Efficiency of different additives in the improvement of the oxidation stability of fatty acid methyl esters with different properties

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Abstract: This study evaluates six formulations in improving the oxidation stability of different fatty acid methyl esters (MEs). Two MEs differed in the unsaturation levels as they were synthesized from different feedstock: a blend of soybean and sunflower oils, and waste cooking oil; they did not fulfill the requirements of the EN 14214 standard concerning their oxidation stability (≈0.6 h) and some impurities. The third MEs (SoSuME-EN) were fully compliant with the standard. Five formulations were phenolic-based, containing single or mixed antioxidant compounds of different molecular structures; one was amine-based. Different dosages of the formulations were added to the ME samples (corresponding to the addition range 50–48300 ppm). The MEs stability expressed as induction periods, *IP*, determined by the Rancimat method, were used for the calculation of stabilization factors, *SF*, indicating the efficiency of the applied formulation. The formulation containing 2-(1,1-dimethyl-ethyl)-1,4-benzenediol was the most efficient concerning the lowest consumption rate and the highest *SF* achieved for the low quality ME. 2,2′-Methylene--bis-(4-methyl-6-*tert*-butylphenol) was linked with higher antioxidant potency than the amine-based formulation and the phenolic compounds with two bulky *tert*-butyl groups. Among the 4 selected phenolic additives, butylated hydroxy-toluene and 2,2′-methylene-bis-(4-methyl-6-*tert*-butylphenol) had similar efficiency in SoSuME-EN (at ≈500 ppm they produced *SF* ≈ 2), while it took twice of this amount for mixed butylphenols to achieve the same effect.

Keywords: biodiesel; phenolic antioxidants; amine-based additive; oxidation stability; Rancimat induction period.

INTRODUCTION

Fatty acid methyl esters (MEs) are main constituents of biodiesel fuels, considered as one of the most promising petroleum diesel fuel substitutes. One of the

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most important characteristics of biodiesel is oxidation stability, which describes its susceptibility to oxidation that results in degradation of the composition and consequently of the fuel properties.[1] In fact, poor oxidation stability is one of main shortages of biodiesel fuels compared to conventional diesel. This is why oxidation stability has been addressed in biodiesel quality standards, such as the European standard EN 14214 and the American standard ASTM D6751. According to the standard EN 14214 from 2012 that defines quality of MEs as automotive fuels, the minimum required value for the oxidation stability expressed as an induction period (or induction time), *IP*, determined by the Rancimat method (EN 14112) is 8 h, which is more rigorous than the previous requirement of 6 h (EN 14214 from 2005). The *IP* represents the time during which changes in the MEs composition induced under elevated temperature (110 °C) and intensive contact with air (accelerated oxidation) in the Rancimat apparatus are not significant for the fuel quality; after this period, serious changes in the composition greatly affect the applicability of the fuel. The longer the induction period is, the better is the oxidation stability. The ASTM D6751 standard specification for use of biodiesel as a blend component with middle distillate fuels is less demanding, setting 3 h as the minimum for oxidation stability.[1]

Degree of oxidative degradation is influenced by a number of factors: the most influential is the fatty acid composition of the MEs inherited from the oily feedstock, preferentially the relative contents of mono- and poly-unsaturated MEs. The trend of increasing oxidation rate among the most abundant unsaturated fatty acid with 18 C-atoms is as follows: oleic (having 1 unsaturated double bond, shortly marked C18:1) < linoleic (with 2 doubled bonds, marked C18:2) << linolenic (with 3 double bonds, C18:3);[2–4] The *IP* measured at 110 °C for the MEs of these fatty acids are: 2.5, 1 and 0.2 h, respectively.[5] A number of excellent reviews on different aspects of biodiesel oxidation have been published.[3,5–10] Apart of the fatty acid composition, the presence of minor components such as diacylglycerols, monoacylglycerols, FFAs, phospholipids, moisture and mineral impurities also affects the antioxidant behavior of biodiesel;[10–13] elevated temperature during storage as well as the exposed surface area between biodiesel and air might also increase the oxidative degradation.[1]

