Hybrid Extraction Method; Innovative Method to Produce Non Oxidative Tuna Oil

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Summary:
The function of Polyunsaturated Fatty Acid such as DHA, EPA are now well known world widely through strong scientific evidences and the importance of nutritional value and popularity are increasing every day all over the world with the name of omega 3 fatty acids.[1),2),3)] However, the fish oil, the source of omega 3 fatty acid had been considered as very vulnerable to oxidation. Many papers say the reason why is because its very easy to oxidize fish oil. The greater the Polyunsaturated Fatty Acid contents in the fish oil is, the faster oxidation takes place.[4),5)]

Here I show that tuna oil extracted from Albacore Tuna Head in closed circuit under low temperature, at first decompressing then pressurizing and heating in a container, is very stable against the oxidation and conserved natural contents of Vitamins richly.

The oil ware kept in a transparent bottle under natural light and some time under the sun light, with room temperature and opened every day for the sensual odor test during three months.

This oil showed a stability of Fatty Acid Composition, Acid Value and Peroxide Value.

That mean omega 3 fatty acids are more easy to handle and ample use as food ingredient to put it in many foods such

as in a ketchup, mayonnaise, bread and noodle, rather than use in the capsules only.

Key words: Hybrid Extraction, Non oxidative tuna oil, POV, AV

Abbreviation: DHA = Docosahexanoic Acid, EPA = Eicosapentaenoic Acid, POV = Peroxide Value, AV = Acid Value, PUFA = polyunsaturated Fatty Acid

Material and Methods

16kgs of frozen albacore tuna head were obtained at Yashima Shoji Co., Ltd. Shishihara plant after trimming whole fish to sashimi ready parts and put them into the double bottom steam vacuum and pressuring cooking container with same amount of water.

Set the automatic temperature control at 55 ° C and start vacuum pump maintaining at -0.088MPa during 1 hour.

This process for boiling water in low temperature is very important to melt solid fat of head easily and not spoil the quality of oil extracted. After 1 hour of extraction under low temperature and pressure, lift the temperature to 103 ° C and pressure 0.02MPa. Holding closed the container to avoid contact to the outside air. It took 16 minutes to reach 103 ° C from 55 ° C. Then 1 hour maintained at 103 ° C.

Under pressurized condition the oil is extracted without boiling water but the pressure come from evaporated steam gradually with volatile ingredients in the liquid. After 1hour, stop steaming and open a valve of container to reduce the pressure causing sudden intense boiling which exhaust vapor with volatile ingredients in the oil and liquids. Separate oil from liquid then filtering obtains pure, transparent and less odor oil. This oil was very stable to oxidation when stored under prohibited condition, before this invention,

at room temperature, contacting with air, in a transparent bottle in a well lighted room.

Results and Discussion

The tuna oil extracted by Hybrid Extraction Method, because of two different stage of pressure and temperature in a shield container the author named it Hybrid Extraction Method, were sent a two different laboratories, one for Dr. T. Hamazaki, Professor of Toyama University, Japan and Dr. K. Fukunaga Associated Professor of Kansai University, Japan and analyzed as below mentioned ways. And both case showed very high stability against oxidation. It was a wholly contradiction of character of PUFA rich fish oil.

Seeking the reason of stability at first discovered this Hybrid extract tuna oil had contained high level of Vitamin E..(Table6). Then estimating existence of the other lipid soluble vitamins, I found Vitamin A and D(Table6). However Vitamin A was not found always.

Antioxidant effect of Vitamin E (alpha-tocopherol) for PUFA:

Harris and Embree recommend an intake of 0.6mg alpha tocopherol equivalents per gram linoleic acid as generally adequate for human adults. [6]

Saito estimates the minimal amount of vitamin E(mg of alpha-tocopherol equivalent) is calculated from a equation,2x {[0.3x5xEPA(g)+[0.3x6xDHA(g)]} , and 1.5 times over this estimate should be secured to sustain nutritional status of vitamin E normal.[7]

Grune, Krämer, Hoppe and Siems say that to prevent the increase of cytotoxic aldehydic lipid peroxidation and storage of n-3 PUFA-enriched eggs, a high vitamin E supplementation with at least 80IU vitamin E/kg is needed.[8]

