Effect of plant phenolics, tocopherol and ascorbic acid on oxidative stability of pork patties

ARTICLE *in* JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE · JUNE 2009

Impact Factor: 1.71 · DOI: 10.1002/jsfa.3595

3 AUTHORS, INCLUDING:

Katleen Raes
Ghent University
103 PUBLICATIONS **2,166** CITATIONS
SEE PROFILE

Stefaan De Smet
Ghent University
232 PUBLICATIONS **3,620** CITATIONS
SEE PROFILE

Research Article

Received: 24 August 2007 Revised: 17 February 2009 Accepted: 3 March 2009 Published online in Wiley Interscience:

(www.interscience.wiley.com) DOI 10.1002/jsfa.3595

Effect of plant phenolics, tocopherol and ascorbic acid on oxidative stability of pork patties

Lindsey Haak, Katleen Raes† and Stefaan De Smet*

Abstract

BACKGROUND: There is great interest in the use of naturally occurring antioxidants to delay oxidation in meat products. The effect of rosemary extract (RE), green tea extract (TE), tocopherol, trolox, ascorbic acid (AA) and ascorbyl palmitate (AP), at levels of 50–200 ppm of antioxidant components, on colour (CIE *L*a*b), lipid (TBARS) and protein oxidation (thiol groups) in fresh, frozen and cooked pork patties during illuminated chill storage was investigated. Individual components of RE and TE were also tested.**

RESULTS: RE, TE, AP, tocopherol and trolox equally inhibited lipid oxidation in fresh and frozen patties, whereas for cooked patties RE was most effective. AA stimulated lipid oxidation. No dose effect in the range of 50–200 ppm was found for fresh and frozen patties, whereas for cooked patties higher doses of RE and TE more efficiently prevented lipid oxidation. Protein oxidation was hardly influenced by antioxidant treatment. Colour stability decreased as follows: tocopherol, AA and AP > RE and TE > trolox. Antioxidant properties of the extracts and their major antioxidant components were comparable.

CONCLUSION: The relative effect of the antioxidants depends on the oxidation parameter assessed, the applied dose and the hydrophilic/lipophilic character.

Keywords: ascorbic acid; green tea; meat; oxidative stability; rosemary; tocopherol

INTRODUCTION

Antioxidants are added to meat products during processing to delay oxidation. Oxidative processes in meat and meat products during storage or cooking lead to the degradation of colour pigments, lipids and proteins which, in turn, can contribute to the deterioration in flavour, texture, colour and nutritional value of the meat.[1] Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and *tert*-butylhydroquinone (TBHQ) have been widely used in the meat industry. However, rising consumer resistance towards the use of synthetic additives, has increased interest in naturally occurring substances.[2] Moreover, given the mounting body of science continuing to report on their antioxidant potential, a variety of extracts of fruits, herbs, vegetables, cereals and other plant material rich in antioxidant substances have been commercialised for food and nutraceutical applications.[3,4] Several studies have demonstrated that their postmortem supplementation to pork, improved its oxidative stability.[5–7] The antioxidant properties of these plant extracts are dedicated to a cocktail of active components, which could act individually as well as in synergy.[8] Among those, are the frequently studied rosemary (*Rosmarinus officinalis*) and green tea (*Camellia sinensis*) extracts (RE and TE, respectively), which have been shown to improve the oxidative stability of meat products.[7,9,10] The major active antioxidant components of RE and TE belong to the polyphenols and are known as phenolic diterpenes [carnosic acid (CA) and carnosol] and epicatechins [epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC)], respectively. The total polyphenol content of plant extracts can be highly variable, however, and depends on the extraction solvent and the plant variety used (e.g. Gramza *et al*.[11] for TE). Therefore, it is important to compare antioxidant extracts based on their active component levels instead of on a w/w basis (weight of substance by weight of meat). By doing so, the superiority of certain antioxidants over others can be properly assessed, and differences can then be explained by qualitative and not quantitative differences in antioxidant compounds present.

In the meat industry, the antioxidants ascorbic acid (AA) and α-tocopherol have been widely considered for extending the retail display life of meat. AA has long been known for protecting the colour of raw red meat during storage.[12] However, depending on its concentration, AA either promoted or inhibited lipid oxidation in muscle foods.[13] The lipid antioxidant α-tocopherol has demonstrated strong antioxidant activity in a wide range of

* *Correspondence to: Stefaan De Smet, Laboratory for Animal Nutrition and Animal Product Quality, Department of Animal Production, Ghent University, Proefhoevestraat 10, 9090 Melle, Belgium. E-mail: Stefaan.DeSmet@UGent.be*

† *Current address: EnbiChem, Department PIH, University College of West-Flanders, Graaf Karel de Goedelaan 5, 8500 Kortrijk, Belgium.*

