Oxidative Stability of Fish Oil Blended with Butter[1]

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ABSTRACT

The oxidative stability of fish oil blended with milk products was evaluated. Oxidation of the oil blend was determined by peroxide value and rancimat test. Improved oxidative stability was observed for fish oil blended with butter. Unsalted and salted butters showed no difference in improvement of effect on oxidative stability of fish oil. No influence on oxidative stability for the fish oil was observed with butter oil, which was the oil fraction of butter. These studies suggested that the improved oxidative stability of fish oil-butter blend was due to the hydrophilic fraction of butter and that butter could improve the oxidative stability of polyunsaturated oils such as fish oil.
(**Key words:** butter, antioxidant, fish oil)

Abbreviation key: DHA = docosahexanoic acid, **PUFA** = polyunsaturated fatty acid, **PV** = peroxide value.

INTRODUCTION

Polyunsaturated fatty acid (**PUFA**), such as eicosapentanoic acid and docosahexanoic acid (**DHA**), has received increasing attention for its nutritional aspects. Recent studies (9, 17) report that DHA plays a key role in brain and retina function and suggest that DHA is important to maintain and improve memory, intelligence, and visual acuity. To enhance these physiological functions, foods that contain PUFA have been developed in recent years, and most of those foods use fish oils as a PUFA source. Some fish such as tuna, bonito, and sardine contain a high amount of PUFA in the body oil fraction; thus, fish oils are an important source of PUFA (19) for food usage. However, fish oil is vulnerable to oxidation. The greater the PUFA concentration in the fish oil is, the faster the oxidation takes place (7). As oxidation progresses, fish oil generates off-flavors characterized as fishy, green, metallic, or burnt and also forms hydroperoxides (12). Hydroperoxides formed by lipid oxidation were reported to be harmful to health (4, 11). Oxidation of fish oil containing the PUFA should be suppressed to maintain the quality and safety for food containing fish oil. Antioxidants are generally used to prevent lipid oxidation. Among antioxidants, tocopherols, which are derived from natural sources such as corn, soybeans, and palms, are widely used because tocopherols are not reported to have the undesirable physiological implications that butylated hydroxytoluene, butylated hydroxyanisole, and butylhydroquinone are reported to have (6, 8, 10, 15, 16). Studies have reported the inhibition effect on lipid oxidation of some milk products such as skim milk and whey. Acid whey and permeate are both reported to inhibit oxidation of several lipids promoted by iron, lipoxidase, photoactivated riboflavin, and hydrogen peroxide-activated metmyoglobin (5). Of milk components, proteins such as casein, α-LA, and enzymes have been previously reported as potent inhibitors of lipid oxidation (1, 3, 13, 20, 21). Minor components such as phospholipids, tocopherols, ascorbic acid, and carotenoids also inhibit oxidation. Milk products could be used as natural antioxidants. However, hydrophilic fractions including butter have not been reported to have an antioxidant effect on lipid oxidation. The aim of this study was to investigate the inhibition effect of milk products on fish oil oxidation in an attempt to exploit the potential of butter as an antioxidant.

MATERIAL AND METHODS

Materials

Bonito oil was obtained from a commercial source as fish oil (Ueda Oil and Fats MGF. Co., Kobe, Japan). The fish oil contained 19.3% DHA, 6.6% eicosapentanoic acid, and 125 ppm of tocopherols. Unsalted and salted butters were prepared by Snow Brand Milk Products Co., Ltd., Tokyo, Japan. Salted butter used in this study contained 1.4% salt. Unsalted butter used in this study contained 0.4% salt. Butter oil was separated from the unsalted butter. Commercial butter oil (Francexpa Co., Paris, France) was also used. Milk protein materials

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TABLE 1. Induction period of fish oil with an addition of 10% unsalted butter as measured by rancimat.

Samples	Induction period
	(h)
Fish oil	2.75
Fish oil + unsalted butter (90/10)	4.05

TABLE 2. Induction period of fish oil with addition of milk products as measured by rancimat.

Samples[1]	Induction period
	(h)
Control	3.14
WPC powder	3.24
WPI powder	3.20
Skim milk powder	3.00
Whole milk powder	2.98
Butter milk powder	2.80

[1]WPC = Whey protein concentrate as AMP 8000 (American Meat Products Co., Ames, IA); WPI = whey protein isolate as Sunlact I-1 (Taiyo Kagaku Co., Yokkaichi, Japan).

were purchased from commercial sources as follows: whey protein concentrate as AMP 8000 (American Meat Products Co., Ames, IA) and whey protein isolate as Sunlact I-1 (Taiyo Kagaku Co., Yokkaichi, Japan). Skim milk powder, whole milk powder, and buttermilk powder were obtained from Snow Brand Milk Co., Ltd.

