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Significant Enrichment of Polyunsaturated Fatty Acids (PUFAs) in the Lipids Extracted by Supercritical CO_2 from the Livers of Australian Rock Lobsters (*Jasus edwardsii*)

Trung T. Nguyen,[†,‡,§] Wei Zhang,*[,†,‡] Andrew R. Barber,[†,‡] Peng Su,[†,‡] and Shan He[†,‡]

[†]Centre for Marine Bioproducts Development and [‡]Department of Medical Biotechnology, School of Medicine, Flinders University, Adelaide, South Australia 5042, Australia

[§]Food Science and Technology Department, Agricultural and Natural Resources Faculty, An Giang University, Long Xuyen, Vietnam

ABSTRACT: Australian rock lobster (*Jasus edwardsii*) liver contains approximately 24.3% (w/w) lipids, which can contain a high amount of polyunsaturated fatty acids (PUFAs). However, this material has been found to be contaminated with arsenic (240 mg/kg) and cadmium (8 mg/kg). The high level of contaminants in the raw material and the large amount of PUFAs in the lipids prove a significant challenge in the extraction of high-quality lipids from this byproduct by conventional methods. Supercritical carbon dioxide (SC-CO_2) extraction is a highly promising technology for lipid extraction with advantages including low contamination and low oxidation. The technique was optimized to achieve nearly 94% extraction of lipids relative to conventional Soxhlet extraction in Australian rock lobster liver at conditions of 35 MPa and 50 °C for 4 h. The extracted lipids are significantly enriched in PUFAs at 31.3% of total lipids, 4 times higher than those in the lipids recovered by Soxhlet extraction (7.8%). Specifically, the concentrations of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in SC-CO_2 extraction are 7 times higher than those obtained by Soxhlet extraction. Moreover, very small amounts of toxic heavy metals such as lead (Pb), arsenic (As), mercury (Hg), and cadmium (Cd) were detected in the SC-CO_2-extracted lipids, 0.5−27 times lower than those in the Soxhlet-extracted lipids, which are 40−200 times lower than the regulatory limit maximum values. The low levels of contaminants and the high proportion of PUFAs (dominated by DHA and EPA) found in the SC-CO_2-extracted lipids from Australian rock lobster liver suggest that the material could potentially be used as a valuable source of essential fatty acids for human consumption.

KEYWORDS: *Australian rock lobster (Jasus ewardsii), rock lobster liver, supercritical CO_2 extraction, ω-3 fatty acids, heavy metal*

INTRODUCTION

Polyunsaturated fatty acids (PUFAs) have been extensively reported in the literature as being beneficial to human health.[1−3] In particular, omega-3 (ω-3) or highly unsaturated fatty acids (HUFAs) are believed to play an important role in the prevention of certain health problems such as diabetes, allergies, and some types of cancers, as well as having antithrombotic, antiarrhythmic, and anti-inflammatory effects.[4] Fishery processing byproducts have been recognized as an important potential source of ω-3 fatty acids, which could be used in the production of ω-3-rich lipids. According to a previous investigation by Tsvetnenko et al.,[5] one major source of PUFAs in the Australian rock lobster (*Panulirus cygnus*) is the hepatopancreas (liver), but this inedible part is usually removed during processing and discarded. Australia is the largest producer and exporter of rock lobster in the world.[5] More than 10000 tons of rock lobster processing wastes including heads, shells, and livers are produced each year, in which the lobster liver accounts for 2−5%. However, only about 25% of these materials are utilized for manufactured products and almost entirely for low commercial value items, such as animal feed, aquafeed, and biofertilizer; the other 75% of the materials are often disposed of as waste with a cost to the processor of up to $150/ton.[6] As a result of the cost and environmental burdens, the Australian lobster processing industry is examining how to utilize these processing coproducts, especially the rock lobster liver, as a valuable potential resource for the production of ω-3-rich lipids.

In recent years, processing coproducts from different types of fish including tuna,[7] herring,[8] salmon,[9] walleye pollock,[10] and shrimp[11] have been extensively studied for lipid extraction; however, very little work has been done on rock lobster liver. It is also important to improve the current extraction procedure of marine coproducts to produce lipids with a high concentration of PUFAs and low contaminants of heavy metals.

