Antioxidant properties of tert-butylhydroquinone and propyl gallate in frozen minced sprats

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Abstract

Relative effects of the combination of tert-butylhydroquinone (TBHQ) and propyl gallate (PG) with or without a sequestering agent (EDTA or citric acid, CA) on rancidity development in minced sprats stored at -15 C were determined by following the changes in peroxide values, thiobarbituric acid values, sensory scores, and fatty acid composition of lipid extracts. The combination of 0.02% TBHQ and 0.02% PG was most effective in reducing the rate of oxidative rancidity development in frozen sprats. It allowed an extended induction period of at least 250 days. Lowering the concentration of the additives from 0.02% to 0.01% significantly reduced their antioxidant effectiveness giving rise to larger losses of polyunsaturated fatty acids. Addition of either EDTA or CA to the mixture of 0.02% TBHQ and 0.02% PG did not prevent significant losses of 20:5 and 22:6 fatty acids.

Introduction

Fish, in general, are highly prone to oxidative rancidity. This may be due to the highly unsaturated fatty acids present in fish lipids. Lipid oxidation in fish may be enhanced by iron and other haematin compounds that are inherent in fish tissues. During mincing, these elements come in close contact with fish lipids. This condition favors oxidation and is exacerbated by the increased surface area of the fish tissues to oxygen.

Minced fish offers a means for optimum utilization of small pelagic species of fish and fish processing wastes intended for human consumption. It is generally used

as a basic ingredient for surimi processing and product development. In view of the high unsaturation of lipids in the mince, its storage quality is critically affected by oxidation.

Antioxidants have been reported to reduce the rate of oxidation in fish. The choice between antioxidants depends on their compatibility and permeability in the food system. Porter (1980) reported that butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are highly soluble in oil but completely insoluble in water. Tert-butylhydroquinone (TBHQ) and propyl gallate (PG) are slightly soluble in water. TBHQ is soluble in oil but not as soluble as BHT and BHA. TBHQ and PG, being amphiphilic, have a high surface energy and are expected to concentrate at the lipid-water interface. Sequestering agents such as ethylenediaminetetraacetic acid (EDTA) and citric acid (CA) are virtually insoluble in triglycerides and hence, effective at the lipid-water interface. However, these agents are believed to be useful only when trace catalysts (e.g., copper or iron) are present, and in their absence EDTA has no protective action (Licciardello *et al.* 1982).

The objective of the study was to determine the relative effectiveness of TBHQ, PG and sequestering agents in inhibiting the development of oxidative rancidity in frozen fatty fish minces stored at -15°C.

Materials and Methods

Antioxidants and sequestering agents used in the study include tert-butylhydroquinone, propyl gallate, ethylenediaminetetraacetic acid, and citric acid.

Sample preparation

Fresh sprats (*Sprattus sprattus*) were obtained frozen within one day of capture from Grimsby, South Humberside, U.K. Sprats were minced using the flesh bone separator (Baader 694) and then separated into 5 batches. The following materials were then added by mixing in a Hobart mixer for exactly 2 minutes.

> Lot A - no additives (control)
> Lot B - 0.02% TBHQ and 0.02% PG
> Lot C - 0.01% TBHQ and 0.01% PG
> Lot D - 0.02% TBHQ, 0.02% PG and 0.01% EDTA
> Lot E - 0.02% TBHQ, 0.02% PG and 0.01% CA

The samples (1 kg) were packed, sealed in low density polyethylene bags, rapidly frozen in a plate freezer, and kept at -30°C for not more than 5 days until all the initial tests had been carried out.

The temperature was then raised to -15°C and oxidative rancidity development was periodically monitored for over 431 days by measuring the peroxide value and thiobarbituric acid value, sensory assessment, and gas chromatographic analysis of the fatty acid composition.

Analytical procedures

Lipid content. Total lipid content was determined by a modification of the method of Bligh and Dyer (1959) in which 0.1 g BHT was added to the chloroform-methanol solvent (20:40). For lipid intended for peroxide value measurement and gas liquid chromatography analysis, care was taken to prevent further oxidation. Care was also taken to exclude light and to remove solvent under vacuum at or below 40°C.

Moisture content. The mince (5 g) was weighed in a tared moisture dish with a purified dry sand. Industrial ethanol (5 ml) was added and the dish was placed in a water bath (80°C) to allow ethanol to evaporate. The sample was dried to constant weight at 105°C.

