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Comparison Study on the Relative Rates in Heat-Driven Oxidation of PUFAs

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Abstract:

Fish oil and fish meal are rich in polyunsaturated fatty acids (PUFAs) which are readily subjected to oxidative deterioration under conditions of increasing temperature and prolonged time. The purpose of the present study was to evaluate the oxidative degradation of PUFAs in fish oil and fishmeal under condition of continuous oven heating at 105 ± 2°C. In this experiment, sampling time for fish oil was set to 0 h, 12 h, 24 h, 36 h, 48 h, 72 h, 84 h, and that for fishmeal was set to 0 h, 12 h, 24 h, 36 h, 48 h, 60 h, 72 h, 84 h, 96 h, and 108 h. The oxidative degradation rates of PUFAs in fish oil and fish meal were evaluated by monitoring the variations of fatty acid compositions with gas chromatography (GC) spectrometry. Data analysis showed that PUFAs (including DHA and EPA) tend to oxidatively decompose at a varying rate in fish oil––under accelerated oxidation conditions, PUFAs in fish oil were oxidated violently within 36 h, and then, their oxidative rates were lowered down gradually to be approximately constant. However, for fishmeal, the oxidative rates of PUFAs were basically linearly proportional to the test time within a period of 108 h. We further deduced that non-lipid compounds (*e.g.* proteins, nucleic acids and carbohydrates) in fishmeal, acting as protective factors, prevent the oxidation of PUFAs through the mechanism of solid absorbing thermal energy.

Keywords*: Fatty acid; Fish meal; Fish oil; Heating; Oxidation; PUFAs*

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I. INTRODUCTION

Lipids serve a number of essential roles in biological cells, and are vital to human nutrition and physiological activity [1]. Fatty acids, the substantial constituent parts in lipids, which include the unsaturated fatty acids (UFAs) especially polyunsaturated fatty acids (PUFAs) are easy to degrade, which causes oxidative rancidity of lipids. Lipid oxidation is a complex process which involves oxidative cleavage of lipid molecules which is accompanied with nutritional loss, flavour deterioration, colour darkening, *etc*. [2]. Generally, this process can produce a series of negative consequences. For example, destructive changes in nutritional value such as damage of essential fatty acids and fat-soluble vitamins, as well as deleterious influences on sensory aspects such as the browned colour and the undesirable off-flavours present in spoiled oils [3]. For many years, oxidized lipids have attracted much interest due to their biochemical and physiological effects, which could be implicated in various human diseases [4, 5]. As it is well known, many considerable physicochemical factors such as heat, light, metal ions, oxygen exposure and enzymes can result in the deterioration of UFAs [6]. Among all types of oil sources, the properties of UFAs, especially the chemical instabilities of PUFAs, have been thought to be a major issue affecting the edible oil quality [7].

Fish oil, as one type of by-product in fishmeal production, is an important industrial material for food, pharmacy, cosmetics, paint products, *etc*. However, fish oil is generally easily prone to oxidative spoilage as a result of its high content of PUFAs (Eicosapentaenoic acid (EPA), Docosapentaenoic acid (DPA) and Docosahexaenoic acid (DHA) included) [8]. Up to now, many sorts of oil sources including fish oil have been studied to assess the extent of oxidative deterioration of lipids [9-11]. Yang and Chiang (2017) applied "the Rancimat test" to investigate the oxidation properties of n-3 PUFAs-rich oil [12]. However, few studies on thermal degradation of PUFAs in fish oil (and/or fishmeal) at a temperature of 105°C have been reported. It should be noted that the temperature parameter is usually considered to be a key factor in such research [13, 14]. Specifically, an increased temperature (*e.g.*, 90°C or 180°C) is the indispensable step for the refining of fish oil [15]. Moreover, foods containing fish oil (*e.g.*, fish oil-fortified extruded products) are often processed between temperatures of 190-215°C [16] which are lower than the temperature during baking, frying and roasting treatment. Consequently, the implications of this study are significantly profound given that it can provide reference data for basic sciences and actual applications. For example, we can compare different kinds of methods for extracting lipid by measuring the changes of fatty acids under heating condition to find their pros and cons.

