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Natural antioxidants in extruded fish feed: Protection at different storage temperatures

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ABSTRACT

This study examines five different specifically designed extruded fish feeds: BD (basal diet), without added antioxidants; BHT, with 200 mg/kg of butylhydroxytoluene; RO, with 600 mg/kg of rosemary extract (*Rosmarinus officinalis*); TC, with 500 mg/kg of thyme essential oil (*Thymbra capitata*, carvacrol chemotype); and TZ, with 500 mg/kg of thyme essential oil (*Thymus zygis*, subspecies *gracilis*, thymol chemotype). The feeds were produced by means of a cooking-extrusion process and were stored under standard conditions at two different average temperatures, either refrigerated (4 ± 1 °C) or at ambient temperature (20–28 °C). Samples were taken during processing (raw mix, recently extruded (moist) and dry feed) and at 4, 8, 12 and 24 weeks of storage. Both TBARS and induced TBARS were determined at each sampling point, as well as phenolic compounds content after feed processing. During the preparation process, the RO feed was the least oxidized and retained the least amount of phenols. The oxidative status of all feeds was maintained in cold storage, although the BD diet became less resistant to induced oxidation. At ambient temperature, the RO and BHT feeds showed the greatest protection against induced oxidation starting at week 8, and at the end of the storage period, the RO feed was the least oxidized. Overall, rosemary extract had a similar effect to that of butylhydroxytoluene on the processing and storage of extruded feed, and better than that of thyme essential oils.

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1. Introduction

The manufacture of fish feed using the extrusion method is widespread around the world. In the food processing industry, this technique has been used during the past century, because of the important benefits it presents. Some of these are starch gelatinization, enzyme inactivation, the elimination of anti-nutritional factors and increased digestibility of vegetable proteins (Cheng and Hardy, 2003).

Extrusion has been shown to have an effect on the lipid oxidation of the feed, both during processing and subsequent storage. The structure of the feed created by this process, with porous surface and internal air chambers, promotes contact between fat and airborne oxygen (Lin et al., 1998). This favors the development of a lipid peroxidation reaction, which consists

Abbreviations: BHT, feed with added butylhydroxytoluene; i-TBARS, induced thiobarbituric acid reactive substances; MDA, malondialdehyde; NFE, nitrogen free extract; NRC, National Research Council; PV, peroxide value index; rANOVA, repeated measures analysis of variance; RO, feed with added rosemary extract; TBARS, thiobarbituric acid reactive substances; TC, feed with added *Thymbra capitata* oil; TZ, feed with added *Thymus zygis* oil.

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of a direct reaction between molecular oxygen and fatty acids, with free radicals acting as catalysts (Alves Martins et al., 2007). Conversely, the denaturation of enzymes involved in lipid oxidation and the inclusion of lipids in the starch matrix protect them during storage (Singh et al., 2007). To this we must add the antioxidant effect of the compounds produced by the Maillard reaction that tends to occur during the extrusion process (Camire et al., 2005).

The oxidative stability of the feed is very important, not only in terms of the growth and health of the fish, but also with regard to their nutritional quality (Sutton et al., 2006; Grigorakis et al., 2010). The supply of oxidized fish oil in the diet impairs the stress response of the fish, as evidenced by their plasma levels of glucose, cortisol and osmolarity (Van Anholt et al., 2004; Alves Martins et al., 2007). In gilthead seabream, it has also been shown to increase the activity of certain liver enzymes with antioxidant activity, such as catalase and superoxide dismutase (Mourente et al., 2002). The immune system of fish is also affected by the oxidation of dietary lipids. A decreased response of the head kidney phagocytes and resistance to infection by *Vibrio anguillarum* have been observed in turbot fed diets including oxidized oil (Obach and Laurencin, 1992). In sea bass, oxidized diets produce more fragile erythrocytes and decrease the activity of lysozyme and the complement system (Obach et al., 1993). Administration of oxidized lipids in the feed also has a clear effect on the occurrence of skeletal deformities, since free radicals damage the membrane of osteoblasts and osteoclasts, interfering with bone development (Lall and Lewis-McCrea, 2007). As for the effect on fillet quality, fish fed oxidized feeds usually show lower levels of PUFA in the muscle, thus reducing their nutritional quality in the eyes of consumers (Alves Martins et al., 2007; Zhong et al., 2008). However, this does not always affect fillet palatability (Koshio et al., 1994).

