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Characterization of purified phospholipids from krill (*Euphausia superba*) residues deoiled by supercritical carbon dioxide

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Abstract−Purification of phospholipids (PL) from the Antarctic krill (*Euphausia superba*) using a two-step extraction process has been investigated. Using supercritical carbon dioxide (SC-CO_2) extraction with optimal extractions conditions of 45 °C, 25 MPa, and CO_2 flow rate of 22 g/min, most of the neutral lipids were extracted. PC, PE and PI were then extracted in a second step conducted with modified existing method using ethanol, hexane and acetone as solvents. The major PL of krill residues was quantified by high performance liquid chromatography (HPLC-ELSD). The fatty acid compositions of total PL, PC, PE and PI were analyzed by gas chromatography (GC). A significant amount of polyunsaturated fatty acids (PUFA) was present in both total and PLs fractions. The purified PLs were characterized by their acid value, peroxide value, and the oxidative stability. The purity of PL ranged between 93 and 97% and was evaluated by spectrophotometry.

Key words: Krill, Phospholipids Purification, Supercritical Carbon Dioxide, Oxidative Stability

INTRODUCTION

As with fish, the Antarctic krill is a rich source of the long-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, unlike fish oil the EPA and DHA of krill oil are in the form of phospholipids (PL), giving it new properties and making it potentially more potent [1]. PL, which is a natural and integral part of cell function and is more readily absorbed increasing bioavailability, is a general term that includes all lipids containing phosphorus. Usually, the analysis of PL is based either on the determination of their total fatty acids by gas chromatography or the determination of the PL classes (phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidic acid (PA), phosphatidylserine, sphingomyelin, lysophosphatidylcholine etc.) with high performance liquid chromatography (HPLC) [2].

Commercially, PL comes from soybeans, egg yolk, or brain tissue [3]. Until now, the soybean is the most frequent and studied source of lecithin. However, lecithin from soybean is rich in mainly saturated fatty acids with some lower unsaturated fatty acids. It does not contain some important polyunsaturated fatty acids (PUFA) including EPA and DHA. Egg yolk has also been used widely as a source of lecithin.

Several methods were compared for recovery and purification of mixtures of lipids and, more specifically, for PL; however, for more difficult isolation, the results and recoveries vary, depending on the type of phase used and the nature of the sample matrix and composition [4]. Some methods that were originally used for PL separation from meat [5] gave low recoveries when applied to other matrixes. In recent years, supercritical fluid extraction technology (SFE), which is used as an alternative for lipid extraction to organic solvent extraction, has received much attention, because it allows a reduction in extraction time, requires little sample manipulation, and involves a much lower solvent consumption, leading to extracts of increased purity [6,7]. Some works refer to the application of SC-CO_2 extraction of marine materials to obtain PUFA. Yamaguchi et al. [8] reported on the extraction of lipids from Antarctic krill. According to their results, only non-polar components such as cholesterol, carotenoid, triacylglycerols and their derivatives were extracted. PL did not appear in the extracted fractions. SC-CO_2 does not provide a means to dissolve PL, but it can be recovered by the addition of a polar entrainer to SC-CO_2 [9]. The choice of a suitable co-solvent must be based on some considerations such as thermodynamics and food safety [10]. Some researchers have already studied the role of ethanol as a co-solvent. Prosise [11] reported that ethanol was an excellent solvent for isolating PL for food use. But all other neutral lipids with PL are also extracted by ethanol. Therefore, further steps are needed to purify the PL.

Our objective was to optimize and improve existing methods to isolate an enriched PL fraction from dry krill and characterize the purified PL. First, SFE conditions were optimized to extract the majority of neutral lipids. Then, in a second processing step, the residual extracted krill, containing PLs, were extracted with ethanol solvent.

MATERIALS AND METHODS

1. Materials and Chemicals

The krill (*Euphausia superba)* were collected from Dongwon F & B Co., S. Korea. The krill blocks were stored at −80 °C for no longer than 1 year before being used experimentally. The carbon dioxide (99.99% pure) was supplied by KOSEM, Korea. Linoleic acid and trolox were purchased from Sigma-Aldrich, Germany. All other chemicals used in different analysis were of analytical or HPLC

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grade.

