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Effect of Treatment with Natural Antioxidant on the Chilled Beef Lipid Oxidation

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Abstract: The effect of superficial treatment of beef trimmings and beef knuckles with bone with taxifolin solution (1 g/L) on the lipid oxidation development during chilled storage was studied. Beef samples were air-packed or packaged in modified atmosphere consisting of 80%O_2/20%CO_2. It was found that the combined application of taxifolin treatment and MAP inhibited effectively formation of secondary derivates of lipid oxidation. The lipid hydroperoxides formation of these samples was reduced by 50-60% and accumulation of TBARS was significantly retarded in comparison with untreated with taxifolin, air-packed control samples. The MAP was found as the crucial factor for minimization of the FAME composition changes of chilled beef.

Key words: Beef, lipid oxidation, quality, shelf life, taxifolin

INTRODUCTION

Lipid oxidation is one of the main processes responsible for off-flavor development and quality loss during the beef chilled storage. The rate of lipid oxidation depends on the muscle type, the presence or absence of activators and inhibitors, and the location within the tissue. In this respect, increasing attention was put on modified atmosphere packaging (MAP) of meat (Narasimha and Sachindra, 2002). In order to increase the chilled beef shelf-life the MAP packaging has been applied in combination with antioxidant treatment. Djenane *et al.* (2003) performed pulverization of beef with rosemary and vitamin C in combination with MAP (70%O_2/20%CO_2/10%N_2). Lund *et al.* (2007) reported for the combined effect of rosemary extract and ascorbate/citrate /1:1/ treatment and MAP (100%N_2 or 80%O_2 /20%N_2) on the chilled beef proteins and lipid oxidation. There are reports for using of some natural antioxidants such as carnosine, carnetine and L-ascorbic acid solutions for beef treatment (Djenane *et al.*, 2004). Antioxidants added reduced the lipid oxidation rate and delayed hydrolysis by sequestering and stabilizing free radicals. Recently, the application of synthetic antioxidants was limited because of their potential health hazards. Therefore, the usage of natural antioxidants could be considered as perspective.

In the available literature we have not found reports for the application of bioflavonoides for pre-treatment of chilled meat. The quercetin, known also as taxifolin is one well-known representative of the flavones (Mitchell *et al.*, 2007).

There are several reports for quercetin application as efficient antioxidant for chilled salmon (Ivanov *et al.*, 2009; Balev *et al.*, 2009), edible oils (Plotnikov *et al.*, 2000), dry milk powder (Rulenko *et al.*, 1995), lard (Tiukavkina *et al.*, 1997), rich of fats sweets (Plotnikov *et al.*, 1993), and poultry fats (Krasiukov *et al.*, 2006). The analysis of the available literature showed that the combination of taxifolin treatment and MAP for inhibition of beef lipid oxidation has not been performed.

The objective of the present study was to establish the effect of treatment with taxifolin, extracted from Siberian larch (*Larix sibirica Ledeb*) on the air-packed and MAP chilled beef lipid oxidation.

MATERIALS AND METHODS

Materials: The beef was supplied by the company "Unitemp" Ltd, Voyvodinovo village, district Plovdiv, Bulgaria. The carcass quarters LOT: L0801300201 were imported from Poland. The carcasses were boned and sorted. The experimental work was performed in the laboratories of the University of Food Technologies, Plovdiv during the 2009.

Taxifolin (2R, 3R-dihydroquercetin) solution preparation: Powder concentrate of taxifolin, extracted from Siberian larch (*Larix sibirica Ledeb*) and produced

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by the company Flavit Ltd., Pushtino (Russia) was used. The concentrate contained: 96% dihydroquercetin, 3% dihydrokempferol and traces of naringenin. One g taxifolin was diluted in 50 cm^3 96% ethyl alcohol and filled up to 1 dm^3 with 950 cm^3 double distillated water. For treatment of fifty kg beef, 1dm^3 of the solution was used.

Modified atmosphere: Some samples of beef cuts were packaged in a modified atmosphere consisting of 80%O_2/20%CO_2 (MAP), according recommendations of Manchini and Hunt (2005).

