Effects of Microflora on Fresh Meat Color

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Color probably is the most frequently used criterion for judging shelf-life and acceptability of fresh meats even though, from a theoretical point of view, the correlation between color and overall quality is of limited value. Surfaces of freshly cut meat, upon exposure to air, change quickly from a purplish-red due to myoglobin to a bright red due to oxymyoglobin. This bright, cherry red color is associated by the consumer with a fresh, wholesome product. This desirable color, however, begins to change immediately as the result of continuous formation of the brown pigment, metmyoglobin (Daun *et al.*, 1971; Fellers *et al.*, 1963). Two types of oxidative changes are mainly responsible for the abnormal brown, grey, and green discoloration of meat. One involves the oxidation of the ferrous iron in the heme compound to the ferric condition; the second is a direct attack by oxygen on the porphyrin ring (Watts, 1954). Under average supermarket storage conditions, packaged meat cuts become unacceptable for sale after approximately 3 days according to Daun *et al.* (1971); these authors fix the onset of unacceptability at a level of 70% metmyoglobin in the surface pigment. In any case, the surface color of fresh meat is determined by the amounts of myoglobin, oxymyoglobin, and metmyoglobin present and, to a lesser extent, by residues of hemoglobin compounds.

Factors affecting color changes on meat surfaces have been listed by Lanier *et al* (1977); these factors include 1) partial pressure of oxygen in the environment, 2) bacterial growth, 3) artificial atmospheres, 4) temperature, 5) tissue lipid oxidation, and 6) drying of the meat surface.

In his book on the microbiology of meats, Jensen (1945) made the observation that organisms, both living and dead, and their enzymes on the surfaces of meat had the potential for oxidizing both fresh and cured meat pigments. A number of other meat scientists and microbiologists also have associated bacterial growth with deterioration of fresh meat color (Butler *et al.*, 1953; Costilow *et al.*, 1955; Robach and Costilow, 1961; Lanier *et al.*, 1978; Lin *et al.*, 1977; Marriot *et al.*, 1967; Ockerman and Cahill, 1977). Therefore, subsequent discussion will deal with the examination of these observations and an assessment of bacteria as agents of color changes in meats.

Butler *et al.* (1953) extended the findings of Brooks (1938) and George and Stratmann (1952) who had previously shown that the rate of metmyoglobin formation from myoglobin increased with decreasing oxygen pressure and increasing temperature of storage. Butler *et al.*(1953) observed that the changes in meats accelerated by bacterial growth included discoloration due to increased rate of metmyoglobin formation, production of off-odors, and slime formation. These authors stated that the changes appeared in the order listed and that any one of them could render the meat unsalable. From their studies, they concluded that the main cause of initial discoloration and formation of metmyoglobin was decreased oxygen pressure as a result of bacterial growth in packages of boneless beefsteaks inoculated with *Pseudomonas*. They found that metmyoglobin percentages increased greatly during the logarithmic growth phase of the *Pseudomonas;* it is well known that the oxygen demand of aerobic organisms, *Pseudomonas* species are aerobic, in the logarithmic growth phase is very high. When the oxygen was reduced to critical pressures, the metmyoglobin percentage increased rapidly. According to Solberg (1968), the critical partial pressure for oxygen is the 4-mm level, below this level rapid oxidation to metmyoglobin occurs. Butler and coworkers indicated that reduction of oxygen beyond the critical point caused the metmyoglobin percentage to be sharply reduced because of reduction to myoglobin. The color that resulted, in this case, was similar to the purple myoglobin color of freshly cut beef before oxygenation had occurred. At this stage, the samples could be brightened to a degree almost equalling that of the original desirable color by exposure to oxygen under pressure; rapid deterioration to metmyoglobin occurred subsequently, however, even though the samples were stored at 32°F. This rapid deterioration was attributed to very large bacterial populations on the meat surface. Costilow *et al.* (1953) did some additional studies with two of the strains of *Pseudomonas* used by Butler *et al.* (1953) known to cause discoloration of beefsteaks. They came to essentially the same conclusion that the rate of discoloration is dependent to a great degree on the activity of the bacteria. In fact, Strange *et al.* (1977) are of the opinion that measurement of meat pigment oxidation and tyrosine values are the most effective monitors of bacterial contamination. On the other hand, Lin *et al.* (1977) found that myoglobin oxidation in hamburger was only weakly correlated with levels of bacterial contamination before and after storage.

