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n-3 Fatty acid content in eggs laid by hens fed with marine algae and sardine oil and stored at different times and temperatures

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Abstract Inclusion of sardine oil (SO) in diets for laying hens significantly increases the *n*-3 polyunsaturated fatty acids (PUFAs) in the egg, but these are more sensitive to oxidation, so the storage time and temperature can cause a decrease in their concentration. Therefore, the objective of this study was to determine the effect of algae *Macrocystis pyrifera*, *Enteromorpha* spp., and *Sargassum sinicola* on *n*-3 PUFA contents in eggs from laying hens fed diets supplemented with sardine oil and stored for different times (0, 15, and 30 days) and temperatures (20°C and 4°C), for 8 weeks. One hundred and twenty hens were divided into four treatments: T1 (commercial diet), T2 (2% SO+10% *M. pyrifera*), T3 (2% SO+10% *Enteromorpha*), and T4 (2% SO+10% *S. sinicola*). At the end, 50 eggs per treatment were collected to quantify total lipids and egg *n*-3 PUFAs at different times (0, 15, and 30 days) and temperatures (20°C and 4°C) of storage. The results were analyzed using a 3×3×2 factorial design, and Tukey test to compare means ($P<0.05$). The results show that *M. pyrifera* and *S. sinicola* had a better effect on eicosapentaenoic acid, while *Enteromorpha* was better for docosahexaenoic acid. In relation to time and temperature, the content of the fractions analyzed in the three treatments at 15 days/4°C had a lower loss compared with eggs analyzed at day 0/20°C.

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Introduction

Changes in food consumption among Mexicans have brought an increase in chronic degenerative diseases, such as diabetes, obesity, and cardiovascular problems, among others (ENN 1999; Bhathena 2008). A serious problem related to obesity is the number of diseases associated with it, such as heart disease and diabetes. Various studies have demonstrated that a diet high in *n*-3 polyunsaturated fatty acids (*n*-3 PUFAs) is beneficial in the treatment of obesity and diabetes and that these compounds also reduce the plasma concentration of triglycerides and insulin. However, these effects depend on the quantity and time of intake of *n*-3 PUFAs as well as on the stage of the disease. Research has indicated that the greatest benefit is achieved when *n*-3 PUFA consumption has occurred for a year (Weisinger et al. 2008).

In non-diabetic but hyperlipidemic patients, it has been observed that consumption of fish oil reduces the risk of heart disease due to its high *n*-3 PUFA content. This effect has been linked specifically to eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) acid because of their antithrombotic and anti-arrhythmic effects and their prevention of atherosclerosis by reducing cholesterol content in plasma (Simopolous 1999; Castro-Gonzalez 2002).

The primary sources of the *n*-3 PUFA alpha-linolenic acid (ALA) are vegetable oils such as linseed oil (53.3%), canola oil (9.3–12%), rapeseed oil (10.9%), walnut oil (10.4%), and soybean oil (6.8–7.3%), while fatty fish such as tuna, trout, and sardines possess high levels of EPA and DHA (33.9%, 21.9%, and 37.10% of total *n*-3 PUFAs,

respectively; Astiasarán and Martínez 2000; Castro-Gonzalez 2002).

However, according to FAO (2003) data, in Mexico, as in other western countries, there is a low consumption rate of fish due to common eating habits and cost, among other factors. For this reason, other mechanisms have been sought to bring the benefits of *n*-3 PUFAs to the Mexican population. One alternative has been the incorporation of fishing industry by-products with high *n*-3 PUFA contents, such as fish oil, into the diet of hens, with the main of goal *n*-3 PUFAs concentrating in the egg yolk.

Several researchers (Leskanich and Noble 1997; Simopolous 2000; González-Esquerra and Leeson 2001) believe that eggs are excellent vehicles to achieve this objective for multiple reasons, including their low calorie content (75 kcal egg^{-1}), protein quality, culinary versatility, and low cost. This use of eggs is of particular interest in Mexico because the country has the highest consumption rate of fresh eggs in the world (21.6 kg per capita annually; UNA 2010). Fresh egg is understood as a food that has not been submitted to any preservation processes whose physical characteristics and chemical and microbiological properties are maintained at an optimal level of edible quality and whose age since laying is not greater than 15 days; included in this classification are products stored in refrigerators for periods no longer than 10 days (NOM-159-SSA1-1996; NMX-FF-079-SCFI-2004), although in practice, housewives are accustomed to store eggs either at room temperature (20°C) or in a refrigerator (4°C) for periods longer than 15 days.

