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Most omega 3 supplements in Israel are highly oxidized

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Abstract
Omega-3 polyunsaturated fatty acids (PUFAs) supplements are widely used in recent years due to their known potential beneficial effect in a variety of neurological disorders and cardiovascular disease. Commercially available omega-3 dietary supplements are known to contain the desired fats, as well as harmful oxidized lipids, derived from the undesired oxidation processes of omega-3. Our study tested 14 most popular commercial brands of fish oil derived PUFAs and 3 non-fish (plant or algae)-derived PUFAs, available in Israel in 2016. The objective of this study was to determine the essential fatty acids content, as well as three known oxidation markers in omega-3 dietary supplement. Our data reveals for the first time that high levels of oxidized saturated fats are found in common n-3 PUFAs dietary supplement commercially available in Israel (fish, plant and algae-derived), as was recently reported in other countries, and may interfere with their intended biological benefits.

Keywords: Omega-3, PUFAs, n-3 PUFAs, Israel

1. Introduction
The long chain omega-3 fatty acid, docosahexaenoic acid (DHA), is a major lipid in the brain, recognized as essential for normal brain function [1]. PUFA composition of cell membranes is, to a great extent, dependent on dietary intake [2]. Insufficient dietary intake of omega-3 fatty acids and excessive intake of omega-6 fatty acids is believed to be a significant contributing factor to aging [3] as well as various diseases [4], neurodegenerative and neurological disorders [5, 6, 7], inflammatory conditions [8], depression and other psychiatric disorders [9], and cardiovascular diseases [10]. Thus, omega-3 dietary supplements have become some of the most popular dietary supplements worldwide and are widely available in Israel without prescription. These supplements are used to prevent and potentially treat a number of serious conditions, such as Alzheimer's disease [11], elevated blood pressure [12], rheumatoid arthritis [13] and inflammatory disorders [14]. There is evidence pointing to omega-3 fatty acids reducing risk of heart attack, stroke, and death from heart disease [15, 16, 17]. Omega-3 fatty acids have been studied extensively in many other conditions, including asthma, cancer, psoriasis and inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis [18, 19, 20, 21]. While some of the studies have been promising, there is no conclusive evidence of the predicted essential role of omega-3 in these conditions.
The variety of different types of omega-3 fatty acids available in the market can be confusing and close attention should be paid to the exact composition and origin of each type. There are the fish and algae oils (marine), which contain docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and there are the plant-derived products containing alpha-linolenic acid (ALA), which is converted to omega-3 fatty acids in the body [22].
Despite their wide availability and broad pharmacological usage, omega-3 products are not regulated as over-the-counter drugs in Israel, and their purity, content, efficacy and safety are not evaluated. It is possible that in order to ingest the recommended values of EPA and DHA n-3 PUFAs using a particular product, the consumer may be required to intake large quantities of capsules or syrup per day. This may in turn result in increased intake of undesired oxidized lipids, and higher product cost.
N-3 PUFA are highly prone to oxidation due to the large number of double bonds within the fatty acid chain [23, 24]. As fish oils oxidise, un-oxidised fatty acids diminish and are replaced by a combination of lipid peroxides and secondary oxidation products [25].
Oxidized lipids have a central role in pathogenic conditions such as atherogenesis and may play a role in vascular injury, inflammation, carcinogenesis and insulin resistance [26, 27, 28, 29]. Consumption of oxidized lipids via dietary supplement may lead to an increase in oxidized lipids levels in the blood, which correlate with increased cardiovascular risk in patients with coronary heart disease [30, 31]. In addition to cell membrane-mediated damage, oxidized lipids associated with low-density lipoprotein (LDL) contribute to endothelial dysfunction, inflammation and atherosclerotic foam cell formation [32, 33]. Thus, oxidative modification of n-3 PUFAs in dietary supplement may interfere with their intended biological or clinical benefits [34, 35].
Free fatty acid content is determined by the relative amount (%) of Oleic acid in the supplement. Oleic acid, also known as Omega-9, is a monounsaturated nonessential fatty acid. The recommended value of oleic acid in dietary supplements should not exceed 3%, according to the American Oil Chemists' Society, official method Ca 5a-40.
Peroxide Value (PV), a measure of how much peroxide is present in oil, serves as a measure of primary oxidation, because when polyunsaturated fatty acids oxidize, the first compounds that are created are peroxides (hydroperoxides). While PV initially increases when oil undergoes oxidation, it can later decrease as peroxides are consumed in the subsequent oxidative reactions. Thus, low PV is not necessarily an indication of high-quality oil. A measure of secondary oxidation is an additional parameter required in order to determine the true "freshness" of an oil product.
P-Anisidine Value (pAV) is appropriate for measuring

