Food Chemistry 115 (2009) 740–744

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

A kinetic model for evaluation of the antioxidant activity of several rosemary extracts

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ARTICLE INFO

Article history:
Received 20 May 2008
Received in revised form 9 October 2008
Accepted 9 December 2008

Keywords:
Rosemary extracts
DPPH·
β-Carotene bleaching test
Emulsion
Reaction rate

ABSTRACT

The antioxidant properties of phenolic diterpenes in several rosemary (*Rosmarinus officinalis*) extract formulations were investigated. Carnosic acid, carnosol and methyl carnosate were identified and quantified by high-performance liquid chromatography (HPLC) and carnosic acid was found to be the most abundant phenolic compound in the rosemary extracts investigated. To describe the antioxidant properties the free radical scavenging activity using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·) test and the antioxidant activity coefficient in the β-carotene–linoleic acid emulsion system, C_{AA}, were assayed. In both assays, extracts with higher total phenolic contents were superior in antioxidant activity. To interpret the antioxidant properties the kinetic approach was used. A power function was found to best represent the time dependence of the content of free radicals scavenged, as well as the content of β-carotene bleached in the presence of rosemary extracts. The rate of free radical scavenging, R_S, and the rate of β-carotene bleaching, R_B, were estimated and suggested as new parameters to describe the antioxidant activity of rosemary extracts. The kinetic data were interpreted in terms of differences in the reactivity of antioxidant compounds.

1. Introduction

The oxidation process of polyunsaturated fatty acids through a free radical chain reaction, so-called autooxidation, has received much attention due to its involvement in food spoilage and the relevance of lipid peroxidation *in vivo*. The introduction of an antioxidant changes the kinetics of this process. The extent of these changes depends on the type of antioxidant, its target molecules and the surrounding conditions. An analytical approach to predicting antioxidant activity that involves a kinetic model should provide a deeper understanding of the mechanisms controlling the process studied.

Among plants reported to have antioxidative activity, rosemary (*Rosmarinus officinalis*) in its ground form or as an extract is widely used in many food applications. A number of phenolic compounds that vary in structure, polarity and mutual interactions have been identified to be responsible for the antioxidative properties of rosemary extracts. Variation in extraction methods gives extracts of different chemical composition. The published data attributed the main antioxidant effect of rosemary extracts to phenolic diterpenes such as carnosol, carnosoic acid and methyl carnosate, and phenolic acids such as rosmarinic and caffeic acids (Cuvelier, Richard, & Berset, 1996; Frankel, Huang, Aeschbach, & Prior, 1996; Huang, Frankel, Schwarz, Aeschbach, & German, 1996; Richheimer, Bernart, King, Kent, & Bailey, 1996).

Many investigators have studied the free radical scavenging activity to better understand the antioxidant properties of rosemary extracts. Recently the capability of rosemary extracts to scavenge free radicals was investigated by Nogala-Kalucka et al. (2005), Almela, Sánchez-Muñoz, Fernández-López, Roca, and Rabe (2006), Yesil Celiktas, Bedir, and Vardan Sukan (2007), Moreno, Scheyer, Romano, and Vojnov (2006). Some investigators (Carvalho, Moura, Rosa, & Meireles, 2005; Cavero et al., 2005; Wellwood & Cole, 2004) also determined the effectiveness of rosemary extracts to interact with free radicals formed in an aqueous emulsion of linoleic acid with β-carotene.

Usually such determinations are based on a fixed endpoint which may not consider the different kinetic behaviour of the antioxidants. Some investigators have proposed kinetic parameters that can provide more complete information about antioxidant behaviour (Perez-Jimenez & Saura-Calixto, 2008; Sanchez-Moreno, Larrauri, & Saura-Calixto, 1998) and suggested that the kinetics could be more important than the total antioxidant capacities determined at a fixed point (Goupy, Dufour, Loonis, & Dangles, 2003). As far as our literature survey could ascertain, an investigation where kinetic data were used as predictors of the antioxidant activity of rosemary extracts has not yet been performed.

