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Effect of hydroxybenzoic acids antioxidants on the oxidative stability of sardine oil

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Abstract

The antioxidant capacities of three derivatives of hydroxybenzoic acids (Gentisic acid, protochatechuic acid and vanillic acid) in sardine oil were compared. Peroxide value, conjugated diene value, p-anisidine value and thiobarbituric acid reactive substances (TBARS) value were assessed to determine the oxidative stability provided by these substances to the sardine oil. Results showed that gentisic acid (2,5 dihydroxy benzoic acid) was the most effective of the chosen hydroxybenzoic acids in imparting oxidative stability to the sardine oil. Protochatechuic acid (3,4 dihydroxy benzoic acid) provided relatively less oxidative stability, while vanillic acid had no effect. Results from this work showed that the position of hydroxylation and methyl substitution influences the antioxidant capacity of the molecules in sardine oil. Furthermore, it was found that the extent of oxidative stability conferred by the antioxidants in lipid systems is influenced by several other physical and chemical factors as well.

Keywords: Antioxidants; Oxidative stability; Sardine oil; Hydroxybenzoic acids; Primary oxidation; Secondary oxidation

1. Introduction

Oils rich in n-3 polyunsaturated fatty acids have been recognised widely for their nutritional significance. However, the high susceptibility of these oils to oxidation, limits their utilisation as processed food and nutritional supplements [1]. The literature on the oxidative stability of n-3 PUFA rich oils are inconsistent, which was attributed to the variable fatty acid composition of oil [2]. Synthetic antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) are generally used to prevent oxidation in fish oil [3]. However, they are not considered safe due to their suspected role as carcinogenic promoters [4]. Hence, the use of natural antioxidants can be considered to improve the oxidative stability of n-3 PUFA rich oil. Studies on the extraction of many types antioxidants from natural sources are continuing to evolve along with their applications in many food products for diminishing free radicals [5]. After successful separation of antioxidants, researchers study the relationship between chemical structures and effectiveness of antioxidants from variable sources which may have anticarcinogenic activity along with antioxidant characters [6]. Though many antioxidants are available in nature, phenolics are found abundantly [5]. They not only provide good sensorial qualities and antioxidant activity, but also impart beneficial properties like antitumour, anti-mutagenic, antiviral, anti-inflammatory [7]. Thus, addition of phenolics to oil results in enhancement of nutritional properties, along with improvement of oxidative stability of oil.

Phenolic acids are one of the most persistent groups of antioxidants in plants and the structure of the antioxidant greatly influences the antioxidant power [6]. Hence, a correlation between their structure and activity in oil could be used to predict the effectiveness of an antioxidant. Further, the activity of antioxidants in lipid systems is influenced by external factors like hydrophilicity, interfacial structures [8]. Hence, it becomes critical to make a comparison of different structured phenolic acids to find the best possible compound. Of the wide variety of phenolic acids available in nature, minor attention has been directed to the antioxidant capacities of simple derivatives of benzoic acids [9]. Since, attempts are being for separating and purifying novel antioxidants from many sources [5], the current study on application of a particular class of antioxidants can help predict the effectiveness of an antioxidant in lipid system after their separation from natural source. This study attempts at

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understanding the effectiveness of the hydroxybenzoic acids in improving the oxidative stability of sardine oil. Because of the limited literature on the hydroxybenzoic acids and the abundance of these compounds in natural resources, current study provides additional choice of antioxidants for imparting stability in fish oil.

2. Materials and methods

Crude sardine oil was procured from a local seafood industry and was refined in our laboratory. The refined oil was stored at −20 °C without any added antioxidants prior to use. Gentisic acid, protochatechuic acid and vanillic acid and 1,1,3,3-tetramethoxypropane (malonaldehyde) were from Sigma (India). Potassium iodide, trichloroacetic acid, sodium thiosulphate and thiobarbituric acid were purchased in analytical grade from Loba Chemie. All solvents were of analytical grade and were purchased from Merck India.

