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Full Length Article

Influence of binary, ternary and quaternary mixtures on oxidative stability and study of kinetics and thermodynamic parameters of the degradation process of soybean biodiesel

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ABSTRACT

Currently, studies show that plant extracts may exert antioxidant action and have aroused great interest because they have the property of preventing or minimizing the oxidative stress caused by free radicals. This study concerning to the binary, ternary and quaternary mixtures of plant extracts and the natural antioxidant quercetin with soybean biodiesel in concentrations ranging from (500 to 3000 ppm), using the acceletated oxidation techniques (Rancimat and PetroOXY), where the temperatures varied from 90 °C to 140 °C. The stabilizing factor was studied and indicated the existence of an antioxidant effect and not an antagonistic effect. Among the extracts the highest quantitative levels of flavonoids and total carotenoids were obtained in the following decreasing sequence: oregano extract (OE) > basil extract (BAE) > bilberry extract (BE), while for antioxidant activity front the DPPH radical the decreasing sequence was: oregano extract (OE) > bilberry extract (BE) > basil extract (BAE). The activation energies of the antioxidant mixtures were obtained by the Arrhenius equation and followed the ascending order: OEQC (62.69 kJ/mol) < BOBE (65.01 kJ /mol) < BAEQC (77.15 kJ/mol) < BEQC (77.73 kJ/mol) < BOBEQC (81.73 kJ /mol) by the technique Rancimat and BEQC (40.90 kJ/mol) < OEQC (41.82 kJ/mol) < BAEQC (42.32 kJ/mol) < BOBEQC (44.48 kJ/mol) < BOBE (45.31 kJ/mol) by the technique PetroOXY. The thermodynamic parameters ΔH* and ΔS*, were obtained by the Eyring equation and the ΔG* by the fundamental equation of thermodynamics, being evident, by the results shown, the existence of an endothermic and non-spontaneous process. The shelf life of antioxidant mixtures with biodiesel followed the descending order: BAEQC > BEQC > OEQC > BOBEQC > BOBE and OEQC > BAEQC > BEQC > BOBEQC > BOBE in the Rancimat and PetroOXY method, respectively. Therefore, the use of mixtures of these natural antioxidants was very promising and of fundamental importance in the control of the oxidative process of soybean biodiesel.

1. Introduction

Recent research has shown that because of the great global development, demand for energy has grown significantly, generating concerns about global warming and the scarcity of non-renewable sources, thus, so many countries have turned to alternative sources, i.e, to exchange fossil energy for renewable energy [1–5]. The use of alternative energy has become a major priority for the world, and biodiesel is becoming increasingly important in this issue, having the advantage of its properties being similar to diesel, which makes it possible to use it in diesel engines [6,7].

To be marketed on a large scale, it is vitally important that the quality of the fuel is guaranteed. The production and use of biodiesel provide the development of a sustainable energy source in the environmental, economic and social aspects, as well as the reduction in the importation of fossil diesel [8].

Soybean has a prominence in the production of oilseeds and oils in the world market, the composition of its oil is approximately 85% of unsaturated carboxylic acids [9]. Due to the high unsaturation content of soybean oil, this may cause inadequacy in the biodiesel industry when used, compromising the storage of biodiesel in the process called autoxidation and consequently leading to the formation of aldehydes, ketones, peroxides and hydroperoxides, negatively affecting their oxidative activity stability [9,10].

The phenolic compounds belong to a class that includes a diversity of simple and complex structures that interact preferentially with the

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peroxyl radical, since it is the most abundant in the autoxidation stage and because it has less energy than other radicals, a fact that favors the abstraction of their hydrogen [10,11]. The antioxidant potential of these compounds is mainly due to redox properties and chemical structure, which play an important role in the neutralization or sequestration of free radicals and the chelation of transition metals [11–14]. These compounds are commonly found in plants and have many biological effects, including antioxidant activity. Tests to check antioxidant properties in plants are important to discover new and promising sources of natural antioxidants [15,16].

The study of antioxidant synergism is of fundamental importance for its quality control, since biodiesel is susceptible to any type of oxidation because it has lipid composition, therefore it is of fundamental importance to know its oxidative mechanisms. The efficiency of any substance that presents antioxidant potential can be improved when, in the same system, there are several compounds that act by different mechanisms to reach the same end [17]. Synergism can be defined as a phenomenon in which a mixture of two or more compounds, when added in the same system, results in a more pronounced effect than that presented by the compounds alone [17,18].

Oxidative stability is a parameter that can be even included in the Rancimat method (EN 14112) and PetroOXY (ASTM D7545). The current specification advocates the accelerated test method EN 14112, using Rancimat equipment, as the official for the evaluation of the susceptibility to oxidation of biodiesel, establishing a minimum period of eight hours as an induction period [19,20]. However, there are few studies employing alternative, including other study done by us, fast and effective methodologies to determine the induction period, such as the PetroOXY method [21,22].

Trying to explore and know more about the study of natural antioxidants acting in the protection of biodiesel, we studied the mixture of several natural antioxidants with the objective of promoting the synergism between them, since an antioxidant alone may not present an effective action against the autoxidation of this biofuel. Thus, we studied the synergism between several antioxidants using the Rancimat and PetroOXY techniques in soybean biodiesel, as well as the kinetics of oxidation reaction retardation.

2. Experimental

All reagents used in this work were of analytical grade (Sigma-Aldrich, Vertec or Merck) and used without further purification. Soybean vegetable oils were purchased at a local market in the city of Teresina-PI, Brazil, being careful to always choose the oil from the same manufacturer and production batch. For the studies carried out, the bilberry leaves (*Plectranthus barbatus*), oregano (*Origanum vulgare* (*L*)) and basil (*Ocimum basilicum* (*L*)), containing substances with antioxidant properties, being cultivated by the authors, while quercetin was acquired by Sigma - Aldrich. It should be noted that the choice of antioxidant and the concentration used was based on previous studies that attest quality, cost, availability and chemical structure [15].

2.1. Production, determination of the fatty acid composition and ^1H nuclear magnetic resonance of biodiesel

The transesterification of biodiesel by homogeneous alkaline catalysis and soybean oil methylation, as well as the quantification of the esters were obtained as described in the literature [10,15]. The ^1H NMR spectra of soybean oil and biodiesel were obtained by an INOVA-500 spectrometer with a resonant frequency of 500 MHz, using the internal standard solvent $CdCl_3$.

2.2. Preparation of extracts

The extracts of bilberry, oregano and basil were prepared according to methodology described in the literature [15].

2.3. Determination of the quantitative content of total carotenoids

The total carotenoids content of ethanolic extract of bilberry, oregano and basil was determined by UV–Vis molecular absorption spectrophotometry, where 2 g of bilberry extracts, oregano or basil were weighed into a 30 mL amber flask with stopper, and then 10 mL of a mixture of acetone: hexane (4:6) was added, and the solution was stirred for 10 min. The extracts were measured on a spectrophotometer using a wavelength of 450 nm. The results were expressed in mg of β-carotene/g of sample according to a standard β-carotene curve [23–25]. The experiments were performed in triplicate.

2.4. Determination of the quantitative content of total flavonoids

The total flavonoid content of ethanolic extract of bilberry, oregano and basil was determined by UV–Vis molecular absorption spectrophotometry using aluminum chloride ($AlCl_3$) methanolic solution. An analytical curve was constructed using rutin as standard. The rutin was dissolved in MeOH/H_2O (7:3) at concentrations of: (3.0, 6.5, 10.0, 13.5, 17.0, 21.0) mg L^{-1}. The samples of antioxidants were preparing using 10 mg of extract and 20% of methanolic solutions of pyridine and $AlCl_3$ (50 mg mL^{-1}), dissolving in MeOH to reach the final concetration of 1000 μg mL^{-1}. An aliquot of 300 μL of the solutin freshly prepared was transferred to flask of 10 mL, and 240 μL of acetic acid, 4 mL of the solution $AlCl_3$ in pirydine and methanol (20%) were added. After 30 min of reaction, the absorbance of the samples was measured at the wavelength of 420 nm. Values were expressed in milligrams of rutin equivalent per gram of sample (mg ER/g AM) [26–28]. The experiments were performed in triplicate.

2.5. Antioxidant activity determination (DPPH)

Antioxidant activity was determined using a mixture of 1.5 mL of a DPPH• ethanolic solution (6×10^{-5} mol L^{-1}) and 0.5 mL of each extract in different specific tests. Readings were taken on a UV–Vis molecular absorption spectrophotometer at 517 nm 30 min after the start of the reaction. As the determinations were made in triplicate, a control sample without antioxidant (only the radical) was used. A linear standard curve was constructed using ascorbic acid solutions (0.98–31.25 μg/mL) as standard. The decrease in optical density reading correlated with the control, establishing a percentage of DPPH discoloration as follows:

$$\%\text{inhibition of DPPH•radical} = [(Abs_{control} - Abs_{blanck})/Abs_{control}] \times 100$$

Abs – absorbance

In addition to the protection percentage was also calculated, the effective concentration to inhibit 50% of the radical (EC_{50}) [29,30].

2.6. Determination of the induction period

The B100 biodiesel samples in mixtures with bilberry extracts, oregano, basil and quercetin were analyzed on Metrohm's Rancimat model 743 equipment, according to the methodology described in EN 14112 and Petrotest's PetroOXY ASTM D7545 equipment [10,15,31,32]. The temperatures analyzed were in the range of 90–140 °C and the concentration of the mixture of the antioxidants ranged from 500 to 3000 ppm [15]. The oxidative stability analyzes were performed as soon as the extracts and quercetin were added to biodiesel. The ratio of bilberry, oregano, basil and quercetin extract for each of the six concentrations used was 1:1 relative to biodiesel mass, ie for binary mixtures, for example, it has been used 250 ppm of each antioxidant to obtain a total concentration of 500 ppm, thus same tendency has been followed for the other concentrations and also for the ternary and quaternary mixtures.

Table 1
Concentration of antioxidants used.

