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EFFECT OF SUPERFICIAL TREATMENT WITH NEW NATURAL ANTIOXIDANT ON SALMON *(SALMO SALAR)* LIPID OXIDATION

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Lipid oxidation is one of the main factors responsible for the quality loss in refrigerated and frozen stored fish products. The aim of the study was to evaluate the effect of superficial treatment of Atlantic salmon *(Salmo salar)* with dihydroquercetin (DHQ) solutions on the hydrolytic and oxidative changes in fish lipids during refrigerated storage. It was found that treatment with DHQ solution (1.0 g l^{-1}) reduced approximately twice the free fatty acids content of chilled stored salmon. After 11 days of storage at 1 °C, the contents of hydroperoxides (HPO) and 2-thiobarbituric acid reactive substances (TBARS) of these samples decreased with 45.00 and 0.91 mg MDA/kg, respectively. The share of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) in control and experimental samples did not differ significantly ($P>0.05$). Results obtained show that the superficial treatment of salmon with DHQ solution (1.0 g l^{-1}) delayed the hydrolytic and oxidative changes in fish lipids significantly, thus preserving the salmon freshness up to 11 days of storage at 1 °C.

Keywords: fish, dihydroquercetin, free fatty acids, hydroperoxides, malondialdehyde, fatty acids

Lipid oxidation is one of the main processes responsible for off-flavour development and quality loss in refrigerated and frozen stored fish products (Hultin, 1994).

Secondary products of lipid oxidation accumulated in fish cause rancid odour, bitter taste, yellowing or darkening of the superficial hypodermic layer and subsequently in depth of the muscle tissue (Tironi et al., 2007). Oxidative deterioration contributes significantly to the limited shelf-life of fatty fishes. Tironi and co-workers (2007) found significant lipid oxidation in minced Argentinian sandperch *(Pseudopercis semifasciata)* muscles during the first 6 days of storage at 1 °C. Ösogul and co-workers (2004) suggested vacuum-packaging of chilled salmon, and Pastoriza and co-workers (1999) packing in carbon dioxide atmosphere to prevent hydrolytic and oxidative deterioration of fish flesh.

Another approach for inhibition of fish lipid oxidation is the addition of antioxidants. They reduce the rate of lipid oxidation and hydrolysis by sequestering and stabilizing free radicals. New natural phenolic compounds are used as antioxidants for stabilising fish products (Pazos et al., 2006). A superficial treatment of fish flesh with phenols isolated from grape (Pazos et al., 2005), mixture of tocopherols and rosemary, sage and black tea extracts (Shahidi, 2000) was suggested. Montero and co-workers (2005) established that the rosemary extract is a more effective antioxidant in fish flesh than quercetin. Becker and co-workers

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(2007) found that rutin is an effective antioxidant only in the liposomes, where it showed synergism with quercetin.

The aim of the present study was to evaluate the effect of superficial treatment with dihydroquercetin solutions on the hydrolytic and oxidative changes of muscle lipids during refrigerated storage of Atlantic salmon *(Salmo salar)* slices.

1. Materials and methods

1.1. Antioxidant dihydroquercetin isolate

The natural antioxidant DHQ was extracted from Siberian larch (*Larix sibirica* Ledeb). The product was supplied by Flavit Ltd (Pushchino, Russia), and it contains 96% dihydroquercetin, 3% dihydrokaempferol, and approximately 1% naringenin.

