A comparative study on the effect of rosemary extract and sodium ascorbate on lipid and pigment oxidative stability of liver pate

Demewez Moges Haile

Revised: 22 June 2013 / Accepted: 1 July 2013 / Published online: 7 July 2013
© Association of Food Scientists & Technologists (India) 2013

Abstract A comparative study on the antioxidant effect of rosemary extract (RE) and sodium ascorbate (SA) on lipid and colour oxidation of liver pâté made of lard and pork liver was done. During the 48 hour experimental time all the pâtés were wrapped in a foil and stored in cold room of 3.5°C under light of 1000 lux. Colour stability was monitored by instrumental colour measurement (CIE L*a*b* colour space) whereas lipid stability was measured by the determination of the 2-thiobarbituric acid reactive substances (TBARS). In the present study RE doses range (0, 125, 250, 375 and 500 ppm) showed no significant ($p>0.05$) and linear effect on colour stability. However the RE revealed a significant effect ($p<0.05$) against lipid oxidation and linearly reduces the TBARS number. The added SA doses (0, 250, 500, 750 and 1000 ppm) revealed significant ($p<0.05$) and linear effect in reducing discoloration. However the studied SA dose ranges showed no significant ($p>0.05$) effect on TBARS number. In this study RE was showed better performance against lipid oxidation and SA was potent against discoloration. The effect of the added spices used in manufacturing of the studied product showed no significant ($p>0.05$) effect against lipid and color oxidation. However the added spices revealed possible antagonistic and synergetic relationship with the studied the antioxidant systems (RE & SA).

Keywords Liver pâté · Pork fat Colour and lipid oxidation · TBARS · CIEL*a*b* · TBARS · Rosemary extract · Sodium ascorbate

D. M. Haile (✉)
Engineering Faculty, School of Chemical and Food Engineering,
Laboratory of Animal products processing, Bahirdar University,
P.O.Box: 2080, Bahirdar, Ethiopia
e-mail: demewez2003@yahoo.com

D. M. Haile
e-mail: hailem@bdu.edu.et

Introduction

Liver pâté, a traditionally cooked and widely consumed meat product in many countries, consists of a comminuted mixture of liver and fat to which additives are added. Due to its chemical composition and manufacturing process, liver pâté is considered as being a product highly liable to oxidation (Estévez et al. 2007). During production, processing, distribution and storage, food undergoes deterioration from chemical and microbial processes. Typically, oxidative deterioration of meat and meat products result from degradative reactions of fats in meat.

Lipid oxidation, leading to rancidity, is one of the major reasons of meat products' quality deterioration, mainly because of their increased fat content, the highly comminuted nature of the raw materials, as well as the possibility of long-term frozen storage of the latter before use. Oxidative processes are also associated with discoloration of meat products, as lipid oxidation results in the formation of pro-oxidants capable of reacting with oxymyoglobin, which lead to metmyoglobin formation (Frankel 1998).

Appearance of meat products is one of the major determinants of their appeal to consumers and, consequently, sales of the product (Clydesdale 1998). Williams et al. (1992) have investigated the economical importance of colour deterioration of meat and meat products. Their data indicated that this problem is associated with a sales reduction of 5.4 % for fresh meat and 3.7 % for meat products, whereas its prevention could lead to a saving of profit.

Cho et al. (1998) ; Jayathilakan et al. (2007a) and McBride et al. (2007) suggested lipid oxidation in turn deterioration of appearance and microbial growth in meat products can be controlled, minimized or delayed by using antioxidants. In order to use any antioxidant in food, it must be safe, easy to incorporate, effective at low concentrations, with no undesirable odor, flavor or colour, heat stable, non-volatile and with good carry through properties and cost-effective (McBride et al. 2007).

Antioxidants used in food system may be either synthetic such as Butylated hydroxyanisole (BHA) or Butylated hydroxytoluene (BHT) or natural food additives. However due to the growing consensus on the potential health hazard caused by synthetic antioxidants and their well known toxicity there is a renewed interest in the use of naturally occurring antioxidants (Antolovich et al. 2002; Yu et al. 2002; Jayathilakan et al. 2007a). Natural antioxidants are primarily plant phenolics that are multifunctional and can act as reducing agents, free radical terminators during the initiation or propagation step of oxidation reaction, metal chelators and singlet oxygen quenchers (Jayathilakan et al. 2007b).

