Research Article

Encapsulated fish oil products available in the UK meet regulatory guidelines with respect to EPA+DHA contents and oxidative status†

Running title: Quality of omega-3 fish oil supplements in the UK

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Abbreviations: EPA, eicosapentaenoic acid, **DHA**, docosahexaenoic acid, **n-3**, omega-3, **LC-PUFA**, long-chain polyunsaturated fatty acids, **EE**, Ethyl Ester, **TAG**, Triacylglycerol, **PV**, peroxide value, ***p*-AV**, para-anisidine value, **TOTOX**, total oxidation, **TBARS**, thiobarbituric acid reactive substances, **FFA**, free fatty acid, **GOED**, Global organization for EPA and DHA omega-3's

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Abstract

Encapsulated fish oil products continue to be of high interest, particularly concerning labelling claims and oxidative status. Thus, the present study analysed twenty-three encapsulated fish oil products from the UK for their lipid and fatty acid composition as well as oxidation parameters. Oil contents ranged from 91.4-118.9% of the manufacturers stated level. Lipid class analyses revealed three different types of oil products consisting of either triacylglycerol (TAG), ethyl ester (EE) or in combination (EE/TAG). Fatty acid profiles varied according to oil form with long-chain omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), more concentrated in EE compared to TAG-based oils with TAG/EE oils containing intermediary levels. Twelve products had EPA+DHA contents lower than advertised, although this was reduced to 11 when the actual measured capsule oil content was taken into consideration. All products had peroxide (PV) and anisidine values below those set by pharmacopeias, although four products had a PV above the industry set limit of 5 meq.kg^{-1}. No relationships were found between oxidative parameters and missing EPA+DHA contents, although a significant relationship was observed between PV and days to expiry. In summary, encapsulated fish oil products on the UK market are not oxidized and meet regulatory guidelines with respect to EPA+DHA contents and oxidative status.

Practical applications

The study highlights the importance of quantifying the actual capsule oil content when determining EPA+DHA levels with respect to label claims. Furthermore, it also places results into context regarding regulatory guidelines demonstrating to regulatory bodies

and consumers alike that UK fish oil products do meet specification and are not oxidised.

1. Introduction

The omega-3 (n-3) long-chain polyunsaturated fatty acids (LC-PUFA), eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3) and, to some extent, docosapentaenoic acid (DPA; 22:5n-3), are widely regarded as being beneficial to human health and development, being key to neural function as well as in reducing the incidence of chronic pathologies including cardiovascular and inflammatory diseases and cancer among others [1,2]. However, humans are inefficient at endogenously producing sufficient quantities of n-3 LC-PUFA and therefore require these beneficial fatty acids in the diet [1]. The main dietary sources of these fatty acids are from fish and seafood, but especially oily fish such as mackerel, sardines and salmon [3,4]. Consequently, global health authorities advocate consuming two portions of fish per week, of which one should be oily, as part of a healthy diet in order to obtain a daily dose of 250-1000 mg EPA+DHA [5]. Nevertheless, the majority of the western world fails to adhere to these guidelines, resulting in low EPA+DHA blood levels that are considered likely to increase the risk of chronic disease [6].

Nutritional supplements are an effective approach for consumers wanting to increase their dietary nutrient intake, and account for 13% of the global market share for EPA/DHA packaged products, excluding fish, behind infant formula and fortified foods [7]. Of these, encapsulated fish oil products are the most popular form of supplement and are widely available throughout developed countries in either natural or concentrated forms. Despite the majority of commercially available fish oil products meeting or exceeding manufacturers' specifications, many studies have reported the EPA and/or DHA content of some products analysed to be lower than that stated on the label, although there is some disagreement as to what is deemed as being reasonably accurate to the label claim [8-24]. Opinions vary as to the exact cause for the missing

EPA and/or DHA contents with autoxidation considered to be the most likely factor. However, the integrity of the analytical techniques employed and the results obtained in some of these studies, most notably Albert et al. [9], has also been disputed with recommendations that laboratories use standardised and/or accredited methods pertinent to the matrix being tested, together with the validation of methods through regular participation in inter-laboratory proficiency tests [16,21,25].

Marine oils are particularly susceptible to oxidative damage, more than other oils, due to their high number of double bonds, and position within the fatty acid chain, as a consequence of their favourable PUFA content [26]. Lipid oxidation results in the formation of free radicals and lipid hydroperoxides that may undergo further reaction into secondary oxidation products such as aldehydes, ketones and alcohols. Consequently, the oxidative status of oils can be evaluated through several methods such as measuring the peroxide value (PV) and *para*-anisidine value (*p*-AV), among others, as primary and secondary oxidative products, respectively. Industry regulators, including the British and European Pharmacopeias, have limits for n-3 LC-PUFA-containing oils at 10 meq.kg^{-1} for PV, 30 for *p*-AV and 50 for the total oxidation value (TOTOX), a combination of both PV and *p*-AV to give an assessment of total oxidation [27,28]. The Global Organization for EPA and DHA omega-3s (GOED), on the other hand, an industry association body commonly representing fish oil manufacturers, have set their own more stringent limits for n-3 LC-PUFA oils, through a Voluntary Monograph of 5 meq.kg^{-1}, 20 and 26 for PV, *p*-AV and TOTOX, respectively, as a means of maintaining products of high quality [28,29].

Encapsulating marine oils is one method of improving the oxidative stability of n-3 LC-PUFA marine oils [26]. Nevertheless, some studies have reported that the oxidative stability of some encapsulated marine oil products may be of concern with regards to

quality and/or safety [9-15,30-32]. In particular, there has been some controversy over the high levels of oxidation found in n-3 LC-PUFA oils from Australia and New Zealand reported by Albert et al. [9] with suggestions that assay interferences due to the types of oils tested together with the methodologies used may have resulted in false positive results [16,21,28]. Nevertheless, the monitoring of fish oil products on the market is of importance in ensuring that products are of a high quality and contain the specified contents to the final consumer.

Thus, the present study looked at the lipid and fatty acid composition, particularly with respect to EPA and DHA in relation to labelled content, as well as the oxidative status, of encapsulated fish oil products available in the UK and discussed how they compare to regulatory guidelines.

