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Processing of pork with colour differences to raw fermented sausages has no impact on the physico-chemical and microbiological characteristics of the products

Verarbeitung von Schweinefleisch mit Farbunterschieden zu Rohwürsten hat keine Auswirkung auf die physikalisch-chemischen und mikrobiologischen Eigenschaften der Produkte

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Summary

Colour of pork is influenced by the myoglobin content, the proportions of myoglobin redox forms and by the intrinsic alterations during muscle to meat transition after slaughter. Meat colour is also related to the microbiological and chemical changes during storage of the meat accompanied with the risk that during processing of different coloured meat to sausages physico-chemical and microbiological characteristics of these products vary. To clarify this assumption, in the present study longissimus muscles from 48 commercial crossbreed pigs were analysed on pH values 45 min after slaughter (p.m.), lightness (L*), redness (a*), yellowness (b*) and electrical conductivity (EC) 24 h p.m. as well as drip loss and shear force. The pork was frozen and sorted prior to processing into colour groups (pale, mean, dark) according to the L* values. Pale pork had higher L*, b* and EC and lower pH and shear force values than dark meat. The sorted meat was subsequently processed to raw fermented sausages using 1.5 % or 2.5 % curing salt (CS). These sausages were analysed on day 1, 7, 14, 21 and 28 after production concerning the parameters L*, a*, b*, pH, water activity (a_w), raw nutrients values as well as thiobarbituric acid reactive substances and total aerobic plate count concentrations. After sorting according to the lightness it became obvious that the colour group only had an influence on the dry matter with higher values in the pale meat. With regard to the impact of the salt concentration, sausages with higher CS concentrations had lower a_w and higher ash concentrations compared to low CS products. In conclusion, processing of different coloured pork does not affect the quality characteristics of raw fermented sausages and therefore sorting of pork prior to production of raw fermented sausages is not necessary.

Keywords: meat quality, TBARS, total aerobic plate count, dry matter

Zusammenfassung

Die Farbe von Schweinefleisch wird durch den Myoglobingehalt, den Anteil der Myoglobin-Redoxformen und durch intrinsische Veränderungen während der Fleischreifung nach der Schlachtung beeinflusst. Fleischfarbe hat auch einen Einfluss auf die mikrobiologischen und (bio)chemischen Veränderungen während der Lagerung des Fleisches, verbunden mit dem Risiko, dass bei der Verarbeitung von Fleisch unterschiedlicher Farbe zu Würsten physikalisch-chemische und mikrobiologische Eigenschaften dieser Produkte variieren. Um diese Annahme abzuklären, wurden in der vorliegenden Studie der *Musculus longissimus dorsi* von 48 kommerziellen Hybridschweinen hinsichtlich des pH-Wertes, gemessen 45 min nach der Schlachtung (p.m.), der Helligkeit (L*), des Rotwertes (a*), des Gelbwertes (b*) und der elektrischen Leitfähigkeit (LF), bestimmt 24 Stunden p.m., sowie des Tropfsaftverlustes und der Scherkraft untersucht. Das Schweinefleisch wurde eingefroren und vor der Verarbeitung anhand der Helligkeitswerte (L*), in Farbgruppen (hell, mittel und dunkel) sortiert. Dabei hatte das helle Fleisch höhere L*-, b*- und LF- und niedrigere pH- und Scherkraftwerte als das dunkle Fleisch. Das sortierte Fleisch wurde anschließend zu Rohwürsten unter Zusatz von 1,5 % und 2,5% Nitritpökelsalz (NPS) verarbeitet. Diese Rohwürste wurden an den Tagen 1, 7, 14, 21 und 28 nach der Herstellung hinsichtlich der Parameter L*, a*, b*, pH, Wasseraktivität (a_w), Rohnährstoffe sowie auf die Konzentrationen Thiobarbitursäure-reaktiver Substanzen (TBARS) und der aeroben Gesamtkeimzahl untersucht. Die Sortierung nach der Helligkeit zeigte, dass die Farbe nur einen Einfluss auf die Trockenmasse hatte, mit höheren Werten im hellen Fleisch. Bezüglich des Einflusses der Salzkonzentration, hatten Würste mit höheren NPS-Konzentrationen niedrigere a_w-Werte und höhere Aschekonzentrationen im Vergleich zu den Produkten, mit niedrigem NPS-Gehalt. Zusammenfassend, die Verarbeitung von farbunterschiedlichem Schweinefleisch beeinflusst nicht die Qualität daraus hergestellter Rohwürste und daher ist eine Sortierung des Schweinefleisches vor der Rohwurstherstellung nicht nötig.

