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RESEARCH ARTICLE

Oxidation of fish oil supplements in Australia

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ABSTRACT

Fish oils oxidise readily, forming primary and secondary oxidation products, which may be harmful for humans. Some recent studies reported that fish oil supplements in Australasia are oxidised above acceptable international limits, however other studies reported low levels of oxidation. This study employed peroxide and p-anisidine values determination to measure primary and secondary oxidation of fish oils in the Australian market. Of 26 supplements tested, 38% exceeded the limit for primary oxidation, 25% exceeded the limit for secondary oxidation and 33% exceeded the limit for total oxidation, according to international recommendations. Four specially marketed supplements were found to deliver significantly lower amounts of fish oil per capsule (165 vs. 577 mg, $p = .007$), yet cost significantly more on a per gram basis ($2.97 vs $0.39, $p < .001$). However, there were no differences in any oxidative markers between regular supplements and the specially marketed products.

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Introduction

Fish is the richest dietary source of omega-3 long-chain polyunsaturated fatty acids (n-3 PUFAs), which have been extensively researched for their health benefits (Grant and Guest 2016). Of the n-3 PUFA family, eicosapentaenoic acid (EPA; C20:5) and docosahexanoic acid (DHA; C22:6) have the most potent immunomodulatory effect, suppressing inflammation mechanistically with potential clinical relevance (Calder 2006; Simopoulos 2002; Wall et al. 2010). As fish oil is both high in n-3 PUFAs and low in omega-6 long-chain polyunsaturated fatty acids (n-6 PUFAs), an anti-inflammatory effect is achieved through the production of n-3 PUFA derived anti-inflammatory mediators and the simultaneous reduction in n-6 PUFA derived pro-inflammatory mediators (Calder 2006; Grant and Guest 2016). As such, recommendations have been made by the National Heart Foundation in Australia to consume 500 mg/day of combined EPA and DHA from two serves of oily fish per week for optimal cardiovascular health, with fish oil supplements, recommended where adequate fish consumption is not possible (National Health and Medical Research Council 2017; National Heart Foundation of Australia 2017) . Similar recommendations have been made in New Zealand and the USA (National Heart Foundation of New Zealand 2017).

The 2011–2012 National Nutrition and Physical Activity Survey indicated that 25% of adult women and 15% of adult men in Australia consume fish oil supplements regularly (Australian Bureau of Statistics 2014; Meyer 2016). Importantly, this number is likely higher in certain groups of the population; for example, 43% of Australian adults over 51 reported taking fish oil supplements (Grieger et al. 2013; Burnett et al. 2017). Dietary intake of n-3 PUFAs in Australian adults was estimated to be 395 mg/day in 2011–2012, with 277 mg/day coming from food and 118 mg/day coming from supplements, which represents approximately one-third of intake coming from supplements (Meyer 2016). This represents a 1.6-fold increase in n-3 PUFA consumption since 1995 (Meyer 2016). The popularity of dietary supplements has seen the supplement industry in Australia valued at $1 billion in 2016, with growth expected in future years (RSM Australia 2016).

Oxidation of oils occurs upon exposure to oxygen, causing molecular changes that result in the initial production of primary oxidation products which eventually break down to secondary oxidation products (GOED and Council For Responsible Nutrition 2015;

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Sottero et al. 2018). EPA and DHA are highly susceptible to oxidation due to the number (five and six, respectively) and type (bis-allylic) of double bonds (Grant and Guest 2016). Studies in fresh fish have shown that oxidation occurs rapidly upon storage, especially with exposure to light (Chaijan et al. 2006; Cardenia et al. 2013). Early studies suggested that oxidation products could be atherogenic or neurodegenerative or at the very least simply reduces the efficacy of the oil (Esterbauer 1993; Haglund et al. 1991; Sayre et al. 1997). Evidence from the most recent randomised controlled trials (RCT) are also mixed. One RCT in young and middle-aged healthy subjects found no difference in the transcriptome between those randomised to high quality or oxidised fish oil in the short term (Myhrstad et al. 2016). However, another RCT in women aged 25–75 found that consuming less oxidised fish oil reduced circulating triglycerides and cholesterol more so than consuming more oxidised fish oil (Garcia-Hernandez et al. 2013). Some studies have shown no difference to the outcome factors with the ingestion of oxidised fish oil compared to a non-oxidised fish oil in the short term, but the effects of long term exposure i.e. months to years, to primary and secondary oxidation products have not been studied rigorously (Garcia-Hernandez et al. 2013; Myhrstad et al. 2016; Ottestad et al. 2016).

