

## CyTA - Journal of Food

ISSN: 1947-6337 (Print) 1947-6345 (Online) Journal homepage: <http://www.tandfonline.com/loi/tcyt20>

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To cite this article: A. Lamas, X. Anton, J.M. Miranda, P. Roca-Saavedra, A. Cardelle-Cobas, J.A. Rodriguez, C.M. Franco & A. Cepeda (2016) Technological development of functional egg products by an addition of n-3 polyunsaturated-fatty-acid-enriched oil, *CyTA - Journal of Food*, 14:2, 289-295, DOI: [10.1080/19476337.2015.1100220](https://doi.org/10.1080/19476337.2015.1100220)

To link to this article: <http://dx.doi.org/10.1080/19476337.2015.1100220>



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Published online: 19 Oct 2015.



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## Technological development of functional egg products by an addition of *n*-3 polyunsaturated-fatty-acid-enriched oil

### Desarrollo tecnológico de ovoproductos funcionales con un alto contenido en ácidos grasos *n*-3

A. Lamas<sup>a</sup>, X. Anton<sup>b</sup>, J.M. Miranda<sup>a\*</sup>, P. Roca-Saavedra<sup>a</sup>, A. Cardelle-Cobas<sup>a</sup>, J.A. Rodríguez<sup>c</sup>, C.M. Franco<sup>a</sup> and A. Cepeda<sup>a</sup>

<sup>a</sup>Laboratorio de Higiene Inspección y Control de Alimentos, Dpto. de Química Analítica, Nutrición y Bromatología, Universidad de Santiago de Compostela, 27002 Lugo, Spain; <sup>b</sup>Clavo congelados, S. A., Caldas de Reis, 36650 Pontevedra, Spain; <sup>c</sup>Centro de Investigaciones Químicas, Universidad Autónoma del Estado de Hidalgo, Carr. Pachuca-Tulancingo Km. 4.5, 42076 Pachuca, Hidalgo, Mexico

(Received 28 May 2015; final version received 22 September 2015)

Eggs are good candidates to be employed as functional food and to provide people with bioactive compounds such as *n*-3 PUFAs. However, in most cases, development of eggs with high content of *n*-3 PUFAs was carried out by modifying the hens' feed. In the present work, egg-derived sticks with high content of *n*-3 PUFAs were technologically developed through addition of three different sources of *n*-3 PUFAs: linseed oil, fish oil and microalgae oil. The developed products were compared to conventional egg-derived products for their proximate composition, fatty acid profile, colour and consumer acceptability. Additionally, lipid oxidation was investigated after 30 days of frozen storage. Nutritional composition, colour parameters and consumer acceptability revealed that egg-derived product obtained by addition of linseed oil was the most adequate. Egg-derived functional foods developed by technological methods are viable and could be considered as an interesting alternative, especially for catering companies.

**Keywords:** omega-3; functional egg product; linseed oil; microalgae oil; fish oil

Los huevos son buenos candidatos para ser empleados como alimentos funcionales y de este modo proporcionar a las personas compuestos bioactivos, tales como los ácidos grasos *n*-3. Sin embargo, en la mayoría de los casos, el desarrollo de huevos con alto contenido en ácidos grasos *n*-3 se realizó mediante la modificación de la alimentación aviar. En el presente trabajo, se desarrollaron ovoproductos con alto contenido en ácidos grasos *n*-3 de modo tecnológico mediante la adición de tres fuentes diferentes de *n*-3: aceite de linaza, aceite de pescado y aceite de microalgas. Los ovoproductos desarrollados se compararon con ovoproductos convencionales en su composición nutricional, perfil lipídico, color y aceptabilidad por parte del consumidor. Además, se investigó su oxidación lipídica después de 30 días de almacenamiento en congelación. Combinando los resultados obtenidos, el ovoproducto elaborado con aceite de linaza resultó ser el más adecuado. De este modo, los ovoproductos funcionales elaborados mediante métodos tecnológicos son viables y pueden ser considerados una alternativa interesante, especialmente para las empresas de restauración colectiva.

**Palabras claves:** omega-3; ovoproducto funcional; aceite de linaza; aceite de microalgas; aceite de pescado

## 1. Introduction

Egg is one of the most appreciated products by consumers. It plays a fundamental role in worldwide diet and, from a gastro-nomic point of view, it is a versatile ingredient, offering multiple possibilities of use and possessing an excellent quality–price ratio. Nutritionally, it is noteworthy that egg supplies the diet with essential nutrients such as vitamins, minerals, fatty acids and essential amino acids and is one of the best and least expensive sources of high-quality dietary protein (Huopalahti et al., 2007; Miranda et al., 2015). Additionally, eggs are well known to contain active compounds that may have a role in essential human nutrition and the prevention and therapy of chronic diseases (Miranda et al., 2015). However, despite all their positive nutritional aspects, egg consumption was traditionally associated with adverse factors for human health, mainly due to their elevated content in saturated fat (about 30 g/kg) and cholesterol (about 2 g/kg) (Li, Zhou, Zhou, & Li, 2013; Weggemans, Zock, & Katan, 2001).

