The Effect of Natural Antioxidants on the Colour of Dried/Cooked Sausages

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Abstract

Rohlík B.-A., Pipek P., Pánek J. (2010): **The effect of natural antioxidants on the colour of dried/cooked sausages**. Czech J. Food Sci., **28**: 249–257.

The colour changes caused by haem pigment oxidation were evaluated in the slices of dried sausages. To slow down these unwanted changes, several colourants and extracts from spices (rosemary, allspice, nutmeg, black pepper, clove, sandal wood, cochineal) were added to the meat product. The colour was evaluated using video image analysis (NIS Elements 2.0 software). The addition of antioxidants showed a positive effect on the colour stability and elimination of colour varieties in different parts of the dried sausage. These colour changes (decrease of redness a^*) in cuts of dry sausages exposed to air and the light are influenced by the concentration of rosemary oleoresin, mostly by its light fraction. Video image analysis proved to be a suitable and simple method to monitor these colour changes of dried sausages.

Keywords: video image analysis; dried sausage; colour; oxidation; rosemary

The colour of meat products (especially dried sausages) is a very important parameter to evaluate. The intensity of the meat product red colour depends on the concentration of haem pigments (myoglobin and haemoglobin) and is also affected by the intravital conditions of the slaughtered animal and technological processes.

The storage conditions of dried sausages play a considerable role. Many chemical reactions can take place affecting the colour of the meat product. The most important chemical reactions that take place are lipid oxidations which in fact affect the oxidation of haem pigments whose red colour turns into brown. These chemical reactions inducing discoloration of the meat products depend on various factors like the ambient temperature, addition of antioxidants (in the occurrence of spices), smoke applied, haem pigment concentration, and others.

The colour changes of dried sausages, caused by the oxidation of haem pigments, can be observed in the slice after several hours of its exposition to the light. These reactions are accelerated by lipid oxidation and can be reduced by using extracts of some spices with antioxidant properties, most commonly sage, oregano, rosemary, etc. (Suhaj 2006).

Rosemary extracts contain specific phenolic compounds, e.g. rosmarinic acid, carnosol, other caffeic acid derivates etc., that are able to stabilise free radicals and break the oxidation chain. The effectiveness of spice extracts is often higher on

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. MSM 6046137305.

the synthetic antioxidants in the dependence on the medium which they should affect in (water, oil, ...) (Yanishlieva *et al*. 2006). Rosemary extracts prove to be efficient antioxidants for dry fermented sausages (Dragojev *et al*. 2007). These antioxidants suppress the lipolytic processes during the production of fermented sausages (Dragojev *et al*. 2006).

The complicated structure of dried sausages (a rough chopped meat product) with different kinetics of the colour changes in separate layers of the sausage makes it very hard to measure and evaluate these changes using classical reflectance spectrophotometry. Therefore, video image analysis is used to divide the sausage into precise parts and to measure and evaluate the colour of the red muscle particles applying a suitable threshold (Pipek *et al*. 2004, 2007).

The colour changes caused by haem pigment oxidation were evaluated in the slices of dried sausages. To slow down these unwanted changes, several colourants and extracts of spices (rosemary, all spice, nutmeg, black pepper, clove, sandal wood, cochineal colourant E120) were added to the meat product.

Various speeds of colour changes were observed in the outer and inner layers of the sausage due to the diverse access of oxygen, diffusion of smoke components into the outer layers, and also to microbiological activity.

MATERIALS AND METHODS

Dried/cooked sausages Vysočina (standard production) were prepared with the addition of different spices with known antioxidative properties (Tables 1–3); the sausage contained 25% of lean beef, 40% of pork, 30% of lard, 2.5% of nitrite-curing salt. The sausages were cooked (70°C/10 min); the interval between the filling and cooking processes was one hour. The final fat particles diameter was 3 mm.

After the production and necessary drying process (under $a_w < 0.93$), the intact sausages were stored at room temperature and analysed in defined intervals. To evaluate the colour stability of the dried sausages, these were cut into slices and exposed to ambient air oxygen in the dark or under the light of two fluorescent tubes.

