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Full Length Article

Evaluation of the antimicrobial activity of synthetic and natural phenolic type antioxidants in biodiesel fuel

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HIGHLIGHTS

- Ten phenolic antioxidants were examined as antimicrobial agents in FAME and B7 blends.
- Two types of FAME were employed in the study based on pomace olive oil and soybean oil.
- Antibacterial activity of the additives generally was not affected by the type of FAME.
- TBHQ, MCT and TBC proved to be effective antimicrobial additives in FAME and B7 blends.

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ABSTRACT

The aim of this study was to investigate the effect of phenolic type antioxidants on the microbial stability of biodiesel fuel along with their relative efficiency to enhance the oxidation and storage stability. Ten commercially available phenolic compounds either of synthetic or natural origin were added separately at concentrations of 1000, 200 and 50 mg/kg in two types of fatty acid methyl esters (FAME) from different source materials, namely soybean oil and pomace olive oil. A selection of the treated FAMEs were also blended with Ultra Low Sulfur Diesel (ULSD) fuel at a concentration of 7% v/v in order to examine the activity of these substances in the final blend. The antimicrobial properties of the phenolic antioxidants were assessed by employing two different methodologies. At first the treated FAMEs and biodiesel blends were examined by detecting their inhibitory potential against the growth of a gram-positive bacterium. Secondly, the ability of the phenolic compounds to suppress bottoms-water microbial activity when added at 1000 mg/kg was assessed, by preparing laboratory-scale challenged microcosms and by measuring the alterations in the cellular Adenosine Triphosphate (cATP) concentration throughout a period of one month. The Rancimat method was employed in order to detect alterations of the relative resistance to deterioration in the presence of the phenolic compounds. The results figured that the hydroquinone and catechol derivatives were the most efficient not only in improving the oxidative behaviour but also in inhibiting the microbial activity in the tested fuels and suppressing the active bioburden in contaminated diesel/biodiesel fuel microcosms. Overall, it was shown that some phenolic antioxidants, primarily added to biodiesel to improve the oxidative stability, could simultaneously enhance the microbial stability of the fuel.

1. Introduction

Biodiesel is a renewable substitute of petroleum diesel fuel, consisting predominantly of fatty acid methyl esters (FAME) of vegetable oil or animal fat origin. It is usually employed as a mixing component in conventional diesel fuel and currently in Europe the maximum allowable concentration of FAME in petroleum diesel is 7% V/V (B7). In spite of the desirable characteristics that biodiesel demonstrates, such as biodegradability, low toxicity, inherent lubricity and alleged benefits on exhaust and greenhouse gas

Abbreviations: ATP, Adenosine Triphosphate; B7, diesel/biodiesel blends with 7% V/V FAME; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; CFA, caffeic acid; CWM, contaminated water microcosms; FAME, fatty acid methyl esters; GA, gentisic acid; MBO, 3,3'-methylenebis[5-methyloxazolidine]; MCT, methyl catechol; IP, induction period; PCA, protocatechuic acid; PG, propyl gallate; POME, pomace olive oil methyl esters; POMO, pomace olive oil; PY, pyrogallol; RM, reference microcosms; TBC, *tert*-butyl catechol; TBHQ, *tert*-butyl hydroquinone; SBO, soybean oil; SBOME, soybean oil methyl esters; ULSD, Ultra Low Sulfur Diesel.

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emissions, (bio-) deterioration issues have been identified as the main concerns associated with the use of FAME as alternative fuel [1]. The lower stability of biodiesel compared to conventional diesel fuels is well documented [2–5]. FAME is susceptible to oxidative deterioration basically due to the unsaturated fatty acid content in the molecule. In specific, FAMEs that comprise of poly-unsaturated fatty acids (PUFA) exhibit lower ageing reserve than those with increased levels of mono-unsaturated (MUFA) ones, since the rate of oxidation is proportional to the number of bis-allylic moieties [6]. Moreover, the hygroscopic characteristics of biodiesel and the chemical structure of FAME, renders it more prone to microbial proliferation and subsequent biodeterioration. Various incidents and arising issues directly from the fuel supply chain have shown that the presence of FAME contributes to reduced microbial stability of the final fuel blend. Additionally, a series of experimental studies support the susceptibility of biodiesel fuel on microbial growth. Consequently, the diesel fuel supply chain is facing the new challenges associated with microbial contamination symptoms in biodiesel fuel, such as filter clogging, microbial induced corrosion in the infrastructure and sludge formation in storage tanks. [7–15].

