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Cochineal as a colorant in processed pork meat. Colour matching and oxidative stability

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In pasteurized, processed pork meat 40 ppm cochineal was found to provide a colour of acceptable similarity to the colour of a standard product with 5·4 ppm erythrosine, as measured by tristimulus colorimetry, and evaluated by a sensory panel. The colour stability of the product with cochineal added was significantly better than the colour stability of the standard product with erythrosine. For the product with cochineal added, photooxidation (combined action of light and oxygen) of the heat-denaturated cured meat pigment nitrosylmyochrome could quantitatively account for the measured colour fading. No evidence for a photosensitizing effect or prooxidative effect of cochineal on lipid oxidation was found during chill storage of the processed pork meat product, exposed to light or protected against light, as evaluated by a combination of sensory analysis of rancidity, and determination of secondary lipid oxidation products by measurement of thiobarbituric acid reactive substances and by fluorescence spectroscopy.

INTRODUCTION

Pasteurized, processed meat products require the use of certain processing aids such as polyphosphates, starch, nitrite and ascorbic acid in order to ensure products with a good water holding capacity and improved oxidative stability. For pork products such as luncheon meat and salami, artificial colouring agents are often included in addition to nitrite, in the recipes in order to provide a constant and uniform appearance to ensure consumer acceptability. In such products, erythrosine has been in common use as a red food colorant. The increased restrictions in the use of synthetic colorants in foods have, however, drawn attention to natural colouring agents, and the 'ancient' colorant cochineal is an interesting alternative to artificial red food colorants (Lloyd, 1980). Cochineal has already been used as a replacement for synthetic colorant in British style sausages and in the brightly coloured Danish salami.

Cochineal is obtained from the scale insect *Dactylopius coccus* Costa (Marmion, 1984), and commercial preparations contain 20–50% of the colouring component carminic acid (Marshall & Horobin, 1974), a β-C-glycosyl derivative of anthraquinone (Fiecchi *et al.*, 1981). The stability of cochineal in food systems is generally considered to be good (Coulson, 1980; Lloyd, 1980; Marmion, 1984; Side, 1987), and in a storage experiment with a salami product, the colour stability of cochineal was found to be even better than the mixture of colorant based on erythrosine, which normally has been used (Ohlen & Bertelsen, 1989). Carminic acid is, however, susceptible to oxidation, and in model experiments the oxidative degradation of carminic acid was found to be strongly dependent on solution pH, on the partial pressure of oxygen and on exposure to light (Jørgensen & Skibsted, 1991).

The oxidative degradation of colorants like carminic acid is of special concern since coupling of pigment oxidation with lipid oxidation in foods under certain circumstances is capable of initiating rancidity (Gutteridge & Quinlan, 1986). Furthermore, the possible role of food colorants as photosensitizers for lipid oxidation should also be considered and tested in the actual products prior to practical use. Baykut *et al.* (1983) found some indication of carminic acid acting as a photosensitizer in the presence of iron salts.

In the present study, we have chosen luncheon meat, for which the annual production in Denmark is approximately 50 000 t, as an important pasteurized cured meat product, and have performed a colour comparison study in order to facilitate the practical replacement of erythrosine with cochineal. This part of the study was followed by a storage experiment for luncheon meat with the 'matching' concentration of

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cochineal, erythrosine or no added colorant. The oxidative changes were determined by objective methods combined with sensory evaluation for the product packed in packaging materials with different oxygen transmission rates and stored in a chill cabinet exposed to light or protected against light.

MATERIALS AND METHODS

Product and packaging

Pork luncheon meat for the colour matching experiment produced at a pilot plant was made from 64% pork meat (fore-end and lard), 3·5% soya protein, 3·0% corn starch, 2·0% NaCl, 0·4% Chark Bouquet (SFK, Copenhagen), 500 ppm ascorbic acid, 129 ppm nitrite (as $NaNO_2$) and 28% water. Five different samples with 0, 20, 40 and 60 ppm cochineal (CC-5000-L-P, Chr. Hansens Lab., Copenhagen, containing 49·4–54·6% carminic acid), and 5·4 ppm erythrosine (SFK), respectively, were produced seperately.

The pork luncheon meat for the storage experiment was manufactured at a conventional meat processing plant, employing the same recipe as used for the colour matching. Two different food colouring agents were added: (1) cochineal at a level of 40 ppm, and (2) erythrosine at a level of 5·4 ppm. A third batch was produced as a control product without any food colour added.

