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ORIGINAL PAPER

Antioxidant activity of rosemary and thyme by-products and synergism with added antioxidant in a liposome system

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Abstract This study describes the use of liposomes as biological membrane models to evaluate the potential of natural antioxidants as inhibitors of lipid peroxidation. Antioxidative effects of by-products from manufacturing of essential oils, i.e., distilled rosemary leaf residues (DRL), distilled thyme leaf residues (DTL), and the combined antioxidative effects of DRL or DTL with α-tocopherol (TOH), ascorbic acid (AA), and quercetin (QC) on peroxidation of L-α-phosphatidylcholine liposomes as initiated by hydrophilic azo-initiators, were investigated. In addition, experiments were repeated with whole thyme leaves (TL) to compare the characteristics of by-products (leaf residues from distillation) with the initial industrial product (leaf for distillation). Extracts from DRL, DTL, and TL all had an obvious antioxidative effect as evidenced by a lag phase for the formation of phosphatidylcholine-derived conjugated dienes. DRL and DTL had similar antioxidative activity, while whole thyme leaves showed superior antioxidant activity compared with distilled thyme residues. Combination of TOH or QC with DRL, DTL, and TL, respectively, showed synergism in prolonging of the lag phase.

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Introduction

Due to growing concern among consumers about adding chemical additives to food, use of synthetic antioxidants is diminishing and focus has shifted toward naturally occurring antioxidants. Implementation of the latter, many of which contribute antimicrobial properties as well, is a promising technology to increase the shelf life of food, including meat products. Particularly, supplementing feed with α-tocopherol was found a simple but convenient way to introduce a natural antioxidant into cellular matrices (such as in muscle food) where initiation of lipid oxidation is expected to occur [1]. Besides α-tocopherol, also other phenolics are assumed to scavenge free radicals before lipid oxidation propagates through unsaturated fatty acids, and accordingly natural antioxidants found in the residues of olive oil production have been implemented [2]. Alternatively, by-products from essential oil production, i.e., distillate residues of aromatic plants, are a potential pool of compounds with strong antioxidant activity [3]. Rosemary (*Rosmarinus officinalis,* L.) and thyme (*Thymus zygis* ssp. *gracilis*, also known as red thyme) are aromatic plants currently exploited in the Region of Murcia (Spain) that generate an excess of residues after the distillation of leaves after manufacturing of essential oils. Although this organic residue is currently underused, there are studies underlining the potential benefits of meat industry of this source of natural antioxidants to prevent lipid oxidation and color changes [4, 5].

Since liposomes mimic cellular structures [6], the feasibility to protect lipid membranes in the presence of

phenolic antioxidants can be investigated in model systems prior to administration through feeding. Such experiments are particularly interesting for meat industry as they furnish preliminary insights with respect to lipid oxidation at relatively short timescales. Thus, extracts made from rosemary and thyme were evaluated, and oxidative conditions, to which food systems as fresh meat and meat products are exposed, were simulated by iron-catalyzed Fenton reactions or by the disintegration of radical initiators. Besides antioxidant activity of by-products from rosemary and thyme leaf distillation (and whole thyme leaves as a reference material), the feasibility of synergy with antioxidants currently used in the meat industry (TOH, AA, and QC) was investigated**.**

Materials and methods

Chemicals

L-α-Phosphatidylcholine (PC) from soybean (99% purity) and *N-tert*-butyl-α-(4-pyridyl) nitrone *N*-oxide (POBN) were purchased from Sigma–Aldrich (Steinheim, Germany), while α-tocopherol was obtained from Fluka (Buchs, Switzerland). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was supplied from Wako Chemicals (Neuss, Germany), and the Folin-Ciocalteu reagent came from Merck (Darmstadt, Germany).

Plant material

Rosemary (*Rosmarinus officinalis* L.) and thyme (*Thymus zygis* ssp. *gracilis*) are the two species that prevail in the Region of Murcia (Spain). The plant material used for this work, i.e., residues after the distillation of essential oils from rosemary leaf and thyme leaf, as well as the initial industrial product of thyme leaf before distillation, was obtained from the local producers of essential oil. Fresh rosemary material was collected from the mountains surrounding Murcia, Spain, while thyme originated from cultivation. Prior to further use, the residue from the distillation of rosemary and thyme is dried at room temperature and leaves and stems are removed.

