JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

PRESERVING THE QUALITY AND PROLONGATION THE SHELF-LIFE OF BEEF PACKED UNDER VACUUM OR MODIFIED ATMOSPHERE USING TERNARY ANTIOXIDANT BLEND

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doi: 10.15414/jmbfs.2016.5.6.617-622

ARTICLE INFO

Received 5. 10. 2014
Revised 28. 2. 2016
Accepted 1. 3. 2016
Published 1. 6. 2016

Regular article

OPEN ACCESS

ABSTRACT

Keeping the quality and prolongation the shelf-life of stored at 0 ± 0.5ºC packed under vacuum or modified (80%O_2/20%CO_2) atmosphere beef *m. semimembranosus* sprayed with 0.02% solution, containing 10 g.l^{-1} dihydroquercetin from Siberian larch (*Larix sibirica Ledeb*), 5 g.l^{-1} rosemary (*Rosmarinus officinalis*) extract and 1 g.l^{-1} L-ascorbic acid was studied. The experiments were carried out with five samples: control - air packaged; vacuum packaged; vacuum packaged and treated with 0.02% ternary antioxidant blend; packaged under modified atmosphere (80%O_2/20%CO_2); and packaged under rich in oxygen modified atmosphere, after spaying with 0.02% ternary antioxidant blend. Samples were stored 28 days (to 32 d *post mortem*) at 0 ± 0.5ºC. The pre-treatment of beef with ternary antioxidant blend preserve the sensory scores and colour properties of beef, and inhibited total microbial growth, and development of *Brochothrix termosphacta* and pathogens to the end of storage (28 d at 0 ± 0.5ºC), was found. The pre-treatment of beef with ternary antioxidant blend was not main factors which can affect the pH and free amino nitrogen changes in fresh beef. The pre-treatment of beef with 0.02% ternary antioxidant blend may be successfully used for preserving the quality and prolonging the shelf-life of beef *m. semimembranosus* packed under modified (80%O_2/20%CO_2) atmosphere. The shelf-life can extend with 75% compared to air packed meat, and with 7 days against only vacuum- or modified atmosphere packed beef.

Keywords: Natural antioxidants, vacuum, modified atmosphere packaging, meat, sensory properties, colour, microbiological status

INTRODUCTION

The main criterions in purchasing fresh beef on the market are its colour, flavour, odour and microbiological status (**Ercolini *et al.*, 2006**). The sensory properties are the most important factor determining the meat quality. That is way, the extension of the quality and shelf-life of packed chilled beef was one of necessity to meet the demands of consumers **(Lagerstedt *et al.*, 2011)**. In this respect, increasing attention was put on the packaging conditions. Vacuum packed meat changes its colour from bright-red to purple-brown during refrigeration **(Blixt and Borch, 2002)**. The reason is conversion of muscle oxymyoglobin in metmyoglobin **(Mancini and Hunt, 2005)**. By controlling the levels of residual oxygen in the package, prevention of this negative phenomenon is achieved. The rich in oxygen modified atmosphere packaging (MAP) preserves muscle oxymyoglobin two times longer, compared to air-packed meat **(Lagerstedt *et al.*, 2011)**. Unfortunately, the high-oxygen MAP system induces lipid and myoglobin oxidation and protein polymerization **(Kim *et al.*, 2010)**. Better oxidative, microbial and colour stability can be achieve when combined the effect of antioxidants and MAP **(Lund *et al.*, 2007; Rojas and Brewer, 2007)**.

For extending the shelf-life of the MAP (70%O_2/20%CO_2/10%N_2) beef was used rosemary or vitamin C solutions **(Djenane *et al.*, 2003)**. **Lund *et al.* (2007)** offered the protein and lipid oxidation in MAP (100% N_2, or 80%O_2/20%N_2) minced beef patties stored for 6 days in the dark at 4°C, to be inhibit by addition of rosemary extract or ascorbate/citrate (1:1) mixture. Natural antioxidants such as rosemary extracts **(Rohlik *et al.*, 2010, 2012)** and dihydroquercetin **(Semenova *et al.*, 2008; Bakalivanova and Kaloyanov, 2012)** have been proposed for prolongation of the shelf-life of meat products and especially the MAP beef **(Fernández-López *et al.*, 2005; Balev *et al.*, 2010)**.

Rosemary extracts (RE) were able to scavenge the free hydroxyl radicals converting them into stable products **(Djenane *et al.*, 2003; Brewer, 2011)**. The aqueous extract of rosemary (*Rosmarinus officinalis Linn.*) contains phenolic diterpenes (carnosic, carnosol, rosmanol, rosmadial, 12-methoxycarnosic acid, epi-, and iso-rosmanol) and phenolic acids (rosmarinic and caffeic) **(Brewer, 2011)**. While carnosic acid also has a single aromatic ring, it has two -OH groups that can serve as H donors. The vicinal -OH groups can chelate pro-oxidative metals thereby preventing oxidation. The polyphenols, rosmarinic acid has two aromatic rings, each with two -OH groups that are capable of donating H^+ and chelating metals. In lipid-based systems, carnosic acid and carnosol effectively chelate iron and scavenge peroxyl radical **(Djenane *et al.*, 2003; Lund *et al.*, 2007)**.

