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Protection of fish feed, made directly from marine raw materials, with natural antioxidants

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ABSTRACT

The present experiments were designed to study the effects of different natural antioxidants in an experimental fish feed, made directly from marine raw materials. A rosemary extract (Herbalox®) and crystalline ascorbic acid were the most effective antioxidants in this feed and the effect of ascorbic acid was enhanced by adding a tocopherol mix. Ascorbyl palmitate, citric acid, a phosphate mix designed to enhance the effect of ascorbic acid, and spermine had minor antioxidant effects, no effect or pro-oxidant effects. It was necessary to add higher concentrations of the rosemary extract than the vitamin C/E combination to obtain an optimal antioxidant effect. A minor effect of adding ethoxyquin to a diet with tocopherol mix and ascorbic acid was detected in one of the experiments, but this effect was not reproduced in the other experiments. It is therefore concluded that the diet used in the present study can be protected against oxidation using natural antioxidants. Since antioxidants must be tested in the oxidising system in which they are going to be used, the present results have to be confirmed before applying them to commercial fish feeds and feed ingredients.

1. Introduction

Fish feeds are partly or fully based on marine ingredients, with high levels of polyunsaturated $n-3$ fatty acids, and are therefore susceptible to lipid oxidation. Presently, fish meal and fish oil used in feeds are protected with synthetic antioxidants, mainly ethoxyquin (EQ) in fish meal and butylated hydroxytoluene (BHT) in fish oil (Lundebye, Hove, Bohne, & Hamre, unpublished). There is a substantial carry-over of these antioxidants to the fish fillet and the mandatory 2 weeks starvation period before slaughter of farmed fish is not sufficient for clearance of these antioxidants from the fillet (Bohne, Lundebye, & Hamre, 2008; Hamre & Bohne, unpublished). Food safety concerns connected to synthetic antioxidants have urged authorities to reduce maximal residual levels of antioxidants allowed in food for human consumption (Bohne et al., 2008) to levels which are just slightly above the levels found in farmed fish (Lundebye et al., unpublished). Therefore, a switch to natural antioxidants in fish feed ingredients would be an advantage both for the aquaculture industry and with regard to consumer health and well-being. The present study was connected to the development of a new method of fish feed production, where whole fish or fish offal were blended with micronutrients and heated using electromagnetic energy (microwaves; Hemre, Sandnes, Lie, Torrisen, & Waagbø, 1995). This method would reduce energy costs, compared to conventional methods, where fish oil and fish meal are first separated and then blended again to make the final feed.

Lipid oxidation is initiated when a free radical ($X^{\cdot}$, $OH^{\cdot}$ or others) abstracts a hydrogen atom from a polyunsaturated fatty acid (PUFA). The PUFA radical formed reacts with oxygen to form a peroxyl radical. The chain reaction propagates when the lipid peroxyl radical abstracts a hydrogen atom from a new PUFA, which enters a new turn in the reaction cycle (Hølmer, 1993). The primary products of lipid oxidation are the conjugated dienes and lipid hydroperoxides, which may undergo cleavage to form different secondary products of low molecular weight, i.e., aldehydes, alkanes, alkenes, alcohols and acids (Horton & Fairhurst, 1987; Hølmer, 1993). Malondialdehyde is often analysed as a representative of the secondary products, for example, by the thiobarbituric acid-reactive substances (TBARS) analyses. Transition metals and reactive oxygen species are known initiators of lipid oxidation.

The antioxidants studied in the present experiments were crystalline ascorbic acid; a mix of phosphates designed to enhance the activity of ascorbic acid; a mix of natural tocopherols with approximately 27%, 1%, 40% and 30% of α, β, γ and δ-tocopherol, respectively; a commercially available rosemary extract, Herbalox®; the lipid-soluble form of vitamin C, ascorbyl palmitate; spermine; and citric acid. Tocopherols act as primary antioxidants by donating a hydrogen to the lipid peroxyl radical, preventing it from starting a new cycle in the auto-oxidation reaction chain. A tocopheoxyl radical is formed in this reaction, which can be reduced to tocopherol by ascorbic acid. Tocopherol also scavenges reactive oxygen

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species in the lipid phase (Frankel, 1998). In addition to the regeneration of tocopherol and perhaps other antioxidants which act as hydrogen donors, ascorbic acid scavenges radicals in the water phase and inactivates metal ions (Frankel, 1998). Antioxidants from rosemary leaves are often extracted by organic solvents (Frankel, 1998), and the main active components are phenolic compounds, the most effective being carnosic acid, carnosol and rosmarinic acid (Erkan, Ayranci, & Ayranci, 2008). Phenolic compounds may act as chain-breaking antioxidants, radical scavengers and metal chelators (Frankel, 1998; Rice-Evans, 1999). Citric acid acts mainly as a metal chelator (Frankel, 1998). Spermine is a polyamine present in all animal tissues and was proposed as an important antioxidant, protecting skin against UV radiation (Løvaas, 1995). The compound was shown to be 30 times more efficient than α-tocopherol in protecting fish oil against lipid oxidation (Løvaas, 1991). Antioxidants such as ascorbic acid and tocopherols may act as pro-oxidants under certain conditions, especially at very high concentrations or when the antioxidants are involved in recycling reactions with transition metals. The antioxidants also show different efficiencies in different oxidising systems, for example in bulk oil, emulsions and complex food/feed systems (Frankel, 1998). It is therefore difficult to predict the efficiencies of different antioxidants and it is necessary to study them in the specific system in which they are going to be used.