One of the most efficient methods for improving the stability and delaying the oxidative degradation of biodiesel is the addition of antioxidants. Antioxidants are chemicals that react with oxidative species more easily than the unsaturated MEs, leading to formation of a stable molecule, which is further resistant to the chain oxidation process.[3] Although effects of various antioxidants on the stability of MEs have often been investigated, a majority of the reported results refer to the addition of a single compound, whereas only few studies considered mixtures that resembled the commercially available formulations.[14–16] Namely, commercial formulations contain one or more active antioxidant compounds in

varying quantities; they have different physical properties (solid state or dissolved, density, viscosity, boiling point, *etc.*) that could affect the compatibility with MEs. Dosing and compatibility of available formulations with a particular biodiesel needs extensive laboratory trials, unless there are comparative information on efficiency of the formulations in improving the stability of fuels similar to the one of interest in a particular case, which can contribute to the more time- and cost-efficient trials. It has to be emphasized that there is no ideal antioxidant that prolongs storage stability of all kinds of biodiesel fuels. Additionally, combination of two or more antioxidants might induce a synergetic effect, enhancing *IP* of biodiesel above the sum of *IP* achievable with each antioxidant separately.[10] On the contrary, there are also examples when antioxidants added to biodiesel were associated with decrease in the oxidation stability due to some antagonistic effects.[16]

The main aim of this study was to test several antioxidant formulations in improving and protecting the stability of MEs of different origin and quality. By measuring *IP* before and after the addition of various formulation dosages (from rather low to massive ones) to MEs, their antioxidative efficiencies were compared, indicating the most efficient active compound(s) in term of the highest enhancement of the stability. Comparison of the results for MEs of varying qualities gave an insight into the antioxidant potencies over a wide range of concentrations in different substrates, also indicating indirectly the influence (or importance) of fuel impurities, the unsaturation level and/or starting *IP* on the protective power of the antioxidants. To the best of the authors' knowledge, this is the first study that compares mono- and multi-active compound-based formulations in different MEs. It contributes to the existing understanding of the protection power of antioxidants in different substrates and also provides valuable information for the selection and application of antioxidants in the fuel sector and other applications in which fatty compounds may be exposed to oxidative stress.

EXPERIMENTAL

Two different raw materials were used for the synthesis of the MEs: a blend (1:1 mass ratio) of domestically produced soybean and sunflower oils, and waste cooking oil collected from a restaurant at the Faculty of Agriculture, University of Novi Sad. The MEs produced from these two feedstock materials are marked as SoSuME and WCOME, respectively. The MEs were produced by a conventional alkali homogeneous transesterification in a laboratory glass reactor (methanol to oil mole ratio 8:1; 1 % KOH catalyst in oil) at a temperature ≈64 °C for 1 h. After the reaction, the product mixture was allowed to settle; the upper layer of MEs was collected, while the lower glycerol layer was discarded. The MEs were purified with distilled water and dried under vacuum. The schematic representation of the experimental setup is given in Fig. S-1a of the Supplementary material to this paper. The MEs were synthesized in successive batches and then homogenized by mixing all batches of a particular ME in one vessel in order to ensure that the total amount was enough for the planned number of experiments.

Before addition of the formulations, the MEs were characterized in accordance to the requirements of EN 14214 and the results are presented in Table S-I of the Supplementary material. The methods used for the characterization of the MEs are also listed in Table S-I. These analyses were performed in SP Laboratorija (Bečej, Serbia) accredited under the ISO//IEC 17025 standard. As could be seen, two quality levels of SoSuME were used: one in compliance with EN 14214 (marked as SoSuME-EN), having initial *IP* of 8.88 h, and the other (SoSuME) that failed the EN 14214 requirements for minimum oxidation stability (the initial *IP* was 0.60 h) and for the methyl ester content, as well as for the maximum content of alkali and earth-alkali metals, phosphorous and water. WCOME was also characterized with low starting oxidation stability (0.63 h) and elevated levels of metals but not so remarkably as in the case of SoSuME. In fact, SoSuME and WCOME were intentionally not purified satisfactory, and they were left for "aging" (under sunlight and in contact with air) for a few months before the addition of the antioxidants. In this way, low quality MEs were produced. Concerning the contents of mono- and poly-unsaturated C18 MEs determined by gas chromatography (GC), SoSuME and WCOME differed: WCOME had more than two times higher content of C18:2 esters (57 %) compared to SoSuME/SoSuME-EN (22 %), while SoSuME//SoSuME-EN (3.2 %) had 32 times higher content of C18:3 than WCOME (0.1 %); the content of C18:1 differed less between MEs (39 % in SoSuME/SoSuME-EN and 31 % in WCOME). In summary, the total C18 unsaturation level (calculated as the sum of the contents of the C18 unsaturated MEs determined by GC method listed in Table S-I) differed markedly between WCOME (88.1 %) and SoSuME (64.2 %). The significant difference was seen also in the content of C16:0 (30 % in SoSuME/SoSuME-EN and 7 % in WCOME). These differences indicated different susceptibilities of these MEs to oxidation. The oxidizability of MEs was calculated using the contents (%) of MEs of the C18:1, C18:2 and C18:3 fatty acids in the following manner:[5] oxidizability = (0.02 · C18:1 % + C18:2 % + 2 · C18:3 %)/100.

