5-1992

Characterization of Pigments Responsible for Red or Pink Discoloration in Cooked Pork

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CHARACTERIZATION OF PIGMENTS RESPONSIBLE FOR RED OR PINK DISCOLORATION IN COOKED PORK

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Nutrition and Food Sciences

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1992

ACKNOWLEDGMENTS

I would like to express appreciation to my major professor, Dr. Daren P. Cornforth, for his valuable guidance and assistance in experimentation and in writing the dissertation. Sincere appreciation is also extended to my graduate committee members: to Dr. Von T. Mendenhall and Dr. Charles E. Carpenter, for their encouragement and advice; to Dr. Deloy G. Hendricks, Dr. David H. Clark, and Dr. Donald V. Sisson, for their helpful suggestions and advice in preparation of this dissertation.

I wish to extend my appreciation to all staff members and fellow graduate students for their helpful assistance, and I also thank Dr. D. K. Salunkhe and Dr. S.S. Kadam for their constant encouragement throughout the study.

This research was funded by the Pork Council of the National Livestock and Meat Board and the Utah State Agricultural Experiment Station.

Finally, I extend my gratitude to my loving parents and family members for making possible this opportunity to study at Utah State University. Also, my heartfelt thanks to my wife, Umadevi, and daughter, Supriya, for their support and patience throughout the investigations.

Viswasrao M. Ghorpade

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ABSTRACT

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by

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Utah State University, 1992

Major Professor: Dr. Daren P. Cornforth
Department : Nutrition and Food Sciences

The pigments responsible for pink or red discoloration in cooked vacuum-packaged meat products {bratwurst (pork and beef), ground pork, and pork roasts} were investigated. In bratwurst, this study attempted to characterize the red pigment that appears upon refrigerated storage, and to determine the effect of pH, cooking and storage temperature, and sodium lactate on incidence of red discoloration. Myoglobin was identified in the exudate of samples with red discoloration. Myoglobin levels of cooked products were significantly lower in samples of low initial pH (5.5) or cooked to higher than normal internal temperature (74°C). Red discoloration was associated with microbial growth. Frozen samples had no red discoloration after 4 weeks storage. Microbial plate count and incidence of red discoloration were lower in samples of lower pH (5.5 vs 5.8 or 6.2), samples cooked to higher internal temperature (74° vs 68°C), and samples containing 3% sodium lactate.

Further, the effects of microbial growth in raw materials (ground pork) on cooked pork color were investigated. In two trials with sow meat held aerobically at 2°C for 3 weeks, microbial load reached spoilage levels (10^7 cfu/g), pH increased to 6.46, and samples cooked to 71°C had red exudate, shown by absorption spectroscopy to contain myoglobin and cytochrome c. Samples cooked to 82°C received high panel ratings for red

color, due to red, flocculent precipitate in exudate, but samples containing undenatured myoglobin levels received low panel ratings. In sow meat held frozen or vacuum packaged at 2°C, pH after 3 weeks was 6.03 and 6.18, and plate counts were 10^4 and 10^7, respectively, but exudates after cooking were much less red. In five trials with fresh U. S. #1 pork legs, plate counts also reached 10^7 cfu/g by 3 weeks storage, and pH increased from 5.99 to 6.37, but cooked samples were not red. Higher myoglobin levels in sow meat probably accounted for the red color and the high level of undenatured myoglobin remaining after cooking of high pH, spoiled samples.

Finally, pink or red discoloration was investigated in the cooked U. S. #1 pork roasts. Myoglobin was the pigment responsible for pink color in pork roasts cooked to 65°C. Roasts cooked to 82°C had gray internal color after cooking, but developed pink internal color after refrigerated storage. Reflectance spectra of pink slices from roasts, cooked to 82°C, then stored for 12 days at 2°C, were characteristic of denatured globin hemochromes or related non-nitrosyl hemochromes.

(122 pages)

INTRODUCTION

It is economically important to solve pink color problems in vacuum-packaged, precooked pork products because of a growing need for convenience products. The vacuum-packed precooked products have several advantages. They are microwaveable and have little or no drip in the package. They also have longer shelflife and are easier to serve. However, many processors of precooked pork have a major problem with pink color formation in the product during refrigerated storage. The major advantages of vacuum packaging are controlling microbial growth, inhibition of oxidiation, and longer shelflife of meat products. Shrinking and oxidation are also reduced. Information on the stability of meat pigments during cooking in vacuum packages is meager. Reduction of undenatured metmyoglobin to myoglobin also occurs during refrigerated storage. This may increase myoglobin content of the stored cooked meat, but more information is needed.

The most widely proposed cause for pink color in fully cooked products is the contamination of meat with nitrate, nitrite, or combustion gases, which leads to the formation of pink color in fully cooked meat products. Cornforth et al. (1986) showed that not all the pink color is formed due to contamination of nitrite. Reflectance spectrophotometry indicated that the pink pigment present in the commercially precooked turkey breast is pink hemochromes. These hemochromes are formed by reactions, under reducing conditions, of heme from myoglobin with nicotinamide or with any denatured globin molecules to form a pink color complex. These pink globin hemochromes are often formed in meat cooked at 71^{o} C and above, the temperature at which the myoglobin is denatured. Meat cooked below 71^{o} C may also be red or pink. This pink color is mainly attributed to undenatured myoglobin. Higher pH (6.5 and above) stabilizes the meat pigment during cooking and storage. Salt decreases heat stability of the pigments and also decreases the pink color formation in fully cooked meat products (Trout, 1989). Many reports are available on heat stability of the meat pigments, but more information is needed

on pigment stability in meats cooked under vacuum. The present study was undertaken with the following objectives:

1. To study the effects of pH, cooking temperature, and sodium lactate on microbial load, color and meat pigments of vaccum-packaged bratwurst.
2. To determine the effects of various fresh meat storage methods on color of cooked ground pork.
3. To identify pigment (myoglobin vs denatured globin hemochromes) responsible for pink discoloration in pork roasts cooked at 65 or 82°C.

PART I. REVIEW OF LITERATURE

INTRODUCTION

Color of the meat is the major criterion used by the consumer to evaluate doneness of cooked meat products. Pork is a major portion of the processed, convenience-type meat products. Precooked vacuum-packaged pork products offer several advantages, including convenience of rapid microwave reheating. However, cooked meat products sometimes develop a red discoloration during refrigerated storage. Consumers are very sensitive to red color in pork because of concern for trichinosis in undercooked pork products. This red or pink discoloration in cooked meats has been a problem for the meat industry for many years. Work on pink color has been reported as early as 1956, and through the years the problem has been studied in many aspects.

MEAT COLOR

Meat color is due to myoglobin, which comprises 80 to 90% of total meat pigment. Other pigments include hemoglobin, cytochromes, flavins, peroxidases, and catalases, but their contribution to color is negligible. Meat color is very important in respect to marketing. Consumers often rate fresh meat with respect to color. Color of fresh bloomed meat is bright red, but color can range from bright red to dark red. Also, discoloration of fresh meat pigments can produce tan, brown, gray, green, or yellow colors due to bacterial growth (AMSA, 1991). Processing conditions such as cooking, smoking, or curing changes meat color and may enhance color stability. Cooked meat color is controlled by the meat pH, cooking temperature, storage conditions, and bacterial growth. Consumers expect cooked meat to be brown, but often cooked refrigerated meat products develop pink or red discoloration. This discoloration is often related to undercooking. Consumer fear about underprocessing decreases sales of the fully cooked products. Through the years,

researchers have identified causes of red discoloration in cooked refrigerated pork products. This review will concentrate on the pigments responsible for pink or red discoloration in cooked pork products and the role of microbial growth and reducing ability in causing discoloration.

MYOGLOBIN

Myoglobin is a water soluble, globular muscle protein that acts as an oxygen storage site in muscle. Myoglobin is dark purplish red and is distributed in heart and skeletal muscle. Myoglobin concentration is dependent on the age, species, muscle fiber type, and genetic and environmental factors.

Age

It has been reported in several studies that myoglobin concentration of muscles increases with the age of the animal. Ledward and Shorthose (1971) studied myoglobin concentration in lambs at ages varying from 98 to 310 days and showed significant difference in myoglobin levels with age and sex. In pigs, myoglobin levels increase after one year and in horses, after two years (Lawrie, 1979). Nishida (1976) reported that myoglobin concentration of the leg, heart, and gizzard of chicken doubled between 6 to 27 weeks after hatching.

Animal species

Myoglobin concentration varies among species: Beef myoglobin ranges from 2 to 5 mg/g of muscle (wet basis) (Hunt and Hedrick,1977; Rickansrud and Hendrickson, 1967); 4 to 7 mg/g in lamb (Ledward and Shorthouse, 1971); 2.4 to 7 mg/g in pork (Topel et al., 1966); 2 to 3 mg/g in dark meat of poultry (Blessing and Muller, 1974; Nishida, 1976); and 0.5 to 1 mg/g in light meat of tuna (Brown, 1962).

Muscle type

Muscle type is more important in determining myoglobin concentration than the animal species. In yellow fin tuna, for example, a typical light meat section will contain approximately 0.7 mg of myoglobin/g of meat whereas dark meat (from the epoxil muscle) can contain greater than 20 mg/g (Brown, 1962). In the chicken, the myoglobin concentration is higher in the gizzard, 19.6-26.4 mg/g, whereas dark leg muscles contain approximately 1.75-2 mg/g; heart typically will have 2.8-2.9 mg per gram of muscle (Nishida, 1976). Also, varying levels of myoglobin in different muscles of beef carcasses have been reported by Hunt and Hedrick (1977).

Genetic and environmental factors

Genetic and certain environmental factors in addition to age, exercise, and the diet of the animal may affect the myoglobin concentration. Myoglobin content of skeletal muscle has been shown to be lowered by iron deficiency in pig tissue (Kainski et al., 1967) or increased in pig muscle by vitamin E deficiency (Bender et al., 1959), and by exercise (Hagler et al., 1980; Pattengale and Holloszy, 1967). In an interesting study, Thomas and Judge (1970) showed that myoglobin in porcine skeletal muscle reached higher concentration in animals reared at constant ambient temperature vs fluctuating temperature environments, provided that the humidity was moderate to high. It has also been shown that animals acclimatized to high altitudes showed increased muscle myoglobin levels (Vaughan and Pace, 1956).

Finally, there is always animal-to-animal variation in muscle myoglobin level for all species, and genetic factors may play an important role. For example, Hart (1961) found lower myoglobin concentration in porcine pale, soft, and exudate (PSE) muscle. However, this finding has been disputed by other workers (Briskey and Wismer-Pederson, 1961).

CHEMISTRY OF MYOGLOBIN WITH RESPECT TO COLOR

There are three major classes of pink or red pigments: undenatured myoglobin, nitrosyl pigments, and reduced hemochromes. Most of the nitrosyl-related work on pink defect in cooked meat has focused on factors affecting pink color without attempting to identify the nature of the pink pigment. It is important to know the nature of the pink pigment in order to prevent its formation. For instance, high cooking temperature will denature oxymyoglobin and destroy pink color, but high cooking temperature enhances formation of denatured globin hemochromes. Thus, it is important to know the chemistry of the various pink pigments. These pigments can be distinguished by their different reflectance and absorbance spectra. Heme iron oxidation state and nature of the ligand (nicotinamide, O_2, NO, CO, etc.) affect spectral characteristics. In order to identify the pigment responsible for the pink color, it is necessary to understand how the spectral characteristics are influenced by heme iron chemistry. Thus, the following sections briefly review heme pigment chemistry and discuss the influence of heme iron chemistry on reflectance and absorbance spectra.

Iron has an atomic number of 26 and is one of the nine transition metals of the first transition series in the Fourth period of the Periodic Table. Iron has 2 electrons in the K shell, eight in the L shell, fourteen in the M shell, and only 2 electrons in the N shell. Thus, the electronic structure of iron in the ground state is $1S^2\ 2S^2\ 2P^6\ 3S^2\ 3P^6\ 3d^6\ 4S^2$. Iron exhibits oxidation states of +2, +3 and +6, and the oxidation state relates to incompletely filled 3d sub shells. The main consideration is for iron with oxidation states of +2 or +3, the reduced and oxidized ionic iron, respectively. Loss of two electrons from elemental iron results in the electronic structure of $1S^2\ 2S^2\ 2P^6\ 3S^2\ 3P^6\ 3d^6$ for ferrous iron. Ferric iron, oxidation number of +3, corresponds to loss of three electrons. The electronic structure is $1S^2\ 2S^2\ 2P^6\ 3S^2\ 3P^6\ 3d^5$ (Nebergall et al.,1976).

Heme, the non-protein unit of myoglobin and hemoglobin, consists of an iron atom and an organic portion called protoporphyrin which is made up of four pyrrole groups linked by methane bridges to form a tetrapyrrole ring. Four methyl, two vinyl, and two propionate side chains are attached to the tetrapyrrole ring. These substituents can be arranged fifteen different ways. Only one of these isomers, known as protoporphyrin IX (Fig. 1), is present in biological systems (Stryer, 1975).

The iron atom in heme binds to the four nitrogen atoms of the tetrapyrrole ring. There are two additional sites for bonding iron, one on either side of the heme plane. These binding sites are known as the fifth and sixth coordination positions (Stryer, 1975).

The d orbitals of iron consist of five lobe-shaped regions, designated d_z2, $d_x2_{-y}2$, dxy,d_{xz}, and d_{zy}. For an octahedral configuration for a metal, i.e., heme iron in myoglobin, the d_z2 and $d_x2_{-y}2$ orbitals which point toward the corners of the octahedral are referred to as antibonding orbitals and the remaining three orbitals, d_{xy}, d_{xz}, and d_{zy}, which point in between the corners of the octahedron, are called nonbonding orbitals. Any electrons which occupy the lobes of the antibonding orbitals will be repelled by the electron pairs belonging to ligand groups located at the corner of the octahedron. On the other hand, electrons in the non bonding orbitals are not directly affected by the electron pair of the reacting ligands. The energy level of antibonding and non bonding orbitals is referred to as the e_g and t_{2g} levels, respectively. The electrons at the e_g level are at higher energy level. The difference in energy between these two levels for a given complex (Fig. 2) is a measure of the energies involved in the movement of the electrons from one energy level to other (referred to as electronic transitions) (Nebergall et al., 1976).

Iron in the oxidized state has five unpaired electrons in its d orbital, and according to crystal field theory, when the iron is coordinated to a ligand producing a weak field, the five electrons remain, one in each of the five d orbitals, i. e., three unpaired electrons at the t_{2g} energy level and two unpaired electrons at the e_g level. However, if the bonding ligand

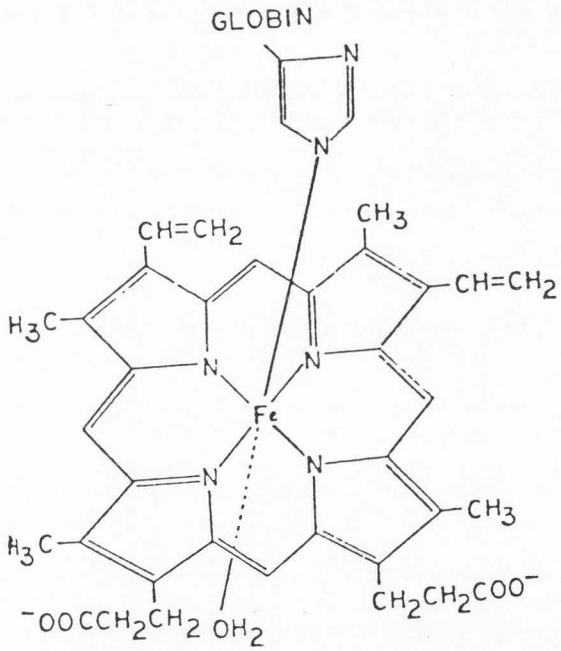

Fig. 1 - Structural representation of myoglobin (Govindarajan, 1973)

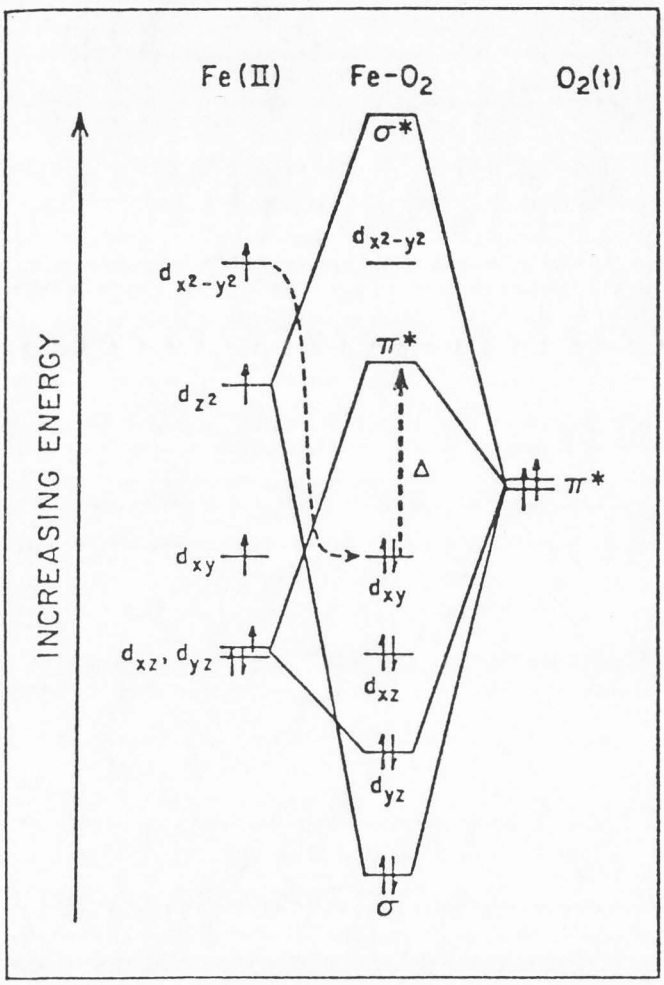

Fig. 2- Energy level diagram for the Fe-O_2 complex (Livingston and Brown, 1981)

produces a strong electrostatic field on the iron, the five unpaired electrons of ferric iron remain at the t_{2g} energy level. The complex of ferric iron with ligands of low field strength is called a high spin compound. The complex of ferric iron with a ligand of high field strength is low spin. The same terminology exists for iron in the reduced state. The strong field, low spin complex in this case is the one in which six electrons of the d orbitals all are at the t_{2g} energy level. Weak field, high spin complexes then are those in which four electrons out of six electrons are at the t_{2g} energy level and the remaining two electrons at e_g energy level (Nebergall et al., 1976). In general, ferrous iron with ligands producing strong field complexes is more stable than those with ligands forming low field complexes (Nebergall et al., 1976). A compound with no unpaired electron(s) is diamagnetic while paramagnetism is characteristic of a substance containing one or more unpaired electrons (Bromberg, 1984). The only diamagnetic heme iron complex is a high field, low spin ferrous iron, i.e., oxymyoglobin (Nebergall et al., 1976; Livingston and Brown, 1981). Complexes of ferric iron (low or strong field) and complexes of low field ferrous iron all have unpaired electrons and, therefore, exhibit characteristics of paramagnetic substances (i.e., metmyoglobin, nitric oxide metmyoglobin, and deoxymyoglobin) (Brill and Williams, 1961; Nebergall et at.,1976).

There are two types of bonding of iron to ligands at the fifth and sixth coordination positions of heme . The sigma bond is formed by donation of electrons from the ligand to the iron (iron acts as electron acceptor). Pi back-bonding occurs when the iron gives up the electrons back to the ligand (iron acts as a electron donor, Williams, 1956). Ferrous iron has a lower charge on the nucleus and more electrons in its d orbitals as compared to ferric iron. This enables ferrous iron to form strong pi back-bonding with suitable ligands. However, ferric iron does not form strong pi back-bonding (Livingston and Brown, 1981). Ligands, such as oxygen and NO, bind to ferrous iron of the heme with strong pi back-bonds. However, the oxygen molecule with its two unpaired electrons cannot bind to

metmyoglobin because ferric iron of the heme in this complex does not engage in strong pi back-bonding. On the other hand, NO has one unpaired electron which participates in sufficiently strong sigma bonding with metmyoglobin to enable stable bonding (Nebergall et al., 1976; Livingston and Brown, 1981). Ligands producing a high electrostatic field by bonding to the sixth position of heme iron can stabilize the ferrous iron toward oxidation. This is important in the case of NO which can labilize the ligand at the fifth position, the histidine group. During heat denaturation the globin histidine ligand may be replaced with a second NO ligand. The resulting di-nitrosohemochrome complex is quite stable to oxidation and ligand exchange (Tarladgis, 1962b; Livingston and Brown, 1981).

As mentioned before, there is an energy gap between e_g and t_{2g} energy levels, and visible light can excite electrons raising them to the higher energy level. The exact wavelength absorbed and visible wavelengths which are transmitted are important for color of an iron complex, i. e., the oxymyoglobin complex having red color. The bonding pattern between oxygen and the iron (pi back-bonding) is also important in forming color in the complex (Livingston and Brown, 1981).

Heme-globin interaction influences not only the overall electronic configuration of the heme, but also its ligand binding properties. The globin histidine imidazole at the fifth position feeds extra electron density into the iron; therefore, the iron has more ability to donate electrons to the ligand at the sixth position via pi back-bonding (Livingston and Brown, 1981). The basicity, or ability to donate electrons, of the ligand at the fifth position can determine the way that other ligands bind to the sixth position (Giddings, 1977). For example, ligands that are less basic than the imidazole group in the fifth position of deoxymyoglobin would raise the possibility of metmyoglobin formation instead of oxymyoglobin formation. On the other hand, a group with a very strong basicity at the fifth position would cause oxygen binding to deoxymyoglobin to become more irreversible. This condition would be undesirable from the respiratory stand point, but

would increase red color stability of fresh meat (Giddings, 1977).

Photoporphyrin IX ring characteristics are also important in binding properties of the iron center. Increasing electron-acceptor properties of the substituents in the porphyrin ring results in the iron becoming less electronegative and, therefore, having more tendency to participate in pi back-bonding with suitable ligands (Williams, 1956).

MYOGLOBIN PIGMENT IDENTIFICATION

Myoglobin pigment can be extracted in phosphate buffer as described by Krzywicki (1982). Relative concentration of myoglobin, oxymyoglobin, and metmyoglobin can be calculated by using formulas described by Trout (1989) for cooked meat products (Appendix C). Use of reflectance spectra for characterization and quantification of myoglobin pigment is common (Stewart et al., 1965; Snyder, 1965; Jermiah et al., 1972; Hunt, 1980). Absorption spectra can be recorded over the visible range from 400-700 nm. Reflectance spectra over the same range can be obtained on a meat slice with a reflectance attachment for identification for various derivatives of myoglobin pigments. All forms of myoglobin exhibit a large absorption peak about 420 nm, the soret bond, due to absorption by the porphyrin ring of heme. Myoglobin also exhibits a large single peak at 550 nm. Oxymyoglobin is usually the predominant pigment observed after blending in air. It exhibits two large absorption peaks, α and β, at 577 and 541 nm, respectively. Metmyoglobin has a broad absorption peak at 505 nm and a small shoulder peak at 630 nm (Fig. 3). All absorption maxima represent reflectance minima in the reflectance spectra. Often these absorption peaks (reflectance minima) were confused over several different pigments. To confirm presence of undenatured myoglobin derivatives Cornforth (1991) suggested addition of 1 drop of 2% ferricyanide and 1 drop of 2% potassium cyanide to the cuvette, converting all myoglobin pigments to cyanometmyoglobin. It exhibits a single peak at 540 nm.