Samples	Additive concentration
B_{100}	Biodiesel without antioxidant
B_{100} + BOBE 500	Biodiesel + (extracts of bilberry + oregano + basil) 500 ppm
B_{100} + BOBE 1000	Biodiesel + (extracts of bilberry + oregano + basil) 1000 ppm
B_{100} + BOBE 1500	Biodiesel + (extracts of bilberry + oregano + basil) 1500 ppm
B_{100} + BOBE 2000	Biodiesel + (extracts of bilberry + oregano + basil) 2000 ppm
B_{100} + BOBE 2500	Biodiesel + (extracts of bilberry + oregano + basil) 2500 ppm
B_{100} + BOBE 3000	Biodiesel + (extracts of bilberry + oregano + basil) 3000 ppm
B_{100} + BOBEQC 500	Biodiesel + (extracts of bilberry + oregano + basil + quercetin) 500 ppm
B_{100} + BOBEQC 1000	Biodiesel + (extracts of bilberry + oregano + basil + quercetin) 1000 ppm
B_{100} + BOBEQC 1500	Biodiesel + (extracts of bilberry + oregano + basil + quercetin) 1500 ppm
B_{100} + BOBEQC 2000	Biodiesel + (extracts of bilberry + oregano + basil + quercetin) 2000 ppm
B_{100} + BOBEQC 2500	Biodiesel + (extracts of bilberry + oregano + basil + quercetin) 2500 ppm
B_{100} + BOBEQC 3000	Biodiesel + (extracts of bilberry + oregano + basil + quercetin) 3000 ppm
B_{100} + BEQC 500	Biodiesel + (extracts of bilberry + quercetin) 500 ppm
B_{100} + BEQC 1000	Biodiesel + (extracts of bilberry + quercetin) 1000 ppm
B_{100} + BEQC 1500	Biodiesel + (extracts of bilberry + quercetin) 1500 ppm
B_{100} + BEQC 2000	Biodiesel + (extracts of bilberry + quercetin) 2000 ppm
B_{100} + BEQC 2500	Biodiesel + (extracts of bilberry + quercetin) 2500 ppm
B_{100} + BEQC 3000	Biodiesel + (extracts of bilberry + quercetin) 3000 ppm
B_{100} + OEQC 500	Biodiesel + (extracts of oregano + quercetin) 500 ppm
B_{100} + OEQC 1000	Biodiesel + (extracts of oregano + quercetin) 1000 ppm
B_{100} + OEQC 1500	Biodiesel + (extracts of oregano + quercetin) 1500 ppm
B_{100} + OEQC 2000	Biodiesel + (extracts of oregano + quercetin) 2000 ppm
B_{100} + OEQC 2500	Biodiesel + (extracts of oregano + quercetin) 2500 ppm
B_{100} + OEQC 3000	Biodiesel + (extracts of oregano + quercetin) 3000 ppm
B_{100} + BAEQC 500	Biodiesel + (extracts de basil + quercetin) 500 ppm
B_{100} + BAEQC 1000	Biodiesel + (extracts de basil + quercetin) 1000 ppm
B_{100} + BAEQC 1500	Biodiesel + (extracts de basil + quercetin) 1500 ppm
B_{100} + BAEQC 2000	Biodiesel + (extracts de basil + quercetin) 2000 ppm
B_{100} + BAEQC 2500	Biodiesel + (extracts de basil + quercetin) 2500 ppm
B_{100} + BAEQC 3000	Biodiesel + (extracts de basil + quercetin) 3000 ppm

Table 1 shows the identification and concentration of the samples used.

3. Results and discussion

3.1. Characterization of oil and biodiesel by ^1H NMR

The signals found in the ^{1}H NMR spectra (Figure S1) of the formed products confirmed the conversion quite efficiently. Soybean oil (Figure S1a) contains triacylglycerides and these compounds are identified in the ^{1}H NMR spectrum by the signs at δ 4.0 (dd), 4.2 (dd) and 5.26 (m) that characterize hydrogens carbinolics of the esterified glycerol portion. These signals were not observed in the obtained product spectra (Figure S1b), indicating the disappearance of the starting material. In addition, the product can be identified by the presence of a singlet at δ 3.58 corresponding to the presence of methyl esters [33].

3.2. Total carotenoids and total flavonoids

The results of the quantitative levels of carotenoids and total flavonoids of the extracts of bilberry, basil and oregano are shown in (Table 2). The quantity of total carotenoid in the extracts ranged from 17.30 ± 2.63 to 23.02 ± 0.77 mg β-carotene/g AM.

Table 2
Quantitative content of bioactive compounds presents in the extracts of bilberry, oregano and basil.

Antioxidants	Total carotenoids mg β-carotene/g AM	Total Flavonoids mg ER/g AM
Bilberry extract (BE)	17.30 ± 2.63	5.78 ± 1.86
Oregano extract (OE)	23.02 ± 0.77	33.71 ± 0.95
Basil extract (BAE)	18.07 ± 1.19	6.56 ± 1.16

± standard deviation of the mean, n = 3.

While the quantification of the total flavonoid content of the extracts is the follow variation: 5.78 ± 1.86 to 33.71 ± 0.95 mg ER/g AM (Table 2). These results showed a linear behavior with coefficient higher than 0.9 and a positive correlation between carotenoid and total flavonoid content.

In general, the higher the content of bioactive compounds present the higher antioxidant activity. Oregano extract presented the highest quantitative levels of total carotenoid and flavonoid compounds followed by basil extract and bilberry extract.

The carotenoids and flavonoids present in the extracts of bilberry, basil and oregano can be able to act as neutralizers of free radicals and other reactive oxygen species, such as singlet oxygen, because they have conjugated double bonds in their structures. Such compounds act as biodegradable antioxidants and can be used in industry in general, especially in the biodiesel industry, to improve oxidative stability [15,30,31,32,33,34].

3.3. Antioxidant activity using the DPPH method

In the evaluation of antioxidant activity by the DPPH method, this radical reacts with the antioxidant, becoming its most stable reduced form. A common way of expressing the results in this assay is to calculate the amount of antioxidant capable of sequestering half of the DPPH• free radical present in the solution. This index is called EC_{50}. The lower the EC_{50} value presented, the less amount of extract and quercetin will be required to reduce 50% of the free radical DPPH•, and the greater its antioxidant activity [30].

In the present study, it has been evaluated the ability of bilberry, oregano and basil extracts to sequester the DPPH• radical in different concentrations, according to the antioxidant capacity of each extract.

The concentration range used for each extract (Bilberry, Oregano, Basil) with quercetin, as well as the linear correlation coefficient (R^2) obtained, are shown in Table 3. All extracts and the positive quercetin and ascorbic acid controls presented correlation coefficient above 0.9,

Table 3
Effective concentration to inhibit 50% of DPPH• radical. Concentration range used for antioxidant activity evaluation by DPPH• radical assay.

Antioxidants	Concentration μg/mL	EC_{50} μg/mL	CI 95%	R^2
Bilberry extract (BE)	250 – 1265.62	1026.0 ± 1.59	1009.0 – 1038.0	0.9408
Oregano extract (OE)	49.37 – 250	134.0 ± 2.36	128.2 – 139.9	0.9834
Basil extract (BAE)	250 – 1265.62	1911.0 ± 2.02	1905.0 – 1941.0	0.9851
Quercetin (QC)	2.8 – 1265.62	7.3 ± 0.07	7.09 – 7.45	0.9709
Ascorbic acid	0.98 – 31.25	22.72 ± 0.13	22.39 – 23.04	0.9946

± standard deviation of the mean, n = 3. 95% CI – 95% confidence interval. R^2 – coefficient of linear correlation.

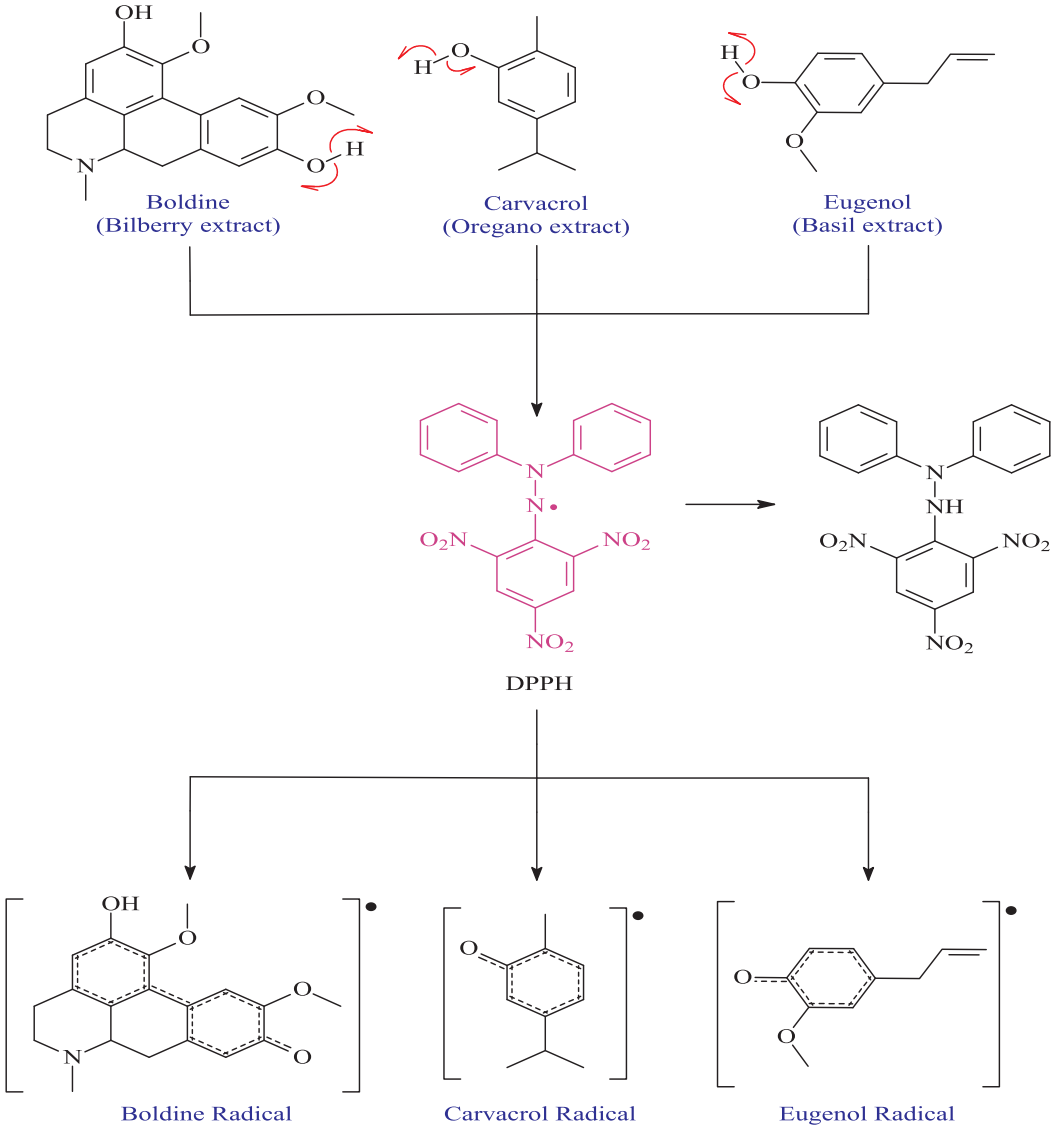

Fig. 1. Mechanism of action of boldine (bilberry extract), carvacrol (oregano extract), eugenol (basil extract) in free radical DPPH.

showing good linearity of the analyzes.

