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Modification ω-6/ω-3 ratio and increase of the shelf life of anchovy oil (*Engraulis ringens*) with addition of olive oil (*Olea europaea*)☆

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Abstract – In the food industry, oil blending represents a simple method for the generation of products with desired nutritional and technological properties. The objective of this work was to blend anchoveta oil (AO) with virgin olive oil (VOO) to modify the ω-6/ω-3 ratio of the product for nutritional purposes and to evaluate its oxidative stability index (OSI) for technological purposes. Four AO/VOO blends were formulated: 50/50, 40/60, 30/70 and 20/80 (w/w), generating a ω-6/ω-3 ratio between 0.610–2.129, nutritionally recommendable. The Rancimat method allowed determining the OSI of the formulations at temperatures of 100, 110 and 120 °C, finding OSI ranges between 0.963–3.410 h, 0.430–1.730 h and 0.183–0.813 h, respectively. The kinetic behavior of the formulations with respect to activation energy (89.622 to 93.414 kJ/mol), entropy (−13.248 to −17.657 kJ/mol), enthalpy (86.455 to 90.248 kJ/mol), Gibbs energy (91.928 to 96.348 kJ/mol) and Q_{10} (2.090 to 2.153) has been described. The formulations are proposed as an alternative to increase the shelf life of AO and increase the nutritional value of VOO.

Keywords: anchovy / olive / oil blends / oxidative stability / Rancimat

Résumé – Modification du ratio ω-6/ω-3 et augmentation de la durée de conservation de l'huile d'anchois (*Engraulis ringens*) *via* l'ajout d'huile d'olive (*Olea europaea*). Dans l'industrie alimentaire, le mélange d'huiles représente une méthode simple pour fabriquer de produits aux propriétés nutritionnelles et technologiques souhaitées. L'objectif de ce travail était de mélanger de l'huile d'anchois (AO) avec de l'huile d'olive vierge (VOO) afin de modifier le rapport ω-6/ω-3 du produit à des fins nutritionnelles et d'évaluer son indice de stabilité oxydative (OSI) à des fins technologiques. Quatre mélanges AO/VOO ont été formulés : 50/50, 40/60, 30/70 et 20/80 (p/p), générant un rapport ω-6/ω-3 compris entre 0,610 et 2,129, recommandable sur le plan nutritionnel. La méthode Rancimat a permis de déterminer l'OSI des formulations à des températures de 100, 110 et 120 °C, avec des valeurs d'OSI comprises entre 0,963–3,410 h, 0,430–1,730 h et 0,183–0,813 h, respectivement. Le comportement cinétique des formulations en ce qui concerne l'énergie d'activation (89,622 à 93,414 kJ/mol), l'entropie (−13,248 à −17,657 J/mol), l'enthalpie (86,455 à 90,248 kJ/mol), l'énergie de Gibbs (91,928 à 96,348 kJ/mol) et Q_{10} (2,090 à 2,153) a été décrit. Les formulations sont proposées comme une alternative pour augmenter la durée de conservation de l'AO et augmenter la valeur nutritionnelle de l'VOO.

Mots clés : anchois / olive / mélanges d'huiles / stabilité oxydative / Rancimat

☆ Contribution to the Topical Issue "Lipids from aquatic environments / Lipides issus des milieux aquatiques".

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Highlights

- Blends of anchoveta oil with olive oil increased the oxidative stability of the product.
- Blends of anchoveta oil and olive oil at 30/70 and 20/80 (w/w) improved the ω-6/ω-3 ratio by 1.3/1 and 2/1.
- Rancimat method allowed estimating the kinetic parameters of the oil blends.