2. Materials and Methods

2.1. Sample collection and preparation

Twenty-three encapsulated n-3 LC-PUFA products, consisting of branded and own-brand standard fish oil and/or fish oil concentrates, were purchased from a variety of retailers and health outlets during January 2017 (see Table 1 for details). Sample bottles remained unopened until the day of analysis and were stored in a cool, dry place in accordance with the manufacturer's recommendations. Prior to the different analytical techniques, several capsules from each product, enough to provide a sufficient quantity of oil for the required methodology (minimum 5 capsules per product), were individually weighed, pierced to remove the oil content before rinsing with isohexane, and leaving overnight *in vacuo* in a desiccator. Empty capsules were reweighed and the capsule oil content calculated. Oil removed from capsules was stored under oxygen-free nitrogen and used the same day to minimize oxidation. For lipid class and fatty

acid analyses, a total lipid solution of 10mg.ml^{-1} was prepared by weighing a known quantity of oil and making up to concentration with chloroform/methanol (2:1, v/v). All analyses were carried out in February 2017 and performed blind and in duplicate, with a relative standard deviation between replicates <10% deemed acceptable, and the mean value reported.

2.2. Reagents and chemicals

All solvents used in the analytical techniques reported were of HPLC grade, with glacial acetic acid and potassium iodide of analytical reagent grade (Fisher Scientific, Loughborough, UK). Concentrated sulphuric acid (Aristar®, sp. gr. 1.84), analytical reagent grade starch (potato), potassium hydrogen carbonate (AnalaR Normapur®) and sodium hydroxide pellets (AnalaR Normapur®) were purchased from VWR International Ltd. (Poole, UK). Both 2-thiobarbituric acid (≥98%) and *p*-anisidine (≥98%) were obtained from Sigma-Aldrich (Poole, UK) together with trichloroacetic acid and phenolphthalein indicator solution, which were American Chemical Society (ACS) grade reagents. Sodium thiosulphate solution (Titrisol®) was purchased from Merck (Darmstadt, Germany). Nanopure water was collected from a Milli-Q ultrapure purification system (0.22 µm; Millipore, UK). Compressed nitrogen (oxygen free, ≥99.99% nitrogen, 300bar EVOS™ Ci 50 L cylinder) was obtained from BOC Ltd (Glasgow, UK).

2.3. Lipid class and fatty acid composition

Lipid classes were separated by single development high-performance thin-layer chromatography (HPTLC) using isohexane/diethyl ether/acetic acid (90:10:1, by vol.) as the development system [33]. Classes were visualised by charring at 160^{o}C for 15

min after spraying with 3% (w/v) aqueous cupric acetate containing 8% (v/v) phosphoric acid and quantified by densitometry using a CAMAG-3 TLC scanner (Version Firmware 1.14.16; CAMAG, Muttenz, Switzerland) with winCATS Planar Chromatography Manager. Identities of individual classes were confirmed by comparison with reference to Rf values of authentic standards run alongside samples.

Fatty acid methyl esters (FAME) from total lipid (1 mg) were prepared by acid-catalysed transmethylation, using 2 ml of 1% (v/v) solution of sulphuric acid in methanol and 1 ml toluene, at 50^{o}C for 16h [34]. The reaction was stopped with 2% (w/v) potassium bicarbonate in nanopure water before FAME were extracted and purified as described previously [35]. FAME were separated and quantified by gas liquid chromatography (GC) using a Fisons GC-8160 (Thermo Scientific, Milan, Italy) equipped with a 30 m x 0.32 mm i.d. x 0.25 µm ZB-wax column (Phenomenex, Cheshire, UK), 'on column' injection and flame ionization detection. Hydrogen was used as carrier gas with the initial oven thermal gradient from 50^{o}C to 150^{o}C at 40^{o}C.min^{-1} to a final temperature of 230^{o}C at 2^{o}C.min^{-1}. Individual FAME were identified by comparison to known standards (Restek 20-FAME Marine Oil Standard; Thames Restek UK Ltd., Buckinghamshire, UK) and published data [35]. The data were collected and processed using Chromcard for Windows (Version 1.19; Thermoquest Italia S.p.A., Milan, Italy). Heptadecanoic acid (17:0) was used as internal standard to calculate fatty acids on an absolute basis (mg.capsule^{-1}).

2.4. Oxidative parameters

Peroxide value (PV) was based upon the Association of Official Analytical Chemists (AOAC) Method 965.33 [36]. Approximately, 1 ml of oil was weighed into a conical flask and dissolved in 20 ml glacial acetic acid/chloroform (2:1, v/v) before

7 drops of saturated potassium iodide (KI) were added, swirled to mix and left in the dark. After 10 min, 15 ml of 10% KI solution together with 1 ml of starch solution was added. The resulting solution was titrated with 0.002 M sodium thiosulphate ($Na_2S_2O_3$) with the endpoint reached when the chalky white residue had dissipated and the solution reverted back to its original colour prior to the addition of starch. PV was calculated as: PV (meq.kg^{-1} oil) = [(Vs-Vb) × M/W] × 1000, where W is oil mass (g), Vs the volume (ml) of 0.002 M $Na_2S_2O_3$ solution titrated, Vb the volume of $Na_2S_2O_3$ titrated for the blank sample and M the molarity of the $Na_2S_2O_3$ solution.

Anisidine value (*p*-AV) was determined by absorbance spectrophotometry after reaction with *para*-anisidine in accordance with the International Union of Pure and Applied Chemistry (IUPAC) Method 2.201 [37]. Briefly, 0.3 g of oil sample was weighed in a 25 ml volumetric flask and made up to volume with isohexane and mixed. The absorbance (A_1) was measured at 350 nm against a reference blank of 5 ml isohexane containing 1 ml glacial acetic acid. A reagent solution comprising 1 ml 0.25% *para*-anisidine in glacial acetic acid was added to the sample and left for 10 min in the dark to react. The absorbance (A_2) was measured against 5 ml isohexane containing 1 ml 0.25% *para*-anisidine in glacial acetic acid and *p*-AV calculated as: *p*-AV = 25 × [1.2 × (A_2-A_1)/W], where W is the oil mass (g).

The total oxidation (TOTOX) value was derived using the results from the PV and *p*-AV analysis, calculated as TOTOX = 2 x PV + *p*-AV.

Thiobarbituric acid reactive substances (TBARS) analysis was based on the method of Sørensen and Jørgensen [38], which measures malondialdehyde (MDA) formed during lipid oxidation as a result of the degradation of PUFA [39]. Approximately, 1 g of oil was weighed into 50 ml centrifuge tubes before the addition of 25 ml 7.5% trichloroacetic acid (TCA) solution, vortex mixed and centrifuged for 5 min at 3399 *g*.

