

Stabilization of fish omega-3 fatty acids using olive oil

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EXECUTIVE SUMMARY

Fish oil and virgin olive oil show markedly different storage lives due to their content of omega-3 fatty acids and polyphenols, respectively. Blending fish oil with highly stable virgin olive oil can be predicted to form combination oils with improved stability compared to fish oil. Prior to testing this hypothesis, we performed a comprehensive review of the literature dealing with the, (i) health benefits of fish oil and virgin olive oil, (ii) conditions influencing the stability of omega-3 fatty acids, (iii) factors influencing the stability of virgin olive oil, (iv) stabilization of edible oils using natural antioxidants, and (v) stabilization of fish oil using plant extracts and oil blends. Laboratory investigations showed that, blending fish oil and virgin olive oil yields combination oils with 16-155% increase in storage life compared to fish oil. The proportions of fish oil and virgin oil required for stabilization depends on the characteristics of the initial base oils.

ABBREVIATIONS

ALA	alpha-linolenic acid
3, 4-DHPEA	3, 4-dihydroxyphenyl ethyl alcohol
DHA	docosahexaenoic acid (C22:6, ω3)
EDA	Elanoic acid
EPA	Eicosapentaenoic acid (C20:5ω3)
LCPUFA	Long chain polyunsaturated fatty acids
LDL	Low density lipoprotein
p-HPEA	p-hydroxyphenyl ethyl alcohol
PUFA	Polyunsaturated fatty acid
ppm	Parts of million (mg/kg)

KEY WORDS

Omega-3 fatty acids, polyunsaturated fatty acids, fish oil, nutritional properties, oxidative stability, dietary fatty acids, health effects, Eskimo diet, Mediterranean diet

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1. 1. HEALTH BENEFITS OF FISH OIL AND OLIVE OIL

1.1.1. Omega-3 fatty acids, polyphenols and health

Consumption of omega-3 long chain polyunsaturated fatty acids is linked with a decreasing risk of, cardiovascular disease [1, 2], Type-2 diabetes [3], mood disorder [4] and inflammatory conditions e.g., rheumatoid arthritis [5-10]^a. Omega-3 fatty acids may have favourable effects also in relation to ulcerative colitis [11], colon cancer [12] and renal health [13] though more supporting evidence is needed in these areas. Fish oil is the main source of long chain omega-3 fatty acids notably, eicosapentaenoic acid (EPA; C20:5 ω 3) and docosahexaenoic acid (DHA, C22:6, ω 3). Long chain omega-3 fatty acids can be produced from alpha-linolenic acid (ALA) within the body but consuming DHA and EPA is considered a more effective strategy for raising plasma levels. At present, the average intake of omega-3 fatty acids in the western diet is considered below requirements. Commercial fish oil supplements and omega-3 fatty acid concentrates help to meet the current RDA [1, 14-17].

Habitual intake of virgin olive oil leads to a declining risk of, cardiovascular disease, cancer, neurodegenerative disease, and immune dysfunction [18-26]. The benefits of virgin olive oil are ascribed to its high levels of oleic acid (C18:1) and phenolic compounds e.g., tyrosol (p-hydroxyphenyl ethyl alcohol; p-HPEA), and 3, 4-dihydroxyphenyl ethyl alcohol (3, 4-DHPEA). The major olive oil phenols occur also as esters (sericoids) with elanolic acid (EDA), i.e. p-HPEA-EDA and 3, 4-DHPEA-EDA^b.

The main focus of virgin olive oil phenols research is currently concerned with; (i) their role in disease prevention, (ii) analysis and characterization in different foods, (iii) their effects on gene expression and (iv) polyphenols-protein binding interactions. Some new application areas for virgin olive oil phenols includes their use as (v) natural antioxidants to stabilize edible oils (cf. section 4) and (vi) as dietary supplements [19, 26-29].

^a Cardiovascular benefits associated with the Mediterranean diet, may include lower incidences of atherosclerosis, hypertension, thrombosis, platelet aggregation and endothelia dysfunction.

^b The polyphenols content of virgin olive oil ranges between 150-700 mg/ Kg (ppm). Hydroxy tyrosol 2-(3,4-Dihydroxyphenyl) ethanol/3,4-DHPEA (0.5-14.4 ppm), Tyrosol or p-Hydroxyphenyl ethanol/4-Hydroxyphenylethanol/ p-HPEA (0.4-14.4 ppm) along with p-HPEA + 3,4-DHPEA (up to 350 ppm each) constitute the most important phenols in virgin olive oil.

1.1.2. Mode of action of fish and olive oil

Virgin olive oil and fish oil share certain health benefits due to their composition (Table 1)^c. Both types of oil have cardio-protective and anti-inflammatory activity. The effects of fish oil are generally ascribed to omega-3 LCPUFA which, (i) inhibit triglyceride and lipoprotein synthesis by the liver and so reduce plasma lipids, (ii) moderate eicosanoid (thromboxane and leucotriene) synthesis, and (iii) replace membrane arachidonic acid [1, 15-17]. Virgin olive oil phenols decrease oxidative stress whilst the MUFA inhibit lipid synthesis and thereby moderate blood lipids [18-26].

1.1.3. Nutritional benefits of combination fish and olive oils

Blends of fish oil and virgin olive oil have been shown to have synergistic benefits related to rheumatoid arthritis [30-32], peripheral vascular diseases [33], ulcerative colitis [34] and colorectal cancer [35]. Blending fish oil with virgin olive oil reduces the LDL-oxidizing effect of the former [33, 36, 37]. Currently, the technological benefits from blending fish oil and virgin olive oil have not been studied in detail [38].

1.2. CONDITIONS INFLUENCING OMEGA-3 FATTY ACID STABILITY

1.2.1. Indices of omega-3 fatty acid autooxidation

The omega-3 LCPUFA are easily oxidized forming lipid hydroperoxides and over 50 volatile products. The autooxidation process for ALA, EPA and DHA increases sample fluorescence [39]. There is also a rise in sample UV absorbance at 232 and 270 nm which provides a highly convenient index for monitoring oil autooxidation [40-42]. Omega-3 fatty acid oxidation is also correlated with the appearance of propanal or propanal measured by headspace gas-liquid chromatography. Finally, autooxidation of lipids forms malonaldehyde which can be measured using the thiobarbituric acid colorimetric assay [43-46]^d. Recent investigations have shown that DHA (C22:6, ω3) is oxidized more rapidly than

^c Tables and Figures are listed at the end of this document.