0.6mgVE/g PUFA is equivalent to 24mg/100g of hybrid oil when this contains about 40% of PUFA. So, as Table 6 shows the tuna extracted oil has enough Vitamin E content to preserve peroxidation. According to NIH information, the synthetic form is only half as active as the natural form.[9]

I'm not sure these experiments to calculate the necessity amount of vitamin E for PUFA were used for the synthetic form but for the dietic supplement synthetic vitamin E is used commonly. However recently I achieved to process the non fish smell and to get such purified level also decreased vitamin E and D contents.[10]

I'm not sure yet if these analysis indicate another unknown antioxidant in the oil or just if purified oil (99.9% of oil contents) delays to cause peroxidation, so far more than 100days stored in a glass transparent opened flask at room temperature in a well lighted and luminous room, the POV were changed from 2meq／kg to 31.8meq/kg. Of course the odor didn't changed and still maintained transparent color.

Analysis of fatty acid (The oil were stored three months at room temperature within a transparent plastic bottle opening at least 5 times per week, in a well lighted room)

The fatty acids composition were analyzed by Gas Chromatography and POV were by Iodometric titration method.

1)Analysis made at the laboratory of Dr. T Hamazaki, Proh. Toyama University, Japan

GC Analytical conditions Model: GC Shimadzu GC-2014, Column: Agilent Technology Capillary Column DB-225(30mx0.25mm), Inj.Temp.:250°C, Carrier Gas: He, Column Temp.: 170°C(5min)--220°C at 4°c/min

< **FA Composition (%)** >

	Sample No.	1	1	2	2	3	3	4	4
	stored at room temperature	day 0	day 0	day 3	day 3	day 10	day 10	day 20	day 20
	Data No.	EO752901	EO752902	EO752903	EO752904	EO760101	EO760102	EO761901	EO761902
1	12 : 0	0.00	0.00	0.03	0.03	0.00	0.00	0.00	0.00
2	14 : 0	4.35	4.43	4.42	4.42	4.51	4.50	4.53	4.37
3	14 : 1 n-5	0.00	0.00	0.00	0.00	0.11	0.11	0.00	0.00
4	16 : 0	18.49	18.38	18.45	18.43	18.48	18.51	18.92	18.70
5	16 : 1 n-7	6.76	6.86	6.80	6.79	6.88	6.90	6.92	6.76
6	18 : 0	3.54	3.44	3.45	3.44	3.42	3.46	3.53	3.57
7	18 : 1 n-9	21.96	21.82	21.90	21.85	22.00	22.03	22.36	22.38
8	18 : 1 n-7	3.17	3.15	3.21	3.19	3.23	3.20	3.29	3.27

9	18 : 2 n-6	1.58	1.57	1.58	1.59	1.67	1.61	1.64	1.65
10	18 : 3 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.19
11	18 : 3 n-3	1.09	1.09	1.09	1.08	1.03	1.10	1.18	1.13
12	20 : 0	0.24	0.23	0.23	0.23	0.13	0.05	0.23	0.24
13	20 : 1 n-9	2.72	2.60	2.65	2.65	2.62	2.51	2.56	2.66
14	20 : 2 n-6	0.25	0.25	0.24	0.24	0.25	0.23	0.24	0.25
15	20 : 3 n-6	0.11	0.15	0.14	0.14	0.13	0.11	0.36	0.11
16	20 : 4 n-6(AA)	1.10	1.11	1.13	1.18	1.07	1.09	1.10	1.07
17	20 : 5 n-3(EPA)	9.24	9.38	9.30	9.30	9.31	9.29	8.95	8.98
18	22 : 0	0.00	0.09	0.10	0.10	0.09	0.10	0.09	0.11
19	22 : 1 n-9	0.45	0.43	0.42	0.42	0.38	0.43	0.00	0.00
20	22 : 4 n-6	0.00	0.15	0.15	0.15	0.11	0.14	0.13	0.15
21	22 : 5 n-6	0.55	0.51	0.52	0.51	0.52	0.50	0.47	0.48
22	22 : 5 n-3	2.15	2.19	2.14	2.16	2.15	2.14	2.17	2.29
23	22 : 6 n-3(DHA)	21.65	21.56	21.43	21.48	21.26	21.33	20.58	20.93
24	24 : 0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25	24 : 1 n-9	0.61	0.63	0.63	0.63	0.65	0.64	0.64	0.71
	EPA/AA	8.42	8.44	8.26	7.91	8.67	8.53	8.12	8.39
	DHA/AA	19.75	19.42	19.03	18.26	19.79	19.58	18.67	19.55
	n-6 / n-3	0.10	0.11	0.11	0.11	0.11	0.11	0.12	0.12
	PUFA	37.7	37.9	37.7	37.8	37.5	37.5	36.9	37.2
	AA/PUFA	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03