Experimental design: The beef cuts were packed at the 6 d *post mortem*. The experiments were performed by using the fallowing samples: control C_A - air-packaged and not treated with taxifolin beef trimmings 90/10%; sample MA_A - MAP and not treated with taxifolin beef trimmings 90/10%; sample T_A - air-packaged and treated with taxifolin beef trimmings 90/10%; sample TMA_A - MAP and treated with taxifolin beef trimmings 90/10%; control C_B - air-packaged and not treated with a taxifolin beef knuckles with bone; sample MA_B - MAP and not treated with taxifolin beef knuckles with bone; sample T_B - air-packaged and treated with taxifolin beef knuckles with bone; TMA_B - MAP and treated with taxifolin beef knuckles with bone.

The samples were strained off for 60 min at 1.2°C. The temperature of air in the premises for packaging was 7.5ºC. Samples were stored at 0±0.5ºC before analysis. The analyses were carried out on 6 d *post mortem* (1st d of the experiment); 10 d *post mortem* (4th d of the experiment - after four days of refrigeration storage), 14 d *post mortem* (8th d of the experiment - after eight days of refrigeration storage), and 18 d *post mortem* (12th of the experiment - after twelve days of refrigeration storage).

Methods:

Sample preparation: The samples were obtained according ISO 3100-1: 1991. Before analysis samples were stored at 0°C for no more than 6 h.

Lipid extraction and determination of peroxide value: Lipids were extracted according to Bligh and Dyer (1959) method.

Peroxide Value (PV) of the extracted lipids was determined iodometrically following EVS-EN ISO 3960:2008. The POV was presented as meqvO_2/kg extracted lipids (Heaton and Uri, 2006).

Determination of thiobarbituric acid reactive substances (TBARS): 2-thiobarbituric acid reactive substances (TBARS) were determined according to the method described by Botsoglou *et al.* (1994). Double beam UV-VIS spectrophotometer Camspec, model M 550 (Camspec Ltd., Kembridge, United Kingdam) was used.

Preparation, purification and measurement of fatty acid methyl esters (FAMEs): The preparation of methyl esters was performed following the procedure of Christie (1984). FAMEs from the separated lipid fractions were obtained after a base-catalyzed trans-esterification with sodium methylate (0.5 mol/L; 1 h; 80ºC). FAMEs were purified by HPLC, using a preparative 100 mm x 10 mm i.d., stainless steel column filled with an SPE Si-NH_2 phase (IST). The polarity gradient was obtained by increasing the proportions of chloroform and methanol in heptane. As above, the column effluent was by-passed so that 90% of the purified FAMEs were recovered for gas chromatography, while the detector measured 10% (Chatelier *et al*., 2005).

Gas chromatography of FAMEs was performed on Shimadzu GC-MS-17A (Shimadzu GmbH, Duisburg, Germany), equipped with CP Wax 52 CB capillary column (30 m x 0.25 mm x 0.25 µm) (Varian Chrompak, Netherland); a Flame-Ionization Detector (FID) and Shimadzu CR-5A integrator (Shimadzu GmbH, Duisburg, Germany). Nitrogen was the carrier gas at flow 0.8 mL/min; split was 80:1. The temperature gradient was from 165 to 230ºC, with 4ºC/min, and kept for 20 min at this temperature. The temperatures of the injector and the detector were set at 260 and 280ºC, respectively. FAMEs were identified by comparing with the retention times of commercial fatty acid methyl ester standards analyzed under the same experimental conditions. The analysis was made in triplicate.