^d The TBCA assay was used in laboratory tests on fish oil and olive oil blends (see part 2 of this report).

EPA (C20:5 ω 3) due to the greater number of double bonds in the former. Both EPA and DHA also oxidize more rapidly compared to ALA [47].

Parameters that affect the rate of oxidation of edible oils have been discussed [48-50]. Oxygen exposure, light, metal ions, and the type of storage container can all affect the rate of autooxidation. The addition of antioxidant as well as the levels of “minor oil components” (e.g. polyphenols and chlorophyll) also affect oil stability. Other factors influencing the stability of omega-3 LCPUFA are discussed in Sections 2.2-2.4.

1.2.2. Physical form of oil

Fish oil emulsions are used for parenteral nutrition [51, 52]. Interestingly, the stability of omega-3 fatty acids emulsions is lower compared to bulk oils [53-55]. The large surface area of DHA emulsions is believed to increase their rate of oxidation [55]. The type and concentration of surfactant used for emulsion formulation also affects DHA oxidation. Methyl esters of omega-3 fatty acids, used in some nutritional supplements, are less stable to autooxidation compared to naturally occurring triglyceride esters [56, 57].

1.2.3. FA position

The stereochemical number (sn), which is the position of fatty acid substitution on the glycerol backbone, affects the rate of autooxidation though conflicting data have been reported. DHA located at sn 1, 3 was more stable compared to DHA located at the sn 1, 2 or Sn 2, 3 positions [53]. However, another study suggested that DHA was more stable in the Sn 2 position [58, 59]. It is feasible that such results may be reconciled by noting that the third fatty acid is usually not identical for different studies.

1.2.4. Tocopherol

Low levels of tocopherol occur naturally in fish oil and may contribute to its stabilization (Table 1). However, high concentrations of added tocopherol appear to *promote* fish oil oxidation though the basis for the pro-oxidant effect remains uncertain [60-62] (see Section 1.3.2).

1.2.4. Phospholipids

The stability of omega-3 fatty acids increases in the presence of phospholipids for reasons that are not yet certain [54, 63].

1.3. FACTORS INFLUENCING VIRGIN OLIVE OIL STABILITY

1.3.1. Appraisal of virgin olive oil stability

Virgin olive oil is considered one of the most stable vegetable oils [64-66]^e. The resistance of virgin olive oil to autooxidation is attributable to its high concentration of MUFA (55-80%) compared to long chain omega-3 fatty acids. A high levels of natural antioxidants also contribute to the resistance of virgin olive oil towards autooxidation; polyphenols (92-850 ppm), α -tocopherol (83-233 ppm), carotenoids (7-21 ppm) and orthodiphenols (4-18 ppm) [66-68]. The stabilizing role of polyphenols explains why virgin olive oil from unripe olive fruit is more stable to autooxidation compared to oil from ripened olive [69-71]. A strong correlation between olive oil stability and total phenols content has been reported [72, 73].

1.3.2. Effect of tocopherol and polyphenols

It is widely recognized that the major natural antioxidants that affect virgin olive oil stability are tocopherols and phenolic compounds [64, 72, 74-77]. However, the *relative* effect of these compounds on olive oil stability is uncertain [67, 78]. Accelerated testing at 60 °C showed that the stability of virgin olive oil is correlated with the total phenols concentration but not with the α -tocopherol concentration, $R= 0.97$ or 0.05 respectively [79]. Bleka et al. found that α -tocopherol stabilizes olive oil when added at 100 ppm (100 mg/ kg oil) but levels >250 ppm decreased oil stability [80]. Olive oil containing the natural range of total phenols did not benefit from further addition of α -tocopherol [76, 81]. Interestingly, one study showed that adding 250-2000 ppm α -tocopherol to cold-

^e The stability of virgin olive oil appears to be second only to coconut and palm oil which have higher levels of saturated fatty acids.

pressed virgin olive oil produced a concentration-dependent increase in stability up to a maximum of 275% [82].

The natural antioxidants within virgin olive oil degrade during the induction phase of autooxidation prior to overt changes in (flavour) quality [42]. Kinetic data suggests that hydroxyl-tyrosol is the first antioxidant lost during autooxidation, followed by α -tocopherol, and then tyrosol [83]. The relative order of autooxidation of virgin olive oil components was also reported as; chlorophyll > total phenols > α -tocopherol > orthodiphenols > carotenoids [84, 85]. According to recent interpretations, the loss of tocopherol and total phenols follow broadly similar time-courses and therefore both antioxidants are involved in virgin olive oil stabilization [65, 86]. Virgin olive oil with adequate levels of natural antioxidants retains oxidative stability for 0.5 and 2-years when stored at 40 °C or 25 °C, respectively [87]. It is now agreed that both α -tocopherol and the phenols contribute to virgin olive oil stability [67, 85, 88-90].

1.4. STABILIZATION OF EDIBLE OILS USING NATURAL ANTIOXIDANTS

1.4.1. Plant extracts

Extracts obtained from a variety of natural products were found to stabilize omega-3 fatty acids against oxidation [91-93], e.g., rosemary [94, 95], grapes, raspberry [96], oregano, parsley, olive oil [97] and also apple [98]. Green tea extracts destabilized fish oil due to the presence of chlorophyll but tea extracts devoid of chlorophyll had a stabilizing effect [99]. Maqsoom et al showed that EPA and fish oil emulsions were stabilized by phenolic extracts [100].

1.4.2. Purified flavonoids

Plant flavonoids function as radical quenching antioxidants [101]. Tests using seal oil and menhaden oil showed that the order of antioxidant activity for a range of flavonoids was, myricetin > quercetin > kaemferol > rutin > naringin > apigenin [102]. Phloretin and phloridzin extracted from apples were shown to stabilize fish oil emulsions to a greater extent compared to tocopherol. A notable disadvantage of flavonoid antioxidants is thought to be their low solubility in edible oils [103].