Rosemary (*Rosmarinus officinalis* L.) extracts have also exhibited potent antioxidant activity and are widely used in the food industry. The antioxidant activity of rosemary extracts has been associated with the presence of several phenolic diterpenes such as carnosic acid, carnosol, rosmanol, rosmariquinone and rosmaridiphenol, which break free radical chain reactions by hydrogen atom donation (Aruoma et al. 1992 and Basaga et al. 1997). A number of researchers have reported the effectiveness of rosemary extracts for achieving higher sensory scores and retarding lipid oxidation (Stoick et al. 1991; Shahidi and Wanasundara 1992). In addition to inhibition of lipid oxidation, Formanek et al. (2003) found that colour changes during storage were inhibited by the addition of rosemary extract in irradiated ground beef.

Ascorbic acid possesses antioxidant properties, although it can act as an antioxidant or as a prooxidant depending on the concentration, the presence of metal ions and the tocopherol content (Schaefer et al. 1995). In a research on ground beef ascorbic acid incorporated at the level of 0.1 % (w/w) was very effective in maintaining redness (a^* values) and addition of ascorbate further increased the reducing power in beef and increased the red colour intensity of ground beef (Ahn and Nam 2004; Nam and Ahn 2003). Alley et al. (1992) also reported colour stability is promoted by residual ascorbate. The colour stabilizing effect of ascorbic acid was derived from the reducing or oxygen scavenging power of ascorbic acid, which maintained the heme pigments in beef in a reduced form (Nam and Ahn 2003; Sa'nchez-Escalante et al. 2001).

Several studies have demonstrated the beneficial effects of these natural antioxidants on lipid oxidation and colour preservation. Due to the interaction of these compounds with their environment, however, the investigation of their activity in a range of food systems is still needed for successful application in meat products. Thus this research has been therefore conducted: first, to determine the optimum concentration of rosemary extract and sodium ascorbate for better lipid and color oxidative stability of liver pate. Secondly, to investigate color and lipid stability of liver pâté enriched with sodium ascorbate and rosemary extract.

Materials and methods

Manufacturing of the model liver pâtés

The model pâtés were prepared at the laboratory of Katholieke Hogeschool Gent, Belgium. For the manufacture of the pâtés a cooking liquid (constituted 30 %) and 10 g/kg sodium caseinate (DMV EMD) were added on subcutaneous fat (constituted 34.8 %) heated for 20 min and mixed in a cutter for 5 min. A fresh and pre-treated liver (constituted 30 %) was cut in a mixer for 8 min and sodium chloride (18 g/kg) and 0.12 g/kg nitrite was added. The nitrified liver emulsion made was kept under refrigeration (7–10 °C). During the manufacturing process additives namely Liver (300 g/kg), Pork fat (348.38 g/kg), Water (300 g/kg), Sodium caseinate (10 g/kg), Sodium ascorbate (0.5 g/kg), NaNO_2^- (0.12 g/kg), NaCl (18 g/kg), Glutamate (5.0 g/kg), Dextrose (5.0 g/kg), White pepper (5.0 g/kg), Nutmeg (5.0 g/kg), Ginger (1.0 g/kg), Cardamom (1.0 g/kg) & Onion powder (1.0 g/kg) were added to the liver-fat emulsion (warmed to 51 °C).

Experimental design

The experiment was designed with different concentrations of antioxidant (six rosemary extract and six sodium ascorbate concentrations) and 2 replications of the entire experiment (Table 1). A total of 28 equal sized pâtés were used. Depending on the experimental batch, different rosemary extract and sodium ascorbate concentrations were incorporated in to the 24 pâtés slices and four pâtés slices were used as a control for each antioxidant system and coded as indicated in Table 1. During the 48 h experimental time all the pâtés were wrapped in a foil and stored in cold room of 3.5 °C under light of 1,000 lux. Colour Measurements were performed at one hr interval in duplicate up to eight hr followed by 24 hr and 48 hr measurement. For the measurement of TBARS samples were kept at −20 °C and TBARS level was determined in duplicate for each samples at times 0 and 48 hr for all antioxidant types and concentrations.

