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Effects of storage temperature and α-tocopherol on oil recovered from sardine mince

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ABSTRACT

Sardine oil released during sardine mince preparation showed good quality with a low hydroperoxide content, excellent color and high content (19.23%) of eicosapentaenoic acid. Recovered sardine oil was stored at two different temperatures (+4 °C and +35 °C) for 28 days with or without the addition of α-tocopherol (50 and 100 ppm). Peroxide values and thiobarbituric acid reactive substances of control sardine oil significantly increased to reach 29.9 meq O_2/kg oil and 46.48 mg MA/kg during storage at +35 °C, but the increase was considerably less (4.36 meq O_2/kg oil and 13.21 mg MA/kg oil respectively) in oil stored at +4 °C. A slight increase (1.5%) in the free fatty acid content was recorded in oil stored at 35 °C. A significant decrease of polyunsaturated fatty acids was recorded after storage, particularly in oil stored at 35 °C, while higher percentages of saturated and mono-unsaturated fatty acids were observed. Storage at +4 °C combined with addition of α-tocopherol (100 ppm) had a beneficial effect on sardine oil stability.

Effets de la température d'entreposage et de l'α–tocophérol sur l'huile récupéréé des sardines hachées

Mots clés : Sardine ; Huile ; Entreposage ; Qualité ; Oxydation ; Acide gras ; Réfrigération

1. Introduction

Fish oil is one of the best sources of dietary polyunsaturated fatty acids (PUFA), especially the n-3 PUFA family including eicosapentaenoic acid [EPA or (20:5 n-3)], docosapentaenoic acid (DPA or 22:5 n-3) and docosahexaenoic acid (DHA or 22:6 n-3). Fish oil has become popular because of its beneficial effects for human health. Several studies have shown that fish oil has important roles in the prevention of cardiovascular diseases and some types of cancer, including colon, breast and prostate cancer (Mori, 2006; Sidhu, 2003).

However, because of its high degree of unsaturation, particular measures should be taken with fish oil including extraction, storage and preservation. During fish oil processing, further oxidative stress might be induced and the levels of natural antioxidants such as α-tocopherol may decrease

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Nomenclature

a^*	redness (+) or greenness (−)
b^*	yellowness (+) or blueness (−)
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
FAMEs	fatty acids methyl esters
FFA	free fatty acids
FTIR	fourier transform infrared spectra
L^*	lightness
MA	malonaldehyde
MUFA	monounsaturated fatty acids
PUFA	polyunsaturated fatty acids
PV	peroxide value
SFA	saturated fatty acids
SL	sardine lipid
SO	sardine oil
TBA	thiobarbituric acid
TBARS	thiobarbituric acid reactive substances
TMP	1,1,3,3-Tetramethoxypropane

considerably (Shahidi et al., 1997). Therefore, the addition of antioxidant is important for the preservation of these products during processing and storage.

Synthetic antioxidants are widely used and effective. However, they pose various problems including toxicological one (Thompson and Trush, 1986). Food safety legislation has gradually become more stringent, requiring the use of toxicity tests for synthetic antioxidants. Additionally, there is a tendency for consumers to use natural products as antioxidants, as these are perceived as safe and do not require prior testing (Giese, 1996).

Hydrolytic and oxidative reactions of fish oil during processing, heat treatment and in the final products during subsequent storage, are among the basic processes causing the production of hydroperoxides (Osman et al., 2001), free fatty acids (Chaijan et al., 2006) and rancidity in food products (Donelli and Robinson, 1995). The basic mechanisms of free radical induced lipid oxidation can be characterised by three distinctive steps: initiation, propagation and termination reactions. This phenomenon can be influenced by both intrinsic and extrinsic factors, such as fatty acid composition, concentration of pro-oxidants, endogenous ferrous iron, myoglobin, enzymes, pH, temperature, ionic strength and oxygen consumption (Andreo et al., 2003).

α-Tocopherol, which is an important natural antioxidant, represents the major tocopherol of common marine oils (Kinsella et al., 1978). The antioxidant activity of tocopherols is mainly due to their ability to donate their phenolic hydrogens to lipid free radicals (Kamal-Eldin and Appelqvist, 1996). Various studies have reported the protective effects of α-tocopherol on oils (Fuster et al., 1998; Zuta et al., 2007) and revealed that α-tocopherol was more effective in animal fats than in vegetable oils.

This study aims to investigate the effects of temperature and antioxidant level in crude sardine *Sardina pilchardus* oil. Fatty acid composition, peroxide value (PV), thiobarbituric acid reactive substances (TBARS), color analysis, free fatty acids and Fourier transform infrared spectra (FTIR) were determined to monitor lipid changes during storage over 28 days.

2. Materials and methods

2.1. Sample collection and preparation

Sardine caught off the Portuguese coast in June 2007, was transported in ice to the laboratory where it was hand eviscerated, beheaded and washed with cold tap water before mincing. The average length and weight of sardine sample (from about 30 kg) were in the range of 17–20 cm and 76–87 g, respectively. Sardine oil obtained from sardine mince processing (washing with $NaHCO_3$/NaCl 0.2%/0.15%) was used to prepare various samples with or without the addition of α-tocopherol (50 and 100 ppm).

α-Tocopherol samples were first solubilized in chloroform, the solvent was evaporated under nitrogen and then 20 g of sardine oil were added to the α-tocopherol. The oil samples (with or without α-tocopherol) are stirred gently for 2 min at +4 °C and then stored in 50 ml beakers at two temperatures (+4 °C and +35 °C) for 28 days. Oil sampling was performed on days 0, 3, 7, 14, 21 and 28.

2.2. Physicochemical analyses

2.2.1. Lipid extraction

Lipid was extracted by the Bligh and Dyer method (1959). Sample (10 g) was homogenized with 60 ml of a chloroform/methanol (1:2) at the speed of 9000 rpm for 8 min at 4 °C using a Polytron homogenizer. The homogenate was treated with 5 ml NaCl saturated solution and 20 ml chloroform with butylated hydroxytoluene (BHT) (50 ppm), the mixture was homogenized at 9000 rpm for 7 and 5 min, respectively. Then, 20 ml of distilled water was added and homogenized again for 1 min. The homogenate was placed in an ultrasound bath for 10 min, then vacuum filtered with a Buchner funnel (Lisbon, Portugal) and the filter was washed with chloroform. The organic layer was extracted with a separating funnel, dried with sodium sulphate and evaporated to dryness in the rotary evaporator. After weighing, the obtained oil was solubilized in a known volume of chloroform with BHT (50 ppm) and stored at −20 °C.

2.2.2. Fatty acid profile

Fatty acid methyl esters (FAMEs) were obtained according to the method of Lepage and Roy (1986) with slight modifications. This determination was performed in a Varian CP-3800 gas chromatograph (Varian Inc Corporate Headquarters, Palo Alto, CA, USA), equipped with an auto-sampler and fitted with a split/splitless injector and a flame ionization detector. The separation was carried out in an Omegawax (SUPELCO, Sigma–Aldrich Group, St. Louis, MO, USA) capillary column (25 m × 0.25 mm idx). Temperature was programmed from 180 °C to 200 °C at 4 °C min^{-1}, holding for 10 min at 200 °C and