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Effect of refrigerated storage on muscle lipid quality of sea bass (*Dicentrarchus labrax*) fed on diets containing different levels of vitamin E

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Abstract

The effect of refrigeration at 1°C on total lipid, phospholipid and fatty acid content of muscle was examined in 4 groups of sea bass (*Dicentrarchus labrax*) fed diets rich in fish oil and supplemented with increasing levels of vitamin E as antioxidant (139, 254, 495, and 942 ppm, respectively). After 1, 3, 6, 9 and 12 days refrigeration, muscle from the 4 sea bass groups contained increasing concentrations of vitamin E (on average 9.4, 13.1, 19.8 and 28.8 ppm) related to diet levels. However lipid parameters were unaffected until the end of the trial. The results indicate that the experimental conditions preserve lipid quality in sea bass muscle at least over the time range examined, irrespective of dietary formula.

1. Introduction

Fish lipids are known to be rich in polyunsaturated fatty acids (PUFA), especially the n-3 PUFA family of linolenic acid (C18:3 n-3) and its derivatives eicosapentaenoic acid (EPA or C20:5 n-3), docosapentaenoic acid (DPA or C22:5 n-3) and docosahexaenoic acid (DHA or C22:6 n-3) which are readily oxidised to form lipid hydroperoxides (Sargent, Henderson & Tocher, 1989). It is well established that during freezing and frozen storage, lipid hydrolysis and PUFA decrease with increasing content of peroxides are two of the most important factors contributing to post mortem deterioration of fish meat quality (Bonnel, 1989). This process is promoted by several factors such as the abundance of PUFA and a high lipid content.

Furthermore tissue vitamin E offers a protective role against lipid peroxidation by scavenging free radicals which are involved in the initiation and propagation of lipid oxidation (Machlin, 1984).

Dietary supplementation with D-α-tocopherol, which carries the highest vitamin E activity in animals (Hamre & Lie, 1995), has been reported to improve the stability of tissue lipids to oxidation in trout (Frigg, Prabucki & Ruhdel, 1990), Atlantic salmon (Waagbø, Sandnes, Torrissen, Sandvin & Lie, 1993), turbot (Stéphan, Guillaume & Lamour, 1995) and sea bass (Messager, Stéphan, Quentel & Baudin Laurencin, 1992; Stéphan, Messager, Lamour & Baudin Laurencin, 1991).

It has been demonstrated that high vitamin E levels preserve trout fillets (Boggio, Hardy, Babbitt & Brannon, 1985), channel catfish (Gatlin, Bai & Erickson, 1992), Atlantic salmon (Waagbø et al.) and turbot (Stéphan et al., 1995) from rancidity during freezing and that they reduce significantly lipid oxidation of Atlantic salmon fillets during refrigeration (Onibi, Scaife, Fletcher & Houlihan, 1996). From this latter point of view, there is no study so far on sea bass, despite its increased production thanks to recent more suitable diets characterized by high energy and high protein content.

These diets nevertheless may change content, composition and distribution of the fat in fish tissues and thus can affect fillet quality during processing and storage. In the present study the effect of different levels of vitamin E in high lipid and PUFA diets on muscle lipid quality in sea bass was investigated during refrigerated storage,

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the most commonly employed means of preservation in the marketing of fish.

2. Materials and methods

2.1. Fish and diets

Six hundred and eighty sea bass (mean weight 208 g) from a commercial producer were randomly subdivided into four groups of 170 individuals and placed into 20 m^3 tanks with a continuous flow of filtered and oxygenated seawater. During the experiment the temperature was ranged from 18.2 to 26.3°C, the salinity from 32 to 40‰ and dissolved oxygen was always $\geqslant 8$ ppm.

For 2 weeks fish were fed basal diet rich in fish oil (Table 1) and then for 87 days experimental diets, obtained by adding to the basal diet, increasing amounts of DL-α-tocopherol acetate (BASF) to reach 100 ppm (Diet A); 200 ppm (Diet B); 400 ppm (Diet C) and 800 ppm (Diet D). Nevertheless the real levels of vitamin E recorded by analysis were 139, 254, 493, 942 ppm, respectively.