As observed in Table 3, all extracts used showed activity in sequestering the free radical DPPH•, however inferior to the ascorbic acid standard. The ethanolic extract of oregano showed the highest antioxidant activity among the extracts with (EC_{50} = 134.0 μg/mL), followed by the bilberry extract (EC_{50} = 1026.0 μg/mL) and basil extract (EC_{50} = 1911. 0 μg/mL).

Fig. 1 outlines a proposal to justify the antioxidant activity of bilberry, oregano and basil extracts. For this, one of the most abundant constituents present in each extract has been chosen according to the literature [5,10,15,37,38,39]. Boldine was chosen for bilberry extract, carvacrol for oregano extract and eugenol for basil extract. In this mechanism, the hydrogen from the active hydroxyl group of boldine, carvacrol and eugenol are abstracted by the free radical of the highly reactive DPPH molecule. The free radical formed is resonant stabilized, not owning the ability to initiate or propagate chain reactions, showing that the antioxidant structure is of fundamental importance for its activity [10].

In general, all extracts are rich in phenolic compounds and have intrinsic antioxidant activity due to phenolic hydrogens. It is noteworthy that phenolic compounds function as radical scavengers and sometimes as metal chelators, acting both in the early stage and in the propagation of the oxidative process [5,10]. The radicals generated from these constituents have high stability due to electron displacement

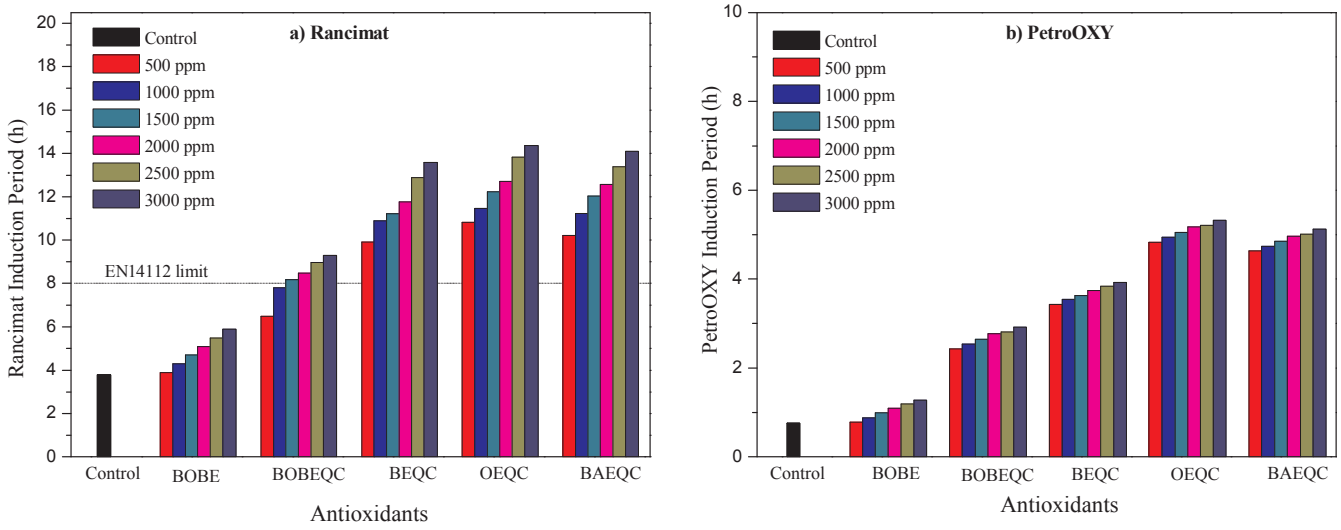

Fig. 2. Period of induction of the mixtures of antioxidants at 110 °C by the technique Rancimat and PetroOXY.

unpaired by the π electron cloud of the aromatic system and are therefore capable of reducing the DPPH• radical (Fig. 1) [10].

3.4. Oxidative stability at 110 °C

The study of binary, ternary and quaternary mixtures was carried out with the objective of evaluating the antioxidant synergism of the mixtures in the oxidative stability of soybean biodiesel. (Table S1 and S2), being the analyzes performed in triplicate. Comparative results were obtained by determination of the induction period using the Rancimat method (EN 14112) and PetroOXY (ASTM D7545), where it was evident that the isolated antioxidants used showed efficiency but did not reach the minimum limit of 8 h provided by the legislation [15,40,41].

The synergistic effect of the mixtures performed at different concentrations (500, 1000, 1500, 2000, 2500 and 3000 ppm) relative to the control sample (B100) at 110 °C resulted in increased oxidative stability of biodiesel (Fig. 2). The reason for working with this wider range of concentrations was also to analyze the prooxidant effects of the mixtures. The data do not suggest such prooxidant properties or at least the antioxidant effect of the mixtures is greater than the prooxidant. These results were of fundamental importance to confirm the efficiency of the additives as well as the combinations proposed in the prevention of degradation of biodiesel under tests of accelerated oxidation.

The presence of quercetin significantly improved the oxidative stability of the samples, being of fundamental importance in the control of the oxidative process of soybean biodiesel, since in many cases an antioxidant alone does not offer a safe protection against oxidation, therefore a mixture of antioxidants may, through the synergistic effect, present a more significant action [15,35,36,37].

Bilberry, oregano and basil extracts (BOBE) mixed with each other increased the oxidative stability of biodiesel in relation to the control sample but did not reach the minimum limit predicted at any concentration tested. When quercetin was added to the biodiesel with the extracts (BOBEQC), it was observed that it showed synergism potentiating the antioxidant effect and considerably increasing the oxidative stability of the samples, varying the induction period of 6.48–9.29 h, at concentrations of 500–3000 ppm (Fig. 2), respectively. Thus, the mixtures of leaf extracts of bilberry, oregano and basil with quercetin proved to be efficient in reducing biodiesel autoxidation.

The values obtained by the antioxidant synergism of the BEQC, OEQC and BAEQC binary mixtures presented significant results in all the concentrations tested, increasing the induction period above 3 times in the concentration of 3000 ppm in relation to the control sample, as well as in relation to the isolated samples [15]. The susceptibility of biodiesel to oxidation is well known and its additivation using potentiated synergistic mixtures of natural antioxidants may lead to economic gains, since the use of natural antioxidants is a viable alternative to retard the oxidative degradation process of biodiesel, since the natural compounds present in quercetin, bilberry extracts, oregano and basil do not cause damage to the environment being biodegradable, non-toxic and easily obtained.

The antioxidant activity was quantified and evaluated by a very important parameter called stabilization factor (SF), where IP_1 and IP_2 are the periods of induction with and without antioxidant, respectively (Eq. (1)). This parameter evaluated the synergistic or antagonistic effect of the isolated and mixed samples (Fig. 3).

$$SF = IP_1/IP_2 \quad (1)$$

According to the results obtained, it was observed that the stabilization factor was superior to 1 for all the samples indicating the existence of an antioxidant effect and not an antagonistic effect [42].

It is noteworthy that there are some considerable differences in antioxidant efficiency between the Rancimat and PetroOXY methods, as can be observed in (Fig. 3a and 3b), which also shows that the antioxidants used clearly showed a much better performance in the PetroOXY method (Fig. 3b) than in the method Rancimat (Fig. 3a). The PetroOXY method (ASTM D7545) is an advantageous tool in the evaluation of oxidative stability of antioxidants, since it presents shorter analysis times than the conventional Rancimat method (EN 14112), it is possible to use smaller amounts of sample and one better temperature control [15,22,43].

The binary mixtures (BEQC, OEQC and BAEQC) provided the greatest stabilization factors when compared to the isolated samples. These same trends were observed in both the Rancimat and PetroOXY accelerated techniques, proving the potential of the antioxidant synergism of the tested samples.

