Evaluation of the Antioxidant Potential of Hyssop (*Hyssopus officinalis* L.) and Rosemary (*Rosmarinus officinalis* L.) Extracts in Cooked Pork Meat

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ABSTRACT: The objective of this work was to study the ability of rosemary and hyssop extracts to inhibit lipid oxidation and metmyoglobin formation in pork meat, thereby stabilizing meat color. We also evaluated their effects on iron release from the heme moiety of pork meat. Meat samples were blended with hyssop and rosemary extracts and cooked. The cooked meat was chopped into pieces and stored for 8 d at 4 °C. Heme iron, TBA values, metmyoglobin percentage, and meat color were calculated. Hyssop and rosemary were seen to inhibit lipid oxidation and degradation of heme pigments caused by cooking and storage. Both spices delayed metmyoglobin formation and stabilized the red meat color during storage of the cooked meat.

Keywords: rosemary, hyssop, antioxidant, meat, lipid oxidation

Introduction

PIGMENT AND LIPID OXIDATION ARE MAJOR DETERIORATIVE REACTIONS in meat and meat products during storage. They are responsible for a significant loss in quality characteristics such as color, flavor, texture, and nutritive value (Wilson and others 1976; Akamittath and others 1990).

In fresh meat and meat products, color is a strong indicator of quality. The color of meat is thought to be due to 3 factors: the concentration of heme pigments, the chemical state of these pigments, and the physical light-scattering properties of the meat structure (MacDougall 1983). Several factors (packaging, oxygen tension, bacteria, pH, temperature, and so forth) affect meat color stability (Cornforth 1994). Oxidation of heme iron from the ferrous state in deoxymyoglobin (Mb) and oxymyoglobin (OMb) to the ferric state in metmyoglobin (MMb) produces the brownish-red color which consumers find undesirable.

Several authors have postulated that pigment and lipid oxidation are interrelated (Anton and others 1993; Yin and Faustman 1993). The presence of free fatty acids (Stewart 1990) or muscle phospholipids (Genot and others 1991) enhances OMb autoxidation. Superoxide anions released from oxidized OMb can generate hydrogen peroxide and hydroxyl radicals, which are potent phospholipid prooxidants (Kanner and others 1990). Radicals generated by lipid oxidation promote the accumulation of MMb (Anton and others 1993; Yin and Faustman 1993).

The rate of lipid oxidation in meat products can be effectively retarded by the use of antioxidants. Although synthetic antioxidants have been widely used in the meat industry to inhibit the development of lipid oxidation (Chastain and others 1982; Chen and others 1984), their use is tending to diminish because of growing concern among consumers about such chemical additives.

This concern has led to increased interest in the use of natural antioxidants. For example, the natural products isolated from spices or aromatic herbs can act as antioxidants and stabilize meat color, thus extending shelf-life of meat and meat products (Greene and others 1971; Mitsumoto and others 1991).

Extracts of aromatic herbs have been described as useful natural foods additives by several authors (Barbut and others 1985; Lai and others 1991; Basaga and others 1997; Murphy and others 1998). Rosemary has been reported to contain certain components (rosmanol, rosmariquinone, rosmaridiphenol, carnosol) which may be up to 4 times as effective as butylated hydroxy anisole (BHA), and equal to butylated hydroxy toluene (BHT) as an antioxidant (Houlihan and others 1984, 1985; Nakatani and Intani 1984). The antioxidative properties of rosemary have been examined in several meat products (Ho and others 1983; Barbut and others 1985; Lai and others 1991), but no data are available for their effects on color, metmyoglobin formation, or the degradation of heme pigments in pork meat. No reference has been found on the use of hyssop as an antioxidant in meat.

The objective of this work was to study the ability of rosemary and hyssop extracts to inhibit lipid oxidation and metmyoglobin formation in pork meat, thereby stabilizing meat color. We also evaluated their effects on iron release from the heme moiety of pork meat.

Material and Methods

Preparation of rosemary and hyssop extracts

Hyssopus officinalis was collected from a commercial plantation located at Castalla (Alicante), Spain, and harvested in summer; *Rosmarinus officinalis* was wild-growth and harvested during summer in Alhama de Murcia (Murcia), Spain. Both towns are located in southeast Spain. For extractive purposes, the leaves and secondary branches were used.