A review of human RCTs found that only two of 18 RCTs with fish oil as the intervention found benefit to the outcome factor, which led researchers to hypothesise that oxidation of fish oil supplements is contributing to poor results in clinical trials (Grey and Bolland 2014). Other researchers have suggested that the treatment of fish oil as a pharmaceutical agent in RCTs is contributing to poor results due to the lack of control over baseline and non-study n-3 PUFA intake (James et al. 2014; McLennan and Pepe 2015).

Three measures have historically been employed to estimate oxidation in fresh oils; Peroxide Value (PV), para-Anisidine Value (pAV), and Totox (GOED and Council For Responsible Nutrition 2015). PV measures hydroperoxides, primary oxidation products, while pAV measures aldehydes, secondary oxidation products (GOED and Council For Responsible Nutrition 2015). Totox combines PV and pAV in an equation to estimate total oxidation (GOED and Council For Responsible Nutrition 2015). International guidelines for oxidation levels in fish oil supplements stipulate that fish oil supplements should have a PV of less than 5 mEq O_2/kg, pAV of less than 20 and Totox less than 26 (GOED 2015). The Therapeutic Goods Administration (TGA) in Australia sets less stringent limits of 10 mEq O_2/kg for PV and 30 for pAV (Department of Health and Ageing 2012). Flavoured fish oils are excluded from the Global Organisation for EPA and DHA Omega 3 (GOED) Voluntary Monograph for pAV due to the known interference of flavours, however, no alternative test is listed (GOED 2015; GOED and Council For Responsible Nutrition 2015).

Globally, there have been numerous studies examining the oxidative status of commercially available fish oil supplements. These studies have shown varying levels of oxidation in fish oil supplements with 12–100% of samples exceeding at least one international limit for oxidation (Fantoni et al. 1996; Halvorsen and Blomhoff 2011; Opperman and Benade 2013; Ritter et al. 2013; Rupp et al. 2013; Albert et al. 2015; Jackowski et al. 2015; Bannenberg et al. 2017; Labdoor 2017; Mason and Sherratt 2017; De Boer et al. 2018). Conversely, other studies have found fish oil products to be minimally oxidised (Kolanowski 2010; Nash et al. 2014; Nichols et al. 2014, 2016; Killeen et al. 2017). A study by Albert et al. (2015) found that the majority of 32 tested fish oil supplements in New Zealand were oxidised above acceptable international limits. This study caused a global media response, prompting an Australian industry-funded study by Nichols et al. (2016) which analysed 10 Australian products and reported that none of them were oxidised above acceptable limits (Krail 2015). The GOED released data from their testing of products globally, which showed only 2–4% of over 2000 products tested were unacceptably oxidised (GOED and Council For Responsible Nutrition 2015). Further to this, a larger industry-funded study was published in 2017 on 47 fish oil supplements available in New Zealand which found that 23% of tested supplements exceeded the limit for total oxidation (Bannenberg et al. 2017). Most recently, Do Boer et al. (2018) published retrospective data from a global supplement testing programme that indicated that approximately 14% of supplements tested exceeded oxidation limits. However, within this sample, it is unclear how the supplements from Australia and New Zealand performed specifically, as the authors didn't separate analysis by country of origin. Evidently, there are conflicting results with respect to fish oils and their oxidative status, globally and more pertinently, within Australasia (Albert et al. 2015, 2017; Bannenberg et al. 2017, Nichols et al. 2016). Consistent with the majority of the literature, we hypothesise that some fish oil supplements in Australia are oxidised above acceptable international limits. As such, the aim of this

study was to provide independent, third-party analysis of the peroxide and p-Anisidine values of a comprehensive range of fish oil supplements available in pharmacies in Australia and to compare the results to international acceptable limits.

Materials and methods

All fish oil capsules and liquid fish oils on sale in major retail outlets in Australia were eligible for inclusion in this study, except for those with the addition of colours, other marine oils e.g. salmon or krill oil or other added products e.g. glucosamine, vitamin D and primrose oil. These products were excluded as these additional ingredients interfere with the pAV determination, giving a falsely high value. This study intended to exclude flavoured products for this reason, however unclear labelling resulted in some flavoured supplements being included in the current study. Gummies and chewable supplements were also ineligible for inclusion in this study, as the tests used have not been validated for these matrices. Nine out of 10 products examined by Nichols et al. (2016) were included, as well as a representative sample of other major brands on the market. Only one liquid fish oil was included in the study. Supplements were purchased in March 2017 from major pharmacy outlets in Sydney. Products were purchased in the smallest available quantity, ensuring the expiry date was at least 12 months away where possible. Three supplements had an expiry date sooner than 12 months, where these were the only batches available. One package of each supplement was purchased, except for one supplement where two packages were required to provide enough fish oil for all testing. The additional package was obtained one month later from the same batch number (#17). The presence or absence of flavours was determined for all supplements via direct contact with the manufacturers. Other information such as country of origin and expiry date, were determined either from labelling or via direct contact with the manufacturers if required. Price was recorded as Recommended Retail Price by the manufacturer at the time of purchase. Once purchased, supplements were removed from packaging, except for the liquid fish oil, blinded to the researcher and were stored at –20 °C protected from light to minimise any further oxidation.