The dihydroquercetin (DHQ) is a dihydroflavonol. It is having properties of a powerful free radical-chain terminator **(Bakalivanova and Kaloyanov, 2012)**. The common characteristic of the flavonoids is the basic 15-carbon flavan structure ($C_6C_3C_6$). These carbon atoms are arranged in three rings (A, B, and C) **(Brewer, 2011)**. The free radical-scavenging potential of the dihydroquercetin includes: (1) Phenolic hydroxyls in flavonoids were the main active groups capable of scavenging $^{\bullet}$OH, (2) Hydroxyl groups in ring B and A were important $^{\bullet}$OH-scavenging active groups, (3) The ortho-dihydroxyl groups in ring A and/or B could greatly enhance the $^{\bullet}$OH-scavenging activity of the rings, (4) The hydroxyl groups on 3',4' position of ring B possessed the highest $^{\bullet}$OH-scavenging activity compared with hydroxyl groups in ring B, and was higher than that of hydroxyl groups in ring A **(Vladimirov *et al.*, 2009)**. The synergism and antagonism between quercetin and other chain-breaking antioxidants is possible, too **(Becker *et al.*, 2007)**.

L-ascorbic acid (AA) is a source of four -OH groups. AA can donate hydrogen to an oxidizing system, to chelate metal ions (i.e. Fe^{2+}), to scavenge free radicals, to quencher the $O_2^{-\bullet}$ radicals, and to acts as a reducing agent **(Brewer, 2011)**. At high levels (>1000 mg.kg^{-1}) AA shifts the balance between ferrous (Fe^{2+}) and ferric (Fe^{3+}) iron, and acts as an oxygen scavenger. However, at low levels (<100 mg.kg^{-1}) it can catalyse oxidation in muscle tissue **(Yetella and Min, 2008)**. L-ascorbic acid can exert a synergistic effect when added along with polyphenolic antioxidants as play role of metals chelators **(Brewer, 2011)**.

The stored meat loses potential to oxygenate deoxymyoglobin. When beef is packaged under vacuum the oxygen content must be less than 0.05%. Addition of reducing agents is recommended to improve oxygen absorption **(Motoyama *et al.*, 2010)**. The inhibition of oxidative processes in beef using combination of

rosemary extracts, as free radical scavengers, which improves and enhances antioxidant effect of dihydroquercetin **(Silva *et al.*, 2002)** was still not discussed.
All the above-mentioned combinations of the treatment of MAP or VP meat with separate antioxidants have not shown a satisfactory effect when it comes to a more prolonged of 21 days storage at 0 - 4°C **(Fernández-López *et al.*, 2005; Kim *et al.*, 2010)**. In the available literature, we did not encounter information on the use of a combination of natural antioxidants and vitamin C, which exhibit a synergistic effect.
That is why, the objective of this study was to determine the effect of surface pre-treatment with of ternary antioxidant blend (TAB) containing dihydroquercetin extracted from Siberian larch *(Larix sibirica Ledeb)*, extract from rosemary (*Rosmarinus officinalis Linn.*), and L-ascorbic acid on the quality and shelf-life of (80%O_2/20%CO_2) modified atmosphere packed (MAP) or vacuum packed (VP) beef.