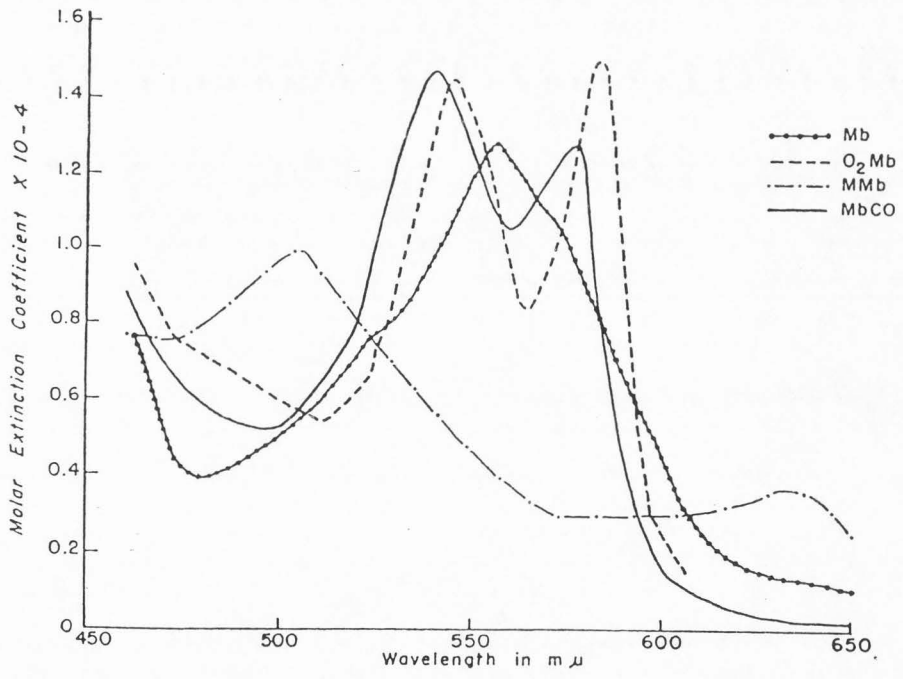

Fig. 3 - Absorption spectra of Mb, O2Mb, MMb and MbCO (El-Badawi et al., 1964)

PIGMENTS RESPONSIBLE FOR PINK COLOR IN COOKED MEATS

Gray brown denatured metmyoglobin is the most probable and expected pigment in cooked meats. Red or pink discoloration in cooked meats leads consumers and inspectors to assume product is undercooked. Over the years researchers have identified undenatured myoglobin, nitritosylhemochrome, carbon monoxide hemochrome, and globin hemochromes as the potentially pink discoloration pigments in cooked meat products. Cytochrome c is the residual pink pigment remaining after oxidation of other pink pigments (Girard et al., 1991). The undesired pink discoloration may be present immediately after cooking, or it may gradually develop during distribution or retail display. Cornforth (1991) has developed a step-wise procedure for identification of pink pigments in cooked meats. Initial meat pH, cooking temperature, cooking methods, processing procedure, packaging, and microbial growth have all been shown to influence pink or red discoloration in cooked meats. Proper changes in product formulation or processing procedures may help to reduce pink discoloration in cooked meats.

Undenatured myoglobin

Cooking temperature and product pH may significantly alter stability of meat pigments. Red discoloration may occur in cooked meat products due to undenatured myoglobin. Schmidt and Trout (1984) and Trout (1989) have studied the effect of the pH on the color of the cooked meat. Beef, pork, and turkey meats were used, and pH of the meat was adjusted to 5.5, 6.0, or 6.5 before cooking. Samples were then cooked at different cooking temperatures (63, 68, or 74°C). The results indicated that meat with higher pH and lower cooking temperature had more pink-red color after cooking. It was suggested that high pH prevented the formation of brown cooked meat color. Mendenhall (1989) also reported that pink-red color found in hard-to-cook hamburger patties was associated with high raw meat pH. This problem occurs in high pH meat (bull meat, dark

cutters), particularly if the patties have been refrigerated before cooking. Refrigeration causes patties of high pH meat (pH 6.0-6.3) to gel. The hot gases formed during cooking do not penetrate these patties, and temperature in the interior of the pattie will not be as high as in normal patties. Thus, myoglobin is not completely denatured.

The effects of cooking temperature and storage period after cooking on color of turkey meat has been studied by Helmke and Froning (1971). Lower redness and higher lightness values were associated with raising cooking temperature from 60°C to 82°C. Occurrence of regenerated pink color within 2 hr storage at refrigeration temperature was also reported. The absorption spectra of extracted pigment from turkey meat cooked to 60° - 77°C was similar to that normally reported for oxymyoglobin. However, the pigment extracted from the meat cooked to 82°C had quite different spectra. Helmke and Froning (1971) pointed out the possibility that myoglobin in turkey meat was partially denatured during cooking, and this may cause the development of the pink in the meat during storage period. Results of the study done by Wierbicki et al. (1957) indicated that heat denaturation of muscle protein begins at about 40°C and is essentially completed at 70°C. Howe et al. (1982) studied development of pink color in cooked pork roasts. Their results from absorption spectra indicated that most of oxymyoglobin was denatured by 60°C. Storage temperature and time had no significant effect on absorption values, indicating that the development of pink color during storage was not a result of oxymyoglobin. Undenatured myoglobin in extracts can be identified by its peculiar absorbance maxima or reflectance minima.

Nitrite and nitrosylhemochrome

The most widely proposed cause for pink color in a fully cooked product is the contamination of meat with nitrite, nitrate or nitrogenous compounds and, subsequently, formation of nitrosylhemochromes. The effect of dietary nitrates and nitrites on color of poultry meat was studied by Froning et al. (1967). Uncooked white meat from chicken fed

nitrates was found to have both significantly higher a_L values and visual score as compared with that from control birds (Froning et al., 1967). The meat from nitrite-fed chickens seemed to have an upward trend of a_L values, but the differences were not significant. Visual scores and a_L values were increased significantly for both uncooked and cooked meat obtained from turkeys having either nitrite or nitrate in their diets (Froning et al., 1967). However, Mugler et al. (1970) reported that various levels of nitrate-nitrogen (75, 150, 300, and 450 ppm) in drinking water did not significantly affect the color of turkey meat. Meat from older birds was found to be significantly redder than the meat from younger birds. Presence of at least 200 ppm nitrate-nitrogen in chill water was necessary to make turkey meat red.

Scriven et al. (1987) found that the starch-based adhesives used on paper tape and some poultry giblet bags often contained high levels of nitrate. They found pink color in steak after cooking. Nitrate may have leached from the wetted tape or the bags. Scriven et al. (1987) found little migration of nitrite or nitrate from giblet paper to chicken breast, and concluded that nitrite or nitrate contamination from packaging was unlikely to be a major cause of residual pink color in processed poultry products.

The effect of exposure to engine exhaust fumes prior to slaughter on color of poultry meat was also studied (Froning et al., 1969). Birds may become exposed to gases such as NO or CO from the truck during transfer to slaughter. With the exception of uncooked white chicken, a_L values were increased significantly in all treatments. The same increase was also noted for visual redness scores.

Froning et al. (1968) found that spray-dried egg albumen caused pink color when added as binder to turkey rolls. Spray-dried albumen was found to increase significantly the redness values of cooked turkey meat, but pan-dried albumen did not affect these values. The pinkness in the meat caused by the spray-dried albumen was decreased significantly by allowing the meat to stand 30 min in the air. More stable pink color in the

meat was produced when the level of spray-dried albumen increased to 10%. This pinkness was present in the samples kept at refrigeration temperature for four or five days. It was suggested that the observed pinkness was possibly caused by either a change in pH or formation of an iron-conalbumin complex. Another possibility is that protein additives added as meat binders might be nitrosylated during drying by an open flame procedure (Cornforth, 1991). Nitrogen oxides (NO_2) are produced in the combustion gases and may directly react with pigments in meat cooked in a gas oven (Pool, 1956), or perhaps react with egg, soy, or milk proteins during drying. Ito et al. (1983) demonstrated that pink nitrosyl hemochrome was formed when myoglobin was incubated with nitrosolated lysozyme or albumin and ascorbic acid. Thus, protein nitrosolated during drying could conceivably release NO under reducing conditions in cooked meat, leading to pink color development.

Nitrosyl pigments can be extracted and measured as described by Hornsey (1956) (Appendix B). Eardman and Watts (1957) measured reflectance spectra for cured meat pigment and observed its fading in presence of air. Cured meat pigment has a broad reflectance minima (absorption maxima) in the wavelength range of 650 to 570 nm (Fig. 4). A reflectance ratio of 650/570 can be used to measure intensity of cured meat pigments. Reference values as reported by Erdman and Watts (1957) for the 650/570 nm ratio and cured color intensity are: no cured color = 1.1; moderate fade 1.6; less intense but noticeable cured color 1.7 to 2.0; and excellent cured color 2.2 to 2.6. Measurements should be taken immediately after cutting the product since exposure to light and air may reduce the color intensity. Barton (1967a,b) also reported use of reflectance wavelengths ratios of 570 and 650, and 540 and 560 nm in measurement of cured meat pigments. Discoloration or fading may occur in the display case. Decreased 'a' (redness) values and increased 'b' (yellowness) Hunter color values have been reported by Barton (1967a) during lighted display. Ratio of a/b is useful in measuring shift of pink color to tan;

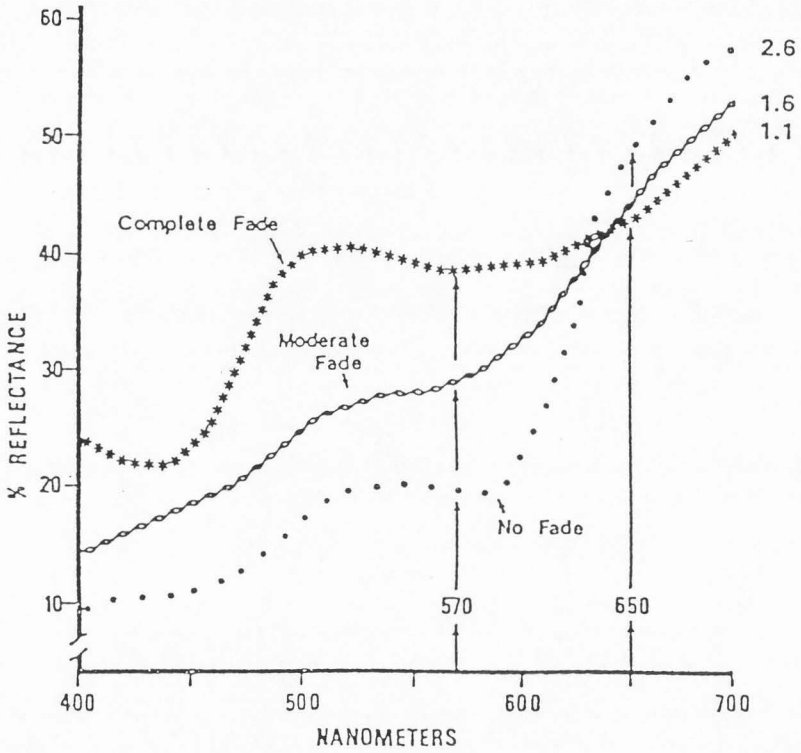

Fig. 4 - Reflectance spectra of nitrosohemochrome with and without fading of color. (Erdman and Watts, 1957)

similarly, sensitivity is obtained by reduction of the a- and b- values to hue angle = $\tan^{-1}$ b/a or cot a/b or saturation index= $(a^2+b^2)^{1/2}$ (Setser, 1984).

Carboxymyoglobin and carbon monoxide hemochrome

Carboxymyoglobin is the red pigment formed when fresh meat is exposed to low levels of carbon monoxide. Upon cooking, pigment will turn brown in color (Watts et al., 1978; Vahabzadeh et al., 1983). Tappel (1957a) reported that cooked beef turned pink upon exposure to carbon monoxide. The reflectance spectra were similar to that of denatured globin hemochromes. The pink pigment was accordingly labelled as denatured globin carbon monoxide hemochrome. Pool (1956) reported that the surface of the turkey breast meat turned pink when cooked in a gas oven. Pink color was less if the breast was covered with the thick skin from an older bird and eliminated in birds roasted in a plastic cooking bag. Pink color was observed when either CO or NO gas was circulated around birds cooked in an electric roaster. Interestingly, birds roasted in some electric tabletop roasters turned pink when the element was set on high and the air in contact with hot elements also circulated past the bird. Although open flames are known to generate both NO and CO, it is likely that NO was the gas responsible for pink color in birds cooked in a gas oven since adjustment of the flame to obtain more complete combustion did not reduce the incidence of the pink color. In birds or the large roasts or rolls exposed to CO or NO gas, a pink ring of 1/4" thick or less will develop. This ring is common and even desired in Texas barbecued beef where meat is slowly cooked in a heavy smoke (Cornforth and Carpenter, 1989).

Carboxymyoglobin has similar absorbance spectra to oxymyoglobin, but they differ in the peak wavelengths. Carboxymyoglobin can be identified by its absorbance maxima (reflectance minima) with α and β peaks at 540 and 575 nm and a soret γ band at 425 (Fig. 3) (Tappel, 1957a; El-Badawi et al., 1964).

Denatured globin hemochromes

Globin hemochromes are formed in meat cooked to an internal temperature of 71°C or greater. In fully cooked meats, myoglobin is denatured, and the heme iron is oxidized. The product is usually brown throughout after cooking. In large roasts or vacuum-packaged products, where oxygen is absent, the denatured metmyoglobin may gradually be reduced (i.e., the heme iron is converted to ferrous form) with refrigerated storage. The reduced or ferrous heme iron will weakly bind many types of nitrogen-containing compounds, including nitrogen groups on denatured proteins, or with nicotinamide, one of the B vitamins. The resultant complex will be pink and will fade upon exposure to air and light (Cornforth and Carpenter, 1988).

Brown and Tappel (1957) studied the properties responsible for the desirable pink color of precooked and canned tuna and it was concluded that the pigment was hemochrome in its nature. Either denatured globin or nicotinamide or both were found to form this pink color pigment in canned tuna. Later, Tappel (1957a) studied the pigments of cooked beef and concluded that the brown pigments were best characterized as mixed denatured globin hemichromes. The reflectance spectra of the cooked meat pigments have also been studied by Tarladgis (1962a). He concluded that the iron of this brown pigment had a denatured globin at the fifth position and the water molecule at the sixth molecule. This complex he concluded to be a high spin ferric iron and named the pigment metmyochromogen. Tarladgis (1962a) argued that presence of nicotinamide, a strong base (good electron donor), as an axial ligand would change the high spin complex to a low spin one. Giddings (1977) pointed out that the presence of an imidazole group, a strong base, at the fifth position of the cooked meat pigment does not change the high spin character of the complex to the low spin one. Therefore, the high spin nature of the cooked meat pigment does not rule out a denatured globin hemochrome having a nitrogenous base as a ligand at the sixth position (Giddings, 1977).

Meat preservation by irradiation causes some changes in myoglobin, which can affect meat color. According to Tappel (1957b) irradiation converts the brown pigment of precooked meat, denatured globin hemichrome, to a red pigment. Satterlee (1972) found that the red myoglobin pigment produced by gamma irradiation of metmyoglobin was oxymyoglobin, but he did not describe the exact mechanism by which oxymyoglobin was formed during the irradiation. One possibility that Satterlee (1972) mentioned was the formation of a reducing radical, during gamma irradiation, with the ability to reduce the iron of metmyoglobin. Irradiation can cause formation of oxymyoglobin from metmyoglobin, when there are high levels of metmyoglobin present initially (Livingston and Brown, 1981). Hansen et al. (1963) reported the development of an objectionable red color in irradiated chicken samples during anaerobic storage at elevated temperature. The irradiated chicken samples, which were partially cooked (68°C for 45 min), become objectionably red when stored for three months in a nitrogen atmosphere at 38°C. Very little red color developed in the samples stored aerobically even though no oxygen remained in the cans after three-month storage (Hansen et al., 1963).

Globin hemochromes are not solubilized by dilute HCl or NaOH. Also, they are not soluble in water, acetone-ether, petroleum ether, NH_3-NH_4Cl buffer (pH 9.0), chloroform, dilute acetic acid, or pyridine (Brown and Tappel, 1957). Figure 5 shows reflectance spectra of hemochromes from chicken breast meat slices and cooked tuna compared to standard nicotinamide hemochrome. This pigment can be identified by its characteristic reflectance minima (absorbance maxima) at 558 (α) and 530 (β) and soret band at 420 (γ) nm (Brown and Tappel, 1957)

Cytochrome c

Cytochrome c has been studied in many organisms and its structure has been determined in over one hundred species. It can be found across the entire spectrum of animals, plants, and anaerobic microorganisms except bacteria, making it an admirable tool

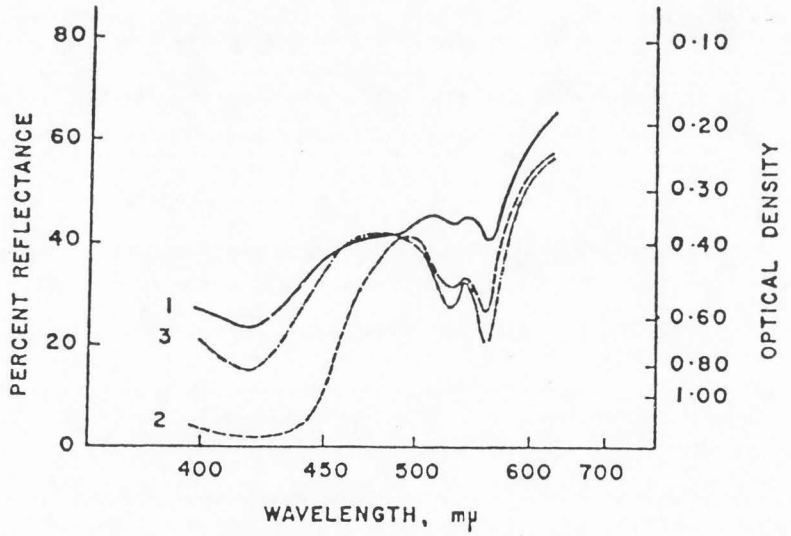

Fig. 5 - Hemochrome reflectance and absorbance spectra. 1. Chicken breast, 2. Tuna, 3. Nicotinamide. (Brown and Tappel, 1957)

for studying the process of molecular evolution. The three-dimensional structure of mitochondrial cytochrome c appears to have been essentially fixed at the time of emergence of the first eucaryotes and is usually considered to be one of the most evolutionarily conservative proteins (Dickerson, 1980).

A schematic representation of a mitochondrial cytochrome c from tuna is shown in Figure 6. The heart of the cytochrome c molecule is the heme group, a porphyrin ring surrounding a central iron atom. The same heme is also present in myoglobin, but there it is held within the protein framework in a different way. The covalent attachment to cysteines, as in cytochrome c, is missing, and only the histidine ligand is present in myoglobin. A heme has two polar propionic acid side chains ($-CH_2-CH_2-COOH$) attached to one edge. In myoglobin these side chains stick out into the aqueous surroundings. In cytochrome c the heme is instead rotated 90 degrees, so that one propionic acid group is just under the surface of the molecule of cytochrome c and in the other group is deeply buried in the globin portion of molecule. Although the overall environment of the heme crevice is hydrophobic due to the packing of aromatic and aliphatic amino side chains with the heme, tyrosine and tryptophan (amino acids No. 48 and 59 in the sequence of cytochrome c) compensate by forming hydrogen bonds with the buried propionic acid. These two hydrogen-bonded side chains are absolutely constant among all eucaryotes (Dickerson, 1980).

Girard et al. (1990) studied residual pink color in cooked turkey and pork. Sliced frozen meat (2 mm) was placed in between glass microscope slides separated with 2 mm spacers before cooking at different temperatures in a water bath. They observed typical globin hemochrome spectra in samples cooked to 85°C. After exposure to air, pink globin hemochromes faded. Absorbance spectra of oxidized samples showed presence of undenatured cytochrome c (Fig. 6). Figure 6 shows that the reduced form of cytochrome c possesses three characteristic peaks at 550, 521,and 415 nm identified by letters α, β, and

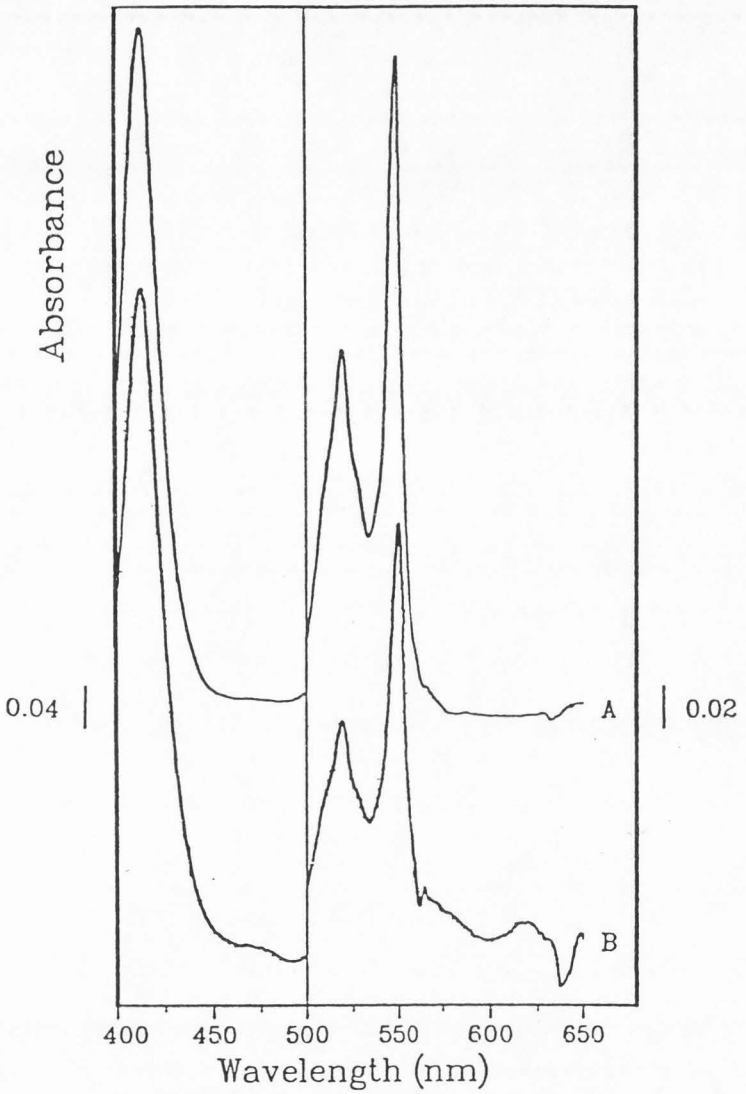

Fig. 6 - Absorption spectra of commercial solution of reduced cytochrome c (A) and cytochrome c from turkey slices cooked at 85°C for 10 min and reduced with sodium dithionite (B) (Girard et al., 1990).

γ, respectively (Girard et al., 1990). Table 1 summarizes absorption maxima (reflectance minima) for various pink or red pigments. Pigments can be identified with respect to their respective absorption maxima or reflectance minima (Cornforth, 1991).

ROLE OF MICROBIAL GROWTH WITH RESPECT TO MEAT COLOR

Bacterial contamination has played a role in fresh meat discoloration during refrigerated storage. Very little attention has been given to the effects of bacterial contamination on meat color. The majority of studies have concentrated on the spoilage bacteria *Pseudomonas spp.*, the predominant psychrotroph found in aerobically packaged meat (Ayres, 1969; Gill, 1983).

Rikert et al. (1957) inoculated ground pork samples with *Achromabactor* and reported improved color for treated versus control samples. However, after the initial 10 days of storage, the noninoculated controls were more red and surpassed the inoculated samples in color desirability. Butlar et al. (1953) inoculated beef steak surfaces with *Pseudomonas* and reported that the metmyoglobin formation rate was maximal during the log phase of the bacterial growth; a higher fading index was reported for the inoculated steaks. Metmyoglobin reduction was observed on steak surfaces and attributed to reducing conditions developed by high bacterial oxygen consumption. The author proposed that initially bacteria would reduce the oxygen partial pressure at the meat surface to the critical level for maximum metmyoglobin formation. As bacterial growth continued, the additional oxygen requirement would further decrease the surface oxygen partial pressure to essentially zero and thus favor the deoxy pigment form.

Faustman et al. (1990) inoculated ground beef homogenates with fluorescent *Pseudomonas* and *Brochotrix thermasphacta.* and stored at 4°C for 10 days. Metmyoglobin formation was increased with the growth of bacteria, but an apparent decrease in metmyoglobin was observed when bacterial population reached approximately

Table 1- Absorption maxima (reflectance minima) for verious pink and red pigments.

PIGMENT	ABSORPTION MAXIMA		
	γ (soret)	β	α
Myoglobin[1]	439	-	555
Oxymyoglobin[1]	420	541	577
Cytochrome c[2]	415	521	550
Nitrosyl hemochrome[3]	-	540	-
Denatured globin carbon[4] monoxide hemochrome	425	540	575
Globin hemochrome[4]	422	530	558
Nicotinamide hemochrome[5,6]	420	529	558

[1]El-Badawi et al., 1964.
[2]Girard et al., 1990.
[3]Hornsey, 1956.
[4]Tappel, 1957a.
[5]Cornforth et al., 1986.
[6] Cornforth, 1991.

10^8 CFU/g. They also showed that the supernatant from *Pseudomonas* cultures caused brown solutions of metmyoglobin to turn red. Kalchayanand et al. (1989) reported pink discoloration in vacuum-packaged beef and proposed that a *Clostridium species* was the causative agent.