Mass of capsule

Capsules were weighed. Its contents were emptied and the capsule washed with hexane and dried. The empty capsule was then weighed and subtracted from the original mass to calculate oil mass in the capsules. Duplicate measures were taken. The liquid fish oil was excluded from capsule mass determination.

Peroxide value determination

Peroxide value was determined according to the European Pharmacopoeia (Ph. Eur.) method 2.5.5 Method A (European Directorate for the Quality of Medicines and Healthcare 2004). Immediately prior to analysis, 4–18 capsules were pierced and the contents were evacuated for analysis. Four grams of oil was weighed in a volumetric flask, from the pooled capsules or directly from the bottle for the liquid fish oil. 24 mL of a 3:2 glacial acetic acid:chloroform solution was added to the flask and swirled to dissolve the sample. Four hundred microlitre of saturated potassium iodide solution was added to the flask and the contents swirled for exactly one minute. Twenty four millilitre of deionised water was immediately added and the contents were shaken vigorously. The solution was titrated with 0.01 M sodium thiosulphate until light in colour, then 5 mL of 1% starch solution was added as an indicator. The titration was continued until the solution was colourless. Duplicate measures were taken from unique samples, of 4–18 pooled capsules, depending on capsule mass. Peroxide value was calculated using the Equation (1);

$$\mathrm{PV} = \ [10 * (V - V_{\mathrm{blank}})]/m \qquad (1)$$

where V is the volume of 0.01 M sodium thiosulphate used to titrate the solution, V_{blank} is the volume used in the blank determination of reagents and m is the mass of the sample in grams.

p-Anisidine value determination

The p-Anisidine value was determined according to the Ph. Eur. method 2.5.36 (European Directorate for the Quality of Medicines and Healthcare 2004). Immediately prior to analysis, two capsules were pierced and the contents were evacuated for analysis. 0.2 g of oil was weighed, from the pooled capsules or directly from the bottle for the liquid fish oil and was dissolved in 10 mL of 2,2,4-trimethylpentane. Absorbance was measured at 350 nm against a blank solution of 2,2,4-trimethylpentane. One mililitre of 2.5 g/L p-anisidine in acetic acid was added to 5 mL of the solution and also to 5 mL of 2,2,4-trimethylpentane. After 10 min, the absorbance of both solutions was measured, using the 2,2,4-trimethylpentane and p-anisidine solution as a blank. Triplicates were

Table 1. Peroxide value (PV) test results.

#	Brand	Product name	Mean ± SD
1	Amcal	Odourless Wild Fish Oil 1000	6.41 ± 0.65[a]
2	Bio Island	DHA for Kids	3.73 ± 0.87
3	Bio Island	DHA for Pregnancy	2.64 ± 0.31
4	Bioceuticals	UltraClean 85	8.24 ± 0.64[a]
5	Bioceuticals	UltraClean DHA Omega	4.84 ± 0.04
6	Bioceuticals	UltraClean EPA/DHA Plus	2.71 ± 0.03
7	Bioglan	Odourless Super Fish Oil 1000	6.34 ± 0.03[a]
8	Bioglan	Odourless Super Fish Oil 2000	2.46 ± 1.03
9	BioSource	Odourless Fish Oil 1000 mg + Omega 3	5.32 ± 0.18[a]
10	Blackmores	Omega Daily Concentrated Fish Oil	3.23 ± 0.28
11	Blackmores	Fish Oil 1000	4.20 ± 0.14
12	Blackmores	Omega Triple Concentrated Fish Oil	2.59 ± 0.17
13	Blackmores	Omega Brain Concentrated Fish Oil	3.64 ± 0.07
14	Cenovis	Odourless Fish Oil 1500 mg High strength	3.34 ± 0.53
15	Healthy Care	Triple Strength Fish Oil	3.81 ± 0.11
16	Healthy Care	Fish Oil 1000mg Omega 3	3.21 ± 0.18
17	Healthy Care	Kid's High DHA	6.98 ± 3.53[a]
18	Nature's Own	Triple Concentrated Fish Oil	3.23 ± 0.44
19	Nature's Way	Kids Smart Drops (liquid)	7.05 ± 1.55[a]
20	Nature's Way	Odourless Fish Oil 1000 mg	7.82 ± 0.04[a]
21	Nature's Way	Odourless Triple Strength Fish Oil 3000	3.84 ± 1.21
22	Swisse	Odourless 4× Strength Wild Fish Oil Concentrate	6.20 ± 0.69[a]
23	Swisse	Odourless Super Strength Wild Fish Oil	4.19 ± 0.07
24	Swisse	Odourless Wild Fish Oil 1000 mg	3.14 ± 0.14
25	Swisse	Odourless High Strength Wild Fish Oil 1500 mg	6.00 ± 0.04[a]
26	Thompsons	Omega-3 Fish Oil	5.59 ± 0.19[a]

