

INHIBITORY EFFECTS OF PHYTIC ACID AS A NATURAL ANTIOXIDANT IN PREVENTION OF FISH OIL PEROXIDATION

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Abstract

The inhibitory effects of endogenous phytic acid extracted from wheat bran (PAE), exogenous phytic acid (PA 4mM) and ascorbic acid (A.A) on oxidative stability in 10% cod liver o/w emulsions were investigated. TBARS values, oxygen absorption and total lipid hydroperoxides content were estimated during storage of emulsions treated with antioxidants. PAE and PA 4mM inhibited TBARS formation in cod liver o/w by 67% and 72% of control after 2 weeks of oxidation at 40°C, respectively. PA 4 mM was highly effective in reducing TBARS values and preventing lipid oxidation compared with ascorbic acid. Residual oxygen content as % in headspace of control vial decreased to about 77% of its initial content (from 20.50 to 4.70%) after 15 days of storage at 40°C. The rate of oxidation for control sample was about 1% O₂/day, while in sample treated with PAE was 0.16% O₂/day; which is about 10 times lower. In the headspace air of the vials containing cod liver o/w emulsion treated with PA (4 mM) was significantly lower than that treated with ascorbic acid (0.1%). Total lipid hydroperoxides content in emulsion treated with PAE were significantly lower than that in the control or emulsion treated with WBE stored at 40°C for 15 days. PA 4 mM inhibited total lipid hydroperoxides formation by 62% of control. Ascorbic acid at concentration 0.1% inhibited hydroperoxidation by 42% at same conditions of storage. In conclusion, phytic acid extracted from wheat bran may be recommended as a food antioxidant that prolongs the stability of fish lipids or fish meats. Moreover, the inhibition of lipid peroxidation by PAE and PA 4 mM, in this investigation was more effective than ascorbic acid.

Key words: phytic acid, wheat bran, fish oil

INTRODUCTION

Lipid oxidation is a major cause of quality changes, involving flavor, texture, and appearance. It can affect, to some extent, the nutritive value and safety of foods [11]. There is also increased evidence that uncontrolled lipid oxidation is involved in the development of many chronic diseases [4]. Fish oil is an object of increasing interest for its nutritional and pharmacological qualities, in particular for its contents of polyunsaturated fatty acid (PUFA)-n3 [18]. The high concentration of PUFAs makes such oil easily oxidizable, and, therefore, it is currently used for identifying substances which improve the shelf-life of fish oil preservation [8]. Antioxidants may inhibit the decomposition of hydroperoxides by acting

as radical scavengers, metal chelators or reducers of hydroperoxides to more stable hydroxyl compounds [12]. Using of natural antioxidants offers the potential advantages of a reduction and/or replacement of synthetic antioxidants, lowered assumed toxicity due to their natural origin as components of foods; enhanced masking of off-flavours arising from oxidation and greater consumer acceptability as natural ingredients in foods [6]. Phytic acid is a common plant constituent, comprising 1–5% by weight of edible legumes, cereals, oil seeds, pollens and nuts [15]. Phytic acid has been reported to be antioxidant [14], anticarcinogenic [26], and hypoglycemic or hypolipidemic [24]. Phytic acid is considered to be an antioxidant agent because it is a

potent inhibitor of iron-catalyzed hydroxyl radical formation by chelating the free iron and then blocking the coordination site [14]. Furthermore, lower inositol phosphates, such as IP4 and IP3, may play roles in mediating cellular responses and have been noted as having a function in second messenger transduction systems [5]. Phytic acid in cereals plays an important role in inhibiting oxidation during preservation or storage [22]. The aim of the present study was to explore the inhibitory effects of phytic acid as a natural anti-oxidant on prevention of fish oil peroxidation in emulsion model systems in comparison with ascorbic acid.

MATERIALS AND METHODS

Materials: Commercial cod liver oil was purchased from Toho Pharmaceutical Co. Inc. (Tokyo, Japan) and purified by silica gel chromatography (2 cm i.d. × 30 cm); briefly, the column was packed with 40-50 μm of spherical silica (kanto Chemical Co. Inc., Tokyo, Japan) and cod liver oil was eluted with a mixture of diethyl ether and *n*-hexan (5:95, v/v). All the standard chemicals were purchased from Tokyo Chemical Industry (Tokyo, Japan), Sigma (St. Louis, Mo, USA) and Wako Pure Chemical Industries (Osaka, Japan).

Preparation of WBE and PAE: Wheat bran extract (WBE) was prepared by extraction of wheat bran with 0.5N HCl and passed through an AGI-X8 chloride anion-exchange column (200-400 mesh) to obtain phytic acid extract (PAE) according to the method of [1].