1 Introduction

Anchoveta (*Engraulis ringens*) oil (AO) is a by-product obtained in the fishing industry (Carvalho *et al.*, 2020). Peru produces on average 230 000 tons of fish oil per year, representing ~23% of the world production (Fréon *et al.*, 2017). It is widely known that anchoveta oil has a high content of poly-unsaturated fatty acids (PUFAS) (Özyurt *et al.*, 2020). It has been reported that these PUFAS would prevent arterial disease, coronary heart disease, arthritis, some cancers and improve neurological functions (Shahidi and Ambigaipalan, 2018; Bi *et al.*, 2019; Rahmawaty and Meyer, 2020). In the food industry, during processing it is very difficult to add fish oil directly to food due to its low solubility, taste and especially because of susceptibility to oxidation (Tatar and Kahyaoglu, 2014). Microencapsulation with the addition of plant-derived antioxidant extracts have been successfully applied to improve the oxidative stability anchovy oil (Wang *et al.*, 2018; Yesilsu and Özyurt, 2019). Because of the relatively low costs oil blending is also used as an industrial strategy to increase the quality of oils, improve the oxidative and nutritional properties of oils by changing the profile of fatty acids, bioactive lipids and natural antioxidants (Hashempour-Baltork *et al.*, 2016). The blending of vegetable oils for food technological purposes has been studied; for example, chia oil (high in ω-3) has been blended with sesame, almond, sunflower and walnut oil (Guiotto *et al.*, 2014; Bordón *et al.*, 2019; Rodríguez *et al.*, 2020), sunflower oil with hydrogenated corn and soybean oil (Naghshineh *et al.*, 2010), walnut/olive oil (Torres *et al.*, 2011), sunflower/sesame oil (Ghosh *et al.*, 2019) and lupin/sacha inchi oil (Rodríguez *et al.*, 2022).

Although the vast majority of studies are oriented to the mixture of oils of vegetable origin, it has been reported that the addition of olive oil with pomegranate extracts in anchovy marinade prolongs lipid oxidation and improves the sensory properties of the marinade (Topuz *et al.*, 2014). Replacement of fish oil with vegetable oils (palm, linseed, corn, olive, sunflower, and coconut) have been used in fish diets (Monge-Ortiz *et al.*, 2017; Agh *et al.*, 2019). The ω-6/ω-3 ratio of fish oil reduces the development of diseases such as atherosclerosis (Alshatwi and Subash-Babu, 2018). Reducing the ω-6/ω-3 ratio by adding fish ω-3 fatty acids in emulsified olive oil may be effective adjunct in the treatment of depressive disorders in children (Jana *et al.*, 2020).

The average international export price for AO is ~ $ 2500/ton, while VOO is ~ $ 4316/ton (FAO, 2022; IMF, 2022). However, the price trend of fish oil will continue to rise, especially AO as it is used in the formulation of novel foods (Ghamkhar and Hicks, 2020). In economic terms, the blending of these raw materials would primarily benefit VOO, as the price of omegas contained in fish oil is attractive to the market. However, in the aquaculture sector where up to 75% of the world's fish oil is used, leveraging the price of fish oil by partial substitution of vegetable oils could show economic incentives in the future. Therefore, studies have already been carried out in this line of research, finding that the substitution of fish oil with vegetable oils, including olive oil, does not significantly affect the nutritional strengths and impact on fish growth (Nasopoulou and Zabetakis, 2012; Sáez-Royuela *et al.*, 2022). On the other hand, although global fish oil production is expected to increase until 2027 (Chouhan *et al.*, 2020), recent studies indicate that global warming is causing substantial decreases of essential fatty acid ω-3 in plankton, especially eicosapentaenoic acid (EPA), which would represent a loss of quality in fish feed and therefore in the nutritional quality of fish (Holm *et al.*, 2022). The economic trend to bet on substitutes such as olive oil, which is derived from olive plant cultivation, in the face of a partial migration from fish oil to vegetable oils is interesting in the face of future climate change (Nasopoulou and Zabetakis, 2012; Hashempour-Baltork *et al.*, 2016).

The objective of this work was to mix AO with VOO for nutritional and technological purposes. From the accelerated reaction study (Rancimat), information was obtained on the OSI, autooxidation kinetic behavior and shelf life of the formulated mixtures, extrapolating the OSI values at usual storage temperatures (25 °C).

2 Materials and methods

2.1 Samples and preparation of blends

VOO was obtained from olive fruits (Criolla variety) harvested in the Tacna Region, Peru. The conventional extraction method was used, which consisted of the following steps: Manual pitting of fruits, crushing of fruit pulp in a blender (Oster, modelBLST4655B, Germany), pressing of the paste in a PH1020 hydraulic press (Neo & Neo Next, China) under a 20-t pressure, clarification of the oil at 4000 rpm ×5 min in a refrigerated centrifuge (SIGMA, model 2–16, USA) to finally store the oil in an amber flask at a temperature of 5.00 ± 0.5 °C. AO was provided by CFG-COPEINCA S.A.C. Fish Processing Company, Chimbote, Peru.

AO/VOO blends were formulated in proportions M1: 50/50, M2: 40/60, M3: 30/70 and M4: 20/80 (w/w), respectively. To achieve adequate homogenization, the methodology described by Rodríguez *et al.* (2020) was followed. Subsequently, the samples were packed in 50 mL dark bottles and sealed with nitrogen to be stored under refrigeration (T ~ 4 °C).