Currently, there are several available technologies for the extraction of lipids and pigments from crustacean processing coproducts. The most common technology is solvent extraction.[3,11,12] The solvent selection, a decisive factor in the quality of the extracted lipids, is crucial. Lipids are generally soluble in nonpolar solvents that have been traditionally used for lipid extraction.[13] Several organic solvents including acetone, ethyl acetate, hexane, isopropanol, methanol, methyl ethyl ketone, and ethanol are used in the food industry, whereas others such as dicloromethane, dimethyl sulfoxide, and chloroform cannot be used due to their toxicity.[14] There is, however, increasing public awareness of the health, environ-

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mental, and safety hazards related to the use of organic solvents for lipid extraction and the possible solvent contamination of the final lipid products. These issues have stimulated the interest in developing alternative extraction methods.[15] As such, supercritical fluid extraction (SFE) has become an important technology for extracting high-quality lipids[16−19] from fishery processing coproducts[20−22] and is also performed as an effective separation technique in the production of nutraceutical supplements and functional foods.[23,24] SFE can be employed at moderate temperature and provides an oxygen-free medium in reducing lipid oxidation during the extraction process. In addition, it allows for the selective extraction of low-polar lipid compounds, avoiding the coextraction polar impurities such as some organic derivatives with heavy metals.[25] Supercritical carbon dioxide (SC-CO_2) extraction is the common SFE technology because this technique is able to extract the heat-sensitive, easily oxidized compounds such as PUFAs and omega-3[17−19,21,22,26] without any contaminated residues in the products. Moreover, CO_2 is a generally recognized as safe (GRAS) solvent type, which is relatively cheap and easy to separate from the solid matrix and extracts.[3,13,23] The excellent extraction performance of the SC-CO_2 extraction is a result of the low viscosity of SC-CO_2, high diffusivity, and high solubility. In contrast, conventional extraction processes such as Soxhlet extraction produce dilute extracts and contain materials that are easily oxidized. Furthermore, the subsequent steps of solvent separations can lead to the degradation of the target products. Several authors have used SC-CO_2 successfully to extract lipids and carotenoids from vegetable[27−30] and animal matrices.[20,30−32] Recent studies have used the SC-CO_2 extraction of lipids and astaxanthin from crustacean processing waste such as shrimp byproducts.[33−36] To our knowledge, no work has been done on the SC-CO_2 extraction of lipids from rock lobster liver. Therefore, the PUFAs proportion and heavy metal contamination, which are major concerns for potential consumers of rock lobster lipids extracted by SC-CO_2 and Soxhlet, have not yet been investigated. The objectives of this study were to optimize the SC-CO_2 extraction of lipids from Australian rock lobster liver, with the aims of obtaining the lipids with enriched PUFAs, comparing them with the lipids extracted from conventional processing methods, and evaluating the PUFA profiles and heavy metal content of the extracted lipids for potential health benefits and to confirm that they meet food safety standards.

MATERIALS AND METHODS

Materials. Fresh livers of Australian rock lobsters were provided by Ferguson Lobster Co. in South Australia. Food grade carbon dioxide (CO_2) gas used for SFE was supplied by CoreGas, South Australia.

Methods. *Proximate Analysis of Lobster Liver.* According to the AOAC (2006) methods, moisture was determined by oven-drying at 105 °C (AOAC 950.46) until a constant weight was obtained, whereas ash content was quantified by incineration in a muffle furnace at 600 °C (AOAC 920.153). Lipid content was determined by using the Soxhlet extraction method (AOAC 991.36), and protein content was determined according to the micro-Kjeldahl method (AOAC 928.08).

Preparation of Rock Lobster Liver for Extraction. One kilogram of fresh lobster liver was held frozen (Thermo Scientific, TSE series -86C Ultra Low Temperature freezer) at −80 °C for 8 h. The frozen lobster liver was transferred to a plastic box with a large surface before it was freeze-dried at −85 °C and 15 mTorr for 48 h by a benchtop freeze-dryer (Virtis model BT6KEL 301804). The freeze-dried lobster liver was collected and stored in the freezer at −80 °C for 1 week before it was used for the lipid extraction.

Supercritical CO_2 Extraction of Lipids from Rock Lobster Liver. Extractions were carried out by using the laboratory supercritical CO_2 extraction system (Applied Separation model Spe-ed SFE-2 7071) equipped with 100 and 1000 mL extraction vessels. The 100 mL extraction vessel was packed with around 10 g of freeze-dried lobster liver for each batch of extraction, and the void volumes at two ends of the extraction vessel were completed with washed sand and glass wool layers. A 20 min static time was used for making a good contact between the sample and the supercritical solvent before the needle valve was opened to release the extracted lipid. For extraction, CO_2 at supercritical state was continuously drained through the extraction vessel, and the extracted lipid was collected in a preweighed dark glass bottle. The CO_2 mass flow rate was maintained at around 0.434 kg/h, and the time for extraction was 240 min. The extraction temperatures of the oven, valve, and vessel were set at 50, 60, and 50 °C, respectively, whereas the extraction pressures were adjusted to 25, 30, and 35 MPa as experimental design. The lipid-collecting bottle was weighed every hour to calculate lipid recovery. Extractions at every condition were carried out in three replicates.

