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Colour changes after carcasses decontamination by steam and lactic acid

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Abstract

The surface decontamination of meat by steaming and by lactic acid prolongs its shelf life. Possible changes of colour were evaluated by reflectance spectrophotometry and video image analysis (VIA). Reflectance spectra were measured using a D_{65} source and CIELab values L^*, a^* and b^* were calculated together with ratios of the different myoglobin forms (red, oxy, met). The same samples were evaluated by video image analysis (software LUCIA). Steaming and spraying with lactic acid increased slightly the lightness (L^*) of the meat surface. Coordinate a^* (redness) decreased slightly after the decontamination treatment. This was confirmed by VIA; brightness increased and red-ratio *r* decreased. Both methods, i.e. reflectance spectrophotometry and VIA, reflect the colour changes in a similar way. The main advantage of reflectance spectrophotometry was its higher sensitivity and the possibility of direct calculation of the ratio of the haem pigment forms. However, video image analysis allows analysing of different particles in the image and this method is therefore a suitable tool for monitoring the changes of the surface appearance.

Keywords: Colour; Myoglobin forms; Steam; Lactic acid; Decontamination

1. Introduction

Surface decontamination of carcasses has been shown many times to prolong the shelf life of meat. Different methods were proposed for this treatment, including the application of organic acids and hot water. Lactic acid is often used, as it is a natural compound produced during postmortem glycolysis in the meat. In addition, the lactate anion inhibits the growth of surviving microbes during storage (Siragusa, 1995). The application of lactic acid was effective under industrial conditions in our studies (Pipek, Izumimoto, & Jelenıková, 2004; Pipek et al., 2005).

Spraying with hot water or steam combines mechanical removal of bacteria stuck on the carcass surface with partial heat decontamination of the surface. Such treatment is limited by possible heat damage caused to the carcass surface but, the water temperature must be high enough to kill the bacteria (Siragusa, 1995). Steaming should have the best decontamination potential for industrial application due to its simplicity (James, Thornton, Ketteringham, & James, 2000). Kozempel, Goldberg, and Craig (2003) explained the advantage of steam as it can enter pores and kill bacteria. The exposure times must be short, of the order of 0.1 s.

The combination of hot steam with spraying by lactic acid solution is another possibility for surface decontamination (see e.g. Dorsa, Cutter, Siragusa, & Koohmaraie, 1996). Such treatment was very effective in the surface decontamination of carcasses under industrial conditions (Pipek et al., 2004, 2005). Kang,

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Koohmaraie, Dorsa, and Siragusa (2001) observed that different combinations of hot water (82 °C) and/or hot air (510 °C) and of lactic acid resulted in a continuous decrease of microbial populations on beef.

An efficient decontamination system should reduce the numbers of bacteria without any detrimental changes to the carcass appearance (James et al., 2000). For this reason, possible effects of the decontamination treatment on the surface colour must be carefully considered as they appear to be the limiting factor for each treatment; the haem pigments can denature and oxidise.

The effect of lactic acid on meat colour is not clear in the literature. van Netten, Mossel, and Huis-in' t-Veld (1995) observed that the application of 2% or 5% lactic acid for 120 s resulted in unacceptable sensory properties including colour of swine carcasses; these changes could be reduced by ascorbic acid. The colour of chicken skin was modified (yellowing and greening) after pulsed-vacuum immersion in lactic acid solution (Deumier, 2004). However, no effect of spraying beef carcasses with lactic acid (1%) was observed by Prasai et al. (1991). Kotula and Thelappurath (1994) observed brighter meat colour after treatment with lactic acid as the immersion of cuts of beef in lactic acid solution for 120 s caused paleness (increase of the brightness, L^* from 33.1 to 35.2 and decrease of redness, a^* from 18.0 to 17.5). Whereas Ellerbroek, Okolocha, and Weise (1997) found that lactic acid treatment caused increased bright discolouration. Uijl (1999) observed only negligible reversible discoloration of beef after 1% lactic acid treatment and no changes in veal even after treatment with 2% lactic acid. The treatment of buffalo steaks with 3% solutions of acetic and lactic acid had no adverse effect on colour (Surve, Sherikar, Bhilegaonkar, & Karkare, 1991). The surface spraying of beef muscle by lactic acid had a negligible effect on the colour (Pipek et al., 2004, 2005); only an insignificant increase in brightness was observed.

