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Antioxidant potential of synthetic and natural antioxidants and its effect on warmed-over-flavour in different species of meat

K. Jayathilakan *, G.K. Sharma, K. Radhakrishna, A.S. Bawa

Defence Food Research Laboratory, Siddarthanagar, Mysore 570 011, India

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Abstract

The role of natural antioxidants, e.g. Maillard reaction products (MRPs 60 mM/2 h), ascorbic acid (500 ppm), cloves (*Eugenia caryophyllata*) (250 mg/100 g), cinnamon (*Cinnamomum zeylanicum*) (250 mg/100 g) and synthetic antioxidants, e.g. tertiary butyl hydroxy quinone (TBHQ), butylated hydroxy anisole (BHA) and propyl gallate (PG), at 0.02% level each, in controlling the warmed-over-flavour (WOF), and non-haem iron release, as well as their potential in cooked and refrigerated stored meats from three common domestic species (sheep, beef and pork) has been investigated. MRPs and TBHQ showed good antioxidant activity (82–91%) and were significantly different ($P < 0.05$) from the other treatments in all three species. Significantly ($P < 0.05$) lower values of hexanal and non-haem iron were obtained for MRPs and TBHQ treated samples, which showed ability to control WOF during refrigerated storage. Non-linear correlation regression analysis was performed between non-haem iron, WOF values and antioxidant activity in all three species. Exponential fit equations were established for beef and pork, while for sheep, the relationship was found to be polynomial with correlation coefficients ranging from 0.90 to 0.97 for non-haem iron and WOF, respectively. The susceptibility of these species to lipid oxidation was in the order, pork > beef > sheep, and the order of antioxidant activity for the natural antioxidants was MRPs > cloves > ascorbic acid > cinnamon; for synthetics it was TBHQ > BHA > PG.

Keywords: Warmed-over-flavour; Antioxidant activity; Non-haem iron; Lipid oxidation; Maillard reaction products; Species; Synthetic; Natural

1. Introduction

Meat flavour deterioration (MFD) is characterised by increased off-flavour and a decline in desirable flavour attributes (Spanier, St Angelo, & Shaffer, 1992). The process of WOF development in meat products, is attributed to the auto-oxidation of meat lipids, which gives rise to hydroperoxides which, via many different pathways, decompose to a large number of volatile compounds (Gray, Gomma, & Buckley, 1996). This is the primary cause of rancidity during frozen storage of meat (from all species) and meat products (Channon & Trout, 2002). Cooked meat is susceptible to lipid oxidation and phospholipids are the primary contributors to lipid oxidation and WOF development (Gandemer, 1999; Mottram, 1991; Pearson & Gray, 1983). There is also a significant variation in total phospholipid content among species, pork being the highest and sheep the lowest (Mottram, 1991).

Flavour is an important quality attribute of muscle foods. The flavour of meat is subject to variability due to both intrinsic and extrinsic factors and it affects the overall acceptability of foods. These factors are of utmost importance because they influence the judgement of the consumer, even before the food is consumed (Shahidi, 2005). The shelf life and acceptability of processed, ready-to-eat uncured meats is limited because of the rapid onset of rancidity, denoted as WOF. This becomes evident during the refrigerated storage of cooked meat products within a few days (Vasundhara & Honikel, 1992).

* Corresponding author. Tel.: +91 0821 2473425; fax: +91 0821 2473468.
E-mail address: dfrlmysore@sancharnet.in (K. Jayathilakan).

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Several adverse health effects, due to the presence of reactive oxygen species (ROS), such as superoxide radical (O_2'), hydroxyl radical (OH′), peroxyl radical (ROO′), during lipid oxidation, have been reported (Fisch, Bohm, Wright, & Konig, 2003; Nakamura, Watanabe, Miyake, Kohno, & Osawa, 2003; Shon, Kim, & Sung, 2003; Valentao et al., 2002). Products of lipid oxidation also interfere with the absorption of protein or folic acid and it has also been found that they can cause pathological changes in the mucous membranes of the digestive tract (Karpinska, Borowski, & Ozieqicz, 2001). To avoid or delay the autoxidation process in the meat, synthetic and natural antioxidants have been successfully utilised (McCarthy, Kerry, Kerry, Lynch, & Buckley, 2001). There is an increasing interest in natural antioxidants because of the safety and toxicity problems of synthetic antioxidants (BHA, TBHQ and PG) that are commonly used in lipid containing/rich foods (Amarowicz, Naczk, & Shahidi, 2000; Ito et al., 1986; Van Esh, 1986).