secondary oxidation in omega-3 oils, according to the American Oil Chemists' Society, official method Cd 18-90. P-Anisidine reacts with the ketones and aldehydes produced by secondary oxidation of omega-3 to form a measurable chemical complex.

Peroxide and p-Anisidine values are defined as the amount of peroxide oxygen per 1 kg of fat or oil and are usually expressed in units of milliequivalents (meq) per kg of oil, according to the American Oil Chemists' Society, official method Cd 8b-90.

Total Oxidation (TOTOX) is an industry-standard indicator of oil oxidation, calculated from the measurements of PV and pAV (TOTOX = 2 x PV + pAV). It is conceived as a way to give a complete view of oxidation by including primary and secondary oxidation measurements.

The maximum recommended levels for these three parameters (Peroxide, para-Anisidine and TOTOX) are determined by the Global Organization for EPA and DHA Omega-3s (GOED) or the International Fish Oil Standards (IFOS).

GOED has advocated for regulatory authorities to codify the limits recommended for PV (<5 meq/kg), pAV (<20 meq/kg) and TOTOX (<26 meq/kg).

IFOS-recommended limits for oxidized omega-3 are somewhat different from those of GOED: PV (<5 meq/kg), pAV (<20 meq/kg) and TOTOX (<19.5 meq/kg). IFOS recommendation for TOTOX is considerably more restricting.

The objective of this study was to test the free fatty acid content (% Oleic acid) and to quantify the levels of the three-standard omega-3 lipid oxidation markers in the 17 most widely available commercial omega-3 dietary supplement products in Israel.

2. Materials and Methods

2.1 Chemical reagents

17 omega-3 dietary supplement products commercially available in Israel, 14 fish-derived and 3 plant or algae-derived, were tested at least 12 months prior to expiration date for the following parameters: free fatty acids content (% Oleic acid), Peroxide value (meq/Kg), p-Anisidine value (meq/Kg) and TOTOX value (calculated). Testing was performed in a specialized certified laboratory (Milouda, Israel).

2.2 Oxidation analysis

For each dietary supplement, 80 gr of pooled extracted oil were analysed for free fatty acids, peroxide and p-Anisidine. Free fatty acids, Peroxide and p-Anisidine tests were performed according to the protocols outlined by the American Oil Chemists Society (AOCS) established procedures [36-38]. TOTOX values were calculated from peroxide and p-Anisidine measures as described. The maximum limit recommended for TOTOX value was <19.5 meq/Kg, according to IFOS.

3. Results

14 fish-derived omega-3 dietary supplements and 3 plant or algae-derived omega-3 dietary supplements were tested for free fatty acids content (% Oleic acid), Peroxide value (meq/Kg) and p-Anisidine value (meq/Kg). TOTOX value was calculated from the Peroxide and Anisidine Values, as mentioned above. Results for fish-derived omega-3 dietary supplement are summarized in Table 1, whereas results for plant or algae-derived omega-3 dietary supplement are summarized in Table 2.

All 14 fish-derived omega-3 dietary supplement contained acceptable levels of Oleic acid. 71% (10/14) of the tested fish-derived omega-3 dietary supplement exceeded the recommended Peroxides Value limit of 5 meq/kg, 36% (5/14) exceeded the recommended p-Anisidine Value limit of 20 meq/kg, and 86% (12/14) exceeded TOTOX value limit of 19.5 meq/kg, as can be seen in Table 1.