MATERIALS AND METHODS

The beef was supplied by the company "Unitemp" Ltd (Voyvodinovo village, district Plovdiv, Bulgaria). The carcass quarters were imported from slaughterhouse SC Nicbac ProdSRL (Loc. Nicolae Balcescu village, district Bacau, Romania). The carcasses were deboned, and the *m. semimembranosus* were packaged on the 4th day *post mortem.* The pH of meat was 5.36, and the temperature at the moment of packaging was around 1°C.
Three-component antioxidants blend solution was prepared as ten g DHQ and one g AA were dissolved in 25 ml 96% ethanol. Five g RE were emulsified in 20 cm^3 96% ethanol. Two liquids were mixed. The mixture was filled up to 1 dm^3 with 950 cm^3 bidistilled water.
The powdered dihydroquercetin extract (DHQ) from Siberian larch (*Larix sibirica Ledeb*) was purchased by Flavit Ltd. (Pushtino, Russia). It contains: 96% dihydroquercetin, 3% dihydrokempferol and 1% naringenin. The powdered rosemary extract (RE) was supplied by Aromena Ltd. (Sofia, Bulgaria). The content of flavonoids was approx. 42 $g.kg^{-1}$, and peroxide value (POV) = 0.658 ± 0.018 meqv $O_2.kg^{-1}$lipids. The L-ascorbic acid (AA) was purchased from Sigma Chemical Group Pty Ltd. (Balcatta, Perth WA, USA). All rest chemicals and reagents were purchased from E. Merck KGaA (Darmstadt, Germany).
The surfaces of the 50 kg beef *m. semimembranosus*, with temperature 6.3ºC, were sprayed with 1 dm^3 0.02% TAB. Samples were strained off for 60 min at 1.2°C, and were packaged in transparent polymer bags. The packaged samples were put into plastic boxes, labelled and stored at 0 ± 0.5°C before analysis. One part of examined samples was MAP (80%O_2/20%CO_2). The other part of experimental samples was VP. A packaging machine Yang SR1, model Polaris VAC, Ductto (Como via al Bassone, Italy) was used.
The experiments were carried out with 5 groups: control samples C - air packaged only, samples VP - vacuum packaged only, samples AVP - vacuum packaged and treated with 0.02% TAB, samples MAP - packaged under modified atmosphere (80%O_2/20%CO_2) only, and samples AMAP - packaged under rich in oxygen (80%O_2/20%CO_2) modified atmosphere, after spaying with 0.02% TAB. Samples were stored 28 days. The analyses were carried out on: 4 day *post mortem* (1 day of experiment), 11 day *post mortem* (7 day of storage), 18 day *post mortem* (14 day of storage), 25 day *post mortem* (21 day of storage), and 32 day *post mortem* (28 day of storage).The samples were obtained according ISO 3100-1:1991. Before analysis samples were stored at 0 ± 0.5°C no more than 6 h.
The panellists making sensory analysis participated in six training sessions and underwent performance testing as specified in guidelines developed by **Meilgaard *et al.* (2006)**. The panellists were passed the triangular test for differentiation of fresh and rancid meat taste, odor and colour. The beef roasts were evaluated by the panellists for aromatics (cooked beef/broth, cooked beef fat, chemical taste, serum/bloody and plum/prune), feeling factors (astringent, metallic and chemical burn) and basic tastes (salt, sour, bitter and sweet). The roast beef samples were also scored using 1-5 scale **(Larick and Turner, 1990)**. Examinations of beef samples were done after the packs opening. The chilled to 0°C samples were put in aluminium foil packs and grilled for 20 min at 200-250°C.
The colour measurement was made by colorimeter Konica Minolta model CR-410 (Konica Minolta Holding, Inc., Ewing, New Jersey, USA), purchased by Sending Inc. (Tokyo, Japan). By it was evaluated the brightness of the colour (L*), red (a*) and yellow (b*) color component **(Hunt *et al.*, 2012)**.
The modified titration method of Sørensen **(Lorenzo *et al.*, 2008)** was used for free amino nitrogen determination in beef samples.
pH value of the beef was determined using pH-meter MS 2004 (Microsyst Ltd., Plovdiv, Bulgaria), equipped by combined pH electrode Sensorex Combination Recorder S 450 CD (Sensorex pH Electrode Station, Garden Grove, CA, USA **(Young *et al.*, 2004)**.
The total aerobic plate count was determined by EN ISO/DIS 4833-2003 **(Cohen *et al.*, 2007)**. The *Escherichia coli* were estimated according ISO 16649-1:2001 **(Nastasijevic *et al.*, 2009)**. The *Salmonella* bacteria determinations were carried out following ISO 6579-2002 **(Piknová *et al.*, 2002)**, and the *Listeria monocytogenes* - using ISO 11290-2002 **(Scotter *et al.*, 2004)**. The *Brochothrix termosphacta* was determined by BSS ISO 13722:2002 **(Russo *et al.*, 2004)**, and of *Enterobacteriaceae spp.* - using BSS ISO 21528-1:2011 **(Ercolini *et al.*, 2006)**.
The data were analysed using factorial analysis of variance procedure (mixed procedure) of SAS Version 8.2 software package **(SAS Institute Inc., 2002)**. The model included the main effects and interactions of nine treatments and four storage times. Multiple tests were used to separate means at 95% significance level for each test.

RESULTS

Sensory evaluated properties

At four (C, VP, AVP, and MAP) from five examined samples steadily decreasing average scores for sensory evaluated beef *m. semimembranosus* surface colour during storage at 0 ± 0.5ºC were assessed. The only exception were samples AMAP, (Fig 1).
The colour of the samples VP was unacceptable after 28 days of refrigeration storage at 0 ± 0.5ºC (32 d *post mortem*). At the end of the experiment (28 d), the colour of the samples AVP and MAP remains acceptable and their marks undergoes reduction by 27 % and 36 % respectively. The reduction in samples VP was 57 %.
During the storage period (28 d at 0 ± 0.5ºC) the odour's scores of samples C, VP, AVP and MAP decreases significantly ($P < 0.05$). The only exception was samples AMAP, where the odour scores were very high throughout the studied period and the average values was not significantly ($P > 0.05$) different to the end of the experiment (Fig 1). Reduction of the smell average scores in samples MAP was 28.0%, while the established decrease in samples AVP was 16.3%.
Similarly to the results obtained for smell, in four (C, VP, AVP and MAP) from the five examined samples the scores for taste decreases during the storage period (28 d at 0 ± 0.5ºC). The only exception were the samples AMAP. In these samples (AMAP) the taste scores were found very high throughout the studied period and the average values did not significantly ($P > 0.05$) different by the end of the experiment (Figure 1). After 28 days of refrigeration storage at 0 ± 0.5ºC (32 d *post mortem*), the flavour of samples VP was completely unacceptable and unpleasant as scores were decreased by 57.1%. The flavour of samples AVP and MAP retained acceptable to the end of the experiment (28 d at 0 ± 0.5ºC). The reduction of the flavour scores in samples MAP was 32.0%, and in samples AVP - 18.4 %. Only the combination of the surface treatment of beef *m. semimembranosus* with 0.02% TAB, and MAP (80%O_2/20%CO_2), was able to preserve the good sensory properties and to extend the shelf-life of meat to 28 day (or up to 32 day *post mortem*). The MAP samples demonstrated good sensory properties up to 21st day after packaging (up to 25 day *post mortem*). During the storage period (28 d at 0 ± 0.5ºC) the highest decrease in sensory evaluated colour was established in vacuum packaged beef (Fig 1).

