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Colour stability and vitamin E content of fresh and processed pork

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Abstract

The effect of increasing muscular content of vitamin E on colour intensity and stability of pork (both fresh and processed) was studied by feeding manipulation. Fresh pork was represented by loin chops and processed pork was represented by a dry cured fermented sausage (salame Milano) and raw cured ham (Parma ham). Dietary vitamin E was increased by feeding the animals with sunflower oil and α-tocopheryl acetate at 100 and 200 ppm. Chops were packed in oxygen-permeable film and in a protective atmosphere (80% O_2 and 20% CO_2) whereas salame Milano and Parma ham were packed under vacuum and in a protective atmosphere (85% N_2 and 15% CO_2). No differences in colour intensity or type were observed in relation to vitamin E content. Colour stability tests suggested, on the other hand, that higher vitamin E contents could be associated with slower myoglobin oxidation rates in fresh chops packed in a protective atmosphere. No effect of vitamin E on colour stability was observed in salame, probably because of the soft fat induced by sunflower oil. Significant differences among dietary groups were also not observed in Parma ham.

Keywords: Pork; Colour; Stability; Vitamin E

1. Introduction

Colour is an important sensory property of meat and, if defective, is a determining factor for consumer's choice at the moment of purchase. Indeed, the lack of what is generally considered as typical fresh meat colour entails a negative judgement on the acceptability, and a mistrust about the safety, of the meat on sale. The world-wide success encountered by meat packed in a protective atmosphere is a clear proof of the importance of preventing or delaying meat discoloration.

Many factors affect meat colour stability and the list includes, among others, pH, temperature, relative humidity, bacterial load, lipid oxidation, partial oxygen pressure, metmyoglobin reducing systems and the type of muscle. No single factor can be held totally responsible for meat discoloration but there is no doubt that the overall strategy for maximising acceptable fresh meat colour must involve delaying pigment oxidation and/or enhancing reduction of oxidised myoglobin (Faustman & Cassens, 1990).

A number of investigations have been carried out in recent years on the protective effect of vitamin E on myoglobin chemical stability and on the possibility of increasing colour shelf life by supplementing animal feeds with supranutritional amounts of vitamin E. A potential mechanism by which the vitamin could act against myoglobin oxidation has been recently suggested (Lynch, Faustman, Chan, Kerry & Buckley, 1998) and appears to be based on the enhancement of cytochrome b_5-mediated reduction of metmyoglobin. Although clear positive effects have been reported on vitamin E supplementation of beef colour stability (Faustman, Cassens, Schaefer, Buege, Williams & Scheller, 1989; Lanari, Schaefer, Liu & Cassens, 1996; Liu, Lanari & Schaefer, 1995; O'Grady, Monahan, Bailey & Dazzi, 1998), similar effects have not been consistently observed in pork and pork products (Honikel, Rosenbauer & Fischer, 1998; Houben & Gerris, 1998; Hoving-Bolink, Eikeleboom, van Diepen, Jongbloed & Houben, 1998; Jensen et al., 1997; Jensen, Flensted-Jensen, Skibsted & Bertelsen, 1998).

The effect of higher-than-usual vitamin E contents on colour features of both fresh and processed pork has

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not been clearly established in the case of the heavy pigs raised in Italy for traditional products such as fermented dry sausages or dry-cured hams. Such pigs are slaughtered at an age of at least 9 months, at a live weight of 150–160 kg, and belong to specific breeds or hybrid lines. An extensive project has been undertaken, inside an extensive European investigation, to evaluate the effect of dietary interventions on meat quality parameters, among which are colour type and stability, of the meat obtained from heavy pigs. This paper reports the results of an experiment on colour attributes of representative types of fresh (loin and green hams) and processed pork (salame Milano and Parma ham).

2. Materials and methods

A set of 84 pigs was raised according to a dietary plan previously described (Zanardi et al., 1998) consisting of 7 different diets (1 control and 6 experimental ones). The 7 diets differed in supplementation with sunflower oil (0 or 6%), copper (35 or 175 ppm) and vitamin E (0, 100, 200 ppm). The two copper levels in the feed did not have significant effects either on animal performances (Bosi, Cacciavillani, Casini, Macchioni, Mattuzzi & Loenibus, 1996) or on analytical parameters, such as muscular copper content, proximate composition, pH, vitamin E content, peroxide and TBARS values (Zanardi et al., 1998). For the sake of the present paper, therefore, dietary copper levels have been ignored and the 84 pigs of the trial have been considered as divided into 4 subgroups of which one, 12 animals, was the control (Diet 1), a second one, 24 animals, received a diet enriched with 6% sunflower oil (78% oleic acid) (Diet 2-basal +6% oil) and two more groups, 24 animals each, were raised on a diet with 6% sunflower oil supplemented with 100 ppm α-tocopheryl acetate in one case (Diet 3-basal +6% oil +100 ppm vitamin E) and 200 ppm α-tocopheryl acetate in the other (Diet 4-basal +6% oil+200 ppm vitamin E). All animals were kept on experimental diets for about 6 months.