1.2. Fish sample preparation and experimental design

Seventy pieces of chilled salmon (6 days post mortem) were transported in 30 min from the fish processor "Nessi 5" Ltd (Plovdiv, Bulgaria) by vehicle transport, equipped with refrigeration system. The fish was cut into 1.5–2.0 cm slices, and was divided into ten equal portions, including pieces from different salmon parts. Four portions (control sample) were packed without antioxidant treatment. Three portions (sample 1) were packed after superficial spraying with a solution containing 0.5 g DHQ diluted in 50 ml ethanol and 950 ml re-distilled water. The remaining three portions (sample 2) were superficially treated with DHQ solution (1.0 g l^{-1}) prepared as described above. The salmon slices were drained away for 15 min at 0±1 °C and packed. The samples were vacuum-packaged, and were quickly chilled at an air temperature of –18 °C until the temperature in the samples' centre reached 2 °C. They were then stored for 11 days at 1±1 °C. The changes of the concentrations of free fatty acids (FFA), lipid hydroperoxides (HPO), free malondialdehyde expressed as 2-thiobarbituric acid reactive substances (TBARS), and fatty acid composition (FAC) of the total lipids were measured after 1, 4, 7, and 11 days of storage (e.g. 6, 10, 13, and 17 days post mortem).

1.3. Lipid extraction

The total light musculature lipids of the fish were extracted by the Bligh and Dyer (1959) method.

1.4. Determination of FFA content

The content of FFA was established indirectly by the acid value (AV). The percentage content of FFA was calculated by the following equation: FFA (g kg^{-1})=5.03×AV (mg KOH/g lipids) (Pomeranz & Meloan, 1994).

1.5. Hydroperoxides and TBARS determination

Hydroperoxides and free MDA from the fish lipids were measured by the TBARS tests, as described by Schmedes and Hølmer (1989), using UV-VIS spectrophotometer Camspec, Model M 550 (Camspec Ltd., Leeds, UK). The 2-thiobarbituric acid was obtained from Sigma-Aldrich (St. Louis, Missouri, USA). The lipid peroxidation MDA colorimetric assay kit was supplied by BioVision (Milpitas, CA, USA).

1.6. Fatty acids composition (FAC) analysis

Fatty acid methyl esters (FAME) were prepared according to the procedure described by Christie (2003). Gas chromatography of fatty acid methyl esters was performed on a Shimadzu 17A gas chromatograph (Shimadzu GmbH, Duisburg, Germany), equipped with CP-Wax 52 CB capillary column (30 m×0.25 mm×0.25 µm) (Varian Chrompack, Netherland); a flame-ionization detector (FID), and Shimadzu CR-5A integrator (Shimadzu GmbH, Duisburg, Germany). FAME were identified by comparing the retention times with FAME standards (Sigma-Aldrich St. Louis, Missouri, USA). Preparation of fatty acid 4,4'-dimethyloxazoline (DMOX) esters was done by the procedure of Christie (2003). An Agilent 6890 Plus System (Agilent Technologies, Santa Clara, CA, USA) equipped with a 5793 mass selective detector (Agilent Technologies, Santa Clara, CA, USA) and a 30 m×0.25 mm×0.25 µm SP-2380 capillary column (Supelco, Bellefonte, PA, USA) was used to examine the DMOX derivatives. Fatty acids were identified by comparing the respective mass-spectra with those of the authentic standard DMOX fatty acid derivatives, Sigma-Aldrich (St. Louis, Missouri, USA).

1.7. Statistical analysis

Data were statistically analysed using the SPSS 11.0 software (SPSS Inc., Chicago, Illinois, USA). All determinations of FFA, HPO, TBARS, and FAC were carried out nine times. Data were analysed by analysis of variance with a significant level of $P \leq 0.05$ (Draper & Smith, 1998). The Duncan's multiple comparison test (SPSS), with a significant difference set at $P \leq 0.05$, was used to compare sample means.