In the present study kinetic data were evaluated to clarify the antioxidant properties of the phenolic diterpenes carnosic acid,

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doi:10.1016/j.foodchem.2008.12.033

carnosol and methyl carnosate in six selected crude rosemary extracts. With that end the rate of DPPH· scavenging and the rate of β-carotene bleaching were estimated. For comparison 2,6-di-*tert*-butyl-4-methylphenol (BHT) was used. Further, the results of antioxidant activity based on kinetic parameters were compared with those commonly used evaluated from measurements at a fixed point.

2. Materials and methods

2.1. Materials and reagents

Six different rosemary extract formulations obtained from rosemary leaves were supplied from the Vitiva Company (Markovci, Slovenia).

The following reagents were obtained from Merck (Darmstadt, Germany): chloroform, ethanol (96%) and sodium carbonate. β-carotene, DPPH· reagent, Folin–Ciocalteu reagent, linoleic acid (95%), and Tween 20 were purchased from Sigma (Sigma–Aldrich GmbH, Steinheim, Germany). All of the reagents were of analytical quality.

2.2. Determination of total phenolic content

The content of total phenolic compounds in the extracts was determined spectrometrically with Folin–Ciocalteu reagent using a slightly modified method by Gutfinger (1981). The reaction mixture contained 200 μL of rosemary extract diluted in 96% ethanol, 125 μL of freshly prepared Folin–Ciocalteu reagent and 125 μL of 20% sodium carbonate solution. The final mixture was diluted to 1 mL with deionized water. The mixture was kept in the dark at ambient temperature for 40 min to complete the reaction. Then the absorbance at 765 nm was measured on a model 8453 Hewlett Packard UV–VIS spectrophotometer (Hewlett Packard, Waldbronn, Germany) with a 1 cm cell. Results were expressed as mg of carnosic acid per g of extract. The test was conducted in triplicate and the results were averaged.

2.3. HPLC analysis

The content of carnosic acid, carnosol and methyl carnosate in rosemary extracts was determined by high-performance liquid chromatography (HPLC). Analyses were performed using a Spectra-Physics pump, a model SpectraSYSTEM, p1500 V3.02, a Spectra-Physics UV–VIS detector (λ = 190–180 nm) with a 6 mm (9 μL) cuvette, a model SpectraFOCUS, a Rheodyne injector (20 μm), model 7725, software PC1000, V3.0, and a LiChrosorb RP-select B, 7 μm column. The mobile phase used was acetonitrile: water = 60:40 (v/v) + 0.5% H_3PO_4 in 1 mM EDTA at a flow rate of 2 mL/min ($\lambda_{detection}$ = 230 nm). The solvent was ethanol: methanol: isopropanol = 90:5:5, 0.5% H_3PO_4 in 1 mM EDTA. The analytical standard was carnosic acid, C1213-50 mg AG, AG Scientific, supplied by MoBiTec.

2.4. Free radical scavenging effectiveness

The free radical scavenging effectiveness of rosemary extracts was determined according to a slightly modified method of Brand-Williams, Cuvelier, and Berset (1995). A 100 μM solution of DPPH· radical in 96% ethanol was prepared. 2.9 mL of this solution was added to 0.1 mL solution of rosemary extract in 96% ethanol at concentrations ranging from 0.1 to 2.0 mg/mL, thus obtaining the desired final concentrations in the reaction mixture. The mixture was shaken vigorously and incubated at room temperature for 30 min. The absorbance at 517 nm was measured at different time intervals. Ethanol (96%) was used as a blank. The control solution consisted of 0.1 mL of 96% ethanol and 2.9 mL of DPPH· solution. Duplicate analyses were run for each extract.