2. Sample Preparation

The krill samples (mean body length, 5.15 cm; mean body weight, 0.65 g) were freeze-dried for about 72 h. Then the dried samples were crushed and sieved (700 μm) by mesh. The dried samples, called raw materials, were used for oil extraction by SC-CO_2 and for PL isolation by organic solvent.

3. SC-CO_2 Extraction

Fifty grams of freeze dried krill sample was loaded into 200 mL stainless steel extraction vessel containing cotton at the bottom of the SFE unit for extracting oil from krill. Before plugging with a cap, another layer of cotton was used at the top of the sample. CO_2 was pumped into the vessel by high pressure pump up to the desired pressure, which was regulated by a back pressure regulator. The vessel temperature was maintained by a heater. Flow rates and accumulated gas volume passing through the apparatus were measured with a gas flow meter. The effects of temperature and pressure on lipid extraction from krill were studied at 35-45 °C and 15-25 MPa at a constant extraction time of 2.5 h. The flow rates of CO_2 were kept constant at 22 g/min for all extraction conditions. The extraction yield was determined and the krill residues remaining in the vessel were stored at −80 °C until further analysis.

4. Purification of PL

The multiple-step procedure for extracting PL from krill is outlined in Fig. 1. The sample (45 g) was extracted according to the procedure described by Palacios and Wang [12], with modification. For the initial extraction, 200 mL of ethanol (95%) was added to 45 g of krill extracted residues (at 45 °C and 25 MPa) in a 300-mL centrifuge bottle and stirred by a magnetic stirrer about 12 h. The mixture was then centrifuged at 1,900 rpm for 10 min, and the supernatant containing ethanol, some polar lipids, and some neutral lipids was transferred to a separator funnel with double volume of hexane. After one hour (1 h), the hexane phase was removed, and the ethanol phase was mixed with an additional double volume of hexane and left for 2 h for phase separation. The ethanol was removed from the ethanol extract by rotary vacuum evaporator at 40 °C, the remaining lipid material was dissolved in 10 mL of hexane and transferred to a 150-mL centrifuge bottle where 60 mL (about fifth volume) of chilled acetone (4 °C) was added and carefully stirred to precipitate the PL. The centrifuge bottle was then placed in an ice-water bath for 15 min and centrifuged at 1,500 rpm. The precipitate was the purified PL. To investigate the effect of the presence of neutral lipids on the ethanol extraction yield, raw krill samples were used in the same purification method. Three replicates of the samples were carried out.

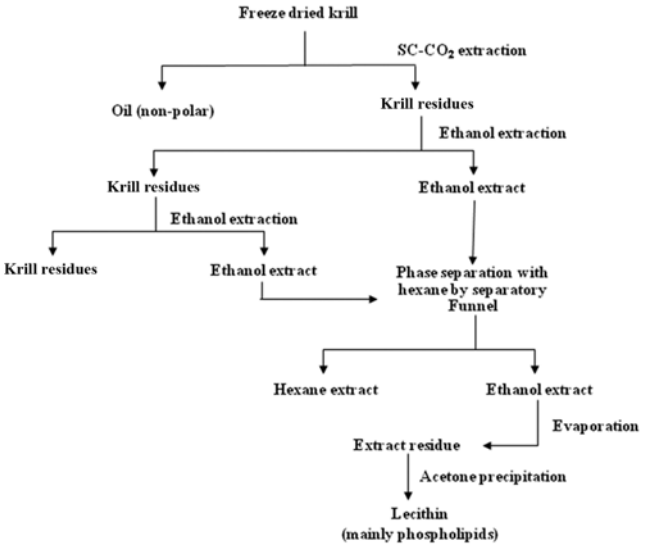

Fig. 1. Summary of the purification steps.

