Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 105 (2007) 1214–1218
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Tocopherol in the lipid stability of tilapia (*Oreochromis niloticus*) hamburgers

Fabíola Helena dos Santos Fogaça *, Léa Silvia Sant'Ana

Universidade Estadual Paulista, UNESP, Centro de Aqüicultura, Botucatu, SP, Brazil

Received 3 August 2006; received in revised form 22 February 2007; accepted 25 February 2007

Abstract

Presence of tocopherol is effective for fish preservation during frozen storage, inhibiting lipid degradation by oxidation. This work evaluated the antioxidant effects of α-tocopherol in diet and postmortem addition on the final quality of hamburgers produced from tilapia fillets kept frozen for zero, 30, 60, and 90 days. Chemical composition varied within the values found for tilapia fish. The increase in α-tocopherol levels reduced the values of thiobarbituric acid reactive substances (TBARS) in the samples at all time intervals. Tocopherol supplementation in diets protected the hamburgers from lipid oxidation more effectively than postmortem addition.

Keywords: Antioxidant; Frozen storage; Supplementation; α-Tocopherol; Tilapia; Chemical composition

1. Introduction

Lipid oxidation is the primary deterioration process of the quality of fish and its products (Jensen, Lauridsen, & Bertelsen, 1998), mainly affecting its acceptability (Nogala-Kalucka et al., 2005). It is intensified immediately after slaughter and during processing, with the destruction of cell membrane integrity as a result of meat cutting, which facilitates the propagation of oxidative reactions (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998).

During storage, lipid oxidation occurs due to an autoxidative mechanism involving free radicals (Hendriks, Cottam, & Thomas, 2006). In this case, the oxidative stability of muscle depends upon the balance between antioxidants, such as vitamin E and some carotenoids, and pro-oxidants including the concentrations of polyunsaturated fatty acids (PUFA) and free iron in the muscle (Monahan et al., 1990).

Vitamin E constitutes the second line of defense in biological systems. It acts mainly by protecting the membranes of oxidizable compounds in the cell cytoplasm by the stabilization of unsaturated fatty acids and the breakdown of peroxide chains ($LOO^{\cdot}$) (Yamamoto, Fujisawa, Hara, & Dunlap, 2001).

Dietary supplementation with α-tocopherol, which has the highest vitamin E activity (National Research Council, 1993), has been the most commonly used form of vitamin E incorporation (Botsoglou, Fletours, Florou-Paneri, Christaki, & Spais, 2003). Its ingestion could be an important pre-slaughter factor in the preservation of fillet quality (Scaife, Onibi, Murray, Fletcher, & Houlihan, 2000), preventing alterations in texture, colour, flavour, and nutritional value (Ruff et al., 2002).

Recently, it has been demonstrated that the supplementation of different levels of vitamin E in diets for Atlantic salmon *Salmo salar* (Hamre et al., 2004; Onibi, Scaife, & Fletcher, 1996), sea bass *Dicentrarchus labrax* (Gatta, Pirini, Testi, Vignola, & Monetti, 2000; Pirini, Gatta, Testi, Trigari, & Monetti, 2000), tilapia (Shiau & Shiau, 2001), "pacu" *Piaractus mesopotamicus* (Sant'Ana, 1998; Sant'Ana & Fernandes, 2000) and turbot *Scophthalmus maximus* L. (Ruff et al., 2002a; Ruff, Fitzgerald, Cross, Hamre, & Kerry, 2003) reduced oxidation rate and promoted preservation of these fishes during storage.

* Corresponding author. Tel./fax: +55 14 38117171.
E-mail address: fabiolafogaca@yahoo.com.br (F.H. dos Santos Fogaça).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.02.032

However, few papers have compared the use of tocopherol in diet with postmortem use, and the interaction between different forms of tocopherol administration on lipid stability of tilapia (*Oreochromis niloticus*) has not been evaluated. Therefore, the objective of this work was to evaluate the antioxidant effects of α-tocopherol, by modifying the diet and/or by the addition of antioxidants after slaughter, on the final quality of hamburgers prepared from tilapia fillets (*Oreochromis niloticus*) kept frozen and analyzed at zero, 30, 60, and 90 days of storage.