Schlüsselwörter: Fleischqualität, TBARS, aerobe Gesamtkeimzahl, Trockensubstanz

Introduction

Raw fermented sausages consist of meat and fat that is preserved by curing, microbiological fermentation and drying. Beside ingredients such as (curing) salt, sugar, starter cultures or the smoking and ripening conditions (Kijowski and Niewiarowicz, 1978; Ruusunen et al., 2003), the "quality" of the meat and fat is also an important factor. After slaughter of pigs specific alterations within the muscle fibres like lactatacidosis or shrinkage and denaturation of the myofibrillar proteins have an impact on parameters (e.g., pH, electrical conductivity, lightness, redness) that are analysed to determine the meat quality (Scheffler and Gerrard, 2007). As lightness of the meat could be determined non-invasive and as it is correlated with pH, drip loss or electrical conductivity of the meat (Huff -Lonergan et al. 2002; Fraqueza et al. 2008), colour is a good parameter to analyse the meat quality. It has been shown that microbiological and chemical spoilage parameters are also related to different meat quality parameters, meaning, for example, that meat with high $pH_{24\,h\,pm.}$ and low L* values is more sensitive to microbiological and chemical alterations during storage (Rey et al., 1976; Ryu and Kim, 2005; Barbut, 2009; Sheard et al., 2012).

As pork shows extreme colour variations either with high lightness (L*) and drip loss and low pH values, named pale, soft and exudative (PSE) meat, or with reversed characteristics, named dark, firm and dry (DFD) pork (Scheffler and Gerrard, 2007), it could be suggested that differences of colour and the accompanied impact on the microbiological and chemical characteristics of the meat have an influence on the "quality" of raw fermented sausages produced from this different coloured meat.

As only studies have been published considering the impact of PSE, DFD and normal pork on processing characteristics (Townsend et al., 1980; Honkavaara 1988), the present study aimed to evaluate the impact of colour differences of pork on the quality of raw sausages produced from this meat, thereby considering normal colour variation, excluding PSE or DFD meat, which is unfit for human consumption.

Material and Methods

Animals and sample collections

The experiments were conducted following the German and European animal welfare regulations for animal transport and slaughter.

Within one year 48 pigs (24 barrows, 24 gilts) of a commercial crossbreed (Piétrain x German Landrace, MHS homozygote negative) were slaughtered in the experimental abattoir on eight slaughter dates in the Department of Animal Sciences in Goettingen, Germany. The pigs were stunned (250 V, 1.3 A, 6 s) and bleeding was initiated by cutting the *A. carotis* within 20 s. Then the carcasses were scalded at 62 °C for 3 min and eviscerated within 30 min. Finally, 35 min after stunning carcasses were transferred to a chilling room and stored at 7 °C (Werner et al., 2010). The pH 45 min. post mortem (pH_{45}) values were determined in the longissimus muscle (LM) between the 13./14. thoracic vertebrae (Th). Approximately 5 h after slaughter, following the veterinary inspection, the right LM was excised between 7th Th and 5th lumbar vertebra from the chilled carcass, individually packed and stored in a chilling room. Twenty four hours post mortem (24 h p.m.) the pH, electrical conductivity and colour values of the LM were analysed at 13th and 14th Th. For drip and grill loss, as well as shear force analysis, a meat piece (2.5 cm thick) was removed at the pH determination location. The rest of the LM muscle was diced (2 x 2 cm^2), packed in plastic bags, vacuumed and stored at 20°C up to six months until sausage production. The least square mean (LSM) and standard deviation (SD) results of all muscle L*24 h values were considered to sort the meat into dark (LSM – 1 SD, N = 15), mean (LSM, N = 15) and pale colour groups (LSM + 1 SD, N = 15). On four different days sausages were produced, as described below. In each experiment, pieces of three or four muscles per colour group, with a total weight of 5 to 7 kg, were removed from the freezer shortly before sausage production. We assured, that the average L* values of the three or four pale, mean and dark pieces, used for sausage production, were comparable between the four production days.