To avoid lipid peroxidation the diets were supplemented with 100 ppm of butylhydroxytoluene and stored at −20°C until fed to the fish.

Table 1
Total lipid and fatty acid composition of diets (means ± s.d.)[a]

	wt%
Total lipid	27.5 ± 0.8
Fatty acid	
14:0	5.8 ± 0.1
16:0	18.8 ± 0.4
16:1 n-7	5.9 ± 0.1
16:4 n-3	1.1 ± 0.0
18:0	3.7 ± 0.0
18:1 n-9	8.9 ± 0.3
18:1 n-7	2.1 ± 0.1
18:2 n-6	7.2 ± 0.3
18:3 n-3	1.5 ± 0.1
18:4 n-3	3.3 ± 0.1
20:1 n-9	2.1 ± 0.0
20:5 n-3	14.4 ± 0.1
22:1 n-9	2.1 ± 0.1
22:5 n-3	1.1 ± 0.0
22:6 n-3	15.3 ± 0.3
Others[b]	6.7 ± 0.0
∑SFA	30.2 ± 0.5
∑MUFA	21.8 ± 0.4
∑PUFA	47.9 ± 0.3
∑n-3	38.0 ± 0.4
∑n-6	8.7 ± 0.3

[a] Values are means of eight determinations.
[b] Sum of 15:0, 16:1 n-9, 16:2 n-7, 16:3 n-7, 17:0, 20:0, 20:4 n-6, 20:4 n-3, 22:4 n-6, 22:4 n-3, 24:1 n-9.

2.2. Sampling procedure and storage conditions

At the end of the feeding period 100 sea bass per diet were sacrificed, distributed into 10 boxes, covered with flake-ice and black nylon bag, and stored in a refrigerated room at 1°C for 12 days. When necessary, ice was replaced so that fish were always covered.

After 1, 3, 6, 9, 12 days, four pools of five fish for each treatment were eviscerated, filleted and, after the skin was removed, were separately homogenised and stored at −80°C until analysed for vitamin E and lipid composition.

2.3. Chemical analyses

Vitamin E analysis was carried out by the HPLC according to Buttriss and Diplock (1984). Total lipids in the diets and fillets were extracted according to Folch, Lees and Sloane-Stanley (1957) and determined gravimetrically. Phospholipids were quantitatively evaluated by colorimetric determination of phosphorus (Bartlett, 1959; Fiske & Subbarow, 1925; Marinetti, 1962). Fatty acid methylesters obtained by transmethylation from total lipids using 14% BF3 in methanol (Morrison & Smith, 1964), were analysed by gas–liquid chromatography (GLC) on a C. Erba Fractovap 2350 gas chromatograph equipped with RTx 2330 capillary column (30 m×0.25 mm) and hydrogen flame-ionisation detector at 280°C. Temperature was programmed by an increase of 5°C/min from 150 to 230°C and final isotherm. Hydrogen was the carrier gas with a flow rate of 1.2 ml/min. Identification of fatty acids was carried out as reported previously (Pagliarani, Pirini, Trigari & Ventrella, 1986). The fatty acid methylesters separated by GLC were expressed as weight percentages of total fatty acids.

The atherogenic index (A.I.) and the thrombogenic index (T.I.) according to Ulbricht and Southgate (1991) were calculated on the basis of fatty acid percentages including saturated (12:0, 14:0, 16:0, 18:0), monounsaturated fatty acids (MUFA) and PUFA:

$$\text{A.I.} = 12{:}0 + 4\,(14{:}0) + 16{:}0/\text{MUFA} + \text{n-3 PUFA} + \text{n-6 PUFA}$$

$$\text{T.I.} = 14{:}0 + 16{:}0 + 18{:}0/0.5\text{MUFA} + 0.5(\text{n-6 PUFA}) + 3(\text{n-3 PUFA}) + (\text{n-3 PUFA}/\text{n-6 PUFA})$$

2.4. Statistical analysis

The results, reported as mean of four pools ± SD, were submitted to ANOVA followed by SNK test when required.