5. Quantification of Purity of Isolated PL

The PL content of lecithin was measured according to Stewart [13] by a colorimetric method based on the formation of a complex between PL and ammonium ferrothiocyanate. Briefly, 0.35 mg of dry lecithin was dissolved in 2 mL of chloroform. Then, 1 mL of a solution prepared from ferric chloride (27 g/L) and ammonium ferrothiocyante (30 g/L) was added. After vortexing, the mixture was centrifuged at 1,000 rpm for 15 min. The lower phase was collected and the absorbance was recorded at 488 nm. A calibration curve was made by standard PC and the result was expressed as gram equivalents of PC per gram of lecithin.

6. Major PL Quantification by HPLC-ELSD

PL composition was determined using a Jasco (LC-Net II/ADC) HPLC system coupled with an evaporative light scattering detector (ELSD), model 400 (Jasco, Japan), model 126 solvent delivery modules. Appropriate dilutions of sample were injected (20 μl injection loop) onto a diol normal-phase silica column (250 mm×4.6 mm i.d., with integral guard column; Advanced Separations Technologies, Waters). The analysis was carried out according to the method of Letter [14]. Extracted lecithin was dissolved in chloroform and injected (20 μL) into injector. The mobile phase was isopropyl alcohol, hexane and water. The drift tube temperature of ELSD was set at 60 °C. The pressure of nitrogen gas as a nebulizer was 50 PSI. The quantification of PL was performed based on the peak area of standard PL, PC, PE and PI. The millennium software was used to analyze the data obtained by HPLC.

7. PLs Characterization

Hexane-insoluble matter, humidity and acid value of the purified PL were determined using the AOCS [15] official methods (Ja 3-87, Ja 2b-87, Ja 6-55). Analyses were performed in triplicate.

7-1. Free Fatty Acids

Free fatty acids of purified PL from krill were analyzed as described by Bernardez et al. [16]. Briefly, 50 mg (approximately) of sample was placed into Pyrex tubes with the addition of 3 ml of cyclohexane, and then 1 ml of cupric acetate-pyridine reagent was added. Tubes were vortexed for 30 s. After centrifugation at 2,000 rpm for 10 min, the upper layer was read at 710 nm. The FFA content was measured on a calibration curve constructed from oleic acid standards. Copper reagent was prepared according to Lowry and Tinsley [17]. A 5% (w/v) aqueous solution of cupric acetate was prepared and filtered. Then the pH of cupric acetate solution was adjusted to 6.1 by using pyridine.

7-2. Peroxide Value

Peroxide value was determined according to AOCS [15] method Cd 8-53 by modified amount. 1.0 g of krill purified PL was dissolved in 6 mL of acetic acid-chloroform (3 : 2) solution. Then 0.1 mL of saturated KI solution was added to the mixtures and the solution was allowed to stand with occasional shaking for 1 min. Distilled water (6 mL) was immediately added to the solution. The solu-

tion was titrated with 0.1 N of sodium thiosulfate until the yellow iodine color had almost disappeared. Then 0.4 mL of starch indicator solution was added and again titrated until the blue color disappeared. A blank determination was conducted with the same procedure. Peroxide value was expressed as milliequivalents peroxide/1,000 g sample.

7-3. Thin Layer Chromatography (TLC) of the Purified PL Fractions

The PL was separated by TLC for the determination of fatty acid compositions of PLs fractions. TLC was carried out using silica gel 60 plates (pre-coated with a 0.20-mm layer of silica gel 60) were obtained from ALUGRAM (Germany). The sample was applied (0.3 mg) on the plate and developed with a solvent system of dichloromethane : methanol : glacial acetic acid (60 : 30 : 10) (18). Spots were visualized by exposure to iodine vapor or by charring with 50% sulfuric acid at 130 °C for 30 min. Standard mixtures of PL were run in parallel with the sample for identification of spots. Spots were then scraped off in a screw cap tube separately and extracted from the silica using the solvent system of chloroform : ethanol : water (2 : 2 : 1, v/v/v). The chloroform phase was collected by phase separation and evaporated by vacuum rotary evaporator. The purity of the remaining residues of each polar lipid was again checked by TLC. PC, PE and PI from the spots were extracted as described above. This purified PC, PE and PI were used for fatty acid compositions.