However, some problems may arise from the hot steam effect and/or from the combination of steam with lactic acid treatment. Under laboratory conditions, the hot steam treatment caused some colour changes of meat surfaces, the effect of heat treatment depended on the surface temperature (Hoke, Houška, Kýhos, Landfeld, & Pipek, 2003). Negligible effect on colour was observed from multihurdle treatment (hot water 65 °C + hot air + lactic acid) on beef trim (Kang et al., 2001).

Surface decontamination of carcasses may impair the appearance, due to denaturation and haem pigment oxidation. The goal of this research was to consider possible colour changes of the carcass surface after decontamination by the combination of steam and lactic acid treatment.

2. Materials and methods

The decontamination of the carcass surfaces by steaming and spraying with lactic acid solution and its effect on shelf life was investigated under industrial conditions (Pipek et al., 2004, 2005). At the same time, the influence of decontamination on the surface appearance was evaluated immediately after treatment and following cold storage.

2.1. Materials

Beef and pork carcasses were decontaminated immediately at the end of slaughter line, before entry into the rapid chilling tunnel, i.e. nearly 30 min postmortem. The surface decontamination included steaming and spraying with the lactic acid solution.

The hot steam (pressure 6 bar) was applied with a special rectangular nozzle 100 mm by 1 mm. The mean period of the steam action on the individual site calculated from stream height, length of the treated area and stream velocity of the nozzle was 0.013 s.

A 2% lactic acid solution (Purac FCC 80) was sprayed by a manual sprayer. Each time the surface was sprayed three times to create a uniform film (consumption of the solution was approximately 1 l per beef carcass and 0.5 l per pork carcass).

The samples of surface tissues (approximately $40 \times 40 \times 5$ mm) were cut from the same carcasses before decontamination ("control"), after steaming ("steam"), after spraying with lactic acid ("steam and lactic acid"). The colour of these samples was measured during cold storage (exact times of measurements are shown in the tables). In the case of pig carcasses the samples of skin over the shoulder were cut; from beef, the surface muscle tissue on the brisket at the 7th rib was used as the sample. Experiments with beef carcasses were carried out in two ways: (a) natural contamination on the surface (Tables 3 and 4), (b) microbial counts on the surface were artificially increased by a solution containing microorganisms collected from the surfaces of beef carcasses (Tables 5 and 6). All samples were prepared simultaneously from 10 carcasses, i.e. each value in the tables represents the average value of 10 samples.

2.2. Methods

The colour was evaluated objectively by reflectance spectrophotometry and by the video image analysis.

For the video image analysis, the pictures of the surface samples, illuminated by two fluorescent tubes, were taken by digital camera (Nikon coolpix 800). The pictures (*.jpg files) were then evaluated by the video image analysis software LUCIA 3.52 (Laboratory Imaging ČR). In the case of pork skin, the whole area of the sample visible on the picture was measured. In the case of beef carcasses, only the colour changes on the muscle tissue visible on pictures were evaluated. For this reason only those parts of the surface representing muscle tissue were thresholded (red area) and their colour was measured. The results were expressed in the RGB system as mean red (R), mean green (G) and mean blue (B), and as brightness (MB) and saturation (MS).

From the values for mean red (R), mean green (G) and mean blue (B), the ratios for red (r), green (g) and blue (b) were calculated using the following ratios:

$$r = R/(R + G + B),$$

$$g = G/(R + G + B),$$

$$b = B/(R + G + B).$$

2.2.1. Reflectance spectrophotometry

The whole visible reflectance spectra were measured in the range from 400 to 700 nm at 10 nm intervals using Chroma Meter Minolta CM-2600d spectrophotometer. The white standard was a piece of tile of known reflectance; the light source D_{65} and the standard observer angle 10° were used.

The measured data were calculated using software Spectra Magic Ver. 3.3 (Minolta 2001, Japan) and the results expressed in terms of lightness L^*, redness a^*, yellowness b^*, chromacity C^* and hue h.

In the case of beef samples the myoglobin form (met, oxy and red) ratios were calculated from the reflectance spectra by the Izumimoto and Ozawa (1993) method.

