DYNAMICS OF ANTIOXIDANT ACTIVITY DURING MANUFACTURING OF MEAT PRODUCTS AND ANTIOXIDANT ACTIVITY PREDICTION

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ABSTRACT

Lipid oxidation causes loss of quality and safety in meat products. This well known fact leads to a broad use of antioxidants in meat products manufacturing. In our research we studied protective effect of natural plant antioxidants and found that there are other factors than amount of antioxidant affecting the course of lipid oxidation. To find out what happens not only with lipids but also with antioxidants during meat products manufacturing, we studied the dynamics of overall antioxidant activity in meat formulas of four different meat products from cutting until marshalling. FRAP method was used for the total antioxidant activity. Results show that there are several independent factors affecting the overall status of lipids. First, it is meat quality, second, antioxidant activity present in all processed material, third, the technology of processing. Meat used in production varies in lipid oxidation development. Generally, the damage cannot be undone. Antioxidant activity of all participating elements differs and the antioxidant activity is provided not only by added antioxidants. The antioxidants have different antioxidative activity dynamics. To compensate a massive peroxide production at cutting of meat, the antioxidants with high reaction speed are important to prevent lipid damage. Later on, more important is overall/total antioxidant activity within which, it seems, antioxidants are functioning synergically in fat protection. Optimal composition of antioxidants in meat products respecting specific composition and manufacturing technology should be estimated for each meat product individually.

Key words: *Antioxidant activity, FRAP, meat products oxidation prediction*

INTRODUCTION

It is well known fact, that lipid oxidation causes organoleptic changes in meat products (Brewer and Vega, 1995; Cross et al., 1987) and that products of the oxidation are hazardous for consumer's health (Addis and Warner, 1991; Bunker, 1992). To minimize both off flavour and health hazards, producers of meat products commonly use antioxidants. Frequently they rely on spices/additives suppliers that add the antioxidants to other substances to simplify their use. Meat used at meat production has also its own oxido-reduction systems which participate on overall lipid oxidation or protection (Kanner et al., 1988; Kohen et al., 1988). Because the compositions of meat preparations differ widely, the effect of all participating compounds on fat oxidation course is uneasy to estimate. A simple method (Benzie and Strain, 1999) can be, however, used to estimate the antioxidant activity (AO activity) in meat products to improve the lipid protection if necessary or to minimise the use additives if possible. This method

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DINAMIKA I PREDVIĐANJE ANTIOKSIDACIJSKE AKTIVNOSTI TIJEKOM PROIZVODNJE MESNIH PROIZVODA

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SAŽETAK

Oksidacija lipida uzrokuje smanjenje kakvoće i sigurnost u proizvodnji mesa. Ova dobro poznata činjenica razlogom je široke upotrebe antioksidanata u proizvodnji mesnih proizvoda. U našem istraživanju proučavali smo zaštitni učinak prirodnih biljnih antioksidanata i utvrdili da uz antioksidante, postoje i drugi faktori koji djeluju na tijek oksidacije lipida. Da bismo utvrdili što se događa ne samo s lipidima nego i antioksidantima tijekom proizvodnje mesnih proizvoda, proučavali smo dinamiku sveukupne antioksidacijske aktivnosti u četiri različita mesna proizvoda od pripreme sirovine do završetka proizvodnje kobasica. Za utvrđivanje ukupne antioksidacijske aktivnost korištena je FRAP- metoda. Rezultati pokazuju da postoji nekoliko nezavisnih čimbenika koji utječu na sveukupni status lipida. Prvo, to je kakvoća mesa, potom, antioksidacijska aktivnost prisutna u svim upotrijebljenim sirovinama, te tehnologija proizvodnje. Ovisno o vrsti mesa razlikuje se i oksidacijski potencijal. Općenito govoreći, šteta se ne može ispraviti. Antioksidacijska aktivnost je različita i nije uzrokovana samo dodanim antioksidantima. Antioksidansi posjeduju različitu dinamiku antioksidacijske aktivnosti. Da bi se nadoknadila proizvodnja peroksida prilikom rasijecanja mesa, uloga antioksidanata s velikom brzinom reakcije je značajna zbog sprječavanja razgradnje lipida. Kasnije je značajnija sveukupna antioksidacijska aktivnost unutar koje, čini se, antioksidanti djeluju kao sinergisti u zaštiti masti. Najbolji sastav antioksidanata u proizvodnji mesa trebao bi se procijeniti za svaki mesni proizvod pojedinačno u odnosu na specifičan sastav i proizvodnu tehnologiju.