The TCA solution containing the extract was transferred into a 7 ml glass vial and 2 ml of 0.02 M 2-thiobarbituric acid (TBA) reagent added and mixed. Samples were left to stand in a water bath set at 100°C for 35 min, cooled and vortex mixed before degassing in a sonicator bath. The samples were centrifuged for 5 min at 3399 *g* before being transferred to cuvettes and the absorbance measured at 532 nm. A blank sample containing 2 ml of distilled water with 2 ml TBA reagent as well as a standard containing 2 ml of 1,1,3,3-tetraethoxypropane (TEP) solution with 2 ml of TBA reagent was also measured. TBARS was calculated as: TBARS (mg MDA.kg^{-1}) = [ABs_{smp} × W_{std} × V_{TCA} × M_{MDA} × 1000]/[Abs_{std} ×W_{smp} × F × M_{TEP}], where Abs_{smp} and Abs_{std} are the absorbances of the sample and the standard at 532 nm, respectively, W_{std} the standard weight (mg), V_{TCA} the volume of TCA added to the sample (ml), M_{MDA} the molecular weight of MDA (72.06 g.mol^{-1}), W_{smp} the sample weight (g), F the dilution factor for the TEP standard (16667) and M_{TEP} the molecular weight of TEP (220.3 g.mol^{-1}).

The free fatty acid (FFA) content of oils was determined according to the American Oil Chemists Society (AOCS) Official Method Ca 5a-40 [40]. Approximately 1 g of oil was weighed into a 250 ml conical flask and dissolved with 75 ml of 95% ethyl alcohol at 50°C before the addition of 2 ml phenolphthalein indicator solution. The sample was titrated with 0.25 M NaOH until the appearance of a permanent pink colour which persisted for 30 sec. FFA was calculated as: FFA (% as oleic acid) = [V × 0.25 × 28.2]/w, where V is the volume of NaOH titrated (ml) and w is the sample weight (g).

2.5. Quality assurance

The method of performance of the analytical procedures described above were further validated through the satisfactory annual performance of interlaboratory proficiency test including: the European Federation for the Science and Technology of Lipids, organised by the German Society for Fat Science (DGF, Frankfurt, Germany), for fatty acid content and oxidative parameters; Masterlab Analytical Services BV (Boxmeer, Netherlands) for analytical methods routinely used in the feed, oil, fish producing and technology sectors; and the American Oil Chemist's Society (AOCS, Illinois, USA) for the fatty acid content of marine oils.

2.6. Statistical tests

Relationships between variables (e.g. oxidative parameters and days to expiry) were assessed using Pearson's correlation coefficient (r). Runs test was used to check linearity with failed data indicating a non-linear relationship (GraphPad Instat® v 3.01). A significance of $P<0.05$ was applied to all tests.

3. Results and discussion

3.1. Oil capsule content

Overall, the oil capsule content of the 23 analysed sample products was found to be relatively consistent with that declared on the product label (Table 1). Only sample 7 contained noticeably less than the manufactures stated level (91.4% of the labelled capsule oil content), whereas samples 11 and 13 were found to be in excess of the advertised capsule oil content (117.1 and 118.9%, respectively) (Figure 1). This finding is similar to that of Albert et al. [9] and Kolanowski [11] who reported an oil capsule content range of 95.6-114.2% and 97.2-101.8% respectively, relative to the product label. Nevertheless, although the oil capsule contents in the present study were >90%

of the manufacturers claimed content such discrepancies will invariably affect the overall fatty acid content (mg.capsule^{-1}) delivered to the consumer.

3.2. Lipid class and fatty acid composition

Lipid class compositions of oil from capsules analysed in the present study revealed three different types of oil products, consisting mainly of triacylglycerol (TAG), ethyl ester (EE) or a combination of both (Table 2). This reflects the wide range of n-3 supplements available on the market in either natural TAG or concentrated forms of fatty acids, particularly with respect to n-3 LC-PUFA content. The standard fish oil products (samples 1-16) were generally comprised of TAG as the main lipid component (81.0-92.0%) and were largely characterized by high levels of myristic (14:0; range 3.6-7.2%), palmitic (16:0; range 9.8-18.8%) and oleic (18:1n-9; range 7.1-17.8%) acids, with EPA+DHA ranging from 19.8-32.0% of total fatty acids (Table 3). In contrast, samples 21-23 were fish oil concentrates with highly elevated levels of n-3 LC-PUFA (47.5-85.6%) with EPA+DHA levels of 38.0-75.2% of total fatty acids and EE as the predominant lipid class (94.6-98.0%). The EEs are chemically produced by trans-esterification of TAG fish oils with ethanol, which allows for the subsequent selective concentration of n-3 LC-PUFA, especially EPA and DHA, to levels greater than that found in natural fish oils [41]. However, the bioavailability and uptake of n-3 LC-PUFA as EEs compared to TAG is widely contested, with greater bioavailability suggested for TAG [42]. Although product packaging may specify that supplements are fish oil concentrates, therefore containing higher amounts of EPA and DHA, the oil form is rarely listed. Concentrated EE forms of EPA and DHA may be re-esterified back to TAG, although the conversion process inevitably increases the cost of the final product and, as such, many concentrates are left in the form of EE as observed in the

present study. Samples 17-20 on the other hand, contained a mix of both fish oil and fish oil concentrates and were therefore comprised of both TAG (21-28.8%) and EE (54.7-69.2%). Subsequently, these products contained intermediary levels of EPA+DHA of 35.7-48.6% of total fatty acids. Nonetheless all profiles reported are comparable to encapsulated fish oil products sampled from Europe [11], America [18,20,23], Brazil [10] and Australasia [17,19,21,43]. The origin of the oils can be further differentiated by their fatty acid profiles using marker fatty acids (Table 3). For example, northern hemisphere fish oils tend to exhibit higher levels of gondoic; (20:1n-9) and cetoleic (22:1n-11) acids, associated with the consumption of calanoid copepods by high-latitude zooplanktonivorous fish [44], as well as lower total monoene levels, and EPA:DHA ratios of around 1.0 [4]. Thus, profiles of samples 1-5, 7-9 and 17-19 all suggest that these products contained northern hemisphere fish oils. Indeed, these samples had cod liver oil as either a component or sole oil source (Table 1). However, sample 6 also had cod liver oil listed as an ingredient although the profile would suggest that this is not the case, as evinced by lower levels of both 20:1n-9 (1.9%) and 22:1n-11 (1.6%) compared to other cod liver oil capsule products (range 5.4-12.0 and 3.7-8.9, 20:1n-9 and 22:1n-11, respectively). It is possible that these marker fatty acids may have been diluted by the inclusion of other fish oils which may have been of southern hemisphere origin. Most encapsulated fish oil products available on the market are blends of fish oils and their derivatives. For example, samples 3-5, 7-9 and 17-19 all contained a combination of cod liver oil and other fish oils and exhibited similar patterns of the marker fatty acids, albeit diluted, compared to samples 1 and 2 which contained only cod liver oil. Additionally, sample 11 contained a higher level of the plant-derived omega-6 fatty acids, linoleic (18:2n-6, 17.0%) and gamma-linolenic acids (18:3n-6, 2.4%). This can be attributed to the inclusion of evening primrose oil,

Oenothera biennis, which is characterized by high levels of these fatty acids and has been used to treat systemic disease marked by chronic inflammation, although its efficacy remains disputed [45,46]. Fish oil capsules, nevertheless, are primarily consumed for their health benefits related to their n-3 LC-PUFA content, specifically EPA and DHA.