3. Results and discussion

The results for the lipid content and the fatty acid composition revealed no significant differences in diets which were supplemented with various levels of vitamin E. For this reason the data shown in Table 1 are the mean values of the various diets. Most notable are the high lipid content (27.5%) and, in contrast to other commercial fish diets, the high level of unsaturation (MUFA 21.8%; PUFA 47.9% of total fatty acids) and of n-3 highly unsaturated fatty acids (HUFA) typical of oils of marine origin (20:5 n-3 = 14.4%; 22:6 n-3 = 15.3%).

As in other studies done on various species of farmed fish (Agradi, Abrami, Serrini, McKenzie, Bolis & Bronzi, 1993; Boggio et al., 1985; Frigg et al., 1990; Krajnovic-Ozretic, Najdek & Ozretic, 1994; Onibi et al., 1996; Stéphan et al., 1995; Waagbø et al., 1993), diet composition seems to have a direct influence on muscle tissue. In fact, the fillets of the different groups show increasing concentrations of vitamin E (Table 2) related to diet levels (on average 9.4, 13.1, 19.8 and 28.8 ppm in groups A, B, C, D, respectively) and comparable to those shown by other authors in sea bass (Stéphan et al., 1991), turbot (Stéphan et al., 1995) and in rainbow trout (Boggio et al., 1985) which had been fed diets supplemented with similar amounts of vitamin E.

This different contents of dietary vitamin E did not significantly influence the amount of total lipids, phospholipids, MUFA, PUFA (Table 2) and general muscle fatty acid composition. For this reason the latter are shown in Table 3 as the mean values obtained from the single groups.

Another noticeable effect of diet composition is the fat content in fillets (average 9.2%) which is much higher than the values for wild sea bass previously reported by other authors and which range between 0.1 and 3.0% (Amerio, Ruggi & Badini, 1996), although it appears consistent with the characteristics found in intensively farmed fish, due to high lipid content of feedstuff and overfeeding to obtain fast growth (Watanabe, 1984). In fact, in the case of farmed sea bass percentages of fat vary from approximately 6.0% (Di Bella, Genovese, Salvo & Dugo, 1992; Nicolosi Asmundo, Scerra, Cataldi Lupo, Arculeo, Sinatra & Campisi, 1993) to 7.62% (Amerio et al.), indicating that sea bass are able to store an appreciable amount of fat in muscle and subcutaneous areas, as well as in visceral and hepatic tissues.

The fatty acid profile (Table 3) also confirms that the assimilation patterns of dietary fatty acids in fish muscle reflect the content of the dietary lipid source. This correspondence with diet content is not only related to the high presence of C16, C18:1 n-9 and C 18 polyunsaturated fatty acids, but also to the high percentage of n-3 HUFA (20:5 and 22:6), definitely of exogenous origin. Accordingly, sea bass like other marine fish (especially carnivorous ones) are not capable of bioconverting linolenic acid (18:3 n-3) to 20:5 and 22:6 n-3

Table 2
Vitamin E, total lipid (TL), phospholipids (PL) content, MUFA and PUFA composition (%) of sea bass fillet during conservation (means ± s.d.)[a]

