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# Extraction of bioactive lipids from *Pleuroncodes monodon* using organic solvents and supercritical $CO_2$

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**SUMMARY:** A huge volume of *Engraulis ringens* (Peruvian anchoveta) is caught together with the species *Pleuroncodes monodon* (munida), whose potential bioactive lipids are not commercially exploited. In the present study, lipid with carotenoid pigment (astaxanthin) and essential fatty acids (EPA+DHA) were obtained from munida lipids extracted with hexane:isopropyl alcohol (He-I), acetone (Ac), ethanol (Et) and supercritical $CO_2$ + ethanol (SC-$CO_2$-Et). The functional quality of the fatty acids was determined by atherogenicity index (AI), thrombogenicity index (TI) and the hypocholesterolemia:hypercholesterolemia (H:H) ratio. The highest astaxanthin (ASTX) contents (4238.65 and 4086.71 µg/g lipid) corresponded to extractions using Ac and SC-$CO_2$-Et. EPA+DHA ranged from 31.15 to 31.85% and the functional quality ranges were between 0.56-0.61 (AI), 0.19-0.21 (TI) and 1.73-1.81 (H:H). Consequently, SC-$CO_2$-Et extraction would be advisable because of its low environmental impact. The IA and IT quality indexes suggest that the consumption of munida lipids would be healthy, although the H:H ratio shows the opposite.

***KEYWORDS:*** *Astaxanthin; EPA+DHA; Fatty acid profile; Functional quality index; Munida; Red squat lobster.*

**RESUMEN:** ***Obtención de lípidos bioactivos de* Pleuroncodes monodon *utilizando solventes orgánicos y $CO_2$ supercrítico.*** Las enormes capturas de *Engraulis ringens* (anchoveta Peruana) son acompañadas por la especie *Pleuroncodes monodon* (munida) cuyo potencial en lípidos bioactivos no es aprovechado comercialmente. En el presente estudio se obtuvo lípidos con pigmentos carotenoides (astaxantina) y ácidos grasos esenciales (EPA+DHA) a partir de lípidos de munida extraídos con hexano:alcohol isopropílico (He-I), acetona (Ac), etanol (Et) y $CO_2$ supercrítico + etanol (SC-$CO_2$-Et). La calidad funcional de los ácidos grasos fue evaluada mediante índices de aterogenicidad (AI), trombogenicidad (TI) y la relación hipocolesterolemia:hipercolesterolémica (H:H). Los mayores contenidos de astaxantina (ASTX) (4238.65 y 4086.71 µg/g de lípido) fueron obtenidos utilizando Ac y SC-$CO_2$-Et. En todas las muestras EPA+DHA osciló entre 31.15 y 31.85% y los rangos de índices de calidad funcional fueron: 0.56-0.61 (AI), 0.19-0.21 (TI) y 1.73-1.81 (H:H). Se concluye que la extracción SC-$CO_2$-Et sería recomendable por su bajo impacto al medio ambiente. Los índices de calidad AI y TI sugieren que el consumo de lípido de munida podría ser saludable, aunque la relación H:H muestra lo contrario.

***PALABRAS CLAVE:*** *Astaxantina; Camaroncito rojo; EPA+DHA; Índice de calidad funcional; Munida; Perfil de ácidos grasos.*

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## 1. INTRODUCTION

The "munida" or red squat lobster (*Pleuroncodes monodon*), is a decapod marine crustacean with an elongated body, belonging to the family Munididae (Santamaría *et al*., 2018). In the Peruvian sea its large biomass accompanies the anchoveta (*Engraulis ringens*), Castillo *et al*. (2020) make estimates of 2,201,712 and 1,687,044 t in summer and spring, 2019, respectively. Despite the abundant biological information on munida, studies on the extraction and quantification of its bioactive components are required in terms of value generation and commercial use.

Marine lipids are known to be the main source of polyunsaturated fatty acids (PUFA), especially ω-3 fatty acids (eicosapentaenoic acid EPA; 20:5 ω-3 and docosahexaenoic acid DHA; 22:6 ω-3) which are considered essential because of their significant influence on biochemical and physiological processes involved in human health (Narayan *et al*., 2006).

