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Rosemary extract can be used as a synthetic antioxidant to improve vegetable oil oxidative stability

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ABSTRACT

The protecting ability of rosemary extract as a plant-based antioxidant and an alternative to synthetic antioxidants against the oxidation of vegetable oils was investigated in the present study. The effect of rosemary extract on improving the oxidative stability of oils was evaluated using the Rancimat and Schaal oven tests. Oils with or without incorporation of rosemary extract or synthetic antioxidants were analyzed using the Rancimat method at 120 °C to determine their induction periods (IP). The changes in antioxidant capacity, total phenolic content, peroxide value, fatty acid composition, and tocopherol content were measured under the Schaal oven test during storage at 62 °C. The IP of oils incorporated with rosemary extract was significantly ($p < 0.05$) higher than blank oils and oils with synthetic antioxidants. The incorporation of rosemary extract into oils effectively prevented the oils by increasing the antioxidant capacity and total phenolic content, decreasing the peroxide value, and delaying the degradation of tocopherols and polyunsaturated fatty acids of oils. Results of this study suggested the potential use of rosemary extract as an effective alternative to synthetic antioxidants.

1. Introduction

Lipid oxidation is one of the most critical factors affecting the shelf life and the quality attributes of oil (da Silva and Jorge, 2014). Lipid oxidation causes undesirable changes in taste, odor, texture, flavor, and appearance of foods, and also destroys fat-soluble vitamins (Gallego et al., 2013; Zeb and Murkovic, 2013). Furthermore, the oxidative degradation of lipids can damage biological membranes, enzymes and proteins, which may pose a direct threat to human health (Malheiro et al., 2013).

In recent decades, synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are extensively used in retarding the oxidative degradation of lipids due to their high oxidative stability and low cost. However, safety concerns regarding the use of synthetic antioxidants have increased globally. Synthetic antioxidants are inappropriate for chronically ill patients, and the prolonged usage of synthetic antioxidants is harmful to humans, potentially provoking the onset of degenerative diseases (Cordeiro et al., 2013a,b; Xu et al., 2001). The benefits of adding natural, plant-based antioxidants to lipids in order to prevent lipid oxidation have been highlighted in recent years. Natural antioxidants retard oxidative rancidity via the following pathways: (1) capturing of free radicals; (2) decomposing/deoxidizing peroxides; and (3) scavenging oxygen (Zheng et al., 2012; Sun et al., 2010, 2011a).

Rosemary (*Rosmarinus officinais* L.) extract is popular as a natural antioxidant due to its strong antioxidant capacity and fat-soluble property, and has been adopted formally into the European regulations (Commission Regulation (EU), 2011). The use of rosemary extract as a natural antioxidant was first reported by Rac and Ostric-Matijasevic in 1955 (Rac and Ostric-Matijasevic, 1955). Wu et al. (1982) found that rosemary extract as a natural antioxidant had a better antioxidant capacity than BHT and BHA. Chen et al. (1992) reported that all rosemary extracts showed strong inhibitory effects on lipid oxidation. The antioxidant capacity of rosemary extract was attributed to the presence of phenolic diterpenes that scavenge singlet oxygen, hydroxyl radicals, and lipid peroxyl radicals, thereby preventing lipid oxidation (Gallego et al., 2013; Zhang et al., 2010; Sun et al., 2011a,b). Three types of phenolic diterpenes are found in rosemary extract: carnosic acid, carnosol, and rosmanol; with carnosic acid present as the major phenolic diterpene (Frankel

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et al., 1996; Thorsen and Hildebrandt, 2003). In addition, a previous study indicated that carnosic acid was the most active antioxidant component in rosemary extracts (Terpinc et al., 2009).

The Rancimat test has been widely used in evaluating the antioxidant capacities of synthetic and natural antioxidants. The Rancimat method offers a real alternative to the active oxygen method for the determination of oxidative stabilities owing to the appreciable saving in labor (Läubli and Bruttel, 1986). The Rancimat test works by measuring the changes in conductivity caused by volatile low-molecular weight organic acids, such as acetic acid and formic acid, which are produced from oil oxidation under elevated temperatures (Cordeiro et al., 2013a,b). The time that elapses until the secondary oxidation products are produced is known as the induction period. This is used to characterize the resistance of oil to oxidation, and the longer the induction time, the more stable the oil is to oxidation.

The purpose of this study was to evaluate the effectiveness of rosemary extract, compared to synthetic antioxidants (BHA and BHT), on retarding the oxidation of three types of vegetable oils–soybean oil, rice bran oil, and cottonseed oil, using the Rancimat method and Schaal oven test.

