. Namely, MH-60 showed much higher amounts of bound phenolic content, $DPPH^{\bullet}$ and $ABTS^{\bullet +}$ radical scavenging capacity compared to remaining mango diets, representing an increase of 72 %, 355 %, and 122 % compared to MNE (Fig. 2). However, in pineapple diets, the highest levels of total bound phenolic content were observed in the PH35 diet, as a result of the interaction between hot extrusion and cold drying, followed by the PH-60 diet, due to the interaction between hot extrusion and hot drying. Respectively, these diets represent an increase of 152 % and 93 % compared to the PNE mixture (Fig. 2). Finally, regarding $ABTS^{\bullet +}$ of free phenolic extracts, Fig. 3 shows that radical scavenging capacity is decreased by the combined effect of hot extrusion and cold drying (PH-35), which in turn represents a decrease of −39 % compared to the PH extrusion (Fig. 2).

Table 5 shows a 1-way ANOVA comparison between all diets. $DPPH^{\bullet}$ radical scavenging capacity of free extracts is significantly higher in the PH-35 diet, followed by the MC-35, PH-60, and MC-60 diets (203.8, 145.6, 140.1, and 131.4 mg TE 100 g^{-1} DM, respectively) compared to all other diets. In terms of bound phenolic extracts, the PH-60, PH-35 and MH-60 diets presented the highest $DPPH^{\bullet}$ radical scavenging capacity values (117.1, 123.0 and 125.3 mg TE 100 g^{-1} DM, respectively). $ABTS^{\bullet +}$ radical scavenging capacity of free phenolic extracts was significantly higher in the MC-60 diet (192.4 mg TE 100 g^{-1} DM) than in diets PC-60, PH-60, PC-35, MC-35, and MH-60 (161.9, 158.7, 147.9, 144.3 and 128.0 mg TE 100 g^{-1} DM, respectively). In bound phenolic extracts, MH-60 had significantly higher values (203.2 mg TE 100 g^{-1} DM) than PH-60 and PH-35 (144.6 and 142.3 mg TE 100 g^{-1} DM, respectively). Finally, the highest values of ORAC in both free and bound phenolic extracts belong to the PH-60 diet (1674.3 and 1216.2 mg TE 100 g^{-1} DM, respectively). Particularly, bound phenolic extracts in PH-60 were significantly higher than in all remaining diets.

Fig. 5 shows a PCA plot of similarities and dissimilarities between diets, explaining 70 % of the data variation. Diets were considerably dispersed, with stronger similarities between the analysed parameters and diets PH-35, PH-60, and MH-60. These three diets were more similar on the F1 axis, which explains 52 % of the variation. Among these three diets, PH-60 was the diet with the highest distance from the control CH-60 on the F2 axis, which explains 18 % of the variation. Pineapple diets with hot extrusion, as well as the mango diet with hot drying, are more closely associated with higher antioxidant capacity in both free and

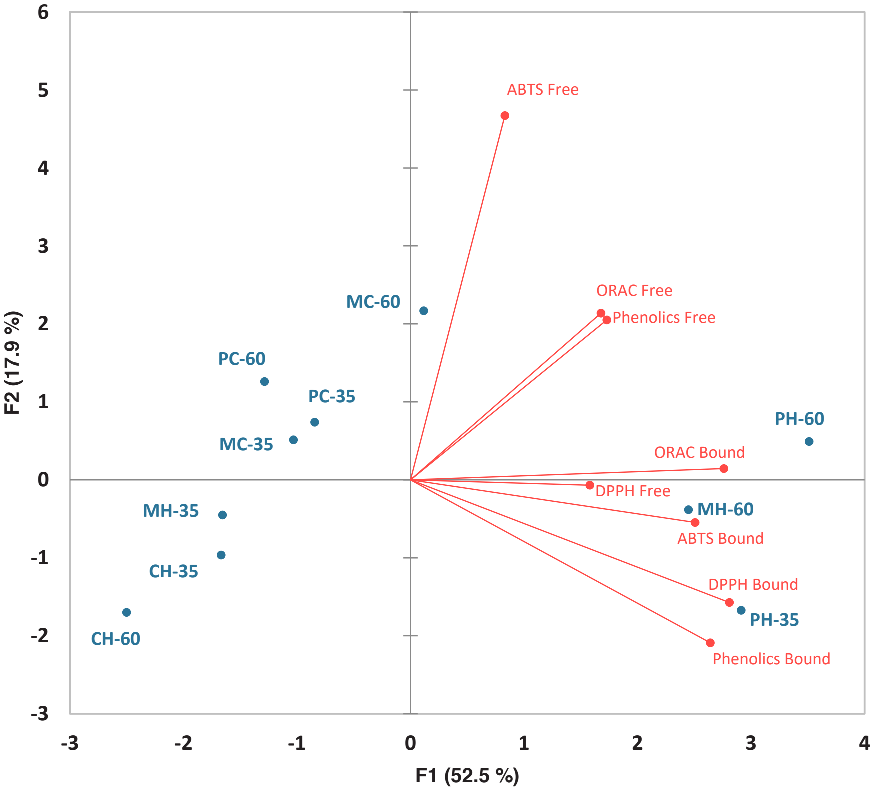


Fig. 5. Principal component analysis applied to phenolic content quantification and antioxidant capacity in dried pellets from control, mango and pineapple diets, at a significance level of 5. Points ascribed to diets (CH-60, CH-35, MH-60, MH-35, MC-60, MC-35, PH-60, PH-35, PC-60, PC-35) were calculated using the mean between observations (n=3). “DPPH”, “ABTS” and “ORAC” stand for the three radical scavenging assays performed in this study. The “Free” and “Bound” suffixes stand for the type of extract where the analysis was performed. “Phenolics Free/Bound” stands for phenolic compounds measured according to the Folin-Ciocalteu method.

bound extracts. While MH-60 and PH-35 were more associated with bound $DPPH^{\bullet}$ and $ABTS^{\bullet +}$, PH-60 and MH-60 were more closely associated with bound ORAC and free $DPPH^{\bullet}$.

4. Discussion

The primary goal of this research is to assess how varying extrusion and drying temperatures influence the radical scavenging capability of aquafeeds enriched with natural antioxidants (*i.e.*, vitamins, carotenoids, and polyphenols) sourced from mango and pineapple peels.