Luncheon meat slices (2·5–3 mm thick, 12–15 g each) were packed under 99% vacuum with 8 slices in each pack. For one half of the packages a laminate foil (Amilon 1550 X, Otto Nielsen Inc., Lyngby) was used, composed of 15 μm biaxial oriented nylon and 50 μm polyethylene (LDPE, tie-layer, EVOH, tie-layer and LDPE) with an oxygen transmission rate (OTR) of 1·5 $cm^3\ m^{-2}\ (24\ h)^{-1}\ atm^{-1}$ (25°C, 75% relative humidity), a carbon dioxide transmission rate of 7·0 $cm^3\ m^{-2}\ (24\ h)^{-1}\ atm^{-1}$ (25°C, 75% relative humidity) and a water vapour transmission rate (WVTR) of 5·5 $g\ m^{-2}\ (24\ h)^{-1}$ (38°C, 90% relative humidity). For the other half, a polyethylene foil, 75 μm (Otto Nielsen Inc., Lyngby) was used, with an OTR of 3800 $cm^3\ m^{-2}\ (24\ h)^{-1}\ atm^{-1}$ (25°C, 75% relative humidity), a carbon dioxide transmission rate of 16 200 $cm^3\ m^{-2}\ (24\ h)^{-1}\ atm^{-1}$ (25°C, 75% relative humidity) and a WVTR of 5·5 $g\ m^{-2}\ (24\ h)^{-1}$ (38°C, 90% relative humidity).

Storage

Half of the packages (randomly selected from each of the six combinations of colouring and packaging material) were placed in the upper layer in a chill cabinet (gondola with forced air circulation). The other half was covered with dark plastic to protect the product against light. The surface temperature of the product was 5°C, which during daily defrosting rose approximately 2°C as measured by continuous registration (Grant data-logger, UK). The chill cabinet was illuminated by fluorescent tubes (Phillips TLD 18W/92). At the surface of the packages, the illumination was measured to an average of 550 lx (Mavolux digital, Gossen, Germany). During storage, samples (one package for each production/storage combination) were analysed at days 2, 9, 17, 23, 29 and 37, respectively.

Colour measurement

The surface colour was measured with a tristimulus colorimeter (Hunterlab D-25 with a D25 M sensory head), calibrated against a white tile (L = 90·7, a = −0·9 and b = −0·1) as previously described (Andersen *et al.* 1988). The tristimulus redness parameter (Hunter a: +/− represents red/green), previously found by multivariate analysis with backward elimination to provide the best correlation with the subjective colour score for cured meat, was combined with the tristimulus brightness parameter (Hunter L: +/− represents white/black) and the tristimulus yellowness parameter (Hunter b: +/− represents yellow/blue) to yield an expression for the total change in colour ΔE (cf. Results section).

Each value presented is an average of four measurements (measurements on two slices from the same package, two measurements per slice). No significant difference was found between measurements at the same slice nor measurements at different slices from the same package.

Sensory analysis

In the colour matching experiment, the luncheon meat was evaluated by a fifteen-membered trained sensory panel, and the panel was asked to identify the cochineal coloured product most similar, concerning colour, to the reference sample produced with erythrosine. The term 'to match' with respect to colour was defined to the panellists as being of near and acceptable similarity. In this respect the term 'to match' is not concerned with the situation of no noticeable difference. The panellists, all with normal colour vision, had been selected for the sensory panel among students of food science in a rigorous testing procedure. The panellists were permanent members of the expert sensory panel of the RVAU Centre for Food Research. The colour evaluation was conducted in a room with fluorescent light (Phillips 30w/82).

In the storage experiment, the degree of discoloration and the degree of off-flavours were evaluated by an eight-membered trained sensory panel, using a 0–15 cm linear scale, where the score 15 was used for no discoloration, and 0 was used to denote severe discoloration in the colour evaluation. Similarly, a score of 15 was used for strong off-flavours, and the score 0 was used for no off-flavours in the flavour evaluation. As panel guidelines, reference samples with no discoloration (score 15) and reference samples with no off-flavour (score 0), and with strong off-flavour (score 15, artificially provoked by UV-light illumination for 1 h) were presented for the panellists at each evaluation.

Determination of thiobarbituric acid reactive substances (TBARS)

The distillation method of Tarladgis *et al.* (1960) was used to measure the development of oxidative rancidity. In order to avoid interference between malondialdehyde and nitrite, the method was modified as described by Zipser & Watts (1962). TBA values (μmoles malondialdehyde per kg meat) were each expressed as means of two analyses.