2.2 Physico-chemical characterization

The acid number (*AV*) was determined by the titration method defined in the official methods Cd 3d-63 of the American Oil Chemists' Society (AOCS, 1998). 7Titration of the oil samples (10 g) dissolved in 50 mL of previously neutralized chloroform–ethanol medium (50:50 v/v) was applied, using a 0.1 N potassium hydroxide (KOH) ethanolic solution as standard reagent to a phenolphthalein endpoint. *AV* was expressed as milligrams of KOH required to neutralize

the free fatty acids present in 1 g of the oil sample (mg KOH/g).

$$AV = G \times N \times 56.1/w,$$

where G is the titratable volume of KOH (mL), N is the normal of KOH (0.1 N) and w is the weight of the sample (g).

The refractive index (*RI*) was measured according to method 921.08 (AOAC International, 2019) working at 25 °C and using a digital A 24051 refractometer (Rudolph Research Analytical, NJ, USA) kept at 20 °C.

The iodine value (*IV*) was determined by the Wijs method, in accordance with method 993.20 (AOAC International, 2019), 0.2 g of oil was dissolved with 10 mL of chloroform and 15 mL of Wijs reagent, after 45 min of rest 10 mL of potassium iodide 15% in 50 mL of distilled water was added, proceeded to titrate with sodium thiosulfate (0.1 N) with a brown to yellow color change, 1 mL of 1% soluble starch was added and titrated again with 0.1 N sodium thiosulfate until the color changed from blue to white. *IV* was expressed as mg I_2/g.

$$IV = (B - M) \times N \times 12.65/w,$$

where B and M are the titratable volumes of sodium thiosulfate (mL) for the blank and oil sample, respectively. N is the sodium thiosulfate normal (0.1 N) and w is the sample weight (g).

The peroxides value (*PV*) was determined following the Cd 8-53 method (AOCS, 1998) with modifications, where 5 g of oil were dissolved in 30 mL of acetic acid-chloroform solution (60/40 v/v), added 0.5 mL of saturated potassium iodide was added and allowed to stand for 1 min in the dark, then 30 mL of distilled water was added and stirred for 5 min. Finally, 0.5 mL of 1% starch solution was added and titrated with 0.01 N sodium thiosulfate solution. PV was expressed in milliequivalents of active oxygen present in 1 kg of oil (meqO_2/kg).

$$PV = G \times N \times 1000/w,$$

where G is the titratable volume of sodium thiosulfate (mL), N is the normal of sodium thiosulfate (0.01 N) and w is the weight of the sample (g).

The p-anisidine (*p-AV*) value was monitored using the Cd 18-90 method (AOCS, 1998) with modifications, two reagent dilutions were prepared, the first consisted of the oil/isooctane mixture (0.5 g/25 mL), and the second was anisidine reagent/acetic acid (0.025 g/25 mL). Subsequently, the absorbances of the dilutions were measured at 350 nm, according to the following description:

$$p - AV = 25 \times (1.2 \times As - Ab)/w$$

where As is absorbance of oil/isooctane minus absorbance of pure isooctane. Ab is absorbance of the oil/isooctane diluted in anisidine/acetic acid (1/1, v/v) minus the absorbance of the anisidine/acetic acid diluted in isooctane (1/1, v/v). w is the weight of the oil (g).

Finally, the total oxidation value (*TotOX*) has been determined as:

$$TotOX = 2PV + p - AV.$$

2.3 Fatty acid profile

The fatty acid composition of the oils was determined according to the fatty acid methyl ester method no. 991.39 (AOAC, 2005), which consisted of weighing 0.025 g of oil and reacting with 1.5 mL of NaOH 0.5 N at 90 °C in a water bath (Foos, mode-loWB1024) for 5 min, then cooling to 30 °C and adding 2.0 mL of boron trifluoride (BF_3) heated to 100 °C for 30 min, again cooled by adding 1 mL of iso-octane and 5 mL of saturated NaCl solution, all under stirring and constantly covered with nitrogen. Identification of the components was determined on gas chromatograph (Shimadzu, model GC-2010, Japan), equipped with a flame ionization detector (FID) and a Shimadzu AOC-20Si autosampler. An SP Rt^{TM}-2560 silica capillary column (100 m × 0.25 mm with 0.20 μm film) was used helium as carrier gas at a flow rate of 30 mL/min and pressure of 261.5 kPa. Injection volume was 1 μL. Injector temperature was programmed at 225 °C (Split mode) and detector at 250 °C. Oven temperature was programmed: initial temperature 100 °C for 4 min, then at 240 °C with a rate of 3 °C/min for 10 min.