Polyphenol extraction

Extracts were prepared by mixing 0.5 g milled spices (distilled rosemary leaves, DRL, distilled thyme leaves, DTL, and thyme leaves, TL) with 4 mL of extraction solvent (water, 70% aqueous acetone or 70% aqueous ethanol). The air in the tube was displaced by nitrogen, and phenolics were extracted at 20 °C by rotating (200 rpm) for 20 min. The mixture was subsequently centrifuged (2 min, 1,100*g*), and the supernatant was evaporated to dryness (~5–20 min) using a rotary vacuum evaporator (at 35 °C). The residue was redissolved in 6 mL of 25% aqueous ethanol and adjusted to pH 2 with acetic acid in order to stabilize the phenolics, and the resulting solutions were covered with nitrogen and were stored at −18 °C until use (maximum 21 days).

Total phenol concentration

The amount of total phenolics in extracts was determined according to the Folin-Ciocalteu method. Samples (200 μL, three replicates) were mixed with 1.0 mL of Folin-Ciocalteu's reagent (diluted 1:10 with water), and 0.8 mL of a 7.5% solution of sodium carbonate was added. The absorption at 765 nm was measured after 30 min with a Cary 3 UV–Vis spectrophotometer (Varian Techtron Pty. Ltd, Mulgrave, Victoria, Australia). The total phenolic content was expressed as gallic acid equivalents (GAE) in mg/L of extract.

Preparation of liposomes

Liposomes were prepared according to a slightly modified literature procedure [3]. A solution (2 mL) containing 1.5 μmol soybean phosphatidylcholine dissolved in chloroform was mixed with 1 mL pure hexane or 1 mL hexane containing α-tocopherol, quercetin, and ascorbic acid. The concentration of α-tocopherol was calculated as mol % of the lipid fraction by using a molecular mass of soybean PC equal to 900 g/mol. Subsequently, the solvent was removed under reduced pressure (approximately 100 mbar) on a rotary evaporator with water bath set at 30 °C. Nitrogen was introduced when returning to atmospheric pressure after complete evaporation. Addition of 10 mL phosphate buffer (10 μM, pH 7.4) followed by 10 min vortex and 30 s sonication to ensure complete suspension yielded a white homogenous suspension of multilamellar liposomes, which was permanently protected from light and kept under nitrogen. Unilamellar liposomes were prepared from the multilamellar liposomes by an Avestin Liposofast Basic small volume (500 μL) extrusion device (Avestin Europe GmbH, Mannheim, Germany). The suspension was passed 21 times through a double layer (polycarbonate membrane, 100-nm pore size) to obtain large unilamellar liposomes. The extracts of the spices were added to the liposome system in the phosphate buffer. Different volumes of 1000-times diluted extract of DRL, DTL, and TL were added to the buffer solution to obtain a concentration corresponding to 1.84×10^{-5} mg GAE according to Graversen et al. [7].

Peroxidation of liposomes

A unilamellar liposome suspension (2.5 mL) was pipetted into quartz cuvettes and incubated for 10 min at 37 °C within the water-jacket cell holder of a Shimadzu UV-2101PC UV–VIS scanning spectrophotometer with automatic cell changer (Kyoto, Japan). Lipid peroxidation was initiated by introducing 25 μL of 75 mM AAPH in sodium phosphate buffer (pH 7.4). The cuvettes were quickly inverted five times and then sealed to avoid evaporation. Up to six samples were measured in each run, using phosphate buffer as blank and the liposome suspension without antioxidant as control. Absorbance was measured at 234 nm, which is the absorption maximum of conjugated dienes, every 10 min for 900 min in total. The lag phase before the onset of oxidation was measured as the time in minutes corresponding to the intercept between the tangent to the propagation phase and the tangent to the lag phase [3].

