Investigation into the factors influencing the oxidative changes in pigment of cooked-cured meat products

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To find the reasons for the occurrence of green spots on the surface of certain cooked-cured meat products, processing and storage experiments were carried out with Ring-Bologna. The aim of the experiments was to find the factors promoting and hindering the green discoloration. The latter phenomenon was promoted by the use of frozen meat, by insufficient heat treatment during smoking and cooking, by higher temperatures during transportation or storage and by crammed storage in unventilated rooms, where products are jammed to each other and other objects. The use of ascorbic acid in a concentration of 500 mg/kg reduced or completely prevented the tendency for greening. In model experiments carried out with the nitroso-pigment produced from horse heart myoglobin the oxidative degradation of the pigment and the development of green colour could be induced by hydrogen peroxide oxidation. By the use of cysteine and ascorbic acid this degradation could be inhibited. According to our findings some oxidative changes in the pigments of meat products are not necessarily of microbiological origin: the peroxides decomposing the pigments may also originate from autoxidation of the tissue.

Untersuchung der die oxidativen Änderungen der Pigmente von gekochten gepökelten Fleischprodukten beeinflussenden Faktoren

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Es wurden Herstellungs- und Lagerungsversuche mit Knackwurst durchgeführt, um die Ursachen für die Entstehung der grünen Flecke auf der Oberfläche von gewissen gekochten gepökelten Fleischprodukten zu untersuchen. Ziel der Versuche war es, diejenige Faktoren aufzufinden, welche auf die Entwicklung der oberflächlichen grünen Flecke fördernd bzw. hemmend wirken. Die Entstehung der grünen Verfärbung förderten die Verwendung von Gefrierfleisch, die unzureichende Wärmebehandlung während der Räucherung und des Kochens, die höhere Temperatur während des Transports und der Lagerung und die Lagerung der Produkte in ungelüfteten überfüllten Räumen, wo sich diese aneinander oder an andere Gegenstände gedrückt werden. Durch die Anwendung von Askorbinsäure in einer Konzentration von 500 mg/kg wurde die Entwicklung von grünen Flecken deutlich geschwächt oder vollständig verhindert. In Modellversuchen, die mit aus Pferdeherz-Myoglobin hergestelltem Nitrosopigment durchgeführt wurden, ist es uns gelungen, die oxidative Veränderung vom Pigment und die Entwicklung der grünen Verfärbung durch Oxidation mit Wasserstoffperoxid hervorzurufen. Der Abbau liess sich mit Anwendung von Cystein und Askorbinsäure hemmen. Nach unseren Versuchen sind manche oberflächliche grüne Verfärbungen von Fleischpigmenten nicht unbedingt auf mikrobiologische Gründe zurückzuführen: die den Abbau der Fleisch-pigmente verursachenden Peroxyde können auch bei der Autoxidation von Geweben entstehen.

Edellinen sivu tyhjä

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L'examen des facteurs qui influencent les changements oxydatifs du pigment des produits de viande fumés et bouillis

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Afin de constater les causes du verdissement superficiel tacheté de certains produits de viande fumés et bouillis nous avons effectué des expériences de production et de stockage de saucisses pour chercher les facteurs qui favorisent ou empêchent le verdissement. Nous avons trouvé que celui-ci est favorisé par l'emploi de la viande frigorifiée, par le traitement thermique insuffisant pendant le fumage et bouillage, par la température plus élevée de transport ou de stockage, par le stockage insuffisament aéré et encombré des produits, par la compression des produits /entre eux ou contre certains objects/.
L'emploi d'acide ascorbique en une concentration de 500 mg/kg a notablement diminué ou complètement arrêté la disposition de verdissement. En expériences de modèle avec du pigment-nitroso gagné de mioglobine extrait de coeur de cheval, il nous réussit par oxydation avec du peroxyde d'hydrogène à opérer la dégradation oxydative du pigment et la production du colorant vert, et par l'usage de cystéine et d'acide ascorbique il nous réussit à empêcher la décomposition. Selon nos recherches certains phénomènes de verdissement des pigments de viande ne sont pas absolument d'origine microbiologique. Les peroxydes qui apportent la dégradation des pigments de viande peuvent être aussi d'origine autooxydative de tissus.