2.4 Oxidative stability index (OSI) and shelf-life

The accelerated stability test was carried out in a Rancimat equipment (Metrohm, model 743, Switzerland) to evaluate the OSI (hours) of the samples according to the AOCS Cd 12b-92 method. Rancimat parameters were programmed at three different reaction temperatures (100, 110 and 120 °C) with an air flow (15 L/h) and constant sample weight (3.00 ± 0.1 g). The temperatures were selected according to the oxidation resistance of AO and VOO (Farhoosh and Hoseini-Yazdi, 2013; Jiang *et al.*, 2020). The OSI values were inversely proportional to the temperatures values and related according to the equation proposed by Heidarpour and Farhoosh (2018);

$$Log(OSI) = \alpha(T) + \beta.$$

Shelf-life prediction (OSI_{25}) was determined by extrapolating the temperature to 25 °C.

2.5 Autooxidation kinetics

The activation energy (Ea) was determined according to:

$$\mathrm{Ln(OSI)} = \mathrm{Ln}\left(\frac{-\mathrm{Ln}(1-\alpha^*)}{\mathrm{Z}}\right) + \left(\frac{\mathrm{E_a}}{\mathrm{R}}\right)\left(\frac{1}{\mathrm{T}}\right),$$

where R = 8.314 J/mol K (universal gas constant), α* is the degree of transformation of unsaturated molecules and Z is factor of Arrhenius equation.

The entropy (ΔS^{++}) and enthalpy (ΔH^{++}) were obtained by regressing Log (k/T) versus (1/T), equation derived from the activated complex theory, according to Heidarpour and Farhoosh (2018);

$$\mathrm{Log}\left(\frac{\mathrm{K}}{\mathrm{T}}\right) = \left[\mathrm{Log}\left(\frac{\mathrm{k_B}}{\mathrm{h}}\right) + \left(\frac{\Delta \mathrm{S}^{++}}{2.303\mathrm{R}}\right)\right] - \left(\frac{\Delta \mathrm{H}^{++}}{\mathrm{R}}\right)\left(\frac{1}{\mathrm{T}}\right),$$

Table 1. Fatty acids composition and physico-chemical characteristics of AO, VOO and their blends oils.