Fenton reaction model system with ESR detection of POBN spin adducts

A total of 4 mL of POBN (0.0032 M) in a 1 M aqueous ethanol solution was mixed with 20 μL of $FeSO_4$ solution (0.022 M) and 50 μL of aqueous, aqueous acetone or aqueous ethanol extract (according to Graversen et al. [7]). As a reference, 50 μL Milli Q water was substituted for extract. The reaction was initiated by the addition of 80 μL H_2O_2 (0.024 M), followed by gentle mixing for 30 s. Subsequently, 50 μL was transferred to ESR micropipettes (Brand, Wertheim, Germany), and the spectra were recorded after 2 min on a Miniscope MS 200 ESR spectrometer (Magnettech, Berlin, Germany) with the following instrument settings: microwave power, 4 mW; sweep width, 7.5 mT; sweep time, 4 min; modulation width, 0.12 mT; amplitude, 500; and time constant, 0.3 s. The degree of inhibition (I_{ESR}) was calculated from the height of the central peak of the ESR signal of the POBN spin adduct by the following formula:

$$I_{ESR} = [1 - (\text{Peak height}_{sample}/\text{Peak height}_{reference})] \times 100\%$$

Statistical analysis

All the experiments were carried out in triplicate. *ANOVA* (Scheffe means test) was carried out for the comparison of the results of the undistilled and the distilled thyme material. Pearson's correlation coefficients were determined between the phenolic content and the antioxidant activity of the plant. A probability of $P < 0.05$ was adopted as the criterion for significant differences. The computer statistics program used was Statistix 8.0 for Windows (Analytical Software, New York, USA).

Results and discussion

Phenolic compounds

Table 1 shows the estimated phenolic content in distilled rosemary and thyme as determined by the Folin-Ciocalteu method. The concentration of phenolic compounds in the rosemary distilled leaves (DRL) extracted with 70% acetone was five times higher than in DRL extracted with water. These measurements are in agreement with the findings of Rødjer et al. [8], who found a higher phenolic content (expressed in mg GAE/L extract) in aqueous acetone (70%) extracts from cherry pomace compared with water or aqueous ethanol (70%) extracts. Likewise, Moreno et al. [9] when investigating the total phenolic content in different extracts of rosemary reported a comparable ratio of phenolic levels obtained from acetone and water extracts, although values were expressed as g GAE per 100 g dry extract (19 ± 8 and 3 ± 2, respectively).

In contrast to DRL extractions, the difference between the amounts of phenolics extracted from the thyme distilled leaves (DTL) using water or acetone was less pronounced (Table 1). In general, the results of phenolic compounds from DTL determined in our study were in line with values reported in the literature. For example, it was reported that phenolic compounds in distilled thyme material vary around 108.5 ± 19.2 mg GAE per g of dry plant [7]. Table 1 shows that *Thymus zygis* (TL) extracted with water, 70% ethanol, and 70% acetone has a higher phenolic content (25.8 ± 2.0, 101.4 ± 2.2, and 97.9 ± 2.9, respectively) compared with other (undistilled) thyme species found in the literature. For example, the phenolic content of *Thymus vulgaris* was studied by Proestos et al.

Table 1 Total phenolics in extracts from rosemary and thyme (mg gallic acid equivalents (GAE) per L)

Extraction mixture	DRL	DTL	TL
Water	23.6 ± 2.5	37.5 ± 3.2	21.5 ± 1.7
	(28.3 ± 3.0)	(45.0 ± 3.8)	(25.8 ± 2.0)
70% ethanol	80.1 ± 1.9	79.6 ± 2.8	84.5 ± 1.8
	(96.1 ± 2.3)	(95.5 ± 3.4)	(101.4 ± 2.2)
70% acetone	118.7 ± 4.3	68.5 ± 3.6	81.6 ± 2.4
	(142.4 ± 5.2)	(82.2 ± 4.3)	(97.9 ± 2.9)

DRL distilled rosemary leaves, *DTL* distilled thyme leaves, *TL* thyme leaves

Values between brackets, representing concentrations of phenolic compounds per g dry plant material (mg GAE/g), were given for comparison with literature values

[10], who found 19.2 ± 0.3 mg of GAE per g dry sample, while Zheng and Wang [11] reported a phenolic content of 2.13 ± 0.11 mg of GAE per g in the fresh leaves.