II. MATERIALS AND METHODS

Mixed standards of 37 fatty acid methyl esters and first chromatogram class of methanol were purchased from Sigma Aldrich (St. Louis, MO, USA). Analytical grade of toluene ("Fuchen", Tianjin,

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China), chromatographic grade of petroleum ether ("Guangzhou", Guangzhou, China), potassium hydroxide ("Guangzhou", Guangzhou, China) were purchased from local marketing agency. Commercially available standard fish oil and steam dried fish meal were produced in Peru (Austral Group, Lima, Peru). Prior to the tests, the samples of fish oil and dried fish meal were both stored in hermetic bag at -20°C.

Equipment and instruments: Varian 431-GC (California, USA); Column: HP-88 (60 m×0.25 mm×0.2 μm), Agilent Technologies Inc.; Electric oven: "Jinghong DHG-9303-1SA" (Shanghai, China); Refrigerated centrifuge (Thermo, Stratos, Germany).

2.1 Thermal Oxidation Tests

The fish oil and fishmeal (the recommended size of fishmeal particle is 60~80 mesh) were uniformly spread on Petri dish with an internal diameter of 6 cm in order to obtain a thin layer of 5~10 mm, and they were exposed to electric oven condition of a constant temperature of 105 ± 2°C. Methyl esterifications of lipids were implemented after heating fish oil specimens for 12 h, 24 h, 36 h, 48 h, 72 h and 84 h, respectively. Similarly, the lipids extracted from fishmeal samples that were heated for 12 h, 24 h, 36 h, 48 h, 60 h, 72 h, 84 h, 96 h, and 108 h, respectively, were methyl-esterified.

2.2 Lipid Extraction and Methyl Esterification

Total lipids in fishmeal were extracted from homogenized samples using chloroform/methanol (2:1, v/v) as previously described [17] with slight modifications. The lipid extracts were dried and concentrated under a stream of nitrogen prior to methyl esterification of fatty acids.

In addition, 0.05 g of lipid samples were placed in a 15 mL tube with screw cap, the tube contained 2 mL mixtures of petroleum ether and toluene (petroleum ether/ toluene=1 / 1). The tube was agitated using a vortex generator in order to fully dissolve the lipids. Then 2 mL potassium hydroxide methanol solutions (0.4 mol/L) were added into the liquid solutions to achieve a state of uniform mixing before standing at room temperature for 30 min. The tube was centrifuged for 10 min at a speed of 2700 g/min; thereafter, 7 mL of double distilled water was added into the tube. A supernatant of approximate 2 mL was collected with a new dry test-tube and a small amount of anhydrous sodium sulfate was added to remove any trace of water. Dewatered supernatant fractions (1-2 mL) were injected into a sampling vial (2 mL) by utilising a disposable syringe with a microporous membrane (0.22 μm), and was made available for gas chromatographic analysis.

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2.3 Gas-Chromatography Analysis of FAMEs

Routine analyses of fatty acid methyl esters (FAMEs) were performed by Varian 431-GC. The GC operating parameters were as follows: Varian 431-GC (California, USA) equiped with capillary column (HP-88, SUPELCO, 60 m×0.25 mm I.D., 0.2 μm film thickness). The oven temperature, maintained at an initial value of 140°C for 5 min, was increased at a rate of 5°C per minute to 240°C, and then was maintained at that temperature for 20 min. The injector and flame ionization detector were set at 260°C. Nitrogen at a pressure of 550 kPa was used as the carrier gas. A split injector (30:1) at 260 °C was used. The temperature of the detector was 260°C.

2.4 Data Analysis and Processing

Peaks of fatty acids were identified by the comparison of retention times with known FAME standards mixture containing 37 Component FAME Mix (Sigma). In relation to the external standard, FAMEs were quantified and presented as "Peak areas". Peak areas were determined using Varian software. Each sample was analysed in triplicates. The results obtained were presented as the "mean ± standard deviation" (X ± SD). Single factor analysis of variance (ANOVA) and regression analyses were performed to analyse the significant differences between data using IBM SPSS Statistics 19.0 (SPSS, IL, USA). Statistically significant differences were determined by Duncan's multiple range tests at the $P < 0.05$ level.