2. Material and methods

2.1. Fish, diets and sampling procedure

This experiment was developed at Universidade Estadual Paulista, UNESP, Brazil. A completely randomized design was used, with six treatments, in a 3×2 factorial arrangement, characterized by the supplementation of two α-tocopheryl acetate levels in the diets, plus a control group, and by the addition or not of 0.1 mg/g α-tocopheryl acetate to the hamburgers.

One hundred and twenty sex-reversed Nile tilapia juveniles were used, with a final mean weight of 490.11 ± 10.41 g. The α-tocopherol supplementation in the feeds was performed in three 200-m^2 nurseries divided into four 50-m^2 sections by 15 mm mesh plastic screens. Three experimental diets (Table 1) containing increasing α-tocopheryl acetate levels (zero, 100, and 200 mg/kg) were used. The α-tocopheryl acetate (Rovimix E-50 adsorbate, Roche commercial product) was diluted into the soybean oil and added after extrusion. The quantities of α-tocopherol determined in the diets were 24.45, 135.98, and 230.74 mg/kg feed. The feed were stored in a freezer (−18 °C) and offered to the fish, without restriction, for 63 days. After that period, the fish were slaughtered in boxes containing ice and water (1:1) and then eviscerated and filleted. The fillets were ground and processed into hamburgers (98.8% fillet and 1.2% condiments), receiving, at this time, the addition or not of α-tocopheryl acetate (0.1 mg/g fillet) postmortem. The hamburgers were molded inside PVC tubes (lined with a polyethylene bag) and then frozen. After 24 h, they were sliced with a vertical band saw and frozen at −18 °C for 90 days.

Table 1
α-Tocopherol concentrations and proximate compositions of the experimental diets

Proximate composition	Diet 1	Diet 2	Diet 3
DM (%)[a]	90.24	89.87	89.85
DE (kcal/kg)[b]	3214	3214	3214
Crude protein (%)	26.38	25.90	25.55
Crude fat (%)	3.27	3.18	3.41
Ash (%)	7.77	7.71	7.72
TNC (%)[c]	36.49	38.16	38.79
Water activity (Aw)	0.572	0.576	0.574
α-Tocopherol (mg/kg)	24.45	135.98	230.74

[a] Dry material.
[b] Digestible energy. Calculated based on DE values for each ingredient.
[c] Total non-structural carbohydrate; TNC% = DM% − (CP% + Fat% + Crude fiber% + Ash%).

2.2. Chemical analysis

Four hamburger samples were taken from each treatment, per time interval, and analyses were performed with triplicates. The chemical composition in terms of moisture (105 °C for 24 h), lipids (extraction with petroleum ether in a Soxhlet device), crude protein (Kjeldahl method, nitrogen × 6.25), and ash (incineration at 550 °C until white ash), was analyzed according to AOAC (1980). High performance liquid chromatography (HPLC) determined tocopherol in the feed, according to the methodology described by Mestre Prates, Quaresma, Bessa, Fontes, and Alfaia (2006). Lipid oxidation was evaluated via the formation of thiobarbituric acid reactive substances (TBARS), according to Vyncke (1970). The oxidation quantification was calculated by the construction of a standard curve with different concentrations of tetraethoxypropane, and the following equation was obtained: $y = 0.1152 \cdot x$ ($r^2 = 0.9962$).

2.3. Statistical analysis

On each sampling occasion, four samples were selected from each treatment batch to be subjected to the different analyses. All measurements were carried out in triplicate. The results were analyzed with the SAS version 6.12 software package. Differences among the mean values of the various treatments and storage periods were determined by Tukey–Kramer test, and the significance was defined either at $P < 0.05$.