Laboratory for Animal Nutrition and Animal Product Quality, Department of Animal Production, Ghent University, Proefhoevestraat 10, 9090 Melle, Belgium

meats when supplemented as α-tocopheryl acetate via the animal diet.[14,15] However, when applied to meat and fish products as α-tocopherol, it was less effective in controlling lipid oxidation.[14,16]

The purpose of this work was to investigate the effectiveness of natural antioxidants and antioxidant extracts (tocopherol, AA, RE and TE) in delaying oxidation (of colour, lipids and proteins) in fresh, frozen and cooked pork patties. All antioxidants/extracts were added on the basis of their active component content (as specified by the manufacturer) in order to effectively compare antioxidants.

MATERIALS AND METHODS

Solvents, standards and plant extracts

Hydrochloric acid and methanol were analytical grade and purchased from Sigma–Aldrich (Bornem, Belgium). Butylated hydroxytoluene (BHT), 2-thiobarbituric acid (TBA), 1,1,3,3-tetramethoxypropane (TMP), tris(hydroxymethyl)aminomethane (TRIS), ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulfate (SDS), and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma–Aldrich as the available standards. Mixed tocopherol (300 g kg^{-1} purity; *R,R,R*-$\alpha,\beta,\gamma,\delta$-tocopherol isomers 50–150 g kg^{-1}, 0–50 g kg^{-1}, 600–700 g kg^{-1}, and 200–300 g kg^{-1} respectively; tocopherol), ascorbic acid (>990 g kg^{-1} purity; AA) and ascorbyl palmitate (>980 g kg^{-1} purity; AP) were kindly donated by DSM Nutritional Products (Deinze, Belgium). All other antioxidative compounds (trolox, CA, EGCG, EGC, ECG, EC; theobromine (TB) and caffeic acid (CAF)) were purchased from Sigma–Aldrich as the available standards. Rosemary extract (Stabiloton® OS; 170 g kg^{-1} CA; RE) and green tea extract (460 g kg^{-1} tea catechins: 500 g kg^{-1} EGCG, 304 g kg^{-1} EGC, 152 g kg^{-1} ECG, 44 g kg^{-1} EC; 7.5 g kg^{-1} TB and 75 g kg^{-1} CAF; TE) were a gift from RAPS (Beringen, Belgium).

Preparation of patties

Frozen and vacuum packed pork meat (*longissimus thoracis*) and fat were weighed in a ratio 3/1 (w/w), chunked, minced through a 10 mm steel plate using a Grindomix GM 200 mincer (Retsch Gmbh Co, Haan, Germany) and held overnight at 0 °C. The meat and fat originated from a previous experiment at our laboratory (unpublished), in which pig diets were supplemented with polyunsaturated fatty acid sources (soybean oil and/or linseed oil). The meat and fat was pooled from several animals and from different dietary treatments to obtain a homogenous batch. Calculated quantities of antioxidants/extracts were first blended with sodium chloride (NaCl, added at 10 g kg^{-1}) and added to the meat before final grinding. To the control patties, only NaCl was added. During preparation, the internal temperature of the patties was monitored by a probe thermometer and did not exceed 4 °C. For the control and each of the antioxidant treatments, meat mixes of 160 g were prepared and formed into four patties (40 g each) using a round shaped mould of 55 mm internal diameter and 20 mm height. The patties were wrapped in an oxygen-permeable polyethylene film and held under refrigerated (4 °C) display conditions (illumination of 900 lux) for 8 days.

Experimental design

In experiment I, the first objective was to test the antioxidant action of four commercially available antioxidants: AA, tocopherol, RE and TE. The second objective was to compare the antioxidant action of tocopherol and its water soluble analogue trolox, and of AA and its lipid soluble analogue AP. Hereby, two supplementation levels [100 and 200 parts per million (ppm) of antioxidant components] were tested for all previously mentioned antioxidant treatments. Thirdly, RE and TE were compared to their major antioxidant components, CA and tea catechins respectively (EGCG, EGC, ECG and EC were added proportionally to their specified occurrence in the TE), at a level of 100 ppm. Carnosol was not added because of its low and unspecified content in the extract and the lack of a stable standard. A last treatment consisted of adding TB + CAF, two minor components of TE, in a dose equal to the one obtained (18 ppm) by adding TE up to the desired catechin level of 100 ppm. The preparation of the patties was done in duplicate in time. For both repetitions, control patties were prepared. Half of the patties were placed in the illuminated chill cabinet to perform oxidative stability measurements on the fresh patties (fresh) and the other half were immediately vacuum packed and stored at −18 °C (4 months) for oxidative stability measurements after freezing and thawing (frozen).