The plant material was dried at 60 °C in an oven (Selecta S.A., Barcelona, Spain) until of constant weight and then ground. Samples were extracted with dimethyl sulfoxide (DMSO) (40 mg of dry weight/mL of DMSO) for 5 h at room temperature with occasional stirring, then left overnight. The mixture was filtered through a filter paper (Albet 385094, Filalbet S.A., Barcelona, Spain) and the extracts were stored at –20 °C until use.

Preparation of meat samples

Pork shoulder meat (subscapularis muscle, 48 h *post-mortem*)

was obtained from a registered abattoir. Samples were prepared following the recommendations of Lee and others (1998). Meat samples (270 g) were blended with 30 mL of test solution, using a Moulinex DB81 blender (Moulinex, Paris, France). Treatment groups included: (1) control (deionized water), (2) DMSO, (3) hyssop extract, (4) rosemary extract. Test solutions were adjusted to 5.8 pH using diluted HCl and NaOH. Aliquots were transferred into 50-mL polypropylene test tubes and stored for 6 h at 4 °C before cooking. The meat was cooked at 70 ± 2 °C (internal temperature) in a water bath, then cooled in an ice bath. The cooked meat was chopped into very fine pieces and stored in a Fibran storage bag (Fibran-Plex II, Fibran S.A., Girona, Spain) for 8 d at 4 °C. Sampling and weighing were performed in a cold room (4 °C).

Analytical methods

Color. Color determinations were made following the recommendations of the American Meat Science Association for color measurements in meat and meat products (Hunt and others 1991). Color determinations were made using a Minolta spectrophotometer CM-2002 R (Minolta Camera Co., Osaka, Japan) with an 11-cm aperture for illumination and 8 mm for measurement, using illuminant D_{65} and a 10° standard observer (Cassens and others 1995). Low reflectance glasses (CR-A571829-752 M, Minolta, Osaka, Japan) were placed between the samples and the equipment.

In all the experiments, lightness (L*), redness (a*), and yellowness (b*) of the CIE LAB color space (CIE 1986) were analyzed.

pH. pH determinations were made using a Crison 507 pH-meter and a Crison CAT 52-32 electrode (Crison Instruments S.A., Alella, Barcelona, Spain).

Thiobarbituric acid value (TBA). Thiobarbituric acid reactive substances (TBARS) assay was performed as described by Rosmini and others (1996). The mixture was heated for 10 min in a boiling water bath (95 to 100 °C) to develop a pink color, cooled with tap water, centrifugated at 5500 rpm for 25 min in a centrifuge (Alresa HZ50, Orto Alresa, Aljavit, Madrid, Spain), and the absorbance of the supernatant was measured spectrophotometrically at 532 nm using a UV spectrophotometer (Unicam Ltd., Cambridge, U.K.). Results were expressed as TBARS (mg malonaldehyde kg^{-1} sample).

Metmyoglobin. Five g of minced meat were used to determine MMb concentration in each sample. Myoglobin was extracted with cold 0.04 M phosphate buffer, pH 6.8, with a sample-to-buffer ratio of 1:10. Samples were homogenized for 15 s with a Polytron homogenizer (Brinkman Instruments, New York, N.Y., U.S.A.) which was set at Speed Setting 4 (10800 rpm). The homogenates were then centrifuged in the Alresa HZ50 for 30 min (50000 *g*) at 5 °C. The absorbance of the filtered supernatant was read at 525, 572, and 730 nm. Percentage of MMb was determining using the formula of Krywicki (1979):

$$\text{MMb (\%)} = 1.395 - ((A_{572} - A_{730}) / (A_{525} - A_{730}))^{*}100$$

Samples were kept on ice at all points during the assay.