Peroxide value (*PV*). Peroxide value v/as determined by following the modified method of Banasihan (1985). Lipid was extracted as described earlier. The chloroform extract containing the lipid was evaporated under reduced pressure to a final volume of 70 to 90 ml. Twenty ml of extract was accurately pipetted into a 500 ml iodine flask and 50 ml of acetic acid:chloroform solution (60:40) was added with shaking to dissolve the lipid. Saturated potassium iodide solution (1 ml) was added, and the mixture was gently mixed for exactly 20 sec and then kept in the dark for 30 min. Distilled water (100 ml) was added and the liberated iodine was titrated with 0.05M sodium thiosulphate using 1 % starch solution as an indicator. A blank run was also carried out.

Thiobarbituric acid value (TBA). Thiobarbituric acid value was determined by a modification of the method of Tarladgis *et al.* (1960). Ten grams of the mince was homogenized with 50 ml distilled water for 2 min. The homogenate was transferred to a 150 ml round bottom flask by washing with an an additional 47.5 ml distilled water. To this mixture, 5 drops of an antioxidant mixture (0.3 g BHA dissolved in 5.4 ml propylene glycol and mixed with 0.3 g BHT dissolved in 4.0 g warm Tween 20) and 1 ml of 0.2% EDTA. The absorbance was measured at 532 nm.

Fatty acid methyl ester preparation and analysis. Methyl esters were prepared based on the modified procedure as described in the IUPAC (1979) standard methods of analysis of fats and fatty oils. Lipid sample (0.1 g) was weighed into a 50 ml pear-shaped flask, tetrahydrofuran (1 ml) and 0.5 M methanolic potassium hydroxide solution (4 ml) were added. The mixture was refluxed for 10 min. The contents were then cooled. Methanolic boron trifluoride (5 ml) was added and the mixture was boiled for 2 to 3 min. The contents were cooled, then petroleum ether (1 ml) and saturated sodium chloride solution (1 ml) were added. Methyl ester solution was washed with 2% potassium carbonate (4 ml) and dried with anhydrous sodium sulphate. The solution was filtered using Whatman filter paper (No. 1) and kept at -30°C until gas chromatographic analysis.

Fatty acid methyl esters were analyzed using an OV-101 WCOT fused silica capillary column with the programmed temperature setting: 50°C for 1 min; ramp rate, 30°C/min; 160°C for 61 min; ramp rate, 30°C/min; and 180°C for 80min. The

detector temperature was 220°C. The pressures used for helium, nitrogen, hydrogen, and air were 10, 60, 20, and 30 psi, respectively.

Methyl esters were identified by plotting the logarithm of the retention time of the fatty acid methyl ester standards against their carbon number. The methyl ester standards used were 14:0; 16:0; 18:0; 18:ln-9; 20:4n-6; 22:ln-9; and 22:6n-3. Identification was confirmed for sprat lipids by comparing with the data given by Hardy and Mackie (1969).

Sensory assessment. The lipid extracted from the stored minced sprats was assessed using a triangle test (Larmond 1982) and linear scaling (Amerine *et al* 1965). The lipid sample (10 ml) was pipetted into a dark sample bottle, stoppered and warmed for about 15 min at 30°C. A panel of 15 trained assessors was asked to assess the lipid samples while still warm.

Results and Discussion

Effect of antioxidants (TBHQ and PG) and sequestering agents (EDTA and CA) on rancidity development in minced sprats was studied by following changes in PV's, TBA values, sensory assessment, and lipid fatty acid composition.