The biological functionality of edible oils is assessed by indexes based on the fatty acid contents, on the AI pro-atherogenic and anti-atherogenic fatty acids ratio, on the TI or ratio of saturated (pro-thrombogenic) and unsaturated (anti-thrombogenic) fatty acids, and H:H index, correlating unsaturated and saturated fatty acids (Chen and Liu, 2020).

Besides of the referred lipids, marine crustaceans are a source of pigments such as astaxanthin (ASTX), a 40-carbon ketocarotenoid (3,3'-dihydroxy-β,β'-carotene-4,4'-dione) belonging to the xanthophyll family (Núñez-Gastélum *et al*., 2016). Natural ASTX has been referred to as a supercarotenoid with high levels of health protection and anti-inflammatory effects among other benefits (Capelli, 2018). In addition, it has a high antioxidant capacity associated with reduced risk of oxidative stress-generated diseases, such as cardiovascular diseases (Régnier *et al*., 2015).

Regarding the extraction of ASTX by solvents, it is known that its high polarity favors the process. Routray *et al*. (2019) used different organic solvents of medium polarity and their mixtures and concluded that hexane was not a good option, although its combination with acetone improved extraction efficiency. An alternative method is the supercritical fluid extraction (SFE) which offers technological and ecological advantages as well as obtaining analytes without exposure to oxygen or thermal damage. Efficient extractions of phospholipids and glycolipids from *Farfantepenaeus paulensis* were conducted using supercritical $CO_2$ + 15% ethanol (Sánchez-Camargo *et al*., 2012).

The objective of this study is focused on the extraction of lipids from munida using solvents hexane + isopropyl alcohol, acetone, absolute ethanol and Supercritical $CO_2$ + ethanol and the evaluation of the quality of their bioactive lipid components (ASTX, EPA and DHA).

## 2. MATERIALS AND METHODS

### 2.1. Characteristics of the raw material

Munida specimens were frozen on board inmediately after caught by the scientific research vessel "Humboldt" belonging to Instituto de Mar del Perú (IMARPE) in June 2019 at the area 18°6' 20.401" S & 70°48' 14.4" W, in front of Caleta Vila Vila (Tacna), 3.5 nm off the southern coast of Peru. Samples were placed in thermal boxes to maintain cooling until arrival to the Bioactive Compounds Laboratory of Instituto Tecnológico de la Producción (ITP). The size distribution of the specimens ranged from 10 to 16 mm cephalothorax length, mean of 12.9 ± 1.2 mm, mode of 13 mm; female specimens represented 54.4% of total samples with a mode of 13 mm, while males registered 14 mm.

### 2.2. Sample preparation

50 kg of "munida" were placed in a cold air dryer (CV-20AN, ASAHI, Japan) at 21 °C for 27 h, then crushed in an analytical mill (A11 basic, IKA, USA) and sieved to obtain a homogeneous material between 0.50 and 0.85 mm particle size. The munida meal (MM) was packed in vacuum-sealed bags and kept at -18 °C until analysis.

### 2.3. Proximal chemical composition

Moisture, fat, ash and protein contents were determined by duplicate determinations of fresh munida and MM according to FAO (1986) methodologies.

### 2.4. MM lipid extraction methods

Four different lipid extraction procedures were performed using a mixture of hexane + isopropyl

alcohol 60:40 (v/v) (He-I), acetone (Ac), absolute ethanol (Et) and Supercritical $CO_2$ + ethanol as cosolvent (SC-$CO_2$-Et).

***He-I:*** The technique described by Sachindra *et al*. (2006) was followed. A mixture 60:40 hexane ACS (Fermont, Mexico) with HPLC grade isopropyl alcohol (Fisher Scientific, Spain) was used to dissolve 8 g MM sample in 50 mL tubes with 40 mL, vortexed for 2 min, sonicated at 25 °C for 10 min and centrifuged (Centrifuge 5804 R, Eppendorf, Brazil) for 40 min at 3200 g at 4 °C after 5 min resting time. The extract was filtered through Whatman Nº 42 filter paper and the residue was subjected to further extraction following the same procedure.

***Ac:*** 8 g MM were placed in 50 mL tubes with 40 mL of Ac (ACS, Merck, 99.5% purity), vortexed for 2 min, sonicated at 25 °C for 10 min and centrifuged for 40 min at 3200 g at 4 °C after 5 min resting time. The extract was filtered using Whatman Nº 42 filter paper and the residue was treated with two additional extractions.