Some studies have been conducted (Van Elswyk 1997; Castillo-Badillo et al. 2005) in which hens' diets were supplemented with fish oil in order to increase the *n*-3 PUFA contents of their eggs (1.02% ALA, 15.54% EPA, and 10.70% DHA/% of total fatty acids). Although the results have been satisfactory, one drawback has been that *n*-3 PUFAs quickly oxidize due to the long-chain hydrocarbons they contain, causing rancidity in the product and, as a result, reducing their shelf life.

Consequently, it has been considered appropriate to add antioxidants to fish oil-supplemented hen diets, with the goal of reducing the risk of oxidation in eggs and concomitant decrease in *n*-3 PUFA contents during storage. The most commonly used antioxidant is vitamin E. However, marine algae are an additional alternative to consider. In the poultry industry, algae have been used as a source of minerals and natural pigments as a means to reduce eggs' cholesterol content (Rodríguez et al. 1995; Herber-McNeill and Van Elswyk 1998; Ramos et al. 1998; Carrillo et al. 1992, 2002, 2008). Additionally, algae are a natural source of antioxidants (Ortega et al. 1993; Allen et al. 2001; Casas-Valdez et al. 2006; Yuan 2008).

Some antioxidants present in marine algae are carotenoids, polyphenols, and vitamins such as E and C (Yuan 2008). The type and content of each varies among different species. The marine algae species *Macrocystis pyrifera* (brown algae), *Enteromorpha* spp. (green algae), and *Sargassum sinicola* (brown algae) are abundant along the west coast of the Gulf of California in Mexico (Hernández et al. 1990; Casas et al. 1993).

Furthermore, the objective of this study was to determine the effect of including different types of algae (*M. pyrifera*, *Enteromorpha* spp., and *Sargassum* spp.) in fish oil-supplemented hen diets on the concentration of three *n*-3 PUFAs—ALA, EPA, and DHA—in eggs stored for different periods of time (0, 15, and 30 days) and at different temperatures (4°C and 20°C).

Materials and methods

Seaweeds were collected in Baja California Sur, Mexico: *M. pyrifera* in Bahia Tortugas and *Enteromorpha* spp. and *Sargassum* spp. on the beaches along La Paz Bay. Samples were collected manually from the intertidal zone at depths between 60 cm and 1.20 m. Afterward, the algae were spread over a cement slab and sun-dried for 2 days. The seaweeds were ground in a hammer mill, and then in a knife mill, using a 1-mm mesh. A sample of 1 kg of each seaweed was taken by quartering and ground; chemical analyses were carried out in triplicate. The sardine oil was from Guaymas, Sonora, México.

The following analyses were carried out in the algae meal using the standard methods of AOAC (2000): moisture content (oven-dried at 60°C to constant weight), ash (ignition at 550°C in an electric furnace), crude fiber (Fibertec apparatus), ether extract (Soxhlet apparatus), and nitrogen content were determined using the Kjeldahl method, and the conversion factor of 6.25 was used to calculate protein content. The sample preparation for mineral content was an acid hydrolysis. Ca, P, Na, K, and Mg were determined by spectrophotometry procedures described by AOAC (2000). Gross energy was determined using a bomb calorimeter; total lipids were determined by following method 923.07 described by AOAC (2000). The fatty acid profiles of seaweeds and sardine oil were analyzed by gas chromatography using a CB23 column (30-m×0.25-mm i.d.) on a Varian 3400 CX gas–liquid chromatograph equipped with an autosampler and a flame ionization detector. Nitrogen was used as a carrier gas at a flow rate of 30 mL min^{-1}. The operation temperatures were: column, 230°C; injector, 150°C; detector, 300°C. The retention times were compared with methyl-fatty acid standards (AOAC method 969.33).

Experimental trial

One hundred and twenty 35-week-old Leghorn hens were randomly distributed into four treatments with three

replicates of ten hens each. The treatments consisted in the inclusion of 2% of sardine oil (SO) and 10% of each alga (*M. pyrifera*, *S. sinicola*, and *Enteromorpha*) into the diet of the hens; a control diet (C) was also prepared. Algae partially substituted sorghum, soybean, and salt in the diets (Table 1). All experimental diets were isonitrogenous (17% PC) and isocaloric (11.50 MJ g^{-1}). The diets were formulated to contain adequate nutrient levels, as defined by NRC (1994). The study lasted 8 weeks, with water and feed ad libitum.

