Short Communication

Oxidative stability of omega-3 tablets

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Preparing solid formulations, as powder and tablets, containing omega-3 can be challenging as the necessary production processes expose the unsaturated omega-3 fatty acids to high temperatures, light, and mechanical stress in presence of air. The present work demonstrates that omega-3 tablets can be prepared with sufficiently low total oxidation values (totox) to satisfy relevant monographs for omega-3 products. The tablets were prepared from spray granulated direct compaction grade 30% w/w triglyceride powders as characterized by Vestland, Jacobsen, Sande, Myrset, and Klaveness, 2015 (Food Chem. 2015. 185: p. 151–158; Food Chem., 2016. 197, Part A: p. 496–502). Addition of ascorbic acid, in combination with EDTA as processing agent, was correlated with lower totox in powders. Spray granulation performed under nitrogen atmosphere contributed to significantly decreased totox in powders after 8 months of storage at accelerated temperature (37°C) compared to spray granulation in air. In long-term stability studies, it was confirmed that coated omega-3 tablets remained at totox <5 after 1 year of storage at ambient temperature.

Practical applications: The confirmation that oxidative stable omega-3 tablets can be produced opens the possibility for a new administration form in the omega-3 field. The use of dry powders in the production process imply more opportunities within combination products as dry powders typically exhibit fewer compatibility issues. Omega-3 tablets are a gelatin- and lactose-free alternative for health conscious individuals all over the world.

Keywords: Compactible powder / Omega-3 / Oxidative stability / Tablets

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1 Introduction

The role of omega-3 fatty acids in health and disease, as reviewed by Riedieger et al., has caused a whole industry around research, development, and sale of various omega-3 products [1]. Marine organisms are currently the main source of the health promoting omega-3 fatty acids, of which the most prominent are docosahexaenoic acid (DHA, 22:6 n-3) and eicosapentaenoic acid (EPA, 20:5 n-3). The basic formulation of omega-3 is pure bulk oil, typically cod liver oil or oil extracted from anchovy and sardine.

Pure oil as omega-3 supplement is accepted by few due to the unpleasant taste and texture of the oil. Oil encapsulated in gelatine, so called soft-gel capsules, are currently the traditional alternative to bulk oil. Lately, several other alternatives have become commercially available, like emulsions, various gel formulations, and fortified food containing omega-3.

Tablets as formulation form for omega-3 is not much explored, as preparing tablets containing omega-3 has been a challenge due to the amounts of oil necessary to include in compactible powder. Previously reported results have,

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Abbreviations: AV, anisidine value; **CD,** cyclodextrin; **DHA,** docosahexaenoic acid (22:6 n-3); **EDTA,** ethylenediaminetetraacetic acid; **EPA,** eicosapentaenoic acid (20:5 n-3); **GOED,** Global organization of EPA and DHA; **Ph. Eur.,** European Pharmacopoeia; **PV,** peroxide value; **PVA,** poly vinyl alcohol; **TG,** triglyceride; **TOTOX,** total oxidation value

however, revealed that direct compactible (DC) grade omega-3 powders can be prepared using β-cyclodextrin (β-CD) as carrier for the omega-3 fatty acid esters [2, 3].

1.1 Aim

A challenge for all suppliers of omega-3 supplements is the high susceptibility to oxidation of the omega-3 products due to the presence of multiple double bonds in the fatty acid chains. The aim of the present work, was to study the oxidative stability of the spray granulated DC grade omega-3 powders and corresponding coated and uncoated tablets where the omega-3 oil was complexed with β-CD. Traditional methods for protection against oxidation were explored to reveal their impact on the intermediate and final products.

2 Materials and methods

2.1 Materials

Triglyceride concentrate Vivomega 3322 TG (EPA as TG 300 mg/g, DHA as TG 200 mg/g, total omega-3 fatty acid esters as TG 600 mg/g, ethyl esters maximum 10% w/w) (GC Rieber, Norway); β-CD (Roquette, France); L-ascorbic acid (Apotek-produksjon, Norway); EDTA-Na_2Ca salt (Sigma–Aldrich); Avicel HFE-102 (IMCD, Sweden); methanol (Fluka/Riedel-de Haën); talc (Fluka, Sigma–Aldrich); magnesium stearate (Ligamed MF-2-V, IMCD, Sweden); Nutraficient (Colorcon, UK); PeroxySafe^{TM} Standard kit (Cat. No. 07KTPR1020), STD Controls (cat no. 07CT1020), and Prep Reagent STD (cat. no. 07PREP1020) (MP Biomedicals, France); propan-2-ol (VWR); acetic acid, $\geq$99.85% (Sigma–Aldrich), p-Anisidine reagent (Sigma–Aldrich); tritridecanoin (Nu-Chec Prep, Inc, USA), dichlorometane (Merck); methanol (Merck); 3N MeOH-HCl (Sigma–Aldrich), and hexane (Merck).