***Et:*** According to the Dalei and Sahoo (2015) methodology 10 g MM were thoroughly homogenized with 100 mL Et (ACS Sharlau, Spain) for 1 hour using a magnetic stirrer. The extract was filtered through Whatman Nº 42 filter paper. Solid recovery was performed on the residue by 4 extractions until the filtrate was colorless.

***SC-$CO_2$-Et:*** A multi-solvent extractor Model 2802.000 (Top Industrie, France) equipped with a $CO_2$ pump (HPFlow Pump 50 - 100), co-solvent pump (90-2491 REV L, SSI), chiller (PCPR 13.02-NED, National Lab), reactor (ø 163 x 353 mm) and a stainless-steel separator (ø 78 x 278 mm) to receive the lipid were used (Barriga-Sánchez *et al.,* 2022). Pressure was manually controlled by a back pressure regulator.

The extraction of 35 g MM by SC-$CO_2$-Et was performed following the reference parameters reported by Sánchez-Camargo *et al*. (2012), 200 bar, temperature 50 °C and a solvent ratio 85/15 ($CO_2$/ethanol) for 2 h. Evaporation of the solvent was carried out using a rotary evaporator (Laborota 4003, Heidolph) at 40 °C and the residue was stored in Ultrapure nitrogen atmosphere (Linde Peru) at -19 °C, until further analysis.

Analyses were conducted in three replicates.

### 2.5. Lipid yield in MM

The MM lipid yield was obtained by calculations according to Equation 1.

$$Yield\ (\%) = \frac{MM\ lipid\ weight}{MM\ weight} x100 \quad (1)$$

### 2.6. Thin layer chromatography (TLC)

The methodology of Núñez-Gastélum *et al*. (2016) was applied in all samples. 1 g lipid was dissolved in 1 mL ACS petroleum ether (Tedia, USA) vortexing for 1 min. 5 µL of each sample were placed on a silica gel 60 $F_{254}$ plate (Merck, Germany) pre-dried at 110 °C for 2 h. The plate was placed in a chamber saturated with 50 mL acetone: hexane (25:75, v/v) as the mobile phase.

Bands were visualized under a 254 nm UV TLC lamp (Merck) and identified by comparing the Retention Factor (Rf) value with the standard ASTX by applying Equation 2. Tests were conducted in triplicate.

$$Rf = \frac{\text{Solute migration distance}}{\text{mobile phase migration distance}} \quad (2)$$

### 2.7. Determination of total carotenoids expressed as ASTX

Total carotenoids expressed as ASTX were determined in all samples according to the methodology of Sánchez-Camargo *et al*. (2011). A standard solution of ASTX (98.6%, Dr Ehrenstorfer) was prepared by diluting 1 to 5 µg/mL of ASTX standard in hexane. 50 mg lipid sample were diluted to 10 mL in hexane. The absorbance value of each solution and the sample were measured at 472 nm (highest absorbance observed) using a UV-200 Spectrophotometer (Shimadzu, Japan) with hexane as the calibration blank**.** Carotenoids were expressed as µg ASTX/g lipid and µg ASTX/g MM.

### 2.8. Fatty acid chromatography

Fatty acids were determined as described by Prevot and Mordret (1976). A gas chromatograph with a FID detector (Autosystem XL, Perkin Elmer, USA) equipped with a Supelcowax 10 column (Merck, Germany) (30 m × 0.25 mm id; film thickness: 0.25

μm) was used. Peak areas were calculated using Total Chrom Navigator software (Version: 6.2.0.0.0:B27, 2001, USA), and each fatty acid percentage was calculated by comparing the individual peak area with the fatty acid total area. The fatty acid peaks were identified by comparison with the retention times of the standard F.A.M.E. Mix C4-C24 (Supelco, Sigma-Aldrich Inc, USA).