1.4.3. Edible oil blends

Blending can increase the concentration of saturated fatty acids and oxidative stability of oils, without recourse to hydrogenation. Most of the current research involves soybean oil blends with a variety of oils, including peanut oil [104], sesame oil [105], high oleic acid sunflower oil [106], palm olein [107] and jojoba oil [108]. Other edible oil blends that exhibit improved stability (compared to one or more components) include, sunflower /palm oil [109, 110], coconut oil / miscellaneous vegetable oils [111], palm/peanut oil [112]. The stability improvements achieved by blending two or more oils is explained by changes in the saturated fatty acid concentration. Test using 7-commercial oils and 21 combinations of these oils showed that oxidative stability was *inversely* related to the number of unsaturated bonds in accordance with equation 1[113];

$$\text{OSI (h)} = 7.51 + m_1 \text{ C16:0} + m_2 \text{ C18:0} + m_3 \text{ C18:1} + m_4 \text{ C18:2} + m_5 \text{ C18:3}.$$

Equation 1

where OSI is the index of oxidative stability measured in hours (h). It can be seen from the coefficient for oxidation (m), that the order of decreasing stability for different fatty acids is, C16:0 ($m_1 = 0.273$) > C18:0 ($m_2 = 0.0797$) > C18:1 ($m_3 = 0.0159$) > C18:2 ($m_4 = -0.01141$) > C18:3 ($m_5 = -0.3962$). A blend containing sesame seed oil and soybean oil was more stable than expected (from equation 1) based on the changes in fatty acid composition, probably due to the high levels of tocopherol in sesame [113].

1.5. OXIDATIVE STABILITY OF FISH AND VIRGIN OLIVE OIL BLENDS

1.5.1. Stabilization of vegetable oils with olive oil

As noted previously (section 3.1) virgin olive oil is one of the more stable edible oils whereas fish oil is regarded as unstable [114, 115]. The low stability of fish oil is attributed to its high content of omega-3 fatty acids, plus a low concentration of natural antioxidants (Table 1). Phenolic extracts *from* olive oil were found to stabilize fish oil against oxidation in a variety of systems including, canned tuna, fish fillets and bulk oils [97, 116, 117]. A blend of canola/palm/olive with corn oil was found to have improved oxidative stability compared to the corn oil [118].

Blending virgin olive oil with borage seed oil was found to protect (n-6) gamma-linolenic acids from autooxidation [119, 120]^f.

1.5.2. Summary and conclusions

Fish oil and virgin olive oil show markedly different stabilities due to the presence of omega-3 fatty acids and polyphenols, respectively. A variety of strategies to preserve the stability and nutritional properties of fish oil have been reported. The use of oils in different physical states (emulsification, encapsulation, dehydration etc), and addition of plant extracts (rosemary, apple etc) as natural antioxidants have met with some success. Based on the preceding desk-top research, blending fish oil and virgin olive oil *is likely* to stabilize the former. Currently there is little or no research in the public domain, on the use of virgin olive oil to stabilize fish oil. An investigation into the effect blending fish and olive oil on the stability of the combined oils is described in Part 2 of this report.

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^f *Borago officinalis* (borage) seeds are rich sources of gamma-linolenic acids (cf. http://en.wikipedia.org/wiki/Borage_seed_oil; accessed June 2010).

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PART 2: LABORATORY INVESTIGATIONS

Stabilization of fish omega-3 fatty acids using olive oil

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2.1. ABSTRACT

Fish oil is unstable to oxidation due to the high concentrations of omega-3 fatty acids and low levels of natural antioxidants. The effect of blending fish oil with virgin olive oil on the oxidative stability of the combined oils was studied. Oils and blends were subjected to accelerated aging at 37 °C for 6 days in the dark and analyzed by the thiobarbituric acid colorimetric assay. The total phenols content of all oils were determined by the Folin reagent. The investigation using two types of fish oils (F301, FO302) and two different virgin olive oils (VOO303 and VOO304) showed that the combination oils had increased stability by 16-76% (FO301) or 47-155% (FO302). The degree of stabilization of blended oils depended on the total phenols content of virgin olive samples (VOO404 > VO303) and the initial quality of the fish oil (FO302 > F301). It may be concluded, that blending fish oil with virgin olive oil is a viable strategy for stabilizing long chain polyunsaturated fatty acids associated with fish oil.

2.2. INTRODUCTION

The benefits of eating fish oil and virgin olive oil is attributed their high content of bioactive components, including omega-3 fatty acids, polyphenols and tocopherol. Commercial fish oil and virgin olive oil blends are now available that provide 2000 mg omega-3 fatty acids per dose (10-ml; 2 tbs) and an unspecified level of polyphenols. In previous work, we assessed the total phenols content of fish oil-virgin olive. Preliminary stability tests were also performed showing that virgin olive oil were up to ~ 80-times more stable to autooxidation compared to fish oil.

Autooxidation of fish oil degrades omega 3 fatty acids leading to off-flavors, a shorter product life and decreased nutritional quality [1-4]. By comparison, virgin olive oil is considered one of the most stable vegetable oils [5, 6]. Investigations from the United States, Spain and Italy have shown that polyphenols extracted from virgin olive oil can retard the oxidation of canned fish, frozen fillets, bulk fish oil as well as fish oil emulsions [7-12].

To our knowledge, the stability characteristics of fish and olive oil blends have not been studied extensively. The aim of these investigations was to determine the effect blending fish oil with virgin olive oil on the stability of the combined oils. The effect of different proportions of two oils on oxidative stability was determined. This investigation showed that oil blends containing 40-60% fish oil had 16-155% greater oxidative stability compared to fish oil alone. The stability of fish oil and olive oil blends depended on the characteristics of the starting oils.

2.3. MATERIALS AND METHODS

Two samples of fish oil (F301 & F302) and two samples of virgin olive oil (V303 & V304) were obtained from an industrial source and stored in dark bottles at 5 °C until use. Each oil sample was analyzed for oxidative stability using the thiobarbituric acid colorimetric test. Total phenols content was determined using the Folin method as described in previous reports. Samples F301 and F302 were blended with 20-80% of V303 or V304 and the resulting blends were subjected to oxidative testing. To ensure rapid oxidation, 1-ml samples of each oil blend were placed in 50-ml PVC tubes. The samples were covered with aluminum foil and

stored in a constant temperature incubator at 37 °C for 6 days. Duplicate PVC tubes were removed after 6 days and the samples were analyzed for the degree of autooxidation using the thiobarbituric acid colorimetric assay with absorbance readings at 535 nm.