Extensive research has demonstrated the antioxidant and immunostimulant properties of certain vitamins, namely vitamins E (tocopherols) and A (retinol) (Udo and Afia, 2013). Consequently, synthetic versions of these vitamins are now widely used as antioxidants in aquafeeds (Morin et al., 2021; NRC, 2011). The present results show that both mango and pineapple peels are natural sources of these vitamins. However, pineapple peels lost much more tocopherols and retinol than mango in the conversion process from peel to flower when compared to mango peels. Ultimately, none of the peel flours used in this study can be considered good sources of these vitamins for aquafeeds, as quantification values were substantially below minimal requirements for cultured fish – namely 10 000 $\mu g\ 100\ g^{-1}$ and 600 $\mu g\ 100\ g^{-1}$, respectively (NRC, 2011).

The antioxidant properties of carotenoids stem from their highly unsaturated structure, making them prone to oxidation. This allows them to neutralise singlet oxygen ($O^{\bullet -}$) ROS and capture free radicals (Aklakur, 2018). However, since fish are not able to synthesize carotenoids, their bioavailability within the organism is dependent on the diet (Aklakur, 2018). Carotenoid inclusion in aquafeeds has shown benefits to the antioxidant and immune systems of cultured fish (Aklakur, 2018; Faehnrich et al., 2016; García-Chavarría and Lara-Flores, 2013; Nakano and Wiegertjes, 2020). However, these beneficial effects are heavily dependent on the carotenoid source, dose, and mode of administration (Carvalho and Caramujo, 2017; Nakano and Wiegertjes, 2020). Although the inclusion levels of carotenoids in aquafeeds as antioxidant sources are not standardized, previous studies have used levels far exceeding those provided by the peels used in this study (Bacchetta et al., 2019; Goda et al., 2018; Saleh et al., 2018; Sallam et al., 2017). Moreover, the present studýs findings demonstrate that converting fruit peels into flour results in losses of over 70 % of total carotenoids, leading to a negligible increase in carotenoid quantities in the feed mixture at a 2 % peel flour inclusion rate. This sensitivity of carotenoids to thermal processing is well-documented (Meléndez-Martínez et al., 2022). Indeed, their highly unsaturated structure makes carotenoids particularly susceptible to degradation when exposed to high temperatures, light, mechanical stress or other pro-oxidant conditions common in feed manufacturing (Achir et al., 2010). Thus, the already low levels of carotenoids in these flours are further reduced by the significant negative impact of common physicochemical conditions during feed manufacturing.

More than 8000 phenolic compounds have been identified from plant sources, most of which exhibit strong antioxidant capacity, *i.e.*, the ability to scavenge free radicals and transform them into more stable molecules via the donation of a hydrogen atom from the phenolic O-H group (Ahmadifar et al., 2021; Brglez Mojzer et al., 2016; Kondo et al., 1999; Williams et al., 2004). Consequentially, natural phenolic compounds can be used as additives in aquafeeds to preserve their quality and prevent rancidity during storage. Additionally, natural phenolic compounds in aquafeeds may also confer supplemental antioxidant protection that might increase the antioxidant defences of farmed fish, as previously reported (Ahmadifar et al., 2021; Maqsood et al., 2013). However, the antioxidant properties of phenolic compounds are highly influenced by their chemical structure and their interactions with other macromolecules, including fibre, protein, lipids, and carbohydrates. Thus, the antioxidant capacity of by-product matrices, such as fruit peels, is determined as much by their phenolic compound profile as by their total phenolic content (Ahmadifar et al., 2021). Overall, the Folin-Ciocalteu method revealed heavy losses of free phenolic compounds in both mango and pineapple peels during their conversion process into flour, with reductions of approximately 50 % and 18 %, respectively. The degradation of these polyphenols due to thermal stress is one of the main mechanisms proposed to explain the yield dynamics of phenolic compounds in plant-based matrices exposed to high temperatures (Antony and Farid, 2022). Alternatively, thermal degradation caused by high temperatures might also release phenolic compounds from lignin, by either breaking the chemical bonds between them or by degrading lignin itself, thus increasing the final amounts of phenolic compounds (Antony and Farid, 2022). The flour manufacturing process used in this study involved drying fruit peels at 60 °C until moisture dropped below 10 %, as higher temperatures are usually considered detrimental to phenolic compounds (Campos et al., 2020b). This highlights the possibility that thermal degradation alone cannot fully explain the behaviour of phenolic compounds observed in this study, as previous studies have shown that thermal degradation of these biomolecules occurs primarily at temperatures above 80 °C (Antony and Farid, 2022). Air exposure during the drying procedure could have been one of the main causes, as oxygen induces the degradation of these biomolecules through oxidation (Tanaka et al., 2009). Moreover, the oxidation of phenolic compounds is accompanied by the reduction of an oxygen molecule, generating the superoxide anion and hydrogen peroxide (Tanaka et al., 2009). These potent ROS further promote the oxidation of macronutrients and phenolic compounds in food matrices such as fruit peels, as well as in the aquafeeds themselves (Colombo et al., 2020; Kurutas, 2015). For example, anthocyanins are known to be present in pineapple and mango peels, albeit in low amounts (Luan et al., 2023; Ranganath et al., 2018). Padayachee et al. (2012) proposed an alternative mechanism for the dynamics of anthocyanins, reporting that these phenolic compounds can significantly bind with primary components of plant cell walls, i.e. cellulose and pectin. This binding occurs through both ionic interactions with pectin and hydrophobic interactions with cellulose, leading to changes in the amounts of free and bound polyphenols, as well as their bioaccessibility (Padayachee et al., 2012).