Changes in peroxide and thiobarbituric values

Similar trends in the PV and TBA values were observed for the untreated mince or control (Figs. 1 and 2). The control generally showed higher PV and TBA values (P < 0.01) throughout the 431-day storage period and a shorter induction period (approximately 20 to 25 days) than any of the treated minces. In addition, the control gave initial PV and TBA values significantly higher (P < 0.05) than the treated minces. This may be due in part to the entrapped oxygen and pro-oxidants in the tissues which were brought in contact with the lipids during mincing, causing significant oxidation to occur in the absence of rancidity inhibitors. Fluctuations in both PV and TBA values of the control are apparent in measurements between approximately 34 and 120 days followed by a gradual increase up to about 431 days. This observation is in contrast to the PV changes found for the model systems (Labuza 1971) and for vegetable oils above 60°C (Ibrahem 1984) where no fluctuations occurred. These erratic variations may partly be due to different rates of hydroperoxide production during extraction and isolation of the fish lipids. Variations in fish may have also contributed to the variability. Hardy and Smith (1976) found erratic PV changes in mackerel fillets stored at - 14°C to -21°C and -29°C which may have been due to the non-stoichiometric production or formation of hydroperoxides and biological variations in fish. Lowering the concentrations of TBHQ and PG from 0.02% to 0.01% increased the PV's and TBA values significantly (Fig. 1 and 2).
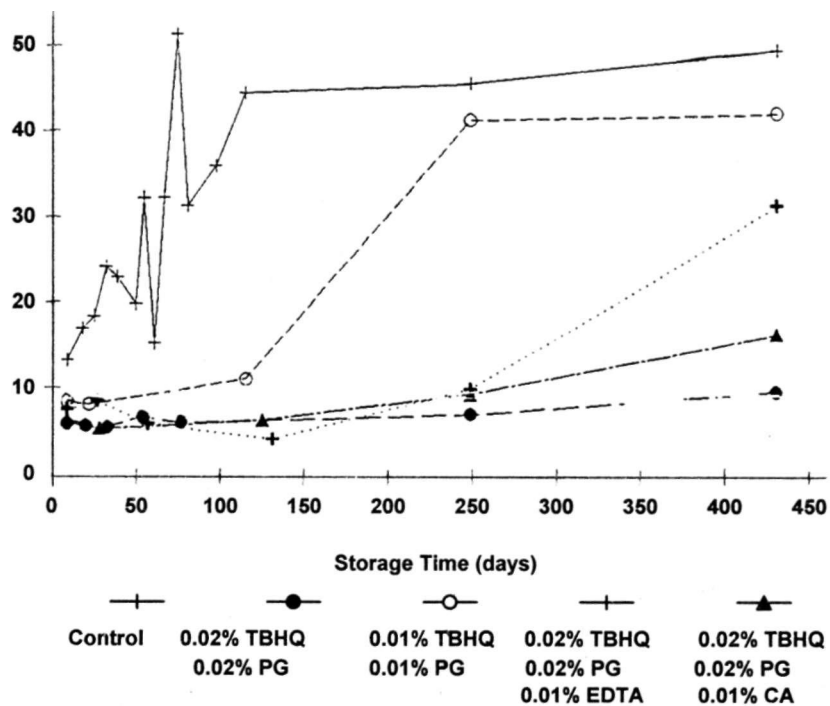
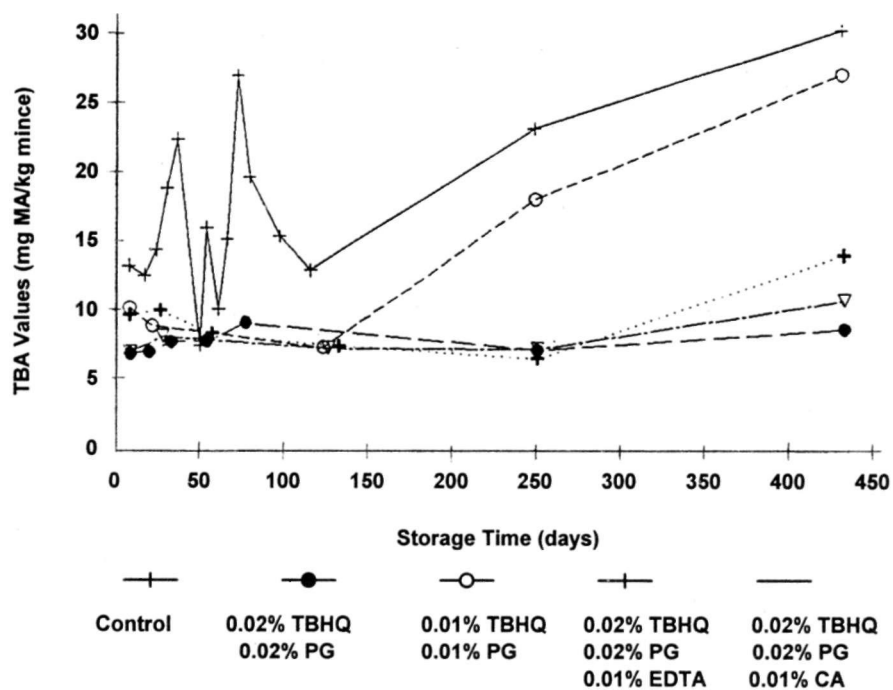Fig. 1.   Effect of antioxidants and sequestering agents on the PV's of minced sprats at -15°C.

Fig. 2.   Effect of antioxidants and sequestering agents on the TBA of minced sprats at -15°C.

