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FATTY ACID COMPOSITION OF EGGS ENRICHED WITH OMEGA –3 FATTY ACIDS ON THE MARKET

Vekoslava STIBILJ[a,b], Mojca KOMAN RAJŠP[c] and Antonija HOLCMAN[b]

[a] Institut "Jožef Stefan", Jamova 39, SI-1000 Ljubljana, Slovenia, Ass.Prof., Ph.D.
[b] Univ. of Ljubljana, Biotechnical Fac., Zootechnical Dept., Groblje 3, SI-1230 Domžale, Slovenia, Ass.Prof., Ph.D.
[c] Same address as b)

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Dedicated to Professor Jasna Stekar, University of Ljubljana, on the occasion of her 70th birthday

ABSTRACT

The aim of our work was to determine fatty acid content in eggs enriched with omega-3 polyunsaturated fatty acids from various producers on the market. Due to simplicity, speed and reduced organic solvent usage, we used the *in situ* trans-esterification ISTE method for the determination of the fatty acid composition in eggs. The method was checked by the analysis of certified reference material CRM 164 (Anhydrous Milk Fat). The analytical results are in good accordance with certified values. The obtained results show that enriched eggs have a better fatty acid composition than average table eggs, regardless the producer. The ratio of FAs ω-6 to ω-3 in enriched eggs is less than 5, being 10 in ordinary eggs. An approximately equal content of alpha linolenic acid (18:3, n-3) was found in the enriched eggs from Jata Reja and Maia, 1.29 wt % and 1.25 wt % respect., while it was 11.28 wt % in the Fisher-Weppler's eggs. The comparison of obtained results, the fatty acid content per 100 g egg content, with literature data is quite difficult, because authors calculate the content of FA in various ways. According to recent data from the literature (Weihrauch *et al.,* 1977, Guardiola *et al.,* 1994, Fatty acids, 1998, Kunachowicz *et al.*, 1998), we have chosen a conversion factor of 0.83 in order to calculate the total fatty acids content in a 100 g egg content. The producers should declare the fatty acid composition, i.e. the content of ω-3 PUFA-s, EPA and DHA on the package so that consumers are informed about the nutritional value of the food.

Key words: poultry / eggs / fatty acids / omega-3 fatty acids / analytical chemistry / gas chromatography

MAŠČOBNOKISLINSKA SESTAVA JAJC OBOGATENIH Z OMEGA –3 MAŠČOBNIMI KISLINAMI NA TRŽIŠČU

IZVLEČEK

Želeli smo ugotoviti vsebnost maščobnih kislin (MK) v jajcih, obogatenih z ω-3 maščobnimi kislinami različnih proizvajalcev na tržišču. Za določitev smo uporabili metodo plinske kromatografije in *in situ* transesterifikacijo maščobnih kislin. Ta tehnika je enostavna, hitra in omogoča majšo porabo organskih topil. Pravilnost in zanesljivost metode smo preverili z analizo certificiranega referenčnega vzorca CRM 164 (Anhydrous Milk Fat) in dobili dobro ujemanje rezultatov s certificiranimi vrednostmi. Ugotovili smo, da imajo vsa obogatena jajca, ne glede na proizvajalca,ugodnejšo maščobnokislinsko sestavo kot običajna jajca. Razmerje MK ω-6/ω-3 je pri obogatenih manj kot 5, pri navadnih pa okrog 10. Nekateri avtorji uporabljajo za izračun vsebnosti MK različne načine, zato je primerjava rezultatov zelo težavna. Glede na novejše literaturne podatke (Weihrauch *et al.*, 1977, Guardiola *et al.*, 1994, Fatty acids, 1998, Kunachowicz *et al*, 1998), smo za izračun vsebnosti MK v jajčni vsebini uporabili konverzijski faktor 0.83. Zaželeno bi bilo, da bi proizvajalci na embalaži navedli maščobnokislinsko sestavo, vsebnost ω-3 večkrat nenasičenih MK, EPK in DHK in tako natančneje seznanili kupca s prehransko vrednostjo živila.

Ključne besede: perutnina / jajca / maščobne kisline / omega-3 maščobne kisline / analitska kemija / plinska kromatografija

INTRODUCTION

Chicken eggs are popular foods due to their high nutritional value, as well as culinary and technological traits.