Production of raw fermented sausages

Four independent replicates of the sausage productions were made. In the meat technological unit of the Institute of Food Quality and Food Safety, a raw sausage batter of each colour group, consisting of approximately 69 % pork, 29.5 % pork backfat, 0.15 % glucose, 0.1 % dextrose and 0.05 % starter culture mixture (Bactoflavour BFL-F05 and SafePro B-LC-20, Chr. Hansen GmbH, Pohlheim, Germany), was produced. The main component of the starter culture B-LC-20 is *Pediococcus acidilactici,* whereas BFL-F05 contains *Lactobacillus sakei* and *Staphylococcus carnosus.* Initially, the selected amount of dark, mean and pale frozen meat and frozen fat were thawed for 15 min. Then the components were minced with a meat grinder (WD 114®, Seydelmann GmbH, Stuttgart, Germany) equipped with a 10 mm cutting plate. The minced meat of each colour group and the frozen fat were separated into two equal amounts of batches (batch I or batch II) and individually transferred to a cutter (SL-11®, ADE, Hamburg, Germany). After adding glucose, dextrose and the starter culture mixture, 1.5 % curing salt (CS: 99.5 % NaCl, 0.5 % $NaNO_2$) was added to batch I and 2.5 % CS to batch II, resulting in initial sodium nitrite concentrations of 75 (batch I) and 125 mg/kg (batch II) $NaNO_2$. The six batches (dark/ 1.5 % CS, dark/ 2.5 % CS, mean/ 1.5 % CS, mean/ 2.5 % CS, pale/ 1.5 % CS, pale/ 2.5 % CS) were afterwards separately homogenised in the cutter for 2 min. The six batches were filled into artificial casings (Naturin R2 (50 mm diameter), Naturin-Viscofan GmbH, Weinheim, Germany). On the production days all sausages (N = 11 to 19 per group) were weighted (250–300 g) and then ripened in a climate chamber until day 28 (relative humidity decrease from 96 % to 84 %, temperature from 22 °C to 15 °C, ventilation between 70 % (1960 U/min) and 50 % (1400 U/min). On days 3, 6 and 11 the sausages were smoked for 10 min at 18 to 22 °C. On days 1, 7, 14, 21 and 28 at least two sausages per group were weighted and homogenised (Grindomix GM 120®, Retsch GmbH, Haan, Germany). The homogenates were either directly used for a_w and pH value determination or frozen in plastic bags (–20 °C) for analysis of thiobarbituric acid reactive substances (TBARS) and raw nutrients (only day 14 samples). Prior to the homogenisation, samples (10 g) were removed for analysis of total aerobic plate count (TPC). Afterwards the colour was determined on the cutting surface of the sausage.

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Analytical methods

The CIE-L* (lightness), a* (redness), b* (yellowness) values of the meat and sausages were evaluated by using a chroma meter (Minolta CR 400®, Konica-Minolta GmbH, Langenhagen, Germany) on the cutting surface of the muscle and the cutting area of the sausage. The surface of the muscle and sausage were exposed to air at room temperature (21 °C ± 2 °C) for 30 min before determining the colour. Each value was a mean of six (meat) or four (sausage) measurements.

The pH values of the meat and sausage were measured by using a portable pH meter (Portamess®, Knick GmbH, Berlin, Germany) combined with a glass electrode (InLab 427®, Mettler-Toledo, Urdorf, Switzerland). Before measuring the pH meter was adjusted to the mean temperature of the meat samples (ca. 7 °C) or sausage homogenates (ca. 21 °C). For the pH determination the electrode was inserted in the center of the muscle and in the homogenates of the sausages.

The electrical conductivity (EC in mS/cm) of the meat was measured with an EC meter equipped with two parallel stainless steel electrodes (LF-Star®, Matthäus GmbH, Poettmes, Germany) until the EC values were stable. Before the measurement the EC meter was calibrated with a specific calibration block (10 mS/cm; Matthäus GmbH, Poettmes, Germany). For the EC determination the electrode was inserted in the center of the muscle. Each value was a mean of at least two measurements.

The muscle was weighted 24 h and 72 h after slaughter and the drip loss was calculated as the loss of weight and expressed in per cent. Between the measurements the muscle was stored in an individual plastic container at 4 °C.

After the drip loss analysis the muscle was used for grill loss determination according to Popp et al. (2013). The grill loss was calculated as the loss of weight during the heating process and expressed in per cent.

The meat samples prepared for the determination of the grill loss were subsequently used for the Warner-Bratzler shear force (WBSF) analysis according to Popp et al. (2013). At least 5 cores with a diameter of 1.27 cm were removed from the sample at different positions parallel to fiber orientation. Shear force determinations were conducted on an Instron universal testing machine (Model 4301, Instron, High Wycombe, United Kingdom) equipped with a WBSF head vertical to the fiber direction. The shear velocity was 200 mm/min. Each value (in N) was expressed as a mean of at least five measurements.

The a_w values of the sausage homogenates were determined by using the a_w-Cryometer AWK 10® (Nagy Instruments GmbH, Gaeufelden, Germany).

The concentrations of TBARS in the sausages were determined photometrically according to Popp et al. (2013). One gram of the sausage homogenate was minced in 10 ml trichloroacetic acid (20 %) for 2 min. After the addition of 0.5 ml butylated hydroxytoluene (0.19 M) and centrifugation for 6 min at 3000 x g (Hermle Z383 K, Hermle GmbH, Wehingen, Germany) the solution was filtered through filter paper (MN 613, Macherey-Nagel GmbH). To 0.7 ml of the filtrate the same volume of thiobarbituric acid (0.02 M) was added and heated for 30 min at 100 °C (LAT GmbH). After cooling the TBARS concentration was determined at 532 nm (Helios ß, Unicam Chromatography GmbH, Kassel, Germany). All experiments were performed in triplicates and results were expressed as µg malondialdehyde (MDA)/g sample.