On the other hand, during the last decades, consumption of functional foods has increased considerably and in consequence, their development and commercialization by food industry has also increased. Nowadays, society is becoming aware of the importance of diet for health and the demand for food products that are associated with well-being and with reducing the risk of disease is increasing. In this context, the polyunsaturated fatty acids (PUFAs), and more specifically, the omega-3 (*n*-3 PUFAs), have been shown to possess positive effects for human health, especially cardio-protective benefits (Lands, 2014). The intake of *n*-3 PUFAs decreases inflammatory markers, blood pressure and serum triglycerides, which have been established as risk factors for cardiovascular diseases (CVD) (Juturu, 2008). In consequence, different *n*-3 PUFA-enriched food products can be found nowadays in the markets of developed countries. The European Commission Regulation 116/2010, relative to the nutritional and health claims in food, establishes that in order to label a product as “high in *n*-3 PUFAs”, the product should contain almost 6 g of

\*Corresponding author. Email: [josemanuel.miranda@usc.es](mailto:josemanuel.miranda@usc.es)

$\alpha$ -linoleic acid (ALA) per kg of food or 100 kcal or 0.80 g of eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) in 1 kg or 100 kcal of food.

Among the different sources that can be employed as sources of  $n$ -3 PUFAs, fish, microalgae and linseed-derived oils are those considered as the best alternative to increase the PUFAs levels in foods (Fraeye et al., 2012). Linseed oil is the major source of ALA, with concentrations of about 50% (Ganesan, Brothersen, & McMahon, 2014), whereas the main sources of DHA and EPA are fish and microalgae-derived oils. Among all the microalgae oils, only those provided from *Schizochytrium* sp. and *Ulkenia* sp. are currently authorized by the European Commission (Decisions 2009/777 and 2009/778) as food ingredients.

As stated before, due to their nutritional properties, good acceptance by people and lack of religious restrictions, eggs are good candidates to be employed as functional food and to provide people with bioactive compounds such as  $n$ -3 PUFAs. In this sense, previous studies have focused their work on supplementing hen feed with different sources of  $n$ -3 PUFAs, including the oils cited before (Carrillo et al., 2008; Fraeye et al., 2012; Lemahieu et al., 2013). However, due to their higher microbiological safety (Rossi, Casiraghi, Primavesi, Pompei, & Hidalgo, 2010) and ease of handling and storing compared to shelled eggs, the food service industry and commercial food manufacturers have shown an increasing interest in the use of liquid-pasteurized egg products instead of whole eggs (Miranda et al., 2015). Additionally, it should be taken into account that according to EC Commission Regulation 1028/2006, egg-derived products can include class “B” eggs, which are much cheaper than fresh class “A” eggs. Nevertheless, the technological development of egg-derived products enriched in  $n$ -3 PUFAs has received little attention (Kassis, Drake, Beamer, Matak, & Jaczynski, 2010).

In order to obtain an egg-derived product with high content of  $n$ -3 PUFAs, while maintaining its main nutritional and sensory characteristics, the idea emerges to partially substitute the yolk

with ingredients enriched in  $n$ -3 PUFAs. Thus, the main objective of this work was to use various  $n$ -3 PUFAs-enriched oil sources: fish, algae and linseed, to supplement egg-derived products with the necessary amount to accomplish the regulation required by the European Union (EU) to be considered as “high in  $n$ -3 PUFAs”. The nutritional and sensory properties of the different egg-derived products developed as well as its lipid stability were also evaluated and discussed.

## 2. Materials and methods

### 2.1. Ingredients and sources of $n$ -3 fatty acids

Pasteurized egg yolk and egg white were separately provided by Derovo<sup>®</sup> (Pombal, Portugal). Modified starch was provided by Huarte (Navarra, Spain). Three types of  $n$ -3 rich oils were obtained from different companies: Fish oil with a minimum of 250 g/kg of DHA+EPA (Eupoly-3 EPA, Biosearch life, Spain), oil obtained from the microalgae *Schizochytrium* spp. containing a minimum of 400 g/kg of DHA+EPA (Life’s omega<sup>®</sup>, DSM, USA) and a linseed oil with a 530 g/kg of  $\alpha$ -linolenic acid (Naturgreen, Murcia, Spain). Fish and microalgae oil contain in their formulation tocopherols and ascorbyl palmitate as antioxidants for a stabilization of the product, namely preventing the  $n$ -3 oxidation.