7-4. Analysis of Fatty Acids Composition by Gas Chromatography

Fatty acids analysis of the oils extracted using SC-CO_2, lecithin, and purified PC, PE and PI was performed by GC. Methyl esters of fatty acids from total lipid extracts were prepared according to AOCS [19]. The gas chromatographic apparatus for the analysis of fatty acid composition was an HP 5790II equipped with a flame ionization detector (FID) and a capillary column DB-wax. Nitrogen was used as a carrier gas (1.0 $mLmin^{-1}$) of fatty acid methyl esters. The oven temperature was programmed, starting at a constant temperature of 130 °C for 3 min, and then increased to 240 °C at a rate of 4 °Cmin^{-1} and hold at 240 °C for 10 min. Injector and detector temperatures were 250 °C. Fatty acid methyl esters were identified by comparison of retention time with standard fatty acid methyl esters mixture (Supleco, USA).

7-5. Oxidative Stability

The oxidative stability was measured by the oxidation of emulsion of purified PL in water (deionized and degassed water) at 37 °C. Four emulsions of PL in water (w/w) (linoleic acid 5%, PL 5%, water 90%; PL 5%, water 95%; torolox 1%, PL 4%, water 95%; linoleic acid 10%, water 90% (control)) were prepared. The mixtures were properly homogenized by a homogenizer. Oxidative stabilities were checked by thiocaynate and thiobarbituric acid method (TBA). Linoleic acid and standard trolox were used to compare oxidative stability of purified PL.

7-5-1. Thiocyanate Method

This method was conducted according to Mitsuda et al. [20]. The peroxide formed by lipid peroxidation reacts with ferrous chloride and forms ferric ions. Ferric ions then unite with ammonium thiocyanate and produce ferric thiocyanate. Briefly, 0.1 mL of emulsion solution was added to 4.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 ml of 0.02 M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance of red color was measured at 500 nm (UVIKON 933, Kontron Instruments). Every 24 h interval during incubation, the absorbance was recorded.

7-5-2. TBA Method

The TBA method measures free radicals present after peroxide oxidation. The TBA value was measured according to Ottolenghi [21]. Briefly, 2 ml of 20% trichloroacetic acid and 2 ml of 0.67% 2-thiobarbituric acid were added to 1 ml of emulsion solution. The mixture was placed in a boiling water bath (100 °C) for 10 min. After cooling, the mixture was centrifuged at 3,000 rpm for 20 min. Absorbance of supernatant was measured at 532 nm by a uv/visible spectrophotometer (UVIKON 933, Kontron Instruments).

8. Statistical Analysis

All experiments were performed in triplicate and each set of results was averaged. The standard deviations were used as the basis for the error bars shown in the figures. The least significant difference at the 95% confident ($P<0.05$) level was calculated by Duncan test using Statistical Analysis System (SAS Ver. 9.1, SAS Institute, USA).

RESULTS AND DISCUSSION

1. SC-CO_2 Extraction

Fig. 2 shows the extraction curves of krill oil by SC-CO_2 at different temperatures (35, 40 and 45 °C) and pressure (15, 20 and 25 MPa) as well as the complete yield data obtained in each experimental run are shown in Fig. 3. Extraction yields varied from 4-6% to 10-11.5%. The highest yield (11.5%) was at 45 °C and 25 MPa. The amount of extracted oil increased with the increasing of CO_2 mass, depending on the pressure and temperature. In this work, the amount of extracted krill oil per solvent (CO_2) mass used was increased over the entire extraction period, until almost all the oil was extracted. On the other hand, the oil extraction yield increased either with pressure at constant temperature or with temperature at constant pressure. By the increase in pressure, the density of the SC-CO_2 was increased and hence the solvating power. Morita and Kajimoto [22] reported that the effect of pressure can be attributed to the increase in solvent power and by the strengthening of inter-