2.2 Methods

2.2.1 Preparation of antioxidant solution

A total of 176.1 g ascorbic acid and 3.74 g EDTA-Na_2Ca were dissolved in 1000 mL purified water.

2.2.2 Preparation of omega-3: β-cyclodextrin powders

An aqueous suspension of β-CD added 0.1 mL antioxidant solution per gram oil to be added was mechanically paddle-stirred at room temperature in an open stainless steel tank (5 L) for 10 min. The weight of water was typically 2–5 times the weight of the β-CD. Triglyceride oil was added and the mixture was further stirred for 30–60 min at 50 rpm at room temperature under air atmosphere until a homogeneous mixture was achieved.

2.2.3 Spray granulation

Spray granulation was performed in a ProCell LabSystem (Glatt GmbH, Germany). Process parameters for the powders prepared for this work was air flow of 100 m^3/h, inlet air temperature of 88°C, spray pressure of 1.2 bars, and spray rate 50–60 g/min. Residence time for individual particles was $\pm$1 h.

2.2.4 Tableting of omega-3 powders

The tableting machine used was a 16 stations rotary press from Kilian, Germany. The punches utilized were oblong shaped, 18$\times$9 mM. A minimum of 400 g formulation comprising the spray granulated omega-3 powder (96% w/w), magnesium stearate (0.5% w/w), talc (1% w/w), and Avicel HFE-102 (2.5% w/w) was prepared by hand-mixing the powders, and the formulation was run in the tablet machine while parameters were adjusted to achieve the best possible tablets.

2.2.5 Friability testing

The friability was measured by spinning close to 6.5 g of tablets in an Erweka TA-UZ friability tester set at 100 rpm. The "before" and "after" weights of the tablets were recorded as described in the European Pharmacopoeia; section 2.9.7 Friability of uncoated tablets.

2.2.6 Crushing strength testing

The hardness was measured utilizing tablet hardness tester Erweka TBH 126. A mean value was calculated on the basis of at least ten individual tablets. The short sides of the oblong tablets were facing the jaws of the hardness tester.

2.2.7 Coating of tablets

Coating was performed in a GMPC 1 Mini-Coater from Glatt. Process parameters included exhaust air temperature fixed at 45°C, process air flow at 35–55 m^3/h, atomizing air pressure and pattern air pressure at 1.0 bar, pan speed at 9–15 rpm, and spray rate at 4–6 g/min.

2.2.8 Implementation of the stability studies

Samples were divided in two batches; one was kept at ambient temperatures exposed to normal, seasonal fluctuations in temperature, light, and residual humidity (RH) in the air (Oslo; Norway), the other batch was kept in a heated cabinet at 37°C. The plastic containers containing the samples were permeable for air, RH, and light and were all filled with 8 g of sample. In section 3.3 the samples were

successive, the powder represented in the analyses was the same as used for pressing tablet cores, and those cores were coated, and represented the coated tablets.

2.2.9 Oxidation analysis

2.2.9.1 Analysis for peroxide values

Determination of primary oxidation was performed analysing the peroxide value (PV), using the AOCS certificated method PeroxySafe^{TM} Standard Test Kit (Certificate No. 030501). This method quantifies PV by transferring a free electron to a metal-chromogen complex whose visible spectrum then changes and can be detected at 570 nm. This is a spectrophotometric method and gives reliable, and reproducible data in the range 0.01–0.05 Meq peroxide/kg sample. Each analysis was performed in triplicates. The oil was extracted from the formulation (powder/tablets crushed with mortar) using Prep Reagent STD (99.9% 2-propanol, antioxidants, and stabilizer), facilitated in ultrasound-bath.

2.2.9.2 Analysis for anisidine values

AOCS official method Cd 18–90 (1997) with modifications was applied to determine the secondary oxidation values. The oil was extracted from the formulation (powder/tablets crushed with mortar) using iso-octane with 5% iso-propanol, facilitated in ultrasound-bath. The anisidine value (AV) is defined by convention as 100 times the optical density measured at 350 nm in a 1 cm cuvette of a solution of 1 g of the oil in 100 mL of a mixture of propan-2-ol (solvent), and p-anisidine (reagent). Each analysis was performed in triplicates.