	Groups	Days of storage at 1°C				
		1	3	6	9	12
Vitamin E mg/kg muscle	A	9.2 ± 0.6a	8.3 ± 0.8a	10.1 ± 0.4a	9.8 ± 0.4a	9.8 ± 1.4a
	B	12.9 ± 0.8b	11.5 ± 1.6b	13.8 ± 0.6b	13.4 ± 0.9b	14.0 ± 1.3b
	C	17.8 ± 0.7c	17.2 ± 1.8c	20.3 ± 1.5c	21.1 ± 2.3c	22.5 ± 3.2c
	D	31.4 ± 2.4d	26.5 ± 1.9d	28.2 ± 2.3d	27.7 ± 2.3d	30.4 ± 2.0d
TL g/100 g muscle	A	9.9 ± 0.5	8.6 ± 0.8	9.1 ± 0.8	10.5 ± 1.2	10.4 ± 1.1
	B	7.7 ± 0.1	7.9 ± 0.3	9.2 ± 0.9	9.4 ± 1.2	9.9 ± 1.5
	C	8.9 ± 0.8	8.6 ± 1.5	8.3 ± 1.9	9.1 ± 0.8	9.7 ± 0.6
	D	9.8 ± 1.5	8.8 ± 0.2	8.8 ± 1.6	9.8 ± 0.6	10.2 ± 1.2
PL g/100 g muscle	A	0.6 ± 0.0	0.7 ± 0.1	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
	B	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
	C	0.7 ± 0.1	0.6 ± 0.0	0.6 ± 0.1	0.6 ± 0.0	0.6 ± 0.0
	D	0.7 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
% MUFA muscle	A	28.5 ± 0.5	27.1 ± 0.4	27.3 ± 0.5	26.9 ± 0.7	27.7 ± 1.4
	B	25.4 ± 0.5	27.7 ± 0.5	27.5 ± 0.8	27.0 ± 0.5	27.4 ± 0.6
	C	25.3 ± 0.8	26.4 ± 0.4	28.0 ± 0.3	25.6 ± 0.5	25.2 ± 0.5
	D	25.6 ± 0.5	25.9 ± 0.4	26.2 ± 0.5	25.8 ± 0.4	26.5 ± 0.5
% PUFA muscle	A	43.0 ± 0.6	44.7 ± 1.0	44.3 ± 0.8	46.0 ± 0.7	46.0 ± 0.1
	B	46.7 ± 1.0	44.9 ± 0.9	44.1 ± 0.7	44.8 ± 0.7	45.3 ± 0.7
	C	46.7 ± 1.0	45.4 ± 0.8	44.9 ± 0.6	47.5 ± 0.7	47.8 ± 0.6
	D	47.0 ± 0.7	47.1 ± 0.6	45.8 ± 0.6	46.6 ± 0.6	46.1 ± 0.5

[a] Within the column values with different letters are significantly different ($p < 0.05$). Values without letters are not significantly different ($P < 0.05$).

Table 3
Fatty acid composition (%) of fillets during conservation (means ± s.d.)[a]