Edible portion of egg content (100 g) contains approximately 11.2 g of fat. There is 0.68 g of polyunsaturated fatty acids (PUFA-s), 4.31 g monounsaturated fatty acids (MUFA-s), and only 3.15 g of saturated fatty acids (SATFA-s) (Fatty acids, 1998). Palmitic acid (16:0), oleic acid (18:1, n-9) and linoleic acid (18:2, n-6) prevail. Long-chain polyunsaturated fatty acids (18 or more carbon atoms in a chain with two or more double bonds) are essential for man, hence they are the most important constituents for the evaluation of various fats (Decker and Cantor, 1992).

Polyunsaturated fatty acids consist of two different groups, omega-3 and omega-6 fatty acids. The human body needs a balance between omega-3 (n-3) and omega-6 (n-6) acids. The ratio of n6 to n3 in an average food intake is 25, the ideal being around 5 (Sinclair, 1991). The composition of omega-3 fatty acids is very important. Alpha linolenic acid, which is the omega-3 fatty acid found mostly in vegetable oils, is converted in the body to the biologically active form and this occurs slowly in healthy people. If a person eats too much vegetable oil rich in omega-6 fatty acids, the ability of the body to make this conversion is reduced (Farell, 1997). The biologically active forms are eicosapentaenoic acid (EPA, 20:5, n-3) and docosahexaenoic acid (DHA, 22:6, n-3). They can also be present in food, so it is important, therefore, to eat foods that are rich in these two biologically active omega-fatty acids. This distinction is so important that British Nutrition Foundation (1991) does not make its recommendations on the basis of total omega-3 fatty acids, but instead on the basis of EPA + DHA (Farell, 1997).

Thus, with proper manipulation of the fatty acid composition of the hens' diet, egg lipids could serve as a source of PUFA-s, EPA and DHA in a human diet.

The aim of our work was to determine the fatty acid composition of eggs enriched with omega-3 polyunsaturated fatty acids from three various producers on the market.

MATERIALS AND METHODS

The fatty acid composition was determined in eggs enriched with omega-3 fatty acids from three producers: Jata Reja, d.d. (Slovenia), Fisher-Weppler, Baden –Baden (Germany) and Maia (Italy). Eggs were bought on the market. The following composite samples of eggs were prepared: Jata Reja, d.d. (2 x 2 eggs in the year 1997 and 3 x 4 eggs in the year 1998), Fisher-Weppler GmbH, Baden-Baden, EiVit (3 x 2 eggs) and MAIA (2 x 2 eggs). We also sampled ordinary table eggs twice (2 x 2 eggs, Jata Reja, d.d.). The composite sample was homogenised and analysed in parallel before the expiration date.

Considering the previous analytical results (Stibilj and Koman Rajšp, 1997), we decided to use the *in situ* transesterification (ISTE) without previous extraction of fats (Park and Goins, 1994).

The fatty acid composition of resulting fatty acid methyl esters (FAME-s) was determined by gas chromatography (GC) using a Hewlett Packard 5890 series I gas chromatograph equipped with a flame ionisation detector (FID) and integrator 3392A under the following conditions (Stibilj and Koman Rajšp, 1997):

Capillary column: HP-20M (Carbowax 20M, 50 m x 0.32 mm x 0.3 μm)
Oven program: 140° C (0 min); 7° C min^{-1} to 215° C (81.29 min)
Injection: Split, 30:1, volume injected 1 μl
Carrier gas: Ar, 2 ml min^{-1}
Makeup gas: N_2, 30 ml min^{-1}
Inlet: 250° C
Detector: 270° C

FAME-s were identified by a comparison of retention times with standards (Nu Check Prep., Inc., Sigma), and their concentrations were calculated by use of adequate response factors (Rf) based on a standard mixture (weight percent - wt % of the total).

GC analysis gave a very good reproducible separation of FAME-s from yolk sample. (Fig.1) The precision and the accuracy of the our analytical method was checked by analysis of BCR Certified Reference Material No. 164 Anhydrous Milk Fat (Pocklington *et al*., 1993).