A total of 15 eggs per treatment were randomly collected from each treatment at the end of week 8 and stored at different temperatures (4°C and 20°C) and for different times (0, 15, and 30 days). They were broken and the yolk and albumen mixed homogeneously. Chemical analyses (total lipids and *n*-3 PUFAs) were carried out. Five 1 g aliquots of whole egg were extracted using chloroform–ethanol (1:1) for lipid analysis (AOAC method 923.07). Esters of fatty acids from all lipid extracts were prepared using 0.2 g aliquot of each extract. Methanolic boron trifluoride was employed as the esterifying agent (AOAC method 969.33).

Statistical analysis

The data were subjected to variance analysis according to a factorial arrangement, 3×3×2. Differences among means were analyzed with Tukey's test, with a confidence level of 95% ($P < 0.05$, SAS v.6.12).

Results

The sardine oil employed in this study had concentrations of *n*-3 PUFAs as follows: 1.02 ALA, 15.54 EPA, and 10.70 DHA as % of total fatty acids (%TFA, Table 2).

In Table 3, the results of the marine algae chemical analysis are presented. The total lipid (TL) contents in the algae were 2.88% of dry weight in *M. pyrifera*, 1.93% in *S. sinicola*, and 2.27% in *Enteromorpha.* The highest content

Table 1 Experimental diets supplemented with sardine oil and seaweeds (%)

Ingredients	Control (T1)	SO+Mp (T2)	SO+Ss (T3)	SO+Espp (T4)
Sorghum (9%)[a]	62.69	50.14	50.14	52.16
Soybean (48%)[a]	23.83	24.37	24.84	23.35
Seaweeds (10%)	0.0	10.00	10.00	10.00
Sardine oil (2%)	0.0	2.00	2.00	2.00
Calcium carbonate	8.82	8.66	7.93	8.42
Soybean oil	2.35	3.05	2.99	2.35
Calcium orthophosphate 1820[b]	1.45	1.12	1.49	1.45
Vitamin and mineral premix[c]	0.25	0.25	0.25	0.25
Salt (NaCl)	0.36	0.15	0.15	0.15
NR micoad (klin-sil)[d]	0.1	0.1	0.1	0.1
Choline chloride 60	0.05	0.05	0.05	0.05
Methionine 98	0.045	0.05	0.046	0.05
Avelut powder[e]	0.005	0.005	0.005	0.005
Avired[f]	0.003	0.003	0.003	0.003
Furacyl[g]	0.001	0.001	0.001	0.001
Calculated nutritive value				
Crude protein (g 100 g^{-1})	17.00	17.00	17.00	17.00
Metabolizable energy (MJ g^{-1})	11.50	11.50	11.50	11.56

SO sardine oil, *Mp M. pyrifera*, *Ss S. sinicola*, *Espp Enteromorpha* spp.

[a] Crude protein content

[b] Calcium orthophosphate P 21% min, Ca 13% min, F 0.21% max

[c] Vitamins and mineral mix, per kg, for laying hens: 12,000 UI vitamin A; 2,500 UIP vitamin D_3; 30 UI vitamin E; 2 mg vitamin K_3; 2.25 mg vitamin B_1; 7.5 mg B_2; 3.5 mg B_6; 0.02 mg B_{12}; 12.5 mg D-pantothenic acid; 0.125 mg biotin; 1.5 mg folic acid. Mineral mix (mg kg^{-1} of complete diet): zinc, 50; copper, 12; iodine, 0.3; cobalt, 0.2; iron, 110; selenium, 0.1; manganese, 110

[d] Mycotoxin sequestrant

[e] Saponified xanthophylls of Azteca marigold (yellow, 15 ppm)

[f] Red xanthophylls (canthaxanthin, 10 ppm)

[g] Furazolidon–bacitracin–zinc

Table 2 Fatty acid composition of SO

Fatty acids (% TFA)	
Saturated fatty acids	28.87
Unsaturated fatty acids	71.13
Monounsaturated fatty acids	39.69
Polyunsaturated fatty acids	31.44
α-linolenic acid (C18:3 ALA) *n*-3	1.02
Eicosapentaenoic acid (C20:5 EPA) *n*-3	15.54
Docosahexaenoic acid (C22:6 DHA) *n*-3	10.70

TFA total fatty acids

of ALA was in *Enteromorpha* (6.4%). EPA and DHA were highest in *M. pyrifera* (4.87% and 1.51%, respectively). In the case of *S. sinicola*, the concentrations of EPA (3.5%) and ALA (2.7%) were similar, but DHA content was the lowest among all three algae.