Total iron. Total iron concentration was determined in wet-ashed samples using the ferrozine assay (Stookey 1970). Pork muscle (0.2 to 0.3 g) was placed in a test tube and digested with concentrated nitric acid and 30% hydrogen peroxide on a hot-plate until a white ash was formed. The ash was dissolved in 0.2 mL of 1.0N HCl and diluted with 0.8 mL deionized water. Ascorbic acid (1 mL, 1%) was added, and the tube contents were mixed with a Vortex (Selecta, Barcelona, Spain). After 20 min, 1 mL 10% ammonium acetate buffer and 1 mL of 1 mM ferrozine color reagent were added and mixed. The mixture was allowed to stand at room temperature for 45 min, then the absorbance was determined from a standard curve made with iron standard solution (Sigma Diagnostics, St. Louis, Mo., U.S.A.).

Heme iron. Heme iron was determined using the method of Hornsey (1956). Pork (2 g) was transferred into a 50-mL polypropylene tube, and 9 mL of acid acetone (90% acetone + 8% deionized water + 2% HCl) was added. The meat was macerated with a glass rod and allowed to stand 1 h in a dark cabinet at room temperature. The extract was filtered with Whatman filter paper nr 42 (Whatman Internations Ltd., Maidstone, England), and the absorbance was read at 640 nm against the acid acetone blank. Total pigments, as acid hematin, were calculated using the formula:

$$\text{Total pigment (ppm)} = A640 \times 680$$

and heme iron was calculated as follows (Clark and others 1997):

Table 1—Effect of rosemary and hyssop on CIELAB color coordinates, Lightness (L*), redness (a*), and yellowness (b*) of cooked pork meat during storage at 4°C

	Storage time (d)	Control	DMSO	Rosemary	Hyssop
L*	Fresh meat	48.10^{aw}	49.40^{aw}	45.50^{aw}	46.30^{aw}
	0	61.72^{bw}	57.82^{bx}	51.75^{by}	55.30^{bz}
	2	65.90^{cw}	60.10^{cx}	53.73^{b}	56.65^{cz}
	4	65.30^{cw}	59.50^{cx}	52.65^{b}	57.36^{cz}
	6	65.50^{cw}	59.68^{cx}	52.20^{by}	57.64^{cz}
	8	65.00^{cw}	59.21^{cx}	53.15^{by}	56.30^{cz}
a*	Fresh meat	8.19^{aw}	7.82^{aw}	8.34^{aw}	8.20^{aw}
	0	6.88^{bw}	7.02^{bw}	7.92^{bx}	7.86^{bx}
	2	5.42^{cw}	5.60^{cw}	6.62^{cx}	6.56^{cx}
	4	5.03^{cw}	5.20^{cw}	6.43^{cx}	6.40^{cx}
	6	5.46^{cw}	5.50^{cw}	6.40^{cx}	6.30^{cx}
	8	4.15^{dw}	4.32^{dw}	5.60^{dx}	5.55^{dx}
b*	Fresh meat	8.12^{aw}	8.26^{aw}	8.32^{aw}	8.35^{aw}
	0	10.52^{bw}	10.62^{bw}	12.50^{bx}	12.39^{bx}
	2	11.46^{cw}	11.60^{cw}	12.46^{bx}	12.29^{bx}
	4	11.33^{cw}	11.42^{cw}	12.32^{bx}	12.12^{bx}
	6	12.86^{dw}	13.02^{dw}	12.28^{bx}	12.08^{bx}
	8	13.02^{dw}	13.12^{dw}	12.40^{bx}	12.22^{bx}

$^{a-d}$Means in same column with different letter different at $p < 0.05$ for each color parameter
$^{w-z}$Means in same row with different letter different at $p < 0.05$ for each color parameter