In experiment II, for AA, RE and TE, the dose effect was examined more closely. Therefore, each of these antioxidants was added at levels of 50, 100, 150 and 200 ppm of antioxidant components. The preparation of the patties was repeated in triplicate in time and again for every repetition, control patties without antioxidant added were prepared. Half of the patties were placed immediately in the illuminated chill cabinet (fresh) and the other half were cooked in a water bath at 70 °C for 40 min (in an impermeable plastic bag) (cooked) before illuminated chill storage and the performance of oxidative stability measurements.

Analyses

Gross and lipid composition

Pooled samples ($n = 4$) of the remainders of the patties were analysed for dry matter, crude protein and crude fat content according to the ISO 1442–1973, ISO 937–1978 and ISO 1444–1973 methods, respectively. The fatty acid composition was not determined on the patties, but was analysed on the fat and meat that was used for preparing the patties. The fatty acid composition of the patties was subsequently estimated from the fatty acid composition of the fat and meat and their contribution to the total lipid content of the patties.

Colour and colour stability

For experiment I, colour stability was measured on day 0 (d0; after a 30 min bloom period), d4 and d8 of illuminated chill storage for both fresh and frozen patties. For experiment II, colour stability was measured on d0, d1, d2, d4 and d8 of illuminated chill storage for the fresh patties. For the cooked patties, colour stability was not measured. Colour coordinates (CIE $L^*a^*b^*$ colour system (1976)) and reflectance spectra (every 10 nm between 400 and 700 nm) were assessed using a HunterLab Miniscan XE plus spectrocolorimeter (light source of D65, standard observer of 10°, 45°/0° geometry, 1 in. light surface, white standard). By means of reflectance values at specific wavelengths, the percentage of metmyoglobin (MetMb%) was calculated according to the method of Krzywicki,[17] modified by Lindahl *et al*.[18]

Lipid oxidation

Lipid oxidation was assessed by 2-thiobarbituric acid-reactive substances measurement (TBARS) using the distillation method as described by Tarladgis *et al*.[19] and was expressed as mg malondialdehyde (MDA) kg^{-1} meat. This method estimates MDA,

a secondary lipid oxidation product that together with TBA forms a coloured complex, which is determined spectrophotometrically at 532 nm. For both experiments, lipid oxidation was measured after 4 and 8 days of illuminated chill storage. For the frozen patties of experiment I, a supplementary assessment of lipid oxidation was done on d1 after thawing.

Protein oxidation

Protein oxidation was assessed by measuring thiol groups using the method of Ellman[20] with slight modifications[21] and was expressed as nmol thiol groups mg^{-1} protein. This method relies on the incubation of a meat homogenate with DTNB, followed by a spectrophotometric measurement of the thiol groups at 412 nm. Protein oxidation was only measured for experiment I, on the same samples and days as for the TBARS measurement.

Statistical analysis

For experiment I, contrast analysis following analysis of variance (ANOVA) was performed to compare antioxidants or to detect differences between antioxidant extracts and their pure antioxidant components. Also, the effect of antioxidant dose was tested by contrast analysis. Data were analysed separately per sampling day. For the analyses performed in experiment II, a general linear model with fixed factors antioxidant and dose was used. The interaction term was not significant and therefore not included in the model. The Bonferroni post hoc comparison of means test was used to compare antioxidants and polynomial contrast analysis was performed to compare doses. All analyses were performed using the statistical software package S-Plus for Windows (version 6.0) and differences were considered significant at the $P < 0.05$ level.

RESULTS

Average dry matter, crude protein and crude fat content of the patties were 40.9, 16.1 and 20.4%, respectively. The proportions of saturated, monounsaturated, n-6 and n-3 polyunsaturated fatty acids were 37.0, 39.4, 19.5 and 4.2%, respectively.

Experiment I

The antioxidant action of AA, tocopherol, RE and TE is shown in Figs 1, 2 and 3 for colour, lipid and protein oxidation, respectively. No difference in a^* values between AA, tocopherol, RE and TE was found immediately after preparing the patties (d0 fresh). However, subsequently, tocopherol and RE addition resulted in the highest and lowest a^* value respectively ($P < 0.05$) (Fig. 1). Tocopherol and AA significantly lowered MetMb% compared to the control (mean MetMb% on d8 for fresh patties 42.4, 44.1 and 47.3, respectively; $P < 0.05$), whereas TE and RE did not significantly decrease MetMb% (45.3 and 44.9, respectively). Throughout the storage period, TBARS values were higher after AA addition and lower after tocopherol, RE or TE addition for both fresh and frozen patties compared to control patties ($P < 0.05$) (Fig. 2). Overall, TE and RE inhibited lipid oxidation to the same extent, whereas tocopherol was less efficient ($P > 0.05$). The antioxidant treatment did not influence protein oxidation as measured by thiol groups except for d8 after frozen storage, when protein oxidation was lower (thiol groups were higher) for tocopherol, RE and TE as compared to the control group and AA ($P < 0.05$). Hereby, TE was superior to RE, whereas tocopherol was intermediate. There was no difference in thiol groups between the control and the AA treatment (Fig. 3).