3. Results and discussion

3.1. Effect of tocopherol on the chemical composition of tilapia hamburgers

The chemical composition of hamburgers is presented in Table 2. The results in chemical composition are in agreement with the values found for tilapia fish. Some factors, like feeding, age, weight, physiological status, and different body regions may influence the chemical composition of fish (Katikou, Hughess, & Robb, 2001). In this work, we used fish from the same origin, cultivated under identical experimental conditions, filleted and processed into hamburgers in the same way, using the same formulation, to avoid factors that could influence the analysis results. According to the results, there was no significant effect of the dietary tocopherol levels on the hamburger's composition. No differences were observed in similar papers that evaluated tocopherol supplementation in feeds with regard to the chemical composition of tilapia (Huang & Huang, 2004), sea bream (Pirini et al., 2000), and trout

Table 2
Hamburger proximate composition of tilapia

Dietary tocopherol (mg/kg)	Moisture (%)	Crude fat (%)	Crude protein (%)	Ash (%)
0	76.12 ± 1.75^{B}	0.63 ± 0.01^{A}	20.67 ± 1.27^{A}	2.33 ± 0.07^{AB}
100	77.96 ± 0.60^{A}	0.56 ± 0.03^{A}	19.97 ± 0.69^{A}	2.21 ± 0.08^{B}
200	75.04 ± 1.09^{B}	0.64 ± 0.02^{A}	20.13 ± 0.74^{A}	2.46 ± 0.15^{A}

Values are mean ± SD of triplicate groups of four hamburgers ($n = 4$). Means in the same column with different superscripts are significantly different at $^{*}P \leqslant 0.05$.

Oncorhynchus mykiss (Chaiyapechara, Casten, Hardy, & Dong, 2003).

3.2. Effect of tocopherol on the lipid stability of tilapia

Table 3 presents the analysis of variance results for the TBARS values of hamburgers according to the supplementation level in the diets (L), postmortem addition (A), and storage time (T). There was an effect of the interaction between the vitamin E supplemented in the diets and the postmortem addition, between addition and supplementation level in the diets and frozen storage time, as well as of the triple interaction (storage time, tocopherol level in diets, and postmortem addition) on hamburger lipid stability.

The TBARS results for the interaction between supplementation level and postmortem addition are presented in Table 4. Treatments that received tocopherol in the diets showed smaller TBARS values when compared against the control group or the group that only received the postmortem addition at all time intervals of frozen storage. The postmortem addition of tocopherol was only effective in the group that did not receive supplementation in the diets, without statistical difference in the other treatments. Lipid peroxidation decreased in muscle tissues when the tocopherol levels in the diets increased, as also observed in others reports (Gatta et al., 2000; Huang & Huang, 2004; Huang, Huang, & Hou, 2004).

In the present work, the postmortem addition of tocopherol in groups that received supplemented feed was performed in order to evaluate the interaction between different forms of tocopherol administration. In the literature, few studies have compared the *in vivo* use of tocopherol with postmortem use. This is because supplementation in the feeds has been demonstrated to be more effective than postmortem addition, due to better tocopherol incorporation into membranes (Buckley & Morrissey, 1992; Morrissey et al., 1998). The results of the present work showed that the interaction between supplementation level and postmortem addition resulted in the same lipid stability of the tilapia hamburger as that of the group who received only dietary supplementation. Therefore, the postmortem addition did not bring any significant advantage over dietary supplementation, as also observed by Kerry, Buckley, Morrissey, O'Sullivan, and Lynch (1998), because of the greater antioxidant efficacy of endogenous rather than exogenous α-tocopherol (Mitsumoto, Arnold, Schaefer, & Cassens, 1993).

Table 3
TBARS values in hamburgers for supplementation level in the diets (L), postmortem addition (A), and storage time (T)

Variation factor	F	p-Value
Tocopherol level (L)	186.35	0.0001
Postmortem addition (A)	23.96	0.0001
Storage time (T)	54.68	0.0001
$L \times A$ interaction	15.8	0.0001
$L \times T$ interaction	8.42	0.0001
$A \times T$ interaction	18.59	0.0001
$L \times A \times T$ interaction	3.87	0.0021
C.V. (%)	14.9	

Table 4
Mean TBARS values (mg/kg) for the interaction between tocopherol supplementation levels and postmortem addition