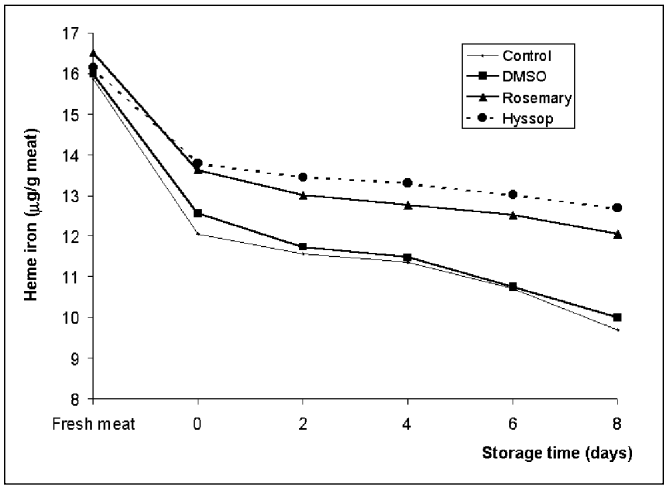

Figure 1—Effect of rosemary and hyssop on heme iron content in cooked pork meat during storage for 8 d at 4 °C

Heme iron (ppm) = total pigment (ppm) × 8.82/100

Statistical analysis

Each parameter was tested in triplicate. Conventional statistical methods were used to calculate means and standard deviations. Statistical analysis (ANOVA) was applied to the data to determine differences ($p < 0.05$). To ascertain significant differences between the levels of the main factor, Tukey's test was applied between means (Afifi and Azen 1979). ANOVAs were made with the following 2 factors: test solution (4 levels; deionized water, DMSO, hyssop extract, and rosemary extract), and time (6 levels; fresh meat, 0, 2, 4, 6, and 8 d of frozen storage). Statistical data analysis was undertaken using the statistical package BMDP Version 9.0 (BMDP 1993).

Results and Discussion

Meat pH

The pH of the control samples increased with storage time at 4 °C, reaching a final value of 5.92, while the pH of the samples with added DMSO, rosemary, and hyssop was not modified during storage, the final pH being higher than the control ($p < 0.05$). There was no difference in pH between treatments.

Corresponding to the report of Rhee (2000), who reported that lipid oxidation decreases as pH increases in meat and poultry, our study's results reflected the degree of lipid oxidation.

In all the treatments, the cooking process itself increased meat pH, probably due to the breaking down of the cellular buffer and to the free fat (Schweigert 1994).

Heme iron content

Muscle tissue contains a considerable amount of iron bound to proteins. Myoglobin is the most abundant hemoprotein in muscle tissue. The total iron and heme iron contents of pork meat were 10.0 ± 3.27 and 4.9 ± 2.16 g/g wet weight, respectively, with heme iron corresponding to 49% of the total iron. These values were similar to those reported by Schricker and others (1982). This heme iron content in pork meat decreased with cooking (Figure 1). There were no differences between the control and DMSO-treated samples regarding heme iron behavior ($p > 0.05$), whereas the content of nonheme iron released by cooking was lower in the samples with rosemary and hyssop ($p < 0.05$). In the control samples, cooking increased nonheme iron release by 3.81 mg/g sample, corresponding to a decrease in heme iron of 24%. Although the mechanism for heme iron release in meat has not been determined, oxidation of the porphyrin ring (Igene and others 1979; Schricker and others 1982) and denaturation of myoglobin (Kristensen and Purslow 2001) are probably involved. Kristensen and Purslow (2001) reported that destruction of the heme molecule will easily lead therefore to denaturation of myoglobin. The lower degradation of heme pigments caused by cooking in the experiments performed with plant extracts may be explained by the presence of chelating agents (o-cathecol phenols), which are found extensively in herbs (Marín and others 2002).

Heme iron content in cooked pork meat decreased with increasing storage time (Figure 1). This decrease of heme iron content was lower with both hyssop and rosemary extracts ($p < 0.05$). The inhibitory effect of hyssop extracts on iron release from degraded pigments was stronger than that of rosemary extracts during storage for 8 d ($p < 0.05$). Although the inhibition mechanisms of rosemary and hyssop on degradation of heme pigments have not been described, their chelating or free-radical scavenging activity might be involved.

Metmyoglobin

Martens and others (1982) reported that native red myoglobin denatures in the 65 to 80 °C range, with 70% remaining at 73 °C. These authors further suggested that red color in cooked meat appears to diminish at lower temperatures, due to a masking effect of myoglobin by aggregation/coprecipitation of other myofibrillar and sarcoplasmic proteins.