Obtaining of the dimethyloxazoline (DMOX) esters and GC-MS analysis: Preparation of fatty acid 4, 4'-DMOX esters was done by procedure described by Christie (1984). An Agilent 6890 Plus System (Agilent Technologies, Santa Clara, CA, USA) equipped with a 5793 mass selective detector (Agilent Technologies, Santa Clara, CA, USA) and a 30m x 0.25mm x 0.25 µm SP 2380 capillary column (Supelco, Bellefonte, PA, USA) were used to examine the DMOX derivatives. The temperature gradient started from 150ºC, and increased with 3ºC/min to 230ºC, and held at this temperature for 15 min; solvent delay was 2.2 min, T_{inj} was 260ºC and T_{aux} - 280ºC. Helium was the carrier gas at 0.8 mL/min. The mass detector was operated at T_{quad} 150ºC and T_{source} at 230ºC. Injection volume was 1.5 µL; split 20:1.

Fatty acids were identified by comparing the respective mass-spectra with those of authentic standard DMOX fatty acid derivatives using the database published in Internet.

Statistical analysis: Data were statistically analyzed by using of the SPSS 11.0 software (SPSS Inc., Chicago, Illinois, USA). Nine repetitions (n = 9) for each sample were carried out. Data were analyzed by the analysis of variance (one-way ANOVA) method with a significant level of $p \leq 0.05$ (Draper and Smith, 1998). The Duncan's

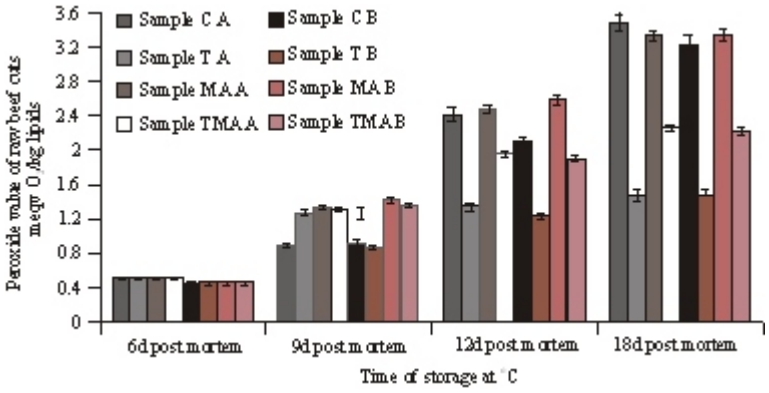

Fig. 1: Changes of the content of lipid hydroperoxides during 8 days refrigeration storage at 0ºC of samples A - beef trimmings 90/10% and samples B - beef knuckle with bones, expressed by changes of peroxide value

multiple comparison test (SPSS) with a significant difference set at $p \leq 0.05$ was used to compare sample means. Significant differences between means less than 0.05 were considered statistically significant (Kenward, 1987).

RESULTS AND DISCUSSIONS

Peroxide Value (PV): The results obtained (Fig. 1) showed, that the PV of all studied samples increased during the chilled storage. It is an evidence for hydroperoxides accumulation in beef. Greater increase of PV was found for C_A, C_B, MA_A and MA_B samples. During the 12 days of child storage, the PV of these samples increased from 0.85±0,07 meqv O_2/kg lipids to 3,28±0,12. At the end of the storage, the PV of C_A, C_B, MA_A and MA_B samples did not differ significantly ($p<0.05$). At the same time, PV of samples TMA_A increased 4.48 times, and of samples T_A - 2.78 times, only (Fig. 1). The highest increase of the PV was registered for the sample MA_B - 8.2 times. In sample TMA_B PV increased 5.45 times, and in samples T_B - 3.65 times.

The hypothesis was suggested that during the twelve days of storage at 0±0.5ºC (to 18d *post mortem*) superficial spraying with taxifolin solution had strong oppressive effect on the prooxidative enzymes of beef muscle tissue. When taxifolin treatment was combined with MAP, the 50-60% reduction of lipid hydroperoxides was determined. Nevertheless, during the refrigeration storage of MAP beef (Barbary and Zeitoun, 1992) or of air-paced beef, intensive oxidative processes were initiated (Kanner, 1994). The hydroperoxides and their derivatives further accelerate development and diffusion of the chain-radical oxidative reactions (Rhee *et al*., 1986).