2)Analysis made at the laboratory of Dr. K.Fukunaga, Kansai University, Japan

Model: GC Shimadzu GC-14B, Column: Supelco Omegawax-250,30m, ϕ 0.25mm, Carrier Gas: He 150kpa 1.5ml/min Inj.Temp.: 250 ° C, Column Temp.: 120 ° C(0min)--240 ° C at 2 ° C/min

Table 2 and 3: Fatty Acid Composition and POV

	purified on 2009 Sep.	purified on 2008 Apr.
Fat content	99.4%(w/w)	98.7%(w/w)
FA composition		
14;0	3.5	3.7
15;0	0.6	0.7
16;0	18.4	18.4
16;1	6.3	6.4
16;2	0.6	0.7
17;0	0.2	0.3
17;1	0.2	0.2
18;0	3.6	3.6
18;1(n-9)	21.6	21.5
18;1(n-7)	3.2	3.1
18;2(n-6)	1.5	1.4
18;3(n-3)	0.7	0.7
18;4(n-3)	0.2	0.2
20;0	0.4	0.4
20;1	2.5	2.5

20;4(n-6)	1.0	1.0	
20;5(n-3)	6.7	6.8	
22;0	0.3	0.5	
22;1	0.9	0.7	
22;5(n-6)	2.0	2.0	
22;5(n-3)	1.7	1.7	
22;6(n-3)	23.0	22.6	
others	1.0	1.0	
	100.0	100.0	
AV	2.35	0.99	(mg/g)
POV	26.3	29.1	

FA Composition	(%)
14;0	3.1
16;0	16.1
16;1	6.2
16;2	0.1
17;0	0.7
17;1	0.6
18;0	3.5
18:1 n-9	20.5
18:1 n-7	3.5
18:2 n-6	1.3
18:3 n-3	0.9
18:4 n-3	1.2
20;0	0.3
20;1	2.6
20:4 n-6	0.9
20:4 n-3	0.6
20:5 n-3	7
22;0	0.1
22;1	1.9
22:5 n-6	0.5
22:5 n-3	1.5
22:6 n-3	24.6
24;1	0.9
others	1.3
POV	1.14meq/kg
Total Fat	93.9%(w/w)

4) Stability test realized at Dr. Fukunaga's laboratory.

Table 4: POV stability test of fish oil

20 ml each of Hybrid extracted oil (A) and Co. A's purified oil (B) were poured in 5 laboratory dishes which has a

diameter of 10cm and stored at temperature of 25° C duri and conditions

by Iodometric titration method. The results were as fo

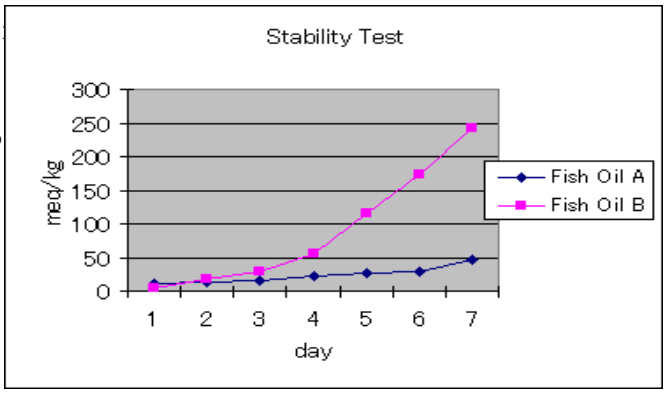

Figure 1: Stability test of A: Hybrid extract tuna oil

Day	A: Extracted oil by Hybrid method	B:Co.A's Purified Fish oil	SD of A	SD of B

0	12.1	5.2	0.3	0.2
0.5	13	17.8	0.5	0.4
1	14.8	29.3	0.3	0.7
2	22.2	56.1	1.2	1.6
4	25.8	116.4	0.9	1.8
8	29.9	174.3	1.3	1.4
16	45.7	242.9	2.6	2.3

5 times analyzed with same conditions.