Preparation of o/w emulsion: Oil-in-water (*o/w*) emulsions consisting of 10% cod liver oil, 1% Tween 20 as emulsifier, and 5ml phytate solution in water phase was homogenized with an UD-200 model of ultrasonic disruptor machine (Tomy Co., Tokyo, Japan) for 10 min in an ice bath.

Oxidation of o/w emulsions: For determination of oxygen absorption, 10 ml of emulsion was placed in a glass vial of 61 ml in volume and subsequently sealed tightly with an aluminum cap with polytetrafluoroethylene/silicone liner (Supelco, Bellefonte, PA). For determination of total hydroperoxide and TBARS contents,

50 ml of control or test emulsion was transferred into Erlenmeyer flask. Vials and flasks were shaken in a dark oven at 40°C.

Analysis of fatty acid composition: Fatty acids of the cod liver oil were transesterified to methyl esters, using a base-catalyzed transesterification, followed by a BF₃-MeOH-catalyzed esterification according to the official method of AOCS Ce Ib-89 [2], to obtain fatty acid methyl ester (FAMES). The FAMES were dissolved in *iso*-octane and injected in to a model GC 14B gas chromatograph (Shimadzu Corp., Kyoto, Japan) equipped with a Supelcowax-10 fused silica wall-coated open tubular column (0.25mm id. × 30 m, 0.25 μm in film thickness; Supelco). The column oven and injection port temperature were held initially at 150°C for 2 min, then programmed to 195°C at a rate of 5°C/min, from 195°C to 250°C at a rate of 1.2°C/min, and finally held at 250°C for 35 min. Helium was used as a carrier gas with an inlet pressure of 2.0 kg/cm².

TBARS determination: The TBARS values were determined in triplicate according to the method of [30]. 0.2 ml oil-water emulsion diluted to 5 ml with distilled water, then 5 ml 0.02 M aqueous solution of TBA in a stoppered test tube, kept at 100°C for 35 min in water bath and subsequently, cooled for 10 min in cold water. Absorbance was measured at 532 nm by UV Spectrophotometer against a blank containing 5 ml distilled water and 5 ml TBA reagent. Results expressed as mg malondialdehyde per kg cod liver oil were calculated from the standard curve of TEP (1.1.3.3.-tetraethoxypropane).

Determination of oxygen absorption: Oxygen absorption was determined according to the method of [21]. A volume of 0.1 ml of the headspace gas in the crimp top glass vial was withdrawn with a gas-tight micro syringe with 24 G needle and immediately injected into shimadzu gas chromatography GC-3BT equipped with a glass column (2.5 mm i.d. x 1.7 m) packed with molecular sieve 5A (80-100 mesh; Nihon Chromato Co. Ltd, Tokyo, Japan) and a thermal conductivity detector. Helium was used as the carrier gas at an inlet pressure of 1.2 kg/cm².

Determination of total lipid hydroperoxides:

Hydroperoxides content was determined by a flow-injection analysis (FIA) HPLC system with a DPPP fluorescent post column detection system according to the method reported by [29]. For the preparation of sample solution, 0.1 ml o/w emulsion was mixed with 1 ml DPPP solution (21.6 nm/ml in methanol) in a 10 ml volumetric flask, and then the mixture was made up to 10 ml using butanol. Next, 20 μ L of the butanol solution was injected into the FIA HPLC system.

Statistical analysis: Measurements of TBARS, oxygen absorption and Total lipid hydroperoxides were carried out in triplicate and represented as mean \pm standard deviation. Microsoft Excel was used for Statistical analysis. Student's *t*-test was used to distinguish significant differences among the mean values. The significance was declared at $P < 0.05$.

RESULTS AND DISCUSSIONS

Fatty acid composition of fish oil: A fatty acid composition of cod liver oil is summarized in Tale (1). The predominant fatty acids were palmitic (C16:0) for saturated fatty acids (11.50 \pm 0.02), Oleic acid (19.91 \pm 0.44) for monounsaturated fatty acid and C20: 5n3 (8.82 \pm 0.60) for polyunsaturated fatty acids which together accounted for 40.23% of the total fatty acids. Total fatty acids were composed of 18.81% of saturated, 51.42% of monounsaturated and 29.80% of polyunsaturated acids.

TBARS values: The effect of wheat bran extract (WBE) and phytic acid extracted from wheat bran (PAE) on TBARS content of emulsified cod liver oil stored at 40°C are shown in Fig. 1. Thiobarbituric acid reactive substances (TBARS) values of the control were higher than those of the samples treated with wheat bran extract or phytic acid extract.