Obviously, since the Folin-Ciocalteu method is a colorimetric assay, it only gives an estimate of phenolic functionalities present and cannot be directly correlated to qualitative composition. However, the technique allows the standardization of extracts, of which antioxidant activity was further investigated in liposome systems.

Antioxidant activity of rosemary and thyme leaves in a liposome system

Phenolic compounds found in rosemary and thyme were previously attributed a role as inhibitors of lipid oxidation, as transition metal chelators, and as scavengers of superoxide radicals [12]. Several studies also demonstrated that this antioxidant capacity could be exploited to protect meat products, particularly after administration through feed [4, 5]. However, to quantify the potential of phenolic compounds extracted from rosemary and thyme to act as food antioxidants, a liposome model system made of soybean phosphatidylcholine was conceived. Inhibition of lipid oxidation, initiated by the hydrophilic radical initiator 2, 2′-azobis (2-amidinopropane) dihydrochloride (AAPH), was followed by monitoring the formation of the conjugated dienes spectrophotometrically. Upon the addition of AAPH to the liposome control (i.e., without phenolic extract added), diene formation occurred immediately (Table 2). On the other hand, a lag phase, indicating the inhibition of lipid oxidation [7], was observed when DRL, DTL, and TL extracts were present (Table 3). Antioxidant effect of extracts from both distilled leaves was similar, corroborating their common descent (i.e., from the *Labiatae* family). The residue obtained after the distillation of rosemary leaves (DRL) has a relative concentration (% dry weight) of 0.01 ± 0.00, 0.07 ± 0.00, 0.13 ± 0.01, 0.15 ± 0.01, 4.39 ± 0.04, and 8.46 ± 0.06 of 12-methylcarnosic acid, carnosol, genkwanin, carnosic acid, hispidulin-7-O-glucoside, and rosmarinic acid, respectively [13]. Carnosic acid is one of the major phenolic constituents in rosemary, and its antioxidant activity is approximately three times higher than carnosol and seven times higher than synthetic antioxidants butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) [14]. Zeng et al. [15] focused on carnosol, rosmanol, and epirosmanol and found that these phenolic diterpenes inhibited the lipid peroxidation of low-density lipoproteins (LDL) in blood and cell membranes.

As for thyme, Jordán et al. [16] demonstrated that caffeic acid, carnosic acid, ferulic acid, rosmarinic acid, and apigenin of thyme leaves (DTL) have a concentration (μg/g dry plant material) of 96.5 ± 20.5, 109.7 ± 28.8, 181.6 ± 96.9, 223.5 ± 113.4, and 225.4 ± 75.4, respectively. Regarding whole thyme leaves (TL), unlike residues after distillation, TL still contain relatively high concentrations of terpenoids, which eventually make up the constituents of extracted essential oils. Yield of the latter is approximately 3%, expressed as mL oil per 100 g dry plant, and major compounds include β-caryophyllene, limonene, methylcarvacrol, terpinen-4-ol, α-pinene, α-thujene, myrcene, linalool, carvacrol, γ-terpinene, ρ-cumenol, and thymol (0.32 ± 0.15, 0.51 ± 0.04, 0.54 ± 0.73, 0.60 ± 0.06, 0.64 ± 0.10, 0.96 ± 0.21, 1.40 ± 0.28, 2.60 ± 0.60, 3.12 ± 0.55, 5.32 ± 2.23, 18.23 ± 3.06, and 58.00 ± 3.90% of extracted essential oil, respectively).