Since bacterial activity was implicated as a factor in pig-

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ment changes in fresh, prepackaged meat, Robach and Costilow (1961) initiated studies to determine the extent to which different bacteria oxidized myoglobin. They used cultures of *Pseudomonas fluorescens, P. aeruginosa,* 2 strains of *P. geniculata,* 3 *Pseudomonas* species, *Achromobacter (Acinetobacter) liquefaciens, Flavobacterium rhenanus, Lactobacillus plantarum,* and *Saccharomyces cereviseae.* At room temperature, the yeast and all the bacteria except *L. plantarum* caused rapid changes. At 4°C, the yeast had little effect on color, but the aerobic bacteria were all active and caused changes from red to brown to purple. Inoculation of surfaces with low numbers of organisms (10^6 to 10^7/g) caused a more rapid appearance of the brown color of metmyoglobin than did inoculation with higher numbers (10^8/g). Inoculation with high cell populations produced the purple color of myoglobin.

In storage experiments in which atmospheres containing different levels of oxygen were used, it was observed that, in an oxygen-free atmosphere, the steaks were the purple color of myoglobin; at 10 mm of oxygen pressure, pigment was oxidized to metmyoglobin and the surface was brown. They observed that no bacterial activity was necessary for pigment oxidation under low oxygen pressure. They concluded that the primary role of bacteria in meat discoloration is the reduction of the oxygen tension in the surface tissue. They based their conclusion on several observations:

1) Pigment oxidation and reduction were controlled by physical adjustments of the oxygen level in the storage atmosphere in the absence of a significant number of bacteria.
2) The oxygen level in the storage atmosphere greatly affected the rate of pigment changes of both inoculated and uninoculated steaks.
3) Oxygen uptake rate of the surface tissue of meat was correlated with microbial activity and with color change.
4) At intermediate levels of oxygen demand of surface tissue, oxidation to metmyoglobin occurred; with high respiration rates, reduction to myoglobin occurred, correlating with similar changes under controlled oxygen atmospheres.
5) Agents inhibiting development of high oxygen uptake rates in surface tissues preserved color under atmospheric conditions but were ineffective under low oxygen pressures.

These authors proposed that the reduction of oxygen in meat tissue by microbial growth or by physical means produced a great increase in reduced myoglobin, which, in turn, was oxidized by metabolic hydrogen peroxide produced either by meat tissue or by bacteria. If the oxygen tension was reduced to a low enough level, little or no hydrogen peroxide was formed, and no oxidation occurred. These observations support the statement of Watts (1954) that the oxymyoglobin of fresh meat and the nitric oxide myoglobin of cured meats must dissociate to myoglobin before oxidation to the brown ferric metmyoglobin takes place. The dissociation of the oxy—compound increases with decreasing oxygen tension. Fresh meat pigments therefore turn brown more readily at oxygen tensions considerably below that of air at atmospheric pressure.

At this point, it may be useful to mention that fresh meat held under refrigeration (0 to 5°C) will develop a predominant flora of psychrotrophic microorganisms. The most frequently observed bacteria are *Pseudomonas, Acinetobacter* (formerly *Achromobacter), and Flavobacterium.* The *Pseudomonas* usually outnumber the others as a result of refrigerated storage; they are obligate aerobes and utilize oxygen during metabolism and growth. These are the reasons for the selection of *Pseudomonas* species for various studies on bacterial growth and discoloration of fresh meat pigments.

The effect of growth of *P. fragi* in aqueous extracts of beef on color, pH, and protein degradation was examined by Bala *et al.* (1977). They prepared sterile extracts of semitendinosus and added 10^5 cells/ml. Samples were stored in the dark at 1°C for time intervals up to 10 days. Samples of sterile beef extract supported no growth during 10 days of storage; the mean aerobic counts of inoculated samples increased from the initial numbers of 10^5 cells/ml to levels of $10^{7.9}$/ml after 10 days. The pH of the sterile extract remained at 5.5, but the pH of the inoculated material increased from 5.5 to 6.0 during storage. The higher pH in the samples inoculated with *P. fragi* was attributed to the production of ammonia and amines from the amino acids in the extract. Color of the beef extract was evaluated by measuring the relative concentrations of myoglobin, oxymyoglobin, and metmyoglobin. A 76% loss of oxymyoglobin occurred in 10 days in inoculated samples; a 45% loss occurred in the sterile samples. Both microbial growth and pH were important in producing discoloration of the extract. Proteolytic activity also was related to the factors of bacterial growth, pH and, discoloration. The globin part of the myoglobin molecule is susceptible to attack by protease and the degradation of the peptide chain alters the reactivity of the heme group and eventually produces discoloration (Fox, 1968). Briskey and Kauffman (1971) have pointed out that the production of metmyoglobin from myoglobin or oxymyoglobin is accelerated by all conditions that cause denaturation of the globin moiety. Also, Watts (1954) has stated that the ability of myoglobin to combine reversibly with oxygen depends upon the specific protein linkage with native globin. Denaturation of the globin destroys the ability of myoglobin to combine reversibly with oxygen and greatly increases the susceptibility of these pigments to true oxidation. Satterlee and Hansmeyer (1974), however, observed very little, if any, proteolytic destruction of surface pigment of meat during storage at 5°C for 18 days and at bacterial counts in excess of 10^8 organisms/cm^2.