Table 1. Fatty acid composition of cod liver oil*

Fatty acids	(% of total fatty acids)
C14:0	4.86 \pm 0.04
C:16:0	11.50 \pm 0.02
C18:0	2.45 \pm 0.04
C16:1n7	12.50 \pm 0.04
C18:1n9	19.91 \pm 0.44
C18:1n7	4.02 \pm 0.07
C18:1n5	0.58 \pm 0.07
C20:1n9	6.03 \pm 0.08
C22:1n11	8.39 \pm 0.60
C16:2n4	1.25 \pm 0.04
C18:2n6	2.14 \pm 0.12
C16:3n4	0.91 \pm 0.07
C16:4n1	0.75 \pm 0.04
C18:3n3	0.82 \pm 0.03
C18:4n3	1.40 \pm 0.08
C20:4n5	0.62 \pm 0.02
C20:4n3	0.92 \pm 0.03
C20:5n3	8.82 \pm 0.60
C22:5n3	3.14 \pm 0.05
C22:6n3	8.72 \pm 0.44
Total saturated	18.81
Total monounsaturated	51.42
Total polyunsaturated	29.80

*Data presented as mean \pm SD

TBARS values of control were increased gradually from 1.21 to 9.54 mg/kg during oxidation of cod liver o/w emulsion at 40 °C for 15 days. The increased rancidity, based on the TBA value is due mainly to the

formation of malondialdehyde decomposed from hydroperoxides of fatty acids which contain three or more double bonds [28]. TBARS values of the wheat bran extract group increased slowly from 1.21 to 5.50

mg/kg during 15 days of storage (oxidation) at 40°C. Wheat bran extract inhibited TBARS formation by 42% of control at the end of oxidation. These affecting may be due to wheat bran extract contains inositol phosphates (fractions of phytic acid) with phytic acid [23] as well as other antioxidants [3]. Phytic acid extracted from wheat bran (PAE) was highly effective in reducing TBARS values and preventing lipid oxidation during oxidation at 40°C. Phytic acid extract inhibited TBARS formation by 67% of control. These results are in

agreement with those reported by [19] and [20]. PA 4 mM (pure phytic acid sodium salt) was highly effective in reducing TBARS values by 72% of control as shown in Fig. 2 and preventing lipid oxidation compared with ascorbic acid. Earlier results reported by [10] revealed that 1 mM phytic acid provided significant protection against oxidative damage and increase shelf-life fourfold. These findings confirmed the results of [17], who found that ascorbic acid ineffective in preventing lipid oxidation on poultry at the end of the storage at -20°C for 6 weeks.

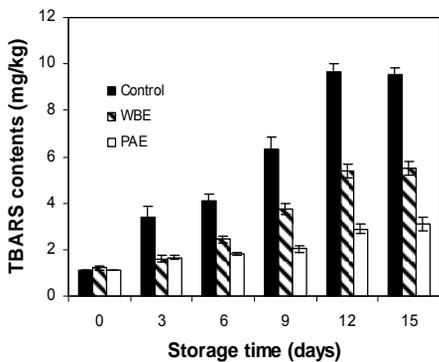


Fig. 1. Changes of TBARS contents during oxidation of cod liver o/w emulsion treated with wheat bran extract (WBE) and phytic acid extract (PAE) at 40°C.

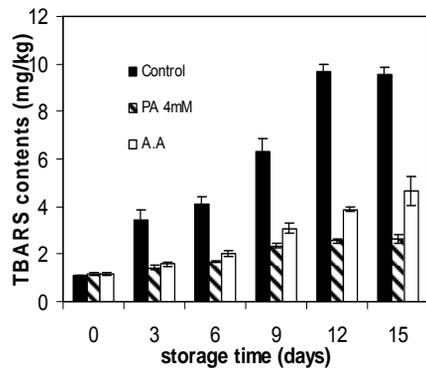


Fig. 2. Changes of TBARS contents during oxidation of cod liver o/w emulsions treated with phytic acid 4mM and ascorbic acid 0.1% (A.A) at 40°C.

Residual Oxygen content: Oxygen in the headspace of emulsion without antioxidants (control) was consumed faster than that with added endogenous phytic acid (Fig. 3). Residual oxygen content as percentage in headspace of control vial decreased to 77% of its initial content (from 20.50 to 4.70%) after 15 days of oxidation at 40°C. Oxygen disappearance in the headspace is directly related to lipid peroxidation of the marine oil because of oxygen reaction with oils to produce peroxy radicals and hydroperoxides [16]. Cod liver o/w emulsion treated with phytic acid extract (PAE) inhibited oxygen consumption during oxidation at 40°C for 15 days. Assuming a zero time reaction, the rate of oxidation for control was about 1% O₂/day, while in sample treated with phytic acid extract was 0.16% O₂/day or about

10 times slower (significant difference). Decreases in residual oxygen contents in the headspace air of the vials containing the cod liver o/w emulsion that had pure phytic acid (4 mM) were significantly lower than that with the emulsion that had ascorbic acid (0.1%) after 2 weeks of oxidation at 40°C as shown in Fig. 4. The rate of oxidation without phytate (control) was 0.0566% O₂/day while with phytate (1 mM) was 0.0127% O₂/day or about 4 times slower [10]. Endogenous phytic acid and exogenous phytic acid (4 mM) had more effect on prevention of oxidation as well as oxygen absorption than ascorbic acid in cod liver o/w emulsion.