Natural antioxidants are primarily plant phenolics that may occur in all parts of plants, such as fruits, vegetables, nuts, seeds, leaves, roots and barks (Pratt & Hudson, 1990). Plant phenolics are multifunctional and can act as reducing agents, free radical terminators, metal chelators and singlet oxygen quenchers (Mathew & Abraham, 2006). Many natural antioxidants have already been isolated from different kinds of plants, such as oilseeds, cereal crops, vegetables, leaves, roots, spices and herbs (Ramarathnam, Osawa, Ochi, & Kawakishi, 1995; Shon et al., 2003; Wettasinghe & Shahidi, 1999). All the phenolic classes have the structural requirements of free radical-scavengers and have potential as food antioxidants (Bandoniene & Murkovic, 2002). Besides this, MRPs formed from the condensation of sugar and amino acids have been reported to possess promising antioxidative activity (Jayathilakan & Sharma, 2006; Mastrocola & Munari, 2000; Morales & Jimenez, 2001). The Maillard reaction produced from an amino acid sugar model system has been associated with the formation of compounds with strong antioxidative activity (Tanaka, Chui, Nagashima, & Taguchi, 1990; Yen & Hsieh, 1995; Yoshimura, Lijima, Watanabe, & Nakazawa, 1997). Antioxidant activity of MRPs derived from a protein-sugar system has also been studied (Jing & Kitts, 2002; Yeboab, Alli, & Yaylauan, 1999). There are reports about the use of preformed MRPs as antioxidants in cooked meats from different species (Bedinghans, 1994; Jayathilakan, Vasundhara, & Kumudavally, 1997). Many meat research scientists have recently made concerted efforts to use L-ascorbic acid as an antioxidant. Use of ascorbic to control lipid peroxidation has been reported in some species of meat (Kim, Lee, & Sung, 1997; Mitsumoto et al., 1991; Okayama, Imai, & Yamanoue, 1987; Sahoo & Anjaneyulu, 1997).

The present study was undertaken to establish the antioxidative potential of natural antioxidants, such as MRPs, ascorbic acid and spices, in sheep, beef and pork in comparison with synthetic antioxidants. Evaluation of the markers, e.g. WOF and non-haem iron, in the presence of antioxidants, elucidates the antioxidant activity. Identifying a natural antioxidant with antioxidant potential similar to those of synthetics, will facilitate the usage of more potent natural antioxidants for meat and meat products, with better shelf stability in terms of decreased WOF and lipid peroxidation. Attempts were also made to correlate the parameters and establish the best fit equations with respect to species variations.

2. Materials and methods

2.1. Materials/chemicals

2.1.1. Meat samples

Fresh mutton (sheep meat) beef and pork (leg portion, 2–3 h *post mortem*) were purchased from the local market, washed thoroughly under running water, deboned, minced and used for analysis.

2.1.2. Spices

The spices (cloves and cinnamon) were obtained from the local market, powdered finely and incorporated into the meat samples from all the species before cooking and refrigerated storage.

2.1.3. Reagents and chemicals

All the chemicals and reagents used in the study were of analytical grade and procured from Sigma Chemicals Corporation, USA and BDH India. The synthetic antioxidants (TBHQ, BHA and PG) employed in the evaluation were obtained from Loba chemie Pvt. Ltd., Mumbai, India. Ascorbic acid was procured from Sarabhai M Chemicals, Baroda, India.

2.2. Preparation of MRPs

MRPs were prepared as described by Jayathilakan and Sharma (2006), by refluxing 60 mM concentration of glucose and lysine in 100 ml of water for 2 h over a sand bath maintained at 100–110 °C and the final volume was maintained by addition of water (100 ml). Several batches of MRPs were prepared by the standardized procedure for this investigation.

2.3. Sample preparation

Synthetic antioxidants (TBHQ, BHA and PG) were incorporated at the permitted level (0.02%). Natural antioxidants, e.g. MRPs (60 mM/2 h), ascorbic acid (500 ppm), and finely powdered spices (250 mg/100 g) cinnamon and cloves were separately added to minced meat samples and thoroughly mixed. Three further sets of samples were prepared for evaluation after 2, 4 and 6 days storage at 5 °C. Treated samples were packed in polypropylene pouches.

2.4. Cooking and storage of samples

All the meat samples, after treatment with natural and synthetic antioxidants (4 sets), were packed in polypropylene pouches (15″ × 9″) and cooked in boiling water bath under atmospheric pressure for 35 min and cooled to room temperature. One set of samples was taken for immediate analysis and the other three were kept at 5 °C for evaluation after 2, 4 and 6 days. All the experiments were repeated five times on the different sets of samples and the values expressed as means ± SD.

2.5. Chemical analysis

The antioxidant potential, expressed in terms of percentage of antioxidant activity, was calculated by the equation (Wijewickreme & Kitts, 1998):

$$\%\,\mathrm{AOA} = \frac{[\text{TBARS value of the control} - \text{TBARS of the test sample}] \times 100}{[\text{TBARS value of the control}]}$$

TBARS values were expressed as mg malonaldehyde/kg sample and estimated colorimetrically using 2-thiobarbituric acid (Taraldgis, Watts, Younathan, & Dugan, 1960) with a chemito UV-visible spectrophotometer, model 160, Chemito Instruments, India. 20 g of blended sample, with 2.5 ml of concentrated HCl and 97.5 ml of distilled water, after adjusting the pH to 1.5, were distilled and 20 ml of the distillate were treated with 5 ml of TBA reagent. After boiling for 35 min the optical density was measured at 538 nm. Along with the samples, separate aliquots of standard tetraethoxy propane were taken. These were treated in the same manner as above and TBARS values were calculated using a standard curve.

