Investigating uncured no nitrate or nitrite added processed meat products

Jeffrey Joseph Sindelar
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Investigating uncured no nitrate or nitrite added processed meat products

by

Jeffrey Joseph Sindelar

A dissertation submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Meat Science

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Iowa State University
Ames, Iowa
2006

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ABSTRACT

Samples of different commercial brands (4 uncured, no-nitrate/nitrite-added and one nitrite-added) of three product types (frankfurters, hams and bacons) were evaluated for color, pigment content, pH, lipid oxidation, residual nitrate and nitrite quantity, and consumer acceptance. All samples contained residual nitrate and residual nitrite except for Brands B and D bacons (<1 ppm nitrite). A large variation in severity of lipid oxidation existed between product types within brands with frankfurters reporting the highest levels. Color measurements indicated the majority of products and brands were similar to the control. Consumer sensory ratings determined that variation existed.

The effects of varying levels of vegetable juice powder (VJP) and incubation times (MIN-HOLD) on quality characteristics and sensory properties of emulsified frankfurter-style cooked (EFSC) sausages and hams over a 90-day storage period were investigated. Four EFSC sausage treatments (TRT) (TRT1: 0.20% VJP, 30 MIN-HOLD; TRT2: 0.20% VJP, 120 MIN-HOLD; TRT3: 0.40% VJP, 30 MIN-HOLD; TRT4: 0.40% VJP, 120 MIN-HOLD) and a sodium nitrite-added (156 mg/kg) control (C) were identified for this study. No differences for lipid oxidation between any TRTs and C or over time were identified. No differences (P>0.05) for Commission International D’Edairerage (CIE) L* values were found between TRTs. CIE a* and reflectance ratio values revealed TRTs 2, 4 and C were redder and had better cured color than TRTs 1 and 3 at Day 0. Trained sensory intensity ratings determined that all TRTs 2, 3, and 4 were similar to the C.
Four ham treatments (TRT1: 0.20% VJP, 0 MIN-HOLD; TRT2: 0.20% VJP, 120 MIN-HOLD; TRT3: 0.35% VJP, 0 MIN-HOLD; TRT4: 0.35% VJP, 120 MIN-HOLD) and a sodium nitrite-added control (C) were identified for this study. No differences (P>0.05) were identified between TRTs and C for CIEL*, a*, b* and cured color measured by reflectance ratio. The C had less (P<0.05) lipid oxidation than TRTs 2 and 4 for combined days. No differences (P>0.05) were reported for cured pigment concentration between TRTs and C. Trained sensory intensity ratings determined that a high level (0.35%) of VJP resulted in the highest scores for undesirable vegetable aroma and flavor.
CHAPTER 1. GENERAL INTRODUCTION

Through the years, the meat industry has seen the development and production of foods to meet the demands of health-conscious consumers. Food products with natural, organic, preservative-free and minimally processed claims are now commonly available to consumers in the market place. These claims are also frequently found for fresh and processed meat products. The association of these claims to healthier, safer and better foods has resulted in a dramatic increase in consumer demands and availability of these products. At the same time, this has allowed the opportunity for larger profit margins for manufacturers as higher prices are often sought and paid for these products. For traditionally cured processed meat products, new choices have become available to health-conscious consumers. Thus, uncured, no-nitrate/nitrite-added meat and poultry products have been developed to satisfy consumer demands for “healthier” and “safer” processed meats.

According to the United States Department of Agriculture (USDA) Code of Federal Regulations Title 9, Part 317.17 and 319.2, meat products which permit or require nitrate or nitrite can also be manufactured without nitrates or nitrites but must be labeled to reflect this. The labeling requirements state the term “Uncured” must precede the common, usual or descriptive name with additional disclaimers including a statement that no nitrates or nitrites were added.

Sodium nitrite has been historically utilized for the purpose of altering the color, flavor, safety and shelf life characteristics. A wealth of research has shown that nitrite is responsible for the development of cured color and flavor, serves as a
strong antioxidant to protect flavor to prevent lipid oxidation and subsequent warmed-over flavors, and perhaps most important, acts as a strong antimicrobial agent to control *Clostridium botulinum* outgrowth. Over the past four decades, nitrite has been scrutinized by media and consumers about the safety concerns of ingesting nitrite relating to potential cancer causing nitrosamine formation. Although a great deal of research has been performed addressing this topic, no link between nitrite and cancer has ever been identified. Perceptions still fresh in consumer minds may result in uncertainty and skepticism about this extremely important yet still controversial substance.

Uncured, no-nitrate/nitrite-added meat products have provided consumers with purchasing choices regardless of what scientific evidences are reported. One would not expect to find any meat product labeled “uncured” having appearance, aroma or flavor characteristics of a nitrite-added cured product. This, however, is not the case as commercially available products labeled “uncured” commonly possess characteristics of a nitrite-cured product. This can lead to a great deal of confusion by consumers.

There are two types of uncured products commonly available to consumers. The first type is where no intention of replacing nitrate or nitrite was made during product manufacture. These products, as would be expected, exhibit appearance, aroma and flavor characteristics of a product in which no nitrate-or nitrite-related curing reactions took place. The second type is where there was an intention of replacing nitrate and nitrite resulting in products possessing attributes similar to those of a nitrate- and nitrite-cured product.
In order to manufacture the latter of the two above products, an ingredient containing nitrite or nitrate must be used so the direct addition of nitrite is avoided. Certain vegetables, found naturally high in nitrate content along with a nitrate reducing starter culture, have successfully been used as an indirect curing source. To complete this unconventional curing system, an incubation of the two above ingredients must take place to result in generated nitrite available for curing reactions to take place. The results of this system are uncured, no-nitrate/nitrite-added meat products with attributes similar to a conventional cured meat products.

Although products with similar characteristics can be produced from this system, little is known on how these products compare to traditional nitrite-added products from a sensory or qualitative standpoint, especially over time. Therefore, the first overall objective of this research was to investigate quality attributes and consumer acceptance of commercially uncured, no-nitrate/nitrite-added hams, frankfurters and bacons compared to a nitrite-added control considered an industry standard for each respective product group. The second overall objective was to investigate the effects of varying levels of vegetable juice powder and incubation times on quality characteristics including lipid oxidation, color and cured meat pigment concentrations of emulsified frankfurter-style cooked sausages and hams over an extended storage period, and to determine if differences exist in finished products determined by trained sensory analysis. The third overall objective was to understand the effects of vegetable juice powder level and incubation time on nitrate and nitrite concentrations during product manufacturing and over the ensuing storage period.
Dissertation Organization

This dissertation is organized into six chapters. The first chapter is a general introduction of uncured, no-nitrate/nitrite-added meat and poultry products. The second chapter is a general literature review of relevant topics pertaining to this research project. Chapters three, four and five are complete manuscripts prepared using the Journal of Food Science Style Guide. The third chapter is a manuscript titled “Investigating quality attributes and consumer acceptance of uncured, no-nitrate/nitrite-added commercial ham, bacon and frankfurters”. The fourth chapter is a manuscript titled “Effects of varying levels of vegetable juice powder and incubation time on color, residual nitrate and nitrite, pigment, pH and trained sensory attributes of ready-to-eat uncured emulsified frankfurter-style cooked sausages”. The fifth chapter is a manuscript titled “Effects of varying levels of vegetable juice powder and incubation time on color, residual nitrate and nitrite, pigment, pH and trained sensory attributes of ready-to-eat uncured ham”. The sixth chapter is a general summary of this research.
CHAPTER 2. LITERATURE REVIEW

I. The History of Nitrites and Nitrates

From the Beginning

The use of nitrites to preserve and cure meats evolved centuries ago (Cassens 1995). Before the discovery of refrigeration, fish and meat were preserved by methods effective for controlling spoilage well past animal harvesting and immediate consumption or to also extend food supplies from times of plenty to times of scarcity. Drying to decrease water activity, smoking, salting, marinating or pickling foods were commonly used methods of preservation (Pegg and Shahidi 2000). The use of salt to preserve fish dates back to 3000 B.C. (Pierson and Smooth 1982) and the practice of salting meat was a common practice by the fifth century B.C. (Pearson and Tauber 1984). The latter three of the five previous mentioned methods were effective principally due to the usage of salt. Modern day curing technologies can be directly related to early salting procedures.

The Discovery

The exact historical origin of meat curing is lost in antiquity but is believed to have been discovered by accident. It is understood and well accepted that impurities in natural salt led to the discovery of modern day meat curing (MacDougall and others 1975; Pierson and Smooth 1982; Price and Schweigert 1987). The history of meat processing refers to several accounts of the contamination of salt with saltpeter (potassium nitrite) resulting in a stable red color in the meat (Cassens
1990). It is unclear whether the saltpeter cured characteristics were deemed desirable before the 10th century B.C., but during the 10th century B.C. and after, the Romans were intentionally adding saltpeter to meat to obtain a wanted red color and distinctive flavor. It was not until the 19th century that several scientific investigations to better understand the curing process were prompted by a discovery that pure salt (sodium chloride) did not produce a “cured color” (Pierson and Smooth 1982).

**Sodium and Potassium Understanding**

Meat curing is defined as the addition of the basic compounds salt, sugar or other sweetener and nitrite into meat to develop distinctive color, flavor and texture properties while aiding in the quality and microbiological aspects of meat products (Pearson and Tauber 1984; Aberle and others 2001). Besides the above mentioned basic compounds, other ingredients such as phosphates, spices, flavorings and sodium erythorbate are also commonly included in cure mixes.

Cassens (1990) and Pierson and Smooth (1982) summarized the work of several pioneers in meat curing research. Slightly before and at the turn of the 20th century, E. Polenske, J. Haldane and K.B. Lehman were among the first scientists to closely examine the curing process and report their scientific findings (Pierson and Smooth 1982). Polenske examined sterilized and unsterilized solutions of saltpeter (potassium nitrate) for the presence of nitrite. Based on his findings, he was able to show that nitrate was reduced to nitrite and subsequently nitric oxide. This phenomenon was attributed to microorganisms. Polenske and Haldane also showed that subsequent heating of those meat products containing nitrite and nitric oxide resulted in the production of cured color as a result of the modifications of
meat pigments during the heating process. Haldane found similar results with the same overall conclusions. Haldane also investigated the pigments that were involved with the red color of cured meats. By adding nitrite to hemoglobin (Hv) to produce nitrosylhemoglobin (NOHb) and then heating these pigments, Haldane was able to generate nitrosylhemochromogen. He then explained that NOHb was the pigment responsible for the red color in cooked cured meat. These early scientists' work established the concept that unpurified salt contaminated with sodium or potassium nitrates was responsible for the color of cured meat. Sebranek (1979) stated that in 1899, K.B. Lehman showed that nitrite and not nitrate was responsible for cured color. His findings were later confirmed by R. Hoagland in 1908 and R.H. Kerr and others in 1926. In an investigation by Hoagland, the author noted that nitrate was converted by bacteria or enzymes to nitrites which subsequently changed to nitric oxide that united with myoglobin in meat to produce a red color (Cerveny 1980).

Hoagland then continued his investigations and in 1914 presented further proof that saltpeter (potassium nitrate) must be reduced to nitrite to have any functionality as a curing ingredient. He also reported that nitrite needed to have a reducing agent present to produce nitric oxide. His investigation of adding nitrite to alkaline meat which resulted in no cured color development supported his theory.

Gray and others (1981) stated that saltpeter (potassium nitrate) or sodium nitrate can be converted to nitrite by the reducing action of bacteria. It was not until the turn of 20th century that it was determined that nitrate did not have a direct influence in the curing process.
II. Regulations

United States Department of Agriculture (USDA)

The scientific knowledge about nitrates and nitrites up to the early 19th century led to the slow and progressive change from nitrate to nitrite usage in cured meats. It was also scientifically shown that lower levels of nitrite than nitrate were needed to achieve the same amount of curing. However, since nitrate was being primarily used up to this time, little knowledge was known about how much nitrite instead of nitrate to use. A survey of 17 different meat plants indicated that the use of nitrate often resulted in extremely variable nitrite contents (Pierson and Smooth 1982).

Due to problems associated with using nitrite and nitrate together, such as spoilage and inconsistent cured color development, on January 19, 1923, the U.S. government authorized experiments to better understand the direct use of sodium nitrite in cured meats. At this same time, the USDA also permitted the direct addition of nitrite into meat and set a limit of 200 ppm (mg/kg) nitrite content in all finished cured meat products. Due to the extensive research to gain a better understanding, sodium nitrite was not approved for meat curing by the U.S. Department of Agriculture until 1925 (Pearson and Tauber 1984).

The experiments authorized by the U.S. government in 1923 investigated nitrate and nitrite usage in cured meats more closely. A better understanding of sufficient levels of both nitrite and nitrates was determined and the current USDA Food Safety Inspection Service (FSIS) regulatory limits for sodium and potassium
nitrate and nitrite curing ingredients are based on the findings from those experiments (Cassens 1990). The conclusions from these experiments stated:

“Sodium nitrite can be substituted successfully for sodium or potassium nitrate in the cure of meat. From ¼ to 1 oz. of sodium nitrite was sufficient to fix the color of 100 lb of meat with the exact quantity depending on the meat to be cured and the process employed. Meats so cured contain no more nitrite than meat cured with nitrate and are free from the unconverted nitrate. The customary curing period may be shortened by using nitrite. Meats cured with sodium nitrite in the proper quantity and in accordance with sound practice are in no way inferior in quality or wholesomeness to meats cured with nitrates.”

The ensuing regulations regarding nitrate and nitrite usage were reiterated and clarified in 1970. Based on these findings as well as further experimentation over time, the maximum ingoing limits of sodium and potassium nitrate and nitrite were determined for various types of curing agents and curing methods. Table 2.1 displays the current regulatory limits for the discussed curing agents.

<table>
<thead>
<tr>
<th>Curing Agent</th>
<th>Immersion Cured</th>
<th>Massaged or pumped</th>
<th>Comminuted</th>
<th>Dry Cured</th>
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<tr>
<td>Sodium Nitrite</td>
<td>200</td>
<td>200</td>
<td>156</td>
<td>625</td>
</tr>
<tr>
<td>Potassium Nitrite</td>
<td>200</td>
<td>200</td>
<td>156</td>
<td>625</td>
</tr>
<tr>
<td>Sodium Nitrate</td>
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<td>700</td>
<td>1718</td>
<td>2187</td>
</tr>
<tr>
<td>Potassium Nitrate</td>
<td>700</td>
<td>700</td>
<td>1718</td>
<td>2187</td>
</tr>
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</table>

Table 2.1. Maximum allowable limits for various curing agents and curing methods cured meat products. (**does not include bacon) All limits are based on total formulation / brine weight for immersion cured or massaged or pumped and green weight for comminuted or dry cured products. Limits are reported in parts per million. This reprint is from USDA FSIS Processing Calculations Inspector's Handbook (FSIS Directive 7620.3).
The FSIS Agency does require a minimum of 120 ppm of ingoing nitrite in all cured meat products which are labeled “Keep Refrigerated” unless a processor is able to validate his/her specific process from a food safety standpoint (USDA 1995). Interestingly, there is no ingoing minimum requirement for the manufacture of shelf stable meat products. Due to the concern of nitrosamine production, bacon follows different and more stringent regulations for nitrite while nitrate is no longer allowed for use in bacon.

The method of bacon manufacture determines the allowable levels of nitrite. For immersion cured or massaged or pumped skin (rind) off fresh bellies, the ingoing sodium nitrite must be 120 ppm (or 148 ppm potassium nitrite) according to USDA FSIS (USDA 1995). There are two cases where these ingoing requirements can result in lower amounts for massaged or pumped bacon. First, if an appropriate partial quality control program is utilized, 100 ppm sodium nitrite (or 123 ppm potassium nitrite) may replace the 120 ppm sodium nitrite (or 148 ppm potassium nitrite) requirement. Secondly, if sugar and a lactic acid starter culture are both used, the required ingoing level of sodium nitrite can drop to 40-80 ppm (or 49-99 ppm potassium nitrite).

If the skin is left on fresh bellies, it is understood that the skin comprises approximately 10% of the weight of the entire belly and therefore 10% less nitrite must be used, or 108 ppm sodium nitrite instead of 120 ppm or 133 ppm potassium nitrate instead of 148 ppm. Bacon can also be dry cured and the required levels for sodium or potassium nitrite are 200 and 246 ppm, respectively. It should also be
noted that nitrate and nitrite are not permitted for use in baby, junior or toddler foods (USDA 1995).

III. Meat Color Chemistry

The value of meat color to the consumer is extremely important. The four determining attributes for consumer purchasing decisions are color, juiciness, flavor and toughness/tenderness. Of these attributes, color is the first and most important factor of the decision making process to purchase meat products (Shahidi 1998; Aberle and others 2001). It is generally believed by consumers that an aesthetically appealing color of a meat product directly relates to the quality of that meat cut (Price and Schweigert 1987).

Pigments of Fresh Meat Color

The concentration and chemical state of hemoproteins or meat pigments are the major contributing factors in the color of raw meat (Pegg and Shahidi 1997). There are several different pigments found in meat. Some of these include myoglobin, hemoglobin, the cytochromes, catalase, the flavins, and other colored substances (Pearson and Tauber 1984). Of those listed, myoglobin and hemoglobin pigments are the two proteins that are principally associated with meat color.

Myoglobin is a globular pigment that contains both a protein and a non-protein portion (Aberle and others 2001). Myoglobin is found in most abundance and is responsible for the red color of fresh meat (Price and Schweigert 1987). The molecular structure of myoglobin consists of 153 amino acids folded around a prosthetic group called heme. The outer ring of the molecule is called porphyrin or 2
tetrapyrrole. Inside the heme ring lies an atom of iron which is where reversible binding of oxygen can take place (Price and Schweigert 1987). Myoglobin has six coordinate positions or bonds. Four of the six coordinate sites are occupied by bonds with porphyrin. The fifth coordinate site is occupied by a bond with imidazole nitrogen of histidine within the molecule. It is the sixth coordination position which is important to the function of the myoglobin. The molecule that is bound to this position determines the color and the properties of the entire complex (Dryden and Birdsall 1980; Price and Schweigert 1987).

Hemoglobin is the pigment of blood and is present in varying amounts. If proper exanguination has taken place during slaughter, little blood containing hemoglobin remains in muscle. However, some hemoglobin will remain in muscle postmortem regardless of the how well exanguination has occurred. When present, hemoglobin also plays a role in color but is much less significant than that of myoglobin. Hemoglobin consists of four protein subunits that are each folded around a central heme (Price and Schweigert 1987).

**Pigments of Cured Meat Color**

The pigments responsible for the color of cooked cured meat are called nitrosylheme pigments (Pegg and Shahidi 1997). Generally speaking, nitrosylhemochrome, or nitrosylhemochromogen, is considered by most Meat and Food Scientists as the stable pink cured color pigment that results from the curing process. This pigment is a result of heating of the nitrosylmyoglobin pigment (Dryden and Birdsall 1980). Nitrosylmyoglobin is the ferrous complex of myoglobin that is coordinated to nitric oxide (NO) which is generated from nitrite (Morita and
others 1998; Wakamatsu and others 2004). The action of the nitrite on color is not to impart a cured pink color but to rather fix the color pigment myoglobin (Dryden and Birdsall 1980). The reaction of myoglobin and nitric oxide results in the nitrosylmyoglobin complex (Morita and others 1998). For uncooked cured meat, the pigment responsible for the bright color is principally nitric oxide myoglobin while nitric oxide haemoglobin also provides small contributions (MacDougall and others 1975).

**Chemical and Physical States of Meat Pigments**

Both the oxidation state and the physical state of the protein are important to meat color. Myoglobin and hemoglobin, which are heme pigments, exist in several semi-stable chemical states. These different states help relate the different colors of fresh and cured meats (Price and Schweigert 1987). Ionic or covalent bond types as well as iron in the ferrous or ferric state play a major role in the color related to myoglobin. It is the prosthetic heme iron group of the sixth coordinate position of myoglobin and hemoglobin which is responsible for the color of myoglobin and hemoglobin (Dryden and Birdsall 1980). This group is able to bind to any atom which has an electron pair to donate. A covalent or ionic bond may be formed at this position of myoglobin. Covalent bonds at this position result in a bright color of fresh and cured meat. An iron covalent complex of oxygen with myoglobin yields the pigment oxymyoglobin. An iron covalent complex of nitric oxide with myoglobin yields the pigment nitrosylmyoglobin. Yet another example of an iron covalent complex is that of carbon monoxide with myoglobin resulting in the pigment
carboxymyoglobin (Dryden and Birdsall 1980). If a covalent complex is not present, the iron can coordinate with water.

The heme iron atom of myoglobin can exist in ferrous (+2) or ferric (+3) states. The state it exists in depends on the amounts of reductants and oxidants in the meat which the myoglobin resides in (Pegg and Shahidi 1997). Some of the naturally occurring reducing compounds present in fresh meat include cysteine, cytochromes, quinones and NADH (reduced from nicotinamide adenine dinucleotide).

When oxygen is not present such as in uncut muscle that is not exposed to oxygen or vacuum packaged meat, deoxymyoglobin appears. This state of myoglobin is a result of reducing conditions that are naturally occurring in meat. Enzymes that are involved with this condition utilize all the oxygen present within the meat creating a ferrous (+2) state. Only water is able to react or bind with the myoglobin resulting in the purplish visible color of deoxymyoglobin (Aberle and others 2001). When molecular or atmospheric oxygen becomes present in meat, reduced pigments will form a relatively stable bright red oxymyoglobin pigment. The myoglobin is converted to oxymyoglobin by covalent binding of oxygen to iron and is in a ferrous (+2) state. Oxymyoglobin remains stable as long as a continuing supply of oxygen is present as an oxygen associated color gradient develops and is established from the surface and inward. Due to this gradient and the rapid use of available oxygen by enzymes involved with oxidative metabolism, the bright red color of oxymyoglobin on the surface directly depends on the available oxygen in the layers below the surface of the meat (Aberle and others 2001). Temperature, pH,
and external oxygen pressure are also contributing factors to the stability of oxymyoglobin (Pegg and Shahidi 1997).

When reducing agents that are generated by the enzymes are depleted, the heme iron of myoglobin is oxidized to the ferric state. The ferric (+3) complex may bind water but can no longer bind oxygen. This state is called metmyoglobin and is depicted by the characteristic brown color of meat. This state is often detrimental to meat as it is nearly always viewed as old or spoiled by consumers. In raw meat, there is a phenomenon that, in the presence of oxygen, the three pigments myoglobin, oxymyoglobin and metmyoglobin are constantly being interconverted (Pegg and Shahidi 1997). However, when heated and denatured, fresh meat in any of the three forms remains brown in color unless compounds such as nitrate or nitrite are added prior to thermal processing.

Fresh meat color is affected by many intrinsic and extrinsic factors that contribute to the oxidative stability of the heme complex. Extrinsic factors include temperature, lighting, microbial contamination and growth, and oxygen availability (Renerre 1999). Intrinsic factors include species and age, pH and muscle metabolic rate.

**IV. Meat Curing**

Meat curing can be defined as the use of both salt and nitrite (the reduced form of nitrate) to chemically alter the physical, chemical and often microbiological properties of meat products (Cassens and others 1979). Historically, meat curing was a practice primarily done to preserve meat. As meat curing progressed, the
definition was understood as the addition of salt, sugar, spices and nitrate or nitrite for aiding in flavor and preservation properties (Pegg and Shahidi 2000). As time passed, various spices and flavorings were added to achieve distinctive product and brand flavor characteristics. Today, meat curing is utilized to achieve consumer demands for products that have unique sensory characteristics and convenience attributes associated with cured meats. Meat curing has traditionally been associated with processed meats for the purpose of altering the color, texture, flavor, safety and shelf life characteristics which makes these products unique from other meat products (Sebranek and Fox 1985).

The curing process is a dynamic, complex and still not fully understood system of reactions, meat pigment changes, chemical state alterations as well as an entire host of secondary reactions. Meat curing results in a vast variety of processed meat products that are available to consumers. Variations in raw materials, formulations and processing technologies and techniques lead to the immense amount of different cured products that are manufactured and available to consumers today (Cassens and others 1979). For whole muscle type products, curing can be accomplished by various methods such as submersion of cure in brines or pickles, injection of cure containing brine or direct addition of dry cure for dry cured type products. Curing comminuted products is most commonly accomplished by direct addition of cure during the grinding, mixing, chopping or by other comminuting processes.
The Curing Reaction

In reviewing nitrite and chloride chemistry, Sebranek and Fox (1985), stated that sodium nitrite and sodium chloride are the two chemicals responsible and “absolutely necessary” to successfully produce cured meat products. Cassens and others (1979) support this statement and add that nitrite is the reactive chemical involved with principal curing reactions. Although nitrates were first discovered as curing agents, research findings have demonstrated that the role of nitrate is to serve as a source of nitrite for curing reactions. While nitrate has the same functionality as nitrite, it acts much slower and therefore is used less seldom (Pearson and Tauber 1984).

The chemistry of nitrite curing is indeed complex and in many cases not clearly understood. The term nitrite is generically used to describe both the anion, NO\textsubscript{2}, and the neutral nitrous acid, HNO\textsubscript{2}. Nitrite curing has been often associated with the production of potentially dangerous compounds formed from nitrosating species of nitrite. In examining the chemistry of meat curing, Pegg and Shahidi (1997) revealed that nitrite itself is not the primary nitrosating species or reactive compound. It was further revealed by Pegg and Shahidi (1997) and Sebranek and Fox (1985) that one of the derivatives, nitrous acid (HNO\textsubscript{2}), actually can form nitrosating (N-nitroso producing) compounds which are the compounds involved in potential nitrosamine formation. These nitrosating compounds enter into a number of complex reactions which, in the end, yield nitrosylmyochromagen. The pKa of a compound represents the acid dissociation constant, the strength of the acid and the ability of the compound to donate protons for affecting reactions. Nitrite has a pK\textsubscript{a}
of 3.36. These authors further explained that since the pH of meat (approximately 5.5-6.5) is clearly above the pKa of HNO₂, the concentration of HNO₂ in cured meat is therefore extremely low. It is believed that the main reactive species of HNO₂ in meat is the anhydride of HNO₂ which is dinitrogen trioxide (N₂O₃). The dinitrogen trioxide reacts with reductants that are naturally found in muscle as well as any ones added such as ascorbates. This compound can readily form nitroso compounds.

Sebranek and Fox (1985) further explain that nitric oxide is another important form of nitrite. Reducing reactions can increase the nitric oxide production which can then form coordinate-covalent complexes of nitric oxide with the heme pigments of meat. These complexes, nitrosylmyoglobin, nitroslhaemoglobin and dinitrosylhaemochrome, form the red and pink colors of cured meats. The amount of nitrous oxide produced during curing is dependent on pH, temperature and time. Beyond the reactions explained, there are also many other complex reactions that can and do occur.

A lower pH will increase the conversion of nitrous acid to nitric oxide. Prusa and Kregel (1985) showed by adding phosphates that increased the pH of poultry frankfurters, less nitrous oxide was produced as supported by a higher residual nitrite concentration in the finished products. Lee and Cassens (1976) concluded that a minimum of two hours is needed to convert 90% of nitrite to nitric oxide and bind with myoglobin for subsequent nitrosomyoglobin formation.

When nitrite is added to comminuted meat, a browning effect occurs due to properties of nitrite acting as a strong heme oxidant (Pegg and Shahidi 2000). Myoglobin and oxymyoglobin are oxidized to metmyoglobin by nitrite. Through the
series of already mentioned, complex reactions involving the reduction of nitrite to nitrous acid, the intermediate pigment, nitrosylmetmyoglobin, is formed. Nitrosylmetmyoglobin is not a stable pigment and therefore autoreduces in the presence of both endogenous and exogenous reductants to form a more stable nitric oxide myoglobin or nitrosylmyoglobin. Upon thermal processing, the globin portion denatures and detaches from the iron atom. The resulting pigment formed from the thermal processing is the stable nitrosylmyochromogen or nitrosylhemochrome (Cassens and others 1979; Shahidi and Pegg 1992). Beyond what has already been discussed about curing reactions, Cassens and others (1979) discuss several other investigated and hypothesized nitrite derivatives, reactions and pathways to possibly explain the complex nitrite chemistry that results in cured meat color and associated characteristics.

For the use of nitrate in a curing system, an additional step of the conversion of nitrate to nitrite is necessary. This step is normally accomplished by the bacterial reduction of nitrate to nitrite (Sebranek 1979; Pinotti and others 2001; MacDougall and others 1975). Bacterial reduction can be accomplished by microorganisms found in the natural flora of meat or by intentional addition of microorganisms with nitrate reducing properties (Sanz and others 1997).