The ratio of the stabilizing factor (SFE) was also determined in order to express the sensitivity of the antioxidants to the test methods used, as shown in (Fig. 3c). In summary, it is important to highlight that the higher the ratio of the stabilizing factor, the greater the sensitivity of the antioxidants to the different conditions established by both accelerated oxidation methods (Rancimat and PetroOXY). Among the samples tested, the mixture of natural antioxidant quercetin with ethanolic basil extract (BAEQC) showed the highest antioxidant sensitivity, followed by the samples (OEQC, BOBEQC, BEQC, QC, OE, BAE and BE). The antioxidant mixtures (BOBEQC, BEQC, OEQC, BAEQC) were much more sensitive to the different test conditions than the

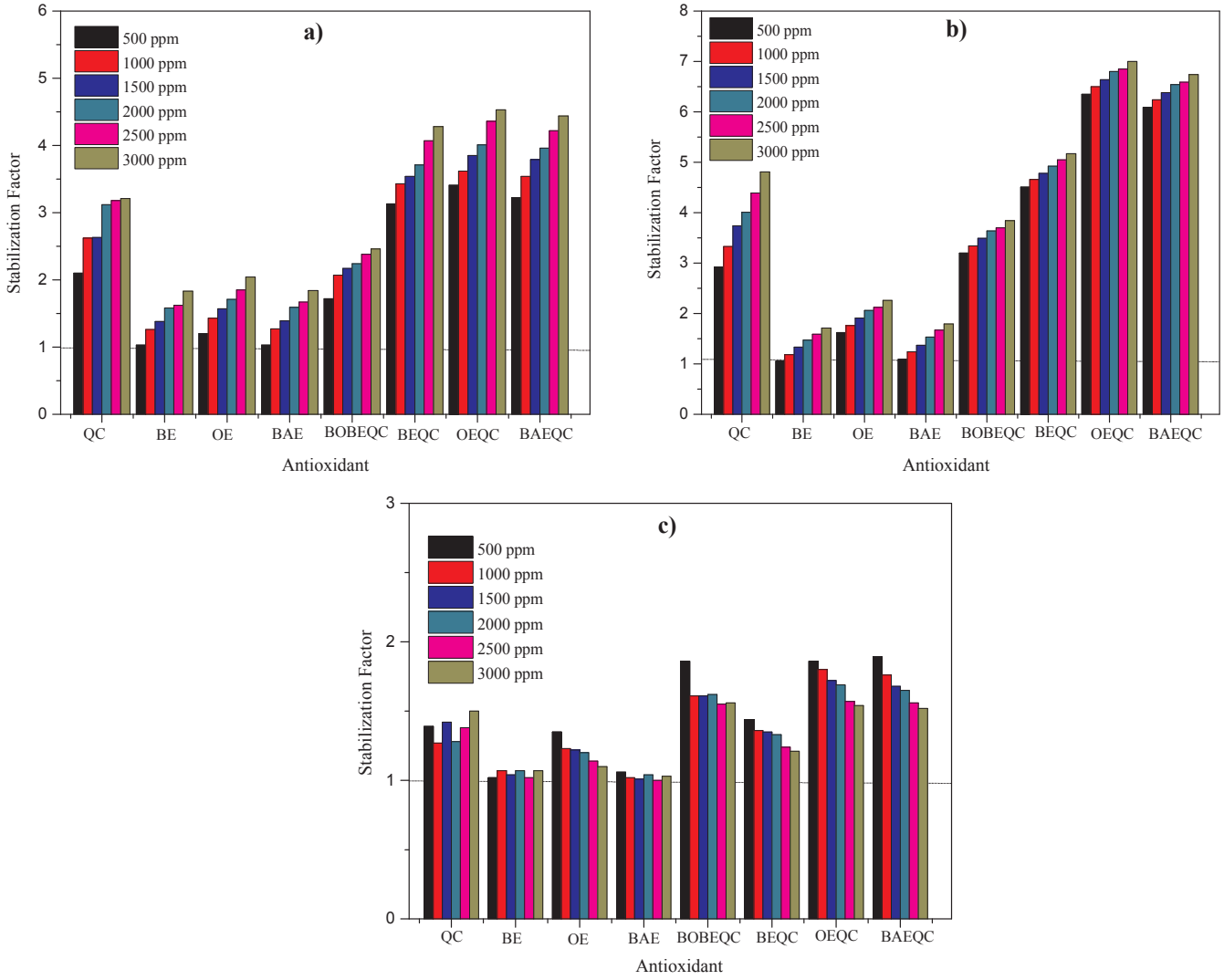

Fig. 3. Stabilization factor versus antioxidant concentration for Rancimat (a), PetroOXY (b) and (c) stabilization factor ratio vs. antioxidant concentration.

isolated samples (QC, BE, OE and BAE) (Fig. 3c). The different test conditions, as well as differences in the principles of measurement, temperatures and partial oxygen pressures of both methods may explain the variable result for both accelerated oxidation techniques [15,21,39,40,41].

In order to evaluate the oxidative stability or its susceptibility to oxidation, the study of the kinetics of delayed oxidation of biodiesel was carried out using binary, ternary and quaternary mixtures of antioxidants in concentrations of 500–3000 ppm.

The temperature is directly linked to the degradation processes, so that the increase in temperature favors the oxidation reactions. In order to evaluate the influence of temperature on the oxidation processes of soybean biodiesel samples with antioxidants, it was decided to make studies varying the temperature from 90 °C to 140 °C, besides showing the existence of synergism or not between the antioxidants tested (Fig. 4).

The addition of antioxidants clearly increased the induction period of the samples (BOBE, BOBEQC, BEQC, OEQC and BAEQC) in both techniques used (PetroOXY and Rancimat) for the measurement of the induction period. As described in the literature, the increase in temperature decreased the induction period of the samples [15,18,20,22,44].

The extract with the highest amount of flavonoids and total carotenoids was also the one that presented the highest antioxidant potential by quantitative analysis of total phenols, and antioxidant activity front DPPH radical [10,15], suggesting that the presence of these compounds can directly influence the antioxidant potential of this extract.

The results evidenced that the law of oxidation retardation speed of soybean biodiesel added with mixtures of different antioxidants (Eq. (2)) is in fact understood as pseudo – first order according to the experimental data obtained. Thus, a graph of the natural logarithm of the concentration of additives verses period of induction was constructed (Fig. 5). The curves presented a certain linearity, due to the good linear correlation coefficient, with R^2 being higher than 0.9109 and 0.9034 in the technique Rancimat and PetroOXY respectively (Table 4). These results allows us to assume that the kinetics of the first order describes the mechanism of degradation [12,15,44,45].

$$\ln[\text{concentration}] = -kt + \ln[\text{concentration}]_{\text{crit}} \quad (2)$$

According to the kinetic studies, the rate constant is temperature dependent. The results showed that as the temperature increases, the values of the reaction constants increase, that is, the higher the temperature, the higher the antioxidant consumption rate and the lower the biodiesel induction period [15,44,45].

The samples containing only plant extracts (BOBE) had the highest values of the rate constants (Table 4). The addition of quercetin in biodiesel with the extracts considerably reduced the values of the rate

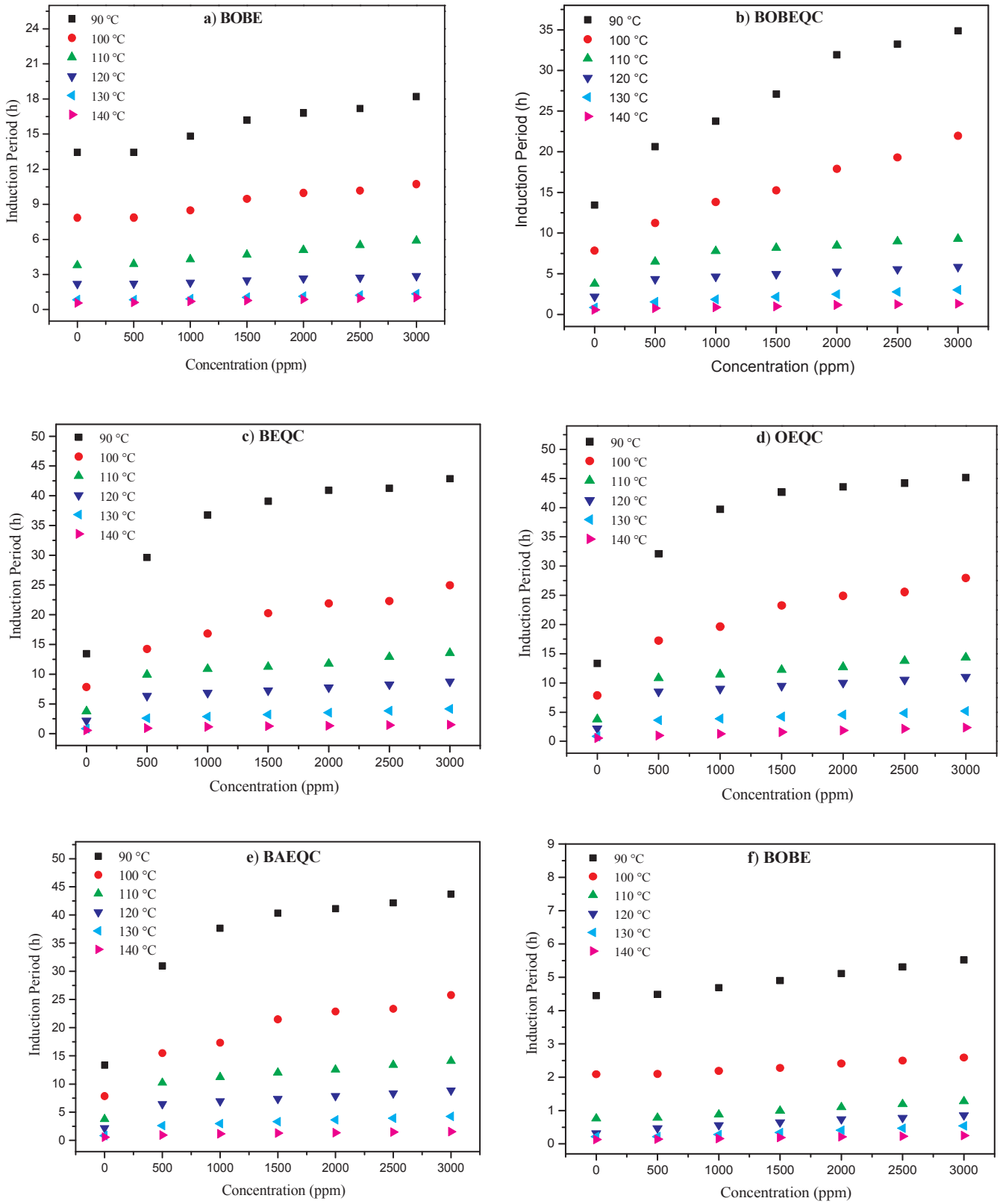

Fig. 4. Effect of concentration and temperature on the oxidative stability of biodiesel doped with mixtures of natural antioxidants by the Rancimat technique (a, b, c, d, e) and PetroOXY (f, g, h, i, j) in the 90–140 °C.

constants (k), it being evident that natural antioxidants have the capacity to promote the removal or inactivation of the free radicals formed during the initiation or propagation of the reaction, donating atoms of hydrogen to these molecules, disrupting the chain reaction [10,15,40].