3.3. EPA and DHA content

Of the 23 capsule products analysed, 12 were found to contain lower EPA+DHA levels than the content stipulated on the product packaging when the expected oil content value was used (Figure 2). However, this was reduced to 11 products when the actual oil capsule content measured was taken into account, with sample 13 increasing from 94.2 to 112.0% of the labelled EPA+DHA content due to a higher than expected capsule oil content (refer to Table 1). Equally, sample 11 would have yielded just 80% of the stated EPA+DHA level had the specified capsule content been used (0.40 g), increasing instead to 94.7% of the labelled value when the actual capsule weight was applied (0.47 g). This further highlighted the importance of quantifying the capsule oil content rather than relying on the declared value when determining the 'true' EPA+DHA content of encapsulated products.

Many studies have reported lower than claimed EPA+DHA levels in marine products available from Europe [8,11], Australasia [9,16,17,19,21,24], North and South America [8,10,15,20,22,23], Korea [12] and Africa [13,14], and the current study is no exception. However, there is general disagreement between studies over what tolerance level is considered as being reasonably accurate to the label claim. Opperman et al. [13] for example, applied a range of 90-100% of the claimed EPA+DHA content as being acceptable. This would have resulted in one sample from the present study

under-delivering (sample 16, 88.9%), whereas 9 samples over-delivered. In Europe, where the current samples tested are marketed, the declared values on product labels are generally based on average values from the manufacturer's analysis in accordance with 200/46/EC directive of the European Parliament and Council [47]. Although the base ingredients may remain the same, e.g. cod liver or fish body oils, their lipid and fatty acid composition is dependent upon a variety of factors including fish species, age and nutritional status, as well as environmental factors [48,49]. Manufacturers and regulators will therefore allow for some variation between fish oil batches when formulating the final product as it is not always possible to contain the exact nutrient level labelled. Accordingly, tolerances of nutrient contents exist for food labelling purposes to ensure that consumers are not being misled. For example, the European Commission's Guidance document relating to food supplements with regards to setting tolerances for nutrient values declared on a label specifies a ± 40% tolerance for polyunsaturated fatty acids [50]. However, this document is only advisory, with no formal legal status, with the ultimate responsibility for the interpretation of the law lying with the European Court of Justice. Interestingly, companies in the UK have for many years tended to work to tolerances of ± 20% as part of their own strict measures for quality control. Comparably, the US Food and Drug Administration's (US FDA) labelling requirements state that "the nutrient content of the composite must be at least equal to 80 percent of the value for that nutrient declared on the label" [51], whereas the Therapeutic Goods Administration (TGA) for Australia indicate that a product should contain at least 90% of its declared content [52]. Thus, despite some encapsulated products falling below the EPA+DHA levels claimed on the label, only one out of twenty-three products (4.3% of total products tested) would have failed the

TGA standards, although all products met the specifications laid out by US and, more importantly, European regulators where all products from the present study are sold.

3.4. Oxidative status of encapsulated oils

In addition to the lower than claimed EPA+DHA levels, several studies have also suggested that the oxidised content of marine oil supplements to be at a level that may be of concern with regards to quality [9-15,30-32]. In the present study, all sample products were found to have PV, *p*-AV and TOTOX values below the respective limits of 10 meq.kg^{-1}, 30 and 50 respectively, set out by the British and European pharmacopeial standards for fish oils [25] (Table 4), although TOTOX is not technically recognized by pharmacopeias as it is only considered a convenient measure of oxidation [39]. Nevertheless, four out of the twenty-three products analysed (17.3%) had a PV greater than the 5 meq.kg^{-1} limit recommended by the more stringent GOED Voluntary Monograph for EPA and DHA oils [28,29]. This is similar to the 28% non-compliance reported by Bannenberg et al. [16], but far fewer than the 83% found by Albert et al [9], who analysed 47 and 36 fish oil products from the Australasian market, respectively. Limits used in the GOED Voluntary Monograph have been voluntarily set by the fish oil manufacturers themselves, and are generally stricter than pharmacopeial standards and regulations to ensure finished fish oil products are of a high quality [28,29]. However, the high non-compliance rate reported by Albert et al. [9] has been challenged by others with particular criticism directed towards the analytical methods and types of fish oil products tested [16,21,25,28]. Similarly, follow-up studies found Australasian fish oil products to have a greater compliance, in terms of EPA+DHA and oxidation status, than previously reported [16,21,24,28]. Many of the samples analysed by Albert et al. [9] for example contained flavourings,

often used to mask the 'fishy' taste, or whose oils were pigmented. Such parameters can interfere with assays, particularly *p*-AV, as flavoured compounds generally contain aldehydes and pigments can directly affect the colormetric measurement that may potentially give false positive readings [16,21,28,53]. Consequently, GOED released technical guidelines to assist in the selection of appropriate methods for analysing n-3 oils [53], with others further recommending methods that are both accredited and validated through regular participation in inter-laboratory ring-tests for the matrix in question [16,21,25]. Thus, despite the availability of pigmented (e.g. virgin salmon oil) and flavoured fish oil products in the UK, all fish oils tested in the present study were intentionally selected to be free of any flavouring or pigmentation to remove any potential conflict with results.