To prevent all these undesirable effects, various synthetic antioxidants are used, primarily butylhydroxytoluene and butylhydroxyanisole in fish oil and ethoxyquin in fish meal (Hamre et al., 2010). These compounds have a strong antioxidant activity. However, it has been proven that they are transferred to the muscle in alarming amounts and have adverse health effects (Ito et al., 1985; Bohne et al., 2008; Lundebye et al., 2010). As a result, natural antioxidants are drawing the attention of the agrifood industry because of their foreseeable safety and wider acceptance by consumers. Although their use in processed foods for human consumption is increasingly being studied (Akhtar et al., 1998; Giménez et al., 2004; Goulas and Kontominas, 2007; Hernández-Hernández et al., 2009; Kostaki et al., 2009; Ozogul et al., 2009, 2010) and their use is regulated as a food additive (European Commission, 2008), few studies have focused on the effect they have on fish feed conservation (Hamre et al., 2010).

In light of this, two essential thyme oils and a rosemary extract were selected, the main components of which have been shown to have a protective effect on food in storage. The aim of our study was to assess the effect of these natural compounds on lipid oxidation in extruded fish feed, considering that this is the main mechanism behind the loss of quality in fish feeds.

2. Materials and methods

2.1. Experimental diets

Five different feeds were prepared: BD (the basal diet), without added antioxidants; BHT, with 200 mg/kg of butylhydroxytoluene (Sigma-Aldrich Quimica, S.L.; Madrid, Spain); RO, with 600 mg/kg of rosemary extract (*Rosmarinus officinalis*; 300 mg/g diterpene content; 1:1, carnosic acid:carnosol; Nutrafur, S.A.; Murcia, Spain); TC, with 500 mg/kg of essential thyme oil (*Thymbra capitata*-carvacrol chemotype; 720 g/kg carvacrol content; Blas Lorente González, S.L.; Murcia, Spain); and TZ, with 500 mg/kg of essential thyme oil (*Thymus zygis*, subspecies *gracilis*, thymol chemotype; 636 g/kg thymol content; Blas Lorente González, S.L.; Murcia, Spain). These diets were formulated according to commercial standards for gilthead seabream, with 450 g protein/kg and 200 g lipid/kg. Fish meal and wheat gluten were used as protein sources, wheat flour as a source of carbohydrates and fish oil as a lipid source, in addition to a vitamin–mineral premix (Table 1). A proximate analysis was conducted on all fish feeds using the routine procedures of AOAC (1997) for dry matter (934.01), protein as 6.25 × nitrogen content (928.08), fat (960.39), ash (942.05) and crude fiber (978.10); these results are shown in Table 1.

2.2. Feed preparation

Two batches of each feed were prepared under the same conditions. The ingredients were blended with a horizontal RM-20 mixer (MAINCA, Barcelona, Spain), first combining the solid ingredients and then adding the fish oil and finally the water (400 g/kg). Due to their liposoluble nature, the aromatic compounds were dissolved in fish oil prior to mixing them with the rest of the ingredients. The diets were produced by an experimental cooking-extrusion process using a single screw semi-industrial extruder (E 19/25 D, Brabender® GmbH and Co.K.G., Duisburg, Germany). This extruder has a screw diameter of 19 mm and a length–diameter ratio of 25:1; its 1.5 kW motor provides a maximum speed of 150 rpm and a nominal compression ratio of 2:1. The extrusion conditions were as follows: feed rate of 40 g/min, screw speed of 50 rpm, temperatures of 50/60/68 °C in the initial, middle and end sections of the extruder barrel, respectively, with a pellet diameter of 4 mm. These temperatures were chosen in order not to exceed a temperature of 70 °C at the extruder output and to maintain the stability of the aromatic compounds. Finally, the feeds were dried in an oven with forced air ventilation (HERAEUS UT12, Thermo Fisher Scientific, Madrid, Spain) for 24 h at 35 °C, until a moisture content of about 90 g/kg was obtained.

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