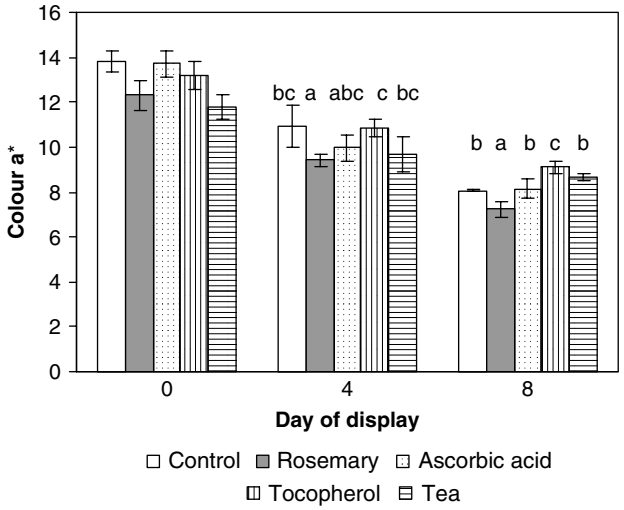

Figure 1. Colour a^* values during illuminated chill storage display of fresh patties after addition of ascorbic acid, tocopherol, rosemary and tea extract. Average values across 100 and 200 ppm dose (error bars represent standard deviations). Within a day, bar charts with different superscripts are significantly different at $P < 0.05$.

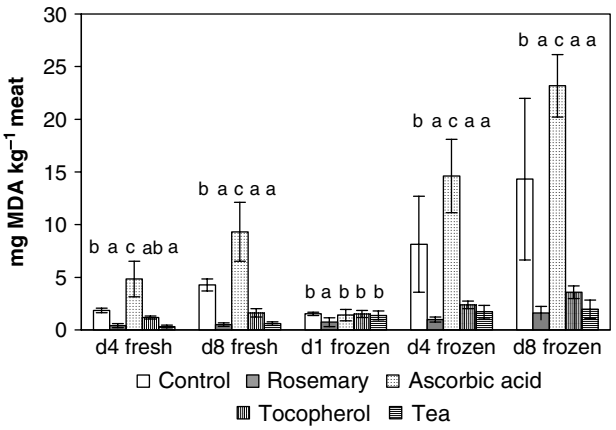

Figure 2. TBARS values (mg MDA kg^{-1} meat) of fresh and frozen patties during illuminated chill storage display after addition of ascorbic acid, tocopherol, rosemary and tea extract. Average values across 100 and 200 ppm dose (error bars represent standard deviations). Within a day, bar charts with different superscripts are significantly different at $P < 0.05$.

Regarding the antioxidant action of the water and lipid soluble analogues of tocopherol and AA (trolox and AP, respectively), the results were as follows: throughout the experiment, AP resulted in an a^* value equivalent to tocopherol and thus higher than AA. The trolox addition resulted in a much lower a^* value than tocopherol, even lower than the control ($P < 0.05$). AA, AP and tocopherol inhibited the formation of MetMb as compared to the control ($P < 0.05$ for the frozen patties and on d8 for the fresh patties), whereas trolox had the opposite effect ($P < 0.05$). TBARS values for the AP treatment were lower than for the control ($P < 0.05$ on d4 and d8 after frozen storage), and thus also lower than for AA (Table 1). Trolox inhibited lipid oxidation more than tocopherol ($P < 0.05$ on d1 after frozen storage) except for d8 after frozen storage, and resulted in lower TBARS values compared to the control at all sampling times (Table 1). Furthermore, on d8 after frozen storage, AP and tocopherol lowered protein oxidation as

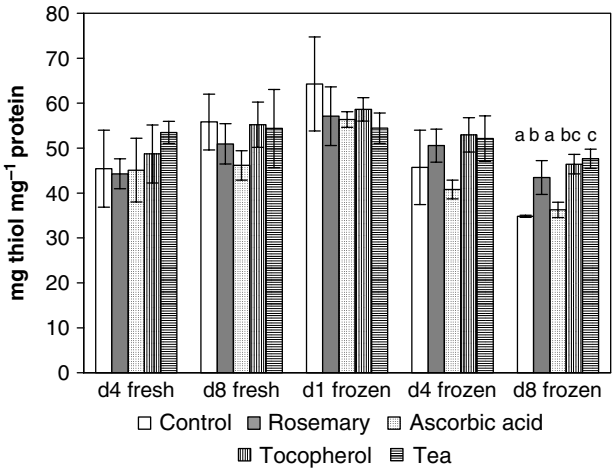

Figure 3. Thiol groups (nmol thiol mg^{-1} protein) of fresh and frozen patties during illuminated chill storage display after addition of ascorbic acid, tocopherol, rosemary and tea extract. Average values across 100 and 200 ppm dose (error bars represent standard deviations). Within a day, bar charts with different superscripts are significantly different at $P < 0.05$.

compared to AA and trolox respectively ($P < 0.05$) (34.8, 36.3, 45.1, 46.4, and 37.1 nmol thiol mg^{-1} protein for control, AA, AP, tocopherol and trolox, respectively).