MMb formation was time-dependent when meat samples were stored for 8 d at 4 °C (Figure 2). Treatment with rosemary and hyssop extracts inhibited MMb formation ($p < 0.05$), an effect which was more pronounced with increasing storage time. Several investigators reported that the susceptibility of myoglobin to autoxidation is the main factor in explaining color stability in meat and meat products (Renerre and others 1992). In meat systems, the identities of the substances capable of oxidizing OMb to MMb are not clear. Several authors (Genot and others 1991) concluded that O_2^- can initiate lipid peroxidation, leading to the formation of prooxidant substanc-

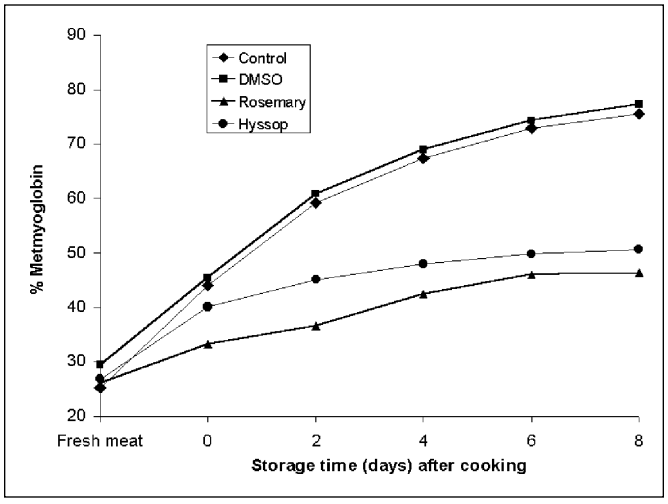

Figure 2—Effect of rosemary and hyssop on metmyoglobin formation (% MMb) in cooked pork meat during storage for 8 d at 4 °C

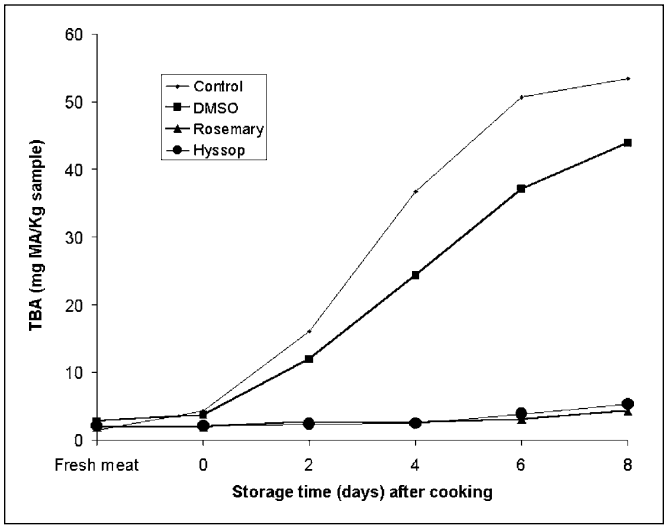

Figure 3—Effect of rosemary and hyssop on TBA values in cooked pork meat during storage for 8 d at 4 °C

es capable of reacting with OMb and resulting in MMb formation. Anton and others (1993) postulated that OMb could be oxidized, not only by lipid-oxy radicals but by other prooxidant radicals generated by O_2^-.

Lipid oxidation

TBARS formation increased sharply with increasing storage time at 4 °C (Figure 3). Both rosemary and hyssop inhibited lipid oxidation in meat samples ($p < 0.05$) to the same extent. The samples with added DMSO decreased TBARS formation, but this effect was lower than that observed in the samples to which rosemary and hyssop were added ($p < 0.05$). The antioxidant effect of DMSO can be explained by the instability of this solvent, which produces SO_2 in the presence of water.