Determination of fluorescent oxidation products

Fluorescent oxidation products were extracted from the meat with chloroform/methanol (2:1) according to the method of Fletcher *et al.* (1973), as modified by Kamarei & Karel (1984). The measurements of fluorescence were carried out with an SLM 48000S spectrofluorometer. Extracts were excited at 360 nm, and the intensity of the emission at 440 nm, was measured relatively to a quinine sulphate standard (1 ppm in 0·5 M H_2SO_4 equals 100).

Statistical analysis

The data for 'total colour change' (ΔE) were analysed by a two-way analysis of variance including the interaction between the two factors: treatment (12 levels) and time (6 levels). The data obtained from the sensory analysis (both evaluation of colour and evaluation of off-flavour) were analysed using a two factor model (storage condition, 4 levels, and time, 6 levels) with interaction and block (number of judges, normally 8) using the general linear model (GLM) option in the SAS computer program (SAS, 1987).

RESULTS

Colour matching

Luncheon meat with 40 ppm cochineal added obtained a colour, which was found to be most similar to the product with 5·4 ppm erythrosine added. As may be seen from Table 1, the same result was obtained both by the objective tristimulus colour measurement and by colour evaluation by the sensory panel. However, as noted by the panellists, cochineal conveyed a more bluish, but acceptable, colour to the product as compared to the erythrosine standard, an observation also confirmed by the tristimulus measurements (decreasing b value).

Oxidative stability during storage

The luncheon meat product with the nearest matching 40 ppm cochineal added was produced in a large scale batch, together with the standard product with 5·4 ppm erythrosine and a control batch without colorant other than nitrite. The oxidative stability of these products was followed during chill storage for 37 days.

Colour stability

High OTR of the packaging material and exposure of the product to light were identified as factors which each, and especially in combination, result in discoloration of the product surface, as shown in Fig. 1. The fading of colour of the luncheon meat product was followed by tristimulus colorimetry and expressed as total change in colour, ΔE:

$$\Delta E(t) = [(L(t) - L(t = 0))^2 + (a(t) - a(t = 0))^2 + (b(t) - b(t = 0))^2]^{1/2}$$

where t is time of measurement.

The products packed in the material with the high OTR and exposed to light had the most significant loss in surface colour (Fig. 1(A)), whereas the product protected against light showed less change in colour during storage (Fig. 1(B)). Correlation analysis showed good correlation between the visual evaluation of colour changes by the panellists and the objective colour measurement. Notably, for luncheon meat stored in packaging material with a high OTR and exposed to light, changes for products without colorants added and for products with erythrosine or cochineal added were very similar, indicating that the colour fading is a result of a common process in all three types of products. The major pigment in heated cured meat

Table 1. Colour of luncheon meat containing cochineal compared to a standard product with 5·4 ppm erythrosine

Colorant	Amount of colour	Tristimulus parameters				Evaluation by sensory analysis Number of times chosen[c]	
		L	a	b	$E_{erythrosine} - E_{cochineal}$[a]	Trial 1	Trial 2
Erythrosine	5·4 ppm	48·72	7·97	8·02	0		
Cochineal	0 ppm	49·69	4·63	10·20	4·28 ± 0·28	0	0
Cochineal	20 ppm	49·23	5·91	8·65	2·89 ± 0·59	4	2
Cochineal	40 ppm	47·68	7·44	7·26	1·63 ± 0·52[b]	11	13
Cochineal	60 ppm	45·42	8·41	6·13	3·93 ± 1·13	0	0

[a] $E_{erythrosine} - E_{cochineal} = [(L_x - L_y)^2 + (a_x - a_y)^2 + (b_x - b_y)^2]^{1/2}$. L_x, a_x and b_x are the Hunter tristimulus parameters for the standard (erythrosine). L_y, a_y and b_y are the Hunter tristimulus parameters for samples containing cochineal.
[b] $E_{erythrosine} - E_{cochineal}$ for this sample was significantly ($p < 0.05$) smaller than for the three other samples containing cochineal.
[c] Number of times the different cochineal samples were chosen as being the one most similar to the standard with respect to colour appearance.