The concentrations of protein, fat, ash and the dry matter were determined in the sausage samples according to the AOAC (1990). The protein concentration was calculated by analysis of the nitrogen concentration of the material (ca. 1 g) using the Kjeldahl method (Vapodest 50s®, Gerhardt Laboratory Systems GmbH, Königswinter, Germany) and multiplying the result by 6.25. Fat was determined after acid hydrolysis of the material (5–10 g) and extraction in a Soxleth equipment (LAT GmbH) by calculating the weight before and after this procedure. The ash concentration was analysed from the weight difference before and after combustion (600 °C, 4 h) of the material (3–5 g) in a muffle furnace (Carbolite®, LAT GmbH, Garbsen, Germany). The dry matter concentration was calculated from the weight before and after drying the muscle sample (3–5 g) in a drying oven (Binder GmbH, Tuttlingen, Germany) at 105 °C for 4 h. All analyses were performed in triplicates.

TPC values of the sausage samples (10 g) were determined using the pour plate technique on plate count agar (Oxoid GmbH, Wesel, Germany). The plates were incubated for 72 h at 30 ± 1 °C (ISO 2293:1976). Counts were expressed as $\log_{10}$ colony forming units (cfu) per gram.

Statistical analysis

The data were analysed with the software Statistica 10.0 (StatSoft, Hamburg, Germany) considering the independent variables colour group (CG) and curing salt concentration (CC) and their interaction (CG x CC). After analysis on normality with the Shapiro-Wilks-test normally distributed data were analysed using ANOVA and the TUKEY-post-hoc-test and non-normally distributed results with the Mann-Whitney-U-Test. A probability error of 0.05 was considered.

Differences between days 1, 7, 14, 21 and 28 were calculated with the t-test for dependent measures considering $P<0.05$.

Results and Discussion

Slaughter characteristics of the pigs

The slaughter characteristics slaughter weight, carcass yield and lean meat percentage of the pigs (N = 48) were 89.2 ± 3.9 kg, 80.3 ± 2.5 % and 62.4 ± 5.3 %, respectively. The slaughter characteristics of the animals, sorted into the dark, mean and pale colour group, did not differ significantly ($P>0.05$) (data not shown).

Quality characteristics of pork

The meat quality characteristics depending on colour are presented in Table 1. Pale, dark and mean coloured meat differed significantly ($P<0.05$) with regard to the $L^*_{24\,h}$. Pale pork had the significantly ($P<0.05$) highest and dark meat the lowest L* values. Dark meat also had significantly ($P<0.05$) lower b* values and higher pH_{45} and shear force values compared to mean and pale coloured meat. The EC results were significantly higher ($P<0.05$) in pale meat in comparison to mean coloured and dark meat. However, no significant influences were observed regarding the a* and drip loss (Table 1) as well as the $pH_{24\,h\ p.m.}$ and grill loss values (data not shown).

A general comparison of meat quality data between different studies is difficult due to differing factors like animal genetic, husbandry and slaughter as well as sample

TABLE 1: *Least square means (LSM) and standard deviations (SD) of different quality parameters of the pork depending on the colour.*

Item	Dark (n = 15)[1]		Mean (n = 15)[1]		Pale (n = 15)[1]	
	LSM	SD	LSM	SD	LSM	SD
L* 24 h p.m.[2]	48.03c	1.69	52.04b	0.75	56.26a	1.77
a* 24 h p.m.[2]	9.19	1.45	8.58	1.39	8.24	1.35
b* 24 h p.m.[2]	2.76b	3.14	7.03a	2.06	7.69a	1.26
pH 45 min p.m.	6.35a	0.26	6.08b	0.15	5.94b	0.26
EC[3] 24 h p.m.	5.33b	3.31	5.17b	2.37	8.85a	3.50
Drip loss [%][4]	3.92	1.42	5.02	2.14	5.25	2.64
Max. shear force [kg][5]	60.85a	12.31	47.74b	15.18	45.47b	8.19

[1]Animals/muscles used for sausage production; [2]Lightness (L*), redness (a*) and yellowness (b*) values of pork 24 h after slaughter (24 h p.m.) on the bone surface; [3]EC = electrical conductivity in mS/cm; [4]Drip loss 24 h and 72 h after slaughter (p.m.); [5]Maximal shear force values using the grill loss samples; [ab]LSM with different letters in the line differ significantly (P<0.05).

preparation, instruments and measurement conditions (Bianchi and Fletcher, 2002).