Ockerman and Cahill (1977) explored the relationship of growth and pH effects of specific bacteria on bovine tissue. They used *P. putrefaciens* because of its ability to increase the pH of meat products, *Leuconostoc mesenteroides* because of its ability to lower tissue pH, and *Bacillus subtilis* because of its ability to maintain a neutral to slightly basic pH. They inoculated sterile bovine muscle tissue with these organisms and held the samples of 2 to 4°C for 21 days. The extent of growth varied for the different organisms. Numbers on the control were constant at less than 10/g. *P. putrefaciens* showed the most growth, followed by *L. mesenteroides* and *B. subtilis.* After 21 days, the pH was highest (7.2 to 7.3) for the *Pseudomonas,* pH 6 for the *Bacillus,* and pH 5.3 for the *Leuconostoc.* The pH of the control remained relatively constant at pH 5.5. *P. putrefaciens,* which produced the greatest numbers of cells and an alkaline pH, caused the greatest deterioration in color after 21 days. A study of the pH values

and reflectance analyses for color at 7 days, however, showed that the color changed in a desirable direction as the pH increased toward pH 6.0, but once beyond pH 6.0, the color became undesirable. They concluded that pH, which can be altered by bacterial growth, can change the chroma and hue in a desirable direction when it is elevated (within limits) and, conversely, that the color will be altered in an undesirable way with a lowered pH or oxygen depletion. It might be added that *Pseudomonas putrefaciens* is proteolytic and that considerable proteolysis could have occurred and contributed to color changes.

Protective water-impermeable coverings used for beef carcasses during shipment protect beef from shrinkage, but also support increased bacterial growth and increased discoloration (Hoke and Smith, 1971). The increased growth, in this instance, was attributed to the high relative humidity within the protective film. Lanier *et al.* (1977) used a model system including a wind tunnel in which they could control temperature, humidity, and air velocity to study the effects of cold storage on the color of lean beef surfaces. They noted that, for short-time storage, low relative humidity yielded more metmyoglobin than did high relative humidity. The effects of low and high relative humidity were reversed, however, on prolonged storage. Evidently, the moist meat surface allowed bacteria to grow readily and to accelerate pigment oxidation. They recommended a relative humidity between 85% and 90%, air movement of 0.5 meters/second and a temperature close to 0°C for maintaining the best surface color. Higher relative humidities and lower air velocities produced a slow initial rate of color change, followed by an accelerated color deterioration.

Certain green discolorations of fresh beef have been observed that have been attributed to the formation of sulfmyoglobin, cholemyoglobin, or verdoheme. During experiments on packaging of fresh beef, Nicol *et al.* (1970) occasionally observed a bright green exudate. This discoloration occurred when oxygen tension was low (~1%) in the atmosphere surrounding the tissue and when the ultimate pH was high (above 6.0). This phenomenon was attributed to the production of hydrogen sulfide by a bacterium identified as *Pseudomonas mephitica*. Under these conditions, the hydrogen sulfide combined with myoglobin to form sulfmyoglobin. They summarized their findings as follows: 1) Hydrogen sulfide was formed from sulfur-containing amino acids under low oxygen tensions, 2) green, reduced sulfmyoglobin was formed only under low oxygen tensions (at high oxygen tensions, oxidation to the red metsulfmyoglobin occurred) and 3) the pH of the meat had to be above 6.0 before formation of the green pigment was observed. This type of color change would be encountered only under rather restrictive conditions.

Because bacterial growth contributes to deterioration of color as well as other spoilage conditions, several studies have been made in which color change was used as an indicator of bacterial growth. The use of ultraviolet, high CO_2 atmospheres, and chlorine rinses have been shown to prolong desirable color and to lower meat surface counts (Reagan *et al.*, 1973; Clark and Lentz, 1972).