Ključne riječi: *Antioksidacijska aktivnost, FRAP, predviđanje oksidacije mesnih proizvoda*

UVOD

Dobro je poznata činjenica da oksidacija lipida uzrokuje organoleptičke promjene u mesnim proizvodima (Brewer i Vega, 1995; Cross i sur., 1987), te da su proizvodi oksidacije štetni za zdravlje potrošača (Addis i Warner, 1991; Bunker, 1992). Proizvođači mesnih proizvoda obično koriste antioksidante da bi smanjili odstupanje u mirisu, a time i rizik za zdravlje. Oni se često oslanjaju na dobavljače mirodija/aditiva koji dodaju antioksidante drugim supstancijama da bi pojednostavili njihovu upotrebu. Meso korišteno u proizvodnji također posjeduje vlastite oksidacijsko-redukcijske sustave koji sudjeluju u sveukopnoj oksidaciji ili zaštiti lipida (Kanneri sur., 1988; Koheni sur., 1988). Budući da je sastav mesnih proizvoda različit, teško je procijeniti učinak svih pripadajućih sastojaka na tijek oksidacije masti. Međutim, može se koristiti jednostavna metoda (Benzie i Strain, 1999) procjene antioksidacijske aktivnosti (AO aktivnost) u mesnim proizvodima

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allows to follow the course of antioxidant depletion during meat products manufacturing and better arranging for either shelf-life estimation or/and use of other protective means to slow down the lipid oxidation in meat products.

MATERIAL AND METHODS

The ferric reducing / antioxidant power assay (FRAP) (Benzie and Strain, 1999) was used to estimate total AO activity of meat preparations and ready made meat products. Because the method was originally developed for use with other biological material, we had to modify it slightly to fit our needs. The FRAP reagent was prepared exactly as described in Benzie and Strain (1999) by mixing 300 mM acetate buffer, ph=3.6 (3.1 g $CH_3COONa \cdot 3H_2O$ (p.a., Lachema, Czech Republic) plus 1.6 ml glacial acetic acid (p.a., Lachema, Czech Republic) made up to 1 l with distilled water), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine, Fluka Chemicals, switzerland) in 40 mM HCl (p.a., Lachema, Czech Republic) and 20 mM $FeCl_3 \cdot 6H_2O$ (p.a., Lachema, Czech Republic) in the ratio 10 : 1 : 1. The reagent was prepared freshly before each experiment.

Because the original assay was developed for different biological fluids, we had to modify sample preparation which was as follows: To 10 g of homogenised meat product 40 ml of 30 % ethanol was added. After 15 minutes of stirring at laboratory temperature, the sample was filtrated.

The estimation was carried out: To 20 ml of the FRAP reagent 250 µl of sample was added. The mixture was incubated at 40 °C and 1 ml from the incubated mixture was withdrawn at the intervals of 1‘, 5‘, 10‘, 15‘, 30‘, 45‘, 60‘, 75‘, 90‘, 105‘ a 120‘ for absorbance measurement at λ=593 nm against water (Spekol 11, Carl Zeiss, DDR). A blank sample was prepared by adding 250 µl of 30 % ethanol to 20 ml of the FRAP reagent. The blank was incubated and measured exactly as the sample.

Results were re-calculated to correspond to 1 g of dried matter in the given meat product.

Maximum antioxidative capacity was calculated as double reciprocal values extension at time = 0’.

To determine the relation between absorbance and concentration of reduced oxygen (nM 1/2 O_2), a calibration curve was prepared by measuring 20, 40, 60, 80 a 100 nM $FeSO_4 \cdot 5H_2O$ at λ=593 nm.

MATERIAL

To test the assay, four meat products were used. Because we wanted to test the method in a long term experiment, we selected long lasting fermented thermally not treated meat products. All meat products used for experiments were labelled as experimental at the very beginning of the sampling and then followed usual technology of processing. For sample preparation whole piece of the meat product was homogenised after peeling its casing off.

Following meat products were used:

„Gombasecká klobása“, sausage with declared use of the antioxidants E 300 and E 301;

„Trebišovská saláma“, salami with declared use of the antioxidants E 300 and E 301;

„Pribina“ salami without declared use of antioxidants;

„Štart“ salami without declared use of antioxidants but with use of the starter microflora.