Fat extraction from the samples was carried out by the method of Folch, Lees, and Sloane Stanley (1957). Fat was extracted using 1:1 and 2:1 chloroform:methanol mixture (v/v). The filtrate was treated with 0.88% aqueous potassium chloride and then with anhydrous sodium sulphate. The concentrate fat was obtained by heating in a rotary vacuum evaporator to constant weight.

WOF profile, expressed in terms of mg of *n*-hexanal/100 g fat, was monitored by the method of Benca and Mitchela (1954). Five gram of the sample were extracted in 50 ml of carbonyl free benzene and 5 ml of the sample filtrate were treated with 3 ml of 3–4% trichloracetic acid in benzene and 5 ml of 0.05% DNPH solution in benzene and incubated at 60 °C for 30 min. After cooling, 4% alcoholic KOH was added and volume made up to 50 ml with ethanol. After 10 min, absorbance was read at 480 nm. A standard curve was drawn, using hexanal in 5 ml benzene. WOF, in terms of hexanal, was calculated with the help of the standard curve.

The catalytic activity of non-haem iron in the presence of antioxidants was estimated by the method of Igene, Yamauchi, Pearson, and Gray (1985). The estimation was carried out by first precipitating the bound haem iron with 10 ml of 40% trichloracetic acid, following centrifugation. The supernatant was removed for determination of free non-haem iron by the colorimetric method using 1,10-phenanthroline.

2.6. Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) and Duncan's multiple range test to evaluate the statistical significance of the treatments and significance was established at $P < 0.01$. Regression analysis for the correlation between antioxidant activity, WOF and non-haem iron was performed and best fit equations as well as correlation coefficients were established using the software curve Expert-1.3 (Hyams, 2003).

3. Results and discussion

3.1. Evaluation of the antioxidant activity of natural and synthetic antioxidants

Data on the antioxidant potential of natural and synthetic antioxidants in cooked meats from sheep, beef and pork, refrigerated at 5 °C for 6 days are depicted in Tables 1–3 respectively. From the Tables it can be seen that MRPs and TBHQ exhibited good antioxidant activity (82–91%) in all three species initially and during storage at 5 °C. MRPs produced good antioxidant activity in cooked refrigerated stored chicken samples (Jayathilakan et al., 1997). The reduction in antioxidative potential of these two antioxidants during storage was not significantly different ($P > 0.01$) among three species, indicating the ability of MRPs in controlling the lipid oxidation by scavenging the free radicals (Benjakul, Lertittikul, & Bauer, 2005) and chelating the metallic catalysts (Wijewickreme & Kitts, 1998). Cloves exhibited a higher antioxidant activity ($P < 0.05$) than did BHA, PG, ascorbic acid or cinnamon. The effectiveness of cloves as an antioxidant in refrigerated stored meat samples was earlier reported by Jayathilakan et al. (1997). Within the treatments, there was a significant difference ($P < 0.01$) observed between MRPs & cinnamon and TBHQ & cinnamon, initially and during storage for 6 days. Within the species, pork was found to be more susceptible to lipid oxidation than were beef and sheep meat. The antioxidant activity values were comparatively lower

Table 1
Antioxidant activity (%)[a,b,c] of natural and synthetic antioxidants in meat from sheep during storage at 5 °C ($n = 5$)

Treatment	Storage period (days)			
	0	2	4	6
Cloves	80.5[c] ± 2.98	78.2[c] ± 3.69	73.9[c] ± 3.36	68.2[c] ± 3.85
Cinnamon	58.4[b] ± 2.94	52.5[b] ± 4.26	45.7[b] ± 3.91	37.2[b] ± 4.16
MRP's	96.3[a] ± 3.16	95.1[a] ± 4.14	93.05[a] ± 3.62	90.5[a] ± 4.05
Ascorbic acid	73.8[c] ± 5.05	71.9[c] ± 3.91	70.9[c] ± 2.90	67.4[c] ± 3.81
TBHQ	98.2[a] ± 1.51	95.1[a] ± 3.06	93.2[a] ± 4.26	91.0[a] ± 4.36
BHA	71.2[c] ± 3.60	60.6[b] ± 3.16	51.5[b] ± 2.61	42.9[b] ± 4.15
PG	68.3[c] ± 4.89	57.9[b] ± 3.86	49.9[b] ± 3.26	40.6[b] ± 2.16

[a] Means ± standard deviation (SD).
[b] Within the column, values superscripted with different letters are significantly different. ab ($p < 0.01$), ac and bc ($p < 0.05$).
[c] Within the rows, cinnamon and PG at 4 and 6 days were significantly different ($p < 0.01$) from initial values. Other treatments were not significantly different ($p > 0.01$) during storage.