On the day of slaughter carcasses were hot-cut into primal cuts according to normal industrial procedures. Among primal cuts, the loins (from 5th dorsal to last lumbar vertebrae included), shoulders, bellies and hams were identified with a numbered tag. Shoulders were hot-deboned and the bellies were skinned. All the cuts were stored in a cold room. The following day the hams were trimmed and subsequently processed according to standard Parma ham procedures. Shoulders, bellies and ham trimmings were used for the production of salame Milano as previously described (Novelli et al., 1998).

The loins were stored in a cold room for 5 days and then cut with a chopping machine into 1.5 cm thick chops. Analyses were carried out after 4 months maturing for salame and 15 months maturing for Parma ham. Matured hams were deboned according to the procedure laid down by the consortium of producers for hams due to be marketed sliced and packed under vacuum or protective atmosphere. Samples for laboratory analyses were taken from cross sections of each product avoiding heads and tails for about 5 cm.

The measurement of pH was carried out with a combined electrode filled with Xerolyt (Ingold 406M6DXKS7/25) on *Longissimus dorsi* muscle (last lumbar vertebrae) of the left loins and on *Semimembranosus* muscle of left and right hams at 1 and 24 h post-mortem. In processed products, pH was measured by homogenising 3 g of the sample with 30 ml distilled water.

The method by Buttriss and Diplock (1984) was used for the determination of vitamin E. The extraction procedure was adapted to the different fat contents of the chops as opposed to salame and ham samples.

In chops, 4 g samples were homogenised for 30 s at medium speed (Ultra Turrax) with 18 ml of an aqueous solution of KCl (1.15%); 2 ml of the homogenate were mixed with 2 ml of an ethanol solution of 0.5% pyrogallol, treated with 0.5 ml KOH (50%) and saponified in a water bath (75–80°C) for 30 min. The samples were cooled in running tap water, treated with 3 ml of hexane containing 3-tert-butyl-4-hydroxy-anisole (BHA) 0.005% (w/v) and centrifuged at 2000 rpm for 10 min. The hexane phase was collected and the extraction repeated with 3 ml of hexane. The two extracts were combined, dried in a nitrogen flow and dissolved in 500 µl of absolute ethanol.

In salame and hams, 2.5 g samples were homogenised, treated with 100 ml pyrogallol solution and mixed for 30 min with a magnetic stirrer. Saponification was performed by adding 20 ml KOH solution and stirring in the dark overnight. Hexane extraction was carried out twice with 40 ml hexane with a separating funnel, the hexane phase was washed with 30 ml distilled water, dehydrated with anhydrous Na_2SO_4 and incompletely dried in rotavapor. Drying was finished with a nitrogen flow and the samples dissolved in 5 ml ethanol.

HPLC analyses were carried out with a Spectra Physics apparatus (Spectra System 2000), UV-VIS detector (Spectra Focus) set at 290 nm, coupled with a fluorescent detector (Spectra System FL 2000) with excitation at 296 nm and emission at 330 nm and a reverse phase column (Supelcosil LC 18, 250 mm length, 4.6 mm internal diameter, 5 µm particle size). The mobile phase was 97% methanol and 3% distilled water with a flux of 1 ml/minute. Chromatographic peaks were managed with a specific software (PC 1000) and vitamin E identification was carried out by comparison with commercial standards. Recovery of internal standards ranged from 97 to 100%.

Colour was sensorially evaluated and objectively measured on *Longissimus dorsi* muscle (last lumbar vertebra), on *Semimembranosus* muscle of green hams and

on *Biceps femoris* of matured Parma hams (Chizzolini et al., 1996). Evaluations and measures were carried out at about 5 days post-mortem for *L. dorsi* and at 24 h post mortem for *Semimembranosus*. In green hams, the measures and evaluations were performed after trimming, a routine operation which takes place approximately 24 h post mortem, so to have a freshly cut surface, whereas a fresh cut was made on the loins. In both cases, enough time was left (between 30 and 60 min) for the oxygenation of myoglobin before measures and sensory evaluations. Measurements could not be performed on *Semimembranosus* muscle in matured hams (as done in green hams) because such a muscle is very dehydrated and excessively dark. The measurements were therefore carried out on *Biceps femoris*, a muscle similar to *Semimembranosus* in composition, fibre types, colour, possibility of colour problems, size and importance. In green hams *Semimembranosus* muscle has the advantage of a wide uncovered surface whereas *B. femoris* only has a small cut end.