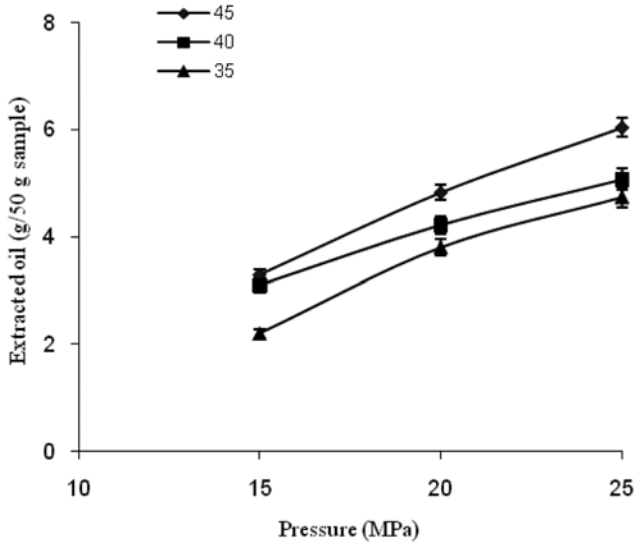

Fig. 2. SC-CO_2 extraction of oil from krill at different temperatures and pressure.

molecular physical interactions. Similar results were found by De Azevedo et al. [23]. The oil yield increased with the increase in temperature. By the increase of temperature, the density of SC-CO_2 was decreased, but it increased the oil component vapor pressure and that enhanced the extraction yield. Macias et al. [24] has also mentioned that the yield depends on a complex balance between the decrease in the SC-CO_2 density and the increase in vapor pressure.

2. Fatty Acids Composition

The fatty acid composition of the lipids of different species of Antarctic krill has already been published [25]. In this study, the fatty acid profile of the oil extracted by SC-CO_2 under different conditions and at different times was slightly similar. Whereas, the most abundant fatty acid was palmitic acid (16 : 0) representing 23.3% of fatty acids in krill oil (Table 1) and the main fatty acids were 18 : 1, 16 : 1, 14 : 0, 20 : 5 n-3, 22 : 6 n-3. The comparison between the fatty acid composition of the total lipids in freeze dried krill and in krill meal [8], as reported in Table 2, shows a significant differences in the 14 : 0, 16 : 1 and in 20 : 3.

3. Organic Solvent Extraction for Comparison and Solvent Selection

To investigate the effect of neutral lipids on the efficiency of the extraction by ethanol, forty-five grams of raw krill was treated using the same purification method, and the purified PL composition was compared with that obtained from SC-CO_2 extracted residues. Also, with the aim of comparing the extraction efficiency of organic solvents in order to select the solvent with highest yield of PL content and safety for human body, different organic solvents were used for the extraction of PL from both raw materials and SC-CO_2 extracted residues. The results of the comparison are reported in Table 2. The highest yield was from SC-CO_2 extracted residues, but the most effective solvent was ethanol with 42.7%.

Table 1. Fatty acids composition from SC-CO_2 extracted krill oil

Fatty acids[a] (%)	SC-CO_2 krill oil	Krill meal [8]
14 : 0	11.2±0.12	19.1
16 : 0	23.3±0.14	18.8
18 : 0	0.87±0.32	1.3
16 : 1	12.31±0.23	16.3
17 : 1	1.02±0.42	1.70
18 : 1	20.11±0.33	22.4
18 : 2 n-6	2.21±0.15	3.56
18 : 3	0.8±0.26	0.26
18 : 4	2.1±0.13	3.21
20 : 1	1.4±0.24	1.86
20 : 3	0.17±0.31	0.09
20 : 4 n-6	0.17±0.43	0.55
20 : 5 n-3	8.24±0.25	6.62
22 : 5 n-3	0.28±0.17	0.18
22 : 6 n-3	3.18±0.25	2.71
24 : 1	0.16±0.22	0.42

[a]The results showed mean value±standard deviation

Table 2. Organic solvent extraction from raw krill and SC-CO_2 extracted residues for comparison and solvent selection