Data analysis

Secondary products of lipid oxidations react with thiobarbituric acid to form a red pigment with an absorbance peak at 535 nm (A535). In this study, the observed colorimetric readings (A535_{OBSVD}) were plotted against % of virgin olive oil present in different oil blends (Figure 1). A535 reading for different samples were also calculated to allow for the expected dilution of fish oil by virgin olive oil, using the relation below;

$$A535_{CALC} = (100/\beta) \cdot \Phi \cdot A535^* \quad \text{Equation 2}$$

where Φ is the weight fraction of fish oil in the blend, and A534* is the A535 reading obtained for 100% fish oil (i.e., with $\Phi=1$). In the above equation, β is a *hypothetical* percentage of samples rejected after testing due to a loss of quality. In the ensuing analysis, β was assigned a value of 5% or 95%^g.

According to the principles of storage life testing, food materials (N) undergo degradation during storage to form a proportionate quantity of defective product, P. Deterioration may be assumed to follow 1st order kinetics, and therefore equation 4 applies;

$$N \rightarrow P \quad \text{Equation 3}$$

$$Q_t = Q_0 e^{-kt} \quad \text{Equation 4}$$

where Q_0 is quality at zero time, Q_t is quality after a fixed time (t) and k (day⁻¹) is the rate constant for deterioration. In accordance with 1st order processes,

$$\text{Ln}(Q_t/Q_0) = -k.t \quad \text{Equation 5}$$

$$\text{Ln}(1 - X) = -k.t \quad \text{Equation 6}$$

$$\text{Ln}(0.5)/k = t \frac{1}{2} \quad \text{Equation 7}$$

^g Accelerated testing requires that instrumental measurements are ultimately translated to “real life” measures of quality. Ideally, instrumental data should be calibrated by matching or comparing with human sensory data. For example, an instrumental reading (A535) = 1 unit might translate to ($\beta =$) 5% rejection of the product by a trained sensory panelists. The value of β can usually be determined empirically. In this work, we apply values of 95% or 5% for β to reflect the two hypothetical extremes of consumer response after 6 days of sample storage.

where $X (= P/ Q_0)$ is the fraction of product deteriorated and product half-life ($t_{1/2}$) is the time required for 50% quality loss to occur. In this study, the fraction of oil degraded was determined from equation 8.

$$X = A535_{\text{OBSVED}} / A535_{\text{CALC.}} \quad \text{Equation 8}$$

The above calculations and numerical simulations were done using MS excel.

2.4. RESULTS

1. Fish oil/virgin olive oil blends were stored in the dark for 6-days at 37 °C and then assessed for autooxidation using the thiobarbituric acid colorimetric assay with absorbance readings at 535 nm ($A535$).
2. **Figure 1** (cf. Section 2.8) shows a declining trend in the observed readings ($A535_{\text{OBSV}}$) as the amount of virgin olive oil in the blends is increased.
3. As an intial stage for data analysis we determined the expected absorbance readings ($A535_{\text{CALC}}$) adjusted for dilution of fish oil by virgin olive oil (cf. dotted lines in Figure 1A and 1B). Values for $A535_{\text{CALC}}$ are the maximum colorimetric readings possible assuming that fish oil is simply diluted by olive oil. Therefore observed $A535$ below calculated values indicate that fish oil is stabilized by blending olive oil (Figure 1A & 1B).
4. Figure 2 (Section 2.8), shows that increasing the proportion of virgin olive oil blended with fish oil increases oil stability until addition levels of 40-60% of sample 304. Interestingly, addition of sample 303 led to increasing stability at all levels of addition.
5. Figures 2, also shows that fish oil sample F301 was degraded more rapidly compared to F302. The differences between these two fish oils persisted, when both oils are blended with virgin olive oil 304.
6. Figure 3 illustrates that the stability of blends increases when fish oil F302 is blended with virgin oil sample VOO303 or VOO304. In contrast, there was little or no stabilization when fish oil F301 was blended with virgin oil 303.
7. Figure 4 and 5 show summary data for oil blends in this study. It is clear from these graphs that sample F301 performed less well compared to F302 in all stability tests. Blends containing FO302 and VOO304 were found to be best combination of oils.
8. The total phenols content of 4-base oils were determined as 16 ppm (FO 301), 18 ppm (F0302), 49 ppm (VOO303) and 126 ppm (VOO304). All base

oils showed significant differences in their apparent phenols content (cf. Table 2). It may be concluded that the high stability of oil blends containing VOO304 is due to its higher total phenols content.

2.5. DISCUSSION

The autooxidation of omega-3 fatty acid lowers the nutritional quality and shelf-life of fish oils. The loss of n-3 fatty acids conforms to 1st or zero order kinetics depending on the prevailing conditions and method of study [13-16]. The low stability of fish oil, compared to virgin olive oil, can be explained by the high level of omega-3 fatty acid and low concentration of naturally occurring antioxidants in the former case. Previous studies showed that the natural antioxidants from virgin olive oil can be lost over time prior to sample deterioration. For virgin olive oil the loss of natural antioxidants was, phenols > α -tocopherol > orthodiphenols > carotenoids. Accordingly, polyphenols and tocopherol were the most important antioxidants in olive oil. Oxidation of olive oil occurs only after the degradation of natural antioxidants. Furthermore, olive oil could be stored for up to 2 years without significant quality loss [17, 18].

The aim of this study was to determine whether olive oil could protect fish oil omega-3 fatty acids from oxidation. Results from this study (Figure 1 -3) are consistent with the initial working hypothesis. Addition of virgin olive oil to fish oil resulted in oil blends with decreased levels of autooxidation measured by the thiobarbituric acid colorimetric assay. To eliminate, the effect of simple dilution, robust stability indices for fish oil and virgin olive oil were estimated using the principles of 1st order kinetics [13, 14]. Product half-life was estimated by applying two hypothetical rejection scenarios namely, sample storage for 6-day under accelerated testing conditions leads to (Condition A) 5% or (Condition B) 95% of rejection of the products. These conditions simulate two hypothetical *consumer* responses following intensive oxidation of fish oil blends.