Of particular interest to note is that products containing EE fish oil concentrates, as either the sole lipid source (samples 22 and 23, 6.84 and 7.04 meq.kg^{-1}, respectively) or in combination with TAG (samples 19 and 20, 5.20 and 6.76 meq.kg^{-1}), tended to be more oxidised, at least in terms of their PV being above the recommended GOED level. Oxidation has been found to occur at a faster rate in EE compared to TAG fish oils containing similar EPA and DHA contents [54]. Furthermore, these four EE products also had the highest levels of DHA (16.8, 19.7, 23.8 and 22.2%, samples 19, 20, 22 and 23, respectively) as compared to TAG-only (samples 1-16, range 6.5-12.9%) and other EE/TAG (15.8 and 16.0%, sample 17 and 18, respectively) and EE oils (12.2%, sample 21), which is more prone to autoxidation than EPA [55]. Tentatively, this would suggest that there is more readily oxidised substrate available, which may subsequently result in a net loss of these fatty acids. In fact, three of these samples with PV > 6.0 meq.kg^{-1}, i.e. samples 20, 22 and 23, also had lower contents of EPA+DHA than stated on the product label. Several authors have speculated that oxidative damage

was most likely the main cause for missing EPA and/or DHA in fish oil products [9,18,23], with Albert et al. [9] observing a relationship of missing EPA+DHA contents with AV and TOTOX values. However, in the current study both the *p*-AV and TOTOX values were below GOED's limits of 20 and 26, respectively (Table 4), and no significant relationships with missing EPA+DHA contents were found ($P>0.05$). Moreover, the additional secondary oxidation products measured, i.e. TBARS, a widely used indicator of MDA formation and lipid peroxidation, together with FFA levels also exhibited no obvious sign of oxidation with values ranging between 0.11-1.39 mg MDA.kg^{-1} and 0.31-1.19%, for TBARS and FFA, respectively (Table 4). Fish oils may undergo clean-up during the manufacturing process, typically by thin-film distillation or deodorization coupled with active carbon, to remove undesirables such as FFA as well as environmental pollutants without affecting fatty acid profiles [12,56]. Moreover, Bannenberg et al. [16] re-examined the oxidative status and EPA and DHA contents of fish oil products one year after they had originally been tested and had passed their expiry dates and found that, although both PV and *p*-AV had increased, EPA and DHA did not differ significantly from the levels when first tested. Similarly, Ritter et al. [54] also found no evidence for a significant decrease in EPA and DHA content when EE and TAG fish oils were incubated at temperatures of 5-60^{o}C for up to 21 days, despite an increase in PV and *p*-AV. Although oils may appear to be of high quality with low oxidative values, they may still contain high molecular weight polymers. Burkow and Henderson [57] for example, found that the polymer content increased rapidly in air-oxidised fish oils and that oils with identical PV and TBARS-values could contain different polymer content. The same authors also observed a correlation between polymer increase and a decrease in total fatty acids, which they suggested indicated that polymers may be the main oxidation product in autoxidised

marine oils. The thin-layer chromatography method used in the present study for separating lipid classes would have been inadequate for visualising polymers, due to insufficient staining caused by the lack of double bonds, instead requiring alternative techniques such as high performance gel permeation chromatography as used by Burkow and Henderson [57]. The additional monitoring for oligomers (cross-reacted oxidised lipids) in n-3 rich marine oils is encouraged by the industry for which the European and British pharmacopeial monographs specify limits of ≤1.5 and 3.0%, respectively [28,58]. Nevertheless, although all oils in the current study exhibited no major signs of oxidation through standard analytical techniques it is unclear whether any other signs of oxidation had occurred, which could contribute to fatty acid losses.

The encapsulation of marine oils together with the addition of antioxidants such as tocopherols (vitamin E) are commonly employed to stabilize n-3 oils [26]. However, exposure to air such as during the preparation of oils prior to analysis renders these processes ineffective. Thus, Bannenberg et al [16] suggest that the PV/*p*-AV ratio should be below 1 as an indicator of good analytical techniques, which was evident in the current study with PV/*p*-AV ranging from 0.11-0.96 (Table 4), as a PV/*p*-AV ratio >1 may indicate that oxidation, specifically primary oxidation, has occurred shortly before analysis due to a prolonged period of air exposure. Irrespective, even under normal storage conditions oxidation will still slowly occur. Accordingly, as in the study of Halvorsen and Blomhoff [30], but in contrast to Albert et al. [9], a significant negative relationship was observed between number of days until expiry and PV ($r = -0.6264$, $P = 0.001$) as well as for the PV/*p*-AV ratio ($r = -0.5832$, $P = 0.004$) (Figure 3). The number of days before expiry varied greatly between products from 122 to 974 days, indicating the long shelf life of encapsulated fish oil products. This extended shelf life also presents a significant challenge in addressing potential variations between

product batches, both with regards to oxidation and fatty acid contents, with products with identical batch numbers being found in multiple stores/retailers regardless of branded or own-brand labels. Consequently, this often restricts studies to single-point analyses on products being tested as in the case of the present study. Nevertheless, collectively these results showed that, although encapsulated fish oil products undergo some form of oxidation during storage, they have the capacity to remain within acceptable levels during the course of their shelf life.

4. Conclusion

In summary, the present study showed that encapsulated fish oil products obtainable in the UK are available in TAG, EE or TAG/EE forms, which predominantly determine the levels of n-3 LC-PUFA offered to the consumer. Moreover, although some products may fall slightly short of the EPA+DHA contents stated on product labels they are, on the whole, compliant with local regulatory guidelines regarding labelling. Nevertheless, it is important that studies verify the actual oil capsule content when determining EPA+DHA contents to ensure an accurate representation of levels available to consumers. Similarly, all products tested were below oxidation limits set out for fish oils by pharmacopeial standards, with only a few samples exceeding the stricter industry imposed limits for PV. Further monitoring studies of this nature are merited as an effective way of maintaining quality control and ensuring products adhere to codes of standards.

Conflict of interest

The authors declare no conflicts of interest.

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Table 1. Sample information, according to manufacturer's label, and the actual measured oil capsule content and EPA+DHA content of the encapsulated fish oil products sampled in the study.

Sample	Brand	Oil Source	No. Capsules	Oil Content (mg.capsule^{-1})		EPA+DHA (mg.capsule^{-1})		Expiry Date (days to expiry[¶])
				Labelled	Actual[*]	Labelled	Actual[#]	
Fish oils								
1	Own Brand	Cod Liver	60	1000	1007	180	173	Aug 2019 (914)
2	Own Brand	Cod Liver	60	410	409	66	77	Aug 2019 (914)
3	Own Brand	Cod Liver / Fish	30	500	504	93	107	Sept 2019 (944)
4	Own Brand	Cod Liver / Fish (body)	60	500	494	93	105	Jun 2019 (852)
5	Own Brand	Cod Liver / Fish	60	500	510	93	109	Sep 2019 (944)
6	Own Brand	Cod Liver / Fish	90	1000	992	187	207	Apr 2019 (791)
7	Own brand	Cod Liver / Fish	30	250	229	46	50	Jul 2018 (518)
8	Branded	Fish / Cod Liver	60	540	540	124	120	May 2018 (457)
9	Own Brand	Cod Liver / Fish	30	1000	967	187	208	Sep 2019 (944)
10	Own Brand	Fish	30	300	294	75	69	Dec 2018 (671)
11	Branded	Fish / Evening Primrose	60	500	469	122	116	Jan 2019 (702)
12	Own Brand	Fish (body)	30	1000	996	250	245	Oct 2019 (974)
13	Own Brand	Fish	30	1000	1189	260	291	Oct 2018 (610)
14	Own Brand	Fish (body)	30	1000	1018	250	269	Sep 2019 (944)
15	Branded	Fish	180	300	301	73	81	Aug 2017 (184)
16	Own Brand	Fish	90	1000	977	300	267	Aug 2019 (914)
Fish oils and concentrates								
17	Branded	Fish Concentrate / Cod Liver / Fish	60	1050	1070	360	326	Jan 2018 (337)
18	Branded	Fish Concentrate / Cod Liver / Fish	30	1050	1053	360	328	June 2017 (122)
19	Branded	Fish Concentrate / Cod Liver / Fish	60	525	532	155	172	May 2018 (457)
20	Branded	Fish Concentrate/ Fish	60	600	597	260	247	Nov 2017 (275)
Fish oil concentrates								
21	Own Brand	Fish Concentrate	60	1500	1509	450	486	Oct 2018 (610)
22	Branded	Fish Concentrate (body)	100	556	562	305	279	Sep 2018 (579)
23	Own Brand	Fish Concentrate	60	1360	1376	950	905	Oct 2018 (610)