Soxhlet Extraction of Lipids from Rock Lobster Liver. Soxhlet extraction of lipids from rock lobster liver was carried out at the National Measurement Institute (NMI) of Australia. Ten grams of freeze-dried rock lobster liver was homogenized thoroughly before it was placed in a Soxhlet thimble. The thimble containing the sample was then placed in a hot extraction beaker while 90 mL of diethyl ether was added and locked into the hot extraction unit. Extraction was carried out using a preprogramed period of approximately 4 h with a temperature of 40 °C. The amount of crude lipids obtained by this method was 2.43 g/10 g of the freeze-dried lobster liver.

Fatty Acid Analysis. The fatty acid composition of the lipids extracted by SFE and Soxhlet was analyzed at NMI.

Extracted fats were converted into fatty acids methyl esters (FAME), separated, and then measured on a Hewlett-Packard 6890 gas chromatograph equipped with a 50 cm capillary column (0.32 mm i.d.; SGE, Victoria, Australia) coated with 70% (w/w) cyanopropyl polysilphenylene-siloxane (BPX-70) (0.25 μm film thickness), which was fitted with a flame ionization detector. Helium was the carrier gas (flow rate = 60 mL/min). and the split ratio was 20:1. The injector temperature was set at 250 °C and the detector temperature at 300 °C. The initial oven temperature was 140 °C and programmed to rise to 220 °C at 5 °C/min and then held for up to 3 min. FAMEs were identified on the basis of the retention time of standards obtained from Nucheck Prep Inc. (Elysian, MN, USA) using Chemstation software. An external standard of 463 from NuCheck Prep Inc. was analyzed and used for calibration.

Heavy Metals and Inorganic Arsenic Analysis. The content of heavy metals and inorganic arsenic was determined using the standard methods VL247 ver. 9.1[37] and NT 2.56[38] described by NMI of Australia.

RESULTS AND DISCUSSION

Composition of Australian Rock Lobster Liver. *Chemical Composition.* The chemical composition of

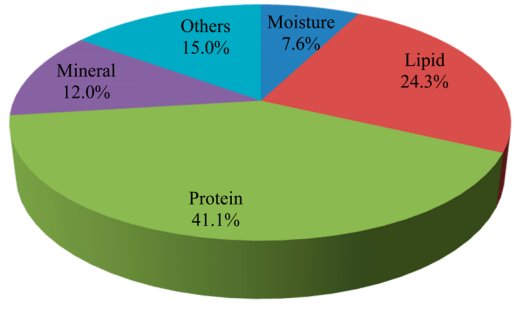

Figure 1. Chemical composition of freeze-dried Australian rock lobster liver (*Jasus ewardsii*).

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Table 1. Heavy Metal Contents[a] in Liver of Australian Rock Lobster (on a Dry Weight Basis), Regulatory Limits, and WHO/FAO Recommendation

trace element	Australian rock lobster liver (mg/kg)	regulatory limits (mg/kg)	WHO/FAO recommendation
antimony	0.028		
arsenic (inorganic form)	0.67	2.0	
arsenic (organic and inorganic forms)	240		
cadmium	8.7	2.0	
copper	1000		10–12 mg/day
lead	0.024	0.5	
mercury	0.28	0.5	
selenium	16.0		46–61 μg/day
tin	<0.01	250	
zinc	170		35–45 mg/day

[a]These analyses were carried out by National Measurement Institute of Australia, following its established standard measurement procedures.

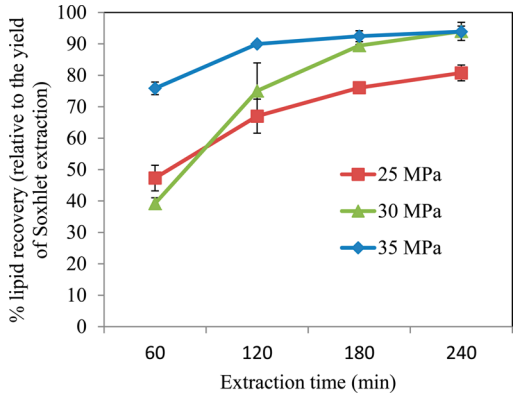

Figure 2. Lipid recovery extracted by SC-CO_2 at different pressures over extraction time.