The explanation of these processes could be found in taxifolin structure and properties (Tiukavkina *et al*., 1997) and its effect on lipid oxidation when the beef is packaged under reach of oxygen modified atmosphere (Zakrys *et al*., 2007). The taxifolin possesses ability to take part in the oxidative-reducing reactions of radical type. It plays role of reducing agent regarding radical substrate, passing over in oxidized form - flavoxile radical (Vladimirov *et al*., 2009).

Changes in TBARS values: Pronounced accumulation of TBARS on 18 d *post mortem* of control samples C_A and C_B was established (Fig. 2). During the chilled storage of beef, TBARS values of these samples increased with 12.8 and 11.9 times respectively. Similar tendency was established during the chilled storage of the samples T_A and T_B. At the end of the experiment, TBARS of these samples increased with 5.8 and 6 times, respectively. Retarded accumulation of TBARS during chilled storage of samples MA_A, MA_B, TMA_A, and TMA_B was found. At the end of the experiment, TBARS values of these samples increased with 2.6, 2.7, 2.3 and 2.4 times, respectively (Fig. 2). There were not statistically significant ($p>0.05$) differences between TBARS values of the MA and TMA samples. These results showed that the superficial spraying of chilled beef with taxifolin solution individually was not able to inhibit effectively the chemical reaction of chain-radical processes and the formation of secondary derivates of lipid oxidation. Therefore, the lipid oxidation processes of beef could be considered in the opening stage of initiation and diffusion of chain-radical reactions affected by enzymatic and non-enzymatic factors (Rhee *et al*., 1986). The spaying of the beef surface also exerts restrictive influence on the antioxidant effect of taxifolin (Vladimirov *et al*., 2009).

Nevertheless, according to the results obtained (Fig. 2) the combined application of antioxidant treatment and MAP inhibited effectively formation of secondary derivates of lipid oxidation.

FAME compositions: FAME compositions of the total beef lipids underwent heterogeneous changes during refrigeration storage depending on the packaging

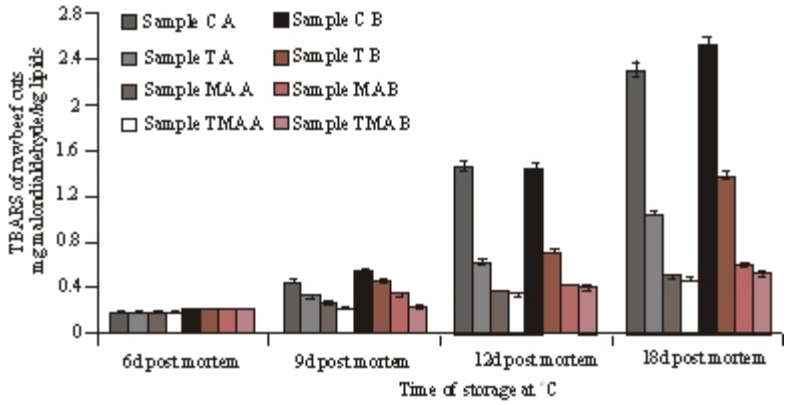

Fig. 2: Accumulation of the secondary derivatives of lipid oxidation during 8 days refrigeration storage at 0ºC of samples A - beef trimmings 90/10% and samples B - beef knuckle with bones, expressed by changes of TBARS

Table 1: Fatty acid compositions of total muscle lipids, extracted form samples A - beef trimmings 90/10% and samples B - beef knuckle with bone during 8 days of refrigeration storage at 0ºC (with normalization of data)