The effect of packaging on meat microbiology may depend on the pack film and the use of different gases. In aerobically packed meats, *Pseudomonas* normally constitute a major portion of the microflora. By vacuum packaging, the accumulation of CO_2 is an important factor in the preservative effects and *Lactobacilli* multiply preferentially in the presence of CO_2. *Lactobacilli* would be able to control the development of spoilage bacteria; therefore lactic cultures could be used to improve the shelf-life of meats. Addition of *Lactobacilli* in vacuum-packaged beef leads to slight inhibitory effects on *Brochothrix thermosphacta,* no green discoloration, and a slight positive effect on color characteristics (Renerre and Montel, 1986).

SODIUM LACTATE AND COLOR

Organic acids such as lactic acid have been used for decontaminating carcasses (Smulders and Woolthuis, 1985; Woolthuis and Smulders, 1985) in fresh meats (Gill and Penney, 1985) but information on use of this salt in controlling microorganism growth and color in meat products is limited. Many food-borne pathogens, including *Clostridium botulinum, Salmonella, E. coli, Staphylococcus aureus,* and *Listeria monocytogens,* are inhibited by natural sodium lactate (Bacus and Bontenbal, 1991). Sodium lactate has been used for more than 20 years in the food industry as a humectant (Reid, 1969) and it has been used in the meat industry as a flavor enhancer and shelflife extender (Duxbury, 1988). Lamkey et al. (1991) have studied sodium lactate as a fresh pork sausage additive and have shown that surface discoloration and microbial growth were reduced by addition of sodium lactate.

Papadopolous et al. (1991a) studied effects of sodium lactate on the color characteristics of cooked meat. Cooked, vacuum-packaged beef top rounds were injected with different levels of sodium lactate (1 to 4%) and stored for 84 days at 0°C. They evaluated color on 0, 14, 28, 42, 56, 70, and 84 days of storage and found that increasing levels of sodium lactate (> 2%) did not significantly affect the surface grey area. Addition of sodium lactate improved flavor and palatability of cooked beef rounds.

Undissociated acids such as sodium lactate have the ability to cross molecular membranes of bacteria and become dissociated inside the cell and acidify the cell interior (Ingram et al., 1956; Hunter and Segal, 1973). Eklund (1983) reported that undissociated weak acids have 10 to 600 times more antimicrobial activity than their dissociated counterparts. Sodium lactate has been shown to inhibit microbial growth in many products (Lamkey et al., 1991; Popadopolous et al., 1991 a,b).

REFERENCES

AMSA. 1991 Guidelines for meat color evaluation. 44th Annual Reciprocial Meat Conference, Manhattan, Kansas.

Ayres, J. C. 1969. Temperature relationship and some other characteristics of the microbial flora developing on the refrigerated beef. Food Res. 25: 1.

Barton, P. A. 1967a. Measurement of colour stability of cooked cured meats to light and air. I. Development of method. J. Sci. Food Agric. 18: 298.

Barton, P. A. 1967b. Measurement of colour stability of cooked cured meats to light and air. II. Testing the procedure. J. Sci. Food Agric. 18: 305.

Bender, A. D., Schottelius, D. D., and Schottelius, B. A. 1959. Effect of short-term vitamin E difficiency on guinea pig skeletal muscle myoglobin. Am. J. Physiol. 197: 491.

Blessing, M. H. and Muller, G. 1974. Myoglobin concentration in the chicken, especially in the gizzard (A. biochemical light and electron microscopy study). Comp. Biochem. Physiol. 47A: 535.

Brill, A. S. and Williams, R. J. P. 1961. The absorption spectra, magnetic moments and the binding of iron in some hemoproteins. Biochem. J. 78: 246.

Briskey, E. J. and Wismer-Pederson, J. 1961. Biochemistry of pork muscle structure. 1. Rate of anaerobic glycolysis and temperature changes versus the apparant structure of muscle tissue. J. Food Sci. 26: 297.

Bromberg, J. P. 1984. "Physical Chemistry," 2nd ed. p. 993. Allyn and Bacon, Inc. Newton, Massachusetts.

Brown, W. D. 1962. The concentration of myoglobin and hemoglobin in tuna flesh. J. Food Sci. 27: 26.

Brown, W. D. and Tappel, A. L. 1957. Identification of the pink pigment of canned tuna. Food Res. 22: 214.

Bucas, J. and Bontenbal, E. 1991. Controlling listeria. Meat & Poultry. June, 64.

Butlar, O. D., Bratzler, L. J., and Mallman, W. L. 1953. The effect of bacteria on the color of prepacked retail beef cuts. Food Technol. 7(10): 397.

Cornforth, D. P. 1991. Methods for identification and prevention of pink color in cooked meat. Reciprocal Meat Conference Proceedings. 44: 53.

Cornforth, D. P., and Carpenter, C. E., 1988. Why cooked meat can be pink. Meat and Poultry, June, 44.

Cornforth, D. P., Vahabzadeh, F., Carpenter, C. E., and Bartholomew, D. T. 1986. Role of reduced hemochromes in pink color defect of cooked turkey rolls. J. Food Sci. 51: 1132.

Dickerson, R. E. 1980. Cytochrome c and the evaluation of energy metabolism. Sci. Am. 242(3): 136.

Duxbury, D. D. 1988. Natural sodium lactate extends shelf life of whole and ground meats. Food processing, Jan. p. 91.

Eardman, A. M. and Watts, B. M. 1957. Spectrophotometric determination of color change in cured meat. J. Agric. Food Chem. 5: 453.

Eklund, T. 1983. The antimicrobial effect of dissociated and undissociated sorbic acid at different pH levels. J. App. Bact. 54: 384.

El-Badawi, A.A., Cain, R.F., Samuels, C.E., and Anglemeier, A.F. 1964. Color and pigment stability of packaged refrigerated beef. Food Technol. May, 159.

Faustman, C., Johnson, J.L., Cassens, R.G., and Doyle, M.P. 1990. Color reversion in beef: Influence of psychrotrophic bacteria. Fleischwirtsch. 70: 676.

Froning, G. W., Hargus, G., and Hartung, T. E. 1968. Color and texture of ground turkey meat products as affected by dried egg white solids. Poultry Sci. 47: 1187.

Froning, G. W., Hartung, T. E., and Sullivan, T. W. 1967. Effects of dietary nitrates and nitrites on color of chicken meat. Poultry Sci. 46: 1261.

Froning, G. W., Mather, F. B., Daddario, J., and Hartung, T. E. 1969. Effect of automobile exhaust fume inhalation by poultry immediately prior to slaughter on color of meat. Poultry Sci. 48: 485.

Giddings, G. W. 1977. The basis of color in muscle foods. Critical Rev. Food Sci. Nutr. 9: 81.

Gill, C. O. 1983. Meat spoilage and evaluation of the potential storage life of fresh meat. J. Food Prot. 46: 444.

Gill, C. O. and Penney, N. 1985. Modification of in-pack conditions to extend the storage life of vacuum-packaged lamb. Meat Sci. 14: 43

Girard, B., Vanderstoep, J., and Richards, J.F. 1990. Characterization of the residual pink color in cooked turkey breast and pork loin. J. Food Sci. 55: 1249.

Govindarajan, S. 1973. Fresh meat color. CRC Critical Reviews in Food Technology. 1: 143.

Hagler, L., Coppes, R. I. Jr., Askew, E. W., and Herman, R. H. 1980. The influence of excercise and diet on myoglobin and metmyoglobin reductase in rat. J. Lab. Clin. Med. 95: 22.

Hansen, H. H., Brushway, M. J., Pool, M. F., and Lineweaver, H. 1963. Factor causing color and texture differences in radiation sterilized chicken. Food Technol. 17: 1188.

Hart, P. C. 1961. Determination of the color of meat by measuring the extinction value. Tijdschr. Diergeneesk 87: 3.

Helmke, A. and Froning, G. W. 1971. The effect of end point cooking temperature and storage on the color of turkey meat. Poultry Sci. 50: 1832.

Hornsey, H. C. 1956. The color of cooked cured pork. 1. estimation of the nitric oxide haem pigments. J. Sci. Food Agric. 7: 534.

Howe, J. L., Gullett, E. A., and Usborne, W. R. 1982. Development of pink color in cooked pork. Can. Inst. Food Sci. Technol. J. 15: 19.

Hunt, M. C. 1980. Meat color measurements. Proc. Recip. Meats Conf. 33: 41.

Hunt, M. C. and Hedrick, B. 1977. Chemical, physical and sensory characteristics of bovine muscle from four quality groups. J. Food Sci. 42: 716.

Hunter, D. R. and Segal, I. H. 1973. Effect of weak acids on amino acid transport by Penicillium chrysogenum: evidence for proton or charge gradient as driving force. J. Bact. 113: 1184.

Ingram, M., Ottaway, F. J. H., and Coppock, J. B. M. 1956. The preservative action of substances in foods. Chem. Ind. 42: 1154

Ito, T. Cassens, R. G., Greaser, M. L., and Izumi, K. 1983. Lability and reactivity of non heme protein bound nitrite. J. Food Sci. 48: 1204

Jermiah, L. E., Carpenter, Z. L., and Smith, G. C. 1972. Beef color related to consumer acceptance and palatability. J. Food Sci. 37: 476.

Kainski, M. H. Zinn, M. E., Merkel, R. A., and Hall, Z. L. 1967. Effect of iron and copper intake on iron, copper and myoglobin levels in skeletal pig tissues. J. Agric. Food Chem. 15: 721.

Kalchayanand, N., Ray, B., Field, R.A., and Johnson, M.C. 1989. Spoilage of vacuum-packaged refrigerated beef by *Clostridium*. J. Food Prot. 52: 424.

Krzywicki, K. 1983. The determination of haem pigments in meat. Meat Sci. 7: 29.

Lamkey, J.W., Leak, F.W., Tuley, W.B., Johnson, D.D., and West, R.L. 1991. Assessment of sodium lactate addition to fresh pork sausage. J. Food Sci. 56: 220.

Lawrie, R. A. 1979. "Meat Science," 3rd ed. Pergamon Press Ltd., Oxford.

Ledward, D. A. and Shorthouse, W. R. 1971. A note on the heme pigment concentration of lamb as influnced by age and sex. Animal Prod. 13: 193.

Livingston, D. L. and Brown, W. D. 1981. The chemistry of myoglobin and its reactions. Food Technol. 35(5): 244.

Mendenhall, V. T. 1989. Effect of pH and total pigment concentration on the internal color of cooked ground beef patties. J. Food Sci. 54: 1

Mugler, D. J., Mitchell, J. D., and Adams, A. W. 1970. Factors affecting turkey meat color. Poultry Sci. 49: 1510

Nebergall, W. H., Schmidt, F. C. and Holtzclaw, Jr., H. F. 1976. "General Chemistry," 5th ed, p. 986. D. C. Health and Company, Lexington, Kentucky.

Nishida, J. 1976. Changes in myoglobin content during development and growth of chicken. Jap. J. Vet. Sci. 38: 299.

Papadopoloulos, L.S., Miller, R.K., Acuff, G.R., Vanderzant, C., and Cross, H.R. 1991a. Effect of sodium lactate on microbial and chemical composition of cooked beef during storage. J. Food Sci. 56: 341.

Papadopoloulos, L.S., Miller, R.K., Ringer, L.J., and Cross, H.R. 1991b. Sodium lactate effect on sensory characteristics, cooked meat color and chemical composition. J. Food Sci. 56: 621.

Pattengale, P. K. and Holloszy, J. O. 1967. Augmentation of skeletal muscle myoglobin by a program of treadmill running. Am. J. Physiol. 213: 783.

Pool, M.F. 1956. Why does some cooked turkey turn pink? Turkey World. Jan., 1968.

Reid, T. F. 1969. Lactic acid and lactates in food products. Food Manufacture. 44(10): 54.

Renerre, M. and Montel, M. C. 1986. Inoculation of steaks with Lactobacillus species and effect on colour and microbial count. In: Proc. of 32nd European Meeting of Meat Research Workers, Ghent, Belgium. p. 213.

Rickansrud, D. A. and Hendrickson, R. L. 1967. Total pigments and myoglobin concentration in four bovine muscles. J. Food Sci. 32: 57.

Rikert, J. A., Bressler, L., Ball, C. O. and Stier, E. F. 1957. Factors affecting quality of prepackaged meat. II. Color studies. B. Effects of storage time, storage temperature, antioxident, bacteria, light, freezing and fat upon color of product. Food Technol. 11: 567.

Satterlee, L. D. 1972. Stability and characteristics of the pigment produced by gama irradiation of metmyoglobin. J. Food Sci. 37: 213.

Schmidt, F. and Trout, G. 1984. pH and color. Meat Industry, August, p 30.

Scriven, F., Sporns, P., and Wolfe, F. 1987. Investigation of nitrite and nitrate levels in paper materials used to package fresh meat. J. Agric. Food Chem. 35: 188.

Setser, C. S. 1984. Color : reflections and transmissions. J. Food Quality. 6: 183

Smullders, F. J. M. and Woolthuis, C. H. J. 1985. Immediate and delayed microbiological effects of lactic acid decontamination of calf carcasses- influence on conventionally boned verses hot boned and vacuum-packaged cuts. J. Food Prot. 48: 838.

Snyder, H. E. 1965. Analysis of pigment at the surface of the beef using reflectance spectrophotometry. J. Food Sci. 457.

Stewart, M. R. Hutehins, B. K., Zipser, W., and Watts, B. M. 1965. Enzymic reduction of metmyglobin in ground beef. J. Food Sci. 30:487.

Stryer, L. 1975. "Biochemistry," p. 877. W. H. Freeman and Company, San Francisco, CA.

Tappel, A. L. 1957a. Reflectance spectral studies of the hematin pigments of cooked beef. Food Res. 22: 404.

Tappel, A. L. 1957b. The red pigment of precooked irradiated meats. Food Res. 22: 408

Tarladgis, B. G. 1962a. Interpretation of the spectra of meat pigments. I. Cooked meats. J. Sci. Food Agric. 13: 481

Tarladgis, B. G. 1962b. Interpretation of the spectra of meat pigments. I. Cured meats. The mechanism of color fading. J. Sci. Food Agric. 13: 485.

Thomas, N. W. and Judge, M. D. 1970. Alteration of porcine skeletal muscle myoglobin by the environment. J. Agric. Sci., Camberra, 74: 241.

Topel, D. G., Merkel, R. A., Mackintosh, D. L., and Hall, J. L. 1966. Variation of some physical and biochemical properties within and among selected porcine muscles. J. Animal Sci. 25: 277.

Trout, G. R. 1989. Variation in myglobin denaturation and color of cooked beef., pork and turkey meat as influenced by pH, sodium chloride, sodium tripolyphosphate. and cooking temperature. J. Food Sci. 54: 536.

Vahabzadeh, F., Collinge, S. K., Cornforth, D. P., Mahoney, A. W., and Post, F. J. 1983. Evaluation of iron binding compounds as inhibitor of gas and toxin production by clostridium botulinum in ground pork. J. Food Sci. 48: 1445

Vaughan, B. E. and Pace, N. 1956. Changes in myoglobin content of the high altitude acclimatized rat. Am. J. Physiol. 185: 549.

Watts, D. A., Wolfe, S. K., and Brown, W. D. 1978. Fate of [^{14}C] carbonmonoxide in cooked or stored groung beef samples. J. Agr. Food Chem. 26: 210

Wierbicki, E., Eunkle, L. E. and Deatherage, F. E. 1957. Changes in water holding capacity and cationic shifts during the heating and freezing and thawing of meat as revealed by a simple centrifugal method for measuring shrinkage. Food Technol. 11: 69.

Williams, R. J. P. 1956. The properties of metalloporphyrins. Chem. Rev. 56: 299.

Woolthuis, C. H. J. and Smullders, F. J. M. 1985. Microbial decontamination of calf carcasses by lactic acid sprays. J. Food Prot. 48: 832.

PART II. EFFECTS OF SODIUM LACTATE, pH, COOKING TEMPERATURE, AND STORAGE TIME ON COLOR AND MICROBIAL LOAD OF VACUUM PACKAGED BRATWURST

ABSTRACT

A red discoloration sometimes occurs in vacuum-packaged bratwurst during storage. This study attempted to characterize the red pigment and determine the effect of pH, cooking temperatures, storage temperature, and sodium lactate on incidence of red discoloration. Myoglobin was identified in the exudate of samples with red discoloration. Myoglobin levels of cooked products were significantly lower in samples of low initial pH (5.5) or cooked to higher than normal internal temperature (74°C). Red discoloration was associated with microbial growth. Frozen samples had no red discoloration after 4 weeks storage. Microbial plate counts and the incidence of red discoloration were lower in samples of lower pH (5.5 vs 5.8 or 6.2), samples cooked to higher internal temperature (74° vs 68°C), or samples containing sodium lactate (0 vs 3%).

INTRODUCTION

Precooked, vacuum-packaged pork products such as bratwurst offer several advantages, including the convenience of rapid microwave reheating. However, the product sometimes develops a red surface discoloration during refrigerated storage. Consumers assume such products are undercooked. In fact, there are several possible causes of pink or red color in cooked meats, other than undercooking. High pH (> 6.0) stabilizes myoglobin to heat, leading to a red color after cooking (Schmidt and Trout, 1984; Trout, 1989). One example is hard-to-cook hamburger patties which result from use of high pH raw materials such as bull meat or dark cutters (Mendenhall, 1989). Pink globin

hemochromes may develop in canned meats (Brown and Tappel, 1957) or well cooked meats such as turkey rolls (Cornforth et al., 1986). Exposure of meat to combustion gases containing carbon monoxide or nitric oxide will result in surface pink color development (Pool, 1956). Nitrate or nitrite contamination of ingredients or water is often suspected of causing unwanted cured meat color. Nitrate contamination of the adhesive used in wrapping tape has been shown to cause undesired pink color in beef roasts, due to migration of nitrate from wetted tape to meat during thawing (Scriven et al., 1987). Undenatured cytochrome c may contribute to pink color of cooked meats (Girard et al., 1990), but its relative role remains unclear since cytochrome c may also be detected in oxidized (and presumably brown-colored) samples. When pink or red discoloration occurs in cooked meats, it is essential to identify the responsible pigment since prevention measures differ for each pigment. A stepwise procedure for red or pink pigment identification in cooked meats has been described (Cornforth, 1991).

Color reversion (brown to red color transformation) has been associated with microbial growth in fresh ground beef homogenates (Faustman et al., 1990) and fresh vacuum-packaged beef (Kalchayanand et al., 1989). Microbial growth may be associated with red discoloration of vacuum-packaged bratwurst during storage. If so, higher cooking temperature, lower product pH, or antimicrobial agents may reduce color problems and extend shelflife. Thus, the objective of this study was to determine the effects of sodium lactate, pH, cooking temperature, and storage time on color and microbial load of vacuum-packaged bratwurst.

MATERIALS AND METHODS

Experimental design and data analysis

The experiment was a split split plot randomized block design. Lactate levels (0 and 3%) were whole plot treatments. The pH levels (5.5, 5.8, 6.5) were subplot

treatments. Cooking temperature (68 or 74°C), storage temperature (-20 or 2°C) and storage time (0, 1-4 weeks) were sub-subplot treatments. There were 10 judges trained for panel evaluation of color. Analysis of variance on treatment means was done using the FCTCVR program (Hurst, 1989).

Bratwurst preparation

Formulation. Bratwurst were prepared using fresh 80:20 pork trim (U. S. # 1) and lean beef trim in equal portions. Meat (13.6 kg beef, 13.6 kg pork) was coarsely ground through a 0.64 cm plate. After thorough mixing, meat was divided into 2 equal portions, and dry ingredients were added as follows: nonfat dry milk (130 g), salt (246 g), black pepper (27 g), mace (13 g), coriander (13 g), nutmeg (9 g), ginger (3 g), monosodium glutamate (21 g), and dextrose (63 g) (Appendix A). Three percent sodium lactate (65% pure sodium lactate in solution) (Archer Daniels Midland Co., Decatur, IL) was then added to half the meat. The other half (control) received an equal volume of distilled water.

pH adjustment. Both control and lactate-treated meats were further divided into 4.5 kg portions. pH was adjusted to 5.5, 6.5, or 5.8 (unadjusted control) by addition of either 1 N HCl or NaOH. After mixing, the meat was equilibrated at 2°C overnight. pH was then checked, and more acid or base was added as needed.

For meat samples without added lactate, pH after equilibration was 5.86, 5.46, and 6.42 for unadjusted samples, or samples adjusted to 5.5 or 6.5, respectively. For samples with 3% lactate, pH after equilibration was 5.80, 5.54, and 6.48 for unadjusted samples, or samples adjusted to 5.5 or 6.5, respectively.

Mixture preparation and cooking. Each pH-adjusted batch (4.6 kg) was coarsely chopped (~ 20 turns) in a bowl chopper (Hely-Joly, Tassin, France) with addition of 7.5% water (.34 kg ice). The mixture was then stuffed (Koch, Kansas City, MO) into 28 mm collagen casings (Koch, Kansas City, MO). Six-inch links were formed and

cooked to an internal temperature of 68 or 74°C in a Vortron, Inc. (Beloit, WI) smokehouse, equipped with a thermocouple probe and digital readout to monitor product internal temperature. A commercial cook schedule was used as follows: 15 min at dry bulb temperature (DBT) of 38°C; 20 min at DBT of 49°C; 15 min at DBT of 54°C; 15 min at DBT of 60°C; 15 min at 66°C; 20 min at DBT of 79°C, to desired internal temperature of 68°C. For product cooked to 74°C internal, an additional cooking step was added: DBT of 88°C until product internal temperature reached 74°C (<1 hr). The cooked bratwurst were showered with cold water for 2 min, then stored at 2°C overnight. After cooling, the bratwurst were removed from the casings, vacuum-packaged (2 per package) in 6x12-inch clear laminated nylon-polyethylene bags (O_2 permeability = 0.6 g O_2/625 cm^2/24 hr at 0°C; Koch, Kansas City, MO), and stored for 4 weeks frozen at -20°C or refrigerated at 2°C.

Visual color ranking

Each week frozen bratwurst were thawed in water and evaluated for visual color compared to refrigerated samples. Treatments were presented to the panel coded, and score was evaluated by a ten-member trained panel, using a 10-point scale where 1=gray and 10=red (undercooked) (AMSA, 1979).

Myoglobin extraction

Total extractable myoglobin was determined using a modification of the procedure of Warriss (1979) (Appendix C). Five gram samples in polyethylene centrifuge tubes were blended with a polytron homogenizer (Brinkmann Instruments, Westbury, NY) for 20 seconds in 25 ml of ice cold 0.04 M phosphate buffer, pH 6.8. After pigment extraction for 1 hr at 4°C, the homogenates were centrifuged at 6500 g for 45 min. The supernatant was further clarified by filtration through Whatman No. 1 filter paper. Visible absorbance spectra were obtained on a recording spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD). Absorbance values at 525, 572, and 700 were used to calculate total

extractable myoglobin and metmyoglobin as percent of total extractable myoglobin (Trout, 1989).

Pigment identification

Pink pigment identification was done by spectrophotometric procedures, as outlined by Cornforth (1991). Red exudate, when present, was collected from the vacuum-packaged sample by syringe and clarified by filtration through a 0.45 mm disposable filter disc (Gelman Sciences, Ann Arbor, MI). Visible absorption spectra were then obtained on a recording spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD). Myoglobin confirmation was done by addition of one drop of 2% potassium ferricyanide to the cuvette (oxidizing all myoglobin or oxymyoglobin to the metmyoglobin form), followed by addition of 1 drop of 2% potassium cyanide. The appearance of a single broad cyanometmyoglobin peak at 540 nm (Warriss, 1979) was further indication that the original sample contained myoglobin.

Reflectance spectra were also obtained for pigment identification in bratwurst, using an integrating sphere reflectance attachment to the spectrophotometer. Cooked bratwurst samples (0.5 cm thick) were placed at the reference port in clear polyethylene pouches (Koch, Kansas City, MO) to prevent soiling of the integrating sphere. There was no difference in baseline of samples with or without polyethylene pouches during baseline correction. The white reference was a similar sample oxidized in hydrogen peroxide for 10-12 min. Visible reflectance spectra were obtained from 450-700 nm to differentiate among possible pink pigments, including nitrosopigment, myoglobin, or globin hemochromes.

Aerobic and anaerobic plate counts

Aerobic and anaerobic plate counts were done by standard procedures (Messer et al., 1978). Twenty five gram samples were taken for the first dilution. Standard plate

count agar (Difco, Detroit, MI) was used as the growth medum. For anaerobic plate counts, plates were incubated in an anaerobic jar (BBL Gas Pack System, Becton Dickinson and Co., Cockeysville, MD). Duplicate plates were counted after incubation at 37°C for 48 hr.

pH measurement

The pH was measured after blending a 10-gram sample with 90 ml distilled water for 1 min with a polytron homogenizer. The pH of homogenates was measured with an Orion pH electrode and Orion Research pH meter Model 601 A (Cambridge, MA) (Appendix D).