Table 2
Antioxidant activity (%)[a,b,c] of natural and synthetic antioxidants in beef during storage at 5 °C ($n = 5$)

Treatment	Storage period (days)			
	0	2	4	6
Cloves	78.3[c] ± 3.86	75.2[c] ± 3.15	70,7[c] ± 3.69	64.4[c] ± 2.89
Cinnamon	58.3[b] ± 3.15	49.4[b] ± 2.91	42.4[b] ± 3.16	33.2[b] ± 1.89
MRP's	93.9[a] ± 3.25	90.3[a] ± 2.49	88.4[a] ± 2.80	86.6[a] ± 3.91
Ascorbic acid	72.3[c] ± 3.61	69.5[c] ± 3.86	65.1[c] ± 3.10	57.7[c] ± 2.16
TBHQ	96.3[a] ± 2.08	93.5[a] ± 3.16	91.6[a] ± 2.98	88.6[a] ± 4.19
BHA	65.3[b] ± 4.10	55.2[b] ± 2.16	47.4[b] ± 2.05	39.4[b] ± 3.89
PG	63.6[b] ± 2.09	54.1[b] ± 4.09	46.4[b] ± 1.98	38.7[b] ± 1.98

[a] Means ± standard deviation (SD).
[b] Within the column, values superscripted with different letters are significantly different. ab ($p < 0.01$), ac and bc ($p < 0.05$).
[c] Within the rows, BHA values at 4 and 6 days are significantly different ($p < 0.01$) from initial values. Ascorbic acid, after 6 days storage is significantly different ($p < 0.01$) from initial values. Other treatments were not significantly different ($p > 0.01$) during storage.

Table 3
Antioxidant activity (%)[a,b,c] of natural and synthetic antioxidants in pork during storage at 5 °C ($n = 5$)

Treatment	Storage period (days)			
	0	2	4	6
Cloves	72.2[c] ± 4.28	68.2[c] ± 2.23	65.3[c] ± 2.86	62.5[c] ± 3.16
Cinnamon	54.8[b] ± 1.89	43.9[b] ± 2.98	36.0[b] ± 1.98	31.4[b] ± 3.61
MRP's	90.2[a] ± 4.10	88.3[a] ± 3.81	85.5[a] ± 2.48	83.0[a] ± 2.46
Ascorbic acid	62.5[c] ± 4.61	65.2[c] ± 3.11	59.4[c] ± 2.98	55.4[c] ± 3.16
TBHQ	94.1[a] ± 3.10	90.5[a] ± 2.11	90.3[a] ± 3.19	86.4[a] ± 3.19
BHA	60.3[b] ± 2.85	51.3[b] ± 1.98	43.7[b] ± 3.98	35.4[b] ± 4.18
PG	58.5[b] ± 2.63	50.1[b] ± 3.09	42.8[b] ± 2.63	34.2[b] ± 1.98

[a] Means ± standard deviation (SD).
[b] Within the column, values superscripted with different letters are significantly different. ab ($p < 0.01$), ac and bc ($p < 0.05$).
[c] Within the rows, BHA values at 4 and 6 days were significantly different ($p < 0.01$) from initial values. Other treatments during storage were not significantly different ($p > 0.01$).

for pork, indicating the particular susceptibility of pork fat to oxidation compared to those of beef and sheep (Buckley et al., 1989; Kanner, 1994; Channon & Trout, 2002). The unsaturation level in fatty acids affects the lipid oxidation profile. The percentage of unsaturated fatty acids is greatest in pork and least in sheep as in the report of Enser, Hallett, Hewitt, Fursey, and Wood (1996). Cinnamon, among natural antioxidants, and PG, among synthetic antioxidants, showed the lowest antioxidant potential in all three species initially and a significant reduction ($P < 0.01$) was observed in antioxidant potential. The order of antioxidant activity for the natural antioxidants was, MRPs > cloves > ascorbic acid > cinnamon, while, for synthetic antioxidants, the order was TBHQ > BHA > PG. From the data obtained, it could also be observed that MRPs, cloves and ascorbic had antioxidant potential almost similar to synthetic antioxidants TBHQ, BHA and PG, respectively, and could be effectively employed as their substitutes for enhancing the shelf life of meat products by controlling lipid oxidation.

3.2. WOF profile of the three species treated with antioxidants during refrigerated storage

WOF profile of sheep meat, beef and pork, with natural and synthetic antioxidants during refrigerated storage for 6 days, was evaluated and the data expressed in terms of mg of hexanal/100 g fat, are shown in Figs. 1–3, respectively. During oxidative degradation of unsaturated fatty acids, present in the meat systems, undesirable flavour and odour occur, due to the formation of aldehydes, ketones and alcohols (Drumm & Spanier, 1991). Expressing these in terms of WOF profile, by determining the hexanal content, is an important tool for evaluating the development of the same during peroxidation of lipids.