The type of alga incorporated into the sardine oil-supplemented diets affected the total lipid and *n*-3 PUFA contents of the eggs (Table 4). The TL content of the eggs from T3 and T4 was significantly lower than those from T2 ($P<0.05$). The ALA concentration was higher in the *S. sinicola* algae treatment (T3) compared with treatments using *M. pyrifera* (T2) and *Enteromorpha* (T4, $P<0.05$). Additionally, the inclusion of brown seaweed (T2 and T3) in the hens' diets resulted in higher egg EPA contents relative to treatment T4 ($P<0.05$), while the DHA concentration was greater when green seaweed *Enteromorpha* (T4) was incorporated ($P<0.05$) into the hens' diets compared with the other treatments.

Table 3 Chemical composition of seaweed meal

Nutrient (g 100 g^{-1})	*M. pyrifera*	*S. sinicola*	*Enteromorpha* spp.
Moisture	9.27	7.40	6.70
Ash	33.47	38.35	32.64
Crude protein	10.50	6.57	14.10
Crude fiber	4.32	6.55	5.17
Total lipids	2.88	1.93	2.27
Metabolizable energy (KJ g^{-1})	9.19	10.53	9.94
Calcium	1.24	3.21	2.49
Phosphate	2.57	0.01	3.50
Sodium	3.11	2.01	9.20
Potassium	5.55	5.77	1.80
Magnesium	0.49	0.90	0.71
Fatty acids (% TFA)			
α-Linolenic acid (C18:3 ALA)	1.67	2.65	6.39
Eicosapentaenoic acid (C20:5 EPA)	4.87	3.53	2.88
Docosahexaenoic acid (C22:6 DHA)	1.51	0.60	0.92

TFA total fatty acids

Table 4 Effect of seaweeds meals, time, and temperature on total lipids and *n*-3 PUFA concentrations in eggs

	Total lipids (g 100 g^{-1})	ALA (% TFA)	EPA	DHA
Seaweeds meals				
Macrocystys pyrifera	10.64a	45.17b	72.31a	434.81b
Sargassum sinicola	10.12b	47.09a	73.49a	437.99b
Enteromorpha spp	10.19b	44.30b	61.45b	482.22a
SEM	0.050	0.511	0.550	3.33
Storage (days)				
0	10.85a	54.12a	96.23a	640.36a
15	10.57b	44.05b	63.31b	406.14b
30	9.53c	38.39c	47.72c	308.53c
SEM	0.050	0.511	0.550	3.33
Storage temperature				
20°C	10.19b	43.80b	63.92b	419.53b
4°C	10.44a	47.24a	74.25a	483.82a
SEM	0.041	0.418	0.449	2.719
Interactions				
Seaweed meals × time	0.0001	0.0001	0.0001	0.0001
Seaweed meals × temperature	0.0001	0.0001	0.0001	0.0001
Time × temperature	0.0001	0.0001	0.0001	0.0001
Seaweed meals × time × temperature	0.0001	0.0001	0.0001	0.0001

Different letters in each column indicate significant differences ($P<0.05$)

ALA α-linolenic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *SEM* standard error of the mean, *TFA* total fatty acids

Storage (0, 15, and 30 days) also had a notable influence on the presence of *n*-3 PUFAs in the eggs. As storage time increased, the egg lipid and *n*-3 PUFA contents were reduced significantly ($P<0.05$, Table 4).

The interaction between algae × time × temperature was detected ($P<0.0001$) in the eggs for total lipid content, ALA, EPA, and DHA. The content of TL in treatment T2 was the most affected by temperature, where values were reduced significantly after 15 days when the eggs were maintained at 20°C ($P<0.0001$). The ALA content was higher in treatments T3 and T4 at 20°C, but under 4°C, treatment T2 maintained more stable ALA concentrations ($P<0.0001$). The EPA concentration remained the most stable in treatments T3 and T4 after 15 and 30 days/20°C, but under 4°C, this fatty acid was better conserved in T2 after 15 days. The DHA content appeared less affected after 15 and 30 days/20°C, when *S. sinicola* was incorporated into the hens' diet (T3); however, when the egg was kept under 4°C, it was the

Enteromorpha treatment (T4) that had the best DHA concentrations after 15 and 30 days ($P < 0.0001$).