AV value has no designation; the value is given by the formula:

$$AV = ((As \times 6) - Ab) \times 4/gmass$$

2.2.10 Determination of totox values

The primary and secondary oxidation values were combined and presented as total oxidation values (totox). Totox combines the peroxide value and the AV as follows:

$$AV + 2PV = Totaloxidation\ value\ (totox)$$

2.2.11 Quantitative fatty acid analysis.

Powder (or crushed tablet) with added internal standard tritridecanoin dissolved in dichloromethane were directly methylated with 3N MeOH HCl. The generated fatty acid methyl esters were then extracted to hexane and injected on GC-FID. Analysis was performed on a 7890A GC with a split/splitless injector, a 7683B automatic liquid sampler, and flame ionization detection (Agilent Technologies, Palo Alto, CA). Separations was performed on a SP-2380 (30 m × 0.25 mM i.d. × 0.25 μm film thickness) column (Supelco, USA). Temperature program as follows; initially 90°C for 0.5 min, then 50°C/min to 150°C, 10°C/min to 225°C, and finally 120°C/min to 245°C for 3 min. Total run time was 12.37 min. Fatty acids were quantified against internal standard tritridecanion. For EPA and DHA, calculated response factors obtained from a mixture of commercial FAMEs (Nu-Check Prep, Inc, USA) were used. For all other fatty acids, theoretical response were used.

3 Results and discussion

3.1 Effect of addition of antioxidants

In multiphase systems such as the intermediate aqueous fish oil: β-CD mixtures prepared in this work the oxidation mechanisms are complex and many factors influence the rate of oxidation [4]. The triglyceride concentrate used for these experiments contained 3 mg/g mixed tocopherols as added by the oil supplier. In addition, the effects of some selected antioxidants in this particular system were screened in initial stability studies using multivariate analysis (Trine Torgersen, Omegatri AS, unpublished results). It was shown that ascorbic acid played an essential role as lower totox were correlated with higher concentrations of ascorbic acid. The data further suggested a superior effect of a combination of ascorbic acid and EDTA.

Ascorbic acid is used in food as preservative and color stabilizer. Ascorbic acid has in several studies been found to have both antioxidant and prooxidant effects in various food systems containing fish oil, hence, it is pointed out at that the effect of the substance in a specific system cannot easily be predicted [5, 6].

EDTA is a synthetic, polar chelator that has its function in the water phase where it sequester metal ions. The presence of metal ions in the water phase of water/oil mixtures is considered a major factor in the promotion of oxidation [7]. EDTA has previously demonstrated protective effect in fish oil emulsions. As reviewed by Jacobsen et al., in a study with mayonnaise containing 12.8% sand eel oil 200 mg EDTA per kg product was included, and shown to completely inhibit oxidative deterioration during storage at 20°C. Lower concentrations, as 6 mg/kg product, was also shown to be sufficient to slow down the oxidation rate of omega-3 oils in mayonnaise [6].

To demonstrate the effect of the antioxidant combination, two spray granulated powders with 30% w/w triglyceride (TG) oil load were prepared, and added, respectively, 0.5% w/w ascorbic acid and 0.5% w/w ascorbic acid in addition to 0.11% w/w EDTA. Both powders were subject to storage at ambient temperature and followed by analyses for oxidation products (Fig. 1).

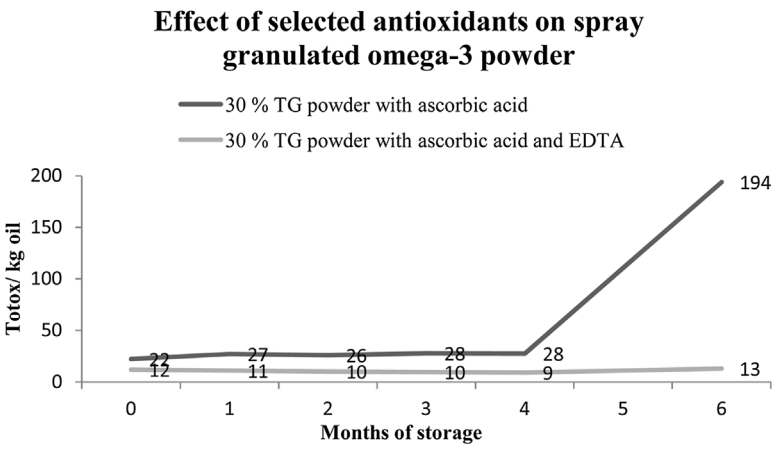

Figure 1. Thirty percent w/w triglyceride powders with ascorbic acid and EDTA, or ascorbic acid only. The powders were stored at ambient temperature. $N = 1$, three analytical replicates, st.dev. for PV, and AV analyses were less than 0.7 for all replicates.