To calculate the weight of each fatty acid the conversion factors for conversion of FAME-s to their corresponding fatty acids were used (AOAC, 1998).

In addition to fatty acids, the total fat in most food includes the glycerol of the triglycerides, phosphate from phospholipids, and unsaponifiable components such as sterols.

For expressing the total fatty acids in a given weight of egg, we used a formula derived from the recent literature for the calculation of mg FA per 100 g of fresh egg (Weihrauch *et al*., 1977, Guardiola *et al*., 1994, Fatty acids, 1998, Kunachowicz *et al*., 1998).

$$\text{g FA } 100\text{g}^{-1} \text{ egg} = \% \text{ total fat egg} \times 0.83 \times \% \text{ FA } 100^{-1}$$

0.83 is the factor expressing the FA fraction in the total fat egg

For the calculation we used 11.1 % fat egg content, which is taken from the Food Composition and Nutrition Tables (1994), for table and enriched eggs of the Slovenian producer, and declared fat value for eggs of the Italian and German producers.

RESULTS AND DISCUSSION

The precision and the accuracy of our analytical method were tested by analysis of BCR Certified Reference Material No. 164 Anhydrous Milk Fat (Table 1).

The U.S. Department of Agriculture's Food Safety and Inspection Service (FSIS) has set specific requirements for using the following terms: high, good source of, free, low, light, reduced/less/fewer, more/added, percent fat-free, lean and extra lean. The definitions match those set by the FDA (The Food and Drug Administration). The term more/added/fortified/enriched food means that an individual food must have at least 10 percent more of the daily value of protein, potassium, dietary fibre, or a vitamin or other mineral per reference amount compared to an appropriate reference food (McCutcheon, 1995). The same definition could be proper for fatty acids.

Table 2 shows the fatty acid composition of table eggs and eggs enriched with omega-3 acids. Fisher-Wepler's eggs contained the highest percentage of alpha linolenic acid (11.28 wt %) and ω-3 fatty acids (14.34 wt %) and this value is more than 10 times higher than its content in table eggs. Fortified eggs of German producer had the lowest percentage of EPA+DHA (2.35 wt %). The percentage of EPA and DHA ranged between 2.35 % (Wisher Weppler) and 4.42 wt % (Jata Reja d.d). EPA+DHA contents and omega-3 FA-s were similar in eggs of Slovenian and Italian producers. The content of EPA was ten times higher in all analysed egg samples than in table eggs. In enriched eggs of all three producers the ratio of fatty acids n-6/n-3 was favourable, being less than 5 (Sinclair, 1991).