For the mixture that only presented vegetal extracts (BOBE) the value of the rate constant increased more than ten times in the temperature of 90 to 140 °C by the technique Rancimat and more than nine

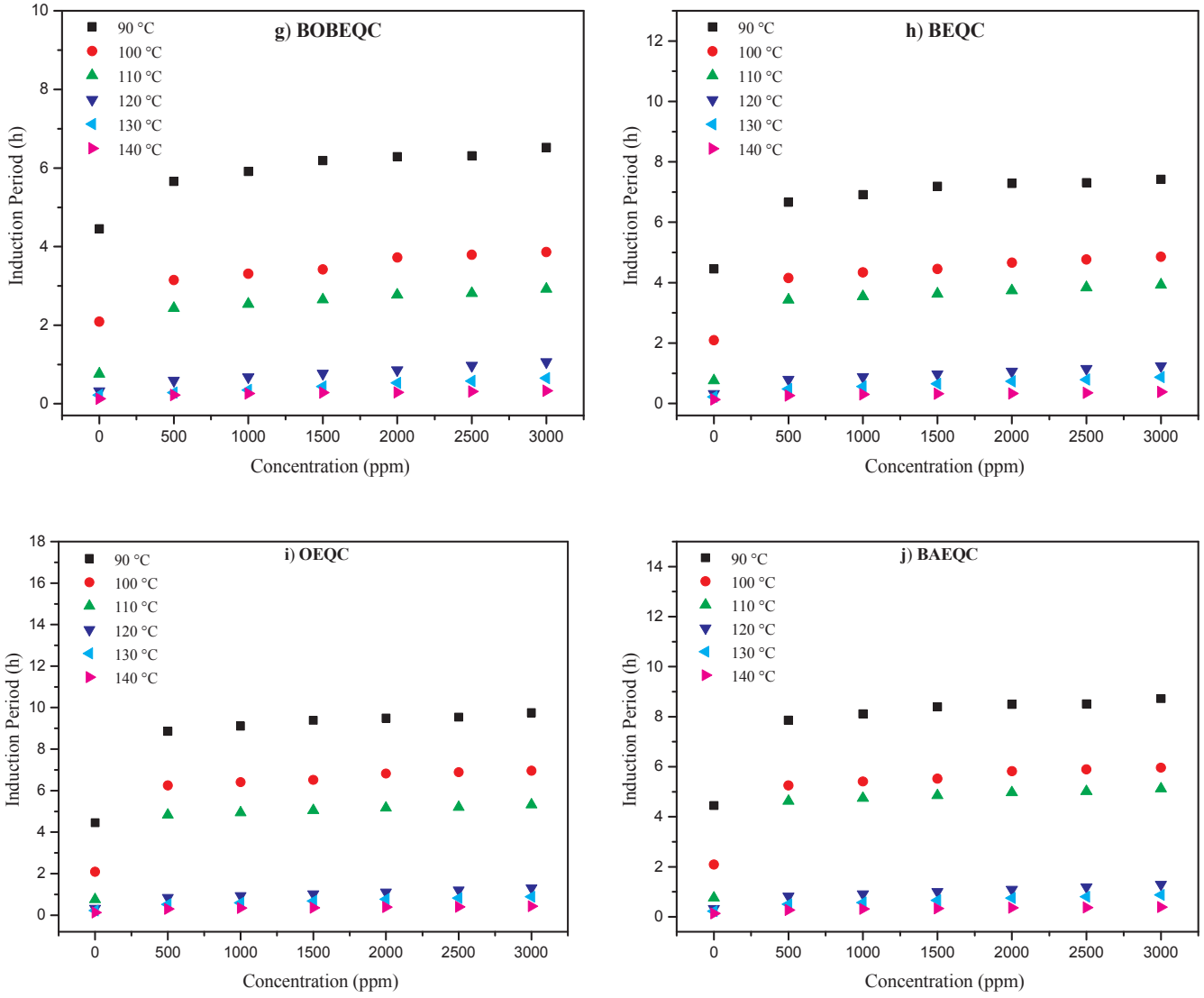

Fig. 4. (*continued*)

times in this same temperature range by the technique PetroOXY. The BOBEQC quaternary mix showed a considerable increase in the value of *k*, increasing about twenty-six times, at temperatures of 90 to 140 °C by the Rancimat technique and eight times in the PetroOXY technique [12,15,19,44,45].

The binary mixtures had an increase in the value of the rate constant somewhat similar to each temperature range, highlighting the BEQC and BAEQC mixtures which increased by about twenty-two and twenty-one times respectively the value of k in the temperature range of 90 to 140 °C by the Rancimat technique, while the OEQC sample increased about eight times in this same temperature range. The efficiency of the binary mixture between oregano extract and the natural antioxidant quercetin (OEQC) was the most significant among the studies carried out. It was also evident that there is a high sensitivity of all these antioxidants studied at high temperatures, since they are more susceptible to oxidative degradation at the higher temperature studied [15,44,45].

The low critical concentration values (a concentration that no longer inhibits oxidation of the methyl esters), show that mixtures with those antioxidants used provide action at low concentrations, (Table 4).

The activation energies (Ea) of biodiesel additived with antioxidant mixtures were determined from the Arrhenius equation to express the quantitative relationship between temperature, reaction constant and activation energy (Eq. (3)).

$$k = A\,e(-E_a/RT)$$

or

$$\ln\ k = \ln\ A - (E_a/R)(1/T) \quad (3)$$

From the Arrhenius equation, a graph of the natural logarithm of the rate constant (ln k) versus the inverse of the absolute temperature (1/T) was constructed by the Rancimat and PetroOXY technique, in which the intercept of the line is equal to lnA, A is designated the frequency factor of collisions between molecules, and the slope is designated as Ea/R, where R is the gas constant (8.314 J.mol^{-1} K^{-1}), (Fig. 6).

In figure (6a), it was observed that the ln *k* versus 1/T follows a good linear relation from the data obtained in the Rancimat technique over the whole temperature range, with the determination coefficients R^2, 0.9513, 0.9340, 0.9599, 0.9626, 0.9489, for the mixtures BOBE, BOBEQC, BEQC, OEQC and BAEQC, respectively. In the PetroOXY test, the R^2 correlation values obtained were 0.8120, 0.7040, 0.6898, 0.7577, 0.7328, following the same sequence of samples cited above.

From the slope of the line (-Ea/R) it was possible to obtain the results of the activation energy for the consumption of antioxidants being classified in the following ascending order OEQC (62.69 kJ/mol) < BOBE (65.01 kJ/mol) BAEQC (77.15 kJ/mol) < BEQC (77.73 kJ/mol) < BOBEQC (81.73 kJ/mol) (Fig. 6a) and BEQC (40.90) kJ/mol) < OEQC (41.82 kJ/mol) < BAEQC (42.32 kJ/mol) <

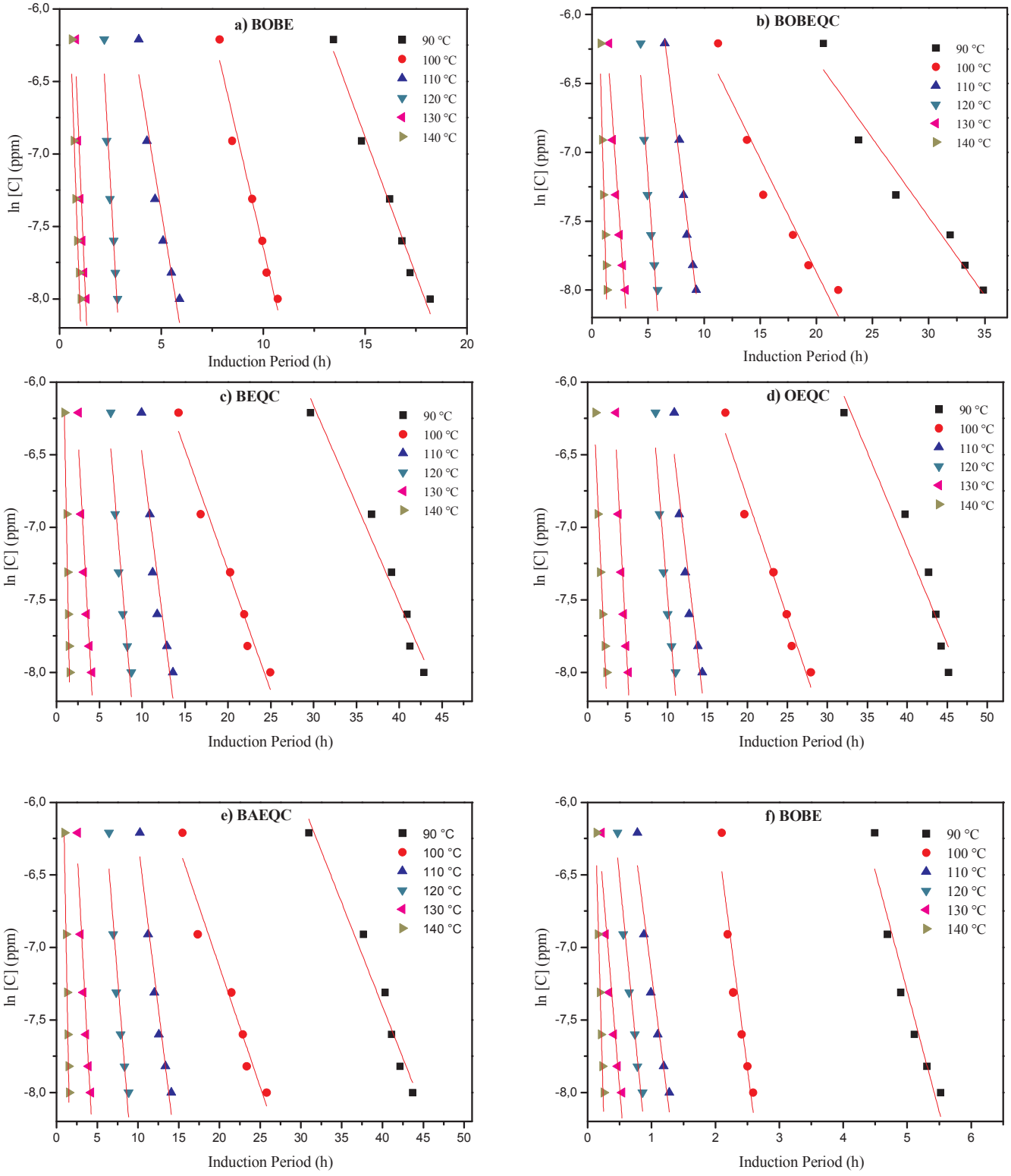

Fig. 5. Natural logarithm of the initial concentration of antioxidant mixtures versus induction time for Rancimat (a, b, c, d, e) and PetroOXY (f, g, h, i, j) tests in the range of 90–140 °C.