Исследование факторов, влияющих на окислительные изменения пигментов варенных, маринованных мясных изделий

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При изыскании причин поверхностного пятнистого позеленения некоторых варенных, маринованных мясных изделий были проведены опыты исследования производства и хранения сардельки с целью исследования факторов, способствующих и замедляющих процесс позеленения. Позеленению способствовало употребление замороженного мяса, недостаточная теплообработка при копчении - варении, более высокая температура при транспортировке или при хранении, а также загромождённое хранение продукта без вентиляции, прижимание продукта друг к другу или к другим предметам. Применение аскорбиновой кислоты в концентрации 500 мг/кг в значительной мере снизило или же совсем прекратило способность к позеленению. При опытах на модели с нитрозопигментом, полученного из миоглобина лошадиного сердца окислением перекисью водорода мы смогли вызвать окислительную деградацию пигмента и образование зелёной краски, а применением цистеина и аскорбиновой кислоты удалось тормозить процесс разложения. На основании наших исследований некоторые явления позеленения пигментов мяса являются не обязательно микробиологического происхождения, а могут происходить от перекисей, вызывающие разложение пигментов мяса и образующиеся при самоокислении тканей.

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Studies on the factors influencing the oxidative changes in the pigments of cooked-cured meat products

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Introduction

In one of our food processing enterprises the surfaces of certain cured, cooked meat products were occassionally observed to turn green in irregular, spotty-striped forms during storage, and especially in warmer weather. This discoloration did not spread over to the entire surface or to the inner parts of the product, and was not accompanied by microbiological deterioration either.

The microbiological discoloration of cured, cooked meat products discovered among the first by NIVEN and associates /NIVEN et al., 1949; NIVEN & EVANS, 1957/ is a well-known product degradation phenomenon investigated earlier in Hungary, too /BIRÓ & INCZE, 1959; INCZE, 1961/. Discoloration, the subject of this study, however, did not seem to be of microbiological origin, the reasons being that no considerable difference was experienced between the viable cell counts of discolored samples and samples showing no discoloration. Lactic acid bacteria producing H_2O_2 could not be isolated even from green samples on Rogosa agar plates containing manganese dioxide /INCZE, 1961/. Furthermore, discoloration could not be spread from one sample to another by laying green and non-green samples on each other. Therefore, production and storage experiments were performed with one of the meat products, with Ring Bologna, subject rather frequently to this discoloration, while oxidation model experiments were performed with nitroso-pigment prepared from horse heart myoglobin to detect factors promoting or hindering its discoloration and also to investigate whether the discoloration in question might be due to autooxidation in the tissues.

Materials and Methods

Analytical Methods

HORNSEY's /1956/ method was applied to determine concentration of meat pigments.

Presence of free nitrite in meat products was checked by a test paper containing sulphanilic acid and alpha-naphthylamine /FEIGL, 1954; ERDMAN & WATTS, 1957/. To the qualitative testing of sulfhydryl groups we applied the sodium nitroprusside color reaction according to WATTS and associates /1955/. Intensity of color reactions was marked by scores 0, 1, 2, 3, 4, or 5.

Peroxide number /Lea number/ of the fat content of raw materials used for the experiments was determined by iodometric titration of the extracts prepared with chloroform, and was characterized by the volume of the 0.002 N sodium thiosulphate solution consumed by the samples, and calculated for 1 g of fat.

Production and Storage Experiments

Ring Bologna samples were prepared in the pilot plant of the Hungarian Meat Research Institute, Budapest. The proportion of raw materials and additives used in the experimental samples was the same as that used by the factory where the discoloration of the products occurred. In the 2^4 factorial production-storage experiment the aim was to investigate the effect of the following factors:

A - addition of ascorbic acid
A_1 - 0 g/10 kg meat emulsion
A_2 - 5 g/10 kg meat emulsion.