The reflectance spectrophotometry data, i.e. lightness L^*, redness a^* and yellowness b^*, were compared with the corresponding results of video image analysis, i.e. brightness MB, and the r, g and b values.

2.2.2. Statistics

Statistical analysis of the measured data was performed using Microsoft Excel, version 2002 at the significance level $P < 0.05$. The Student's t test between corresponding values was carried out.

3. Results

The surface appearance changes in the case of pork and beef are discussed separately, due to the different tissues involved in pork carcasses (skin) and beef carcasses (muscle).

3.1. Decontamination of pork carcasses

The external surface of a pork carcass is covered by skin containing stromatic proteins (mostly collagen) and fat; these components are without any apparent colour and are mostly white. The colour of skin on the surface of pork carcasses at the end of the slaughter line, i.e. at the moment of decontamination, is usually of a white–pink–yellow colour. Slight reddening on the skin surface may be due to haem pigments from blood residues.

In our experiments, decontamination by steaming and lactic acid caused a slight reduction of skin surface red colour, which became lighter. These facts were documented by both methods, i.e. by video image analysis (Table 1) and by reflectance spectrophotometry (Table 2).

Video image analysis showed that a slight reduction of the red ratio r occurred after decontamination, while the brightness (MB) increased. This increase of brightness can be explained not only by the removal of haem pigments, but also by a decrease of the pH value below the isoelectric point, allowing swelling of the skin proteins. During subsequent cold storage all samples (treated and control) became darker, i.e. brightness MB decreased in all samples as a consequence of water evaporation from the carcass surface. The treatment by steam and lactic acid caused a decrease in saturation (MS) of pork skin. The slight decrease in saturation together with the decrease of the red ratio again suggests the elimination of haem pigments.

However, as evident from Table 1, all these differences were very small and most of them insignificant ($P < 0.05$). Only differences in r-ratio were statistically significant – the samples treated with both steam and lactic acid had lower r-ratios in comparison with the controls (untreated) during the whole storage period.

Similar changes were also documented by reflectance spectrophotometry (Table 2). The coordinate a^* (redness) decreased similarly to the red ratio r, but these differences were not significant. The lightness L^* was slightly increased by the decontamination treatments; the steaming seems to affect the colour more than spraying by lactic acid. This suggests mechanical removal of the pigments rather than chemical changes. Colour changes measured by reflectance spectrophotometry were insignificant ($P < 0.05$).

It can be concluded that the decontamination treatments have only minor effects on the colour of pork skin. Usually, the skin is not noticeably coloured and the treatment by steam and lactic acid solution actually represents the washing off of remnants of blood from the surface rather than any oxidative changes. The significant reduction of red colour (evaluated by video image analysis) supports this hypothesis. The decontamination of pork carcasses slightly improves their appearance and therefore does not limit the practical use of surface decontamination by the steaming and the lactic acid treatment.

Table 1
The effect of decontamination by steam and lactic acid on the colour of pork carcasses (skin over the shoulder)

Time after the treatment (h)	Treatment	r	g	b	MS	MB
6	Control	0.448[a]	0.334	0.217	89	0.55
	Steam	0.434	0.339	0.227	82	0.55
	Steam + lactic acid	0.429[a]	0.341	0.23	79	0.57
26	Control	0.443[a]	0.339	0.218	88	0.55
	Steam	0.430	0.342	0.228	81	0.57
	Steam + lactic acid	0.429[a]	0.344	0.227	80	0.56
29	Control	0.445[a]	0.34	0.215	91	0.5
	Steam	0.434	0.345	0.221	86	0.52
	Steam + lactic acid	0.427[a]	0.344	0.229	81	0.53

Results of measurement by video image analysis.
[a] Statistically significant differences between values within column and time.

Table 2
The effect of decontamination by steam and lactic acid on the colour of pork carcasses (skin over the shoulder)

Time after the treatment (h)	Treatment	L^*	a^*	b^*
6	Control	66.8	0.80	17.4
	Steam	66.7	0.40	17.4
	Steam + lactic acid	69.5	−0.90	15.7
26	Control	66.7	−0.20	17.9
	Steam	68.0	0.10	15.3
	Steam + lactic acid	68.4	−0.09	16.3
29	Control	66.1	−0.80	14.3
	Steam	66.3	1.40	16.4
	Steam + lactic acid	68.9	−0.08	16.1

Results of measurement by reflectance spectrophotometry.