FAME, mg/100g total lipids	Samples A - beef trimmings 90/10%				Samples B - beef knuckle with bone			
	C_A	T_A	MA_A	TMA_A	C_B	T_B	MA_B	TMA_B
$C_{12:0}$	0.44±0.10	0.06±0.00	0.12±0.00	0.31±0.12	0.34±0.11	0.42±0.11	0.29±0.12	0.38±0.14
$C_{14:0}$	1.97±0.32	2.01±0.42	1.65±0.35	1.82±0.32	1.88±0.31	1.98±0.31	1.66±0.32	1.87±0.34
$C_{14:1}$ cis-9	0.03±0.00	0.37±0.12	0.16±0.15	0.24±0.12	0.03±0.00	0.22±0.08	0.29±0.12	0.37±0.14
$C_{15:0}$	0.53±0.10	0.47±0.14	0.52±0.09	0.49±0.03	0.55±0.11	0.48±0.11	0.48±0.13	0.52±0.12
$C_{16:0}$	25.20 ±0.56	24.66±0.64	23.94±0.59	22.35±0.53	23.77±1.01	24.10±0.61	23.53 ±1.07	23.14±0.89
$C_{16:1}$ cis-9	2.58±0.25	2.61±0.11	2.41±0.24	2.39±0.32	2.40±0.21	2.42±0.20	2.27±0.21	2.29±0.25
$C_{17:0}$	1.57±0.34	0.98±0.64	1.34±0.29	1.52±0.33	1.29±0.10	1.41±0.41	1.15±0.37	1.27±0.26
$C_{17:1}$ cis-10	1.57±0.34	0.98±0.64	1.33±0.59	1.52±0.53	1.29±0.48	1.41±0.31	1.15±0.27	1.24±0.36
$C_{18:0}$	16.72 ±0.45	15.61±0.21	16.56±0.24	16.17±0.11	15.88 ±0.21	16.26±0.20	15.32 ±0.11	16.18±0.22
$C_{18:1}$ cis-9	30.61±0.42	27.32±0.54	28.63 ±0.59	31.20 ±0.42	28.90 ±0.42	29.1±0.41	30.59± 0.43	29.46±0.39
$C_{18:1}$ trans-9	3.33±0.39	0.06±0.00	2.45±0.59	1.62±0.42	3.11±0.32	0.42±0.41	2.38±0.43	3.00±0.39
$C_{18:1}$ cis-11	1.59±0.25	1.95±0.22	1.71±0.29	1.64±0.22	1.84±0.24	1.87±0.41	1.86±0.23	1.73±0.39
$C_{18:1}$ trans-11	3.31±0.25	2.85±0.37	2.46±0.59	2.43±0.42	3.11±0.33	2.89±0.41	2.86±0.43	3.78±0.39
$C_{18:2}$ (CLA) cis-9. trans-11	0.14±0.00	0.23±0.04	0.18±0.00	0.18±0.02	0.18±0.04	0.20±0.04	0.19±0.03	0.14±0.00
$C_{18:2}$ w-6	3.26±0.29	12.59±0.32	8.40±0.35	8.30±0.42	8.61±0.42	8.81±0.41	7.99±0.33	6.36±0.41
$C_{18:3}$ w-6	0.05±0.00	0.08±0.00	0.08±0.00	0.10±0.00	0.08±0.00	0.09±0.00	0.08±0.00	0.06±0.00
$C_{18:3}$ w-3	1.55±0.22	1.16±0.11	1.30±0.24	1.19±0.21	1.41±0.10	1.21±0.31	1.44±0.11	1.41±0.12
$C_{20:0}$	0.88±0.12	0.05±0.00	0.65±0.12	0.46±0.11	0.40±0.10	0.52±0.10	0.56±0.11	0.45±0.10
$C_{20:2}$ w-6	0.09±0.00	0.13±0.01	0.08±0.00	0.09±0.11	0.10±0.00	0.11±0.00	0.12±0.00	0.13±0.00
$C_{20:3}$ w-6	0.33±0.00	0.63±0.05	0.38±0.12	0.50±0.11	0.49±0.11	0.49±0.10	0.50±0.05	0.52±0.11
$C_{20:4}$ w-6	0.80±0.10	3.07±0.12	2.84±0.12	2.52±0.11	1.10±0.21	2.41±0.10	2.19±0.25	2.71±0.11
$C_{20:5}$ w-3	0.83±0.10	0.61±0.13	0.77±0.12	0.76±0.11	0.84±0.21	0.64±0.10	0.71±0.11	0.76±0.12
$C_{24:0}$	0.65±0.10	0.04±0.00	0.58±0.12	0.47±0.11	0.39±0.10	0.41±0.10	0.37±0.11	0.40±0.00
$C_{22:1}$ cis-13	0.69±0.32	0.11±0.00	0.17±0.17	0.33±0.12	0.90±0.12	0.70±0.11	0.56±0.06	0.27±0.05
$C_{22:4}$ w-6	0.18±0.00	0.24±0.02	0.23±0.02	0.25±0.02	0.15±0.04	0.23±0.00	0.28±0.11	0.24±0.02
$C_{22:5}$ w-3	0.56±0.10	1.04±0.25	0.83±0.24	0.90±0.21	0.64±0.12	0.98±0.10	0.80±0.11	0.91±0.25
$C_{22:6}$ w-3	0.54±0.10	0.10±0.00	0.23±0.04	0.25±0.00	0.31±0.02	0.32±0.10	0.38±0.00	0.41±0.05
Total fatty acids	100±0.11	100±0.21	100±0.18	100±0.15	100±0.19	100±0.17	100±0.18	100±0.16
Total saturated fatty acids	47.96 %	43.87 %	45.36 %	43.59 %	44.50 %	45.58 %	43.36 %	44.21 %
Total monounsaturated fatty acids	43.71 %	36.25 %	39.32 %	41.37 %	41.59 %	38.94 %	41.96 %	42.14 %
Total polyunsaturated fatty acids	4.85 %	19.88 %	15.32 %	15.04 %	13.91 %	15.48 %	14.68 %	13.65 %
Ratio w-6 /w-3 PUFA	1.39	5.33	3.89	3.85	3.35	3.91	3.41	2.91
Ratio PUfA/SFA	0.17	0.45	0.34	0.34	0.31	0.34	0.34	0.31