RESULTS

Treatment main effects

Bratwurst is an uncured product with the typical gray color of cooked meat. However, a light red surface discoloration appeared on some vacuum-packaged, precooked bratwurst during storage, accompanied by a red-colored exudate. After 4 weeks storage, panelists rated refrigerated bratwurst significantly ($P < 0.05$) more red (Appendix F, Table 8) than frozen control samples (Table 2). PH, cooking temperature, and storage temperature also significantly affected color score (Table 2). Bratwurst at pH 6.5 was significantly more red than samples made at lower pH. Panelists only evaluated bratwurst in vacuum packages. However, the interior of bratwurst at pH 6.5 was also very noticeably more red than bratwurst at pH 5.8 or 5.5.

Bratwurst cooked to an internal temperature of 68°C was significantly more red than samples cooked to 74°C (Table 2). Refrigerated bratwurst were rated significantly more red than frozen samples (Table 2). Sodium lactate had no significant effect on bratwurst color score when data were pooled over all storage times (Table 2) (Appendix F, Table 8). Total extractable myoglobin is the fraction of the pigment that is soluble and undenatured

Table 2.- Means for main effects of lactate, pH, cooking temperature, storage temperature and storage time on color score, myoglobin content, percent metmyoglobin and microbial load of vacuum-packaged bratwurst.

Variable		Color[1] score	Mb[2] (mg/g)	MMb[3] (%)	APC[4] (logCFU/g)	ANPC[5] (logCFU/g)	pH
sodium lactate (%)	0	3.9	1.20	58.5	5.86	5.23	5.78
	3	3.3	1.22	55.5	5.31	4.61	5.87
pH	5.5	2.2[a]	0.48[a]	80.3[a]	5.53	4.86	5.47[a]
	5.8	3.0[b]	0.85[b]	53.4[b]	5.61	4.98	5.78[b]
	6.5	5.7[c]	2.30[c]	37.4[c]	5.63	4.91	6.23[c]
cooking temp. (°C)	68	3.8[a]	1.42[a]	54.2[a]	5.82[a]	5.27[a]	5.85
	74	3.4[b]	0.99[b]	59.8[b]	5.35[b]	4.56[b]	5.81
storage temp. (°C)	2	3.7[a]	1.17[a]	58.6	5.78[a]	5.11[a]	5.66[a]
	-20	3.5[b]	1.27[b]	56.8	4.45[b]	3.17[b]	5.99[b]
storage time (wks)	0	3.6[a]	1.18[a]	57.2	4.21[a]	2.84[a]	6.06[a]
	1	3.6[a]					
	2	3.6[a]	1.12[b]	54.6	6.08[b]	5.95[b]	5.89[a]
	3	3.2[b]					
	4	4.1[c]	1.16[a]	59.9	7.37[c]	7.38[c]	5.59[b]

[1] 1 = gray, 10 = red
[2] Mb = Myoglobin. Total Mb extracted in cold 0.4 M phosphate buffer, pH 6.8.
[3] MMb = Metmyoglobin, % of total extractable Mb.
[4] APC = aerobic plate count. CFU/g = colony forming units/gram meat
[5] ANPC = anaerobic plate count
[a-c] Mean values for treatments in each variable with different letter superscripts are significantly different ($p < 0.05$, a, b, c), the absence of superscripts indicates, nonsignificance.

and includes myoglobin, oxymyoblobin, and metmyoglobin. Total extractable myoglobin levels of cooked bratwurst were significantly affected by meat pH, cooking temperature, storage temperature, and storage time (Table 2). Extractable myoglobin levels were much higher in samples of pH 6.5 than in bratwurst at pH 5.8 or 5.5. Extractable myoglobin levels were also higher in samples cooked at lower temperatures (68 vs 74°C). Refrigerated (2°C) samples had less extractable myoglobin than frozen samples had due to prior loss of myoglobin to the exudate that appeared in refrigerated samples during storage. Extractable myoglobin levels decreased with storage time, probably also due to loss of myoglobin to exudate. The fraction of brown metmyoglobin was higher at low pH (5.5 vs 6.5), or in bratwurst cooked at higher temperature (74 vs 68°C).

Total plate counts were higher in bratwurst cooked to lower internal temperature (68°C) or in refrigerated samples as compared to frozen samples. As expected, plate counts increased significantly with storage time (Table 2). Mean aerobic and anaerobic plate counts of raw emulsions were 1.4×10^5 and 6.3×10^3, respectively. After cooking to 68°C, aerobic and anaerobic plate counts were reduced to 3.2×10^4 and 1.2×10^3, respectively. After cooking to 74°C, aerobic and anaerobic plate counts were reduced to 3.8×10^3 and $<1 \times 10^2$, respectively. pH of cooked bratwurst were affected by meat pH, storage temperature and storage time. Bratwurst emulsions with adjusted pH of 5.5 or 6.5 resulted in cooked bratwurst with mean pH of 5.47 and 6.23, respectively. Refrigerated samples had mean pH of 5.66 vs 5.99 for frozen bratwurst. PH declined significantly during storage, from an overall mean of 6.06 initially to pH 5.59 after 4 weeks storage (Table 2), probably due to effects of microbial growth.

Treatment interactions

The effects of pH and storage time on color score of vacuum-packaged bratwurst are shown in Figure 7. Initially, bratwurst at pH 6.5 was more red than bratwurst at pH 5.8 or 5.5. Color scores did not change appreciably through 3 weeks storage. However, a

significant increase in red discoloration was observed by the fourth week of storage. Color reversion (brown to red transformation) was especially noticeable in bratwurst at pH 5.8. Samples at pH 6.5 were rated more red than others after 4 weeks storage, but color reversion was less noticeable since samples at pH 6.5 were quite red initially (Figure 7). Bratwurst formulated with 3% sodium lactate remained brown-colored even through 4 weeks storage while samples without lactate had a significant ($p < 0.05$) (Appendix F, Table 13) increase in red discoloration from the third to fourth week (Figure 8). Bratwurst with 3% lactate and cooked to 74°C internal temperature was rated by the panel as significantly ($p < 0.05$) more brown colored than samples without lactate and cooked to 68°C (Figure 9). After cooking, mean aerobic and anaerobic plate counts were about 1.5×10^4 and 6.9×10^2, respectively, and no differences in microbial plate counts were apparent in samples with or without sodium lactate (Figure 10). After 4 weeks storage, mean aerobic and anaerobic plate counts had increased to 2.3×10^7 and 2.4×10^7, respectively. Samples containing lactate had significantly lower plate counts. After 4 weeks storage, mean aerobic plate count was 9.3×10^7 and 5.6×10^6 for samples without or with lactate, respectively. Mean anaerobic plate count was 7.8×10^7 and 7.2×10^6 for samples without or with 3% lactate. Microbial load was also significantly affected by the interaction of storage time and cooking temperature. After cooking, mean aerobic plate counts were 4.1×10^4 and 6.2×10^3 for bratwurst cooked to 68 and 74°C, respectively. After 4 weeks storage, aerobic plate counts were 1.5×10^6 and 7.6×10^5, respectively. Anaerobic plate counts were similarly lower for bratwurst cooked at 74 vs 68°C.

Total extractable myoglobin (Mb) of cooked bratwurst were affected by the interaction of meat pH and cooking temperature (Figure 11). At pH 5.5, 0.48 mg Mb/g was present, and there was no difference in myoglobin content of bratwurst cooked to 68 vs 74°C. As meat pH increased, undenatured myoglobin content of bratwurst also significantly increased. Highest extractable myoglobin levels (2.74 mg Mb/g) were found

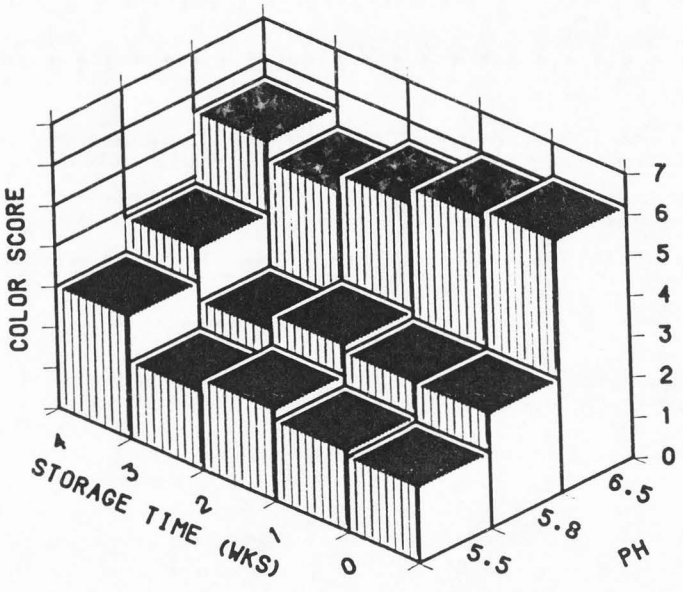

Fig. 7- Effects of pH and storage time on color score of vacuum-packaged bratwurst.

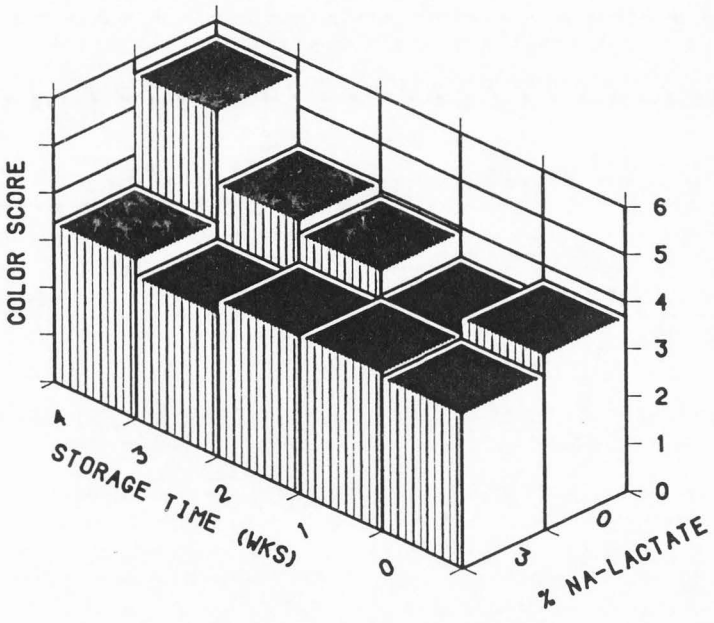

Fig. 8- Effects of sodium lactate and storage time on color score of vacuum- packaged, refrigerated bratwurst.

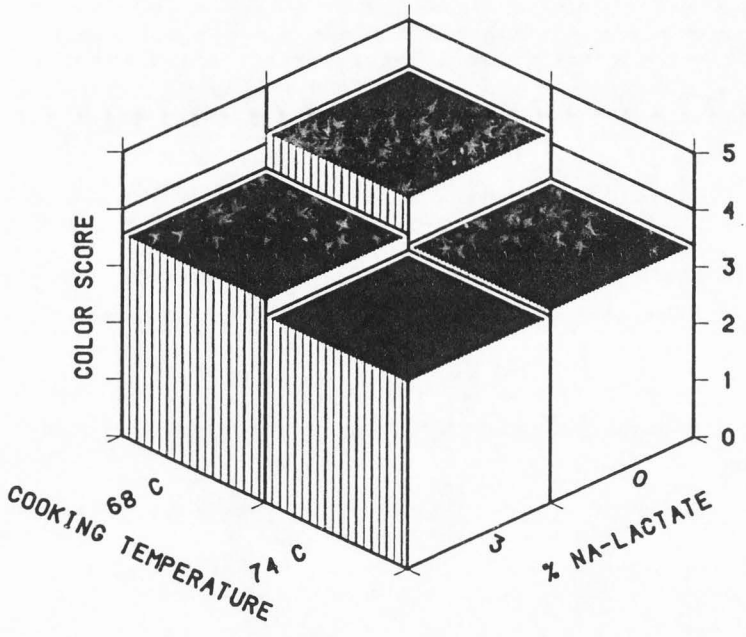

Fig. 9 - Effects of sodium lactate and cooking temperature on color score of vacuum-packaged bratwurst.

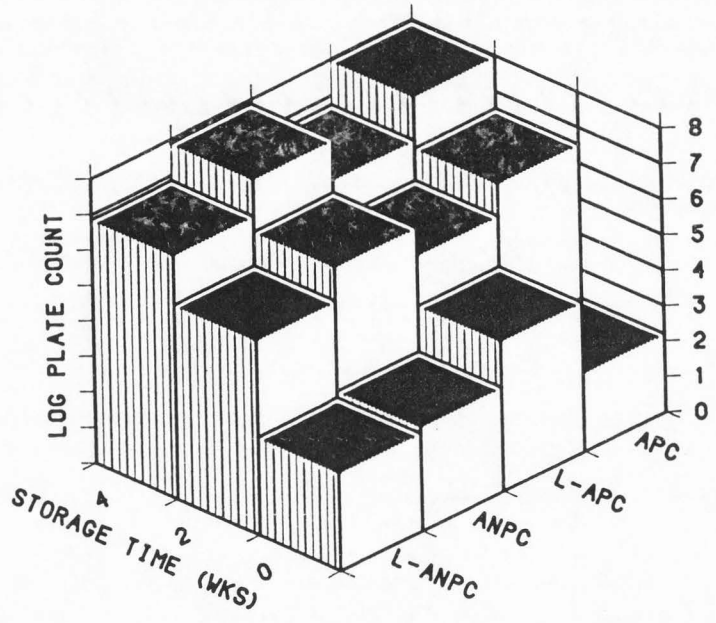

Fig. 10 - Effects of sodium lactate and storage time on microbial plate count of vacuum-packaged bratwurst.

in samples of pH 6.5, cooked to 68°C. The percent metmyoglobin in myoglobin extracts decreased with increasing pH. For example, bratwurst cooked to 68°C with initial pH of 5.5, 5.8, or 6.5 had percent metmyoglobin values of 78, 46, and 36%, respectively. At 74°C, percent metmyoglobin values were 82, 62, and 46%, respectively.

Pigment identification

Red surface discoloration, accompanied by a red exudate, was most apparent in bratwurst after 4 weeks storage. Figure 12 shows the spectra for exudate from bratwurst cooked to 68°C, then refrigerated for 4 weeks. Large absorption peaks were apparent at 542 and 580 nm, with a small peak at 627 nm. The b and a absorption peaks of oxymyoglobin are at 544 and 582 nm, respectively (Bowen, 1949; El-Badawi et al., 1964). Metmyoglobin has a small absorption peak at 627-630 nm. It thus appears that the red exudate in refrigerated bratwurst were due to myoglobin which was oxygenated to oxymyoglobin during filtration. The presence of a cyanometmyoglobin peak in exudates treated with potassium ferricyanide and cyanide is further indication that myoglobin was present in the original exudate (Figure 12).

The reflectance spectra of bratwurst with red discoloration are shown in Figure 13. The presence of a single broad reflectance minima at about 555 nm was further indication for the presence of undenatured myoglobin in the sample. The lower reflectance curve in Figure 13 has two minima at about 578 and 548 nm, indicative of oxymyoglobin. This sample apparently formed some oxymyoglobin during the course of reflectance measurement.

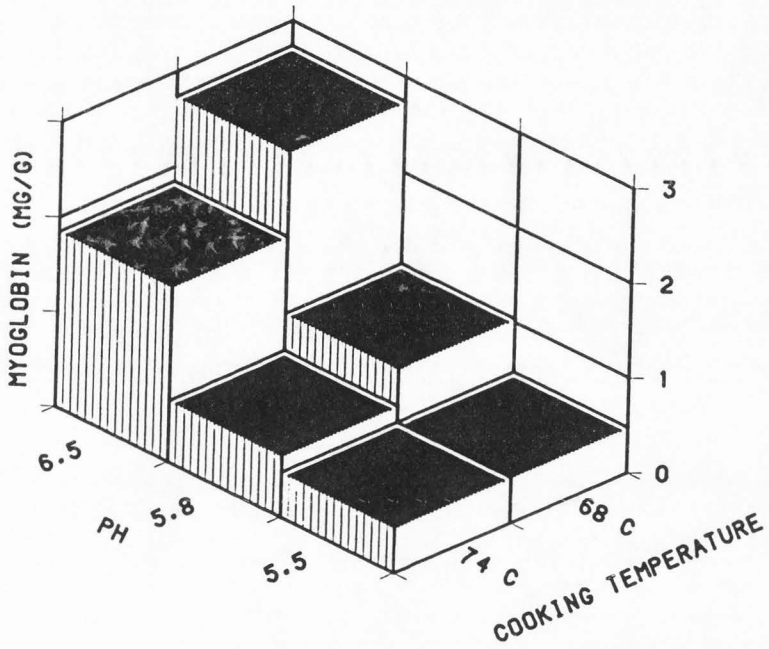

Fig. 11- Effects of cooking temperature and pH on myoglobin content of cooked, vacuum-packaged bratwurst.

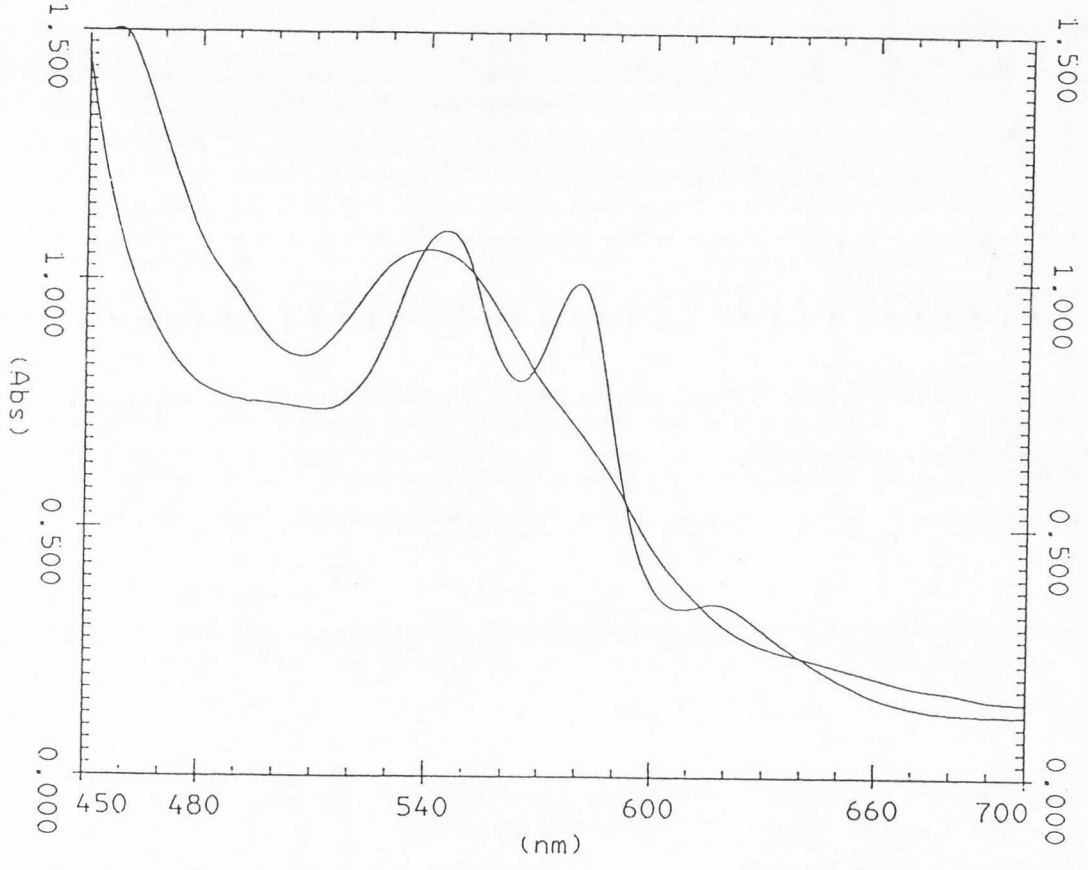

Fig. 12 - Visible absorption spectrum of red exudate from bratwurst cooked to 68°C. Also shown is the visible absorption spectrum of red exudate after addition of 2% potassium ferricyanide and 2% potassium cyanide, resulting in a peak of cyano-metmyoglobin at 540 nm. The sample was bratwurst cooked to 68°C, then vacuum-packaged and stored at 2°C for 4 weeks.

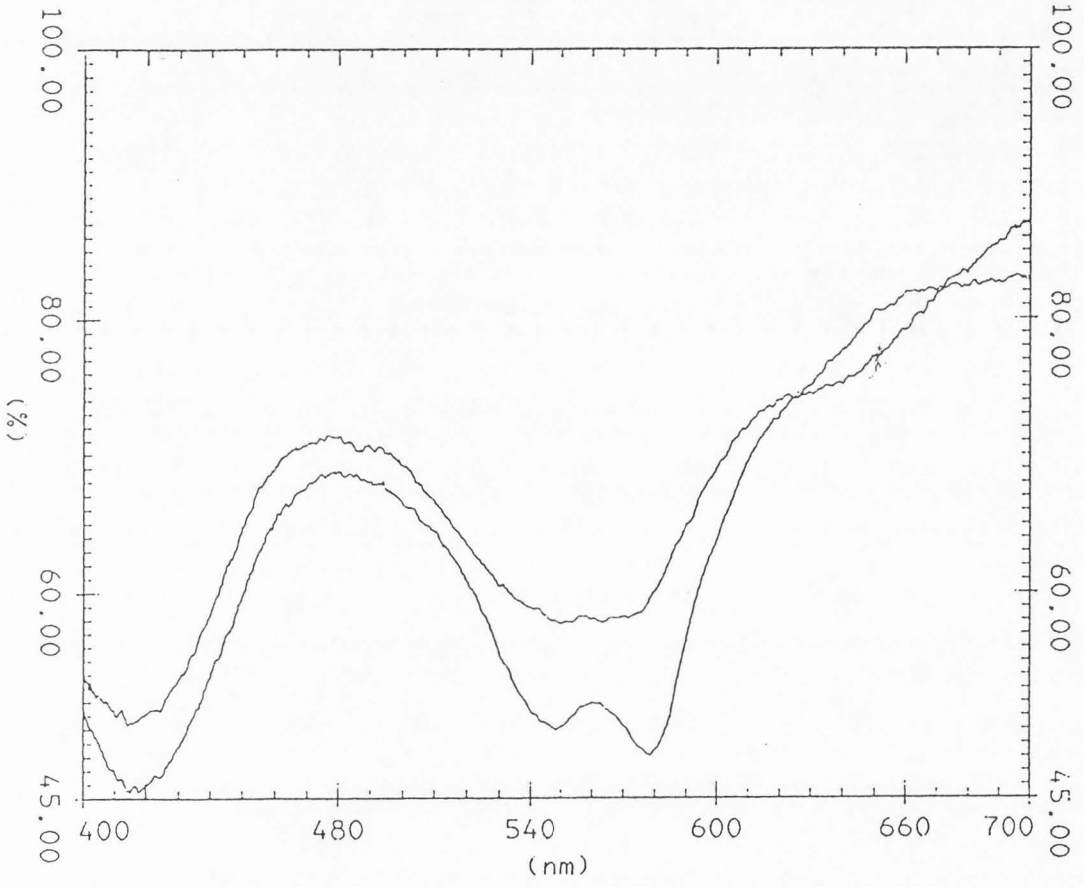

Fig. 13 - Reflectance spectra of bratwurst cooked at 68°C and at pH 5.8 (top line) or pH 6.5.

DISCUSSION

In this study, initial color of cooked bratwurst were greatly influenced by pH and cooking temperature. Bratwurst adjusted to pH 6.5 before cooking had a noticeably red internal color after cooking and actually had a cured appearance. After cooking, bratwurst at pH 6.5 had a brown exterior, but upon vacuum packaging even the exterior turned red and remained noticeably red for about 1-2 days at 2°C. Frozen bratwurst of pH 6.5 remained red until thawed. Schmidt and Trout (1984) have previously demonstrated that high pH meat has noticeable red color after cooking to temperatures as high as 74°C. The pH effect was most noticeable in beef which has higher myoglobin content than pork or turkey. Trout (1989) demonstrated that high pH increased myoglobin stability to heat, as indicated by the marked decrease in myoglobin denaturation with cooking of high pH beef, pork, or turkey.