Storage time (days)	mg tocopherol/kg	Postmortem addition	
		Yes	No
Initial ($P1$)	0	3.47 ± 0.60 bB	2.78 ± 0.50 aB
	100	1.34 ± 0.46 aA	1.26 ± 0.10 aA
	200	1.67 ± 0.37 bA	0.94 ± 0.24 aA
30 ($P2$)	0	3.03 ± 0.19 aC	5.16 ± 0.57 bC
	100	2.41 ± 0.62 aB	3.20 ± 0.30 bB
	200	1.45 ± 0.37 aA	1.63 ± 0.17 aA
60 ($P3$)	0	2.38 ± 0.11 aB	3.21 ± 0.35 bC
	100	2.33 ± 0.80 aB	2.38 ± 0.26 aB
	200	1.60 ± 0.18 aA	1.42 ± 0.21 aA
90 ($P4$)	0	3.22 ± 0.40 aB	4.79 ± 0.65 bC
	100	3.02 ± 0.17 aB	3.34 ± 0.48 aB
	200	2.12 ± 0.28 aA	2.47 ± 0.48 aA

Means followed by the same letters are not different by Tukey test at 5% probability (lower case letters in the rows and upper case letters in the columns). Analyses of four samples with three replicates ($n = 12$).

Table 5 summarizes the interaction effect of tocopherol level in the diets and storage period. There was an oxidation rate increase during the first 30 days of storage in all treatments. The results also showed that the highest tocopherol supplementation level in the diets (200 mg/kg) was the most effective in the preservation of tilapia hamburger quality, when compared with other groups. The group receiving 100 mg α-tocopheryl acetate/kg, which is the amount representing the species requirements, according to the National Research Council (1993) (50–100 mg of vitamin E/kg diet), also demonstrated a decrease in oxidation, of smaller intensity, however. Other authors have also observed this protective effect of vitamin E during

Table 5
Mean TBARS values (mg/kg) in Nile tilapia fillet hamburgers for the interaction between tocopherol level/kg feed and storage time

Interval (days)	mg vitamin E level/kg diet		
	0	100	200
0	2.78 ± 0.50 bA	1.26 ± 0.10 abA	0.94 ± 0.24 aA
30	5.16 ± 0.57 cB	3.20 ± 0.30 bC	1.63 ± 0.17 aB
60	3.21 ± 0.35 cA	2.38 ± 0.26 bB	1.42 ± 0.21 aAB
90	4.79 ± 0.65 cB	3.34 ± 0.48 bC	2.47 ± 0.48 aC

Means followed by the same letters are not different by Tukey test at 5% probability (lower case letters in the rows and upper case letters in the columns). Analyses of four samples with three replicates ($n = 12$).

storage (Ruff, Fitzgerald, Cross, & Kerry, 2002b). Diets containing 167 mg vitamin E/kg feed showed a significant influence on lipid preservation in Atlantic salmon fillets maintained at 4 °C for 12 days (Onibi et al., 1996). Tocher et al. (2002) also observed higher TBARS values in fishes fed vitamin E-deficient diets.

The effects of the interaction between postmortem addition and storage period are presented in Table 6. During storage, postmortem addition of α-tocopherol decreased TBARS values at all time intervals in groups that did not receive the dietary supplementation of vitamin E. A protective effect of the postmortem addition of vitamin E on the reduction of oxidation was also reported in carp cells by George, Riley, McEvoy, and Wright (2000).

Tocopherol is known to be effective in increasing muscle α-tocopherol levels and improving oxidative stability after slaughter and during frozen storage. Monahan et al. (1990) demonstrated that dietary vitamin E supplementation result in elevated concentrations of α-tocopherol in the cell membranes. They also observed a linear relationship between dietary vitamin E and muscle α-tocopherol levels.

Fig. 1 better illustrates the antioxidant effect of tocopherol during the 90 days of storage on the lipid stability of tilapia hamburgers. The decrease in TBARS values in the period between 30 and 60 days of frozen storage suggests a reduction in substrates for reaction with TBA, which may mean either the end of the chain reaction of PUFAs or the consumption of reactive oxygen species. In this study, there appeared to be some fluctuations in TBARS values. Other authors have also observed similar trend in oxidation rates during frozen storage. Tokur, Polat, Beklevik, and Ozkutuk (2004) found TBARS values of 0.045 at 30 days, 0.059 at 60 days, and 0.041 mg malonaldehyde equivalents/kg at 90 days, in analyses performed in tilapia hamburgers. Sant'Ana and Fernandes (2000) evaluated lipid oxidation in pacu (*Piaractus mesopotamicus*) fillets and observed a gradual increase-decrease-increase of the amount of malonaldehyde equivalents around 0.62; 0.59; and 1.49 mg/kg fillet after 30, 60, and 90 days of storage.