[*]Based on the analysis of individual capsules (minimum 15 per sample product)

[#]EPA+DHA content calculated using actual measured oil content (mg.capsule^{-1}) from pooled capsule oil (minimum 5 capsules per sample product)

[¶]Based on date analysed to label expiry date

Table 2. Lipid class composition (% of total lipid), as determined by HPTLC, of encapsulated fish oil products sampled in the present study.

	Sample Product																						
	Fish oils																**Fish oil / concentrate**				**Concentrates**		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**
Lipid class (% total lipid)																							
Wax/Steryl ester	1.1	1.7	1.5	1.0	1.4	1.6	2.6	0.3	1.5	1.7	3.0	2.0	1.1	2.0	1.9	2.5	1.9	1.6	0.8	1.7	-	-	-
Ethyl ester	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	57.0	54.7	55.3	69.2	97.4	94.6	98.0
Triacylglycerol	88.3	92.0	89.4	87.5	86.2	87.9	85.2	89.2	85.4	83.5	86.1	83.9	81.0	84.0	83.8	85.1	35.2	36.2	38.8	21.5	-	-	-
Free Fatty Acid	0.8	0.5	0.9	1.2	0.8	0.8	1.8	0.9	0.6	1.2	0.6	0.7	1.1	0.6	1.0	0.6	0.3	0.7	0.4	0.4	0.9	0.7	0.6
Cholesterol/Sterol	2.3	1.8	1.6	2.5	2.6	2.4	1.7	2.8	5.2	2.4	1.8	3.4	3.4	3.4	2.9	2.4	1.3	1.7	1.2	1.6	1.0	1.4	0.4
Diacylglycerol	5.6	2.7	5.3	6.3	7.2	5.7	5.9	5.4	6.9	7.3	7.4	8.1	9.4	7.9	8.4	7.6	2.7	3.9	1.5	4.1	-	-	-
Total neutral	***98.1***	***98.7***	***98.7***	***98.5***	***98.2***	***98.4***	***97.2***	***98.6***	***99.6***	***96.1***	***98.9***	***98.1***	***96.0***	***97.9***	***98.0***	***98.2***	***98.4***	***98.8***	***98.1***	***98.5***	***99.3***	***96.7***	***99.0***
Pigmented/polar material	***1.9***	***1.3***	***1.3***	***1.5***	***1.8***	***1.6***	***2.8***	***1.4***	***0.4***	***3.9***	***1.1***	***1.9***	***4.0***	***2.1***	***2.0***	***1.8***	***1.6***	***1.2***	***2.0***	***1.5***	***0.7***	***3.3***	***1.0***

- not detected

Table 3. Fatty acid composition (% of total fatty acids) of oil from encapsulated fish oil products sampled in the study.