Color reversion (brown to red transformation) has been associated with high numbers of pseudomonads in fresh ground beef homogenates (Faustman et al., 1990) and with spoilage due to Clostridia in vacuum-packaged fresh beef (Kalchayanand et al., 1989). In this study, color reversion in cooked bratwurst were also associated with microbial growth. Pink color development was greatest between the third and fourth week of refrigerated storage when plate counts were at spoilage levels (10^7/g). Bacterial genus was not identified, but facultative bacteria were likely responsible since both aerobic and anaerobic plate counts were high. Frozen control samples, of course, had no microbial growth during storage, and no pink discoloration occurred in frozen samples.

Sodium lactate has been shown to reduce microbial growth and increase color stability of cooked, vacuum-packaged beef roasts during refrigerated storage (Papadopoulos et al., 1991a; 1991b). Similar results have been reported for sodium lactate in fresh pork sausage chubs (Lamkey et al., 1991). However, sodium lactate did not improve color stability of fresh pork sausage patties in retail display, apparently because

color deterioration was due to factors other than microbial growth (Lamkey et al., 1991). In this study, sodium lactate did not significantly improve color score or lower plate counts of precooked bratwurst when compared to means taken over all storage times. Bratwurst color score did not usually increase until the fourth week of storage. However, the interactions of sodium lactate with storage time and cooking temperature were significant. After 4 weeks storage, bratwurst with lactate and cooked to 74°C had significantly less red discoloration and lower plate counts than samples without lactate.

CONCLUSIONS

The red surface discoloration appearing in cooked, vacuum-packaged bratwurst with storage was associated with an increase in microbial plate count. Undenatured myoglobin was identified in samples with red discoloration. Higher cooking temperature (74°C), lower product pH (5.5), and addition of 3% sodium lactate interacted to lower microbial load and the incidence of red discoloration. Thus, higher than normal cooking temperature and use of acids or microbial inhibitors may be needed to extend shelflife and color stability of precooked, vacuum-packaged pork products.

REFERENCES

AMSA. 1979. Guidelines for cookery and sensory evaluation of meat. American Meat Science Association, Chicago, IL.

Bowen, W. J. 1949. The absorption spectra and extinction coefficients of myoglobin. J. Biol. Chem. 179: 235.

Brown, W. D. and Tappel, A. L. 1957. Identification of the pink pigment of canned tuna. Food Res. 22: 214.

Cornforth, D. P. 1991. Methods for identification and prevention of pink color in cooked meat. Reciprocal Meat Conference Proceedings. 44: 53.

Cornforth, D. P., Vahabzadeh, F., Carpenter, C. E., and Bartholomew, D. T. 1986. Role of reduced hemochromes in pink color defect of cooked turkey rolls. J. Food Sci. 51: 1132.

El-Badawi, A. A., Cain, R.F., Samuels, C.E., and Anglemeier, A. F. 1964. Color and pigment stability of packaged refrigerated beef. Food Technol. May, 159.

Faustman, C., Johnson, J. L., Cassens, R. G., and Doyle, M.P. 1990. Color reversion in beef: influence of psychrotrophic bacteria. Fleischwirtsch. 70: 676.

Girard, B., Vanderstoep, J., and Richards, J. F. 1990. Characterization of the residual pink color in cooked turkey breast and pork loin. J. Food Sci. 55: 1249.

Hurst, R. 1989. FCTCVR program for analysis of variance. Utah State University, Logan, UT.

Kalchayanand, N., Ray, B., Field, R. A., and Johnson, M. C. 1989. Spoilage of vacuum-packaged refrigerated beef by clostridium. J. Food Prot. 52: 424.

Lamkey, J. W. Leak, F. W., Tuley, W.B., Johnson, D. D., and West, R. L. 1991. Assessment of sodium lactate addition to fresh pork sausage. J. Food Sci. 56: 220.

Mendenhall, V. T. 1989. Effect of pH and total pigment concentration on the internal color of cooked ground beef patties. J. Food Sci. 54: 1.

Messer, J. W., Peeler, J. T., and Gilchrist, J. E. 1978. Aerobic plate count, Ch. 4. In "FDA Bacteriological Analytical Manual," p. VI-1. 5th ed. AOAC, Washington, D.C.

Papadopoulos, L. S., Miller, R. K., Acuff, G. R., Vanderzant, C., and Cross, H. R. 1991a. Effect of sodium lactate on microbial and chemical composition of cooked beef during storage. J. Food Sci. 56: 341.

Papadopoulos, L. S., Miller, R. K., Ringer, L. J., and Cross, H.R. 1991b. Sodium lactate effect on sensory characteristics, cooked meat color and chemical composition. J. Food Sci. 56: 621.

Pool, M. F. 1956. Why does some cooked turkey turn pink? Turkey World. Jan., 1968.

Schmidt, G. and Trout, G. 1984. pH and color. Meat Industry, August, p. 30.

Scriven, F., Sporns, P., and Wolfe, F. 1987. Investigation of nitrite and nitrate levels in paper materials used to package fresh meat. J. Agric. Food Chem. 35: 188.

Trout, G. 1989. Variation in myoglobin denaturation and color of cooked beef, pork and turkey meat as influenced by pH, sodium chloride, sodium tripolyphosphate and cooking temperature. J. Food Sci. 54: 536.

Warriss, P. D. 1979. The extraction of haem pigments from fresh meat. J. Food Technol. 14: 75.

PART III. EFFECTS OF VARIOUS FRESH MEAT STORAGE METHODS ON COLOR OF COOKED GROUND PORK

ABSTRACT

The effects of microbial growth in raw materials on cooked pork color were investigated. In two trials with sow meat held aerobically at 2°C for 3 weeks, microbial load reached spoilage levels (10^7 cfu/g), pH increased to 6.46, and samples cooked to 71°C had red exudate, shown by absorption spectroscopy to contain myoglobin and cytochrome c. Samples cooked to 82°C also received high panel ratings for red color, due to red, flocculent precipitate in exudate, but undenatured myoglobin levels were low. In sow meat held frozen or vacuum-packaged at 2°C, pH after 3 weeks was 6.03 and 6.18, and plate counts were 10^4 and 10^7, respectively, but exudates after cooking were much less red. In five trials with fresh U. S. # 1 pork legs, plate counts also reached 10^7 cfu/g by 3 weeks storage, and pH increased to 6.37, but cooked samples were not red. Higher myoglobin levels in sow meat probably accounted for the red color and high level of undenatured myoglobin remaining after cooking of high pH, spoiled samples.

INTRODUCTION

Consumers usually interpret pink or red color in uncured cooked meats as an indication of undercooking. Consumers are especially sensitive to red color in pork due to concern with trichinosis in undercooked pork. Several conditions other than undercooking may cause pink or red color in cooked meats, including high meat pH (Schmidt and Trout, 1984; Trout, 1989), contamination with nitrite or nitrate (Scriven et al., 1987), exposure of cooked meat to CO or NO gases (Pool, 1956), or the development of pink globin hemochromes in canned or well cooked meats under anaerobic conditions (Brown and

Tappel, 1957; Cornforth et al., 1986).

Microbial growth has been associated with color reversion (brown to red) in fresh ground beef homogenates (Faustman et al., 1990), in fresh vacuum-packaged beef (Kalchayanand et al., 1989), and in precooked, vacuum-packaged bratwurst (Ghorpade et al., 1992).

Jacob (1970) has shown that both aerobic and anaerobic microbial growth are associated with a significant lowering of oxidation-reduction potential (ORP) in microbial media. The drop in ORP occurred early in the log phase of growth. This suggests that reducing conditions associated with microbial growth in raw meat ingredients might promote metmyoglobin reduction and red color development in the cooked products. Thus the objective of this study was to determine the effects of raw meat pH, storage time, and storage method (frozen control, vacuum refrigerated, or aerobic refrigerated) on microbial plate count, reducing ability, pH, and color of cooked pork. A second objective was to identify the pigment responsible for red color, if red coloration were apparent.

MATERIALS AND METHODS

In Experiment 1, sow carcasses were deboned, trimmed of visible fat, ground through a 0.64 cm plate, vacuum-packaged (21 kg/bag), and frozen for later use. For storage treatments, thawed meat was first mixed with 2% salt. Two-thirds of the meat (14 kg) was then vacuum-packaged (-0.8 bar) in 120 g portions in 12 x 24 cm polyethelene bags (Koch, Kansas City, MO) and stored frozen (-20°C) or at 2°C for 1, 2, or 3 weeks. The remaining one third of the meat was stored aerobically in bulk at 2°C for 1, 2, or 3 weeks, loosely covered with plastic wrap to retard surface dehydration. At 1, 2, or 3 weeks storage, 120 g portions of bulk refrigerated meat were also vacuum-packaged in preparation for cooking. Three bags from each storage condition were then cooked in a water bath for 40 min at 71 or 82°C, then evaluated by panel for cooked meat color. At 1,

2, and 3 weeks storage uncooked meat samples from each storage treatment were taken for measurement of microbial plate count, reducing ability, and pH. Experiment 1 has two replicates.

In experiment 2, fresh pork legs were deboned, trimmed, and immediately used in preparation of the raw meat storage treatments, as described in experiment 1. Experiment 2 was replicated once. In experiment 3, fresh pork legs were again used as the meat source. To evaluate possible effects of raw meat pH, meat (30 kg) was divided into two equal portions, and pH was adjusted to 5.5 or 6.0 with 1N HCl or NaOH. After mixing, meat was equilibrated at 2°C overnight. Then pH was measured and more acid or base was added as needed. For each pH, meat samples were stored frozen, vacuum refrigerated, or aerobically refrigerated as described in experiment 1. In experiment 3, cooking temperatures were reduced to 65 and 71°C to more closely approximate commercial cooking temperatures. Experiment 3 has three replicates.

Panel evaluation of color

After cooling for 30 min in cool running tap water, a 5-member trained panel (10 members for experiment 1 and 2) evaluated coded samples for cooked meat color on a 10-point scale, where 1 = gray, well cooked color (no red color), 4 = slightly red, 7 = moderately red, and 10 = very red color of meat and exudate. In a prior training session, panelists were familiarized with the cooked meat color scale and 8 x 10 color photos from preliminary experiments were provided to illustrate gray, well cooked samples (color score = 1) and very red cooked samples (color score =10). The photos were also posted for comparison when cooked samples were evaluated.

Reducing ability

Reducing ability was measured as described by Lee et al. (1981). A two-gram sample was blended with 10 ml of 25 mM PIPES {Piperazine-n, n-bis (2-ethanesulfonic

acid)} buffer, pH 5.8, with a polytron homogenizer (Brinkmann Instruments, Westbury, NY). Five ml of the homogenate was transferred to a 10 ml volumetric flask, mixed with two ml of 5 mM potassium ferricyanide, and chilled at 2°C with occasional stirring for 1 hr. Then 0.1 ml of 0.5% ammonium sulfamate and 0.2 ml of 0.5 M lead acetate were added. The mixture was held at room temperature for 5 min followed by the addition of 2.5 ml of 20% trichloroacetic acid (TCA). The solution was brought to volume (10 ml) with distilled water. After 5 min, the solution was filtered through Whatman No. 42 filter paper and absorbance of filtrate was read at 420 nm. Sample reducing ability was expressed as absorbance of 1 mM ferricyanide minus absorbance of the filtrate of sample plus ferricyanide.

Microbial plate counts

Aerobic and anaerobic plate counts were done by standard procedures (Messer et al., 1978). Twenty-five gram samples were taken for the first dilution. Standard plate count agar (Difco, Detroit, MI) was used as the growth medium. For anaerobic plate counts, plates were incubated in an anaerobic jar (BBL gas pack system, Becton Dickinson and Co., Cockeysville, MD). Duplicate plates were counted after incubation at 37°C for 48 hr.

pH measurements

pH was measured after blending a 10-gram sample with 90 ml distilled water for 1 min with a polytron homogenizer. The pH of filtered homogenate was measured with an Orion Research pH meter model 601A (Orion, Inc., Cambridge, MA)(Appendix D).

Pigment identification

Pigment identification on samples with red-colored exudate was done by spectrophotometric procedures as outlined by Cornforth (1991). Red exudate was collected from vacuum-packaged cooked samples and clarified by centrifugation of filtrate

in 50 ml polyallomer tubes at 38,700 g for 1 hr in a Sorvall centrifuge using rotor SS-34 (Dupont, Wilmington, DE). Visible absorption spectra were then obtained on a recording spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD).

Experimental design and data analysis

Experiments 1 and 2 were 3 x 3 factorial designs, with 3 raw meat storage methods (vacuum, -20°C, vacuum, 2°C, or aerobic, 2°C), and 3 storage times (1, 2, or 3 weeks). Experiment 3 was a 3 x 3 x 2 factorial design, with 3 storage methods and 3 storage times as described, and with two raw meat pH values (5.5 or 6.0). Analysis of variance of treatment means for aerobic plate count, reducing ability, and raw meat pH were done using the FCTCVR program (Hurst, 1989). For panel color scores, cooking temperature was also included as an experimental variable as previously described. For panel color scores, a nested factorial design was used, where the number of panelists was nested among the treatment cells and sample numbers (triplicate samples) were nested among the panelists.

RESULTS

In experiment 1, there were no significant differences in the color score of samples by storage method or cooking temperature after 1 week of storage (Fig. 14). However, after 3 weeks storage, color differences were apparent in cooked samples (Fig. 15). Samples that were aerobically refrigerated for three weeks, then cooked at 71°C were rated ($p < .05$) (Appendix F, Table 14) more red colored than samples stored in vacuum bags or frozen. Noticeable red color was also observed in aerobically refrigerated samples cooked to 82°C. In experiment 1, aerobic and anaerobic plate counts had increased to spoilage levels (10^7 CFU/g of meat) in aerobic refrigerated samples after 3 weeks storage (Table 3). There were no significant differences among storage methods for raw meat reducing ability, but reducing ability of all samples was ($p < 0.05$) (Appendix F, Table 16) higher

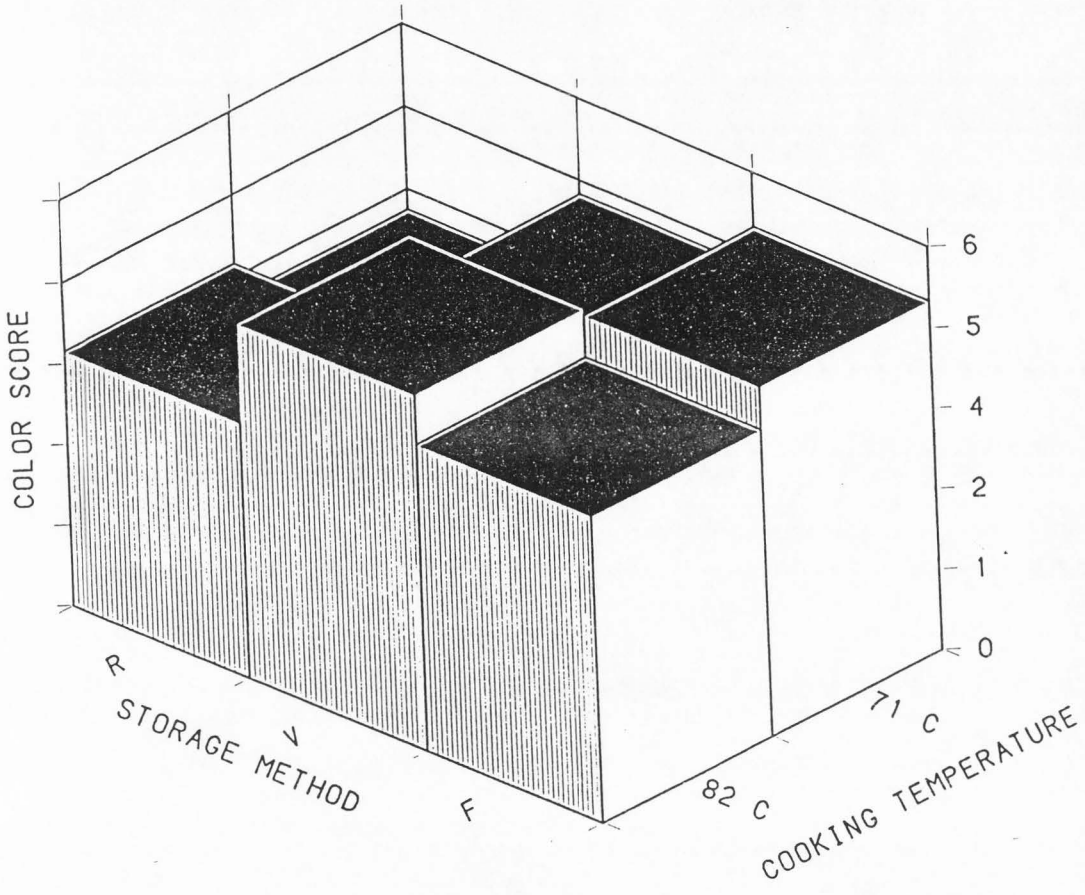

Fig. 14 - Effects of storage method and cooking temperature on color score of cooked ground pork (Experiment 1). Raw meat was held one week frozen, refrigerated, or vacuum-refrigerated, then cooked.

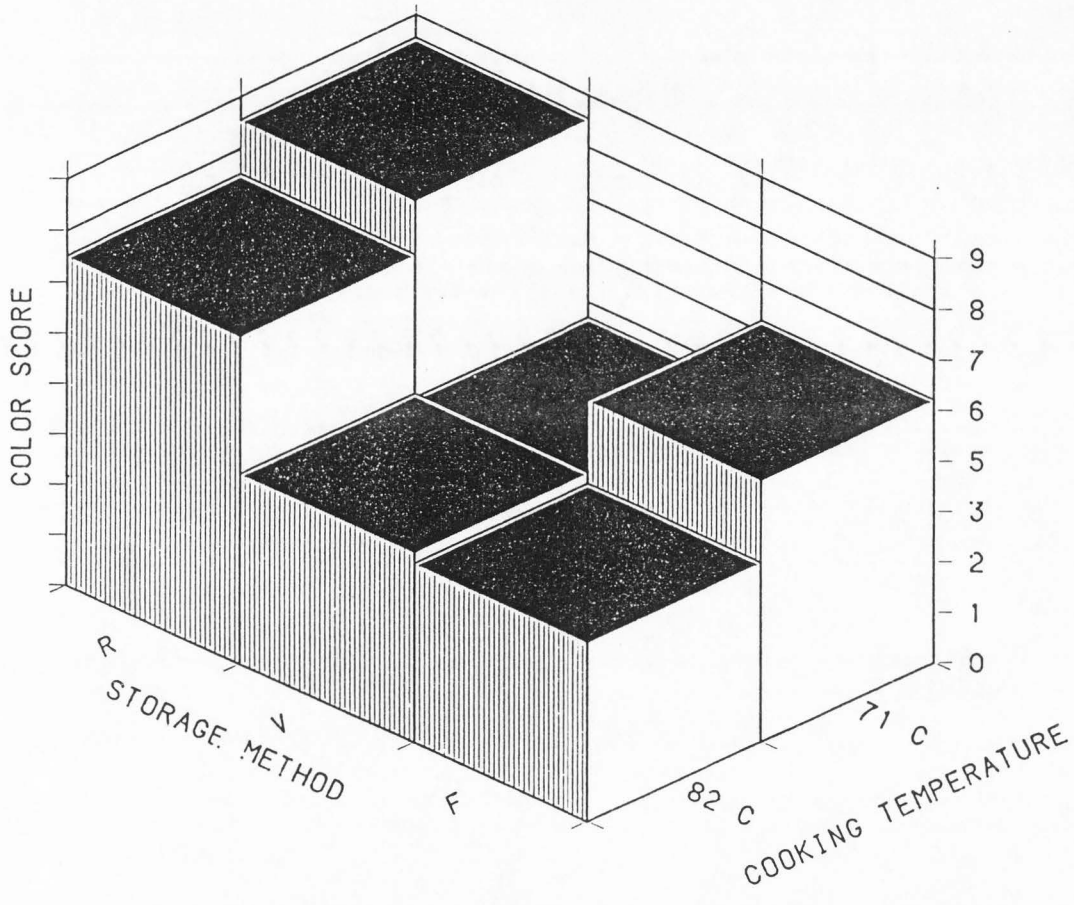

Fig.15 - Effect of storage method and cooking temperature on color score of cooked ground pork(Experiment 1). Raw meat was held three weeks frozen, refrigerated, or vacuum-refrigerated, then cooked.

Table 3- Effects of raw meat storage method and storage time on mean[1] microbial plate count, reducing ability, raw meat pH, and cooked meat color score in experiment 1.

Treatments		APC[3] (log CFU/g)	ANPC[4] (log CFU/g)	Reducing Ability	Meat pH	Cooked meat color score[5]	
Storage Time (wks)	Storage[2] Method					71°C	82°C
0	Initial	4.34	4.43		6.08		
1	V -20°C	4.35		0.19	6.03	5.20	4.57
	V 2°C	4.56	4.23	0.17	6.00	4.60	5.30
	A 2°C	4.71		0.23	6.00	3.30	3.80
2	V -20°C	4.58		0.42	6.00	7.83	4.60
	V 2°C	7.02	7.04	0.45	6.15	4.37	4.07
	A 2°C	7.20		0.55	6.24	8.27	5.30
3	V -20°C	4.30		0.50	6.05	5.87	4.03
	V 2°C	7.45	7.15	0.55	6.18	4.17	4.27
	A 2°C	7.79		0.60	6.46	8.50	7.33

1 Each value in the table is the mean of 2 replications. Microbial plate count, reducing ability, and pH measurement were all done in duplicate for each replicate.

2 V= vacuum, A= aerobic

3 APC= aerobic plate count

4 ANPC= anaerobic plate count

5 Panel color score; 1= gray, well cooked color, 10= very red, uncooked color.

for raw meat samples after 3 weeks storage (data not shown). pH increased from 6.0 initially to 6.46 after 3 weeks storage of aerobic refrigerated samples (Table 3).

In experiment 2, aerobic and anaerobic plate counts reached levels of 10^7 CFU/g by 2 weeks refrigerated storage, and actually decreased somewhat to 10^6 CFU/g by 3 weeks storage. Aerobically refrigerated samples increased in pH from 5.99 initially to 6.33 after 1 week storage and declined somewhat thereafter (Table 4). Raw meat reducing ability and cooked meat color score were not significantly affected by storage time or storage method in experiment 2.

In experiment 3, microbial plate counts significantly increased with refrigerated storage. Reducing ability was ($p < 0.05$)(Appendix F, Table 22) higher for aerobically refrigerated samples than for frozen or vacuum-refrigerated samples. The reducing ability of aerobically refrigerated samples increased to 0.65 by 3 weeks storage (Table 5), and the interaction of storage time and storage method on reducing ability was significant ($p < .05$). Raw meat pH was significantly affected by storage method, storage time, and initial meat pH (Table 6). Storage method, storage time, cooking temperature, and initial meat pH significantly affected cooked meat color scores (Table 6). However, cooked meat color scores only ranged from 1 (gray, well cooked) to 4 (slightly red color), in contrast to experiment 1 where samples developed much redder color (color score of 7-8 for some samples after 3 weeks storage; Table 3).

Visible absorption spectra were obtained on filtered extracts of red colored cooked samples in experiment 1 after 3 weeks storage. A large peak at 550 nm was observed and was characteristic of undenatured myoglobin (Fig. 16). The peak at 577 nm was characteristic of the alpha peak of oxymyoglobin. The beta peak of oxymyoglobin normally occurs at 541 nm, but was obscured by the broad myoglobin peak. A small peak was also observed at about 625 nm indicative of metmyoglobin. Pigment concentrations were not determined, but it was visually apparent that pigment levels were highest in

Table 4 - Effects of raw meat storage method and storage time on mean[1] microbial plate count, reducing ability, raw meat pH, and cooked meat color score in experiment 2.

Treatments		APC[3] (log CFU/g)	ANPC[4] (log CFU/g)	Reducing Ability	Meat pH	Cooked meat color score[5]	
Storage Time (wks)	Storage Method[2]					71°C	82°C
0	Initial	4.73	4.43	0.24	5.99		
1	V -20°C	4.77		0.39	6.02	4.17	4.60
	V 2°C	5.63	4.23	0.31	5.97	3.80	5.13
	A 2°C	5.43		0.32	6.33	2.98	5.78
2	V -20°C	4.77		0.34	6.04	3.75	5.00
	V 2°C	7.23	7.04	0.33	5.96	4.36	5.33
	A 2°C	7.37		0.37	6.30	3.38	7.30
3	V -20°C	4.76		0.32	6.04	5.90	3.88
	V 2°C	5.91	7.15	0.33	5.92	4.80	5.11
	A 2°C	6.06		0.37	6.37	6.61	5.98

1 Each value in the table is the mean of 2 replications. Microbial plate count, reducing ability, and pH measurement were all done in duplicate for each replicate.