III. RESULTS AND ANALYSIS

This study offered an indirect method for measuring the degree of lipids oxidation, given the significant compositional differences of oxidation process of UFAs and PUFAs [18]. Here in our trials, the fatty acid profiles of fish oil and fishmeal during the heated period are presented in Table I and Table II, respectively.

As shown in Fig 1, the degradation of fish oil proceeded rapidly within 36 h. The oxidation curves of DHA and EPA during this period could be fitted with straight line, and the slope values (represented with the letter 'K') are given as follows: $|K_1| = 3.54$ (for DHA) and $|K_2| = 2.15$ (for EPA) (Table III). Similarly, throughout the whole heating period of 108 h for fishmeal trial (Fig 2), we acquired two slope's numerical values $|K_3| = 1.35$ (for DHA) and $|K_4| = 0.43$ (for EPA) (Table III), respectively. It is easy to find that $|K_1| > |K_2| \gg |K_3| > |K_4|$, which indicated that the oxidative rates of DHA and EPA in fish oil decrease more rapidly than those in fishmeal. By comparison of the thermo stabilities of DHA and EPA, we concluded that EPA is more resistant to thermo-deterioration (at oven temperature of 105 ± 2°C) than DHA. From the point of view of physico-chemical structure, the spatial configuration of DHA

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is distinct from that of EPA due to the length of its carbon backbone and extent of unsaturation. DHA possesses the architectural feature of 22 carbon atoms and 6 double bonds. By contrast, EPA loses two carbon atoms and only has five double bonds. Thus, DHA molecule, with more double bond and longer carbon chains, is structurally more stable than EPA. In fish oil, DHA and EPA degraded more slowly between 36 h and 84 h in comparison with the testing time of 36 h, which is probably due to the presence of oxidized PUFAs which prevented them from being oxidized.

In fish oil (Fig 1), the oxidative damage curves of PUFAs were similar to ω-3 PUFAs during the whole period of the test. Within 36 h, the oxidation rates of PUFAs and ω-3 PUFAs were both applied to straight line fitting, and two slope's numeric values were obtained: $|K_5|$ = 6.43 (for PUFA) and $|K_6|$ = 6.00 (for ω-3 PUFA) (Table III). The same analysis approach for fishmeal was used within the period of testing (Fig 2), and two slope values were as follows: $|K_7|$ = 1.81 (for PUFA) and $|K_8|$ = 1.69 (for ω-3 PUFA) (Table III). Obviously, in both fish oil and fishmeal, the oxidative decomposition curve of PUFAs had a similar trend as ω-3 PUFAs.