Sebranek (1979), examining the importance of salt or sodium chloride in meat curing, summarized that chloride ions could actually help catalyze nitrosation reactions. He went on to state that the significance of these reactions in practical meat processing may have little influence on nitrite behavior since very acidic conditions would be required for this occurrence. Additionally, salt levels used in the
curing process are generally not high enough to provide complete preservation but at levels typically used in conjunction with nitrite, salt does offer preservative effects. Synergistic effects between salt and nitrite aid in preservation from both a microbiological as well as storage stability standpoint (Romans and others 1985). Interestingly, since salt is known to be a strong pro-oxidant, nitrite can also be described as a compound which combats the oxidation effects of salt on meat proteins and stabilize color.

Cassens and others (1979) in fact suggested that a portion of nitrite added to meat during the curing process is actually converted to nitrate. Their review of several nitrite investigators provided support to this phenomenon. One theory was that a secondary oxidation involving nitrous acid could be involved in the converting of nitrite to nitrate. This theory was further supported by comments from Dethmers and Rock (1975) who proposed that nitrous acid could yield nitric oxide and nitrate from the oxidation of nitric acid by oxygen to yield nitrite which could subsequently react with water to yield nitrite and nitrate. It was also suggested that sodium ascorbate could play a role in the converting of nitrite to nitrate. Price and Schweigert (1987) went as far as saying that the inert properties of nitrate allow a large portion of it to be converted from nitrite in a cooked cured meat system. Work by Pérez-Rodriguez and others (1996) monitoring nitrite and nitrate in frankfurters reported that in nitrite cured, cooked and packaged frankfurters, approximately 50% of ingoing nitrite was present while about 10-15% of added nitrite was found as nitrate. Although these events have been reported to occur during the curing process, the significance of these occurrences has not been well established.
Cure Accelerators

As advancements in meat curing were occurring in the early second quarter of the 20th century, high levels of remaining nitrate and nitrite found in cooked cured meat products led way to the needed discovery of a compound effective in reducing these finished product levels. In the 1930s, ascorbic acid was discovered to be effective in reducing nitrite to nitric oxide and thus reducing finished product levels of nitrite and nitrate (Pegg and Shahidi 2000). In the 1950s, ascorbic acid, ascorbate and their isomers, erythorbic acid and erythorbate were authorized for use in cures by the USDA. USDA FSIS states that since cure accelerators are used in conjunction with nitrite and nitrate, they are not permitted for use in non meat curing systems (USDA 1995). Table 2.2 displays the current regulatory limits for the discussed cure accelerators in cured meat products. Beyond those listed in Table 2.2, glucono delta lactone (GDL) and sodium acid pyrophosphate (SAPP) are each permitted cure accelerators at a maximum finished product level of 5000 ppm for only cured comminuted meat products. For bacon manufacture, USDA FSIS regulates the amount of cure accelerator used which must be exactly 550 ppm of either sodium ascorbate or sodium erythorbate.

<table>
<thead>
<tr>
<th>Cure Accelerator</th>
<th>Maximum Limit</th>
</tr>
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<tbody>
<tr>
<td>Ascorbic Acid</td>
<td>469 ppm</td>
</tr>
<tr>
<td>Erythorbic Acid</td>
<td>469 ppm</td>
</tr>
<tr>
<td>Sodium Ascorbate</td>
<td>547 ppm</td>
</tr>
<tr>
<td>Sodium Erythorbate (isoascorbate)</td>
<td>547 ppm</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>Up to half of any listed above</td>
</tr>
<tr>
<td>Sodium Citrate</td>
<td>Up to half of any listed above</td>
</tr>
<tr>
<td>Fumaric Acid</td>
<td>650 ppm</td>
</tr>
</tbody>
</table>
Table 2.2. Maximum allowable limits for various cure accelerators for cured meat products. (**specific regulations address bacon) All limits are based on total formulation / brine weight for immersion cured or massaged or pumped products and green weight for comminuted or dry cured products. Limits are reported in parts per million. This reprint is from USDA FSIS Processing Calculations Inspector’s Handbook (FSIS Directive 7620.3).

Since nitrite must be reduced to nitric oxide and the iron portion of muscle pigments be reduced from the ferric (+3) to the ferrous (+2) state, compounds classified as cure accelerators are often incorporated in curing practices. There are several important functions of cure accelerators. The first is to promote the reduction of nitrite to accelerate curing reactions and speed the synthesis of cured meat pigments (Borenstein 1976; Lee and Shimaoka 1984; Izumi and others 1989). These compounds also known as reductants are capable or donating electrons which enable curing related reactions to occur much more readily and rapidly. The reducing conditions that are developed due to the addition of cure accelerators provide favorable conditions for the chemical conversion of nitrous acid to nitric oxide and subsequent reactions with reduced myoglobin. Secondly, excess cure accelerators can act as an antioxidant to help stabilize the color and flavor of cured meat products. Cure accelerators can serve as oxygen scavengers to help prevent cured meat color fading from light and air (Pegg and Shahidi 2000).

Although erythorbate (the erythro isomer of ascorbate) and ascorbate are used interchangeably in the meat industry, Lee and Shimaoka (1984) noted that ascorbate is more stable in aqueous solutions than erythorbate and also may function as a better antioxidant. An example of the reducing effects of ascorbate and erythorbate was shown in work by Lee and Shimaoka (1984). In their work of
investigating iron bioavailability while already understanding the successful effectiveness of ascorbate to complex or reduce iron to a more available ferrous form, they reported that erythorbate was also effective in reducing or complexing iron to result in more bioavailable forms. This work helps demonstrate the reducing ability of both ascorbate and erythorbate.

Izumi (1992) investigated the reactions of ascorbic acid and ascorbic acid derivatives. Izumi proposed that the addition of ascorbic acid to nitrate resulted in intermediates between nitrous acid and ascorbic acid which may involve reactions for cured color development. Ascorbic acid-2-derivatives (phosphate and sulfate) were investigated for reaction properties with nitrite. The findings showed that both ascorbic acid-2-derivatives not only reacted with nitrite but their nitrate reducing ability was also much less than that of ascorbic acid itself.

Borenstein (1976) studied the effects of various compounds alone or combined with sodium ascorbate to accelerate cured pigment development. Citric acid, malic acid, levulenic acid, alpha-tocopherol and monobasic ammonium phosphate were all shown to have no effect in accelerating cured pigment development either alone or in conjunction with ascorbates. It was discovered that salts of EDTA combined with ascorbate did have a significant effect in speeding up cured color development. Based on the chemical properties of EDTA and the excellent potentiating properties of EDTA, Borenstein was able to summarize that pigment reduction and not nitric oxide production is the rate limiting step in cured meat pigment synthesis. Izumi and others (1989) stated that ascorbic acid measured spectrophotometrically was effective in deceasing free nitrite in an
aqueous solution. The reaction occurred more rapidly as pH reduces from 5.49 to 3.63. They also disclosed that nitrosating compounds formed between ascorbic acid and nitrite play a significant role in developing nitroso compounds in cured meat.

Although the effectiveness of cure accelerators in cured pigment synthesis is well documented, a research note by Sebranek and others (1977), clearly explains the importance of using appropriate levels of nitrite in cured meat products regardless of levels of cure accelerator used. In their investigation of varying sodium nitrite levels (26, 52 and 156 ppm) and sodium erythorbate levels (0 or 546 ppm) in frankfurters, the authors revealed that frankfurters manufactured with 156 ppm sodium nitrite and interestingly either 0 or 546 ppm sodium erythorbate were rated significantly higher than all other combinations for color, flavor and acceptability sensory scores. Frankfurters manufactured with 52 ppm sodium nitrite and either 0 or 546 ppm sodium erythorbate also rated significantly higher for color and acceptability but not for flavor compared to frankfurters manufactured with 26 ppm sodium nitrite and either 0 or 546 ppm sodium erythorbate. The significant difference in acceptability was believed to be due in part by the acceptable color of those frankfurters. In summary, the authors determined that nitrite concentrations relating to cured meat characteristics are extremely critical and more so than concentrations of erythorbate used for the consumer acceptability of frankfurters.

V. Factors Affecting Cured Meat Color

The color of cured meat products is very important from a customer standpoint. Cured meat color can be affected by biological factors and extrinsic
factors resulting in undesirable fading or discoloration that consumers could deem unattractive. The most common biological factor affecting cured meat color stability is microbiological contamination and growth. Extrinsic factors include oxygen, light and dehydration (Draudt and Deatherage 1956). Nitrosylhemochrome pigment and the less stable nitrosyl myoglobin are both vulnerable to oxidation when oxygen and light are present. Møller and others (2003) showed the significance that oxygen had on color stability. By investigating residual oxygen, oxygen transmission rates of packaging films, product to headspace volume ratio, illuminance level and nitrite level during curing, they observed significant effects on cured color stability for all listed factors. In modified atmosphere packaged ham, the researches noted strong interactions between headspace oxygen level, product to headspace ratios and level of illuminance concluding that not controlling any one of the three will result in loss of color stability. Due to oxygen in headspace levels over 0.5% showing noticeable color fading in modified atmosphere packaged ham, Pexara and others (2002) recommended the use of vacuum packaging for cured turkey fillets and pork-piroski sausages. These researchers showed overall faster discoloration of a* color values over time of four different modified atmosphere combinations of carbon dioxide, nitrogen and oxygen in the packaged fillets and sausages than compared to vacuum packaged treatments held at 4ºC.

VI. Benefits of Using Nitrates / Nitrites

Beginning at the time of discovering nitrite and nitrate, taking advantage of the benefits of using them as well as the purpose of adding them to meat products
over time has both changed and stayed the same. Upon the discovery of these compounds, sausage and cured meat products that were being heavily spiced and cured for preservation reasons were able to be refined to meet flavor characteristics that were demanded by consumers (Cerveny 1980). These compounds allowed for the emergence of early ready-to-eat type meat products. By using significantly less salt and/or other preservation methods due to the introduction and incorporation of nitrite or nitrates, meat products began to move from a state of unsatisfactory quality and poor shelf life to improved quality and longer shelf life. Of interest to cured meat products are the benefits of using nitrite and nitrite from both a microbiological as well as a qualitative standpoint. Bauermann (1979) showed a higher coliform level for uncured, no-nitrate/nitrite-added poultry frankfurters compare to nitrite-added counterparts. Thus, sodium nitrite-added poultry frankfurters were shown to have an increased shelf life versus no-nitrite-added poultry frankfurters.

**Microbiological Benefits of Nitrate / Nitrite Addition**

The microorganism *Clostridium botulinum* is derived from the Latin word for sausage, botulus (Archer 2002). It was discovered and first isolated by Van Ermengem in 1896 in an outbreak associated with raw salted pork (Jay 2000) and incidences have occurred worldwide (Sofos and others 1979). *C. botulinum* is a gram positive, anaerobic spore forming rod (Jay 2000). Based on the serological specificity of *C. botulinum* toxins, there are seven toxigenic types that are recognized. Those include types A, B, C, D, E, F and G. In foods with low acid, such as meat products, spores of *C. botulinum* can germinate, grow and produce toxin. An important and dangerous characteristic of *C. botulinum* spores is that once
present, they are extremely heat resistant. *C. botulinum* grows without the presence of oxygen making vacuum packaged meat products an ideal medium. If consumed, the resulting toxin causes the disease known as botulism. Botulism is the most lethal of all food-borne diseases carrying a 20 to 50% mortality rate. Clinical symptoms of the disease include double vision, drooping upper eye lid, difficulty swallowing, nausea, vomiting, and abdominal cramping. Botulism toxins prevent certain nerves from functioning resulting in muscle paralysis and respiratory impairment.

From 1793 to 1990, 688 outbreaks, 1,784 cases and 978 deaths in the United States have been attributed to botulism as reported by the Center for Disease Control (Tompkin 1980). From 1990 to 2000, 160 outbreaks, 263 cases and 11 deaths from botulism occurred in the United States (Sobel and others 2004). Interestingly, 39% of those cases occurred in Alaska. *C. botulinum* grows well in nutrient rich meat and isn’t considered a good competitor if other microorganisms are present. Its optimum growth temperature is near 37ºC and some strains can grow at temperatures as low as 3.3ºC up to 55ºC. The necessary pH range for growth lies between 4.6 and 8.3 (Jay 2000). Although *C. botulinum* is a strict anaerobe, most commonly associated with growth in anaerobic conditions of canned products or vacuum packaged products, the interior portion of sausage and cured meat products that have a low enough oxidation-reduction environment can also result in growth and toxin production (Sofos and others 1979).

The effects nitrite has on *C. botulinum* are indeed quite interesting. Nitrite is exceptionally effective against *C. botulinum* (Archer 2002; Huhtanen and others
1985; Hustad and others 1973; Lövenklev and others 2004; Pierson and Smooth 1982; Sofos and others 1979). Botulism was considered a serious problem associated with meat and sausages until nitrite was utilized as a curing ingredient. The role nitrite and nitrate have on preventing and controlling microbial growth has been examined by numerous researchers. Less nitrite is needed to provide for color development than to control bacteria (Roberts 1975). The main portion of nitrite added to cured meats is for \textit{C. botulinum} control whereas only a small portion (roughly 25 ppm or less) is needed for color development (Sofos and others 1979). It has been suggested that as little at 5 ppm is actually needed for satisfactory cured color development, yet 20 ppm may be needed for cured color stability. Sofos and others (1979) stated that as nitrite levels increase, control of \textit{C. botulinum} growth and toxin production also increases. It is of value to this topic to mention the importance of salt (NaCl) in \textit{C. botulinum} control. Salt works synergistically with nitrite and other factors such as pH, meat type and heat treatment to control spore outgrowth and thus greatly aid in \textit{C. botulinum} control. Salt levels at 5% (wt/vol) were shown to completely inhibit \textit{C. botulinum} under optimal growth conditions (Lövenklev and others 2004). However, salt at this high level would be deemed too salty by consumers. Thus, the salt and nitrite synergistic interaction should be explored in greater detail.

Bayne and Michener (1975) reported that nitrite concentrations present in commercial meat products are not sufficient to effectively inhibit the growth of \textit{Staphylococcus} or \textit{Salmonella}. Investigations by Buchanan and Solberg (1972) disagreed. At pH 7.2, Buchanan and Solberg (1972) demonstrated that sodium
nitrite levels ranging between 0 and 2000 ppm in Brain-Heart Infusion Agar had no inhibiting effect on *S. aureus* aerobically. Oxygen pressure and pH influence the bacteriostatic action of sodium nitrite on *Staphylococcus aureus*. Nitrite inhibits bacteria more effectively at low pH or acid values (Roberts 1975). The same results were found under anaerobic conditions with the exception of a significant extended lag phase found at a level comparable to prepared curing brines of 2000 ppm. Interestingly, at pH 6.3, increasing ppm of sodium nitrite increased the lag phase and generally reduced the growth phase aerobically and even more drastically during anaerobic conditions. The authors suggest their results offer evidence that *S. aureus* could be significantly controlled in cured meats with 200 ppm sodium nitrite ingoing levels, especially if vacuum packaging is used.

Giusti and De Vito (1992) reported that the results nitrate and nitrate have on inactivating *Yersinia enterocolitica* between *in vitro* and treated pork can actually be difficult to compare. However, the researchers found that 100, 150 and 250 ppm sodium nitrite mixed with pork mince and inoculated with *Y. enterocolitica* resulted in no colony forming units present after 24 hours incubation.

Modified-atmosphere packaging is a technology often used to replace oxygen present in a package with other gases. Lövenklev and others (2004) investigated the gene expression of *C. botulinum* and concluded that exposure to oxygen had no effect on *C. botulinum* gene expression. The authors also discovered that gene expression was actually stimulated in the presence of carbon dioxide ranging from a 10 to 70% atmosphere with the highest growth levels at near 70%. Modified
atmosphere packaging was suggested to actually promote and not inhibit *C. botulinum* growth.

Most *C. botulinum* challenge studies follow the concept of adding spores to manufactured meat products, incubating and measuring growth or toxin production at various time points and usually with variables in holding temperature. Since interpretation and application to various products is not always easy, estimating the probability of spore outgrowth can also be accomplished by a probability modeling system (Hauschild 1982).

The properties of curing with nitrite that make it an effective antibotulinal compound are dependent on interactions of nitrite with several other factors. Those factors that nitrite interacts with include salt, pH, heat treatment, spore level, ingoing nitrite level during manufacture and residual nitrite levels in the meat (Archer 2002). The nature of the competing flora, available iron in the product and other present additives such as ascorbate, erythorbate, phosphate, etc. are other additional factors (Roberts and Gibson 1986). Cooked, cured and packaged storage temperatures are yet another important factor (Roberts 1975). The antibotulinal effects of nitrite in thermal processed meat product systems takes place at two different stages in the life cycle of *C. botulinum*. The first *C. botulinum* controlling effect of nitrite is the inhibition of vegetative cells emerging from surviving spores. The second controlling effect is preventing cell division in any vegetative cells that do emerge from surviving spores (Pierson and Smooth 1982). Inoculum levels may play a role in the accuracy of scientific results. Investigations studied at very high levels may not correlate well with large-scale commercial production scenarios (Roberts 1975).
Sofos and others (1979b) found that heat shocked (80°C for 15 minutes) *C. botulinum* spores added to poultry emulsion that was cooked to an internal temperature of 68.5°C and incubated at 27°C grew to toxic levels during 3 or 4 days of incubation when no nitrite or sorbic acid was added. When 156 ppm nitrite was added, that time doubled to 6 days. However, an increase in the delay of toxin production was found when sorbic acid was added in conjunction with sodium nitrite. Sorbic acid added at 0.2% and sodium nitrite at 40 ppm controlled *C. botulinum* growth up to 10 to 17 days of incubation. Even longer delays (31 days) were shown when sodium nitrite was added at 156 ppm. The authors summarized that a four to ten-fold increase in botulinal safety was shown by the combination of sorbic acid and nitrite. This variation was directly related to the amount of nitrite added (40 or 156 ppm). No sensory evaluation was performed in this experiment to determine the acceptance of these combinations but results do support the importance of sodium nitrite on the control of *C. botulinum*.

Nitrite cured bacon was found to be considerably more resistant to *C. botulinum* outgrowth which was attributed to higher salt content as well as the additive effect of nitrite and sodium chloride (Huhtanen and others 1981). Huhtanen and others (1983) reported that the use of potassium sorbate alone or when combined with hydrochloric, phosphoric acid, acetic, citric, lactic, succinic, or sorbic acids was not as effective as 120 ppm sodium nitrite in inhibiting *C. botulinum* injected bacon. The authors data did, however, state that the control of *C. botulinum* in ham, chicken and turkey frankfurters with the combination of sorbic or potassium sorbate and phosphoric acid was effective. The levels necessary for this control
may be high enough to result in negative acceptance by consumers although this was not suggested in this study. Nelson and others (1983) showed that a combination of potassium sorbate at 0.26% and 40 ppm nitrite was effective in inhibiting *C. botulinum* spore germination and sorbic acid plus sodium nitrite treatments offered comparable results contrary to results by Huhtanen and others (1983). Product pH differences between studies are believed to be responsible for the discrepancies and support the importance of pH in the control of *C. botulinum*. Sodium acid pyrophosphate, sodium hexametaphosphate and sodium tripolyphosphate in combination with sorbic acid (0.20%) or potassium sorbate (0.26%) and sodium nitrite (40 ppm) also showed inhibitory effects on botulinal toxin production by extending the delay phase.

Hustad and others (1973) stated the development of *C. botulinum* toxin in wieners was directly influenced by the level of nitrite added to the meat. They reported that nitrate had little effect on controlling *C. botulinum* toxin production, yet nitrite added at 50 ppm only resulted in 2 toxic samples of 110 tested and nitrite levels higher than 50 ppm ingoing resulted in zero toxic samples. This work is in agreement with Lövenklev and others (2004) who noted that 45 ppm sodium nitrite was effective in suppressing *C. botulinum* gene expression.

**Irradiation Interactions with Nitrite**

Investigating irradiation and nitrite effects on *C. botulinum* toxin formation, Rowley and others (1983) reported that a 0.0 Mrad radiation dose and zero ppm added nitrite to temperature abused bacon (27ºC) resulted in 20 swollen pouches out of 20 tested and 12 of 20 positive for viable toxin when inoculated at 2 spores/g
of bacon. The researchers found that adding 40 ppm nitrite to those conditions resulted in both a delay in pouch swelling as well as a decrease in toxin formation. Furthermore, it was noted that sour/off odor probably due to spoilage bacteria in non-irradiated bacon stored at 5°C occurred at 35 days with 0 ppm nitrite, 61 days with 40 ppm and no evidence of occurrence was noted during the 61 day evaluation when 120 ppm level was used.

**Qualitative Aspects of Nitrate / Nitrite Addition**

Nitrite possesses several characteristics that improve meat products from a qualitative standpoint. Besides the previously discussed desirable color fixation and anti-bacterial effects from the addition of nitrite, it is also extremely effective in controlling lipid oxidation (Roberts and Gibson 1986). Nitrite increases the qualitative shelf life of cured meat products (Erduran and Hotchkiss 1995; Price and Schweigert 1987; Pearson and Tauber 1984) by controlling and stabilizing the oxidative states of lipids in meat products (Shahidi and Hong 1991). Lipid oxidation is considered to be a major reason for the deterioration in the quality of meat products often resulting in the development of rancidity and subsequently warmed over flavors (Vasavada and Cornforth 2005; Yun and others 1987). The rate and degree of lipid oxidation is related to the amount of unsaturated fats present as well as oxygen exposure, the removal of oxygen and the addition of antioxidants and/or reducing agents (Shahidi 1998). Salt is known as a strong pro-oxidant and thus adds difficulty of controlling lipid oxidation. The effect of nitrite on controlling lipid oxidation is explained by Price and Schweigert (1987) by controlling the iron that would be otherwise serve as a catalyst for lipid oxidation reactions. As nitrite
reactions form cured pigments, iron present in the meat is retained by these reactions, reduced to the Fe$^{+2}$ form and are thus inactive or not available as a catalyst for lipid oxidation reactions. Erduran and Hotchkiss (1995) supported this and stated the importance of the above reactions had on preventing Fe$^{+2}$ from being released and available for lipid oxidation reactions during the heating or thermal processing or meat products. Once oxidative reactions begin, auto-oxidation normally entails, however, the use of vacuum packaging has been shown to be an effective intervention to these quality problems by helping prevent or delay rancidity (Chang and Chen 1998). However, even when vacuum packaging is utilized, no-nitrite-added bacon has been shown to yield rapid off-flavor development likely due to the exclusion of nitrite (Gray and others 1981).

The role nitrite has on meat flavor is a complex stimulus involving characteristics such as aroma/odor, texture, taste and temperature (Gray and others 1981). The chemistry behind the composition and formation of cured meat flavor is not clearly understood (Shahidi 1998; Gray and others 1981). Shahidi (1998) explained the nature of cured meat flavor is unknown but the inhibitory effects nitrite has on lipid oxidation aids in the development of the unique flavor. In addition, sensory research has suggested that cured flavor is not solely a result of retarding lipid oxidation but a combination of a complex cured aroma and flavor in cooperation with a lack of rancid flavors. Many of the same compounds that may contribute to aroma and flavor are present in both uncured and cured cooked meat products. The volatile compounds 4-methyl-2-pentanone, 2,2,4-trimethylhexane and 1,3-
dimethylbenzene are three components identified to possibly contribute directly or indirectly to cured meat aroma (Shahidi 1998).

Ramarathnam and others (1991a) studied aroma concentrates from cooked, uncured and cured pork. The authors identified 50 hydrocarbons, 37 carbonyls, six acids and two alcohols present in both the uncured and cured pork that may contribute to cured meat flavor. By utilizing purge-and-trap methods of gas chromatography, 32 new meat-flavor compounds for uncured and cured pork (Ramarathnam and others 1993a) and 12 new meat flavor compounds for beef and chicken (Ramarathnam and others 1993b) were identified. One component of interest, hexanal, was found in both cured and uncured pork but was reported to be at a considerably lower level in cured pork (0.24% of amount found in uncured).

A similar pattern for hexanal has also been shown for both cooked uncured and cured beef and chicken (Ramarathnam and others 1991b). Hexanal is an oxidation product of lipid and may play a role in understanding flavor from a lipid oxidation effects standpoint. The authors also observed many volatiles were either absent or present in lower concentrations in cured vs. uncured pork possibly providing initial explanation of cured meat flavor. Difficulty in identifying specific components involved in cured meat flavor has been attributed to the formation and interference of carbonyls and hydrocarbons during isolation and detection that make it difficult to isolate many minor in concentration yet possibly significant components (Ramarathnam and others 1991b).

In sensory studies, panelists were able to differentiate between samples manufactured with different levels of nitrite (10, 156 and 200 ppm) (Gray and others
Olesen and others (2004) reported considerable differences in the production of volatile compounds in fermented dry sausages manufactured with nitrite, nitrate or nitrite/ascorbate combinations and two different starter culture combinations. The authors believed besides curing compounds, starter culture microorganisms also play significant roles in the generation of important fermented sausage volatile compounds. Also investigating nitrite versus no nitrite usage in dry sausage, Noel and others (1990), concluded nitrite plays an extremely important role in the development of specific flavor notes as supported by sensory analysis. Dethmers and Rock (1975) stated the addition of nitrite above 50 ppm in thuringer sausage reduced off-flavor development and improved the flavor quality whereas treatments with no nitrite added were considered to be the most rancid and had the poorest flavor quality (p<0.05). Investigating the role of nitrite addition in ham, Froehlich and others (1983) reported a significant (p<0.05) improvement in trained sensory cured meat flavor intensity scores as ingoing nitrite levels increased from 0, 50 and 100 ppm. As levels of nitrite increased (0 to 150), significant differences (p<0.05) were also reported for Hunter a* color values indicating that higher ingoing amounts of nitrite yielded objectively redder (pinker) colored ham.

The presence of nitrites and nitrates in meat products can also be considered a hindrance. In uncured cooked meat products such as poultry rolls manufactured from chicken or turkey or precooked roast beef, difficulties can result from trace levels of nitrite or nitrate contamination present during the manufacturing process (Heaton and others 2000). Although not fully understood, ingredient and packaging contamination are two of several possibilities for the source of nitrates and nitrites.
Very low levels of nitrite have been shown to cause the pinking phenomenon. These trace levels can cause pinking in uncured products while cooking which in turn can be interpreted by purchasing consumers as uncooked or not fully cooked meat products.

Ahn and Maurer (1989) reported pinking effects in oven-roasted turkey breasts with as little as 1 ppm added sodium nitrite. Heaton and others (2000) also found similar results in cooked turkey rolls, chicken rolls and pork shoulder rolls. The authors reported that sensory panelists detected pinkness or pink color in turkey, chicken and pork rolls at 2, 1 and 4 ppm, respectively. The authors also stated that meat products with higher pigment concentrations (pork) required higher nitrite levels for panelists to detect the visual pinking effects. In a separate study investigating pinking and poultry, Ahn and Maurer (1987) noted that microbial contamination from equipment and human handling can offer a source of pinking if trace amounts of nitrite or nitrate are present.

**VII. Nitrates / Nitrites and Humans**

To this point, the discussion of nitrite and nitrate has been centered on the manufacture and consumption of cured meat products. The importance of these compounds has been repeatedly discussed as a compound accidentally discovered and later utilized for the primary purpose of producing products with the beneficial properties associated with the use of these compounds. However, the whole story of this unique compound has not yet been told. Knowingly or not, all humans consume, synthesize and utilize nitrate and nitrite on a daily basis. Nitrite is actually
excreted in sweat. Nitrite and/or nitrate present in humans can be derived from endogenous or exogenous sources. Where those sources originate is of particular interest. Sodium nitrite is found throughout the environment and is a primary component of the global nitrogen cycle. The major source of human exposure to nitrite is by oral intake of food and water (Abuharfeil and others 2001; Chung and others 2004; White 1975). It has been suggested that 40% of absorbed nitrite is unchanged and passes through the body by urinary excretion, however the fate of the remaining 60% is not precisely known.

**Endogenous and Exogenous Sources**

Nitrite can be synthesized endogenously in the human body by enzymatic and other possible reactions. Nitrites play profound roles in normal body functions. As a product of enzymatic synthesis in humans, nitric oxide from the synthesis of nitrite, controls blood pressure, immune response, wound repair and neurological functions (Archer 2002). Nitric oxide has even been believed to act as a defensive weapon by inhibiting metabolic pathways to block growth or kill cells of certain diseases (Cassens 1995). Thus, nitrite can be viewed as a natural metabolite vital to the repair, function and survival of human biological function.