Due to the low oxidation stability of biodiesel, the treatment with antioxidant agents is a common practice. Oxidation inhibitors are categorized as either primary antioxidants (radical scavengers) or secondary antioxidants (peroxide decomposers). The agents that are usually added in order to upgrade the ageing reserve of biodiesel belong to the class of hindered phenols, with the following being representative examples: BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), TBHQ (*tert*-butyl hydroquinone), PG (propyl gallate) and PY (pyrogallol) [16–21]. Phenolic compounds are widely used as lipid auto-oxidation inhibitors and a large number of these substances have been investigated concerning their antioxidant activity, such as hydroxybenzoic and hydroxycinnamic acids and their derivatives [22–23]. Hindered phenols act as primary antioxidants, i.e., these agents interrupt the free-radical chain of auto-oxidative reactions by donating hydrogen atoms to terminate alkoxy and alkyl peroxy radicals and form stable free radicals which do not initiate or propagate further oxidation of lipids [24].

Apart from their antioxidant activity, it is suggested that many of these phenolic compounds also possess antibacterial and antifungal properties [25]. Several publications can be found in the literature regarding the antimicrobial performance of phenolic antioxidants but the overwhelming majority is associated to lipid-food systems and foodborne pathogens. [26–30]. It has been found that the mechanism of their antimicrobial action interferes with the function and the composition of the cellular membrane as well as with the synthesis of the nucleic acids, proteins and lipids in microorganisms [26]. Nevertheless, limited data exist on the effect of phenolic type antioxidants on the microbial stability of biodiesel fuel. In a recent study two types of hindered phenols, TBHQ and BHT, were evaluated as antimicrobial agents and it was found that the former was a more efficient inhibitor [31].

Based on the above, and by taking into account that the addition of antioxidants is a regular practice in biodiesel, this study focused on the examination of substances that could exhibit a dual function, in other words a simultaneous antioxidant and antimicrobial activity. The detection of those kind of additives could be beneficial in terms of biodiesel's stability against deterioration mechanisms. Therefore, in the present study ten phenolic type substances were evaluated regarding their antimicrobial properties, when added in biodiesel fuel originally as oxidation inhibitors. Pomace olive oil methylesters and soybean oil methylesters were produced and were utilized as representative mono-unsaturated and poly-unsaturated FAMEs, respectively.

2. Experimental

2.1. Materials and reagents

2.1.1. Fatty acid methyl esters

Refined pomace olive oil (POMO) and soybean oil (SBO) were obtained from a Greek oil producer (Kore S.A.) and were used as starting materials without further purification. Table 1 presents the physicochemical properties of the oils engaged in FAME production. Methanol, 99.99% purity and sodium methoxide, pure, anhydrous powder, were sourced from Fischer Scientific Inc.

Pomace olive oil methylesters (POME) and soybean oil methylesters (SBOME) were produced from the previously mentioned parent oils by alkaline transesterification reaction. Methanolysis was carried out for 2 h in a 2 L flask at 65 °C using a 6:1 methanol/oil molar ratio. Sodium methoxide (CH_3ONa) was employed as catalyst at a concentration of 0.75 wt%. After the completion of the reaction, the upper methyl esters phase was separated from the glycerol phase and was purified by washing with 5% w/w sulfuric acid (H_2SO_4) followed by warm water. The excess of methanol was removed by rotary evaporator. The purified methyl esters were dried over anhydrous sodium sulfate (Na_2SO_4) and after vacuum filtration the final FAMEs were obtained. The quality parameters of the produced methyl esters are given in Table 2 and were analyzed according to the applicable requirements and test methods indicated in the European Standard EN14214:2012.

Their fatty acid composition, as listed in Table 3, was determined by gas chromatography using a DANI Master GC apparatus in accordance with EN14103 standard method. POME is abundant in oleic acid (C18:1), whereas SBOME consists predominantly of linoleic acid (C18:2). Considerably higher levels of linolenic acid (C18:3) were detected in SBOME.

2.1.2. B7 blends

FAME samples were blended with an ultra-low sulfur diesel (ULSD) at a concentration of 7% V/V (B7), equal to the maximum allowable mixing ratio according to EN590:2013. The ULSD base fuel sample was a hydrotreated atmospheric straight run gasoil, it was supplied from a local refinery (Hellenic Petroleum, S.A.) and it was additive-free. The prepared blends were examined regarding their basic physicochemical properties as listed in Table 4.

2.1.3. Phenolic antioxidants

Ten different types of commercially available phenolic antioxidants compounds were tested for their effectiveness in the

Table 1
Physicochemical properties of the parent oils.

Property	Units	POMO	SBO	Standard Method
Density @ 15 °C	kg/m^3	917.7	923.2	EN ISO 12185
K. Viscosity @ 40 °C	mm^2/s	40.38	32.49	EN ISO 3104
Water Content	mg/kg	300	300	EN ISO 12937
Acid Value	mg KOH/g	0.35	0.30	EN 14104
Saponification Value	mg KOH/g	194	202	AOAC CD3-25