Analysis of Fatty Acid and Tocopherol

Contents of the fatty acids were measured by gas chromatography. Oil samples were transmethylated by potassium hydrate to obtain the fatty acid methyl esters. The methyl esters were dissolved in n-hexane and injected into the gas chromatography system. The fatty acid content was measured with a HP 5890 series II (Hewlett-Packard, Palo Alto, CA); flame-ionization detector; and a fused-silica capillary column, DB-WAX (30 m, 0.25 mm i.d.), 0.25 μM (film) (J & W Scientific Inc., Folsom, CA). Helium was used as carrier gas. The tocopherol content was measured by HPLC with a Zorbax NH2 column (250 mm × 4.6 mm; Hewlett-Packard, Palo Alto, CA), and a fluorescence detector (Jasco Corp., Hachioji, Japan). The mobile phase was n-hexane/2-Propanol = 980:20. Wavelength was set at an excitation of 298 nm and emission of 325 nm.

Preparation of Oil Blend

Fish oil was added to butter, butter oil, or other powdered milk products then homogenized (Ultraturrax T-25; Janke and Kunkel Co., Staufen, Germany) at 20,500 rpm for 1 min at 20°C.

Preparation of the Hydrophilic Fraction of Butter

Unsalted butter was melted at 50°C in an oven for 1 h and then centrifuged. The centrifuged butter was separated into three phases: the oil phase, the aqueous (middle solution) phase, and the precipitate fraction. The oil phase was removed. The aqueous phase and precipitate were combined and dispersed in water, then the aqueous phase was collected, followed by freeze-drying to obtain the hydrophilic fraction of butter.

Oxidation of Oil Blends

For the oven storage test, oil blends (25 g of each) were placed in 50-ml brown glass vials and were kept in the dark at 37°C. Two samples per oil blend were prepared for each oxidation time. The progress of the oxidation was monitored by following the peroxide formation, determined by peroxide value (**PV**). The PV was determined by a colorimetric ferric thiocyanate method (2). The rancimat test was carried out with oil blends at 90°C (Rancimat E679; Metrohm Co., Herisau, Switzerland). The oxidation stability of the oil blends was evaluated with induction time determined by rancimat test (14, 18). The oven storage test by PV and the rancimat test were duplicated.

RESULTS AND DISCUSSION

The induction time of fish oil blend with butter was determined by rancimat test, as shown in Table 1. The induction time of fish oil blended with butter was about 1.5 times longer than that of fish oil, under the conditions used in this study.

The induction times of the fish oil blend to which other milk products were added were determined by rancimat test, as shown in Table 2. Each oil blend contained 20% butter oil and 80% fish oil and milk products were added at 1.1%. The control sample contained 20% butter oil and 80% fish oil. Fish oil blends with milk products had similar induction times to that of the control fish oil blend. These results indicated that butter provided antioxidant activity for fish oil.

The influence of salt contained in butter on the oxidative stability of the fish oil blend by rancimat test was evaluated. Table 3 shows that the rancimat induction times of salted butter and unsalted butter were almost the same.

The effect of butter content in the fish oil blend was investigated by an oven storage test. Fish oil blends that contained butter ranging from 0 to 50% were pre-

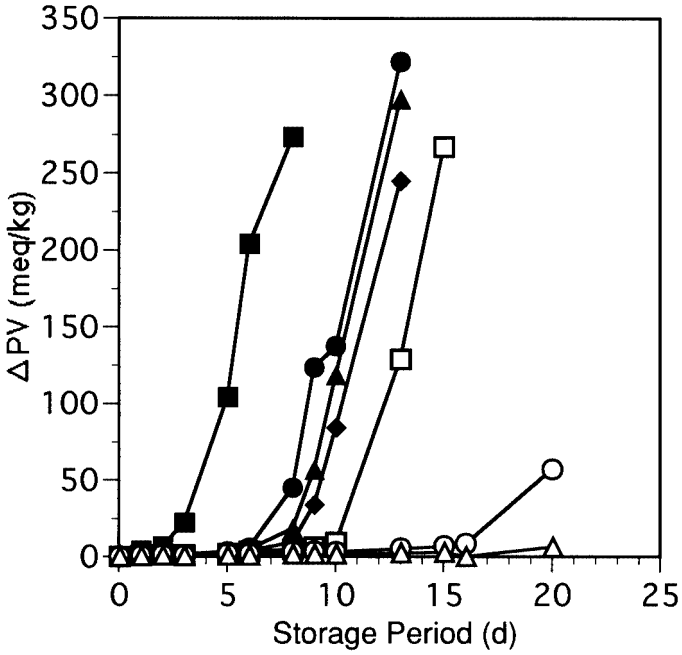

Figure 1. Effect of unsalted butter concentration on the oxidation of fish oil as measured by changes in peroxide value (ΔPV). Unsalted butter concentration in fish oil blend: 0.0%, ■; 2.5%, ●; 5.0%, ▲; 7.5%, ◆; 10.0%, □; 20.0%, ○; 50%, △.