Table 2 Lag phase of α-tocopherol, quercetin, and ascorbic acid found by the spectrophotometric measurement of the formation of conjugated dienes in soybean phosphatidyl choline liposomes in aqueous solution with free radical initiation of oxidation in the aqueous phase using 75 mM AAPH

Sample	Lag phase time (min)
Control	4 ± 3
AA	64 ± 2
QC	171 ± 3
TOH	100 ± 2

AA ascorbic acid, *QC* quercetin, *TOH* α-tocopherol

Table 3 Lag phase found in distilled rosemary leaves, distilled thyme leaves, and thyme leaves by the spectrophotometric measurement of the formation of conjugated dienes in soybean phosphatidyl choline liposomes in aqueous solution with free radical initiation of oxidation in the aqueous phase using 75 mM AAPH

Sample	Lag phase time (min)					
	Water		Ethanol		Acetone	
	E	*C*	*E*	*C*	*E*	*C*
DRL	85 ± 7	–	65 ± 3	–	66 ± 3	–
DRL + AA	146 ± 2	150	146 ± 6	153	125 ± 3	129
DRL + QC	276 ± 4	256	277 ± 2	260	256 ± 3	236
DRL + TOH	197 ± 3	185	203 ± 2	189	186 ± 2	166
DTL	65 ± 2	–	59 ± 2	–	65 ± 6	–
DTL + AA	125 ± 2	129	122 ± 2	153	135 ± 2	130
DTL + QC	259 ± 4	235	262 ± 2	230	256 ± 2	237
DTL + TOH	187 ± 3	165	175 ± 2	160	186 ± 2	167
TL	74 ± 2	–	102 ± 6	–	82 ± 5	–
TL + AA	132 ± 2	139	163 ± 2	166	142 ± 3	146
TL + QC	256 ± 4	245	285 ± 2	273	265 ± 2	233
TL + TOH	196 ± 3	174	215 ± 2	202	207 ± 2	183

DRL distilled rosemary leaves, *DTL* distilled thyme leaves, *TL* thyme leaves: extracts corresponding to a concentration of 1.84×10^{-5} g GAE/L. *AA* ascorbic acid, *QC* quercetin, *TOH* α-tocopherol: 1 mol % *TOH, QC,* and *AA* relative to phosphatidyl choline. *E* experimental, *C* calculated

Antioxidant activity of the extract from thyme leaf was higher than that of the distilled leaf (Table 3), most likely because phenolic monoterpenes, thymol and carvacrol, were removed by distillation. The latter are known to inhibit lipid peroxidation and are responsible for the longer lag phase of ethanol extracts from TL compared with aqueous extracts [17]. On the other hand, essential oil of thyme, which is particularly abundant in thymol, carvacrol, and cumenol, showed only moderate inhibition of LDL oxidation (20–27%), while deodorized aqueous extracts of rosemary and thyme led to varying degrees of radical scavenging and were capable of prolonging the lag time in the LDL oxidation assay.

Comparison between the undistilled and the distilled thyme plant material (TL and DTL, respectively) within each employed method was carried out by *ANOVA*. Thus, although the total phenolic content of the DTL extracted with water (37.5 mg GAE/L extract) was found to be higher ($P < 0.05$) than that of the undistilled TL leaf (21.5 mg GAE/L extract), the lag phase was longer ($P < 0.05$) in the TL than in the DTL experiment (Fig. 1). This suggests that TL contains more powerful antioxidants. However, extracts are complex mixtures in which synergy or adverse effects due to prooxidant activity can produce different results than the sum of individual compounds would give. In addition, Tepe et al. [18] claimed that the antioxidative potential of thyme extract was particularly due to polar fractions, which were found most effective.

Interaction of rosemary and thyme with other antioxidants

α-Tocopherol (TOH)