In this study, the free phenolic extracts of both flours were found to be rich in gallic acid (3,4,5-trihydroxybenzoic acid), a potent antioxidant and free radical scavenger that can protect biological cells, tissues, and organs from damage caused by oxidative stress (Badhani et al., 2015; Gao et al., 2019). They also revealed the presence of rutin (quercetin-3-O-rutinoside), whose antioxidant and anti-inflammatory effects have been extensively studied (Afanas'eva et al., 2001). Mango flour was also found to contain mangiferin, a glucosylxanthone with specific antioxidant properties related to mango products (Mei et al., 2021). Additionally, pineapple flour showed high levels of gentisic and ferulic acids, alongside isoferulic acid. These phenolic compounds are proven to have potent anti-inflammatory, antirheumatic, and antioxidant properties, mainly exerted via their phenoxyl group (Amić et al., 2020; Joshi et al., 2012; Mei et al., 2021; Wang et al., 2011; Zduńska et al., 2018). However, further *in vivo* studies with the present diets will be required to fully demonstrate this potential on fish. Both flours showed distinct profiles of bound polyphenol. Mango flour contained the highest concentration of gallic acid among all the antioxidant polyphenols, followed by epicatechin gallate, which also has proven antioxidant properties (He et al., 2018), and mangiferin. The increase in gallic acid levels in mango flour compared to its levels in the peel might be related to the presence of bound epicatechin gallate in mango peel. Specifically, thermal degradation of the catechin dimer has been suggested as the mechanism responsible for generating gallic acid from catechin derivatives during the drying process (Lee et al. (2010). Meanwhile, pineapple flour revealed high amounts of bound transferulic acid, followed by 4-coumaric acid, both of which exhibit potent radical-scavenging activity (Rezaeiroshan et al., 2022; Shen et al., 2019). Despite both fruit flours having a similar proximate composition, pineapple peel flour contains more neutral detergent fibre, potentially explaining the higher levels of fibre-bound phenolic compounds found in pineapple flour compared to mango flour (Jakobek and Matić, 2019). This increase in fibre-bound polyphenols in both flours, relative to their respective peels, may be attributed to a bioencapsulation effect, where phenolic compounds bind with lignin, pectin, and cellulose. This effect might have facilitated the extraction of bound polyphenols following the thermal stress induced during the peel-to-flour phase (Antony and Farid, 2022). Despite the vulnerability of these phenolic compounds to high extrusion and drying temperatures, several studies have demonstrated that heat treatments can enhance the antioxidant content and capacity of fruit peels, such as citrus peel (Xu et al., 2007). Furthermore, the increased bioavailability of phenolic compounds, facilitated by the weakening of chemical bounds between fibre and phenolic content in the fish digestive tract, could potentially benefit fish, as previously reported in humans (Campos et al., 2020a). Indeed, in humans, Jakobek and Matić (2019) showed that dietary fibres might function as a regulatory mechanism for modulating phenolic bioaccessibility in different parts of the digestive tract, potentially increasing the amounts that reach the lower intestinal sections, where phenolic compounds, once released, can exert beneficial effects (Barba et al., 2020; Călinoiu and Vodnar, 2019; Juániz et al., 2015).

Compared to the peels, DPPH$^\bullet$ increased in the bound phenolic extracts of pineapple flour. Moreover, both the bound phenolic extracts of mango and the free phenolic extracts of pineapple flour increased ABTS$^{\bullet+}$ activity. However, there was an overall decrease in antioxidant capacity, measured by DPPH$^\bullet$, ABTS$^{\bullet+}$, and ORAC radical scavenging capacity assays, after converting both peels into flour, indicating the need for optimization efforts to maximize this parameter. Methods for measuring the ability of compounds to act as free radical scavengers or hydrogen donors, widely used in food science, play a crucial role in assessing antioxidant content and efficacy for preservation and/or protection against oxidative damage (Kedare and Singh, 2011). All three methods used in this study to assess antioxidant capacity - ABTS$^{\bullet+}$, DPPH$^\bullet$, and ORAC - can be used in aqueous and nonpolar organic solvents, enabling examination of both hydrophilic and lipophilic antioxidants. However, ABTS$^{\bullet+}$ diluted in water, as employed here, has an affinity for both hydrophilic and lipophobic compounds, including hydrogen atom donors, while DPPH$^\bullet$ shows a higher affinity for lipophilic compounds (Floegel et al., 2011). The prevalence of hydrophilic phenolic acids across all samples throughout the manufacturing chain accounts for the generally higher ABTS$^{\bullet+}$ values compared to DPPH$^\bullet$ in most samples. Moreover, while the DPPH$^\bullet$ and ABTS$^{\bullet+}$ assays mimic free radicals, the ORAC assay uses a biological radical ($O_2^{\bullet -}$), naturally formed within cells, making it more relevant to measure radical scavenging capacity in *in vitro* experiments (Ratnavathi and Komala, 2016). Therefore, the ORAC assay provides a more realistic assessment of biological models. Nonetheless, all three assays are essential for a comprehensive evaluation of a sample's antioxidant capacity. However, it is worthy of note that the antioxidant capacity of these extracts depends on the yield of phenolic compounds during extraction.

The experimental mixtures CNE, PNE, and MNE are isoproteic, isolipidic, and isoenergetic, and these properties were maintained in the corresponding diets. Although PNE and MNE are richer in fibre compared to CNE, they contain less starch, possibly due to the replacement of wheat meal with peel flour, as wheat is mostly composed of starch (Hu et al., 2023).

In terms of phenolic content, mangiferin was detected in both free and bound forms across the entire mango manufacturing chain. Indeed, this phenolic compound was found in mango peel, in the MNE mixture, and in extruded pellets MH and MC, as well as in the dried pellets from MH-35 and MC-35. However, only trace amounts were found in all samples after the extrusion process. In addition to its strong radical-scavenging properties, mangiferin can modulate important metabolic pathways such as glycolysis, the tricarboxylic acid cycle, lipid and amino acid metabolism, and energy biosynthesis (Antony and Farid, 2022; Mei et al., 2021). However, its low solubility leads to poor transmembrane permeability and increased intestinal metabolic instability, which can directly impact its bioavailability and biological activity in farmed fish (Mei et al., 2021). On the other hand, the MNE mixture showed high amounts of bound gallic acid, consistent with the expected 2 % content observed in mango peel flour. This highlights that mango extruded pellets were the only samples capable of retaining gallic acid in the bound form. Moreover, the bound phenolic content of the PNE mixture matches the expected 2 % increase conferred by pineapple peel flour content, in terms of 4-coumaric and transferulic acids. Diets PH-60 and PH-35, containing pineapple, showed double the maximum amounts of these polyphenols compared to other diets. We can thus conclude that mango peel flour is an effective source of bound gallic acid when included in aquafeeds, while pineapple flour is an effective source of 4-coumaric and transferulic acids.