The means indicated with different letters are statistically significant different ($p \leq 0.05$). Results in the table are presented as relative percentages of the peak area, received after integration.

conditions and taxifolin treatment (Table 1). The FAME compositions of the total lipids extracted from beef trimmings 90/10% were significantly differed from those of beef knuckles.

The optimal ratio of n-6/n-3 Polyunsaturated Fatty Acids (PUFA) - 5.33 and the highest percentage of total PUFA - 19.88% were determined in samples T_A. The PUFA/SFA ratio of these samples was 0.45, which value

was higher in comparison with the other experimental samples. The FAME composition of MA_A and TMA_A samples did not differ significantly ($p>0.05$). The n-6/n-3 PUFA ratio of samples MA_A and TMA_A was 3.89 and 3.85, respectively. The total PUFA content of these samples was 15.32 and 15.04%, and the PUFA/SFA ratio was 0.34 and 0.35, respectively. These results showed that the MAP is the crucial factor for minimization of the FAME composition changes. On the other hand, taxifolin treatment of air-packed beef trimmings 90/10% stabilizes the PUFA, and especially the PUFA with a proven dietetic and healthy effect (Gray *et al*., 1996). The smallest levels of n-6/n-3 PUFA ratio - 2.91, total PUFA content - 13.651%, and PUFA/SFA ratio - 0.31 were determined for samples TMA_B (Table 1).

The differences of fatty acid composition of total lipid from two types beef muscles established in the present study were in accordance with the results of Kanner (1994). Oxidative quality and shelf-life of beef, including FAME composition of the total lipids, depend from oxidative or glycolytic muscle type (Gray *et al*., 1996). Taxifolin treatment was more effective for inhibition of lipid oxidation development in oxidative muscles (Vladimirov *et al*., 2009) than in rich of oxygen modified atmosphere packaged meat (Zakrys *et al*., 2007).

CONCLUSION

The results obtained and their analysis let a conclusion to be made that the superficial treatment of beef with taxifolin solution (1 g/L) combined with packaging in modified atmosphere consisting of $80\%O_2/20\%CO_2$ delayed significantly lipid oxidation development during chilled storage. Combined application of taxifolin treatment and MAP reduced the lipid hydroperoxides formation by 50 to 60% and retarded accumulation of TBARS in chilled beef. The MAP of chilled beef was found as the crucial factor for minimization of the FAME composition changes.

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