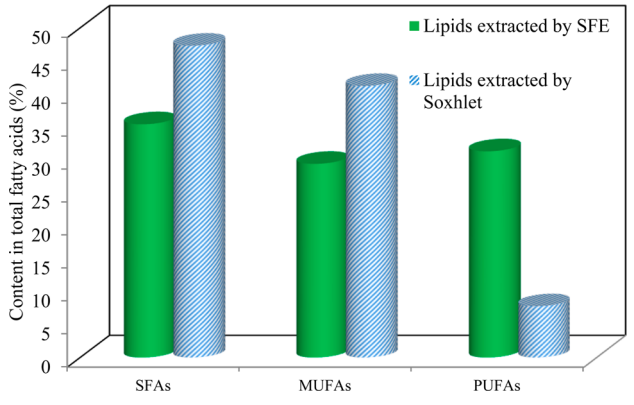

Figure 3. Differences in fatty acid composition of the lipids extracted by SC-CO_2 and Soxhlet method.

Australian rock lobster liver was analyzed. As shown in Figure 1, the two major components of the Australian rock lobster livers obtained in this study are protein (41.1% by dry weight) and lipids (24.3% by dry weight). The lipid content obtained in this study is higher than the 19.4% lipid content reported in the study by Tsvetnenko et al.[5] This could be caused by different species of lobster being used in the experiments or by

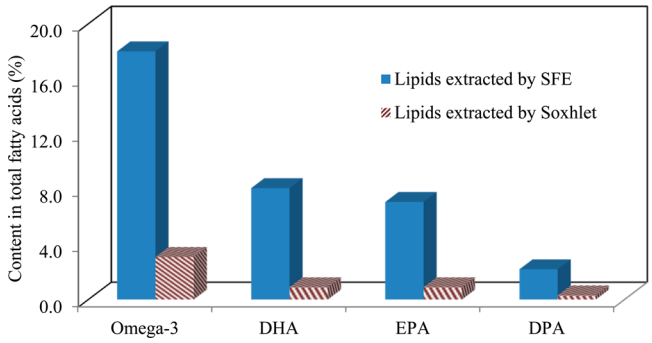

Figure 4. Contents of omega-3 in the lipids extracted by SC-CO_2 and Soxhlet method.

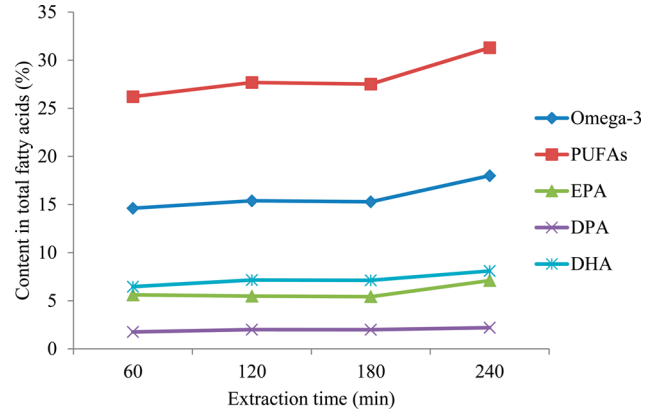

Figure 5. Evolution of PUFAs, omega-3 fatty acids, DHA, EPA, and DPA over the extraction time.

Table 2. Heavy Metal Contents[a] in the Lipids Extracted by SC-CO_2 (50 °C, 35 MPa, 4 h, CO_2 Flow Rate = 0.434 kg/h) and Soxhlet (40 °C, 4 h) as well as Regulatory Limits

trace element	lipids extracted by SC-CO_2 (mg/kg)	lipids extracted by Soxhlet (mg/kg)	regulatory limits (mg/kg)
antimony	<0.01	<0.01	
arsenic (inorganic form)	0.05	0.22	2.0
arsenic (organic and inorganic forms)	2.2	31	
cadmium	<0.01	0.27	2.0
copper	13	150	
lead	0.012	0.022	0.5
mercury	<0.01	0.012	0.5
selenium	0.25	2.6	
tin	0.19	<0.01	250
zinc	12	18	

[a]These analyses were carried out by National Measurement Institute of Australia, following its established standard measurement procedures.

environmental or other factors. Compared with the lipid content of menhaden, which is an Atlantic fish caught primarily for production of fish oil and meal (9.13%), the lipid content in rock lobster liver was nearly 3 times higher. Moreover, Tsvetnenko et al.[5] also reported that the lipids recovered from rock lobster cephalothorax were rich in PUFAs, of which HUFAs accounted for 50.8%. The results indicated that Australian rock lobster liver, a relatively readily available lobster

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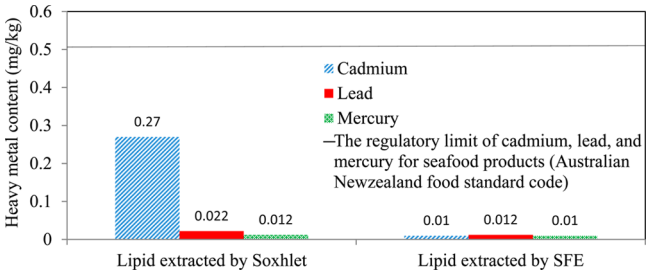

Figure 6. Contents of cadmium, lead, and mercury in the lipids extracted by SC-CO_2 and Soxhlet methods.