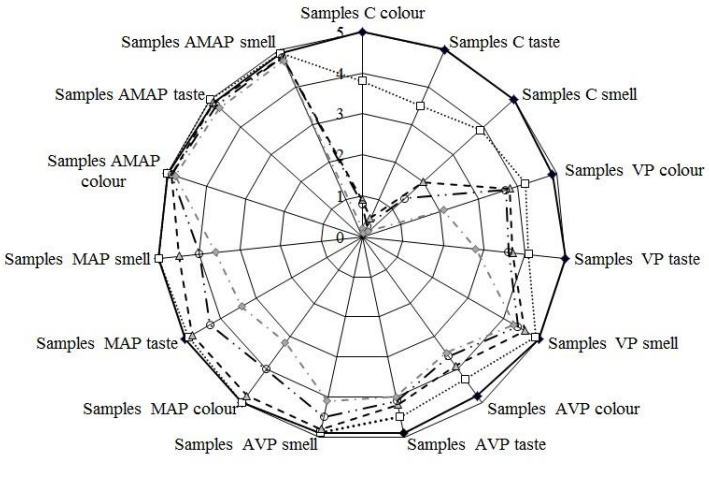

Figure 1 Effect of the ternary antioxidant blend superficial treatment on the sensory characteristics of beef *m. semimembranosus* packaged under vacuum and rich of oxygen modified atmosphere during storage at 0 ± 0.5ºC.
Legend: **samples C** - controls only air packaged; **samples VP** - vacuum packaged only; **samples AVP** - vacuum packaged and treated superficially with 0.02% tertiary antioxidant blend; **samples MAP** - packaged under modified atmosphere

($80\%O_2/20\%CO_2$) only, **samples AMAP** - packaged under rich in oxygen ($80\%O_2/20\%CO_2$) modified atmosphere, after superficial spaying 0.02% tertiary antioxidant blend

Instrumentally measured colour characteristics

The results obtained for instrumentally measured colour characteristics (Table 1) of beef *m.–semimembranosus* during storage at 0 ± 0.5°C correspond with the data from sensory analysis (Fig 1). During the refrigeration storage (28 d at 0 ± 0.5ºC) the colour lightness (L*) of control samples C significantly ($P \leq 0.05$) decrease with 7.33% comparing with initial values.

Table 1 Effect of the three-component antioxidant blend superficial treatment on the colour characteristics of the external surface of beef *m. semimembranosus* packaged under vacuum and rich of oxygen modified atmosphere during storage at 0 ± 0.5ºC