Six antioxidant formulations, marked from A to F, were available for testing. Five formulations were phenolic-based (A-E) and one was amine-based (F); five contained one active antioxidant compound (*i.e.*, mono-active compound-based formulations: A-D and F), while one represented a mixture of different phenolic compounds (*i.e.*, multi-active compound-based formulation: E). The main antioxidant compounds in the formulations are listed in Table I.

TABLE I. Composition of the commercial formulations A-F used in this study based on the Material Safety Data Sheets (MSDSs) provided by the producers

Formulation	Composition	Type
A	2,6-Di-*tert*-butyl-*p*-cresol (butylated hydroxytoluene – BHT >99.7 %)	Phenolic
B	2-*tert*-Butylhydroquinone (TBHQ, 10–20 %), butene, homopolymer (2.5–5 %), solvent naphtha, heavy aromatics (0.25–1 %)	Phenolic
C	2,2'-Methylene-bis-(4-methyl-6-*tert*-butylphenol) (20 %)	Phenolic
D	2,2'-Methylene-bis-(4-methyl-6-*tert*-butylphenol) (96–99 %)	
E	2,6-Di-*tert*-butylphenol (>75 %), 2,4,6-tri-*tert*-butylphenol (15 %), 2-*tert*-butylphenol (7 %), 2,4-di-*tert*-butylphenol (2 %), 2,5-di-*tert*--butylphenol (1.7 %), phenol 0.2 %, 4-*tert*-butylphenol (0.2 %)	Phenolic
F	Amine substituted resin (60-100 %), heavy aromatic naphtha (0–30 %), naphthalene (0–5 %), 1,2,4-trimethylbenzene (0–5 %)	Amine

The A additive was based on a compound with two bulky *tert*-butyl groups linked to an aromatic ring in the ortho positions (2,6-) to the OH-group. The main antioxidant compound

in B consisted of two OH groups linked to aromatic ring in the para position (1,4-) and one *tert*-butyl group in the ortho position (2-). The C and D formulations had the same main active compound (with two methyl-butylphenol rings bridged by a CH_2-group) but in different concentrations prepared by the producer. The E formulation had a unique composition compared to the other phenolic formulations as it represented a mixture of various phenols with one, two or three *tert*-butyl groups. The additive F contained mainly an amine-based resin. All of them were tested for improving the oxidation stability of SoSuME and WCOME, while four phenolic-based formulations, found to be less effective for these low quality MEs, were further assessed in maintaining the initially high stability of SoSuME-EN under the accelerated oxidation test. Some properties of the formulations are given in Table II. Different dosages of the formulations were added to 100 mL of MEs, as presented in Table III.

TABLE II. Properties of the commercial formulations A–F used in this study; properties except boiling points were taken from the MSDSs. Boiling points are not given in the MSDSs, but they were taken from Wikipedia pages, considering the main components of the tested formulations: BHT for A; TBHQ for B; and 2,6-di-*tert*-butylphenol for E; the boiling point for 2,2′-methylene-bis-(4-methyl-6-*tert*-butylphenol), the main compound in C and D, was taken from https://www.alfa.com/en/catalog/H36783/

Property	Formulation					
	A	B	C	D	E	F
Physical state	Solid	Liquid	Liquid	Solid	Liquid	Liquid
Density, kg dm^{-3}	1.03 at 20 °C	0.914 at 15 °C	0.89 at 20 °C	1.04–1.1 at 20 °C	0.94 at 20 °C	0.92 at 20 °C
Flash point, °C	127	62	180	185	114	64
Viscosity, kinematic, mm^2 s^{-1}	–	605 at 20 °C 135 at 40 °C	–	–	–	157 at 100 °C
Solubility in water, mg dm^{-3}	0.76	0.03	Insoluble	0.007	0.4	–
Boiling point, °C	265	273	187	187	253	–