### 2.2. Development of experimental egg-derived products

Egg-derived products were prepared as sticks and subsequently cut at pilot plant level in a local food industry (Clavo Congelados, Caldas de Reis, Spain) following the instructions of the egg-derived product manufacturer (Figure 1), with slight modifications. Thus, for the case of the product enriched with linseed oil (LE) 18 g/kg were added, for the case of the product enriched with microalgae oil (AE) 3.5 g/kg were added and for the case of the product enriched with fish oil (FE) 4.2 g/kg were added. In the egg-derived product used as control (CE) no

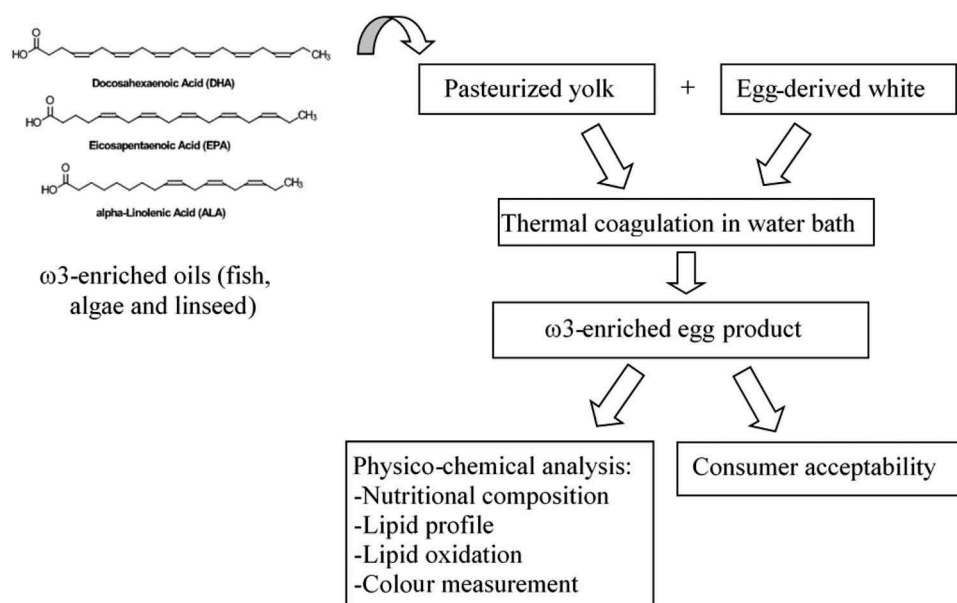


Figure 1. Schematic procedure of the development and analysis of enriched omega-3 egg sticks.

Figura 1. Procedimiento esquemático del desarrollo y análisis de palitos de huevo enriquecidos en omega-3.

addition of any oil was carried out. The amount of oil required to obtain the adequate amount of *n-3* to the egg to be labelled as “with high content in *n-3*” following the EU legislation was calculated from the levels of DHA+EPA and ALA declared by the oil’s manufacturers and the levels of the control egg previously determined. Samples were subsequently maintained under refrigeration until analysis.

### 2.3. Nutritional composition and lipid profile determination

All analyses for proximate composition, fatty acid profile and cholesterol content were performed using standard methods (AOAC, 2002). The moisture content was determined by drying samples in a Selecta oven (Barcelona, Spain) to a constant weight. Total protein was determined by the Kjeldahl method and a factor of 6.25 was used to convert total nitrogen to crude protein. The samples were digested using a Kjeltex 1007 digester (Tecator, Höganäs, Sweden) and distilled using a Kjeltex 1026 distilling unit (Tecator). Lipid content was determined by extraction with diethyl ether/petroleum benzene (1/1, v/v) in a Soxtec System HT 1043 (Tecator). Ash content was determined by incineration in an Omega 8–2/1100–1 muffle furnace (Utena, Lithuania). Carbohydrate quantity and energy content were calculated according to the procedure recommended by Allison and Senti (1983).