However, the significant relations between L* on the one hand and the b*, EC and pH values on the other hand as well as the not significant relation between L* and a* and $pH_{24\ h\ p.m.}$ results mainly agree with studies of Fernandez et al. (2002), Huff-Lonergan et al. (2002), Hammelman et al. (2003), Ryu and Kim (2005) or Schubert-Schoppmeyer et al. (2008). Meat colour, which is related to the light absorption, scattering and reflectance of the tissue, is not only influenced by the concentration and redox state of myoglobin or the muscle structure, but also in particular by the rate and extent of pH decrease (Scheffler and Gerrard, 2007). After slaughter of the pigs accumulation of lactate reduces pH values accompanied with denaturation and shrinkage of the myofibrillar proteins and release of water and electrolytes. These effects are clearer, if the rate of pH reduction increases. Therefore it could be suggested that the higher reflectance of pale meat is due to increasing myofibrillar shrinkage/ denaturation and liquid/electrolyte accumulation in comparison to dark meat. This explains why increasing lightness is accompanied with a higher rate of pH decline and increasing conductivity (Sielaff and Hoeft, 1979; Scheffler and Gerrard, 2007).

The not significant (P>0.05) relation between lightness and drip loss was also presented by Zelechowska et al. (2012). In contrast to this, other studies showed higher drip loss values in pale meat and vice versa (Fernandez et al., 2002; Huff-Lonergan et al., 2002; Ryu and Kim, 2005, Werner et al., 2010) or presented differing drip loss values in pork with comparable L* values (Hammelmann et al., 2003). The contradictory publications are difficult to explain, but we suggest that the generally low drip loss differences between the pale, mean and dark pork in the present study may be related to the sorting and the exclusion of PSE or DFD meat, since many studies also analysed PSE or DFD pork.

With regard to the shear force values, the studies of Ryu and Kim (2005) and Copenhafer et al. (2006) support our results showing that meat with low L* values is more tough due to higher shear force values.

Quality characteristics of the pig sausages

We considered in the following part of the discussion due to lack of comparable investigations also studies that used PSE pork for sausage production to show that colour effects could be found, although normal colour variation might not have an effect on the sausage quality parameters.

The weight of the sausages decreased significantly (P<0.05) from day 1 (96.7 %) to day 28 (59.2 %) of storage, but there was no significant (P>0.05) difference between the sausages made of dark, mean and pale pork (data not shown). A weight reduction was also stated by Townsend et al. (1980) or by Muguerza et al. (2002) and is due to the drying of the raw fermented sausages. It is interesting to note, that the use of PSE meat with extremely high L* values resulted in higher weight losses in comparison to sausages produced with normal meat (Townsend et al.; 1980; Honkavaara, 1988).

Water activity (a_w), an indicator for the liquid content and storage dependent liquid loss, decreased significantly (P<0.05) between day 1 (0.97) and day 28 (0.87) of ripening (data not shown). Sausages with 2.5 % CS showed significantly (P<0.05) lower a_w values compared to products with 1.5 % CS on all investigation days. An impact of the colour on the a_w values could not be found on any day (Table 2). The presented effect of salt concentration on a_w values was also shown by Olesen et al. (2004) and Stollewerk et al.

TABLE 2: *Least square means (LSM, upper value) and standard deviations (SD, lower value) of the lightness (L*) and a_w values of pork sausages, depending on colour group (CG) and curing salt concentration (CC).*

Item	Dark (n = 4)[1]		Mean (n = 4)[1]		Pale (n = 4)[1]		CG	CC	CG x CC
	1.5 % CS[2]	2.5 % CS[2]	1.5 % CS	2.5 % CS	1.5 % CS	2.5 % CS			
L* day 1	58.86 2.63	58.05 2.60	59.15 3.70	57.39 2.32	58.76 3.97	58.33 2.73	0.98	0.43	0.90
L* day 7	65.27 1.15	64.35 1.59	65.36 1.76	64.41 1.43	66.81 1.25	65.20 1.18	0.19	0.06	0.86
L* day 14	61.59 1.00	60.33 1.99	61.65 1.38	60.55 1.44	62.99 1.02	61.93 1.58	0.10	0.07	0.98
L* day 21	59.18 2.16	58.47 2.52	59.35 1.88	58.28 1.82	60.38 1.65	59.11 3.03	0.64	0.28	0.97
L* day 28	56.70 1.10	56.45 1.69	56.68 0.92	56.63 1.53	57.67 1.10	58.02 1.70	0.15	0.97	0.91
a_w day 1	0.97 0.001	0.96 0.006	0.97 0.001	0.97 0.001	0.97 0.002	0.96 0.003	0.32	<0.05	0.56
a_w day 7	0.95 0.003	0.94 0.003	0.95 0.004	0.95 0.005	0.95 0.005	0.94 0.002	0.39	<0.05	0.71
a_w day 14	0.93 0.003	0.92 0.007	0.93 0.003	0.92 0.005	0.93 0.006	0.92 0.007	0.71	<0.05	0.69
a_w day 21	0.91 0.006	0.90 0.007	0.92 0.008	0.90 0.003	0.91 0.006	0.90 0.020	0.87	<0.05	0.55
a_w day 28	0.87 0.015	0.86 0.019	0.89 0.016	0.86 0.023	0.88 0.016	0.87 0.023	0.71	<0.05	0.89