TABLE III. The mass concentrations (in ppm$_m$) of the main active compounds added to the methyl esters (MEs) in different amounts (in mL) of the formulations A–F; for liquid formulations, it is calculated by taking into account the volumes of the formulations added to 100 mL of MEs, the average value of the MEs densities given in Table S-I (886 kg m^{-3}), the densities of the formulations presented in Table II, and the percentage share of the main active compound. The measured mass of solid additives A and D were taken for the calculation of the relevant ppm$_m$ concentrations

Formulation dosage in mL added to 100 mL of MEs	A	B	C	D	E	F
0.05	564	51–102	100	604	530	519
0.10	1129	102–203	201	1208	1061	1038
0.20	2257	203–406	402	2415	2122	2077
0.50	5643	508–1016	1005	6038	5305	5192
1.0	11287	1016–2032	2009	12077	10609	10384
3.0	33860	3047–6095	6027	36230	31828	31151
4.0	45147	4063–8126	8036	48307	42438	41535

Before the addition, the liquid formulations were heated up to 60 °C in order to reduce the viscosity and make easier pipetting.[17] In the case of the solid additives (A and D), the adequate quantity was first dissolved in toluene, added to 100 mL of MEs and mixed, followed by evaporation of the solvent. As ≈4 % is the limit for constituents other than MEs in accordance to the limit for the MEs content (96.5 %) requested by EN 14214, the higher dosages were not tested. Although massive dosages of the antioxidants (*i.e.*, those ≥1 %) could be considered neither economically nor technically feasible for the biodiesel industry,[18] they were applied in order to investigate their protective potency over a wide range of concentrations, particularly to comparatively investigate their ability to increase the low initial stability of poor quality substrates with different unsaturation levels.

The efficiency of additives was expressed as a stabilization factor, SF,[19] calculated by dividing IP obtained for the additized MEs (*i.e.*, MEs with added antioxidant formulation, IP_X) with the initial (starting) IP of the untreated (neat) MEs (*i.e.*, MEs without the additive, IP_0). The IPs were determined in accordance to the method EN 14112 using Metrohm 743 Rancimat equipment (Herisau, Switzerland). The principle of the method for the measurement of the IPs is illustrated in Fig. S-1b of the Supplementary material. Three grams of sample were placed in the heating block at 110 °C and intensively oxidized with an air flow (10 L h^{-1}), which resulted in formation of volatile products (mainly formic and acetic acids)[4] that were transferred to a flask containing 50 mL of distilled water. After some time, large quantities of volatiles dissolved in water induce a significant increase in the measured conductivity. The time corresponding to the inflection point in the oxidation curve (conductivity *vs.* time) is the IP. Each sample was analyzed in duplicate and the results on IP are presented as averages.

RESULTS AND DISCUSSION

Impacts of the antioxidant formulations added in different dosages on the IPs of studied MEs are presented in Fig. S-2 of the Supplementary material. The lowest dosages of the antioxidants that provided IPs above the limit of 8 h for the low quality MEs (SoSuME and WCOME) are listed in Table IV. The stabilization factors, SF, calculated for the tested formulations and the added amounts, are presented in Fig. S-3 of the Supplementary material. The highest SF and the corresponding IP achieved for each of the additized MEs are given in Table V.

TABLE IV. The lowest dosages of the formulations A–F (in mL) followed by the corresponding active antioxidant compound (in ppm$_m$) that provided oxidative stability of low quality methyl esters (SoSuME and WCOME) above the EN 14214 limit of 8 h; NA: the EN 14214 limit is not achieved (IP was < 8 h) at any of the tested dosages

Ester	A	B	C	D	E	F
SoSuME	1/11287	0.5/508-1016	1/2009	3/36230	1/10609	0.5/5192
WCOME	NA	1/1016-2032	NA	3/36230	NA	NA

Concerning MEs that were not in compliance with EN 14214, *i.e.*, SoSuME and WCOME, the lowest additions of 0.05, 0.10 and 0.20 mL, corresponding up to about 2500 ppm$_m$ of the main antioxidant compounds (Table III), were insufficient to improve the very low starting IP to be in compliance with the standard thresholds (Fig. S-2), even though these dosages increased the initial IP by factor of 2–4 (Fig. S-3). It could be seen that the IP of these MEs increased above the