### 2.9. Functional quality of MM lipid

The fatty acid profile of the MM lipid was used to determine its functional quality by means of the AI and TI according to equations 3 and 4, respectively (Ulbricht and Southgate, 1991). The H:H was evaluated in accordance with equation 5 as defined by (Santos-Silva *et al*., 2002).

$$AI=\frac{(C12:0)+4(C14:0)+(C16:0)}{(\sum MUFA)+(\sum \omega\text{-}6)+(\sum \omega\text{-}3)} \quad (3)$$

$$TI=\frac{(C14:0)+(C16:0)+(C18:0)}{0.5(\sum MUFA)+0.5(\sum \omega\text{-}6)+3(\sum \omega\text{-}3)+(\frac{\sum \omega\text{-}3}{\sum \omega\text{-}6})} \quad (4)$$

$$H:H=\frac{(C18:1\omega\text{-}9)+(C18:2\omega\text{-}6)+(C20:4\omega\text{-}6)+(C18:3\omega\text{-}3)+(C20:5\omega\text{-}3)+(C22:5\omega\text{-}3)+(C22:6\omega\text{-}3)}{(C14:0)+(C16:0)} \quad (5)$$

Where: C12:0 (lauric acid); C14:0 (myristic acid); C16:0 (palmitic acid); C18:0 (stearic acid); C18:1 ω-9 (oleic acid); C18:2 ω-6 (linoleic acid); C18:3 ω-3 (linolenic acid); C20:4 ω-6 (arachidonic acid); C20:5 ω-3 (eicosapentaenoic acid); C22:5 ω-3 (docosapentaenoic acid); C22:6 ω-3 (docosahexaenoic acid); MUFA (monounsaturated Fatty Acids).

### 2.10. Statistical analysis

Minitab version 17 was used for analysis of variance and Tuckey's comparison test for lipid yield data, ASTX contents and fatty acid profile obtained for each extraction procedure.

## 3. RESULTS AND DISCUSSION

### 3.1. Proximal Chemical Composition (PCC)

Table 1 shows similar fat content in munida fresh samples to the data obtained by Albrecht-Ruiz and Cueto (2006) and *P. planipes* fresh samples (Fonseca-Rodríguez and Chavarría-Solera, 2017) and meal data (Civera *et al*., 2000) showing the higher yield oil in munida as an advantage.

Fat is one of the most variable components in marine animals and is influenced by biotic and abiotic factors (age, catching area, time and depth of capture). Bascur *et al*. (2017) investigated the effect of seasonal variations and food availability to which *P. monodon* ovigerous females were exposed during their reproductive period (February to December) and during winter. The results indicated that these organisms adjusted their biochemical processes to ensure their survival and that of their embryos.

### 3.2. Thin Layer Chromatography (TLC)

Table 2 shows that ASTX (Rf 0.53) was identified in all *P. monodon* oil extracts. The values obtained were 0.63 and 0.81, which would evidence the esterified form of this molecule (monoesters and

Table 1. Munida proximal chemical composición (g/100g sample)

| | Moisture | Fat | Protein | Ashes |
|---|---|---|---|---|
| Fresh munida (*P. monodon*)[1] | 73.57 ± 0.07 | 6.06 ± 0.08 | 10.69 ± 0.11 | 7.59 ± 0.09 |
| Fresh munida (*P. monodon*)[2] | 74.2 | 6.50 | 10.60 | 4.70 |
| Fresh munida (*P. planipes*) [3] | 83.12 ±1.66 | 1.16 ± 0.28 | 13.52 ± 1.15 | 1.51 ± 0.44 |
| Munida meal (MM)[1] | 9.17 ± 0.02 | 23.16 ± 0.26 | 34.5 ± 0.12 | 15.15 ± 0.06 |
| *P. planipes* meal [3] | 7.83 ± 1.44 | 8.04 ± 1.42 | 40.45 ± 2.56 | 39.00 ± 1.55 |

Values in the Table are mean ±SD of duplicate analyses. [1]Results obtained in the present study, [2] Albrecht-Ruiz and Cueto, (2006); [3]Civera *et al.,* (2000).

Table 2. Retention factors (Rf) of munida *(P. monodon*) lipid extracted with different solvents

| Sample | Rf |
|---|---|
| ASTX standard | 0.53 |
| Lipid extracted by Et | 0.53, 0.63, 0.81 y 0.99 |
| Lipid extracted by Ac | 0.53, 0.63, 0.81 y 0.99 |
| Lipid extracted by He-I | 0.53, 0.63, 0.81 y 0.99 |
| Lipid extracted by SC-$CO_2$-Et | 0.53, 0.63, 0.81 y 0.99 |

Et = absolute ethanol, He-I = hexane and isopropyl alcohol 60:40 (v/v), Ac = acetone, SC-$CO_2$-Et = Supercritical $CO_2$ + ethanol.

diesters) as typical forms which are characteristic in crustaceans (Hornero-Méndez, 2019). The Rf values obtained also agree with the results obtained by Dalei and Sahoo (2015) in crustacean shell residues. These authors also refer to the fact that Rf 0.99 evidences the presence of β-carotene, a molecule that would also be present in the munida lipids.