2 V= vacuum, A= aerobic

3 APC= aerobic plate count

4 ANPC= anaerobic plate count

5 Panel color score; 1= gray, well cooked color, 10= very red, uncooked color.

Table 5 - Effects of raw meat storage method and storage time on microbial plate count, reducing ability, raw meat pH, and cooked meat color score in experiment 3.

Treatments			Log APC (CFU/g)[3]	Log ANPC[4] (CFU/g)	Reducing Ability	Meat pH	Cooked meat color score[5]	
Storage Time (wks)	Storage Method[2]	pH					65°C	71°C
0	Initial		4.97	5.01	0.34	5.86		
1	V -20°C	5.5	5.31		0.35	5.67	1.70	2.63
		6.0	5.27		0.38	6.12	3.30	3.87
	V 2°C	5.5	5.01	4.98	0.37	5.65	2.40	2.80
		6.0	5.45	5.19	0.44	6.22	2.90	4.07
	A 2°C	5.5	5.27		0.40	5.71	1.63	2.67
		6.0	5.53		0.39	6.30	1.87	3.00
2	V -20°C	5.5	5.21		0.34	5.72	2.63	2.67
		6.0	4.95		0.36	6.28	5.00	4.17
	V 2°C	5.5	6.00	5.92	0.38	5.70	2.83	3.2
		6.0	6.52	6.40	0.40	6.35	4.43	3.67
	A 2°C	5.5	6.16		0.42	6.16	2.20	3.47
		6.0	6.46		0.41	6.48	1.87	2.13
3	V -20°C	5.5	5.40		0.36	5.63	2.10	2.80
		6.0	4.87		0.36	6.57	4.00	3.90
	V 2°C	5.5	7.04	6.63	0.38	5.61	2.40	3.57
		6.0	8.04	7.29	0.38	6.38	3.70	4.27
	A 2°C	5.5	7.88		0.41	6.26	1.43	2.67
		6.0	8.05		0.65	6.74	1.20	1.60

1 Each value in the table is the mean of 2 replications. Microbial plate count, reducing ability, and pH measurement were all done in duplicate for each replicate.

2 V= vacuum, A= aerobic

3 APC= aerobic plate count

4 ANPC= anaerobic plate count

5 Panel color score; 1= gray, well cooked color, 10= very red, uncooked color.

Table 6 - Means for the main effects of storage time, storage method, raw meat pH and cooking temperature on raw meat microbial plate count, reducing ability, meat pH and cooked meat color score in experiment 3.

Treatments	APC[2] (log CFU/g)	ANPC[3] (log CFU/g)	Reducing Ability	Meat pH	Color Score[4]
Storage Time (Wks)					
1	5.26[a]	5.08[a]	0.38	5.96[a]	2.73[a]
2	5.88[b]	6.16[b]	0.39	6.12[b]	3.19[b]
3	6.88[c]	6.96[c]	0.42	6.20[c]	2.77[a]
Storage Method[1]					
V -20°C	5.17[a]		0.36[a]	5.99[a]	3.23[a]
V 2°C	6.34[b]	6.07	0.39[b]	5.99[a]	3.34[b]
A 2°C	6.56[c]		0.45[c]	6.28[b]	2.13[c]
Initial Meat pH					
5.5	5.92	5.84	0.37[a]	5.79[a]	2.51[a]
6.0	6.12	6.29	0.42[b]	6.38[b]	3.28[b]
Cooking Temperature					
65°C					2.64[a]
71°C					3.15[b]

1 V= vacuum, A= aerobic

2 APC= aerobic plate count

3 ANPC= anaerobic plate count

4 Panel color score; 1= gray, well cooked color, 10= very red, uncooked color.

a-c Mean values within each column with different letter superscripts are significantly different ($p < 0.05$).

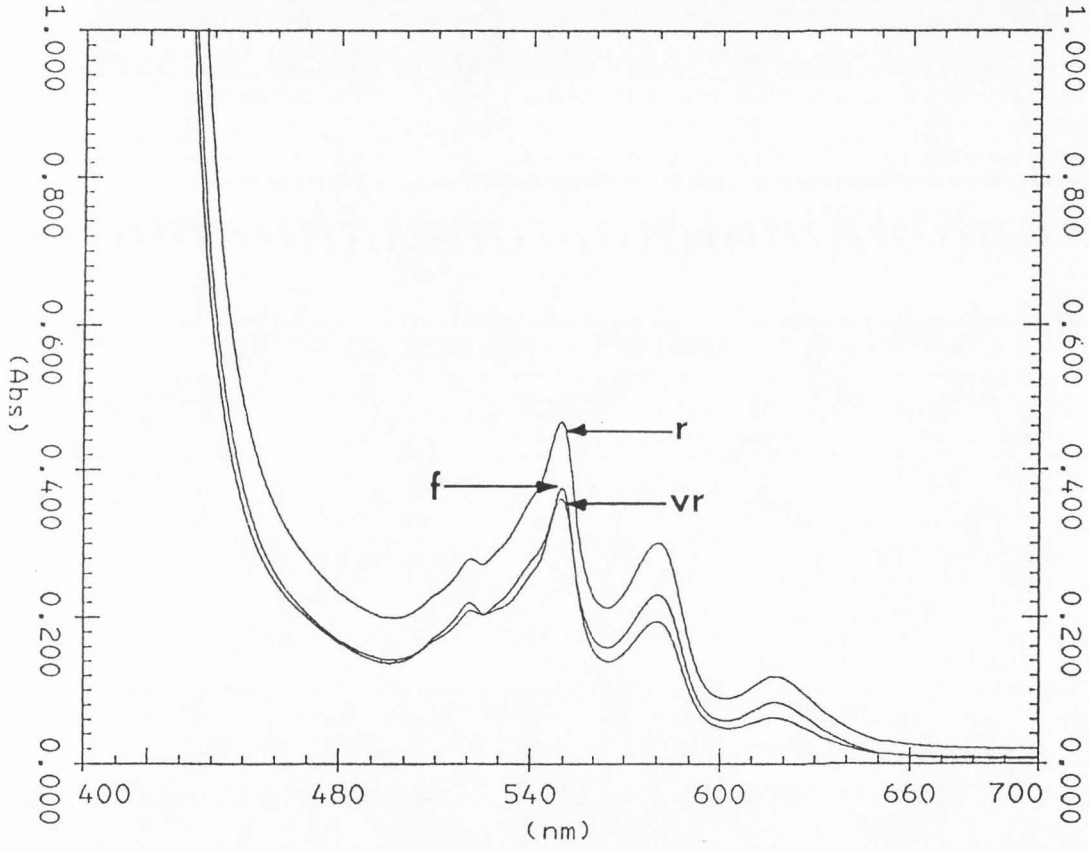

Fig. 16 - Visible absorption spectra of extracts of cooked pork (Experiment 1) at 71°C, after storage for three weeks as follows: frozen (f), refrigerated (r), vacuum-refrigerated (vr). The extract was red.

exudate from refrigerated samples (Fig.16 and 17). Smaller absorption peaks characteristic of myoglobin and oxymyoglobin were detected in samples cooked at 82°C. Refrigerated samples cooked at 82°C had red colored particulates that were removed by filtration. After filtration all samples cooked at 82°C had a slight yellowish exudate (Fig. 17).

Visible absorption spectra were also obtained on filtered extracts of cooked samples in experiments 2 and 3. Although panelists did not rate cooked samples as red as in experiment 1, some extracts were light red colored. Figure 18 shows a representative spectrum. Similar to Figure 16, undenatured myoglobin, oxymyoglobin, and metmyoglobin were present, as indicated by peaks at about 550 nm (myoglobin), 570 nm (oxymyoglobin), and 625 nm (metmyoglobin). A prominent peak was also apparent at 520 nm. Cytochrome c absorbs at 521 and 550 nm (Girard et al., 1990). Thus cytochrome c was also probably present in cooked meat extracts. Myoglobin content of uncooked sow meat and fresh pork leg meat were 6.9 and 2.5 mg/g, respectively.

DISCUSSION

In all three experiments, microbial plate counts significantly increased in refrigerated samples, accompanied by a rise in pH. This result is in agreement with much previous work (Ingram and Dainty, 1971) showing an increase in raw meat pH from 5.5 in fresh meat to about 6.5 in meat at or near spoilage. High raw meat pH (6.0 - 6.5) is known to increase stability of myoglobin to heat denaturation, and is thus associated with red color in cooked meats (Schmidt and Trout, 1984; Trout, 1989). However, in the seven trials among 3 experiments in this study, only in two trials (Experiment 1) was red color observed in cooked meat samples of high pH. In experiments 2 and 3 (a total of 5 replications) no red color was observed in cooked samples, even though pH was usually above 6.0 and reducing ability was higher than initial values. In experiment 1 sow meat was used. The higher myoglobin content of sow meat vs fresh pork legs is likely the major

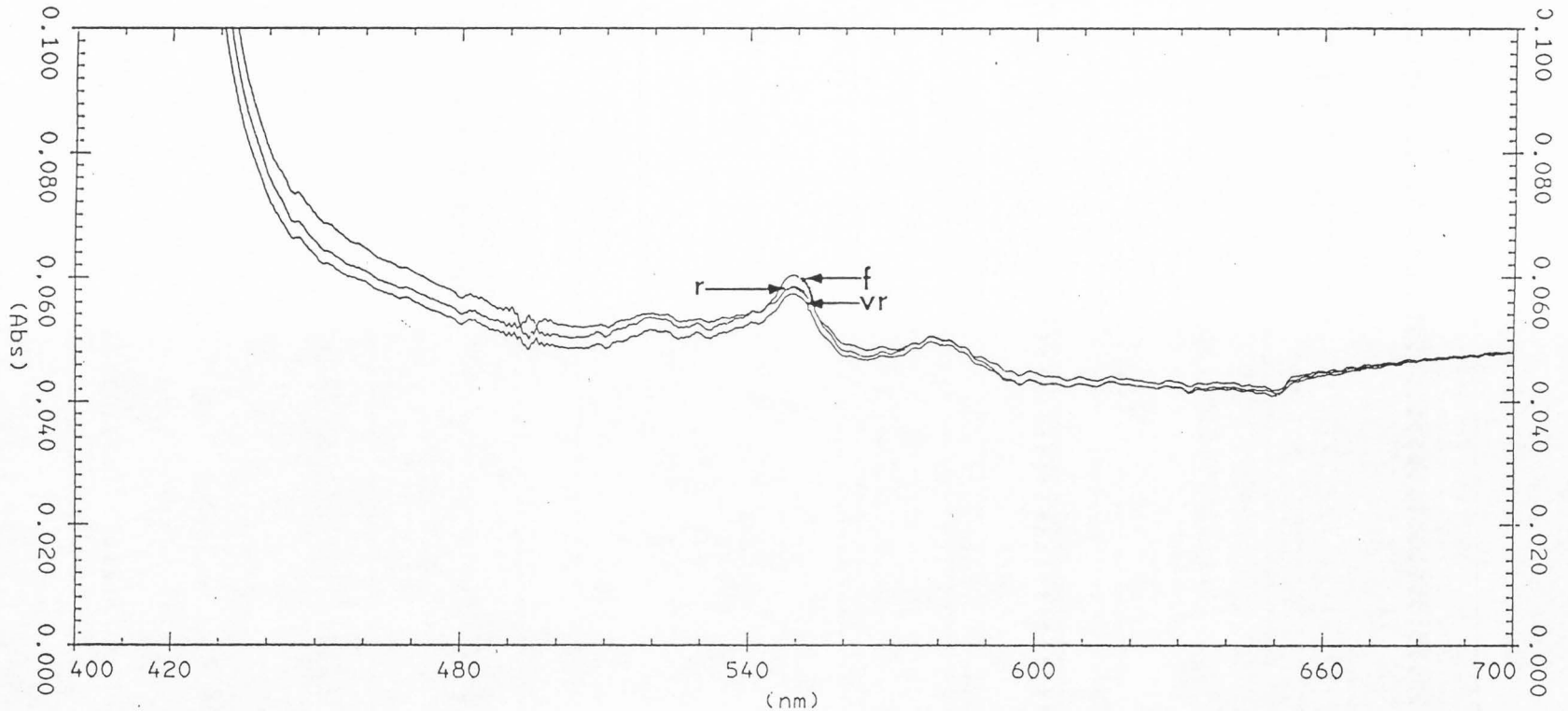

Fig. 17 - Visible absorption spectra of extracts of cooked pork (Experiment 1) at 82 °C, after storage for three weeks as follows: frozen (f), refrigerated (r), vacuum-refrigerated (vr). The extract was yellowish-brown.

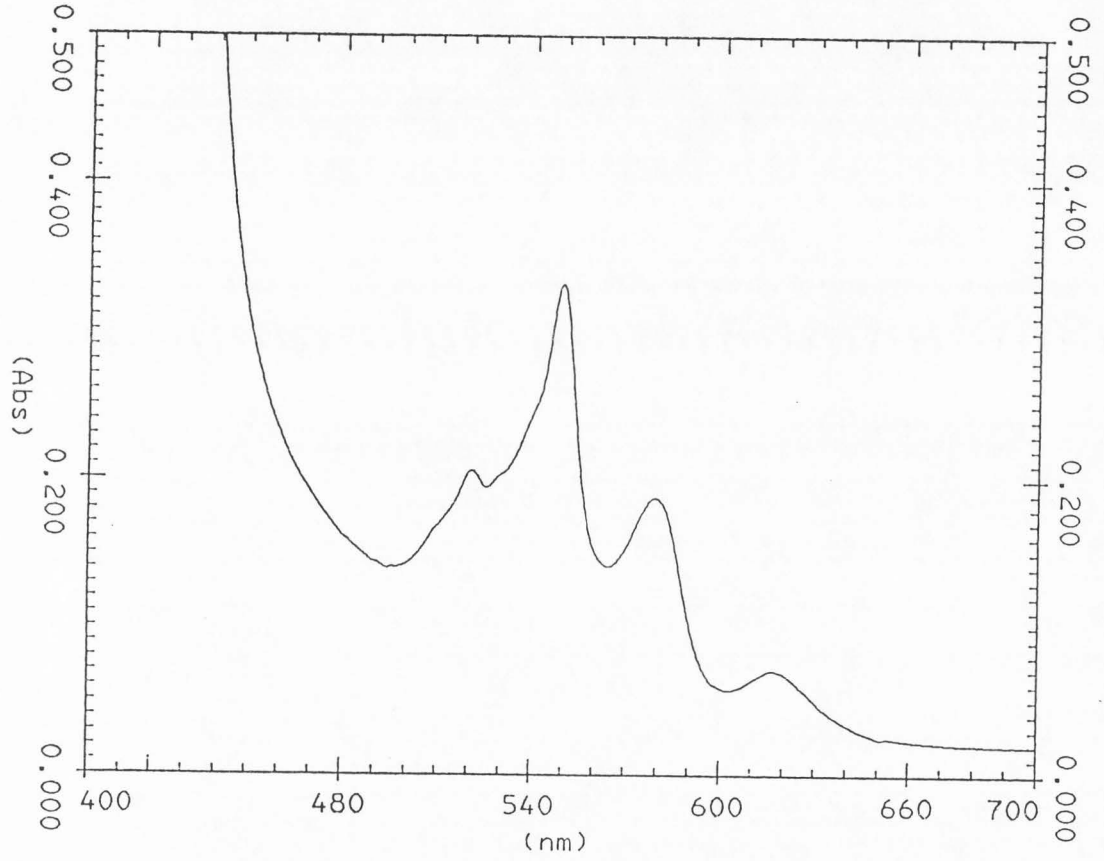

Fig. 18 - Visible absorption spectrum of extracts of pork cooked at 71°C, after vacuum refrigeration for 1 week (Experiment 2). The extract was pale red.

reason cooked meat color scores were higher for spoiled samples in experiment 1. Microbial growth was also required to raise pH, stabilizing myoglobin to heat. In experiments 2 and 3 with fresh pork legs, microbial growth also raised pH, but apparently myoglobin levels were not sufficiently high for red color to persist after cooking. Also in experiments 2 and 3, the raw meat developed a noticeable surface slime on aerobically refrigerated samples after 3 weeks storage. Microbial species identification was not determined in this study. However, differences in microbial proteolysis may have contributed to the difference in slime development and cooked meat color observed in this study between experiment 1 vs experiments 2 and 3.

It is well known that *Pseudomonads* are often the predominant species in aerobically refrigerated fresh meat while *Lactobacillus species* usually predominate in anaerobically refrigerated fresh meat (Gill, 1983). Faustman et al. (1990) showed that *Pseudomonas* and *Brochothrix spp.* inoculated in fresh beef homogenates were associated with red colored supernatant, but red color was only observed when bacterial numbers were in excess of 10^9 CFU/g meat. Kalchayanand et al. (1989) concluded that *Clostridium spp.* were responsible for pink discoloration in fresh vacuum-packaged beef steaks.

In agreement with the results of Faustman et al. (1990), the red color of cooked samples in experiment 1 of this study were only observed after 2 or 3 weeks of refrigerated storage when the microbial load was at spoilage levels. Certainly, meat with high microbial load (10^7 - 10^9 CFU/g) is not suitable for commercial meat processing. Thus, it is concluded that microbial growth during refrigerated storage of raw meat is not a cause of red discoloration in cooked pork products in commercial situations.

CONCLUSION

In experiment 1, red discoloration resulted from the higher myoglobin content of

the sow meat. Myoglobin and cytochrome c were indentified in the red exudate of the cooked meat samples in experiment 2 and 3. Thus, it is concluded that microbial growth during refrigerated storage of raw meat is not a cause of red discoloration in cooked pork products in commercial situations.

REFERENCES

Brown, W.D. and Tappel, A.L. 1957. Identification of the pink pigment of canned tuna. Food Res. 22: 214.

Cornforth, D.P. 1991. Methods for identification and prevention of pink color in cooked meat. Reciprocal Meat Conference Proceedings, 44: 53.

Cornforth, D.P., Vahabzadeh, F., Carpenter, C.E., and Bartholomew, D.T. 1986. Role of reduced hemochromes in pink color defect of cooked turkey rolls. J. Food Sci. 51: 1132.

Faustman, C., Johnson, J.L., Cassens, R.G., and Doyle, M.P. 1990. Color reversion in beef: influence of psychrotrophic bacteria. Fleischwirtsch. 70: 676.

Ghorpade, V. M., Cornforth, D. P., and Sisson, D. V. 1992. Effects of sodium lactate, pH, cooking temperature, and storage time on color and microbial load of vacuum-packaged bratwurst. J. Food Sci. In press.

Gill, C. O. 1983. Meat spoilage and evaluation of the potential storage life of fresh meat. J. Food Prot. 46: 444.

Girard, B., Vanderstoep, J., and Richards, J.F. 1990. Characterization of the residual pink color in cooked turkey breast and pork loin. J. Food Sci. 55: 1249.

Hurst, R. 1989. FCTCVR program for analysis of variance. Utah State University, Logan, UT.

Ingram, M. and Dainty, R. H. 1971. Changes caused by microbes in spoilage of meats. J. Appl. Bact. 34:21.

Jacob, H. E. 1970. Redox potential. Ch. 4. In "Methods in Microbiology," p. 91. Vol. 2 J. Norris and D. Ribbons, (Ed.). Academic Press, New York.

Kalchayanand, N., Ray, B., Field, R.A., and Johnson, M.C. 1989. Spoilage of vacuum-packaged refrigerated beef by *Clostridium*. J. Food Prot. 52: 424.

Lee, M., Cassens, R. G., and Fennema, O. R. 1981. Effect of metal ions on residual nitrite. J. of Food Proc. and Preserv. 5: 191.

Messer, J.W., Peeler, J.T., and Gilchrist, J.E. 1978. Aerobic plate count. Ch. 4. In FDA "Bacteriological Analytical Manual," p. IV-1. 5th Ed. AOAC, Washington, D.C.

Pool, M.F. 1956. Why does some cooked turkey turn pink? Turkey World. Jan., 1968.

Schmidt, G., and Trout, G. 1984. pH and color. Meat Industry, August, p. 30.

Scriven, F., Sporns, P., and Wolfe, F. 1987. Investigation of nitrite and nitrate levels in paper materials used to package fresh meat. J. Agric. Food Chem. 35: 188.

Trout, G. 1989. Variation in myoglobin denaturation and color of cooked beef, pork and turkey meat as influenced by pH, sodium chloride, sodium tripolyphosphate and cooking temperature. J. Food Sci. 54: 536.

PART IV. CHARACTERIZATION OF PIGMENTS RESPONSIBLE FOR PINK COLOR IN PORK ROASTS COOKED TO 65 OR 82°C

ABSTRACT

Myoglobin was the pigment responsible for pink color in pork roasts cooked to 65°C. Roasts cooked to 82°C had gray internal color after cooking, but the cooked meat developed pink internal color after refrigerated storage. Reflectance spectra of pork roast slices cooked to 82°C were characteristic of denatured globin hemochromes or related non-nitrosyl hemochromes.

INTRODUCTION

Pink or red color in cooked meats may result from a number of factors other than undercooking. Trout (1989) found that both beef and pork retained some pink color due to the presence of undenatured myoglobin when cooked to internal temperatures as high as 76°C if meat pH was 6.5 or 7.0. Scriven et al. (1987) reported that pink color in cooked beef steak was due to contamination of nitrate derived from the wrapping adhesive. Presumably, cured meat pigment, nitrosylhemochrome, was responsible for the pink color after cooking. Pool (1956) reported that turkeys roasted in gas ovens developed a surface pink color when exposed to combustion gases containing NO and CO. Pink globin hemochromes have been reported to cause pink color in canned tuna (Brown and Tappel, 1957) or in the interior of cooked turkey rolls (Cornforth et al., 1986). Ghorpade et al. (1992) found that microbial growth was associated with development of red discoloration in pre-cooked vacuum-packaged bratwurst, and myoglobin was the responsible pigment. Girard et al. (1990) concluded that cytochrome c was responsible for residual pink color remaining in cooked turkey or pork slices after other pink pigments had faded. Howe et al. (1982) reported pink color development in well-cooked pork roasts following refrigerated

or frozen storage. The pigment faded quickly upon exposure to light and oxygen and was not oxymyoglobin. The purpose of this study was to further characterize the pigment(s) responsible for pink color in well-cooked (82°C) pork roasts compared to roasts cooked to lower temperature (65°C).

MATERIALS AND METHODS

Product preparation

Four fresh pork legs (right and left legs from two carcasses) were deboned and trimmed of visible fat. Twenty small roasts (2-3 lb each) were wrapped individually in aluminium foil and roasted in a convection oven at 177°C. Five roasts from each animal were removed from the oven when internal temperature reached 65 +/-2°C. The remaining roasts were cooked to an internal temperature of 82 +/- 2°C. After cooling for 30 min, some roasts were evaluated for cooked meat color, and the rest were vacuum-packaged (-0.8 bar) in polyethylene bags and stored at 2°C.

Panel color evaluation and hunter color measurement

On 0, 1, 4, 8, and 12 days of storage, roasts were sliced for panel color evaluation and measurement of Hunter color values. For 0 time, slices were taken about 30 min after cooking. The first slice (1.3 cm thick) was held in air for 15 min prior to color evaluation or measurement. A second slice was taken in the presence of panelists. Fading of pink color was determined as the difference in color scores between sample the evaluated at 10 sec vs 15 min after slicing. A color scale of 1-7 was used, where 1 = no pink color (entirely gray) and 7 = very pink. For instrumental color measurement, the Hunter Lab Digital Color Difference meter (D25D2A) (Hunter Associates Laboratory, Inc., Reston, VA.) was standardized with the pink standard plate ('L' (lightness) =66.8, 'a' (redness) =21.4, 'b' (yellowness) = 12.0). Hunter color values were recorded on 1.3 cm thick

slices from each roast immediately after slicing, and on the same slice 15 min after slicing.

Pigment identification

Total extractable myoglobin was determined using a modification of the procedure of Warriss (1979). Five gram samples in polypropylene centrifuge tubes were blended with a polytron homogenizer (Brinkmann Instruments, Westbury, NY) for 20 sec in 25 ml of ice cold 0.04 M phosphate buffer. After pigment extraction for 1 hr at 4°C, the homogenates were centrifuged at 6500g for 45 min. The supernatant was further clarified by filtration through Whatman No. 1 filter paper. Visible absorption spectra were obtained on a recording spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD). Absorption values at 525, 572, and 700 nm were used to calculate total extractable myoglobin, metmyoglobin, and denatured myoglobin as percent of total extractable myoglobin (Trout, 1989) (Appendix C). Acetone soluble nitroso pigment was measured by the procedure of Hornsey (1956) (Appendix B). Globin hemochromes were identified by their unique reflectance characterized by reflectance minima at or near 530 and 558 nm (Brown and Tappel, 1957). Standard globin hemochrome solution was prepared as follows. Denatured bovine serum albumin (BSA; 1mg/mL diluted in 0.1 M potassium phosphate, pH 7.5) was prepared by heating 10 mL of solution in a water bath for 30 sec at 70°C. Two mL of denatured BSA and 0.1 mL of hematin solution (0.5mg/mL) were mixed together and diluted to 5 ml final volume with 0.1 M sodium borate (pH 12.5). A few grains of sodium dithionite were added to reduce the solution. For reflectance spectroscopy a 3 mm slice of cooked meat was rapidly placed in a 5x8 cm polyethylene bag to exclude air from the surface, and a reflectance spectrum was rapidly obtained using an integrating sphere attachment to the recording spectrophotometer. Reflectance spectra were also obtained on samples after exposure to air for 15 min or after reduction in sodium dithionite (2%).