Solvent	Total PL purified[a] (%)	
	Raw krill	SC-CO_2 treated krill
Petroleum ether	26.5±0.52	29±0.41
Acetone	18.6±0.35	20±0.24
2-Propanol	33.5±0.43	36±0.35
Ethanol	37.4±0.51	42.7±0.16

[a]The results showed mean value±standard deviation

Table 3. Major PLs composition

PLs distribution[a] (%)	
PC	80.4±0.64
PE	14.9±0.46
PI	0.7±0.72

[a]The results showed mean value±standard deviation

4. Major PL Composition

Major PL compositions are shown in Table 3. The main PLs of krill were PC and PE. PC and PE content of krill was 80.4% and 14.9% of total PL, respectively. PI was also present but with very low percentage (0.7%) of total PL. Kusumoto et al. [26] reported that PL from krill (*Euphausia pacifica*) contained (36.2-53.8%) of PC and (3.4-17.5%) of PE. Gordeev et al. [27] and Fricke et al. [18] also reported that PC content of PL from krill (*Euphausia superba*) was 3 to 5 times higher than PE content, which was similar to this study. The PL content of krill from different catches may vary considerably. The variations are probably due to both differences in nutritional status and maturity of the roe of krill, as well as to different isolation and quantification process used.

5. PLs Characterization

The isolated PL was almost pure (97%). It was found that the deoiled krill residues contained 8.06% of total PL. Analysis of the purified krill PL shows a composition in agreement with the range reported in the literature for krill PL [18]. PL content variation in krill may due to different factors, such as sample age, fishing area, season, extraction process and time, extraction efficiency by different solvents etc. The results from krill PL characterization are summarized in Table 4. Hexane-insoluble matter of PL was low (<1%) indicating almost the purity of PL.

5-1. Free Fatty Acids

FFAs are responsible for the acidity of oil. Changes of FFA content are mainly related to hydrolytic reactions in the lipid. FFA content of the purified PL was 2.34 g/100 g of PL. The amount of FFA and peroxide value agreed with the results of Ackman et al. [28] and Sargent and Falk-Petersen [29], who reported values in the range of 2-8% of the dry weight. But contrast the result (0.6%) reported by Saether et al. [30], who mentioned the special precautions taken

Table 4. Characterization of the purified PLs

Paramater	Value
Hexane-insoluble matter[a] (%)	0.91±0.73
Moisture[a] (%)	2.13±0.65
Acid value[a] (mg KOH/g)	23.07±0.24
Peroxide value[a] (milliequivalent/1,000 g)	4.66±0.28
Free fatty acids[a] (g/100 g)	2.34±0.34

[a]The results showed mean value±standard deviation

in their study to avoid postmortem lipolysis. Also, higher temperature and storage time caused a significant increment of the FFA in the hake byproducts oil [31]. Previous studies reported that the oxidation rate of Antarctic krill (*Euphausia superba*) lipid is very slow and no peroxides are accumulated even after a long-term storage [32].

5-2. Acid Value and Peroxide Value

The acid value and peroxide values of purified PL from SC-CO_2 extracted krill residues are given in Table 4. Acid value was used to determine the acidity of PL. On the other hand, peroxide value is used as a measurement of rancidity of lipids which occurs by auto oxidation. Generally, peroxide and acid values of commercially available soy lecithin (mainly PL) are found up to 12 milliequivalent/1,000 g and 30 mg KOH/g of lecithin, respectively. The PL isolated from krill contained free fatty acids which increased its acid value.

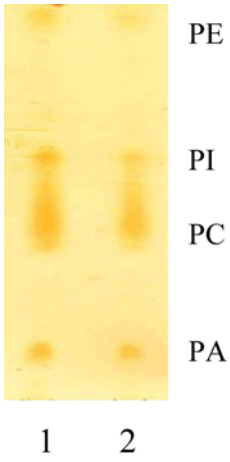

Fig. 3. Thin-layer chromatograms of PL purified from SC-CO_2 extracted residues (1) and from raw krill (2): PA, PC, PI, and PE.