All three plant or algae derived omega-3 dietary supplement contained acceptable levels of Oleic acid. 33% (1/3) of the tested non-fish-based omega-3 dietary supplement exceeded the recommended Peroxides Value limit of 5 meq/kg, 66% (2/3) exceeded the recommended p-Anisidine Value limit of 20 meq/kg, and 66% (2/3) exceeded TOTOX value limit of 19.5 meq/kg, as can be seen in Table 2.

As can be seen in Figure 1, in all 17 omega-3 dietary supplement the % Oleic acid are found under the acceptable level of 3% oleic acid.

As can be seen in Figure 2, 14 out of the 17 (83%) omega-3 dietary supplement exceeded the IFOS-recommended TOTOX value limit (bottom graph). 6 out of the 17 (59%) dietary supplement exceeded the IFOS-recommended limit by more than x3 fold.

The results are summarized in Table 3. The tested omega-3 dietary supplement contained an acceptable level of % Oleic acid. Of the fish-derived omega-3 dietary supplement tested, 71% exceeded the recommended limit for Peroxide Value, 36% exceeded the recommended limit for p-Anisidine Value, whereas the calculated TOTOX value exceeded recommendations in most (~86%) of the tested fish-derived omega-3 dietary supplement. Of the plant or algae-derived omega-3 dietary supplement tested, 33% exceeded the recommended limit for Peroxide Value, 66% exceeded the recommended limit for p-Anisidine Value, and 66% exceeded the recommended limit for TOTOX.

To summarize, the majority of all tested omega-3 dietary supplement products exceeded the recommended limits for Peroxide Value (65%) and TOTOX value (~82%).

4. Discussion

We tested 17 various omega-3 dietary supplement products commercially available in Israel, fish-derived as well as plant or algae-derived. Our study shows that most of the widely popular Omega-3 dietary supplement in Israel contained acceptable levels of % Oleic acid. However, significant levels of peroxides and secondary oxidation products that exceeded, some even considerably, the international industry standards were found in most of these dietary supplements. Our data, which support other studies performed in recent years in markets throughout the world (e.g. USA [34], New Zealand [26, 29], North America [36] and South Africa [37]), point to and emphasize the problem of high levels of oxidized lipids present in commonly available omega-3 dietary supplement, that may be harmful and/or interfere with the dietary supplement potential health benefits, such as antioxidant activity [34].

The high levels of oxidation shown in this study are broadly consistent with other surveys, which have shown that about 65% of fish oil products are oxidized above international recommendations. As oil oxidizes, the concentrations of EPA and DHA (the purported active compounds) decrease, suggesting reduced efficacy [25]. The health implications of consuming oxidized fish oil remain unclear, but recent

studies are beginning to shed some light on this issue. Evidence from animal studies show that large doses of oxidized lipids may cause organ toxicity, growth retardation and accelerated atherosclerosis [33].

Consumers are often unable to identify oxidized oil in the commercially available omega-3 dietary supplement, and may be exposed to unacceptable levels of oxidized lipids via consumption of purportedly beneficial dietary supplement, in particular because the "best before" date stated by the manufacturer on the packaging is not an appropriate indication of the level of product oxidation. Dietary supplement product cost is also not an indicator, since some of the more expensive supplements had greater oxidation.

In addition, it is likely that the omega-3 supplements used in many clinical trials have also been significantly oxidized, which may have had an effect on the published results. We feel it is particularly important to test the state on oxidation of omega-3 supplements used in any clinical research, and report these findings along with the other relevant study data.

Ours is the first study which analysed and compared three different sources (fish, plant and algae) of omega-3 supplements in Israel. Our results indicate that despite the possible beneficial effects of omega-3 in many disorders, consumption of commercially available unregulated dietary supplements may be unsafe due to the high levels of potentially harmful oxidized lipids found in many of such products, when tested.

Thus, we believe that recommendations regarding consumption of such over-the-counter products should be reconsidered, quality monitoring regulations should be applied and recommendations for conditions of transportation and storage of omega-3 products must be assessed.