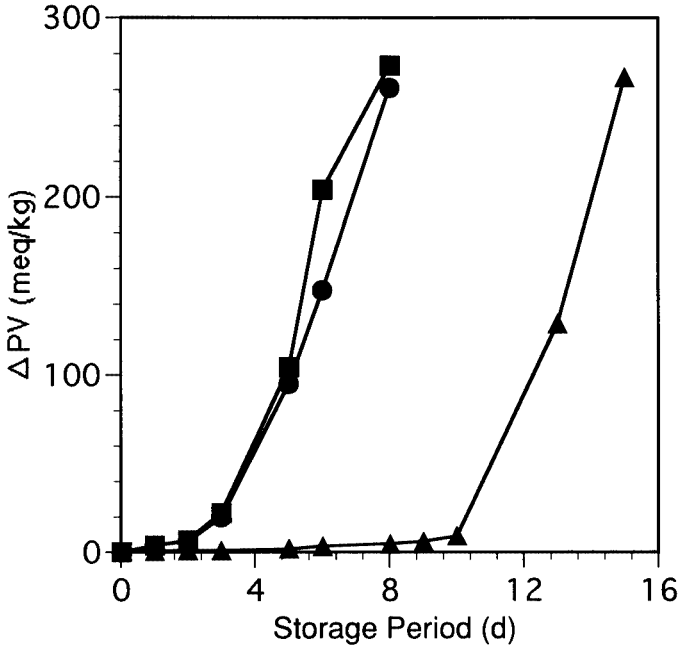

Figure 2. Effect of unsalted butter and butter oil on the oxidation of fish oil as measured by changes in peroxide value (ΔPV). Key: fish oil, ■; fish oil blends with 10% butter oil, ●; fish oil blends with 10% unsalted butter, ▲.

pared and stored in an oven at 37°C. The period for which PV maintained baseline, which is defined as the PV induction time, depended on the percentage of unsalted butter and was extended with increased percentages of unsalted butter, as shown in Figure 1. Addition of unsalted butter was so effective in increasing the PV induction time of the fish oil blend that 2.5% butter extended the PV induction time to approximately two times that of the control fish oil. The fish oil blend with 50% butter did not increase in PV for 20 d. These results indicated that butter significantly heightened the oxidative stability of the fish oil blend.

The influence of butter oil on the oxidative stability of fish oil was investigated by oven storage test. Figure 2 shows that the PV induction time of fish oil was increased by blending with unsalted butter, but that of fish oil blended with butter oil was not increased. The effect of butter oil content on fish oil blend was investigated by rancimat test. Figure 3 shows that the induction time measured by rancimat of the fish oil blend did not increase with <50% butter oil in the fish oil blend, but increased induction time was observed with

TABLE 3. Induction period of fish oil with an addition of 10% salted or unsalted butter as measured by rancimat.

Samples	Induction period
	(h)
Unsalted butter	4.05
Salted butter	4.19

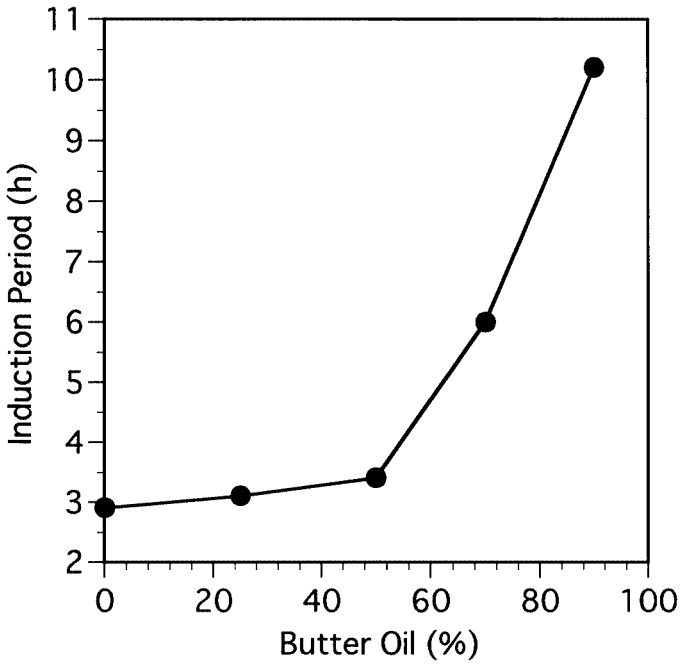

Figure 3. Effect of butter oil concentration on the oxidation of fish oil as measured by rancimat.