less stringent ASTM D6751 limit with the 0.50-mL dosages of the formulations (Fig. S-2), except for C and D. The dosage of 0.50 mL increased *IP* of SoSuME above the EN 14214 limit of 8 h only in the case of B and F (Fig. S-2). Taking into account the concentrations of the main active compounds in 0.50 mL of B and F (Table III), it could be concluded that the more potent antioxidant was the TBHQ present in B than the amine substituted resin in F. Looking further into the contents of the active compounds in other formulations needed to be added to SoSuME to overcome the 8-h limit (Table IV), it could be observed that the active compound of C (2,2′-methylene-bis-(4-methyl-6-*tert*-butylphenol)) also had marked antioxidant effect as its concentration was only 2 times that of TBHQ, while approximately 10–20 times more of the active compounds in A (2,6-di--*tert*-butyl-*p*-cresol) and E (2,6-di-*tert*-butylphenol and other phenols with one, two or three *tert*-butyls) were needed compared to TBHQ. Even though D contained the same active compound as C but in a higher amount, it showed very low antioxidant efficiency as a very large quantity was needed to be added to SoSuME to reach the 8-h limit (35–70 times more than in the case of TBHQ). As D is a solid substance that needed to be dissolved before addition to MEs (toluene was used), while C was a solution (20 %) of the same compound prepared by manufacturer (using biodiesel as the solvent), it could be only presumed that the method of the dissolution and/or mixing of the D solutions with MEs, particularly in the lower addition volumes, was the reason for its low efficiency. Furthermore, the lower boiling point of the active compound in D could be the reason for its higher losses during the solvent evaporation step after addition to MEs than for the active compound in A, which was also prepared by dissolving in toluene since it was provided in the solid state as D (Table II). Hence, the increasing quantities of the relevant active compounds necessary to enhance the *IP* of SoSuME above 8 h, ordered the formulations as B < C < F < E=A (D was excluded here due to the probable mixing problem that impaired its protective efficiency).

TABLE V. The highest stabilization factors (*SF*)/induction periods (*IP* in h) achieved for the antioxidants A–F added to the tested methyl esters SoSuME, WCOME and SoSuME-EN (numbers in parentheses are the antioxidant dosages in ppm_m that provided the specified values of *SF*/*IP*)

Formulation	SoSuME	WCOME	SoSuME-EN
A	74.2/44.5 [11287]	11.9/7.5 [11287]	11.1/98.3 [45147]
B	116.8/70.0 [1016-2032]	27.2/17.2 [1016-2032]	–
C	24.6/14.8 [2009]	7/4.4 [8036]	5.7/50.7 [8036]
D	43.2/25.9 [48307]	28.8/18.4 [48307]	18.0/159.5 [48307]
E	52.7/31.6 [10609]	6.4/4.0 [5305]	6.26/55.6 [42438]
F	22.1/13.3 [5192]	10.2/6.4 [5192]	–

The highest *IP* of SoSuME were not measured for the highest applied dosages of the formulations, except in the case of D (Fig. S-2, Table V). This *IP* was several dozen times higher than the starting *IP* of SoSuME and the formulations could be order according to the highest achieved *SF* as follows (Table V): B (116.8) > A (74.2) > E (52.7) > D (43.2) > C (24.6) > F (22.1). The main antioxidant concentrations that induced these highest *IP*/*SF* varied significantly (Table V): B and C reached their maximum antioxidative potency in SoSuME at similar concentration of up to ≈2000 ppm$_m$, whereas the maximum for A and E was at higher levels ≈11000 ppm$_m$. It could be stated that the most efficient antioxidants for SoSuME were TBHQ and 2,2′-methylene-bis-(4-methyl-6-*tert*-butylphenol), with the later applied as the original solution (prepared in biodiesel by the producer, C). Difference seen between C and D regardless of the same active compound they contained could be ascribed to different states in which they were provided by the producer that further implied different ways of the application.

In the case of WCOME, an *IP* above 8 h was achieved only with B and D (Table IV). In fact, TBHQ and 2,2′-methylene-bis-(4-methyl-6-*tert*-butylphenol) were the only compounds that enabled improvement of the oxidation stability of the highly unsaturated WCOME to be in compliance with EN14214, while BHT in A, mixed mono-, di- and tri-*tert*-butylphenols in E, and amine substituted resin in F failed. These two compounds were also successful in making SoSuME compliant with the EN standard at the lowest consumption rates, but WCOME required larger dosages. As the highest dosage of the C formulation (4 mL) that contained 8036 ppm$_m$ of the same active compound as D did not increase the *IP* above 8 h (Fig. S-2), it could be assumed that the massive consumption rate of the D formulation was the consequence of both the lower ability of 2,2′-methylene-bis-(4-methyl-6-*tert*-butylphenol) to protect MEs with high unsaturation, and the possible problems of mixing with the MEs, previously also suspected in the case of SoSuME. Generally, it could be observed that all formulations were less efficient for WCOME compared to SoSuME, as the *IP* and *SF* of the additized WCOME were lower than in the case of SoSuME (Figs. S-2 and S-3). This could be ascribed to higher unsaturation level in WCOME and, as expected, its much higher oxidative instability than SoSuME: the oxidizability[5] for WCOME was double the one for SoSuME (0.59 and 0.29, respectively). This further implied that the unsaturation level has a more important effect on reducing stability than the level of impurities found in low quality MEs.