BOBEQC (44.48 kJ/mol) < BOBE (45.31 kJ /mol) (Fig. 6b). The activation energies of most reactions are between 40 and 400 kJ/mol. Thus, the activation energies found for the oxidation retardation reaction of soybean biodiesel additived with different antioxidant mixtures are consistent with the literature [15,18,45,46,47].

The thermodynamic parameters were also calculated, for this purpose, the equation derived from the theory with activated complex was used (Eq. (4)).

$$k = (k_B/h)T\, e^{\Delta S^*/R}\, e^{-(\Delta H^*/RT)}$$

ou:

$$\ln(k/T) = (-\Delta H^*/R)(1/T) + \{\ln(k_B/h) + (\Delta S^*/R)\} \quad (4)$$

where *kb* is Boltzmann's constant ($1.380658 \times 10^{-23}\,J\,K^{-1}$), *h* is

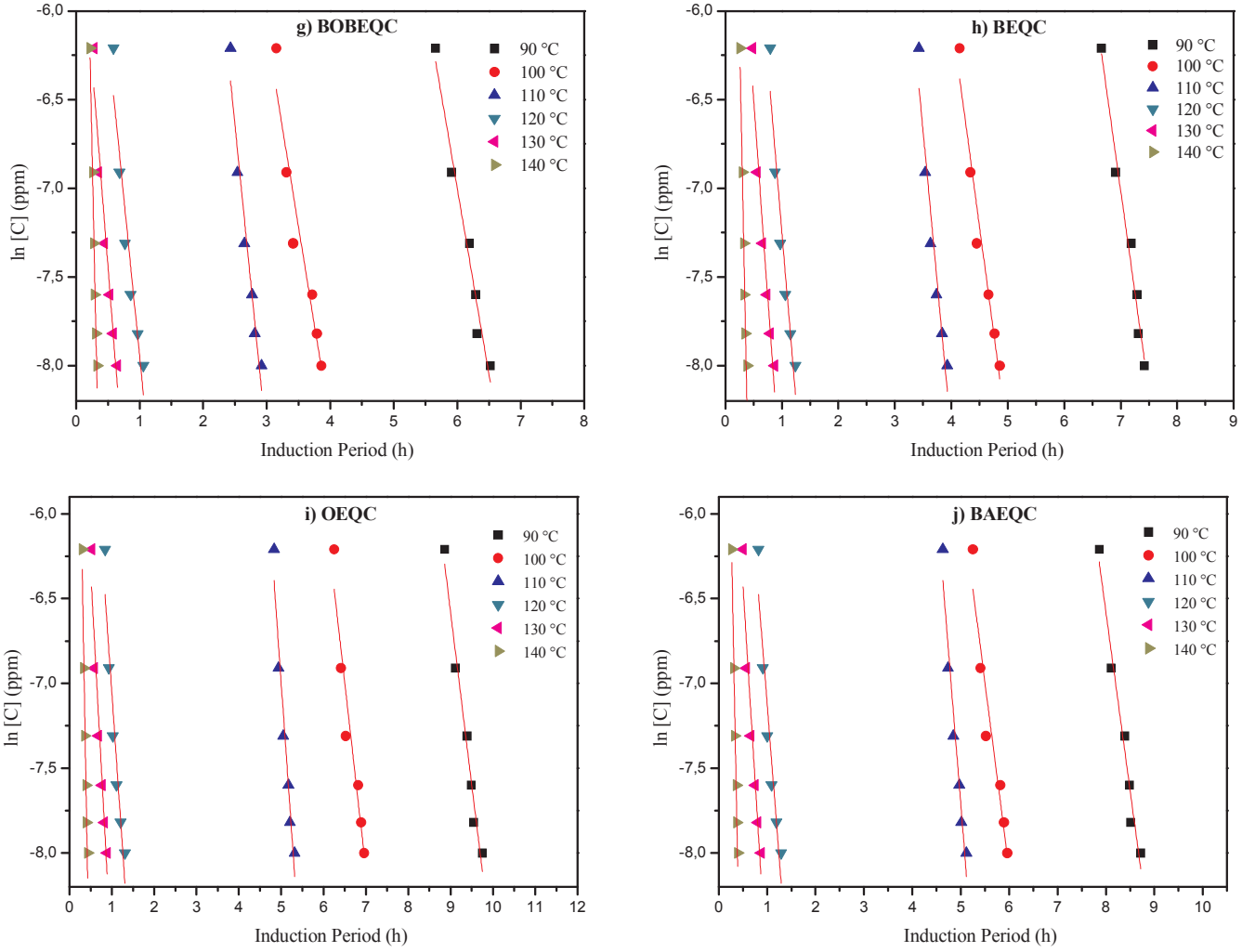

Fig. 5. (*continued*)

Planck's constant ($6.6260755 \times 10^{-34}$ J s). A graph ln *(k/T)* vs. *1/T* is constructed, in which the angular coefficient provided the enthalpy value of activation and the intercession of the line to the activation entropy (Fig. 7). The activation enthalpy (ΔH*) and activation entropy values (ΔS*) allowed the calculation of the activation free energy (ΔG*) by the fundamental equation of thermodynamics. (Eq. (5)). The results of the thermodynamic parameters are described in (Table 5).

$$\Delta G* = \Delta H* - T\Delta S* \quad (5)$$

Regarding the thermodynamic parameters, it was observed that the results obtained from the determination coefficient (R^2) were more significant in the Rancimat technique than in the PetroOXY technique, as evidenced in (Table 5).

It was observed that all the samples presented negative values of activation entropy (ΔS*), which can be associated to the formation of the species in the activated state, where the reactants bind to form complexes and decrease the number of free species in the medium. It is

Table 4
Rate constant k (h^{-1}), critical concentration of Ccr (ppm) and coefficient of determination (R^2) for the consumption of selected antioxidant mixtures in the 90 – 140 °C range for Rancimat[a] and PetroOXY[b] tests.

T	BOBE			BOBEQC			BEQC			OEQC			BAEQC		
	k	Ccr	R^2	k	Ccr	R^2	k	Ccr	R^2	k	Ccr	R^2	k	Ccr	R^2
90 °C[a]	0.3814	3.1899	0.9784	0.114	5.3974	0.944	0.136	8.0045	0.9581	0.1323	6.3598	0.9252	0.1425	5.529	0.9589
100 °C[a]	0.6042	50.0028	0.9624	0.1641	98.4944	0.9164	0.1662	52.9845	0.9589	0.1636	34.124	0.9589	0.1655	45.6042	0.9495
110 °C[a]	0.855	22.874	0.9181	0.6574	6.8209	0.981	0.3974	10.9134	0.9921	0.3593	4.1371	0.9555	0.337	17.1158	0.977
120 °C[a]	2.5316	2.4109	0.93	1.1387	4.4817	0.9247	0.7125	6.9587	0.9109	0.68	1.9542	0.9191	0.7125	6.4883	0.9109
130 °C[a]	3.3684	40.4473	0.9117	1.159	103.5443	0.9338	1.0844	38.8613	0.9149	1.0844	13.0658	0.9149	1.0598	38.4747	0.935
140 °C[a]	4.0703	58.557	0.9193	2.9769	64.7154	0.9312	3.0602	30.5694	0.9893	1.2256	44.7012	0.9325	3.0602	28.5027	0.9893
90 °C[b]	1.6567	2.6644	0.9166	2.1035	0.0036	0.9654	2.273	0.0001	0.9737	2.0371	0.0001	0.9691	2.1035	0.0001	0.9654
100 °C[b]	3.3897	1.8965	0.9077	2.2115	1.682	0.9131	2.3842	0.03	0.9499	2.2115	0.0006	0.9131	2.2115	0.0057	0.9131
110 C[b]	3.3985	43.816	0.9321	3.5629	9.5831	0.9478	3.4207	0.005	0.9263	3.5629	0.0002	0.9478	3.5629	0.0004	0.9478
120 °C[b]	4.4769	72.2404	0.9548	3.5914	78.2571	0.9034	3.8	31.5004	0.9181	3.6255	30.5694	0.9037	3.6255	33.1154	0.9037
130 °C[b]	5.3109	202.35	0.9047	4.5708	172.4315	0.9361	4.4311	72.9665	0.9378	4.5708	57.3974	0.9361	4.5708	63.434	0.9361
140 °C[b]	15.419	71.521	0.9351	16.933	12.6797	0.9746	15.66	9.39	0.9443	14.0278	8.3311	0.9629	14.9311	9.5831	0.982

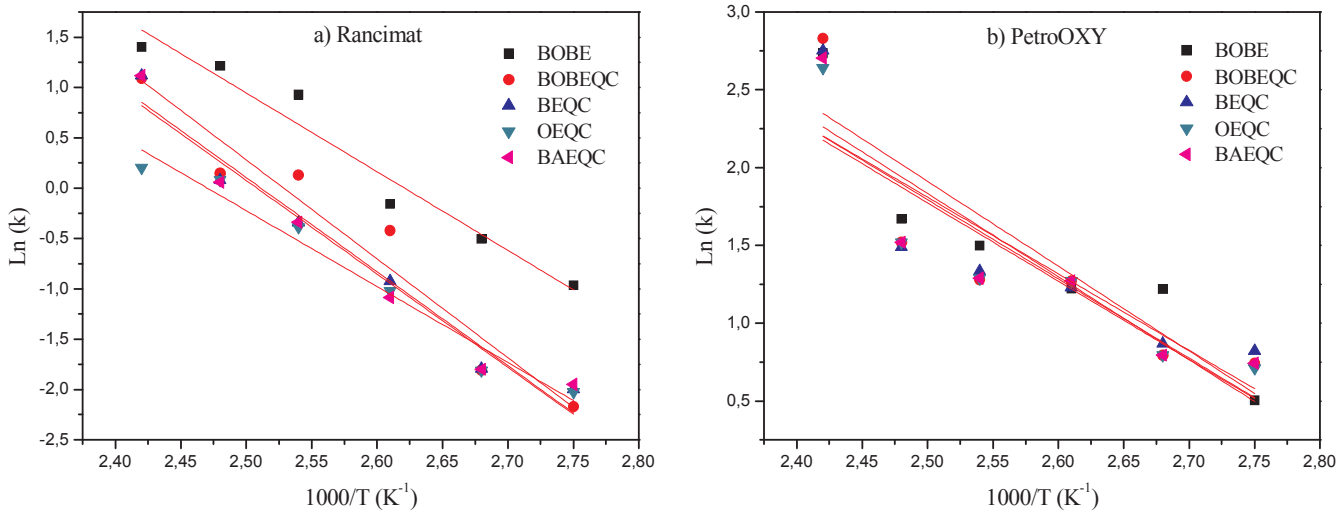

Fig. 6. Temperature dependence of k for the antioxidants consumption for (a) Rancimat and (b) PetroOXY tests.

