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Oxidative degradation and non-enzymatic browning due to the interaction between oxidised lipids and primary amine groups in different marine PL emulsions

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ABSTRACT

Due to the beneficial health effects of marine phospholipids (PL) there is an increasing industrial interest in using them for nutritional applications including emulsified foods. This study was undertaken to investigate both oxidative and hydrolytic stability of marine PL emulsions in relation to the chemical composition of the marine PL used. Moreover, non-enzymatic browning reactions were also investigated. Emulsions were prepared by high pressure homogenizer using different concentrations and sources of marine PL. In some formulations, fish oil was added in order to study the effect of increasing levels of triglycerides in the emulsions. The oxidative and hydrolytic stability of emulsions was investigated through measurement of peroxide value, free fatty acids, and ^{31}P NMR during storage at 2 °C for up to 32 days. The oxidative stability of marine PL emulsions during storage was further investigated through the measurement of secondary volatile compounds by solid-phase microextraction (SPME) and dynamic headspace (DHS) connected to gas chromatography (GC–MS). Non-enzymatic browning reactions were investigated through the measurement of Strecker derived volatiles, colour changes and pyrrole content. The results suggested that the oxidative stability of marine PL emulsions was significantly influenced by the chemical composition and the concentration of marine PL used to prepare them. Emulsions with good oxidative stability could be prepared from marine PL of high purity and high content of PL and antioxidant and low TAG content.

1. Introduction

Many studies have shown that marine phospholipids PL provide more advantages than marine triglycerides (TAG) available from fish oil. Marine PL have higher content of physiologically important *n*-3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) than fish oil (Peng, Larondelle, Pham, Ackman, & Rollin, 2003). EPA and DHA have better bioavailability when provided by PL as compared to TAG (Wijendran et al., 2002). In addition, marine PL have a broad spectrum of health benefits including those from *n*-3 PUFA, their polar head groups and the combination of the two in the same molecule. The health benefits of marine PL have been demonstrated in recent study on krill oil (Ierna, Kerr, Scales, Berge, & Griinari, 2010).

The current knowledge about the oxidative stability of marine PL was recently reviewed by Henna Lu, Nielsen, Timm-Heinrich, and Jacobsen (2011), who reported that several studies have shown that marine PL have better oxidative stability than fish oil regardless of their high degree of unsaturation (Boyd, Nwosu, Young, & MacMillian, 1998). Recent studies have particularly focused on the oxidative stability of marine PL in liposomal form (Moriya et al., 2007; Mozuraityte, Rustad, & Storro, 2008). It has been suggested that the good oxidative stability of marine PL might be due to (a) their tight intermolecular packing conformation at the sn-2 position (Applegate & Glomset, 1986) and (b) synergism between the phospholipids and α-tocopherol, which is also present in marine PL (Moriya et al., 2007). Furthermore, some studies (Hidalgo, Mercedes Leoan, Nogales, & Zamora, 2007; Hidalgo, Nogales, & Zamora, 2005) showed that slightly oxidised phospholipids in the presence of amino compounds had a better oxidative stability as compared to non-oxidised phospholipids. This was suggested to be due to the formation of antioxidative carbonyl–amine compounds resulting from the reaction between oxidised amino phospholipids/amino acids and fatty acid oxidation products. Similar to the Maillard reaction, the reaction between lipid oxidation products and proteins/PE may result in browning due to formation of pyrroles and both types of reactions are therefore termed as non-enzymatic browning (Zamora, Nogales, & Hidalgo, 2005).

Due to the numerous health benefits of marine PL, there is an increasing desire to use marine PL emulsion as *n*-3 delivery systems with the purpose to increase the *n*-3 PUFA content in foods. A good delivery system is characterised by having a good physical and oxidative stability. To the best of our knowledge, only one study has so far been carried out to investigate the feasibility of

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marine PL emulsion as delivery system for food enrichment (Lu, Nielsen, Baron, & Jacobsen, in press). However, this study mainly focused on the physicochemical properties of marine PL emulsions and not on their oxidative stability during storage. Therefore, the main objective of this study was to investigate the oxidative stability of marine PL emulsions during storage. We hypothesise that the oxidative stability of marine PL emulsions vary depending on the chemical composition of marine PL used for their preparation. Therefore, the oxidative stability of emulsions prepared with different types of marine PL and with or without addition of fish oil (triglycerides) was investigated. In addition, most of the marine PL that are available in the market are not solely containing PL but also containing residues of amino acids, protein or reducing sugar. The presence of these residues even in small amounts may react with lipid oxidation products in marine PL emulsions as previously mentioned. Therefore, we also measured colour changes, which can be attributed to PL pyrrolisation and Strecker derived volatiles, which can be attributed to amino acids degradation in marine PL emulsions.

2. Materials and methods

2.1. Materials

Three different marine phospholipids (LC, MPW and MPL) were obtained from PhosphoTech Laboratoires (Saint-Herblain Cedex, France) and Triple Nine (Esbjerg, Denmark), respectively. Fish oil (Maritex 43-01) was supplied by Maritex (Subsidiary of TINE BA, Sortland, Norway). This fish oil had low initial PV (0.16 meq/kg) and contained 240.4 mg/kg α-tocopherol, 99.3 mg/kg γ-tocopherol and 37.9 mg/kg δ-tocopherol. Sodium acetate and imidazole were obtained from Fluka (Sigma–Aldrich Chemie GmbH, Buchs, Spain) and Merck (Darmstadt, Germany), respectively. All solvents were of HPLC grade (Lab-Scan, Dublin, Ireland).