The antioxidant dose did not influence the extent of oxidation considerably. Only a few differences were noticed. For AA, the MetMb% was lower for the 200 ppm than for the 100 ppm dose, whereas for RE and trolox the opposite was found (e.g. for fresh patties on d8 mean MetMb% for 100 and 200 ppm was 45.4 and 42.7, 43.8 and 45.9, 60.2 and 63.0 for AA, RE and trolox, respectively). Only for AP after frozen storage, there was a dose effect on lipid oxidation, with the highest TBARS value for 200 ppm ($P < 0.05$; e.g. mean value for frozen patties on d8 was 2.36 and 4.46 mg MDA kg^{-1} meat for 100 and 200 ppm, respectively). Furthermore, there was hardly any effect of the antioxidant dose on thiol groups. Only for RE on d8 after frozen storage, thiol groups were lower for the 200 ppm as compared to the 100 ppm dose (46.1 vs. 40.8 nmol thiol groups mg^{-1} protein) ($P < 0.05$). Given the lack of significance for the interaction between antioxidant treatment and dose, it can be said that the antioxidant or pro-oxidant character of a specific antioxidant was unaltered, independent of dose.

The comparison between the antioxidant extracts and their major antioxidant components did not reveal differences in their antioxidant action. Hence, the catechins and the carnosic acid resulted in significantly lower TBARS values compared to the control treatment at most sampling times (Table 1). The minor components of TE, TB + CAF, resulted in markedly lower TBARS values compared to the control treatment ($P < 0.05$ at d8 for the fresh patties and at d4 and d8 for the frozen patties). However, they inhibited lipid oxidation to a smaller extent compared to the TE or the catechins. These differences were not significant due to the high variability encountered.

Table 1. Mean values for TBARS (mg MDA kg^{-1} meat) of fresh and frozen patties during illuminated chill storage display for 1, 4 and 8 days, for ascorbic acid vs ascorbyl palmitate and tocopherol vs trolox (means across 100 and 200 ppm dose) and for tea and rosemary extract (100 ppm) vs their respective components

	Fresh		Frozen		
	Day 4	Day 8	Day 1	Day 4	Day 8
Control	1.85^{a}	4.28^{a}	1.53^{a}	8.15^{a}	14.3^{a}
Hydrophilic vs lipophilic variants					
Ascorbic acid	4.84^{c}	9.32^{c}	1.42^{a}	14.6^{c}	23.2^{c}
Ascorbyl palmitate	1.10^{a}	1.84^{a}	1.57^{a}	2.40^{b}	3.41^{b}
P	<0.05	<0.05	NS	<0.05	<0.05
Tocopherol	1.18^{a}	1.62^{b}	1.50^{a}	2.38^{b}	3.58^{b}
Trolox	0.51^{b}	1.48^{b}	0.61^{b}	0.84^{b}	4.24^{b}
P	NS	NS	<0.05	NS	NS
Extracts vs components					
Tea extract	0.32^{b}	0.60^{b}	1.38^{a}	1.73^{b}	1.97^{b}
Catechins	0.42^{b}	1.62^{a}	1.50^{a}	2.08^{b}	2.69^{b}
Theobromine and caffeic acid	1.15^{a}	2.65^{a}	1.27^{a}	3.14^{b}	7.17^{b}
P	NS	NS	NS	NS	NS
Rosemary extract	0.41^{b}	0.51^{b}	0.74^{b}	0.98^{b}	1.60^{b}
Carnosic acid	0.58^{a}	0.78^{b}	1.27^{a}	1.32^{b}	1.20^{b}
P	NS	NS	NS	NS	NS
RMSE	0.66	1.36	0.37	1.94	2.52

^{a,b,c} Within days, superscript letters refer to: a = not significantly different from the control ($P > 0.05$), b = lower than the control at $P < 0.05$, and c = higher than the control at $P < 0.05$.
NS = $P > 0.05$ for means within days of the comparisons of hydrophilic versus lipophilic variants and extracts versus components