Experimental design and data analysis

The experiment was a split plot randomized block design with cooking temperature (65 or 82°C) and storage time (0, 1, 4, 8,12 days) as a whole plot treatment and time after slicing (0 or 15 min) as a subplot treatment. There were 5 trained judges to evaluate pork roast color. The experiment was replicated once. All analyses were done in duplicate. Statistical analyses were done separately for color score, Hunter color 'a' (redness) values, and pigment analysis. Analysis of variance was done using the Stat View TM 512+ statistical package on a Macintosh SE computer (Anonymous, 1986).

RESULTS AND DISCUSSION

Slices of pork roasts cooked to 65°C were light pink colored with panel color score of 4.4-5.0 (Table 7). The pink color did not fade after 15 min exposure to air. The interior of pork roasts cooked to 82°C were brown colored after cooking (0 time), with mean panel color score of 2.9. A pink color developed during refrigerated storage of roasts cooked to 82°C as indicated by an increase in panel color scores and Hunter color 'a' (redness) values (Table 7). However, pink color of well cooked roasts quickly faded after slicing as previously noted by Howe et al. (1982). In roasts cooked to 82°C, undenatured myoglobin levels were lower ($p < 0.05$) (Appendix F, Table 27) and metmyoglobin (as % of undenatured myoglobin) levels were higher than for roasts cooked to 65°C. Nitrosopigment levels were essentially zero in all roasts, confirming that pink color was not due to presence of contaminating nitroso compounds (Appendix E).

Undenatured deoxymyoglobin and oxymyoglobin were the pigments responsible for pink color in roasts cooked to 65°C, as confirmed by reflectance spectra on pink slices, exhibiting reflectance minima at 545 and 579 nm, characteristic of oxymyoglobin (Fig. 19A). The spectrum was essentially unchanged after exposure of the slices to air for 15 min (Fig 19B). Slices from roasts cooked to 82°C, then sliced 30 min after cooking,

Table 7 - Effect of cooking temperature and refrigerated storage time on panel color score, Hunter 'a' values, myoglobin and nitrosopigment content of pork roasts

Cooking Temperature	Storage Time	Color Score[2]		Hunter 'a' Values[3]		Mb[1] (mg/g)	MMb[1] (%)	PMD[1] (%)	NOMb[1] (ppm)
		Time after slicing		Time after slicing					
		0	15	0	15				
65°C	0	4.4[4]	4.8	7.65[4]	7.45	0.80[4]	61.97[4]	82.94[4]	1.09[4]
	1	4.7	5.2	8.30	7.95	1.77	7.465	39.96	0.50
	4	5.0	4.7	4.60	7.45	1.07	19.74	63.93	0.83
	8	4.8	4.2	4.10	4.70	0.85	19.50	71.43	0.00
	12	4.5	4.2	5.05	4.60	0.78	39.11	73.68	0.46
	Mean	4.7	4.6	5.94	6.43	1.05	42.99	66.39	0.58
82°C	0	2.9	2.3	3.60	4.30	0.56	76.09	81.08	0.50
	1	4.3	2.8	7.80	0.95	0.47	69.70	84.14	0.68
	4	5.3	1.7	9.10	1.00	0.53	77.90	82.14	0.39
	8	5.1	1.6	10.00	0.20	0.46	80.16	84.61	0.00
	12	5.0	1.1	10.75	0.70	0.33	81.78	88.72	0.00
	Mean	4.52	1.9	8.25	1.43	0.47	77.13	84.14	0.31

[1]Mb= total extractable myoglobin; MMb= metmyoglobin, % total extractable myoglobin; PMD= myoglobin denatured, as % of myoglobin content of uncooked meat; NOMb= nitrosomyoglobin.
[2]1 = very gray, 7 = very pink
[3] 'a' = redness
[4]$LSD_{0.05}$ for color score= 0.76; $LSD_{0.05}$ for 'a' values=4.99; $LSD_{0.05}$ for Mb= 0.43; $LSD_{0.05}$ for MMb= 16.42; $LSD_{0.05}$ for PMD= 11.03; For NOMb, the F test was not significant.

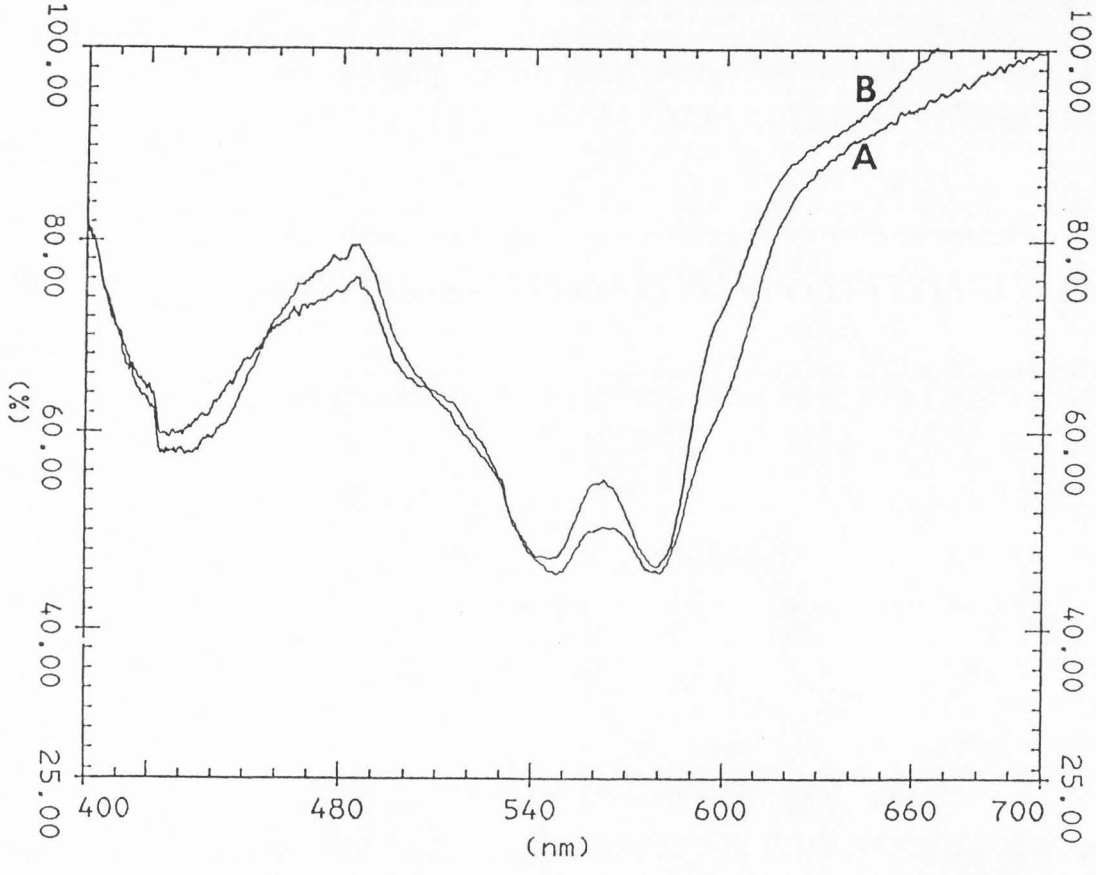

Fig. 19 - Reflectance spectra of pork roasts cooked to 65°C, then vacuum-packaged and refrigerated 12 days at 3°C. Spectra were taken on 3 mm slices immediately after slicing (A) or 15 min after slicing (B).

were not pink (Fig. 20A). However, pink color developed during refrigerated storage of roasts cooked to 82°C. Spectra from pink slices of roasts cooked to 82°C exhibited a shoulder at 529 nm and a prominent minima at 557 nm (Fig. 20B). After 15 min in air, the pink color faded, and the shoulder at 529 nm disappeared (Fig. 20C). Reflectance spectra of denatured globin hemochrome solution (denatured bovine serum albumin and hematin reduced with sodium hydrosulfite) was pink colored, with reflectance minima at 529 and 557 nm (Fig. 21). Thus, it is concluded that denatured globin hemochromes or related non-nitrosyl hemochromes were responsible for the pink color that developed with refrigeration of roasts cooked to 82°C. A number of pink non-nitrosyl hemochromes have previously been characterized, including mixed nicotinamide-denatured globin hemochrome (Brown and Tappel, 1957), nicotinamide hemochrome (Cornforth et al., 1986), and denatured cytochrome c-bovine serum albumen hemochromes (Ahn and Maurer, 1990). All had spectra characterized by dual reflectance minima or absorption maxima near 530 and 558 nm.

In conclusion, undenatured deoxymyoglobin and oxymyoglobin were the pigments responsible for the pink color of pork roasts cooked to 65°C. Denatured globin hemochrome or related non-nitrosyl hemochromes were responsible for the pink color that developed during refrigerated storage of roasts cooked to 82°C.

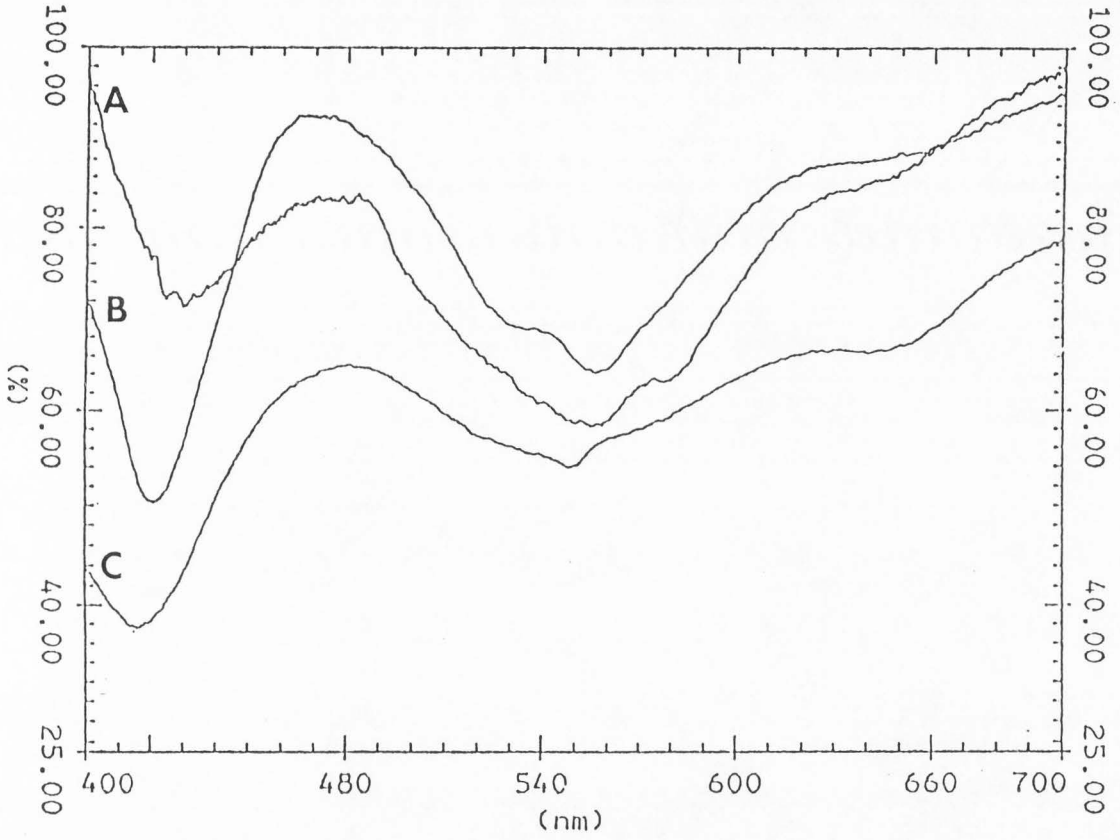

Fig. 20 - Reflectance spectra of pork roasts cooked to 82°C. Spectra were taken on 3 mm slices taken 30 min after cooking (A: brown slices), and on slices from roasts that were vacuum packaged and held at 3°C for 12 days. For refrigerated samples, spectra were obtained immediately after slicing (B: pink slices), or after 15 min exposure to air (C: faded brown slices).

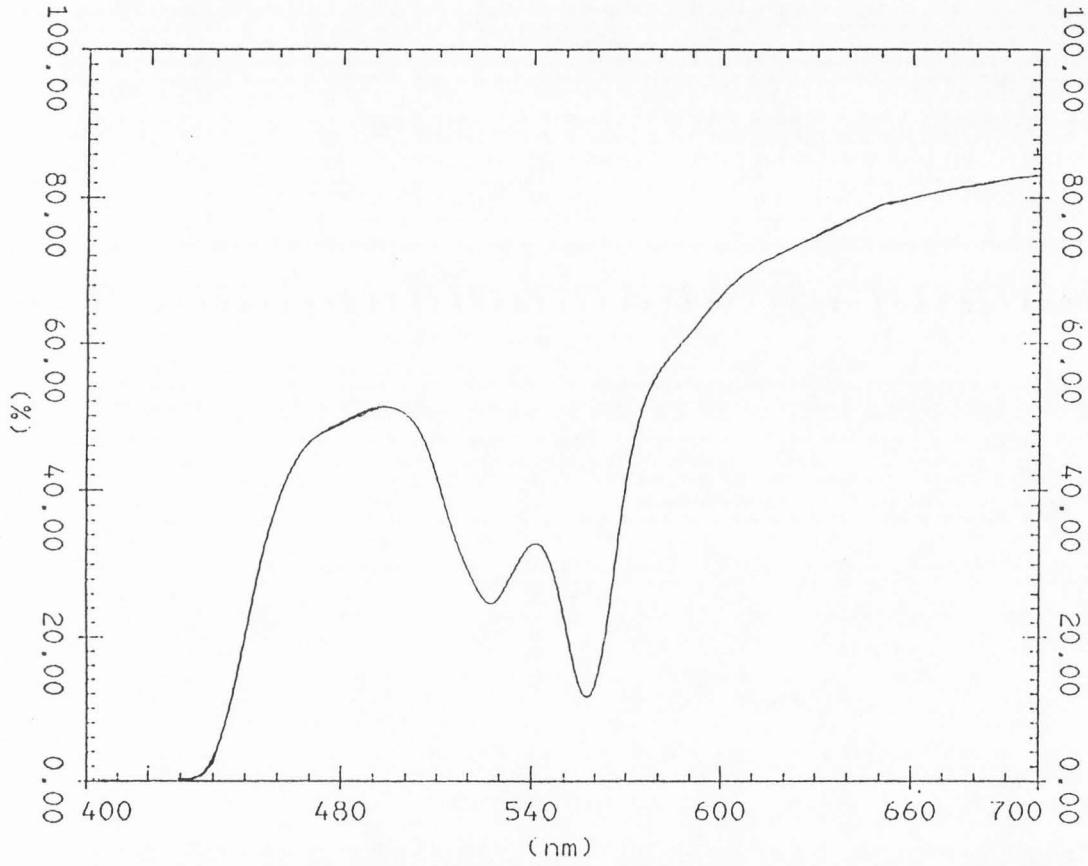

Fig. 21- Reflectance spectra (converted from absorption spectra) for pink-colored denatured globin hemochrome solution, consisting of denatured bovine serum albumin, hematin and sodium dithionite. Pink color did not develop until reduction with sodium dithionite.

REFERENCES

Ahn, D. U. and Maurer, A. S. 1990. Poultry meat color: Kinds of meat pigments and concentrations of the ligands. Poultry Sci. 69: 157.

Anonymons. 1986. Stat View^{TM} 512+, Brain Power, Inc. Calabasas, CA 91302.

Brown, W. D. and Tappel, A. L. 1957. Identification of the pink pigment of canned tuna. Food Res. 22: 214.

Cornforth, D. P., Vahabzadeh, F., Carpenter, C. E., and Bartholomew, D. T. 1986. Role of reduced hemochromes in pink color defect of cooked turkey rolls. J. Food Sci. 51: 1132.

Ghorpade, V. M., Cornforth, D. P., and Sisson, D. V. 1992. Inhibition of red color development in cooked, vacuum-packaged bratwurst. J. Food Sci. In press.

Girard, B., Vanderstoep, J., and Richards, J. F. 1990. Characterization of the residual pink color in cooked turkey breast and pork loin. J. Food Sci. 55: 1249.

Hornsey, H. C. 1956. The color of cooked cured pork. 1. Estimation of the nitric oxide haem pigments. J. Sci. Food Agric. 7: 534.

Howe, J. L., Gullett, E. A., and Usborne, W. R. 1982. Development of pink color in cooked pork. Can. Inst. Food Sci. Technol. J. 15: 19.

Pool, M.F. 1956. Why does some cooked turkey turn pink? Turkey World. Jan., 1968.

Scriven, F., Sporns, P. and Wolfe, F. 1987. Investigation of nitrite and nitrate levels in paper materials used to package fresh meat. J. Agric. Food Chem. 35: 188.

Trout, G. 1989. Variation in myoglobin denaturation and color of cooked beef, pork and turkey meat as influenced by pH, sodium chloride, sodium tripolyphosphate and cooking temperature. J. Food Sci. 54: 536.

Warriss, P.D. 1979. The extraction of haem pigments from fresh meat. J. Food Technol. 14: 75.

PART V. RESEARCH NEEDS

Denatured globin hemochromes cause pink color in meat cooked anaerobically to temperature > 74° C. In canned baby food, pink color is prevented by precooking ground meat aerobically, then canning meat in the regular manner (Urbain, 1992). Oxidizing conditions such as cooking meat in the presence of KIO_3 prevented pink color development in experimental conditions (Cornforth et al., 1986). However, this treatment would not be commercially feasible. Ascorbic acid is a widely used oxidiation-reduction compound, normally used to scavenge O_2 to create reducing conditions in canned foods, including sea foods and meats (Bauernfeind, 1982). Perhaps ascorbate could be oxidized to dehydroascorbate, which then could be added to meat during vacuum cooking, oxidizing heme pigment and preventing pink discoloration. Dehydroascorbate is not commercially available, but may be prepared by oxidizing ascorbic acid in presence of active charcoal using methanol as a solvent in 100% O_2 environment. Dehydroasorbic acid may be collected by filtering and evaporating methanol by rotary evaporator (Ohmori and Takagi, 1978).

The pink color remaining after cooking of high pH meat may be prevented by recognizing the importance of raw meat pH and implementing procedures to control product pH. For example, a buyer of beef patties might specify that patties have pH > 6.0, which is easily achieved by blending high pH meat with normal meat to obtain final blends of desired pH. Acid marinade (cooking in vinegar, etc.) might also lower pH so that myoglobin is fully denatured during cooking. Work is needed to demonstrate feasibility of pH control and acid cooking procedures to prevent this problem in commercial situations.

The pink color attributed to microbial growth in vacuum-packaged bratwurst may be prevented by use of antimicrobial such as sodium lactate and higher cooking temperature to reduce bacterial load of the cooked product. More work is needed to identify a specific microorganism responsible for pink discoloration in vacuum-packaged products.

REFERENCES

Bauernfeind, J. R. 1982. Ascorbic acid technology in agricultural pharmaceutical foods and industrial application. Ch.20. In "Ascorbic Acid Chemistry, Metabolism, Uses," P. A. Seib and B. M. Tolbert (Ed.), p.395. American Chemical Society, Washington, D. C.

Cornforth, D. P., Vahabzadeh, F., Carpenter, C. E., and Bartholomew, D. T. 1986. Role of reduced hemochromes in pink color defect of cooked turkey rolls. J. Food Sci. 51: 1132.

Ohmori, M. and Takagi, M. 1978. A facile preparation of dehydroascorbic acid-methanol solution and its stability. Agric. Biol. Chem. 40: 173.

Urbain, 1992. Personal communication. M. S. U. Ag. Expt. Station, East Lansing, MI.

SUMMARY

In bratwurst study, we attempted to characterize the red pigment that appears upon refrigerated storage, and to determine the effect of pH, cooking and storage temperature, and sodium lactate on incidence of red discoloration. The red surface discoloration appearing in cooked, vacuum-packaged bratwurst with storage was associated with an increase in microbial plate count. Undenatured myoglobin was identified in samples with red discoloration. Higher cooking temperature (74°C), lower product pH (5.5), and addition of 3% sodium lactate interacted to lower microbial load and the incidence of red discoloration. Thus, higher than normal cooking temperature and use of acids or microbial inhibitors may be needed to extend shelflife and color stability of precooked, vacuum-packaged pork products.

Further, the effects of microbial growth in raw materials (ground pork) on cooked pork color were investigated. In experiment 1, red discoloration resulted from the higher myoglobin content of the sow meat. Myoglobin and cytochrome c were indentified in the red exudate of the cooked meat samples in experiment 2 and 3. Thus, it is concluded that microbial growth during refrigerated storage of raw meat is not a cause of red discoloration in cooked pork products in commercial situations.

Finally, pink or red discoloration was investigated in the cooked U. S. #1 pork roasts. Myoglobin was the pigment responsible for pink color in pork roasts cooked to 65°C. Roasts cooked to 82°C had gray internal color after cooking, but developed pink internal color after refrigerated storage. Reflectance spectra of pink slices from roasts, cooked to 82°C, then stored for 12 days at 2°C, were characteristic of denatured globin hemochromes or related non-nitrosyl hemochromes.

APPENDICES

APPENDIX A

Bratwurst Spice Formula

Ingredients	Weight
Meat	
Lean beef	4.5 lbs
Pork trim (80:20)	5.5 lbs
Additives	
Water+ Ice	0.75 lb
NFDM	
Pepper	9.0 g
Mace	4.5 g
Coriander	4.5 g
Ginger	3.0 g
Nutmeg	3.0 g
Mono sodium glutamate	7.0 g
Dextrose	21 g

APPENDIX B

Nitrosoheme Pigment

Reagents:

1. Acetone-a: Place 90 ml distilled water in a one liter volumetric flask; add acetone, mix and bring it to volume.
2. Acetone-b: Mix water with 20 ml concentrated HCl and bring to 100 ml volume. Transfer the diluted HCl to a 1 liter volumetric flask, add acetone, mix and bring it to volume with additional acetone.

Procedure:

Do the following in subdued light to lessen fading of pigment:

1. Weigh out 2.0 g sample in 50 ml polypropylene centrifuge tube.
2. Pipet 9.0 ml acetone-a into centrifuge tube.
3. Macerate meat mass for 2-3 min with a glass rod.
4. Stopper centrifuge tube and mix by gentle swirling.
5. Let stand 10 min, then filter through two Whatman # 42 filter papers into a test tube.
6. Transfer filtrate into 1 cm cuvette and read absorbance within 1 hr at 540 nm. Calculate as nitrosopigment.
7. Prepare another 2.9 g sample, using acetone-b
8. Macerate and allow to stand 1 hr before filtering.
9. Filter the extract into another test tube and read absorbance at 640 nm and calculate as total pigment.

Calculations:

The calculations were made using extinction coefficients fron Hornsey (1956).

ppm nitoso pigment = $A_{540} \times 290$

ppm total pigment= $A_{640} \times 680$

APPENDIX C

Pigment Analysis In Cooked Meats

Determinaton of myoglobin (Mb), % metmyoglobin (MetMb), and % myoglobin denatured (PMD) in cooked meats.

1. Grind meat through a 1/8" plate or mince in to 3mm cubes.
2. Weigh out duplicate samples of about 2 grams of meat. Place the samples in 50 ml polypropylene centrifuge tubes.
3. Add 50 ml ice cold phosphate buffer (pH 6.8, 0.04M) per 5 gram of sample.
4. Homogenize sample for 40 to 45 sec at low speed, using the small diameter head of a polytron homogenizer. Ensure that meat pieces are mixed in solution.
5. Hold the sample at 4°C (on ice) for one hr.
6. Centrifuge sample at 9000 RPM for 45min at 4°C. Filter supernatant through Whatman # 1 filter paper.
7. Take individual absorbance at 700, 572, and 525 nanometers (nm) using phosphate buffer as the blank.

Calculate Mb, % denatured Mb, % MetMb using the following formulas (Trout, 1989).

