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Influence of the addition of antioxidants in vivo on the fatty acid composition of fish fillets

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Abstract

In this study, the influence of the addition of antioxidants in vivo on the fatty acid composition of the flesh of a freshwater fish known as "pacu" (*Piaractus mesopotamicus*) is verified. Four groups (one being the control group) of juvenile "pacu" were cultured on isocaloric and isoproteic diets. The lipid source was soybean oil and diets were added with either 100 ppm of α-tocopheryl acetate, or 100 ppm of BHT or 1.4 g of rosemary extract (Herbalox®)/kg diet. The fatty acid composition of the lipids of the different groups was determined before and after irradiation at 2 and 3 kGy, respectively, for the evaluation of the protective effects of the different antioxidants. Similarly, thiobarbituric acid reactive substances (TBARS) were determined from irradiated and non-irradiated samples. The results showed that the use of antioxidants altered the fatty acid composition of the fillets. TBARS and irradiation confirmed their important role in protecting against lipid oxidation. Among all the antioxidants used, tocopherol was the most efficient, as shown by the highest percentage of polyunsaturated fatty acids (PUFA), by the lowest values of TBARS and by the analyses of the individual fatty acid levels at different irradiation doses. Significant statistical differences were observed only in 17% of the fatty acids in the fillets of the groups.

Keywords: Antioxidants; Fatty acid; Freshwater fish; Lipid oxidation

1. Introduction

Some of the major changes that occur during processing, distribution, and final preparation of food are due to oxidation. Oxidation of lipids initiates other changes in the food system which affect nutritional quality, wholesomeness, safety, colour, flavour, and texture (Shahidi & Wanansudara, 1992).

Antioxidants are used by the food industry to delay the oxidation process (Brand-Williams, Cuvelier & Berset, 1995). One way to improve product stability prior to processing is by dietary manipulation. An example is the increase in the concentration of natural antioxidant vitamin E in the tissues through an elevated dietary supplementation (Gatlin, Bai & Erikson, 1992).

The activity of an antioxidant can be estimated by quantitatively determining primary or secondary products from the autoxidation of lipids or by monitoring other variables, such as the studies on accelerated oxidation conditions or model systems tests (Shahidi & Wanasundara, 1992).

Careful consideration concerning the applicability of the method to biological systems and data interpretation are essential, as the complexities of food and/or biological systems can often contradict the validity of the existing analytical systems (Namiki, 1990).

Irradiation is a physical method of food processing consisting of exposing food to radiation during a limited period of time (Olszyna-Marzys, 1992). The autoxidative process induced by irradiation in fat is much the same as that which occurs without irradiation. However, with irradiation, it is quite accelerated (IGGFI, 1992).

Irradiation continues to offer a well-known and very useful method of producing radicals and of studying their important reactions for biology, both in vitro and in vivo (Pryor, 1978).

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In the present study, we have verified the efficiency of in vivo use of three antioxidants: α-tocopheryl acetate, 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT) and rosemary extract Herbalox®. Irradiation was used to evaluate protective effects of all those different antioxidants.

2. Material and methods

During 63 days of the most appropriate period — July–September — (Carneiro, Chain & Dias, 1995), the experiments were carried out in the Aquaculture Centre in UNESP, Brazil. It was demonstrated that, from April to September, when the temperature was more agreeable, fish fed on pelleted diet gained more weight.

With an average weight of 198.89 ± 39.6 g, 80 juvenile "pacu" (*Piaractus mesopotamicus*) were randomly divided into four groups of 20 fish, one group being control. The fish were cultured for 9 weeks (63 days) in 50 m^2 tanks with brick walls and a ground earth bottom supplied with continuous water. The juvenile "pacu" were fed on isocaloric and isoproteic diets. The composition of the experimental diets was identical for all groups with the presence of added antioxidants in the different groups, except in the control. The lipid source was soybean oil and the diet for each group was added with either 100 ppm of α-tocopheryl acetate, or 100 ppm of 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT) or 1.4 g of rosemary extract Herbalox®/Kg diet.

Each sample was individually packed in a commercial polyethylene bag with a sealing clamp and maintained under iced conditions. They were transported to the irradiation facility Embrarad S. A., São Paulo, cooled with packs of ice and irradiated to average doses of 2 and 3 kGy using a ^{60}Co irradiator (Dose rate: 3 kGy/h).

The TBARS were analysed from nonirradiated and irradiated samples to determine the extent of lipid oxidation on an acid extract, according to Wyncke (1970).

The lipids were extracted by the method of Folch, Lee and Sloane Stanley (1957). The fatty acid composition was determined on the lipid extracts after methylation with sulfuric acid and ammonium chloride (Hartman & Lago, 1973). The fatty acid methyl esters (FAME) were analysed using a CG-500 chromatograph, equipped with fused silica capillary column Carbowax-20 M (30 m, 0.25 mm ID) and flame ionization detector (FID). Nitrogen was used as the carrier gas. Thermal gradient from 150 to 230°C at 6°C/min. Injector and FID temperature were 250°C. Heptadecanoic acid (C17, Sigma) was added to all samples as an internal standard before preparation of FAME.