2.5. Antioxidant activity in the β-carotene–linoleic acid emulsion system

The antioxidant activity of rosemary extracts in an aqueous emulsion system of linoleic acid and β-carotene was determined according to a slightly modified method of Moure et al. (2000). The β-carotene (2 mg) was mixed with 10 mL of chloroform. A 2 mL aliquot was put into a round-bottomed flask and 40 mg linoleic acid and 400 mg Tween 20 were added. Chloroform was removed using a rotavapor. After evaporation, 100 mL oxygenated distilled water was added and mixed well using a vortex mixer. Aliquots of 5 mL of this emulsion and 0.2 mL solution of rosemary extract in 96% ethanol were placed in tubes, capped and mixed thoroughly. The final concentration of rosemary extract in the emulsion was 0.04 mg/mL. As a control mixture, 0.2 mL of 96% ethanol and 5 mL of the above emulsion was used. The tubes were maintained at 50 °C in a water bath. The absorbance was measured at 470 nm, immediately after their preparation (t = 0 min) and at incubation times t = 20, 40, 60, 80, 100 and 120 min against the blank. The blank was prepared by adding 0.2 mL of 96% ethanol to an emulsion consisting of linoleic acid, Tween 20 and distilled water. Duplicate analyses were run for each extract.

3. Results and discussion

3.1. Determination of phenolic compounds in rosemary extracts

The content of total phenolics expressed as carnosic acid equivalents in the extracts determined according to the Folin–Ciocalteu assay and the results of the HPLC analysis are presented in Table 1. As we can see from this table, rosemary extracts differed in the content of total phenolics and also in the relative amounts of carnosic acid, carnosol and methyl carnosate. The most abundant compound found in the extracts was carnosic acid, ranging between 13.4% and 70.0%.

3.2. Kinetic studies

During lipid oxidation many free radical species are formed. The DPPH· radical has been widely used to evaluate the free radical scavenging capacity of antioxidants. The free radical is scavenged by an antioxidant that donates an electron or hydrogen atom to a radical and therefore a stable molecule is formed. The remaining level of DPPH· in the reaction medium was calculated using the following relation:

$$\%\ \text{of remaining DPPH}^{\cdot} = 100 \cdot [A_{s\,517\ \text{nm}(t=30)}/A_{c\,517\ \text{nm}}], \quad (1)$$

Table 1
The contents of total phenolic compounds (expressed as carnosic acid equivalents), carnosic acid, carnosol and methyl carnosate in rosemary extracts.

Rosemary extract formulation	Total phenolic content (mg/g)*	Carnosic acid (%)**	Carnosol (%)**	Methyl carnosate (%)**
No. 1	300 ± 1	15.6	3.2	1.5
No. 2	317 ± 8	19.7	5.1	0.7
No. 3	668 ± 4	40.7	9.7	1.9
No. 4	966 ± 4	70.0	3.7	1.9
No. 5	278 ± 5	13.4	4.2	0.5
No. 6	330 ± 7	18.8	6.4	0.8

* Average of three replicates ± standard deviation.
** The weight percentage of total phenolic content.

where $A_{s\,517\ nm(t=30)}$ is the absorbance of the sample measured at $t = 30$ min and $A_{c\,517\ nm}$ is the absorbance of the control. It was found that the % of remaining DPPH· linearly decreased with increased rosemary concentration to a certain point then levelled off. The effectiveness of rosemary extracts in scavenging free radicals was evaluated as the concentration of rosemary extracts in the reaction mixture that caused a decrease in the initial DPPH· concentration by 50%, defined as EC_{50}. A higher EC_{50} value indicates a weaker capability to scavenge DPPH· radicals. Table 2 presents the EC_{50} values for the investigated rosemary extracts and BHT. The investigated rosemary extract formulations, with the exception of extract formulation No. 5, showed stronger free radical scavenging effectiveness than BHT.

