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Comparative Study on the Effect of Rosemary Extract and Sodium Ascorbate on Lipid and Pigment Oxidative Stability of Liver Pate

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ABSTRACT
A comparative study on the antioxidant effect of rosemary extract (RE) and sodium ascorbate (SA) on lipid and color oxidation of liver pâté 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. Color stability was monitored by instrumental color measurement (CIE L*a*b* color space) 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 color 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 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.

Key words: *liver pâté; Color and lipid oxidation; TBARS; CIE L*a*b*; TBARS; Rosemary extract; Sodium ascorbate.*

INTRODUCTION

Lipid oxidation, leading to rancidity, is one of the major reasons of meat products' quality deterioration. This irreversible change contributes to the development of unacceptable organoleptic characteristics and is more important in frozen preserved meat products, where microbiological spoilage is not common. 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).

Cho *et al*. (2004) ; 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

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 & 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 color changes during storage were inhibited by the addition of rosemary extract in irradiated ground beef.

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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 color intensity of ground beef (Ahn and Nam, 2004 & Nam and Ahn, 2003). Alley *et al*. (1992) also reported color stability is promoted by residual ascorbate. The color 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 and Sánchez-Escalante *et al*., 2001).

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 10g/ 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 minute and sodium chloride (18 g/kg) and 0.12g/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), sater (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) and 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, rosemary extract and sodium ascorbate (Kerry ingredients and flavors,Bornem,Belgium) were incorporated at different concentrations 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 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. Color Measurements were performed at one hr interval in duplicate up to eight hr followed by 24hr 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 0 and 48 hr for all antioxidant types and concentrations.

Instrumental color measurement: Surface color 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 inch 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 color following curing with nitrite) was calculated by using the ratio of reflectance values at wave lengths of 650 and 570 (650/570nm). The percentage of metmyoglobin (MetMb %) at the surface of the samples was also determined from the measurement of reflex attenuance at the iso-bestic point by using the method of Krzywickii (1979): % MetMb = 237.5 (1-[-0.3 log R^{473}-0.7 log R^{473}+log R^{730}/-0.5log R^{525}

-0.5log R^{530} +log R^{730}] %Metmb =1.395. (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 100ml and homogenized with 30 ml cold perchloric acid ($HClO_4$,0.6 M) and 1ml of BHT (absolute ethanol) using ultra-turrax at 1300 r/min for about 30 seconds. The slurry was filtered through a folder filter (S & S 595 1/2) and centrifuged (5min, 2500r/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 1ml TBA reagent. The test tubes were placed in boiling water bath (100°C) for 35 minutes 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 14.4µl 1, 1, 3, 3-tetramethoxypropane (Sigma T-1642) in 0.6 M $HClO_4$.

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

TBA number (µg MDA/g sample) = C x 6 x 72/G x 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 hour) the difference in TBARS between 0 hour (start) and 48 hour (end) was used to see the effect of the treatments.

***Data handling and statistical analysis*:** Data obtained from instrumental measurement and chemical analysis was all submitted to General Linear Model (GLM) procedure from SPSS 15.0 (SPSS, 2006) statistical soft ware 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 found necessary, Pearson correlation coefficient was also calculated.

RESULTS AND DISCUSSIONS

Rosemary extracts optimization against color and lipid stability: The mean rate of color variables change (slope) and P-value of the studied RE concentrations are summarized in Table 2.The five RE concentration levels showed no significant

Table 1. Antioxidants formulations used for the antioxidant experiment

Sodium Ascorbate (SA)			Rosemary extracts (RE)		
Treatment	Dose (ppm)	Spices	Treatment	Dose (ppm)	Spices
SAB0	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
B=Showed no spice

(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 RE concentration on color stability (a*) was observed (Table 2). This inconsistent behavior towards concentration of RE was also reported by McBride *et al*. (2007) during a study on comparative addition of RE 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 RE 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 RE concentration and the lowest rate of discoloration was observed at 500 ppm. Sanchez-Escalante *et al*. (2001) advised that the effectiveness of rosemary on stabilizing color 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 RE (Table 2). Development of TBARS was greatest in samples containing no RE and addition of RE 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 375ppm. 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 RE indicates a possible pro-oxidant effect of RE 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 RE 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.*,