The highest efficiency of B could be explained by the unique dihydroxyl aromatic structure of TBHQ that more easily donates hydrogen atoms (radical) to free radicals of esters produced during the oxidation stress of MEs than monohydroxyl structures present in A and E, interrupting the propagation step of the free radicals chain more readily, and making it a more powerful and protective antioxidant.[5,20] Interestingly, 2,2′-methylene-bis-(4-methyl-6-*tert*-butylphenol)

in C and D also contains 2 OH-groups per molecule, but each of them is linked to one of two aromatic rings in the bisphenyl structure, which seems to render them less protective than 2 OH-groups linked to an aromatic ring in para-position, as in TBHQ. Besides the number of OH-groups in the molecule, presence of alkyl groups is also important for antioxidant behavior, as hydroxyl groups on benzene ring do not show antioxidant behavior unless its ortho or para positions were occupied by alkyl groups; in this way, the electron density on the OH-groups is increased, which makes the release of a hydrogen radical easy.[5] Both compounds, TBHQ and 2,2′-methylene-bis-(4-methyl-6-*tert*-butylphenol), contain one bulky butyl group in the ortho position to an OH-group. On the other hand, the main active compounds in A and E had two tertiary butyl groups in ortho position to OH-group that possibly induced more steric hindrance, suppressing the electron-release from the OH group and making A and E less efficient, especially if compared to TBHQ.[16] Apart of the favorable structure, the highest antioxidant efficiency of TBHQ could be additionally related to the products known to be formed during the antioxidative activity of TBHQ that may also have antioxidant properties, synergistically retarding the oxidation.[20] Besides the chemical structure, difference in the antioxidant efficiency of the phenolic compounds could also be attributed to physical properties of the formulations that might influence compatibility and mixing with the substrates as was suggested for the poor performances of D (originally provided as a solid substance).

The unique composition of the additive F (the only amine-based additive among the tested ones) influenced the oxidation stability of the treated low quality MEs in a rather different manner in comparison to the phenolic additives. It seemed to be moderately efficient in the stability enhancement of SoSuME compared to other phenolic-based formulations. At the level of 0.50 mL that contained 5192 ppm_m of the active compound (Tables IV and V, Fig. S-2), an *SF* of 22.1 was achieved (Fig. S-3), which was enough to surpass the threshold of 8 h required by EN14214. However, the same dosage failed to increase *IP* of WCOME above the lower limit. Further increase of the dosages actually fully destabilized SoSuME and WCOME as the *IP* was measured to be ≈0. This might be interpreted in the following way: interaction of large dosages of the formulations with the low quality MEs constituents induced an almost instantaneous increase of the conductivity in the measuring cell of the Rancimat apparatus. This observation will be further explained hereafter in comparison to the results obtained for high additions to SoSuME-EN. It is known that the antioxidant function of an aromatic amine is superior to that of phenols as many more radicals per molecule could be trapped than in the case of phenols and therefore, the usually the required quantity of amine-based antioxidants is lower than for the phenolic ones,[10] which was the case in this study if less efficient phenolic formulations A and E were taken into account. Additionally, it is worth noting that the addition

of amine-based antioxidants leads to the introduction of nitrogen into the bio-diesel composition, diminishing one of its advantages over fossil fuels of being fuel without heteroatoms such as S and N. This was the reason why it was excluded from the further testing performed in this study.