Lipids were extracted from the egg yolks using the method described by Folch, Lees, and Sloane-Stanley (1957), and the fatty acid methyl esters were prepared as described by ISO method 5509 (1978). Quantification was carried out using a gas Hewlett-Packard 6890 chromatograph equipped with a flame ionization detector (FID) and separation was achieved in a DB-WAX capillary column (60 m × 0.25 mm, id., 0.25 mm film thickness). Injection of 1 µL was made in the splitless mode with an injection temperature of 230°C. Detector temperature was 250°C. Hydrogen was used as gas carrier at a flow rate of 1.9 mL/min. The identification of fatty acids methyl esters was performed by comparison with the retention time of a standard prepared with a mixture of fatty acids previously injected. Recovering of the methyl esters was calculated by adding a known amount of an internal standard (C:23) that is not naturally present in the samples. Samples were picked in triplicate and values were expressed as g of fatty acids/ kg of total fat.

### 2.4. Lipid oxidation

Lipid oxidation was assessed by measuring the thiobarbituric acid reactive substances (TBARs), which represents the levels of malondialdehyde (MDA) produced (end product of lipoperoxidation substances), using the method adapted from Martinez et al. (2012). Thus, 2 g of sample was homogenized by vortexing in 10 mL of 100 g/kg of trichloroacetic acid (TCA, Merck, Darmstadt, Germany) and 5 mL of 0.02 M 2-thiobarbituric acid (TBA, Merck). Then, it was centrifuged at 2100 × *g* for 20 min in an H-103-N Series centrifuge (Kokusan, Tokyo, Japan). The supernatant was collected and filtered, heated in boiling water for 35 min at 100°C, and chilled in iced water for 10 min. Finally, absorbance at 532 nm was measured in a Jasco V-630 Spectrophotometer (Navarra, Spain). 1,1,3,3-tetraethoxypropane (Sigma Aldrich) was used as a standard in the range 1 × 10<sup>-6</sup>–14 × 10<sup>-6</sup> mol/L. The TBARs concentration was

expressed as mg malondialdehyde/ kg of sample. Each egg-derived product developed was analysed in triplicate immediately after their production (day 0) and after 30 days of freezing storage.

### 2.5. Colour measurement

Colour parameters were determined by measuring the yolk according to the methodology described by Abularach, Rocha, and de Felício (1998), using a digital CR-300 Konica colorimeter (Minolta, Osaka, Japan). A cylindrical piece of 25 mm diameter of each yolk treatment was analysed at room temperature in specular exclusion mode. The parameters’ lightness (*L\**), redness/greenness (*a\**) and yellowness/blueness (*b\**) were determined using D65 as illuminant, 8° viewing angle and standard 10° observer angle. Determinations in each egg-derived products were performed in triplicate.

### 2.6. Consumer acceptability

Determination of the consumer acceptability was performed by a panel of 10 hedonic tasters using a 7-point hedonic scale where 1 was “disgusted me a lot” and 7 was “I really like it”. Sessions took place in a sensory evaluation laboratory equipped with individual assessment booths and uniform lighting conditions. Five attributes were analysed: appearance, odour, texture, flavour and overall acceptability. Each taster was supplied with four samples that each had a diameter of 42 mm and a thickness of 10 mm. Each sample corresponded to a different type of egg bar coded with a three-digit number. Spring water and unsalted bread were provided so that the panellists could clean their mouths between samples.

### 2.7. Statistical analysis

A Student’s *t*-test was used to identify differences in the proximate composition, fatty acid profile, consumer acceptability and colour evaluation between the modified and conventional egg-derived products. The evolution of TBARs during storage were statistically analysed using a two-way analysis of variance (ANOVA), with the type of egg-derived product and storage time as factors. Differences were considered significant for *P* < 0.05. All analyses were conducted using the statistical package SPSS 16.0 for windows (SPSS, Chicago, IL, USA).

## 3. Results and discussion

### 3.1. Nutritional composition and lipid profile determination

Nutritional compositions obtained for the different egg-derived formulations are shown in Table 1. All modified egg-derived products (LE, FE or AE) showed significant differences in most parameters determined from the results obtained for CE samples. With respect to moisture content, LE samples were found to be significantly lower than CE, AE and FE samples. Taking into account that the moisture of oils employed in the formulation of modified products is less than 10 g/kg, the higher addition of oil in LE samples could be the cause of a final egg by-product with lower moisture content than CE, FE or AE.

Protein content was significantly higher in CE than the other modified egg-derived products, which presumably indirectly caused the addition of fat in the modified products.

Table 1. Nutritional composition obtained for all egg-derived product formulations.

Tabla 1. Composición nutricional obtenida para los ovoproductos desarrollados.