[a]Indicates a result over the GOED Voluntary Monograph limit of 5 mEq O_2/kg. Results reported are the average of tests performed in duplicate on unique pooled samples of 4–18 capsules.

performed from unique samples of two pooled capsules. p-Anisidine value was calculated using the Equation (2);

$$\mathrm{pAV} = [10(1.2 * A_2 - A_1)]/m \quad (2)$$

where A_2 is the absorbance of the oil + p-anisidine solution, A_1 is the absorbance of the oil solution and m is the mass of the sample in grams.

Totox

Totox was calculated using the Equation (3);

$$\mathrm{Totox} = 2 * \mathrm{PV} + \mathrm{pAV}.$$

Data analysis

Data was analysed using SPSS Version 24 (SPSS, Chicago, IL). Results are reported as means of duplicate or triplicate tests. Results for oxidative markers were compared to both the GOED Voluntary Monograph, as well as the TGA Guidelines (Department of Health and Ageing 2012; GOED 2015). Differences between groups were assessed using Student's t-test. Associations between variables were assessed using Pearson's correlation. Statistical significance of $p < .05$ was used.

Results

Results for the oxidative markers of supplements tested are shown in Tables 1–3. Oxidative status is compared to the GOED Voluntary Monograph in tables. The less stringent Australian TGA guidelines are also compared in text only.

Peroxide value (PV)

Thirty-eight per cent of supplements tested exceeded the GOED Voluntary Monograph limit of 5 mEq O_2/kg (Table 1). Zero per cent of supplements exceeded the less stringent Australian TGA limit of 10 mEq O_2/kg.

p-Anisidine value (pAV)

The pAV test is only valid for unflavoured supplements, so analysis excludes flavoured supplements. Of the unflavoured supplements, 25% exceeded both the GOED Voluntary Monograph limit of 20 and the TGA limit of 30 (Table 2). Data for flavoured supplements is shown in Table A1.

Totox

As Totox includes pAV in the calculation, it is only valid for unflavoured supplements. Of the unflavoured

Table 2. p-Anisidine value (pAV) test results.

#	Brand	Product name	Mean ± SD
2	Bio Island	DHA for Kids	5.60 ± 0.86
3	Bio Island	DHA for Pregnancy	5.64 ± 1.77
7	Bioglan	Odourless Super Fish Oil 1000	78.3 ± 1.28[a]
9	BioSource	Odourless Fish oil 1000 mg + Omega 3	122 ± 6.81[a]
11	Blackmores	Fish Oil 1000	11.6 ± 0.94
15	Healthy Care	Triple Strength Fish Oil	5.06 ± 0.51
16	Healthy Care	Fish oil 1000 mg Omega 3	9.54 ± 1.22
19	Nature's Way	Kids Smart Drops (liquid)	4.45 ± 0.50
21	Nature's Way	Odourless Triple Strength Fish Oil 3000	90.8 ± 7.77[a]
22	Swisse	Odourless 4X Strength Wild Fish Oil Concentrate	7.64 ± 1.03
23	Swisse	Odourless Super Strength Wild Fish Oil	18.1 ± 2.86
26	Thompson's	Omega-3 Fish Oil	7.50 ± 1.27

[a]Indicates a result over the GOED Voluntary Monograph limit of 20. Results reported are the average of tests performed in triplicate on unique pooled samples of two capsules. Note that as the pAV test is invalid for flavoured supplements due to interference, results are shown only for unflavoured supplements.

Table 3. Totox results.