The presence of nitrates and nitrites in the human body can also result from exogenous sources. Nitrate is commonly ingested when people consume vegetables (Archer 2002) and perhaps certain fruits (Hardisson and others 1996). It is well known that leafy green or root vegetables and drinking water are sources of nitrate that humans are exposed to (Cassens 1997b). Nitrates and nitrites are part of the nitrogen cycle of plants and are by-products of green plant photosynthesis.
(Bednar and Kies 1994). Fertilizer used on vegetables with nitrogen compounds, the genetics of plants, the maturity and environmental conditions plants grow in (lack of water, soil fertility) also can play a role in the amount of nitrate found in these foods (Wolff and Wasserman 1972). It has been proposed that food sources that contain nitrate are the primary source of human nitrate intake from which vegetable and water consumption can account for 80% to 95% of total ingested nitrate. White (1975) estimated 81.2% of nitrate intake and 1.6% of nitrite intake is derived from vegetable consumption. The National Academy of Sciences (1981) stated that vegetables account for 85% of dietary nitrate reporting levels of 2600 ppm nitrate found in beets, 1500 ppm in celery and 1700 ppm in lettuce. Knight and others (1987) found that vegetables in Great Britain contributed to over 90% of nitrate intake. The authors additionally noted a greater reduction in human exposure of nitrate is more feasible by reducing the intake of vegetables and water than cured meat products.

The National Academy of Sciences (1981) reported that 39% of dietary nitrite was from cured meat, 34% was from baked goods and cereals and 16% was from vegetables. Nitrite consumption from water and vegetables has also been proposed to account for roughly 2% total ingested while cured meats may account for approximately 4%. American Meat Institute Foundation scientists reported less than 5% of nitrite intake comes from cured meats (AMI 2003). Surprisingly, saliva can easily account for over 90% of ingested nitrite, presumably due to the reduction of nitrate to nitrite by the bacteria present in the oral cavity and the acidic properties of saliva (Archer 2002; Cassens and others 1979). Dietary nitrite has been proposed
to act as a strong bacteriostatic or bactericidal antimicrobial when it is present in an acidified form protecting from microbial infections from the ingesting of pathogenic microorganisms. The pathway of salivary nitrate reduced to nitrite, acidified in the stomach, and nitric oxide and other oxides of nitrogen may provide for beneficial physiological effects.

**VII. Residual Nitrite**

When nitrite is added to meat systems, it reacts chemically or is bound to components such as protein. Heat during thermal processing serves to speed up these reactions. After normal manufacturing processes, the amount of detectable nitrite is usually only approximately 10-20% of the initial added amount when analytically measured (Cassens 1997a). These levels of nitrite or also called residual nitrite decline over the storage life of cured meat products until they are often non detectable (Skjelkvåle and Tjaberg 1974; Eakes and Blumer 1975).

In a comprehensive study of nitrite and cure accelerator levels for cooked sausages and dry and semi-dry sausages, the Nitrite Safety Council (1980) reported “highly variable” residual nitrite and cure accelerator levels not only within product categories but also between specific types of sausages (beef, pork, poultry, etc.). They also reported that generally 25-50% of added nitrite remained in the product during 24-48 hours after processing. Purchasing and analyzing local retail commercial cured bacon, sliced ham, and wieners and analyzing for nitrite, Cassens (1997a) reported residual nitrite in bacon to range between 1 and 15 ppm, sliced ham to range between 3 and 9 ppm and wieners to range between 1 and 9 ppm.
Investigating retail commercial cured bacon, bologna, ham and wieners, Cassens (1997a) reported a range of 0 to 48 ppm in residual nitrite for all four products with an overall product average of 10 ppm.

In Thuringer sausage, researchers discovered residual nitrite concentrations varied based on amount of ingoing (50, 100, 150 ppm), finished product storage time and storage temperature (Dethmers and Rock 1975). Initial ingoing nitrite levels compared to raw emulsion levels showed a nitrite decrease of approximately 40%, approximately 80% at 0 weeks of storage and approximately 86 and 92% at 1 and 4 weeks of storage, respectively held at 7.5°C. A similar rapid decrease was observed at a 27°C storage temperature at all ingoing levels.

**Importance / Significance**

Nitrite (more specifically residual nitrite) has been criticized and associated with cancer formation in humans. Epidemiological data generated by human study participants who were instructed to recall past cured meat products brought the concern of nitrite usage to a high level of public concern. Researchers reviewed these epidemiological reports and concluded inaccurate reporting and stated: 1. Methodology problems existed resulting in misleading conclusions. 2. Nitrite is not a carcinogen. 3. Nitrosamines are not found in hot dogs. 4. Most of the patients in the study were from low income groups, not clearly depicting the entire population (Cassens 1990; NAS 1981). Nonetheless, the importance of residual nitrite is not only important from a qualitative perspective but also from a food safety standpoint.

Szczawinski and others (1989) discussed the importance of residual nitrite in nitrite-cured, pasteurized and irradiated pork meat stating the extent of *C. botulinum*
spore inhibition is related to the rate of nitrite depletion which in turn, can allow the germination of spores. These authors maintain that botulinal spores can germinate in the presence of nitrite but their growth is clearly inhibited by that same presence of nitrite. These residual nitrite levels can adversely be affected by irradiation. High dose irradiation (10-50 kGy) on *C. botulinum* spores can severely deplete nitrite levels and reduce the inhibition of the *C. botulinum* spore outgrowth.

**Factors Affecting Levels**

There are several factors that can affect the amount of residual nitrite found in cured, cooked meat products. Those factors can include non-meat ingredients added in conjunction with nitrite or nitrate such as cure accelerators, physical processing procedures. The factors can result in physical nitrite loss during processing, packaging characteristics such as packaging type and method, and storage parameters such as storage time and storage temperature.

The use of cure accelerators such as erythorbates and ascorbates are the largest factor that contribute to the levels of residual nitrite found in cured meat products. The purpose and function of cure accelerators is well known and documented (Lee and Shimaoka 1984; Pegg and Shahidi 2000; Pearson and Tauber 1984; Price and Schweigert 1987). Lee and Shimaoka (1984) clearly demonstrated the substantial depletion of nitrite in sausage where erythorbate was added compared to those where erythorbate was not added. Brown and others (1974) also showed lower residual nitrite levels in cured ham when ascorbate was added. As ascorbate levels were increased, lower residual nitrite levels were found. The authors reported day 0 storage asorbate values of 49.7, 43.0, 18.0 and 14.0
ppm when 0, 227, 455 and 568 ppm asorbate was added to hams containing 182 ppm nitrite at manufacture. This pattern continued at day 2, 5, 8 and 16. The authors also observed superior color stability and uniformity when ascorbate was added during the manufacture of cured hams. Perhaps not often thought as an ingredient that would affect residual nitrite, frankfurter formulations containing liquid smoke have also been reported to increase the depletion of residual nitrite (Pérez-Rodríguez and others 1998).

In a review by Sebranek (1979), the importance of pH on the residual nitrite levels was discussed. A small pH decrease, as low as 0.2 pH units, during product manufacture can result in a doubling in the rate of color formation due to more favorable nitrite-myoglobin interactions. Due to these more favorable reactive conditions, subsequent lower residual nitrite concentrations were found. Research by Kilic and others (2001) supported these statements. In their investigations on the influence turkey meat had on residual nitrite in cured meat products, the authors reported treatments with higher pH values to also had higher residual nitrite levels.

Different raw materials and non-meat ingredients can also contribute to pH and the effects pH has on residual nitrite levels. Kilic and others (2002) found a relationship between residual nitrite and pH values. Including poultry meat in treatments was found to alter overall product pH and thus affect residual nitrite levels, which is agreement with Prusa and Kregel (1985). The addition of phosphates that altered product pH was also found to affect residual nitrite levels. The authors data indicated a pH change (decrease) as little as 0.2 pH units significantly (p<0.05) reduced residual nitrite levels at day 0, 14 and 49 in wiener
with varying raw material and non-meat ingredient formulations. Investigating residual nitrite properties associated with white and red muscles in a model system, Lee and others (1976) determined lower pH directly related to lower levels of residual nitrite regardless of muscle type. Generally, red muscles were found to have higher residual nitrite levels, however, this was normally found to be due partially to pH and concentration of myoglobin differences.

Jantawat and others (1993) stated the initial ingoing nitrite level, extent of the thermal process and the storage time of canned ham rolls all significantly decreased residual nitrite levels. Interestingly, these authors reported that the heating time of the canned ham rolls was more detrimental to residual nitrite levels than the heating temperature. The authors also found a decreasing residual nitrite level with increased storage time relationship. Kilic and others (2001) conversely found that heating temperature during thermal processing affected residual nitrite concentration by lowering levels as temperatures increased. Lee and Cassens (1983) indicated that the length of thermal processing can cause different depletion rates of residual nitrite. The authors reported significantly (p<0.05) more residual nitrite depletion in hams during slow heating (30°C slowly raised to 72°C; 95 minute time) than fast heating (72°C continuous for 42 minutes). Hustad and others (1973) reported that both nitrite and nitrate concentration are affected by both storage time and storage temperature. The authors investigating nitrite and nitrate properties in frankfurters stated that nitrite and nitrate reduction post processing was less rapid at 7°C vs. 27°C. The length of storage also was determined to be a factor in residual levels. Storage time at 14 days resulted in approximately a 90% reduction of the ingoing
nitrite levels that ranged between 30 and 300 ppm nitrite. Levels of nitrate remained comparable to ingoing levels up to approximately 21 days when frankfurters were stored at 7°C. At 27°C storage temperatures, approximately one-half of initial ingoing nitrate levels were present at 14 days storage. This was believed to be due to the microbial activity at higher storage temperatures on the conversion of nitrate to nitrite. Ahn and others (2002b) support the storage time effects but also noted packaging effects. Sausage samples stored in vacuum packages had lower residual nitrite values (68.1 vs 81.8 ppm) than samples stored in aerobic conditions. The authors believed this phenomenon was caused by the environment being in the reduced state thus allowing the conversion of nitrite to nitric oxide and the lower residual nitrite levels found. The authors also showed that gamma irradiation significantly (p<0.05) reduced N-nitrosamines in 20 kGy-irradiated samples for both aerobic and vacuum packaged samples. Additionally, irradiation levels of 10 kGy or higher were shown to decrease N-nitrosamines.

Several authors reported the reduction in residual nitrite by irradiation. Nitrite reduction from irradiation may be explained due to its reaction with the hydroxyl radical formed by the radiolysis of water. Thus, irradiation can be an effective method to reduce residual nitrite and consequently N-nitrosamine formation in products that are exposed to high temperature heating. Ahn and others (2002b) reported that irradiation was effective in reducing residual nitrite in both aerobic and vacuum packaged samples, yet was more effective in vacuum packaged samples over the 5, 10, 20, and 30 kGy doses investigated. These findings were supported with similar irradiation research findings on emulsion type sausages (Ahn and others
2003), with pork sausage (Ahn and others 2002a) and with ground pork meat (Szczawinski and others 1989). These authors also reported a reduction of red color in emulsified sausages modified atmosphere packaged and theorized to be caused by the denitrosylation of nitrosomyoglobin by irradiation. This is in disagreement with Houser and others (2003) who did not find redness differences in irradiated ham but instead reported lightness (L*) differences between ham irradiated at 4.5 kGy and non-irradiated control treatments. Houser and others (2005b) also found no color change differences in ham and frankfurters irradiated at 1.6 kGy. Houser and others (2005a) also showed that irradiation dose level (0, 1.2, 2.3 and 4.5 kGy) significantly (p<0.05) affected residual nitrite level in cured ham. Irradiation above 5 kGy has even been shown to be effective in reducing or destroying potentially present volatile N-nitrosamines (Ahn and others 2002c).

**IX. Concerns of Using Nitrates and Nitrites**

The concern about the intake of nitrates and nitrites in humans is centereded on the possibility that these two compounds may be a source of nitrosating compounds leading to the subsequent and toxic development of carcinogenic N-nitrosocompounds such as N-nitrosamines (Walker 1990). Fiddler and others (1992) stated that the first incident involving a nitrosamine was associated with a fish-derived food product. The presence of carcinogenic N-nitrosamines in meat products, particularly in bacon, has caused concern about the use of nitrite in meat products (Shahidi 1988; Cassens 1995; Cassens 1997a). Carcinogenic volatile N-nitrosamines have been suggested to induce tumors in many organs in the human
body (Ahn and others 2002b). The formation and presence of these dangerous compounds can be caused by high temperature exposure to foods containing sodium nitrite (Ahn and others 2002b). Nitrates have also been suggested to react with amines present in gastric acids to form carcinogenic nitrosamines and possibly various cancers (Archer 2002). However, no association or link between this has been identified. In fact, no epidemiological study has linked nitrate or nitrite consumption to a specific cancer or cancer risk to date. Eichholzer and others (1998) investigating dietary nitrates, nitrites and N-nitroso compounds, summarized that no epidemiologic evidence was found linking brain, esophageal and nasopharyngeal cancers to the intake of these compounds. An association between them, however, was not ruled out. Maekawa and others (1982) investigated the carcinogenicity of sodium nitrite and sodium nitrate in Fischer-344 rats (n=240). Over a two year study of continuously administering rats sodium nitrite and sodium nitrate in dietary drinking water, these authors found no carcinogenic activity from these ingredients or nitrosamines generated. Furthermore, two summary reports generated from an immense amount of research and testing published by the National Academy of Sciences (NAS 1981; NAS 1982) conclusively asserted that nitrite cured meat did not pose as a human health risk. A study by García Roché and others (1987), estimating the intake of nitrates and nitrites of 12000 12-17 year old students in Havana, Cuba reported that daily consumed levels of 65 to 79 mg of nitrate and 2.3 to 4.8 mg of nitrite were lower than the acceptable maximum daily intake recommended by the Food and Agriculture Organization of United Nations and the World Health Organization. From these findings, the authors stated that
there was not a health risk associated with these common levels of nitrate and nitrite consumption.

**Nitrate / Nitrite Poisoning**

When not used or controlled properly, nitrite and nitrate can be a dangerous and often times lethal chemical ingredient. It is reported that levels above 300 mg/kg (ppm) of body weight is considered the lethal dose for nitrite (Pierson and Smooth 1982). For this reason, the USDA considers nitrite and nitrite restricted ingredients and regulates usage levels (Pearson and Tauber 1984). Under these USDA regulations, nitrite used in meat at regulated levels and subsequent ingestion from these products is not known to not present a known health hazard (Pierson and Smooth 1982). Nitrite, if consumed by itself, can cause serious health effects, which if not treated or too severe, can be fatal. At high ingested doses, nitrite and/or nitrate can cause acute fatal poisoning characterized by cyanosis due to formation of methemoglobinemia (Gautami and others 1995). Cyanosis is the characteristic blue color of the skin observed when the amount of unoxygenated hemoglobin is abnormally high. This signifies that not enough oxygen is circulating in the blood to organs and tissues in the body. Methemoglobinemia is a condition where hemoglobin is oxidized to the methemoglobin form in which the iron in the heme component of the molecule has been oxidized from the ferrous (+2) to the ferric (+3) state. When this occurs, the hemoglobin molecule becomes incapable of effectively transporting and releasing oxygen to organs and tissues in the body. The tissues and organs are thus “starved” for oxygen and can slow or stop function depending on the severity of incident.
Dietary methemoglobinemia is a condition in infants less than 6 months of age referred to as “Blue Baby syndrome” where babies that have an underdeveloped protective enzyme system are exposed to nitrite poisoning causing skin and other organs to turn blue in color due to a lack of oxygen transported to these organs (Archer 2002). It may also be caused by the infant ingestion of foods containing nitrates, the high pH condition of their gastrointestinal tracts and a greater abundance of nitrate reducing bacteria present (Hardisson and others 1996). Most methemoglobinemia cases have been caused by mix ups of ingredients (i.e. salt and sodium nitrite) and most are reported as accidental in nature.

**Nitrosamine and Cancer Relationship**

Nitrite is a reactive chemical and controlled restricted ingredient and must be used with caution. It can act as a nitrosating compound under certain conditions producing nitroso compounds of which some are classified as carcinogens (Cassens 1997b). It is suggested that nitrite converted to nitric oxide can react with certain classes of secondary amines to result in the formation of carcinogenic nitrosamines, specifically a problem with bacon (Wolff and Wasserman 1972). For this reason, the presence and formation of nitrosamines in meat products containing nitrate or nitrite have been considered of significant importance to investigate and understand. A common belief of the danger of nitrosamine presence or production from cured meat products is that they can potentially be carcinogenic (Brown and others 1974). Thus, a great deal of research has been done to reduce residual nitrite levels in cured meat products.
Bacon has been a product showing the most difficulty in eliminating nitrosamine presence and formation (Pearson and Tauber 1984). The combination of high cooking temperatures (i.e. frying) and the presence of secondary amines and residual nitrite in bacon have proven difficulties to addressing the nitrosamine issue (Pearson and Tauber 1984). Therefore, special regulations by USDA FSIS requiring lower ingoing nitrite levels of 120 ppm and not allowing nitrate in bacon were implemented. Additionally, reducing ingoing nitrite levels as well as nitrite and nitrosamine reducing technologies have been investigated for their effectiveness.

In an effort to reduce ingoing nitrite in bacon, Tanaka and others (1985b) investigated the use of lactic acid starter culture and lower sodium nitrite levels. These investigators reported that bacon manufactured with 40 or 80 ppm sodium nitrite, lactic acid starter culture and sucrose had better antibotulinal protection and less nitrosamine formation than control bacon manufactured with 120 ppm sodium nitrite, no lactic acid starter culture and no sucrose. In addition, this process also known as the “Wisconsin Process” resulted in bacon with sensory characteristics similar to traditional manufactured (120 ppm) bacon (Tanaka and others 1985a).

Ivey and others (1978) indicated that fried bacon containing potassium sorbate and increasing levels of nitrite did not result in increased occurrences of nitrosopyrrolidine formation during nitrosamine analysis. On the contrary, Fiddler and others (1992) found increased levels of nitrosamine formation in fish-meat frankfurters when heat treated by frying (171°C). The authors investigated nitrosamine formation from boiling, microwave, roller grill, broiling and frying cookery methods. The authors reported the least amount of nitrosamines was formed from
microwaving and the most formed by frying. Optimal nitrosamine formation
temperatures during frying of 100ºC were suggested to explain their findings. Ahn
and others (2002a) monitored nitrite and N-nitrosamine levels in irradiated pork
sausage and reported that irradiation effectively reduced residual nitrite levels as
well as eliminated any N-nitrosamines present over a 4 week storage period.

Lowering the ingoing nitrite level has also been considered a possible answer
to controlling the nitrosamine problem. This approach often affects the quality,
consumer acceptability and potentially food safety of these types of meat products
(Pierson and Smooth 1982). Thuringer sausage manufactured with 50, 100 and 150
ppm nitrite showed no nitrosamines detected over 4 weeks of 7.5 and 27ºC storage
temperatures (Dethmers and Rock 1975). Sebranek (1979) supported the
effectiveness of reduced levels of ingoing nitrite in product formulations as well as
use of cure accelerators such as erythorbate and ascorbate in effectively reducing
both residual nitrite as well as potential nitrosamines in cured meat products.
Rywotycki (2002) investigating ingredient and heat effects on nitrosamine formation
concluded that curing with sodium nitrite can increase nitrosamines but these effects
can be negated by the addition of sodium ascorbate in the formulation. This
phenomenon was also shown when investigating the presence and formation of
nitrosamines regarding the production of soy sauce. Soy sauce found to have nitrite
present from contamination of water containing nitrate and fermented to yield nitrite
was shown to effectively reduce nitrosamine presence by the addition of ascorbic
acid (Nak-Ju and others 1991). With a more common occurrence of nitrate/nitrite
poisoning incidents (Hardisson and others 1996), perhaps the concerns over these
compounds should shift from disease causing (cancer) to human poisoning (methemoglobinemia).

**X. Uncured, No-Nitrate/Nitrate-Added Meat Products**

**The Rationale and Concept**

Although there are several advantages of using nitrite in meat curing systems, there is one disadvantage that has plagued this compound for over 30 years. The implications surrounding the presence and formation of $N$-nitrosamines in cured meat products, specifically bacon, has continuously held nitrite at the forefront of carcinogen related concerns (Shahidi 1988). Health concerns may have, in part, prompted consumers to shift purchasing decisions to uncured, no-nitrate/nitrite-added meat and poultry products. The perceptions that uncured, no-nitrate/nitrite-added meat products are “safer” and “healthier” are sensible reasons for the consumer demands of these products. Creative marketing and labeling may also play a role in this perception. Commercial uncured, no-nitrate/nitrite-added meat products are commonly manufactured with organic or natural raw materials and ingredients.

**Uncured, No-Nitrate/Nitrite-Added Meat Product Labeling**

Meat products to which nitrate or nitrite is permitted or required can also be manufactured without nitrates or nitrites but must be labeled according to the USDA Code of Federal Regulations Title 9, Part 317.17 and 319.2. These regulations state:

“Any product which is required to be labeled by a common or usual name to which nitrate or nitrite is permitted or required to be added may be
prepared without nitrate or nitrite and labeled with such common or usual name or descriptive name when immediately preceded with the term ‘Uncured’ as part of the product, provided that the product is found by the Administrator to be similar in size, flavor, consistency, and general appearance to such product as commonly prepared with nitrate or nitrite, or both.

Products which contain no nitrate or nitrite shall bear the statement ‘No Nitrate or Nitrite Added.’

Products described shall also bear the statement ‘Not Preserved-Keep Refrigerated Below 40 °F At All Times’ unless they have been thermally processed to Fo 3 or more; they have been fermented or pickled to pH of 4.6 or less; or they have been dried to a water activity of 0.92 or less.

Products shall not be subject to the above mentioned labeling requirements if they contain an amount of salt sufficient to achieve a brine concentration of 10 percent or more."

The terms organic and natural found on labels can often lead to additional confusion when paired with the term uncured. Products labeled organic or natural are not allowed to have added nitrates or nitrites by definition. Regulations for organic and natural serve a distinctly different category of products governed by separate labeling policies. Those policies and differences are beyond the scope of this discussion but a generalization is that all organic and natural labeled products are uncured but not all uncured products are necessarily natural or organic.

Concerns

Uncured, no-nitrate/nitrite-added meat and poultry products can carry higher risks for botulism than nitrite-added products. These products lack the barriers that help ensure the safety associated with these types of products, especially if temperature abused (Miller and others 1993). Miller and others (1993) discovered while investigating various organic acid salts (lactate, pyruvate and citrate) for C.
*botulinum* control in uncured, no-nitrate/nitrite-added turkey product that inoculated samples became toxic before off-odors and soft textures were apparent to sensory panelists. This fact is alarming in that the traditional method of spoilage microorganisms used as an indicator of early warnings of more harmful pathogenic growth is not necessarily applicable with uncured, no-nitrate/nitrite-added meat and poultry products. On the other hand, Bayne and Michener (1975) stated that products manufactured with or without nitrite can become hazardous if contaminated with *Staphylococcus* or *Salmonella*. Temperature abuse may be an important factor in regard to the growth of these two microorganisms. Microorganisms that survive thermal processing or are a result of post lethality contamination did not significantly grow faster in uncured, no-nitrate/nitrite-added frankfurters than conventional ones. Hustad and others (1973) investigating *C. botulinum* toxin production in wieners reported 79 of 220 samples that did not contain nitrite or nitrate were positive for the presence of *C. botulinum* toxin after 56 days of storage at 7 and 27°C storage conditions.

Trends in the 1990s of the introduction and production of preservative-free, low salt food products packaged in air-tight containers has sparked concern since little or no intrinsic control of *C. botulinum* spore germination and growth is present in these products (Sobel and others 2004). Since the sole control method for *C. botulinum* germination and growth in these consumer-perceived, uncured, “healthier” products is refrigeration, concerns about these types of products are relevant to food safety discussion.
It has been stated before that no compound has been found to date that replaces all the functions that nitrite plays in cured meats. Nitrite is responsible for the development of cured color and flavor, serves as a strong antioxidant to protect flavor and acts as a strong antimicrobial to control *C. botulinum* outgrowth (Shahidi and Pegg 1992). Efforts to replace nitrite with a compound(s) that serves those important functions have been difficult and often unsuccessful. Yun and others (1987) reported that combinations of chelators and antioxidants can successfully duplicate the action of sodium nitrite in cooked pork. However, microbial safety or consumer visual acceptance was not addressed. Huhtanen and Feinberg (1980) reported that sorbic acid can successfully be used to inhibit *C. botulinum* but neither lipid oxidation nor consumer acceptance of the uncured, no-nitrate/nitrite-added poultry frankfurters were addressed in their study. Huhtanen and others (1983) also found success in inhibiting *C. botulinum* in bacon research by replacing nitrite with alkynoic and alkenoic acids and esters yet these researchers did not address lipid oxidation or consumer acceptance. Wasserman and others (1977) investigated the consumer acceptance of bacon cured with and without nitrite. The authors reported no significant differences (p>0.05) for preference between the two treatments, however this study did not consider the risk of *C. botulinum* growth or the oxidative stability over time of the nitrite free bacon.

**Consumer Acceptance**

From a sensory standpoint, consumer preferences for meat products are influenced by appearance, flavor, tenderness and juiciness (Resurreccion 2003). Heath concerns (desire for healthy foods), the need for convenience and price can
often be factors in consumer demands for meat products. Bauermann (1979) stated that uncured, no-nitrate/nitrite-added chicken frankfurters have an extremely undesirable gray-green color under normal daylight. Uncured, no-nitrate/nitrite-added turkey frankfurter color was described as even less desirable in appearance. Huhtanen and others (1981) reported that flavor scores for nitrite cured and uncured, no-nitrate/nitrite-added sorbic acid containing bacon were not significantly different from one another but large variations of flavor scores within the treatments were shown. The authors summarized that uncured, no-nitrate/nitrite-added sorbic acid containing bacon presented acceptable sensory quality attributes and satisfactory *C. botulinum* spore outgrowth prevention. Results from cured ham research performed by Brown and others (1974) revealed that hams manufactured without nitrite had lower flavor intensity sensory scores (P<0.05) than hams cured with either 91 or 182 ppm nitrite.

Investigating the “plate waste” or how much nitrite-cured and uncured, no-nitrate/nitrite-added bacon remained on the breakfast plates of students in a dining hall, Williams and Greene (1979), reported no significant difference of the means between the two types of bacon served four times over a two and one-half hour testing period. However, 2-thiobarbituric acid (TBA) test numbers revealed that nitrite cured bacon had TBA numbers of 0.4 and 0.6 on the first and last days of testing respectively. TBA numbers for uncured, no-nitrate/nitrite-added bacon were 0.8 and 1.7 on the first and last days of testing respectively. A TBA number of 0.5 to 1.0 is considered to be the threshold for oxidized odor and 1.0 to 2.0 for oxidized flavor (Tarladgis and others 1960).
Hustad and others (1973) reported that flavor quality scores for wieners with varying levels of nitrite added (50 to 300 ppm) were all significantly higher (P<0.05) that others with 0 ppm added (no nitrite added). The color of the wieners between all nitrite levels (50, 100, 150, 200 and 300) was not deemed different but all were considered different when compared to 0 ppm added nitrite. In this study, the uncured, no-nitrate/nitrite-added wiener’s internal surface was described as brown and grey. Noel and others (1990) looked at similar criteria for uncooked dry sausage. They reported objective color differences between nitrite-added and no-nitrite-added sausages. Utilizing sensory triangle tests, the authors showed that flavor properties could be significantly (P<0.05) differentiated between nitrite-added and uncured, no-nitrite-added treatments. Another sensory triangle test showed that attempts of using hardwood smoke intentionally to mask both cured meat flavors in nitrite cured pork roast and flavors present in uncured, no-nitrite cured pork roast (Cho and Bratzler 1970). The effort to possibly improve the acceptance of uncured, no-nitrite-added cured pork roast were, however, determined unsuccessful.

Gray and others (1981) noted that acceptable bacon could be manufactured without using nitrite according to sensory preference testing but this study did not consider the oxidative stability or C. botulinum possible risks of the uncured bacon. Contrarily, ham samples with no added nitrite were disliked by a consumer sensory panel compared to nitrite-added ham samples (Froehlich and others 1983). Interestingly, 50 or 150 ppm added nitrite had no effect on overall desirability of ham suggesting that how intense or pink the color or cured meats may not necessarily be as important as the presence of pink or cured color in cured meat products.
Judge and Cioch (1979) produced uncured, no-nitrate/nitrite-added hams injected with a solution of water, sodium chloride, brown sugar, sodium tripolyphosphate and sodium erythorbate. Sensory panelist scores for palatability were deemed acceptable. Flavor and texture attributes were considered not to resemble that of nitrite cured hams and were generally described as that of uncured roasted pork. Wasserman and others (1977) showed no difference in consumer preference between uncured, no-nitrate/nitrite-added bacon and nitrite cured bacon.

The Future

For uncured, no-nitrate/nitrite-added products to become successful, an acceptable food additive/ingredient must be found that produces the characteristic cured flavor and appearance with the antibotulinal role of nitrite-added products (Tompkin 1980). In addition, oxidative stability must also be addressed. Wolff and Wasserman (1972) commented on removing nitrite and nitrite completely from cured meat products: “We could be replacing one hazard by another, more serious one”. Addressing these issues is critical for the manufacture of safe and consumer desirable uncured, no-nitrate/nitrite-added meat products.