2. Results and discussion

2.1. Determination of free fatty acids (FFA)

An increase of FFA was found in all samples, indicating for lipolysis development during chilled storage at 1 °C (Fig. 1). Pronounced lipolysis was established in the control sample, where the FFA content increased with 11.6 g kg^{-1} at the end of the experiment (11th day) (Fig. 1). These results were in agreement with the findings of Tironi and co-workers (2007), who established pronounced lipolysis of minced sea salmon *(Pseudopercis semifasciata)* after 9 days of storage at 1 °C. There are various endogenous enzymes in the fish skin and muscles, which could initiate and spread lipid hydrolysis, followed by lipid oxidation. Significantly ($P<0.05$) lower accumulation of FFA was found during storage of the experimental samples in comparison with the controls. After 11 days of the experiment, FFA content increased with 3.9 g kg^{-1} and 3.1 g kg^{-1} for the experimental samples 1 and 2, respectively. Such slight increase of FFA content indicates that the lipolytic processes were at their initial stage. The results obtained show that superficial treatment of salmon slices with DHQ solutions (0.5 or 1.0 g l^{-1}) significantly delays lipolysis of the fish muscle lipids. Probably, the natural dihydroquercetin isolate inhibits lipoxygenase and cyclooxygenases of the salmon muscle. The FFA contents of experimental samples 1 and 2 were lower than the control by 7.7 g kg^{-1} and 8.5 g kg^{-1}, respectively. Moreover, the FFA content of all experimental samples remained significantly lower than the critical for the quality value of 12.5 g kg^{-1}. After 11 d of storage at 1 °C, the lipolysis of the salmon slices sprayed superficially with DHQ solution (1.0 g l^{-1}) was delayed nearly twice compared to the control sample.

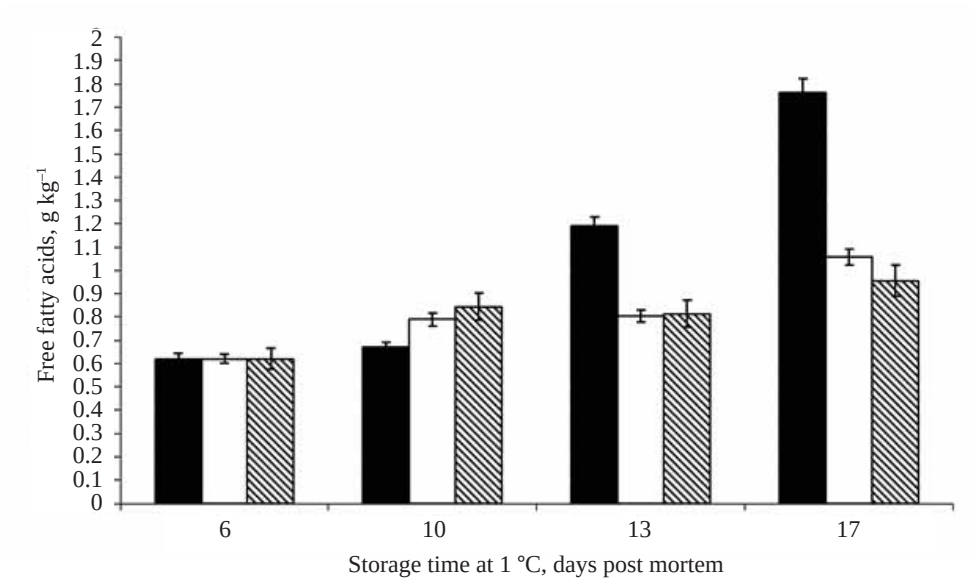

Fig. 1. Effect of superficial treatment with DHQ solutions (0.5 g l^{-1} or 1.0 g l^{-1}) on the free fatty acid formation (g kg^{-1} total extracted light muscle lipids) during storage at 1 °C. Symbols: ■: Control sample; □: sample 1; ▨: sample 2. Remark: FFA of sample 1 and sample 2 on d 13 post mortem are significantly ($P<0.05$) lower than of the control sample, but did not differ significantly among themselves ($P>0.05$)

2.2. Hydroperoxides (HPO)

During the chilled storage of the samples, an exponential increase of lipid HPO was established (Fig. 2). Results showed that initial HPO content of the control sample was 25.5 mg MDA/kg. After 11 d of storage at 1 °C, the HPO content of the control sample increased significantly ($P<0.05$) and reached the value of 897 mg MDA/kg (Fig. 2). At the end of experiment (11 days), the HPO of experimental samples 2 and 1 was 47.5 mg and 32.5 mg MDA kg^{-1}, respectively, significantly lower than the control sample (Fig. 2). However, the effectiveness of superficial treatment with DHQ solution of the salmon slices is limited by the fact that the free lipid hydroperoxides are fat soluble and those are relatively stable at low temperature (Zarzycki & Swiniarska, 1993). Thus, they could spread the reaction in depth of the fish flesh, where the antioxidant solution cannot penetrate.