Discussion

The fact that the sardine oil used in this study had a higher content of EPA than DHA agrees with the data obtained by Cachaldora et al. (2006) who indicated that the proportion of these two fatty acids in fish oil varies by species. In anchovy oil, the EPA concentration is greater than the DHA concentration (approximately 185 gkg^{-1} vs. 85 gkg^{-1}), while the opposite relationship exists in tuna oil (roughly 80 gkg^{-1} EPA vs. 220 gkg^{-1} DHA).

The high EPA and DHA contents in sardine oil justify its use as an alternative for enriching eggs with *n*-3 PUFAs (González-Esquerra and Leeson 2001). It is known that EPA and DHA fatty acids from fish oil or from some algae rich in DHA are deposited directly into the egg yolk and in other tissues (Grobas and Mateos 1996). In fact, greater increases have been achieved in egg DHA content (3.5%) and EPA content (0.52%) with lower amounts of fish oil than with greater numbers of oleaginous seeds (Hargis and Van Elswyk 1993).

In general, the lipid content in marine algae is low and can fluctuate between 0.65% and 3% (Carrillo 1999; Jiménez-Escrig and Goñi 1999); nevertheless, these algae constitute an important source of PUFAs (Kumari et al. 2010). The results obtained in this study concerning the *n*-3 PUFA composition of different algae agree with results obtained by other authors in the sense that brown algae are a good source of C20:5, while green algae are a good source of C18:3 (Jamieson and Reid 1972; Ragan 1981; Shameel 1990; Kumari et al. 2010). The fatty acid composition in marine algae is very complex and can vary even within the same species, given that the major variations occur according to the time of the year, collection zone and depth, drying method, etc. (Floreto et al. 1993; Jiménez-Escrig and Goñi 1999; Nelson et al. 2002; Sánchez-Machado et al. 2004).

The effect of algae type on the lipid and *n*-3 PUFA contents in the eggs

The fact that the eggs in treatment T2 (with *M. pyrifera*) had a greater TL content could be a result of the greater concentration of TL in this alga compared with the other species.

Additionally, the fact that the greatest deposition of DHA in the eggs was obtained when hens consumed *Enteromorpha* (an alga with higher ALA content), and not when they consumed brown algae (with more EPA), agrees with the results of other authors (Caston and Leeson 1990; Jia et al. 2008) when they incorporated different amounts of linseed (an ingredient with a high ALA content) into the diet of laying hens. This result could be explained, as indicated by Cherian and Sim (1997), by the fatty acid composition of an egg yolk which is the result of a combination of fatty acids, both formed de novo through lipogenesis and provided in the diet. In poultry, as in humans, ALA can be converted into EPA and DHA through various processes of elongation and desaturation. This conversion is slow and can be affected by various factors, including competition between *n*-6 and *n*-3 fatty acids for the delta-desaturase enzyme, given the conversion of ALA to EPA and LA to arachidonic acid (Yannakopoulos 2007). In the present case, it was evident that DHA enrichment of the egg was more effective when the green alga *Enteromorpha* was incorporated into the hens' diet than when brown algae were included. It is now recognized that DHA's biosynthesis is more complex than once thought and that more than one enzyme is required in the synthesis of polyunsaturated fats (Jia et al. 2008). It is possible that the green algae possess (1) compounds that facilitate the synthesis or formation of ALA and DHA in the yolk (2) or antioxidant compounds (not present in brown algae) that prevent the loss of DHA in eggs (Yuan 2008).

The effect of storage time

It was observed that the greatest concentration was achieved in fresh eggs at 0 day; as time passed, this concentration diminished. The fact that the quantified variables (TL and *n*-3 PUFAs) decreased over time, both at 20°C and at 4°C, could have been due to lipid oxidation and auto-oxidation occurring in the eggs. The factors involved in lipid oxidation are O_2, certain minerals (mainly Fe and Cu), storage time, temperature, and pH. It is logical to expect that as storage time increases, so will the level of lipid degradation in the eggs. Because the eggshell is a porous structure, it allows O_2 to enter; there was a modification in the pH. This effect is reflected by increasing the pH over storage time (Li-Chan et al. 1995; Oliveira et al. 2009). Another factor that influences changes in egg composition is the hydrolysis of albumen proteins. Belitz and Grosch (1997) reported that, between the first and second weeks of storage, the movement of water from the albumen to the yolk is observed as the flattening of the yolk and greater fluidity in the albumen due to changes in pH, altering the natural conditions of the egg.