Analysis and measurements

Instrumental colour measurement

Surface colour of the slices was measured in duplicate using a tristimulus colorimeter (Hunter Lab Mini scan device: D65 illuminant, 10° standard observer, 45°/0° geometry, 1 in. light surface) and an average value was used in further calculations. Before each measurement the apparatus was standardized against a black and white calibration tiles. $L^*a^*b^*$ values and reflectance values at different wave lengths from 470 to 700 nm were obtained from instrumental measurement. The percentages of nitrosomyoglobin (Reddish colour following

Table 1 Antioxidants formulations used for the antioxidant experiment

Sodium Ascorbate (SA)			Rosemary extracts (RE)		
Treatment	Dose (ppm)	Spices	Treatment	Dose (ppm)	Spices
SABO	0	With out	REB0	0	With out
SAB500	500	With out	REB250	250	With out
SA0	0	With	RE0	0	With
SA250	250	With	RE125	125	With
SA500	500	With	RE250	250	With
SA750	750	With	RE375	375	With
SA1000	1000	With	RE500	500	With

SA sodium ascorbate

RE Rosemary extracts

curing with nitrite) was calculated by using the ratio of reflectance values at wave lengths of 650 and 570 (650/570 nm). The percentage of metmyoglobin (Metmb %) at the surface of

the samples was also determined from the measurement of reflex attenuation at the iso-bestic point by using the method of Krzywickii (1979):

$$\% \text{Metmb} = 237.51 - (1 - [-0.3 \log R^{473} - 0.7 \log R^{473} + \log R^{730} / -0.5 \log R^{525} - 0.5 \log R^{530} + \log R^{730}])$$

$$\% \text{Metmb} = 1.395 \cdot (1 - (A^{572} - A^{730} / A^{525} - A^{730}))$$

Where: A=Millmolar absorbance & R=reflectance

Lipid oxidation analysis

Lipid oxidation was evaluated through the determination of TBARS using the method of Salih et al. (1987). The frozen sample weighed 5 g was grinded and dispensed in a plastic pot of 100 ml and homogenized with 30 ml cold perchloric acid (HClO₄, 0.6 M) and 1 ml of BHT (absolute ethanol) using ultra-turrax at 1,300 r/min for about 30 s. The slurry was filtered through a folder filter (S & S 595 1/2) and centrifuge (5 min, 2,500 r/min). Five ml meat sample extract (aliquots), distilled water (blank) and standard working solution were transferred in heat resistance glass test tubes in duplicate and mixed with 1 ml TBA reagent. The test tubes were placed in boiling water bath (100 °C) for 35 min together with tubes for the standard curve. After cooling to room temperature in tap water, the absorbance was measured spectrophotometrically at 532 nm against a blank. The standard curve was prepared by using dilutions prepared from a 20 times diluted 14.4 µl 1, 1, 3, 3-tetramethoxypropane (Sigma T-1642) in 0.6 M HClO₄.

The MDA concentration was converted to TBAR number (µg MDA/g meat sample) as follows:

$$\text{TBA number}(\mu\text{g MDA/g sample}) = C \times 6 \times 72 / G \times 1000$$

Where: 72=molecular weight of MDA

G Weight of the sample in g and

C Concentration of MDA per 5 ml extract based on the regression line the standard curve.

The sample was kept as cold as possible during the treatment to prevent oxidation. During the analysis standard curves were prepared for each TBARS measurement to avoid the effect of variations during pipetting, weighing, incubation and other steps. Because of the variability in TBARS value observed at the start (0 h) the difference in TBARS between 0 h (start) and 48 h (end) was used to see the effect of the treatments.

Data handling and statistical analysis

Summary statistics (mean and standard error of the mean) were computed for each dependent variable. Variability was observed in the aforementioned colorimetric variables and TBARS value at the start (0 h). Thus for this experiment the rate of change (slope) of Metmb %, redness (a*) value and nitrosomyoglobin (650/570 nm) percentage were used to determine surface discoloration. Similarly the difference in TBARS values between 0 h and 48 h were used as a variable to determine lipid oxidation and Mean values are reported as µg MDA/ g of meat.

Data obtained from instrumental measurement and chemical analysis was all submitted to General Linear Model (GLM) procedure from SPSS 15.0 (SPSS 2006) statistical software package. The treatments whenever found significant, the tukey test was used for pair wise comparisons among the different treatments at the 5 % ($p < 0.05$) significant level.

Whenever it was found necessary Pearson correlation coefficient was also calculated.