Sensory evaluations of fresh meat colour were performed using a 5 points scale with the aid of colour standards from 1 (very pale) to 5 (very dark) under standard illumination obtained with fluorescent lamps (Philips TLD 18W/96: 6500 K, 97 chromatic index, 65 ratio lm/W) at a light intensity of about 1000 lux. Similarly, the colour of freshly cut salame and ham was sensorially evaluated according to a 5 points scale for colour intensity and colour homogeneity from 1 (very low) to 5 (very high).

Colour measures were carried out with a Minolta Chromameter Reflectance II CR200/08 using the white tile provided by the manufacturer as the internal standard and set to use Illuminant D65 (day light, 6500 K). Triplicate measures were taken on *Longissimus dorsi*, *Semimembranosus* and *Biceps femoris* muscles. The measures were expressed as L*a*b* (C.I.E. 1976) and the variables Psychometric Chroma (Chroma or Saturation Index) and Hue Angle (Hue) (MacLaren, 1980) were calculated.

Colour stability trials were carried out in the following way. Chops were packed in oxygen-permeable film (Weegal film KEA/216, water vapour trans. rate 534 g/m^2/24 h, O_2 trans. rate 14000 cm^3/m^2/24 h/atm, CO_2 trans. rate 80000 cm^3/m^2/24 h/atm) and under protective atmosphere with 80% O_2 and 20% CO_2. The bottom of the trays was made with a laminated film, Formivel/PVC HB-XX 470B (PVC/EVOH/PE 470 μm, O_2 trans. rate 2.5 cm^3/m^2/24 h/atm) and the top of the trays was made with Univel/M HB-X (PET-PVCD coated/adhesive/LDPE 114 μm, O_2 trans. rate 8–10 cm^3/m^2/24 h/atm). Salame Milano and ham slices were packed in protective atmosphere (85% nitrogen and 15% CO_2) and under vacuum (Univel/M HB-X PET-PVCD coated/adhesive/LDPE 114 m, O_2 trans. rate 8–10 cm^3/m^2/24 h/atm). The packs were placed in a refrigerated display cabinet (4°C) exposed to illumination with fluorescent lamps (Philips TLD 18W/96: 6500 K, 97 chromatic index, 65 ratio lm/W) at a light intensity of about 1000 lux.

The appearance of the brown colour of metmyoglobin was evaluated by a panel according to a 5 points scale for brown colour (1 = very light; 5 = very deep). The test lasted until average scores were higher than 3, considered to be the level above which the consumers would not buy the packets. Some of the results dealing with chops colour stability have been reported previously by Zanardi et al. (1998).

3. Results and discussion

Average pH values (Table 1) of *L. dorsi* and *Semimembranosus* muscles at 1 and 24 h post-mortem were in the normal range for all dietary groups. No difference was observed among the groups. The measures of pH were taken just to check for possible PSE and DFD cases, which could induce changes in colour perception independently from feed manipulation.

The determination of vitamin E (Table 2) in fresh *L. dorsi* samples show an increase of the vitamin in the muscles of all dietary groups compared with the control one. The biggest relative increase takes place with diet 2, i.e. the one with oil but without vitamin E supplementation. The diets supplemented with vitamin E did not differ between themselves, although their vitamin content was significantly higher than those observed in diet 1 and diet 2. The distribution among the dietary groups was confirmed when the results were expressed on a dry matter basis to compensate for the different moisture contents. Vitamin E content in matured salame Milano confirms the differences previously observed in chops among the four dietary groups and vitamin E distribution does not change if the results are expressed on a dry matter basis without NaCl to avoid the distorting effects of moisture and NaCl contents. Parma hams had a distribution similar to those observed for chops and salame. Notably, significant differences were observed between control, oil supplemented and oil + vitamin E-supplemented groups. The groups supplemented with 100 and 200 ppm had very similar vitamin E values. On a dry matter basis, without

Table 1
Mean ± standard deviation of pH measures of *L. dorsi* (LD) and *Semimembranosus* (SM) muscles

Diet	pH 1 h LD	pH 24 h LD	pH 1 h SM	pH 24 h SM
1	6.32 ± 0.16	5.62 ± 0.09	6.19 ± 0.33	5.63 ± 0.21
2	6.27 ± 0.21	5.56 ± 0.11	6.16 ± 0.24	5.56 ± 0.05
3	6.27 ± 0.26	5.58 ± 0.08	6.07 ± 0.25	5.60 ± 0.07
4	6.21 ± 0.34	5.63 ± 0.15	6.14 ± 0.28	5.65 ± 0.16

Table 2
Vitamin E content (mg/kg) of fresh *L. Dorsi*, salame Milano and Parma ham (dry matter without NaCl for salame and ham) (mean ± standard deviation)[a]