Parameters*	Samples			
	CE	LE	AE	FE
Moisture (g/kg)	778.60 ± 5.60 <sup>a</sup>	758.10 ± 4.60 <sup>b</sup>	781.50 ± 3.50 <sup>a</sup>	781.70 ± 3.40 <sup>a</sup>
Protein (g/kg)	105.60 ± 2.10 <sup>a</sup>	98.50 ± 3.10 <sup>b</sup>	95.2 ± 4.00 <sup>b</sup>	96.80 ± 5.30 <sup>b</sup>
Ash (g/kg)	13.50 ± 2.40 <sup>a</sup>	14.50 ± 0.18 <sup>a</sup>	11.50 ± 0.20 <sup>b</sup>	12.20 ± 0.31 <sup>b</sup>
Carbohydrates (g/kg)	21.20 ± 2.90 <sup>c</sup>	36.60 ± 0.24 <sup>a</sup>	27.70 ± 0.27 <sup>b</sup>	22.90 ± 0.34 <sup>c</sup>
Total Fat (g/kg)	81.10 ± 6.00 <sup>c</sup>	92.30 ± 0.63 <sup>a</sup>	84.10 ± 0.55 <sup>b</sup>	86.40 ± 0.43 <sup>b</sup>
ΣSFA (g/kg)	31.30 ± 1.50	29.30 ± 1.9	32.00 ± 1.80	32.80 ± 1.60
ΣUFA (g/kg)	49.80 ± 1.10 <sup>c</sup>	62.00 ± 1.70 <sup>a</sup>	54.00 ± 1.10 <sup>b</sup>	54.50 ± 0.80 <sup>b</sup>
ALA (g/kg)	0.40 ± 0.04 <sup>b</sup>	10.00 ± 2.10 <sup>a</sup>	0.38 ± 0.02 <sup>b</sup>	0.37 ± 0.01 <sup>b</sup>
EPA + DHA (g/kg)	0.41 ± 0.02 <sup>c</sup>	0.32 ± 0.19 <sup>d</sup>	2.00 ± 1.30 <sup>a</sup>	1.82 ± 1.10 <sup>b</sup>
ω-6/ω-3 ratio	24.59 ± 0.92 <sup>a</sup>	1.69 ± 1.15 <sup>d</sup>	5.39 ± 1.31 <sup>c</sup>	6.33 ± 1.18 <sup>b</sup>
UFAs/SFAs ratio	1.55 ± 0.15 <sup>b</sup>	2.11 ± 0.19 <sup>a</sup>	1.69 ± 0.13 <sup>b</sup>	1.68 ± 0.11 <sup>b</sup>
Energy content (kcal/kg)	1237.1 ± 22.1 <sup>b</sup>	1371.1 ± 18.7 <sup>a</sup>	1248.5 ± 18.60 <sup>b</sup>	1256.4 ± 15.40 <sup>b</sup>

Notes: \*Results are expressed as average ± standard deviation (SD). Different letters (<sup>a-d</sup>) in the same row differ significantly ( $P < 0.05$ ). CE: Control egg-derived products; LE: linseed-oil-enriched egg-derived products; AE: Algae-oil-enriched egg-derived products; FE: fish-oil-enriched egg-derived products.

Nota: \*Los resultados se muestran como media ± desviación estándar (SD). Letras diferentes (<sup>a-d</sup>) en la misma fila muestran diferencias estadísticamente significativas ( $P < 0,05$ ). CE: ovoproductos control; LE: ovoproductos elaborados con aceite de linaza; AE: ovoproductos elaborados con aceite de microalgas; FE: ovoproducto elaborado con aceite de pescado.

Carbohydrates and ash content was significantly higher in LE samples than in all the other egg-derived products. Total fat content was also higher in LE samples than in AE or FE samples, but the fat content in AE and FE samples was significantly higher than obtained in CE samples. LE samples were formulated with a higher amount of *n*-3-enriched oil than the others formulations to meet the requirements established by European legislation. On the other hand, this addition causes a significant increase of fat (higher in LE samples than all the other ones and higher in AE and FE with respect to CE) and the energy content (only significantly higher in LE samples than all the other ones).

As it was expected, addition of *n*-3 high content oil to LE, AE and FE products changed its lipid profile (Table 1) with respect to CE. Thus, significant differences were observed in the ALA content between LE and the other egg-derived products. For LE, the obtained results indicated an ALA content of 10 g/kg. This content significantly overcomes the value indicated in the EU legislation (8110 mg ALA/kg, taking into account that it must contain 6 mg/kcal). This ALA content in LE samples was significantly higher than the values obtained for all other egg-derived products, which reported values of about 370–400 mg/kg.