Results and discussions

Optimization of sodium ascorbate and rosemary extract doses for lipid and colour stability of liver pâté

Rosemary extracts optimization against colour and lipid stability

The mean rate of colour variables change (slope) and P-value of the studied rosemary extract concentrations are summarized in Table 2. The five rosemary extract concentration levels showed no significant ($p>0.05$) difference for the rate of decrease of the hunter a^* value (redness). In the present study inconsistent pattern for the efficacy of the rosemary extract concentration on colour stability (a^*) was observed (Table 2). This inconsistent behavior towards concentration of rosemary extract was also reported by McBride et al. (2007) during a study on comparative addition of rosemary extract in beef.

Rate of change of MetMb formation showed no significant ($P>0.05$) difference among the doses and all treated samples showed higher rate of MetMb formation than the control. The present study demonstrated consistent increase in rate of MetMb formation which may indicating possible pro-oxidant effect of rosemary extract at higher concentration as revealed by an increase in the rate of MetMb formation (Table 2).

The rate of loss of nitrosomyoglobin (650/570 nm) during storage also showed no significant ($p>0.05$) differences. However unlike the Hunter a^* value nitrosomyoglobin showed linear and gradual decrease where its stability increase with increasing the rosemary extract concentration and the lowest rate of discoloration was observed at 500 ppm. Sa'nchez-Escalante et al. (2001) advised that the effectiveness of rosemary on stabilizing colour in beef can be enhanced through the addition of reducing agents such as rosemary powder.

TBARS showed significant ($p<0.05$) difference among the different concentrations of rosemary extract (Table 2). Development of TBARS was greatest in samples containing no rosemary extract and addition of rosemary extract showed a gradual and linear ($P=0.019$) decrease in TBARS value up to a concentration of 375 followed by increase at a concentration above 375 ppm. Gutensperger and Escher (1994) also reported RE significantly decreased TBARS formation at all storage times at levels of 250 and 500 ppm, but not at 100 ppm. This increase of TBARS above 375 ppm rosemary extract indicates a possible pro-oxidant effect of rosemary extract at higher concentration. McBride et al. (2007) also reported promotion of Warmed over flavor development as quantified by TBARS value and pro-oxidant effect at a concentration of 1 % (w/w) of rosemary extract as compared to 0.25 %, 0.1 % and the control during a study conducted on comminuted beef. It has been demonstrated that a variety of antioxidant compounds may be pro-oxidant (Yoshida et al. 2003) and such activity is system and concentration dependent. Given the requirement for non-organoleptic interference by antioxidant compounds this effectiveness of rosemary extract at lowest concentration is perceived as advantageous.

Table 2 Effect of different concentrations of Rosemary Extract (RE) & Sodium Ascorbate (SA) on colour and lipid stability of pâté

Antioxidant system	Doses	Rate of change values			
		Δa^*	$\Delta 650/570 \text{ nm}$	$\Delta \text{MetMb}\%$	ΔTBARS
Rosemary Extract	0	-1.3 ± 0.12^a	-0.16 ± 0.02^a	4.0 ± 0.13^a	0.12 ± 0.05^a
	125	-1.23 ± 0.14^a	-0.15 ± 0.01^a	4.19 ± 0.26^a	0.08 ± 0.02^a
	250	-1.3 ± 0.11^a	-0.16 ± 0.01^a	4.22 ± 0.31^a	0.07 ± 0.02^a
	375	-1.3 ± 0.09^a	-0.15 ± 0.009^a	4.32 ± 0.19^a	0.04 ± 0.03^a
	500	-1.25 ± 0.1^a	-0.14 ± 0.02^a	4.37 ± 0.2^a	0.06 ± 0.05^a
	p-value	0.881	0.563	0.616	0.005
Sodium Ascorbate	0	-1.18 ± 0.37^a	-0.152 ± 0.007^a	4.1 ± 0.23^a	0.13 ± 0.01^a
	250	-1.12 ± 0.086^a	-0.149 ± 0.004^{abc}	3.84 ± 0.20^{ab}	0.105 ± 0.01^a
	500	-1.08 ± 0.03^a	-0.147 ± 0.004^{abc}	3.7 ± 0.06^b	0.10 ± 0.1^a
	750	-1.08 ± 0.08^a	-0.139 ± 0.009^{bc}	3.50 ± 0.03^b	0.07 ± 0.06^a
	1000	1.07 ± 0.08^a	-0.135 ± 0.004^{bc}	3.43 ± 0.07^b	0.10 ± 0.04^a
	p-value	0.141	0.002	0.000	0.94