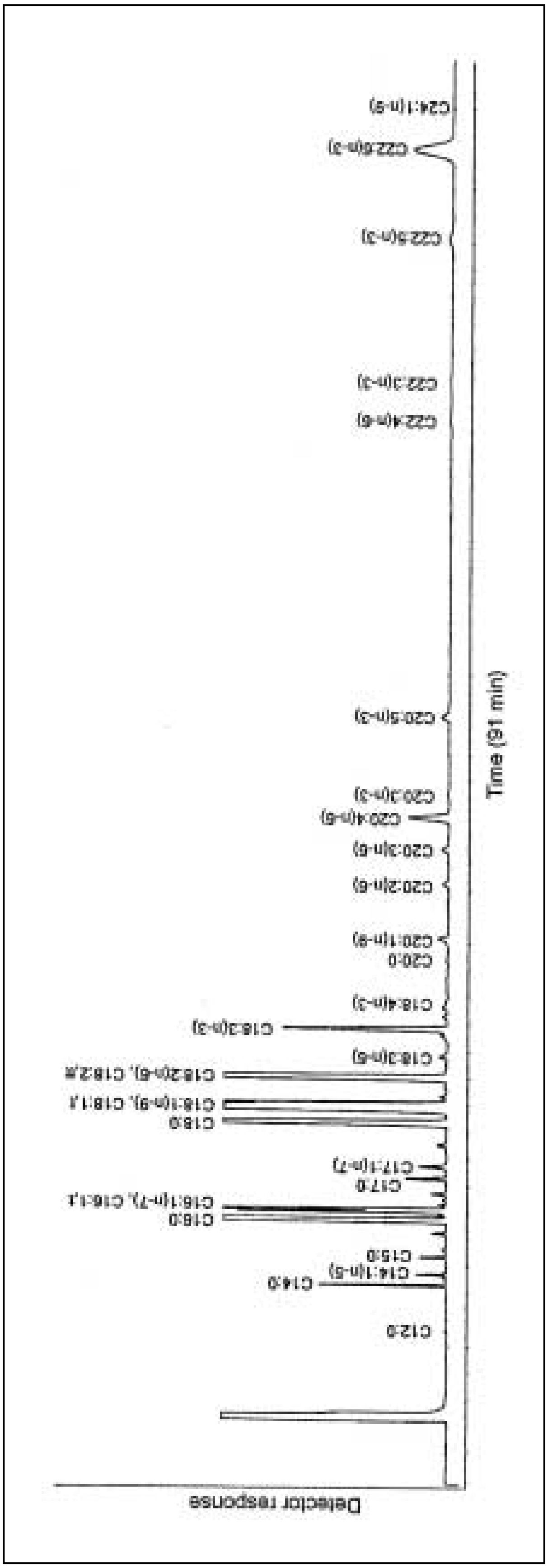

Fig.1. Chromatogram of fatty acid profile of yolk sample.

Table 1. Results for fatty acid profile in the BCR Certified Reference Material No. 164 Anhydrous Milk Fat

Fatty acid	Obtained results Average± standard deviation (n=4)	Certified value
	(g 100g^{-1} of the total fatty acids)	
C10:0	3.19 ± 0.31	2.89 ± 0.12
C12:0	4.34 ± 0.31	4.03 ± 0.10
C 14:0	11.16 ± 0.44	10.79 ± 0.35
C15:0 iso	0.32 ± 0.01	0.30*
C15:0 anteiso	0.54 ± 0.02	0.50*
C15:0	1.12 ± 0.02	1.00*
C16:0 iso	0.25 ± 0.01	0.20*
C 16:0	26.62 ± 0.21	26.91 ± 0.84
C 16:1 (n-7), C 16:1, t	1.78 ± 0.04	1.50*
C 17:0 iso	0.59 ± 0.01	0.50*
C 17:0	0.51 ± 0.01	0.50*
C 18:0	9.88 ± 0.34	10.51 ± 0.40
C 18:1	24.56 ± 0.62	24.82 ± 0.61
C 18:2	2.04 ± 0.06	2.68 ± 0.40
C 18:3 (n-3)	0.63 ± 0.02	0.51 ± 0.04**
C 20:0	0.21 ± 0.02	0.10*
C 20:11 (n-9)	0.20 ± 0.01	0.20*

* informative value, **only cis isomer

In the literature the contents of fatty acids are quoted as ″mg FA per 100 g of edible portion of egg″, ″per 100 g of egg content″, ″per yolk″, ″per g of yolk″ but the authors never stated how the values were calculated. Therefore, a direct comparison of our results with those from the literature cannot be made. Using the formula quoted by literature (Weihrauch *et al.,* 1977, Guardiola *et al*., 1994, Fatty acids, 1998, Kunachowicz *et al*., 1998) the results were expressed in g of fatty acids per 100 g of egg content (Table 3).

The Slovenian producer declares that one enriched egg covers the recommended daily intake of omega-3 fatty acids and that the content of EPA is 10-times higher than in ordinary table eggs. The German and Italian producers report the content of total SATFA-s, MUFA-s, PUFA-s and of the ω-3 fatty acids. The content of omega-3 PUFA-s is not mentioned on the Slovenian package. On Maia's declaration the content of DHA and EPA is stated (Fig. 2). It is evident that the total FA-s content on the declarations (9.3 and 9.5 g 100g^{-1} respect.) nearly equals the content of fat (9.5 g 100g^{-1}), hence the comparison of results is difficult.

The obtained results were compared with those from the literature, calculated according to the above formula. The eggs of the German producer contained 185.3mg EPA+DHA and the most ω-3 FA, 1130 mg per 100 g of egg content. (Table 4).