5. Tables and Figures

Table 1: Fish-derived omega-3 oxidation markers

Sample number	Free fatty acids % Oleic acid	Peroxide Value (meq /Kg)	p-Anisidine Value (meq/Kg)	TOTOX Value (meq/Kg)
dietary supplement 1	0.49	47.3	38	132.6
dietary supplement 2	0.17	7.8	12.8	28.4
dietary supplement 3	0.28	15.7	13.3	44.7
dietary supplement 4	0.21	13.1	25	51.2
dietary supplement 5	0.11	7.7	16.1	31.5
dietary supplement 6	0.38 (Average)	4 (Average)	12.9 (Average)	20.9 (Average)
dietary supplement 7	0.09	22.5	13.8	58.8
dietary supplement 8	0.37	10.3	13.8	34.4
dietary supplement 9	0.11	48.4	47.3	144.1
dietary supplement 10	0.08	11.3	8.4	31
dietary supplement 11	<0.5	4.8	20.7	30.3
dietary supplement 12	0.55	26.4	37.6	90.4
dietary supplement 13	0.4	3.9	10.8	18.6
dietary supplement 14	0.14	3	13.1	19.1

Table 2: Plant or Algae-derived Omega-3 oxidation markers.

Sample number	Free fatty acids % Oleic acid	Peroxide Value (meq/Kg)	Anisidine Value (meq/Kg)	TOTOX Value (meq/Kg)
dietary supplement 15	2.19	3.2	3.4	9.8
dietary supplement 16	0.33	2.8	55.8	61.4
dietary supplement 17	0.25	96.30	40	232.6

Table 3: Percentage of dietary supplement products that exceeded IFOS-recommended oxidation value limits.

Parameter	Fish Oil-derived Omega 3 (n=14)	Plant or algae-derived Omega 3 (n=3)	Total (n=17)
FFA% Oleic acid	0% (0/14)	0% (0/3)	0% (0/17)
Peroxide Value (PV)	71.4% (10/14)	33% (1/3)	65% (11/17)
Anisidine Value (AV)	35.7% (5/14)	66% (2/3)	41% (7/17)
TOTOX Value	85.7% (12/14)	66% (2/3)	82% (14/17)

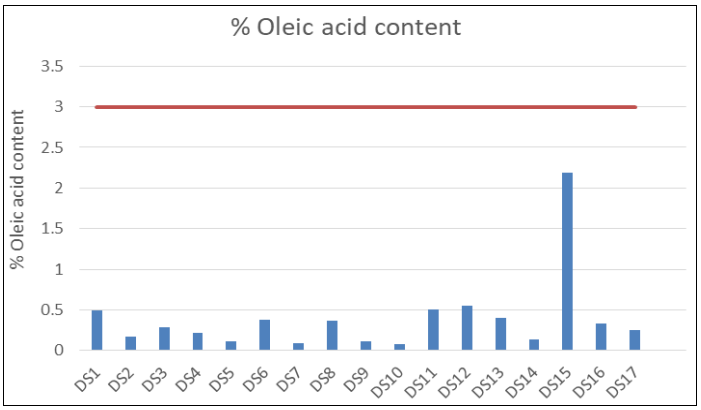

Fig 1: % Oleic acid in 17 omega-3 dietary supplement tested. Actual measured oleic acid content (%) in each supplement (blue bars) is compared to the limit content of 3% (red line).