In the presence of AAPH, a combination of TOH and each extract studied showed a lag phase that was longer than the sum of lag phases of individual components (Table 3). The observation of these synergistic effects, which is in agreement with previous reports on the combination of α-tocopherol and polyphenols in heterogeneous systems [7], probably depends on different solubilities of the antioxidants in the two phases of the heterogeneous systems. Indeed, α-tocopherol, a major natural chain-breaking antioxidant in cell membranes, provides protection against early events in lipid oxidation at the site of radical initiation. Plant extracts on the other hand proved effective in blocking the peroxidation process in both phases by scavenging free radicals, by inhibiting catalysis by iron, and through chain-breaking activity [19]. In this respect, Fang and Wada [20] reported that a mixture of α-tocopherol and rosemary extract in a sardine model system exerted a stronger antioxidant effect than either α-tocopherol or rosemary extract alone. It was proposed that the synergistic action of rosemary is related to the regeneration of α-tocopherol through the donation of a hydrogen atom to the tocopheroxyl radical. A similar mechanism was suggested by Trojakova et al. [21], who reported that rosemary efficiently protects tocopherols naturally present in rapeseed oil. It should be noted, however, that Wong et al. [22] failed to observe a synergistic effect between TOH and rosemary extracts.

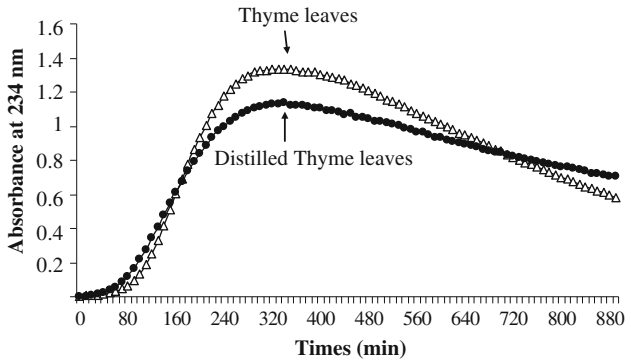

Fig. 1 Formation of conjugated dienes in liposomes determined by measuring the absorbance at 234 nm at 37 °C. A 70% acetone extracts from distilled and undistilled thyme leaves, adjusted to a concentration of 1.84×10^{-5} mg GAE/L, were evaluated

Quercetin (QC)

Incorporation of 1 mol% QC in the liposomes followed by AAPH-induced oxidation showed a lag phase (171 ± 3 min), which was significantly extended on the addition of DRL, DTL, and TL extracts (Table 3). Moreover, since the lag phase of the combination of QC with respective extracts was found to be longer than the sum of lag phases of each individual component, synergism must be active (Fig. 2). Similar effects were observed by Altunkaya et al. [23], who found that antioxidants from a lettuce extract acted synergistically with quercetin in a liposome system.

Ascorbic acid (AA)

The combination of AA with DRL, DTL, or TL showed a lag phase that was approximately the same as when AA was present alone (Table 3); hence, synergism was absent (except in the DRL ethanol extract and DTL acetone extracts). Although AA can react with radicals generated in the water phase by AAPH disintegration or it can regenerate polyphenols from their oxidized forms due to its low oxidation potential [23], it is likely that kinetic factors inhibited further effects in the liposome system. Still, a pronounced synergism was previously observed between ascorbyl palmitate, a lipophilic ascorbate derivative, and oregano extract (to preserve α-tocopherol in sunflower oil)

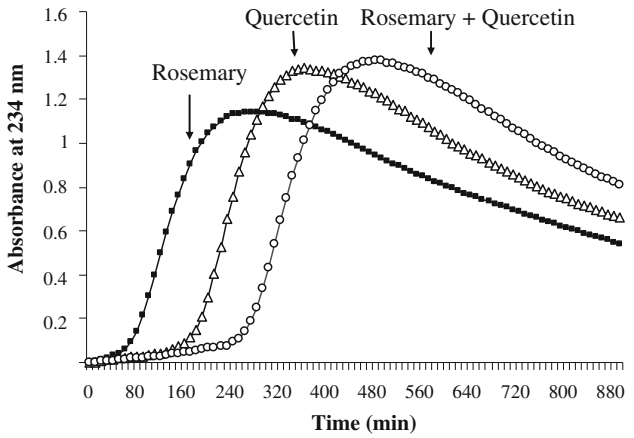

Fig. 2 Formation of conjugated dienes in liposomes determined by measuring the absorbance at 234 nm at 37 °C. A 70% acetone extract from distilled rosemary leaves, adjusted to a concentration of 1.84×10^{-5} mg GAE/L, was evaluated

[24], suggesting solubility issues hamper synergy in our experiments.