The experiments were divided into three phases and repeated twice. First, the spices with expected antioxidative properties were added to the meat product and their effects on haem oxidation were observed. Secondly, the spices that effectively slowed down the haem pigment oxidation in the first part were used in the following experiment and their optimal concentrations were tested. In the last phase, different derivates of rosemary extract were tested for the slowing down the colour changes and lipid oxidation in dried sausages.

Table 1. Contents of added spices with antioxidant properties

Sample	Content	Addition (g/kg)
KA1	cochineal 2% – powder	1
KA2		2
SE1	sandal wood 10% extract – powder	1
SE2		0.7
SX	santex – sandal wood extract	1.9
SE 3	sandal wood 10% extract – powder	1.4
PC	black pepper	0.5
MO	nutmeg	0.3
NK	allspice	0.3
HR	clove	0.8
Control		–

Preparation of samples. The production of dried sausages was conducted as follows. Beef, curing mixture, and a small amount of ice were mixed in a cutter. After that pork, pork fat, and basic spices were added into the mixture and chopped in the cutter at a high speed of the knife blades

Table 2. Added spices with antioxidant effects – different concentrations

Sample	Content	Addition (g/kg)
B6	rosemary Bordantix	0.6
B3		0.3
M6	nutmeg	0.6
M3		0.3
NK6	allspice	0.6
NK3		0.3
R6	rosemary Trumf	0.6
R3		0.3
Control	control	–

Table 3. Different concentrations of added rosemary extract and fractions of the rosemary extract

Sample	Antioxidant	Addition (g/kg)
R1	standard rosemary oleoresin	0.6
R2		0.4
R3		0.3
R4		0.2
R5		0.1
R6	heavy fraction	0.3
R7	light fraction	0.3
Control	control	–

until the particles of meat and fat achieved 6 mm in diameter. This meat dough was divided into different batches and into each batch specific spices with antioxidant properties were added (Tables 1–3).

Colour evaluation using VIA. The surfaces of the exposed slices were scanned using scanner HP Scanjet 5470c and the images were evaluated using VIA software NIS-Elements 2.20 (Laboratory Imaging, Prague, Czech Republic). First of all, it was necessary to eliminate the background and measure the radius of the slice. After the radius was measured, the image was divided into three concentric circles. After the threshold of the muscle particles, their colours were measured and expressed as lightness L^*, redness a^*, yellowness b^*, mean red (R), mean green (G), and mean blue (B); the ratio of red $r = R/(R + G + B)$ and hue h = arctg (a^*/b^*) were calculated.

RESULTS AND DISCUSSION

The use of video image analysis (VIA) proves to be a suitable method to observe the colour changes in dried sausages. This method enables, in contrast to classical reflectance spectrophotometry, to analyse separate parts of the meat product. The images of the dried sausages obtained from the scanner were divided into different parts of interest without damaging the sample. VIA makes it possible to observe the colour changes in different layers of the dried sausage in detail by applying a suitable threshold and to distinguish the muscle particles from fat parts. It also enables to observe the colour changes of the meat product due to specific recipes (different spices with antioxidant properties added) and specific storage conditions.

The comparison between the effectiveness of colourants and of extracts from spices with antioxidative properties

The addition of antioxidants and colourants to dried sausages affects their colour in different ways. Separate slices were exposed to different thermal and light conditions, in order to determine the efficiency of the antioxidants added. During the exposure of the slices, the proportion of the red colour rapidly changed. The colourants logically increased the value of redness a^* (CIELab) and decreased the lightness of the sample. On the other hand, the spice extracts with antioxidative properties prevented the oxidation of haem pigments, thus the red colour of the muscle particles did not turn into yellowish brown hue. This is the reason why the samples with cochineal colorant and rosemary extract Bordantix (KA1, KA2) had the highest redness a^*. The lowest value of redness a^* was observed in the control sample (without any colourants and antioxidants).

The evaluated hue h was the lowest in the samples with cochineal colourant and rosemary extract Bordantix (KA1, KA2). This supports the previously discussed values of the redness a^*. After 72 h of the light exposure at 25°C, the hue value increased rapidly in all samples except samples KA1 and KA, which indicates the colour change into yellow brown. The difference in hue was small between samples KA1 and KA2, not only due to the content of cochineal colourant but also due to the presence of Bordantix (rosemary antioxidant) (Table 4).