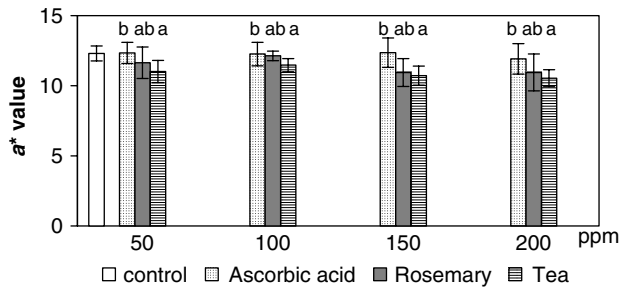

Figure 4. a^* values of fresh patties (d0) after addition of ascorbic acid, rosemary and tea extract at doses of 50 to 200 ppm. Within one dose, bar charts with different superscripts are significantly different at $P < 0.05$ (error bars represent standard deviations).

Experiment II

There was no effect of antioxidant treatment on the a^* value of the patties, except for d0 where the a^* value was lowest for TE, intermediate for RE and highest for AA and the control ($P < 0.05$) (Fig. 4). On d1 and d2, MetMb% was highest for AA ($P < 0.05$; data not shown). No important dose or interaction effect was found for the colour parameters.

For the fresh patties, TBARS values were much higher when AA was added compared to RE or TE ($P < 0.001$; data for d4 in Fig. 5). For the cooked patties, TBARS values were highest for AA, lower for TE and lowest after RE addition ($P < 0.05$; data for d4 in Fig. 5). Only after cooking, was there a linear effect of antioxidant dose on lipid oxidation ($P < 0.05$ and $P < 0.001$ for d4 and d8, respectively) with decreasing TBARS values for increasing doses of RE and TE, and increasing TBARS values for increasing doses of AA.

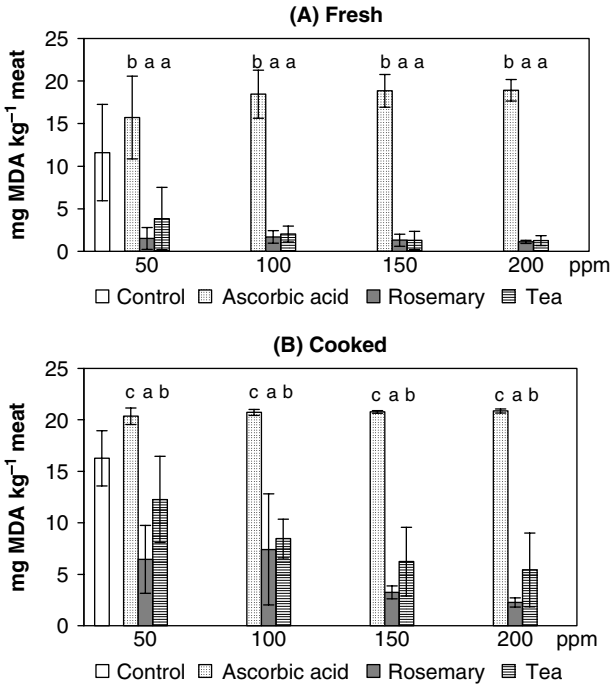

Figure 5. TBARS values (mg MDA kg^{-1} meat) of fresh (A) and cooked (B) patties after 4 days of illuminated chill storage following addition of ascorbic acid, rosemary and tea extract at doses of 50 to 200 ppm. Within one dose, bar charts with different superscripts are significantly different at $P < 0.05$ (error bars represent standard deviations).

DISCUSSION AND CONCLUSIONS

Tocopherol and AP resulted in the best colour stability (a^* value, MetMb%) whereas trolox negatively influenced colour of the patties. The effect of AA on colour stability was inconclusive. AA inhibited the formation of MetMb and was most effective at the highest dose in our first experiment; however, this could not be confirmed in the second experiment. The action of AA as an antioxidant in terms of colour oxidation was reported by Mitsumoto *et al*.[22] There was no obvious effect of the extracts or their individual components on colour of the patties. For TE, this was in line with results of Martinez *et al*.[7] for fresh pork sausages, whereas contrary to our results showing the lowest a^* value for RE, these authors found an improvement of colour by RE. In beef patties packaged in a modified atmosphere, Sanchez-Escalante *et al*.[23] found a highly effective inhibition of metmyoglobin formation by RE, either alone or with AA, whereas AA alone had a lower inhibitory effect on myoglobin oxidation.