Means followed by different superscripts within row show statistically significant ($p<0.05$) differences

($n=4$) $\pm$ Standard deviation

In previous studies in general the loss of redness is reflected on the increase of MetMb formation which was not the case in this rosemary extract doses study. Generally as the a^* value was behaved inconsistently and 650/570 nm showed linear relation up to the higher level founding an adequate RE level for colour stability was difficult in the present study. But regarding lipid oxidation the present study found 375 ppm was adequate to retard lipid oxidation of liver pate. Previously colour and lipid oxidation effect of rosemary extract on meat products has been reported on the basis of comparison with other potential antioxidants (Djenane et al. 2002 and Sa'nchez-Escalante et al. 2001) thus little information is available on concentration effect of rosemary extract.

Sodium ascorbate optimization against colour and lipid oxidation of liver pâté

The mean rate of colour variables change (slope) and P-value of the studied sodium ascorbate concentrations are summarized in Table 2.

Redness showed non significant ($p>0.05$) difference but linear ($p=0.012$) effect among the doses and the control sample (SA0). The higher rate of a^* value loss was observed in the control and redness stability increase as concentration increase where the higher stability was observed at 1,000 ppm (Table 2). A continuous decrease in loss of redness in response to sodium ascorbate treatment was also reported from previous works (Zuckerman and Mannheim 2001; Sahoo and Anjaneyul 1997). However Sahoo and Anjaneyul (1997) reported higher redness stability at 500 ppm and lowest at 600 ppm during a study on ground buffalo meat which is not in agreement with this study where a gradual and linear decrease was observed to the highest level.

There was a significant ($P<0.05$) difference in rate of MetMb formation between the control and 500, 750, and 1,000 ppm levels (Table 2). From the linear ($p=0.000$) decrease in the rate of MetMb formation we noticed that the difference may have been owing to the effect of sodium ascorbate as all the sodium ascorbate treated samples had a lower rate of MetMb formation than the control. The same decrease in MetMb formation due to sodium ascorbate enrichment was also noticed by Sahoo and Anjaneyul (1997). In the present study addition of extra sodium ascorbate on the control showed consistent decrease in the rate of MetMb formation where the lowest observed at 1,000 ppm level. This result was however not in agreement with Sahoo and Anjaneyul (1997) report of sodium ascorbate at 500 ppm was adequate to retard discoloration and further increasing of sodium ascorbate to 600 ppm had no added advantage for lowering of MetMb content.

The rate of decrease of nitrosomyoglobin showed significant ($p<0.05$) difference between both 1,000 ppm and the control (Table 2). However there was no significant difference

between the other doses. The rate of loss of nitrosomyoglobin was linear ($p=0.000$) in response to an increase in doses of sodium ascorbate where the lower decrease (higher stability) was observed at 1,000 ppm. The higher discoloration revealed by loss of nitrosomyoglobin was observed for the untreated sample (SA0). This value (650/570 nm) confirmed the maintenance of the red colour of the liver pâté (Cured product) owing to sodium ascorbate treatment.

In general previous works on sodium ascorbate level recommend different concentration. Shivas et al. (1984) reported vitamin C at 500 and 1,000 ppm prolonged the colour shelf life of ground beef as compared to 100 ppm and control. Sahoo and Anjaneyul (1997) found 500 ppm adequate to retard discoloration. Because of the linear effect of sodium ascorbate doses on the rate of change of colorimetric variables (a^* , MetMb% and 650/570 nm) to the higher concentration level in this study it is difficult to get a clear cut SA level for colour stability.

All the treated samples showed lower but non significant TBARS value than the control. Ascorbic acid treatment was also reported to decrease lipid oxidation in ground beef (Shivas et al. 1984) and beef steaks (Okayama et al. 1987). Sodium ascorbate at 750 ppm contributes to the lowest TBARS value may indicate that it inhibits the lipid oxidation to the maximum extent. However this study demonstrates non linear effect of SA level against lipid oxidation where SA at 1,000 ppm gave the higher TBARS number as compared to 250, 500 & 750 ppm but less than the control (Table 2). Despite the inconsistent behavior of TBARS the Value at 1,000 ppm which is higher TBARS value than 750 ppm may indicate the possible pro-oxidant effect of sodium ascorbate at higher concentration level.