#	Brand	Product name	Totox
2	Bio Island	DHA for Kids	13.1
3	Bio Island	DHA for Pregnancy	10.9
7	Bioglan	Odourless Super Fish Oil 1000	91.0[a]
9	BioSource	Odourless Fish Oil 1000 mg + Omega 3	133[a]
11	Blackmores	Fish Oil 1000	20.1
15	Healthy Care	Triple Strength Fish Oil	12.7
16	Healthy Care	Fish Oil 1000 mg Omega 3	16.0
19	Nature's Way	Kids Smart Drops (liquid)	18.6
21	Nature's Way	Odourless Triple Strength Fish Oil 3000	98.4[a]
22	Swisse	Odourless 4X Strength Wild Fish Oil Concentrate	20.0
23	Swisse	Odourless Super Strength Wild Fish Oil	26.5[a]
26	Thompson's	Omega-3 Fish Oil	18.7

[a]Indicates a result over the GOED Voluntary Monograph limit of 26. Note that as the pAV test is invalid for flavoured supplements due to interference, and Totox is a function of pAV, Totox is an invalid measure for flavoured supplements. As such, results are shown only for unflavoured supplements.

supplements, 33% exceeded the GOED Voluntary Monograph limit of 26, while 25% exceeded the TGA limit of 50 (Table 3). Data for flavoured supplements is shown in Table A2.

PV/pAV ratio

The PV/pAV ratio is used to assess whether oxidation has occurred shortly before testing or alternatively at a point early in sample processing or storage (Bannenberg et al. 2017). A PV/pAV ratio greater than one suggests oxidation has occurred very recently and is an atypical result indicative of methodological issues (Bannenberg et al. 2017). Only two supplements had a PV/pAV ratio greater than one (7.7%).

Capsule mass

Measured capsule mass was highly correlated (0.996) with labelled capsule mass ($p < .001$) (Figure 1).

Cost and concentration

EPA + DHA content was negatively correlated (−0.439) with price per g n-3 ($p = .025$), such that price per gram decreased as concentration of EPA + DHA increased, however, this data appears to be skewed by the four products specifically marketed for children and pregnant women, as analysis without these four speciality products only shows a weak non-significant positive correlation ($p = .224$; Figure 2). The specialty supplements had significantly lower mean EPA + DHA content per capsule than the rest of the supplements, 165 vs. 577 mg ($p = .007$) and were also found to be significantly more expensive than the rest of the supplements on a per gram basis, $0.39 vs. $2.97 per g n-3 ($p < .001$). However, there were no differences in any of the oxidative markers between the two groups. Also, EPA + DHA content was not correlated with any of the oxidative markers.

Discussion

This study evaluated the oxidative state of 26 fish oil supplements widely available in Australia. Of the supplements tested, 38% exceeded the limit for primary oxidation, 25% exceeded the limit for secondary oxidation and 33% exceeded the limit for total oxidation, according to the GOED Voluntary Monograph (GOED 2015). However, if the less stringent TGA guidelines are applied, none exceeded the limit for primary oxidation and 25% exceeded the limit for both secondary and total oxidation (Department of Health and Ageing 2012). Supplements tested were bought off the shelf, as consumers would buy them and mostly had at least one year until their expiry date. This indicates that consumers are at risk in one in three purchases of exposing themselves to primary and/or secondary oxidation products when consuming

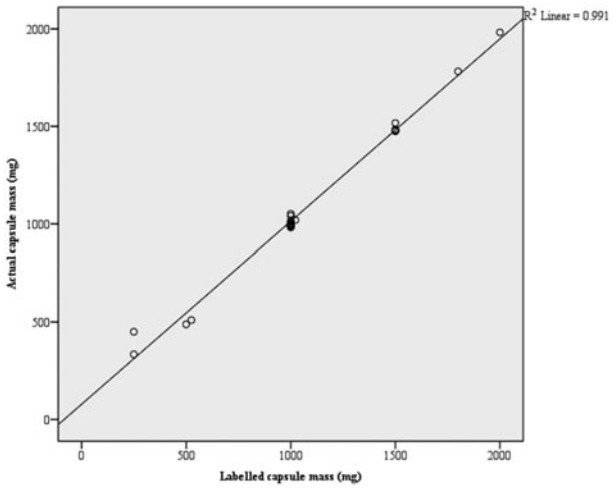

Figure 1. Labelled capsule mass (mg) vs. measured capsule mass (mg). Capsule mass was measured by weighing the capsule before evacuation, then emptying the capsule, washing with hexane, drying completely and then re-weighing. Capsule mass was determined to be the pre-evacuation weight minus the post-evacuation weight.

fish oil supplements purchased from their local retailer, according to international standards.