3.2. Decontamination of beef carcasses – natural contamination

In beef carcasses, their external surface is covered by muscle tissue and some parts are covered by connective or adipose tissue. Muscle tissue has a red colour and its chemical composition differs from that of the other two tissues.

The reflectance spectrophotometry proved that the surface lightness L^* increased after steaming as well as after the spraying with lactic acid; no further changes were observed during subsequent cold storage (Table 3). At the same time, the redness a^* was reduced after each decontamination treatment but during subsequent cold storage the redness of all samples increased. Changes of the b^* coordinate were not consistent, this value had a tendency to decrease after both decontamination treatments, and to increase during storage.

After the steaming and spraying with lactic acid, the form of haem pigments (myoglobin and/or haemoglobin) changed slightly from the reduced form to other two types. Although the haem pigments were predominantly in the oxygenated form immediately before the decontamination treatment, a small increase of this form was observed after decontamination (Table 3) together with a decrease of the other two forms. It is evident that decontamination (steaming and lactic acid) accelerated the transformation to the oxy-form from the other forms, i.e. from the red and met forms (Figs. 1(a)–(c)). This can be related to the higher temperature and decreased pH. The decrease in the met form seems opposite to that expected due to denaturation, but the extent is small and muscle reduction activity may prevent the oxidation.

The colour changes of the surface of carcasses after decontamination by steam and lactic acid can be explained by the denaturation of surface layers followed by oxidation of haem pigments and to changes of protein hydration, due to reduction of pH after lactic acid treatment. However, the changes in the myoglobin forms (Table 3) indicated that no noticeable oxidation occurred, on the contrary, there is a slight increase in the oxy-form and a decrease in the red-form. A faster loss of the reduced form of myoglobin after the treatment with lactic acid was also observed in earlier experiments (Pipek et al., 2004, 2005).

The video image analysis similarly proved that the red ratio *r* decreased slightly after the hot steam treatment (Table 4) but were increased by the spraying with lactic acid. The differences in the *r* ratios between the control samples and samples treated with steam and lactic acid were significant.

The brightness MB increased after the steam treatment as well as after spraying with lactic acid. These differences were insignificant during first 9 h; the differences in brightness between untreated samples and samples decontaminated using both operations (steam + lactic acid) became statistically significant after 25 h cold storage.

The saturation MS decreased after decontamination; this decrease was significant between samples decontam-

Table 3
The effect of decontamination by steam and lactic acid on the colour of beef carcasses with natural contamination on the surface (muscle surface on the shoulder)

Time after the treatment (h)	Treatment	L^*	a^*	b^*	C^*	hue	oxy	red	met
4	Control	50.7	5.89	4.05	7	36	43	46	11
	Steam	53.5	4.52	2.63	6	48	47	41	13
	Steam + lactic acid	53.9	4.01	−0.60	6	26	55	35	10
9	Control	48.9	9.58	6.88	12	33	76	17	7
	Steam	48.6	8.02	3.91	10	28	71	20	9
	Steam + lactic acid	54.7	5.47	−1.99	7	25	78	16	6
25	Control	46.8[a]	11.6	6.26	14	33	87	3	10
	Steam	52.1	9.21	4.45	11	33	80	7	13
	Steam + lactic acid	58.4[a]	7.49	1.69	9	28	92	4	5
27	Control	48.8[a]	11.7	7.76	14	33	88	4	8
	Steam	54.9	9.59	7.45	13	39	84	6	10
	Steam + lactic acid	59.4[a]	8.50	1.91	9	33	99	0	1
29	Control	47.3[a]	12.3	8.77	15	35	88	2	10
	Steam	55.5	9.36	7.31	13	46	86	1	13
	Steam + lactic acid	56.8[a]	8.01	1.44	9	31	93	2	6

Results of measurement by reflectance spectrophotometry.
[a] Statistically significant differences between values within column and time.