2.3. Thiobarbituric acid reactive substances (TBARS)

TBARS values of all studied salmon samples increased significantly ($P<0.05$) during the chilled storage (Fig. 3). Pronounced accumulation of TBARS in control samples was established. The TBARS values of these samples increased from 0.15 mg MDA/kg during the first four days of storage to 1.29 mg MDA/kg after 11 days of storage. Tironi and co-workers (2007) also found significant increase of TBARS values of Argentinian sandperch *(Pseudopercis semifasciata)* during the first 6 days of storage at 1 °C. Retarded TBARS accumulation during the chilled storage of the experimental samples was established (Fig. 3). TBARS values of these samples did not exceed 0.3 mg MDA/kg at the 7th day of storage. Therefore, it can be assumed that the lipid oxidation of the experimental samples is in the

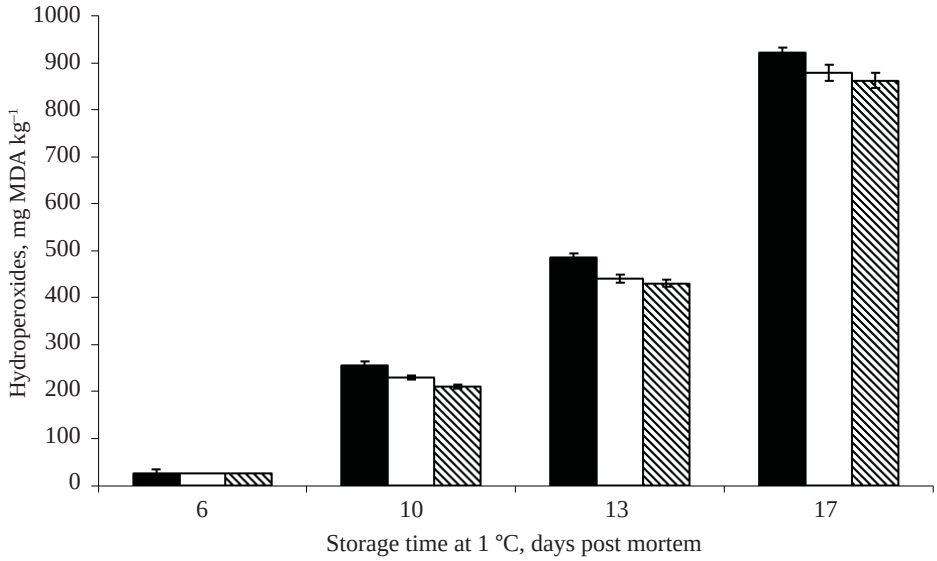

Fig. 2. Effect of superficial treatment with DHQ solutions (0.5 g l^{-1} or 1.0 g l^{-1}) on the lipid hydroperoxides formation (mg MDA/kg total extracted light muscle lipids) during storage at 1°C. Symbols: ■: Control sample; □: sample 1; ▨: sample 2. Remark: HPO of sample 1 and sample 2 on d 13 and d 17 post mortem are significantly ($P<0.05$) lower than of the control sample, but did not differ significantly among themselves