Diet	*L. dorsi*		Salame Milano		Parma ham	
	Fresh tissue	Dry matter	Fresh tissue	Dry matter	Fresh tissue	Dry matter[a]
1	1.44 ± 0.22c	5.18 ± 0.69c	3.91 ± 0.29d	6.45 ± 0.71c	2.34 ± 0.57c	5.98 ± 1.48c
2	3.41 ± 0.62b	12.31 ± 2.23b	7.76 ± 0.82c	13.57 ± 1.82b	5.47 ± 1.00b	14.17 ± 2.72b
3	4.31 ± 0.68a	15.48 ± 2.36a	9.73 ± 1.15b	16.73 ± 2.23ab	7.38 ± 0.99a	18.73 ± 1.98a
4	4.65 ± 0.81a	16.87 ± 2.81a	11.46 ± 0.97a	19.65 ± 2.06a	7.48 ± 1.41a	19.41 ± 3.55a

[a] Different letters in columns stand for significant differences, $P \leq 0.05$, Scheffè's test.

Table 3
Colour measures of fresh *L. dorsi* muscle (mean ± standard deviation)

Diet	L*	a*	b*	S.I.[a]	Hue
1	46.01 ± 2.78	6.66 ± 2.34	3.72 ± 2.01	7.67 ± 2.96	0.47 ± 0.13
2	47.06 ± 2.63	7.50 ± 2.56	4.59 ± 2.20	8.85 ± 3.24	0.52 ± 0.13
3	47.40 ± 2.86	7.20 ± 2.71	4.49 ± 2.39	8.56 ± 3.44	0.52 ± 0.17
4	46.73 ± 3.63	8.40 ± 3.98	4.82 ± 2.76	9.72 ± 4.77	0.50 ± 0.10

Table 4
Colour measures of fresh *Semimembranous* muscle (mean ± standard deviation)

Diet	L*	a*	b*	S.I.[a]	Hue
1	52.18 ± 3.48	9.89 ± 1.89	5.87 ± 1.60	11.55 ± 2.26	0.53 ± 0.10
2	52.40 ± 3.15	10.06 ± 2.12	5.83 ± 1.18	11.66 ± 2.29	0.53 ± 0.08
3	51.24 ± 2.65	10.15 ± 1.91	5.58 ± 1.22	11.60 ± 2.17	0.50 ± 0.06
4	50.06 ± 3.68	10.23 ± 2.13	5.29 ± 1.30	11.56 ± 2.30	0.48 ± 0.09

[a] S.I.: saturation index.

Table 5
Colour measures of Parma ham (mean ± standard deviation)

Diet	L*	a*	b*	S.I.[a]	Hue
1	39.13 ± 2.48	16.99 ± 2.60	6.56 ± 1.24	18.29 ± 2.29	0.37 ± 0.10
2	39.78 ± 3.13	17.00 ± 1.73	6.14 ± 1.44	18.14 ± 1.61	0.35 ± 0.09
3	41.58 ± 2.75	18.25 ± 1.91	5.91 ± 1.79	19.28 ± 1.76	0.31 ± 0.10
4	40.64 ± 3.47	18.00 ± 1.93	5.64 ± 1.25	18.91 ± 1.80	0.31 ± 0.08

[a] S.I.: saturation index.

NaCl, values ranged from about 6 ppm in the control group to 14 ppm in the oil-supplemented group and to 19 ppm in the vitamin E-supplemented ones. Such values were very similar to those of salame.

Colour measures of fresh chops, green hams and matured hams (Tables 3, 4 and 5) did not reveal significant differences among the groups. Variations in colour measures among samples have obviously been observed but, in fresh meat (*L. dorsi* and *Semimembranosus*), were related to pH values, in L* and hue, just as had been shown in other studies (Chizzolini et al., 1996), but not with diets. The biggest variations were observed in *L. dorsi* measurements which showed average values of the a* coordinate ranging from 6.66 ± 2.34 of group 1 to 8.40 ± 3.98 of group 4, whereas the saturation index ranged from 7.67 ± 2.96 of the first group to 9.72 ± 4.77 of the fourth one. In the same samples, L* values varied from a minimum of 46.01 ± 2.78 in group 1 to a maximum of 47.40 ± 2.86 in group 3 and Hue varied from 0.47 ± 0.13 in group 1 to 0.52 ± 0.17 in group 3. According to previous experiences (Chizzolini et al., 1996; Joo, Kauffman, Kim & Kim, 1995), human perception of pork colour is strictly linked with L* and Hue values, in their turn significantly affected by pH mainly through effects on water holding capacity, whereas a* and saturation index

appear to be less important. Variations in average values of L*a*b* colour measurements among the groups were smaller in *Semimembranosus* of green hams and in *B. femoris* of matured hams. Differences were more evident, although not significant, in hue values which varied from 0.53 ± 0.10 in group 1 to 0.48 ± 0.09 of group 4 in *Semimembranosus* and from 0.37 ± 0.10 in group 1 to 0.31 ± 0.08 in group 4 in *B. femoris*.