Myoglobin (mg/g)= (A525 - A700) x 2.303 x dilution factor.

$$\text{(PMD)}\ \%\ \text{denatured myoglobin} = \left(1 - \frac{\text{Myoglobin conc. after heating}}{\text{Myoglobin conc. before heating}}\right) \times 100$$

$$\%\ \text{MetMb} = \left(1.395 - \frac{(A_{572} - A_{700})}{(A_{525} - A_{700})}\right) \times 100$$

APPENDIX D

pH Determination of Meat

Procedure

1. Grind or mince meat sample.
2. Take duplicate samples (10 g) and add 90 ml distilled water.
3. Mix well, then filter.
4. Calibrate a pH meter to pH 4 and pH 7.0.
5. Measure sample pH on filtrate.
6. Record an average of duplicate readings.

APPENDIX E

Nitrite Analysis

Reagents and Apparatus:

1. NED reagent: Dissolve 0.2g N-(1-napthyl)-ethylenediamine 2HCL in 150 ml 15% (v/v) acetic acid. Filter if necessary, and store in brown glass bottle.
2. Sulfanilamide reagent: Dissolve 0.5 g sulfanilamide in 150 ml 15% (v/v) acetic acid. Filter, if necessary, and store in brown glass bottle.
3. Nitrate standard:
   a. Stock solution; 1,000 ppm sodium nitrite, Dissolve 1.0 g sodium nitrite in water and dilute to one liter.
   b. Intermediate solution; 100 ppm sodium nitrite. Dilute 100 ml stock solution to 1 liter with water.
   c. Working solution; 1 ppm sodium nitrite. Dilute 10 ml intermediate solution to 1 liter with water.
4. Test filter paper for nitrite contamination by analyzing 3-4 sheets from box. Filter about 40 ml water through each sheet. Add 4 ml sulfanilamide reagent, mix, let stand 5 min, add 4 ml NED reagent, mix and wait 15 min. If any sheets are positive, discard entire box.

Procedure:

1. Weigh 5 g finely comminuted and thoroughly mixed sample in to 50 ml beaker.
2. Add about 40 ml of 80°C water. Mix thoroughly with glass rod, breaking up all lumps, and transfer to 500 ml volumetric flasks.
3. Wash beaker and rod with successive portions of the hot water, adding all washings to the flask.

4. Add enough hot water to bring volume to about 300 ml, transfer flask to 80°C water bath and let stand for 2 hr, shaking occasionally.
5. Cool to room temperature, dilute to volume with water and remix.
6. Filter, add 2.5 ml sulfanilamide reagent to aliquot containing 5-50 µg sodium nitrite in 50 ml volumentric flask and mix.
7. After 5 min, add 2.5 ml NED reagent, mix, dilute to volume, mix and let color develop for 15 min.
8. Transfer portion of solution to photometer cell and read A_{540} against blank of 45 ml water, 2.5 ml sulfanilamide reagent, and .5 ml NED reagent.
9. Prepare standard curve by adding 10, 20, 30, and 40 ml of working sodium nitrite solution to 50 ml volumetric flasks, add 2.5 ml sulfanilamide reagent, mix and proceed as above, beginning with step 7. Standard curve is a straight line to 1 ppm sodium nitrite in final solution.

Calculations

$$\text{ppm } NaNO_2 \text{ (In sample)} = \mu g\ NaNO_2/g \text{ sample} = \text{ppm } NaNO_2 \times \frac{50}{\text{aliquot size (ml)}} \times \frac{500 \text{ ml}}{\text{sample wt (g)}}$$

APPENDIX F

Analysis of Variance Tables

Table 8 - Analysis of variance for color score of cooked vacuum-packaged bratwurst as influenced by sodium lactate, meat pH, cooking and storage temperature during storage.

SOURCE	DF	MS	F
Replications	1	471.71	81.33
Lactate (L)	1	178.22	30.73
Error (a)	1	5.80	
pH (p)	2	2696.26	25.34**
Lxp	2	4.75	00.05
Error (b)	4	106.40	
Cooking Temp. (T)	1	81.40	15.05**
Storage Temp. (S)	1	39.01	07.21**
Storage Wks. (W)	4	55.88	10.33**
T x S	1	23.21	04.29*
T x W	4	21.84	04.04**
S x W	4	52.34	09.68**
L x T	1	40.56	07.50**
L x S	1	60.16	11.12**
L x W	4	21.09	03.90**
p x T	2	19.31	03.56*
p x S	2	0.80	00.15
p x W	8	24.13	04.46**
T x S x W	4	4.09	00.78
L x T x S	1	42.14	07.79**
L x T x W	4	3.79	00.70
L x S x W	4	20.06	03.71**
L x p x T	2	2.89	00.53
L x p x S	2	3.70	00.68
Lx p x W	8	12.58	02.33*
p x T x S	2	2.61	00.48
p x T x W	8	5.04	00.93
p x S x W	8	7.81	01.44
L x T x S x W	4	1.19	00.22
L x p x T x S	2	2.37	00.44
L x p x T x W	8	5.19	00.96
Lx p x S x W	8	2.22	00.41
p x T x S x W	8	1.14	00.21
L x p x T x S x W	8	2.05	00.38
Error (c)	114	5.41	
Judges (cells)	2160	2.18	00.40
Total	2399		

* Significant at $p < .05$

** Significant at $p < .01$

Table 9 - Analysis of variance for myoglobin in cooked vacuum-packaged bratwurst as influenced by sodium lactate, meat pH, cooking and storage temperature during storage.

SOURCE	DF	MS	F
Replications	1	0.777	9.65
Lactate (L)	1	0.016	3.47
Error (a)	1	0.002	
pH (p)	2	29.616	81.20**
Lx p	2	0.122	0.34
Error (b)	4	0.360	
Cooking Temp. (T)	1	4.722	59.42**
Storage Temp. (S)	1	0.257	3.24
Storage Wks. (W)	1	0.159	2.06
T x S	1	0.000	0.00
T x W	1	0.057	0.71
S x W	1	0.258	3.24
L x T	1	0.000	0.00
L x S	1	0.032	0.40
L x W	1	0.002	0.03
p x T	2	1.828	23.00**
p x S	2	0.022	0.27
p x W	2	0.019	0.24
T x S x W	1	0.000	0.00
L x T x S	1	0.006	0.08
L x T x W	1	0.000	0.00
L x S x W	1	0.032	0.40
L x p x T	2	0.006	0.08
L x p x S	2	0.006	0.08
Lx p x W	2	0.064	0.82
p x T x S	2	0.008	0.11
p x T x W	2	0.130	1.64
p x S x W	2	0.022	0.27
L x T x S x W	1	0.006	0.08
L x p x T x S	2	0.044	0.56
L x p x T x W	2	0.007	0.09
Lx p x S x W	2	0.005	0.07
p x T x S x W	2	0.008	0.11
L x p x T x S x W	2	0.045	0.56
Error (c)	42	0.075	
Total	95		

* Significant at $p < .05$

** Significant at $p < .01$

Table 10 - Analysis of variance for metmyoglobin in cooked vacuum-packaged bratwurst as influenced by sodium lactate, meat pH, cooking and storage temperature during storage.

SOURCE	DF	MS	F
Replications	1	190.801	0.89
Lactate (L)	1	6.688	0.02
Error (a)	1	334.400	
pH (p)	2	14837.799	22.83**
Lx p	2	56.29	0.09
Error (b)	4	625.444	
Cooking Temp. (T)	1	512.450	2.39
Storage Temp. (S)	1	76.827	0.36
Storage Wks. (W)	1	17.442	0.08
T x S	1	8.449	0.04
T x W	1	35.996	0.17
S x W	1	80.446	0.38
L x T	1	22.952	0.11
L x S	1	114.975	0.54
L x W	1	352.896	1.65
p x T	2	953.868	4.46*
p x S	2	6.585	0.03
p x W	2	43.813	0.20
T x S x W	1	7.304	0.03
L x T x S	1	4.833	0.02
L x T x W	1	111.672	0.52
L x S x W	1	119.394	0.56
L x p x T	2	11.599	0.05
L x p x S	2	10.314	0.05
Lx p x W	2	30.926	0.14
p x T x S	2	14.554	0.07
p x T x W	2	171.267	0.08
p x S x W	2	6.762	0.03
L x T x S x W	1	3.977	0.02
L x p x T x S	2	11.346	0.05
L x p x T x W	2	266.185	1.24
Lx p x S x W	2	9.786	0.05
p x T x S x W	2	13.172	0.06
L x p x T x S x W	2	10.469	0.05
Error (c)	42	209.38	
Total	95		

* Significant at $p < .05$

** Significant at $p < .01$

Table 11 - Analysis of variance for aerobic plate counts in cooked vacuum-packaged bratwurst as influenced by sodium lactate, meat pH, cooking and storage temperature during storage.

SOURCE	DF	MS	F
Replications	1	0.444	1.58
Lactate (L)	1	2.593	21.24
Error (a)	1	0.122	
pH (p)	2	0.027	0.14
Lx p	2	0.094	0.49
Error (b)	4	0.192	
Cooking Temp. (T)	1	7.283	25.93**
Storage Temp. (S)	1	42.762	152.23**
Storage Wks. (W)	1	80.048	284.96**
T x S	1	0.146	0.52
T x W	1	1.665	5.93
S x W	1	42.76	152.23**
L x T	1	0.080	0.29
L x S	1	1.802	6.42*
L x W	1	2.769	9.86**
p x T	2	0.119	0.43
p x S	2	0.058	0.21
p x W	2	0.465	1.66
T x S x W	1	0.146	0.52
L x T x S	1	0.000	0.00
L x T x W	1	0.131	0.47
L x S x W	1	1.802	6.42*
L x p x T	2	0.024	0.08
L x p x S	2	0.046	0.16
Lx p x W	2	0.173	0.62
p x T x S	2	0.028	0.10
p x T x W	2	0.106	0.38
p x S x W	2	0.059	0.21
L x T x S x W	1	0.000	0.00
L x p x T x S	2	0.056	0.20
L x p x T x W	2	0.140	0.50
Lx p x S x W	2	0.046	0.16
p x T x S x W	2	0.029	0.10
L x p x T x S x W	2	0.056	0.20
Error (c)	42	0.281	
Total	95		

* Significant at $p < .05$

** Significant at $p < .01$

Table 12 - Analysis of variance for anaerobic plate counts in cooked vacuum-packaged bratwurst as influenced by sodium lactate, meat pH, cooking and storage temperature during storage.

SOURCE	DF	MS	F
Replications	1	3.592	9.30
Lactate (L)	1	4.442	6.79
Error (a)	1	0.654	
pH (p)	2	0.269	1.34
Lx p	2	0.260	1.30
Error (b)	4	0.200	
Cooking Temp. (T)	1	15.796	40.89**
Storage Temp. (S)	1	90.137	233.32**
Storage Wks. (W)	1	162.176	419.80**
T x S	1	0.000	0.00
T x W	1	0.841	2.18
S x W	1	90.137	233.32**
L x T	1	0.012	0.03
L x S	1	0.665	1.72
L x W	1	1.630	4.22*
p x T	2	0.214	0.56
p x S	2	0.048	0.12
p x W	2	0.194	0.50
T x S x W	1	0.000	0.00
L x T x S	1	0.000	0.00
L x T x W	1	0.912	2.36
L x S x W	1	0.665	1.72
L x p x T	2	0.026	0.07
L x p x S	2	0.180	0.05
Lx p x W	2	0.052	0.14
p x T x S	2	0.008	0.02
p x T x W	2	0.101	0.26
p x S x W	2	0.048	0.12
L x T x S x W	1	0.000	0.00
L x p x T x S	2	0.129	0.34
L x p x T x W	2	0.118	0.31
Lx p x S x W	2	0.180	0.47
p x T x S x W	2	0.008	0.02
L x p x T x S x W	2	0.129	0.34
Error (c)	42	0.386	
Total	95		

* Significant at $p < .05$

** Significant at $p < .01$

Table 13 - Analysis of variance for pH in cooked vacuum-packaged bratwurst as influenced by sodium lactate, meat pH, cooking and storage temperature during storage.

SOURCE	DF	MS	F
Replications	1	0.459	7.92
Lactate (L)	1	0.203	25.23
Error (a)	1	0.008	
pH (p)	2	4.593	22.36**
Lx p	2	0.006	0.03
Error (b)	4	0.205	
Cooking Temp. (T)	1	0.038	0.66
Storage Temp. (S)	1	2.620	45.17**
Storage Wks. (W)	1	5.444	93.83**
T x S	1	0.006	0.11
T x W	1	0.001	0.02
S x W	1	2.620	45.17**
L x T	1	0.028	0.48
L x S	1	0.445	7.68**
L x W	1	0.519	8.95**
p x T	2	0.002	0.05
p x S	2	0.138	2.37
p x W	2	0.126	2.18
T x S x W	1	0.006	0.11
L x T x S	1	0.025	0.44
L x T x W	1	0.040	0.69
L x S x W	1	0.445	7.68**
L x p x T	2	0.002	0.04
L x p x S	2	0.002	0.04
Lx p x W	2	0.007	0.12
p x T x S	2	0.000	0.00
p x T x W	2	0.000	0.00
p x S x W	2	0.138	2.37
L x T x S x W	1	0.025	0.44
L x p x T x S	2	0.005	0.10
L x p x T x W	2	0.005	0.08
Lx p x S x W	2	0.003	0.04
p x T x S x W	2	0.000	0.00
L x p x T x S x W	2	0.005	0.10
Error (c)	42	0.058	
Total	95		

* Significant at $p < .05$

** Significant at $p < .01$

Table 14 - Analysis of variance for color score in cooked ground pork as influenced by pH, storage weeks, storage method, and cooking temperature in experiment 3.

SOURCE	DF	MS	F
Replications	2	5.67	2.26
pH (p)	1	160.24	63.84**
Error A	2	2.51	
Storage Method (M)	2	162.35	66.67**
p x M	2	88.32	36.35**
Error B	8	2.43	
Storage Wks.(W)	2	23.46	5.48*
p x W	2	0.78	0.18
M x W	4	11.79	2.76
p x M x W	4	5.35	1.25
Error C	24	4.28	
Cooking Temp.(T)	1	68.50	36.05**
p x T	1	14.70	7.74*
M x T	2	8.98	4.73*
p x M x T	2	0.60	0.32
W x T	2	15.85	8.34*
p x W x T	2	7.75	4.08
M x W x T	4	3.23	1.70
p x M x W x T	4	0.97	0.51
Error D	36	1.90	
Duplicate (Judges)	108	1.12	
Judges (Cells)	864	1.52	
Total	1079		

* Significant at $p < .05$

** Significant at $p < .01$

Table 15 - Analysis of variance for aerobic plate counts in stored ground pork as affected by storage weeks and method in experiment 3.

source	df	MS	F
Storage Wks (W)	2	7.257	486.675**
Storage Method (S)	2	7.876	528.201**
W x S	4	1.501	100.645**
Error	9	0.015	
Total	17		

* Significant at $p < .05$
** Significant at $p < .01$

Table 16 - Analysis of variance for reducing ability in stored ground pork as affected by storage weeks and method in experiment 3.

source	df	MS	F
Storage Wks (W)	2	0.204	29.579**
Storage Method (S)	2	0.014	1.99
W x S	4	1.47E-3	0.213
Error	9	6.911E-3	
Total	17		

* Significant at $p < .05$
** Significant at $p < .01$

Table 17 - Analysis of variance for meat pH in stored ground pork as affected by storage weeks and method in experiment 3.

source	df	MS	F
Storage Wks (W)	2	0.072	86.584**
Storage Method (S)	2	0.064	78.758**
W x S	4	0.027	32.685**
Error	9	8.27E-4	
Total	17		

* Significant at $p < .05$
** Significant at $p < .01$

Table 18 - Analysis of variance for color score in cooked ground pork as influenced by storage weeks, storage method, and cooking temperature in experiment 2.

SOURCE	DF	MS	F
Replications	1	152.63	
Storage Wks.(W)	2	85.08	1.68
Storage Method (S)	2	69.18	1.37
Cooking Temp.(T)	1	216.90	4.29*
W x S	4	22.27	<1
W x T	2	193.18	3.82
S x T	2	93.57	1.65
W x S x T	4	27.03	<1
Error	17	50.47	
Judges (Cells)	324	5.02	
Triplicate (Judges)	720	1.1	
Total	1079		

* Significant at $p < .05$

** Significant at $p < .01$

Table 19 - Analysis of variance for aerobic plate counts in stored ground pork as affected by storage weeks and method in experiment 2.

source	df	MS	F
Storage Wks (W)	2	2.262	225.672**
Storage Method (S)	2	4.527	451.650**
W x S	4	0.578	57.691**
Error	9	0.01	
Total	17		

* Significant at $p < .05$
** Significant at $p < .01$

Table 20 - Analysis of variance for reducing ability in stored ground pork as affected by storage weeks and methods in experiment 2.

source	df	MS	F
Storage Wks (W)	2	7.22E-5	0.48
Storage Method (S)	2	1.71E-3	1.14
W x S	4	2.32E-3	1.55
Error	9	1.49E-3	
Total	17		

* Significant at $p < .05$
** Significant at $p < .01$

Table 21 - Analysis of variance for meat pH in stored ground pork as affected by storage weeks and method in experiment 2.

source	df	MS	F
Storage Wks (W)	2	1.06E-4	0.528
Storage Method (S)	2	0.249	1248.028**
W x S	4	1.96E-3	9.778
Error	9	2.00E-4	
Total	17		

* Significant at $p < .05$
** Significant at $p < .01$

Table 22 - Analysis of variance for color score in cooked ground pork as influenced by storage weeks, storage method, and cooking temperature in experiment 1.

SOURCE	DF	MS	F
Replications	1	811.20	
Storage Wks.(W)	2	175.91	2.98
Storage Method (S)	2	41.35	<1
Cooking Temp.(T)	1	82.22	1.39
W x S	4	12.04	<1
W x T	2	20.73	<1
S x T	2	53.95	<1
W x S x T	4	31.09	<1
Error	17	59.02	
Judges (Cells)	324	4.82	
Triplicate (Judges)	720	0.59	
Total	1079		

* Significant at $p < .05$

** Significant at $p < .01$

Table 23 - Analysis of variance for aerobic plate counts in stored ground pork as affected by storage weeks and method, and meat pH in experiment 1.

source	df	MS	F
Storage Wks (W)	2	11.277	56.15**
Storage Method (S)	2	10.128	50.25**
W x S	4	3.389	16.87**
Meat pH (p)	1	0.550	2.74
W x p	2	5.24E-4	2.60E-3
S x p	2	0.936	4.66*
W x S x p	4	0.126	0.627
Error	36	0.201	
Total	53		

* Significant at $p < .05$
** Significant at $p < .01$

Table 24 - Analysis of variance for reducing ability in stored ground pork as affected by storage weeks and method, and meat pH in experiment 1.

source	df	MS	F
Storage Wks (W)	2	8.15E-3	2.677
Storage Method (S)	2	0.036	11.83**
W x S	4	0.012	4.050**
Meat pH (p)	1	0.019	6.325*
W x p	2	6.46E-3	2.123
S x p	2	3.27E-3	1.074
W x S x p	4	0.014	4.543**
Error	36	3.04E-4	
Total	53		

* Significant at $p < .05$
** Significant at $p < .01$

Table 25 - Analysis of variance for meat pH in stored ground pork as affected by storage weeks and method, and meat pH in experiment 1.

source	df	MS	F
Storage Wks (W)	2	0.300	51.10**
Storage Method (S)	2	0.484	82.41**
W x S	4	0.073	12.15**
Meat pH (p)	1	4.723	803.53**
W x p	2	0.067	11.34**
S x p	2	0.058	9.81**
W x S x p	4	0.037	6.36**
Error	36	5.87E-3	
Total	53		

* Significant at $p < .05$
** Significant at $p < .01$

Table 26 - Analysis of variance for anaerobic plate counts in stored ground pork as affected by storage weeks and method, and meat pH in experiment 1.

source	df	MS	F
Storage Wks (W)	2	5.302	15.903**
Meat pH (p)	1	0.925	2.774
W x p	2	0.078	0.235
Error	12	0.333	
Total	17		

* Significant at $p < .05$
** Significant at $p < .01$

Table 27 - Analysis of variance of quantity of myoglobin (Mb), percent metmyoglobin (MMb), percent myoglobin denatured (PMD) and ppm nitroso pigments, as affected by storage time and cooking temperature.

		Mb (mg/g)		MMb (%)		PMD (%)		Nitroso pigment (ppm)	
Source	DF	MS	F	MS	F	MS	F	MS	F
Block	1	0.00	<1	0.78	<1	11.02	<1	0.42	6.00
Storage Time (S)	4	0.19	4.75*	547.18	9.08**	267.93	10.31**	0.41	5.85**
Cooking Temp (C)	1	1.71	42.75**	11315.00	187.8**	1574.49	60.55**	0.34	4.85*
SxC	4	0.17	4.25*	410.46	6.81**	277.79	10.68**	0.10	1.42
Error	9	0.04		60.24		26.00		0.07	
Total	19								

* Significant at $p < .05$
** Significant at $p < .01$

Table 28 - Analysis of variance of pork roasts color score values, as affected by storage time, cooking temperature, and time after slicing.

Source	DF	MS	F
Block	1	0.84	
Storage Time (S)	4	2.45	1.65
Cooking Temperature (C)	1	105.13	71.11**
SxC	4	0.95	<1
Error (a)	9	1.47	
Time After Slicing (T)	1	117.04	289.00*
Error (b)	1	0.41	
SxT	4	7.82	5.27**
CxT	1	88.44	59.65**
SxCxT	4	3.74	2.53
Error (c)	9	1.48	
Judge (cells)	160	0.61	<1
Total	199		

* Significant at $p < .05$
** Significant at $p < .01$

Table 29 - Analysis of variance of Hunter color 'a' or redness values, as affected by storage time, cooking temperature, and time after slicing.

Source	DF	MS	F
Block	1	12.88	2.29
Storage Time (S)	4	2.48	0.44
Cooking Temperature (C)	1	18.09	3.22
SxC	4	10.11	1.80
Error (a)	9	5.62	
Time After Slicing (T)	1	100.17	14.03
Error (b)	1	7.14	
SxT	4	9.27	1.90
CXT	1	133.59	27.36**
SxCxT	4	12.01	2.46
Error (c)	9	4.88	
Total	39		

* Significant at $p < .05$
** Significant at $p < .01$

VITA

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Candidate for the Degree

of

Doctor of Philosophy

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Ghorpade, V. M., Kadam, S. S., and Salunkhe, D. K. (1986). Thermal Stability and changes in trypsin inhibitor during germination and cooking of horse gram. *Journal of Food Science and Technology*. Vol 23 May-June pp. 164-165.

Ghorpade, V. M., Chavan, J. K., and Kadam, S. S. (1986). Studies on preservation of moth bean sprouts. *Indian Food Packer* , Jan- Feb. pp. 49-59.

Ghorpade, V. M., and Kadam, S. S. (1988). Chapter Germination' is included in *Hand Book of World Food Legumes : Nutritional chemistry, Processing Technology and Utilization.*Volume III by Salunkhe D. K. and Kadam S. S. (editors) C. R. C. Press Inc., U.S.A.

Ghorpade, V. M., and Cornforth, D. P., and Sisson, D. V. (1992). Inhibition of red discoloration in cooked, vacuum-packaged bratwurst. *Journal of Food Science.* In press.

Ghorpade, V. M., and Cornforth, D. P. (1992). Characterization of pigment responsible for pink color in pork roast cooked to 65 or 82°C. *Journal of Food Science.* (submitted).

Kadam, S. S., Ghorpade, V. M., Adsule, R. N., and Salunkhe, D. K. (1986). Trypsin inhibitor in moth bean, Thermal stability and changes during germination and cooking. *Qual Plant Foods Hum. Nutr.* 36 pp. 43- 46.

Cornforth, D. P., Ghorpade, V. M., and Kim, Y. (1992). Effect of various meat storage methods on color of cooked ground pork. *Journal of Muscle Foods.* In press.

Presentations:

Kadam S. S., Ghorpade V. M., and Salunkhe D. K. (1983). Toxic constituents in horse gram; changes during germination and cooking. *5th toxicology Conference* at Pune (India) on December 20, 1983.

Ghorpade, V. M., Cornforth, D. P., and Sisson, D. V. (1991). Effects of sodium lactate, pH, cooking temperature, and storage time on color and microbial load of vacuum-packaged bratwurst. Presented at Graduate student poster competition AMSA, Kansas State University, Manhattan, KS on June 9-13, 1991.

Cornforth, D. P., Ghorpade, V. M., and Kim, Y., (1991). Effect of various meat storage methods on color of cooked ground pork. Presented at IFT meeting Dallas, Texas, June, 20-24, 1991.