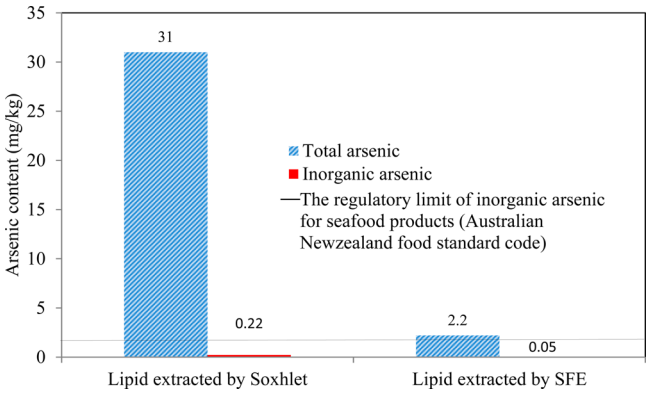

Figure 7. Contents of total and inorganic arsenic in the lipids extracted by SC-CO_2 and Soxhlet methods.

processing byproduct, could be a valuable but inexpensive resource for the extraction of ω-3-rich lipids.

Heavy Metal Composition. The concentration of heavy metals found in Australian rock lobster liver is shown in Table 1. Copper was detected in very high amounts of approximately 1000 mg/kg. This value for copper is several times higher than 151 mg/kg in veal liver, which in turn is regarded as a very high copper content food.[39] Consuming just 10−15 g of rock lobster liver could meet the daily requirement for copper suggested by the WHO/FAO. Copper is an essential mineral required for bone and connective tissue production and for coding specific enzymes that possess the function of eliminating free radicals. A deficiency in copper can lead to osteoporosis, joint pain, and lowered immunity because copper is essential for the absorption of iron.[40] Apart from having a rich copper content, Australian rock lobster liver also has high contents of zinc (170 mg/kg) and selenium (16 mg/kg), which are beneficial for human health.[39] Whereas zinc is good for skin care, healing of wounds, prostate disorders, colds, weight loss, appetite loss, pregnancy, diarrhea, respiratory infections, and malaria,[41] selenium is one of the most effective mineral antioxidants because it actually prevents the formation of new free radicals by participating in various cellular reactions that lower the peroxide concentration in the cellular body.[42] Therefore, Australian rock lobster liver could be an excellent dietary source of zinc and selenium for human demands.

However, four other elements including lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) that are generally considered to be hazardous to human health at low to medium concentrations[43] have also been found in Australian rock lobster liver. The amounts of Pb and Hg were just half of the regulatory limit (0.5 mg/kg) of seafood products, but the total As and Cd were quite high, at contents of 240 and 8.7 mg/kg,

Table 3. Fatty Acid Composition[a] of the Lipids Extracted from Liver of South Australian Rock Lobster Using SC-CO_2 (50 °C, 35 MPa, 4 h, CO_2 Flow Rate = 0.434 kg/h) and Soxhlet (40 °C, 4 h)

fatty acid		lipids extracted by SC-CO_2 (%)	lipids extracted by Soxhlet (%)
saturated fatty acids (SFAs)		35.4	47.3
C4:0	butyric	<0.1	<0.1
C6:0	caproic	<0.1	0.3
C8:0	caprylic	<0.1	<0.1
C10:0	capric	<0.1	<0.1
C12:0	lauric	<0.1	0.1
C14:0	myristic	4.3	5.7
C15:0	pentadecanoic	1.9	2.2
C16:0	palmitic	16.7	22.5
C17:0	margaric	2.0	2.6
C18:0	stearic	8.1	10.6
C20:0	arachidic	1.4	1.9
C22:0	behenric	0.7	0.9
C24:0	lignoceric	0.1	0.3
monounsaturated fatty acids (MUFAs)		29.4	41.2
C14:1	myristoleic	<0.1	<0.1
C16:1	palmitoleic	5.5	7.6
C17:1	heptadecenoic	0.3	0.3
C18:1	oleic	20.5	28.4
C20:1	eicosenic	2.3	3.4
C22:1	docosenoic	0.5	0.8
C24:1	nervonic	0.4	0.7
polyunsaturated fatty acids (PUFAs)		31.3	7.8
C18:2ω-6	linoleic	1.8	1.8
C18:3ω-6	γ-linolenic	<0.1	<0.1
C18:3ω-3	α-linolenic	0.5	0.2
C20:2ω-6	eicosadienoic	1.0	0.9
C20:3ω-6	eicosatrienoic	0.2	<0.1
C20:3ω-3	eicosatrienoic	0.2	0.8
C20:4ω-6	arachidonic	8.0	1.2
C20:5ω-3	eicosapentaenoic (EPA)	7.1	0.9
C22:2ω-6	docosadienoic	0.3	0.4
omega 3	fatty acids (ω-3)	18.0	3.1
omega 6	fatty acids (ω-6)	13.3	4.7
C22:4ω-6	docosatetraenoic	2.0	0.4
C22:5ω-3	docosapentaenoic (DPA)	2.2	0.3
C22:6ω-3	docosahexaenoic (DHA)	8.1	0.9
total mono trans fatty acids		0.6	0.5
total poly trans fatty acids		3.2	3.2
P:M:S ratio		0.9:0.8:1	0.2:0.9:1