In summary, bacterial growth can contribute significantly to color deterioration of fresh meat. Growth of bacteria causes a reduction in oxygen concentration in the environment and consequently modifies the color of the meat pigment. Changes in pH, production of proteolytic enzymes, and the realtive humidity in the system also contribute to the problem. Species and strains of organisms differ in their ability to produce changes or to grow under the prevailing conditions, adding to the variability in pigment alteration. Discoloration can be an indicator of growth of bacteria and of subsequent spoilage; therefore, any measures that can be taken to delay the development of high numbers of bacteria are desirable. These measures include proper packaging, refrigeration, humidity, storage conditions, and above all, the handling of the product during processing under good sanitary conditions to keep the numbers of initial bacteria at a minimum.

Discussion

H. N. Draudt, Peter Eckrich & Sons: Is there an organism that has been associated with gassiness in pork? It seems that you can keep the microorganisms down in pork when you're holding it and keep total counts level but you still might get gassiness. Is this, in your knowledge, or anyone else's knowledge, a phenomenon involved with microorganisms or is it something chemical. It's kind of a hydrogen sulfide odor and they call it gassiness. I think most people are familiar with it, but is it microbiological?

H. W. Walker: I have to admit, I'm not familiar with this phenomenon. Is this in refrigerated pork?

H. N. Draudt: Yes, refrigerated pork. Even though your total counts will be low, you can get what's commonly known, and known to a lot of packing house people, as gassiness. It's a hydrogen sulfide, it's a sulfide-type odor and I wondered if anybody has any clues on this.

H. W. Walker: When you said total count, were there any anaerobic counts done?

H. N. Draudt: Yes, I recently held material for five or six days at 30 degrees. Counts were level, but I still got gassiness in pork. And I thought, well how can I get gassiness? It is microbiological but there is no change in the general microbiological picture. I was hoping that someone might have an idea on what might cause this particular phenomenon.

H. W. Walker: I'm at a loss for an answer. Maybe somebody else in the audience has some familiarity with this.

W. H. Kennick, Oregon State University: It's been reported rather casually in the literature about three times now that there is a rather significant reduction in microbial counts in electrical stimulation carcasses. Do you have any comment on this? Any cause and effect type of thing?

H. W. Walker: Not really. Maybe the first author who reported on electrically stimulated beef knows some changes that have taken place. Is there a change in pH or any differences in pH or anything like that?

D. L. Huffman, Auburn University: Dr. Dutson's right here. I think he can speak to that much better than I could.

T. R. Dutson, Texas A&M University: Maybe Dr. Vanderzant would be the best one to answer this because we have been conducting some studies on this at A&M and definitely there's a change in pH and I think, according to some of the studies we've done, it depends on when the pH changes and

when the analysis occurs and when the inoculation occurs relative to that pH. But in addition to what I've said, maybe Dr. Vanderzant has some other comments.

C. Vanderzant, Texas A&M University: It would be a good time to pass along to somebody else. I'm very fortunate in sitting next to Dr. Gill who has published a recent note in the *Journal of Protection* if I'm not mistaken. He disagrees with previous findings by Henrickson and Riker about the effect of electrical stimulation in which he showed at least to a limited number of microorganisms that there was no effect. In our own studies, there is very limited effect of electrical stimulation on microorganisms in meat. Sometimes you find some differences between different carcasses, but I think that some of the conditions prior to slaughter might have a greater effect on the microorganisms than the electrical stimulation itself. Maybe Dr. Gill wants to reinterpret my description of this particular paper.

C. O. Gill, Meat Industry Research Institute of New Zealand: Not really. As far as I can see, there's no reason to suppose this electrical stimulation should have any effect whatsoever upon the microflora. If anybody can give me a good reason for it happening, perhaps we will be able to conduct some more experiments, but there's just no reason why it should effect the microflora.

D. L. Huffman, Auburn University: I had no intention of getting involved in electrical stimulation in microbial counts. Dr. Walker, I wonder if you could expand just a little bit on the last paper you reviewed showing the low oxygen tensions and the greenish discoloration. If you recall, the last slide or next to last slide that I had, we thought we had relatively sanitary conditions. Now we didn't do any bacterial counts, but we still get this greenish discoloration particularly with the inside top rounds.

H. W. Walker: As I understand it, they have associated this color change specifically with pseudomonas that can produce H_2S. You say you had relatively low counts. Maybe you had a majority of them that were hydrogen sulfide producing organisms. That would be my first guess or something in there that is producing hydrogen sulfide. And whether it's with pseudomonas or some other organisms would be open to question.

D. L. Huffman: Changes could be brought about by something other than bacterial conditions.

H. W. Walker: I didn't run across that, but I see no reason why it couldn't if you had a source of some chemical compound that could bring this about. Because you do get chemical changes under other conditions and changes in color due to chemicals.

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