Data is averaged data and Standard Deviation

N=5

5) Stability test after using as cooking oil frying croquettes at 180 ˚ C

Table 5: heating test

	non heating	after heat	fry	1day after	2days	3days	4days	10days
EPA	6.7	6.6	7	7.1	7.1	7.1	7.1	6.2
DHA	20.1	20.3	21.3	20.9	21.2	20.9	20.8	19
AV	1.18	1.41	1.33	1.23	1.24	1.37	1.37	1.72
POV	1.58	0.87	1.31	1.17	1.22	1.08	1.38	0.6
Conjugated diene	0.054	0.062	0.089	0.091	0.109	0.113	0.114	0.129

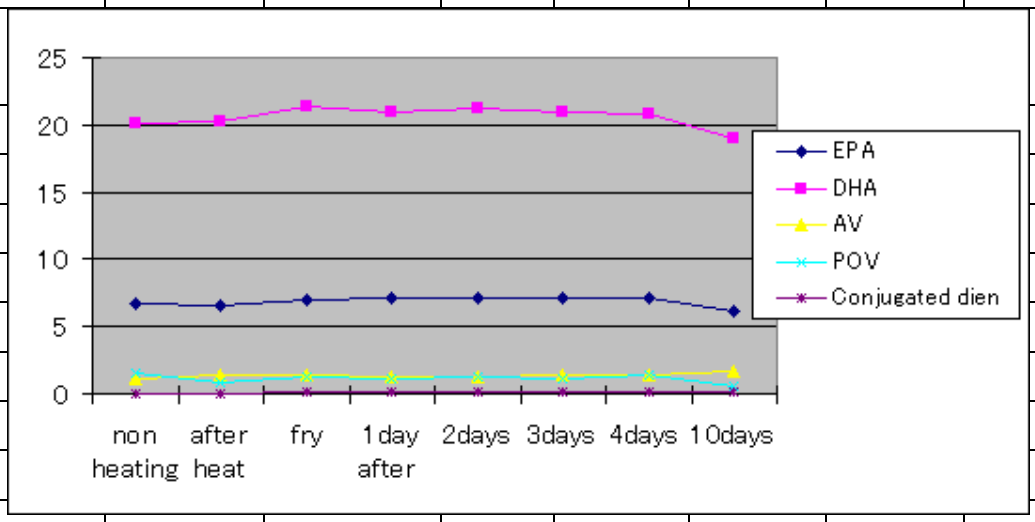

Figure 2: Heating test

note: DHA,EPA and conjugated diene %, AV mg/kg, POV meq/kg

After 10days making fried croquettes the oil didn't oxidized neither spoiled

No change in the quality and quantity of omega 3 PUFA.				

Table 6: High vitamin D and E contents of hybrid extract tuna oil

Vitamin contents ware analyzed at Japan Inspection Association of Food and Food Industry Environment

By High performance liquid chromatography.

Vitamin D (µg/100g)		Vitamin E (mg/100g)	
Mixed Blue Fin & Albacore Oil	146,	Big Eye Tuna Oil	43.7
Yellow Fin Oil	92.5	Yellow Fin Tuna Oil	34.6
Mixed Yellow Fin & Albacore	64.4		

Table 7: The result of analysis realized at Japan Inspection Association of Food and Food Industry Environment

Tuna Deodorant Oil

Vitamin A (retinol equivalent) : 0µg/100g High performance liquid chromatography				
Vitamin D : 7.31µg/100g High performance liquid chromatography				
Vitamin E(α-tocopherol) : 5.1mg/100g High performance liquid chromatography				
Acid Value : 0.2 Neutralization titration method				
Peroxide Value : 2 meq/kg Iodometric titration method				
EPA : 6.28 g/100g gas chromatography				
DHA : 24.3g/100g gas chromatography				
Total mercury : Not detected Atomic absorption spectrophotometry				
Arsenic : Not detected Atomic absorption spectrophotometry with hydride generator				
Heavy metals (as Pb) : Not detected Sodium sulfide method				
PCB : 0.18ppm gas chromatography				

Conclusions

Tuna oil extracted by hybrid extraction method, boiled at low temperature and low pressure then increasing temperature and pressure until over 100 ° C without boiling in a shielded container cut off from air, is very stable to oxidization.

One reason is very rich Vitamin E in the oil however there is another possibility of unknown antioxidant and need further deep study.

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