Fenton reaction model system with ESR detection of POBN spin adducts

The Fenton model system used to assess the protective properties of plant extracts (including also adverse prooxidative effects) is based on the basic Fenton assay. Antioxidative potential of distilled rosemary, distilled and undistilled thyme leaf extracts was quantified by indirectly measuring their ability to scavenge 1-hydroxyethyl radicals ($CH_3 \cdot CHOH$), which result from the interaction of ethanol with the highly reactive hydroxyl radical ($^{\cdot}OH$) generated by Fenton chemistry. The $CH_3 \cdot CHOH$ species was trapped by POBN producing a spin adduct, the formation was monitored using ESR spectroscopy [8]. Antioxidants, present after the addition of small amounts of plant extracts to the Fenton mixture, compete for $CH_3 \cdot CHOH$ and $^{\cdot}OH$, and the level of detectable POBN spin adducts decreases according to the radical scavenging activity of the different extracts (Fig. 3). Aqueous rosemary or thyme extracts reduced the spin adduct formation more than the corresponding acetone or ethanol extracts, and the antioxidant effects were already observed at phenolic concentrations <3.87 mg GAE/L, but whole plant material (TL) was even more effective with <2.12 mg GAE/L for the aqueous ethanol extract.

Antioxidative effects per phenol unit, α^{anti}, of extracts from mixed solvents were, despite their higher concentration of phenolics, markedly lower than those of 100% aqueous extracts (Fig. 4a). Indeed, high concentrations of plant extracts eventually gave rise to prooxidant effects that were observed as relative concentrations of spin adducts higher than 100% (Fig. 3). Similar prooxidative effects in