[a]These analyses were carried out by National Measurement Institute of Australia, following its established standard measurement procedures.

respectively (Table 1). The high contamination of these toxic heavy metals could be caused by a large amount of As and its derivatives present in seawater due to natural processes[44] and pollution as many species of fish and shellfish can bioaccumulate As and toxic heavy metals.[45] Despite contamination with high levels of As, the vast majority of As and its derivatives in Australian rock lobster liver are in organic forms, which are harmless,[45] and its limit is not specified in the food code. By contrast, soluble inorganic arsenic compounds, which

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are highly toxic,[46] are present in very small amounts of about 0.67 mg/kg compared with the regulatory limit of 2 mg/kg. However, the content of Cd in rock lobster liver is 4 times higher than the maximum value recommended for seafood according to our study. This high Cd content should be brought into account when rock lobster liver is directly consumed or utilized as a raw material for food. As a result of this, rock lobster liver is usually removed during lobster processing to avoid cross-contamination of toxic heavy metals. An advanced technology is required to ensure the extracted lipids have significantly reduced contaminants.

Optimization of SC-CO_2 Extraction of Lipids from Rock Lobster Liver. SC-CO_2 extraction has been applied to several materials to investigate extraction yield. A number of processing parameters including pressure, temperature, CO_2 rate, and extraction time have been reported to have significant influence on lipid recovery, with extraction pressure and temperature being the most important factors. Several optimum conditions have been suggested for SC-CO_2 extraction of lipids from different materials, but these conditions could be changed depending upon the nature of the materials being treated. In the literature, the suggested pressure usually ranges from 20 to 40 MPa and the temperature varies from 40 to 60 °C. In the present work, extractions of lipids from Australian rock lobster liver were carried out with pressures from 25 to 35 MPa for 1−4 h at 50 °C and the CO_2 flow rate of 0.434 kg/h in order to find the efficient condition for lipid extraction from this material. Although the SC-CO_2 extraction can be carried out at the mild temperature for better prevention of lipid oxidation, the results from initial studies carried out at the same temperature as the Soxhlet extraction (40 °C) indicated that lipid recovery was very low (only 32.17% after 2 h of extraction). Therefore, a slightly higher extraction temperature of 50 °C was chosen to minimize negative effects on the PUFA profile but to achieve a high enough lipid recovery (>90%).

As shown in Figure 2, the extraction curves obtained in this study fit well with the empirical model suggested by Kandiah and Spiro,[47] with an assumption that two diffusion stages, which are based on the amount of lipids accessible to the supercritical CO_2, could control the process. At the early stage, the content of most accessible lipids is high and, thus, the extraction rate is high. As can be seen in Figure 2, the initial extraction rate, or the lipid recovery, increases significantly with applied pressure. When the extraction pressure rose from 25 to 35 MPa, the lipid recovery increased significantly by about 30% (from 47.30 to 75.86%) during the first 2 h of extraction. Up to 90% of the lipids in rock lobster liver relative to the Soxhlet extraction could be recovered in this stage once the pressure of 35 MPa was applied, but for the extraction pressure of 30 and 25 MPa these values were just 75 and 67%, respectively. This result indicates that the internal mass transfer is negligible and the process is controlled by the lipid solubility in SC-CO_2. Because both solvent density and capacity increase with pressure, a rise in pressure leads to an increase in the lipid solubility of the solvent. However, at the second stage, the remaining lipids, which are less accessible to the solvent, were extracted much more slowly because of the considerably higher internal mass transfer resistance.[21] This can be clearly observed in Figure 2 when only around 4, 13, and 19% of the lipids were recovered during the last 2 h of extraction with pressures of 35, 30, and 25 MPa, respectively. The results indicate that using high pressure for SC-CO_2 extraction of lipids from rock lobster liver can shorten the extraction time because the extraction rate increases with pressure.