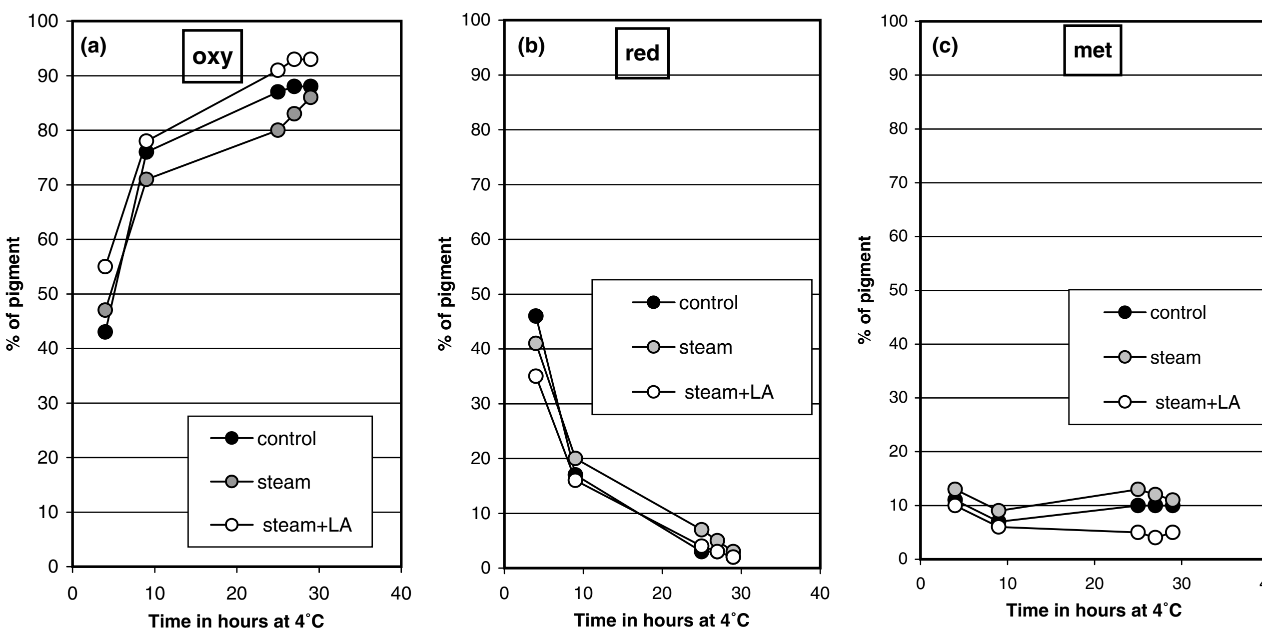

Fig. 1. (a)–(c) The effect of decontamination by steam and lactic acid on the ratio of different forms of haem pigments on the surface of the beef carcasses with the natural contamination on the surface (muscle surface on the shoulder).

inated by both methods and the control. During storage (after 25 h), the differences in saturation between steamed only samples and samples treated with both steam and lactic acid became significant.

Although these changes suggest oxidation of reduced haem pigments, the oxy form increases and the met form decreases as shown by reflectance spectrophotometry and discussed above.

Both video image analysis and reflectance spectrophotometry proved that treatment by hot steam made the surface of the carcasses lighter and reduced the red hue. The subsequent spraying by lactic acid increased these effects.

Table 4
The effect of decontamination by steam and lactic acid on the colour of beef carcasses with natural contamination on the surface (muscle surface on the shoulder)

Time after the treatment (h)	Treatment	r	g	b	MS	MB
4	Control	0.525[a]	0.264	0.216	94[a]	0.36
	Steam	0.490	0.276	0.238	75	0.37
	Steam + lactic acid	0.416[a]	0.298	0.288	40[a]	0.44
9	Control	0.533[a]	0.237	0.230	88[a]	0.38
	Steam	0.498	0.249	0.259	72	0.40
	Steam + lactic acid	0.422[a]	0.272	0.306	50[a]	0.44
25	Control	0.588[a]	0.222	0.191	114[a]	0.35[a]
	Steam	0.518	0.246	0.236	112[b]	0.37
	Steam + lactic acid	0.450[a]	0.265	0.285	56[ab]	0.45[a]
27	Control	0.549[a]	0.245	0.221	93[a]	0.40 [a]
	Steam	0.496	0.245	0.258	91[b]	0.40
	Steam + lactic acid	0.430[a]	0.268	0.302	51[ab]	0.50 [a]
29	Control	0.551[a]	0.242	0.207	100[a]	0.36[a]
	Steam	0.513	0.255	0.232	80[b]	0.41
	Steam + lactic acid	0.453[a]	0.271	0.270	57[ab]	0.43[a]

Results of measurement by video image analysis.
[a,b] Statistically significant differences between values within column and time.