Samples	Storage time, days	The colour lightness, (L*)	Redness (a*)	Yellowness (b*)
Control samples C	1d	38.36 ^{a} ± 0.47	23.55 ^{a} ± 0.49	9.65 ^{a} ± 0.33
	7d	38.37 ^{a} ± 0.46	23.28 ^{a} ± 0.52	9.50 ^{a} ± 0.34
	14d	37.02 ^{b} ± 0.39	14.37 ^{b} ± 0.48	10.44 ^{b} ± 0.46
	21d	36.40 ^{b} ± 0.38	12.83 ^{c} ± 0.47	11.83 ^{c} ± 0.37
	28d	35.55 ^{c} ± 0.43	11.36 ^{d} ± 0.39	12.67 ^{d} ± 0.36
Samples VP	1d	38.36 ^{a} ± 0.47	23.55 ^{a} ± 0.66	9.65 ^{a} ± 0.33
	7d	38.14 ^{a} ± 0.42	23.26 ^{a} ± 0.50	9.63 ^{a} ± 0.59
	14d	37.27 ^{b} ± 0.38	20.40 ^{b} ± 0.63	6.77 ^{b} ± 0.49
	21d	37.09 ^{b} ± 0.34	20.19 ^{b} ± 0.53	6.65 ^{b} ± 0.37
	28d	36.36 ^{c} ± 0.33	19.14 ^{c} ± 0.45	5.90 ^{c} ± 0.35
Samples AVP	1d	38.36 ^{a} ± 0.47	23.55 ^{a} ± 0.49	9.65 ^{a} ± 0.33
	7d	38.47 ^{a} ± 0.44	23.63 ^{a} ± 0.51	10.58 ^{b} ± 0.50
	14d	38.36 ^{a} ± 0.51	22.40 ^{b} ± 0.50	10.51 ^{b} ± 0.44
	21d	37.86 ^{b} ± 0.45	21.30 ^{c} ± 0.43	9.39 ^{c} ± 0.39
	28d	36.89 ^{c} ± 0.42	20.85 ^{d} ± 0.48	8.67 ^{d} ± 0.32
Samples MAP	1d	38.36 ^{a} ± 0.47	23.55 ^{a} ± 0.39	9.65 ^{a} ± 0.33
	7d	39.96 ^{b} ± 0.46	24.09 ^{b} ± 0.36	10.26 ^{b} ± 0.56
	14d	39.33 ^{b} ± 0.42	23.86 ^{b} ± 0.45	11.49 ^{b} ± 0.58
	21d	38.42 ^{c} ± 0.32	21.01 ^{c} ± 0.46	12.64 ^{c} ± 0.47
	28d	34.77 ^{d} ± 0.39	16.78 ^{d} ± 0.48	14.53 ^{d} ± 0.68
Samples AMAP	1d	38.36 ^{a} ± 0.47	23.55 ^{a} ± 0.39	9.65 ^{a} ± 0.33
	7d	38.87 ^{a} ± 0.49	23.56 ^{a} ± 0.36	10.21 ^{a,b} ± 0.38
	14d	38.49 ^{a} ± 0.47	23.51 ^{a} ± 0.41	10.77 ^{b} ± 0.47
	21d	38.26 ^{a} ± 0.43	23.42 ^{a} ± 0.39	11.01 ^{b} ± 0.43
	28d	37.98 ^{a} ± 0.42	23.18 ^{a} ± 0.33	11.34 ^{b} ± 0.39

Means ± standard divinations
$^{a, b, c, d}$ – indexes show data with statistical different value in columns ($p < 0.05$)

Similarly to the control samples C, the colour lightness (L*) in samples VP decreases with 5.21%. However, on 28th day of storage the colour lightness (L*) of samples MAP was reduced with 9.36% ($P \leq 0.05$). A decrease by 3.83% in the L* value of samples AVP was estimated. Only the lightness (L*) of the samples AMAP does not change significantly ($P > 0.05$) throughout the 28 day period of the refrigeration storage at 0 ± 0.5°C. Our results was similar to the data obtained by **Ahn *et al.* (2002)** which determined beef patties containing antioxidants had higher L* values after 9th day of storage.

The colour redness (a*) in four of examined samples (C, VP, AVP, and MAP), decreases significantly ($P \leq 0.05$) during 28 days of storage at 0 ± 0.5ºC. The established decreases were with 51.76%, 18.73%, 11.46%, and 28.75% respectively, compared with initial values. For the studied period (28 days at 0 ± 0.5ºC) only the a*-values was of samples AMAP was unchanged ($P > 0.05$).

The results obtained for colour yellowness (b*) indicate different trends between beef samples during 28 days storage at 0 ± 0.5°C (Table 1). The b* values of control samples C and samples MAP increased significantly ($P \leq 0.05$) with 31.3%, and with 50.6%. Superficial pre-treatment of beef with 0.02% natural antioxidant mixture increases the b* values of rich in oxygen modified packaged samples AMAP only to 17.5%. Contrary, a decreasing of the b* values was observed in VP samples, and was less pronounced in samples AVP (Table 1). After 28th day of storage at 0 ± 0.5°C the colour yellowness (b*) in samples VP decrease with 38.9%, and in samples AVP – with 10.2% **(**Table 1).

pH value

The pH of experimental samples VP, AVP, MAP and AMAP increases very slightly, but significantly ($P \leq 0.05$) during 28 days of storage at 0 ± 0.5ºC (to 32nd day *post mortem*) (Table 2). The only exception were control samples C, with 18.21% increase of pH. At the end of the experiment (28 d at 0 ± 0.5ºC) maximal increase of the pH was established in samples MAP and AMAP ($P > 0.05$). For the studied period (28 d at 0 ± 0.5ºC) the pH of samples VP and AVP increase with 6.38% and 6.92% respectively.

Free amino nitrogen

A significant ($P \leq 0.05$) increasing of FAN was determined in all samples (Table 2). After 28 days of storage (32 d *post mortem*) FAN content in control samples C raised 27.8 times. The increase in experimental samples was from 15.2 (samples VP) to 15.8 times (sample AVP). During 28 days of refrigeration storage (0 ± 0.5ºC) the FAN content in test samples does not exceed 10 $mg.100g^{-1}$ meat. These results confirm the data from sensory estimated beef odour and flavour and were evidence for a proteolysis.