2.1. Analytical methods

In order to perform the oxidation studies of sardine oil, 100 ppm of antioxidants dissolved in solvent were taken in glass vials. The solvent was evaporated by flushing with nitrogen and carefully calculated amount of oil was added. The samples were then homogenised for 15 minutes. The sample vials were stored at 37 °C and covered with aluminium foil and kept in contact with atmospheric air for 14 days. Analysis was performed for all samples periodically.

2.1.1. Peroxide value

Peroxide value measurement was done based on the standard AOCS method [10]. The peroxide value of oil is measured by Eq. (1) and expressed as milli equivalents of peroxide per 1000 g of oil.

$$Peroxide\ value = \frac{(S-B)*M*1000}{m} \quad (1)$$

S is the sample titre value, B is the blank titre value, M is the molarity of sodium thiosulfate used, m is the mass of test in g.

2.1.2. p-Anisidine value (pAV)

p-Anisidine value was determined according to AOCS [10]. The anisidine values were calculated as in Eq. (2)

$$pAV = 25*\frac{1.2As-Ab}{m} \quad (2)$$

As is the absorbance of oil after reaction with p-anisidine, Ab is the absorbance of oil in isoocatane, m is the weight of sardine oil used for analysis (g).

2.1.3. Conjugated diene (CD) value

Conjugated dienes are formed during oxidation of fats or oils and can be analysed by measurement of absorbance at 230–235 nm. The CD value in bulk sardine oil was measured based on the method described by Hopia et al. [11]. The absorbance of the lipid – isooctane solution was measured at 234 nm. Oxidation is directly proportional to increase in absorbance.

2.1.4. Thiobarbituric acid reactive substances (TBARS) value

Another significant assay for monitoring secondary oxidation products is the thiobarbituric acid reactive substances (TBARS) assay and it was performed as described by Buege and Aust [12] with minor modifications. The experiment is based on the reaction between malonaldehyde (MAD) which is a product of oxidation and thiobarbituric acid to give a red colour complex. Oil sample was mixed with TBARS reagent containing 0.375% thiobarbituric acid, 15% trichloroacetic acid and 0.25 N HCl. A slight pink colour was developed on heating the mixture. The samples were then cooled under running water and centrifuged. The absorbance of the supernatant was read at 532 nm. A standard graph was constructed using 1,1,3,3-tetramethoxypropane (MAD).

2.1.5. Statistical analysis

The experimental data in triplicates were analysed by one way analysis of variance (ANOVA) using MiniTab17 software and samples with $p < 0.05$ were significant.

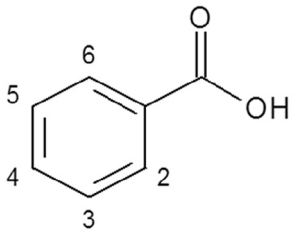
3. Results and discussion

Based on detailed literature study, the antioxidants were chosen from hydroxybenzoic acid group. The three chosen antioxidants from benzoic acid group showed variation in the structure based on the degree of hydroxylation and methylation and the position of functional group substitution (Fig. 1).

3.1. Peroxide value

The production of complex array of secondary products results from these colourless and odourless labile hydroperoxides [13]. Hence, determination of hydroperoxides was used to monitor primary oxidation of sardine oil in the presence of three derivatives of hydroxy benzoic acid (Fig. 1). All samples, including control showed a steady increase in the peroxide value during initial six days (Fig. 2). However, a faster increase was noted in later days, with control and vanillic acid sample showing maximum of 22.09 and 21.63 respectively.

Of all the benzoic acids tested, gentisic acid showed highest reduction in peroxide value of 25.13% (Table 1) which is consistent with the results obtained by Ashidate et al. [14] when



Gentisic	2 – OH, 5 – OH
Protochatechuic	3 –OH, 4 – OH
Vanillic	3 – OCH_3, 4 - OH

Fig. 1. Structure of hydroxybenzoic acids.