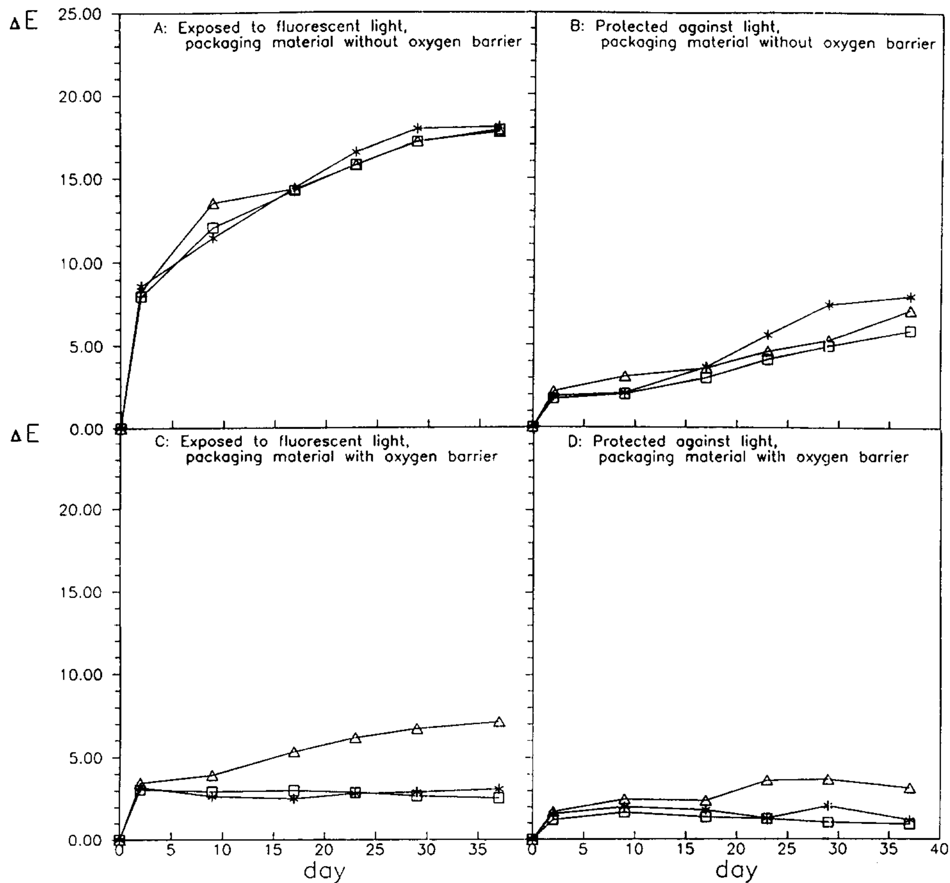

Fig. 1. Colour change (ΔE) during chill storage of luncheon meat. $\Delta E = [(L(t) - L(t = 0))^2 + (a(t) - a(t = 0))^2 + (b(t) - b(t = 0))]^{1/2}$. $L(t{=}0)$, $a(t{=}0)$ and $b(t{=}0)$ are the Hunter tristimulus parameters at day 0. $L(t)$, $a(t)$ and $b(t)$ are the Hunter tristimulus parameters at the time t. (-✱-✱-, control; -□-□-, cochineal; -△-△-, erythrosine.)

products is nitrosylmyochrome, and the common fading appears to be the result of photooxidation of this heat-denaturated pigment (Skibsted, 1992).

The packaging material with a very low OTR clearly protects luncheon meat against colour fading both for products exposed to light (Fig. 1(C)) and for products protected against light (Fig. 1(D)). For packages stored in light, a small but significant colour loss ($p < 0{\cdot}05$) was observed during the initial 1—2 days of storage, presumably due to residual oxygen. This is in accordance with previous findings (Andersen *et al.*, 1988), where light in combination with residual oxygen in sliced vacuum-packed ham caused the most severe colour loss. For samples packed in the material with oxygen barrier, a significant higher colour loss ($p < 0{\cdot}05$) was observed for luncheon meat with erythrosine added compared to the two other products, indicating that bleaching of erythrosine is less dependent on availability of oxygen.

Lipid oxidation

Lipid oxidation was significantly affected both by the packaging material used and by the storage conditions, but not by the presence of cochineal. Similar to what was found for the discoloration process, the combination of light and oxygen was found to accelerate lipid oxidation in the products, as determined both by objective analyses and by sensory evaluation. For samples packed in the material with the high OTR and stored in light, the rapidly increasing amount of TBARS and of fluorescent oxidation products during storage (Fig. 2), was paralleled by the development of a rancid taste as indicated by an increasing score for off-flavour (Fig. 3). Notably, only a minor increase in lipid oxidation products was detected in samples packed in the material with the low OTR and stored in the dark. For samples packed in a material with a high OTR and stored in the dark, a lag phase for lipid oxidation of approximately 20 days was observed. For samples packed in the material with the low OTR, the exposure to light had little if any effect on lipid oxidation, although a transient formation of TBARS was noted (Fig. 2(A,B)).