Sensory colour evaluation scores (Table 6) performed on the same muscles at the same time were in agreement with objective measures. Similarly, the sensory evaluation of salame Milano did not show differences linked with vitamin supplementation but colour intensity and colour homogeneity were lower in oil-supplemented groups. The results agree with the data on colour stability (see below). Limited variations were observed in colour intensity of Parma ham but they did not appear to be specifically linked with any individual diet. The results of sensory evaluations of the colour of Parma hams were in agreement with objective colour measurements.

Colour stability of fresh chops packed in oxygen-permeable film did not show significant differences among dietary groups, whereas differences have emerged in chops packed under protective atmosphere (Zanardi et al., 1998) (Fig. 1). As vitamin E content increased, the rate of brown metamyoglobin formation decreased. The group supplemented with 200 ppm vitamin E, in particular, was associated with a significantly lower rate of brown metmyoglobin formation.

Evaluations of colour stability of salame (Figs. 2 and 3), both for vacuum packaging and protective atmosphere,

Table 6
Sensory evaluation scores of the colour of fresh meat (*L. Dorsi*, *Semimembranosus* muscles), salame Milano and Parma ham (mean ± standard deviation)

Muscle	Diet 1	Diet 2	Diet 3	Diet 4
L. dorsi	2.92 ± 0.18	2.79 ± 0.29	2.83 ± 0.28	3.06 ± 0.34
Semimem	2.91 ± 0.31	2.85 ± 0.36	2.97 ± 0.36	2.99 ± 0.37
Salame				
Colour-intens.	3.36 ± 0.45	2.86 ± 0.28	2.73 ± 0.31	2.77 ± 0.12
Colour-homog.	2.96 ± 0.35	2.61 ± 0.15	2.55 ± 0.30	2.53 ± 0.39
Ham				
Colour-intens.	3.20 ± 0.20	3.04 ± 0.43	3.19 ± 0.40	3.23 ± 0.35
Colour-homog.	3.50 ± 0.20	3.63 ± 0.51	3.66 ± 0.39	3.76 ± 0.41

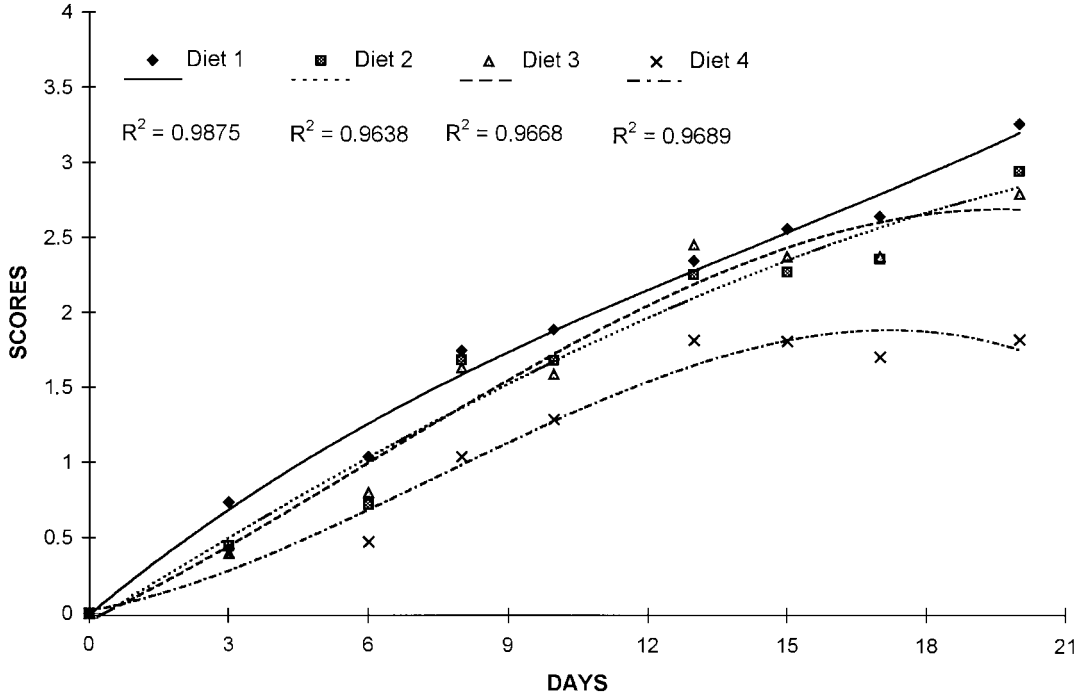

Fig. 1. Rate of brown colour appearance in fresh chops packed in protective atmosphere.