Regarding the other egg-derived modified products (AE and FE), the status of products that are “high in *n*-3 PUFAs” was achieved through its content in DHA+EPA (0.8 mg/k g and 100 kcal) because the oil employed in their formulations contains high amount of these fatty acids. Table 1 shows that concentrations of DHA+EPA obtained for FE (1.36 g/kg) and AE (1.25 g/kg) were significantly higher than those obtained for LE (0.32 g/kg) or CE (0.41 g/kg), respectively.

It is very important to underline that although previous works obtained eggs enriched in *n*-3 PUFAs by modifying hens’ feed or entirely replacing egg yolk with emulsion oils with *n*-3 PUFAs, the results obtained were less effective than technological methods used in this work. For example, Petrovic et al. (2012) added linseed oil (10–40 g/kg) to feeding hens and obtained eggs with higher content of *n*-3 PUFAs. When 40 g/kg of linseed oil was added to hens’ diet, egg yolks obtained showed values of about 32.2 g/kg of ALA in total fat. In this

work, an addition of 1.8 g/kg of linseed oil in the yolk resulted in values of 110 g/kg ALA in total fat. Thus, we tripled the ALA concentration in the final egg-derived product employing less than a half of linseed oil. This demonstrated that fortification is a more cost effective and practical way to provide micronutrients to communities in need without substantially increasing the total cost of the food product at the point of manufacturing (Wimalawansa, 2013), especially when manufacturing large quantities of foods. Scientific opinion from the European Food Safety Authority (EFSA, 2009) recommended an intake of 2 g of ALA per person per day. Thus, taking into account the results obtained here, eating 100 g of LE egg-derived product, consumers can achieve about 50% of ALA recommended per person per day.

In the same way, technological methods were more cost effective to obtain egg-derived products’ “high in *n*-3 PUFAs” based on their DHA+EPA content. Thus, Gonzalez-Esquerria and Leeson (2000) developed feed for laying hens containing about 20–60 g/kg of menhaden oil with the aim of obtaining *n*-3 PUFAs-enriched eggs. Employing feed with 2% of this oil, they achieved eggs with contents of DHA+EPA at about 1.96 g/kg. However, in this work contents of 1.82 g/kg of egg-derived product were obtained by adding a much lower concentration (0.42%) of fish oil. Bruneel et al. (2013) and Lemahieu et al. (2013) used different species of microalgae to manufacture feed for hens with different contents of EPA+DHA. After feeding hens with this *n*-3 PUFAs-enriched feeds, the maximum content of DHA+EPA found in eggs was 100 g/kg. In this work, egg-derived products supplemented with a 3.5 g/kg of microalgae oil gave a result of 2 g/kg of DHA+EPA in the product. Intake of 100 g of the AE product provides 80% of DHA+EPA recommended per person per day, according to EFSA (2009).

Another important factor to consider for products enriched with *n*-3 PUFAs is the *n*-6/*n*-3 ratio. According to the European Food Safety Agency (2009), in European countries, the intake of *n*-3 PUFAs is usually lower than recommended, whereas consumption of *n*-6 PUFAs is higher than recommended. As it is well known, the main *n*-6 PUFA in eggs is linoleic acid. After ingestion, linoleic acid is converted by enzymatic desaturation



and elongation in *n*-6 long chain PUFAs and eicosanoids (Rose & Holub, 2006), which have detrimental health effects due to pro-aggregatory and pro-inflammatory activity (Lands, 2014). In contrast, *n*-3 PUFAs possess positive effects such as those related with the inhibition of the synthesis of thromboxane A2 and the production of prostaglandins that cause vasodilation (Chow, 2008). As was stated in Table 1, it is possible to observe significant differences in the *n*-6/*n*-3 ratio between all the developed egg-derived products; LE samples showed the lower ratio (1.69) and CE samples showed the higher ratio (24.59).

Unlike PUFAs, saturated fatty acids (SFAs) have been linked to an increased risk of cardiovascular disease (Siri-Tarino, Sun, Hu, & Krauss, 2010). The unsaturated fatty acids (UFAs)/SFAs ratio increased as the amount of added oil increased. The LE samples had the highest UFAs/SFAs ratio (2.11) and significant differences with respect to all other egg-derived products. Therefore, consumption of LE samples could be considered more adequate for human health than those the other egg-derived foods developed due to a reduction of the levels of SFAs and increasing the content of UFAs (Chow, 2008).