The results showed that supplementation with 200 mg tocopherol/kg feed; regardless of the addition or not of 0,1 mg/g tocopherol during processing had a similar effect in the preservation of Nile tilapia hamburgers under frozen storage for 90 days. However, even at low concentrations, tocopherol supplementation also prevented hamburger lipid oxidation when compared with the control group.

Usually, α-tocopheryl acetate is used in dietary supplementation of vitamin E for animals, while α-tocopherol is the option for meat. In this work, α-tocopheryl acetate was used in hamburgers, but it could hardly or only partially be hydrolyzed during the frozen storage and may not provide enough protection to the hamburger against oxidation. Thus, in future works, the exogenous (postmortem) and endogenous (in diets) utilization of α-tocopheryl acetate or α-tocopherol must be compared. In addition, tocopherol determination content must be performed for hamburgers, to give the exact concentrations of tocopherol present in hamburger regardless of the loss of tocopherol in vivo during the feeding trial and the loss during postmortem process. By doing so, the efficacy of endogenous and exogenous antioxidant could be better evaluated.

Table 6
Mean TBARS values for the interaction between storage time and postmortem addition of tocopherol

Tocopherol (mg)	Time (days)	Postmortem addition	
		Yes	No
0	0	3.47 ± 0.60 bB	2.78 ± 0.50 aA
	30	3.03 ± 0.19 aAB	5.16 ± 0.57 bB
	60	2.38 ± 0.11 aA	3.21 ± 0.35 bA
	90	3.22 ± 0.40 aB	4.79 ± 0.65 bB
100	0	1.34 ± 0.46 aA	1.26 ± 0.10 aA
	30	2.41 ± 0.62 aC	3.20 ± 0.30 bC
	60	2.33 ± 0.80 aB	2.38 ± 0.26 aB
	90	3.02 ± 0.17 aC	3.34 ± 0.48 aC
200	0	1.67 ± 0.37 aA	0.94 ± 0.24 aA
	30	1.45 ± 0.37 aA	1.63 ± 0.17 bB
	60	1.60 ± 0.18 aA	1.42 ± 0.21 bAB
	90	2.12 ± 0.28 Aa	2.47 ± 0.48 bC

Means followed by the same letters are not different by Tukey test at 5% probability (lower case letters in the rows and upper case letters in the columns). Analyses of four samples with three replicates ($n = 12$).

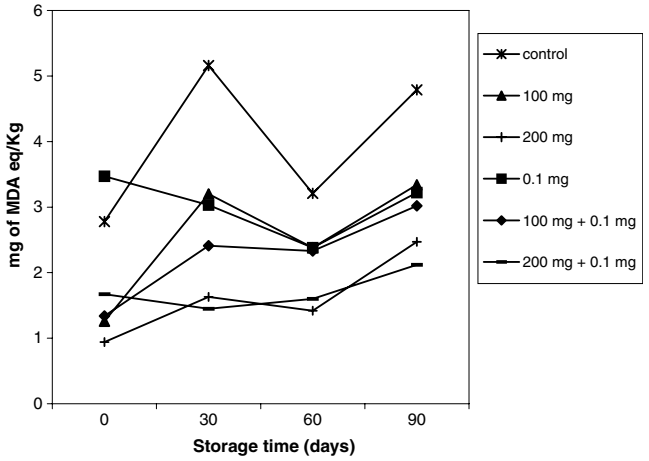

Fig. 1. TBARS values determined in tilapia hamburgers during frozen storage for 90 days (−18 °C).

4. Conclusions

Tocopherol supplementation in the diet protected hamburgers from lipid oxidation more effectively than the post-mortem addition. Incorporation of vitamin E into membrane lipids via the diet might be the most effective means for extending the shelf life of restructured meat products.

Acknowledgement

The authors thank the Conselho Nacional de Pesquisa (CNPq).

References

AOAC (1980). In Association of Official Analytical Chemists International: *Official methods of analysis*. Arlington, Virginia, USA.

Botsoglou, N. A., Fletours, D. J., Florou-Paneri, P., Christaki, E., & Spais, A. B. (2003). Inhibition of lipid oxidation in log-term frozen stored chicken meat by dietary oregano essential oil and α-tocopheryl acetate supplementation. *Food Research International, 36*, 207–213.