### 3.3. Lipid extraction yield

The highest efficiency in lipid extraction from the MM sample was obtained using Et as solvent. Likewise, Xie *et al*. (2018) reported the advantage of Et in SC-$CO_2$ extraction compared to 3 solvents per step working with krill meal. This result could be explained by the SC-$CO_2$ increased polarity and its ability to dissociate protein-phospholipid complexes (Hardardottir and Kinsella, 1988). However, Ali-Nehari *et al*. (2012) reported higher efficiency in krill meal lipid extraction using hexane (16.2% lipids) compared to SC-$CO_2$-Et (12.2%), although Xie *et al*. (2017) reported higher yields with Et (16.33%) in comparison to hexane yields (12.18%), explaining that alcoholic solvents are more efficient for krill meal lipid extraction.

### 3.4. Content of carotenoids expressed as ASTX

Carotenoid values expressed as ASTX and extracted under the conditions established in the present study ranged from 2998.01 to 4238.65 µg /g lipid. The highest values were obtained using Ac and SC-$CO_2$-Et (Table 3). The higher efficiency of Ac compared to Et for extracting ASTX from crustacean lipids has been demonstrated by Dalei and Sahoo (2015) and Xie *et al*. (2018). The lower polarity of Ac facilitates its penetration through the hydrophobic mass surrounding the pigment and favors its miscibility (Dalei and Sahoo, 2015).

All ASTX values obtained in MM by means of the proposed treatments exceeded those reported in residues of *Farfantepenaeus paulensis*, a species belonging to the genus *Penaeus*, (1074 µg ASTX/g lipid) (Sánchez-Camargo *et al*., 2011) and those obtained in krill oil extracted with SC-$CO_2$-Et (86.2 µg ASTX/g lipid) and hexane (103.2 µg ASTX/g lipid) by Ali-Nehari *et al*. (2012). These results suggest not only the affinities of the solvent and extraction conditions but that munida would represent a source of higher contents of ASTX compounds compared to similar species.

Typically, the choice of solvent is made according to the polarity of the target compound. Routray *et al*. (2019) reported improved ASTX extraction efficiency when using hexane combined with other solvents, although in the present work the use of He-I mixture extracted the lowest ASTX values indicating that isopropyl alcohol did not improve ASTX extraction efficiency (Table 3).

The results of SC-$CO_2$-Et extraction are in agreement with Routray *et al*. (2019) research data on the significant improvement in this extraction technology to recover ASTX using Et as cosolvent. Also,

Table 3. Lipid yields (%) in munida meal (MM) and ASTX in munida (*Pleuroncodes monodon*) lipid as extracted by different solvents

| Extraction methods | Yield (g lipids/100 g MM) | Content of carotenoids (µg ASTX /g lipid) |
|---|---|---|
| He-I | 17.29 ± 0.45 $^{c}$ | 2998.01 ± 81.54 $^{c}$ |
| Ac | 14.71 ± 0.29 $^{d}$ | 4238.65 ± 21.04 $^{a}$ |
| Et | 22.93 ± 0.71 $^{a}$ | 3443.23 ± 126.30 $^{b}$ |
| SC-$CO_2$-Et | 18.90 ± 0.36 $^{b}$ | 4086.71 ± 80.11 $^{a}$ |

He-I = hexane and isopropyl alcohol 60:40 (v/v), Ac = acetone, Et = absolute ethanol, SC-$CO_2$-Et = Supercritical $CO_2$ + ethanol. Data are shown as mean ± standard deviation. Different letters in the same column indicate significant difference ($p < 0.05$). Tukey test ($p < 0.05$) was used for the comparison of means. All experiments were carried out in duplicate.

Sánchez-Camargo *et al*. (2011) reported 15% Et as co-solvent to substantially improve ASTX extraction, and highlighted the advantage of the solubilization of polar compounds such as phospholipids and glycolipids.