Profile	Anchovy	Olive	M1	M2	M3	M4
C14:0	8.179 ± 0.026^{a}	0.000 ± 0.000^{f}	4.156 ± 0.049^{b}	3.172 ± 0.011^{c}	2.554 ± 0.008^{d}	1.836 ± 0.005^{e}
C16:0	20.389 ± 0.148^{a}	16.750 ± 0.171^{e}	18.434 ± 0.119^{b}	18.336 ± 0.010^{b}	17.872 ± 0.016^{c}	17.540 ± 0.045^{d}
C16:1	8.620 ± 0.183^{a}	1.628 ± 0.136^{f}	5.052 ± 0.084^{b}	4.458 ± 0.014^{c}	3.526 ± 0.104^{d}	3.027 ± 0.145^{e}
C18:0	3.743 ± 0.030^{a}	2.910 ± 0.046^{e}	3.360 ± 0.025^{b}	3.343 ± 0.036^{b}	3.160 ± 0.038^{c}	3.050 ± 0.026^{d}
C18:1	14.802 ± 0.277^{f}	54.013 ± 0.100^{a}	34.141 ± 0.023^{e}	38.262 ± 0.012^{d}	42.249 ± 0.140^{c}	46.037 ± 0.129^{b}
C18:2 (ω-6)	1.212 ± 0.201^{f}	19.498 ± 0.024^{a}	10.304 ± 0.070^{e}	12.183 ± 0.094^{d}	14.012 ± 0.077^{c}	15.841 ± 0.059^{b}
C18:3 (ω-3)	0.102 ± 0.008^{f}	1.021 ± 0.010^{a}	0.562 ± 0.001^{e}	0.654 ± 0.003^{d}	0.746 ± 0.005^{c}	0.838 ± 0.006^{b}
C20:0	1.783 ± 0.015^{a}	0.522 ± 0.003^{f}	0.166 ± 0.029^{b}	1.021 ± 0.007^{c}	0.896 ± 0.005^{d}	0.772 ± 0.004^{e}
C20:5 (ω-3, EPA)	19.520 ± 0.124^{a}	0.117 ± 0.002^{f}	9.994 ± 0.063^{b}	8.018 ± 0.051^{c}	6.043 ± 0.039^{d}	4.068 ± 0.027^{e}
C22:6 (ω-3, DHA)	12.669 ± 0.083^{a}	0.000 ± 0.000^{f}	6.335 ± 0.041^{b}	5.068 ± 0.033^{c}	3.801 ± 0.025^{d}	2.534 ± 0.017^{e}
PUFAS	33.854 ± 0.151^{a}	20.636 ± 0.031^{f}	27.194 ± 0.090^{b}	25.923 ± 0.079^{c}	24.602 ± 0.067^{d}	23.280 ± 0.055^{e}
MUFAS	23.422 ± 0.452^{a}	55.641 ± 0.226^{f}	39.192 ± 0.105^{e}	42.720 ± 0.026^{d}	45.775 ± 0.176^{c}	49.064 ± 0.141^{b}
Other fatty acids	8.643 ± 0.762^{a}	3.546 ± 0.469^{d}	6.498 ± 0.105^{cd}	5.485 ± 0.122^{c}	5.142 ± 0.273^{bc}	4.459 ± 0.243^{b}
ω-3	32.642 ± 0.050^{a}	1.138 ± 0.008^{f}	16.890 ± 0.021^{b}	13.740 ± 0.015^{c}	10.589 ± 0.010^{d}	7.439 ± 0.004^{e}
ω-6	1.224 ± 0.200^{f}	19.498 ± 0.024^{a}	10.304 ± 0.070^{e}	12.183 ± 0.094^{d}	14.012 ± 0.077^{c}	15.841 ± 0.059^{b}
ω-6/ω-3	0.037 ± 0.001^{f}	17.129 ± 0.093^{a}	0.610 ± 0.004^{e}	0.887 ± 0.008^{d}	1.323 ± 0.008^{c}	2.129 ± 0.009^{b}
IA (mg KOH/g)	0.430 ± 0.010^{c}	0.590 ± 0.010^{a}	0.500 ± 0.000^{b}	0.530 ± 0.010^{b}	0.530 ± 0.010^{b}	0.580 ± 0.010^{a}
Refraction Index	1.477 ± 0.001^{a}	1.469 ± 0.002^{a}	1.475 ± 0.001^{a}	$1.475 \pm 0.001a^{a}$	1.471 ± 0.002^{a}	1.470 ± 0.001^{a}
IV (mg I_2/g)	178.12 ± 1.120^{a}	89.120 ± 0.890^{f}	134.79 ± 0.990^{b}	127.46 ± 0.260^{c}	116.23 ± 0.100^{d}	107.82 ± 0.76^{b}
PV (meqO_2/kg)	4.890 ± 0.040^{a}	10.070 ± 0.040^{f}	6.920 ± 0.260^{e}	7.250 ± 0.120^{d}	8.170 ± 0.060^{c}	8.850 ± 0.160^{b}
p-AV	15.260 ± 0.550^{a}	4.990 ± 0.510^{f}	10.450 ± 0.070^{e}	8.940 ± 0.060^{d}	7.120 ± 0.100^{c}	5.460 ± 0.240^{b}
TotOx	24.990 ± 0.132^{a}	25.047 ± 0.180^{a}	24.233 ± 0.125^{b}	24.320 ± 0.243^{c}	23.369 ± 0.220^{c}	23.120 ± 0.233^{c}

Different letters in the same row indicate significant differences ($p < 0.05$).

where $k_B = 1.380658 \times 10–23$ J/K (Boltzmann constant), $h = 6.6260755 \times 10–34$ Js (Planck's constant) and $K = 1/OSI$. The Gibbs free energy (ΔG^{++}) was calculated according to equation: $\Delta G^{++} = \Delta H^{++}\text{-}T\Delta S^{++}$.

The value of Q_{10} was determined according to Farhoosh (2007);

$$Q_{10} = \frac{\text{OSI at time T}}{\text{OSI at T} + 10°\text{C}}.$$

2.6 Statistical analysis

Data processing was performed in Minitab statistical software version 18 (Softonic, USA). Analysis of variance (ANOVA) was used to determine significant differences between treatment means using Tukey's test ($p < 0.05$). Before the ANOVA, the normal distribution was verified and, when necessary (OSI_{25}), the data underwent logarithmic transformation. Means were calculated by triplicate analysis of the samples.