[1]Sausages were produced with dark (L* = 48.5), mean (L* = 50.9) and pale coloured pork (L* = 53.7); [2]CS = Curing salt (0.5 % sodium nitrite ($NaNO_2$), 99.5 % sodium chloride (NaCl)), 1.5 % CS = 15 g NaCl or 75 mg $NaNO_2$ per kg sausage, 2.5 % CS = 25 g NaCl or 125 mg $NaNO_2$ per kg sausage; effects of colour group (CG), curing salt concentration (CC) or interaction (CG x CC) are significant if P<0.05.

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(2012). Higher salt contents, for example of sodium chloride, increase the polarity and the water holding capacity of proteins (Ruusunen and Puolanne, 2005). This reduces the amount of free water and the related water activity (a_w value).

The pH values decreased significantly (P<0.05) in all sausages between day 1 (5.21) and 7 (4.87) followed by a significant increase (P<0.05) up to day 28 (5.13, data not shown). The 1.5 % CS sausages showed lower pH values only on day 1 compared to the high CS products. An impact of the colour on the pH values could not be found on any day (data not shown). An initial decrease of pH values followed by an increase was also shown in other studies (Marco et al., 2006; Fernández-López et al., 2008; Ercoşkun and Özkal, 2011). The decrease of pH is related to the increasing lactate production of the starter culture bacteria (Pérez-Alvarez et al., 1999). The following pH increase might be due to the accumulation of non-protein nitrogen and amino acid catabolism products from proteolytic processes during dry sausage ripening (Hughes et al., 2002; Fernández-López et al., 2008; Kaban and Kaya, 2009). However, Salgado et al. (2005) stated that the pH increase during progressed ripening appears to be more related to the decrease in lactic acid content than to the formation of nitrogen compounds. Other authors reported that both processes are related to pH increase during ripening (Bruna et al., 2001; Lücke, 2008). The influence of the higher salt concentration on pH values at day 1 was also shown by Olesen et al. (2004) and Stollewerk et al. (2012) and seems to be due to the inhibition of growth and/or metabolic activity of the starter culture bacteria by the high salt contents (Olesen et al., 2004). To make more accurate statements, why the pH value rose during ripening and to explain the influence of the higher salt concentration, further analysis would be necessary.

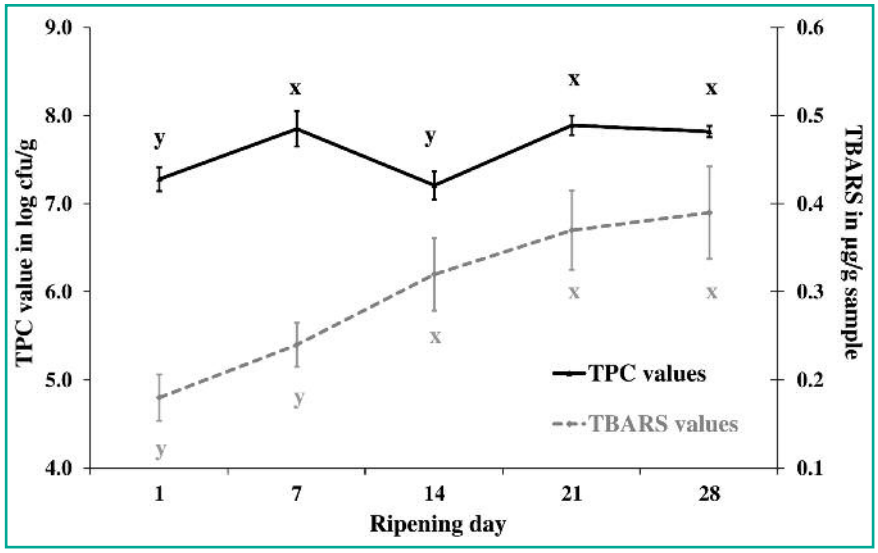

FIGURE 2: *Least square means and standard errors of the means of the total plate count (TPC) and thiobarbituric acid reactive substances (TBARS) results of all pork sausages depending on the ripening day. [vwxyz]LSM with different letters between the ripening days differ significantly (P<0.05).*