The purpose of the present study was to evaluate the protective effects of seven different natural antioxidant preparations on a fish feed produced directly from marine raw materials. Two multivariate reduced factorial designs were used to compare the main effects of different antioxidants and study their interactions. The most active preparations were then tested individually and together for optimal concentrations. Feeds protected with tocopherol mix and ascorbic acid were finally compared to feeds containing added ethoxyquin and phosphate in addition to the natural antioxidants.

2. Materials and methods

2.1. General

2.1.1. Origin of antioxidants

Rosemary: Oleoresin Rosemary Herbalox®, Brand HT-W, Kalsec, Kalamazoo, MI.

Tocopherol mix: MTS-70 (70% tocopherols), Archer Daniels Midland Company, Illinois.

Ascorbic acid, ascorbyl palmitate, citric acid, spemine, ethoxyquin: Sigma Aldrich Inc. St. Luis, MO.

Phosphates: Merck, Darmstadt, Germany.

2.2. Diets

An outline of the production and storage plan for the diets is given in Fig. 1 and diet compositions in Table 1. Whole herring or herring offal was finely minced in a food processor and divided into diet batches according to the number of diets that were going to be produced. The batches of mince were then blended thoroughly with Suprex® wheat (Codrico BV, Rotterdam, the Netherlands), vitamin (Hoffmann-La Roche, Basel, Switzerland) and mineral (Merck, Darmstadt, Germany) mixes according to National Research Council, NRC (1993), and the different combinations of antioxidants. The diet blends were divided into finger-thick strings and cooked for 2 min in a microwave oven to obtain 35–45% dry matter. The strings were then cut into pellets.

Subsamples of each diet, according to the number of sampling points during the frozen storage period and the number of analyses planned, were put into Nunc boxes (Nunc GmbH and Co. Ltd., Langenselbold, Germany) and frozen at −20 °C where they were stored for different periods of time. If not analysed immediately at the sampling day, the boxes were cooled to −80 °C and stored there until analyses. Dry matter and lipid levels in the diets, sampled after the heat treatment, are also given in Table 1.

2.3. Antioxidant concentrations and experimental designs

Four experiments were conducted and the antioxidant combinations added to the diets are given in Table 2, while Table 3 gives an overview of the experimental designs. In all experiments, a diet without added antioxidants (blank) and a diet with 0.7 g/kg tocopherol mix, 1.0 g/kg ascorbic acid, 0.85 g/kg phosphate mix and 0.12 g/kg ethoxyquin (dry matter basis, control) were included. The choice of control diet was based on previous experience at NIFES.

Experiments 1 and 2 were carried out as a $2^{(7-3)}$ and $2^{(6-2)}$ reduced factorial designs, respectively (Table 3a and b). In the case of Experiment 1, this means that the seven different antioxidants were added at two levels (1, −1) and that the number of antioxidant combinations (cases) was systematically reduced from 128 to 16, according to Box, Hunter, and Hunter (1978). With a full factorial design it would be possible to calculate effects of the single antioxidants (main effects) and all the interactions, but with seven variables one would need 128 treatments. Reducing the design to $2^{(7-3)}$ and 16 treatments gives loss of resolution, since the main effects and interactions overlap with one another. In this particular design, it is possible to calculate the main effects separated from each other and from the effects of two-factor interactions. There are three possible interpretations (overlap) of each two-factor interaction (Tables 4 and 5) and the three-factor interactions

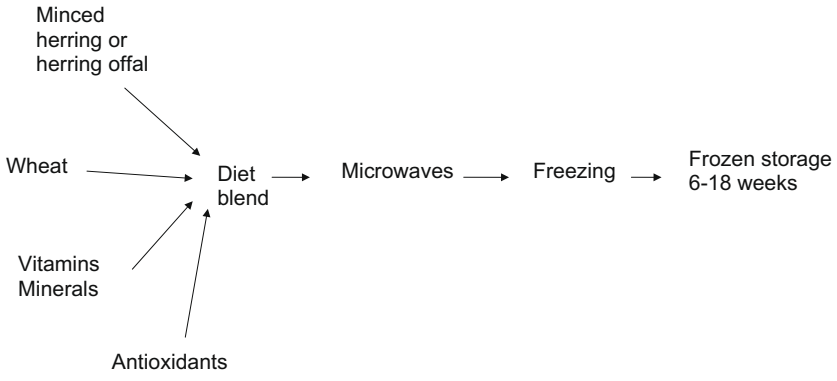

Fig. 1. Overview of the feed production and storage.