Although the amount of recovered lipids can be increased with an extended extraction time at all observed pressures, the maximum value of lipid recovery was found at 4 h of extraction. The lipid recovery for 4 h of extraction at 35 and 30 MPa was approximately 94.0%, whereas this value for 25 MPa was reduced to 80.76%. The result, that lipid recovery increased with higher extraction pressure, is in agreement with the previous investigation by Sahena et al.[48] Therefore, SC-CO_2 extraction of lipids from rock lobster liver using high pressure not only shortened the extraction time but also improved the extraction yield.

It is also observed in Figure 2 that the appropriate time for extraction is significantly different among the extraction pressures. Although 4 h of extraction seems to be an appropriate time for the pressure of 30 MPa, this time is clearly not suitable for 35 MPa. This is because the lipid recovery for the pressure of 35 MPa at 2 and 4 h was not significantly different (nearly 90.0 and 94.0%). The prolonged extraction time could lead to a considerable increase in processing costs, and in the case of extraction at 35 MPa, 2 h of extraction is clearly more economical than others. In the overall consideration of extraction yield, extraction time, and PUFA content, the SC-CO_2 extraction at 35 MPa and 50 °C for 2 h of extraction was chosen as the efficient condition for lipid extraction from freeze-dried rock lobster liver.

Significant Improvement in Nutritional Profile of the SC-CO_2-Extracted Lipid. *PUFA-Rich Lipid Extracted by SC-CO_2 Extraction.* Lipid extraction with SC-CO_2 has been suggested as a promising alternative for producing lipids with a high amount of PUFAs. CO_2 at supercritical condition possesses superior mass transfer characteristics, and this technique involves the use of a nonoxidant atmosphere and mild temperature, which could prevent the oxidation of PUFAs. In current research, the lipids extracted from Australian lobster liver by SC-CO_2 extraction and Soxhlet were analyzed, and their fatty acid profiles were compared. It can be observed in Figure 3 that there are significant differences in the fatty acid composition of the two extracted lipid mixtures. Whereas the majority of total fatty acids in the Soxhlet-extracted lipids are saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs), its PUFAs are very low, at only around 7.8%. In contrast, the SC-CO_2-extracted lipids have a very high proportion of PUFAs (31.3%), which is greater than the level of MUFAs (29.4%) and equivalent to SFAs. Notably, the level of PUFAs in the SC-CO_2-extracted lipids is 4 times higher than that in the Soxhlet-extracted lipids and considerably higher compared to 22.6% of PUFAs in the previous work of Tsvetnenko et al.,[5] who extracted lipids from rock lobster (*Panulirus cygnus*) cephalothorax using a mixture of methanol/chloroform solvent that was then evaporated under vacuum. This result is also supported by the study of Cheung et al.,[49] who compared the fatty acid composition of lipids extracted from brown seaweed by SC-CO_2 and Soxhlet. Their work also showed that the lipid oxidation of PUFAs had been minimized under low oxidizing conditions used in SC-CO_2 extraction and, therefore, the obtained lipids contained higher PUFAs.

Apart from significantly enriching PUFAs, the SC-CO_2-extracted lipids also have a significantly higher proportion of ω-3 fatty acids, which account for 18% of total fatty acids, 6 times higher compared with the 3.1% of ω-3 fatty acids found in the lipids recovered by the Soxhlet method (Figure 4). This result

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is similar to the study of Cheung et al.,[49] who discovered that ω-3 fatty acid levels in the SC-CO_2-extracted lipids from brown seaweed were significantly higher than those in the Soxhlet-extracted lipids and concluded that ω-3 fatty acids were extracted more effectively with SC-CO_2 extraction than with the Soxhlet. More specifically, ω-3 HUFAs such as DHA, EPA, and DPA, which have biochemical activities that may be useful in the prevention and treatment of several disorders and diseases including coronary heart disease, rheumatoid arthritis, asthma, cancers, diabetes, and others,[50] were the most dominant PUFAs of the lipids extracted by SC-CO_2 extraction. These ω-3 HUFA concentrations were 7 times higher than those in the Soxhlet-extracted lipids, and they comprised >95% of the total ω-3 fatty acids in the SC-CO_2-extracted lipids. The maintenance of a high amount of PUFAs in the composition of the extracted lipids with the vast majority of these being ω-3 HUFAs provides a significant advantage for the SC-CO_2 extraction technique for extracting ω-3 rich lipids from the liver of Australian rock lobster.

The investigation on the evolution of PUFAs and ω-3 fatty acids of the SC-CO_2-extracted lipids over the extraction time (Figure 5) indicated that these fatty acid contents are relatively constant in the first 3 h and slightly increase in the fourth hour. The extraction time does not have a significant impact on the fatty acid profile.