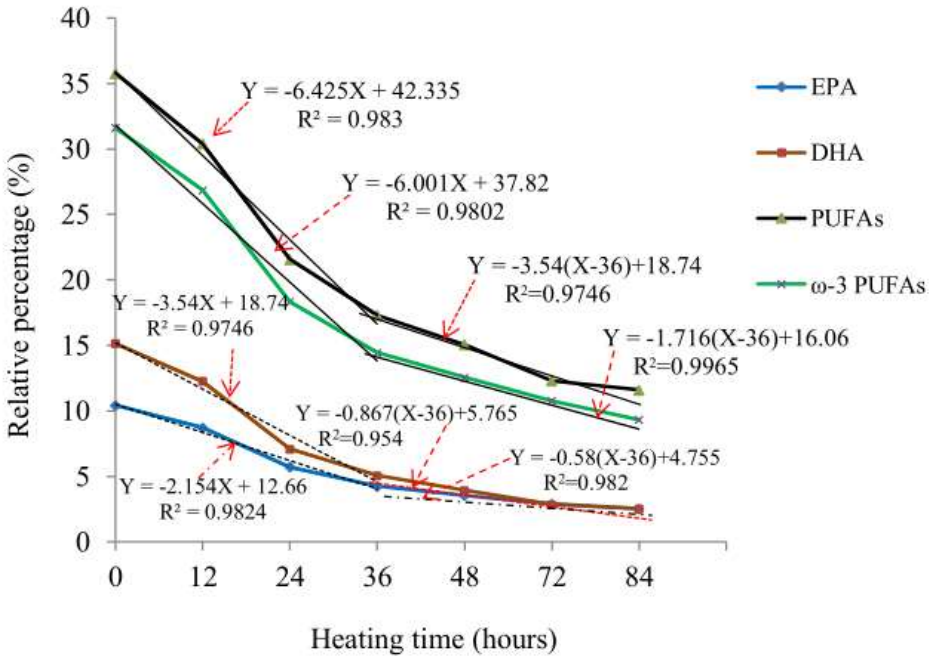

Fig 1: Oxidative degradation curves of DHA, EPA, PUFAs and ω-3 PUFAs in fish oil

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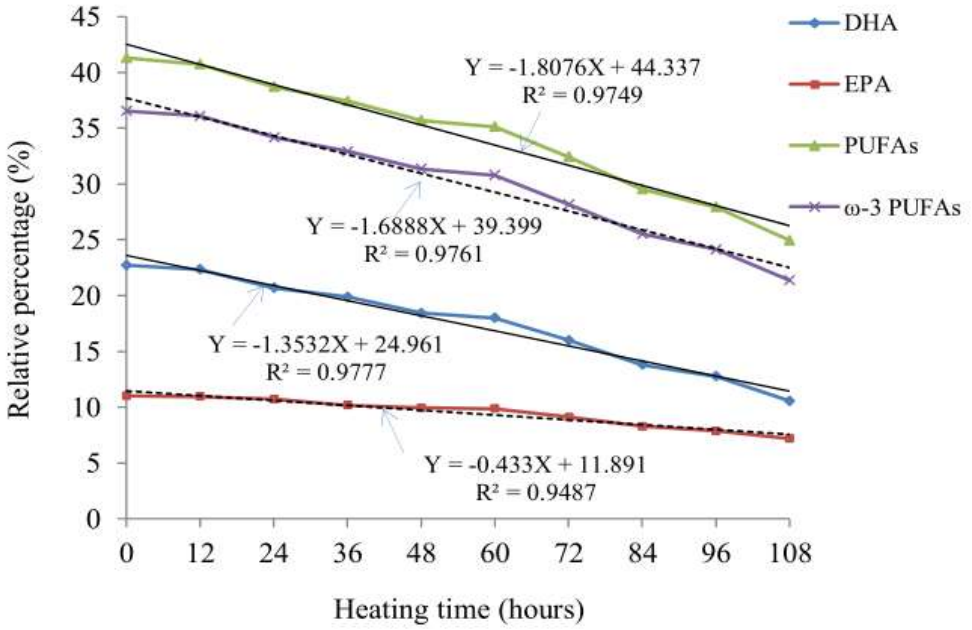

Fig 2: Oxidative degradation curve of DHA, EPA, PUFAs and ω-3 PUFAs in fishmeal

Table I. Changes in fatty acid composition of fish oil with different heat treatments

Fatty acids (%)	0h	12h	24h	36h	48h	72h	84h
14:0	7.00±0.03	7.92±0.01	9.27±0.01	9.61±0.01	10.20±0.01	10.24±0.11	10.86±0.04
15:0	1.05±0.01	1.12±0.01	1.34±0.01	1.40±0.01	1.50±0.02	1.53±0.04	1.58±0.01
16:0	19.86±0.24	22.22±0.01	25.99±0.01	27.87±0.02	28.48±0.03	29.24±0.04	30.48±0.03
17:0	1.00±0.13	1.15±0.03	1.00±0.01	1.00±0.11	1.11±0.01	1.26±0.04	1.47±0.04
18:0	3.24±0.50	3.54±0.04	4.16±0.05	4.27±0.02	4.48±0.02	4.67±0.00	4.85±0.50
20:0	0.57±0.06	0.63±0.03	0.75±0.03	0.78±0.01	0.82±0.01	0.86±0.01	0.86±0.01
∑Saturated	32.72±0.97	36.58±0.13	42.51±0.12	44.93±0.18	46.59±0.10	47.80±0.24	50.10±.63
15:1	0.96±0.04	0.99±0.02	1.07±0.02	1.06±0.01	1.04±0.01	1.06±0.04	1.05±0.02
16:1	6.43±0.21	7.23±0.02	8.22±0.01	8.41±0.01	8.74±0.01	8.84±0.02	8.92±0.03
18:1n-9	11.56±0.15	12.43±0.03	13.93±0.03	14.24±0.01	14.67±0.00	15.08±0.01	15.32±0.01
20:1	2.17±0.11	2.15±0.05	1.97±0.00	1.77±0.01	1.67±0.02	1.55±0.05	0.89±0.01
22:1	4.66±0.12	4.63±0.03	5.23±0.01	5.26±0.01	5.51±0.01	5.75±0.05	5.73±0.04

