Food Chemistry 139 (2013) 837–844

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Inhibition kinetics of lipid oxidation of model foods by using antioxidant extract of fermented soybeans

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ARTICLE INFO

Article history:
Received 4 October 2012
Received in revised form 13 January 2013
Accepted 15 January 2013
Available online 29 January 2013

Keywords:
Lipid oxidation
Antioxidant of fermented soybeans
Logistic equation
Linoleic acid
Sunflower oil
Emulsion

ABSTRACT

Fermentation by using *Aspergillus oryzae* has been reported to increase antioxidant activity of soybeans significantly. The effectiveness of the extract from fermented soybeans was studied in 3 model foods with different complexities, i.e., linoleic acid emulsion, sunflower oil emulsions and bulk sunflower oil. For the emulsion systems, oxidation at two different pH values (4.5 and 7) was also compared. A reparameterised logistic equation was used to describe and to predict the experimental data. In general, a good agreement between experimental trends and simulated data from the model was found. A crude antioxidant extract (5 mg/g) showed a comparable antioxidant activity to 0.26 mg/g of butylated hydroxytoluene (BHT) in the linoleic acid emulsions. The extract exhibited a better capability to retard primary products in the linoleic acid systems than the secondary products. The opposite effect was observed in the bulk sunflower oil and its emulsion systems.

1. Introduction

Lipid autoxidation is an autocatalytic reaction that leads to the development of rancidity. This process, which is the major cause of quality losses in the food industry, could take place during preparation, processing and storage of many food products. The oxidation changes appearance, taste, odour and shelf life of food. These changes degrade functional and nutritional compounds of food, damage essential fatty acids and produce oxidised polymers which could raise safety concerns (Crapiste, Brevedan, & Carelli, 1999; Frankel, 1996; Paiva-Martins, Correia, Felix, Ferreira, & Gordon, 2007). Consumers' rejection causes considerable losses in the food industry. Hence, susceptibility of food towards lipid oxidation affects quality and consumer acceptability and limits its application (van Ruth, Roozen, & Jansen, 2000).

Oxidative stability and deterioration of lipids depends on the initial composition, concentration of minor compounds (including anti-oxidant or pro-oxidant substances), preparation and processing, and storage conditions (Crapiste et al., 1999; Frankel, 1996). The use of synthetic antioxidants in food has become a concern due to their potential health hazards. For this reason, studies of antioxidants from natural sources have been intensively carried out in recent years.

Unsaturated fatty acids are primary targets for free radical reactions of autoxidation. Their double bonds are highly reactive due to a low activation energy (Min & Boff, 2002). The number of studies on oxidation stability of PUFAs are high, due to the abundance of polyunsaturated fatty acids (PUFAs) in foodstuffs, their relatively high susceptibility to oxidation, the increasing use of polyunsaturated vegetable oil and iron fortification in lipid-containing foods (Frankel, 1996). Linoleic acid is one of the essential fatty acids and plays an important role in human nutrition. Despite its health benefits, this unsaturated fatty acid is sensitive to oxidative deterioration (Ohkawa, Ohishi, & Yagi, 1978) and thus needs to be stabilised to delay the onset of oxidation.

Although a wide range of model systems is available for evaluation of antioxidant substances, the choice depends mainly upon the nature of the substances under investigation (Wettasinghe & Shahidi, 1999). Many studies have reported the oxidation inhibition of model systems by using pure compounds such as tocopherols (Huang, Frankel, & German, 1994). However, only a few studies on the use of natural antioxidant extracts to prevent lipid oxidation are available. Moreover, discrepancies in the activity of the same natural antioxidant are found depending on the model systems used (Wettasinghe & Shahidi, 1999).

The objective of this work is to determine the inhibition effects of an antioxidant extracted from soybeans fermented with

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http://dx.doi.org/10.1016/j.foodchem.2013.01.049

Aspergillus oryzae on model lipid foods. It has been reported that the antioxidant activity of soybeans considerably increases after fermentation (Romero, Doval, Sturla, & Judis, 2004; Wardhani, Vazquez, & Pandiella, 2009). Although the antioxidant activity of unfermented and fermented soybeans has been studied, information related to their application in different model foods remains limited.

In this research three model foods were chosen to represent low and high lipid content foods. For the purpose of comparison of the performance of the extract in different foods, all the model foods contained PUFA as oxidisable lipids. For the reason mentioned before, linoleic acid was chosen as the major PUFA of the model foods. In order to avoid interferences from other lipid compounds, a linoleic acid emulsion was selected to illustrate the simplest model. Plant lipids usually contain more PUFA than those of animal origin (Pokorny & Trojakova, 2001). Hence, an oil of vegetable origin was used as a model to simulate a more real food in this study. Sunflower oil was chosen due to its high concentration of linoleic acid and relative low content of γ and δ-tocopherol, which makes it rather unstable during storage. The reported range of linoleic acid content in sunflower oil is 64.3–71.6% (Crapiste et al., 1999; Van Ruth et al., 2000).

In the present work, an evaluation of antioxidant ability of crude extracts from fermented soybeans on model lipid systems is reported. The effect of two different pH values of the emulsions on the inhibition of lipid oxidation during storage time was also described. Lipid oxidation was determined by following the formation of conjugated dienes as the primary oxidation products and thiobarbituric acid reactive substances as the secondary products. A logistic model was used to fit and predict the profile of the kinetics of oxidation development as well as to generate significant parameters for comparative and predictive purposes.