Assuming ~5% of product rejection after 6-days accelerated storage, the half-life of fish oil sample FO301 and FO302 was predicted as ~80 days and 95 days, respectively (Figure 2C and 2D). Blending with fish oil with 40-60% virgin olive oil increased the half-life of FO301 by 6-17% (Figure 4).. By comparison the half-life of fish oil FO302 increased by 47-70% after blending with 40-60%

olive oil (Figure 4). These results suggest that initial characteristics quality of fish oils (FO301 and FO302) is important in terms of the quality of final blends.

Assuming 95% product rejection after 6 days, the half-life of fish oil at 37 °C was estimated at 1.3-2.5 days. Under such circumstance, the estimated stability increase for blends prepared using 40-60% olive oil VOO304 was 79% (FO301) and 152% (F302) (Figure 5). A second olive oil sample (VOO303) had only a modest stabilizing effect on F302 and no effect on the stability of FO301 in the short term (Figure 5).

The current results indicate that the benefits of blending fish oil with olive oil could be substantial and that the storage stability of fish oils could be doubled under appropriate conditions. There are some uncertainties in the present analysis. First, the preceding calculations are based on samples containing 40-60% of fish oil by mass. The stability characteristics of blends containing greater than 60% virgin olive oil are possibly of less interest because such blends are unlikely to deliver prescribed amounts of omega-3 fatty acids. Second, the improvements in half-life have been stated in relation to possible consumer expectations (5% or 95% rejection after accelerated storage). Such an approximation is necessary to emphasize that instrumental measurements need always to be calibrated against human trials.

2.6. CONCLUSIONS

The optimum proportions of oil required to be blended and the degree improvements in storage life achievable were found to both depend on the initial fish oil quality. We found that FO301 was less stable compared to FO302 for reasons that have yet to be divulged by the client.

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PART 3: APPENDICES: FIGURES AND TABLES

Figure 1A

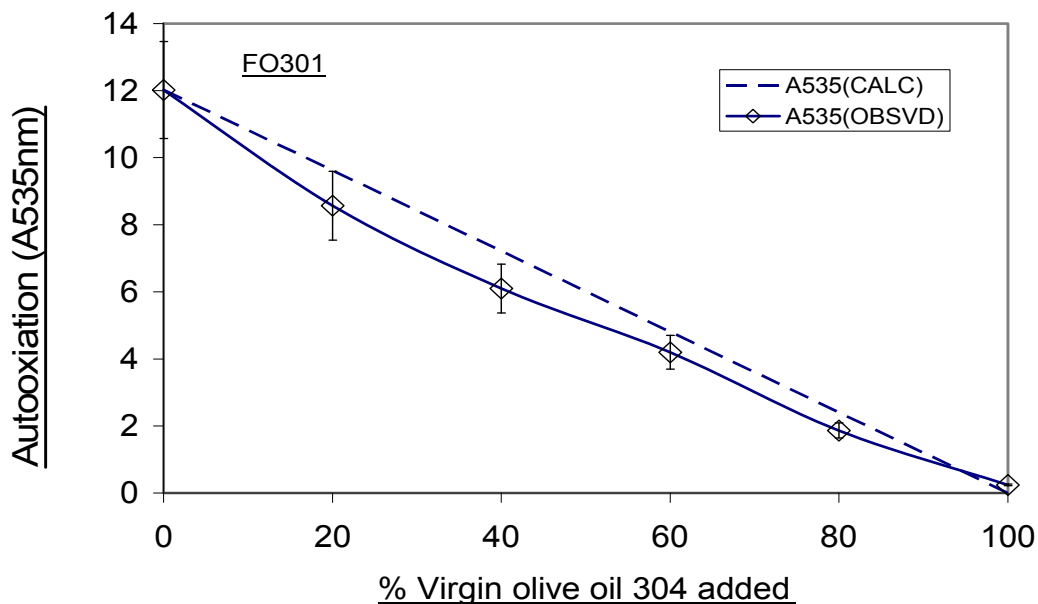


Figure 1B

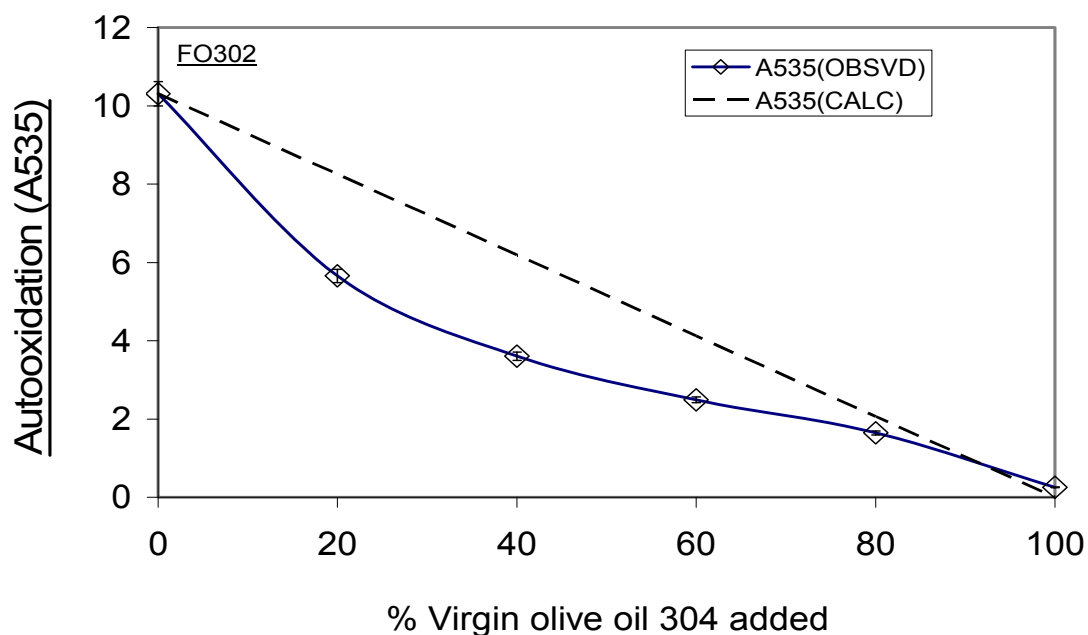


Figure 1: Effect of blending fish oils with olive oil on autooxidation.