Metal ions play a role in the propagation stage of oxidation fatty acids, catalyzing the breakdown of lipid hydroxiperoxides to produce the radicals acoxyl or peroxyl, which themselves may initiate new oxidation chains. Fe in eggs is bound to proteins in the albumen, which, in the presence of thiol, induce auto-oxidation, producing H_2O_2, an oxidant agent that promotes lipid peroxidation of unsaturated fatty acids. Another oxidation reaction occurring in the egg is the

production of hydroxyl radicals (the Fenton reaction; Wong 1989).

The effect of temperature

Temperature also influences TL and *n*-3 PUFA contents. The values that were maintained were for eggs stored at 4° C; however, ALA did not exhibit differences in samples stored at either 4°C or 20°C. In the presence of heat and the absence of O_2, unsaturated fatty acids give rise mainly to dimers and cyclic compounds that generate more free radicals. The combination of these fragments gives rise to short- and long-chain fatty acids as well as linear-chain dicarboxylic acids and hydrocarbons (Figge 1971). Belitz and Grosch (1997) mentioned that eggs are best stored between 0°C and 1.5°C, with 85–95% relative humidity.

The observed algae×storage time×temperature interaction showed that ALA and EPA contents were greater in treatments T3 (with *S. sinicola*) and T4 (with *Enteromorpha*) after 15 and 30 days at 20°C, but under 4°C, the T2 (*M. pyrifera*) treatment maintained the most stable ALA and EPA concentrations. The DHA content appeared less affected after 15 and 30 days at 20°C with T3, but at 4°C, the T4 treatment showed the greatest fatty acid concentrations after 15 and 30 days. The explanation behind this phenomenon could be that, in general, marine algae contain compounds (tocopherols, carotenoids, phenols, and vitamins E and C) that protect fatty acids from oxidation.

The role of tocopherols as antioxidants centers on their capacity to increase the period of induction or the initiation of unsaturated fatty acid oxidation in eggs (Coultate 1998). A content of 107.14 mg tocols/100 g lipids has been reported for green algae, while for brown algae is 111.29 mg tocols/100 g lipids (Yuan 2008).

For carotenoids, it has been reported that green algae contain 180 mg β-carotene/100 g dry algae and that brown algae contain 97 mg β-carotene/100 g dry algae (Yuan 2008). For the species of the genus *Enteromorpha* and *Eisenia bicyclis*, a hydrophobic antioxidant identified as pheophytin *a*, has been isolated, which is a magnesium-lacking chlorophyll (Nakamura et al. 1996).

The stability of the ALA and EPA contents in eggs laid by hens fed with T3 (*S. sinicola*) could also be due to the fact that marine algae contain phenolic compounds, as tannins and phlorotannins, represented by phloroglucinol and the derivatives polymerized from it. The antioxidant activity of these compounds is similar to that of α-tocopherol in impeding oxygen capture. The intensity of this effect depends on the degree of polymerization; less polymerized compounds have greater efficacy. The free radical sequestering capacity and the inhibition of lipoxygenase activity exhibited by these phenolic compounds may be due to the fact that they impede oxygen capture by the substrate (fatty acids), thereby inhibiting the formation of peroxides and/or acting as electrons or hydrogen atom donors (Jiménez-Escrig and Goñi 1999; Yuan 2008).

Chan et al. (1997) reported that *S. sinicola* contains 51.99 mg vitamin C/100 g dry weight, a potent antioxidant that reactivates the α-tocopherol radical such that it regains its original structure and can continue its antioxidant activity. However, the algae were sun-dried, so vitamin C is not present in dehydrated algae because it is extremely sensitive to temperature (Yuan 2008).

In the case of green algae, it is important to point out that different antioxidant compounds can act or work synergistically to achieve an efficient antioxidant effect in eggs enriched with *n*-3 PUFAs. In conclusion, the green alga *Enteromorpha* had a protective effect on the DHA content of eggs, while the brown algae *M. pyrifera* and *S. sinicola* had a similar effect on EPA content. Additionally, eggs were better preserved at 4°C, but decreases of these fatty acids were observed over storage time.

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