Table 2. Effect of different concentrations of Rosemary Extract (RE) and Sodium Ascorbate (SA) on color and lipid stability of pate

AntioxidantSystem	Rate of change values			
	“ a*	**“ 650/570nm**	**“ MetMb%**	**“ TBARS**
RE0	-1.3 ± 0.12^{a}	-0.16 ± 0.02^{a}	4.0 ± 0.13 ^{a}	0.12 ± 0.05^{a}
RE125	-1.23 ± 0.14^{a}	-0.15 ± 0.01^{a}	4.19 ± 0.26^{a}	0.08 ± 0.02^{a}
RE250	-1.3 ± 0.11^{a}	-0.16 ± 0.01^{a}	4.22 ± 0.31^{a}	0.07 ± 0.02^{a}
RE375	-1.3 ±0.09^{a}	-0.15 ± 0.009^{a}	4.32 ± 0.19^{a}	0.04 ± 0.03^{a}
RE500	-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
SA0	-1.18 ± 0.37^{a}	-0.152 ± 0.007^{a}	4.1 ± 0.23 ^{a}	0.13 ± 0.01^{a}
SA250	-1.12 ± 0.086^{a}	-0.149 ± 0.004^{abc}	3.84 ± 0.20^{ab}	0.105 ± 0.01^{a}
SA500	-1.08 ± 0.03^{a}	-0.147 ± 0.004^{abc}	3.7 ± 0.06^{b}	0.10 ± 0.1^{a}
SA750	-1.08 ±0.08^{a}	-0.139 ± 0.009^{bc}	3.50 ± 0.03^{b}	0.07 ± 0.06^{a}
SA1000	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

SA = Sodium Ascorbate

RE =Rosemary extracts

Means followed by different superscripts within row show statistically significant (p<0.05) differences (n=4) ± Standard deviation

2003) and such activity is system and concentration dependent. Given the requirement for non-organoleptic interference by antioxidant compounds this effectiveness of RE at lowest concentration is perceived as advantageous.

In previous studies in general the loss of redness is reflected on the increase of MetMb formation which was not the case in this RE doses study. Generally as the Hunter a* value was behaved inconsistently and 650/570 nm showed linear relation up to the higher level founding an adequate RE level for color 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 color and lipid oxidation effect of RE on meat products has been reported on the basis of comparison with other potential antioxidants (Djenane et al., 2002 and Sanchez-Escalante *et al.*, 2003) thus little information is available on concentration effect of RE.

Sodium ascorbate optimization against color and lipid oxidation of liver pâté: The mean rate of color variables change (slope) and P-value of the studied SA 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 1000 ppm (Table 2). A continuous decrease in loss of redness in response to SA treatment was also reported from previous works (Zuckerman and Mannheim 2001; Sahoo and Anjaneyulu, 1997). However Sahoo and Anjaneyulu (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 1000 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 SA as all the SA treated samples had a lower rate of MetMb formation than the control .The same decrease in MetMb formation due to SA enrichment was also noticed by Sahoo and Anjaneyulu (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 1000ppm level .This result was however not in agreement with Sahoo and Anjaneyulu (1997) report of SA at 500 ppm was adequate to retard discoloration and further increasing of SA 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 1000ppm 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 SA where the lower decrease (higher stability) was observed at 1000ppm. 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 color of the liver pâté (Cured product) owing to SA treatment.

In general previous works on SA level recommend different concentration. Shavis *et al*. (1984) reported vitamin C at 500 and 1000 ppm prolonged the color 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 SA 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 color stability.

CONCLUSIONS

Comparison of RE 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 RE doses revealed linear effect against lipid oxidation and the optimum was found at 375

ppm. SA 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 color stability and it is difficult to establish the optimum level of SA 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 RE as a potent antioxidant against lipid oxidation where as SA was better against discoloration. We suggest testing of combination of RE and SA 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.

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