Notes: Deterioration was measured by the TBCA assay. The dotted line shows a maximum response expected for the blends. Figure 1A shows fish oil 301, Figure 1 B shows Fish oil 302. Stability test were performed at 37 oC for 6-days (dark).

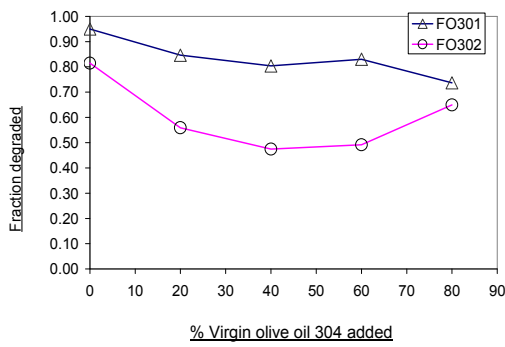


Figure 2A

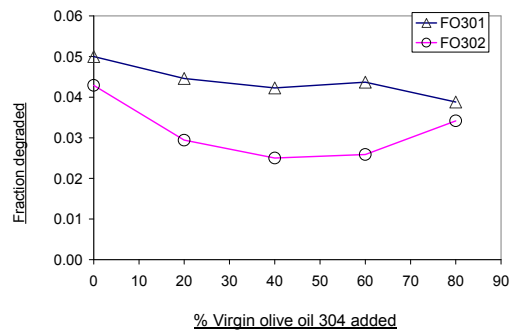


Figure 2B

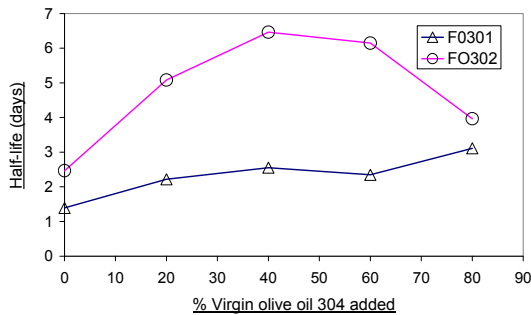


Figure 2C

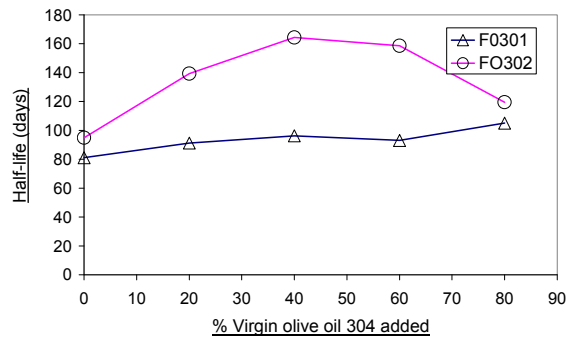


Figure 2D

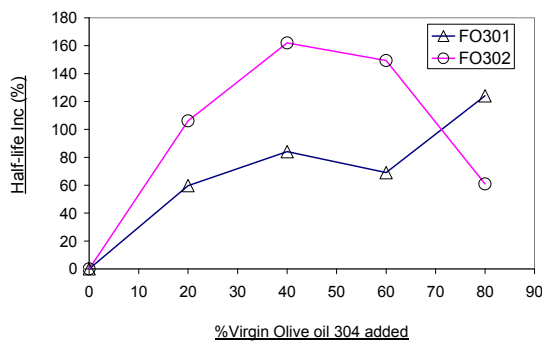


Figure 2E

Assume: 95% Rejection in 6 days

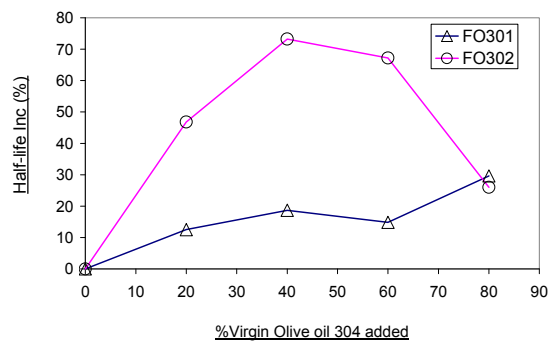


Figure 2F

Assume: 5% Quality loss in 6 days

Figure 2: Effect of blending fish oils with virgin olive oil 304 on autooxidation indices.

Notes: From top to bottom graphs show fraction degraded after 6 days, half-life (days) and % increase in half-life. Right panel (A, C, &E) and left panels (B, D, F) show responses if 95% or 5% quality losses in occur in 6-days, respectively.