3 Results and discussion

3.1 Physico-chemical characterization and ω-6/ω-3 ratio

Table 1 shows the physicochemical characteristics of AO, VOO and their blends. The *PV* (< 15 meqO_2/kg) of VOO were within the range established by Codex Alimentarius (2015) for vegetable oils, in the case of *AV* this was slightly higher than the 0.11 mg KOH/g of the extra VOO presented by Özkan and Özcan (2016). The *PV* of AO was close to the 3.03 meqO_2/kg shown by Yesilsu and Özyurt (2019) for AO (*Engraulis encrasicolus*), while *AV* was similar to the 0.43–0.44 mg KOH/g shown by Park *et al.* (2021) in commercial anchoveta fish oil. The IR of the blends of the oils with the controls did not show significant differences; therefore the mixture was physically homogeneous. The *IV* for AO was higher than that reported by Fadhil *et al.* (2015) and Mata *et al.* (2017) who indicated values of 86.36 and 146 mgI_2/g, respectively. On the other hand, the *IV* found for olive oil and blends was between the permissible limits (75–94 mgI_2/g) for edible oils (Codex Alimentarius, 2015). The *p-AV* in VOO was low compared to AO, since AO tends to oxidize much faster due to the amount of unsaturated fatty acids it presents, the value of 15.260 shown in Table 1, is similar to that presented by Yesilsu and Özyurt (2019) for OA but with approximately 15 days of storage. The TotOx value of AO and VOO did not present significant differences; likewise the blends did not present significant differences between them ($p < 0.05$).

The oil blends presented, in a majority way, significant differences with respect to their physicochemical properties ($p < 0.05$). Anchovy oil presented 33.85% PUFAS, mainly composed of DHA and EPA (ω-3), while olive oil presented 20.67% PUFAS, mainly composed of linoleic acid (ω-6), both fatty acid profiles were confirmed with the reports of Özyurt *et al.* (2020) and Xiang *et al.* (2017), respectively. The ratio of ω-6/ω-3 in of the M3 and M4 formulations were between 1/1 and 4/1, meeting the recommended nutritional requirements to

Table 2. OSI of AO, VOO and their blends.

Oils	Temperature (°C)		
	100	110	120
Anchovy	0.320 ± 0.020^{a}	0.177 ± 0.006^{a}	0.103 ± 0.006^{a}
Olive	18.677 ± 0.297^{e}	7.537 ± 0.186^{e}	3.513 ± 0.058^{e}
Blends			
M1	0.913 ± 0.011^{b}	0.430 ± 0.173^{b}	0.210 ± 0.010^{b}
M2	1.257 ± 0.011^{b}	0.527 ± 0.015^{b}	0.283 ± 0.006^{b}
M3	2.327 ± 0.085^{c}	1.103 ± 0.006^{c}	0.520 ± 0.020^{c}
M4	3.767 ± 0.032^{d}	1.730 ± 0.046^{d}	0.813 ± 0.023^{d}

Different letters in the same column indicate significant difference between the OSI (hours) of the oils ($p < 0.05$).

reduce risks of chronic diseases (Simopoulos, 2002; FAO, 2012; Rodríguez *et al.*, 2020). However, Alshatwi and Subash-Babu (2018) indicate that ω-6/ω-3 ratios of canola (5.6/1) and fish (1/2) oils prevent the development of atherosclerosis and macrophage-induced foam cell formation, opposite case high ω-6/ω-3 ratio as in corn (52/1), olive (13.4/1) and coconut (highly saturated) produce negative effects such as lipid accumulation in macrophages and foam cell conversion. The present study presented in all anchoveta and olive oil blends ratios of ω-6/ω-3 within the range recommended by Alshatwi and Subash-Babu (2018).

3.2 Oxidative stability index and shell life

The OSI of the oil blends is shown in Table 2. As expected, the OSI of AO was lower than that of VOO and the blends, this was due to the fact that anchoveta oil presented a high PUFAS composition that makes it highly sensitive to oxidation and deterioration. The OSI of AO at temperature of 100 °C (0.32 h) was slightly lower than the OSI of mackerel fish oil (0.47 h) presented by Yang and Chiang (2017), who conducted the study under the same parameters of temperature and air flow in Rancimat, this difference in OSI is explained in the PUFAS composition of the fish species, since in the study of mackerel oil the PUFAS contents were 17.5%, while those presented in this study for AO was 30.85%.