The meat products were acquired at manufacturing plant and sampled at specified stages of manufacturing:

1st sampling was done after immediately meat preparation mixing, prior to its stuffing into casings - 1st day of manufacturing.

2nd sampling was done after “smoking” (after incubation period during which the lactic acid producing bacteria are multiplied to occupy the environment and lower its pH) - 4th day of manufacturing. “Trebišovská saláma” salami, however, was sampled at 7th day of processing due to different production technology.

3rd sampling was done after finishing, at the day of meat products marshalling from the manufacturing facility – 12th day of processing. “Trebišovská saláma” salami, however, was sampled at 26th day to estimate AO activity at the last day of consumption of the meat product.

radi poboljšanja zaštite lipida, ako je potrebno, ili za smanjenje primjene aditiva, ako je moguće. Ova metoda omogućava uvid u tijek antioksidacijske aktivnosti za vrijeme proizvodnje mesnih proizvoda kao i bolju procjenu roka održivosti i/ili primjene drugih zaštitnih sredstava radi usporavanja oksidacije lipida u mesnim proizvodima.

MATERIJAL I METODE

Za procjenu ukupne AO aktivnosti sirovina i gotovih mesnih proizvoda korišten je FRAP test (ferric reducing / antioxidant power assay) (Benzie i Strain, 1999). Budući da je ova metoda prvotno bila namijenjena za primjenu kod drugih bioloških materijala, istu smo modificirali kako bi odgovarala našim potrebama. FRAP reagens je pripremljen točno prema uputi Benzie i Strain-a (1999), tako da je pomiješano 300 mM acetatnog pufera, pH=3.6 (3.1 g $CH_3COONa \cdot 3H_2O$ (p.a., Lachema, Czech Republic) 1.6 ml ledne octene kiseline (p.a., Lachema, Czech Republic) i dopunjeno do 1 l s destiliranom vodom), 10 mM TPTZ (2,4,6-tripiridil-s-triazin, Fluka Chemicals, Švicarska) u 40 mM HCl (p.a., Lachema, Češka) i 20 mM $FeCl_3 \cdot 6H_2O$ (p.a., Lachema, Češka) u omjeru 10 : 1 : 1. Reagens je pripreman prije svakog pokusa.

Budući da je originalni postupak razvijen za različite biološke tekućine, morali smo modificirati način pripreme uzorka. Tako je u 10 g homogeniziranog mesnog proizvoda dodano 40 ml 30-% etanola. Uzorak je filtriran nakon 15 minuta miješanja na sobnoj temperaturi.

Procjena je izvedena na slijedeći način: U 20 ml FRAP reagensa dodano je 250 μl uzorka. Mješavina je zatim inkubirana na 40 °C i iz inkubirane mješavine uzimano je po 1 ml u intervalima od 1‘, 5‘, 10‘, 15‘, 30‘, 45‘, 60‘, 75‘, 90‘, 105‘ i 120‘ za mjerenje absorbancije kod λ=593 nm nasuprot vode (Spekol 11, Carl Zeiss, DDR). Slijepa proba je pripremljena dodavanjem 250 μl 30-postotnog etanola u 20 ml FRAP reagensa. Slijepa proba je inkubirana i izmjerena jednako kao i uzorak.

Rezultati su preračunati da bi odgovarali 1 g suhe tvari u određenom mesnom proizvodu.

Maksimalni antioksidacijski kapacitet izračunat je kao dvostruke recipročne vrijednosti ekstenzije kod vremena nula.

Za utvrđivanje odnosa između absorbancije i koncentracije reduciranog kisika (nM 1/2 O_2), pripremljena je kalibracijska krivulja mjerenjem 20, 40, 60, 80 i 100 nM $FeSO_4 \cdot 5H_2O$ kod λ=593 nm.

MATERIJAL

Za procjenu testa korištena su 4 mesna proizvoda. Budući da smo željeli ispitati metodu u dugotrajnom pokusu, odabrali smo trajne, fermentirane, toplinski neobrađene mesne proizvode. Svi mesni proizvodi upotrijebljeni u pokusima, označeni kao eksperimentalni na samom početku uzorkovanja proizvedeni su uobičajenom tehnologijom. Za pripremu uzorka homogeniziran je cijeli komad mesnog proizvoda nakon skidanja ovitka.