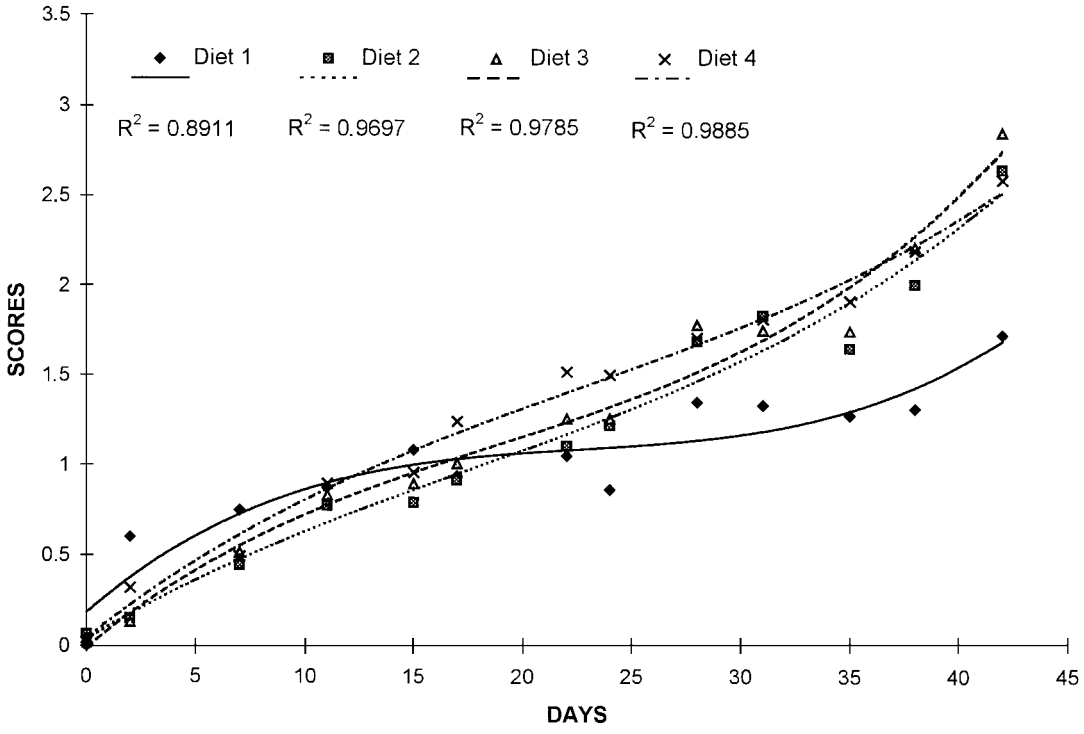

Fig. 2. Rate of brown colour appearance in salame Milano packed in protective atmosphere.