Extruded pellets from diets MH-60, PH-60, and PH-35 showed approximately double the amount of free phenolic compounds than the control diet CH-60. This includes significantly higher levels of gentisic acid, known for its anti-inflammatory, antimutagenic, hepatoprotective, neuroprotective, antimicrobial, and antioxidant properties (Abedi et al., 2020). Moreover, the bound phenolic compounds in MH-60, PH-60, and PH-35 diets also showed higher levels compared to both control diets. These diets demonstrated significant increases in specific fibre-bound phenolic compounds with potent antioxidant activity, namely syringic acid (Srinivasulu et al., 2018), sinapic acid (Chen, 2016), and transferulic acid (Srinivasan et al., 2007). Additionally, 4-coumaric acid, recognised for its potent antioxidant and ROS scavenger capabilities (Shen et al., 2019), was one of the most prevalent bound phenolic acids in diet PH-60, while both control diets showed only trace amounts.

The effects of thermal stress on the profile and bioavailability of phenolic compounds, as well as their ability to bond with fibre, have been extensively studied (Arfaoui, 2021; D'Archivio et al., 2010). While some studies have reported detrimental changes in the nutritional and antioxidant profiles of vegetable-based food matrices at higher temperatures (Juániz et al., 2015), others have observed beneficial effects (Xu et al., 2007). Indeed, the outcomes of these studies are highly influenced by the specific food matrix and thermal processing methods used (Arfaoui, 2021). In this study, results revealed that conventional extrusion temperatures (~100 °C) and drying temperatures (60 °C), typically used in aquafeed manufacturing, are more effective in preserving dietary antioxidants and overall feed antioxidant properties compared to colder extrusion processes (~25 °C) and significantly longer drying periods at lower temperatures (35°C). Indeed, since diets must be dried to < 12 % moisture (NRC, 2011), short-term drying at high temperatures seems to be more effective than prolonged drying at lower temperatures. Lower drying temperatures require extended exposure to oxygen, light, and heat, which are influential factors in polyphenol degradation. Higher extrusion and drying temperatures may have affected the structural stability of fibre or its chemical bonds with phenolic compounds, making them more extractable with the solvents used in this study (Arfaoui, 2021). Moreover, while higher temperatures may be damaging to free phenolic compounds, potentially disrupting the bioencapsulation effect provided by fibre and making bound phenolic compounds more vulnerable to degradation, they could also possibly increase the bioavailability of bound phenolic compounds (D'Archivio et al., 2010).

In our inter-diet comparison using principal component analysis (PCA), we found that pineapple diets subjected to hot extrusion (PH-60 and PH-35), along with the mango diet that underwent both hot extrusion and hot drying (MH-60), were more closely associated with higher DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and ORAC values. Specifically, PH-60 showed a pronounced association with ORAC values. Furthermore, a One-Way ANOVA comparison showed that the PH-60 diet had significantly higher ORAC values than all other diets containing pineapple and mango peel. These results suggest that the PH-60 diet may provide the most effective antioxidant supplement for mitigating oxidation in aquafeeds. Compared to both control diets, CH-60 and CH-35, all three diets (PH-60, PH-35, and MH-60) showed higher phenolic content and increased antioxidant capacity in terms of DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and ORAC. Overall, the present results support the incorporation of pineapple and mango flour, concomitantly with higher extrusion and drying temperatures, as biopreservatives and nutraceuticals in aquafeeds. Phenolic compounds have been extensively studied and used in the food industry to reduce the reliance on synthetic antioxidants, thereby enhancing the shelf-life of perishable products such as animal feeds, and inhibiting lipid oxidation chain reactions (Ullah et al., 2022). This approach has been recognised as viable for preserving the quality of fish and fish products over time (Maqsood et al., 2013), and it has shown resistance to deactivation during the high-temperature extrusion-cooking process (Oniszczuk et al., 2019). While the tested diets have not yet been evaluated in fish, several studies have reported positive effects associated with the inclusion of polyphenol-rich fruit by-products in aquafeeds. For instance, incorporating orange peel in diets for Gilthead seabream, *Sparus aurata,* resulted in increased antioxidant enzyme activity and decreased lipid peroxidation in the liver (Salem et al., 2019). Similar benefits were reported with the inclusion of pomegranate peel in diets for Nile tilapia, *Oreochromis niloticus* (Hamed and Abdel-Tawwab, 2021), whilst lemon peel extract increased liver total antioxidant capacity in tambaqui (*Colossoma macropomum*) (Lopes et al., 2020). Moreover, pineapple peel flour has been reported to increase bacterial infection survival rates and disease resistance in Nile tilapia, *Colossoma macropomum* (Attalla et al., 2022; Van Doan et al., 2021). It can be inferred that the biological properties of polyphenols in organisms are heavily dependent on factors such as the phenolic concentration of the natural antioxidant source, dosage, and method of inclusion. Additionally, synergies between different antioxidants, as well as the bioaccessibility and bioavailability of phenolic compounds in the organism, can influence their effectiveness (Lizárraga-Velázquez et al., 2019). Therefore, conducting an *in vivo* trial with these specific diets is essential to evaluate their practical efficacy in enhancing fish antioxidant defences.

5. Conclusions

Our study demonstrated that incorporating 2 % pineapple or mango peel flour in aquafeeds increases their antioxidant content and capacity compared to a control diet subjected to the same manufacturing conditions. Conventional temperatures proved more effective in increasing feed antioxidant capacity by releasing phenolic compounds from antioxidant fibre. These compounds were more abundant and evidenced greater resistance throughout the feed manufacturing process compared to vitamins and carotenoids. These findings are a significant contribution to understanding how heat treatments impact natural antioxidant potency in aquafeeds and highlight pineapple and mango peel flours as effective sources of additional antioxidants in aquafeeds, supporting a circular economy approach. However, conducting an *in vivo* trial with these diets is crucial to determine whether these enhanced antioxidant properties translate into tangible benefits for farmed fish.

CRediT authorship contribution statement

Cristina Velasco Rubial: Writing – review & editing, Validation, Supervision, Methodology, Investigation. **Ricardo Pereira:** Writing – original draft, Visualization, Investigation, Formal analysis. **Luisa Valente:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Manuela Pintado:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Jorge Dias:** Writing – review & editing, Validation, Methodology. **Ricardo Goméz-Garcia:** Writing – review & editing, Validation, Formal analysis.

