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Original article

Oxidative stabilization of RBD palm olein under forced storage conditions by old Cameroonian green tea leaves methanolic extract

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ABSTRACT

Background: Lipid oxidation is responsible for the deterioration of the nutritional and organoleptic qualities of foods. It also leads to the formation in these products of compounds which are harmful for the consumer's health. The synthetic antioxidants used by industries in order to solve this problem are also prone to be toxic and quite volatile at high temperatures. On the other hand, the trend among researchers to replace the synthetic antioxidants with natural ones is increasing because of their potential health benefits. In this study, old Cameroonian green tea leaf methanolic extracts were added in palm olein and its efficiency in delaying the oxidation of oil under forced storage conditions was assessed.

Methods: The plant material was extracted with methanol and its total phenolics content evaluated by colorimetry, followed by the qualitative identification of some phenolic antioxidants by HPLC-DAD and ESI-MS. After preliminary antioxidant studies, the extract was added in oil at concentrations that ranged from 200 to 1800 ppm. Oil containing butylated hydroxytoluene (BHT) and oil without antioxidant served as positive and negative controls, respectively. The oxidative stability of these oil samples was evaluated by determining their oxidation induction times on Rancimat (at 110 °C) and measuring their oxidative state by the Schaal oven test method during 30 days of storage at 70 °C. Here, oil samples were collected every 10 days and their peroxide, *p*-anisidine, total oxidation, thiobarbituric acid, iodine values as well as changes in their linoleic acid profile gas chromatography coupled to flame ionization detector (GC/FID) were evaluated.

Results: The outcomes showed the total phenolics content of the extract to be 53.5 mg GAE/g. Gallic acid, epicatechin gallate, gallocatechin and epigallocatechin gallate were the phenolic antioxidants detected. The induction times of palm olein supplemented with the extract (200–1800 ppm) were found to be in the range of 24.8–28.9 h, while those of control and oil containing BHT were 20.1 and 22.7 h, respectively. The extracts, at all concentrations, were also found to be efficient like butylated hydroxytoluene in inhibiting the oxidation of palm olein during 30 days of storage at 70 °C.

Conclusion: The investigations showed that old Cameroonian green tea leaves are a viable source of natural antioxidants for delaying palm olein oxidation.

1. Introduction

Lipid oxidation that results from the reaction between unsaturated fatty acids and molecular oxygen is one of the culprits of deterioration in fats and oils. It decreases the nutritional properties of foods since it involves the loss of essential fatty acids, essential amino acids, destruction of vitamins, and reduction of protein digestibility [1]. It also alters the organoleptic properties of foods as well as changes in colour, texture, appearance of rancid odours and undesirable flavours [2]. Besides affecting the nutritional and organoleptic qualities of the products, during this process, it may generate potential toxic compounds through the action of free radicals and reactive oxygen species that are harmful to human health and are implicated in degenerative diseases such as cancer, cardiovascular diseases, and early ageing [3]. Due to these changes, consumers do not accept oxidized products and industries suffer from economic losses [4].

Abbreviations: DAD, diode array detector; ESI-MS, electrospray ionization-mass spectrometry; GAE, gallic acid equivalent; PO, palm olein; Ca.S, *Camellia sinensis*.

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In order to delay lipid oxidation, synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and *tert*-butyl hydroquinone (TBHQ) have been used to maintain the quality and extend the shelf-life of oils. However, their use is increasingly contested or even banned in certain countries, due to their potential health risks [3]. As a consequence, they are negatively perceived by consumers. Besides, according to Chang et al. [5] and Thorat et al. [6], BHA and BHT are quite volatile and easily decompose at high temperatures. In order to overcome this challenge, food industries have started to search for alternative antioxidants that are more stable and that originate from natural sources, which in general are supposed to be safer.

Many studies have been previously conducted in view of finding natural sources of antioxidants which can limit vegetable oil oxidation [7,8,9,10,11]. However, recently, only a few natural sources have been authorized for industrial purposes (case of rosemary) [1]. It is necessary to find other natural sources of antioxidants. According to the literature, the antioxidant potential of natural plant extracts is mainly contributed by their phenolic compounds. Tea leaves are well-known for their high antioxidant activity and phenolic content. Up to now, only few studies have described the efficiency of old green tea leaf extracts in stabilizing vegetable oils. In one study, Navas et al. [12] employed black tea extract to stabilize corn oil under accelerated storage conditions. The results of their study suggest that black tea leaf extracts are an effective source of antioxidant for stabilization of corn oil. However, it has also been proven that green tea contains more antioxidant compounds than black tea leaves [13].

Green tea tree (*Camellia sinensis* L.) belongs to the family of Theaceae. Its leaves contain a variable amount of natural antioxidants, mainly phenolics, among which (+)-catechin, (+)-gallocatechin, (−)-epicatechin gallate, (−)-epicatechin, (+)-gallocatechin gallate, (−)-epigallocatechin, and (−)-epigallocatechin gallate represent up to 90%. The strongest antioxidant among these molecules has been reported to be epigallocatechin gallate [14]. In Cameroon, old tea leaves (matures, found at the base of the tree) are generally considered as waste, and are always eliminated from the tree during cleaning to improve the development of young leaves, which are used for tea manufacturing. These old leaves, instead of being thrown away, could be exploited for other purposes. Hence, in this study, old Cameroonian green tea leaf methanolic extracts were added to palm olein and its effectiveness in delaying oil rancidity under forced storage conditions was investigated.