According to Capelli (2018) the recommended daily intake of ASTX (4 mg) can be provided by one gram of munida lipid obtained by Ac and SC-$CO_2$-Et extractions (4238.65 and 4086.71 µg/g lipid, respectively); nevertheless, considering Ac toxicity, the use of SC-$CO_2$-Et is considered the best extraction option for safety concerns among the extraction methods evaluated.

### 3.5. Fatty acids

Table 4 shows the fatty acid profile of munida lipids obtained by the different lipid extraction methods. Among the saturated fatty acids (SFA) C16:0 represented the highest percentage in all the extracts while C18:1 ω-9 was the most abundant among monounsaturated fatty acids (MUFA) and its quantity was not affected by the extraction method applied.

A predominant presence of long-chain PUFA acids was observed, and ranged from 40.79 to 41.51% with high values of EPA and DHA. No significant

Table 4. Fatty acids content in munida (*Pleuroncodes monodon*) lipid (%) extracted with different solvents

| Fatty acids | Extraction Procedures | | | |
|---|---|---|---|---|
| | He-I | Ac | Et | SC- $CO_2$-Et |
| C14:0 (Myristic) | 4.04±0.17 $^{b}$ | 4.38±0.15 $^{ab}$ | 4.48±0.03 $^{a}$ | 4.38±0.00$^{ab}$ |
| C14:1 (Myristoleic) | 0.19±0.00 | Nd | Nd | Nd |
| C15:0 (Pentadecaenoico) | 0.41±0.01 $^{a}$ | 0.42±0.01 $^{a}$ | 0.42±0.00 $^{a}$ | Nd |
| C16:0 (Palmitic) | 21.76±0.40 $^{b}$ | 21.99±0.23 $^{ab}$ | 21.95±0.02 $^{ab}$ | 22.71±0.01 $^{a}$ |
| C16:1 (Palmitoleic) | 7.14±0.16 $^{c}$ | 7.80±0.10 $^{a}$ | 7.54±0.01 $^{ab}$ | 7.19±0.03 $^{bc}$ |
| C17:0 (Heptadecaenoic) | 2.76±0.01 $^{ab}$ | 2.79±0.13 $^{ab}$ | 2.60±0.11 $^{b}$ | 2.96±0.02 $^{a}$ |
| C17:1 (Cis-10-Heptadecenoic) | 0.84±0.02 $^{a}$ | 0.17±0.01 c | 0.36±0.00 $^{b}$ | 0.16±0.00 $^{c}$ |
| C18:0 (Estearic) | 2.96±0.01 $^{a}$ | 2.69±0.01 $^{b}$ | 2.67±0.01 $^{a}$ | 2.92±0.02 $^{a}$ |
| C18:1 ω-9 (Oleic) | 12.92±0.05 $^{a}$ | 13.15±0.03 $^{a}$ | 12.89±0.02 $^{a}$ | 12.9±0.02 $^{a}$ |
| C18:1 ω-7 (Vaccenic) | 4.36±0.04 $^{a}$ | 4.40±0.01 $^{a}$ | 4.36±0.02 $^{a}$ | 4.46±0.01 $^{a}$ |
| C18:2 ω-6 (Linoleic) | 1.43±0.00 $^{a}$ | 1.38±0.00 $^{b}$ | 1.43±0.00 $^{a}$ | 1.38±0.01 $^{b}$ |
| C18:3 ω-6 (γ-Linolenic) | 0.74±0.01 $^{b}$ | 0.81±0.00 $^{a}$ | 0.73±0.01 $^{b}$ | 0.74±0.01 $^{b}$ |
| C18:3 ω-3 (α-Linolenic) | 0.99±0.00 $^{a}$ | 1.00±0.00 $^{a}$ | 1.00±0.02 $^{a}$ | 0.89±0.00 $^{b}$ |
| C18:4 ω-3 (Stearidonic) | 5.71±0.35 $^{a}$ | 5.81±0.00 $^{a}$ | 5.81±0.02 $^{a}$ | 5.36±0.01 $^{a}$ |
| C20:0 (Arachidic) | 0.47±0.00 $^{a}$ | 0.46±0.00 $^{a}$ | 0.38±0.00 $^{b}$ | 0.47±0.02 $^{a}$ |
| C20:1 ω-9 (Eicosaenoic) | 0.92±0.00 $^{a}$ | 0.96±0.02 $^{a}$ | 0.83±0.00 $^{b}$ | 0.85±0.01 $^{b}$ |
| C20:2 (Eicosadienoic) | 0.26±0.06 $^{a}$ | Nd | Nd | 0.22±0.01 $^{b}$ |
| C20:3 ω-6 (Eicosatrienoic) | Nd | Nd | Nd | 0.66±0.00 |
| C20:3 ω-3 (Eicosatrienoic) | 0.64±0.01$^{b}$ | 0.58±0.01$^{c}$ | 0.70±0.03$^{a}$ | Nd |
| C20:4 ω-6 (Araquidonic) | 0.31±0.01 | Nd | Nd | Nd |
| C20:5 ω-3 (EPA) | 11.32±0.07 $^{b}$ | 11.26±0.07 $^{b}$ | 11.82±0.02 $^{a}$ | 11.31±0.01 $^{b}$ |
| C22:6 ω-3 (DHA) | 19.83±0.27 $^{a}$ | 19.96±0.22 $^{a}$ | 20.04±0.04 $^{a}$ | 20.43±0.01 $^{a}$ |
| SFA | 32.39±0.60 $^{a}$ | 32.73±0.24 $^{a}$ | 32.51±0.16 $^{a}$ | 33.44±0.03 $^{a}$ |
| MUFA | 26.38±0.15 $^{a}$ | 26.48±0.05 $^{a}$ | 25.98±0.03 $^{b}$ | 25.56±0.02 $^{c}$ |
| PUFA | 41.23±0.75 $^{a}$ | 40.79±0.30 $^{a}$ | 41.51±0.13 $^{a}$ | 41.00±0.05 $^{a}$ |
| ∑ ω-3 | 38.48±0.70 $^{a}$ | 38.60±0.30 $^{a}$ | 39.36±0.13 $^{a}$ | 37.99±0.04 $^{a}$ |
| EPA + DHA | 31.15±0.35 $^{a}$ | 31.21±0.29 $^{a}$ | 31.85±0.06 $^{a}$ | 31.75±0.03 $^{a}$ |