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24:1	0.43±0.06	0.32±0.01	0.53±0.03	0.52±0.01	0.53±0.01	0.53±0.01	0.54±0.04
18:1n-7	3.38±0.46	3.43±0.04	3.81±0.01	3.85±0.01	3.98±0.02	4.08±0.01	4.14±0.04
∑MUFA	29.59±1.15	31.18±0.20	34.76±0.11	35.11±0.07	36.14±0.08	36.89±0.19	36.59±0.19
18:2n-6	1.96±0.11	1.83±0.01	1.82±0.04	1.74±0.06	1.63±0.01	0.79±1.11	1.56±0.04
18:3n-3	2.80±0.13	3.03±0.03	3.42±0.01	3.47±0.01	3.62±0.01	3.75±0.04	3.15±0.04
20:3n-3	3.24±0.01	2.86±0.03	2.13±0.03	1.66±0.01	1.46±0.01	1.25±0.01	1.11±0.04
20:4n-6	1.23±0.02	0.86±0.04	0.67±0.01	0.51±0.02	0.45±0.01	0.42±0.01	0.42±0.01
20:5n-3	10.43±0.14	8.71±0.02	5.71±0.01	4.25±0.04	3.54±0.02	2.90±0.01	2.53±0.04
22:5n-6	0.99±0.03	0.86±0.04	0.72±0.01	0.63±0.02	0.41±0.02	0.34±0.01	0.31±0.02
22:6n-3	15.14±0.17	12.26±0.01	7.10±0.01	5.06±0.08	3.94±0.01	2.86±0.04	2.53±0.01
∑PUFA	35.79±0.50	30.41±0.17	21.57±0.08	17.32±0.18	15.05±0.08	12.31±1.23	11.61±0.16
∑n-3PUFA	31.61±0.34	26.86±0.08	18.36±0.02	14.44±0.08	12.56±0.04	10.76±0.10	9.32±0.09
∑n-6PUFA	4.18±0.16	3.55±0.09	3.21±0.06	2.88±0.10	2.49±0.04	1.55±1.13	2.29±0.07

Table II. Alternations in fatty acid profile of fishmeal under oven-heated treatments

Fatty acids (%)	**0h**	**12h**	**24h**	**36h**	**48h**	**60h**	**72h**	**84h**	**96h**	**108h**
14:0	7.54± 0.01	7.57± 0.01	7.59± 0.01	7.71± 0.07	8.06± 0.01	7.78± 0.28	8.63± 0.00	8.87± 0.00	9.39± 0.00	9.66± 0.01
15:0	0.67± 0.01	0.70± 0.02	0.67± 0.01	0.68± 0.00	0.68± 0.02	0.71± 0.01	0.77± 0.01	0.78± 0.00	0.83± 0.00	0.86± 0.04
16:0	19.75 ±0.00	20.19 ±0.00	20.22 ±0.02	20.72 ±0.00	20.07 ±0.00	22.22 ±0.00	22.85 ±0.00	23.10 ±0.00	24.69 ±0.00	25.66 ±0.71
17:0	1.13± 0.00	1.19± 0.00	1.17± 0.00	1.16± 0.04	1.22± 0.00	1.27± 0.03	1.30± 0.02	1.48± 0.03	1.60± 0.01	1.81± 0.07
18:0	4.10± 0.00	4.23± 0.00	4.21± 0.00	4.31± 0.00	4.55± 0.18	4.55± 0.18	4.84± 0.00	4.97± 0.35	5.29± 0.00	5.51± 0.14
∑Saturated	33.19 ±0.02	33.88 ±0.03	33.86 ±0.04	34.58 ±0.11	34.58 ±0.21	36.53 ±0.50	38.39 ±0.03	39.2± 0.38	41.80 ±0.01	43.50 ±0.97
15:1	0.72± 0.00	0.73± 0.00	0.71± 0.01	0.75± 0.00	0.69± 0.00	0.67± 0.00	0.72± 0.00	0.71± 0.00	0.68± 0.00	0.65± 0.00
16:1	6.50± 0.01	6.50± 0.01	6.51± 0.04	6.65± 0.00	6.97± 0.14	6.76± 0.14	7.37± 0.07	7.55± 0.00	7.89± 0.01	7.94± 0.01
18:1n-	0.41±	0.40±	0.37±	0.39±	0.37±	0.35±	0.36±	0.36±	ND	ND