XI. Alternatives to Nitrates / Nitrites in Cured Meat Products

In order to identify alternatives to adding nitrite itself to cured meat products, a more thorough understanding of the chemical nature of the inhibitor that is formed by heating nitrite in meat products is necessary (Roberts 1975). Nitrite use dates back to antiquity, however, regulated use has been in effect since 1925 (Shahidi and Pegg 1992). To date, no replacement for nitrite has been discovered that effectively
produces the characteristic cured meat aroma and flavor of meat products it is used in (Gray and others 1981). Shahidi and Pegg (1992) stated: “It is unlikely that a single compound will be found that can perform all the functions of nitrite”. Furthermore, the National Academy of Sciences committee on nitrite and alternative curing agents in food stated the following from a food safety perspective (NAS 1981):

“The committee believes that the degree of protection against botulism is likely to decrease if the essential preservative uses of nitrite are substantially reduced without introducing an efficacious, but safer alternative.”

**Sources to Replace Nitrite**

With the continued demand for “safer” and alternative uncured, no-nitrate/nitrite-added meat products, research revolving around the replacement of sodium nitrite has been performed in great depth. Results and information gathered from the numerous attempts of nitrite replacement have improved both the knowledge about the importance of sodium nitrite as well as the difficulty of removing it from cured meats. Two approaches can be viewed for removing and replacing nitrite. Direct replacement is defined as complete removal of nitrate and nitrite from a curing system while indirect replacement is the process of removing some or all nitrate and nitrite from the curing system and replacing it with another source. Direct and indirect replacement of nitrite will thus be discussed as two potential and even theoretical approaches.
**Direct Replacement**

Sorbic acid and its alkaline salts have been used to control spoilage by inhibiting yeast and mold in food products (Sofos and others 1979b). Investigating canned uncured, no-nitrate/nitrite-added poultry frankfurters, Huhtanen and Feinberg (1980) stated that sorbic acid can be used to inhibit *C. botulinum* spore outgrowth. Growth and toxin production times, which were measured by the time it took for the cans to swell from gas production, were longer for poultry (chicken and turkey) frankfurters at 0.40% added sorbic acid then compared to none added. This effective level was suggested to be comparative to commercial products containing 135 ppm nitrite. However, Bauerman (1979) found that using sorbic acid to replace sodium nitrite at intended usage level in chicken and turkey frankfurters imparted flavor acceptance problems.

Sofos and others (1979b) found similar delays in toxin production (6 or 7 days incubated at 27°C) at 0.2% added sorbic acid to poultry emulsions. Below this level, no effect on toxin growth prevention was shown. Furthermore, the antimicrobial activity of sorbic acid is dependent on the pH of the meat product (Sofos and others 1979a). Since free undissociated acid at low pH (5.0 to 5.5) is the effective form of sorbic acid, its application to an array of meat products that have large variations in pH may be in question. Huhtanen and others (1981) speculated that the decrease in pH from the addition of sorbic acid of around 0.14% aided in the control of *C. botulinum* spore outgrowth in uncured, no-nitrate/nitrite-added bacon. Thus the lowering of pH had positive effects on controlling *C. botulinum* spore outgrowth.
Huhtanen and others (1985) and Huhtanen (1983) investigated the potential replacement of nitrite for controlling *C. botulinum* by the action of several short-chain alkynoic and alkenoic acids and esters added to bacon inoculated with *C. botulinum* spores. Several of these compounds were shown to have effective properties when compared with a control manufactured with 120 ppm sodium nitrite. However, the authors stated that organoleptic and physical properties of the products would need to be addressed and evaluated to determine product acceptance.

Organic acid salts of propionate, citrate, acetate, lactate and pyruvate were tested for effectiveness in suppressing *C. botulinum* growth in uncured, no-nitrate/nitrite-added turkey product. Although results varied on the effectiveness of each specific compound, the general conclusion was these compounds may contribute to improving the food safety of these products by acting as secondary barriers to *C. botulinum* outgrowth (Miller and others 1993). No reduction in toxin formation was shown at sample pH levels of 5.5, 6.0 or 6.5. These compounds were described as “attractive alternatives” to other potential compounds possessing natural antimicrobial properties.

Sodium acid pyrophosphate, sodium hexametaphosphate and sodium tripolyphosphates investigated and added to uncured, no-nitrate/nitrite-added chicken emulsions were shown to have no effect on controlling toxin production by *C. botulinum* (Nelson and others 1983).

Cooked cured-meat pigment (CCMP) has been investigated as a replacement for nitrite in great detail. Dinitrosyl ferrohemochrome (DNFH) is generally accepted to be the pigment associated with the pink color of cured meats (O’Boyle and others
CCMP of DNFH has been developed and studied as a synthetic replacement of DNFH (O'Boyle et al. 1992; Shahidi et al. 1985). Shahidi and Pegg (1990b; 1991; 1991) demonstrated that CCMP prepared from hemin and nitric oxide and added to ground pork could replace the cured color development of sodium nitrite. In their research, the authors reported that Hunter $L$, $a$, $b$ color values were not significantly different between treatments containing cooked cured-meat pigment and those containing sodium nitrite. Shahidi and Pegg (1992) were also successful in reproducing nitrite cured color by substitution of nitrite with CCMP prepared from beef red blood cells. The authors noted that success of color with CCMP was dependent on the myoglobin content of the raw materials into which the CCMP was incorporated. Stevanović et al. (2000) support the substitution of sodium nitrite with CCMP as an effective replacement for nitrite. Shahidi and Pegg (1994) also investigated nitrosamine formation with nitrite free CCMP added cured pork systems and demonstrated that none was present or formed. Work by O'Boyle et al. (1992) demonstrated that CCMP could be successfully incorporated into hams. From these findings, the authors concluded that marketable nitrite-free hams and other similar cured-meat products could be successfully manufactured with CCMP. Wood et al. (1986) utilized several antioxidants and antimicrobials with CCMP to study the success of uncured, no-nitrate/nitrite added products from an antitoxin aspect. The authors reported that a treatment containing ascorbate, sodium tripolyphosphate, tertiary butyl hydroquinone, sodium hypophosphite and DNFH possessed antitoxin control equal to the control (150 ppm nitrite and
ascorbate), noting the significant accomplishment for developing a nitrite-free curing system.

**Indirect Replacement**

Skjelkvåle and Tjaberg (1974) investigating salami sausage produced with and without sodium nitrite or nitrate showed that a nitrite or nitrate source must be used to generate cured product characteristics. These authors reported that treatments containing micrococi and lactobacilli starter cultures without the addition of nitrite resulted in no residual nitrite at day 1 or any time after. Morita and others (1998) reported that salami manufacture with no nitrate or nitrite but containing a starter culture (*Staphylococcus xylosus*) resulted in product color similar to salami manufactured with nitrite. A possible cause was theorized to be the possible presence of nitric oxide producing bacteria present in the product. This theory, however, is in disagreement with Morita and others (1996) who investigated the bacterial influence on pigment in parma ham and found no association.

Studies by Tanaka and others (1985a) as well as Tanaka and others (1985b) have demonstrated that lactic acid starter cultures could be utilized to replace a portion of ingoing nitrite in bacon. The next question to be answered is whether lactic acid starter culture can be incorporated into an uncured, no-nitrate/nitrite-added curing system to completely replace sodium nitrite. The possibilities exist for utilizing nitrate from indirect sources in curing systems with the addition of nitrate reducing microorganisms in lactic acid starter cultures that could successfully accomplish complete replacement of sodium nitrite.
The possibility of replacing nitrite indirectly through use of naturally nitrate containing ingredients is an interesting point of discussion. Vegetables are well known to contain reasonably significant amounts of nitrate. Cabbage, lettuce, spinach, carrots, beets and radishes are several vegetables reported to naturally contain nitrate (anonymous). Those amounts were reported to be 200 to 352 ppm (cabbage), 600 to 1700 ppm (lettuce), 500 to 1900 ppm (spinach), 100 to 900 ppm (carrots), 1200 to 1300 ppm (beets) and 1500 to 1800 ppm (radishes). Ranges in levels can partially be explained by fertilizing, maturity and climate variations. Similar nitrate levels of these and many other vegetables were also reported by Walker (1990), Fujihara and others (2001), Santamaria and others (1999), White (1975) and Cieslik and Sikora (1998).

Certain vegetables, beets and spinach, with exceptionally high nitrate levels have been specifically investigated due to possible health concerns (Bednar and others 1991; Lee and others 1971). Chung and others (2004) found concern with high levels of nitrate in spinach and cabbage. Kolb and others (1997) specifically investigated the potential nitrosamine formation and prevention from vegetable juice manufactured from beets high in nitrate.

Huate-Mendicoa and others (1997) reported that a blanching/freezing process could affect nitrate levels in broccoli and demonstrated another source of nitrate accumulation. The authors reported significantly higher (p<0.05) levels of nitrate in blanched frozen (127 to 231 ppm) versus fresh (49 to 97 ppm) analyzed samples. The unexpected increase was believed to be due to nitrate contamination during the blanching process since Santamaria and others (1999) stated that
cooking removes approximately 50% of accumulated nitrate. High nitrate levels are obviously not a positive attribute from a human health perspective but do spark thought provoking ideas about this possibly underutilized component of vegetables.

**XII. Summary of Literature**

The concept of uncured, no-nitrate/nitrite-added meat products is still not well understood. A great deal of confusion overshadows these uncured products with disclaimers stating no nitrite or nitrate added. The labeling word “uncured” could, in reality, describe products without any intention of having cured characteristics. This can lead to an abundance of confusion to consumers on what types of products they are purchasing or even what might be in them. The labeling of many current commercially available uncured meat products may be more accurate if called “no-nitrite added.....” or “naturally cured.....” as commercial product evaluation may question a product with nitrite-added product characteristics being labeled as uncured or no-nitrate/nitrite-added. It is clear that a source of nitrite or nitrate is necessary to produce all the characteristic properties associated with cured meat products. Whether that source must originate from direct addition of nitrite or indirect addition from naturally nitrate containing ingredients is an unanswered question.

Work focusing on reducing nitrate with starter cultures has shown effectiveness (Tanaka and others 1985a; Tanaka and others 1985b; Andres 1979) that can be possibly transferred to no-nitrite-added concepts. From the vast knowledge from research relating to sodium nitrite, meat curing and cured meat properties, a solid platform has been built to investigate this area. There is no doubt that these
products are of utmost importance for investigations of quality and safety. The safety of our food supply depends on the knowledge about the safety of our foods. Uncured, no-nitrate/nitrite-added meat products clearly fall into this need. Based on commercial uncured, no-nitrate/nitrite-added product availability and variety, consumer demand for these products is likely to increase over time.
References


García Roché MO, García A, Torres O. 1987. Estimation of the daily intake of nitrates and nitrites which may be consumed by students 12-17 years old in secondary schools in the City of Havana. Nahrung 31:217-220.


Sources of nitrite. (anonymous).


CHAPTER 3. INVESTIGATING QUALITY ATTRIBUTES AND CONSUMER ACCEPTANCE OF UNCURED, NO-NITRATE/NITRITE-ADDED COMMERCIAL HAM, BACON AND FRANKFURTERS

A paper submitted to the Journal of Food Science

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Abstract

Increasing demands for natural, organic and/or preservative-free foods have prompted the manufacture and consumer availability of uncured, no-nitrate/nitrite-added processed meat and poultry products. Quality and sensory attributes of these uncured products is unclear. The objective of this study was to determine if quality and sensory differences exist between uncured and cured meat products. Five different commercial brands (four uncured, no-nitrate/nitrite-added and one nitrite-added) of three product types (frankfurters, hams and bacons) were obtained from retail supermarkets. The samples were evaluated for color, pigment content, pH, lipid oxidation, residual nitrate and nitrite content, and consumer acceptance. All brands from all product types evaluated, except for one bacon (Brand B), had cured color, aroma and flavor attributes similar to the nitrite-added control (Brand E). All product types and all brands contained residual nitrate and residual nitrite except for Brands B and D bacons (<1 ppm nitrite). Lipid oxidation as measured by 2-thiobarbituric acid reactive substances (TBARS) revealed a large variation in the occurrence of lipid oxidation between product types within brands with frankfurters reporting the highest levels. Color measurements indicated the majority of products and brands were similar to the control. Consumer sensory ratings for surface/lean
color, aroma, flavor, texture and overall acceptance determined that variation did exist. Brand E (nitrite-added control) and one uncured brand for each product type were not different (P>0.05) for overall acceptance but received higher scores (P<0.05) than all other brands within each product type.

Keywords: uncured, residual nitrate, residual nitrite, ham, frankfurter, bacon

Introduction

Meat curing has traditionally been associated with processed meats for the purpose of altering the color, flavor, safety and shelf life characteristics which makes these products unique compared to other meat products (Sebranek and Fox 1985). Today, meat curing is utilized to achieve consumer demands for products that have unique sensory characteristics and convenience attributes associated with cured meats. Nitrite is responsible for the development of cured color and flavor, serves as a strong antioxidant to protect flavor and acts as a strong antimicrobial to control Clostridium botulinum outgrowth (Shahidi and Pegg 1992). Nitrite controls and stabilizes the oxidative states of lipids in meat products (Shahidi and Hong 1991) thus preventing lipid oxidation and subsequent warmed-over flavors (Vasavada and Cornforth 2005; Yun and others 1987). To date, no replacement for nitrite has been discovered that effectively produces the characteristic cured meat aroma and flavor of nitrite containing meat products (Gray and others 1981).

Recent consumer interest for natural, organic and healthier foods has prompted consumer demands for uncured, no-nitrate/nitrite-added meat and poultry products. Concerns about nitrate and nitrite intake may also play a role in demand
for these products. However, human nitrate and nitrite intake can take place by several avenues. Nitrate is commonly ingested when people consume vegetables (Archer 2002) and it is well-known that leafy green or root vegetables and drinking water are sources of nitrate to which humans are exposed (Cassens 1997). The National Academy of Sciences (1981) reported that 39% of dietary nitrite was from cured meat, 34% was from baked goods and cereals and 16% was from vegetables which also account for 85% of dietary nitrate. White (1975) estimated 81.2% of nitrate intake and 1.6% of nitrite intake is derived from vegetable consumption. Knight and others (1987) suggest a greater reduction in human exposure of nitrate is more feasible by reducing the intake of vegetables and water than cured meat products.

Two classifications of uncured, no-nitrate/nitrite-added meat and poultry products currently exist in the market place: those that do not include nitrate or nitrite (uncured products), and those with the intention of replacing nitrate and nitrite to simulate typical curing. Uncured meat products that do not include nitrate or nitrite commonly reveal undesirable quality characteristics of appearance (Bauermann 1979) and sensory aroma and flavor (Hustad and others1973; Brown and others 1974; Froehlich and others 1983). Uncured meat products with the intention of replacing nitrate and nitrite often possess quality and sensory attributes similar to those found in nitrite cured products. However, little information is available on the qualitative or sensory attributes these types of product compared to conventional nitrite-added products.
Therefore, the objectives of this research were to first determine if quality differences of lipid oxidation, color and cured pigment concentration exist between commercially available uncured, no-nitrate/nitrite-added frankfurters, bacons and hams and nitrite-added products, and secondly, to determine the acceptability of those products by a consumer sensory analysis from the perspective of “consumer purchased” market place available meat products.

Materials and Methods

Experimental Design and Data Analysis

The experimental design was a randomized complete block design using a mixed effects model. Statistical analysis was performed for all measurements using the Statistical Analysis System Mixed Model procedure (SAS Inst. 2003; version 9.1, SAS Institute Inc., Cary, N.C., U.S.A.). The model included the random main effect of replication (n=2), the fixed main effect of brand (A-E) where Brand A-D = uncured, no-nitrate/nitrite-added and Brand E = nitrite-added, and the random effects of the interaction of replication x brand. The significant main effect means were separated and least significant differences were found using Tukey-Kramer multiple pair wise comparison method. Significance level was determined at P<0.05. The experiment was replicated two times.

Product Procurement

Five different commercial brands (four uncured, no-nitrate/nitrite-added and one nitrite-added) of frankfurters, hams and bacons were obtained from retail supermarkets and transported under refrigeration to the Iowa State University Meat
Laboratory (Ames, IA., U.S.A.) on two separate trips two weeks apart (replication). No stipulations were placed on selecting of the product except that each brand of products shared the same sell by date. Brand E (nitrite-added control) selection was based on consideration of a high-quality standard for that product group (ham, bacon, frankfurter). After the products arrived at the Iowa State Meat Laboratory, they were assigned to brand designations (A-E) and stored under refrigeration (0-2 °C). Un-sliced ham treatments were sliced to comparable thickness as the remainder of treatments and placed into barrier bags (Cryovac B540, Cryovac Sealed Air Corp., Duncan, S.C., U.S.A.) and vacuum packaged (Multivac Model A6800 vacuum packager, Multivac Inc., Kansas City, Mo., U.S.A.). Bacon was repackaged and 10 randomly selected slices needed for sensory evaluation were individually placed into barrier bags and vacuum packaged. The packaging film for the vacuum packaged slices had an O₂ transmission rate of 3-6 cc/m²/24 h at 1 atm, 4.4 °C, and 0% RH, and a water vapor transmission rate of 0.5-0.6 g/645 cm²/24 h and 100% RH. The slicing and subsequent packaging of the samples was conducted in as little light as possible to minimize light-induced cured color fading.

**Color Measurements**

Color measurements were conducted using a Hunterlab Labscan spectrocolorimeter (Hunter Associated Laboratories Inc., Reston, Va., U.S.A.). The Hunterlab Labscan was standardized using the same packaging material as used on the samples, placed over the white standard tile. Values for the white standard tile were X=81.72, Y=86.80 and Z=91.46. All of the measurements were taken while products were maintained in vacuum packaged conditions with the exception of the
internal color of frankfurters. Internal color of frankfurters was measured immediately after slicing the frankfurters lengthwise.

Illuminant A, 10° standard observer with a 1.27 cm viewing area and 1.78 cm port size was used to analyze frankfurter samples and a 4.45 cm viewing area and 5.08 cm port size was used to analyze internal surface color of ham samples. Commission International d’Eclairage (CIE) L* (lightness), a* (redness), and b* (yellowness) and cured meat color determined by reflectance ratio of wavelengths 650/570 nm (Hunt and others 1991). Measurements were taken at 4 randomly selected areas on the samples and the resulting average was used in data analysis.

Lean portion only and entire exposed area (fat and lean) color measurements were determined for 3 randomly selected and individually vacuum packaged bacon slices from each treatment. Illuminant A, 10° standard observer with a 0.64 cm viewing area and a 1.02 cm port size, was used to analyze the lean portion only and a 2.54 cm viewing area and 3.05 cm port size was used to analyze the entire exposed area of bacon slices. Commission International d’Eclairage (CIE) L* (lightness), a* (redness), and b* (yellowness) and cured meat color determined by reflectance ratio of wavelengths 650/570 nm (Hunt and others 1991). Measurements were taken at 8 to 12 randomly selected areas of the lean portion and 6 randomly selected areas of the entire exposed area for bacon slice samples and the resulting average was used in data analysis.

**Total and Cured Pigment Analysis**

Mononitrosylhemochrome (cured meat pigment) and total pigment concentrations were measured after extraction in 80% acetone and acidified
acetone, respectively (Hornsey 1956). The experiment including sample preparation was done in subdued light, to reduce pigment fading. Samples were finely ground/chopped using a food processor (Sunbeam-Oskar Model 4817, Sunbeam Products Inc., Delray Beach, Fla., U.S.A.).

Cured pigment analysis was conducted using a modified method of Hornsey (1956). Duplicate 10 g samples were mixed with 40 ml of acetone and 3 ml of distilled, de-ionized water with a Poltroon mixer (PT 10/35, Kinematical GmbH, AG, Switzerland) for 1 min at speed setting 7. The sample was immediately filtered through a Whatman 42 filter paper, and the absorbance (540 nm) measured on the filtrate. Nitrosylhemochrome concentration was calculated as $A_{540} \times 290$ and was recorded in parts per million (ppm).

Total pigment analysis was conducted using a modified method of Hornsey (1956). The same finely ground/chopped samples used for cured pigment analysis were utilized for total pigment analysis. Duplicate 10 g samples were mixed with 40 ml of acetone, 2 ml of distilled, de-ionized water and 1 ml of concentrated hydrochloric acid using a Polytron mixer (PT 10/35, Kinematica GmbH, AG, Switzerland) for 1 min at speed setting 7. The samples were allowed to stand for 1 h, then filtered through a Whatman 42 filter paper and immediately analyzed. Absorbance was measured at 640 nm. Total pigment concentration was calculated as $A_{640} \times 680$ and was recorded in parts per million (ppm).

**pH Determination**

The pH of the frankfurter, ham and bacon samples was determined by blending the samples with distilled, de-ionized water in a 1:9 ratio, then measuring
the pH with a pH/ion meter (Accumet 925: Fisher Scientific, Fair Lawn, N.J., U.S.A.) equipped with an electrode (Accumet Flat Surface Epoxy Body Ag/AgCl combination Electrode Model 13-620-289, Fisher Scientific, Fair Lawn, N.J., U.S.A) calibrated with phosphate buffers 4.0 and 7.0, according to the method of Sebranek and others (2001). For each brand, measurements were made in duplicate.

**TBARS Analysis**

Lipid oxidation was measured by the modified 2-thiobarbituric acid reactive substances (TBARS) test as described for cured meats (Zipser and Watts 1962). TBARS values were reported as mg of malonaldehyde equivalents/kg of meat sample. Products were measured concurrently with trained sensory panel evaluation. For each brand, measurements were made in duplicate.

**Residual Nitrite Analysis**

Residual nitrite was determined by the AOAC method (AOAC 1990). The same finely ground/chopped samples that were used for pigment analysis were also used for residual nitrite measurement. All residual nitrite assays were done in duplicate and all brands within a block were analyzed at the same time to minimize variation in the analysis due to time.

**Residual Nitrate Analysis**

Sample preparation and nitrate determination methods were modifications of Ahn and Maurer (1987). Five g of meat product samples were weighed in a 50-ml test tube and homogenized with 20 ml of distilled, de-ionized water (DDW) using a Polytron homogenizer (Type PT 10/35, Brinkmann Instruments Inc., Westbury, N.Y., USA) for 10 s at high speed. The homogenate was heated for 1 h in a 80 °C water
bath. After cooling in cold water for 10 min, 2.5 ml of the homogenate was transferred to a disposable test tube (16 x 100 mm). Carrez II (dissolve 10.6 g potassium ferrocyanide in 100 ml DDW) and Carrez I (dissolve 23.8 g zinc acetate in 50 ml DDW, then add 3 ml glacial acetic acid and dilute to 100 ml with DDW) reagents were added (0.1 ml each) to precipitate proteins. The solution was diluted with 2.3 ml of DDW and mixed well. After precipitation, the supernatant was centrifuged at 10,000 x g for 20 min and the clear upper layer was used for nitrate measurement by high performance liquid chromatography (Agilent 1100 Series HPLC system, Agilent Technologies, Wilmington, Del., U.S.A.). The column used was Agilent Zorbax SAX (analytical 4.6 x 150 mm, 5-micron) (Agilent, Wilmington, Del., U.S.A.) and the elution buffer was 15 mM phosphate buffer, pH 2.35, with isocratic elution. The flow rate was 1.0 ml/min and the sample volume was 25 µL. The wavelength used was 210 nm. The area of the nitrate peak was used to calculate nitrate concentration (ppm) using a nitrate standard curve.

Consumer Sensory Panel

Frankfurter, ham and bacon samples were presented to consumer sensory panelists at Iowa State University (Ames, IA., U.S.A) on two consecutive days, collecting data from 55-57 panelists each day. Six one-hour sessions containing 8-10 panelists were held each day. Expectorant cups were provided to prevent taste fatigue and distilled, de-ionized water was provided to clean the palate between samples. The presentation order was randomized for each session (group of panelists). A computer ballot was constructed and data was collected using a computerized sensory scoring system (COMPUSENSE five, Compusense, Inc.
A 9-point hedonic scale was used where 1=dislike extremely and 9=like extremely. Demographic questions were asked about purchasing decisions.

Six frankfurters per brand (N=5) were added to boiling water that was removed from the heat source. The covered pans containing the frankfurters immediately removed from the heat source and allowed to rest for 7 min. Frankfurters (n=6) were cut into five, 1.9 cm pieces with 1.27 cm pieces from each end being discarded. Panelists were presented three, 1.9 cm randomly selected heated pieces in covered containers and were asked to determine desirability of aroma, internal color, flavor, texture and overall acceptance of the frankfurter samples.

Refrigerated (3.3-5.6 ºC), sliced ham samples were evaluated by the panelists without reheating, the samples which would be characteristic for this product. Panelists were presented three, 4 x 4 cm pieces of each brand in a covered Styrofoam container. Panelists were asked to determine desirability of aroma, surface color, flavor, texture and overall acceptance of the ham samples.

Bacon slices were layered with paper towels in pans and pre-cooked at the ISU Meat Laboratory using a convection oven (ALTO-SHAMM Combitherm Model 10.10ML/IN, ALTO-SHAAM, INC., Menomonee Falls, Wis., U.S.A.) for 20 min at 112.8 ºC then cooled prior to transporting to sensory laboratory. Pre-cooking was done on the day of the sensory evaluation to minimize sample preparation time at the sensory laboratory. During the sensory evaluation, panelists were presented vacuum packaged uncooked slices of bacon to evaluate the color of lean in the
bacon slice. Uncooked vacuum packaged bacon slices were held in refrigerated temperatures (3.3-5.6 °C) before and after sample evaluation. Bacon slices were cooked in a microwave on setting “high” for 1.5-5 min, rotated 180°, and cooked for an additional 1.5-5 min until the fat in the slices was yellowish brown. One entire strip of each bacon brand was served uncovered on a white plate to panelists. Panelists were asked to determine desirability of color of the lean in the uncooked bacon slice and aroma, flavor, texture and overall acceptance of the cooked bacon samples.

**Results and Discussion**

**Ham**

Commercial uncured, no-nitrate/nitrite-added hams (Brands A-D) and a nitrite-added control (Brand E) were obtained from retail supermarkets, transported to the Iowa State University Meat Laboratory (Ames, IA., U.S.A.) and evaluated for various attributes (Appendix 1). Brand E was selected based on being considered a high-quality standard by consumers. Brands A-D had cured color, aroma and flavor attributes similar to Brand E (control).

**Color Measurements**

The internal surface of ham slices was evaluated for CIE L*, a*, b* and cured meat color by reflectance ratio (Table 1). Brands A, B and D were lighter in color than Brand E according to L* values and Brand B was significantly (P<0.05) lighter in color than Brand E. No differences were found between Brands A, C or E for a* values, however, Brand B and D were significantly (P<0.05) less red than Brand E.
TABLE 1: Least squares means for objective color ($L^*$, $a^*$, $b^*$) values; reflectance ratio ($R_{ratio}$) values; and pigment analysis (total pigment and cured pigment, nitrosylhemochrome) for uncured, no-nitrate/nitrite-added (Brand A-D) and nitrite-added (Brand E) commercial hams.

<table>
<thead>
<tr>
<th>Product</th>
<th>OBJECTIVE COLOR$^b$</th>
<th>CURED COLOR$^c$</th>
<th>SPECTROPHOTOMETRIC MEASUREMENTS$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$L^*$</td>
<td>$a^*$</td>
<td>$b^*$</td>
</tr>
<tr>
<td>Brand A</td>
<td>66.26$^j$</td>
<td>18.86$^{kl}$</td>
<td>12.77$^g$</td>
</tr>
<tr>
<td>Brand B</td>
<td>69.44$^{gl}$</td>
<td>16.64$^{jm}$</td>
<td>10.99$^h$</td>
</tr>
<tr>
<td>Brand C</td>
<td>59.54$^h$</td>
<td>22.01$^g$</td>
<td>13.05$^g$</td>
</tr>
<tr>
<td>Brand D</td>
<td>65.41$^{gl}$</td>
<td>17.57$^{km}$</td>
<td>12.86$^g$</td>
</tr>
<tr>
<td>Brand E</td>
<td>62.72$^{lj}$</td>
<td>20.52$^{gl}$</td>
<td>12.23$^g$</td>
</tr>
<tr>
<td>SEM$^e$</td>
<td>1.09</td>
<td>0.56</td>
<td>0.22</td>
</tr>
</tbody>
</table>

$^a$ Product: Brand A-D = different brands of commercial uncured, no-nitrate/nitrite-added ham products and Brand E = commercial nitrite added ham product (control).

$^b$ Commission International D’Edairerage (CIE) $L^*$,$a^*$,$b^*$ were $L^*$ = lightness, $a^*$ = redness, and $b^*$ = yellowness on a 0-100 white scale.

$^c$ Cured meat color measurement by reflectance ratio of wavelengths 650/570 nm where 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.

$^d$ Total pigment and cured pigment (nitrosylhemochrome) analysis.

$^e$ SEM = Standard error of the means for uncured, no-nitrate/nitrite-added and nitrite-added commercial ham products.