	Sample Product																						
	Fish Oils																Fish oil / Concentrates				Concentrates		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**
Fatty acid (% total lipid)																							
14:0	3.6	3.6	5.1	5.2	4.9	7.6	5.4	4.9	5.3	7.2	3.6	5.8	6.8	6.4	7.0	7.2	2.2	2.3	2.2	2.2	0.4	0.2	0.1
16:0	9.8	9.9	13.3	13.3	13.2	18.8	13.7	12.9	12.4	17.8	11.6	15.1	16.1	16.1	16.7	16.1	7.4	8.8	7.8	7.6	7.6	0.6	0.2
18:0	2.1	2.1	2.7	2.8	2.7	3.8	2.8	2.7	2.4	3.7	3.2	3.1	3.3	3.3	3.4	3.3	2.8	2.9	2.7	3.6	5.4	4.5	0.5
20:0	0.1	0.0	0.3	0.3	0.3	0.6	0.2	0.3	0.3	0.5	0.3	0.5	0.5	0.5	0.3	0.2	0.5	0.5	0.5	0.8	0.4	1.0	0.3
Total saturated[1]	***15.9***	***16.0***	***21.9***	***22.2***	***21.5***	***31.7***	***23.1***	***21.6***	***21.1***	***30.1***	***19.3***	***28.4***	***27.8***	***27.0***	***28.4***	***27.4***	***15.1***	***14.9***	***13.4***	***15.0***	***14.0***	***7.0***	***1.5***
16:1n-7	7.6	9.0	8.6	8.5	8.4	10.4	8.6	8.6	9.0	9.7	6.4	8.4	9.2	8.7	8.7	8.7	4.7	4.9	4.6	3.7	2.5	0.5	0.1
18:1n-9	17.8	17.1	13.7	13.3	14.2	11.3	12.8	13.2	12.7	10.9	7.1	9.3	10.2	8.8	8.6	8.0	11.9	11.8	11.6	7.3	17.5	8.3	2.0
18:1n-7	4.4	4.9	3.9	3.9	3.8	3.5	3.8	4.0	3.7	3.4	2.9	2.9	3.0	3.1	3.2	3.1	3.5	3.6	3.4	3.0	5.2	3.3	0.9
20:1n-11	1.8	1.1	1.0	0.9	0.9	0.2	1.6	0.6	0.9	0.2	0.2	0.2	0.2	0.2	0.2	0.2	1.2	1.0	1.0	0.3	0.4	0.3	0.1
20:1n-9	12.0	11.9	6.7	6.9	6.8	1.9	5.4	7.1	6.0	1.8	1.5	1.7	1.6	1.8	1.4	1.1	6.9	6.4	7.1	2.6	2.0	3.0	1.0
22:1n-11	8.9	6.5	5.1	5.2	5.1	1.6	4.1	3.7	4.3	1.5	1.3	1.8	1.3	1.8	1.1	0.8	6.1	6.5	5.9	2.3	1.3	2.0	0.4
22:1n-9	0.9	0.7	0.6	0.6	0.6	0.3	0.5	0.5	0.5	0.3	0.2	0.3	0.3	0.3	0.3	0.2	0.8	0.8	0.8	0.6	0.2	0.6	0.1
Total monoenes[2]	***55.0***	***52.7***	***41.0***	***40.6***	***41.2***	***30.7***	***38.2***	***39.0***	***38.4***	***29.1***	***20.6***	***25.7***	***27.0***	***26.0***	***24.7***	***23.5***	***36.7***	***36.6***	***35.8***	***21.4***	***29.9***	***19.3***	***4.8***
18:2n-6	1.6	2.1	1.6	1.5	1.5	1.5	1.8	2.1	1.6	1.4	17.0	1.6	1.4	1.3	1.2	1.3	1.4	1.2	1.3	1.0	4.1	1.2	0.6
18:3n-6	0.1	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.3	2.4	0.3	0.3	0.3	0.3	0.3	0.1	0.2	0.2	0.2	0.3	0.2	0.1
20:2n-6	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3
20:4n-6	0.4	0.4	0.8	0.8	0.8	1.5	0.8	0.8	0.9	1.4	1.3	1.1	1.3	1.2	1.3	1.1	1.3	1.4	1.3	2.1	2.0	2.0	5.0
Total n-6 PUFA[3]	***2.5***	***3.1***	***3.1***	***3.1***	***3.1***	***4.2***	***3.4***	***3.7***	***3.3***	***4.0***	***21.4***	***3.9***	***3.7***	***3.5***	***3.5***	***3.5***	***3.9***	***3.6***	***3.7***	***4.6***	***7.4***	***4.8***	***8.1***
18:3n-3	0.8	0.9	0.8	0.8	0.8	0.9	0.9	0.9	0.9	0.8	0.7	0.8	0.8	0.8	0.7	0.9	0.7	0.7	1.0	0.6	1.7	0.9	0.3
18:4n-3	2.6	2.6	2.8	2.9	2.8	1.8	2.8	3.0	3.0	2.2	2.7	3.4	2.9	3.4	2.7	3.1	2.2	2.1	2.5	1.8	4.1	3.1	1.0
20:4n-3	0.8	0.7	0.9	0.9	0.9	1.0	0.7	0.8	1.0	0.9	1.0	1.0	0.8	1.0	0.8	0.8	1.1	1.0	1.2	1.3	1.2	1.9	2.0
20:5n-3 (EPA)	8.6	8.8	13.5	13.5	13.6	14.6	13.9	13.8	15.0	16.5	22.1	17.9	18.1	18.7	19.2	19.5	19.9	20.3	20.6	28.9	25.8	33.8	53.0
22:5n-3 (DPA)	1.2	1.2	1.5	1.5	1.6	1.8	1.4	1.5	1.7	1.9	1.3	1.8	1.9	1.9	2.1	2.2	2.7	2.6	2.7	3.9	1.7	3.5	4.7
22:6n-3 (DHA)	11.2	12.9	11.1	11.2	11.2	10.2	11.8	12.4	12.4	11.3	6.5	12.0	11.8	12.5	12.5	12.5	15.8	16.0	16.8	19.7	12.2	23.8	22.2
Total n-3 PUFA[4]	***25.7***	***27.4***	***31.3***	***31.5***	***31.6***	***31.0***	***32.1***	***33.1***	***34.7***	***34.3***	***35.1***	***37.7***	***36.9***	***39.0***	***38.7***	***39.9***	***43.5***	***43.9***	***46.0***	***57.8***	***47.5***	***68.7***	***85.6***
Total PUFA[5]	***29.1***	***31.3***	***37.1***	***37.2***	***37.2***	***37.6***	***38.7***	***39.4***	***40.5***	***40.8***	***60.1***	***45.9***	***45.2***	***47.1***	***46.9***	***49.1***	***48.2***	***48.5***	***50.8***	***63.6***	***56.1***	***73.7***	***93.7***
EPA+DHA	***19.8***	***21.7***	***24.6***	***24.7***	***24.8***	***24.8***	***25.7***	***26.2***	***27.4***	***27.8***	***28.6***	***29.9***	***29.9***	***31.2***	***31.7***	***32.0***	***35.7***	***36.3***	***37.4***	***48.6***	***38.0***	***57.6***	***75.2***
EPA+DPA+DHA	***21.0***	***22.9***	***26.1***	***26.2***	***26.4***	***26.6***	***27.1***	***27.7***	***29.1***	***29.7***	***29.9***	***31.7***	***31.8***	***33.1***	***33.8***	***34.2***	***38.4***	***38.9***	***40.1***	***52.5***	***39.7***	***61.1***	***79.9***

[1]includes 15:0, 22:0 and 24:0

[2]includes 16:1n-9, 17:1, 20:1n-7 and 24:1n-9

[3]includes 20:3n-6, 22:4n-6 and 22:5n-6

[4]includes 20:3n-3 and 21:5n-3
[5]includes 16:2, 16:3 and 16:4

Table 4. Primary (PV, meq.kg^{-1}) and secondary oxidation markers (*p*-AV; TBARS, mg MDA.kg^{-1}; FFA, %), total oxidation (TOTOX; 2PV +AV) and ratio of PV/AV of encapsulated fish oil products sampled in the study.

	Sample Product																						
	Fish oils																**Fish oils / Concentrates**				**Concentrates**		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**
PV (meq.kg^{-1})	2.46	3.57	2.60	3.88	2.67	2.59	3.70	3.97	1.44	4.43	4.37	1.74	2.86	2.20	3.96	2.08	4.52	4.26	5.20	6.76	4.15	6.84	7.04
AV	9.83	6.11	12.70	9.58	11.90	9.88	10.59	7.26	13.63	13.56	9.24	8.63	15.63	12.20	11.37	14.34	6.43	8.64	10.41	7.03	11.54	7.59	9.54
TOTOX	14.75	13.25	17.90	17.34	17.24	15.06	17.99	15.20	16.51	22.42	17.98	12.11	21.35	16.60	19.29	18.50	15.47	17.16	19.84	20.81	20.55	21.27	23.62
PV/AV	0.25	0.58	0.20	0.41	0.22	0.26	0.35	0.55	0.11	0.33	0.47	0.20	0.18	0.18	0.35	0.15	0.70	0.49	0.50	0.96	0.36	0.90	0.74
TBARS (mg MDA.kg^{-1})	0.27	0.19	0.19	0.46	0.17	0.56	0.26	0.16	0.27	1.37	0.11	0.29	1.39	0.15	0.31	0.41	0.99	0.33	2.49	0.51	0.52	0.56	1.32
FFA (%)	0.33	0.31	0.57	0.35	0.39	0.42	1.19	0.36	0.38	0.66	0.42	0.38	0.52	0.38	0.60	0.38	0.38	0.40	0.54	0.35	0.40	0.36	0.45

Limit of PV, AV and TOTOX for marine oils of 10 meq.kg^{-1} 30 and 50, respectively, by the British and European pharmacopeias or 5 meq.kg^{-1} 20 and 26, respectively, by GOED [25,26].