These results indicate a slightly higher percentage of products exceeding the oxidation limits than found by Bannenberg et al. (2017) and De Boer et al. (2018) yet a far lower percentage than found by Albert et al. (2015). Unfortunately, direct comparisons with results from some previous studies were not altogether possible. For example, the study by Albert et al. (2015) did not exclude flavoured products or even report which products were flavoured, thus comparisons of secondary and total oxidation are not possible. The current study only had six products in common with Bannenberg et al. (2017) and it is unknown how many were in common with Albert et al. (2015) and De Boer et al. (2018), thus few direct comparisons between results for specific products can be made. In addition, De Boer et al. (2018) analysed samples shortly after manufacture, while this study, consistent with others, analysed samples after dispatch to retail outlets (Albert et al. 2015; Nichols et al. 2016; Bannenberg et al. 2017).

It is important to note that the products found to be exceeding oxidation markers may have been within the acceptable oxidation limits at manufacture. Oxidation may have occurred post-manufacture i.e. in transit to or storage by the retailer, as it is known that fish oxidises rapidly upon storage, particularly when exposed to light (Chaijan et al. 2006; Cardenia et al. 2013). It is perplexing that storage in darkness and at low temperatures is recommended for bulk fish oil before and during manufacture, yet no such recommendation exists for finished encapsulated fish oil products (EFSA Panel on Biological Hazards 2010). Evidently, somewhere along the supply chain, oxidation of a significant percentage of commercially available fish oil products to an unacceptable level does occur. Thus, further investigation into optimal, yet feasible, batching, transport and storage conditions is required to minimise oxidation along the entire supply chain, enabling supply of a minimally oxidised product to the consumer. Importantly, the low incidence of samples with a PV/pAV ratio greater than one (8%) indicates that minimal oxidation occurred during sample processing in the laboratory and that oxidation measured was largely indicative of oxidation that occurred, somewhere in the supply chain, prior to purchase (Bannenberg et al. 2017).

This study sampled 26 supplements, yet more than half of these were unable to be validly tested for secondary oxidation due to flavoured additives. For those not tested for secondary oxidation, this yields an incomplete picture of the oxidative state of the supplement, which is a major limitation of the current study. Subsequently, as a result of the small sample size for pAV and Totox, few associations could be made between variables and significance could not be reached. There are currently no publicly available, validated methods to test for secondary oxidation in flavoured fish oils and no alternative method is offered in any regulatory framework (Department of Health and Ageing 2012; Nutrasource Diagnostics Inc 2013;GOED 2015; Bannenberg et al. 2017). A low PV can represent either low or high total oxidation, as PV is seen to increase initially and then decrease as secondary oxidation occurs (Ritter and Budge 2012; Jackowski et al. 2015). For this reason, while a high PV indicates that oxidation has occurred, a low PV alone cannot be used to make inferences about the oxidative state of an oil. Therefore, a test for secondary oxidation needs to be developed to allow flavoured products to undergo the same stringent testing that unflavoured products are subjected to and to allow for inferences to be made about the total oxidative state of the supplement (GOED and Council For Responsible Nutrition 2015). Our findings with regards to pAV of flavoured supplements agree with the findings of De Boer et al. (2018) in that a greater percentage of flavoured supplements exceeded the pAV limit than unflavoured supplements and that citrus flavourings affected the pAV more than other (vanillin) flavourings (Appendix A). These results, in conjuction with those of Bannenberg et al. (2017) and Nichols et al. (2016) confirm that future studies