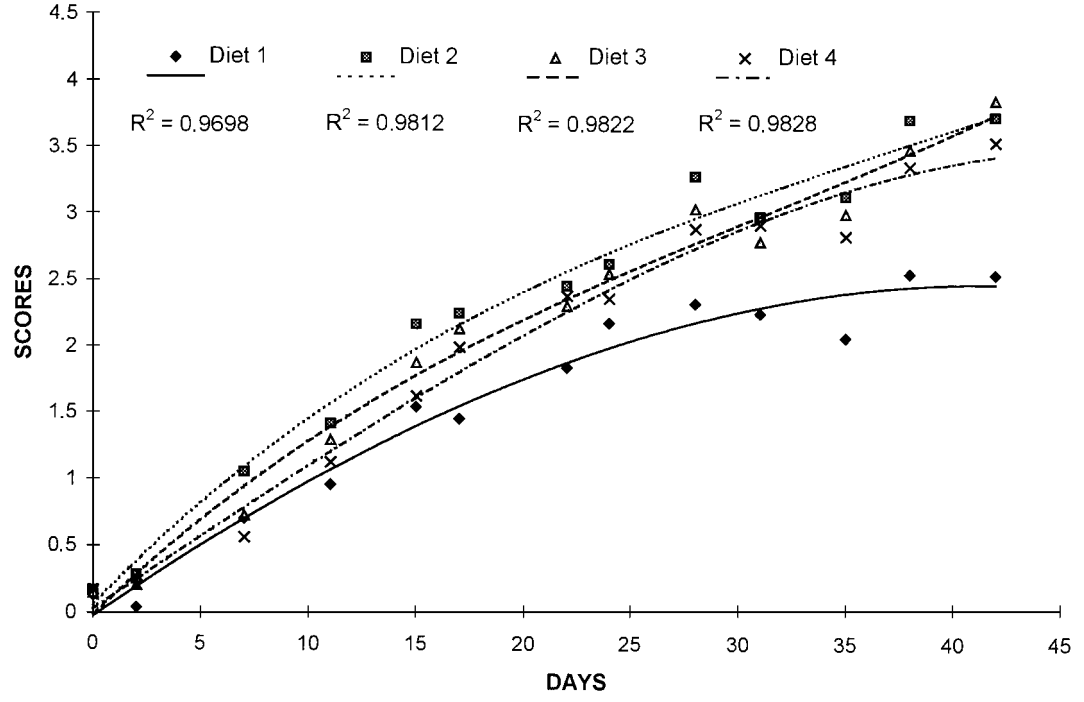

Fig. 3. Rate of brown colour appearance in salame Milano packed under vacuum.

show a quicker rate of oxidation for the diets with oil supplementation, compared with the control diet. No differences were observed on the basis of vitamin E supplementation. The lower colour stability of salame Milano produced with meat of the animals from oil-supplemented diets could be due to the detrimental effect of soft fat deriving from oil supplementation. The fat in such a case tended to smear during mincing and mixing and the matured salami presented frequent cracks on slicing. Sensory evaluation by a panel confirmed the lower colour

attributes (red intensity and homogeneity) of salame produced with oil-supplemented meat. Soft fat might have hampered nitrite diffusion and reaction with myoglobin.

Colour stability trials of ham slices packed under vacuum and in protective atmosphere and exposed to fluorescent light lasted for nearly three months (Figs. 4 and 5). In all cases, about 2 months were required before evaluation scores approached a value of 3. Vacuum packaging appeared to be slightly more protective than modified atmosphere as a score of 3 points was reached about 10 days later. Control samples showed a modest tendency towards quicker oxidation rates. The difference from the other samples, though, was very limited and not significant. The oil- and vitamin

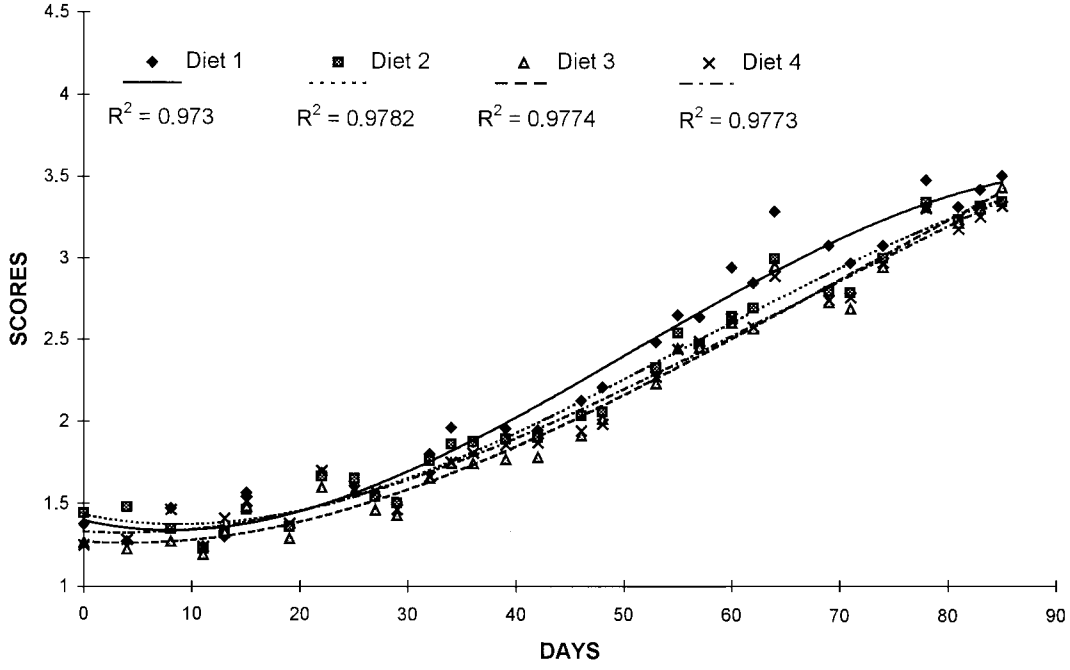

Fig. 4. Rate of brown colour appearance in Parma ham packed in protective atmosphere.