Table 2. Average fatty acid composition of table and enriched eggs on the market

Fatty acid	Fatty acid composition (wt % -percentage of total present fatty acids)#***				
	Table eggs	Enriched egg			
	Jata, d.d. (2)**	Maia (2)**	Fisher-Weppler (3)**	Jata, d.d.	
				1997 (2)**	1998 (3)**
C12:0	<0,01	<0.01	<0.01	<0.01	<0.01
C14:0	0.28 ± 0.06	0.40 ± 0.01	0.21 ± 0.02	0.26 ± 0.01	0.35 ± 0.03
C14:1(n-5)	0.08 ± 0.02	0.11 ± 0.02	0.03 ± 0.00	0.07 ± 0.01	0.09 ± 0.02
C15:0	0.07 ± 0.01	0.12 ± 0.05	0.07 ± 0.00	0.07 ± 0.01	0.08 ± 0.00
C16:0	21.67 ± 1.63	21.90 ± 0.53	19.35 ± 0.67	21.37 ± 0.51	25.21 ± 0.14
C16:1(n-7), C16:1,t	3.58 ± 0.33	3.34 ± 0.10	2.82 ± 0.14	3.30 ± 0.16	3.90 ± 0.39
C17:0	0.22 ± 0.02	0.32 ± 0.06	0.21 ± 0.01	0.21 ± 0.01	0.22 ± 0.02
C17:1(n-7)	0.24 ± 0.02	0.28 ± 0.03	0.23 ± 0.01	0.19 ± 0.01	0.20 ± 0.01
C18:0	9.80 ± 0.22	8.50 ± 0.25	8.48 ± 0.12	8.83 ± 0.23	8.21 ± 0.49
C18:1(n-9), C18:1,t	43.86 ± 0.99	38.05 ± 1.09	37.82 ± 0.74	41.35 ± 0.56	40.31 ± 0.47
C18:2(n-6), C18:2,tt	**14.92 ± 1.26**	**19.75 ± 1.07**	**15.02 ± 0.49**	**16.11 ± 0.1**	**14.04 ± 0.65**
C18:3(n-6)	**0.09 ± 0.02**	**0.03 ± 0.02**	**0.08 ± 0.00**	**0.02 ± 0.01**	**0.08 ± 0.01**
C18:3(n-3)	**0.52 ± 0.09**	**1.25 ± 0.07**	**11.28 ± 0.21**	**1.73 ± 0.02**	**1.29 ± 0.39**
C18:4(n-3)	/	/	0.16 ± 0.01	/	0.10 ± 0.00
C20:0	0.02 ± 0.01	0.07 ± 0.10	<0.03	0.03 ± 0.01	<0.03
C20:1(n-9)	0.31 ± 0.04	0.31 ± 0.02	0.14 ± 0.01	0.31 ± 0.02	0.23 ± 0.03
C20:2(n-6)	**0.22 ± 0.02**	**0.20 ± 0.03**	**0.12 ± 0.01**	**0.18 ± 0.01**	**0.11 ± 0.01**
C20:3(n-6)	**0.18 ± 0.02**	**0.20 ± 0.03**	**0.13 ± 0.02**	**0.18 ± 0.01**	**0.15 ± 0.01**
C20:4(n-6)	**2.60 ± 0.17**	**1.47 ± 0.10**	**0.93 ± 0.04**	**1.32 ±0.04**	**0.93 ± 0.10**
C20:3(n-3)	**<0.01**	**0.01 ± 0.01**	**0.19 ± 0.03**	**0.04 ± 0.01**	**<0.04**
C20:5(n-3)	**<0.01**	**0.19 ± 0.03**	**0.24 ± 0.03**	**0.29 ± 0.01**	**0.34 ± 0.06**
C22:4(n-6)	/	/	*	/	**0.09 ± 0.03**
C22:5(n-3)	/	/	**0.44 ± 0.04**	/	**0.32 ± 0.09**
C22:6(n-3)	**1.34 ± 0.15**	**3.40 ± 0.55**	**2.10 ± 0.13**	**4.13 ± 0.27**	**3.73 ± 0.42**
Total SATFA	32.07	31.41	28.34	30.77	34.08
Total MUFA	48.07	42.09	41.04	45.23	44.72
Total PUFA	19.87	26.50	30.62	24.00	21.20
Total n-6	18.01	21.65	16.28	17.81	15.39
Total n-3	1.86	4.85	14.41	6.19	5.77
n-6/n-3	**9.68**	**4.46**	**1.13**	**2.87**	**2.67**
n-6/n-3(L PUFA)	13.44	6.01	7.10	3.99	3.51
EPA+DHA	**1.34**	**3.59**	**2.35**	**4.42**	**4.06**