Comparisons of rosemary extract and sodium ascorbate and the effect of added spices on lipid and colour stability of liver pâté

Different concentrations of rosemary extract and sodium ascorbate were tested for the reduction of discoloration and lipid oxidation of liver pâté during the light displayed time. A slope comparison of the two antioxidant systems at the same concentration and the effect of added spices was done and the results are presented in Table 3.

At 250 ppm all measured physical colour variables showed no significant ($P>0.05$) difference. Even though statistically not significant samples treated with sodium ascorbate showed better colour stability as compared to rosemary extract treated samples. Similarly Lipid oxidation showed no significant difference ($p>0.05$) between the two antioxidant system tested at 250 ppm. However the higher TBARS value (0.1 ± 0.09) was observed in sodium ascorbate treated samples.

At 500 ppm of the two antioxidant systems significant differences ($P<0.05$) were observed on Hunter a^* value, MetMb % and 650/570 nm values. As indicated in Table 3 RE treated

Table 3 Mean rate of change (slope) of colorimetric variables and TBARS values from comparison of SA and RE at 250 ppm

Variables	Mean rate of Change (Slope)		P-value
Rosemary extract and sodium ascorbate at 250 ppm			
	RE250	SA250	
a*	1.28±0.11 ^a	1.12±0.09 ^a	0.062
MetMb%	4.22±0.3 ^a	3.84±0.2 ^a	0.086
650/570	0.16±0.01 ^a	0.15±0.004 ^a	0.268
TBARS	0.07±0.02 ^a	0.1±0.09 ^a	0.540
Rosemary extract and sodium ascorbate at 500 ppm			
	RE 500	SA500	
a*	1.2±0.05 ^a	1.09±0.03 ^b	0.008
MetMb%	4.37±0.2 ^a	3.7±0.06 ^b	0.001
650/570	0.14±0.02 ^a	0.14±0.004 ^a	0.933
TBARS	0.06±0.05 ^a	0.1±0.12 ^a	0.561
Rosemary extract at 500 ppm and sodium ascorbate at 500 ppm			
	RE 500	SA500	
a*	1.2±0.05 ^a	1.09±0.03 ^b	0.008
MetMb%	4.37±0.2 ^a	3.7±0.06 ^b	0.001
650/570	0.14±0.02 ^a	0.14±0.004 ^a	0.933
TBARS	0.06±0.05 ^a	0.1±0.12 ^a	0.561

Means followed by different superscripts within row show statistically significant ($p<0.05$) differences

($n=4$)±Standard deviation

Δ650/570 nm=nitrosomyoglobin value

samples showed higher rate of decrease (discoloration) in a* value (1.2±0.05) during the 3 h display as compared to SA treated samples (1.09±0.03). Thus similar to the comparison at 250 ppm sodium ascorbate treated samples display better red colour stability than rosemary extract treated samples.

Regarding increase in proportion (%) of MetMb the higher significant rate of increase (4.37±0.2) was observed from rosemary extract treated samples as compared to sodium ascorbate treated group (3.7±0.06). Thus in this experiment sodium ascorbate gave the best result with regard to preventing over all visual discoloration. The finding that sodium ascorbate treated samples was redder (lower rate of loss of a* and 650/570 nm) and least discolored (low rate of MetMb formation) was in agreement with previous reports by Lund et al. (2007) where they reported ascorbate better preserved red colour compared to rosemary. However previous works by Djenane et al. (2002) reported the opposite higher protection of colour fading in using rosemary in combination with vitamin C followed by rosemary alone whereas ascorbate exerted only small effect. Sa'nchez-Escalante et al. (2001) also reported no antioxidant effect of ascorbate during the work on beef pâté.

Lipid oxidation showed no significant difference ($p>0.05$) between the two antioxidant system tested at 500 ppm even though rosemary extract treated samples were given lower (0.06±0.05) TBARS numbers than the sodium ascorbate treated samples (0.1±0.12). This effectiveness of rosemary extract in inhibiting lipid oxidation was also reported by Sa'nchez-Escalante et al. (2003) and Djenane et al. (2002)

and has been attributed to a number of phenolic compound with multifunctional modes of antioxidant activities. Trout and Dale (1990) also reported effectiveness of RE for minimizing lipid oxidation like BHT and suggested rosemary extract has better dispersibility in aqueous solution and more readily incorporate in to meat products.