From the Figures it could be observed that it is possible to decrease the oxidation significantly ($P < 0.05$), and thereby WOF development of the meat samples during refrigerated storage, compared to control. Within the treatments, MRPs and TBHQ were very effective in decreasing the WOF development and the hexanal values in fresh meat samples and, after storage, did not differ significantly ($P > 0.01$) among the three species. The effectiveness of TBHQ in retarding lipid oxidation in restructured beef steaks was earlier reported by Crackel, Gray, Booren, Pearson, and Buckley (1988). MRPs have also been found to contain stable free radicals and it is possible that these interact with the peroxy radicals, causing an inhibition of the lipid oxidation (Bailey, Shin-Lee, Dupuy, St Angelo, & Vercelloti, 1987; Namiki & Hayashi, 1975). Cloves produced a greater decrease in oxidation and thereby WOF than did BHA, PG, ascorbic acid or cinnamon, indicating the positive effect of spices and herbs in controlling the same by inhibiting deteriorative changes in lipids (Barbut, Josephson, & Maurrer, 1985). In all the three species of meat, significant variation ($P < 0.01$) in the values of hexanal was observed between MRPs and TBHQ, with all the three control samples and cinnamon, further substantiating, the effectiveness of MRPs and TBHQ in sheep, beef and pork, as well as their ability to inhibit the WOF. The hexanal values of ascorbic acid also showed significant changes ($P < 0.05$), underlining the ability of ascorbic acid to effectively reduce the WOF during refrigerated storage in the three species of meat. The ability of ascorbic acid to inhibit the oxidation of lipids, by interfering in the chain propagation step, was earlier reported by Kim, Cho, Lee, Kim, and Sung (1998). The trend in the results of WOF values was in accordance with the values for antioxidant activity of these treatments. The percentage of antioxidant activity showed a direct correlation with WOF in all three species and MRPs among the natural antioxidants and TBHQ, among the synthetic antioxidants, elucidated the effectiveness (Figs. 1–3) in arresting the WOF development, as reflected by their antioxidant activities (Tables 1–3). Cloves, ascorbic acid and cinnamon were also effective in retarding the WOF development, thus indicating the usefulness of these natural antioxidants as substitutes for synthetic antioxidants.

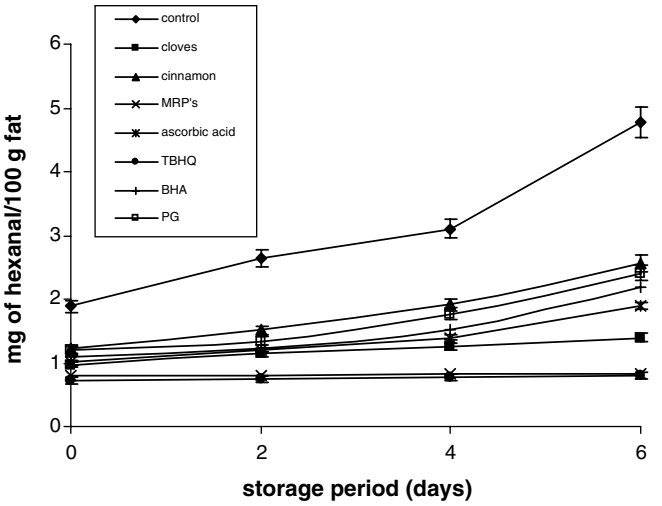

Fig. 1. WOF profile (mg of hexanal/100 g fat) of meat from sheep in the presence of synthetic and natural antioxidants during storage at 5 °C ($n = 5$). TBHQ and MRP's are significantly different ($p < 0.01$) at 0, 2, 4, and 6 days with control. Cloves, ascorbic acid, BHA, PG and cinnamon are significantly different ($p < 0.05$) at 0, 2, 4, and 6 days, for control. MRP's and TBHQ's are significantly different ($p < 0.05$) at 4 and 6 days, from PG and cinnamon.

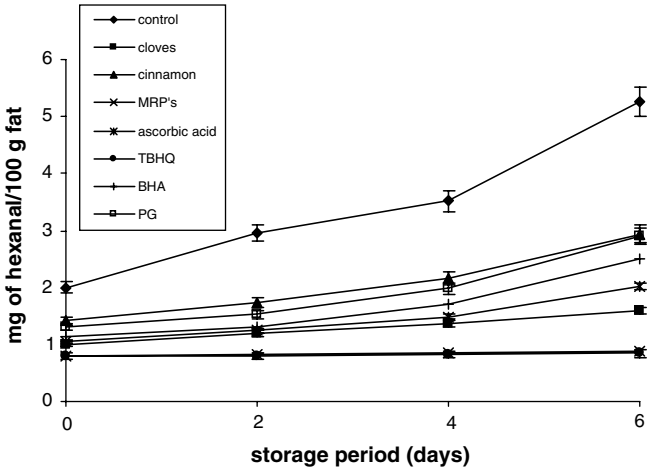

Fig. 2. WOF profile (mg of hexanal/100 g fat) of beef in the presence of synthetic and natural antioxidants during storage at 5 °C ($n = 5$). TBHQ and MRP's are significantly different ($p < 0.01$) at 0, 2, 4, and 6 days with control. Cloves, ascorbic acid, BHA, PG and cinnamon are significantly different ($p < 0.05$) at 0, 2, 4, and 6 days, for control. MRP's and TBHQ's are significantly different ($p < 0.05$) at 4 and 6 days, from PG and cinnamon.