Korišteni su sljedeći mesni proizvodi:

„Gombasecká klobása“, kobasica na kojoj je deklarirana primjena antioksidanata E 300 i E 301;

„Trebišovská saláma“, salama s deklariranom primjenom antioksidanata E 300 i E 301;

„Pribina“ salama bez deklarirane primjene antioksidanata;

„Štart“ salama bez deklarirane primjene antioksidanata na dodatak starter-kulture.

Mesni proizvodi iz proizvodnog pogona uzorkovani su u određenim fazama proizvodnje:

1. uzorkovanje je uslijedilo odmah nakon pripreme mesne smjese, a prije nadijevanja u crijeva – 1. dan proizvodnje.

2. uzorkovanje je bilo nakon dimljenja (nakon razdoblja inkubacije tijekom kojeg dolazi do razmnažanja bakterija, mliječne kiseline i snižava pH vrijednost) – 4. dan proizvodnje. “Trebišovská saláma” je uzorkovana 7. dana proizvodnje zbog drugačije tehnologije proizvodnje.

3. uzorkovanje je uslijedilo nakon što su mesni proizvodi bili gotovi i spremni za otpremu iz proizvodnog pogona – 12. dan obrade. Međutim “Trebišovská” salama je uzorkovana 26-tog dana radi procjene AO aktivnosti, zadnjeg dana roka valjanosti mesnog proizvoda.

RESULTS

Values of estimated dry matter in meat products are in table 1.

Relation between absorbance and concentration of reduced oxygen was determined by a calibration curve estimated as described by Benzie and Strain (1999) by measuring of absorbance of 20, 40, 60, 80 and 100 nM $FeSO_4 \cdot 5H_2O$ at 593 nm, respectively. Concentration of the Fe^{2+} is measured as the final product of the TPTZ reduction. Because in reduction of Fe^{3+} to Fe^{2+} equimolar concentration of oxygen (1/2 O_2) is involved, calibration dependence can be expressed as dependence of absorbance on reacted oxygen.

Table 1. Values of estimated dry matter in meat products (%)

	Gombasecká klobása	Trebišovská saláma	Pribina salami	Štart salami
Meat preparation (1st day)	57,187	56,807	57,187	56,807
After „smoking“ (4th day)	65,363	69,58	65,363	69,58
At marshalling (12th day)	69,295	76,44	69,295	76,44

Table 2. Dependence of absorbance at 593 nm on $FeSO_4 \cdot 5H_2O$ concentration

nM $FeSO_4 \cdot 5H_2O$	A_{593}
0	0
20	0,14
40	0,337
60	0,489
80	0,642
100	0,77

Table 3. AO activity in „Gombasecká klobása" sausage (nM ½ O_2)

Time (min.)	Meat preparation (1st day)	After „smoking" (4th day)	At marshalling (12th day)
1	26,56	16,36	12,14
5	30,88	39,81	13,06
10	33,87	45,04	26,95
15	37,64	45,23	40,84
30	54,14	54,34	42,67
45	52,92	57,64	49,61
60	54,80	60,15	52,90
75	56,46	60,93	56,01
90	56,57	66,55	57,29
105	61,45	67,91	59,48
120	63,88	68,29	62,41

REZULTATI

Vrijednosti suhe tvari u mesnim proizvodima prikazani su u tablici 1.

Odnos između absorbancije i koncentracije reduciranog kisika određivan je kalibracijskom krivuljom dobivenom, kao što su opisali Benzie i Strain (1999), mjerenjem absorbancije od 20, odnosno 40, 60, 80 i 100 nM $FeSO_4 \cdot 5H_2O$ kod 593 nm. Koncentracija Fe^{2+} je mjerena kao gotovi proizvod TPTZ redukcije. Zbog redukcije Fe^{3+} u Fe^{2+} potrebna je ekvimolarna koncentracija kisika (1/2 O_2), te se kalibracijska ovisnost može izraziti kao ovisnost

Table 1. Vrijednosti suhe tvari u mesnim proizvodima (%)

	Gombasecká klobása	Trebišovská saláma	Pribina salami	Štart salami
Priprema mesa (1. dan)	57,187	56,807	57,187	56,807
Nakon "dimljenja" (4. dan)	65,363	69,58	65,363	69,58
Završetak proizvodnje (12. dan)	69,295	76,44	69,295	76,44