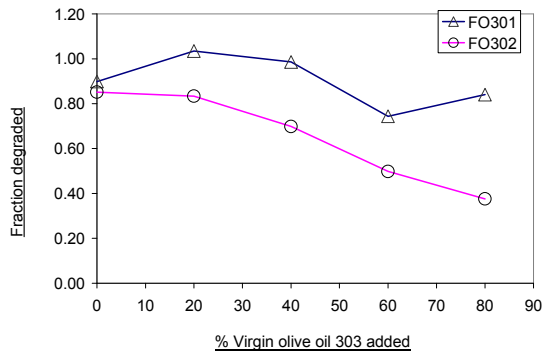


Figure 3A

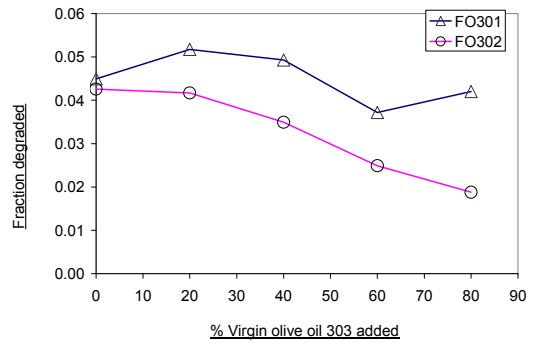


Figure 3B

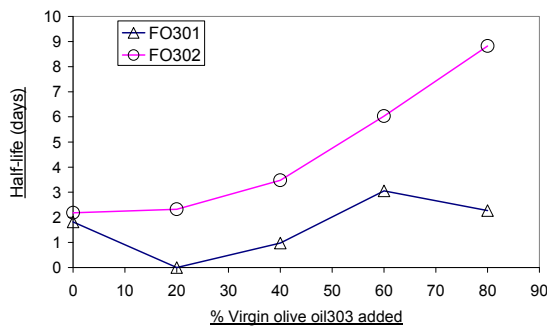


Figure 3C

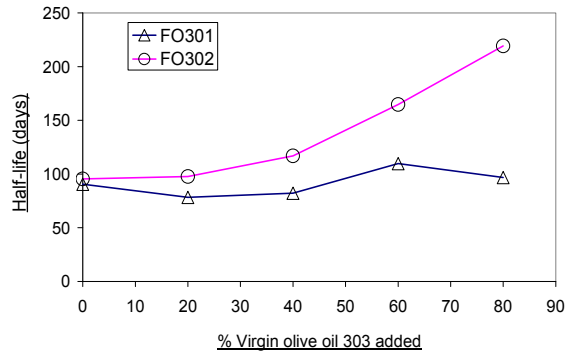


Figure 3D

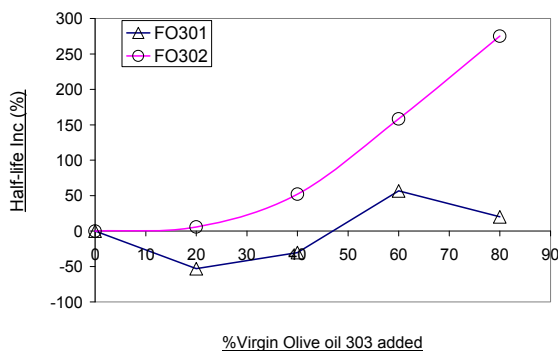


Figure 3E

Assume: 95% Rejection in 6 days

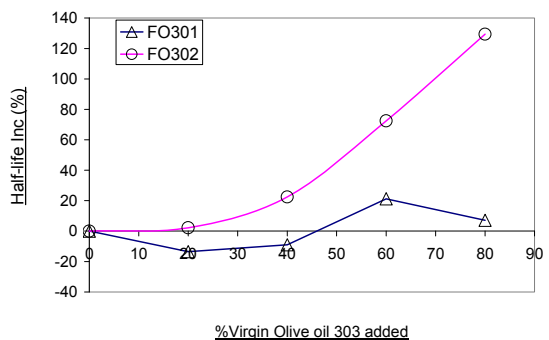


Figure 3F

Assume: 5% Rejection in 6 days

Figure 3: Effect of blending fish oils with virgin olive oil 303 on autooxidation indices.

Notes: From top to bottom graphs show fraction degraded after 6 days, half-life (days) and % increase in half-life. Right panel (A, C, & E) and left panels (B, D, F) show responses if 95% or 5% quality losses in occur in 6-days, respectively.

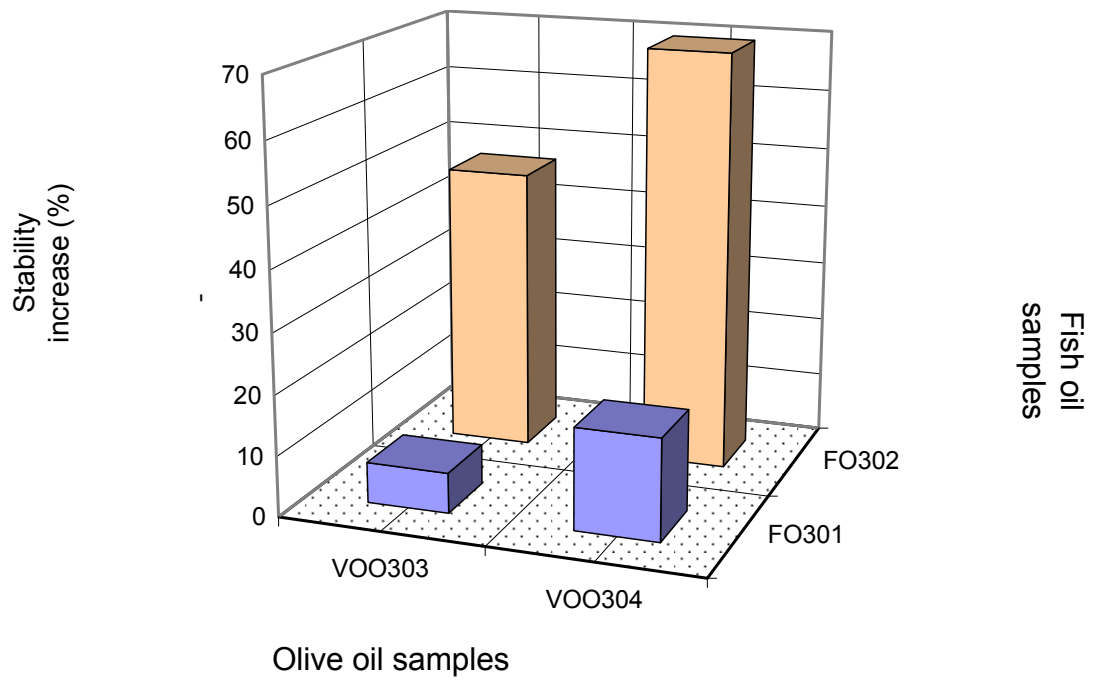


Figure 4: Effect of blending fish oils with virgin olive oil on stability.

Data shows % increase of stability for blends containing 40-60% fish oil (FO301 or FO302) mixed with virgin olive oil (VOO303 or 304). Calculations assume 5% consumer rejection at the end of testing (i.e. sample half-lives are 81-161 days).