The lightness L^* of the samples which had been exposed to the light gradually decreased. The smallest decline in lightness L^* was observed in the samples with cochineal colourant and rosemary (KA2), and the largest decrease in the sample with sandal wood (SX) and the control sample (K). The decrease of lightness L^* was affected by the drying of the sample, which obviously led to a darker colour due to a higher concentration of haem pigments.

On the basis of these measurements, which tested the antioxidant capacity of different spices and colourants, only some selected spices were tested in the second experiment (rosemary, nutmeg, all-

Table 4. Change of redness a^* (samples kept at 25°C)

Sample	0 h				Sample	72 h			
	L^*	a^*	r	h		L^*	a^*	r	h
KA 1	76.03	15.81	0.464	29.10	KA 1	70.02	13.51	0.455	31.42
KA 2	75.96	17.27	0.465	23.55	KA 2	71.01	15.32	0.461	26.02
SE 1	76.49	13.18	0.455	37.76	SE 1	72.68	11.03	0.447	42.53
SE 2	77.51	13.28	0.449	34.24	SE 2	71.95	10.88	0.443	40.29
SX	77.42	12.85	0.453	39.03	SX	68.89	11.71	0.460	43.16
SE 3	78.42	12.22	0.444	37.96	SE 3	70.74	11.61	0.454	41.96
PC	80.21	11.23	0.434	38.63	PC	73.25	9.78	0.438	45.01
MO	77.41	12.90	0.450	36.95	MO	71.51	11.02	0.451	43.85
NK	78.97	10.86	0.434	39.53	NK	72.93	9.70	0.439	45.97
HR	80.05	11.16	0.439	41.64	HR	73.44	10.41	0.442	43.90
Control	79.12	12.16	0.444	38.37	Control	71.61	9.85	0.442	46.04

spice, and two rosemary extracts: Bordantix and Trumf). The cochineal colourant was excluded due to its capability to hold the red colour of the meat product and to mask the eventual discoloration.

The comparison of selected spices with antioxidative properties

Due to the results obtained in the first experiment, the antioxidative properties of the following additives, i.e. rosemary (Bordantix and Trumf), nutmeg, and allspice, were studied in intact sausages and in separate slices of the sausages. In the second experiment, the effects of these antioxidants were observed in terms of:

– The effect of storage duration of intact sausages;
– The effect of different exposure conditions on colour changes of sliced dried sausages;
– Colour changes in different parts of dried sausages;
– Colour changes affected by additives.

The effect of storage duration

The samples (intact sausages) prepared for the second experiment were stored in a drying room (18°C, 85% relative humidity as in the first experiment) and were subsequently delivered to the laboratory for analysis. The colour changes were observed in terms of the storage duration in the drying room and were measured directly on the samples delivery to the laboratory. The samples were also studied after having been exposed to the light and dark at 4°C for a 48 h period.

The addition of the antioxidants tested and different storage conditions affected the colour of the dried sausages in different ways.

The lightness of intact sausages stored in the drying room had a decreasing trend due to the water loss and the increasing concentration of haem pigments (Table 5). During the storage, all samples seemed to darken at the same rate but at the end of 8-week storage time, the control sample appeared to have the lowest lightness L^*.

The red colour of the intact sausage changed rapidly during storage in the drying room. No obvious oxidation reaction took place whereas the concentration of haem pigment slightly increased due to the drying process. Therefore, the value of redness a^* and the ratio of the red colour r increased. The highest values were obtained with sample B6 that contained 0.6 g/kg Bordantix, which is the most effective antioxidant. Bordantix is an extract from rosemary purified of natural colourants and aromatic substances (Anonymous 2003). The effectiveness of Bordantix depends on its ability to affect lipid oxidation and subsequently slow down haem pigment oxidation.