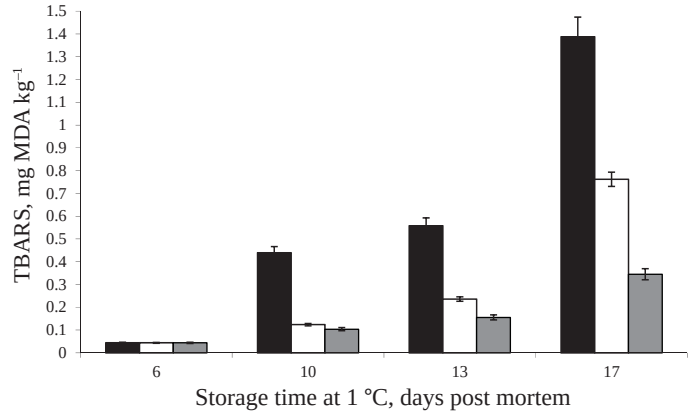

Fig. 3. Effect of superficial treatment with DHQ solutions (0.5 g l^{-1} or 1.0 g l^{-1}) on the TBARS (mg MDA/kg) content during storage at 1 °C. Symbols: ■: Control sample; □: sample 1; ▩: sample 2. Remark: TBARS of control sample, sample 1 and sample 2 are significantly ($P<0.05$) different throughout the studied period, with the exception of the initial values – d 6 post mortem ($P>0.05$)

initial stage till the 7th day of the storage. At this stage, the TBARS values of experimental samples 1 and 2 were 0.37 mg and 0.45 mg MDA/kg, respectively, lower than the control sample. Our results were in accordance with the data reported by Vareltzis and co-workers

(1997), who established significantly lower concentrations of free MDA in frozen stored horse mackerel *(Trachurus trachurus)* and Mediterranean hake *(Merluccius mediterraneus)* treated with rosemary *(Rosmarinus officinalis)* extracts. The significant delay of the lipid oxidation development in salmon samples treated with DHQ solution (1.0 g l^{-1}) could be explained by the powerful antioxidant capacity of DHQ (Shahidi, 2000). It is well known that the antioxidant activity of flavonoids is due to their ability to reduce free radical formation and to scavenge free radicals (Cao et al., 1997). Thus, they react as hydrogen donating free radical scavengers.

2.4. Fatty acids composition (FAC) of fish lipids

It was established that the FAC of the salmon light musculature lipids underwent heterogeneous changes during the storage at 1 °C (Table 1). At the end of the experiment (11th day), FAC of experimental sample 2 was similar to the FAC of the control sample, determined at the beginning of experiment (day 1).

Table 1. Fatty acid compositions (g kg^{-1}), fatty acid classes (% of fatty acids), ω3/ω6 and C16:0/(C20:5+C22:6) ratios of light muscle lipids (% of fatty acids)

Fatty acid	Control sample 1 d	Control sample 4 d	Sample 1 4 d	Sample 2 4 d	Control sample 7 d	Sample 1 7 d	Sample 2 7 d	Control sample 11 d	Sample 1 11 d	Sample 2 11 d
14:0	36.9	36.5	36.8	36.7	33.8	33.4	34.3	37.7	37.8	37.7
15:0	3.1	3.1	3.1	3.3	2.9	2.7	3.1	2.9	3.0	3.2
16:0	120.6	120.3	121.5	118.8	121.6	119.1	118.1	121.6	120.9	120.2
16:1ω7	39.0	38.7	38.9	39.1	37.6	38.2	40.2	36.8	39.1	44.0
16:2ω4	4.7	4.5	4.9	5.1	4.6	5.0	5.0	4.8	4.9	5.0
17:0	2.0	1.9	1.8	1.7	2.0	1.8	1.7	2.2	2.0	1.8
16:3ω3	2.8	2.7	2.8	2.2	2.6	2.5	2.3	2.8	2.7	2.2
17:1ω9	3.0	2.8	2.7	3.1	3.1	3.0	2.8	3.0	2.9	2.8
16:4ω3	3.4	3.4	3.3	3.2	3.4	3.3	3.2	3.4	3.4	3.3
18:0	39.9	39.0	38.8	37.2	38.5	37.3	37.0	36.3	35.4	35.1
18:1ω9	419.1	412.0	411.5	411.2	418.3	410.4	412.1	399.0	398.1	393.8
18:1ω7	31.8	31.6	30.8	29.6	31.5	30.7	29.4	31.2	30.5	29.3
18:2ω9	1.8	1.8	2.3	2.4	1.9	2.4	2.6	2.4	2.7	2.8
18:2ω6	73.0	73.1	73.5	74.9	75.4	72.8	75.0	64.6	71.0	72.1
18:3ω3	16.2	16.5	17.0	17.1	13.9	14.2	16.8	12.3	17.2	17.2
18:4ω3	11.6	11.5	11.8	12.0	11.4	11.6	12.0	11.3	12.5	12.8
20:0	4.7	4.5	4.6	4.4	4.7	4.5	4.5	4.4	4.3	4.2
20:1ω9	73.1	72.0	70.3	74.2	74.6	74.1	72.3	89.2	75.4	73.0
20:2ω9	8.1	7.9	8.6	8.9	7.9	8.1	8.2	8.0	8.1	8.5
20:4ω6	3.3	3.4	4.3	4.2	3.9	3.6	4.4	3.0	5.6	6.2
20:4ω3	13.0	13.4	13.7	14.0	13.1	13.0	14.1	13.5	14.8	14.9