Statistical analyses were developed using a GraphPad Instat, 2.01 version, GraphPad Software. Differences between mean numbers were determined by analysis of variance, followed by the Tukey test.

3. Results and discussion

The fatty acid composition after different irradiation doses for each treatment is present in Tables 1, 2, 3 and 4. The results of the Tukey tests applied to individual fatty acids at different irradiation doses demonstrated significant differences in 75% of the fatty acids in the control group.

The Tukey test applied to the groups with BHT or rosemary extract Herbalox® in the diet showed significant difference in 42% of the fatty acids. However, for the group with tocopherol, only 17% of fatty acids had a significant difference. Similar work on antioxidant activities of sage and rosemary extracts and tocopherol has been done in a model meat system showing that all three antioxidants were effective as antioxidants, although vitamin E was more effective than the plant extracts (Wong, Hashimoto & Shibamoto, 1995).

Saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) revealed similar results for the different antioxidants. In the control group differences were observed between the irradiation doses for SFA, MUFA and PUFA, whereas no significant differences were observed for the tocopherol group in any class of fatty acid. Similarly, *n*-3, *n*-6 and *n*-9 fatty acid showed the same results.

Table 1
Fatty acid composition of "pacu" flesh fed (control diet) before and after irradiation[a]

Fatty acid	0 kGy[f]	2 kGy[f]	3 kGy[f]	AV[b]
14:0	2.06 ± 0.07a	2.52 ± 0.23b	2.28 ± 0.15a,b	*
16:0	18.00 ± 0.06a	20.77 ± 1.58b	19.77 ± 0.39a,b	*
16:1	4.44 ± 0.22a	5.88 ± 0.22b	6.18 ± 0.33b	***
18:0	7.84 ± 0.06	9.48 ± 0.96	8.22 ± 0.60	NS
18:1 n-9	39.86 ± 0.19	37.79 ± 2.18	39.70 ± 1.28	NS
18:2 n-6	21.54 ± 0.07a	17.85 ± 0.12b	20.87 ± 0.51c	***
18:3 n-3	1.40 ± 0.06	1.45 ± 0.15	1.46 ± 0.02	NS
20:3 n-6	1.03 ± 0.01a	0.86 ± 0.07b	0.66 ± 0.03c	***
20:4 n-6	2.06 ± 0.02a	1.73 ± 0.19b	1.05 ± 0.07c	***
20:5 n-3	0.22 ± 0.01a	0.29 ± 0.04b	0.17 ± 0.02a	**
22:5 n-6	0.75 ± 0.03a	0.64 ± 0.09a	0.30 ± 0.01b	***
22:6 n-3	0.82 ± 0.02a	0.71 ± 0.18a	0.29 ± 0.02b	**
SFA[c]	27.90 ± 0.10a	32.77 ± 2.77b	30.28 ± 1.12a,b	*
MUFA[d]	44.30 ± 1.18a	43.67 ± 2.40b	45.88 ± 1.57a	***
PUFA[e]	27.81 ± 0.09a	23.55 ± 0.36b	24.20 ± 0.47b	***
n9	39.86 ± 0.19	37.79 ± 2.18	39.70 ± 1.28	NS
n6	25.38 ± 0.10a	21.08 ± 0.47b	22.29 ± 0.48c	***
n3	2.43 ± 0.05a	2.47 ± 0.12a	1.92 ± 0.05b	***

[a] Mean ± standard deviation ($n = 3^1$).
[b] AV, analysis of variance, NS = not significant *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$.
[c] SFA, Saturated fatty acid.
[d] MUFA, Monounsaturated fatty acid.
[e] PUFA, Polyunsaturated fatty acid.
[f] Within each row, means followed by different letters are significantly different. Tukey–Kramer test ($p < 0.05$).

Table 2
Fatty acid composition of "pacu" flesh fed (BHT diet) before and after irradiation[a]