Buckley, D. J., & Morrissey, P. A. (1992). Vitamin E and meat quality. In F. Hoffmann (Ed.), *Vitamins and fine chemicals*. Irlanda: La Roche Ltda.

Chaiyapechara, S., Casten, M. T., Hardy, R. W., & Dong, F. M. (2003). Fish performance, fillet characteristics, and health assessment index of rainbow trout (*Oncorhynchus mykiss*) fed diets containing adequate and high concentrations of lipid and vitamin E. *Aquaculture, 219*, 715–738.

Gatta, S. S., Pirini, S., Testi, S., Vignola, S., & Monetti, P. G. (2000). The influence of different levels of dietary vitamin E on sea bass (*Dicentrarchus labrax*) flesh quality. *Aquaculture Nutrition, 6*, 47.

George, S., Riley, C., McEvoy, J., & Wright, J. (2000). Development of a fish in vitro cell culture model to investigate oxidative stress and its modulation by dietary vitamin E. *Marine Environment Research, 50*, 541–544.

Hamre, K., Christiansen, R., Waage, A., Torstensen, B. E., Ujgren, B., Lie, O., et al. (2004). Antioxidant vitamins, minerals and lipid levels in diets for Atlantic salmon (*Salmo salar*, L.): Effects on growth performance and fillet quality. *Aquaculture Nutrition, 10*, 113–123.

Hendriks, W. H., Cottam, Y. H., & Thomas, D. V. (2006). The effect of storage on the nutritional quality of meat and bone meal. *Animal Feed Science and Technology, 127*(1–2), 151–160.

Huang, C. H., & Huang, S. L. (2004). Effect of dietary vitamin E on growth, tissue lipid peroxidation and liver glutathione level of juvenile hybrid tilapia, *O. niloticus* × *O. aureus*, fed oxidized oil. *Aquaculture, 237*, 381–389.

Huang, C.-H., Huang, M.-C., & Hou, P.-C. (2004). Effect of dietary vitamin E level on growth, tissue peroxidation, and erythrocyte fragility of transgenic coho salmon, *Oncorhynchus kisutch*. *Comparative Biochemistry and Physiology, 139 A*, 199–204.

Jensen, C., Lauridsen, C., & Bertelsen, G. (1998). Dietary vitamin E: Quality and storage stability of pork and poultry. *Trends in Food Science and Technology, 9*, 62–72.

Katikou, P., Hughess, S. I., & Robb, D. H. F. (2001). Lipid distribution with Atlantic salmon (*Salmo salar*) fillets. *Aquaculture, 202*, 80–99.

Kerry, J. P., Buckley, D. J., Morrissey, P. A., O'Sullivan, K., & Lynch, P. B. (1998). Endogenous and exogenous α-tocopherol supplementation: Effects on lipid stability (TBARS) and warmed-over flavour (WOF) in porcine *M. longissimus dorsi* roasts held in aerobic and vacuum packs. *Food Research International, 31*(3), 211–216.

Mestre Prates, J. A., Quaresma, M. A. G., Bessa, R. J. B., Fontes, C. M. G. A., & Alfaia, C. M. P. M. (2006). Simultaneous HPLC quantification of total cholesterol, tocopherols and beta-carotene in Barrosã – PDO veal. *Food Chemistry, 94*, 469–477.

Mitsumoto, M., Arnold, R. N., Schaefer, D. M., & Cassens, R. G. (1993). Dietary versus postmortem supplementation of vitamin E on pigment and lipid stability in ground beef. *Journal of Animal Science, 71*(1), 105–118.

Monahan, F. J., Buckley, D. J., Gray, J. I., Morrissey, P. A., Asghar, A., Hanrahan, T. J., et al. (1990). Effect of dietary vitamin E on the stability of raw and cooked pork. *Meat Science, 27*, 99–108.

Morrissey, P. A., Sheehy, P. J. A., Galvin, K., Kerry, J. P., & Buckley, D. J. (1998). Lipid stability in meat and meat product. *Meat Science, 49*(1), 73–86.

National Research Council (1993). *Nutrient requirements of fish*. Washington: Academic Press, 114 p.