Tocopherol, RE and TE strongly inhibited lipid oxidation, whereas AA clearly showed a lipid pro-oxidant character in pork patties. Tocopherol and trolox showed a comparable lipid antioxidant activity. This was somewhat unexpected, given the different isomeric configuration of the analogues – α for trolox vs. α, β, γ and δ for tocopherol – favouring the *in vitro* antioxidant activity of the last.[24] The pro-oxidant character of AA was reverted to antioxidant when it was added as its lipid soluble analogue AP; however, only at the lowest dose (100 ppm). The increased TBARS value for the highest dose of AP indicated a dose-dependent transition towards a lipid pro-oxidant character as reported by Yen *et al*.[13] for AA. The pro-oxidant character of AA seen for lipid oxidation did not appear in terms of protein oxidation, and as already mentioned, neither for colour oxidation. In meat products, sodium ascorbate is more frequently used than AA. The sodium salt is preferred for some applications due to its slower reaction rate with nitrite and the neutral pH value in aqueous solutions. Sodium ascorbate has the same reducing properties as AA. Therefore, it is unlikely that the results of the present study would change if sodium ascorbate had been used instead of AA. For cooked patties, RE was a more effective inhibitor of lipid oxidation compared to TE. For stabilising pork lard and chicken fat, Chen and Chan[25] concluded that RE was less effective than TE. In beef patties, Sanchez-Escalante *et al*.[23] found that RE, either alone or with AA, was very effective in inhibiting lipid oxidation whereas AA had no effect. Although a different antioxidant composition and content of the extracts might be responsible for opposite outcomes in different studies, to our view this also stresses the need to test antioxidants in the food matrix in which they will be applied.

Given the lack of difference in the oxidation inhibiting effect of either the extracts (RE and TE) or their major antioxidant components, it can be said that the antioxidant properties of the extracts can be attributed to these individual components. For tea catechins, this is consistent with Tang *et al*.,[26] reporting on their excellent lipid oxidation inhibiting effect. Despite their low dose, the minor antioxidant components of TE, TB + CAF, clearly lowered lipid oxidation and might therefore together with the catechins contribute to the global oxidation inhibiting effect of the extract. The lack of dose effect for the antioxidants in fresh and frozen patties implies that there was no supplementary lipid oxidation inhibiting effect of any of the antioxidants above the level of 100 ppm. After cooking, however, higher antioxidant doses (as measured up to 200 ppm) more effectively inhibited oxidation.

Throughout the storage period of the fresh patties, there was no indication for a protein oxidation inhibiting effect of the added substances. Moreover, only at the end of the display period of the frozen patties, was it shown that AP and tocopherol were more effective inhibitors of protein oxidation compared to their water soluble analogues and only then was the antioxidant activity of tocopherol, RE and TE as shown for lipid oxidation apparent. This poor protein oxidation inhibiting effect of RE (added at 0.4 g kg^{-1}) corresponds with findings for cooked pork patties.[27] However, it is in contrast to Estevez *et al*.[28] who stated that the addition of rosemary essential oil (0.6 g kg^{-1}) in refrigerated frankfurters inhibited protein oxidation. For TE, no study on the protein oxidation inhibiting effect in meat products could be found to compare with. Furthermore, Vuorela *et al*.[29] reported that polyphenolic plant components were excellent inhibitors of protein oxidation in cooked pork patties. Another explanation might be that more severe protein oxidation was only setting in late during the storage of frozen patties, so that differences between antioxidant treatments became clear only then.

ACKNOWLEDGEMENTS

L. Haak is grateful for the grant by Ghent University. The authors thank RAPS (Beringen, Belgium) and DSM Nutritional Products (Deinze, Belgium) for supplying the antioxidants. S. Lescouhier, S. Coolsaet, D. Baeyens are thanked for their technical support.

REFERENCES

1 Kanner J, Oxidative processes in meat and meat products: Quality implications. *Meat Sci* **36**:169–189 (1994).
2 Pokorny J, Natural antioxidants for food use. *Trends Food Sci Technol* **2**:223–227 (1991).
3 Shahidi F, Antioxidants in food and food antioxidants. *Food* **44**:158–163 (2000).