Table 1. Continued

Fatty acid	Control sample 1 d	Control sample 4 d	Sample 1 4 d	Sample 2 4 d	Control sample 7 d	Sample 1 7 d	Sample 2 7 d	Control sample 11 d	Sample 1 11 d	Sample 2 11 d
20:5ω3	8.1	8.6	8.0	9.1	8.6	8.1	8.9	10.6	10.8	11.6
22:1ω11	60.8	68.1	67.7	65.5	67.2	67.0	63.6	69.4	67.0	62.2
22:3ω6	3.8	3.8	3.9	3.6	4.1	3.9	4.3	5.0	5.2	5.6
22:5ω3	traces	traces	traces	traces	traces	traces	traces	traces	traces	traces
22:6ω3	16.0	14.9	13.3	14.5	15.1	20.2	20.1	20.4	20.3	25.0
24:1ω9	0	4.0	4.1	4.5	4.3	4.1	4.0	4.2	4.4	5.0
SFA	207.2	205.3	206.6	202.1	203.5	198.8	198.7	205.1	203.4	202.2
MUFA	627.0	629.2	621.9	627.2	636.6	627.5	624.4	629.8	617.4	610.1
PUFA	165.8	165.5	171.5	170.7	159.9	173.7	176.9	165.1	179.2	187.7
EPA + DHA	24.1	23.5	21.3	23.6	23.7	28.3	29.0	31.0	31.1	36.6
ω3/ω6	0.84	0.88	0.86	0.87	0.81	0.91	0.92	1.02	1.00	1.04
Polyene index [16:0/ (EPA+ DHA)]	5.00	5.12	5.70	5.03	5.13	4.21	4.07	3.92	3.89	3.28
Total fat	12.85	12.89	12.81	12.83	12.95	12.89	12.88	12.99	12.92	12.95

MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EPA: eicosapentaenoic acid (20:5ω3); DHA: docosahexaenoic acid (22:6ω3)

It was found that FAC, as well as the percentage of the saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) of salmon superficially treated with DHQ solution (1.0 g l^{-1}) did not change significantly ($P>0.05$) during cold storage.

3. Conclusion

The results of the present study revealed that the superficial treatment of Atlantic salmon *(Salmo salar)* slices with DHQ solution (1.0 g l^{-1}) could be used for preserving their freshness up to 11 days (17 days post mortem) of storage at 1 °C. Such treatment delays significantly the lipolytic and oxidative changes in fish. However, the antioxidant solution could not penetrate deep into the fish flesh, due to its specific muscle tissue structure, limiting the effectiveness of superficial DHQ treatment.

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