Table 2 Effect of the three-component antioxidant blend superficial treatment on the pH and free amino nitrogen content of beef *m. semimembranosus* packaged under vacuum and rich of oxygen modified atmosphere during storage at 0 ± 0.5ºC

Samples	Storage time, d	pH	Free amino nitrogen, $mg.kg^{-1}$ meat
Control samples C	1d	5.49 ^{a} ± 0.03	0.22 ^{a} ± 0.09
	7d	5.83 ^{b} ± 0.02	4.15 ^{b} ± 0.52
	14d	5.83 ^{b} ± 0.04	5.07 ^{c} ± 0.48
	21d	6.09 ^{c} ± 0.05	8.75 ^{d} ± 0.47
	28d	6.49 ^{d} ± 0.06	10.04 ^{e} ± 0.39
Samples VP	1d	5.49 ^{a} ± 0.03	0.22 ^{a} ± 0.09
	7d	5.54 ^{a} ± 0.04	3.86 ^{b} ± 0.50
	14d	5.56 ^{a} ± 0.05	4.69 ^{c} ± 0.63
	21d	5.61 ^{b} ± 0.04	5.29 ^{d} ± 0.53
	28d	5.62 ^{b} ± 0.02	6.04 ^{e} ± 0.45
Samples AVP	1d	5.49 ^{a} ± 0.03	0.22 ^{a} ± 0.09
	7d	5.63 ^{b} ± 0.03	3.94 ^{b} ± 0.51
	14d	5.64 ^{b} ± 0.04	4.87 ^{c} ± 0.50
	21d	5.66 ^{b} ± 0.04	5.83 ^{d} ± 0.43
	28d	5.75 ^{c} ± 0.04	6.42 ^{e} ± 0.48
Samples MAP	1d	5.49 ^{a} ± 0.03	0.22 ^{a} ± 0.09
	7d	5.47 ^{a} ± 0.04	3.68 ^{b} ± 0.36
	14d	5.78 ^{b} ± 0.03	4.63 ^{c} ± 0.45
	21d	5.81 ^{b,c} ± 0.04	5.32 ^{d} ± 0.46
	28d	5.87 ^{c} ± 0.04	6.01 ^{e} ± 0.48
Samples AMAP	1d	5.49 ^{a} ± 0.03	0.22 ^{a} ± 0.09
	7d	5.64 ^{b} ± 0.03	3.89 ^{b} ± 0.36
	14d	5.75 ^{c} ± 0.05	4.75 ^{c} ± 0.41
	21d	5.78 ^{c} ± 0.04	5.52 ^{d} ± 0.39
	28d	5.84 ^{d} ± 0.04	6.31 ^{e} ± 0.33

Means ± standard divinations
$^{a, b, c, d, e}$ – indexes show data with statistical different value in columns ($p < 0.05$)

Microbiological examinations

The standard examined microbiological indices for freshness and hygiene of samples were in the norms during whole 28th day period of storage at 0 ± 0.5°C (Table 3). These results was evidence that all examined samples meet the requirements of Regulation (EC) № 1441/2007. The only exception was found in control samples C on the 28th day of storage. In these samples a triple increase of the *E.coli* growth was determined.
The similar results were observed for the *Enterobacteriaceae spp.* In samples VP, AVP, MAP and AMAP the absence or very limited growth of *Enterobacteriaceae spp.*, determined between 14th and 28th day of storage at 0 ± 0.5ºC were found. In contrast, in control samples C was established systematic irregular increase in the *Enterobacteriaceae spp*, more pronounced after 21st day of storage when viewed 128.6 times more colonies, and further – on the 28th day – 653.6 times. However, the *Brochothrix thermosphacta* colonies increased significantly ($p \leq 0.05$), but on the end of storage (28th day) not exceed 50000 - 1890000 cfu.g in different samples. The most pronounced increase was rapidly detected in control samples C - 9450 times from the initial number. Ten-fold smaller number of *Brochothrix thermosphacta* colonies was determined in samples AMAP. The slowest increase of the *Brochothrix thermosphacta* colonies – only 250 times was found in samples AVP.

Table 3 Effect of the three-component antioxidant blend superficial treatment on the microbiological status of beef *m. semimembranosus* packaged under vacuum and rich of oxygen modified atmosphere during storage at 0 ± 0.5ºC