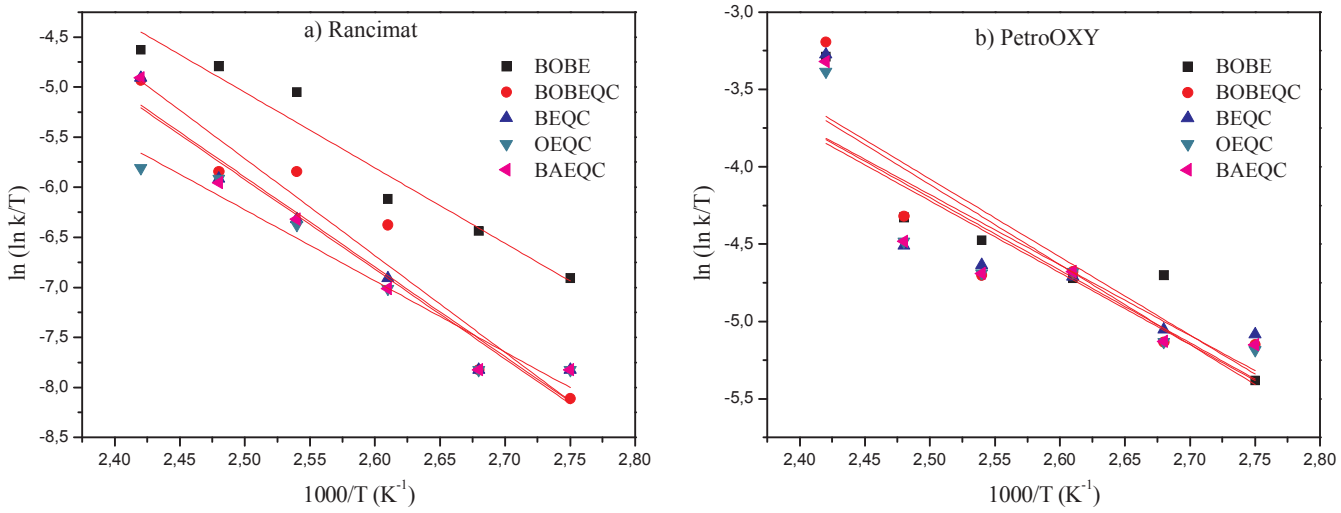

Fig. 7. Temperature dependence of the ln k/T to biodiesel using (a) Rancimat and (b) PetroOXY tests.

Table 5
Values of enthalpy (ΔH*), entropy (ΔS*), activation free energy ((ΔG*) and determination coefficient (R^2) of soybean biodiesel added with different antioxidant mixtures using Rancimat and PetroOXY techniques.

B_{100} + Antioxidants	ΔH* (kJ mol^{-1})	ΔS* (J mol^{-1} K^{-1})	R^2	ΔG* at 25 °C (kJ mol^{-1})
BOBE[a]	65.01	−95.19	0.9513	93.39
BOBEQC[a]	81.73	−58.94	0.9340	99.30
BEQC[a]	77.73	−70.42	0.9599	98.72
OEQC[a]	62.69	−110.74	0.9626	95.71
BAEQC[a]	77.15	−72.00	0.9489	98.62
BOBE[b]	41.98	−194.63	0.7877	100.01
BOBEQC[b]	42.98	−192.38	0.7292	100.34
BEQC[b]	37.83	−205.77	0.6561	99.18
OEQC[b]	38.58	−204.19	0.7248	99.46
BAEQC[b]	38.99	−203.03	0.6965	99.52

important to note that the more negative the activation entropy (ΔS*), more species of activated complexes are being formed and consequently the greater the reactivity of the médium [15,43].

The enthalpy of activation (ΔH*) represents the energy consumed by the reactions that occur during the oxidation, that is, the higher this value, the less reactive the medium becomes.

The positive values of free energy (ΔG*) at 25 °C show that the oxidation of soybean biodiesel in the presence of mixtures with different antioxidants is not spontaneous, ie, the reaction is occurring inversely, in the sense of inhibiting oxidation, proving the antioxidant character of the binary mixtures (BEQC, OEQC and BAEQC), ternary (BOBE) and quaternary (BAEQC) [15,43,48].

3.5. Shelf life of antioxidant mixtures at 25 °C

In order to verify the temperature dependence with the induction period, graphs of ln of the induction period versus the temperature (°C) were constructed for soybean biodiesel additived with antioxidant mixtures (BOBE, BOBEQC, BEQC, OEQC and BAEQC) at different concentrations (500, 1000, 1500, 2000, 2500 and 3000 ppm), also using Rancimat and PetroOXY techniques (Fig. 8). It is important to note that both accelerated oxidation methods have very different aging conditions. In the Rancimat method, the test conditions are performed at atmospheric pressure, where oxidative stability is determined by increasing conductivity, and only highly volatile oxidation products are detected. Nonvolatile oxidation products such as gums remain in the sample and the results obtained using this method provide an incomplete analysis of the oxidative stability of the sample. In PetroOXY method there is one variable a plus the pressure, in fact this method detects the result directly of the pressure drop of the oxidation process,

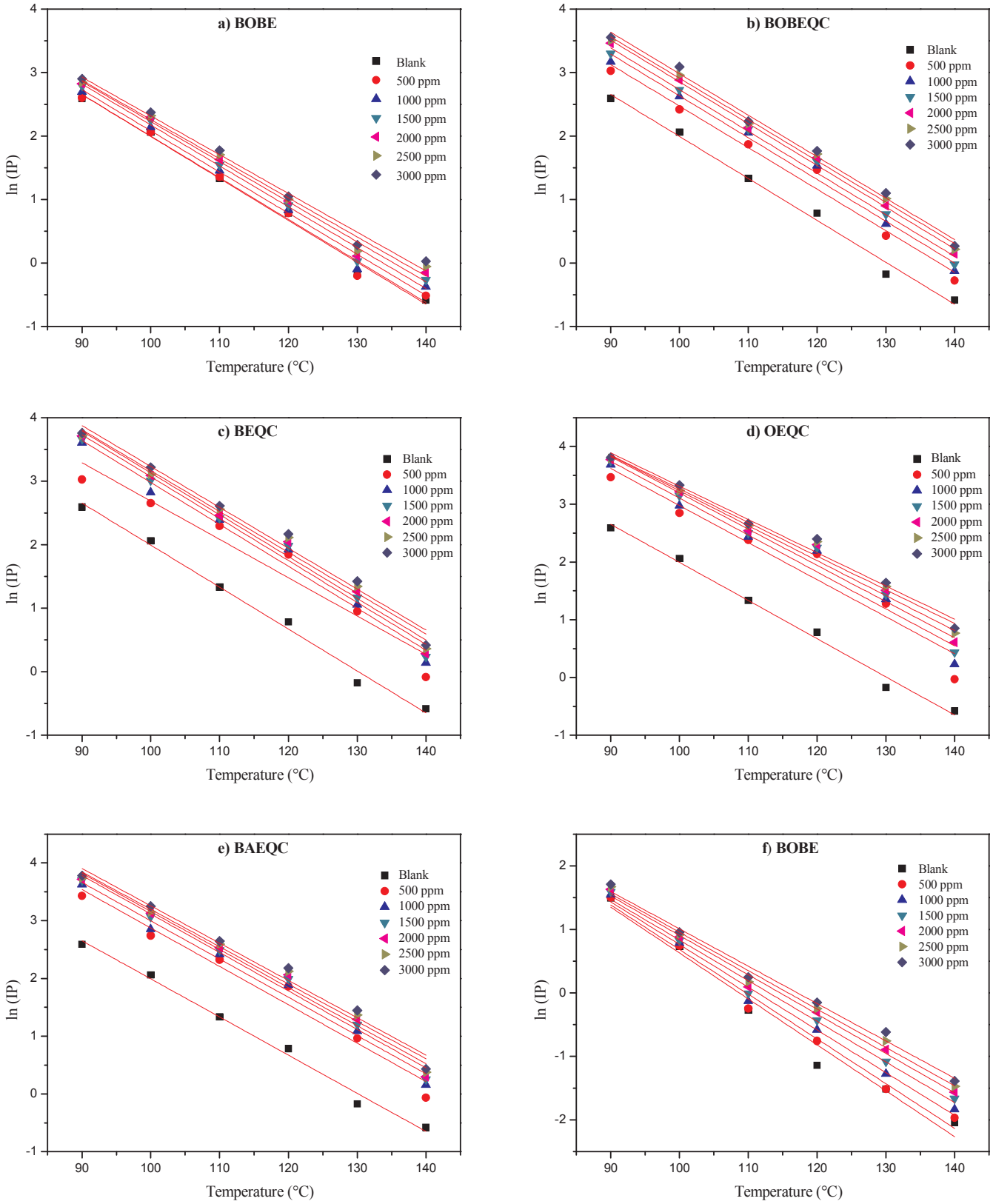

Fig. 8. Natural logarithm of the induction period as a function of temperature (90–140 °C) for biodiesel containing different mixtures of antioxidants using the techniques Rancimat (a, b, c, d, e) and PetroOXY (f, g, h, i, j).

accelerated by heat and oxygen pressure. Unlike EN14112, proponents of the methodology argue that PetroOXY includes all oxidation products, volatile and non-volatile, providing a complete analysis of oxidation sample stability [10,15].