Table 5. Fatty acid composition of the PLs fraction (% (w/w))

F.A. as methyl esters[a]	Total PL	PC	PE	PI
14 : 0	1.12	2.78	4.32	10.15
15 : 0	1.50	1.21	1.55	5.63
16 : 0	4.69	18.05	25.66	36.02
16 : 1	0.21	0.70	1.16	19.33
18 : 0	10.28	6.20	1.33	8.72
18 : 1	29.16	33.09	16.77	20.15
18 : 2 n-6	3.50	11.54	2.17	ND
20 : 4 n-6	18.07	24.35	9.50	ND
20 : 5 n-3	20.57	2.08	26.30	ND
22 : 6 n-3	10.90	ND	10.79	ND
Total (%)	100.0	100.0	100.0	100.0
Saturated	11.59	28.24	33.31	60.52
Unsaturated	82.41	71.76	66.69	39.48

[a]Results were average of two determinants. Standard error of the fatty acid constituents was on the order of about ±2%. For total PL, fatty acids showed which was present more than 1% of total fatty acids
ND: not detected

5-3. Fatty Acid Composition of PL

To analyze fatty acid compositions of major PL, the PL were separated by preparative TLC (Fig. 3). The fatty acid compositions of isolated PL, PC, PE and PI are shown in Table 5. The predominant fatty acid in total PL and in PC subfraction was oleic acid (18 : 1) (29.16% and 33.9%, respectively). Arachidonic acid (20 : 4 n-6) was abundant in PC subfraction (24.35%) and in total PLs in different percentages (18.07%). Palmitic acid (16 : 0) was predominant in PI subfraction (36.02%), but also was highly present in all subfractions. Stearic acid (18 : 0) varied from 1.33% to 10.28% in all subfractions. PC can be an important source of essential PUFA; the present study showed that PC contained almost 40% of PUFA. Several works have confirmed that in marine crustaceans PE is a highly unsaturated PL [33]. Because of the high levels of PUFA that contain, particularly EPA and DHA, PE has been considered as a reserve for the adaptation of membrane fluidity to changes in temperature [34]. In agreement with these previous studies, these results showed that PE contained 26.30% of EPA and 10.79% of DHA.