2.2. Preparation of marine PL emulsion

Different formulations of marine PL emulsion (300 ml for each formulation) were prepared either with PL alone or with PL and fish oil (Table 1). Emulsions were prepared in two steps; pre-emulsification and homogenisation. For the preparation of emulsions comprising both fish oil and marine PL, marine PL in liquid form (MPL, MPW) was first mixed with fish oil whereas marine PL in solid form (LC) was first dissolved in 10 mM acetate-imidazole (pH 7) buffer solution prior to pre-emulsification with fish oil. In the pre-emulsification step, marine PL or a combination of fish oil and marine PL were added to the buffer over 1 min under vigorous mixing (19,000 rpm) with an Ultra-Turrax (Ystral, Ballrechten-Dottingen, Germany) followed by 2 min of mixing at the same speed. All pre-emulsions were subsequently homogenised in a Panda high pressure table homogenizer (GEA Niro Soavi SPA, Parma, Italy) using a pressure of 800 bar and 80 bar for the first and second stages, respectively. After homogenisation, 1 ml of sodium azide (10%) was added to each emulsion (220 g) to inhibit microbial growth. Emulsions (220 g for each formulation) were stored in 250 ml blue cap bottles at 2 °C in darkness for 32 days. Samples were taken on day 0, 4, 8, 16 and 32, flushed with nitrogen and stored at −40 °C until further analysis. Samples were analysed for their oxidative stability, which included measurement of peroxide value (PV) and measurement of secondary volatiles through Solid Phase Microextraction (SPME) GC–MS (day 16 and 32). In addition to SPME GC–MS analysis, dynamic headspace (DHS) GC–MS analysis was performed on selected samples, namely MPW and F-MPW emulsions (day 16 and 32). In order to study non-enzymatic browning of marine PL emulsion, pyrrole content and colour change (lightness and Yellowness Index, YI) of marine PL emulsions were determined on day 0 and day 32.

Table 1
Experimental design for marine PL emulsions.

Formulations/ emulsions	%Fish oil	%Phospholipids			%Total lipids	Acetate-imidazole buffer (%)
		MPL	MPW	LC		
MPL		10.0			10.0	90.0
F-MPL	7.0	3.0			10.0	90.0
MPW			10.0		10.0	90.0
F-MPW	7.0		3.0		10.0	90.0
LC				10.0	10.0	90.0
F-LC	7.0			3.0	10.0	90.0

2.3. Characterisation of marine phospholipids

2.3.1. Determination of ethoxyquin and tocopherol

Approximately 0.5 g of marine PL was used for extraction with heptane (5 ml) and the extract was analysed for tocopherol and ethoxyquin content by HPLC analysis (Agilent 1100 series, Agilent Technologies, Palo Alto, CA, USA). For determination of tocopherol, a Water Spherisorb (R) silica column (4.6 × 150 mm, i.d. = 3 μm) was used. The mobile phase consisted of heptane and iso-propanol (100:0.4, respectively) and was introduced at a flow rate of 1 ml/min. Tocopherols were detected with a fluorescence (FLD) detector at 290 nm as excitation wavelength and at 330 nm as emission wavelength according to the AOCS Official method Ce 8-89 (1998).

For determination of ethoxyquin, the heptane extract was evaporated under nitrogen to dryness and the obtained residue was redissolved in acetonitrile and analysed using a C18 Thermo hypersil ODS column (250 mm, i.d. = 4.6 μm). Ethoxyquin was detected with a UV detector at 362 nm and the mobile phase consisted of acetonitrile and 1 mM ammonium acetate (80:20, respectively), and was introduced at a flow rate of 0.8 ml/min.

Two extractions were made from each sample and the measurement was performed in duplicate and quantified by authentic standards.

2.3.2. Determination of fatty acid and phospholipids composition

For fatty acids composition in polar lipids and neutral lipids, approximately 0.5 ml marine phospholipids in chloroform (with a concentration of 10–20 mg/ml) was transferred to a Sep-pak column containing 500 mg aminopropyl-modified silica (Waters Corporation, Milford, MA, USA) for lipid separation. A mixture of 2 × 2 ml choloroform and 2-propanol (ratio 2:1) was used to elute the neutral lipid fraction (NL), whereas 3 × 2 ml methanol were used to elute the PL fraction by gravity. Eluates were evaporated under nitrogen and methylated according to AOCS Official method Ce 2-66 (1998), followed by separation through gas chromatography (HP 5890 A, Hewlett Packard, Palo Alto, CA) with a OMEGA-WAX™ 320 column according to the method described by AOCS Official method Ce 1b-89 (1998). The analysis was performed in duplicate. The PL composition of marine PL was determined through ^{31}P NMR by Spectra Service GmbH (Cologne, Germany). All spectra were acquired using an NMR spectrometer Avance III 600 (Bruker, Karlsruhe, Germany), magnetic flux density 14.1 Tesla QNP cryo probe head and equipped with automated sample changer Bruker B-ACS 120. Computer Intel Core2 Duo 2.4 GHz with MS Windows XP and Bruker TopSpin 2.1 was used for acquisition, and Bruker TopSpin 2.1 was used for processing.

2.3.3. Determination of lipid classes by thin layer chromatography

The different lipid classes of marine PL were measured by TLC-FID Iatroscan MK-V (Iatron Laboratories, Inc., Tokyo, Japan) with Chromo Star v3.24S software (Bruker-Franzen & SCAP, Germany).