Table 5. The change of lightness L^* during storage in the drying room (18°C, 85% relative air humidity)

Sample	Storage time (weeks)					
	1	2	3	4	6	8
B6	77.89	75.29	75.34	71.96	69.84	70.18
B3	77.45	76.17	76.01	74.26	72.49	70.99
M6	79.10	75.36	74.17	74.12	71.88	70.97
M3	78.46	75.84	75.17	73.53	72.29	69.45
NK6	78.25	76.45	75.89	73.29	71.89	69.79
NK3	78.56	76.73	77.08	74.74	71.39	70.76
RZ6	79.61	76.71	77.14	73.70	72.92	69.09
RZ3	78.01	76.91	74.84	72.94	71.56	69.39
Control	78.80	76.34	74.69	73.76	72.47	69.16

To study the effect of the added antioxidants for another point of view, the samples were sliced and kept at 4°C exposed to the light and dark. The slices of the samples kept in the dark at 4°C for 48 h showed to have higher values of redness a^* than those kept at the same temperature but exposed to the light (Table 6). The presence of the light initiates the lipid and haem pigment oxidation.

The colour of samples containing rosemary extracts (B and R) was relatively stable under the light exposure at 4°C. The best effect was observed in the sample with added Bordantix (0.6 g/kg), which slowed down the oxidative reactions to minimum. On the other hand, the samples with nutmeg and allspice had lower values of redness due to the small antioxidative capacities of these two spices. In the case of the control sample, it is obvious that the meat products without any antioxidants cannot retain their colour under the light exposure.

The effect of different exposure conditions on colour changes of sliced sausages

It is undoubted that different storage conditions affect the chemical reactions in dried sausages and thus can lead to undesirable colour changes. Sliced samples were therefore exposed to different thermal and light conditions. The aim was to observe the effectiveness of the added spices with antioxidative properties.

Some of the samples (slices) from the second experiment were kept in the dark and others were exposed to the light at 4°C. A certain amount of samples (slices) were kept at 25°C exposed to the light (5000 K).

A decrease of redness a^*occurred in all slices; the most remarkable changes were observed with the slices exposed to the light at 25°C. This is due to the higher temperature and light conditions that accelerated the oxidation reactions of haem pigments and so caused the discoloration of the samples. Similar colour changes were also observed in slices stored at 4°C exposed to the light. The changes were not so fast as in the samples mentioned before, because the lower temperature slightly slowed down the oxidation reactions. The redness values a^* of the slices kept in the dark had an increasing tendency due to the drying process that subsequently increased the haem pigments concentration. The absence of light and low temperature minimised the extent of the oxidation reactions and supported the increase of redness a^* (Figure 1).

To explain the effects of the added antioxidants and storage conditions on the colour changes of dried sausages, lightness L^*, redness a^*, and the ratio of the red colour r were observed an example obtained with sample B6 (Bordantix 0.6 g/kg) and the control sample (without any antioxidant) can be seen in Figure 2. When the samples were stored at both 25°C and 4°C, sample B6 showed to be coloured more red than the control sample. The stability of the red colour in sample B6 was caused by the addition of antioxidants from rosemary (Bordantix), which slowed down the oxidative reactions of haem pigments. Consequently,

Table 6. The change of redness a^* during storage in the drying room (18°C, 85% relative air humidity), slices exposed to the light for 48 h at 4°C

Sample	Storage time (weeks)					
	1	2	3	4	6	8
Fresh cut						
B6	12.73	14.16	14.07	15.50	16.12	15.83
B3	12.91	13.17	12.17	13.60	15.02	15.07
M6	12.00	13.37	14.06	14.14	14.83	15.84
M3	12.10	13.24	13.39	14.35	14.28	16.55
NK6	12.87	13.28	13.48	14.51	14.82	16.25
NK3	12.49	13.42	12.09	13.73	14.78	15.34
R6	11.92	12.97	12.72	13.92	13.69	15.92
R3	12.90	13.16	13.48	13.80	14.73	15.91
Control	12.22	12.89	13.23	13.79	14.57	15.78
48-h exposed to light at 4°C						
B6	8.23	8.12	7.46	7.72	7.76	6.91
B3	7.17	6.92	5.65	6.03	6.37	5.45
M6	6.14	7.50	6.97	6.21	5.95	6.16
M3	6.48	6.70	6.19	5.25	5.65	5.16
NK6	5.18	5.57	5.64	5.61	5.42	6.19
NK3	5.46	5.26	5.01	4.99	5.12	6.04
R6	5.01	5.43	5.00	5.81	4.41	6.73
R3	4.84	5.01	4.74	4.91	4.66	5.00
Control	4.56	4.73	4.97	4.77	6.55	7.63

sample B6 showed to be redder than the control sample (Figure 2).