Table 2. Ovisnost absorbancije kod 593 nm o $FeSO_4 \cdot 5H_2O$ koncentraciji

nM $FeSO_4 \cdot 5H_2O$	A_{593}
0	0
20	0,14
40	0,337
60	0,489
80	0,642
100	0,77

Table 3. AO aktivnost u kobasici „Gombasecká klobása"(nM ½ O_2)

Vrijeme (min.)	Priprema mesa (1. dan)	Nakon dimljenja (4. dan)	Završetak proizvodnje (12. dan)
1	26,56	16,36	12,14
5	30,88	39,81	13,06
10	33,87	45,04	26,95
15	37,64	45,23	40,84
30	54,14	54,34	42,67
45	52,92	57,64	49,61
60	54,80	60,15	52,90
75	56,46	60,93	56,01
90	56,57	66,55	57,29
105	61,45	67,91	59,48
120	63,88	68,29	62,41

Table 4. AO activity in „Trebišovská saláma” salami (nM ½ O_2)

Time (min.)	Meat preparation (1st day)	After „smoking“ (7th day)	At marshalling (26th day)
1	40,4586	17,02687	15,88695
5	51,09787	27,28617	18,92674
10	56,67083	34,50568	19,0534
15	57,68409	52,49111	21,71322
30	64,52362	56,54417	27,15951
45	75,92284	65,7902	29,43936
60	79,9759	70,09657	30,83259
75	83,39566	73,38968	32,73246
90	88,58864	75,03624	33,49241
105	90,48851	76,55613	34,63233
120	91,1218	79,72258	37,54547

Table 5. AO activity in „Pribina” salami (nM ½ O_2)

Time (min.)	Meat preparation (1st day)	After „smoking“ (4th day)	At marshalling (12th day)
1	12,7205	8,667443	10,56731
5	17,02687	14,11374	17,15353
10	19,0534	14,11374	19,0534
15	21,83988	14,11374	21,83988
30	26,52622	17,40685	25,51296
45	32,35249	21,33324	28,04612
60	34,1257	22,47317	29,43936
75	36,15223	24,37304	30,57928
90	39,572	24,87967	31,33923
105	40,07863	25,63962	31,33923
120	42,23181	31,21257	33,49241

Table 4. AO aktivnost u salami „Trebišovská saláma"(nM ½ O_2)

Vrijeme (min.)	Priprema mesa (1. dan)	Nakon dimljenja (7. dan)	Završetak proizvodnje (26. dan)
1	40,4586	17,02687	15,88695
5	51,09787	27,28617	18,92674
10	56,67083	34,50568	19,0534
15	57,68409	52,49111	21,71322
30	64,52362	56,54417	27,15951
45	75,92284	65,7902	29,43936
60	79,9759	70,09657	30,83259
75	83,39566	73,38968	32,73246
90	88,58864	75,03624	33,49241
105	90,48851	76,55613	34,63233
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Table 5. AO aktivnost u „Pribina“ salami (nM ½ O_2)

Vrijeme (min.)	Priprema mesa (1. dan)	Nakon dimljenja (4. dan)	Završetak proizvodnje (12. dan)
1	12,7205	8,667443	10,56731
5	17,02687	14,11374	17,15353
10	19,0534	14,11374	19,0534
15	21,83988	14,11374	21,83988
30	26,52622	17,40685	25,51296
45	32,35249	21,33324	28,04612
60	34,1257	22,47317	29,43936
75	36,15223	24,37304	30,57928
90	39,572	24,87967	31,33923
105	40,07863	25,63962	31,33923
120	42,23181	31,21257	33,49241

Table 6. AO activity in „Štart” salami (nM ½ O_2)

Time (min.)	Meat preparation (1st day)	After „smoking“ (4th day)	At marshalling (12th day)
1	8,920759	8,160811	8,794101
5	13,60711	14,74703	18,04014
10	17,15353	15,127	19,94001
15	19,94001	15,127	22,72648
30	24,11972	16,90021	26,9062
45	30,95925	18,67343	29,05938
60	32,35249	21,07993	30,07265
75	32,73246	23,35977	30,83259
90	35,77226	24,24638	31,84586
105	35,89891	25,25964	32,85912
120	36,78552	29,3127	33,2391