B - using frozen meat stored in this state for longer periods
B_1 - applying entirely frozen meat,
B_2 - applying merely pre-cooled fresh meat,

C - intensity of heat treatment during production
C_1 - smoking up to 55°C, cooking up to 65°C /core temperature/,
C_2 - smoking up to 60°C, cooking up to 70°C /core temperature/,

D - storage conditions
D_1 - bulk storage at 24°C throughout 16 h, continued by storage at 12°C,
D_2 - loose, airy storage at 12°C from the beginning.

The Lea number of lipids extracted from industrial fat used for producing the experimental samples was 1.5±0.36, that of lipids extracted from non-frozen beef was 7.9±0.42, while that of lipids extracted from frozen stored beef was 12.0±3.89.

With each experimental treatment, changes taking place during storage were studied using 20 samples per treatment. Frequency of surface discoloration was recorded daily during storage.

Model Experiments to Oxidize Meat Pigments

To investigate peroxide oxidation of pure meat pigment, we suspended a horse-heart myoglobin preparation in a solution containing potassium hydrogen-sulphite as well $NaNO_2$ so that the concentration of the solution was 0.4 µmol/ml as regards myoglobin while 0.1 mg/ml as regards $NaNO_2$. Insoluble parts removed by filtration, the dark reddish-brown filtrate /the solution of nitroso-myoglobin/ was distributed in to centrifuge tubes and treated by heat. Two different heat treatments were applied: one at 70°C lasting for 15 minutes and the other at 85°C lasting for the same period, then after cooling, the nitroso-myochromogen precipitates were separated by centrifuge. The total haem pigment content of nitroso-myochromogen precipitates, and the nitroso-pigment content was determined by HORNSEY's /1956/ methods, by spectrophotometry.

We tried to oxidize nitroso-myochromogen precipitate washed with distilled water with hydrogen peroxide after suspending it with 5 % NaCl solution and to analyse the effect of nitrite, cysteine and ascorbic acid on the stability of nitroso-pigment against oxidation.

Comparative tests were carried out using the following scheme:

- pigment suspension containing 45 mg/l of sodium nitrite,
- pigment suspension containing 45 mg/l of sodium nitrite and 3300 µmol/l of cysteine,
- pigment suspension containing 45 mg/l of sodium nitrite, and 427 mg/l of ascorbic acid,
- pigment suspension containing 45 mg/l of sodium nitrite, 3300 µmol/l of cysteine and 427 mg/l of ascorbic acid,
- pigment suspension containing no free nitrite, cysteine or ascorbic acid.

Concentration of H_2O_2 was 280 µM in the reaction mixtures. After treatment with hydrogen peroxide determined the remaining concentration of nitroso-pigment and total pigment content measurable in haematin while with qualitative tests we checked the presence of free nitrite and sulfhydryl groups in the suspending medium.

After separating the precipitate received by a heat treatment of 15 min at 70°C, we performed H_2O_2 treatment of pigment left over in the supernatant. To study the protective effect of reducing materials, reaction mixtures were supplemented by only half of the concentration of the same additives. In this case changes were followed up directly by recording the absorption spectra.

Results

For each treatment, percentage frequency of samples discolored during seven days of storage, as well as the viable cell counts measured on Rogosa agar plates are shown in Fig. 1. In conformity with practical experience also the greening of the experimental samples was observed mainly around the pressed surface area in forms of spots. On the basis of greening frequencies, analysed by factor analysis, we established that application of meat stored in frozen condition as well as insufficient smoking-cooking temperatures predisposed the product to greening, especially if /temporarily/ exposed to storage conditions leading to muggyness. The effect of these three factors contributing to green discoloration could be decreased substantially by the addition of ascorbic acid. With ascorbic acid containing samples where not all of the production - storage treatments were unfavourable 500 mg/kg ascorbic acid prevented greening entirely. According to factor analysis, all four technological and storage factors had a statistically significant effect upon greening and, except for the intensity of the heat treatments involved in smoking and cooking, the remaining factors significantly influenced each others' effect: there was an interaction. Colony counts on Rogosa agar plates did not show considerable differences; microbial contamination of greened samples similarly resembled that of non-greened ones. On the manganese-dioxide containing culture medium, none of the colonies indicated hydrogen peroxide production.