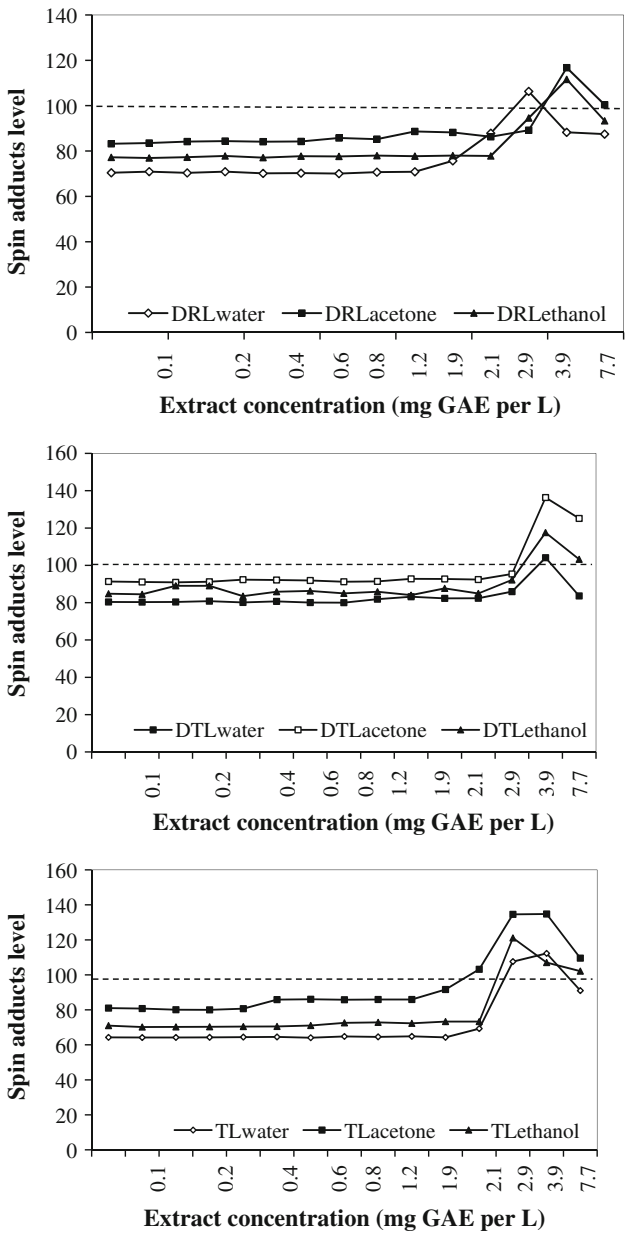

Fig. 3 The effect of aqueous, aqueous acetone and aqueous ethanol extracts on the formation of POBN spin adducts in the Fenton assay. *Upper panel* Distilled rosemary leaves (DRL); *Middle panel* Distilled thyme leaves (DTL); *Lower panel* Thyme leaves (TL). Spin adduct levels were normalized to the control experiment in the absence of antioxidant (representing the 100% level)

the Fenton assay were previously reported with highly concentrated extracts, for example with cherry pomace [8] and with the plant phenol oleuropein [25]. Cao et al. [26] used the ORAC assay to conclude that flavonoids change from antioxidants at low concentrations to prooxidants at high concentrations. The effect of polarity of extraction solvent on prooxidative effect is shown in Fig. 4b, and except for the aqueous DRL extract and the ethanol TL extract, activities were identical. Indeed, compounds with

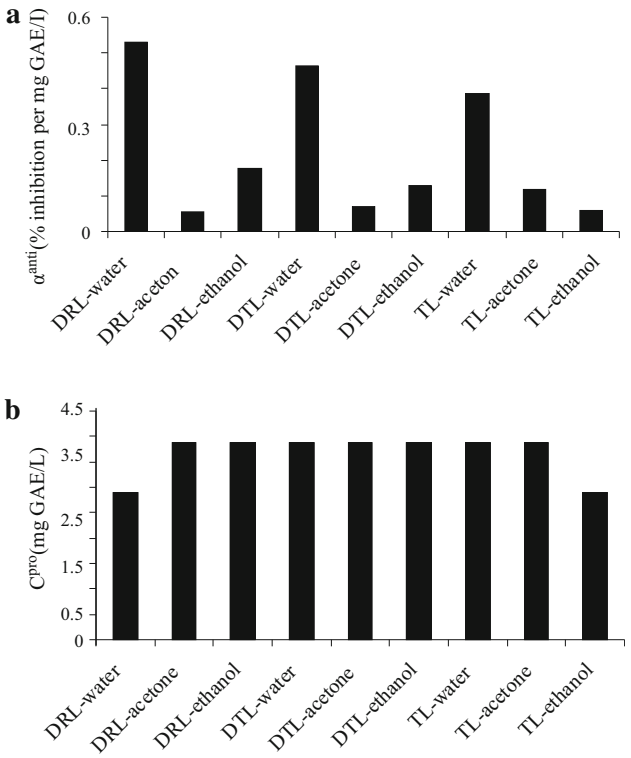

Fig. 4 **a** Antioxidative activity, α^{anti}, of various rosemary (DRL) and thyme (DTL and TL) extracts as determined by the Fenton/ethanol model system. **b** The concentrations, C^{pro}, of phenolics in different solvent extraction systems corresponding to the transition from an overall antioxidative activity to a prooxidative activity in the Fenton/ethanol model system

high reducing power not only scavenge radicals but also reduce Fe^{3+} to Fe^{2+} and thereby support hydroxyl radical formation by Fenton chemistry.

Conclusion

Distilled leaves of rosemary and thyme were found to be a rich source of antioxidants as shown by the inhibition of the formation of conjugated dienes in a liposome system. Comparing the residues of both distilled leaves, the major concentrations of phenolic compounds were found in rosemary, although extracts from undistilled leaves (from thyme) contained more phenolic substances than those from the distilled residue. Generally, aqueous extracts had a lower phenolic content than extracts from mixed solvents (i.e., aqueous ethanol or acetone). Compounds in resulting extracts worked synergistically in combination with α-tocopherol and quercetin, but not with ascorbic acid. Based on this study, it can be concluded that rosemary and thyme residues, as by-products from distillation of essential oils, are a readily accessible source of natural antioxidants, which possibly provides a good alternative to using synthetic antioxidants in the protection of foods and meat products in particular.

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