Table 7. Calculated values of AO activities in “Gombasecká saláma” salami

	Meat preparation	After “smoking”	At marshalling
Dependence equation	AOA = 8,4034Ln(time) + 20,466	AO activity = 10,2692Ln(time) + 19,1546	AO activity = 11,6601Ln(time) + 4,67801
R^2 (%)	91,06	98,21	93,47
Calculated immediate AOA (½O_2 nM O_2)	20,47	19,15	4,68
Calculated maximum AOA (½O_2 nM O_2)	50,22	60,92	43,82

Table 8. Calculated values of AO activities in “Trebišovská saláma” salami

	Meat preparation	After “smoking”	At marshalling
Dependence equation	AOA = 19,9879Ln(time) + 59,0691	AOA = 20,4733Ln(time) + 14,7684	AOA = 6,12328Ln(time) + 15,7062
R^2 (%)	93,44	96,20	90,80
Calculated immediate AOA (½O_2 nM O_2)	59,07	14,77	15,71
Calculated maximum AOA (½O_2 nM O_2)	131,54	41,91	37,24

Table 6. AO aktivnost u „Štart" salami (nM ½ O_2)

Vrijeme (min.)	Priprema mesa (1. dan)	Nakon dimljenja (4. dan)	Završetak proizvodnje (12. dan)
1	8,920759	8,160811	8,794101
5	13,60711	14,74703	18,04014
10	17,15353	15,127	19,94001
15	19,94001	15,127	22,72648
30	24,11972	16,90021	26,9062
45	30,95925	18,67343	29,05938
60	32,35249	21,07993	30,07265
75	32,73246	23,35977	30,83259
90	35,77226	24,24638	31,84586
105	35,89891	25,25964	32,85912
120	36,78552	29,3127	33,2391

Table 7. Izračunate vrijednosti AO aktivnosti u salami "Gombasecká saláma"

	Priprema mesa	Nakon dimljenja	Završetak proizvodnje
Jednadžba ovisnosti	AOA = 8,4034Ln(vrijeme) + 20,466	AO aktivnost = 10,2692Ln(vrijeme) + 19,1546	AO aktivnost = 11,6601Ln(vrijeme) + 4,67801
R^2 (%)	91,06	98,21	93,47
Izračunata trenutna AOA (˝O_2 nM O_2)	20,47	19,15	4,68
Izračunata maksimalna AOA (˝O_2 nM O_2)	50,22	60,92	43,82

Table 8. Izračunate vrijednosti AO aktivnosti u salami "Trebišovská saláma"

	Priprema mesa	Nakon dimljenja	Završetak proizvodnje
Jednadžba ovisnosti	AOA = 19,9879Ln(vrijeme) + 59,0691	AOA = 20,4733Ln(vrijeme) + 14,7684	AOA = 6,12328Ln(vrijeme) + 15,7062
R^2 (%)	93,44	96,20	90,80
Izračunata trenutna AOA (½O_2 nM O_2)	59,07	14,77	15,71
Izračunata maksimalna AOA (½O_2 nM O_2)	131,54	41,91	37,24

Table 9. Calculated values of AO activities in "Pribina" salami

	Meat preparation	After "smoking"	At marshalling
Dependence equation	AOA = 10,7764Ln(time) + 12,3848	AOA = 6,50773Ln(time) + 9,63144	AOA = 7,22398Ln(time) + 14,2184
R^2 (%)	91,99	87,18	98,95
Calculated immediate AOA (½O_2 nM O_2)	12,38	9,63	14,21
Calculated maximum AOA (½O_2 nM O_2)	50,01	32,96	41,83

Table 10. Calculated values of AO activities in "Štart" salami

	Meat preparation	After "smoking"	At marshalling
Dependence equation	AOA = 11,7313Ln(time) + 9,09162	AOA = 6,37595Ln(time) + 11,5827	AOA = 8,04962Ln(time) + 14,227
R^2 (%)	95,15	87,71	99,63
Calculated immediate AOA (½O_2 nM O_2)	9,09	11,58	14,23
Calculated maximum AOA (½O_2 nM O_2)	51,33	35,35	41,37

Measured values are in table 2.

Therefore the calculated dependence of the absorbance on reacted oxygen was

A_{593} = 0.00290476 · nM 1/2 O_2 + 0.00278657

r = 0.998306 (r^2 = 99.6514 %)

SE = 0.0191861

Antioxidant capacity of the meat products, expressed as nM of 1/2 O_2 that can be eliminated from the meat products are in tables 3—6.