Declaration of Competing Interest

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

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References

Abedi, F., Razavi, B.M., Hosseinzadeh, H., 2020. A review on gentisic acid as a plant derived phenolic acid and metabolite of aspirin: comprehensive pharmacology, toxicology, and some pharmaceutical aspects. Phytother. Res. 34 (4), 729–741.

Achir, N., Randrianatoandro, V.A., Bohuon, P., Laffargue, A., Avallone, S., 2010. Kinetic study of β-carotene and lutein degradation in oils during heat treatment. Eur. J. Lipid Sci. Technol. 112 (3), 349–361.

Afanas’eva, I.B., Ostrakhovitch, E.A., Mikhal’chik, E.V., Ibragimova, G.A., Korkina, L.G., 2001. Enhancement of antioxidant and anti-inflammatory activities of bioflavonoid rutin by complexation with transition metals. Biochem. Pharmacol. 61 (6), 677–684.

Ahmadifar, E., Yousefi, M., Karimi, M., Fadaei Raieni, R., Dadar, M., Yilmaz, S., Dawood, M.A.O., Abdel-Latif, H.M.R., 2021. Benefits of dietary polyphenols and polyphenol-rich additives to aquatic animal health: an overview. Rev. Fish. Sci. Aquac. 29 (4), 478–511.

Aklakur, M., 2018. Natural antioxidants from sea: a potential industrial perspective in aquafeed formulation. Rev. Aquac. 10 (2), 385–399.

Amić, A., Marković, Z., Dimitrić Marković, J.M., Milenković, D., Stepanić, V., 2020. Antioxidative potential of ferulic acid phenoxyl radical. Phytochemistry 170, 112218.

Anderson, J.S., Sunderland, R., 2002. Effect of extruder moisture and dryer processing temperature on vitamin C and E and astaxanthin stability. Aquaculture 207, 137–149.

Antony, A., Farid, M., 2022. Effect of temperatures on polyphenols during extraction. Appl. Sci. 12, 2107.

AOAC, 2006. Official Methods of Analysis of AOAC International, 18 ed. Gaithersburg, USA.

Arfaoui, L., 2021. Dietary plant polyphenols: effects of food processing on their content and bioavailability. Molecules 26 (10), 2959.

Attalla, R., Fadda, S., El-Serafy, S., 2022. Investigation of pineapple remnants used in fish aqua feeds. Egypt. J. Aquat. Biol. Fish. 26, 1–22.

Bacchetta, C., Rossi, A.S., Cian, R.E., Drago, S.R., Cazenave, J., 2019. Dietary β-carotene improves growth performance and antioxidant status of juvenile *Piaractus mesopotamicus*. Aquac. Nutr. 25 (4), 761–769.

Badhani, B., Sharma, N., Kakkar, R., 2015. Gallic acid: a versatile antioxidant with promising therapeutic and industrial applications. RSC Adv. 5 (35), 27540–27557.

Bai, S.C., Katya, K., Yun, H., 2015. 7 - Additives in aquafeed: an overview. In: Davis, D.A. (Ed.), Feed and Feeding Practices in Aquaculture. Woodhead Publishing, Oxford, pp. 171–202.

Baiano, A., Del Nobile, M.A., 2016. Antioxidant compounds from vegetable matrices: biosynthesis, occurrence, and extraction systems. Crit. Rev. Food Sci. Nutr. 56, 2053–2068.

Barba, F.J., Tonello-Samson, C., Puértolas, E., Lavilla, M., 2020. Present and future of high pressure processing: a tool for developing innovative. Sustainable, Safe and Healthy Foods. Elsevier, pp. 221–244.

Bharathi, S., Cheryl, A., Rajagopalasamy, C., Uma, A., Ahilan, B., Aanand, S., 2019. Functional feed additives used in fish feeds. Int. J. Fish. Aquat. Stud. 3 (7), 44–52.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci. Technol. 28 (1), 25–30.

Brglez Mojzer, E., Knez Hrnčič, M., Škerget, M., Knez, Ž., Bren, U., 2016. Polyphenols: extraction methods, antioxidative action, bioavailability and anticarcinogenic effects. Molecules 21 (7), 901.

Călinoiu, L.F., Vodnar, D.C., 2019. Thermal processing for the release of phenolic compounds from wheat and oat bran. Biomolecules 10 (1), 21.

Campos, D.A., Coscueta, E.R., Vilas-Boas, A.A., Silva, S., Teixeira, J.A., Pastrana, L.M., Pintado, M.M., 2020a. Impact of functional flours from pineapple by-products on human intestinal microbiota. J. Funct. Foods 67, 103830.

Campos, D.A., Ribeiro, T.B., Teixeira, J.A., Pastrana, L., Pintado, M.M., 2020b. Integral valorization of pineapple (*Ananas comosus* L.) by-products through a green chemistry approach towards added value ingredients. Foods 9 (1), 60.

Carvalho, C.C.C.R., Caramujo, M.J., 2017. Carotenoids in aquatic ecosystems and aquaculture: a colorful business with implications for human health. Front. Mar. Sci. 4, 93.

Chen, C., 2016. Sinapic acid and its derivatives as medicine in oxidative stress-induced diseases and aging. Oxid. Med. Cell. Longev. 2016, 3571614.

Chowdhury, S., Saikia, S., 2020. Oxidative stress in fish: a review. J. Sci. Res. 12, 145–160.

Colombo, S.M., Foroutani, M.B., Parrish, C.C., 2020. Fats and oils in aquafeed formulations. In: Shahidi, F. (Ed.), Bailey’s Industrial Oil and Fat Products. John Wiley & Sons, Ltd., New Jersey, U.S.A, pp. 1–28.

Coscueta, E.R., Pellegrini Malpiedi, L., Nerli, B.B., 2018. Micellar systems of aliphatic alcohol ethoxylates as a sustainable alternative to extract soybean isoflavones. Food Chem. 264, 135–141.

D’Archivio, M., Filesi, C., Varì, R., Scazzocchio, B., Masella, R., 2010. Bioavailability of the polyphenols: status and controversies. Int. J. Mol. Sci. 11 (4), 1321–1342.

Dávalos, A., Gómez-Cordovés, C., Bartolomé, B., 2004. Extending applicability of the oxygen radical absorbance capacity (ORAC−Fluorescein) assay. J. Agric. Food Chem. 52 (1), 48–54.