Attempts to increase the OSI of AO have been evaluated by Ogrodowska *et al.* (2020) who added pumpkin oil in fish oil in a ratio (50/50), subjected to a temperature of 110 °C in Rancimat, successfully achieving results of up to ~1.5 h similar to the 1.55 reported for the M4 blend of the present study. The VOO presented OSI values similar to those presented by Wang *et al.* (2020) who determined OSI values of 3.14 and 3.35 h at a temperature of 120 °C in Rancimat for different varieties of olive oils from Israel and planted in China. Another similar study in Rancimat for olive oil was reported by Heidarpour and Farhoosh (2018) who determined in two olive varieties (Zard and Roghani) OSI values between 25.0–28.4, 11.2–11.6 and 4.6–4.9 h for temperatures of 100, 110 and 120 °C, respectively.

The shelf life of AO projected by extrapolation in Rancimat (OSI_{25}) was less than one day (Tab. 3), this result coincides with that determined by Paucar-Menacho *et al.* (2015) who reported that for crude fish oil the shelf life is less than two days using the Rancimat method. However, the shelf life of fish oil (cod liver) can reach 2 months after opening the container (Mozuraityte *et al.*, 2016), studies of continuous storage at 10 °C indicate that after opening the bottles the content reaches a $PV = 8$ meqO_2/kg at 36 days (Chol, 2005), while Boran *et al.* (2006) reaches that same PV between 60 and 90 days at 4 °C. He *et al.* (2020) indicates that fish oil studied for 14 days at room temperature reduces 38% of its ω-3 content, techniques such as lyophilization and encapsulation allow maintaining ω-3 contents. The incorporation of medicinal plant extracts on fish oil can preserve it without problems for 6 months under ambient conditions (Jacob and Mathew, 2019). On the other hand, Table 3 presented significant differences regarding shelf-life projections (OSI_{25}) of the blends versus AO ($p < 0.05$), this indicates that the presence of VOO exerts positive influence on the preservation of the final oil. The addition of VOO in AO was able to increase the shelf life in a range of approximately 8 to 48 days. On the other hand, the shelf life of olive oil was 401.6 days (~ 13 months), these results were in the range of 8 to 28 months proposed by Guillaume and Ravetti (2016). Shelf-life studies with non-accelerated techniques are recommended to demonstrate more accurately (days) the positive effect of olive oil addition on anchoveta oil preservation at industrial scale.

3.3 Autooxidation kinetics

The ki parameters (Tab. 4) evidenced the high susceptibility of AO to oxidation, for example, the Ea value indicates the behavior of the oxidation reaction with respect to its delay, it has been suggested that a high unsaturated fatty acid content in an oily matrix decreases the Ea values and on the contrary an accumulation of saturated fatty acids increases the Ea values (Adhvaryu *et al.*, 2000). AO presented a value of Ea = 68.8 kJ/mol lower than the values reported by Yang and Chiang (2017) for fish oils (82.84–96.97 kJ/mol) in which the mackerel variety is included. For the case of VOO, the value of Ea = 101.87 kJ/mol was close to the 89–94 kJ/mol presented by Gharby *et al.* (2021) for virgin and refined olive oil. The blends of the oils generated an orderly tendency to increase Ea as the proportions of olive oil increased, although there were no significant differences between blends clearly showing that the presence of olive oil in the various

Table 3. Shelf life (OSI_{25}) and Q_{10} of AO, VOO and their blends.

Oils	α	β	R^2	OSI_{25}	Q_{10}
Anchovy	−0.02 ± 0.00	1.95 ± 0.09	0.998 ± 0.03	0.92 ± 0.14^{a}	1.76 ± 0.09^{a}
Olive	−0.03 ± 0.00	4.89 ± 0.07	0.997 ± 0.00	401.62 ± 49.33^{f}	2.31 ± 0.20^{b}
Blends					
M1	−0.03 ± 0.00	3.15 ± 0.08	0.998 ± 0.00	9.46 ± 1.38^{b}	2.09 ± 0.14^{b}
M2	−0.03 ± 0.00	3.32 ± 0.06	0.990 ± 0.00	13.46 ± 1.51^{c}	2.12 ± 0.30^{b}
M3	−0.03 ± 0.00	3.62 ± 0.03	0.999 ± 0.00	26.77 ± 1.53^{d}	2.12 ± 0.07^{b}
M4	−0.03 ± 0.00	3.90 ± 0.05	0.999 ± 0.00	49.22 ± 4.75^{e}	2.15 ± 0.07^{b}

Different letters in the same row indicate significant difference among samples at $p < 0.05$; α and β are constants; R^2: coefficient of determination. OSI_{25} in days.