$^{g-m}$ Means within same column with different superscripts are different (P<0.05).
For b* values, Brand B was significantly (P<0.05) less yellow than all other brands. Measuring cured color fading by reflectance ratios (Hunt and others 1991; Erdman and Watts 1957), Brand E had a significantly (P<0.05) higher reflectance ratio than Brand D and Brand B. Although not significantly different, Brand C revealed a higher reflectance ratio than Brand E indicating that this uncured, no-nitrate/nitrite-added ham had better cured color than the nitrite-added control (Brand E). All brands except Brand B would be considered to have excellent cured color according to the reflectance ratio ratings.

**Total and Cured Pigment Analysis**

Least squares means for total pigment and cured pigment concentrations are reported in Table 1. Total pigments ranged between 58.14 and 92.46 ppm. This large variation could be attributed to differences in raw materials (among species or within species) used during product manufacture as well as differences in formulation proportions of raw materials. Cured pigment concentrations (range of 36.80 to 62.64 ppm) must then be proportionally compared to the total pigment concentrations for each Brand for reasonable comparison between Brands. Significant (P<0.05) replication x brand interactions were found for both total and cured pigment measurements (Table 2). Brand C had a higher (P<0.05) cured pigment concentration than all other Brands and also had the highest total pigment concentration. However, when comparing cured and total pigment concentrations for Brands C and E, trends show that Brand C actually had a higher percentage of cured pigment than Brand E when compared to the respective total pigment concentrations.
TABLE 2: Least squares means for replication x brand interaction (P<0.05) of attributes for uncured, no-nitrate/nitrite-added (Brand A-D) and nitrite-added (Brand E) commercial hams.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Brand A rep 1</th>
<th>Brand A rep 2</th>
<th>Brand B rep 1</th>
<th>Brand B rep 2</th>
<th>Brand C rep 1</th>
<th>Brand C rep 2</th>
<th>Brand D rep 1</th>
<th>Brand D rep 2</th>
<th>Brand E rep 1</th>
<th>Brand E rep 2</th>
<th>SEM^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.07</td>
<td>5.98</td>
<td>6.22*</td>
<td>6.04*</td>
<td>6.15</td>
<td>6.27</td>
<td>6.48</td>
<td>6.37</td>
<td>6.55*</td>
<td>6.38*</td>
<td>0.03</td>
</tr>
<tr>
<td>TBARS</td>
<td>0.17*</td>
<td>0.13*</td>
<td>0.17*</td>
<td>0.12*</td>
<td>0.20</td>
<td>0.20</td>
<td>0.37*</td>
<td>0.27*</td>
<td>0.14</td>
<td>0.14</td>
<td>0.01</td>
</tr>
<tr>
<td>Total Pigment</td>
<td>67.22</td>
<td>69.02</td>
<td>56.17</td>
<td>60.11</td>
<td>106.11*</td>
<td>78.81*</td>
<td>79.53*</td>
<td>66.61*</td>
<td>81.19*</td>
<td>94.55*</td>
<td>1.84</td>
</tr>
<tr>
<td>Cured Pigment</td>
<td>42.65</td>
<td>43.62</td>
<td>34.66*</td>
<td>49.75*</td>
<td>71.50*</td>
<td>53.78*</td>
<td>31.70*</td>
<td>41.91*</td>
<td>34.08*</td>
<td>44.47*</td>
<td>0.87</td>
</tr>
<tr>
<td>Residual Nitrite</td>
<td>5.02*</td>
<td>6.81*</td>
<td>3.78*</td>
<td>6.05*</td>
<td>6.84*</td>
<td>9.47*</td>
<td>7.41*</td>
<td>11.05*</td>
<td>23.69*</td>
<td>35.65*</td>
<td>0.21</td>
</tr>
<tr>
<td>Surface Color</td>
<td>6.65</td>
<td>5.93</td>
<td>5.68</td>
<td>4.89</td>
<td>6.91</td>
<td>7.00</td>
<td>5.14</td>
<td>5.18</td>
<td>6.61</td>
<td>6.74</td>
<td>0.22</td>
</tr>
<tr>
<td>Aroma</td>
<td>6.42</td>
<td>5.75</td>
<td>5.33</td>
<td>4.39</td>
<td>6.74</td>
<td>7.28</td>
<td>3.75</td>
<td>3.61</td>
<td>6.77</td>
<td>6.46</td>
<td>0.25</td>
</tr>
</tbody>
</table>

^a Attribute with significant (P<0.05) replication x brand model interaction.

^b SEM = Standard error of the means for uncured, no-nitrate/nitrite-added and nitrite-added (control) commercial ham products.

* Replication (rep) means between rep 1 and rep 2 within row for each brand are different (P<0.05).
pH Determination and TBARS Analysis

Measurements for pH and lipid oxidation (measured by TBARS) are reported in Table 3. There were significant (P<0.05) pH differences between all brands except for Brands D and E. Brands D and E also show higher (P<0.05) pH values than all other brands. However, a replication x brand interaction was identified and is reported in Table 2. Measurements of lipid oxidation revealed that Brand E had a significantly (P<0.05) lower TBARS value than Brands C and D. Although not significant, Brand E also revealed a lower TBARS value than Brands A and B. A replication x brand interaction was also present (Table 2) indicating variance in the samples between replications, however, all values were low indicating that very little lipid oxidation had occurred in the tested products.

Residual Nitrate and Nitrite Analysis

No differences (P>0.05) were found between Brands A, B and D for residual nitrate, however, Brands C and D had significantly (P<0.05) less residual nitrate than Brands A, B and D (Table 3). Brand E had a significantly (P<0.05) higher amount of residual nitrite than all other Brands while Brand B had significantly (P<0.05) less than all other Brands (Table 3). A significant (P<0.05) replication x brand interaction was present for residual nitrite and those least squares means are reported in Table 2. The results of this analysis indicated that both nitrite and nitrate were present in the uncured, no-nitrate/nitrite-added hams tested in this experiment.

Consumer Sensory Panel

Consumer panelists were administered demographic questions to better understand consumer purchasing decisions. The age of participants was as follows:
TABLE 3: Least squares means for pH, lipid oxidation (TBARS), residual nitrites and residual nitrates for uncured, no-nitrate/nitrite-added (Brand A-D) and nitrite-added (Brand E) commercial hams.

<table>
<thead>
<tr>
<th>Product&lt;sup&gt;a&lt;/sup&gt;</th>
<th>TBARS&lt;sup&gt;b&lt;/sup&gt; mg/kg</th>
<th>pH&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Residual Nitrite&lt;sup&gt;d&lt;/sup&gt; ppm</th>
<th>Residual Nitrate&lt;sup&gt;e&lt;/sup&gt; ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand A</td>
<td>0.1488&lt;sup&gt;jk&lt;/sup&gt;</td>
<td>6.03&lt;sup&gt;j&lt;/sup&gt;</td>
<td>5.92&lt;sup&gt;i&lt;/sup&gt;</td>
<td>17.60&lt;sup&gt;jk&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brand B</td>
<td>0.1414&lt;sup&gt;jl&lt;/sup&gt;</td>
<td>6.13&lt;sup&gt;i&lt;/sup&gt;</td>
<td>4.91&lt;sup&gt;k&lt;/sup&gt;</td>
<td>19.91&lt;sup&gt;gij&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brand C</td>
<td>0.1973&lt;sup&gt;i&lt;/sup&gt;</td>
<td>6.21&lt;sup&gt;h&lt;/sup&gt;</td>
<td>8.15&lt;sup&gt;i&lt;/sup&gt;</td>
<td>11.37&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brand D</td>
<td>0.3198&lt;sup&gt;g&lt;/sup&gt;</td>
<td>6.43&lt;sup&gt;g&lt;/sup&gt;</td>
<td>9.23&lt;sup&gt;i&lt;/sup&gt;</td>
<td>10.00&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brand E</td>
<td>0.1375&lt;sup&gt;hkl&lt;/sup&gt;</td>
<td>6.47&lt;sup&gt;g&lt;/sup&gt;</td>
<td>29.67&lt;sup&gt;g&lt;/sup&gt;</td>
<td>20.04&lt;sup&gt;ik&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.0045</td>
<td>0.03</td>
<td>0.17</td>
<td>0.55</td>
</tr>
</tbody>
</table>

<sup>a</sup> Product: Brand A-D = different brands of commercial uncured, no-nitrate/nitrite-added ham products and Brand E = commercial nitrite-added ham product (control).

<sup>b</sup> 2-Thiobarbituric acid test reported as mg malonaldehyde/kg of sample.

<sup>c</sup> pH of commercial ham products.

<sup>d</sup> Residual nitrite determination reported in ppm of sample.

<sup>e</sup> Residual nitrate determination reported in ppm of sample.

<sup>f</sup> SEM = Standard error of the means for uncured, no-nitrate/nitrite-added and nitrite-added commercial ham products.

<sup>g</sup>-<sup>l</sup> Means within same column with different superscripts are different (P<0.05).
18-24 (13%), 25-34 (20%), 35-44 (15%), 45-54 (30%), 55-64 (18%) and >64 (4%). Panelists were asked how often they consumed ham and the responses were as follows: at least once per week (25%), two times per month (45%), once per month (18%) and less than once a month (12%). Panelists were also asked what the most important consideration was for ham purchasing decisions and the responses were: price (46%), brand name (30%), nutritional value (11%) and ingredients (13%).

Consumer sensory panel acceptability scores for surface color, aroma, flavor, texture and overall acceptance are found in Table 4. Brand E was not found different (P>0.05) than Brands A or C for surface color but Brand E showed a higher (P<0.05) score than Brands B and D. For aroma, flavor and texture, Brand E had a significantly (P<0.05) higher sensory scores than Brands B and D but was not found different (P>0.05) than Brands A or C. Least squares means for overall acceptance show Brands C and E were not significantly (P>0.05) different, however, both revealed higher (P<0.05) scores than Brands A, B and D. A significant (P<0.05) replication x brand interaction was present for both surface color and aroma (Table 2). These results indicate sensory differences do exist between uncured, no-nitrate/nitrite-added and nitrite-added hams.

After ham evaluations, consumers were asked if they would purchase (yes or no) each brand and those results are as follows: Brand A (63% yes), Brand B (42% yes), Brand C (79% yes), Brand D (28% yes) and Brand E (74% yes). These results indicate that a large variation in consumer acceptance for uncured, no-nitrate/nitrite-added commercially available products does exist. Interestingly Brand C (uncured) received a higher yes/no rating than Brand E (cured).
TABLE 4: Least squares means for sensory attributes of surface color, aroma, flavor, texture and overall acceptance for uncured, no-nitrate/nitrite-added (Brand A-D) and nitrite-added (Brand E) commercial hams.

<table>
<thead>
<tr>
<th>Product</th>
<th>Surface Color</th>
<th>Aroma</th>
<th>Flavor</th>
<th>Texture</th>
<th>Overall Acceptance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand A</td>
<td>6.29&lt;sup&gt;f&lt;/sup&gt;</td>
<td>6.09&lt;sup&gt;f&lt;/sup&gt;</td>
<td>6.15&lt;sup&gt;f&lt;/sup&gt;</td>
<td>6.58&lt;sup&gt;f&lt;/sup&gt;</td>
<td>6.09&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brand B</td>
<td>5.29&lt;sup&gt;g&lt;/sup&gt;</td>
<td>4.86&lt;sup&gt;g&lt;/sup&gt;</td>
<td>5.13&lt;sup&gt;g&lt;/sup&gt;</td>
<td>5.77&lt;sup&gt;g&lt;/sup&gt;</td>
<td>5.22&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brand C</td>
<td>6.96&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.84&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.89&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brand D</td>
<td>5.16&lt;sup&gt;eg&lt;/sup&gt;</td>
<td>3.68&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.22&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.99&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.45&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brand E</td>
<td>6.68&lt;sup=df&lt;/sup&gt;</td>
<td>6.61&lt;sup&gt;df&lt;/sup&gt;</td>
<td>6.72&lt;sup&gt;df&lt;/sup&gt;</td>
<td>6.78&lt;sup&gt;df&lt;/sup&gt;</td>
<td>6.77&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.16</td>
<td>0.18</td>
<td>0.19</td>
<td>0.17</td>
<td>0.17</td>
</tr>
</tbody>
</table>

<sup>a</sup> Product: Brand A-D = different brands of commercial uncured, no-nitrate/nitrite-added ham products and Brand E = commercial nitrite-added ham product (control).

<sup>b</sup> SENSORY ATTRIBUTES = Consumer panel scores using a 9 point hedonic scale where 1 = dislike extremely, 9 = like extremely.

<sup>c</sup> SEM = Standard error of the means for uncured, no-nitrate/nitrite-added and nitrite-added commercial ham products.

<sup>d-g</sup> Means within same column with different superscripts are different (P<0.05).
**Frankfurters**

Commercial uncured, no-nitrate/nitrite-added frankfurters (Brands A-D) and a nitrite-added cured control (Brand E) were obtained from retail supermarkets, transported to the Iowa State University Meat Laboratory (Ames, IA., U.S.A.) and evaluated for various attributes (Appendix 2). Brand E was selected based on being considered a high-quality standard by consumers. Brands A-D had cured color, aroma and flavor attributes similar to Brand E (control). Products selected were formulated with 100% beef to standardize comparisons. Replication x brand significant (P<0.05) interactions were observed for all experimental attributes with the exception of consumer sensory aroma (Table 5). This indicated that variation between replication (day products acquired) existed for the attributes tested. With all significant differences found for the fixed main effect of brand, the effect of replication must also be considered when interpreting the results.

**Color Measurements**

The internal surface of frankfurters was evaluated for CIE L*, a*, b* and cured meat color by reflectance ratio (Table 6). Brand E had a significantly (P<0.05) lighter internal surface color (L*) than all other Brands. No differences for a* values existed between Brands D and E, however, both revealed a higher (P<0.05) a* value (redder) than Brands A and B and a lower (P<0.05) a* value than Brand C. Brands B and E also showed a significantly (P<0.05) higher b* than Brands A, C and D but no differences were identified between Brands B and E. For cured color fading measured by reflectance ratios (Hunt and others 1991; Erdman and Watts 1957), Brand E had a lower (P<0.05) reflectance ratio than Brands C and D yet a higher
TABLE 5: Least squares means for replication x brand interaction (P<0.05) of attributes for uncured, no-nitrate/nitrite-added (Brand A-D) and nitrite-added (Brand E) commercial frankfurters.

| Attribute                             | Brand A       | Brand B       | Brand C       | Brand D       | Brand E       | SEM
|---------------------------------------|---------------|---------------|---------------|---------------|---------------|-----
| pH                                    | 5.41*         | 5.98*         | 6.08*         | 6.09          | 6.05          | 6.00* 5.88* 0.03 |
| TBARS                                 | 1.35*         | 0.69*         | 0.38          | 0.38          | 0.37*         | 0.45 0.41 0.39* 0.78* 0.01 |
| Total Pigment                         | 202.91        | 194.58        | 188.77        | 218.79        | 210.46        | 235.35 212.47 162.08 106.15 9.68 |
| Cured Pigment                         | 94.31         | 95.50         | 29.50         | 35.28         | 130.45        | 112.25* 134.80* 100.05* 89.14* 1.78 |
| Residual Nitrite                      | 3.94*         | 8.61*         | 1.46          | 1.41          | 8.16          | 7.52* 8.62* 2.17 2.21 0.20 |
| Residual Nitrate                      | 9.86          | 16.35         | 7.23          | 6.35          | 43.01         | 42.49 46.35 24.52* 38.81* 1.76 |
| L* color value                        | 59.48         | 59.30         | 58.74*        | 54.98*        | 57.26         | 59.00 59.29 72.52 73.65 0.52 |
| a* color value                        | 15.15*        | 17.48*        | 14.57         | 14.11         | 20.59         | 20.41* 18.07* 19.02 18.41 0.30 |
| b* color value                        | 15.14*        | 17.25*        | 19.82         | 18.96         | 16.40         | 16.53 17.77 19.44 19.72 0.29 |
| Reflectance Ratio                     | 1.86*         | 2.08*         | 1.67          | 1.65          | 2.74          | 2.68* 2.33* 2.04 1.98 0.04 |
| Surface Color                         | 4.98*         | 6.09*         | 3.49          | 3.42          | 5.81          | 5.96 6.58 5.89 5.55 0.23 |
| Flavor                                | 3.26*         | 5.18*         | 4.43          | 3.98          | 4.75          | 3.75 5.00 6.34 5.60 0.30 |
| Texture                               | 4.36*         | 5.64*         | 4.45          | 4.27          | 4.66          | 4.81* 6.44* 5.74 4.87 0.27 |
| Overall Acceptance                    | 3.53*         | 5.49*         | 4.23          | 3.73          | 4.75          | 4.23* 5.51* 6.04 5.27 0.27 |

*a Attribute with significant (P<0.05) replication x brand model interaction.

b SEM = Standard error of the means for uncured, no-nitrate/nitrite-added and nitrite-added commercial frankfurter products.

* Replication (rep) means between rep 1 rep 2 within row for each brand are different (P<0.05).
TABLE 6: Least squares means for objective color (L*, a*, b*) values; reflectance ratio (Rratio) values; and pigment analysis (total pigment and cured pigment, nitrosylhemochrome) for uncured, non-nitrate/nitrite-added (Brand A-D) and nitrite-added (Brand E) commercial frankfurters.

<table>
<thead>
<tr>
<th>Product^a</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Rratio</th>
<th>Total Pigment</th>
<th>Cured Pigment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand A</td>
<td>59.39^i</td>
<td>16.31^i</td>
<td>16.19^h</td>
<td>1.97^i</td>
<td>198.75^g</td>
<td>94.90^i</td>
</tr>
<tr>
<td>Brand B</td>
<td>56.86^h</td>
<td>14.34^j</td>
<td>19.39^g</td>
<td>1.66^k</td>
<td>203.78^g</td>
<td>32.39^k</td>
</tr>
<tr>
<td>Brand C</td>
<td>58.40^i</td>
<td>20.74^g</td>
<td>17.08^h</td>
<td>2.72^g</td>
<td>203.10^g</td>
<td>133.34^g</td>
</tr>
<tr>
<td>Brand D</td>
<td>59.14^i</td>
<td>19.24^h</td>
<td>16.28^hk</td>
<td>2.50^h</td>
<td>223.91^g</td>
<td>123.52^h</td>
</tr>
<tr>
<td>Brand E</td>
<td>73.09^j</td>
<td>18.72^h</td>
<td>19.58^l</td>
<td>2.01^i</td>
<td>134.12^h</td>
<td>94.59^l</td>
</tr>
<tr>
<td>SEM^e</td>
<td>0.38</td>
<td>0.21</td>
<td>0.21</td>
<td>0.03</td>
<td>6.84</td>
<td>1.36</td>
</tr>
</tbody>
</table>

^a Product: Brand A-D = different brands of commercial uncured, no-nitrate/nitrite-added frankfurter products and Brand E = commercial nitrite-added frankfurter product (control).

^b Commission International D’Edairerage (CIE) L*a*b* were L* = lightness, a* = redness, and b* = yellowness on a 0-100 white scale.

^c Cured meat color measurement by reflectance ratio of wavelengths 650/570 nm where 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.

^d Total pigment and cured pigment (nitrosylhemochrome) analysis.

^e SEM = Standard error of the means for uncured, no-nitrate/nitrite-added and nitrite-added commercial frankfurter products.

^f-k Means within same column with different superscripts are different (P<0.05).
(P<0.05) ratio than Brand B. According to reflectance ratio ratings, Brands C and D had excellent cured color while Brands A and B had a less intense, but noticeable cured color. A significant (P<0.05) replication x brand interaction was present for all color measurements. These results indicate redness (a*) values may positively correspond to reflectance ratios while an increase in lightness (L*) values may negatively affect cured pigment measurements determined by reflectance ratio.

**Total and Cured Pigment Analysis**

Least squares means for total and cured pigment concentrations are reported in Table 6. Brand E revealed the lowest (P<0.05) concentration of total pigment compared to all other brands. Interestingly, no significant differences were observed between Brands A-D for total pigment concentration. These results indicated that differences in total pigments did exist and were only associated with the control. Since all Brands were all beef frankfurters, a possible explanation of the differences could be related to the formulation of the frankfurters. Brand E may have been higher in fat or had more added formulation added water or non-meat ingredients which would affect the amount of pigment in the finished product.

For cured pigments, no differences (P>0.05) were found between Brands A or E, however, both were significantly higher (P<0.05) than Brand B and significantly (P<0.05) lower than Brands C and D. These results for cured pigment concentrations indicate that differences did exist between brands. Interestingly, Brand B had a clearly lower cured pigment concentration than all other brands. The ingredient statement for Brand B (not shown) does not indicate any ingredients with curing ability, however, paprika was added possibly serving as a colorant. Brand B
showed properties of cured color \((a^* = 14.34)\) but also revealed a low cured pigment concentration supporting this theory.

**pH Determination and TBARS Analysis**

Measurements for pH and lipid oxidation (measured by TBARS) are reported in Table 7. No significant \((P>0.05)\) pH differences were identified between Brands C or D but both Brands had a significantly \((P<0.05)\) higher pH than the other brands. For lipid oxidation, Brand A had the highest \((P<0.05)\) reported TBARS value compared to all other brands. Brand E had a higher \((P<0.05)\) TBARS value than Brands B, C and D but a lower \((P<0.05)\) value than Brand A. Since a TBARS value of 0.5 to 1.0 is considered to be the threshold for oxidized odor and 1.0 to 2.0 for oxidized flavor \((\text{Tarladgis and others 1960})\). Brands A and E were approaching those levels. Sensory results might be expected to reflect those lipid oxidation effects. Brand E (nitrite-added control) was not expected to have a higher TBARS value than other Brands (uncured, no-nitrate/nitrite-added) indicating that Brand E may have either had a higher percentage formulated fat or may have been further in its shelf life compared to Brands A-D.

**Residual Nitrate and Nitrite Analysis**

Brand E displayed a significantly \((P<0.05)\) lower level of residual nitrite than Brands C and D while also having a higher \((P<0.05)\) level than Brand B (Table 7). Jantawat and others \((1993)\) found a decreasing residual nitrite level with increased storage time relationship and Hustad and others \((1973)\) reported that nitrite concentration is affected by both storage time and storage temperature. The results suggested Brand E may have been far into the shelf life for that product.
### TABLE 7: Least squares means for pH, lipid oxidation (TBARS), residual nitrites and residual nitrates for uncured, no-nitrate/nitrite-added (Brand A-D) and nitrite-added (Brand E) commercial frankfurters.

<table>
<thead>
<tr>
<th>Product</th>
<th>TBARS mg/kg</th>
<th>pH ppm</th>
<th>Residual Nitrite ppm</th>
<th>Residual Nitrate ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand A</td>
<td>1.0187g</td>
<td>5.70j</td>
<td>6.27i</td>
<td>13.10j</td>
</tr>
<tr>
<td>Brand B</td>
<td>0.3834hm</td>
<td>5.85i</td>
<td>1.43l</td>
<td>6.79i</td>
</tr>
<tr>
<td>Brand C</td>
<td>0.4068lm</td>
<td>6.08g</td>
<td>7.71g</td>
<td>41.26i</td>
</tr>
<tr>
<td>Brand D</td>
<td>0.4333jl</td>
<td>6.04g</td>
<td>8.07g</td>
<td>44.42gi</td>
</tr>
<tr>
<td>Brand E</td>
<td>0.5848k</td>
<td>5.94h</td>
<td>2.19h</td>
<td>31.66h</td>
</tr>
<tr>
<td>SEM</td>
<td>0.0060</td>
<td>0.02</td>
<td>0.14</td>
<td>1.30</td>
</tr>
</tbody>
</table>

| a Product: Brand A-D = different brands of commercial uncured, no-nitrate/nitrite-added frankfurter products and Brand E = commercial nitrite-added frankfurter product (control).
| b 2-Thiobarbituric acid test reported as mg malonaldehyde/kg of sample.
| c pH of commercial frankfurter products.
| d Residual nitrite determination reported in ppm of sample.
| e Residual nitrate determination reported in ppm of sample.
| f SEM = Standard error of the means for uncured, no-nitrate/nitrite-added and nitrite-added commercial frankfurter products.
| g-m Means within same column with different superscripts are different (P<0.05). |
when it was acquired. Since temperature has also been found to affect residual nitrite levels, temperature during storage at the retail supermarket meat cases is a variable we must consider when interpreting these results. The higher the storage temperature, the more rapidly residual nitrite is depleted (Hustad and others 1973). The results found indicated that nitrite was likely added during the manufacture of these products, however the amounts and the source are not clearly known.

The results for residual nitrate show that Brand E had significantly (P<0.05) lower level than Brands C and D while also having a higher (P<0.05) level than Brands A and B (Table 7). The ingredient statement for Brand E does not indicate the addition of nitrate but did indicate the addition of sodium nitrite. Several researchers have reported the presence of nitrate in products of which only nitrite was added. Cassens and others (1979) suggest that a portion of nitrite added to meat during the curing process is actually converted to nitrate which may explain this phenomenon. Similar findings with Brands A-D could also be explained by this statement. Since production dates of the products are not known, it is difficult to compare Brands for residual nitrite and nitrate at the measured time. It is clear, however, that residual nitrate and nitrite are present in those products labeled as uncured.

*Consumer Sensory Panel*

Consumer panelists were administered demographic questions to better understand consumer purchasing decisions. The age of participants was as follows: 18-24 (8%), 25-34 (27%), 35-44 (22%), 45-54 (27%), 55-64 (14%) and >64 (2%). Panelists were asked how often they consumed frankfurters and the responses were
as follows: at least once per week (12%), two times per month (33%), once per month (29%) and less than once a month (26%). Panelists were also asked what the most important consideration was for frankfurter purchasing decisions and the responses were: price (26%), brand name (28%), nutritional value (20%) and ingredients (26%). Consumer sensory panel acceptability scores for surface color, aroma, flavor, texture and overall acceptance are found in Table 8. For surface color acceptability scores no differences (P>0.05) were found between Brands C, D and E while all brands rated significantly (P<0.05) higher than Brand B. Brand E revealed a higher (P<0.05) aroma score than Brand B and, although not significant, had higher scores than all other Brands. For flavor, Brand E had a higher (P<0.05) score than all other brands. All Brands were similar in texture according to sensory scores except for Brand B have a significantly (P<0.05) lower score than Brands C, D and E. Finally, no differences for overall acceptance were found between Brands C and E; however, Brand E received a significantly (P<0.05) higher score than Brands A, B and D. These results indicate that although Brand E did not receive the highest scores for all consumer attributes, those same attributes allowed it to receive the highest overall acceptance score. Those scores are supported by a post sensory question measuring the likelihood of purchase of each of the Brands based on the sensory evaluation. The same panelists were asked if they would purchase (yes or no) each brand and those results are as follows: Brand A (33% yes), Brand B (19% yes), Brand C (41% yes), Brand D (35% yes) and Brand E (50% yes). These results indicate that a large variation in consumer acceptance of uncured, non-nitrate/nitrite-added commercially available products exists. Interestingly Brand E
<table>
<thead>
<tr>
<th>Product</th>
<th>Surface Color</th>
<th>Aroma</th>
<th>Flavor</th>
<th>Texture</th>
<th>Overall Acceptance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand A</td>
<td>5.54&lt;sup&gt;egh&lt;/sup&gt;</td>
<td>5.18&lt;sup&gt;ed&lt;/sup&gt;</td>
<td>4.22&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.00&lt;sup&gt;de&lt;/sup&gt;</td>
<td>4.51&lt;sup&gt;lg&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brand B</td>
<td>3.45&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4.47&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.21&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4.36&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.98&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brand C</td>
<td>6.07&lt;sup&gt;dg&lt;/sup&gt;</td>
<td>4.98&lt;sup&gt;ed&lt;/sup&gt;</td>
<td>5.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.15&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.00&lt;sup&gt;deg&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brand D</td>
<td>6.27&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.70&lt;sup&gt;ed&lt;/sup&gt;</td>
<td>4.38&lt;sup&gt;df&lt;/sup&gt;</td>
<td>5.62&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.87&lt;sup&gt;dg&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brand E</td>
<td>5.72&lt;sup&gt;dh&lt;/sup&gt;</td>
<td>5.73&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.97&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.30&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.66&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>0.16</td>
<td>0.19</td>
<td>0.21</td>
<td>0.20</td>
<td>0.19</td>
</tr>
</tbody>
</table>

<sup>a</sup> Product: Brand A-D = different brands of commercial uncured, no-nitrate/nitrite-added frankfurter products and Brand E = commercial nitrite-added frankfurter product (control).

<sup>b</sup> SENSORY ATTRIBUTES = Consumer panel scores using a 9 point hedonic scale where 1 = dislike extremely, 9 = like extremely.

<sup>c</sup> SEM = Standard error of the means for uncured, no-nitrate/nitrite-added and nitrite-added commercial frankfurter products.

<sup>d-h</sup> Means within same column with different superscripts are different (P<0.05).
(nitrite-added control) received the highest yes/no rating suggesting that Brand E was considered a higher-quality product compared to the other brands tested.