	Days of storage at 1°C				
Fatty acid	1	3	6	9	12
14:0	4.8 ± 0.2	4.8 ± 0.1	4.2 ± 0.2	4.6 ± 0.1	4.3 ± 0.2
16:0	18.0 ± 0.5	17.9 ± 0.3	18.0 ± 0.1	17.9 ± 0.4	18.3 ± 0.3
16:1 n-7	6.1 ± 0.3	6.3 ± 0.6	5.7 ± 0.4	5.6 ± 0.2	5.6 ± 0.3
18:0	3.6 ± 0.3	3.5 ± 0.1	4.4 ± 0.6	3.7 ± 0.4	3.7 ± 0.2
18:1 n-9	13.8 ± 1.3	14.4 ± 0.1	15.2 ± 0.3	14.7 ± 0.4	14.6 ± 0.8
18:1 n-7	2.1 ± 0.2	1.7 ± 0.2	2.0 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
18:2 n-6	3.6 ± 0.1	3.7 ± 0.1	4.1 ± 0.3	3.8 ± 0.1	3.6 ± 0.1
18:3 n-3	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
18:4 n-3	2.2 ± 0.2	2.2 ± 0.1	2.0 ± 0.1	2.2 ± 0.1	2.2 ± 0.1
20:1 n-9	2.3 ± 0.1	2.3 ± 0.2	2.2 ± 0.2	2.1 ± 0.1	2.4 ± 0.1
20:4 n-6	1.5 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
20:5 n-3	14.1 ± 0.3	13.8 ± 0.7	13.5 ± 0.3	14.4 ± 0.3	14.2 ± 0.2
22:1 n-9	1.4 ± 0.2	1.5 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.6 ± 0.2
22:5 n-3	1.6 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.8 ± 0.1
22:6 n-3	18.0 ± 1.0	17.6 ± 0.8	17.2 ± 1.1	17.6 ± 0.8	17.8 ± 1.4
Others[b]	6.0 ± 0.3	6.3 ± 0.3	5.7 ± 0.1	6.1 ± 0.1	6.1 ± 0.3
∑SFA	28.0 ± 0.3	27.7 ± 0.6	27.8 ± 0.5	27.4 ± 0.6	27.4 ± 0.5
∑MUFA	26.2 ± 1.5	26.8 ± 0.8	27.3 ± 0.8	26.3 ± 0.7	26.7 ± 1.1
∑PUFA	45.9 ± 1.9	45.5 ± 1.1	44.8 ± 0.8	46.2 ± 1.1	46.3 ± 1.1
∑n-3	38.7 ± 2.0	38.4 ± 1.3	37.4 ± 1.1	39.3 ± 1.2	39.0 ± 1.6
∑n-6	5.9 ± 0.1	5.8 ± 0.2	6.2 ± 0.4	5.7 ± 0.3	5.5 ± 0.1
A.I.	0.51	0.51	0.48	0.50	0.49
T.I.	0.18	0.18	0.19	0.18	0.18

[a] Values are means of 16 determinations.
[b] Sum of 15:0, 16:1 n-9, 16:2 n-7, 16:3 n-7, 16:4 n-3, 17:0, 20:0, 18:2 n-3, 20:2 n-6, 20:4 n-3, 22:4 n-6, 22:4 n-3, 24:1 n-9.

by elongation and desaturation due to a lack of the necessary enzymes (Sargent et al., 1989; Trigari, Pirini, Ventrella, Pagliarani, Trombetti & Borgatti, 1992).

The percentage of n-3 HUFA in farmed marine fish is often lower than in their wild relatives because the manufactured feeds usually contain high amounts of lipids rich in SFA and MUFA, but deficient in n-3 HUFA (Ackman & Takeuchi, 1986). This can significantly reduce the nutritional quality of lipids in farmed fish. The diets rich in n-3 HUFA are, in fact, suitable for reducing the risk of several chronic cardiovascular and other human diseases (Ulbright & Southgate, 1991). However, the fatty acid percentages which we found in muscle tissue lipids confirm that diets which contain marine oils are able to guarantee, in farmed sea bass as well, a pattern of fatty acids, and particularly n-3 HUFA, which is quite similar to that found in wild specimens (Krajnovic-Ozretic, Najdek & Ozretic, 1994), with excellent atherogenic (0.50) and thrombogenic (0.18) indexes (Amerio et al., 1996).

It must be noted that all of the lipid characteristics described did not undergo significant changes during the refrigeration period. No apparent decreases in the α-tocopherol, TL, PL, MUFA and PUFA content were seen during refrigerated storage (Table 2); likewise the general fatty acid composition was not affected (Table 3).

Contrary to what could have been expected, this indicates that even in the fillets with a lower quantity of vitamin E, significant processes of phospholipid hydrolysis and degradation of unsaturated fatty acids with peroxide formation did not occur. On the other hand, previously published TBARS values (Monetti, Gatta, Trigari, Pirini & Testi, 1997) found in the same specimens had already shown very slow lipid changes which are practically negligible. In *Salmo salar*, instead, different dietary quantities of vitamin E (167; 521; 786 ppm) have been shown to significantly influence the state of preservation of lipids in fillets kept at 4°C for 12 days (Onibi et al., 1996). It is however significant that the sea bass used in our study were refrigerated at a temperature of 1°C with ice and protected from factors (light, oxygen) capable of starting lipid oxidation, by a dark sheet of plastic. These conditions most likely contributed to inhibiting the lipid degradation phenomena even at the lowest concentrations of vitamin E.