The effect of rosemary extracts and BHT on the kinetics of free radical scavenging for the investigated antioxidants is compared in Fig. 1. As shown, the values of $A_{c\,517\ nm} - A_{s\,517\ nm(t=x)}$ as a function of time are presented at a concentration of antioxidant in the reaction mixture amounting to 0.01 mg/mL. The value $A_{c\,517\ nm} - A_{s\,517\ nm(t=x)}$ refers to the content of DPPH· scavenged at $t = x$. As we can see in Fig. 1, in the presence of rosemary extracts a rapid initial decrease of DPPH· content is followed by slow subsequent disappearance of DPPH·. Antioxidants can deactivate (scavenge or quench) free radicals by two major mechanisms: by reduction via electron transfer or by hydrogen atom transfer that may also occur in parallel (Huang, Ou, & Prior, 2005). The end result is the same, regardless of the mechanism, but the kinetics differ (Prior, Wu, & Schaich, 2005). DPPH· scavenging is considered to be mainly based on electron transfer whilst hydrogen atom transfer is a marginal reaction pathway (Foti, Daquino, & Geraci, 2004). The contribution of a particular pathway depends on the species involved. The initial step includes the reactions of electron and/or H atom transfer from an antioxidant to the free radical. Different kinetic models were used to analyse the first rapid step (Goupy et al., 2003). As we can see in Fig. 1 there are significant differences between the slopes after the end of the initial fast step that do not rank in the same manner as the EC_{50} values do. These differences are related to the role of secondary slow reactions (dimerization or disproportionation) of the phenol-derived radicals initially formed. Saguy and Karel (1980) proposed some mathematical models that describe the dynamic behaviour of the system being analysed. The dependence of $A_{c\,517\ nm} - A_{s\,517\ nm(t=x)}$ on time in this investigation could be best expressed as a power function:

$$A_{c\,517\ nm} - A_{s\,517\ nm(t=x)} = a \cdot t^{b}, \tag{2}$$

where a and b are parameters that were obtained by non-linear regression analysis. The corresponding determination coefficients are presented in Table 2. The curves in Fig. 1 are plotted on the basis of the parameters in Eq. (2).

To quantify the kinetic behaviour of the investigated antioxidants, the rate of DPPH· scavenging, R_S, was estimated as the first derivative of the function represented by Eq. (2):

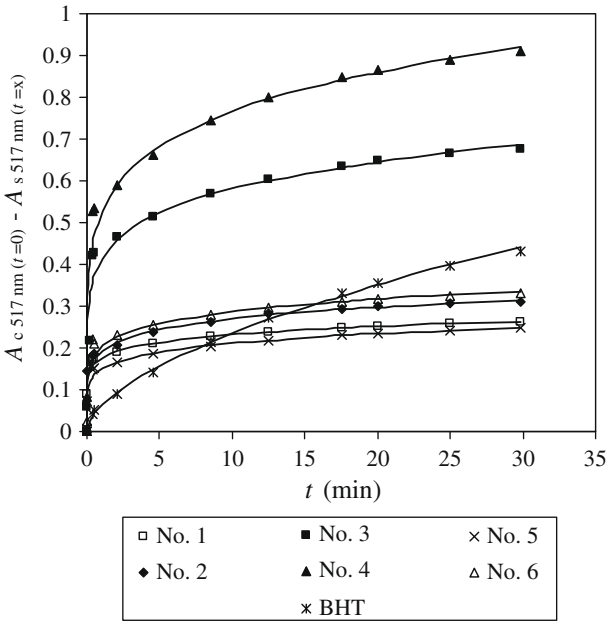

Fig. 1. The dependence of $A_{c\,517\ nm} - A_{s\,517\ nm(t=x)}$ on time of incubation at a rosemary extract concentration in the reaction mixture of 0.01 mg/mL. Symbols represent experimental values, curves are plotted according to the parameters from Eq. (2).