Fatty acid	0 kGy[g]	2 kGy[g]	3 kGy[g]	AV[b]
14:0	2.66 ± 0.29	2.40 ± 0.24	2.64 ± 0.10	NS[c]
16:0	21.27 ± 0.55	20.25 ± 0.53	21.03 ± 0.01	NS
16:1	2.95 ± 0.42	4.83 ± 0.31	5.41 ± 0.27	NS
18:0	9.26 ± 0.24	8.90 ± 0.64	9.66 ± 0.13	NS
18:1 n-9	35.47 ± 1.19	36.82 ± 0.89	36.97 ± 0.29	NS
18:2 n-6	18.89 ± 0.22	19.38 ± 0.31	19.20 ± 0.08	NS
18:3 n-3	1.60 ± 0.06	1.63 ± 0.21	1.47 ± 0.04	NS
20:3 n-6	1.02 ± 0.09a	0.97 ± 0.08a,b	0.80 ± 0.05b	*
20:4 n-6	2.59 ± 0.16a	2.53 ± 0.13a	1.54 ± 0.22b	***
20:5 n-3	0.41 ± 0.03a	0.38 ± 0.04a	0.24 ± 0.01b	*
22:5 n-6	0.92 ± 0.14a	0.87 ± 0.17a	0.49 ± 0.08b	**
22:6 n-3	1.00 ± 0.02a	1.06 ± 0.10a	0.57 ± 0.14b	**
SFA[d]	33.19 ± 0.85	31.55 ± 1.32	33.33 ± 0.09	NS
MUFA[e]	40.42 ± 1.08	41.65 ± 1.10	42.38 ± 0.54	NS
PUFA[f]	26.41 ± 2.23a	26.80 ± 0.50a	24.31 ± 0.54b	***
n9	35.47 ± 1.68	36.82 ± 0.89a	36.97 ± 0.29b	NS
n6	23.40 ± 0.25a	23.74 ± 0.47a	22.03 ± 0.42b	**
n3	3.01 ± 0.02a	3.06 ± 0.17a	2.28 ± 0.12b	***

[a] Mean ± standard deviation ($n = 3^1$).
[b] AV, analysis of variance.
[c] NS, not significant $*p < 0.05$ $**p < 0.01$ $***p < 0.001$.
[d] SFA, Saturated fatty acid.
[e] MUFA, monounsaturated fatty acid.
[f] polyunsaturated fatty acid.
[g] Within each row, means followed by different letters are significantly different. Tukey-Kramer test ($p < 0.05$).

Table 3
Fatty acid composition of "pacu" flesh fed (rosemary extract Herbalox® diet) before and after irradiation[a]

Fatty acid	0 kGy[f]	2 kGy[f]	3 kGy[f]	AV[b]
14:0	2.52 ± 0.23	2.36 ± 0.12	2.31 ± 0.11	NS
16:0	20.77 ± 1.58	20.73 ± 0.11	20.08 ± 0.31	NS
16:1	4.88 ± 0.22a	5.36 ± 0.57a,b	4.74 ± 0.12b	*
18:0	9.48 ± 0.96	8.89 ± 1.09	9.71 ± 0.26	NS
18:1 n-9	37.79 ± 2.18	37.94 ± 0.32	39.55 ± 0.47	NS
18:2 n-6	18.85 ± 0.13	19.16 ± 0.35	19.25 ± 0.40	NS
18:3 n-3	1.45 ± 0.15	1.59 ± 0.20	1.39 ± 0.10	NS
20:3 n-6	0.86 ± 0.07a	0.83 ± 0.01a,b	0.74 ± 0.03b	*
20:4 n-6	1.73 ± 0.19a	1.70 ± 0.07a	1.34 ± 0.10c	*
20:5 n-3	0.29 ± 0.04	0.28 ± 0.07	0.16 ± 0.01	NS
22:5 n-6	0.64 ± 0.09a	0.59 ± 0.02a	0.36 ± 0.02b	**
22:6 n-3	0.74 ± 0.09a	0.38 ± 0.10b	0.59 ± 0.02a	**
SFA[c]	32.77 ± 2.77	31.99 ± 0.95	32.11 ± 0.43	NS
MUFA[d]	43.67 ± 2.40	43.30 ± 0.25	44.29 ± 0.59	NS
PUFA[e]	23.55 ± 0.37a	24.74 ± 0.70b	23.60 ± 0.20a,b	*
n9	37.79 ± 2.18	37.94 ± 0.32a	39.55 ± 0.47b	NS
n6	21.08 ± 0.47a	22.27 ± 0.42b	21.68 ± 0.26a,b	*
n3	2.47 ± 0.11a	2.46 ± 0.28a	1.92 ± 0.20b	*

[a] mean ± standard deviation ($n = 3.^1$).
[b] AV, Analysis of variance, NS, not significant $*p < 0.05$ $**p < 0.01$ $***p < 0.001$.
[c] SFA, saturated fatty acid.
[d] MUFA, monounsaturated fatty acid.
[e] PUFA, polyunsaturated fatty acid.
[f] Within each row, means followed by different letters are significantly different. Tukey–Kramer test ($p < 0.05$).