3.3. Evaluation of the catalytic activity of non-haem Fe in the presence of natural and synthetic antioxidants in sheep meat, beef and pork

Figs. 4–6 depict the data pertaining to the non-haem iron for all seven treatments (cloves, cinnamon, MRPs, ascorbic acid, TBHQ, BHA and PG) and for the control samples of sheep, beef and pork, respectively, during refrigerated storage. Since lipid oxidation is a major problem in meat, much effort has been devoted to identifying the catalysts of lipid oxidation in meat (Wettasinghe & Shahidi,

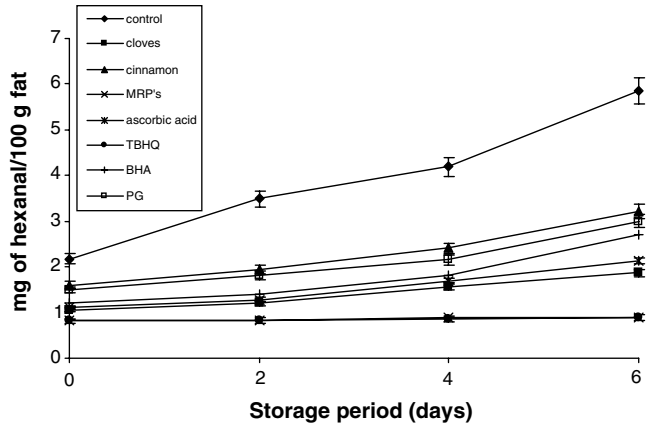

Fig. 3. WOF profile (mg of hexanal/100 g fat) of pork in the presence of synthetic and natural antioxidants during storage at 5 °C ($n = 5$). TBHQ and MRP's are significantly different ($p < 0.01$) at 0, 2, 4, and 6 days with control. Cloves, ascorbic acid, BHA, PG and cinnamon are significantly different ($p < 0.05$) at 0, 2, 4, and 6 days, for control. MRP's and TBHQ's are significantly different ($p < 0.05$) at 4 and 6 days, from PG and cinnamon.

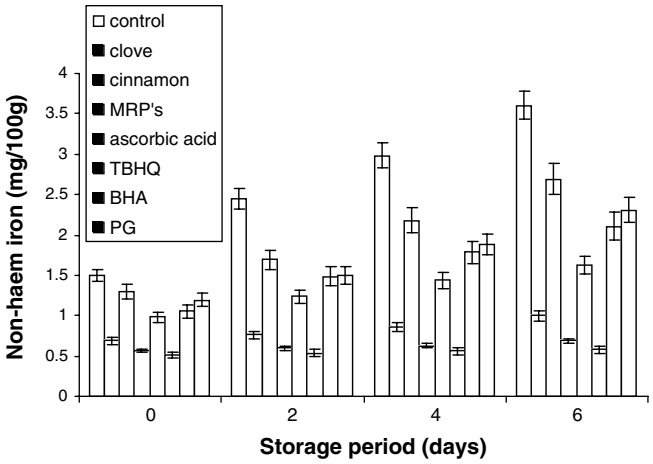

Fig. 5. Non-haem iron values of beef in the presence of natural and synthetic antioxidants during storage at 5 °C ($n = 5$). Control is significantly different ($p < 0.05$) from all the treatments. MRP's are significantly different ($p < 0.01$) from other treatments, within the storage period.

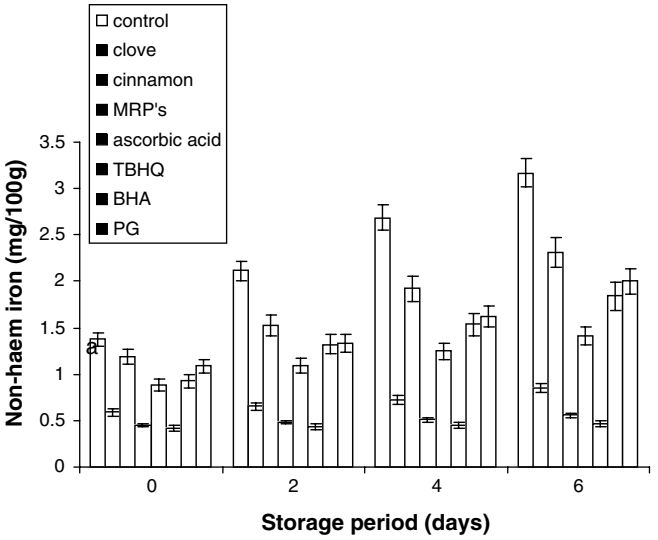

Fig. 4. Non-haem iron values of meat from sheep in the presence of natural and synthetic antioxidants during storage at 5 °C ($n = 5$). Control is significantly different ($p < 0.05$) from all the treatments. MRP's are significantly different ($p < 0.01$) from other treatments, within the storage period.