$$R_S = a \cdot b \cdot t^{b-1}. \tag{3}$$

The values of R_S calculated at $t = 0.25$ min (the initial rate), at $t = 10$ min (after the end of the initial rapid reaction step) and at $t = 30$ min (at the end of the observation period when the reactions are presumably completed) are given in Table 2. A decrease of R_S for the investigated antioxidants with time of incubation was observed. Table 2 presents the ratio between R_S at $t = 30$ and 10 min (γ). As we can see this ratio was considerably higher for BHT (the relative decrease of R_S with time is smaller) than it was for rosemary extracts. This means that the situation when the reaction is completed is reached later in the case of BHT. Assuming the EC_{50} and R_S values in Table 2, we can show as presented in Fig. 2 that the results determined at a fixed end point do not strictly relate to the results obtained from the kinetic data; BHT, despite its moderate free radical scavenging effectiveness (EC_{50}), at the initial stage of rapid reaction exhibited the lowest R_S value compared to rosemary extracts, whilst after the end of the initial rapid reaction step its R_S value was the highest. The correlation between EC_{50} and R_S calculated at $t = 10$ min gives an r^2 value of 0.722. This situation is probably a consequence of the fact that at $t = 30$ min (when the EC_{50} value is evaluated) in the presence of BHT, and also in the presence of rosemary extract formulation No. 4, the reaction might not have been

Table 2
The antioxidant concentration in the reaction mixture that caused a decrease in the initial DPPH· concentration of 50%, EC_{50}, the antioxidant activity coefficient, C_{AA}, the determination coefficients, r^2 determined by fitting data through Eqs. (2) and (7), the rates of free radical scavenging, R_S, at $t = 0.25$ and 10 min, the ratio between R_s at $t = 30$ and 10 min, γ, the rate of β-carotene bleaching, R_B, of rosemary extracts and the ratio between R_B at $t = 120$ and 10 min, χ.

Rosemary extract formulation	EC_{50} (mg/mL)	C_{AA}	r^2 (Eq. (2))	$R_S \times 10^{-3}$ at $t = 0.25$ min (min^{-1})	$R_S \times 10^{-3}$ at $t = 10$ min (min^{-1})	γ	r^2 (Eq. (7))	$R_B \times 10^{-3}$ (min^{-1})	χ
No. 1	0.0227 ± 0.0005	0.59 ± 0.01	0.986	70.3	2.7	0.38	0.985	3.0	0.2
No. 2	0.0188 ± 0.0001	0.74 ± 0.01	0.988	91.3	3.9	0.39	0.980	2.0	0.2
No. 3	0.0092 ± 0.0002	0.79 ± 0.02	0.955	200.7	8.7	0.39	0.992	1.5	0.2
No. 4	0.0066 ± 0.0001	0.86 ± 0.01	0.974	275.8	12.7	0.40	0.975	1.0	0.2
No. 5	0.0274 ± 0.0001	0.60 ± 0.01	0.989	72.5	3.1	0.39	0.987	3.0	0.2
No. 6	0.0193 ± 0.0005	0.64 ± 0.01	0.959	97.2	4.2	0.39	0.993	2.5	0.2
BHT	0.0129 ± 0.0005	0.96 ± 0.02	0.998	64.8	13.5	0.63	0.980*	0.10	4.5

* Average of two replicates ± standard deviation.
** r^2 was determined by fitting data through Eq. (9).

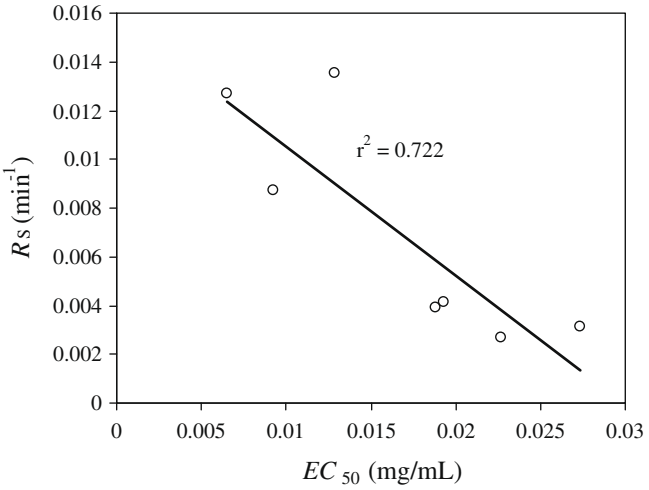

Fig. 2. The dependence of the rate of free radical scavenging, R_S, on antioxidant concentration in the reaction mixture that caused a decrease in the initial DPPH· concentration by 50%, EC_{50}.