Figure Legends

Figure 1. The actual capsule oil content measured of fish oil products relative to the claimed content (dotted line).

Figure 2. The EPA+DHA content of encapsulated fish oil products relative to the labelled content (dotted line) based on claimed and actual capsule oil contents.

Figure 3. The relationship between days to expiry and (a.) PV and (b.) PV/*p*-AV ratio of encapsulated fish oil products analysed in the present study.

Figure 1.

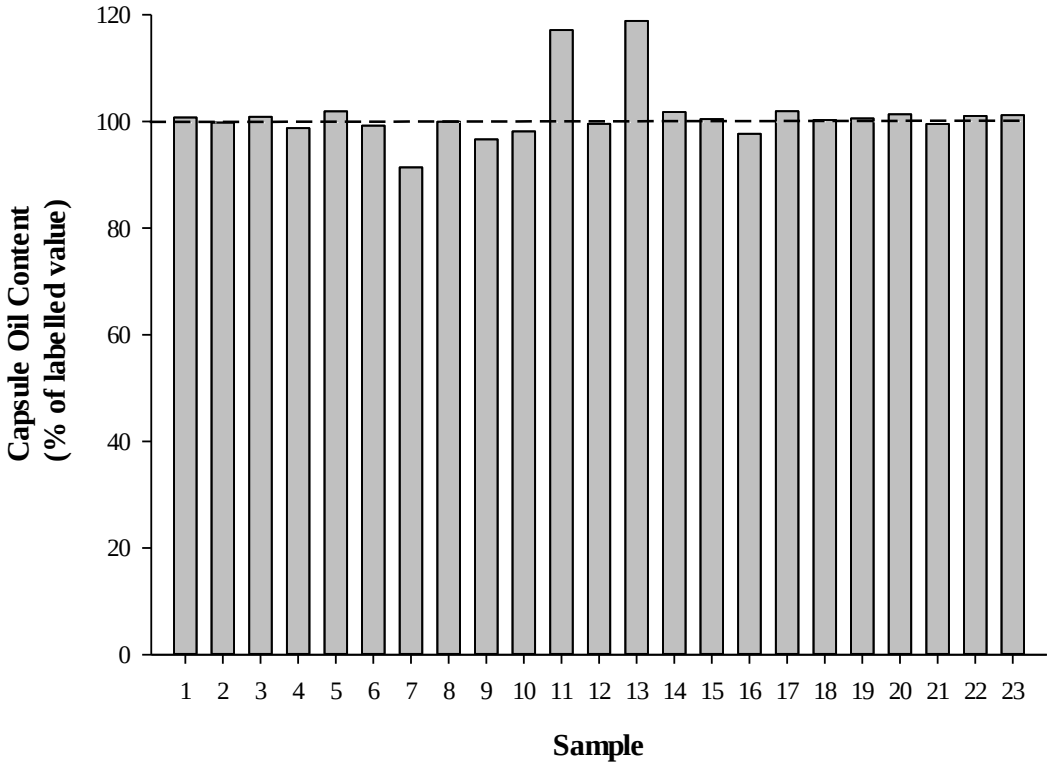

Figure 2.

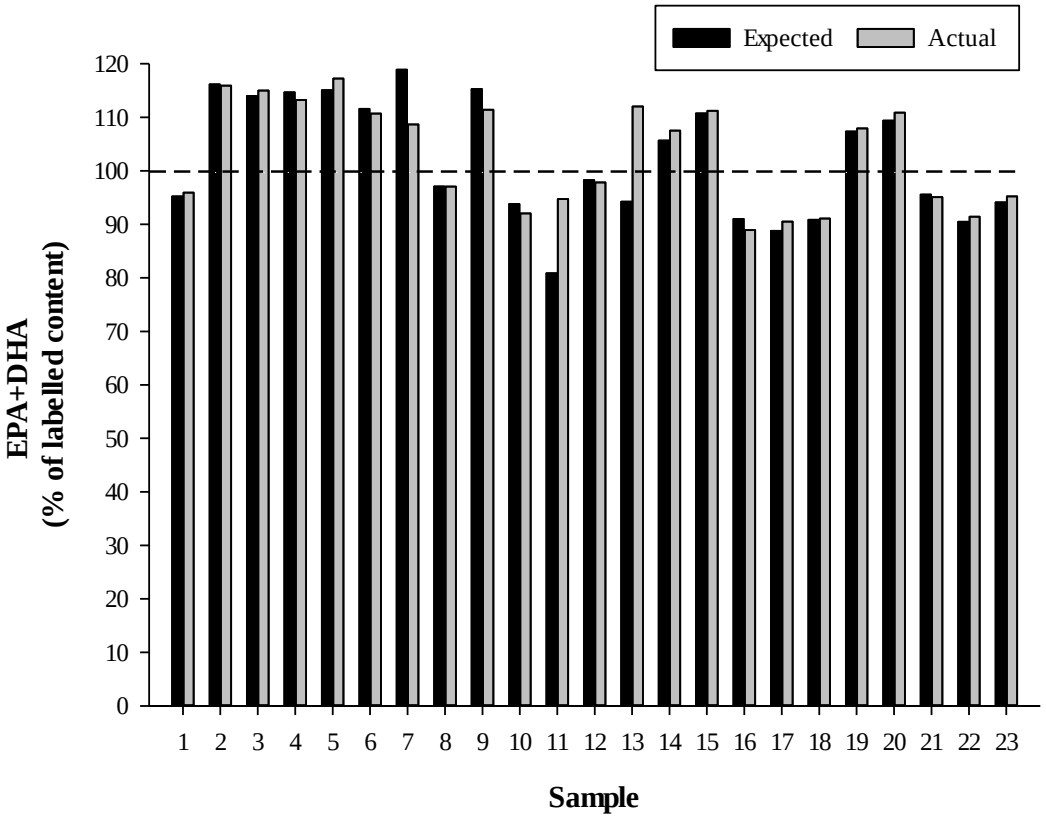

Figure 3.