Samples	Storage time at 0 ± 0.5ºC, d	Total mesophilic aerobic and facultative anaerobic microorganisms, cfu/g Norm *5.10⁵- 5.10⁶* cfu/g	*Brochothrix termosphacta*, cfu/g Norm is not listed	*Enterobacteriaceae*, cfu/g Norm is not listed	*Escherichia coli*, cfu/g Norm *500 - 5000* cfu.g^{-1}	*Salmonella* spp., Presence in 25 g Norm: Absence in *25 g sample*	*L. monocytegenes*, Presence in 1 g Norm: Absence in *1 g sample*
Control samples C	**1d**	534 ^{a} ± 38	200 ^{a} ± 24	28 ^{a} ± 15	20 ^{a} ± 8	-	-
	7d	1938 ^{b} ± 28	970 ^{b} ± 37	50 ^{a,b} ± 8	40 ^{b} ± 7	-	-
	14d	321000 ^{c} ± 592	130000 ^{c} ± 146	60 ^{b} ± 10	50 ^{b} ± 10	-	-
	21d	962400 ^{d} ± 2608	784000 ^{d} ± 569	3600 ^{c} ± 187	3300 ^{c} ± 155	-	-
	28d	2314000 ^{e} ± 4183	1890000 ^{e} ± 1258	18300 ^{d} ± 433	15200 ^{d} ± 373	-	-
Samples VP	**1d**	534 ^{a} ± 38	200 ^{a} ± 24	28 ^{a} ± 15	20 ^{a} ± 8	-	-
	7d	1606 ^{b} ± 36	760 ^{b} ± 30	-	-	-	-
	14d	20300 ^{c} ± 384	25000 ^{c} ± 133	-	-	-	-
	21d	71400 ^{d} ± 548	50000 ^{d} ± 487	-	-	-	-
	28d	195200 ^{e} ± 837	130000 ^{e} ± 792	26 ^{a} ± 13	19 ^{a} ± 8	-	-
Samples AVP	**1d**	534 ^{a} ± 38	200 ^{a} ± 24	28 ^{a} ± 15	20 ^{a} ± 8	-	-
	7d	960 ^{b} ± 24	850 ^{b} ± 32	-	-	-	-
	14d	12300 ^{c} ± 348	6200 ^{c} ± 90	-	-	-	-
	21d	44600 ^{d} ± 894	7000 ^{d} ± 355	-	-	-	-
	28d	179000 ^{e} ± 1000	50000 ^{e} ± 669	14 ^{a} ± 9	10 ^{a} ± 7	-	-
Samples MAP	**1d**	534 ^{a} ± 38	200 ^{a} ± 24	28 ^{a} ± 15	20 ^{a} ± 8	-	-
	7d	1380 ^{b} ± 27	1240 ^{b} ± 95	-	-	-	-
	14d	4118 ^{c} ± 93	3200 ^{c} ± 55	-	-	-	-
	21d	321000 ^{d} ± 837	68000 ^{d} ± 852	-	-	-	-
	28d	981800 ^{e} ± 2049	121000 ^{e} ± 1653	-	-	-	-
Samples AMAP	**1d**	534 ^{a} ± 38	200 ^{a} ± 24	28 ^{a} ± 15	20 ^{a} ± 8	-	-
	7d	820 ^{b} ± 26	400 ^{b} ± 23	-	-	-	-
	14d	980 ^{c} ± 286	640 ^{c} ± 28	-	-	-	-
	21d	104200 ^{d} ± 707	12500 ^{d} ± 410	-	-	-	-
	28d	878800 ^{e} ± 3564	189000 ^{e} ± 806	27 ^{a} ± 11	16 ^{a} ± 6	-	-

Means ± standard divinations.
$^{a, b, c, d}$ – indexes show data with statistical different value in columns ($p < 0.05$) ***Legend:*** With the sign "**-**" is result marked "***is not detectable***".

DISCUSSION

Similarly to our results **Djenane *et al.* (2003)** found a significant reduction of the **sensory evaluated colour scores** after addition of rosemary and ascorbic acid mixture. Those authors **(Djenane *et al.*, 2003)** explain their findings by the rates of metmyoglobin formation and the extended shelf-life from about 10 to 20 days. Our results about sensory evaluated odor were confirmed and by findings of **Rojas and Brewer (2007)** which were determined the effect of 0.02% rosemary oleoresin on colour stability of cooked beef patties stored at 4ºC for 8 days. At the end of storage (28d at 0 ± 0.5°C) the determined light off-odor in samples VP, AVP and MAP, were associated with lipid oxidation rancidity, and described as a smell of wet cardboard. The MAP of beef gives brightly red colour on the meat surface, but high oxygen content in MAP leads to rancid taste, while meat is still attractive red. The high oxygen atmosphere promotes the myoglobin oxidation and prolongs the time for metmyoglobin formation on meat surface **(Kim *et al.*, 2010).** However, up to 21st day of storage samples AVP received comparatively higher sensory evaluated flavor scores. The main reason was the use of DHQ as inhibitor of lipid oxidation **(Semenova *et al.*, 2008)**, which in combination with rosemary **(Ahn *et al.*, 2002)** and ascorbic acid effectively preserve the bright red beef colour and reduce the worm over-flavor scores. The results obtained for colour brightness (L*) can be explained with properties of antioxidants used in TAB. Rich in phenolic components rosemary extract show strong activity and separating H^+ ions removed hydrogen peroxide and reactive oxygen radical species **(Brewer, 2011)**. L-ascorbic acid donate hydrogen, chelate metal ions (i.e. Fe^{2+}), scavenge free radicals and quencher the peroxyl ($O_2^{\bullet}$) radicals **(Brewer, 2011)**. On the other hand, DHQ release H^+ protons and reduce prooxidant activity of metal ions with mobile valence such as iron and copper ions, "free" and heme iron **(Silva *et al.*, 2002)**.