It is in any case reasonable to consider that different quantities of vitamin E in muscle tissue could better exhibit their protective effects in the various methods of preservation (i.e. cooked, canned, smoked, vacuum packed), which modern food technologies are using ever more widely with aquaculture products (Nicolosi Asmundo et al., 1993; Sensidoni & Dalla Rosa, 1991).

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References

Ackman, R. G., & Takeuchi, T. (1986). Comparison of fatty acid and lipids of smolting hatchery-fed and wild Atlantic salmon (*Salmo salar*). *Lipids, 21*, 117–120.

Agradi, E., Abrami, G., Serrini, G., McKenzie, D., Bolis, C., & Bronzi, P. (1993). The role of dietary n-3 fatty acid and vitamin E supplements in growth of sturgeon (*Acipenser naccari*). *Comparative Biochemistry and Physiology, 105A*, 187–195.

Amerio, M., Ruggi, C., & Badini, C. (1996). Meat quality of reared fish: nutritional aspects. *Italian Journal of Food Science, 3*, 221–229.

Bartlett, G. R. (1959). Phosphorus assay in column chromatography. *Journal of Biological Chemistry, 234*, 466–468.

Boggio, S. M., Hardy, R. W., Babbitt, J. K., & Brannon, E. L. (1985). The influence of dietary lipid source and alpha-tocopherol acetate level on product quality of rainbow trout (*Salmo gairdneri*). *Aquaculture, 51*, 13–24.

Bonnel, A. D. (1989). *Quality assurance in seafood processing*. New York: Chapman and Hall.

Buttriss, J. L., & Diplock, A. T. (1984). High performance liquid chromatography methods for vitamin E in tissues. *Methods in Enzymololgy, 105*, 131–147.

Di Bella, G., Genovese, L., Salvo, F., & Dugo, G. (1992). Fatty acid composition of reared and wild mediterranean ichthyofauna. *Italian Journal of Food Science, 1*, 17–23.

Fiske, C. G., & Subbarow, Y. (1925). The colorimetric determination of phosphorus. *Journal of Biological Chemistry, 66*, 375–400.

Folch, J., Lees, M., & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry, 226*, 497–509.

Frigg, M., Prabucki, A. L., & Ruhdel, E. U. (1990). Effect of dietary vitamin E levels on oxidative stability of trout fillets. *Aquaculture, 84*, 145–158.

Gatlin, D. M., Bai, S. C., & Erickson, M. C. (1992). Effect of dietary vitamin E and synthetic antioxidants on composition and storage quality of channel catfish, *Ictalurus punctatus*. *Aquaculture, 106*, 323–332.

Hamre, K., & Lie, Ø. (1995). α-Tocopherol levels in different organs of Atlantic salmon (*Salmo salar* L.) — effect of smoltification, dietary levels of n-3 polyunsaturated fatty acids and vitamin E. *Comparative Biochemistry and Physiology, 111A*, 547–554.

Krajnovic-Ozretic, M., Najdek, M., & Ozretic, B. (1994). Fatty acid in liver and muscle of farmed and wild sea bass (*Dicentrarchus labrax* L.). *Comparative Biochemistry and Physiology, 109A*, 611–617.

Machlin, L. J. (1984). Vitamin E. In L. J. Machlin, *Handbook of vitamins. Nutrition, biochemical and clinical aspects* (pp. 99–145). New York: Marcel Dekker.

Marinetti, G. V. (1962). Chromatographic separation, identification and analysis of phosphatides. *Journal of Lipid Research, 3*, 1–20.