Significantly Lower Toxic Heavy Metals in the SC-CO_2 Extracted Lipids. More than 50 different arsenic species have been found in marine environments, and arsenic derivatives, such as arsenobetaine, being the main species and water-soluble form, have been detected in fish.[51] Therefore, seafood is considered as a major dietary source for arsenic exposure.[46] In our current work, the heavy metal content of the extracted lipids have been especially taken into account because some toxic heavy metals were detected over the regulatory limit in the raw materials used for extracting the lipids. This becomes more necessary because a considerable amount (4.3−10.5 ppm) of arsenolipids, which are nonpolar lipids bound with As, have been found in 10 different fish lipids.[52] Table 2 shows the concentration of heavy metals detected in the lipids extracted by the SC-CO_2 extraction and by the Soxhlet. The lipids extracted by both methods contain very small amounts of heavy metals compared with the raw materials and the regulatory limits, whereas their beneficial elements such as Zn and Se are still quite high. The content of these heavy metals in the extracted lipids changed significantly from 0.5 to 870 times depending on the method used for the lipid extraction and the types of trace elements. The low contamination of heavy metals in the extracted lipids is a result of these methods utilizing nonpolar solvents for extraction, which usually solubilize mainly the nonpolar compounds and leave behind the polar compounds.

However, this work has been specifically focused on an investigation of toxic heavy metals because contamination with these compounds can render the material unusable due to potential risks of excessive ingestion, which may lead to a decline in mental, cognitive, and physical health. As shown in Figures 6 and 7, there are significant differences in the amounts of toxic heavy metals in the lipids extracted by SC-CO_2 and Soxhlet techniques. The contents of all toxic heavy metals in the SC-CO_2-extracted lipids were considerably lower than those in the Soxhlet-extracted lipid. Whereas the concentration of Pb in the SC-CO_2-extracted lipids was just half of that in the Soxhlet-extracted lipids (0.022 mg/kg), the content of inorganic arsenic in the former was 4 times lower compared with the amount of 0.22 mg/kg in the latter. Notably, the content of Cd in the SC-CO_2-extracted lipids (<0.01 mg/kg) was 27 times lower than that in the Soxhlet-extracted lipids, whereas the concentration of total arsenic was nearly 15 times lower (2.2 mg/kg compared with 31 mg/kg). This trend is strongly supported by the recent investigations by Rubio-Rodríguez et al.[25] Their results indicate that SC-CO_2 extraction is an effective technology for extracting fish lipids with greatly reduced contamination with toxic heavy metals.

Lipid Profiles. Table 3 illustrates the fatty acid composition of the lipids extracted by SC-CO_2 (50 °C, 35 MPa, 0.434 kg/h) and by Soxhlet from Australian rock lobster liver. It needs to be highlighted that there are significant differences in the fatty acid compositions of the lipids extracted by these two methods. Whereas the highest proportion in the Soxhlet-extracted lipids was SFAs (47.3%) followed by MUFAs (41.2%) and PUFAs (7.8%), these contents in the SC-CO_2-extracted lipids were 35.4% of SFAs, 29.4% of MUFAs, and 31.1% of PUFAs, respectively. The former was rich in SFAs and MUFAs but very poor in PUFAs, whereas the latter has very high amounts of PUFAs dominated by ω-3 (18%) and ω-6 (13.3%). The differences in fatty acid profiles of these two lipids could be explained by differences in conditions used for extracting the lipids. Whereas the Soxhlet method employed organic solvents such as diethyl ether for extraction in this study, the SC-CO_2 extraction used CO_2 at supercritical condition that has high diffusion and very low surface tension compared to liquid solvents.[53] These novel properties could be a favorable condition for PUFA extraction. This point, that the yield of omega-3 fatty acids extracted by SC-CO_2 extraction at higher pressure (31.0 and 37.9 MPa) was significantly higher than that in the Soxhlet, was confirmed by Cheung et al.[49]However, both lipids have diversified fatty acids in which oleic is the most dominant in their composition (20.5% for SC-CO_2-extracted lipids and 28.4% for Soxhlet-extracted lipids). Palmitic is abundant in SFAs (16.7% for SC-CO_2-extracted lipids and 22.5% for Soxhlet-extracted lipids), whereas DHA, EPA, and arachidonic are three main fatty acids in PUFAs, accounting for up to 75%.

AUTHOR INFORMATION

Corresponding Author

*(W.Z.) Phone: +61-8-7221-8557. Fax: +61-8-7221-8555. E-mail: wei.zhang@flinders.edu.au.

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ABBREVIATION USED

AOAC, Association of Official Analytical Chemists; WHO, World Health Organization; FAO, Food and Agriculture Organization

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