complete, therefore the antioxidant activity expressed as the EC_{50} value in this case is underestimated.

Real food generally consists of multiple phases in which lipids and water coexist with some emulsifier, therefore an antioxidant assay using a heterogeneous system such as an emulsion is also required. Heat-induced oxidation of an aqueous emulsion system of linoleic acid was used to estimate the antioxidant activity of the investigated extracts. β-carotene has been used as a target molecule. With the aim of minimising side reactions that could give misleading results in this assay, a temperature that did not exceed 50 °C was chosen. Free radicals (peroxyl radicals) (ROO·) formed by oxidation of linoleic acid attack the β-carotene molecule and, as a result, it undergoes rapid degradation (decolorization). The extent of β-carotene bleaching can be slowed down by the presence of an antioxidant (AH) that donates a hydrogen atom to quench the free radical what results in antioxidant radical (A·) and lipid derivative (ROOH) formation. The antioxidants compete with β-carotene for peroxyl radicals and the following reactions should be considered to occur:

$$\mathrm{ROO}^{\cdot} + \beta - \text{carotene} \rightarrow \text{bleaching} \quad (4)$$

$$\mathrm{ROO}^{\cdot} + \mathrm{AH} \rightarrow \mathrm{A}^{\cdot} + \mathrm{ROOH}. \quad (5)$$

The antioxidant activity coefficient, C_{AA} was calculated according to the following relation:

$$C_{AA} = 1 - [A_{s\,470\ \mathrm{nm}(t=0)} - A_{s\,470\ \mathrm{nm}(t=80)}/A_{c\,470\ \mathrm{nm}(t=0)} - A_{c\,470\ \mathrm{nm}(t=80)}] \quad (6)$$

where $A_{s\,470\ \mathrm{nm}(t=0)}$ is the initial absorbance of the sample containing antioxidant, $A_{c\,470\ \mathrm{nm}(t=0)}$ is the initial absorbance of the control, $A_{s\,470\ \mathrm{nm}(t=80)}$ is the absorbance of the sample at $t = 80$ min and $A_{c\,470\ \mathrm{nm}(t=80)}$ is the absorbance of the control at $t = 80$ min. As can be seen in Table 2 where the values of C_{AA} are presented, all of the rosemary formulations behaved similarly as in the DPPH· test, and thus the extracts most active as free radical scavengers also provide the best results against the oxidation of linoleic acid. Contrary to the results obtained with the DPPH· test, BHT in comparison to rosemary extract formulations was found to have the highest C_{AA} (be more active) in the β-carotene–linoleic acid emulsion system.

The effect of rosemary extracts on the kinetics of autooxidation of polyunsaturated fatty acids was evaluated using the measured data from the β-carotene bleaching test. As already mentioned, in that system β-carotene as a target molecule was exposed to free radicals formed by linoleic acid oxidation in the presence of a free radical scavenger, i.e., rosemary extract compounds. The change in concentration of β-carotene was monitored by measurement of the absorbance of the sample, $A_{s\,470(t=x)}$ at $t = 20$, 40, 60, 80, 100 and 120 min. In Fig. 3 the values of $A_{s\,470(t=0)} - A_{s\,470(t=x)}$ at a rosemary extract concentration in the emulsion of 0.04 mg/mL are presented against time. The difference $A_{s\,470(t=0)} - A_{s\,470(t=x)}$ is referred to as the content of β-carotene bleached. As in the case of DPPH· scavenging kinetics, the mathematical model that most satisfactorily describes the time dependence of $A_{s\,470(t=0)} - A_{s\,470(t=x)}$ for rosemary extract formulations is the power function:

$$A_{s\,470\ \mathrm{nm}(t=0)} - A_{s\,470\ \mathrm{nm}(t=x)} = a \cdot t^{b}. \quad (7)$$