Sensory assessment

Results for the rancid odor sensory assessment of lipids extracted from the minced sprats are given in Fig. 3. Fluctuations in the mean values and a high standard deviation of about 3.0 (90% coefficient of variation) were observed during the first 56 days of storage. This may be due in part to the initial sample presentation used, i.e. comparing the samples with sunflower oils containing 1% rancid sprat oil (the odd sample for the triangle tests) and 40% rancid sprat oil as reference for linear scaling. However, results improved (standard deviation < 2; coefficient of variation < 50%) when the rancid odor of the samples was subsequently assessed and compared simultaneously in pairs, using one as the odd sample. Therefore, the assessments for up to about 100 days of storage must be regarded as suspect and only the subsequent results will be discussed.

The scores for the control sample were generally higher (P < 0.05) than the treated samples over the period of 431 days. The control sample was assessed to have an odor value of 3.7 after 431 days which corresponds to a "very rancid" odor. The perceived rancidity in the initial samples illustrates the difficulty that assessors had in distinguishing a 'rancid' from a 'fishy' odor. However, the assessors rated the control sample as being significantly more rancid than in any treated samples after about 431 days of storage. The control sample showed large changes in PV and TBA values over 431 days of storage (Figs. 1 and 2) whereas the rancid odor scores changed little over the storage period (Fig. 3). The sensory testing of the samples appeared less sensitive to changes in the mince than the PV and TBA value determinations. However, sensory testing could assess the relative rancid odors of a set of samples.
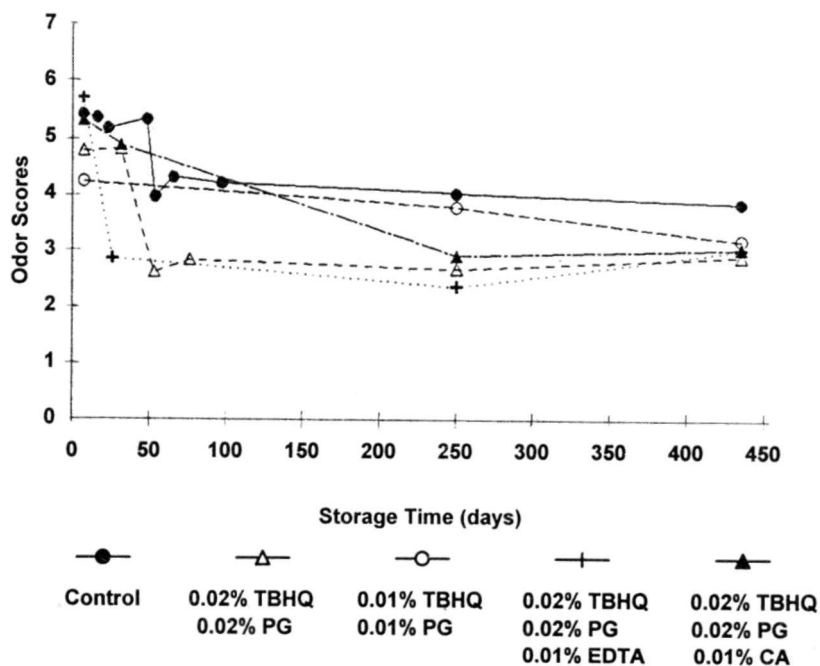

Fig. 3.   Sensory scores for lipids from minced sprats at -15°C with added antioxidants and sequestering agents.

Fatty acid composition

Analytical results in Table 1 give the following initial major fatty acid components in decreasing order of concentrations: 18:1 (20%), 16:0 (19%), 22:6 (12%), 22:1 (12%), 20:5 (8%), 14:0 (6%), 16:1 (7%), 20:1 (6%), 18:0 (3%), 18:3 (2%) and less than 1% of 20:4 and 22:5. These concentrations are in close agreement with those of Hardy and Mackie (1976). The 20:5 and 22:6 fatty acids were generally most affected by oxidative rancidity in minced sprats stored at -15°C. This is not surprising since fatty acids having several double bonds in their carbon chains are known to undergo rapid oxidation. The control sample showed significant losses (P < 0.05) in the polyenes (16%), 20:5 (17%) and 22:6 (19%) after 251 days. However, losses were expectedly smaller than those of the mince treated with lower concentration of anti-oxidants. The control showed larger or similar losses of polyenes, 20:5 and 22:6 as those observed for the rest of the treated minces. The Student's T-test indicates that these losses observed for the control were not significantly different (P > 0.05) from those found in any other samples. No significant losses of the 16:1, 18:3, and 20:4 fatty acids were observed for the control and all other minces.