Oxidation of muscle lipids involves the oxidation of membrane polyunsaturated fatty acids (Keller and Kinsella 1973). Transition metals, such as iron and copper, and heme moiety are important factors in this reaction (Sato and Hegarty 1971; Kanner and others 1988). Rosemary and hyssop extracts are rich in phenol compounds (Shahidi 2000). Other studies suggest that polyphenols might inhibit free radical formation and the propagation of free radical reactions through the chelation of transition-metal ions, particularly those of iron and copper (Brown and others 1998). The phenolic compounds of hyssop and rosemary extracts may be involved in the inhibition of lipid oxidation. There are reports on the antioxidant activity of rosemary extracts in several foods: cured pork fat (Iriarte and others 1992), beef patties (Formanek and others 2001), chicken meat (Nissen and others 2000), fish products (Duxbury 1992; Pokorny and others 1998), and dehydrated egg (Duxbury 1992). The inhibition of lipid oxidation by hyssop and rosemary extracts may be largely due to their ability to chelate transition metals and/or facilitate the oxidation of ferrous ions.

Lipid oxidation as expressed by TBA number increased with cooking and with storage after cooking ($p < 0.05$). The inhibitory effect of rosemary and hyssop extracts on this index was the same. Sato and Hegarty (1971) reported that nonheme iron was the active catalyst in cooked meats. Chen and others (1984) also reported that iron was released from heme pigments during cooking and proposed that the resultant increase in nonheme iron was responsible for lipid oxidation.

There was a negative relationship between heme iron content (Figure 1) and TBA number (Figure 3) of cooked pork ($r = -0.97$; $p < 0.01$), which supports the view that nonheme iron in cooked meat is responsible for catalyzing lipid oxidation.

Meat color

Lightness of pork meat increased with storage time at 4 °C ($p < 0.05$) (Table 1). Fernández-López and others (2000) reported that an increase in MMb increases lightness in fresh meat. As can be seen in Figure 2, MMb increases during storage time.

In all samples, lightness increased with cooking ($p < 0.05$). This effect of cooking upon L* in meat and meat products has been reported by Phillips and others (2001), who attributed it to meat protein denaturation by heat. This denaturation provokes a release of water from the meat. The free water on the meat surface is also related to L* values (the more free water, the greater the lightness) (Hunt and Kroft 1985).

Samples treated with rosemary and hyssop extracts showed lower L* values than the control samples ($p < 0.05$), with rosemary having a stronger effect in this respect. Mínguez and others (1992) reported that L* values in meat could be influenced by lipid oxidation. The inhibitory effect upon lipid oxidation by rosemary and hyssop extracts described in this work could explain the L* values obtained in these samples.

Redness decreased with storage time at 4 °C ($p < 0.05$) (Table 1). The redness of the control sample faded very rapidly. Phillips and others (2001) reported a decrease in a* values of cooked ground beef with storage time. This is not surprising since meat which is stored longer would be expected to have either predominantly OMb or MMb (as opposed to DMb), which in turn would predispose the meat to faster browning (Hunt and others 1995, 1999).

In all samples a* values fell with cooking ($p < 0.05$). In general, higher a* values would indicate that the reddest meat could be interpreted as indicating a less thoroughly cooked appearance. Phillips and others (2001) reported a decrease in a* values by cooking in partly raw beef, and Hunt and others (1999) reported it in ground beef.

There were differences ($p < 0.05$) between the results obtained with the rosemary and hyssop treatments (Table 2). The protective effect on redness in pork meat was higher with rosemary. Several authors have studied the effect of different antioxidants on the color of meat and meat products (Higgins and others 1998; Lee and others 1998) and have reported that meat oxidation provokes a decrease in a* values.

Yellowness in the control samples increased with storage time at 4 °C, but remained unaltered in the samples with added rosemary and hyssop extracts. The b* values obtained in the added-rosemary and hyssop samples were higher than control samples. So rosemary and hyssop extracts clearly had a protective effect on the b* coordinate. The increase in b* values observed in the control samples during storage time could be related to the increase in MMb (Figure 2). The cooking process increased b* values in all samples.

Conclusions

HYSSOP AND ROSEMARY INHIBITED LIPID OXIDATION AND THE degradation of heme pigments caused by cooking and storage. Hyssop and rosemary delayed MMb formation during storage in cooked meat and stabilized the red meat color. These findings indicate that rosemary and hyssop may be useful as additives for meat processing to prevent lipid oxidation and decoloration, thus increasing shelf-life.

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