4 Pellegrini N, Serafini M, Colombi B, Del Rio D, Salvatore S, Bianchi M, *et al*, Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different *in vitro* assays. *J Nutr* **133**:2812–2819 (2003).
5 McCarthy TL, Kerry JP, Kerry JF, Lynch PB and Buckley DJ, Evaluation of the antioxidant potential of natural food/plant extract as compared with synthetic antioxidants and vitamin E in raw and cooked pork patties. *Meat Sci* **57**:45–52 (2001).
6 Rey AI, Hopia A, Kivikari R and Kahkonen M, Use of natural food/plant extracts: cloudberry (*Rubus chamaemorus*), beetroot (*Beta vulgaris* 'Vulgaris') or willow herb (*Epilobium angustifolium*) to reduce lipid oxidation of cooked pork patties. *Food Sci Technol* **38**:363–370 (2005).
7 Martinez L, Cilla I, Beltran JA and Roncales P, Antioxidant effect of rosemary, borage, green tea, pu-erh tea and ascorbic acid on fresh pork sausages packaged in a modified atmosphere: Influence of the presence of sodium chloride. *J Sci Food Agric* **86**:1298–1307 (2006).
8 Halliwell B, The antioxidant paradox. *Lancet* **355**:1179–1180 (2000).
9 Madsen HL, Andersen L, Christiansen L, Brockhoff P and Bertelsen G, Antioxidative activity of summer savory (*Saruteja hortensis* L.) and rosemary (*Rosmarinus officinalis* L.) in minced, cooked pork meat. *Z Lebensm Unters Forsch* **203**:333–338 (1996).
10 Sebranek JG, Sewalt VJH, Robbins KL and Houser TA, Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. *Meat Sci* **69**:289–296 (2005).
11 Gramza A, Khokhar S, Yoko S, Gliszczynska-Swiglo A, Hes M and Korczak J, Antioxidant activity of tea extracts in lipids and correlation with polyphenol content. *Eur J Lipid Sci Technol* **108**:351–362 (2006).
12 Greene BE, Hsin IM and Zipser MW, Retardation of oxidative color changes in raw ground beef. *J Food Sci* **36**:940–942 (1971).
13 Yen GC, Duh PD and Tsai HL, Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. *Food Chem* **79**:307–313 (2002).
14 Higgins FM, Kerry JP, Buckley DJ and Morrissey PA, Effect of dietary alpha-tocopheryl acetate supplementation on alpha-tocopherol distribution in raw turkey muscles and its effect on the storage stability of cooked turkey meat. *Meat Sci* **50**:373–383 (1998).
15 Kerry JP, Buckley DJ, Morrissey PA, O'Sullivan K and Lynch PB, Endogenous and exogenous alpha-tocopherol supplementation: effects on lipid stability (TBARS) and warmed-over flavour (WOF) in porcine M-longissimus dorsi roasts held in aerobic and vacuum packs. *Food Res Int* **31**:211–216 (1998).
16 He Y and Shahidi F, Antioxidant activity of green tea and its catechins in a fish meat model system. *J Agric Food Chem* **45**:4262–4266 (1997).
17 Krzywicki K, Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of beef. *Meat Sci* **3**:1–10 (1979).
18 Lindahl G, Lundström K and Tornberg E, Contribution of pigment content, myoglobin forms and internal reflectance to the colour of pork loin and ham from pure breed pigs. *Meat Sci* **59**:141–151 (2001).
19 Tarladgis BG, Watts BM and Younathan MT, A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J Am Oil Chem Soc* **37**:44–48 (1960).
20 Ellman GL, Tissue sulfhydryl groups. *Arch Biochem Biophys* **82**:70–77 (1959).
21 Batifoulier F, Mercier Y, Gatellier P and Renerre M, Influence of vitamin E on lipid and protein oxidation induced by H_2O_2-activated MetMb in microsomal membranes from turkey muscle. *Meat Sci* **61**:389–395 (2002).
22 Mitsumoto M, Faustman C, Cassens RG, Arnold RN, Schaefer DM and Scheller KK, Vitamins E and C improve pigment and lipid stability in ground beef. *J Food Sci* **56**:194–197 (1991).
23 Sánchez-Escalante A, Djenane A, Torrescano G, Beltrán JA and Roncalés P, The effects of ascorbic acid, taurine, carnosine and rosemary powder on colour and lipid stability of beef patties packaged in modified atmosphere. *Meat Sci* **58**:421–429 (2001).
24 Dziezak JD, Antioxidants:The ultimate answer to oxidation. *Food Technol* **40**:94–102 (1986).
25 Chen ZY and Chan PT, Antioxidative activity of green tea catechins in canola oil. *Chem Phys Lipids* **82**:163–172 (1996).
26 Tang SZ, Sheehan D, Buckley DJ, Morrissey PA and Kerry JP, Anti-oxidant activity of added tea catechins on lipid oxidation of raw minced red meat, poultry and fish muscle. *Int J Food Sci Technol* **36**:685–692 (2001).
27 Salminen H, Estevez M, Kivikari R and Heinonen M, Inhibition of protein and lipid oxidation by rapeseed, camelina and soy meal in cooked pork meat patties. *Eur Food Res Technol* **223**:461–468 (2006).
28 Estevez M, Ventanas S and Cava R, Protein oxidation in frankfurters with increasing levels of added rosemary essential oil: Effect on color and texture deterioration. *J Food Sci* **70**:427–432 (2005).
29 Vuorela S, Salminen H, Makela M, Kivikari R, Karonen M and Heinonen M, Effect of plant phenolics on protein and lipid oxidation in cooked pork meat patties. *J Agric Food Chem* **53**:8492–8497 (2005).