Based on the changes of intensity of free sulfhydryl reaction, carried out on various parts of sausage samples, it could be stated that, in samples containing frozen meat, the concentration of free sulfhydryl groups decreased more rapidly than in samples prepared of fresh meat. Disappearance of free nitrite was accelerated by keeping the sausage samples at 24°C for 16 hours at the beginning of storage.

In samples remaining red, surface parts in direct contact with the skin gave free sulfhydryl reaction of smaller or greater intensity even on the 7th day of storage. Discolored surfaces did on no occasion give color reactions indicating presence of free nitrite and free sulfhydryl groups.

According to the results of analyses performed by HORNSEY's method the quantity of total haem pigments precipitated, after nitrite treatment, from horse heart myoglobin solution by a 15-min treatment at 70°C was merely one third of the total haem pigment content precipitating after a heat treatment of 15 min at 85°C. But in both cases, about 75 % of the total haem pigment in the separated precipitate proved to be nitroso pigment. Pigment decomposition experienced in the course of oxidation by hydrogen peroxide, carried out after suspending the precipitates in a solution containing nitrite and sodium chloride, resulted in the ratios indicated in Fig. 2. These ratios depended on the presence of anti-oxidative additives.

As shown in the figure, in pigment suspensions not containing anti-oxidative additives 94-99 % of the nitroso-myochromogen was decomposed similarly, the total pigment content, expressed in haematin, of such suspensions was only about half of the total pigment content of the suspension not treated with hydrogen peroxide.

Separation of nitroso-myochromogen precipitated by a 15-min heat treatment at 85°C resulted in a colourless supernatant, indicating that the pigment was completely precipitated. A heat treatment of 15 min at 70°C, on the other hand, resulted in only partial precipitation of the pigment, after the separation of which the supernatant remained reddish-brown in color. Therefore, the hydrogen peroxide treatment was also performed with this non-denatured pigment solution. At the same time, the protective effect of cysteine and ascorbic acid was also studied. With the exception of H_2O_2, the concentration of additives was half of that in oxidation experiments with pigment precipitates. As a result of H_2O_2 treatment, the pink

color of the samples containing reducting additives turned yellow, while the brown color of the samples containing no reducing agent turned green. Absorption spectra registered before and after color changes and oxidation treatment are shown in Fig. 3.

The absorption spectrum registered before oxidation indicated nitroso-myoglobin /WOLF et al., 1978/. After oxidation, the spectrum of the pigment samples prepared by adding both cysteine and ascorbic acid or containing only cysteine indicated haematin formation during the oxidation. The spectrum of the green sample containing no reducing agent differed from the spectra of both nitroso-myoglobin and haematin, and the spectrum of the sample supplemented only by ascorbic acid as an anti-oxidant, was also somewhat similar to the spectra of greenish discolored samples after hydrogen peroxide oxidation.

Discussion and Conclusions

Based on our experiments we can state that the investigated discoloration of meat products is the consequence of oxidative degradation of the meat pigments probably as an effect of peroxides /TARLAGDIS, 1961; 1962/. We have not had so far identified the green pigment. Based on data of the relevant literature, we have come to the conclusion that greening phenomenon that occurred during the model-experiment might be the result of reactions connected with the formation of ferryl-myoglobin /GEORGE, 1952; GEORGE & IRVINE, 1955; KING & WINFIELD, 1963; PEISACH et al., 1968; GIDDINS & MARKAKIS, 1973/. According to our observations, the discoloration investigated by us and the assumed peroxides are not of microbiological origin but probably the consequence of autoxidation of components of meat products. Discoloration seems to be promoted by the use of frozen stored meat, probably as a consequence of its more advanced autoxidation state, by insufficient heat treatment in the course of smoking and cooking as well as by the elevated delivery and storage temperature. The striped-spotty discoloration indicates the inhomogeneous distribution of meat product components decisive from the point of view of discoloration. Since in the storage experiment discoloration took place after pressing sausages to each other or to other objects, and around the pressed surface, presumably it is somehow connected also with fat and moisture migration which might be the consequence of pressure. It is significant from this aspect, too, that the water binding capacity of frozen meat is very low /ANON., 1978/. Products with significant redox-poising capacity are less liable to discoloration, in this respect great significance can be attributed to sulfhydryl groups and to the addition of an anti-oxidant, suitably ascorbic acid.