Comparison rosemary extract 250 and sodium ascorbate 500 ppm was done and the result showed significant ($p<0.05$) effect of sodium ascorbate at 500 ppm on all colorimetric parameters than rosemary extract 250 ppm. Samples treated with 500 ppm sodium ascorbate revealed better colour stability as evident from their low rate of decrease in a* and nitrosomyoglobin loss and low rate of Metmb formation (Table 3). Lipid oxidation showed no significant difference between the two antioxidant systems. However 250 ppm rosemary extract revealed low level of TBARS (0.07±0.02) as compared to 500 ppm sodium ascorbate (0.10±0.12).

The effect of the added spices used during the manufacturing of liver pâté showed statistically non significant ($p>0.05$) difference in minimizing colour and lipid oxidation. However mean values of this comparisons revealed consistent relationship between samples with and without spices. TBARS values of samples without spices (REB0 and SAB0) were higher than samples containing spices (RE0 and SA0). Samples treated with 500 ppm SA (SA500) and with 250 ppm RE (RE250) also showed lower TBARS value than samples treated with the same SA and RE but contain no spice (SAB500 and REB250). These results give a hint that even though the difference is not significant the added spices inhibited lipid oxidation as assessed by

TBARS and possible synergetic effect between the added spices with SA and antagonistic relation with rosemary extract against lipid oxidation.

Similarly the added spices showed non significant effect on colour of the product. Without rosemary extract and sodium ascorbate (RE0 and SA0) generally the presence of spices was revealed by better colour stability. However when rosemary extract and sodium ascorbate added on samples containing spices their effect was negative and cause higher MetMb formation. Higher MetMb formation rate was observed from samples containing spices and treated with sodium ascorbate and rosemary extract as compared to samples treated with sodium ascorbate and rosemary extract but no spice. This may be due to a possible presence of antagonistic relation between the rosemary extract and sodium ascorbate with the spices.

Conclusions

Comparison of rosemary extract doses revealed non linear effect of doses on over all discoloration as evident from the rate of loss of a^* and 650/570 nm values. Whereas rosemary extract doses revealed linear effect against lipid oxidation and the optimum was found at 375 ppm. Sodium ascorbate doses gave linear and significant effect against over all discoloration of the liver pâté. In the present study the highest concentration level showed significantly better colour stability and it is difficult to establish the optimum level of sodium ascorbate to retard discoloration and lipid oxidation and we propose to extend further levels till better retardation is observed. Generally in the present study we found rosemary extract as a potent antioxidant against lipid oxidation where as sodium ascorbate was better against discoloration. We suggest testing of combination of rosemary extract and sodium ascorbate for better antioxidant potential. During testing of antioxidants on products contain possible antioxidant sources such as spices attention has to be given for possible antagonistic and synergetic effect.