L* and a* analysis is a good method to follow quality changes of cured meat products. The L* values (Figure 1) of all sausages increased significantly (P<0.05) between day 1 and 7 followed by a decrease (P<0.05) up to day 28. The L* results were comparable at days 1 and day 21. The a* values increased continuously (P<0.05) during the ripening period (Figure 1). The colour group and curing salt concentrations had no effect on the L*(Table 2) and a* values of the pork sausages (data not shown). The time-dependent alterations of L* and a* results during storage of the raw fermented sausages were also shown by Acton and Dick (1977), Townsend et al. (1980) or Muguerza et al. (2002). The decrease of L* and increase of a* values might be caused by the formation of nitroso-myoglobin, which is related to the characteristic red colour of dry fermented sausages (Wirth, 1986). Klettner and Troeger (2000) and Sammet (2004) also found that the salt, especially the nitrite concentration, had no effect on the colour results of raw fermented sausages. The nitroso-myoglobin formation in the sausages seems to eliminate the initially high L* differences of pork before sausage production.

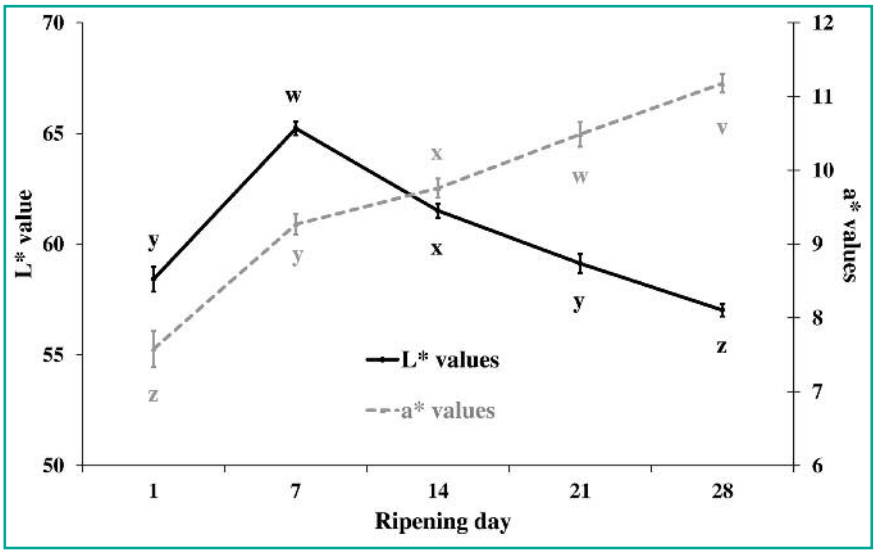

FIGURE 1: *Least square means and standard errors of the means of the lightness (L*) and redness (a*) results of all pork sausages depending on the ripening day. [vwxyz]LSM with different letters between the ripening days differ significantly (P<0.05).*

The interaction of different factors (hurdles), like starter cultures, pH or a_w, determine microbial stability and safety of fermented sausages (Työppönen et al. 2003). In the present study TPC values of all pork sausages increased (P<0.05) from day 1 to 7 followed by a significant decrease (P<0.05) from day 7 to day 14. Until day 21 the TPC values increased again (P<0.05) remaining on comparable levels until day 28 (Figure 2). Table 3 shows that on day 28 sausages with 2.5 % CS had lower (P<0.05) TPC values compared to low CS products. The colour group did not influence TPC results on any storage day. The microbial safety of dry fermented sausages depends on the synergistic effects pH and water activity

values as well as sodium chloride (NaCl) and curing salt contents ($NaNO_3$). Consequently the microflora changes during ripening (Heir et al., 2013). The initial increase might be a result of the predominant selection growth of the starter culture, which caused the pH decrease and inhibits the growth of spoilage and pathogenic bacteria (Leroy and de Vuyst, 1999). The significant decrease between day 7 and day 14 seems to be related to the drying process and a_w reduction during ripening. The increasing pH from day 7 to 28 might be advantageous for other bacteria than starter cultures. It could be suggested that the reduced temperature from 22 °C to 15 °C at the end of ripening also influences preferred growth of psychrophilic bacteria. However, further investigations are necessary to clarify these suggestions. The inhibitory effects of higher CS content on the TPC at day 28 might be related to the salt and probably nitrite effect of the CS (Olesen et al., 2004). Salt, especially nitrite, has an inhibitory effect on bacterial growth. There is a relation between the inhibitory effect of nitrite with its initial concentrations and the reduction in pH (González-Fernández et al., 2006).