It is known that antioxidants efficiently function in an optimal range of concentrations between the critical and the finite saturation levels beyond which no further improvement in the antioxidant activity could be achieved.[10] For the low quality MEs tested in this study, it was found that there was a dosage that induced maximum ("peak") of *IP* for all the tested phenolic formulations except for D (probably because of the mentioned problem of its mixing), Fig. S-2. This maximum *IP* might be regarded as the "saturation" level for SoSuME and WCOME. After the observed "peaks", the reduced *IP*, *i.e.*, antioxidant power was seen with further increase of dosages (Fig. S-2) that could be considered as counter-intuitive behavior. In some cases, the high dosages even resulted in full disturbance of the oxidation stability manifested as $IP \approx 0$ (Fig. S-2). The null value of *IP* was not seen in the additized good quality MEs (SoSuME-EN). It might be only suspected that all the antioxidants added at the higher dosages displayed some pro-oxidative behavior in the presence of impurities in SoSuME and WCOME, which was more pronounced for monoaromatic phenolic and amine structures in the MEs with higher unsaturation level (WCOME), but this observation requires further study.

As the B formulation was found to be the most efficient antioxidant for the low quality MEs (Tables IV and V), the formulation was presumed to be highly likely the most protective also in the case of SoSuME-EN. Thus it was excluded from the further analysis of the phenolic antioxidants, and only A, C, D and E that were found to be less effective for low quality MEs, were added to SoSuME--EN in order to examine their capability to retain oxidative degradation of biodiesel with an initial *IP* above the EN threshold. The following levels led to a doubling of the initial *IP* of SoSuME-EN: 500–1000 ppm$_m$ of BHT in A and of mixed butylphenols in E, and ≈400 ppm$_m$ (≈2400 ppm$_m$) of 2,2′-methylene-bis--(4-methyl-6-*tert*-butylphenol) in C (and D). Thus, the efficiency of BHT, 2,2′--methylene-bis-(4-methyl-6-*tert*-butylphenol), and mixed butylphenols added to high quality MEs was similar (500–1000 ppm$_m$); D might be excluded from this observation due to already mentioned possible problem of the mixing.

Generally, the *SF* achieved for SoSuME-EN were lower than for SoSuME and WCOME (Fig. S-3). The *SF* calculated for the low dosages were in the narrow range up to 5.3, whereas more pronounced differences among *SF* (2.7––116.8) were seen for the higher dosages (1, 3 and 4 mL), probably because differences in antioxidation mechanisms and specific interactions of the formulation with the substrate were more pronounced at higher concentrations. Additionally, the Rancimat method has its own limitations in measuring long *IP*, when the

results may not be accurately obtained because of water evaporation in the conductivity cell.[20] Different trends between *IPs* and the dosages could be observed among the formulations added to SoSuME-EN. Namely, almost the same linear trend of the *IP* increase with the dosage was seen for additions of A and E (Fig. S-2); for C and D, a slight increase of *IP* was observed up to the 0.20-mL dosage followed by the sharp trend of the *IP* increase with the higher dosages. The saturation levels as maximum *IP* ("peak") were not seen for any of the tested formulations in SoSuME-EN, contrary to those observed for low quality MEs (Fig. S-2), which may be the consequence of specific interactions of impurities in the latter with active compounds in higher levels during accelerated oxidation.

Literature data also evidenced high potency of TBHQ. The comprehensive study of Mittelbach and Schober on the efficiency of 10 synthetic antioxidants (besides 10 mixtures of natural antioxidants also included in the study) added at 1000 ppm to four different MEs (rapeseed oil-, waste frying oil-, sunflower oil-, and tallow-based) also revealed TBHQ to be among the most efficient synthetic antioxidants, producing the greatest enhancement of the *IP*; *i.e.*, at the level of 1000 ppm of TBHQ, the *SF* obtained for undistilled waste frying oil-based biodiesel with an initial oxidation stability of 5.9 h was about 5.[19] The widely used BHT was also tested in that study, but it was not as efficient as TBHQ: at a concentration of 1000 ppm, it led to an *SF* of ≈2 for the same biodiesel,[19] coinciding with the finding for the same dosage of the A formulation added to SoSuME-EN in the present study. The relative inefficiency of BHT compared to TBHQ was suspected to be the consequence of partially evaporation during measurements at 110 °C, failing to protect the MEs from oxidative degradation.[19] Again, the higher efficiency of TBHQ than BHT was proven in the study of Ryu: TBHQ at 300 ppm and BHT at 1000 ppm provided sufficient resistance to oxidation of soybean MEs (initial *IP* of 1.36 h) taking into account the previous EN 14214 requirement of 6 h; TBHQ at a dosage of 2000 ppm enhanced the *IP* over 40 h, while BHT did not provide *IP* over 10 h at the same dosage.[21] Yang *et al.* investigated the efficiency of several antioxidants with dosages up to 8000 ppm to improve very low starting *IP* (0.7 h) of the soybean MEs.[22] They also reported TBHQ as more efficient than BHT: at 3000 ppm TBHQ improved initial *IP* to ≈6 h, while BHT did not increase *IP* to 6 h even at 8000 ppm. Domingo *et al.* studied the effect of BHT and TBHQ on soybean oil ethyl esters with very low initial *IP* of 0.16 h at dosages ranging from 200 to 8000 ppm: the highest efficiency was observed for TBHQ at 8000 ppm with *SF* of 52.52; at the same dosage, BHT provided a *SF* of 35.59.[23,24] Lapuerta *et al.* found that BHT amounts of ~4000 ppm and ≈1500 ppm in biodiesel from used cooking oil and soybean oil (initial *IP* of 0.7 and 3.61 h), respectively, were enough to achieve the limit of 8 h at 110 °C.[18]