* under detection limit, which is 0.01 wt % of total FAME-s, # average± SD, **each sample was analysed in duplicate, / FA was not determined, ***omega-3 and omega-6 fatty acids are bolded

Fig.2. Declarations on the packages of enriched eggs.

Table 3. The comparison of obtained results and those from the declarations

Components	Composition of enriched eggs (g $100g^{-1}$ egg content)						
	Maia		Fisher-Weppler		Jata Reja d.d.		
	Found	Declared value	Found	Declared value*	Found# 1997	Found# 1998	Declared value
Fat		9.5		9.5			
Total SATFA	2.5	3.1	2.2	2.8	2.8	3.1	
Total MUFA	3.3	4.6	3.2	4.2	4.1	4.1	
Total PUFA	2.1	1.6	2.4	2.5	2.2	2.0	
Total n-3**	382.4	408	1100	1100	570	531.6	
EPA**	15	33	19		27	31.3	&
DHA**	268	285	166		380	344	

*g $100g^{-1}$ egg, **mg, # eggs were sampled twice, 1997 and 1998, & ten times higher value than in table egg

Table 4. The average content of (EPA + DHA) and ω-3 fatty acids in eggs from markets and the comparison with the data from the literature

EGGS	EPA+DHA		Σ OMEGA-3		References
	ut. %	mg $100g^{-1}$ egg content	ut. %	mg $100g^{-1}$ egg content	
Table (Jata Reja)	1.34	123.5	1.86	181.7	This study
Enriched (Jata Reja) , 1997	4.42	407.2	6.19	570.3	
Enriched (Jata Reja),1998	4.06	375.3	5.77	531.6	
Enriched (Maia)	3.59	283.1	4.85	382.4	
Enriched (Fisher-Weppler)	2.35	185.3	14.34	1130	
Eggs (feed with 3 % fish oil with natural content of carotenoids)	3.8	284.1	4.6	342.8	Hammershoj (1995)
Eggs from small farms	1.4	133	2.1	195	Guardiola *et al*. (1994)
Eggs (feed with 1.5 % menhaden oil)	/	188	/	/	Marshall *et al.* (1994)
Greek eggs from free range (pasture+insects+dry figs+barley+maize)	/	282	/	530	Simopoulus (1989)
Table eggs	/	35		52	Simopoulus (1989)

/ - no data

Recommended daily amounts of omega-3 fatty acids ranges between 0.1 and 2 g day^{-1} (Omegatech, 1997).

CONCLUSIONS AND RECOMMENDATIONS

The obtained results show that enriched eggs have a better fatty acid composition than average table eggs, regardless of the producer. The ratio of n-6 to n-3 in enriched eggs is less than 5, being 10 in ordinary eggs.

- An approximately equal content of alpha linolenic acid (18:3, n-3) was found in the enriched eggs from Jata Reja and Maia, 1.29 wt % and 1.25 wt % respect., while it was 11.28 wt % in the Fisher-Weppler's eggs.
- The contents of EPA+DHA and omega-3 FAs were similar in the eggs of Slovenian and Italian producers.
- The content of EPA in enriched eggs from the Slovenian market was approx. 30 mg, while in the ordinary eggs it is 1 mg, all per 100 g of egg content.
- Some authors calculate the content of FA in various ways, therefore the comparison of the results is quite difficult. According to recent data from the literature (Weihrauch *et al.,* 1977, Guardiola *et al.,* 1994, Fatty acids, 1998, Kunachowicz *et al.*, 1998), we have chosen a conversion factor of 0.83 in order to enable the calculation of the total fatty acids in a 100 g egg content.
- The producers should declare the fatty acid composition, i.e. the content of ω- 3 PUFA-s, EPA and DHA, on the package so that consumers are informed about the nutritional value of the food.

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