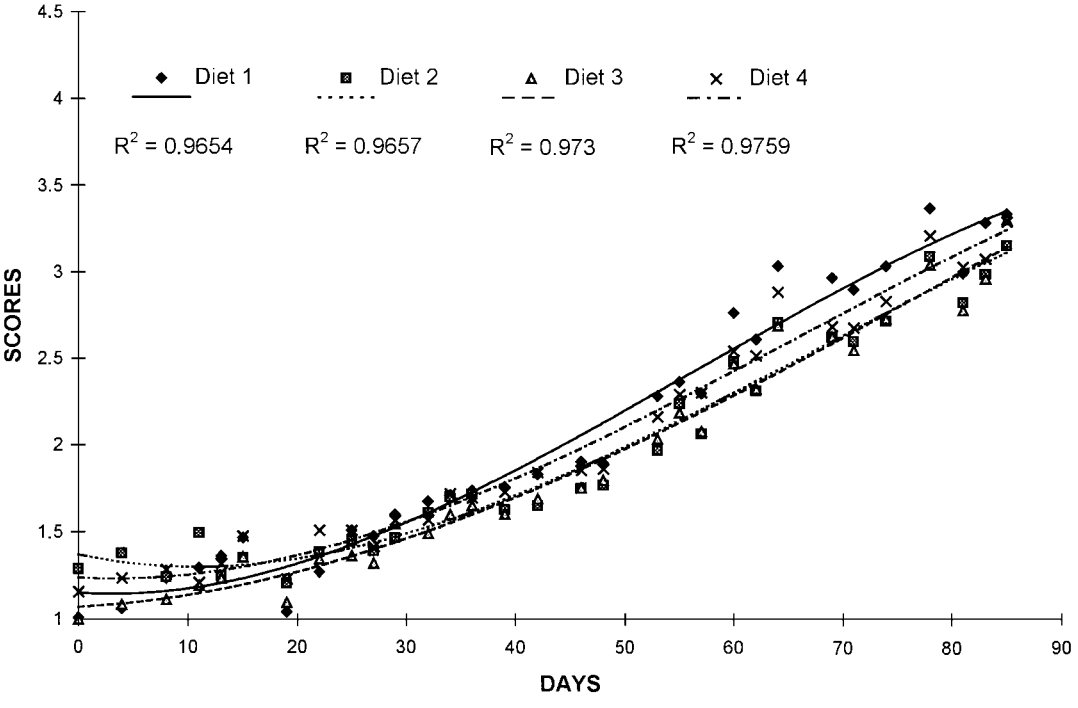

Fig. 5. Rate of brown colour appearance in Parma ham packed under vacuum.

E-supplemented samples showed equivalent changes with storage times. The results do not openly disagree with what had been observed with chops where vitamin E appeared to have some, although limited, effect. The myoglobin chemical status in ham is not yet known and the exact compound responsible for Parma ham colour in the absence of nitrate/nitrite has not been identified (Parolari, Chizzolini, Bellatti & Dazzi, 1983). There are reports suggesting that some Staphylococcus strains can produce red myoglobin derivatives from metmyoglobin in meat systems such as Parma ham (Morita, Sakata & Nagata, 1996; Sakata, Morita & Nagata, 1998). Such molecules have not been fully investigated and their oxidative stability and possible interactions with vitamin E are not known.

Although there appears to be a substantial consensus on the positive effect of increased vitamin E contents on colour stability of beef, the same does not apply to pork. Most of the reports deal with fresh meat, such as that by Asghar et al. (1991), who failed to observe significant improvements, following vitamin E supplementation, in L* a* b* values of *L. Dorsi*, stored for over 10 days. On the other hand, Monahan, Asghar, Gray, Buckley & Morrissey (1992) reported a better stability of a* values of chops thawed and stored for 8 days after 4 months frozen storage. Honikel et al. (1998) reported a tendency, although not always significant, towards higher a* values in chops of supplemented animals. Hoving-Bolink et al. (1998) did not report significant effects of supranutritional levels of vitamin E on colour stability of fresh *L. Dorsi* and *Psoas* major muscles and similar results had been reported by Jensen et al. (1997, 1998) on the same muscles and on chops packed in air and in protective atmosphere. A positive effect of high muscular levels of vitamin E has been reported by Corino, Paganini & Pastorelli (1997) in fresh pork, salame and hams. Houben and Gerris (1998), on the other hand, working with pasteurised ham reported that dietary supplementation of pigs with vitamin E did not appear to offer significant advantages over currently used feeding regimes. Moreover, as observed in the present investigation for Parma hams, vacuum packaging appeared to be more colour protective compared with protective atmosphere.

In conclusion, colour stability trials have suggested that higher vitamin E contents could be associated with slower myoglobin oxidation rates in fresh chops packed in protective atmosphere. Salame colour stability tests, on the other hand, probably could not show any effect of vitamin E due to the presence of oil in the diet. Significant differences among dietary groups were not observed in Parma ham but, in this case, slices were packed either under vacuum or in protective atmosphere (85% nitrogen and 15% CO_2), both systems being characterised by the absence of oxygen.

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