Effect of TBHQ and PG

The combined 0.02% TBHQ and 0.02% PG was the most effective in inhibiting oxidative changes in minced sprats stored at -15°C since the changes of the PV and TBA values were consistently and significantly lower (P < 0.05) than those observed in other samples (Figs. 1 and 2). These same additives at the 0.02% level showed an extended induction period of at least 250 days. However, lowering the concentrations of these additives to 0.01% significantly reduced (P < 0.01) their effectiveness. The combined 0.01% TBHQ and 0.01% PG gave a shorter induction period of about 120 days. Thereafter, the mince treated with combined 0.01% TBHQ and 0.01% PG exhibited a significant increase in PV and TBA values (P < 0.01). The PV found for the latter reached a maximum of about 41 mEq/kg lipid after about 251 days and was maintained up to 431 days. The TBA values reached a maximum of about 26 mg MA/kg mince at approximately 431 days. These results seem to follow the expected pattern for lipid oxidation. The PV reaches its maximum before the TBA values since hydroperoxides are intermediates in the formation of malonaldehyde in the system.

In terms of sensory assessment, from about 140 days of storage, the rancid odor scores observed for all treatments with 0.02% TBHQ and 0.02% PG were generally lower than for the control and the 0.01% TBHQ and 0.01% PG. No significant difference (P > 0.05) between the scores for lipid samples containing 0.02% TBHQ and 0.02% PG with or without a sequestering agent was detected even after 431 days of storage. The assessors rated the samples treated with 0.02% TBHQ and 0.02% PG as approximately similar to the sample containing 0.01% TBHQ and 0.01% PG (about 3.0) after about 431 days when the PV and TBA values were significantly lower (P < 0.01) than those observed in the mince treated with 0.01% TBHQ and 0.01% PG (Fig. 3).

Losses in fatty acids are generally in agreement with the PV and TBA results after 251 days (Table 1). The mince treated with combined 0.01% TBHQ and 0.01% PG showed a highly significant reduction (P < 0.01) in the polyenes, 20:5 and 22:6 fatty acids. However, increasing the levels of TBHQ and PG to 0.02% significantly reduced (P < 0.05) the losses in the polyenes, 20:5, and 22:6 from 21 to 11%, 21 to 13% and 25 to 12%, respectively. TBHQ combined with ascorbic acid was also found effective in reducing losses of both ω-6 and ω-3 fatty acids (Boyd *et al.* 1993).

Overall, TBHQ and PG are effective in reducing rancidity development and from preventing fatty acid losses when present at concentrations of 0.02%, Polyenes, 20:5 and 22:6 were the fatty acids most affected by oxidative rancidity in minced sprats stored at -15°C. In addition, neither EDTA nor CA significantly increased the antioxidant effectiveness of the combination of TBHQ and PG.

Table 1. Percentage fatty acid composition of lipids, sensory scores, average PV 6k TBA values and % loss of fatty acids from minced sprats stored at -15°C for 251 days*.

		After 251 days of storage				
Acids	Initial Values	Control	0.02% TBHQ 0.02% PG	0.01% TBHQ 0.01% PG	0.02%TBHQ 0.02% PG 0.01% EDTA	0.02% TBHQ 0.02% PG
14:0	6.35	6.47	6.16	6.25	5.81	5.96
16:1	6.78	6.29	6.51	6.54	6.41	6.35
16:0	18.76	18.41	18.09	18.91	17.93	18.03
18:3	1.65	1.44	1.54	1.45	1.55	1.47
18:1	20.34	18.10	18.62	19.04	20.07	20.01
18:0	2.79	2.80	2.85	3.38	3.21	3.20
20:5	8.11	6.51	6.80	6.48	6.98	6.64
20:4	0.66	0.71	0.68	0.67	0.78	0.64
20:1	6.16	6.65	6.11	6.75	6.65	6.48
Sensory scores	3.9	2.6	3.6	2.3	2.8
PV (mEq/kg lipid)	28.7	7.1	17.7	8.1	8.0
TBA(mg MA/kg mince)	6.1	8.1	11.3	8.8	7.9
% loss of 16:1	1	0	2	0	2
% loss of 18:3	0	0	5	7	1
% loss of 20:4	11	3	3	22	3
% loss of 20:5	17	13	21	10	15
% loss of 22:6	19	12	25	15	18
% loss of polyenes	16	11	21	11	15

*Values given in the table are means of triplicate determinations.

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