Initial totox of the powder containing ascorbic acid only was almost twice the totox of the powder also added EDTA (Fig. 1). The trend continued until a sudden and persistent rise in totox of the powder with only ascorbic acid could be observed after 4 months of storage. The importance of EDTA, as processing agent during the aqueous phase of the production process was hence firmly emphasized. These findings underline the significance of a combination of the two types of antioxidants for a superior protection against oxidation in these omega-3 powders.

3.2 The effect of inert atmosphere under spray granulation of powders

Oxygen is necessary for oxidation and all handling of omega-3 containing products should ideally be performed under inert atmosphere to reduce access for oxygen [8, 9]. Oxygen has been reported to be up to three times more soluble in food oils than in water [10, 11]. During the preparation of the products in this work, the oil was introduced to an aqueous mixture of purified water, antioxidants, and β-CD. The mechanical stirring caused the oil to divide into smaller droplets in the aqueous phase, closely resembling an O/W emulsion. The actual oil surface exposed to the ambient environment was hence small compared to the volume of the liquid mixture, and the risk for extensive initiation of oxidation at this stage may be considered small.

However, during spray granulation there is a massive introduction of air in the drying chamber, in parallel with increased temperature, and mechanical stress. The residence time in the drying chamber was typically approximately 1 h. It was therefore, assumed that spray granulation in inert atmosphere will result in increased oxidative stability.

Thirty percent w/w TG oil powders were prepared utilizing nitrogen (inert atmosphere) or air as atomizing, and drying gas during spray granulation. The oxidative stability of the resulting powders were compared in a stability study (Fig. 2).

The results showed, that the use of nitrogen under spray granulation significantly decreased the oxidation rate in powder samples kept at 37°C (Fig. 2). The initial phase of oxidation can be of various length depending on surrounding conditions and it is probable that less oxidation was initiated during spray granulation in nitrogen [12]. The powders spray granulated in air probably had both more hydroperoxides initially and more oxygen embedded in the powder after spray granulation, resulting in exponential rate of oxidation in the samples kept at 37°C, observable already after 4 months into the study. The oxygen permeation during storage did not annihilate the initial differences in the powders, despite permeable storage containers and relatively large surface/volume ratio of samples (8 g).

It has been shown in numerous studies that oxidation cannot be stopped, but it can be slowed down, among other by decreasing temperature [13, 14]. After 8 months of storage, also the powder sample spray granulated in nitrogen stored at 37°C started to rise in totox (Fig. 2). This underlines the powerful influence elevated temperatures have on oxidation rate, as both powder samples kept at ambient conditions were still fluctuating around steady state in totox. Whether similar differences can be observed in powders stored under ambient temperatures is yet to be seen in this study.

3.3 Impact of processing on oxidative stability of powder and tablets – long-term and accelerated stability studies

Powders to be tableted undergo several unit operations being transferred between containers, mixed into a tablet formulation and finally pressed into tablets. During these processes, the powder is exposed to atmospheric conditions, mechanical stress, light, and heat. As observed by Anwar et al. [15], more than one production process steps often includes increased

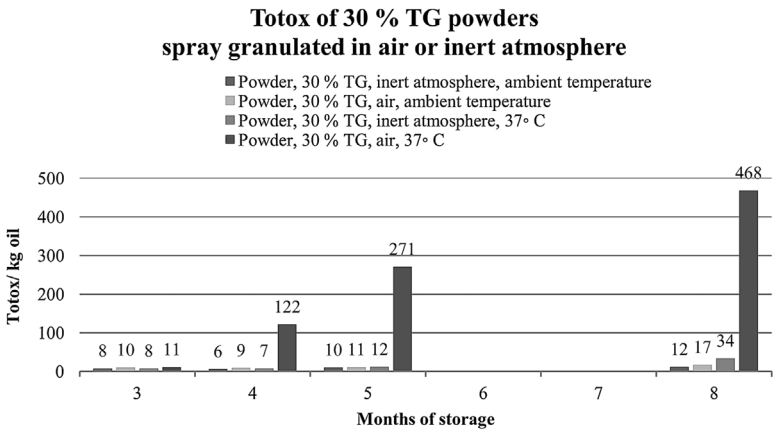

Figure 2. The indicated atmosphere (air/inert) was different under spray granulation only. $N = 1$, three analytical replicates, st.dev. for PV, and AV analyses were less than 0.7 for all replicates.

initiation of oxidation in fish oil containing products. However, a powder has a high surface to volume ratio compared to a tablet, decreasing the surface of a powder by compressing it into a tablet may actually provide protection against oxidation due to reduced access to the fatty acid esters for triggers of oxidation.