Values calculated from these measurements are in tables 7—10.

DISCUSSION

From the results obtained by the FRAP method it is possible to derive very useful information about antioxidant activity within meat products. Noticeable are several important facts:

The antioxidant activity can be estimated as either "immediate" antioxidant activity at time 0' of the estimation or as "maximum" antioxidant activity at determined time interval.

The "immediate" antioxidant activity plays an important role during and soon after meat cutting when high levels of oxidants are present in the environment meat preparation and readily detectable as increased peroxide value level (Smith et al., 1996; Bystrický et al., 1998; Korimová et al., 2000, 2001; Marcinčák et al., 2003) and antioxidants, if present at proper level, can decrease/slow down speed of lipid oxidation at this point.

The "maximum" antioxidant activity differs from the "immediate" antioxidant activity and depends on presence of other antioxidants regenerating each other (Palozza and Krinsky, 1992) and passing electrons along redox potential. The "maximum" antioxidant activity can be calculated as all values measured in our experiment gave good correlation for the logarithmic dependence between time of measurement and antioxidant activity. Level of the "maximum" antioxidant activity determines how will be the meat product environment able to

Table 9. Izračunate vrijednosti AO aktivnosti u salami "Pribina"

	Priprema mesa	Nakon "dimljenja"	Završetak proizvodnje
Jednadžba ovisnosti	AOA = 10,7764Ln(vrijeme) + 12,3848	AOA = 6,50773Ln(vrijeme) + 9,63144	AOA = 7,22398Ln(vrijeme) + 14,2184
R^2 (%)	91,99	87,18	98,95
Izračunata trenutna AOA (½O_2 nM O_2)	12,38	9,63	14,21
Izračunata maksimalna AOA (½O_2 nM O_2)	50,01	32,96	41,83

Table 10. Izračunate vrijednosti AO aktivnosti u salami "Štart"

	Priprema mesa	Nakon "dimljenja"	Završetak proizvodnje
Jednadžba ovisnosti	AOA = 11,7313Ln(vrijeme) + 9,09162	AOA = 6,37595Ln(vrijeme) + 11,5827	AOA = 8,04962Ln(vrijeme) + 14,227
R^2 (%)	95,15	87,71	99,63
Izračunata trenutna AOA (½O_2 nM O_2)	9,09	11,58	14,23
Izračunata maksimalna AOA (½O_2 nM O_2)	51,33	35,35	41,37

absorbancije o reagiranom kisiku.

Izmjerene vrijednosti su prikazane u tablici 2.

Prema tome, izračunata ovisnost absorbancije o reagiranom kisiku bila je

A_{593} = 0.00290476 · nM 1/2 O_2 + 0.00278657

r = 0.998306 (r^2 = 99.6514 %)

SE = 0.0191861

Antioksidacijska sposobnost mesnih proizvoda, izražena kao nM od 1/2 O_2 koja se može eliminirati iz mesnih proizvoda prikazana je u tablicama 3—6.

Vrijednosti izračunate na temelju tih mjerenja prikazane su u tablicama 7—10.

DISKUSIJA

Iz rezultata dobivenih primjenom FRAP metode mogu se dobiti vrlo korisne informacije o antioksidacijskoj aktivnosti mesnih proizvoda. Uočeno je nekoliko značajnih činjenica:

Antioksidacijska aktivnost može se procijeniti bilo kao "trenutna" antioksidacijska aktivnost kod vremena nula procjene ili kao "maksimalna" antioksidacijska aktivnost u određenom vremenskom intervalu.

"Trenutna" antioksidacijska aktivnost ima važnu ulogu tijekom i odmah nakon rasijecanja mesa kada se velike koncentracije oksidanata lako otkrivaju kao povećana razina vrijednosti peroksida (Smith i sur., 1996; Bystrický i sur., 1998; Korimová i sur., 2000, 2001; Marcinčák i sur., 2003) a antioksidanti, ako su prisutni u odgovarajućoj koncentraciji, u tom času mogu smanjiti/usporiti brzinu oksidacije lipida.