Dawood, M.A.O., Habotta, O.A.E., Elsabagh, M., Azra, M.N., Van Doan, H., Kari, Z.A., Sewilam, H., 2022. Fruit processing by-products in the aquafeed industry: a feasible strategy for aquaculture sustainability. Rev. Aquac. 14 (4), 1945–1965.

Ehsani, A., Jasour, M.S., Agh, N., Hashemi, M., Khodadadi, M., 2018. Rancidity development of refrigerated rainbow trout (*Oncorhynchus mykiss*) fillets: comparative effects of *in vivo* and in vitro lycopene. J. Sci. Food Agric. 98, 559–565.

Elseady, Y., Zahran, E., 2013. Ameliorating effect of β-carotene on antioxidant response and hematological parameters of mercuric chloride toxicity in Nile tilapia (*Oreochromis niloticus*). Fish. Physiol. Biochem. 39 (4), 1031–1041.

Encarnação, P., 2016. Functional feed additives in aquaculture feeds. In: Nates, S.F. (Ed.), Aquafeed Formulation, 5. Academic Press, San Diego, pp. 217–237.

European Commission, 2004. List of the authorised additives in feedingstuffs published in application of Article 9t (b) of Council Directive 70/524/EEC concerning additives in feedingstuffs (Text with EEA relevance).

European Commission, 2017. Suspending the authorisation of ethoxyquin as a feed additive for all animal species and categories. 962.

European Commission, 2022. Commission Implementing Regulation (EU) 2022/1375 of 5 August 2022 concerning the denial of authorisation of ethoxyquin as a feed additive belonging to the functional group of antioxidants and repealing Implementing Regulation (EU) 2017/962 (Text with EEA relevance), in: Union, E. (Ed.).

Faehnrich, B., Lukas, B., Humer, E., Zebeli, Q., 2016. Phytogenic pigments in animal nutrition: Potentials and risks. J. Sci. Food Agric. 96, 1420–1430.

FAO, 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. 211.

Floegel, A., Kim, D.-O., Chung, S.-J., Koo, S.I., Chun, O.K., 2011. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. J. Food Compos. Anal. 24 (7), 1043–1048.

Gao, J., Hu, J., Hu, D., Yang, X., 2019. A role of gallic acid in oxidative damage diseases: a comprehensive review. Nat. Prod. Commun. 14 (8), 1934578X19874174.

Goda, A.M.A., Sallam, A.E., Srour, T.M., 2018. Evaluation of natural and synthetic carotenoid supplementation on growth, survival, total carotenoid content, fatty acids profile and stress resistance of european seabass, *Dicentrarchus labrax*, Fry. aquaculture. Studies 18 (1), 27–39.

Gómez-García, R., Campos, D.A., Oliveira, A., Aguilar, C.N., Madureira, A.R., Pintado, M., 2021. A chemical valorisation of melon peels towards functional food ingredients: bioactives profile and antioxidant properties. Food Chem. 335, 127579.

Gonçalves, B., Falco, V., Moutinho-Pereira, J., Bacelar, E., Peixoto, F., Correia, C., 2009. Effects of elevated CO2 on grapevine (*Vitis vinifera* L.): volatile composition, phenolic content, and in vitro antioxidant activity of red wine. J. Agric. Food Chem. 57 (1), 265–273.

Gunathilake, T., Akanbi, T.O., Suleria, H.A.R., Nalder, T.D., Francis, D.S., Barrow, C.J., 2022. Seaweed phenolics as natural antioxidants, aquafeed additives, veterinary treatments and cross-linkers for microencapsulation. Mar. Drugs 20 (7), 445.

Hamed, H.S., Abdel-Tawwab, M., 2021. Dietary pomegranate (*Punica granatum*) peel mitigated the adverse effects of silver nanoparticles on the performance, haemato-biochemical, antioxidant, and immune responses of Nile tilapia fingerlings. Aquaculture 540, 736742.

He, J., Xu, L., Yang, L., Wang, X., 2018. Epigallocatechin gallate is the most effective catechin against antioxidant stress via hydrogen peroxide and radical scavenging activity. Med. Sci. Monit. 24, 8198–8206.

Hernández, A., García García, B., Jordán, M.J., Hernández, M.D., 2014. Natural antioxidants in extruded fish feed: Protection at different storage temperatures. Anim. Feed Sci. Technol. 195, 112–119.

Hu, H., Qiu, M., Qiu, Z., Li, S., Lan, L., Liu, X., 2023. Variation in wheat quality and starch structure under granary conditions during long-term storage. Foods 12 (9), 1886.

Jakobek, L., Matić, P., 2019. Non-covalent dietary fibre - polyphenol interactions and their influence on polyphenol bioaccessibility. Trends Food Sci. Technol. 83, 235–247.

Joshi, R., Gangabhagirathi, R., Venu, S., Adhikari, S., Mukherjee, T., 2012. Antioxidant activity and free radical scavenging reactions of gentisic acid: in-vitro and pulse radiolysis studies. Free Radic. Res. 46 (1), 11–20.

Juániz, I., Ludwig, I., Huarte, E., Pereira Caro, G., Moreno-Rojas, J., Cid, C., De Peña, M.-P., 2015. Influence of heat treatment on antioxidant capacity and (poly) phenolic compounds of selected vegetables. Food Chem. 197, 466–473.

Kedare, S.B., Singh, R.P., 2011. Genesis and development of DPPH method of antioxidant assay. J. Food Sci. Technol. 48 (4), 412–422.

Kondo, K., Kurihara, M., Miyata, N., Suzuki, T., Toyoda, M., 1999. Scavenging mechanisms of (-)-epigallocatechin gallate and (-)-epicatechin gallate on peroxyl radicals and formation of superoxide during the inhibitory action. Free Radic. Biol. Med. 27 (7), 855–863.

Kousoulaki, K., Sæther, B.-S., Albrektsen, S., Noble, C., 2015. Review on European sea bass (*Dicentrarchus labrax*, Linnaeus, 1758) nutrition and feed management: a practical guide for optimizing feed formulation and farming protocols. Aquac. Nutr. 21 (2), 129–151.

Kumar, H., Bhardwaj, K., Sharma, R., Nepovimova, E., Kuča, K., Dhanjal, D.S., Verma, R., Bhardwaj, P., Sharma, S., Kumar, D., 2020. Fruit and vegetable peels: utilization of high value horticultural waste in novel industrial applications. Molecules 25 (12), 2812.