Total lipid hydroperoxides: In control sample of cod liver oil o/w emulsion, total hydroperoxides content were increased up to

198 $\mu\text{mol/kg}$ in the first 6 days of oxidation then increased up rapidly to 680 $\mu\text{mol/kg}$ after 15 days of oxidation and storage at 40°C as shown in Fig. 5. The marine oil is rich in PUFA, especially those of the ω -3 family, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid, and these PUFA are highly sensitive to peroxidation [7]. Total lipid hydroperoxides contents in emulsion treated with phytic acid extract were significantly lower than that in control. In cod liver oil *o/w* emulsion oxidized at 40°C under dark, phytic acid at

concentration of 4 mM (PA 4mM) was more effective on the formation of hydroperoxides compared with control (Fig. 6). Phytic acid 4 mM inhibited total hydroperoxides formation by 62% of control. In contrast, ascorbic acid at concentration 0.1% was slightly reduced of hydroperoxides formation at 40°C compared with PA 4 mM (Fig. 6). [17] reported that ascorbic acid ineffective in preventing lipid oxidation on poultry at the end of the storage at -20°C for 6 weeks

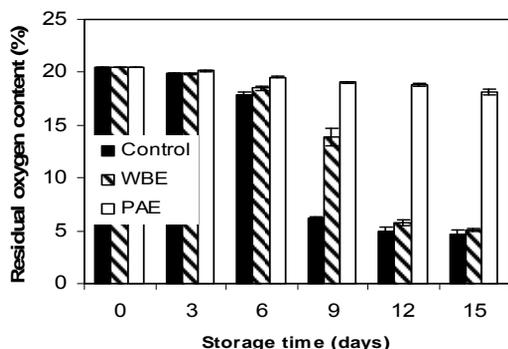


Fig. 3. Changes in residual oxygen contents in the headspace air of vials during oxidation of cod liver oil *o/w* emulsion treated with wheat bran extract (WBE) and phytic acid extract (PAE) at 40°C.

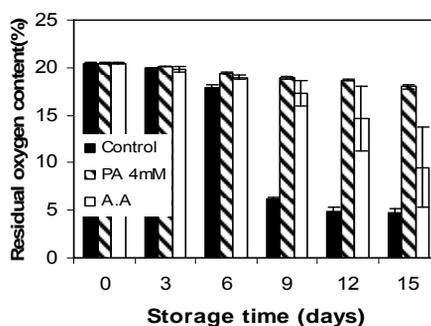


Fig. 4 Changes of residual oxygen contents in the headspace air of vials during oxidation of cod liver oil *o/w* emulsion treated with phytic acid 4mM and ascorbic acid 0.1% (A.A) at 40°C

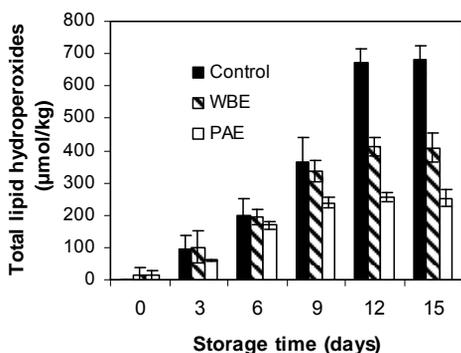


Fig. 5. Changes of total lipid hydroperoxide contents during oxidation of cod liver oil *o/w* emulsion treated with wheat bran extract (WBE) and phytic acid extract (PAE) at 40°C

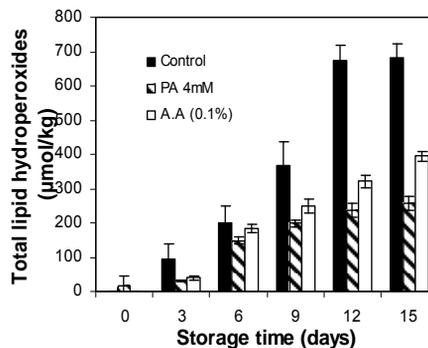


Fig. 6. Changes of total lipid hydroperoxide contents during oxidation of cod liver oil 10% *o/w* emulsions treated with phytic acid 4mM and ascorbic acid 0.1% (A.A) at 40°C.

CONCLUSIONS

In conclusion, phytic acid extracted from wheat bran substantially inhibited

malondialdehyde formation, oxygen uptake, as well as warmed-over flavor development during storage of fish lipids or fish meat

products. The inhibition of lipid peroxidation by phytic acid was more effective than ascorbic acid. Our results suggest that phytic acid as a natural food antioxidant may be prolongs the stability of fish lipids during storage.

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