Palm olein has been used in this study as an oxidation substrate because it is consumed in most the countries all over the world, and is used for food formulations, cooking, and fast food manufacturing. It also contains non-negligible amounts of unsaturated fatty acids, among which linoleic acid, an essential fatty acid, is the most represented. However, relatively fewer studies have been reported on the effect of natural antioxidants in stabilizing palm olein under accelerated storage, probably because it belongs to the same oils as palm stearin, which is highly stable toward oxidation, so there is less interest to stabilize them with antioxidants. Only the effectiveness of synthetic antioxidants in retarding palm olein deterioration during accelerated storage and the effectiveness of some natural antioxidants on the palm olein stability during deep-fat frying have been reported in some papers [15,16].

2. Material and methods

2.1. Material

Refined, bleached, and deodorized palm olein, free from additives, was obtained from SCS/RAFCA Palm Oil Industry Company Ltd., Bafoussam, West Cameroon. Old green tea leaves (matures, found at the base of the tree) were freshly collected from Cameroon Tea Estate (CTE) industry's farm based in Djuttitsa, West Cameroon, on April 2013. All the chemicals and reagents used were of analytical reagent grade.

3. Methods

3.1.1. Extraction of tea leaves antioxidants

The old tea leaves freshly collected from the farm were cleaned and dried in an electric air-dried oven at 50 °C for 48 h. The dried leaves were ground to pass through a 1 mm sieve. About 100 g of the obtained powder was extracted into 800 ml of methanol for 48 h at room temperature. The mixture was regularly subjected to shaking during the extraction. The extract was filtered with a Whatman No. 1 filter paper, and residue was again extracted with 400 ml of methanol to ensure maximum extraction of phenolic compounds. Methanol was used for extraction because of its good antioxidant extraction power compared to other solvents [4]. The combined filtrates were subjected to rotary evaporation at 40 °C under reduced pressure for the removal of the solvent, and the solvent residue was removed by drying the extract in the oven at 45 °C until the extract became solid and the weight became constant. The dried extract was stored at 4 °C prior to further analysis.

3.1.2. Phytochemical characterization of the extract

3.1.2.1. Colorimetric determination of the total phenolic content

The total phenolic content of the extract have been evaluated using the Folin–Ciocalteu colorimetric method, as described by Gao et al. [17]. Briefly, in a test tube of 5 ml volume, 20 μl of a 2 mg/ml extract solution was added, followed by the Folin–Ciocalteu reagent (0.2 ml) and distilled water (2 ml). After 3 min incubation of the solution mixture at room temperature, 1 ml of 20% sodium carbonate solution was added and the mixture re-incubated for 2 h under the same conditions. The absorbance of the resulting blue-coloured solution was measured at 765 nm using a spectrophotometer. The total phenolic content of the extract was calculated from the gallic acid standard curve, and expressed as milligrams equivalents gallic acid per gram of extract.

3.1.2.2. High-performance liquid chromatography analysis

Reverse-phase HPLC was used to analyse the composition of phenolics in the extract (1 mg/ml in methanol). The HPLC Agilent system 1200 series used was equipped with a quaternary pump model G11311A and diode array detector (DAD) model G11315B in combination with Chemstation software. The column type was an RP-C18 Lichrospher column, 5 μm, 4.0 mm internal diameter × 250 mm length. Separations were done in the isocratic mode, using acetonitrile-1% orthophosphoric acid in water (70:30 *v*/v) at a flow rate of 1 ml/min; with an injection volume of 20 μl. DAD detection was at 280 nm. Identification of the antioxidants was achieved qualitatively by comparing their retention time to those of standards.

3.1.2.3. Electrospray ionization-mass spectrometry analysis (ESI-MS)

A qualitative ESI-MS analysis of the extract (1 mg/ml in methanol) was performed on a QUATTROMICRO instrument, equipped with an ESCI multimode ionizator, operating in negative ionization mode, in 100% methanol. The injection volume in the system was 50 μl. ESI-MS parameters were optimized by direct infusion with tuning mix. The other optimum values of the ESI-MS parameters were: temperature, 120 °C; desolvation temperature, 300 °C; desolvation gas flow, 500 l/h; RF lens, 0.2 V; extractor, 3 V; cone, 25 V; and capillary, 3.41 KV (setting); and temperature, 119 °C; desolvation temperature, 317 °C; desolvation gas flow, 423 l/h; RF lens, 0.2 V; extractor, 3.34 V; cone, 28.45 V; and capillary, 3.41 KV (readbacks). The accurate mass data for molecular ion were processed using the software Masslynx V4.1. The identification of compounds was made qualitatively by comparing $[M-1]^-$ fragment ions in samples with those of standards.