**Bacon**

Commercial uncured, no-nitrate/nitrite-added bacon (Brands A-D) and a nitrite-added control (Brand E) were obtained from retail supermarkets, transported to the Iowa State University Meat Laboratory (Ames, IA., U.S.A.) and evaluated for various attributes (Appendix 3). Brand E was selected based on being considered a high-quality standard by consumers. Brands A, C and D had cured color, aroma and flavor attributes similar to Brand E (control). Brand B had cured color, aroma and flavor attributes similar to an uncured product with no intentions of replacing sodium nitrite. Replication x brand significant (P<0.05) interactions were observed several experimental attributes. This indicates that variation between replication (day products acquired) existed for the many of the attributes tested. For all significant differences found for the fixed main effect of brand when an interaction is present, the effect of replication must also be considered when interpreting the results.

**Color Measurements**

Color measurements was evaluated by CIE L*, a*, b* and cured meat color by reflectance ratio (Table 9). Color measurements were taken for both the entire sliced surface (lean and fat) and also the lean only portion of the sliced surface. For the entire slice, no differences were found for either L* or a* color. A significant (P<0.05) replication x brand interaction, however, was present for a* color (Table 10). Objective b* values show that Brand B was significantly (P<0.05) different than all other brands. No differences were found for cured color fading as measured by
TABLE 9: Least squares means for objective color (L*, a*, b*) values and reflectance ratio (Rratio) values for uncured, no-nitrate/nitrite-added (Brand A-D) and nitrite-added (Brand E) commercial bacon.

<table>
<thead>
<tr>
<th>Product</th>
<th>OBJECTIVE COLOR</th>
<th>CURED COLOR</th>
<th>OBJECTIVE COLOR</th>
<th>CURED COLOR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L*</td>
<td>a*</td>
<td>b*</td>
<td>Rratio</td>
</tr>
<tr>
<td>Brand A</td>
<td>74.37</td>
<td>13.90</td>
<td>14.14</td>
<td>1.81</td>
</tr>
<tr>
<td>Brand B</td>
<td>73.94</td>
<td>11.09</td>
<td>16.21</td>
<td>1.55</td>
</tr>
<tr>
<td>Brand C</td>
<td>71.20</td>
<td>12.69</td>
<td>13.81</td>
<td>1.72</td>
</tr>
<tr>
<td>Brand D</td>
<td>72.58</td>
<td>13.68</td>
<td>13.38</td>
<td>1.74</td>
</tr>
<tr>
<td>Brand E</td>
<td>73.42</td>
<td>13.61</td>
<td>14.43</td>
<td>1.89</td>
</tr>
<tr>
<td>SEM</td>
<td>1.46</td>
<td>0.78</td>
<td>0.41</td>
<td>0.13</td>
</tr>
</tbody>
</table>

a Product: Brand A-D = different brands of commercial uncured, no-nitrate/nitrite-added bacon products and Brand E = commercial nitrite-added bacon product (control).

b Color measurement inclusive of fat and lean portions of bacon slice. Commission International D’Edairerage (CIE) L*a*b* were L* = lightness, a* = redness, and b* = yellowness on a 0-100 white scale.

c Cured meat color measurement inclusive of fat and lean portion of bacon slice by reflectance ratio of wavelengths 650/570 nm where 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.

d Color measurement of only lean portion of bacon slice. Commission International D’Edairerage (CIE) L*a*b* were L* = lightness, a* = redness, and b* = yellowness on a 0-100 white scale.

e Cured meat color measurement of only lean portion of bacon slice by reflectance ratio of wavelengths 650/570 nm where 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.

f SEM = Standard error of the means for uncured, no-nitrate/nitrite-added and nitrite-added commercial bacon products.

g-j Means within same column with different superscripts are different (P<0.05).
TABLE 10: Least squares means for replication x brand interaction (P<0.05) of attributes for uncured, no-nitrate/nitrite-added (Brand A-D) and nitrite-added (Brand E) commercial bacon.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Brand A rep 1</th>
<th>Brand A rep 2</th>
<th>Brand B rep 1</th>
<th>Brand B rep 2</th>
<th>Brand C rep 1</th>
<th>Brand C rep 2</th>
<th>Brand D rep 1</th>
<th>Brand D rep 2</th>
<th>Brand E rep 1</th>
<th>Brand E rep 2</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.70*</td>
<td>6.11*</td>
<td>5.72*</td>
<td>5.87*</td>
<td>5.87*</td>
<td>5.62*</td>
<td>6.16*</td>
<td>6.31*</td>
<td>6.14</td>
<td>6.15</td>
<td>0.02</td>
</tr>
<tr>
<td>TBA</td>
<td>0.20</td>
<td>0.16</td>
<td>0.86*</td>
<td>1.76*</td>
<td>0.57</td>
<td>0.53</td>
<td>0.23</td>
<td>0.24</td>
<td>0.09*</td>
<td>0.15*</td>
<td>0.01</td>
</tr>
<tr>
<td>Cured Pigment</td>
<td>44.37*</td>
<td>74.87*</td>
<td>9.04</td>
<td>10.70</td>
<td>44.05</td>
<td>50.32</td>
<td>44.32</td>
<td>47.82</td>
<td>64.18</td>
<td>67.40</td>
<td>1.30</td>
</tr>
<tr>
<td>Residual Nitrite</td>
<td>2.81*</td>
<td>6.28*</td>
<td>0.00</td>
<td>0.00</td>
<td>0.04</td>
<td>0.14</td>
<td>4.70*</td>
<td>7.47*</td>
<td>4.75*</td>
<td>13.10*</td>
<td>0.37</td>
</tr>
<tr>
<td>Texture</td>
<td>5.96</td>
<td>5.47</td>
<td>4.14</td>
<td>3.69</td>
<td>6.36</td>
<td>6.24</td>
<td>6.41</td>
<td>7.02</td>
<td>7.11</td>
<td>7.27</td>
<td>0.24</td>
</tr>
<tr>
<td>[Reflectance Ratio]</td>
<td>1.96</td>
<td>1.66</td>
<td>1.44</td>
<td>1.66</td>
<td>1.50</td>
<td>1.94</td>
<td>1.65</td>
<td>1.82</td>
<td>2.19</td>
<td>1.60</td>
<td>0.18</td>
</tr>
</tbody>
</table>

* Attribute with significant (P<0.05) replication x brand model interaction.

b Values resulting from measurements taken inclusive of both fat and lean portion of bacon slice.

c SEM = Standard error of the means for uncured, no-nitrate/nitrite-added and nitrite-added (control) commercial bacon products.

* Replication (rep) means between rep 1 and rep 2 within row for each TRT or C are different (P<0.05).
reflectance ratios (Hunt and others 1991; Erdman and Watts 1957). Notably, all brands rated as less intense but noticeable cured color except for Brand B having a rating of moderate fade. A replication x brand interaction (P<0.05) was also present for reflectance ratio (Table 10).

Least squares means for lean only color measurements are found in Table 9. No significant differences were observed for L* values. For a* values, Brand A revealed the highest numeric value that was only significantly (P<0.05) higher than Brand B. Brand E had a significantly (P<0.05) different b* value than Brands C and D but was not found different than Brands A or B. For reflectance ratio of the lean only portion of the bacon slices, no differences were found between Brands A, C, D and E. Brand B was significantly (P<0.05) lower for reflectance ratio than Brands A, C and D while Brand D again revealed the highest numeric value. Lean only portion reflectance ratios were higher for all brands compared to lean and fat combined measurements. Interestingly, Brand B rated as moderate fade even though only the lean portion was measured indicating a noticeable light cured meat color.

Total and Cured Pigment Analysis

Total and cured meat concentration means are reported in Table 11. Brand E was significantly (P<0.05) lower in total pigments than Brand A. Total pigment concentrations of Brands ranged from 62.14 to 108.77 ppm. This range could be attributed to different proportions of different muscles within a slice as well as varying proportions of lean and fat within slices. For cured pigment, Brand E had a significantly (P<0.05) higher concentration than all other Brands. Additionally, Brand B had a lower (P<0.05) cured pigment concentration than all other brands.
<table>
<thead>
<tr>
<th>Product</th>
<th>Total Pigment</th>
<th>Cured Pigment</th>
<th>TBARS&lt;sup&gt;c&lt;/sup&gt; mg/kg</th>
<th>pH&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Residual Nitrite&lt;sup&gt;e&lt;/sup&gt; ppm</th>
<th>Residual Nitrate&lt;sup&gt;f&lt;/sup&gt; ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand A</td>
<td>108.77&lt;sup&gt;hj&lt;/sup&gt;</td>
<td>59.62&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.1779&lt;sup&gt;k&lt;/sup&gt;</td>
<td>5.91&lt;sup&gt;l&lt;/sup&gt;</td>
<td>4.54&lt;sup&gt;l&lt;/sup&gt;</td>
<td>11.37&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brand B</td>
<td>79.31&lt;sup&gt;ik&lt;/sup&gt;</td>
<td>9.87&lt;sup&gt;i&lt;/sup&gt;</td>
<td>1.3106&lt;sup&gt;h&lt;/sup&gt;</td>
<td>5.79&lt;sup&gt;k&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;k&lt;/sup&gt;</td>
<td>11.44&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brand C</td>
<td>62.14&lt;sup&gt;il&lt;/sup&gt;</td>
<td>47.18&lt;sup&gt;l&lt;/sup&gt;</td>
<td>0.5492&lt;sup&gt;i&lt;/sup&gt;</td>
<td>5.74&lt;sup&gt;k&lt;/sup&gt;</td>
<td>0.09&lt;sup&gt;k&lt;/sup&gt;</td>
<td>13.73&lt;sup&gt;hi&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brand D</td>
<td>97.22&lt;sup&gt;ik&lt;/sup&gt;</td>
<td>46.07&lt;sup&gt;kl&lt;/sup&gt;</td>
<td>0.2352&lt;sup&gt;j&lt;/sup&gt;</td>
<td>6.24&lt;sup&gt;h&lt;/sup&gt;</td>
<td>6.09&lt;sup&gt;h&lt;/sup&gt;</td>
<td>13.81&lt;sup&gt;hi&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brand E</td>
<td>84.85&lt;sup&gt;jkl&lt;/sup&gt;</td>
<td>65.79&lt;sup&gt;l&lt;/sup&gt;</td>
<td>0.1242&lt;sup&gt;l&lt;/sup&gt;</td>
<td>6.15&lt;sup&gt;i&lt;/sup&gt;</td>
<td>8.93&lt;sup&gt;i&lt;/sup&gt;</td>
<td>18.38&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.52</td>
<td>0.92</td>
<td>0.0051</td>
<td>0.01</td>
<td>0.26</td>
<td>1.42</td>
</tr>
</tbody>
</table>

<sup>a</sup> Product: Brand A-D = different brands of commercial uncured, no-nitrate/nitrite-added bacon products and Brand E = commercial nitrite-added bacon product (control).

<sup>b</sup> Total pigment and cured pigment (nitrosylhemochrome) analysis.

<sup>c</sup> 2-Thiobarbituric acid test reported as mg malonaldehyde/kg of sample.

<sup>d</sup> pH of commercial bacon products.

<sup>e</sup> Residual nitrite determination reported in ppm of sample.

<sup>f</sup> Residual nitrate determination reported in ppm of sample.

<sup>g</sup> SEM = Standard error of the means for uncured, no-nitrate/nitrite-added and nitrite-added commercial bacon products.

<sup>h-l</sup> Means within same column with different superscripts are different (P<0.05).
A replication x brand significant (P<0.05) interaction was present for cured meat pigment concentration (Table 10). Trends between total and cured pigments indicated Brand E had the highest percentage of cured pigment conversion compared to total pigment and Brand B revealed the lowest. These results are in agreement with similar patterns displayed with objective color measurements.

**pH Determination and TBARS Analysis**

Measurements for pH and lipid oxidation (measured by TBARS) are reported in Table 11. Brand E revealed a higher (P<0.05) pH than Brands A, B and C and a lower (P<0.05) pH value than Brand D. For lipid oxidation, Brand E had a lower (P<0.05) TBARS value than all other brands. Brand B revealed the highest (P<0.05) TBARS value than all other Brands and at a level (1.3106) where lipid oxidation may be detectable to consumers. Brand E would be expected to have the lowest TBARS value since sodium nitrite was utilized, however the level reported was lower than expected with the high amount of compositional fat normally found in bacon. Perhaps the bacon purchased was early in the shelf life (since manufacture) or proactive methods to control lipid oxidation by the manufacturer were taken. It should be noted that a significant (P<0.05) interaction of replication x brand was present for both pH and TBARS analysis (Table 10).

**Residual Nitrate and Nitrite Analysis**

Residual nitrite and nitrate values are displayed in Table 11 and a significant replication x brand interaction was observed for residual nitrite (Table 10). As expected, Brand E had the highest (P<0.05) amount residual nitrite compared to all other brands. Both Brands B and C reported having non-detectable (<1.0 ppm)
levels of residual nitrite and both were significantly (P<0.05) lower than all other brands. Interestingly, both Brands B and C revealed cured color as indicated by cured pigment even though we would not expect this since non-detectible levels of nitrite were discovered. In addition, no differences (P>0.05) for CIE L* or a* color values were determined between any Brands. All Brands revealed residual nitrate and Brand E showed a significantly (P<0.05) higher level than Brand A or B. The presence of residual nitrite for all brands indicates that curing reactions may have occurred yet it is unclear to what extent. A possible explanation for the non-detectible nitrite levels of Brands B and C is they may have been further along in their shelf life than the other brands. As the length of shelf life increases, decreasing levels of nitrite would be expected (Jantawat and others 1993), especially with varying or high storage temperatures (Hustad and others 1973).

**Consumer Sensory Panel**

Consumer panelists were administered demographic questions to better understand consumer purchasing decisions. The age of participants was as follows: 18-24 (5%), 25-34 (23%), 35-44 (21%), 45-54 (31%), 55-64 (20%) and >64 (1%). Panelists were asked how often they consumed bacon and the responses were as follows: at least once per week (18%), two times per month (44%), once per month (23%) and less than once a month (14%). Panelists were also asked what the most important consideration was for bacon purchasing decisions and the responses were: price (16%), brand name (17%), lean to fat ratio (62%) and ingredients (5%). Consumer sensory panel acceptability scores for lean color, aroma, flavor, texture and overall acceptance are found in Table 12.
TABLE 12: Least squares means for sensory attributes of lean color, aroma, flavor, texture and overall acceptance for uncured, no-nitrate/nitrite-added (Brand A-D) and nitrite-added (Brand E) commercial bacon.

<table>
<thead>
<tr>
<th>Product</th>
<th>Lean Color</th>
<th>Aroma</th>
<th>Flavor</th>
<th>Texture</th>
<th>Overall Acceptance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand A</td>
<td>6.61&lt;sup&gt;hij&lt;/sup&gt;</td>
<td>6.52&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.17&lt;sup&gt;eg&lt;/sup&gt;</td>
<td>5.72&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.91&lt;sup&gt;fh&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brand B</td>
<td>4.60&lt;sup&gt;g&lt;/sup&gt;</td>
<td>5.02&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3.46&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3.92&lt;sup&gt;g&lt;/sup&gt;</td>
<td>3.58&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brand C</td>
<td>6.98&lt;sup&gt;deh&lt;/sup&gt;</td>
<td>6.80&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.13&lt;sup&gt;g&lt;/sup&gt;</td>
<td>6.30&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.35&lt;sup&gt;eh&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brand D</td>
<td>6.59&lt;sup&gt;di&lt;/sup&gt;</td>
<td>7.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.97&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.71&lt;sup&gt;de&lt;/sup&gt;</td>
<td>6.89&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brand E</td>
<td>7.12&lt;sup&gt;ej&lt;/sup&gt;</td>
<td>6.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.83&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.19&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.05&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.16</td>
<td>0.16</td>
<td>0.18</td>
<td>0.17</td>
<td>0.16</td>
</tr>
</tbody>
</table>

<sup>a</sup> Product: Brand A-D = different brands of commercial uncured, no-nitrate/nitrite-added bacon products and Brand E = commercial nitrite-added bacon product (control).

<sup>b</sup> SENSORY ATTRIBUTES = Consumer panel scores using a 9 point hedonic scale where 1= dislike extremely, 9= like extremely.

<sup>c</sup> SEM = Standard error of the means for uncured, no-nitrate/nitrite-added and nitrite-added commercial bacon products.

<sup>d-j</sup> Means within same column with different superscripts are different (P<0.05).
For lean color acceptability scores, trends indicate that Brand E had the highest score while Brand E had a significantly (P<0.05) higher score than Brands B and D. Brand B revealed the lowest (P<0.05) lean color score compared to all other brands. Aroma scores show that Brand B was significantly (P<0.05) lower than all other brands. Brand B also had the lowest (P<0.05) score for flavor while Brand E had one of the highest. Brand B also had the lowest (P<0.05) texture score of all brands while Brand E again had one of the highest although a significant (P<0.05) replication x brand interaction was identified. For overall acceptance, no differences (P>0.05) were found between Brands D and E or Brands C and D, however, Brand E was significantly (P<0.05) higher in overall acceptability compared to Brands A, B and C while Brand D had a higher (P<0.05) score than Brands A and B. Brand B also revealed the lowest (P<0.05) overall acceptance score compared to all other brands. From consumer sensory analysis, it can be concluded that Brand B might be considered a low-quality bacon as compared to the Brands tested. This is supported by a post sensory question measuring the likelihood of purchase of each of the brands based on the sensory evaluation. The same panelists were asked if they would purchase (yes or no) each brand and those results are as follows: Brand A (57% yes), Brand B (14% yes), Brand C (64% yes), Brand D (84% yes) and Brand E (88% yes). Brand E (nitrite cured control) received the highest yes/no rating closely followed by Brand D suggesting both Brand D and E would be considered highly acceptable compared to the other brands tested while Brand B would be considered unacceptable most consumers as demonstrated by consumer sensory evaluation.
Conclusions

Commercial uncured, no-nitrate/nitrite-added hams, bacons and frankfurters were compared against nitrite cured products considered to be industry standards in their respective product category. Variation between brands as well as variation between replications as indicated by replication x brand interactions existed for all products and product categories tested.

All uncured, no-nitrate/nitrite-ham Brands (A-D) tested appear to have been manufactured with the intention of replacing sodium nitrite without the direct addition of nitrite as indicated on the ham labels. Generally, Brands A, B, C and D were comparable in color, total and cured pigment, lipid oxidation, residual nitrite and residual nitrate measurements to Brand E (nitrite-added control). Although residual nitrite measurements were considerably lower for Brands A-D compared to Brand E, the levels reported were not necessarily unexpected. Depending on the length of time since manufacture and storage temperature conditions, variations between Brands A-D as well as compared to Brand E could be normally expected. Consumer sensory differences did exist between the Brands (A-E), however, no scores were extremely low indicating that all hams tested would be considered acceptable by a majority of consumers.

A greater amount of variation was identified between frankfurters (Brands A-D) than hams. The large reported differences in cured meat pigment concentrations indicated a large variation in the amount of curing during product manufacture had taken place. Significant CIE a* values support this occurrence. Lipid oxidation appears to be approaching detectable levels for Brands A-D, however, those levels
were similar to Brand E (nitrite-added control) so little can be deducted from that measurement.

Consumer sensory scores may provide the most definitive answers. Brands A, C, D and E received similar scores for most sensory attributes while Brand B was clearly rated the lowest for all sensory attributes. In some instances, Brands C and D actually rated higher than Brand E (nitrite-added control) and this may be explained by the differences in raw materials and/or non-meat ingredients used during product manufacture. Another possible explanation is a masking or overpowering effect of spices typically used in frankfurters that may effect consumer sensory perception and subsequently their scores.

The largest evaluation differences were identified with bacon products investigated. Interestingly, in general, little variation was found for color measurements with the exception of a* (redness) color and reflectance ratios for the lean only portion assessment. Brand B, the uncured, no-nitrate/nitrite-added bacon appearing to intentionally not replace sodium nitrite during manufacture, had the lowest a* and reflectance ratio values. This was not unexpected as residual nitrite values for Brand B were also non-detectable. Although differences existed between brands for total pigment, cured pigment and lipid oxidation, the most notable differences existed between Brand B compared to all other brands. Consumer sensory results report that Brand B had the lowest scores for all sensory attributes tested while all other brands (A,C,D) were similar to the Brand E (nitrite-added control).
This research provides a snapshot of products available to consumers and better insight on consumer reactions to those products as well as possible analytical reasoning why consumer differences did or did not exist. Although replication brand interactions existed for many attributes investigated making data interpretation more difficult, it was clearly demonstrated that variation in uncured, no-nitrate/nitrite-added processed meat products does in fact exist. Further research regarding reasons for investigated variation existed as well as methods to reduce this variation is needed. Further research to better understand consumer acceptability thresholds for uncured, no-nitrate/nitrite-added processed meat products is also needed.

Acknowledgement

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References


Abstract

Uncured, no-nitrate/nitrite-added meat products can be manufactured with vegetable juice powder (VJP) and a starter culture containing *Staphylococcus carnosus* and possess quality and sensory attributes similar to traditional cured products. The first objective of this study was to determine the effects of varying concentrations of VJP and incubation times (MIN-HOLD) on quality characteristics including lipid oxidation, color and cured meat pigment concentrations of emulsified-frankfurter-style-cooked (EFSC) sausages over a 90-day storage period. The second objective was to compare residual nitrate and nitrite content and the third objective was to determine if differences exist in sensory properties of finished products. Four EFSC sausage treatments (TRT) (TRT1: 0.20% VJP, 30 MIN-HOLD; TRT2: 0.20% VJP, 120 MIN-HOLD; TRT3: 0.40% VJP, 30 MIN-HOLD; TRT4: 0.40% VJP, 120 MIN-HOLD) and a sodium nitrite-added control (C) were used for this study. No differences for lipid oxidation as measured by 2-thiobarbituric acid reactive substances (TBARS) between any TRTs and C or over time were observed. No differences (P>0.05) for CIE L* values were found between TRTs. CIE a* and reflectance ratio values revealed that TRTs 2, 4 and C were redder than TRTs 1 and...
3 at Day 0. Trained sensory intensity ratings for cured aroma, color and flavor, uniform color and firmness determined that all but TRT 1 were similar to C. These results indicate a longer incubation time (120 min) was found more critical than VJP level (0.20% or 0.40%) to result in products comparable to a sodium nitrite-added control.

Keywords: uncured, residual nitrate, residual nitrite, vegetable juice powder, emulsified

**Introduction**

Meat curing can be defined as the use of both salt and nitrite (the reduced form of nitrate) to chemically alter the physical, chemical and often microbiological properties of meat products (Cassens and others 1979). The use of sodium or potassium nitrates and nitrites to preserve and cure meats evolved centuries ago (Cassens 1995). Although used for hundreds of years, sodium nitrite was not approved for meat curing by the United States Department of Agriculture until 1925 (Pearson and Tauber 1984). It was not until the turn of 20th century when scientists determined that nitrate did not have a direct role in the curing process and the conversion of nitrate to nitrite was necessary for curing reactions. This step is normally accomplished by the bacterial reduction of nitrate to nitrite (Sebranek 1979; Pinotti and others 2001; MacDougall and others 1975, Gray and others 1981). Bacterial reduction can be accomplished by microorganisms found in the natural flora of meat or by intentional addition of microorganisms with nitrate-reducing properties (Sanz and others 1997).
Nitrite is responsible for the development of cured color and flavor, serves as a strong antioxidant to protect flavor and acts as a strong antimicrobial to control *Clostridium botulinum* outgrowth (Shahidi and Pegg 1992). Nitrite controls and stabilizes the oxidative state of lipids in meat products (Shahidi and Hong 1991) thus preventing lipid oxidation and subsequent warmed-over flavors (Vasavada and Cornforth 2005; Yun and others 1987). Less nitrite is needed to provide for color development than to control bacteria (Roberts 1975).

The value of meat color to the consumer is extremely important. The four determining attributes for consumer purchasing decisions are color, juiciness, flavor and toughness/tenderness. Of these attributes, color is the first and primarily most important factor of the decision-making process to purchase meat products (Aberle and others 2001). Recent consumer interest for natural, organic and healthier foods has prompted consumer demands for uncured, no-nitrate/nitrite-added meat and poultry products.

Two classifications of uncured, no-nitrate/nitrite-added meat and poultry products currently exist in the market place: those that do not utilize nitrate or nitrite (uncured products) and those with the intention of replacing nitrate and nitrite to simulate nitrite-added cured product properties. In order to manufacture cured products without direct addition of sodium nitrite, a nitrate source and reducer must be utilized. Vegetables are well-known to contain significant amounts of nitrate (Walker 1990; Fujihara and others 2001) and when added at high enough levels with a nitrate reducer may provide adequate amounts of nitrite to accomplish curing reactions.
Therefore, the objectives of this research were to first determine the effects of varying concentrations of commercial vegetable juice powder and incubation times on quality characteristics including lipid oxidation, color and cured meat pigment concentrations of emulsified-frankfurter-style cooked (EFSC) sausages over an extended storage period, and secondly, to determine if differences exist in finished products as determined by trained sensory analysis. A third objective was to determine the effects of vegetable juice powder concentration and incubation time on nitrate and nitrite concentrations during product manufacture and over an ensuing storage period.

**Materials and Methods**

**Experimental Design and Data Analysis**

Varying concentrations of vegetable juice powder (VJP) and incubation (MIN-HOLD) times (30 or 120 min) for the manufacture of emulsified-frankfurter-style cooked (EFSC) sausage were investigated. Four EFSC sausage treatments (TRT1: 0.20% VJP, 30 MIN-HOLD; TRT2: 0.20% VJP, 120 MIN-HOLD; TRT3: 0.40% VJP, 30 MIN-HOLD; TRT4: 0.40% VJP, 120 MIN-HOLD) and a sodium nitrite-added control (C) were used for this study.

Statistical analysis was performed for all measurements using the Statistical Analysis System (version 9.1, SAS Institute Inc., Cary, N.C., U.S.A.) Mixed Model procedure (SAS Inst. 2003). The experimental design was a 2 (VJP level) x 2 (MIN-HOLD time) factorial design. The main plot consisted of 3 blocks (replication) and 5 EFSC sausage treatments resulting in 15 observations for trained sensory and
proximate composition. The model included the fixed main effects of treatment and replication. The random effect was the interaction of treatment x replication.

Within the main factorial design was a split plot for measurements over time. The split plot contained 5 sampling periods (day 0, 14, 28, 56 and 90) and combined with the main plot resulted in a total of 75 observations for color, nitrite, nitrate, pH, cured pigment, total pigment and lipid oxidation. The model included the fixed main effects of treatment, replication, day and the interaction of treatment x day. The random effect was the interaction of treatment x replication.

The significant main effect means for all experiments were separated and least significant differences were found using Tukey-Kramer multiple pairwise comparison method. Significance level was determined at P<0.05. For all other experiments, main effects were tested for significance using a mixed effects model.

Product Procurement and Manufacture

Ready-to-eat EFSC sausages were manufactured with 85% coarse-ground (9.5 mm) fresh beef trimmings and 50% lean fresh pork trimmings obtained from a local supplier. The 50% pork trimmings were ground (Biro MFG Co. Marblehead, Ohio, U.S.A.) using a 1.27 mm plate. Samples (5.90 kg) of both beef and pork trimmings were taken to determine the fat content using an Anyl Ray Fat Analyzer (Kartrig Pak, Model 316-48, Davenport, IA., U.S.A.) to formulate a finished lean content of 72%. The beef trimmings and pork trimmings were separated into five batches (11.34 kg each). Treatments (TRT 1-4) and Control (C) were randomly assigned to the batches. The beef and pork EFSC sausage formulation for TRT 1 and TRT 2 consisted of the following ingredients: 59.4% beef trimmings, 19.8% pork
trimmings, 15.8% ice/water, 1.77% salt, 1.58% dextrose, 1.42% spices (Blend TG-05-405-000 (mustard, spices, garlic powder), A.C. Legg Packing Co., Calera, Ala., U.S.A.), 0.20% vegetable juice powder (Vegetable Juice PWD NAT, Chr. Hansen Inc., Gainesville, Fla., U.S.A), and 0.0256% starter culture containing *Staphylococcus carnosus* (CS 299 Bactoferm™, Chr. Hansen Inc., Gainesville, Fla., U.S.A). TRT 3 and TRT 4 consisted of the following ingredients: 59.3% beef trimmings, 19.8% pork trimmings, 15.8% ice/water, 1.77% salt, 1.58% dextrose, 1.41% spices (Blend TG-05-405-000, A.C. Legg Packing Co., Calera, Ala., U.S.A.), 0.40% vegetable juice powder (Vegetable Juice PWD NAT, Chr. Hansen Inc., Gainesville, Fla., U.S.A), and 0.0256% starter culture (CS 299 Bactoferm™, Chr. Hansen Inc., Gainesville, Fla., U.S.A). The control consisted of 59.4% beef trimmings, 19.8% pork trimmings, 15.9% ice/water, 1.77% salt, 1.58% dextrose, 1.42% spices (Blend TG-05-405-000, A.C. Legg Packing Co., Calera, Ala., U.S.A.), 0.0436 sodium erythorbate and 0.0124% sodium nitrite. No phosphates were added to any TRTs or C because the EFSC sausages were intended to be similar to natural or organic products which restrict phosphate usage.