The amount of the unsaturated fatty acids in total PL was very

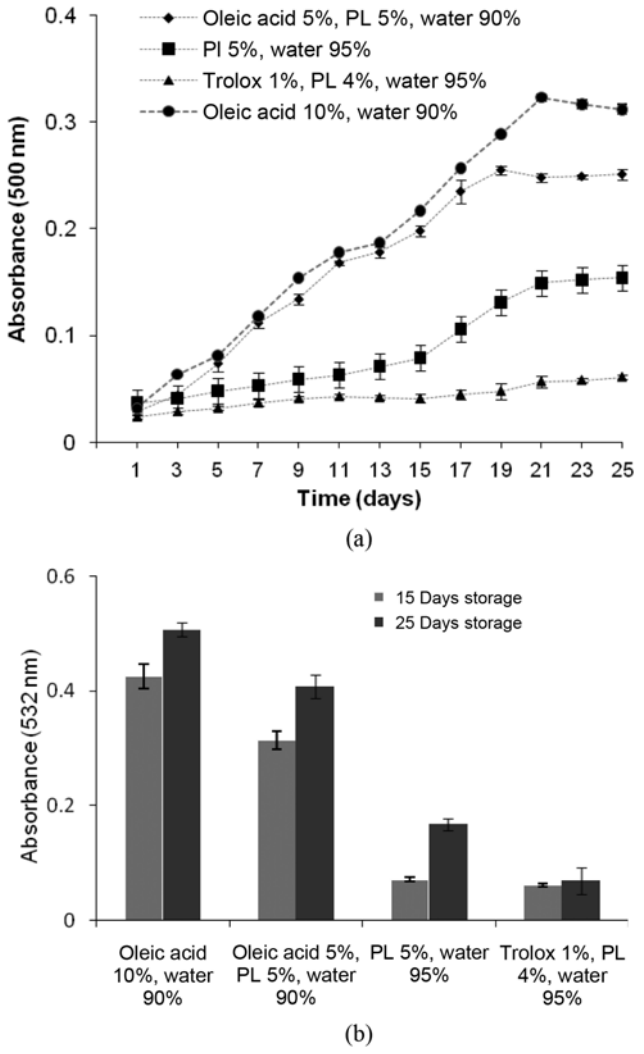

Fig. 4. Oxidative stability of the purified krill PL. (a) Thiocyanate method, (b) Thiobarbituric acid method.

high (82.41%), while the percentage of the saturated fatty acids was found 11.59%. The unsaturated/saturated ratio varied from 0.6 to 7.11 for the total PL and for all subfractions. The PUFA were the most abundant of the unsaturated fatty acids of total PL, PC, and of the PE subfraction. The results obtained are in agreement with those of other authors [27].

5-4. Oxidative Stability

Instead of determining the absolute state of oxidation of incubated sample, the oxidation trend was evaluated. The oxidative stabilities of PL are shown in Fig. 4(a) and (b). It has been reported that the oxidation rate of Antarctic krill (*Euphausia superba*) lipid is very slow [32]. In this study, PL with linoleic acid showed increase in absorbance values from first day. The increase in absorbance value was an indicator of auto oxidation by formation of peroxides during incubation. PL showed low absorbance value, indicating low level of lipid peroxidation until 17 days (Fig. 4(a)). And it showed increased oxidation after 19 days. On the other hand, PL with trolox showed higher oxidative stability than that of the other emulsions. Trolox, which is an antioxidant, inhibited the peroxide formation by lipid peroxidation in a certain period. The control showed increase in absorbance values from day 1 and reached on day 19 and dropped on day 23. In addition, the PL showed slightly high absorbance compared to PL with linoleic acid. It might be due to the presence of peroxide from the oxidation of some neutral lipid (impurities) existing in the emulsion of PL. In TBA method, the absorbance measured on day 15 was slightly similar between PL and PL with trolox emulsion. However, it was high on PL with linoleic acid emulsion, indicating low oxidative stability (Fig. 4(b)). On the other hand, a significant increase in absorbance was found on day 25 for PL emulsion. It has also been shown that the major constituents of the PUFA of PL in krill are EPA and DHA, which were the most susceptible to oxidation; in soybean, linoleate, and in egg yolk, arachidonate. Therefore, it can be supposed that the oxidative deterioration of PL proceeds most rapidly in krill, less rapidly in egg yolk and least rapidly in soybean [35]. However, PL showed high oxidative stability, which can be explained by the presence of the natural antioxidant, astaxanthin in PL, since Krill is a major source of astaxanthin, which has strong antioxidant activity [36]. Gogolewski et al. [37] also reported that long chain polyunsaturated fatty acid esterified with polar lipids had synergistic effect with antioxidant. Previous studies reported the high oxidative stabilities of PL from animal and plant sources by applying different methods [12,38].

CONCLUSION

The suitable conditions for the purification of PL from krill were examined. With a two-step extraction process, it is possible to isolate a PL-rich extract from krill. By SFE with CO_2 most of the neutral lipids were extracted from the Antarctic krill. The functional lipids in the spent solids from this first stage SFE were enriched with the removal of the neutral lipids. PC, PE and PI were then extracted in a second step using ethanol, hexane and acetone as solvents. Under optimum conditions, the final yield estimated was about 42.7% (w/w) of krill lipid. EPA formed the most abundant molecular species in PE, but not in PC and PI. While, arachidonic acid (20 : 4 n-6) was mainly present in PC and PE but was absent in PI. Also, the purified PL showed a potent antioxidant activity. Therefore, further work could be done on the isolation and characterization of the individual compounds in the extracts, which are responsible for antioxidant activity. These results show a great potential for utilization of SFE to obtain a functional, value-added ingredient from marine product, with great economic significance.

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