Et = absolute ethanol, He-I = hexane and isopropyl alcohol 60:40 (v/v), Ac = acetone, SC-$CO_2$-Et = Supercritical $CO_2$ + ethanol. Data are shown as mean ± standard deviation. Different letters in the same row indicate significant difference ($p < 0.05$), Tukey test ($p < 0.05$) was used for the comparison of means. All experiments were carried out in duplicate.
Nd: no detected.

differences ($p > 0.05$) regarding the extraction methods were observed. The values reported in the present study exceeded those obtained by Xie *et al*. (2017) using Ac as solvent in three krill species as well as by Ali-Nehari *et al*. (2012) in krill oil. The later one found higher efficiency by using SC-$CO_2$-Et compared to hexane. These high long-chain PUFA contents are considered essential for membrane fluidity and inflammatory mediator functionality and show potential benefits in neuronal development and cardiovascular health (Janssen and Kiliaan, 2014).

The high C16:0 and C20:5 ω-3 values in munida lipid samples were similar to those reported by Ali-Nehari *et al*. (2012) in oily extracts of krill, in residues of *Farfantepenaeus paulensis* (Sánchez-Camargo *et al*., 2011) and in *Litopenaeus vannamei*; while the C22:6 ω-3 contents were higher in the samples of the present study.

The EPA+DHA contents in *P. monodon* showed no significant differences, and the values obtained by the extraction techniques ranged from 31.15 to 31.85%. ω-6 fatty acids were not detectable in some cases, as in the case of C20:3 ω-6 and C20:4 ω-6. C18:2 ω-6 was the omega-6 fatty acid with the highest content. The results obtained in the present study show similarity to those reported by Ali-Nehari *et al*. (2012) and Xie *et al*. (2017) for krill oil.

### 3.6. Functional quality of MM lipid

No differences were observed among the AI values of the MM lipids obtained with all the solvents used in the present study (Table 5), although these values were lower than those calculated from studies carried out by Xie *et al*. (2017) and Sánchez-Camargo *et al*. (2012) for *Euphausia superba* and *Penaeus paulensis* respectively. This would be advantageous in the case of munida, considering that Turan *et al*. (2007) refer to AI values and also thrombogenicity indexes (TI) close to zero, which are considered favorable for preventing coronary heart disease.