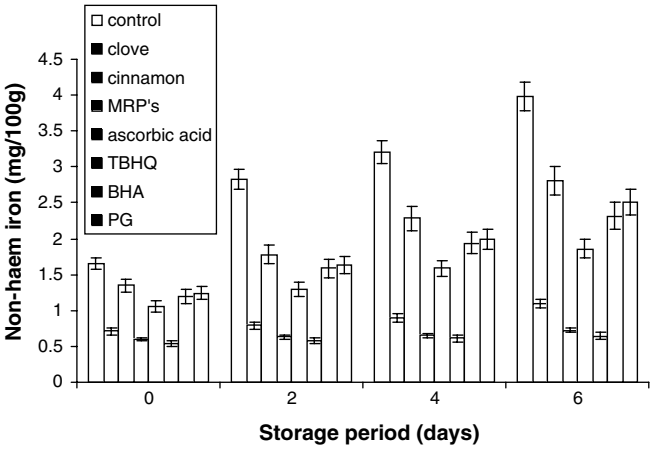

Fig. 6. Non-haem iron values of pork in the presence of natural and synthetic antioxidants during storage at 5 °C ($n = 5$). Control is significantly different ($p < 0.05$) from all the treatments. MRP's are significantly different ($p < 0.01$) from other treatments, within the storage period.

1997). It has been generally accepted that iron, in some form, promotes the oxidation of meat lipids. Within the various forms of iron non-haem iron has been reported to play a major role in accelerating lipid oxidation in cooked meat (Chem, Pearson, Gray, Fooladi, & Ku, 1984). The release of non-haem iron in the presence of salt and its role in enhancing lipid oxidation in pork were studied by Hsing-Feng-Lin (1997). Therefore, the release of non-haem iron, after cooking and refrigerated storage of the three species in the presence of natural and synthetic antioxidants, was evaluated, as presented in Figs. 4–6.

The increase in the values of non-haem iron is clear, indicating the catalytic activity in promoting the lipid oxidation and WOF. From the Figures it could be seen that, as in earlier findings, the treatments with MRPs and TBHQ showed smaller values for non-haem iron, in all the species, but were not significantly different ($P > 0.01$) during refrigerated storage for 6 days. The value for cloves was different ($P < 0.05$) from those of BHA, PG, ascorbic acid and cinnamon, indicating the ability of cloves to inhibit the release of non-haem iron during cooking and storage in all the three species. From the data depicted in Figs. 4–6, it could be concluded that all the antioxidants had significant ability ($P < 0.05$) to inhibit/suppress the catalytic activity of non-haem iron and thus the WOF and rancidity of the meat samples when compared to control samples of all

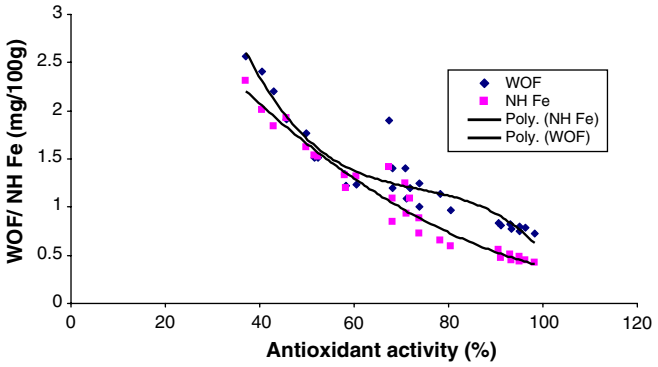

Fig. 7. Variation in WOF and NH Fe in the presence of synthetic and natural antioxidants in meat from sheep.

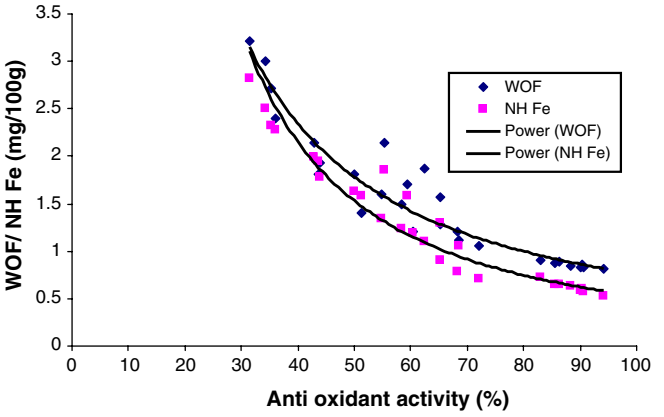

Fig. 9. Variation of WOF and NH Fe in the presence of synthetic and natural antioxidants in pork meat.