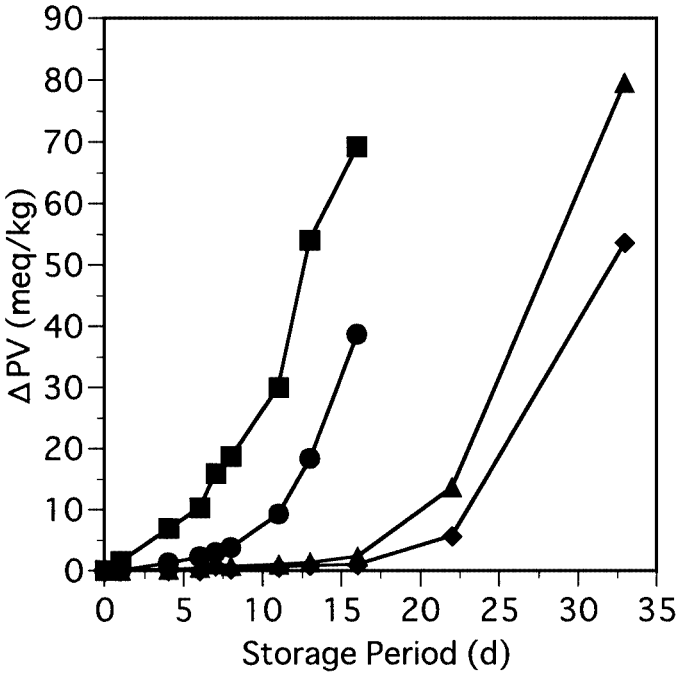

Figure 4. Effect of hydrophilic fraction of butter on the oxidation of fish oil blend containing 20% butter oil as measured by changes in peroxide value (ΔPV). Hydrophilic fraction of butter concentration in fish oil blend: 0.00%, ■; 0.48%, ●; 0.96%, ▲; 1.44%, ◆.

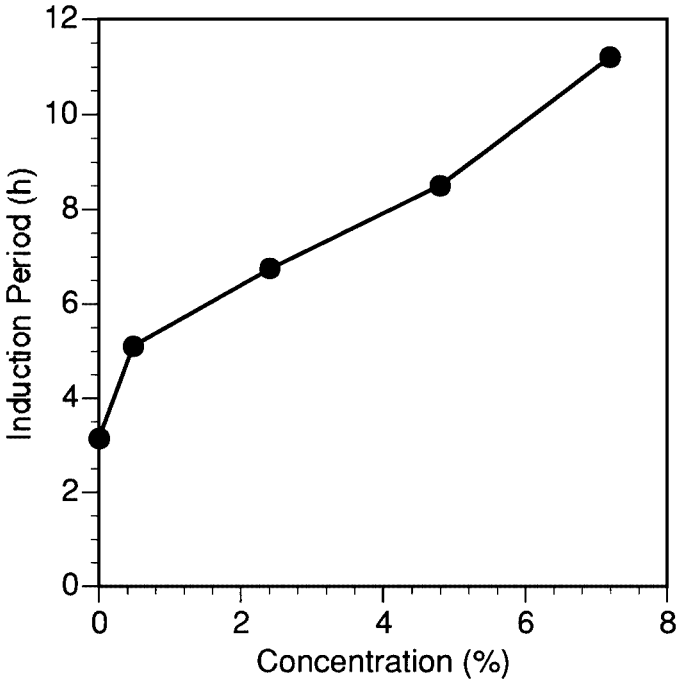

Figure 5. Effect of hydrophilic fraction of butter on the oxidation of fish oil blend containing 20% butter oil as measured by rancimat.

>50% added butter oil. The results indicated that decreased concentrations of polyunsaturated fatty acids in the fish oil blend showed insignificant effect at <50% butter oil. The results associated with the effect of butter on the oxidative stability of the fish oil blend described previously suggest that the hydrophilic component of butter plays an important role in the increased oxidative stability of the butter and fish oil blend.

The effect of the hydrophilic fraction of butter on the oxidative stability of the fish oil blend was evaluated by an oven storage test and a rancimat test. The freeze-dried hydrophilic fraction of butter was added to the fish oil blend containing 20% butter oil, which was the same oil composition as that used in our experiments on oxidation stability of milk products. The PV induction time increased with greater concentrations of the hydrophilic fraction of butter added to the fish oil blend (Figure 4); the rancimat induction time also increased under these conditions (Figure 5). Both tests supported that the hydrophilic fraction of butter contributed significantly to the increased oxidative stability of the fish oil blend that contained butter.

CONCLUSIONS

With the results presented in this study, we concluded that addition of butter resulted in increased oxidative stability of fish oil, and the hydrophilic fraction of butter played an important role as an antioxidant. The hydrophilic fraction of butter consisted mainly of proteins and conjugated lipids, including maillard reaction products generated during the manufacturing process of butter. The antioxidative activity of the hydrophilic fraction of butter was seemingly due to these components. However, more a precise investigation is required to clarify the impact of these components.

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