The results obtained about colour redness (a*) confirm the hypothesis of **Lund *et al.* (2007)** who claim that in high oxygen atmospheres rosemary extract protected the fresh red meat colour. Our results are similar to **Akarpat *et al.* (2008)** research about use of hot-water rosemary extracts for prevention of colour changes in beef patties, and confirm **Rohlik *et al.* (2012)** findings about positive effect of RE addition on colour in the dried/cooked sausages. A TAB which is a powerful polyphenolic antioxidant and plays role of an inhibitor of free radical formation **(Silva *et al.*, 2002)** probably reduced velocity of the oxymyoglobin oxidation in AMAP samples. Similarly to our findings about colour stability **Berruga *et al.* (2005)** confirmed optimal levels in VP and MAP lamb *m. longissimus dorsi*. **Berruga *et al.* (2005)** confirm that after 7th day of refrigeration storage at 2°C, the b* value increased and a* value decreased. The results of the **Berruga *et al.* (2005)** regarding colour lightness (L*) were opposite of our findings. The analysis of those results showed that the combination between superficial treatment with 0.02% TAB, and MAP can stabilize L*, and a* values, and was able to minimize the increase of b* value of fresh beef comparing with vacuum and air packaging.
When commenting the pH of the control samples C it should be noted that they were in the process of deep deterioration with a strong sour smell, probably caused by the development of lactic acid bacteria **(Blixt and Borch, 2002)**. Under similar conditions, a higher pH can accelerate the respiratory activity of the muscle tissue and to form a purple deoxymyoglobin **(Rhee *et al.*, 2004)**. This explains the purple colour of VP samples. On the other hand, most myoglobin-reducing activity was recorded at pH 7.4 **(Hutchison *et al.*, 2010)**. The myoglobin reduction decreased with pH decline to 5.7 (the pH established at the end of the storage). The lower pH values in samples VP can be caused by more rapid development of anaerobes **(Blixt and Borch, 2002)**, or comparatively high activity of lactic acid bacteria **(Borch *et al.*, 1996)** under those conditions. Our results showed that the beef treatment with 0.02% TAB had no effect on the pH

changes of meat and the main factor for MAP beef spoilage was the growth of lactic acid bacteria. At comparatively low pH and higher osmotic pressure proteolytic enzymes, such as calcium-neutral proteases **(Hool and Corry, 2007)**, calpains **(Sazili *et al.*, 2004)**, or cathepsins **(Polidori *et al.*, 2001)** have been activated, in a result of exempted calcium ions in sarcoplasmic reticulum **(Geay *et al.*, 2001)**. Under those conditions bacteria associated with the spoilage of chilled meat, had negative effects such as sour off-flavour, discoloration, gas production, slime production and decrease in pH, and consist except of *Brochothrix thermosphacta*, *Carnobacterium spp.*, *Lactobacillus spp.*, *Leuconostoc spp.* and *Weissella spp.* **(Borch *et al.*, 1996)**.While the *Brochothrix thermosphacta* dominates in MAP beef **(Baranyi *et al.*, 1996)**, the main microflora in VP beef was probably lactic acid bacteria **(Blixt and Borch, 2002)**. More favourable microbiological results in VP beef can be explained by the suppression of *Brochothrix thermosphacta*, anaerobic, and lactic acid bacteria, *Pseudomonas* and *Enterobacteriaceae spp*. to levels that would not cause meat spoilage **(Blixt and Borch, 2002)**. The growth of aerobic microflora decreases in VP meat **(Motoyama *et al.*, 2010).** Compared to the control samples C, MAP ($80\%O_2/20\%CO_2$) with or without TAB treatment extend the shelf-life of beef with 14 days. Similarly to our findings **Djenane *et al.* (2003)** significantly reduced the rates of microbial growth using the mixture of rosemary and ascorbic acid. The rosemary extract influenced only on the growth of lactic acid bacteria **(Fernández-López *et al.*, 2005).**

CONCLUSIONS

The results obtained allowed us to conclude that the packaging of beef in modified atmosphere ($80\%O_2/20\%CO_2$), combined with preliminary superficial treatment with 0.02% TAB containing DHQ, RE, and AA may extend the shelf-life of beef stored at 0 ± 0.5°C till 32nd day *post mortem* (to 28th day of storage). This shelf-life is with 7 days (25%) longer in comparison with beef packed under rich in oxygen modified atmosphere or under vacuum. The combination of pre-treatment of beef with TAB and rich in oxygen MAP preserve the sensory properties, stabilize the L* and a* values, and provide slower increase of the b* value. The pre-treatment of beef with TAB and packaging in rich oxygen MAP inhibit the microbial growth and prevent meat spoilage.

ACKNOWLEDGEMENTS

The author expresses his gratitude to the teams of the Unitemp Ltd., Voyvodinovo village, Plovdiv region, Vitalife Ltd., Sofia, and Alimenti Ltd., Tsaratsovo village, Plovdiv district for the co-operation and support rendered.

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