Development and stabilization of nitroso-pigments take place mainly in the heat treatment of meat products as a phase of production. The importance of cooking and that of free sulfhydryl groups in stabilizing pigments of cured meats was pointed out already by HORNSEY /1956/ and WATTS and associates /1955/. Possibly, when producing meat products, the concentration of components with redox-poising capacity, the extent of denaturation and coagulation of protein, the stability or the oxidation tendency of pigments may be different depending on raw-material conditions, salt concentration, intensity of heat effect in the course of production, etc.

Depletion of sulfhydryl /reducing/ groups is accompanied by the oxidation of the ferrous atom of haem, and the decomposition of the porphyrin ring. The sulfhydryl groups or other reducing agents, e.g. added ascorbic acid, protect product color, in case sufficient nitrite is present.

Based on the above, it is clear that effects resulting in protein denaturation - and heat treatment is the most important among these -, are significant not only in developing good color of a cured meat color but also in stabilizing the nitroso-pigment. In the protein denaturation process sulfhydryl groups are liberated /WATTS et al., 1955/ and it is their reducing effect that can be the cause of the above favourable effect.

Numerous other researchers, too, have sucessfully applied ascorbic acid to prevent oxidative rancidity and discoloration of raw meat /WATTS & LEHMAN, 1952; BAUERNFEIND et al., 1954; WATTS, 1954/. Application of ascorbic acid is advantageous for cured meat products not only due to its effect of preventing oxidation in the course of storage but also because more nitrite is reduced with it to nitrogen oxide and this way more myoglobin can be transformed into nitroso-myoglobin. At the company mentioned in the Introduction of this study, application of ascorbic acid introduced upon experiences and results of our experiments practically diminished the problem of discoloration.

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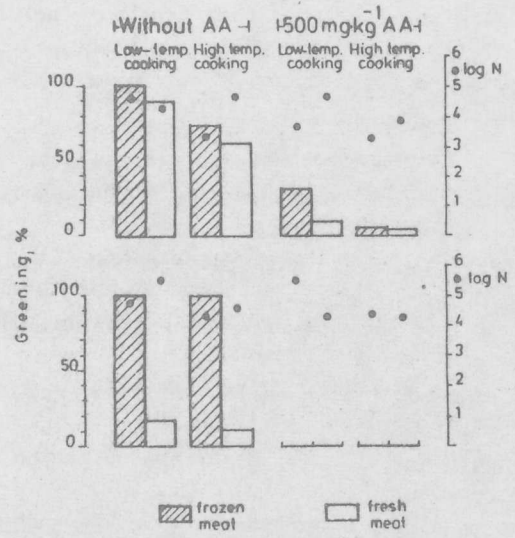

fig. 1 Discoloration frequency and viable cell count /determined on Rogosa agar/ of experimental sausage samples after 7 days of storage, as a function of production and storage conditions. Legend: columns represent percentage frequency of greened samples, • N marks viable cell count per g, AA = ascorbic acid; low-temperature cooking = smoking up to 55°C, cooking up to a core temperature of 65°C; high-temperature cooking = smoking up to 60°C, cooking up to a core temperature of 70°C. Top diagram: storage at 12°C after keeping at 24°C for 16 h until muggy. Bottom diagram: storage at 12°C.