"Maksimalna" antioksidacijska aktivnost se razlikuje od "trenutne" antioksidacijske aktivnosti i ona ovisi o prisustvu drugih antioksidanata koji jedan drugoga obnavljaju (Palozza i Krinsky, 1992) i prenose elektrone dok ima redoks potencijala. "Maksimalna" antioksidacijska aktivnost može se izračunati jer su sve vrijednosti izmjerene u našem pokusu pokazale dobru korelaciju za logaritamsku ovisnost između vremena mjerenja i antioksidaeliminate oxygen coming from outside into inside environment and thus slow down lipid oxidation process within product's shelf life.

As our experimental results show, meat products under investigation differ in both "immediate" and "maximum" activities.

From the course of antioxidant activity in the „Gombasecká klobása" sausage it is possible to deduce that added antioxidants were spent at meat preparation mixing and regenerated until "smoking" technological step. The "maximum" antioxidant activity, being virtually unchanged during all production steps suggests that the regeneration of antioxidants is well balanced.

The course of antioxidant activity in the „Trebišovská saláma" salami shows that abundant of antioxidant was used providing high both "immediate" and "maximum" antioxidant activity at the "mixing" technological step. Decrease in the "immediate" antioxidant activity with subsequent fast regeneration of the "maximum" antioxidant activity at the "smoking technological step suggests unbalanced, low, level of regenerating antioxidants that take over in the "maximum" antioxidant activity. Decrease of the "maximum" antioxidant activity until the "marshalling" of meat product suggests depletion also of regenerating antioxidants.

Both "Pribina" and "Štart" salami are possessing similar both "immediate" and "maximum" antioxidant activities. Dynamics in antioxidant activities show lack of the "immediate" antioxidant activity.

CONCLUSION

FRAP assay is very simple and potentially very useful tool for improving quality and safety of meats products through better protection of lipids. Easily interpreted results allow to improve effect of antioxidants while minimising their use. With several simple measurements and calculation it is possible predict the necessary amounts of antioxidants with fast and lower reaction speeds to efficiently protect meat products against oxidation within their shelf-life.

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cijske aktivnosti. Razina "maksimalne" antioksidacijske aktivnosti određuje kako će sastojci nadjeva reagirati s kisikom i tako usporiti proces oksidacije lipida do kraja vremena održivosti proizvoda.

Kao što naši eksperimentalni rezultati pokazuju, ispitivani mesni proizvodi se razlikuju kako po "trenutnim" tako i po "maksimalnim" aktivnostima.

Iz tijeka antioksidacijske aktivnosti u kobasici „Gombasecká klobása" može se zaključiti da su se dodani antioksidansi istrošili kod pripreme mesne smjese i regenerirali do tehnološke faze dimljenja. "Maksimalna" antioksidacijska aktivnost, koja je praktički ostala nepromijenjena tijekom svih faza proizvodnje, ukazuje da je regeneracija antioksidanata dobro izbalansirana.

Tijek antioksidacijske aktivnosti proizvoda "Trebišovská saláma" pokazuje da je upotrebljena znatna količina antioksidanta koja je osigurala visoku kako "trenutnu" tako i "maksimalnu" antioksidacijsku aktivnost u tehnološkoj fazi miješanja. Smanjenje "trenutne" antioksidacijske aktivnosti a potom brza regeneracija "maksimalne" antioksidacijske aktivnosti u fazi dimljenja ukazuje da neuravnotežena niska razina regenerirajućih antioksidanata sudjeluje u "maksimalnoj" antioksidacijskoj aktivnosti. Smanjivanje "maksimalne" antioksidacijske aktivnosti do završetka proizvodnje mesnog proizvoda ukazuje također na smanjenje regenerirajućih antioksidanata.

"Pribina" i "Štart" salame posjeduju slične "trenutne" i "maksimalne" antioksidacijske aktivnosti. Dinamika antioksidacijskih aktivnosti pokazuje nedostatak "trenutne" antioksidacijske aktivnosti.

ZAKLJUČAK

FRAP metoda je jednostavna i potencijalno vrlo korisno sredstvo ocjene kakvoće i sigurnosti mesnih proizvoda. Laka interpretacija rezultata omogućava poboljšanje učinaka antioksidanata uz njihovu minimalnu upotrebu. S nekoliko jednostavnih mjerenja i izračunom moguće je predvidjeti koje su količine antioksidanata s velikom i malom brzinom reakcije potrebne za djelotvornu zaštitu mesnih proizvoda od oksidacije tijekom njihovog vremena održivosti.

PRIZNANJE

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