Kurutas, E.B., 2015. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutr. J. 15, 71.

Lanigan, R.S., Yamarik, T.A., 2002. Final report on the safety assessment of BHT. Int. J. Toxicol. 21 (2), 19–94.

Lee, R.-J., Lee, V.S.Y., Tzen, J.T.C., Lee, M.-R., 2010. Study of the release of gallic acid from (–)-epigallocatechin gallate in old oolong tea by mass spectrometry. Rapid Commun. Mass Spectrom. 24 (7), 851–858.

Liu, K., Frost, J., Welker, T.L., Barrows, F.T., 2021. Comparison of new and conventional processing methods for their effects on physical properties of fish feed. Anim. Feed Sci. Technol. 273, 114818.

Lizárraga-Velázquez, C.E., Hernández, C., González-Aguilar, G.A., Heredia, J.B., 2019. Effect of dietary intake of phenolic compounds from mango peel extract on growth, lipid peroxidation and antioxidant enzyme activities in zebrafish (*Danio rerio*). Lat. Am. J. Aquat. Res. 47, 602–611.

Lopes, J.M., Marques, N.C., dos, Santos, M.Dd.M.C., Souza, C.F., Baldissera, M.D., Carvalho, R.C., Santos, L.L., Pantoja, B.T.S., Heinzmann, B.M., Baldisserotto, B., 2020. Dietary limon *Citrus latifolia* fruit peel essential oil improves antioxidant capacity of tambaqui (*Colossoma macropomum*) juveniles. Aquac. Res. 51 (12), 4852–4862.

Luan, A., Zhang, W., Yang, M., Zhong, Z., Wu, J., He, Y., He, J., 2023. Unveiling the molecular mechanism involving anthocyanins in pineapple peel discoloration during fruit maturation. Food Chem. 412, 135482.

Maqsood, S., Benjakul, S., Shahidi, F., 2013. Emerging role of phenolic compounds as natural food additives in fish and fish products. Crit. Rev. Food Sci. Nutr. 53 (2), 162–179.

Mei, S., Perumal, M., Battino, M., Kitts, D.D., Xiao, J., Ma, H., Chen, X., 2021. Mangiferin: a review of dietary sources, absorption, metabolism, bioavailability, and safety. Crit. Rev. Food Sci. Nutr. 1–19.

Meléndez-Martínez, A.J., Mandić, A.I., Bantis, F., Böhm, V., Borge, G.I.A., Brnčić, M., Bysted, A., Cano, M.P., Dias, M.G., Elgersma, A., Fikselová, M., García-Alonso, J., Giuffrida, D., Gonçalves, V.S.S., Hornero-Méndez, D., Kljak, K., Lavelli, V., Manganaris, G.A., Mapelli-Brahm, P., Marounek, M., Olmedilla-Alonso, B., Periago-Castón, M.J., Pintea, A., Sheehan, J.J., Tumbas Šaponjac, V., Valšíková-Frey, M., Meulebroek, L.V., O'Brien, N., 2022. A comprehensive review on carotenoids in foods and feeds: *status quo*, applications, patents, and research needs. Crit. Rev. Food Sci. Nutr. 62 (8), 1999–2049.

Morin, P., Gorman, A., Lambrakis, L., 2021. A literature review on vitamin retention during the extrusion of dry pet food. Anim. Feed Sci. Technol. 277, 114975.

Nakano, T., Wiegertjes, G., 2020. Properties of carotenoids in fish fitness: a review. Mar. Drugs 18 (11).

NRC, 2011. National Research Council (NRC): Nutrient requirements of fish and shrimp.

Olmos-Soto, J., 2015. Functional feeds in aquaculture. In: Kim, S.W. (Ed.), Handbook of Marine Biotechnology. Springer, pp. 1303–1319.

Oniszczuk, T., Oniszczuk, A., Gondek, E., Guz, L., Puk, K., Kocira, A., Kusz, A., Kasprzak, K., Wójtowicz, A., 2019. Active polyphenolic compounds, nutrient contents and antioxidant capacity of extruded fish feed containing purple coneflower (*Echinacea purpurea* (L.) Moench.). Saudi J. Biol. Sci. 26 (1), 24–30.

Ortak, M., Caltinoglu, C., Sensoy, I., Karakaya, S., Mert, B., 2017. Changes in functional properties and in vitro bioaccessibilities of β-carotene and lutein after extrusion processing. J. Food Sci. Technol. 54, 3543–3551.

Padayachee, A., Netzel, G., Netzel, M., Day, L., Zabaras, D., Mikkelsen, D., Gidley, M.J., 2012. Binding of polyphenols to plant cell wall analogues – part 1: anthocyanins. Food Chem. 134 (1), 155–161.

Pereira, R., Costa, M., Velasco, C., Cunha, L.M., Lima, R.C., Baião, L.F., Batista, S., Marques, A., Sá, T., Campos, D.A., Pereira, M., Jesus, D., Fernández-Boo, S., Costas, B., Pintado, M., Valente, L.M.P., 2022. Comparative analysis between synthetic vitamin E and natural antioxidant sources from tomato, carrot and coriander in diets for market-sized *Dicentrarchus labrax*. Antioxidants 11 (4), 636.

Ranganath, K.G., Shivashankara, K.S., Roy, T.K., Dinesh, M.R., Geetha, G.A., Pavithra, K.C., Ravishankar, K.V., 2018. Profiling of anthocyanins and carotenoids in fruit peel of different colored mango cultivars. J. Food Sci. Technol. 55 (11), 4566–4577.

Ratnavathi, C.V., Komala, V.V., 2016. Chapter 1 - Sorghum Grain Quality. In: Ratnavathi, C.V., Patil, J.V., Chavan, U.D. (Eds.), Sorghum Biochemistry. Academic Press, San Diego, pp. 1–61.

Resende, D., Velasco, C., Pereira, M., Sá, T., Rocha, C., Cunha, L., Lima, R., Brazinha, C., Pintado, M., Valente, L., 2024. Incorporating sardine cooking water aromas into plant-based diets for European seabass: effects on appetite regulation, growth and sensory properties of fish flesh. Anim. Feed Sci. Technol. 314, 116017.

Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B., Sasal, P., 2014. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. Aquaculture 433, 50–61.