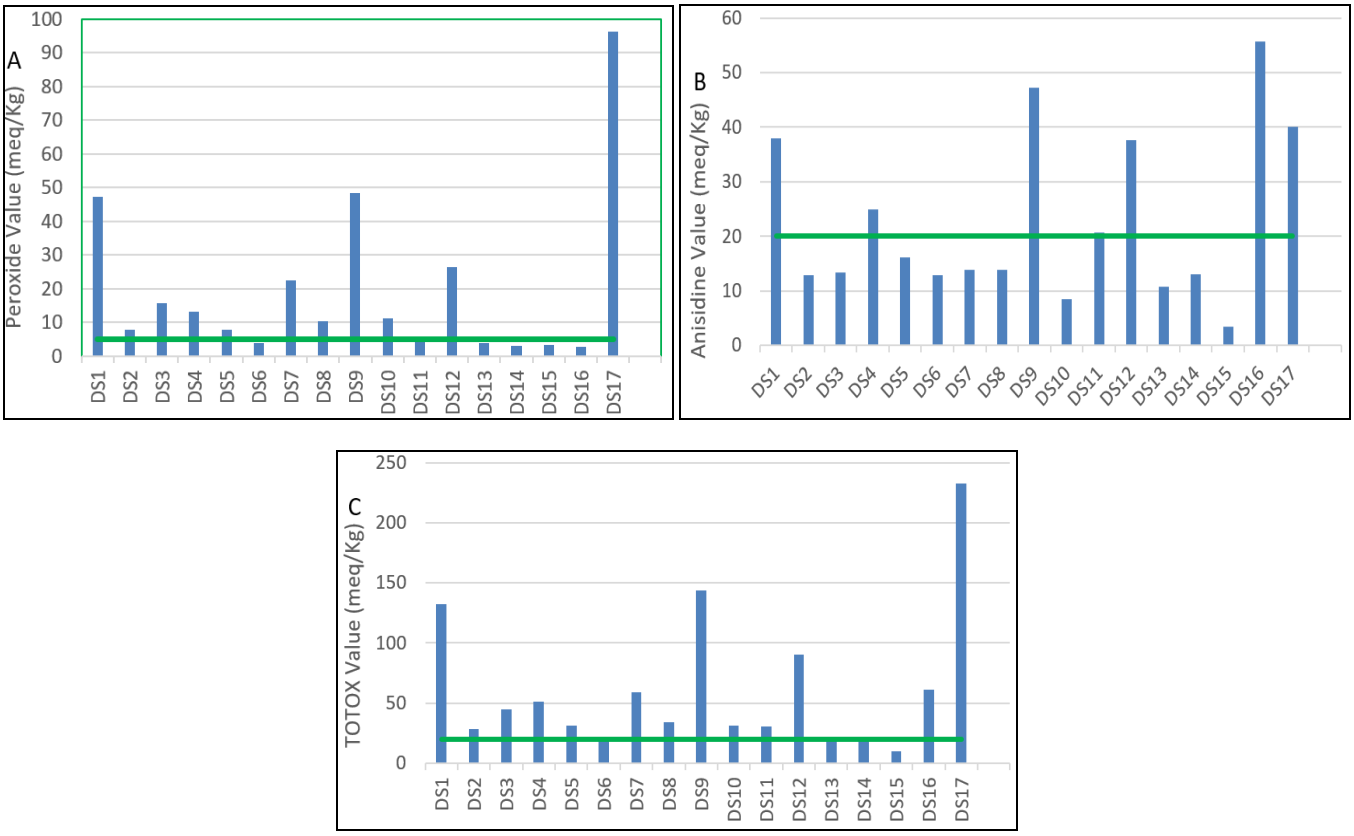

Fig 2: Oxidation markers levels in the tested omega-3 dietary supplement as compared to IFOS-recommended international limits (green line). A. Peroxide Value (meq/Kg oil, limit <5 meq/Kg). B. p-Anisidine Value (meq/Kg oil, limit <20 meq/Kg). C. TOTOX calculated value (meq/Kg oil, limit <19.5 meq/kg).

6. Conclusion

Our study shows that the majority of omega-3 dietary supplement available for purchase in Israel have acceptable oleic acid content; however, they are significantly oxidized, with PV, pAV and TOTOX values exceeding recommended limits. We propose that oleic acid content alone in not a sufficient and accurate indication of quality, and that all three oxidation markers should be utilized in quality testing and regulation of these products. The health implications of oxidized lipids consumption are investigated but still unclear. Further testing is required to examine and better understand the effects of storage and handling conditions on oxidation of encapsulated fish oils and other omega-3 supplements. Formulation studies and additives that prevent primary and secondary oxidation reactions and optimize omega-3 long-term stability and quality should be considered.

Future safety and efficacy trials, particularly in humans, should assess the oxidative state of omega-3 dietary supplement as standard practice. This can be easily done by measuring and calculating the peroxide, p-Anisidine, and TOTOX values for each dietary supplement.

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