3.3. Decontamination of beef carcasses artificially contaminated

The appearance of beef carcasses after decontamination was studied in a separate experiment, in which the initial surface microbial counts were artificially increased by a solution of microorganisms swabbed from the surface of other beef carcass. The results are in Tables 5 and 6 and are very similar to those shown with natural contamination (Tables 3 and 4).

Reflectance spectrophotometry showed that the surface lightness L^* increased after both steaming and spraying by lactic acid; the samples treated with both steam and lactic acid were significantly lighter than the controls, the differences between these samples and samples that were only steamed were insignificant.

Table 5
The effect of decontamination by steam and lactic acid on the colour of beef carcasses with artificially increased contamination on the surface (muscle surface on the shoulder)

Time after the treatment (h)	Treatment	L^*	a^*	b^*	C^*	hue	oxy	red	met
8	Control	44.5[a]	3.91[ab]	0.94	5	26	47	33	20
	Steam	53.6	0.78[a]	−2.54	4	36	68	18	10
	Steam + lactic acid	58.3[a]	0.38[b]	−0.07	3	40	70	18	12
18	Control	42.3[a]	5.38	4.44	9	38	65	24	11
	Steam	54.2	3.62	0.64	5	36	87	3	8
	Steam + lactic acid	56.6[a]	3.10	2.10	5	34	83	4	13
21	Control	47.6[a]	5.78	2.95	6	37	51	30	19
	Steam	57.3	3.44	−0.49	4	24	79	13	9
	Steam + lactic acid	57.9[a]	2.62	0.10	3	18	81	10	10
44	Control	47.9[a]	6.68	5.55	9	38	76	5	18
	Steam	56.9	4.08	2.58	5	38	79	4	10
	Steam + lactic acid	57.2[a]	4.35	4.10	7	44	86	3	11
91	Control	47.1[a]	7.99	6.25	10	40	90	1	9
	Steam	55.9	5.19	4.14	7	44	88	2	10
	Steam + lactic acid	57.2[a]	3.77	3.95	6	45	88	1	11

Results of measurement by reflectance spectrophotometry.
[a,b] Statistically significant differences between values within column and time.

Table 6
The effect of decontamination by steam and lactic acid on the colour of beef carcasses with artificially increased contamination on the surface (muscle surface on the shoulder)

Time after the treatment (h)	Treatment	*r*	*g*	*b*	MS	MB
8	Control	0.475[ab]	0.256	0.269	66[ab]	0.36[a]
	Steam	0.403[a]	0.293	0.303	36[a]	0.46
	Steam + lactic acid	0.397[b]	0.296	0.307	33[b]	0.49[a]
18	Control	0.520[a]	0.251	0.229	89	0.37[a]
	Steam	0.447	0.285	0.268	54	0.46
	Steam + lactic acid	0.422[a]	0.272	0.306	50	0.47[a]
21	Control	0.524[ab]	0.247	0.228	88	0.33[a]
	Steam	0.445[a]	0.280	0.275	53	0.43
	Steam + lactic acid	0.437[b]	0.289	0.274	52	0.46[a]
44	Control	0.557	0.243	0.200	97	0.35[a]
	Steam	0.472	0.274	0.254	66	0.44
	Steam + lactic acid	0.443	0.285	0.272	54	0.46[a]
91	Control	0.526	0.256	0.218	92	0.37[a]
	Steam	0.453	0.280	0.267	57	0.47
	Steam + lactic acid	0.440	0.286	0.275	48	0.48[a]

Results of measurement by video image analysis.
[a,b] Statistically significant differences between values within column and time.

The redness a^* decreased after each decontamination treatment, but during subsequent cold storage the redness of all samples increased. The differences in redness a^* between treated and untreated samples were statistically significant only at the beginning; they became insignificant during storage.

The changes in haem pigments were similar as in the previous experiment although the increase in oxymyoglobin content was greater on both treated samples (steamed and steam + lactic acid treated; Figs. 2(a)–(c)).