Rezaeiroshan, A., Saeedi, M., Morteza-Semnani, K., Akbari, J., Hedayatizadeh-Omran, A., Goli, H., Nokhodchi, A., 2022. Vesicular formation of trans-ferulic acid: an efficient approach to improve the radical scavenging and antimicrobial properties. J. Pharm. Innov. 17 (3), 652–661.

Riaz, M., Ali, R., 2009. Stability of vitamins during extrusion. Crit. Rev. Food Sci. Nutr. 49, 361–368.

Saleh, N., Wassef, A., E, M., Shalaby, S., 2018. The role of dietary astaxanthin in European sea bass (*Dicentrarchus labrax*) growth, immunity, antioxidant competence and stress tolerance. Egypt. J. Aquat. Biol. Fish. 22 (5 (Special Issue), 189–200.

Salem, M.E.-S., Abdel-Ghany, H.M., Sallam, A.E., El-Feky, M.M.M., Almisherfi, H.M., 2019. Effects of dietary orange peel on growth performance, antioxidant activity, intestinal microbiota and liver histology of Gilthead sea bream (*Sparus aurata)* larvae. Aquac. Nutr. 25 (5), 1087–1097.

Sallam, A.E., Mansour, A.T., Srour, T.M., Goda, A.M.A., 2017. Effects of different carotenoid supplementation sources with or without sodium taurocholate on growth, feed utilization, carotenoid content and antioxidant status in fry of the European seabass, *Dicentrarchus labrax*. Aquac. Res. 48 (7), 3848–3858.

Sanches-Silva, A., Costa, D., Albuquerque, T.G., Buonocore, G.G., Ramos, F., Castilho, M.C., Machado, A.V., Costa, H.S., 2014. Trends in the use of natural antioxidants in active food packaging: a review. Food Addit. Contam.: Part A: Chem., Anal. Cont. Expo. Risk Assess. 31 (3), 374–395.

Sánchez-Moreno, C., 2002. Review: methods used to evaluate the free radical scavenging activity in foods and biological systems. Food Sci. Technol. Int. 8 (3), 121–137.

Schafberg, M., Loest, K., Müller-Belecke, A., Rohn, S., 2020. Impact of processing on the antioxidant activity of a microorganism-enriched fish feed and subsequent quality effects on fillets of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 518, 734633.

Schieber, A., Weber, F., 2016. Carotenoids. In: Carle, R., Schweiggert, R.M. (Eds.), Handbook on Natural Pigments in Food and Beverages, 5. Woodhead Publishing, pp. 101–123.

Shahbandeh, M., 2019. Leading Global Producers of Fresh Vegetables, 2017. FAO, Rome, Italy.

Shen, Y., Song, X., Li, L., Sun, J., Jaiswal, Y., Huang, J., Liu, C., Yang, W., Williams, L., Zhang, H., Guan, Y., 2019. Protective effects of p-coumaric acid against oxidant and hyperlipidemia-an *in vitro* and *in vivo* evaluation. Biomed. Pharmacother. 111, 579–587.

Slavin, M., Yu, L.L., 2012. A single extraction and HPLC procedure for simultaneous analysis of phytosterols, tocopherols and lutein in soybeans. Food Chem. 135 (4), 2789–2795.

Srinivasan, M., Sudheer, A.R., Menon, V.P., 2007. Ferulic acid: therapeutic potential through its antioxidant property. J. Clin. Biochem. Nutr. 40 (2), 92–100.

Srinivasulu, C., Ramgopal, M., Ramanjaneyulu, G., Anuradha, C.M., Suresh Kumar, C., 2018. Syringic acid (SA) – a review of its occurrence, biosynthesis, pharmacological and industrial importance. Biomed. Pharmacother. 108, 547–557.

Tanaka, T., Matsuo, Y., Kouno, I., 2009. Chemistry of secondary polyphenols produced during processing of tea and selected foods. Int. J. Mol. Sci. 11 (1), 14–40.

Thivend, P., Mercier, C., Guilbot, A., 1972. Determination of starch with glucoamylase. In: Whistler, R.L., Bemiller, J.N. (Eds.), Methods in Carbohydrate Chemistry. Academic Press, New York, pp. 100–105.

Udo, I., Afia, O., 2013. Optimization of dietary vitamin-E (tocopherols) in fish: a review. Nig. J. Agric. Food Environ. 9.

Ullah, H., Hussain, Y., Santarcangelo, C., Baldi, A., Di Minno, A., Khan, H., Xiao, J., Daglia, M., 2022. Natural polyphenols for the preservation of meat and dairy products. Molecules 27 (6), 1906.

Van Doan, H., Hoseinifar, S.H., Harikrishnan, R., Khamlor, T., Punyatong, M., Tapingkae, W., Yousefi, M., Palma, J., El-Haroun, E., 2021. Impacts of pineapple peel powder on growth performance, innate immunity, disease resistance, and relative immune gene expression of Nile tilapia, *Oreochromis niloticus*. Fish. Shellfish Immunol. 114, 311–319.

Van Soest, P.J., Robertson, J.B., 1985. Analysis of forages and fibrous foods. Cornell University, Ithaca, N.Y.

Wang, X., Li, X., Chen, D., 2011. Evaluation of antioxidant activity of isoferulic acid *in vitro*. Nat. Prod. Commun. 6 (9), 1285–1288.

Williams, R.J., Spencer, J.P.E., Rice-Evans, C., 2004. Flavonoids: antioxidants or signalling molecules? Free Radic. Biol. Med. 36 (7), 838–849.

Wu, D., 2016. Recycle technology for potato peel waste processing: a review. Procedia Environ. Sci. 31, 103–107.

Xie, P.J., Huang, L.X., Zhang, C.H., Zhang, Y.L., 2015. Phenolic compositions, and antioxidant performance of olive leaf and fruit (*Olea europaea* L.) extracts and their structure–activity relationships. J. Funct. Foods 16, 460–471.

Xu, G., Ye, X., Chen, J., Liu, D., 2007. Effect of heat treatment on the phenolic compounds and antioxidant capacity of citrus peel extract. J. Agric. Food Chem. 55 (2), 330–335.

Zduńska, K., Dana, A., Kolodziejczak, A., Rotsztejn, H., 2018. Antioxidant properties of ferulic acid and its possible application. Ski. Pharmacol. Physiol. 31 (6), 332–336.