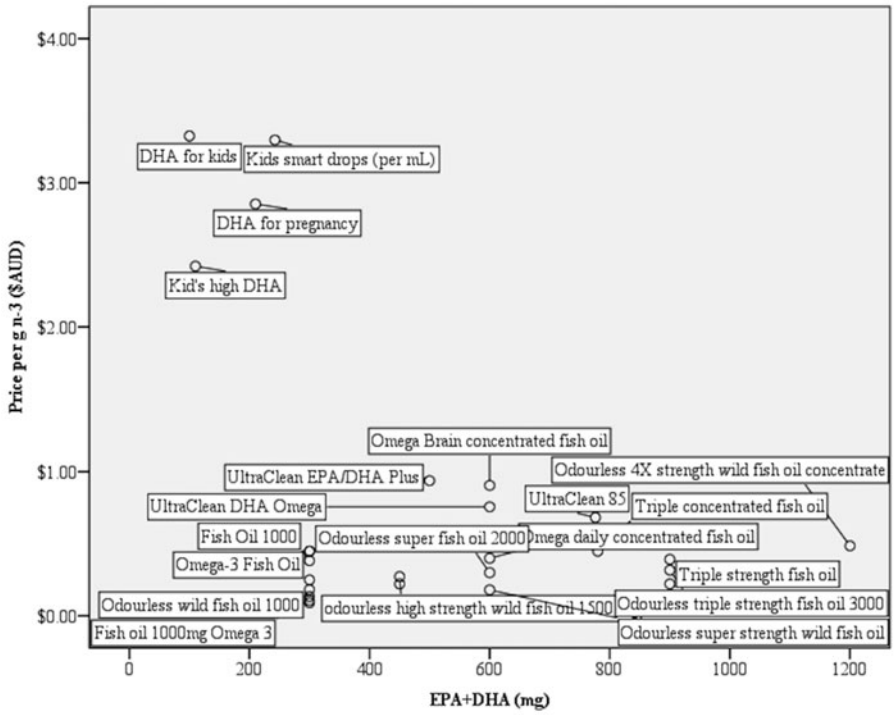

Figure 2. EPA + DHA per capsule (mg) vs. price per gram of n-3 PUFAs. EPA + DHA content is reported according to label claims. Price is recommended retail price in $AUD at time of purchase.

should routinely separate analysis of pAV in flavoured and unflavoured supplements.

Price was not found to be correlated with oxidative markers. However, four products targeted at specific groups (one at pregnant women and three at children) were found to deliver significantly lower amounts of n-3 PUFAs per capsule than regular supplements and cost significantly more per gram (Figure 2). There was, however, no difference in any oxidative markers between these four products and the rest of the supplements tested. On face value, this suggests a degree of unfair marketing to these vulnerable groups of consumers, based on the assumption that price is largely related to the amount of active ingredient delivered. In addition, the promotion of these "specialised" products suggests that these groups benefit from specialised supplements and fails to encompass the conflicting evidence around the role of fish oil in pregnancy and that exposure to lipid oxidation products during this vulnerable period of gestation could have detrimental effects on the developing foetus (Albert et al. 2016, 2017; Rice et al. 2017; Saccone et al. 2016) Marketing to these specific groups needs to be regulated with respect to the health claims made and must be in line with the most current scientific evidence. As such, the supplement industry may need to more closely monitor health claims made for fish oil supplements, especially those targeted to vulnerable groups of consumers.

Limitations are noted in this study. Firstly, this study did not evaluate the effects of antioxidant concentration on oxidation markers. As the inclusion of antioxidants is not required and is not routinely reported on supplement packaging in Australia, future studies could help establish the relative value of adding antioxidant, of various types, to the preservation of omega 3 from oxidation. As with previous reports, this study also collected data at a single time point and did not monitor the levels of oxidation in products at different time points e.g. from manufacture to delivery and during storage under various conditions, thus yielding a static measure of oxidation, rather than a richer, more kinetic measure of the likely causes and time course of oxidation in fish oil supplements.

However, some of the limitations outlined above can be overcome by the development of newer methods of detecting oxidation. Thankfully, new methods are emerging to detect oxidation in oils based on spectroscopy, that are quick, cheap and non-destructive (Cebi et al. 2017; Killeen et al. 2017). In addition to this, new methods are emerging for the production of stable fish oil supplements. For example, the use of coated triglyceride powders and natural odourless antioxidants offer promising directions to produce stable fish oil supplements with more resistance to oxidation than is currently seen (Fhaner et al. 2016;

Vestland et al. 2017). It is hoped that together, these developments will ensure a high quality non-oxidised omega-3 product to the consumer in the near future.

However, in the present, due to the lack of valid testing for flavoured supplements, consumers should be advised to choose an unflavoured supplement to ensure that it has been validly tested according to the GOED Voluntary Monograph (GOED 2015). Choosing an unflavoured supplement also allows the consumer to determine when the oil is rancid by smelling for odour, as sensory evaluation can at least as sensitive as laboratory testing (Ritter and Budge 2012). At present, natural flavours used in fish oil supplements are regarded as excipient ingredients that are not required to be declared on packaging in Australia (Therapeutic Goods Administration 2016). To allow consumers to choose an unflavoured product, revision of the regulation with respect to labelling of added flavours needs to occur.

In conclusion, it is well established that increased serum n-3 PUFAs, in relation to n-6 PUFAs, confer anti-inflammatory benefits (Calder 2006; Simopoulos 2002; Wall et al. 2010). As such, the guidelines in Australia promote the intake of 500 mg EPA + DHA daily, ideally from oily fish or from supplements if fish intake is not possible (National Heart Foundation of Australia 2017). Fish oil intakes in Australia are currently below the Suggested Dietary Target for reduced chronic disease risk (National Health and Medical Research Council 2017; Meyer 2016). Supplementation is a cheap, quick and easy way of increasing one's n-3 PUFA intake. Yet, the evidence for the benefits of fish oil supplementation for the general population remains unclear due to poor evidence from RCTs (Nestel et al. 2015; Siscovick et al. 2017). So too, the evidence for the effects of long term exposure to lipid oxidation products is poor (Turner et al. 2006). However, the current body of evidence suggests long term exposure to lipid oxidation products in the doses seen in fish oil supplements are likely to have deleterious effects on inflammation, oxidative stress and lipid metabolism (Turner et al. 2006). To tease apart the effects of fish oil and the effects of oxidation products, RCTs should routinely report the oxidative state of the supplement administered and also seek to minimise subject's exposure to oxidation products from the fish oil. Clinical trials also need to better control background n-3 PUFA intake and exclude subjects with high baseline intake to allow for distinct characterisation of the effects of fish oil supplementation (McLennan and Pepe 2015). More research is needed to elucidate methods to produce efficacious, stable, non-oxidised n-3 PUFAs to increase population n-3 PUFA intake.