Tablets can be coated and poly vinyl alcohol (PVA) co-polymers are mentioned in literature as film forming polymers with extremely low oxygen permeability. In a study by Fujii, Noami, Tomita, and Furuya (2008), tablet cores containing ascorbic acid coated with a PVA co-polymer stored under normal atmospheric conditions were compared to uncoated tablet cores kept under nitrogen atmosphere. The oxidation rates of ascorbic acid in the two samples were similar, implying equal protection against oxidation provided by inert atmosphere, and the PVA film coating [16]. The tablets prepared for this work were therefore, coated with PVA based commercially available coating material; a 6% w/w coating layer was applied to all coated tablets.

According to European Pharmacopoeia (Ph.Eur.), the maximum acceptable level for PV is 10 Meq/kg and AV 30; totox value has no defined limits, but if the above mentioned values were taken into consideration the maximum totox level would reach 50 [17]. Values set by the Global Organization for EPA and DHA (GOED) are somewhat lower; PV (meq/kg) is expected to be <5, AV value <20, and the combined totox value <26 [18]. However, following these recommendations is voluntary for suppliers of health supplements and the producers have the responsibility to develop their own internal specifications. Unacceptable totox in this study was defined as totox >30.

For health supplement products it is possible to use stability study models based on pharmaceutical research; for tablets this would typically imply long-term and accelerated studies at various humidities, and temperatures as detailed in ICH guidelines [19]. However, it is known that for foods containing fats and oils applying elevated temperatures can influence the oxidation process in an unpredictable manner. Heat can impact not only the rate of oxidation, but also the favored oxidation reaction patterns, and the activity of the different pro- and antioxidants present [20]. Results gained from extrapolation of accelerated studies may, hence, not necessarily be consistent with result from real-time stability studies at ambient temperatures.

Despite this fact, it is common to use accelerated stability studies as an indicator for shelf-life among producers of omega-3 supplements, often because it is desirable to launch the product before a real-time stability study at ambient temperatures can be finalized.

Typical limitations set by practical considerations in a production environment were applied to the product samples prepared for this study, including production in bright light at ambient temperatures and no use of inert atmosphere. 30% w/w TG powder was prepared and compacted to tablet cores; all cores achieved a crushing strength of >80 N, and a friability of less than 1% w/w. The tablet cores were coated. Samples of powder, cores, and coated tablets were included in long-term and accelerated stability studies.

The results from the stability studies showed that 13 months after production, the totox for coated TG tablets had just reached unacceptable levels for samples kept at 37°C while, totox for samples kept at ambient conditions were still at steady state (Fig. 3).

The storage conditions in this study were chosen to mimic realistic storage for the coated tablets, on a store shelf, or at the end consumer. The normal storage conditions for the powder as a raw material for inclusion in tablets would, however, be in a sealed container, kept below 15°C and protected from direct light. Storage under such conditions have shown to keep totox below 15 in powders stored for 20 months (unpublished results, ongoing study).

The reduction of access for oxygen by compressing the powder to tablets, followed by coating, proved to decrease the oxidation rate. However, the protective effect of the mere encapsulation of the fatty acid esters by β-CD and the effect of the combination of ascorbic acid, and EDTA added to the powder can also be observed as delayed oxidation of the