Messager, J. L., Stéphan, G., Quentel, C., & Baudin Laurencin, F. (1992). Effects of dietary oxidised fish oil and antioxidant deficiency on histopathology haematology, tissue and plasma biochemistry of sea bass *Dicentrarchus labrax*. *Aquat. Living Resour., 5*, 205–214.

Monetti, P. G., Gatta, P. P., Trigari, G., Pirini, M., & Testi, S. (1997). Effetti dell'integrazione del mangime con vitamina E su alcune caratteristiche qualitative della spigola (*Dicentrarchus labrax*) (pp. 405–406). Atti XII Congresso Nazionale ASPA, Pisa, 23–26 giugno.

Morrison, W. R., & Smith, L. M. (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *Journal of Lipid Research, 5*, 600–608.

Nicolosi Asmundo, C., Scerra, V., Cataldi Lupo, M.C, Arculeo, M., Sinatra, M. C., & Campisi, S. (1993). Composizione e aspetti nutrizionali di *Dicentrarchus labrax* in relazione all'accrescimento. *La Rivista di Scienza del'Alimentazione, 4*, 459–467.

Onibi, G. E., Scaife, J. R., Fletcher, T. C., & Houlihan, D. F. (1996). Influence of α-tocopherol acetate in high lipid diets on quality of refrigerated Atlantic salmon (*Salmo salar*) fillets. In *Refrigeration and aquaculture* (pp. 145–152). Proceedings of the 1996 Bordeaux aquaculture conference.

Pagliarani, A., Pirini, M., Trigari, G., & Ventrella, V. (1986). Effects of diets containing different oils on brain fatty acid composition in sea bass (*Dicentrarchus labrax*). *Comparative Biochemistry and Physiology, 83B*, 277–282.

Sargent, J., Henderson, R. J., & Tocher, D. R. (1989). The lipids. In J. E. Halver, *Fish nutrition* (pp. 153–218). San Diego: Academic Press.

Sensidoni, A., & Dalla Rosa, M. (1991). Qualità dei prodotti dell'acquacoltura destinati alla trasformazione alimentare. *Rivista Italiana di Acquacoltura, 26*, 71–81.

Stéphan, G., Guillaume, J., & Lamour, F. (1995). Lipid peroxidation in turbot (*Scophthalmus maximus*) tissue: effect of dietary vitamin E and dietary n-6 or n-3 polyunsaturated fatty acids. *Aquaculture, 130*, 251–268.

Stéphan, G., Messager, J. L., Lamour, F., & Baudin Laurencin, F. (1991). Interaction between dietary alpha-tocopherol and oxidised oil on sea bass *Dicentrarchus labrax* (pp. 215–218). Proceedings of the IV international symposium on fish nutrition and feeding, 24–27 June. Paris: INRA.

Trigari, G., Barbani, R., Marcheselli, M., Ventrella, V., Pagliarani, A., Trombetti, F., & Pirini, M. (1993). Effect of storage at −20°C on muscle lipids of sole (*Solea solea*) and mullet (*Mullus barbatus*). *Atti SISvet, XLVII*, 719–723.

Trigari, G., Pirini, M., Ventrella, V., Pagliarani, A., Trombetti, F., & Borgatti, A. R. (1992). Lipid composition and mitochondrial respiration in warm- and cold-adapted sea bass. *Lipids, 27*, 371–377.

Ulbricht, T. L. V., & Southgate, D. A. T. (1991). Coronary heart diseas: seven dietary factors. *The Lancet, 338*(19), 985–994.

Waagbø, R., Sandnes, K., Torrissen, O. J., Sandvin, A., & Lie, Ø. (1993). Chemical and sensory evaluation of fillets from Atlantic salmon (*Salmo salar*) fed three levels of n-3 polyunsaturated fatty acids at two levels of vitamin E. *Food Chemistry, 46*, 361–366.

Watanabe, T. (1984). Fish feeds and their quality. Proceeding of the 2nd international conference on aquafarming "Acquacoltura 84", Verona, Italy, 12–13 October.