Table 4
Fatty acid composition of "pacu" flesh fed (tocopherol) diet before and after irradiation[a]

Fatty acid	0 kGy[f]	2 kGy[f]	3 kGy[f]	A.V.[b]
14:0	2.14 ± 0.06	2.34 ± 0.09	2.20 ± 0.16	NS
16:0	20.18 ± 0.99	21.10 ± 0.40	20.87 ± 0.56	NS
16:1	4.91 ± 0.25	4.88 ± 0.21	5.13 ± 0.26	NS
18:0	8.54 ± 0.89	8.99 ± 0.98	7.97 ± 0.41	NS
18:1 n-9	36.15 ± 1.54	36.47 ± 0.39	37.83 ± 0.27	NS
18:2 n-6	19.27 ± 0.73	19.23 ± 0.27	19.42 ± 1.07	NS
18:3 n-3	1.35 ± 0.07	1.28 ± 0.07	1.47 ± 0.10	NS
20:3 n-6	1.21 ± 0.12	1.09 ± 0.14	1.01 ± 0.06	NS
20:4 n-6	3.40 ± 0.79a	2.51 ± 0.33a,b	2.09 ± 0.12b	*
20:5 n-3	0.34 ± 0.05	0.30 ± 0.03	0.34 ± 0.08	NS
22:5 n-6	1.30 ± 0.19a	0.85 ± 0.19b	0.68 ± 0.03b	**
22:6 n-3	1.30 ± 0.11	0.97 ± 0.15	1.00 ± 0.016	NS
SFA[c]	30.86 ± 1.91	32.42 ± 1.44	31.05 ± 0.81	NS
MUFA[d]	41.06 ± 1.78	41.36 ± 0.49	42.95 ± 0.08	NS
PUFA[e]	28.10 ± 1.39	26.23 ± 0.97	26.02 ± 0.24	NS
n9	36.15 ± 1.54	26.47 ± 0.39	37.830 ± 0.27	NS
n6	25.12 ± 1.42	23.68 ± 0.92	23.20 ± 0.98	NS
n3	2.99 ± 0.10	2.55 ± 0.06	2.82 ± 0.29	NS

[a] Mean ± standard deviation ($n = 3^1$).
[b] AV, analysis of variance, NS, not significant.
[c] SFA, saturated fatty acid.
[d] MUFA, monounsaturated fatty acid.
[e] PUFA, polyunsaturated fatty acid.
[f] Within each row, means followed by different letters are significantly different. Tukey–Kramer test ($p < 0.05$).

In the tocopherol group, a significant difference was observed only in the highly unsaturated fatty acid. Wolters, Tilbury and Konings (1987) suggested that fatty acyl chains containing more double bonds were more radiosensitive.

Oxidative changes in fish flesh are quantified by the measurement of TBARS, in nonirradiated and irradiated samples. The results are shown in Table 5.

The values of TBARS evaluated in μg malonaldehyde/g fish fillet, and the standard curve were $y = 0.168 \times (r = 0.9968)$.

The data show that TBARS in the fish flesh of control, BHT and rosemary extract had statistically significant differences between nonirradiated and irradiated samples. However, for the tocopherol group a significant difference was observed only at the 3 kGy dose.

These results suggest that the tocopherol had the best protection against oxidation when compared with the effects of other antioxidants. Buckley and Morrissey (1992) suggest that because α-tocopherol is an integral part of the membrane, it consequently stabilises membrane lipids.

Galvin, Morrissey and Buckley (1998) suggests that in chicken, supplementation at levels of at least 200 mg of α-tocopheryl acetate/kg diet is required to stabilise irradiated breast and thigh meat (dose 2.5 and 4 kGy) following cooking and during storage.

Table 5
TBARS levels (μg malonaldehyde/g fish fillet) in irradiated and non-irradiated fish[a,c]

Treatment	0 kGy	2 kGy	3 kGy	AV[b]
Control	0.401 ± 0.048a	0.681 ± 0.092b	0.634 ± 0.091b	*
Tocopherol	0.176 ± 0.055a	0.364 ± 0.098a,b	0.460 ± 0.103b	*
BHT	0.200 ± 0.037a	0.349 ± 0.014b	0.393 ± 0.058b	**
Rosemary extract	0.135 ± 0.050a	0.388 ± 0.111b	0.462 ± 0.076b	**

[a] Means ± standard deviation ($n = 3$).
[b] AV, analysis of variance.
[c] Within each row, means followed by different letters are significantly different ($p < 0.05$).

Various researchers have also found that feed which includes tocopherol supplementation decreases lipid oxidation in the flesh of various fish species, such as trout (Frigg, Prabuck & Ruhdel, 1990) and channel catfish (Gatlin III et al., 1992; O'Keefe & Noble, 1987).

Hampson, Fox, Lakritz and Thayer (1996) showed a dose dependence of malonaldehyde production due to irradiation in pork, lamb, beef and turkey muscle. However, no significant statistical difference was found in the range 0–10 kGy.

According to the results, the use of antioxidants was important in protecting against lipid oxidation and of all the three antioxidants used in this trial, tocopherol was the most efficient.

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