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9t	0.01	0.04	0.03	0.03	0.02	0.06	0.01	0.05		
18:1n-9	6.07± 0.00	6.17± 0.00	6.14± 0.00	6.24± 0.00	6.51± 0.21	6.51± 0.21	6.79± 0.09	7.06± 0.07	7.38± 0.00	7.58± 0.14
20:1	1.24± 0.07	2.06± 0.00	1.58± 0.59	1.28± 0.14	1.22± 0.01	1.31± 0.21	1.34± 0.06	1.33± 0.13	1.35± 0.16	1.42± 0.04
22:1	3.93± 0.00	3.86± 0.07	4.00± 0.01	4.11± 0.00	4.25± 0.00	4.24± 0.07	4.47± 0.04	4.77± 0.00	4.79± 0.00	4.91± 0.00
24:1	0.85± 0.01	0.89± 0.00	0.92± 0.00	0.98± 0.00	1.10± 0.00	1.09± 0.07	1.18± 0.00	1.23± 0.01	1.35± 0.00	1.36± 0.01
18:1n-7	2.41± 0.00	2.48± 0.00	2.44± 0.00	2.48± 0.00	2.60± 0.07	2.57± 0.05	2.72± 0.02	2.75± 0.00	2.92± 0.00	2.97± 0.00
∑MUFA	22.13 ±0.10	23.09 ±0.12	22.67 ±0.68	22.88 ±0.17	23.71 ±0.45	23.50 ±0.81	24.95 ±0.29	25.76 ±0.26	26.39 ±0.18	26.85 ±0.21
18:2n-6	1.49± 0.00	1.41± 0.00	1.38± 0.00	1.39± 0.00	1.44± 0.00	1.40± 0.00	1.45± 0.02	1.45± 0.04	1.46± 0.04	1.48± 0.03
18:3n-3	2.54± 0.00	2.54± 0.01	2.54± 0.00	2.61± 0.07	2.77± 0.06	2.70± 0.09	2.81± 0.05	3.16± 0.07	3.26± 0.14	3.39± 0.14
20:3n-3	0.26± 0.01	0.26± 0.01	0.25± 0.04	0.27± 0.01	0.23± 0.01	0.22± 0.01	0.26± 0.01	0.27± 0.02	0.24± 0.04	0.24± 0.01
20:4n-6	1.27± 0.00	1.25± 0.35	1.25± 0.00	1.23± 0.00	1.11± 0.00	1.18± 0.07	1.14± 0.04	1.10± 0.00	0.96± 0.00	0.83± 0.07
20:5n-3	11.01 ±0.00	10.95 ±0.06	10.71 ±0.14	10.18 ±0.00	9.94± 0.00	9.87± 0.05	9.10± 0.00	8.29± 0.00	7.87± 0.00	7.18± 0.00
22:5n-6	2.01± 0.04	1.98± 0.03	1.90± 0.04	1.89± 0.02	1.78± 0.01	1.77± 0.06	1.70± 0.02	1.48± 0.01	1.40± 0.03	1.27± 0.02
22:6n-3	22.73 ±0.00	22.35 ±0.72	20.69 ±0.00	19.88 ±0.00	18.43 ±0.35	18.00 ±0.71	15.99 ±0.00	13.80 ±0.00	12.75 ±0.00	10.56 ±0.00
∑PUFA	41.31 ±0.05	40.74 ±1.18	38.72 ±0.22	37.45 ±0.10	35.70 ±0.43	35.14 ±0.99	32.45 ±0.14	29.55 ±0.14	27.94 ±0.25	24.95 ±0.27
∑n-3PUFA	36.54 ±0.01	36.1± 0.79	34.19 ±0.18	32.94 ±0.08	31.37 ±0.42	30.79 ±0.86	28.16 ±0.06	25.52 ±0.09	24.12 ±0.18	21.37 ±0.15
∑n-6PUFA	4.77± 0.04	4.64± 0.39	4.53± 0.04	4.51± 0.02	4.33± 0.01	4.35± 0.13	4.29± 0.08	4.03± 0.05	3.82± 0.07	3.58± 0.12