Nogala-Kalucka, M., Korczak, J., Dratwia, M., Lampart-Szczapa, E., Siger, A., & Buchowsk, M. (2005). Changes in antioxidant activity and free radical scavenging potential of rosemary extract and tocopherols in isolated rapeseed oil triacylglycerols during accelerated tests. *Food Chemistry, 93*, 227–235.

Onibi, G. E., Scaife, J. R., & Fletcher, T. C. (1996). Influence of α-tocopherol acetate in high lipid diets on quality of refrigerated Atlantic salmon (*Salmo salar*) fillets. *Refrigeration and Aquaculture, 33*, 145–152.

Pirini, M., Gatta, P. P., Testi, S., Trigari, G., & Monetti, P. G. (2000). Effect of refrigerated storage on muscle lipid quality of sea bass (*Dicentrarchus labrax*) fed on diets containing different levels of vitamin E. *Food Chemistry, 68*, 289–293.

Ruff, N., Fitzgerald, R. D., Cross, T. F., Hamre, K., & Kerry, J. P. (2003). The effect of dietary vitamin E and C level on market-size turbot (*Scophthalmus maximus*) fillet quality. *Aquaculture Nutrition, 9*, 91–97.

Ruff, N., Fitzgerald, R. D., Cross, T. F., & Kerry, J. P. (2002b). Fillet shelf-life of Atlantic halibut *Hippoglossus hippoglossus* L. fed elevated levels of α-tocopheryl acetate. *Aquaculture Research, 33*, 1059–1071.

Ruff, N., Fitzgerald, R. D., Cross, T. F., Teurtrie, G., & Kerry, J. P. (2002a). Slaughtering method and dietary tocopherol acetate supplementation affect *rigor mortis* and fillet shelf-life of turbot (*Scophthalmus maximus*). *Aquaculture Research, 33*(9), 703.

Sant'Ana, L.S. (1998). *Influência da Fonte Lipídica da dieta e da Adição de Antioxidantes in vivo na Composição em ácidos graxos de filés de Pacu (Piaractus mesopotamicus) cultivados*. Tese (Doutorado em Ciências Farmacêuticas – Faculdade de Ciências Farmacêuticas da Universidade de São Paulo, USP). São Paulo (157 p).

Sant'Ana, L.S., & Fernandes, J.B. (2000). Efeito do armazenamento na composição em ácidos graxos de filés de peixes da espécie pacu (*Piaractus mesopotamicus*). In *Proceedings of congresso brasileiro de ciência e tecnologia de alimentos*, Vol. 4. (pp. 272). Fortaleza: SBCTA.

Scaife, J. R., Onibi, G. E., Murray, I., Fletcher, T. C., & Houlihan, D. F. (2000). Influence of α-tocopherol acetate on the short- and long-term storage properties of fillets from Atlantic salmon *Salmo salar* fed a high lipid diet. *Aquaculture Nutrition, 6*, 65–71.

Shiau, S. Y., & Shiau, L. F. (2001). Re-evaluation of the vitamin E requirement of juvenile tilapia (*Oreochromis niloticus* × *O. aureus*). *Animal Science, 72*, 529–534.

Tocher, D. R., Mourente, G., Van Der Eecken, A., Evjemo, J. O., Diaz, E., Bell, J. G., et al. (2002). Effects of dietary vitamin E on antioxidant defense mechanism of juvenile turbot (*Scophthalmus maximus* L.) and sea bream (*Sparus aurata* L.). *Aquaculture Nutrition, 8*(3), 195.

Tokur, B., Polat, A., Beklevik, G., & Ozkutuk, S. (2004). Changes in the quality of fishburger produced from tilápia (*Oreochromis niloticus*) during frozen storage (−18 °C). *European Food Research and Technology, 218*, 420–423.

Vyncke, W. (1970). Direct determination of the TBA value in trichloroacetic acid extract of fish as a measure of oxidative rancidity. *Fette-Scifen Anstrichmittel, 72*, 1084–1087.

Yamamoto, Y., Fujisawa, A., Hara, A., & Dunlap, W. C. (2001). An unusual vitamin E constituent provides enhanced antioxidant protection in marine organisms adapted to cold-water environments. *Procedures of National Academic of Sciences, 98*, 13144–13148.