References

- Ahn DU, Nam KC (2004) Effects of ascorbic acid and antioxidants on colour, lipid oxidation and volatiles of irradiated ground beef. *Radiat Phys Chem* 71:149–154
- Alley G, Cours D, Demeyer D (1992) Effect of nitrate, nitrite and ascorbate on colour and colour stability of dry, fermented sausage prepared using 'Back sloping'. *Meat Sci* 32:279–287
- Antolovich M, Prenzler PD, Patsalides E, McDonald S, Robards K (2002) Methods for testing antioxidant activity. *Analyst* 127:183–198
- Aruoma OI, Halliwell B, Aeschbach R, Lo' liger J (1992) Antioxidant and pro-oxidant properties of active rosemary constituents: carnosol and carnosic acid. *Xenobiotica* 22(2):257–268
- Basaga H, Tekkaya C, Acitel F (1997) Antioxidative and free radical scavenging properties of rosemary extract. *Lebensm Wiss Technol* 30(1):105–108
- Cho YI, No HK, Meyers SP (1998) Physicochemical characteristics and functional properties of various commercial chitin and chitosan products. *J Agric Food Chem* 46(9):3839–3843
- Clydesdale FM (1998) Color: origin, stability, measurement and quality. In: Irwin AT, Singh RP (eds) *Food storage stability*. CRC Press Boca Raton, Florida, pp 175–190
- Djenane D, Sánchez-Escalante A, Beltrán JA, Roncalés P (2002) Ability of atocopherol, taurine and rosemary, in combination with vitamin C, to increase the oxidative stability of beef steaks packaged in modified atmosphere. *Food Chem* 76:407–415
- Estévez M, Ramírez R, Ventanas S, Cava R (2007) Sage and rosemary essential oils versus BHT for the inhibition of lipid oxidative reactions in liver pâté. *LWT* 40:58–65
- Formanek Z, Lynch A, Galvin K, Farkas J, Kerry JP (2003) Combined effects of irradiation and the use of natural antioxidants on the shelf life stability of overwrapped minced beef. *Meat Sci* 63(4):433–440
- Frankel EN (1998) *Lipid oxidation*. The Oily Press Ltd., Dundee
- Gutensperger B, Escher FE (1994) Oxidative changes of heat sterilized meat in trays. *J Food Sci* 59:689–692
- Jayathilakan K, Sharma GK, Radhakrishna K, Bawa AS (2007a) Effect of natural antioxidants on the lipid stability of fluidized bed-dried mutton. *Food Chem* 100:662–668
- Jayathilakan K, Sharma GK, Radhakrishna K, Bawa AS (2007b) Antioxidant potential of synthetic and natural antioxidants and its effect on warmed-over-flavour in different species of meat. *Food Chem* 105:908–916
- Krzywick K (1979) Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of meat. *Meat Sci* 3:1–10
- Lund MN, Hviid MS, Skibsted LH (2007) The combined effect of antioxidants and modified atmosphere packaging on protein and lipid oxidation in beef patties during chill storage. *Meat Sci* 76(2):226–233
- McBride NTM, Hogan SA, Kerry JP (2007) Comparative addition of rosemary extract and additives on sensory and antioxidant properties of retail packaged beef. *Int J Food Sci Tech* 42:1201–1207
- Nam KC, Ahn DU (2003) Effects of Ascorbic Acid and Antioxidants on the colour of irradiated ground beef. *J Food Sci* 68:1686–1690
- Okayama T, Imami T, Yamanoue M (1987) Effect of ascorbic acid and alpha-tocopherol on storage stability of beef steaks. *Meat Sci* 21:267–273
- Sahoo J, Anjaneyul ASR (1997) Quality improvement of ground buffalo meat by preblending with sodium ascorbate. *Meat Sci* 46:231–241
- Salih AM, Smith DM, Price JF, Dawson LE (1987) Modified Extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. *Poult Sci* 66:1483–1488
- Sánchez-Escalante A, Djenane D, Torrecano G, Beltrán JA, Roncalés P (2001) 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
- Sánchez-Escalante A, Djenane D, Torrecano G, Beltrán JA, Roncalés P (2003) Antioxidant Action of Borage, Rosemary, Oregano, and Ascorbic Acid in Beef Patties Packaged in Modified Atmosphere. *J Food Sci* 68:339–344
- Schaefer DM, Liu Q, Faustman C, Yin MC (1995) Supranutritional administration of vitamins E and C improves oxidative stability of beef. *J Nutr* 125:1792S–1798S
- Shahidi F, Wanasundara PKJP (1992) Phenolic antioxidants. *Crit Rev Food Sci* 32(1):67–103
- SPSS (2006) *SPSS for windows: Statistical software*, version 15.0

- Stoick SM, Gray JL, Booren AM, Buckley DJ (1991) Oxidative stability of restructured beef steaks processed with oleoresin rosemary, tertiary butylhydroquinone and sodium tripolyphosphate. *J Food Sci* 56(3):597–600
- Shivas SD, Kropf DH, Hunt MC, Kastner CL, Kendall JL, Dayton AD (1984) Effects of ascorbic acid on display life of ground beef. *J Food Prot* 47:1–5
- Trout GR, Dale S (1990) Prevention of warmed-over flavor in cooked beef: effect of phosphate type, phosphate concentration, a lemon juice/phosphate blend and beef extract. *J Agric Food Chem* 38:665–669
- Williams SN, Frye TM, Frigg M, Schaefer DM, Scheller KK, Liu Q (1992) Vitamin E. *Meat Focus Int* 3(2):22–23
- Yoshida Y, Niki E, Noguchi N (2003) Comparative study on the action of tocopherols and tocotrienols as antioxidants: chemical and physical effects. *Chem Phys Lipids* 123:63–75
- Yu L, Scanlin L, Wilson J, Schmidt G (2002) Rosemary extracts as inhibitors of lipid oxidation and colour change in cooked turkey products during refrigerated storage. *J Food Sci* 67:582–585
- Zuckerman H, Mannheim CH (2001) Colour improvement of kosher beef using sodium ascorbate and erythorbate. *J Muscle Foods* 12:137–151