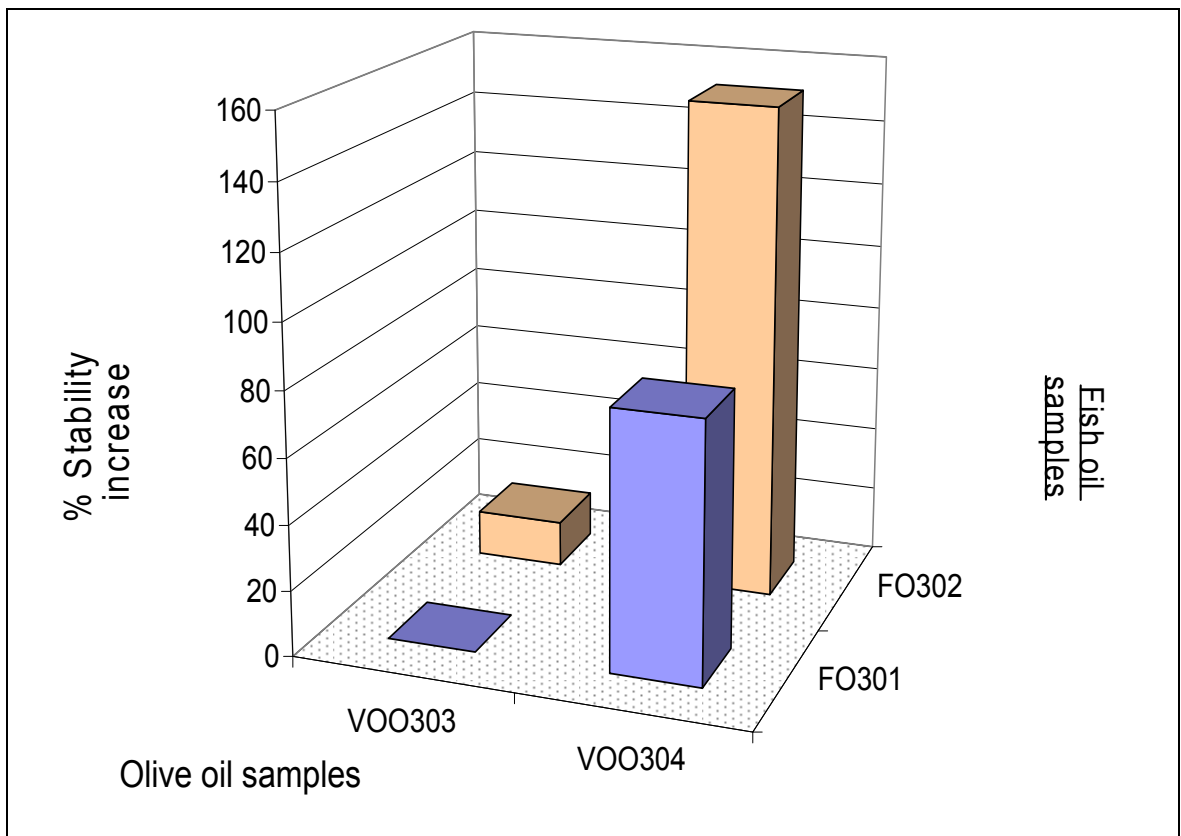


Figure 5: Effect of blending fish oils with virgin olive oil on stability.

Data shows % increase in stability for blends containing 40-60% fish oil (FO301 or FO302) mixed with virgin olive oil (VOO303 or 304). Calculations assume 95% consumer rejection at the end of 6-days accelerated testing (i.e. half-life is 1.4-6 days).

Table 1: A list of fish and virgin olive oil composition and functional properties

Parameter	Fish oil*	Virgin Olive oil	Comments
SFA	19-26	8-20%	Contributes to the stability of oils
MUFA C18:1	33-56%	55-80%	Contributes to the stability of oils
PUFA (Omega-3)	24-28%	~0-1%	Essential fatty acid, susceptible to oxidation
o C18:3 (ALA)	1%	0.5%	Contributes to the low stability of fish oil
o C20:5 (EPA)	7-8%	-	Contributes to the low stability of fish oil
o C22:6 (DHA)	13-15%	-	Contributes to the low stability of fish oil
Polyphenols (mg/kg)	0	92-850	Antioxidants to stabilize virgin olive oil
Tocopherol (mg/kg)	20	83-233	Antioxidant, ≤250 ppm added to fish oil
Lipid, and LDL synthesis	↓	↓	Effect of MUFA and PUFA
Eicosanoid metabolism	↓	—	Anti-inflammatory action of omega-3 PUFA
Gene expression	↓↑	↑↓	Fish oil → eicosanoid pathway, cell proliferation. Olive oil phenols → Antioxidant responsive transcription factors

Abbreviations: SFA = saturated fatty acids, PUFA (omega 3 FA) polyunsaturated fatty acid, omega-3 Fatty acids, MUFA = mono unsaturated fatty acids, ALA = alpha-linolenic acid (C18:3, ω3), EPA = eicosapentaenoic acid (C20:5ω3), DHA = docosahexaenoic (DHA, C22:6, ω3). *Summarized from this review.

*Average values for wild and farmed salmon (see footnote ^h).

^h Blanchet, C. et al. (2005). Fatty acid composition of wild and farmed Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). *Lipids* 40(5), 529-531.

Table 2: Effect of olive oil blends on fish oil stability

5% rejection @6days

Sample	Half-life (days)	%	%change
FO301	~81	100	0.0
FO301+ VOO 303	96	107	6.7
FO301+ VOO 304	95	117	16.9
FO302	95	100	0.0
FO302 + VOO 303	140	147	47
FO302+ VOO 304	161	169	69

95% rejection @6 days

Sample	Half-life (a) (days)	%	%change
FO301	1.4	100	0
FO301+ VOO 303	2.4	96	-4
FO301+ VOO 304	2.5	179	79
FO302	2.5	100	0
FO302 + VOO303	2.4	114	14
FO302+ VOO 304	6.3	252	152

Notes: Average values are reported for blends containing 40-60% fish oil

Table 3: Total phenols content of fish and olive oils

Total phenols (mg/ kg)				
	FO301	FO302	VOO303	VOO304
Av	15.9	18.5	49.1	125.9
SD	3.7	4.2	15.1	16.0

T-test results, p values**				
Samples	FO301	FO302	VO303	VO304
FO301	x	1.53E-02	3.92E-07	6.64E-14
FO302	1.53E-02	x	2.75E-08	1.57E-13
VO303	3.92E-07	2.75E-08	x	2.60E-09
VO304	6.64E-14	1.57E-13	2.60E-09	x

Notes: **Comparison of samples using t-tests. P <0.5 shows significantly different total phenols content.