The results from both the sensory analyses and objective methods reveal no significant difference between luncheon meat containing cochineal and luncheon meat without any colorant added with respect to lipid oxidation and development of rancidity.

DISCUSSION

In order to replace erythrosine with natural colorants such as cochineal in processed foods, colour matching

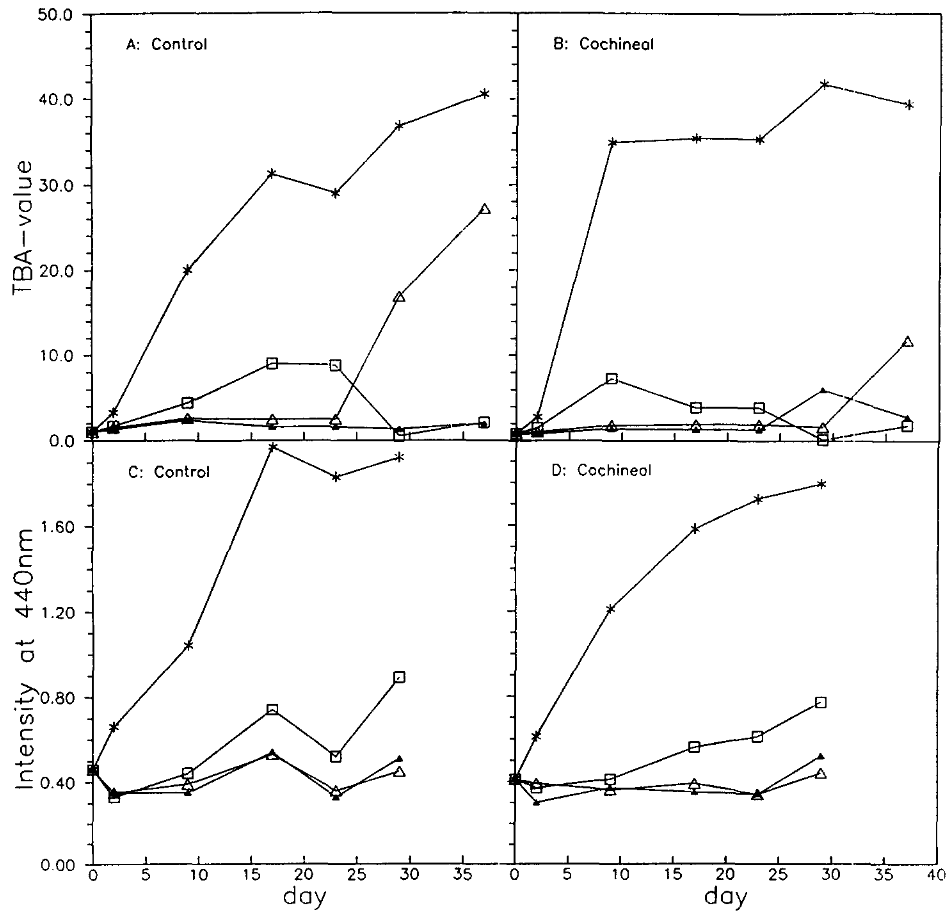

Fig. 2. Lipid oxidation measured as TBA value (μmoles malondialdehyde per kg meat) and fluorescence (intensity of emission at 440 nm relative to 1 ppm quinine sulphate) during chill storage of luncheon meat without colorant (A, C) and with cochineal as colorant (B, D). (*—*, exposed to fluorescent light, packaging material without oxygen barrier; □—□, exposed to fluorescent light, packaging material with oxygen barrier; △—△, protected against light, packaging material without oxygen barrier; ▲—▲, protected against light, packaging material with oxygen barrier).