2. Materials and methods

2.1. Production and extraction conditions of antioxidant extract

Soybeans were fermented with *A. oryzae* as described in a previous work (Wardhani et al., 2009). The antioxidant of the fermented soybeans was extracted using 67% v/v ethanol in a 3-h Sohxlet extraction at 65 °C (Wardhani, Vazquez, & Pandiella, 2010).

2.2. Preparation of linoleic acid emulsion

Linoleic acid (97% purity) was obtained from Sigma (Poole, UK). The linoleic acid emulsions (30 g) were prepared based on the method of Romero et al. (2004) by mixing 0.168 g of linoleic acid, 0.168 g of Tween 20 (Fluka, Gillingham, UK), phosphate buffer (50 mM, pH 4.5 and 7.0) and the antioxidant extract. Phosphate buffer was used because it has a buffering capacity between pH 4.5 and 7 and does not change the nature of the acid radicals that may affect the rate of oxidation (Mabrouk & Dugan, 1960). The extracts were added to give 0.1, 0.5, 1, 2, 5 and 10 mg/g of the extract concentration in the final emulsion. The mixture was homogenised at 13,000 rpm for 5 min using an Ultra-Turrax T25 basic (IKA-Werke, Staufen, Germany). A commercial antioxidant, 0.26 mg/ml BHT (Aldrich, Steinheim, Germany), was dissolved in methanol and used as reference for comparison. A sample without addition of antioxidant was used a control. The emulsions were kept in closed clear bottles and incubated in the oven at 60 °C.

2.3. Preparation of sunflower oil emulsion and bulk sunflower oil

Sunflower oil (Fluka) was selected for the more complex model because it is a mixture of oils dominated by linoleic acid. The sunflower oil had a peroxide value (PV) of 1.3 and consisted of linoleic acid 62.2%, oleic acid 26.4%, palmitic acid 6.2% and stearic acid 3.4%.

The sunflower oil emulsion systems (30 g) consisted of 10% w/w oil, 1% w/w Tween 20 (Fluka), the corresponding extract, and the phosphate buffer solution (50 mM, pH 4.5 and 7.0). The extracts were added to give 0.1, 0.5, 1, 2, 5 and 10 mg/g of the extract concentration in the final emulsion. The mixture was homogenised as for the linoleic acid emulsions.

The bulk oil sample (30 g) was prepared by adding the extract to the sunflower oil to give the same final extract concentrations as in the sunflower oil emulsions. In the emulsion and bulk oil cases, controls without the extract and with commercial antioxidant (BHT) were also prepared. All samples were placed in closed clear bottles and incubated in the oven at 60 °C.

2.4. Quantification of the oxidation products

Oxidation products were measured as primary and secondary products. The primary oxidation products were monitored through conjugated diene production and secondary products were measured as thiobarbituric acid reactive substances.

2.4.1. Determination of conjugated dienes (CD)

The conjugated diene measurement in the emulsion samples was carried out according to the procedure described by Huang, Frankel, Schwarz, and German (1996). The sample (10 μl) was dispersed in 1 ml of methanol and the absorbance was measured at 234 nm. For the oil substrate, samples (100 mg) were dissolved in 10 ml cyclohexane (Merck, Darmstadt, Germany) and the absorbance was measured at the same wavelength (Van Ruth et al., 2000). Samples were diluted necessarily to maintain the reading under one unit of absorbance.

2.4.2. Determination of thiobarbituric acid reactive substances (TBARS)

TBARS method was modified from the method of Ohkawa, Ohishi, and Yagi (1978). Into capped test tubes, samples (100 μl) were mixed with 1.5 mL of 0.5% TBA (Sigma–Aldrich) and 1.5 mL of 20% acetic acid (Fisher Scientific, Loughborough, UK). The pH of the mixture solution was adjusted to 4.0 with NaOH. Tubes were boiled for 15 min, in a water bath and cooled at room temperature subsequently. After the addition of 1 mL of chloroform (Merck), the tubes were centrifuged at 2028 *g* (Heraeus Labofuge 300; Thermo, Waltham, MA) for 10 min. The chloroform layer was separated and the absorbance of the aqueous phase was measured at 532 nm.

2.5. Mathematical modelling

The reaction mechanism of lipid oxidation is well understood. However, the rate of lipid oxidation for these elementary steps cannot be used for modelling purposes, since it is very complex and difficult to measure all the compounds involved in the reaction as reactants, intermediary components and products separately. The most common approach of kinetic modelling of lipid oxidation, although little used, is by means of logistic equation (Ozilgen & Ozilgen, 1990). This mathematical model has been broadly employed for modelling sigmoidal profiles of chemical and biological processes (Murado, González, & Vázquez, 2002). In the integrated form it can be formulated as:

$$L = \frac{L_m}{1 + \exp(b - kt)}, \text{with } b = \ln\left(\frac{L_m}{L_o} - 1\right) \quad (1)$$

where L is measured as the extent of lipid oxidation, L_o and L_m are the initial and the maximum lipid oxidation, k is the specific maximum reaction rate constant (with $time^{-1}$ units) and t is the time.