The parameters a and b obtained by regression analysis (the corresponding determination coefficients are presented in Table 2) were used to evaluate the rate of β-carotene bleaching, R_B, as the first derivative of the function represented by Eq. (7)

$$R_B = a \cdot b \cdot t^{b-1}. \quad (8)$$

In the presence of BHT the dependence of $A_{s\,470\ \mathrm{nm}(t=0)} - A_{s\,470\ \mathrm{nm}(t=x)}$ on t followed the second order polynomial:

$$A_{s\,470\ \mathrm{nm}(t=0)} - A_{s\,470\ \mathrm{nm}(t=x)} = a \cdot t + b \cdot t^{2}. \quad (9)$$

The parameters a and b obtained by regression analysis (with a determination coefficient amounting to 0.980) were used to calculate the rate of β-carotene bleaching, R_B, as a derivative of the function described by Eq. (9)

$$R_B = a + 2b \cdot t. \quad (10)$$

The curves in Fig. 3 are plotted on the basis of the parameters in Eqs. (7) and (9). A higher R_B value indicates weaker antioxidant activity in the β-carotene–linoleic acid emulsion system. As is shown in Table 2 where the R_B values at $t = 10$ min are collected, the presence of rosemary extract in the oxidising lipid system decreased the kinetics of β-carotene degradation. Namely, in comparison to the control, i.e., without antioxidant ($R_B = 5.3 \times 10^{-3}$ min^{-1}), R_B values of rosemary extracts displayed much lower values. For BHT a notably lower R_B at $t = 10$ min that amounted to

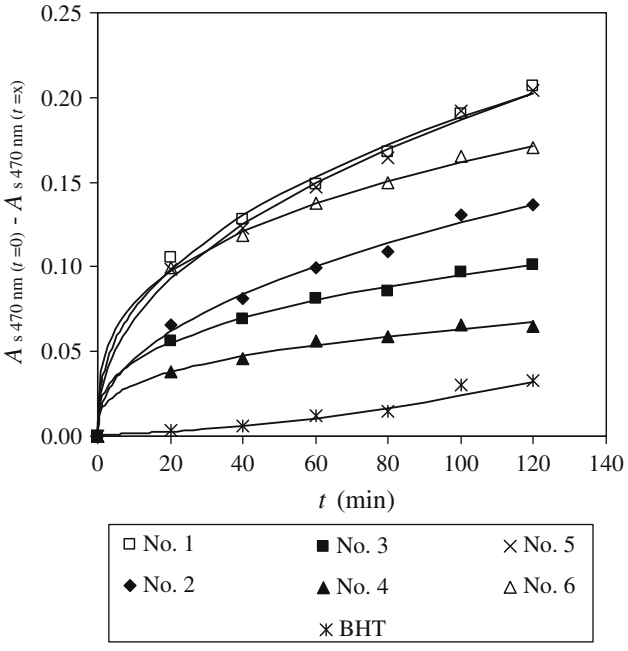

Fig. 3. The dependence of $A_{s\,470\ \mathrm{nm}(t=0)} - A_{s\,470\ \mathrm{nm}(t=x)}$ on time of incubation at an antioxidant concentration in emulsion of 0.04 mg/mL. Symbols represent experimental values, curves are plotted according to the parameters from Eqs. (7) and (9).