Emulsions were produced using methods described by Rust (1987). The EFSC sausages were manufactured using a vacuum bowl cutter (Krämer & Grebe Model VSM65, Krämer & Grebe GmbH & Co. KG., Biendenkopf-Wallau, Germany). The beef trim was chopped with salt, vegetable juice powder or nitrite (depending on treatment) and half of ice/water under vacuum until 3 °C was achieved. The bowl cutter was scraped and the pork, dextrose, spices, starter culture or sodium erythorbate (depending on treatment) and remaining water was added and chopped
under vacuum until 14 °C was achieved. After chopping was completed, the meat batter was transferred to a rotary vane vacuum-filling machine with linking attachment (Risco vacuum stuffer, Model RS 4003-165, Stoughton, Mass., U.S.A) and stuffed into 33 mm impermeable plastic casings (WP-E Clear 35 Micron, World Pac USA International Inc., Sturtevant, Wis., U.S.A.). The impermeable casings were used to control cross-contamination effects that any environmentally released nitric oxide gas could have on the TRTs during thermal processing. The casings had an O₂ permeability rate of 6-7 cm³/m²/24 h at 1 atm and a water vapor permeability of 130 g/m²/24 h.

TRTs were placed on separate smokehouse trucks to allow separate incubation (MIN-HOLD) times (30 min or 120 min). The stuffed EFSC sausages were transferred to two single truck thermal processing ovens (Maurer, AG, Reichenau, Germany; Alkar, Model MT EVD RSE 4, Alkar Engineering Corp., Lodi, Wis., U.S.A). Incubation was conducted at 40.6 °C dry bulb and 39.4 °C wet bulb temperatures. MIN-HOLD times started when the internal temperature of the EFSC sausages reached 37.8 °C. The control was added to the thermal processing oven after incubation steps were complete. Cooking was accomplished using a common frankfurter smokehouse schedule reaching an internal temperature of 71.1 °C. After thermal processing, the EFSC sausages were chilled for 12 h at 0-2 °C. The EFSC sausages were placed in barrier bags (Cryovac B540, Cryovac Sealed Air Corp., Duncan, S.C., U.S.A.) and vacuum packaged. The packaging film had an O₂ transmission rate of 3-6 cc/m²/24 h at 1 atm, 4.4 °C, and 0% RH, and a water vapor transmission rate of 0.5-0.6 g/645 cm²/24 h and 100% RH.
*Color Measurements*

Color measurements were conducted using a Hunterlab Labscan spectrocolorimeter (Hunter Associated Laboratories Inc., Reston, Va., U.S.A.). The Hunterlab Labscan was standardized using the same packaging material as used on the samples, placed over the white standard tile. Values for the white standard tile were X=81.72, Y=86.80 and Z=91.46. Exterior color of the EFSC sausage was measured immediately after removing from the packaging material and internal color was measured immediately after slicing the EFSC sausages lengthwise.

Illuminant A, 10° standard observer with a 1.27 cm viewing area and 1.78 cm port size, was used to analyze EFSC sausage samples. Commission International d’Eclairage (CIE) L* (lightness), a* (redness), and b* (yellowness) and cured meat color were determined by reflectance ratio of wavelengths 650/570 nm (Hunt and others 1991; Erdman and Watts 1957). Measurements were taken at 6 randomly selected areas on the samples (n=2) and the resulting average was used in data analysis.

*Proximate Composition*

Proximate composition was determined for the EFSC sausage samples including crude fat (AOAC 1990), moisture (AOAC 1990a) and crude protein (AOAC 1993).

*pH Determination*

The pH of the EFSC sausage samples was determined by blending the samples with distilled, de-ionized water in a 1:9 ratio, then measuring the pH with a pH/ion meter (Accumet 925: Fisher Scientific, Fair Lawn, N.J., U.S.A.) equipped with
an electrode (Accumet Flat Surface Epoxy Body Ag/AgCl combination Electrode Model 13-620-289, Fisher Scientific, Fair Lawn, N.J., U.S.A) calibrated with phosphate buffers 4.0 and 7.0, according to the method of Sebranek and others (2001). For each treatment, measurements were made in duplicate.

**TBARS Analysis**

Lipid oxidation was measured by the modified 2-thiobarbituric acid reactive substances (TBARS) test as described for cured meats (Zipser and Watts 1962). TBARS values were reported as mg of malonaldehyde equivalents/kg of meat sample. For each treatment, measurements were made in duplicate.

**Total and Cured Pigment Analysis**

Mononitrosylhemochrome (cured meat pigment) and total pigment concentrations were measured after extraction in 80% acetone and acidified acetone, respectively (Hornsey 1956). The experiment, including sample preparation, was done in subdued light, to reduce pigment fading. Samples were finely ground/chopped using a food processor (Sunbeam-Oskar Model 4817, Sunbeam Products Inc., Delray Beach, Fla., U.S.A.).

Cured pigment analysis was conducted using a modified method of Hornsey (1956). Duplicate 10 g samples were mixed with 40 ml of acetone and 3 ml of distilled, de-ionized water with a Polytron mixer (PT 10/35, Kinematica GmbH, AG, Switzerland) for 1 min at speed setting 7. The sample was immediately filtered through a Whatman 42 filter paper, and the absorbance (540 nm) measured on the filtrate. Nitrosylhemochrome concentration was calculated as $A_{540} \times 290$ and was recorded in parts per million (ppm).
Total pigment analysis was conducted using a modified method of Hornsey (1956). The same finely ground/chopped samples used for cured pigment analysis were utilized for total pigment analysis. Duplicate 10 g samples were mixed with 40 ml of acetone, 2 ml of distilled, de-ionized water and 1 ml of concentrated hydrochloric acid using a Polytron mixer (PT 10/35, Kinematica GmbH, AG, Switzerland) for 1 min at speed setting 7. The samples were allowed to stand for 1 h, then filtered through a Whatman 42 filter paper and immediately analyzed. Absorbance was measured at 640 nm. Total pigment concentration was calculated as $A_{640} \times 680$ and was recorded in parts per million (ppm).

**Residual Nitrite Analysis**

Residual nitrite was determined by the AOAC method (AOAC 1990b). The same finely ground/chopped samples that were used for pigment analysis were also used for residual nitrite measurement. All residual nitrite assays were done in duplicate and all treatments within a block were analyzed at the same time to minimize variation in the analysis due to time.

**Residual Nitrate Analysis**

Sample preparation and nitrate determination methods were modifications of Ahn and Maurer (1987). Five grams of meat product samples were weighed in a 50-ml test tube and homogenized with 20 ml of distilled, de-ionized water (DDW) using a Polytron homogenizer (Type PT 10/35, Brinkmann Instruments Inc., Westbury, N.Y., USA) for 10 s at high speed. The homogenate was heated for 1 h in 80 °C water bath. After cooling in cold water for 10 min, 2.5 ml of the homogenate was transferred to a disposable test tube (16 x 100 mm). Carrez II (dissolve 10.6 g
potassium ferrocyanide in 100 ml DDW) and Carrez I (dissolve 23.8 g zinc acetate in 50 ml DDW, then add 3 ml glacial acetic acid and dilute to 100 ml with DDW) reagents were added (0.1 ml each) to precipitate proteins. The solution was diluted with 2.3 ml of DDW and mixed well. After precipitation, the supernatant was centrifuged at 10,000 x g for 20 min and the clear upper layer was used for nitrate measurement by high performance liquid chromatography (Agilent 1100 Series HPLC system, Agilent Technologies, Wilmington, Del., U.S.A.). The column used was Agilent Zorbax SAX (analytical 4.6 x 150mm, 5-micron) (Agilent, Wilmington, DE, USA) and the elution buffer was 15 mM phosphate buffer, pH 2.35, with isocratic elution. Flow rate was 1.0 ml/min and sample volume was 25 µL. The wavelength used was 210 nm. The area of nitrate peak was used to calculate nitrate concentration (ppm) using a nitrate standard curve.

Trained Sensory Panel

EFSC sausages were evaluated by a trained sensory panel for color, aroma, flavor and texture characteristics. Ten trained panelists, made up of Iowa State University students and staff, were used for each session. For training, three one-hour sessions were held using commercial and experimental products to develop descriptive terms for the desired attributes. EFSC sausages were evaluated for cured frankfurter aroma, internal cured frankfurter color, uniformity of internal frankfurter color, cured frankfurter flavor and firmness.

Attributes were measured using a line scale (numerical value of 15 units) with graduations from 0 to 15 where 0 represented none (aroma and flavor), not uniform
(color), low (color) and soft (firmness) and 15 represented intense (aroma and flavor), high (color), uniform (color) and hard (firmness).

Expectorant cups were provided to prevent taste fatigue and distilled de-ionized water and unsalted soda crackers were provided to clean the palate between samples. The presentation order was randomized for each session. A computer ballot was constructed and data was collected using a computerized sensory scoring system (COMPUSENSE five, Compusense, Inc. v.4.4, Guelph, Ontario, Canada, N1H3N4).

EFSC sausage samples were removed from vacuum packages and added to 2 quarts of boiling water in 3-quart sauce pans. The covered pans containing EFSC sausage were immediately removed from the heat source and allowed to rest for 7 min. EFSC sausages were cut into 1.9 cm pieces with 1.27 cm pieces from each end being discarded. Panelists were presented two, 1.9 cm randomly selected heated pieces in a covered container and asked to determine intensity of aroma, internal cured color, cured flavor, uniformity of internal color, and texture of the EFSC sausage samples.

Results and Discussion

Product Processing Attributes

Various product and processing parameters were recorded during the manufacture of the EFSC sausages (Appendix 4 and 5). The means for beef trim characteristics were as follows: 16.5% fat, pH of 5.53 and a temperature of -0.67 ºC. The means for pork trim were as follows: 55.8% fat, pH of 6.24 and a temperature of
3.33 °C. The average pH of water used in the formulation was 8.88 with a temperature of 5.0 °C. Temperature and pH of TRTs and C batches were measured after stuffing and before incubation (pre-incubate). Pre-incubate pH ranged from 5.44 to 5.46 and no differences were found between any TRTs or C. The pH was also measured after the incubation step but prior to the cooking steps (post-incubate). Post-incubate pH ranged from 5.41 to 5.54 and no differences were found between the TRTs.

The time needed, at incubation temperatures, for the internal temperature of the EFSC sausage TRTs to reach optimum conditions (37.8 °C) ranged between 21 and 35 min. The difference in time was due to slight variation in stuffing temperatures between replications (n=3) as well as performance differences between the two thermal processing ovens. Total average thermal processing times (including come-up time to optimum incubation temperature) for TRTs 1 and 3 was 109 min and for TRTs 2 and 4 was 208 min. The control was added to the thermal processing oven after incubation of the TRTs was completed and had a total average thermal processing time of 83 min.

**Color Measurements**

Both external and internal color measurements were measured for EFSC sausage TRTs and C. Since EFSC sausages were cooked in impermeable casings with no smoke treatment applied, external color demonstrated color development on the outside surface of uncured, no-nitrate/nitrate EFSC sausages relative to the nitrite-added control. As meat pigment (myoglobin) concentration is increased in a formulation, cured pigment formation near/on the external surface may lessen in an
uncured, no-nitrate/nitrite-added system as thermal processing conditions and lower concentrations of nitrate-converted-nitrite may adversely effect this formation.

A significant (P<0.05) interaction was observed between treatment and day for objective external CIE a* values as reported in Table 1. As incubation times increased at each VJP concentration, redness values increased (P<0.05) at days 0 and 14. Although not significant, this trend was also observed at days 28, 56 and 90. All TRTs were significantly different (P<0.05) than C at day 0 and 14 except for TRT 4. Fernández-Ginés and others (2003) reported a decrease in a* values in bologna over 28 days of shelf life. Our observations did not agree with these findings as a* values increased or remained similar over time.

No interaction was present for treatment*day for external CIE L*, b* but the main effect of day was significant (P<0.05) and the corresponding least squares combined means for TRTs and C are reported in Table 2. CIE L* values generally increased over time with the largest significant (P<0.05) increase in lightness occurring between days 0 and 90.

A significant (P<0.05) interaction was observed for treatment*day for external cured color fading as indicated by measurements of reflectance ratio (Hunt and others 1991; Erdman and Watts 1957) and are reported in Table 3. TRT 4 and C had significantly (P<0.05) greater reflectance ratios than TRTs 1, 3 and 4 at days 0 and 14. However, no differences (P>0.05) were found between TRT 4 and C on any day. Interestingly, as incubation times increased, reflectance ratio values increased, regardless of VJP level. Exterior reflectance ratios generally increased over time
TABLE 1: Least squares means for the interaction of treatment combination (TRT 1-4, C) with storage time (Day 0, 14, 28, 56, 90) for objective external surface color (a*) \(^a\) of no-nitrite-added (TRT 1-4) and nitrite-added control (C) EFSC sausages.

<table>
<thead>
<tr>
<th>TRT(^b)</th>
<th>0</th>
<th>14</th>
<th>28</th>
<th>56</th>
<th>90</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>(q)13.08(^e)</td>
<td>(p)15.62(^f)</td>
<td>(p)16.18(^f)</td>
<td>(n)15.96(^f)</td>
<td>(n)16.83(^f)</td>
</tr>
<tr>
<td>2</td>
<td>(n)16.39</td>
<td>(q)17.66</td>
<td>(o)p17.52</td>
<td>(n)o17.20</td>
<td>(n)o17.69</td>
</tr>
<tr>
<td>3</td>
<td>(o)q13.86(^e)</td>
<td>(n)p16.26(^f)</td>
<td>(n)p16.50(^f)</td>
<td>(n)o16.56(^f)</td>
<td>(n)o17.40(^f)</td>
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<tr>
<td>4</td>
<td>(p)18.09</td>
<td>(o)18.22</td>
<td>(o)p17.68</td>
<td>(n)a17.44</td>
<td>(n)a18.30</td>
</tr>
<tr>
<td>C</td>
<td>(p)18.64</td>
<td>(o)18.43</td>
<td>(o)18.11</td>
<td>(o)18.07</td>
<td>(o)18.60</td>
</tr>
</tbody>
</table>

SEM\(^d\) = 0.28

\(^a\)Commission International D’Edairerage (CIE) a* were a* = redness on a 0-100 white scale measured on the external surface.

\(^b\)Treatment combinations: TRT 1=low VJP + short MIN-HOLD; TRT 2=low VJP + long MIN-HOLD; TRT 3=high VJP + short MIN-HOLD; TRT 4=high VJP + long MIN-HOLD; C=156 ppm (mg/kg) sodium nitrite.

\(^c\)DAY = 0, 14, 28, 56, 90 day vacuum packaged samples held at 0-2 °C.

\(^d\)SEM = Standard error of the means for no-nitrite-added and nitrite-added EFSC sausages.

\(^e-f\)Means within same row with different superscripts \(a\) are different (P<0.05).

\(^n-q\)Means within same column with different superscripts are different (P<0.05).
TABLE 2: Least squares means\textsuperscript{a} for the main effects of time (Day 0, 14, 28, 56, 90) for objective internal color (L*)\textsuperscript{b}, objective external color (L*,b*)\textsuperscript{c} and total pigment\textsuperscript{d} of no-nitrite-added (TRT 1-4) and nitrite-added control (C) EFSC sausages.

<table>
<thead>
<tr>
<th>DAY\textsuperscript{e}</th>
<th>0</th>
<th>14</th>
<th>28</th>
<th>56</th>
<th>90</th>
<th>SEM\textsuperscript{f}</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*\textsuperscript{b}</td>
<td>63.38\textsuperscript{k}</td>
<td>65.75\textsuperscript{gij}</td>
<td>65.29\textsuperscript{l}</td>
<td>65.73\textsuperscript{ij}</td>
<td>67.99\textsuperscript{h}</td>
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</tr>
<tr>
<td>L*\textsuperscript{c}</td>
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<td>68.48\textsuperscript{i}</td>
<td>67.96\textsuperscript{l}</td>
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<td>70.76\textsuperscript{h}</td>
<td>0.25</td>
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<tr>
<td>b*\textsuperscript{c}</td>
<td>16.36\textsuperscript{gh}</td>
<td>16.47\textsuperscript{g}</td>
<td>15.98\textsuperscript{gh}</td>
<td>15.80\textsuperscript{h}</td>
<td>16.18\textsuperscript{gh}</td>
<td>0.15</td>
</tr>
<tr>
<td>Total Pigment\textsuperscript{d}</td>
<td>151.9\textsuperscript{gi}</td>
<td>145.7\textsuperscript{ij}</td>
<td>139.8\textsuperscript{hj}</td>
<td>139.8\textsuperscript{hj}</td>
<td>138.3\textsuperscript{h}</td>
<td>1.85</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Combined means of TRT (treatments 1-4 and control).
\textsuperscript{b} Commission International D’Edairerage (CIE) L*a*b* where L* = lightness on a 0-100 white scale for the internal surface color of EFSC sausages.
\textsuperscript{c} Commission International D’Edairerage (CIE) L*a*b* where L* = lightness and b* = yellowness on a 0-100 white scale for the external surface color of EFSC sausages.
\textsuperscript{d} Total pigment analysis.
\textsuperscript{e} DAY = 0, 14, 28, 56, 90 day vacuum packaged samples held at 0-2 °C.
\textsuperscript{f} SEM = Standard error of the means for DAY for no-nitrite-added and nitrite-added EFSC sausages.
\textsuperscript{g} Means within same row with different superscripts are different (P<0.05).
TABLE 3: Least squares means for the interaction of treatment combination (TRT 1-4, C) with storage time (Day 0, 14, 28, 56, 90) for external surface reflectance ratio (R/ratio)\(^a\) of no-nitrite-added (TRT 1-4) and nitrite-added control (C) EFSC sausages.

<table>
<thead>
<tr>
<th>TRT(^b)</th>
<th>DAY(^c)</th>
<th>0</th>
<th>14</th>
<th>28</th>
<th>56</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(1.61^e)</td>
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<td>(1.94^f)</td>
<td>(1.99^f)</td>
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</tr>
<tr>
<td>2</td>
<td>(1.99^q)</td>
<td>(2.09^q)</td>
<td>(2.11^{op})</td>
<td>(2.07^{np})</td>
<td>(2.06^{np})</td>
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</tr>
<tr>
<td>3</td>
<td>(1.70^{or})</td>
<td>(1.94^{ql})</td>
<td>(1.99^{np})</td>
<td>(2.02^{lf})</td>
<td>(2.02^{np})</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>(2.21^q)</td>
<td>(2.17^o)</td>
<td>(2.11^{op})</td>
<td>(2.12^{op})</td>
<td>(2.16^{op})</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>(2.25^q)</td>
<td>(2.20^o)</td>
<td>(2.19^{op})</td>
<td>(2.18^{np})</td>
<td>(2.18^{np})</td>
<td></td>
</tr>
</tbody>
</table>

SEM\(^d\)= 0.03

\(^a\)Cured meat color measurement by reflectance ratio of wavelengths 650/570 nm where 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.

\(^b\)Treatment combinations: TRT 1=low VJP + short MIN-HOLD; TRT 2=low VJP + long MIN-HOLD; TRT 3=high VJP + short MIN-HOLD; TRT 4=high VJP + long MIN-HOLD; C=156 ppm (mg/kg) sodium nitrite.

\(^c\)DAY = 0, 14, 28, 56, 90 day vacuum packaged samples held at 0-2 ºC.

\(^d\)SEM = Standard error of the means for no-nitrite-added and nitrite-added EFSC sausages.

\(^e-f\)Means within same row with different superscripts are different (P<0.05).

\(^n-p\)Means within same column with different superscripts are different (P<0.05).
(day) with the exception of the control, which was unexpected. This may be explained by residual nitrate present in TRTs acting as a reservoir for nitrite-related reactions during storage. The phenomenon is supported by Houser and others (2005) who reported color fading and regeneration occurring in ham over time.

Internal CIE $a^*$ values of EFSC sausages for which a significant ($P<0.05$) treatment*day interaction was observed are listed in Table 4. TRTs 1 and 3 were significantly ($P<0.05$) less red than TRTs 2, 4 and C at day 0. TRT 4 and C were also redder ($P<0.05$) than TRTs 1, 2 and 3 at day 14. These results indicate the development of $a^*$ redness was more dependent on the length of incubation rather than the concentration of VJP when compared to a nitrite-added control. Internal CIE $L^*$ values for the main effect of day (time) are reported in Table 2. Combined means for the 90-day storage period showed an increase ($P<0.05$) in lightness between day 0 compared to days 14, 28, 56, and 90. The main effects of treatment and day were significant ($P<0.05$) for internal $b^*$ values and are reported in Table 5.

Table 6 reports a significant ($P<0.05$) interaction of treatment and day for internal cured meat color measurements determined by reflectance ratio. Trends similar to external reflectance ratio results were observed. At day 0, a significantly ($P<0.05$) greater reflectance ratio was observed for TRT 2 compared to TRT 1 and also for TRT 4 compared to TRT 3. Further, no differences at day 0 were found between TRTs 2, 4 and C indicating that those treatment combinations possessed excellent cured color (Hunt and others 1991). It is worthy to mention that all treatment combinations over all days received at least a “less intense but noticeable cured color” rating and all measurements over time for TRTs 2, 4 and C received an
**TABLE 4:** Least squares means for the interaction of treatment combination (TRT 1-4, C) with storage time (Day 0, 14, 28, 56, 90) for objective internal surface color \((a^*)^a\) of no-nitrite-added (TRT 1-4) and nitrite-added control (C) EFSC sausages.

<table>
<thead>
<tr>
<th>TRT(^b)</th>
<th>DAY(^c)</th>
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<th>(14)</th>
<th>(28)</th>
<th>(56)</th>
<th>(90)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>(*q17.25^f)</td>
<td>(*n17.56^f)</td>
<td>(*n17.73^f)</td>
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</tr>
<tr>
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<td>(nq18.69^o)</td>
<td>(nno18.61)</td>
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</tr>
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</tr>
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<td>19.59</td>
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</tr>
<tr>
<td>C</td>
<td>(p19.69)</td>
<td>(o19.63)</td>
<td>(o19.18)</td>
<td>(o19.28)</td>
<td>19.84</td>
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</tr>
</tbody>
</table>

SEM\(^d\) = 0.27

\(^a\)Commission International D’Edairerage (CIE) \(L^*a^*b^*\) where \(a^*\) = redness on a 0-100 white scale measured on the internal surface.

\(^b\)Treatment combinations: TRT 1=low VJP + short MIN-HOLD; TRT 2=low VJP + long MIN-HOLD; TRT 3=high VJP + short MIN-HOLD; TRT 4=high VJP + long MIN-HOLD; C=156 ppm (mg/kg) sodium nitrite.

\(^c\)DAY = 0, 14, 28, 56, 90 day vacuum packaged samples held at 0-2 \(^o\)C.

\(^d\)SEM = Standard error of the means for no-nitrite-added and nitrite-added EFSC sausages.

\(^e-f\)Means within same row with different superscripts are different (P<0.05).

\(^n-o\)Means within same column with different superscripts are different (P<0.05).
<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>0</th>
<th>14</th>
<th>28</th>
<th>56</th>
<th>90</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.88&lt;sup&gt;g&lt;/sup&gt;</td>
<td>5.97&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.00&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.01&lt;sup&gt;o&lt;/sup&gt;</td>
<td>6.00&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>b*</td>
<td>16.60&lt;sup&gt;g&lt;/sup&gt;</td>
<td>17.29&lt;sup&gt;e&lt;/sup&gt;</td>
<td>16.77&lt;sup&gt;eg&lt;/sup&gt;</td>
<td>16.76&lt;sup&gt;eg&lt;/sup&gt;</td>
<td>17.12&lt;sup&gt;eg&lt;/sup&gt;</td>
<td>0.15</td>
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</table>

**Objective Color**

<table>
<thead>
<tr>
<th>TRT&lt;sup&gt;e&lt;/sup&gt;</th>
<th>pH</th>
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</thead>
<tbody>
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<td>16.54&lt;sup&gt;opq&lt;/sup&gt;</td>
</tr>
<tr>
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<td>5.95&lt;sup&gt;o&lt;/sup&gt;</td>
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<td>5.95&lt;sup&gt;o&lt;/sup&gt;</td>
<td>16.71&lt;sup&gt;nqs&lt;/sup&gt;</td>
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<td>4</td>
<td>5.94&lt;sup&gt;o&lt;/sup&gt;</td>
<td>17.21&lt;sup&gt;r&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>5.97&lt;sup&gt;o&lt;/sup&gt;</td>
<td>17.15&lt;sup&gt;r&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.07</td>
</tr>
</tbody>
</table>

<sup>a</sup> pH of EFSC sausage samples.<br>
<sup>b</sup> Commission International D’Edairerage (CIE) L*a*b* where b* = yellowness on a 0-100 white scale.<br>
<sup>c</sup> DAY = 0, 14, 28, 56, 90 day vacuum packaged samples held at 0-2 ºC.<br>
<sup>d</sup> SEM = Standard error for DAY for the combined means of TRT for no-nitrite-added and nitrite-added EFSC sausages.<br>
<sup>e</sup> Treatment combinations: TRT 1=low VJP + short MIN-HOLD; TRT 2=low VJP + long MIN-HOLD; TRT 3=high VJP + short MIN-HOLD; TRT 4=high VJP + long MIN-HOLD; C=156 ppm (mg/kg) sodium nitrite.<br>
<sup>f</sup> SEM = Standard error for TRT for the combined means of DAY for no-nitrite-added and nitrite-added EFSC sausages.<br>
<sup>g-o</sup> Means within same row with different superscripts are different (P<0.05).<br>
<sup>n-s</sup> Means within same column with different superscripts are different (P<0.05).
TABLE 6: Least squares means for the interaction of treatment combination (TRT 1-4, C) with storage time (Day 0, 14, 28, 56, 90) for internal surface reflectance ratio (R/ratio)\(^a\) of no-nitrite-added (TRT 1-4) and nitrite-added control (C) EFSC sausages.

<table>
<thead>
<tr>
<th>TRT(^b)</th>
<th>DAY(^c)</th>
<th>0</th>
<th>14</th>
<th>28</th>
<th>56</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(q1.92^e)</td>
<td>(p2.08^f)</td>
<td>(n2.13^f)</td>
<td>(n2.16^f)</td>
<td>2.19(^f)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>(n^p2.37)</td>
<td>(o^p2.25)</td>
<td>(n^o2.27)</td>
<td>(n^o2.24)</td>
<td>2.26</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>(o^q2.01^e)</td>
<td>(n^p2.13^ef)</td>
<td>(n^o2.17^f)</td>
<td>(n^o2.21^f)</td>
<td>2.21(^f)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>(p2.37)</td>
<td>(o2.35)</td>
<td>(o^o2.30)</td>
<td>(n^o2.27)</td>
<td>2.30</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>(p2.43)</td>
<td>(o2.37)</td>
<td>(o2.34)</td>
<td>(o2.34)</td>
<td>2.35</td>
<td></td>
</tr>
</tbody>
</table>

SEM\(^d\) = 0.03

\(^a\) Cured meat color measurement by reflectance ratio of wavelengths 650/570 nm where 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.

\(^b\) Treatment combinations: TRT 1=low VJP + short MIN-HOLD; TRT 2=low VJP + long MIN-HOLD; TRT 3=high VJP + short MIN-HOLD; TRT 4=high VJP + long MIN-HOLD; C=156 ppm (mg/kg) sodium nitrite.

\(^c\) DAY = 0, 14, 28, 56, 90 day vacuum packaged samples held at 0-2 \(^\circ\)C.

\(^d\) SEM = Standard error of the means for no-nitrite-added and nitrite-added EFSC sausages.

\(^e-f\) Means within same row with different superscripts are different (P<0.05).

\(^n-d\) Means within same column with different superscripts are different (P<0.05).
“excellent cured color” rating according to cured color intensity ratings outlined by Hunt and others (1991). This would indicate that length of incubation has more effect on cured meat measurements of reflectance ratio than VJP level.

**Proximate Composition**

Proximate composition for EFSC sausages (Appendix 6) for moisture ranged from 61.36 to 61.98% (standard error of the means = 0.11), and C was significantly (P<0.05) higher in moisture than TRTs 2 and 3 (data not shown). Fat ranged from 21.09 to 21.65% (standard error of the means = 0.16) and protein ranged from 13.24 to 13.54% (standard error of the means = 0.23). These results show that treatment combinations were uniform in proximate composition.