The AI and TI values obtained in the present study are slightly higher than those obtained by Lopes *et al*. (2014) for grape pomace oil (AI= 0.18-0.32, TI= 0.06-0.17). Studies by Pinto *et al*. (2020) on *Endopleura uchi* oil reported AI values similar to those obtained in our work, although their TI values were higher (AI=0.44, TI=1.32) than ours.

Regarding the use of solvents, the highest H:H ratio was observed in the extracted munida lipids using the He-I mixture (1.81). This value exceeded those obtained by Xie *et al*. (2017) and Xie *et al*. (2018), although it was lower than that obtained by Sánchez-Camargo *et al*. (2012) for *Penaeus paulensis* when using SC-$CO_2$-Et. Low H:H values are considered unfavorable as they may induce an increase in cholesterolemia (Santos-Silva *et al*., 2002); while high values‒like 2.66 in uxi (*Endopleura uchi*) are recommended by Pinto *et al*. (2020).

Our results suggest that the SC-$CO_2$-Et lipid extraction method applied in Peruvian marine species of commercial importance contributes to quantifying carotenoid pigments and essential fatty acids (EPA and DHA) among other bioactive comounds. *Argopecten purpuratus* (scallops), *Romaleon setosum*, *Cancer porteri*, *Platymera gaudichaudii*, *Paralomis longipes* (crabs) *Loxechimus albus* (sea urchin) and many other fish species represent a promising dietary source.

Table 5. Functional Quality Indexes in munida lipid compared to lipid data from other species data

| Solvent | Crustacean | AI | TI | H:H |
|---|---|---|---|---|
| He-I[1] | *Pleuroncodes monodon* | 0.56 | 0.20 | 1.81 |
| Ac[1] | | 0.59 | 0.20 | 1.77 |
| Et[1] | | 0.59 | 0.19 | 1.78 |
| SC-$CO_2$-Et[1] | | 0.61 | 0.21 | 1.73 |
| Ethanol[2] | *Euphausia superba* | 1.96 | 0.24 | 1.32 |
| Acetone[2] | | 2.85 | 0.31 | 1.14 |
| Ethanol[3] | *Euphausia superba* | 1.24 | 0.17 | 1.64 |
| Supercritical $CO_2$ + ethanol[4] | *Penaeus paulensis* | 0.97 | 0.40 | 2.16 |

[1]Our data; calculated based on the results of: [2] Xie *et al*. (2017), [3]Xie *et al*. (2018), [4]Sánchez-Camargo *et al*. (2012). He-I = hexane and isopropyl alcohol 60:40 (v/v), Ac = acetone, Et = absolute ethanol, SC-$CO_2$-Et = Supercritical $CO_2$ + ethanol, AI: atherogenicity index, TI: thrombogenicity index, H:H: Hypocholesterolemia: hypercholesterolemia ratio.

## 4. CONCLUSIONS

Higher ASTX contents were obtained from munida using Ac and SC-$CO_2$-Et, followed by Et extractions. On the other hand, the contents of SFA, PUFA, omega-3, EPA+DHA in munida lipids with all extraction solvents showed no significant differences. PUFA were the most predominant in the lipid. Among SFA and MUFA, C16:0 and C18:1 ω-9 were prevalent. The sum of C20:5 ω-3 and C22:6 ω-3 varied from 31.15 to 31.85%.

The functional quality indexes AI and TI for munid lipid were favorable; while the H:H values were low. The results of the present study suggest that it is an important source of lipids which contain ASTX, EPA and DHA. The extraction of munida lipids with Et or SC-$CO_2$-Et in further studies are suggested for possible application in the food industry.

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## CONFLICTS OF INTERESTS

No potential conflict of interest was reported by the authors**.**

## DECLARATION OF ETHICS

The authors hereby declare their agreement with this publication and their contributions to justify their authorship; that there is no conflict of interest; and that they have complied with all relevant ethical and legal requirements and procedures. All sources of funding are fully and clearly detailed in the acknowledgement section. The respective signed legal document is on file with the journal.

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