The lightness of both samples had a decreasing character, but the lightness of sample B6 decreased faster. The drying process accelerated the decline of lightness at 25°C. It is obvious that the colour values decrease (L^*, a^*, r) faster when the samples are exposed to the light that initiates the oxidative

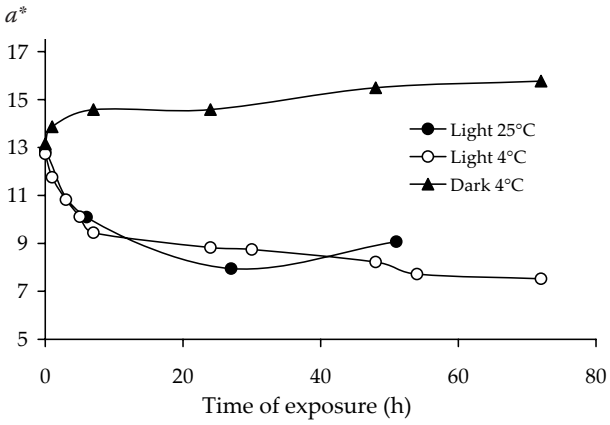

Figure 1. Effects of the exposure conditions on the colour changes (redness a^*) of the sausage cuts – example of sausage with rosemary extract Bordantix (B6)

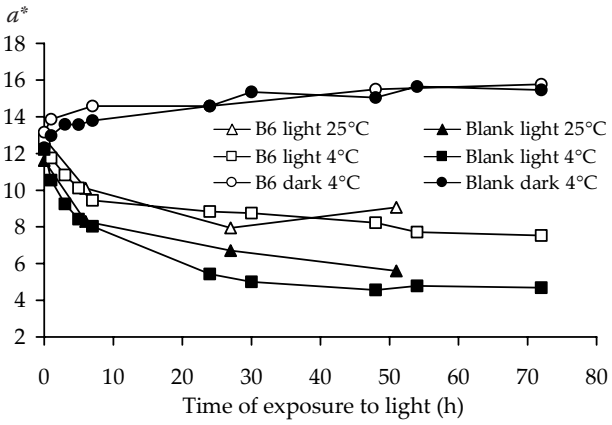

Figure 2. Colour changes of redness a^* – sample with Bordantix (B6) and control sample

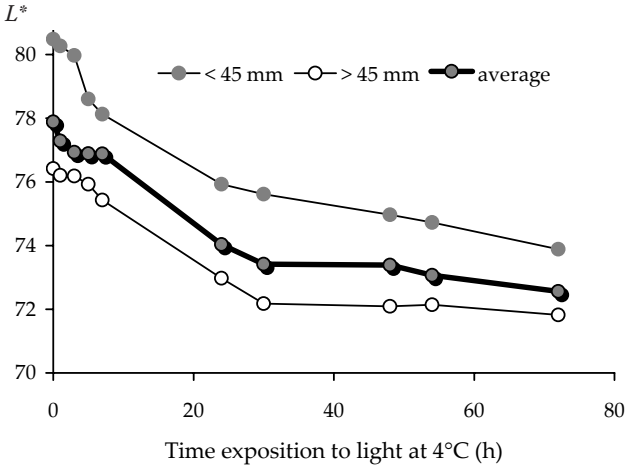

Figure 3. Colour changes of lightness L^* exposed to the light at 4°C – sample with Bordantix (B6)

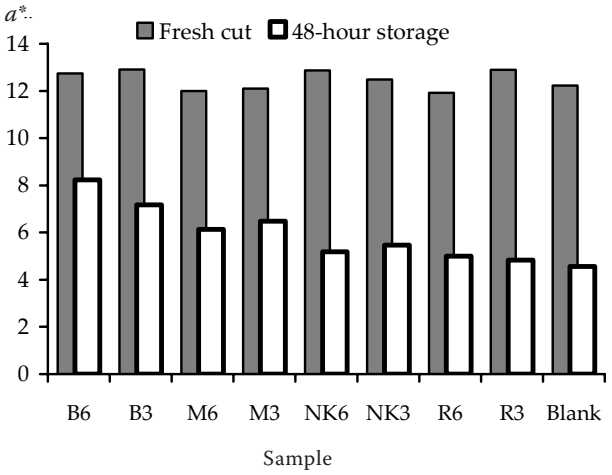

Figure 4. Changes of redness a^* – samples kept at 4°C

reactions of lipids and haem pigments while heat accelerates these reactions.