The oxidative degradation of lipids during storage is indicated by the concentrations of TBARS. Oxidative changes should be considered to characterise quality and shelf life of dry fermented sausages (Kameník et al., 2012). The TBARS values increased ($P<0.05$) in all sausages between day 7 and 14. At days 1 and 7 and days 14, 21 and 28 TBARS values were comparable (Figure 2). No influence of the colour of pork or CS concentrations on the TBARS content could be shown on all storage days (data not shown). Bruna et al. (2001), Marco et al. (2006) or Olivares et al. (2011) also showed an increase of TBARS values during the early 28 days of ripening indicating higher oxidative lipolysis during storage (Olivares et al., 2011). With regard to the CS concentration, Coutinho de Oliveira et al. (2012) also presented no impact of this parameter on the TBARS results. Studies using PSE pork for sausage production showed either no impact (Kuo and Chu, 2003), or increasing effects of this pale pork on the TBARS concentrations (Townsend et al., 1980).

Pork sausages, produced with pale meat, had significantly ($P<0.05$) higher dry matter values than products made of mean and dark pork that showed comparable values (Table 3). Moreover the ash percentages were influenced by CS content with significantly ($P<0.05$) higher values in 2.5 % CS compared to 1.5 % CS products (Table 3). However, no significant effects of the colour group or CS content regarding protein and fat concentrations could be obtained (Table 3). The colour effect on the dry matter results agrees with the results from Honkavaara (1988) and Kuo and Chu (2003), who found higher dry matter percentages in sausages produced with PSE than normal meat. The effect of the CS concentration on the ash content could be explained by the fact that salt is the main component of the ash portion during nutrient analysis (Stollewerk et al., 2012).

In conclusion, despite the differences in the meat quality of the different coloured pork before processing there was only an impact of the colour group on the dry matter percentages of raw fermented sausages. No effect of the colour group could be obtained on the other physico-chemical and microbiological characteristics. The curing salt concentration showed effects on the a_w and the ash results. These data indicate that sorting of pork with regard to the lightness values prior to further processing to dry fermented sausages is not necessary.

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TABLE 3: *Least square means (LSM, upper value) and standard deviations (SD, lower value) of the total aerobic plate count (TPC) values and of the raw nutrient contents of the pork sausages, ripened up to day 14, depending on colour group (CG) and curing salt concentration (CC).*

Item	Dark (n = 4)[1]		Mean (n = 4)[1]		Pale (n = 4)[1]		CG	CC	CG x CC
	1.5 % CS[2]	2.5 % CS[2]	1.5 % CS	2.5 % CS	1.5 % CS	2.5 % CS			
TPC[3] day 1	7.38 0.88	7.12 0.55	7.27 0.74	7.03 0.63	7.77 0.05	7.12 0.53	0.71	0.21	0.81
TPC day 7	7.77 1.32	7.86 0.17	8.00 1.10	7.65 1.16	7.95 0.84	7.89 0.97	0.98	0.83	0.93
TPC day 14	7.08 0.65	7.18 1.01	7.29 0.72	7.22 0.57	7.24 0.88	7.28 0.88	0.95	0.96	0.98
TPC day 21	7.81 0.59	7.96 0.82	8.09 0.46	7.64 0.17	8.07 0.17	7.79 0.57	0.98	0.44	0.58
TPC day 28	7.90 0.19	7.63 0.22	7.92 0.12	7.73 0.43	8.13 0.22	7.64 0.14	0.72	<0.05	0.55
Dry matter in %	62.11 0.41	61.78 0.69	62.15 0.37	63.38 1.17	63.50 0.82	64.08 0.86	<0.05	0.20	0.25
Protein in %	27.29 0.20	26.75 0.10	26.61 0.36	26.58 1.92	27.64 0.40	28.13 2.53	0.26	0.97	0.77
Fat in %	31.09 0.52	30.22 0.71	31.39 0.40	32.50 3.08	31.25 1.18	30.26 0.85	0.27	0.72	0.39
Ash in %	3.35 0.38	4.52 0.22	3.97 0.94	4.26 0.25	3.87 0.77	4.97 0.24	0.34	<0.05	0.34

[1]Sausages produced with dark (L* = 48.5), mean (L* = 50.9) and pale coloured pork (L* = 53.7); [2]CS = Curing salt (0.5 % sodium nitrite ($NaNO_2$), 99.5 % sodium chloride (NaCl)), 1.5 % CS = 15 g NaCl or 75 mg $NaNO_2$ per kg sausage, 2.5 % CS = 25 g NaCl or 125 mg $NaNO_2$ per kg sausage; [3]TPC in log colony forming units (cfu) per g sample; effects of colour group (CG), curing salt concentration (CC) or interaction (CG x CC) are significant if $P<0.05$.

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