### 3.2. Lipid oxidation

An important aspect that should be taken into account when fortifying foods with *n*-3 PUFAs is the possibility of increased lipid oxidation risk, because *n*-3 PUFAs are highly susceptible to oxidation (Meynier et al., 2014). The oxidative decomposition of PUFAs result in aldehydes and unsaturated carbonyls, which are harmful to human health and could generate unpleasant flavours that make the product unacceptable to the consumer (Brewer, 2009). Table 2 shows the results obtained for the levels of oxidation on day 0 and day 30 or frozen storage. On day 0, MDA levels were directly proportional to the amount of oil added and to the presence not of antioxidants in the oil formulation. Thus, LE samples had the highest level of MDA probably due to the absence of antioxidants in the oil formulation causing a partial oxidation of the *n*-3 PUFAs after its production and commercialization. FE and AE however had values near to the CE sample, which had the lowest value of MDA. This can be explained by the fact that these oils present tocopherols and ascorbyl palmitate in their formulation by stabilizing the *n*-3 PUFAs. After storage, however, the presence or non-presence of antioxidants in the oil formulation did not influence the levels of MDA. Thus, at day 30, CE samples still had less MDA than the *n*-3 PUFAs-enriched samples. However, for all formulations the obtained MDA values were low, not affecting to the quality of the product since it should be noted that only values from 0.002 g MDA/kg can be sensory detected (Martinez et al., 2012).

Comparing the obtained results, it was found that LE and AE samples had significantly higher levels of MDA than FE or CE samples. This fact can be due to CE samples having the lowest UFAs/SFAs ratio, which makes it less susceptible to oxidation than the others. On the other hand, microalgae oil employed for AE elaboration was rich in DHA, which makes it more susceptible to lipid oxidation by the large presence of double bonds. This would explain why the level of oxidation is very similar between AE samples and LE samples, despite the fact that AE samples contain five times less *n*-3 PUFAs than LE samples. Additionally, the results obtained were consistent with those found by Kassis, Gigliotti, Beamer, Tou, and Jaczynski (2012), in which the egg product made from algae oil had a higher degree of lipid oxidation than products made from fish oil and linseed oil.

### 3.3. Colour measurement

With respect to colour parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ), CE samples showed the highest value of  $L^*$  (Table 3) with significant differences compared to AE and LE samples. LE samples showed the lowest level of brightness (58.16), so adding linseed oil reduced the brightness of the yolk. On the other hand, CE and LE samples had the highest redness ( $a^*$ ), which was significantly higher than AE and FE samples. Yellowness ( $b^*$ ) was significantly higher in CE samples than all other samples. Although the consumer preferences of egg yolk colour is generally linked to the geographical location, culture and traditions, it is true that in most parts of the world, consumers prefer deeply orange yolks (Beardswort & Hernandez, 2004). Consequently, CE and LE products are more adequate than AE or FE products for their commercialization because of their colour parameters.

### 3.4. Consumer acceptability

The results of consumer acceptability analyses are shown in Table 4. Although results obtained for CE samples were slightly higher than those obtained for LE, FE or AE samples, significant differences were not observed in any of the parameters tested. This is a positive aspect because adding oil can affect flavour and odour and consequently cause the rejection of the product by the consumer. In addition, the score values obtained for all samples were above average. These results are in concordance with those obtained by Kassis et al. (2012) for an egg product also enriched in  $\omega$ -3 rich. However, although results are similar it should be noted that they do not include appearance into the parameters to be evaluated and from our point of view, this is important since

Table 2. Lipid oxidation (mg MDA/kg egg yolk) of different treatments on day 0 and day 30.

Tabla 2. Oxidación lipídica (mg MDA/kg yema de huevo) de los diferentes ovoproductos desarrollados en el día 0 y día 30.

Storage time*	Samples			
	CE	LE	AE	FE
Day 0	1.57 ± 0.03 ( $\times 10^{-4}$ ) <sup>b</sup>	2.39 ± 0.02 ( $\times 10^{-4}$ ) <sup>a</sup>	1.65 ± 0.04 ( $\times 10^{-4}$ ) <sup>b</sup>	1.73 ± 0.03 ( $\times 10^{-4}$ ) <sup>b</sup>
Day 30	3.04 ± 0.05 ( $\times 10^{-4}$ ) <sup>b</sup>	3.66 ± 0.05 ( $\times 10^{-4}$ ) <sup>a</sup>	3.73 ± 0.03 ( $\times 10^{-4}$ ) <sup>a</sup>	3.22 ± 0.03 ( $\times 10^{-4}$ ) <sup>b</sup>

Notes: \*Results are expressed as average ± standard deviation (SD). Different letters (<sup>a-c</sup>) in the same row differ significantly ( $P < 0.05$ ). CE: Control egg-derived products; LE: linseed-oil-enriched egg-derived products; AE: Algae-oil-enriched egg-derived products; FE: fish-oil-enriched egg-derived products.