CONCLUSIONS

The range of the results confirmed that MEs of different compositions required different dosages of the analyzed formulations, *i.e.*, the potency of an antioxidant, including its consumption rate and the efficiency, differed among substrates. With respect to MEs with very low initial oxidation stability, the results showed the advantage of TBHQ over the other tested phenolic anti-oxidants, while the amine-based formulation showed moderate protection pot-ency in the narrowest range of the applied concentrations. These findings implied that for low quality substrates, molecules with two OH groups per molecule could be linked with higher antioxidant potency than those with one OH-group and two bulky *tert*-butyl groups or amines. Higher unsaturation levels required higher dosages of the antioxidants to induce the 8-h threshold. Concerning the high quality MEs, BHT and 2,2′-methylene-bis-(4-methyl-6-*tert*-butylphenol) ensured $SF \approx 2$ with the lowest quantity among the four tested phenolic formul-ations. As the main differences between SoSuME and SoSuME-EN were the impurity levels (alkali and earth-alkali metals, phosphorous, water) and the initial *IP*, the different trends observed for them in the *IP* increase with the antioxidant levels ("peaks" in the curve of *IP vs.* dosage observed for SoSuME, but not for SoSuME-EN) suggested the possible role of impurities in pro-oxidative behavior of the higher antioxidant dosages under accelerated oxidation, but this obser-vation requires further studies.

SUPPLEMENTARY MATERIAL

Additional data are available electronically at the pages of journal website: https:////www.shd-pub.org.rs/index.php/JSCS/index, or from the corresponding author on request.

ИЗВОД
ЕФИКАСНОСТ АНТИОКСИДАНТНИХ ФОРМУЛАЦИЈА У МЕТИЛ ЕСТРИМА МАСНИХ КИСЕЛИНА РАЗЛИЧИТИХ КАРАКТЕРИСТИКА

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У раду је процењен утицај шест формулација на побољшање оксидационе стабил-ности различитих метил-естера масних киселина (МЕ). Два испитана МЕ се разликују у нивоима засићености, јер су синтезовани из различитих сировина: мешавине соје и сунцокретовог уља (SoSuME) и од отпадног уља за кување (WCOME); они не испуњавају услове стандарда EN 14214 у погледу оксидационе стабилности (~0,6 h) и неких при-меса. Трећи МЕ (SoSuME-EN) је у потпуности усаглашен са стандардом EN 14214. Пет антиоксидативних формулација је на бази фенола, са једном или више активних суп-

станци различите молекулске структуре, док је једна на бази амина. Формулације су додате у 7 нивоа (у опсегу садржаја активних супстанци 50-48300 ppm) Стабилност МЕ је изражена као период индукције, *IP*, одређен Ранцимат методом, и коришћен за израчунавање фактора стабилизације, *SF*, који указује на ефикасност примењене формулације. Формулација која садржи TBHQ била је најефикаснија у погледу најмањих количина и највећих *SF* постигнутих код МЕ лошијег квалитета. 2,2′-метилен-бис-(4-метил--6-*терц*-бутилфенол) је показао бољу антиоксидациону моћ од аминске формулације, као и фенолних формулација са две терц-бутил групе. Од 4 изабрана фенолна адитива, BHT и 2,2′-метилен-бис-(4-метил-6-терц-бутилфенол) су показали сличну ефикасност у SoSuME-EN (при ~500 ppm постигнут је *SF* ≈ 2), док је била потребна двоструко већа количина формулација са мешаним бутилфенолима за постизање истог ефекта.

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