three species. Earlier studies on the catalytic activity of non-haem iron in chicken samples also yielded similar results (Jayathilakan et al., 1997). Among the species, pork was found to be more susceptible to the catalytic action of non-haem iron, as indicated by the higher values in all treatments. These findings are in accordance with the earlier results on antioxidant activity (Tables 1–3) and WOF (Figs. 1–3). The susceptibility of this species may be due to the greater concentration of polyunsaturated fatty acids, which are the main substrate for lipid oxidation and WOF (Kanner, 1994; Channon & Trout, 2002). From the results, it could be concluded that MRPs, cloves and ascorbic acid could act as good inhibitors of the catalytic activity of non-haem iron in all the three species and could well substitute for synthetic antioxidants. The WOF profile and the non-haem iron values clearly established the antioxidative activity of these additives.

3.4. Correlation of WOF and non-haem iron with antioxidant activity in sheep meat, beef and pork during storage

To establish a correlation between the different parameters studied, e.g. antioxidant activity, WOF profile and non-haem iron, in the three species of meat, non-linear correlation regression analysis was performed. Regressions of the data in Figs. 1–6 were carried out in relation to the data in Tables 1–3, using the software curve expert 1.3; these are represented in Figs. 7–9. Exponential fit equations were established for beef and pork and polynomial equations for sheep. The equations for the variation of WOF and non-haem iron with antioxidant activity in beef were $y = 5.1292e^{-0.0202x}$ and $y = 6.3806e^{-0.0264x}$ with correlation coefficients of 0.92 and 0.97, respectively, and for pork $y = 220.54x^{-1.2318}$ and $y = 602.49x^{-1.5275}$, with correlation coefficients of 0.92 and 0.94, respectively. The polynomial equations for the variation of WOF and non-haem iron with antioxidant activity for sheep meat were found to be $Y = -2E-05x^3 + 0.0048x^2 - 0.3613x + 10.55$ and $Y = 0.003x^2 - 0.065x + 4.2471$, with correlation coefficients of 0.96 and 0.95, respectively. From the regression analysis, it could be concluded that positive correlation existed for WOF and non-haem iron with antioxidant activity in all the three species. Evaluation of these parameters and the establishing of the correlation pattern clearly indicate the lipid oxidation profile of these species with the antioxidants.

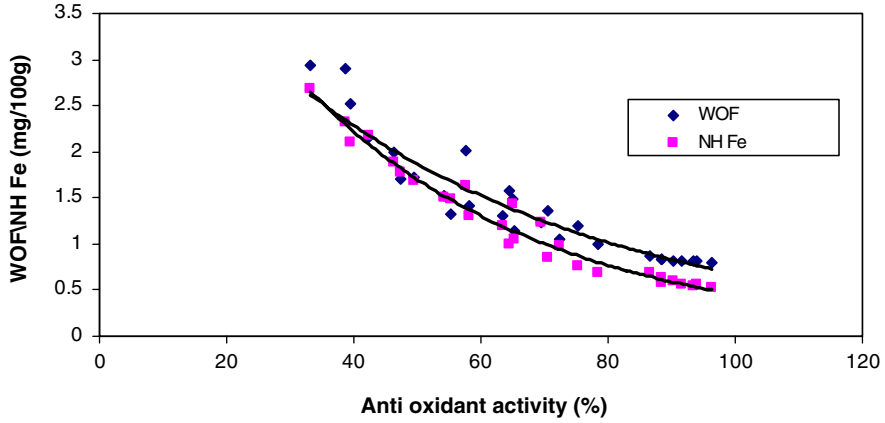

Fig. 8. Variation of WOF and NH Fe in the presence of synthetic and natural antioxidants in beef meat.

4. Conclusions

The studies revealed variation in the antioxidant potential of various synthetic and natural antioxidants in three common domestic meat species (sheep, beef and pork). The importance of early MRPs as natural antioxidants and their potential to control WOF, lipid oxidation and inhibition in the release of non-haem iron, equivalent to TBHQ in all the species of meat, has been established in the study. MRPs, cloves and ascorbic acid could well be used as substitutes for the synthetic antioxidants TBHQ, BHA and PG, respectively to control the WOF development in these meat species. Individual antioxidant potentials of natural and synthetic antioxidants were established with reference to the three species and correlation with WOF profile and non-haem iron catalysis. WOF and non-haem iron could be taken as markers for the evaluation of antioxidant activity. The data obtained for the non-haem iron and WOF have illuminated the inhibition mechanisms of the antioxidants in the oxidation of lipids during refrigerated storage of these meat species. Regression analysis of the data on antioxidant activity with WOF and non-haem iron revealed exponential fit equations for beef and pork and a polynomial equation for sheep. The susceptibility of these species to lipid oxidation was in the order, pork > beef > sheep and the order of antioxidant activity for the natural antioxidants was MRPs > cloves > ascorbic acid > cinnamon; for synthetic antioxidants, it was TBHQ > BHA > PG.

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