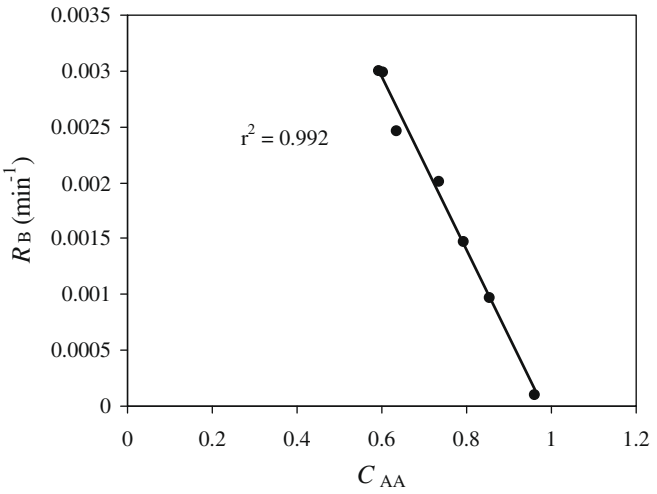

Fig. 4. The dependence of the rate of β-carotene bleaching, R_B, on antioxidant activity coefficient, C_{AA}, at an antioxidant concentration in the emulsion of 0.04 mg/mL.

0.10×10^{-3} min^{-1} was determined. As could be obtained from Table 2 and seen in Fig. 4, an excellent correlation between C_{AA} and R_B exists ($r^2 = 0.992$). The high degree of correlation presented in Fig. 4, in contrast to the low degree of correlation between EC_{50} and R_S values (Fig. 2), could be explained by considering that R_S and R_B determinations were assayed under different experimental conditions that are based on different reaction mechanisms. These differences could also reflect differences in radical stability. The DPPH· radical, contrary to the peroxyl radical is a long-lived radical.

Kinetic analysis makes it possible to demonstrate differences in reactivity between antioxidant compounds. The downward curvature of plots in Fig. 3 as observed for phenolic diterpenes in rosemary extracts suggested that the content of β-carotene bleached in the early stage of the assay is formed more rapidly than later what means that rather low level of peroxyl radicals was consumed by phenolic diterpenes. In the presence of BHT the upward curvature of the plot indicated that in the initial step BHT more effectively competed with β-carotene for peroxyl radicals than phenolic diterpenes, suggesting the weaker availability of phenolic diterpenes to peroxyl radicals. This situation was quantified by calculation of R_B at $t = 120$ min and estimated as the ratio between R_B at $t = 120$ and 10 min (χ). As presented in Table 2 for rosemary extracts the value of χ amounted to 0.2 but for BHT it was 4.5 suggesting that BHT was consumed more rapidly than phenolic diterpenes.

4. Conclusions

The rate of free radical scavenging and the rate of β-carotene bleaching were proposed as new parameters to describe the antioxidant activity of phenolic diterpenes in rosemary extracts. The values for the reaction rate showed the expected dependence on the concentration of total phenolic compounds in the rosemary extract. Rosemary extract formulations with a higher content of phenolic compounds provide a higher rate of scavenging, whilst the rate of β-carotene bleaching process decreased with increased content of phenolic compounds. When BHT was taken into consideration, the results for free radical scavenging determined at a fixed end point (expressed as EC_{50}) did not strictly relate to the results obtained from the kinetic data; BHT after the end of the initial fast step, despite its moderate antioxidant activity when expressing results as EC_{50}, exhibited the highest R_S value. In an aqueous emulsion system a much better correlation between the C_{AA} and R_B was determined. If antioxidant activity was determined at a fixed point when the steady state of reaction had not yet been reached, the kinetic approach may give a more comprehensive understanding about the behaviour of antioxidants. Expression of the results in terms of the kinetic approach does not take into account only the activity of an antioxidant but also provide information on how quickly the antioxidant acts. Therefore it would be advisable that both the results of antioxidant activity based on kinetic data together with those based on measurements at a fixed end-point be combined, so as to provide comprehensive information on the total antioxidant capacity of a sample.

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