2. Materials and methods

2.1. Materials

Soybean oil, rice bran oil, and cottonseed oil without added antioxidants were provided by Hao Koufu Co., Ltd., Shanxi, China. Commercial rosemary extract with a very high carnosic acid content of 70% were purchased from Hainan Shupu Science and Technology Ltd. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tocopherol standards (α-tocopherol, γ-tocopherol, and δ-tocopherol) were purchased from Sigma–Aldrich (St Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

2.2. Rancimat analysis

The Rancimat analysis was conducted using a Rancimat 892 (Metrohm, Herisau, Switzerland) instrument. Rosemary extract was directly added at a concentration of 400 mg/kg to the oils and stirred for 10 min at room temperature. A mixture of synthetic antioxidants (50% BHA and 50% BHT) was added at their legal limit of 200 mg/kg (Duh and Yen, 1997) as a positive control. Oils without added antioxidants were considered as blank controls. Oils (5 g each) were accurately weighed into each reaction vessel. The target temperature was set at 120 °C and airflow rate was 20 L/h. The results were expressed as induction period (IP), which was automatically determined from the inflection point of the curve using the software supplied by the company.

2.3. Schaal oven storage stability test

In the processing and marketing of vegetable oils, the shelf life of oils (period of time until an oil develops rancidity) is an important quality factor. This is measured by its oxidative stability, and methods for evaluating oxidative stability of oils are therefore necessary. One widely used method for evaluating oils is the Schaal oven test, for which elevated storage temperatures was used to destroy the original physicochemical attributes of oils (Antolovich et al., 2002). The Schaal oven test was employed in the current study to evaluate the effectiveness of rosemary extract in retarding the oxidative deterioration of oils. Blank oils and oils incorporated with rosemary extract and synthetic antioxidants (BHA + BHT) were accurately weighed (50 g ± 0.01 g) into amber bottles without headspace and stored in an oven at the constant temperature of 62 ± 1 °C for 24 days, with samples taken every 6 days for DPPH, ABTS, total phenolic content, and peroxide value analyses. Oil samples taken on Day 0 and 24 were analyzed for their fatty acid composition and tocopherol content.

2.4. DPPH and ABTS antioxidant assay

The DPPH antioxidant assay was performed according to Sui and Zhou (2014). A spectrophotometer (UV Mini 1240, Shimadzu, Kyoto, Japan) blanked using methanol was used in the analysis. A volume of sample or Trolox standard (0.1 mL) was added into 3.9 mL of DPPH stock solution (6×10^{-5} M), and left to stand for two hours in the dark at room temperature. The absorbance of the mixture was measured at the end of the two hours using the spectrophotometer at 515 nm. Total antioxidant capacity measured using DPPH assay was reported as mg Trolox equivalents per mL of sample.

The ABTS assay was performed following the procedure of Sui et al. (2014). ABTS radical cation solution was produced by reacting potassium persulfate (2.45 mM) with ABTS stock solution (7 mM) in equal volumes, and the reaction was left to stand for 12–16 h in the dark before use. ABTS radical cation solution (1 mL) was diluted with 60 mL methanol to achieve an absorbance reading of 0.70 (±0.02) at 734 nm. An aliquot of sample or Trolox (2.5 μL) was allowed to react with the diluted ABTS radical cation solution (1 mL) for seven minutes before measuring its absorbance at 734 nm using the spectrophotometer. The total antioxidant capacity obtained using the ABTS assay was reported as mg Trolox equivalents per mL of sample.

2.5. Peroxide value measurement

The peroxide value (PV) indicates the state of primary oil oxidation. The factors that result in a high PV value are the history of oil and exposure to factors that cause oxidation, such as high temperatures during production and storage (Hui and Chandan, 2007). The peroxide value was determined according to our previous work (Li et al., 2014). Oil samples (300 mg) were accurately weighed and dissolved in 9.9 mL of chloroform/methanol mixture (7:3, v/v) followed by the addition of 50 mL of xylenol orange (10 mM) and 50 μL of iron (II) chloride solution. The mixture was left to react for 5 min at room temperature before centrifuging at 1000 g for 5 min. The absorbance was measured at 560 nm using the spectrophotometer. Results were expressed in miliequivalents of active oxygen per kg of oil.

2.6. Total phenolic content measurement

The total phenolic content of oils was measured using the Folin–Ciocalteau method according to the work of Zheng et al. (2012) with some modifications. A 100 mg aliquot of oil sample was mixed with the Folin–Ciocalteau reagent (0.5 mL) and methanol (2 mL). The mixture was shaken and 1.5 mL of 15% Na_2CO_3 was added into the mixture followed by shaking the mixture for 30s. Distilled water was added into the mixture to make a final volume of 7 mL. The mixture was then incubated at 50 °C for 20 min before centrifuging at 2000 g for 10 min. After centrifugation, the supernatant was transferred into a cuvette and its absorbance measured at 750 nm. The total phenolic content of the oil samples was expressed as gallic acid equivalents.

2.7. Fatty acid composition analysis

The fatty acid composition was analyzed using an Agilent 7890 gas chromatograph coupled with an Agilent 5975 mass spectrometer (GC–MS; Agilent Technology, CA, USA), and equipped with an HP-88 capillary column (100 mm × 0.25 mm id, 0.2 μm