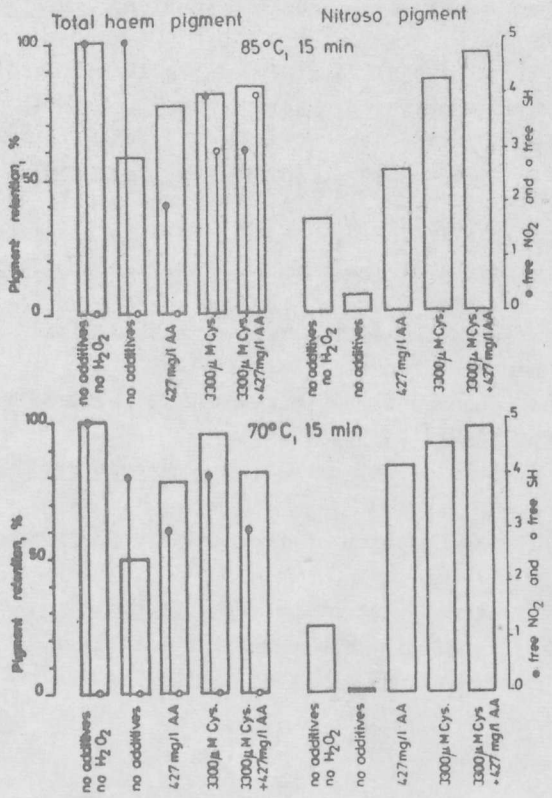

fig. 2 Effect of cysteine and ascorbic acid on the decomposition by H_2O_2 of suspensions of nitroso-myochromogen precipitates formed from horse heart myoglobin in a solution containing 5 % NaCl and 45 mg/l $NaNO_2$. The figure illustrates pigment quantities measured after 90 min of shaking at room temperature in the presence of 280 µM H_2O_2. Data are given in percentage of the original pigment content of the suspension not containing additives and hydrogen peroxide. Columns in the upper diagram illustrate stability of pigment precipitated by a heat treatment of 15 min at 85°C, while those in the lower diagram show stability of pigment precipitated during 15 min of heat treatment at 75°C. Symbols • and o mark scores for observed intensity of free $-NO_2$ and free -SH color reactions performed after shaking the suspensions. AA = ascorbic acid; Cys = cysteine.

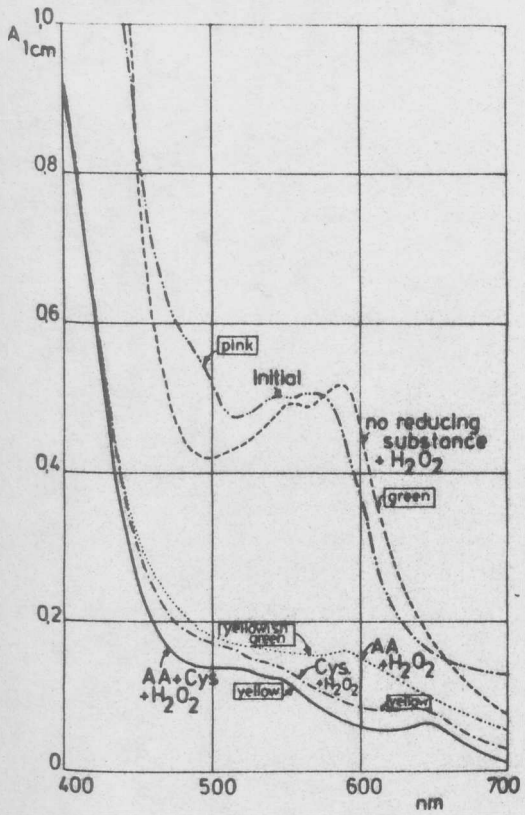

fig. 3 Color and absorption spectrum of supernatants after removing nitroso-myochromogen precipitates produced by a 15-min /"insufficient"/ heat treatment at 70°C, before /initial/ and after treating with 280 /µM hydrogen peroxide and in the presence and absence, resp., of reducing additives.
AA = 213 mg/l ascorbic acid;
Cys = 1650 /µM cysteine.