Colour changes in different parts of dried sausages

Haem pigments occurring in the meat products such as dried sausages oxidise due to the heat treatment and release iron, which can easily react with low molecular substances (amino acids, nucleotides etc.). The chalets formed initiate the oxidation of lipids which has an adverse impact on the sensory properties of the meat products (Monahan *et al*. 1993; Morissey *et al*. 1998).

The colour changes in different layers of dried sausages were studied to find out if any diversity exists between different layers. Figure 3 (sample B6 as an example) shows that the outer layer (> 45 mm) of the dried sausage is darker than the inner part (< 45 mm). This apparently depends on the higher concentration of haem pigments due to the drying process. The substances of the smoke medium may diffuse into the sausage and influence its colour changes.

The inner part of the dried sausage is lighter than the outer layer due to the higher water content in the inner part. In this part of the sausage, most of the smoke substances are absent, thus no chemical interactions of this kind can change the colour. Both factors (higher water content and lower smoke diffusion) may also influence the effects of antioxidants.

Colour changes affect by additives

Different spices and their extracts with antioxidative properties were added to dried sausages (Table 2) to avoid the oxidative reactions that lead to discoloration. To verify the antioxidative properties of the added extracts, the colour changes of the samples were studied.

Based on the values measured, it is possible to find that each additive (spice extract) affected the colour changes in the dried sausage in a different way. The samples exposed both to the light and dark at 4°C had a decreasing trend of the lightness. The highest value was observed in the sample with Trumf rosemary (R6) and the lowest in sample B3. After 48 h of storage under the same conditions, sample R6 showed again to be the lightest sample.

Considering redness a^*, the samples containing Bordantix (B6 and B3) and rosemary extract Trumf (R3) had the highest values of a^* (Figure 4). However, after 48-h exposure to the light at 4°C, only samples B6 and B3 showed to be the reddest. In the case of the red colour ratio r, the same finding was made, samples B6 and B3 exhibiting the smallest loss of red colour when stored at 4°C and exposed to the light.

These data imply that rosemary extracts added to dried sausages (Bordantix and Trumf) can slow, down the oxidation reactions of haem pigments that lead to discoloration. On the other hand, the samples containing nutmeg and allspices seemed to be less affected. In the case of the control sample, it is obvious that it lost its red colour because

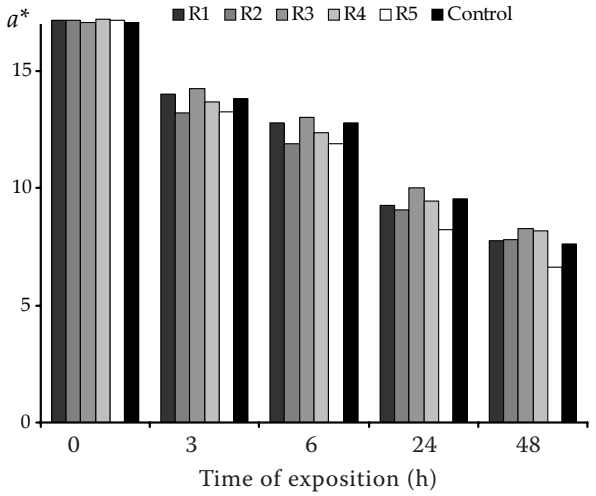

Figure 5. Effects of different concentrations of rosemary extract on dry sausage colour during exposition to air and the light

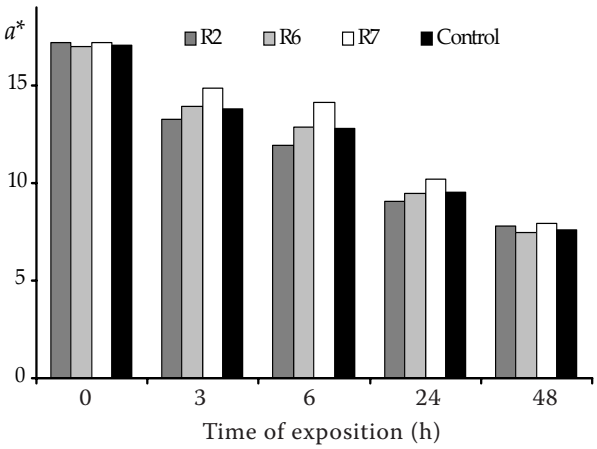

Figure 6. Effects of different fractions of rosemary extract on dry sausage colour during exposition to air and the light

it did not contain any antioxidant to avoid these unwanted reactions.