The results were obtained in hours and months and showed good linear correlation with linear regression coefficients (R^2) varying 0.9433–0.9317 by the Rancimat and PetroOXY techniques respectively (Table 6). If the mechanism of consumption of antioxidants in biodiesel

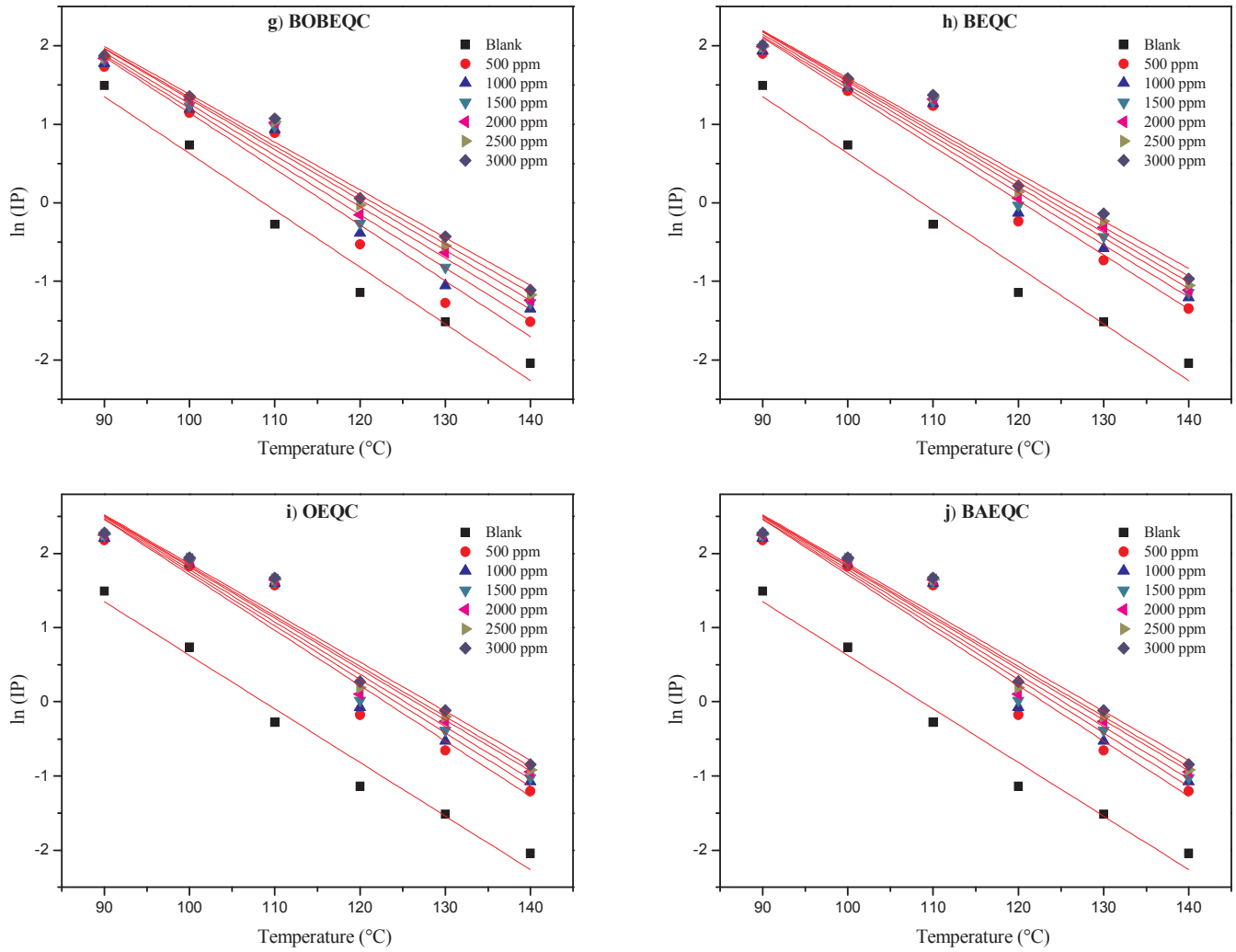

Fig. 8. (*continued*)

does not change, the extrapolation of this line provides the period of induction of biodiesel added with mixtures of antioxidants at any temperature, thus the temperature used was 25 °C [15,43,45].

It is important to note that an increase of 10 °C dramatically reduced the induction period of the samples. Another factor that also interferes in the induction period and deserves attention is the nature and the concentration of the antioxidant used.

According to the results presented in Table 6, the largest values of shelf life were obtained for the binary samples (BEQCE, OEQC and BAEQC). Among these samples, the highest shelf life was obtained for the sample B100 + BAEQC 1500, presenting an estimated shelf life of 3323.48 h (4.61 months) by the Rancimat technique at a temperature of 25 °C. For the PetroOXY test, the longest estimated shelf life was obtained by the sample B100 + OEQC 500 (1500.51 h 2.08 months), confirming that the mixtures of vegetable extracts with the natural antioxidant quercetin showed synergism, providing significant increases in the induction period of these samples.

The lowest shelf life results were obtained by ternary mixtures containing only plant extracts as antioxidants (BOBE), proving the need to add quercetin (additive) in these samples in order to increase their shelf life. The quaternary mixture (BOBEQC) showed an increase in the induction period in relation to the control sample, with the highest increase obtained in the concentration of 3000 ppm with a useful self life of (2623.52 h, 3.64 months) in the Rancimat technique. In the PetroOXY test the the longest estimated shelf life was obtained by the sample B100 + BOBEQC 500 with induction period 653.42 h which is equivalent to 0.91 months.

Another important point was observed in the PetroOXY technique, where in all cases the longest shelf life was obtained at the lowest antioxidant concentration (500 ppm). This leads us to believe that at concentrations above 500 ppm the shelf life is independent of the antioxidant concentration but is dependent on the type of antioxidant used.

Thus according to the results obtained in Table 4 and Table 6 the useful shelf life in descending order of these samples was the following: BAEQC > BEQC > OEQC > BOBEQC > BOBE by the results obtained in the technique Rancimat and OEQC > BAEQC > BEQC > BAEQC > BOBE by the results obtained in the PetroOXY technique, the chemical environment might differ in both cases and thus might affect the rate of aging and the prediction of shelf life.

4. Conclusion

The study of oxidative stability through mixtures of antioxidants at different temperatures and concentrations using the Rancimat and PetroOXY accelerated techniques, showed to be efficient in the protection of biodiesel, being observed the prevalence of a synergism among antioxidants, evidenced by the increase of the period of induction of the samples analyzed.

The kinetic and thermodynamic parameters of the binary, ternary and quaternary mixtures of plant extracts with the natural antioxidant quercetin showed a kinetics of delayed oxidation of pseudo - first order

Table 6
Useful life at 25 °C of different mixtures of antioxidants by Rancimat and PetroOXY techniques.

Samples	Rancimat (a)			PetroOXY (b)		
Induction time	Hours	Months	R^2	Hours	Months	R^2
B_{100}	1034.24	1.42	0.9922	422.15	0.59	0.9766
B_{100} + BOBE 500	997.14	1.38	0.9890	386.11	0.54	0.9880
B_{100} + BOBE 1000	1027.28	1.43	0.9891	329.90	0.46	0.9913
B_{100} + BOBE 1500	1096.91	1.53	0.9886	277.80	0.38	0.9917
B_{100} + BOBE 2000	1049.68	1.46	0.9890	256.62	0.36	0.9921
B_{100} + BOBE 2500	972.11	1.33	0.9880	240.30	0.33	0.9914
B_{100} + BOBE 3000	981.99	1.36	0.9887	225.02	0.31	0.9890
B_{100} + BOBEQC 500	1589.00	2.21	0.9828	653.42	0.91	0.9569
B_{100} + BOBEQC 1000	1932.68	2.68	0.9885	531.39	0.74	0.9633
B_{100} + BOBEQC 1500	2100,47	2.92	0.9925	472.86	0.66	0.9724
B_{100} + BOBEQC 2000	2387.49	3.31	0.9963	453.91	0.63	0.9772
B_{100} + BOBEQC 2500	2406.66	3.34	0.9955	405.87	0.56	0.9810
B_{100} + BOBEQC 3000	2623.52	3.64	0.9930	379.98	0.53	0.9821
B_{100} + BEQC 500	2213.71	3.07	0.9661	723.36	1.00	0.9563
B_{100} + BEQC 1000	2770.27	3.85	0.9815	619.97	0.86	0,9595
B_{100} + BEQC 1500	3031.80	4.21	0.9865	572.67	0.79	0.9636
B_{100} + BEQC 2000	3175.98	4.41	0.9845	552.05	0.77	0.9639
B_{100} + BEQC 2500	2936.80	4.08	0.9822	507.60	0.70	0.9639
B_{100} + BEQC 3000	3152.39	4.38	0.9815	455.58	0.63	0.9643
B_{100} + OEQC 500	2408.49	3.34	0.9365	1500.51	2.08	0.9386
B_{100} + OEQC 1000	2692.65	3.73	0.9615	1288.94	1.79	0.9418
B_{100} + OEQC 1500	2695.24	3.74	0.9737	1179.28	1.64	0.9466
B_{100} + OEQC 2000	2370.27	3.29	0.9786	1061.89	1.47	0.9480
B_{100} + OEQC 2500	2067.00	2.87	0.9828	1006.36	1.40	0.9521
B_{100} + OEQC 3000	2054.42	2.85	0.9836	921.87	1.28	0.9547
B_{100} + BAEQC 500	2587.36	3.59	0.9714	1214.32	1.69	0.9362
B_{100} + BAEQC 1000	2881.28	4.00	0.9850	1035.12	1.44	0.9393
B_{100} + BAEQC 1500	3323.48	4.61	0,9871	946.73	1.31	0.9435
B_{100} + BAEQC 2000	3308.24	4.60	0.9842	852.14	1.18	0.9446
B_{100} + BAEQC 2500	3115.25	4.33	0.9826	802.83	1.11	0.9478
B_{100} + BAEQC 3000	3295.66	4.58	0.9815	755.14	1.05	0.9498

due to the good linear correlation presented. The values obtained for enthalpy, entropy and free energy of Gibbs in the activated state indicated an endothermic, non-spontaneous process, showing that the use of these antioxidant blends as a way to retard or inhibit oxidation was quite effective.

The results obtained in this study showed that the extracts of bilberry, oregano and basil presented a very promising potential in synergisms with the natural antioxidant quercetin, being an economically and environmentally interesting alternative, because it comes from a renewable source, low cost and ecologically correct.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fuel.2019.116235.

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