Disclosure statement

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Appendix

Table A1. p-Anisidine Value (pAV) test results of flavoured supplements.

#	Brand	Product	Mean ± SD	Flavour
1	Amcal	Odourless Wild Fish Oil 1000	97.5 ± 5.39[a]	Vanillin
4	Bioceuticals	UltraClean 85	6.16 ± 0.93	Vanillin
5	Bioceuticals	UltraClean DHA Omega	30.7 ± 2.50[a]	Vanillin
6	Bioceuticals	UltraClean EPA/DHA Plus	4.42 ± 1.19	Vanillin
8	Bioglan	Odourless Super Fish Oil 2000	56.8 ± 0.63[a]	Citrus
10	Blackmores	Omega Daily Concentrated Fish Oil	19.7 ± 0.71	Vanillin
12	Blackmores	Omega Triple Concentrated Fish Oil	22.3 ± 1.59[a]	Vanillin
13	Blackmores	Omega Brain Concentrated Fish Oil	25.8 ± 0.79[a]	Vanillin
14	Cenovis	Odourless Fish Oil 1500 mg High Strength	71.5 ± 5.84[a]	Citrus
17	Healthy Care	Kid's High DHA	217[a,b]	Citrus
18	Nature's Own	Triple Concentrated Fish Oil	9.99 ± 0.84	Citrus
20	Nature's Way	Odourless Fish Oil 1000 mg	107 ± 8.68[a]	Citrus
24	Swisse	Odourless Wild Fish Oil 1000 mg	26 ± 0.44[a]	Vanillin
25	Swisse	Odourless High Strength Wild Fish Oil 1500 mg	31.1 ± 2.62[a]	Vanillin

[a]Indicates a result over the GOED Voluntary Monograph limit of 20. Results reported are the average of tests performed in triplicate on unique pooled samples of two capsules. Note that as the pAV test is invalid for flavoured supplements due to interference, results are shown here for flavoured supplements only for interest. Type of flavour is recorded as per communication with company representatives. Mean pAV were different between citrus and vanillin flavourings, however, this did not reach significance.

[b]No SD for pAV of product Z as only one reading could be taken. Two subsequent readings were taken and were too high to be read by the spectrophotometer.

Table A2. Totox results of flavoured supplements.

#	Brand	Product	Totox	Flavour
1	Amcal	Odourless Wild Fish Oil 1000	110[a]	Vanillin
4	Bioceuticals	UltraClean 85	22.6	Vanillin
5	Bioceuticals	UltraClean DHA Omega	40.4[a]	Vanillin
6	Bioceuticals	UltraClean EPA/DHA Plus	9.83	Vanillin
8	Bioglan	Odourless Super Fish Oil 2000	61.7[a]	Citrus
10	Blackmores	Omega Daily Concentrated Fish Oil	26.2[a]	Vanillin
12	Blackmores	Omega Triple Concentrated Fish Oil	27.5[a]	Vanillin
13	Blackmores	Omega Brain Concentrated Fish Oil	33.1[a]	Vanillin
14	Cenovis	Odourless Fish Oil 1500 mg High Strength	78.1[a]	Citrus
17	Healthy Care	Kid's High DHA	231[a]	Citrus
18	Nature's Own	Triple Concentrated Fish Oil	16.5	Citrus
20	Nature's Way	Odourless Fish Oil 1000 mg	123[a]	Citrus
24	Swisse	Odourless Wild Fish Oil 1000 mg	32.3[a]	Vanillin
25	Swisse	Odourless High Strength Wild Fish Oil 1500 mg	43.1[a]	Vanillin

[a]Indicates a result over the GOED Voluntary Monograph limit of 26. Note that as the pAV test is invalid for flavoured supplements due to interference and Totox is a function of pAV, Totox is an invalid measure for flavoured supplements. As such, results are shown here for flavoured supplements only for interest. Type of flavour is recorded as per communication with company representatives. Mean Totox was different between citrus and vanillin flavourings, however, this did not reach significance.