The comparison of rosemary extracts

As found in the second experiment, the extract of rosemary (oleoresin) effectively inhibited the oxidation reactions of haem pigments. The next steps was to find out the ideal concentration for the addition to dried sausages. Further, to find out if among different fractions of the rosemary extract some can have even better antioxidative properties.

The storage time of whole intact sausages had a small effect on the colour, mostly due to the water evaporation and haem pigment concentration, which resulted in a darker colour (decrease of lightness L^*). The addition of rosemary extract had minimal influence on this darkening.

Considering the sausage cuts kept at 4°C, the colour of these samples changed if exposed to air and the light.

The decrease of redness a^* was most apparent during the first 48 hours after cutting. The addition of rosemary (0.2–0.3 g/kg) influenced the decrease of redness a^* in the most effective way (Figure 5). Comparing the individual fractions, the most effective in suppressing the redness decrease seems to have been the light fraction (Figure 6), whereas the heavy fraction of oleoresin showed no effect on preventing the oxidative reactions of haem pigments. Nearly the same results were found with the ratio of red colour r.

The best antioxidant to prevent discoloration of dried sausages stored at low temperatures and exposed to the light seems to be the light fraction of oleoresin (rosemary extract).

CONCLUSIONS

The addition of antioxidants showed a positive effect on the colour stability and elimination of colour varieties in different parts of the dried sausage. Colour changes (redness a^* decrease) during the exposition of dry sausages cuts to air and the light were influenced by the concentration of rosemary oleoresin, mostly its light fraction, but at higher concentration of oleoresin the carry-through effect is supposed. To monitor these colour changes of dried sausages, video image analysis proved to be a suitable and simple method.

References

Anonymous (2003): Bordantix liquid C-5, Technical Sheet. Extractos vegetales s.a. – EVESA Spain.

Dragojev S., Balev D. (2006): Effect of natural antioxidants on lipolysis and pH of dry-fermented sausages "Lukanka" type. In: Proceedings 52nd International Congress of Meat Science and Technology, August 13–18, 2006, Dublin, Ireland. Wageningen Academic Publishers, the Netherlands: 451–452.

Dragojev S., Balev D., Vulkova-Jorgova K. (2007): Inhibition of lipid oxidation in dry-fermented sausages

using rosemary extracts. In: Proceedings 53rd International Congress of Meat Science and Technology, August 5–10, 2007, Beijing, China. China Agricultural University Press: 421–422.

Monahan F.J., Crackel R.L., Gray J.I., Buckley D.J., Morrissey P.A. (1993): Catalysis of lipid oxidation in muscle model systems by haem and inorganic iron. Meat Science, **34**: 95–106.

Morrissey P.A., Sheehy P.J.A., Galvin K., Kerry J.P., Buckley D.J. (1998): Lipid stability in meat and meat products. Meat Science (Supplement 1), **49**: S73–S86.

Pipek P., Jeleníková J., Sarnovský L. (2004): The use of video image analysis for fat content estimation. Czech Journal of Animal Sciences, **49**: 115–120.

Pipek P., Lojková A., Bělková B.-A., Staruch L. (2007): Analýza obrazu při hodnocení kvality masa a masných výrobků. In: Zborník XVI. Medzinárodnej konferencie LABORALIM 2007. Banská Bystrica 7.–8. 2. 2007. STU Bratislava: 16–21.

Suhaj M. (2006): Spice antioxidants isolation and their antiradical activity, a review. Journal of Food Composition and Analysis, **19**: 531–537.

Yanishlieva N.V., Marinova E., Pokorný J. (2006): Natural antioxidants from herbs and spices. European Journal of Lipid Science and Technology, **108**: 776–793.

Received for publication December 24, 2009
Accepted after corrections January 25, 2010

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