Nota: \*Los resultados muestran como media ± desviación estándar (SD). Letras diferentes (<sup>a-c</sup>) en la misma fila muestran diferencias estadísticamente significativas ( $P < 0,05$ ). CE: ovoproductos control; LE: ovoproductos elaborados con aceite de linaza; AE: ovoproductos elaborados con aceite de microalgas; FE: ovoproducto elaborado con aceite de pescado.

Table 3. Colour parameters obtained for all egg-derived products developed.

Tabla 3. Parámetros de color obtenidos para todos los ovoproductos desarrollados.

Parameters*	Samples			
	CE	LE	AE	FE
<i>L</i> *	66.48 ± 0.68 <sup>a</sup>	58.16 ± 0.14 <sup>c</sup>	65.07 ± 0.45 <sup>b</sup>	65.8 ± 0.54 <sup>a,b</sup>
<i>a</i> *	22.04 ± 0.64 <sup>a</sup>	21.73 ± 0.33 <sup>a</sup>	18.94 ± 0.25 <sup>b</sup>	19.21 ± 0.33 <sup>b</sup>
<i>b</i> *	62.52 ± 2.12 <sup>a</sup>	58.35 ± 0.57 <sup>b</sup>	50.81 ± 0.85 <sup>c</sup>	50.20 ± 1.06 <sup>c</sup>

Notes: \*Results are expressed as average ± standard deviation (SD). Different letters (<sup>a-c</sup>) in the same row differ significantly ( $P < 0.05$ ). CE: Control egg-derived products; LE: linseed-oil-enriched egg-derived products; AE: Algae-oil-enriched egg-derived products; FE: fish-oil-enriched egg-derived products.

Nota: \*Los resultados muestran como media ± desviación estándar (SD). Letras diferentes (<sup>a-c</sup>) en la misma fila muestran diferencias estadísticamente significativas ( $P < 0,05$ ). CE: ovoproductos control; LE: ovoproductos elaborados con aceite de linaza; AE: ovoproductos elaborados con aceite de microalgas; FE: ovoproducto elaborado con aceite de pescado.

Table 4. Consumer acceptability attributes obtained for all egg-derived products developed.

Tabla 4. Parámetros de aceptabilidad del consumidor obtenidos en los diferentes ovoproductos desarrollados.

Consumer acceptability*	Samples			
	CE	LE	AE	FE
Appearance	5.25 ± 1.04	5.13 ± 1.13	4.75 ± 1.16	5.13 ± 1.13
Odour	5.13 ± 0.84	4.75 ± 0.71	4.75 ± 1.04	4.63 ± 0.74
Flavour	4.63 ± 0.52	3.88 ± 0.83	4.00 ± 0.93	3.63 ± 1.19
Texture	4.38 ± 0.74	4.00 ± 0.76	3.25 ± 1.28	4.13 ± 0.83
Acceptability	4.63 ± 1.06	3.88 ± 0.99	3.75 ± 1.04	4.00 ± 1.07

Notes: \*Results are expressed as average ± standard deviation (SD). CE: Control egg-derived products; LE: linseed-oil-enriched egg-derived products; AE: Algae-oil-enriched egg-derived products; FE: fish-oil-enriched egg-derived products.

Nota: \*Los resultados muestran como media ± desviación estándar (SD). CE: ovoproductos control; LE: ovoproductos elaborados con aceite de linaza; AE: ovoproductos elaborados con aceite de microalgas; FE: ovoproducto elaborado con aceite de pescado.

in our process one of the objectives was reproducing the visual appearance of an egg.

#### 4. Conclusions

The results obtained here indicate that the technological development of egg-derived products enriched with *n*-3 PUFAs provide a wide range of advantages. First, it is noteworthy that the developed egg-derived products required less than 2% of oil employed as sources of *n*-3 PUFAs to accomplish the requirements of the EU legislation to label the product as “high in *n*-3 PUFAs”, much less than similar products obtained through modification of hens’ feed. According to the combined results of nutritional composition, colour and consumer acceptability, egg-derived products supplemented with linseed oil seem to be the most adequate. Analysis of lipid oxidation during freezing storage and consumer acceptability did not reveal important disadvantages with respect to CE products. Consequently, the technological methods developed in this work could be a good alternative for food industries to obtain egg-derived products with a high content of *n*-3 PUFAs. It could be especially interesting for catering companies that can easily develop a product with high added value, and with little increase in cost, without depending on external providers (such as hen farms).

#### Disclosure statement

No potential conflict of interest was reported by the authors.

#### Funding

The authors wish to thank the Centro para el Desarrollo Técnico Industrial (CDTI) from the Ministerio de Economía y Competitividad for financial support.

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