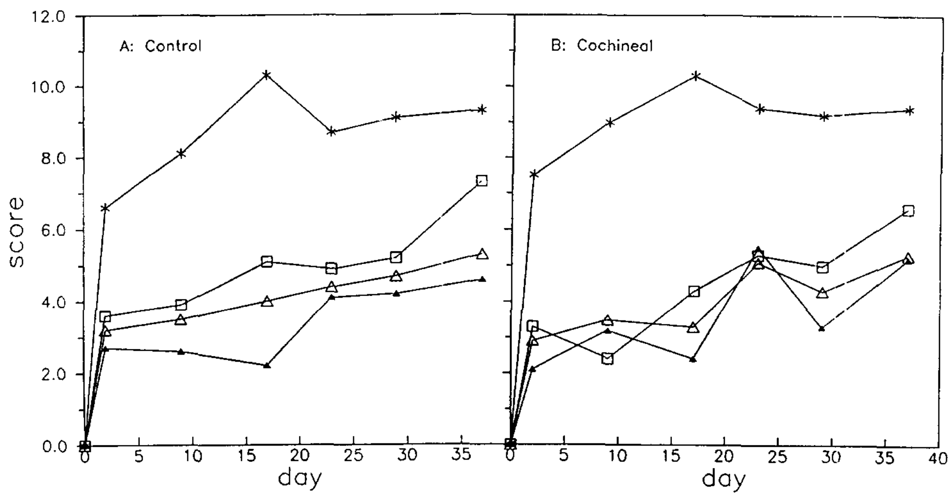

Fig. 3. Sensory score (development of rancid taste) during chill storage of luncheon meat without colorant (A) and with cochineal as colorant (B). (*—*, exposed to fluorescent light, packaging material without oxygen barrier; □—□, exposed to fluorescent light, packaging material with oxygen barrier; △—△, protected against light, packaging material without oxygen barrier; ▲—▲, protected against light, packaging material with oxygen barrier).

experiments have to be performed for each type of product. For batches of pasteurized, processed pork (luncheon meat) with increasing amounts of cochineal added, colour matching to a standard product based on a combination of the tristimulus colorimetric parameters Hunter *a*, *b* and *L*, and the derived 'total colour difference', ΔE, was found to provide the same result as evaluation by a fifteen-member sensory panel. The objective method, as used in the present study, is inexpensive and fast, when compared to sensory colour evaluations, and may be a useful tool for colour matching of other products. It should, however, be noted that a meat colour similarity by instruments to the level obtained in the present study (ΔE of approaching 2) is not always acceptable. Sensory evaluation and acceptability trials would still have to be done for colour matching of several products.

The most significant colour fading of each of the three different products used in the storage experiment was found to be caused by the combined action of light and oxygen. For products packed in materials with the high OTR and exposed to light during storage, colour fading could quantitatively be accounted for as photooxidation of nitrosylmyochrome. As is well known, nitrosylmyochrome is very sensitive to light in the presence of oxygen (Andersen *et al.*, 1988), and for samples packed in materials with low OTR, the discoloration is expected to be halted, when the residual oxygen is depleted (Skibsted, 1992). For concentrations of residual oxygen below a critical limit, diffusion of oxygen across the packaging material

$$O_2(\text{atm}) \xrightarrow{k_{\text{diff}}} O_2(\text{product})$$

becomes rate-determining, rather than the photooxidation process

$$O_2(\text{product}) + \text{nitrosylmyochrome} \xrightarrow{h\nu} \text{metmyoglobin}$$

The result obtained in the present study, revealing a fast initial discoloration, is quantitatively in agreement with this model. Moreover, this pattern is similar both for the product with cochineal added and for the product without cochineal added, indicating that the contribution to the overall colour of the product from cochineal is constant during the storage period. Cochineal may thus be concluded to be a stable colorant for the actual product, in agreement with the previous finding in aqueous model systems in the pH region of relevance for meat products (Jørgensen & Skibsted, 1991). In contrast, erythrosine was found to be very unstable in the actual product when exposed to light, irrespective of packaging material OTR. Thus, parallel photochemical reactions of non-oxidative nature seems to be of importance for deterioration of erythrosine in meat products.

The combined action of light and oxygen was likewise found to accelerate lipid oxidation. However, carminic acid did not show any prooxidative effect, when development of rancidity in the product with cochineal added was compared with the product without cochineal added. Notably, oxygen activation ascribed to the simultaneous presence of quinone and hydroquinone functions in carminic acid, has been demonstrated in aqueous solution model systems (Baykut *et al.*, 1983). The hydroquinone functions are, however, likely to react with proteins in meat during heat treatment, in effect blocking the oxygen activation (Lloyd, 1980). Heat treatment is likewise expected to inactivate meat enzymes, which, as suggested by Gutteridge & Quinlan (1986), are capable of reducing carminic acid in one-electron transfer processes producing free radicals, which would be capable of initiating oxidative deterioration processes of biomolecules.

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