Note: ND, not detected.

Table III. Regression coefficients and coefficients of determination (R2) of the linear regression of the fitting curves in heated fish oil and fishmeal

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	Rate of DHA	**Rate of EPA**	**Rate of PUFA**	**Rate of ω-3 PUFA**
Fish oil (Within 36 hours)	Y = –3.54X + 18.74 R2 = 0.9746	Y = –2.154X + 12.66 R2 = 0.9824	Y = –6.425X + 42.335 R2 = 0.983	Y = –6.001X + 37.82 R2 = 0.9802
Fish oil (After 36 hours)	Y = –0.867(X – 36) + 5.765 R2 = 0.954	Y = –0.58(X – 36) + 4.755 R2 = 0.982	Y = –3.54(X – 36) + 18.74 R2 = 0.9746	Y = –1.716(X – 36) + 16.06 R2 = 0.9965
Fishmeal	Y = –1.3532X + 24.961 R2 = 0.9777	Y = –0.433X + 11.891 R2 = 0.9487	Y = –1.8076X + 44.337 R2 = 0.9749	Y = –1.6888X + 39.399 R2 = 0.9761

IV. DISCUSSION

The health benefits of PUFAs to humans have been demonstrated by several studies both *in vivo* and *in vitro* [18-21]. For example, DHA plays an important role in the maintenance of normal neural functions [22]. EPA has many vital physiological properties, such as affecting immune system, regulating blood flow, and supporting immune response, *etc.* [21]. However, in foods containing PUFAs, PUFAs act as a two-edged sword, they are capable of producing potential adverse effects on human health by generating oxidative compounds including alcohols, aldehydes, ketones, hydrocarbons, esters, furans, lactones epoxides, hydroxy chemicals, hexanal, pyrroles and malondialdehyde [23, 24].

According to relevant research literatures, the rate of oxidation of fish oil is significantly different from other fats and oils [25]. In addition, some studies associated with anti-oxidation of fish oil have been reported [24].

In the fishmeal trial, the oxidation curve of DHA or EPA can be approximately linearly fitted as a result of the heat that is evenly dispersed by other structural components of fish meal. However, no articles have been published to report such phenomenon.

V. CONCLUSION

This study focused on the fundamental properties of heat-induced degradation of PUFAs in fish oil and fishmeal. Essentially, it is to study the thermodynamic changes of fish oil fatty acids in two different forms. In the present study, the thermo-oxidation curves of PUFAs in fishmeal, including DHA and EPA, highly approached linearity under such heating condition. In comparison with

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fishmeal, the contents of PUFAs (including DHA and EPA) in fish oil decreased rapidly within 36 h, and then the oxidation rate slowed down, which showed that fish oil is labile to oxidative decay at a varying rate under a constant temperature as previously reported. For fishmeal, non-fat substances (*e.g.*, proteins, carbohydrates and nucleic acids) protected PUFAs from excessive destruction by partially absorbing thermal energy, and maintained the oxidation rate to be roughly constant during this experiment. Our study extended the understanding of dynamic shifts of fish oil fatty acids under oven-heated condition. Moreover, it can really provide reliable data references for fish oil production and processing, the design and production of fish oil pills, as well as processing and preservation of marine products. Therefore, much more works should be carried out to avoid oxidation and promote the processing and preservation of fish oil in the future.

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