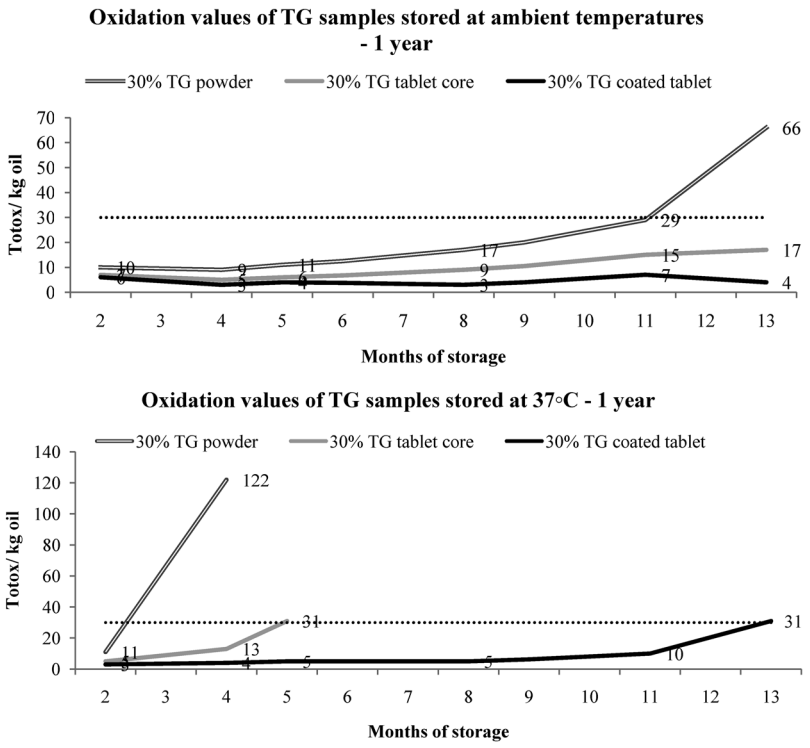

Figure 3. All samples (8 g) were kept in closed plastic containers permeable for light and atmospheric gases. Totox 30 (dotted line) was the maximum accepted totox in this study. $N = 1$, three analytical replicates, st.dev. for PV were less than 0.2, and less than 0.6 for AV (for samples below totox 30).

omega-3 fatty acid esters in the powder samples. The powder samples in the current study, stored at ambient temperature kept below totox 30 for 11 months while a 20 g sample of pure omega-3 fatty acid ethyl ester 60% concentrate was stored under similar conditions, and reached totox 58 after 1 week (Omegatri AS, unpublished results).

Totox of the powder samples kept at 37°C reached unacceptable levels at some point between the 2nd and 4th months of storage. The powder samples kept at ambient temperature reached totox 29 11 months into the study; hence, a delay of approximately 9 months compared to the accelerated samples.

The corresponding values for tablet cores were totox at 31 after 5 months at 37°C; the samples kept at ambient temperatures would probably reach totox 30, 14–15 months into the stability study, considering the oxidative status of these samples at 13 months. Experience with development of totox values has shown that, once totox starts increasing from the initial steady state level, the increase is steep, and irreversible [14, 21]. Hence, an estimated 9–10 months delay can be expected for tablet core samples.

If this were to apply also for the coated tablets, an estimated shelf-life would be 13 + 9 months; 22 months. A shorter or longer shelf-life cannot be ruled out as long as the real-time results are yet to be seen. The significant added oxidative stability represented by the application of a coating, may prove to significantly prolong the shelf-life of this formulation.

3.4 Quantitative fatty acid analysis of coated tablets

In the study performed by Albert et al., on all encapsulated fish oil products available in retail and online in New Zealand, it was observed that 69% of the encapsulated fish oil products had less than 67% of the labelled content of EPA, and DHA. Typically encapsulated fish oil have a stated shelf life of 2 years, however, the authors noted that among other the best-before date proved less relevant as marker for quality (quantitative content of EPA and DHA, and oxidation levels) [22].

The coated omega-3 tablets participating in the long-term stability study described in section 3.3 were subjects to a quantitative fatty acid analysis on EPA and DHA after 1 year of storage at ambient temperature.

It was found that the content of EPA and DHA was at 100 and 98% w/w, respectively, of the content at time of manufacture of the oil. The analyzed contents were compared to the calculated amounts of EPA and DHA from the certificate of analyses from the omega-3 oil supplier, hence, differences in analytical methods cannot be ruled out as cause for the observed differences in quantitative content of DHA.

4 Conclusions

The results observed show that oxidative stable omega-3 tablets can be produced. The quantitative content of EPA

and DHA was confirmed in coated tablets after 1 year of storage, and the tablets were smell and odor-free. The possibility to optimize the production process further by spray granulating under nitrogen atmosphere may significantly add time to the estimated shelf-life of the coated tablet products, as indicated in this study.

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Conflict of interest statement: Tina Lien Vestland is a shareholder and employee in Omegatri AS. Jo Klaveness and Astrid Hilde Myrset are shareholders in the company. Lizette Balle Petersen is a previous employee of the company.

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