The video image analysis gave results very similar (Table 6) to those found by reflectance spectrophotometry. Brightness (MB) increased on decontaminated samples in comparison to the controls during the whole storage time; the differences between the samples treated with a combination of steam and lactic acid and the control ones were significant.

The differences of *r* ratio between controls and decontaminated samples were significant for the first 21 h after treatment.

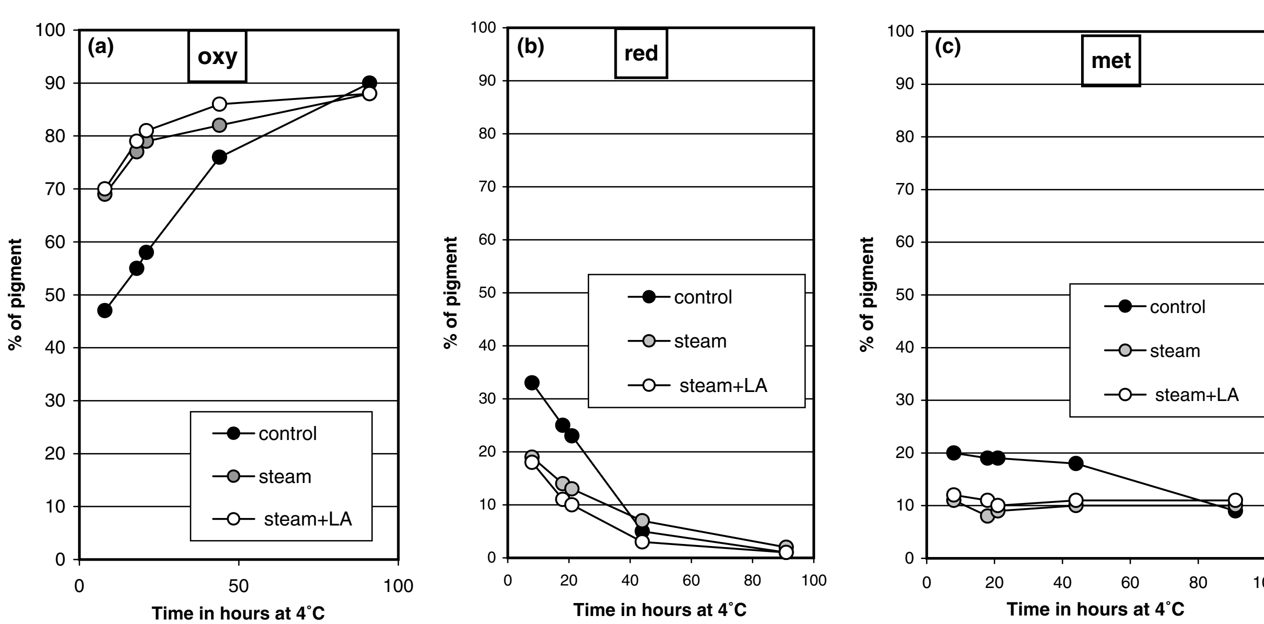

Fig. 2. (a)–(c) The effect of decontamination by steam and lactic acid on the ratio of different forms of haem pigments on the surface of the beef carcasses with artificially increased initial contamination on the surface (muscle surface on the shoulder).

The saturation MS decreased after decontamination; this decrease was however significant only at the beginning of the storage.

3.4. Comparison of reflectance spectrophotometry and video image analysis

Both methods reflect the changes in surface appearance similarly. Although reflectance spectrophotometry is more sensitive for subtle colour changes, video image analysis is more suitable in those cases where various small parts of different tissues, i.e. different areas on the picture, have to be distinguished. For this reason, some differences in colour between the samples were found to be significant only by video image analysis. The main advantage of the reflectance spectrophotometry was that it enabled direct calculation of haem pigment changes. At present, this is not possible by VIA.

4. Conclusions

The surface decontamination of carcasses by the combination of steaming and spraying with the lactic acid caused only subtle colour changes. In the case of muscle tissue, they are hardly noticeable; on pork skin, the appearance is improved.

The surface colour changes were similarly described by both methods used, i.e. by the reflectance spectrophotometry and by the video image analysis.

As the decontamination by steaming and by lactic acid spraying did not negatively influence the surface appearance, it can be used to prolong the shelf-life of carcasses and to increase the safety of meat.

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