Table 4. Thermodynamic study of AO, VOO and their blends.

Oils	Ea (kJ/mol)	ΔH^{++} (kJ/mol)	ΔS^{++} (J/mol K)	ΔG^{++} (kJ/mol)
Anchovy	68.880 ± 1.595^{a}	65.709 ± 1.595^{a}	−61.138 ± 4.048^{a}	89.125 ± 0.120^{a}
Olive	101.866 ± 1.595^{c}	98.701 ± 1.596^{c}	−8.401 ± 4.220^{b}	101.157 ± 0.02^{f}
Blends				
M1	89.622 ± 1.795^{b}	86.455 ± 1.795^{b}	−14.289 ± 4.747^{b}	91.928 ± 0.025^{b}
M2	90.893 ± 1.272^{b}	87.727 ± 1.272^{b}	−13.248 ± 3.298^{b}	92.800 ± 0.010^{c}
M3	91.287 ± 0.640^{b}	88.121 ± 1.640^{b}	−17.657 ± 1.703^{b}	94.883 ± 0.068^{d}
M4	93.414 ± 1.309^{b}	90.248 ± 1.309^{b}	−15.925 ± 3.512^{b}	96.348 ± 0.038^{e}

Identical letters in the same column indicate no significant differences among samples ($p < 0.05$). Activation energy (Ea), enthalpy (ΔH^{++}), entropy (ΔS^{++}) and Gibbs energy (ΔG^{++}).

matrices would delay the oxidation process, thus increasing the shelf life of the samples.

Enthalpy (ΔH^{++}) is another thermodynamic parameter that indicates the endothermic ($\Delta H^{++} > 0$) or exothermic ($\Delta H^{++} < 0$) nature of the autooxidation reaction (Farhoosh and Hoseini-Yazdi, 2013). All samples were endothermic in nature, AO presented lower enthalpy values than VOO because its endothermic behavior required less energy to oxidize due to the influence of different temperatures in Rancimat; this also translates into a reduction of OSI values. VOO presented enthalpy values within the range 91–103 kJ/mol presented by Heidarpour and Farhoosh (2018).

Regarding entropy, the values were negative ($\Delta S^{++} < 0$), indicating that activated complexes tend to be more ordered than reactive complexes (Rodríguez *et al.*, 2020). Studies in VOO show negatives de ΔS^{++} values, although slightly away from our results, *e.g.*, Heidarpour and Farhoosh (2018) determined values between −78.8 and −95.4 J/mol K. On the other hand, Gharby *et al.* (2016) reported values between −14.55 and −50.55 J/mol K. Finally, positive Gibbs free energy values ($\Delta H^{++} > 0$) have been reported which are interpreted as a non-spontaneous process the autooxidation reaction in Rancimat. VOO presented higher values (101.157 kJ/mol) than the blends and AO, showing its higher resistance to the autooxidation reaction. In general, the oil blends did not show significant differences with respect to the thermodynamic parameters of activation energy, enthalpy and entropy.

The Q_{10} values represent the doubling of the reaction rate for every 10 °C increase in temperature (Redondo-Cuevas *et al.*, 2018), thus all mixtures had values around two (1.76, fish oil – 2.3, olive oil). In the case of VOO, this can be corroborated with the reports of Gharby *et al.* (2021) who determined in virgin and refined oil values of $Q_{10} = 2.0–2.1$. Likewise, Gharby *et al.* (2016) determined for four varieties of VOO from Morocco values of $Q_{10} = 2.0–2.0$. Even mixtures of sunflower and sesame oils with the addition of antioxidants obtained values between 2.03 and 2.19 (Ghosh *et al.*, 2019), the same for the blends of chia and sesame 1.96 to 2.12 (Rodríguez *et al.*, 2020).

4 Conclusions

The present study demonstrated that it is possible to significantly increase the shelf life of AO with the addition of VOO, achieving an extension between 8 to 48 days under accelerated study conditions (Rancimat), projected at normal storage temperatures (25 °C), without involving significant changes at the level of physicochemical characteristics of the product ($p < 0.05$). The AO/VOO formulations of 30/70 and M4: 20/80 (w/w), produced nutritionally attractive ω-6/ω-3 ratios. The advances obtained and developed in this study can be applied to the food industry as a strategy to improve preservation, nutrition and development of new products.

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Conflicts of interest

The authors declare no conflicts of interest in relation to this article.

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