**pH Determination and TBARS Analysis**

No significant differences were observed for the treatment*day interaction for pH, however, the main effect of day was significant (P<0.05). Combined least squares means of combination treatments for pH are displayed in Table 5. Day 0 showed significantly (P<0.05) lower pH values than days 14, 22, 56 and 90. Actual changes in pH were small so the overall importance relative to this work should be minimal because a change of pH in these products over time was not expected.

No significant differences were observed for any interaction or main effects for lipid oxidation measured by TBARS. Least squares means of treatment*day interaction for TBARS values ranged between 0.208 and 0.285 which are well below detectable levels for lipid oxidation. A TBARS value of 0.5 to 1.0 is considered to be the threshold for oxidized odor and 1.0 to 2.0 for oxidized flavor (Tarladgis and others 1960). TBARS values were low for the control, as expected, because sodium
Nitrite was added. Nitrite has been shown to be an effective antioxidant (Shahidi and others 1991). Higher values and greater differences for TBARS values, especially over time, were expected for TRTs 1, 2, 3 and 4 because sodium nitrite was not added. These expectations, however, were not realized suggesting that TRTs containing VJP were comparable to the sodium nitrite control for lipid oxidation over time.

**Total and Cured Pigment Analysis**

Least squares means for combined treatment combinations of total pigments are reported in Table 2. Analysis for total pigments revealed that concentrations decreased over time. Because the measurements were conducted on different days (0, 14, 28, 56 and 90) error may have been introduced into the testing procedures because total pigment concentration would not be expected to change with time (day).

A significant (P<0.05) difference for the interaction of treatment*day was observed for cured pigment concentration (Table 7). TRTs 2, 4 and C had significantly (P<0.05) greater cured pigment concentrations at days 0, 14 and 28 than TRTs 1 and 3 and slightly, though not significantly (P>0.05) greater at days 56 and 90. However, no differences between TRTs 2, 4 and C were found at any day. Trends indicated that as incubation times increased, cured pigment concentrations also increased regardless of VJP level. Thus, the level of formulated VJP does not appear to be as important as the amount of time allowed for the VJP nitrate-to-nitrite conversion to result in cured pigment development. Trends also showed that cured
TABLE 7: Least squares means for the interaction of treatment combination (TRT 1-4, C) with storage time (Day 0, 14, 28, 56, 90) for cured pigment$^a$ of no-nitrite-added (TRT 1-4) and nitrite-added control (C) EFSC sausages.

<table>
<thead>
<tr>
<th>TRT$^b$</th>
<th>DAY$^c$</th>
<th>0</th>
<th>14</th>
<th>28</th>
<th>56</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>q76.4$^g$</td>
<td>q93.8$^e$</td>
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<td>n97.1$^{ef}$</td>
<td>101.9$^f$</td>
</tr>
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<td>np108.1</td>
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<td>no105.9</td>
<td>107.9</td>
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<td></td>
<td>oq81.9$^e$</td>
<td>oq97.7$^f$</td>
<td>nq100.4$^f$</td>
<td>no101.4$^f$</td>
<td>103.5$^f$</td>
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<td>p105.0</td>
<td>p107.6</td>
<td>nq108.1</td>
<td>o106.6</td>
<td>108.7</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>p108.4</td>
<td>p110.0</td>
<td>oq110.1</td>
<td>o109.5</td>
<td>110.2</td>
</tr>
</tbody>
</table>

SEM$^d$ = 1.68

$^a$ Cured pigment (nitrosylhemochrome) analysis.

$^b$ Treatment combinations: TRT 1=low VJP + short MIN-HOLD; TRT 2=low VJP + long MIN-HOLD; TRT 3=high VJP + short MIN-HOLD; TRT 4=high VJP + long MIN-HOLD; C=156 ppm (mg/kg) sodium nitrite.

$^c$ DAY = 0, 14, 28, 56, 90 day vacuum packaged samples held at 0-2 °C.

$^d$ SEM = Standard error of the means for no-nitrite-added and nitrite-added EFCS sausages.

$^{e-g}$ Means within same row with different superscripts are different (P<0.05).

$^{n-s}$ Means within same column with different superscripts are different (P<0.05).
pigment development generally increased over time for TRTs 1, 2, 3 and 4. This may be explained by residual nitrate present in VJP formulated TRTs with residual nitrates serving as a reservoir for nitrite-related reactions during storage.

*Residual Nitrite Analysis*

Residual nitrite in EFSC sausages was determined at pre-incubate, post-incubate and throughout a 90-day storage period (Table 8). The control compared to all TRTs (1-4) was significantly (P<0.001) higher in residual nitrite at pre-incubate and no residual nitrite was detected in any TRTs (1-4). At post-incubate, all TRTs contained residual nitrite and all TRTs had different (P<0.05) amounts except for TRTs 1 and 3. From this table, the importance of incubation time for the conversion of nitrate to nitrite is clear. As incubation time was increased, residual nitrite levels also increased. It should be noted that residual nitrite was not measured for the control at post-incubate since no incubation step was applied to the control.

Significant (P<0.05) interactions of treatment*day (time) for treatment combinations were present for residual nitrite and are found in Table 9. As expected, residual nitrite levels diminished over time for all treatment combinations. This observation has been well documented by Jantawat and others (1993) who found a decreasing residual nitrite level with increased storage time relationship and by Hustad and others (1973) who reported that nitrite concentration was affected by both storage time and storage temperature. Since storage temperature was held constant in this study, storage time would be believed to be the principle factor in nitrite concentration decreases. Another explanation was suggested by Ahn and


TABLE 8: Least squares means for the main effect of treatment combination (TRT 1-4, C) for residual nitrite and nitrate attributes during the manufacture of no-nitrite-added (TRT 1-4) and nitrite-added control (C) EFSC sausages.

<table>
<thead>
<tr>
<th>TRT&lt;sup&gt;a&lt;/sup&gt;</th>
<th>PPM Residual Nitrite&lt;sup&gt;b&lt;/sup&gt;</th>
<th>PPM Residual Nitrate&lt;sup&gt;c&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Incubate&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Post-Incubate&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
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<td>5.6&lt;sup&gt;lij&lt;/sup&gt;</td>
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<td>7.7&lt;sup&gt;l&lt;/sup&gt;</td>
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<td>0&lt;sup&gt;h&lt;/sup&gt;</td>
<td>46.0&lt;sup&gt;g&lt;/sup&gt;</td>
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<tr>
<td>C</td>
<td>59.1&lt;sup&gt;g&lt;/sup&gt;</td>
<td>NA</td>
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<tr>
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<td>1.22</td>
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</table>

<sup>a</sup>Treatment combinations: TRT 1=low VJP + short MIN-HOLD; TRT 2=low VJP + long MIN-HOLD; TRT 3=high VJP + short MIN-HOLD; TRT 4=high VJP + long MIN-HOLD; C=156 ppm (mg/kg) sodium nitrite.

<sup>b</sup>Residual nitrite determination reported in ppm (mg/kg) of sample.

<sup>c</sup>Residual nitrate determination reported in ppm (mg/kg) of sample.

<sup>d</sup>Pre-Incubate = Samples collected randomly during stuffing and before incubation.

<sup>e</sup>Post-Incubate = Samples collected randomly after incubation (MIN-HOLD) prior to cooking.

<sup>f</sup>SEM = Standard error of the means for TRT for no-nitrite-added and nitrite-added EFSC sausages.

<sup>g-j</sup>Means within same column with different superscripts are different (P<0.05).
TABLE 9: Least squares means for the interaction of treatment combination (TRT 1-4, C) with storage time (Day 0, 14, 28, 56, 90) for residual nitrite (ppm)\(^a\) of no-nitrite-added (TRT 1-4) and nitrite-added control (C) EFSC sausages.

<table>
<thead>
<tr>
<th>TRT(^b)</th>
<th>DAY(^c)</th>
<th>0</th>
<th>14</th>
<th>28</th>
<th>56</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.1(^e)</td>
<td>10.6(^f)</td>
<td>8.8(^{fh})</td>
<td>4.9(^{gh})</td>
<td>4.9(^{gh})</td>
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</tr>
<tr>
<td>2</td>
<td>21.7(^e)</td>
<td>17.2(^f)</td>
<td>12.0(^{gh})</td>
<td>9.1(^{h})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>21.3(^e)</td>
<td>16.6(^f)</td>
<td>12.9(^{fh})</td>
<td>9.9(^{gh})</td>
<td>8.5(^g)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>58.5(^e)</td>
<td>44.3(^f)</td>
<td>33.1(^g)</td>
<td>22.3(^{h})</td>
<td>16.3(^l)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>46.9(^e)</td>
<td>31.4(^f)</td>
<td>22.0(^{g})</td>
<td>12.4(^{hi})</td>
<td>8.7(^i)</td>
<td></td>
</tr>
</tbody>
</table>

SEM\(^d\)= 1.02

\(^a\) Residual nitrite determination reported in ppm (mg/kg) of sample.
\(^b\) Treatment combinations: TRT 1=low VJP + short MIN-HOLD; TRT 2=low VJP + long MIN-HOLD; TRT 3=high VJP + short MIN-HOLD; TRT 4=high VJP + long MIN-HOLD; C=156 ppm (mg/kg) sodium nitrite.
\(^c\) DAY = 0, 14, 28, 56, 90 day vacuum packaged samples held at 0-2 \(^\circ\)C.
\(^d\) SEM = Standard error of the means for no-nitrite-added and nitrite-added EFSC sausages.
\(^e\)\(^i\) Means within same row with different superscripts are different (P<0.05).
\(^n\)\(^r\) Means within same column with different superscripts are different (P<0.05).
others (2002) who noted packaging effects in sausage samples stored in vacuum packages vs. aerobic packages. These authors reported that vacuum packaged sausages had lower residual nitrite than samples stored in aerobic conditions. The authors suggested that this phenomenon was caused by the product environment being in the reduced state thus allowing the conversion of nitrite to nitric oxide and resulting in the lower residual nitrite levels found.

Within each pair of treatment combinations (TRT 1 & 2; TRT 3 & 4) where VJP level was held constant, residual nitrite levels were significantly (P<0.05) higher when the incubation time increased (Table 9). This pattern occurred at all days over the 90-day storage time. Also, TRT 4 resulted in higher (P<0.05) residual nitrite values than the control on all days. This indicates that either the VJP concentration of 0.40% resulted in a greater amount of nitrite converted from nitrate during 120 min of incubation compared to the nitrite-added control or a higher proportion of the nitrite in the control was reacted through curing reactions resulting in the low C nitrite values observed.

Residual Nitrate Analysis

Residual nitrate in EFSC sausages was also determined at pre-incubate, post-incubate and throughout a 90-day storage period. Least squares means for pre-incubate and post-incubate are reported in Table 8. TRTs 1 & 2 and TRTs 3 & 4 were formulated with VJP levels of 0.20% and 0.40%, respectively. Pre-incubate values for TRTs 1 & 2 or TRTs 3 & 4 for nitrate would then be expected to be very similar. Both TRT 1 and TRT 2 were significantly (P<0.05) lower in residual nitrate than TRT 3 and TRT 4, while the control was different (P<0.05) than all TRTs.
Overall, residual nitrate differences were not surprising. Also, residual nitrate was detected in the control to which only nitrite was added. Cassens and others (1979) suggest that a portion of nitrite added to meat during the curing process is actually converted to nitrate.

Post-incubate values for all TRTs for residual nitrate are significantly (P<0.05) different from one another. Again, residual nitrate was not measured in C because it was not incubated. Post-incubate values for all TRTs were lower than pre-incubate values indicating that nitrate to nitrite conversion occurred (Table 8). As incubation time was increased (30 to 120 min) post-incubate residual nitrate levels were significantly (P<0.05) lower. These values along with the presence of residual nitrite in TRTs at post-incubation signify that conversion of nitrate to nitrite occurred. As residual nitrate for each TRT decreased from pre-incubate to post-incubate, residual nitrite increased. These results show that nitrate available in VJP was reduced to nitrite by the starter culture containing \textit{S. carnosus}. Although this action can be accomplished by natural microorganisms found in the natural flora of the meat (Sanz and others 1997; Sebranek 1979; Pinotti and others 2001; MacDougall and others 1975), it can also be more effectively accomplished by intentional addition of microorganisms with nitrate-reducing properties (Sanz and others 1997). This additional reduction step in the curing reaction is critical and necessary for a curing system with nitrate-containing VJP.

A significant (P<0.05) difference for the interaction of treatment*day was also observed for residual nitrate and the corresponding values are found in Table 10. As incubation time increased, and VJP level remained constant, significantly
TABLE 10: Least squares means for the interaction of treatment combination (TRT 1-4, C) with storage time (Day 0, 14, 28, 56, 90) for residual nitrate (ppm)\(^a\) of no-nitrite-added (TRT 1-4) and nitrite-added control (C) EFSC sausages.

<table>
<thead>
<tr>
<th>TRT(^b)</th>
<th>DAY(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>pq25.8(^e)</td>
</tr>
<tr>
<td>2</td>
<td>r9.3</td>
</tr>
<tr>
<td>3</td>
<td>n73.6(^e)</td>
</tr>
<tr>
<td>4</td>
<td>oqr12.2</td>
</tr>
<tr>
<td>C</td>
<td>p33.0</td>
</tr>
</tbody>
</table>

SEM\(^d\) = 2.67

\(^a\) Residual nitrate determination reported in ppm of sample.
\(^b\) Treatment combinations: TRT 1=low VJP + short MIN-HOLD; TRT 2=low VJP + long MIN-HOLD; TRT 3=high VJP + short MIN-HOLD; TRT 4=high VJP + long MIN-HOLD; C=156 ppm (mg/kg) sodium nitrite.
\(^c\) DAY = 0, 14, 28, 56, 90 day vacuum packaged samples held at 0-2 °C.
\(^d\) SEM = Standard error of the means for no-nitrite-added and nitrite-added EFSC sausages.
\(^e-f\) Means within same row with different superscripts are different (P<0.05).
\(^n-r\) Means within same column with different superscripts are different (P<0.05).
(P<0.05) less residual nitrate was present at all days for the corresponding TRTs (TRT 1 vs. 2; TRT 2 vs. 4). As could be expected, TRT 3 revealed the highest residual nitrate value because TRT 3 was comprised of a high VJP level and a low incubation time resulting in less nitrate-to-nitrite conversion. As with pre-incubate measurements, the control showed residual nitrate values at each measured day over the 90-day storage period.

Several researchers have reported the presence of nitrate in products of which only nitrite was added. Pérez-Rodríguez and others (1996), monitoring nitrite and nitrate in frankfurters, reported that in nitrite-cured, cooked and packaged frankfurters, approximately 50% of ingoing nitrite was present while about 10-15% of the added nitrite was found as nitrate. Interestingly, one theory is that a secondary oxidation involving nitrous acid could be involved in the conversion of nitrite to nitrate. This theory is also supported by Dethmers and Rock (1975) who proposed nitrate formation from the simultaneous oxidation-reduction of nitrous acid to yield nitric oxide and nitrate and from the oxidation of nitric acid by oxygen to yield nitrite which could subsequently react with water to yield nitrite and nitrate. Interestingly, little change in residual nitrate level over time for the TRTs or C was observed and may be explained by the previous comments. This is further supported by noting that residual nitrite levels generally decreased over time while residual nitrate levels generally remained constant. Research on nitrite to nitrate reactions performed by Hustad and others (1973) could possibly explain the constant nitrate levels found in this study. Instead of both residual nitrite and nitrate levels diminishing over time, a
portion of nitrite may have been continually converted to nitrate, helping to maintain the levels of nitrate observed.

The residual nitrate of VJP was measured and found to be 27,462 ppm (mg/kg) \((n=3)\) or 2.75% of the VJP \((w/w)\). Therefore, formulation nitrate (when added to bowl cutter) was approximately 69 ppm for TRTs 1 & 2 and 139 ppm for TRTs 3 & 4. Hustad and others (1973) discussed loss of nitrite during processing and reported an average nitrite reduction of 16% after the meat was added to the bowl cutter. Similar trends were found in our results for both residual nitrate (Table 8 & 10) and nitrite (Table 8 & 9) indicating that both nitrite and nitrite were either physically lost during the manufacturing process or unavailable (in reactive form) for measurement due to involvement in curing reactions.

Besides storage time and temperature, pH has also been suggested to affect residual nitrite and nitrate levels. Sebranek (1979) noted the importance of pH on residual nitrite and indicated that a pH decrease as little as 0.2 pH units during manufacture can result in a doubling in the rate of color formation due to more favorable nitrite-myoglobin interactions. This effect of pH is supported by Kilic and others (2001) and Prusa and Kregel (1985). However, no differences in pH between any treatment combination was found in the present study which indicated that pH did not play a role in differences of residual nitrite and nitrate found or for any observed differences in cured characteristics.

*Trained Sensory Panel*

Trained sensory analysis was performed on day 14 following product manufacture. Significant differences \((P<0.05)\) for the main effects of treatment
TABLE 11: Least squares means for sensory attributes of cured frankfurter aroma (cured aroma), internal cured frankfurter color (cured color), uniformity of internal frankfurter color (Uniform Color), cured frankfurter flavor (cured flavor) and firmness for no-nitrite-added (TRT 1-4) and nitrite-added control (C) EFSC sausages.

<table>
<thead>
<tr>
<th>SENSORY ATTRIBUTES^b</th>
<th>Cured Aroma</th>
<th>Cured Color</th>
<th>Uniform Color</th>
<th>Cured Flavor</th>
<th>Firmness</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8.41</td>
<td>7.76^f</td>
<td>8.22^f</td>
<td>8.10^e</td>
<td>7.05^e</td>
</tr>
<tr>
<td>2</td>
<td>8.77</td>
<td>9.33^de</td>
<td>9.62^g</td>
<td>8.66^e</td>
<td>7.69^e</td>
</tr>
<tr>
<td>3</td>
<td>8.12</td>
<td>8.42^df</td>
<td>9.06^g</td>
<td>8.57^e</td>
<td>7.62^e</td>
</tr>
<tr>
<td>4</td>
<td>8.45</td>
<td>9.16^d</td>
<td>9.69^g</td>
<td>9.01^de</td>
<td>8.02^e</td>
</tr>
<tr>
<td>C</td>
<td>9.33</td>
<td>10.47^e</td>
<td>10.05^g</td>
<td>9.93^d</td>
<td>9.35^d</td>
</tr>
<tr>
<td>SEM^c</td>
<td>0.40</td>
<td>0.43</td>
<td>0.48</td>
<td>0.38</td>
<td>0.43</td>
</tr>
</tbody>
</table>

^a Treatment combinations: TRT 1=low VJP + short MIN-HOLD; TRT 2=low VJP + long MIN-HOLD; TRT 3=high VJP + short MIN-HOLD; TRT 4=high VJP + long MIN-HOLD; C=156 ppm (mg/kg) sodium nitrite.

^b SENSORY ATTRIBUTES = Trained panel scores using a line scale (numerical value of 15 units) with graduations from 0 to 15 where 0 represented none (aroma, flavor), low (cured color) not uniform (uniformity of color) and soft (firmness) and 15 represented intense (aroma, flavor), high (cured color), uniform (uniformity of color) and hard (firmness).

^c SEM = Standard error of the means for sensory attributes for no-nitrite-added and nitrite-added EFSC sausages.

^d-j Means within same column with different superscripts are different (P<0.05).
combinations for sensory attributes are reported as least squares means in Table 11. The control revealed the highest scores for all sensory attributes. No off-flavor/aroma or vegetable aroma/flavor attributes were included in the sensory evaluation because sensory training did not indicate any objectionable flavors or aromas present in the products used to develop the descriptive terms for the sensory ballot. This may have been due to the frankfurter spices used which could provide a predominant aroma/flavor and result in a flavor masking in the EFSC sausages. No differences for cured aroma were found between any treatment combinations but trends indicated that increasing incubation time improved cured aroma sensory scores.

The control had a significantly ($P<0.05$) higher score for cured color than TRTs 1, 3 and 4. TRT 1 was significantly ($P<0.05$) lower for cured color than TRTs 2, 4 and C, indicating that visual cured color was affected by incubation time and VJP level. For uniformity of color, TRT 1 was different ($P<0.05$) than TRTs 2, 4 and C while no differences ($P>0.05$) were observed between TRT 1 and TRT 3 or between TRTs 2, 3, 4 and C. No differences ($P>0.05$) for cured flavor were found between any TRTs, however C had a higher ($P<0.05$) score for cured flavor than all TRTs. Additionally, C was firmer ($P<0.05$) than all TRTs. It is unclear why these differences were found unless sensory perception of firmness was affected by the other sensory attributes. However, nitrite-curing reactions have been suggested to increase firmness (Pegg and Shahidi 2000).
Conclusions

Treatment combinations containing vegetable juice powder (VJP) and starter culture containing *S. carnosus* were shown to be comparable to a sodium nitrite-added control for color, lipid oxidation, cured pigment and trained sensory measurements. No differences in TBARS values between any treatment*day combination were observed and all values were below the detectable threshold of lipid oxidation, indicating that all treatment combinations were effective in controlling lipid oxidation. Differences in color, cured pigment, residual nitrate and residual nitrite measurements for the TRTs validated that curing reactions occurred. Incubation time was found to be a more critical factor than VJP concentration for cured meat properties similar to the control for objectively measured attributes. This was also the case for sensory results; however, sensory differences were not as definitive as the objective measurements suggesting that differences may not be as easily detected by consumers. It is worthwhile to note that the sodium nitrite-added control had the highest sensory scores for all attributes measured although differences were not significant in all cases.

The ingoing nitrate levels of 69 ppm (TRTs 1 & 2) and 139 ppm (TRTs 3 & 4) as used in this study would result in less nitrite than the USDA FSIS maximum allowable limit of 156 ppm nitrite, even if the nitrate was 100% converted. Since USDA FSIS requires a minimum of 120 ppm nitrite in cured meat products labeled “Keep Refrigerated” (USDA 1995), the low ingoing levels found in this study could result in microbiological concerns, specifically relative to *C. botulinum* survival and outgrowth.
The VJP used in this study was an effective replacement for sodium nitrite at the tested levels for the manufacture of uncured, no-nitrate/nitrite-added EFSC sausages. At the VJP concentrations (0.20% and 0.40%) tested, a longer incubation time (120 min) was found more critical than the VJP concentration for results comparable to a sodium nitrite-added control.

Further research regarding additional increased or decreased incubation times and VJP levels is needed to generate a better understanding of the relationship and effects, especially at higher VJP concentrations, that they may have on the quality characteristics of EFSC sausages. Further research regarding the effects of this technology on microbiological control and shelf life of these products at the concentrations tested in this study is also needed.

**Acknowledgement**

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References


CHAPTER 5. EFFECTS OF VARYING LEVELS OF VEGETABLE JUICE POWDER AND INCUBATION TIME ON COLOR, RESIDUAL NITRATE AND NITRITE, PIGMENT, pH AND TRAINED SENSORY ATTRIBUTES OF READY-TO-EAT UNCURED HAM

A paper submitted to the Journal of Food Science

Jeffrey J. Sindelar, Joseph C. Cordray, Joseph G. Sebranek, Jane A. Love and Dong U. Ahn

Abstract

Vegetable juice powder (VJP) and a starter culture containing Staphylococcus carnosus have been identified as necessary ingredients for the manufacture of un cured, no-nitrate/nitrite-added meat products with quality and sensory attributes similar to traditional cured products. The objectives of this study were to determine the effects of varying concentrations of VJP and incubation time (MIN-HOLD) on quality characteristics including lipid oxidation, color and cured meat pigment concentrations of ham over a 90-day storage period, compare residual nitrate and nitrite content, and determine if differences exist in sensory properties of finished products. Four ham treatments (TRT) (TRT1: 0.20% VJP, 0 MIN-HOLD; TRT2: 0.20% VJP, 120 MIN-HOLD; TRT3: 0.35% VJP, 0 MIN-HOLD; TRT4: 0.35% VJP, 120 MIN-HOLD) and a sodium nitrite-added control (C) were used for this study. No differences (P>0.05) were observed between TRTs and C for CIE L*, a*, b* and cured color measured by reflectance ratio. Lipid oxidation as measured by 2-thiobarbituric acid reactive substances (TBARS) for combined TRTs and C revealed little change over time while the C had less (P<0.05) lipid oxidation than TRTs 2 and 4 for combined days. No differences (P>0.05) were reported for cured pigment
concentration between TRTs and C. Trained sensory panel intensity ratings for ham and vegetable aroma, and flavor, color and firmness showed that a high concentration (0.35%) of VJP resulted in the highest scores for undesirable vegetable aroma and flavor. Treatment combinations with a low concentration (0.20%) of VJP were comparable to a sodium nitrite-added control for all sensory attributes.

Keywords: uncured, residual nitrate, residual nitrite, vegetable juice powder, ham

**Introduction**

Meat curing is defined as the addition of salt, sugar or other sweetener, and nitrite into meat to develop distinctive color, flavor and texture properties while aiding in the quality and microbiological aspects of meat products (Pearson and Tauber 1984; Aberle and others 2001). It is understood and well accepted that impurities in natural salt led to the discovery of modern day meat curing (MacDougall and others 1975). Meat curing has traditionally been associated with processed meats for the purpose of altering the color, texture, flavor, safety and shelf life characteristics which makes these products unique compared to other meat products (Sebranek and Fox 1985).

Today, meat curing is utilized to achieve consumer demands for products that have unique sensory characteristics and convenience attributes associated with cured meats. Nitrite is responsible for the development of cured color and flavor, serves as a strong antioxidant to protect flavor and acts as a strong antimicrobial to control *Clostridium botulinum* outgrowth (Shahidi and Pegg 1992). Nitrite controls
and stabilizes the oxidative states of lipids in meat products (Shahidi and Hong 1991) thus preventing lipid oxidation and subsequent warmed-over flavors (Vasavada and Cornforth 2005; Yun and others 1987).

Recently, increasing consumer interest for natural, organic, preservative-free and healthier foods have prompted consumer demands for the availability of uncured, no-nitrate/nitrite-added meat and poultry products. However, to date, no replacement for nitrite has been discovered that effectively produces the characteristic cured meat aroma and flavor of meat products in which it is used (Gray and others 1981). Ham samples with no added nitrite were disliked by a consumer sensory panel compared to nitrite-added ham samples (Froehlich and others 1983). Brown and others (1974) discovered that hams cured without nitrite had lower flavor intensity sensory scores (P<0.05) than hams cured with either 91 or 182 ppm nitrite.

Two classifications of uncured, no-nitrate/nitrite-added meat and poultry products currently exist in the market place: those that do not include nitrate or nitrite (uncured products), and those with the intention of replacing nitrate and nitrite to simulate typical curing. In order to manufacture cured products without the direct addition of sodium nitrite, a nitrate source and reducer must be utilized. Vegetables are well known to contain significant amounts of nitrate (Walker 1990; Fujihara and others 2001). Nitrate, however, is not a reactive species for curing reactions and must be first reduced to nitrite to enter into curing reactions. Nitrate-to-nitrite reduction can be accomplished by microorganisms found in the natural flora of meat
or by intentional addition of microorganisms with nitrate-reducing properties (Sanz and others 1997).

Therefore, the objectives of this research were to first determine the effects of varying concentrations of commercial vegetable juice powder and incubation time on quality characteristics including lipid oxidation, color and cured meat pigment concentrations of hams over an extended storage period, and secondly, to determine if differences exist in finished products as determined by trained sensory analysis. A third objective was to assess the effects of vegetable juice powder concentration and incubation time on nitrate and nitrite concentrations during product manufacture and over an ensuing storage period.

**Materials and Methods**

*Experimental Design and Data Analysis*

Varying concentrations of vegetable juice powder (VJP) and incubation (MIN-HOLD) times (0 or 120 min) for the manufacture of ham was investigated. Four ham treatments (TRT1: 0.20% VJP, 0 MIN-HOLD; TRT2: 0.20% VJP, 120 MIN-HOLD; TRT3: 0.35% VJP, 0 MIN-HOLD; TRT4: 0.35% VJP, 120 MIN-HOLD) and a sodium nitrite-added control (C) were used for this study. Statistical analysis was performed for all measurements using the Statistical Analysis System (version 9.1, SAS Institute Inc., Cary, N.C., U.S.A.) Mixed Model procedure (SAS Inst. 2003). The experimental design was a 2 (VJP level) x 2 (MIN-HOLD time) factorial design. The main plot consisted of 3 blocks (replication) and 5 ham treatments resulting in 15 observations for trained sensory and proximate composition. The model included
the fixed main effects of treatment and replication. The random effect was the interaction of treatment x replication.

Within the main factorial design was a split plot for measurements over time. The split plot contained 5 sampling periods (day 0, 14, 28, 56 and 90) and combined with the main plot resulted in a total of 75 observations for color, nitrite, nitrate, pH, cured pigment, total pigment and lipid oxidation. The model included the fixed main effects of treatment, replication, day and the interaction of treatment x day. The random effect was the interaction of treatment x replication.

The significant main effect means for all experiments were separated and least significant differences were found using Tukey-Kramer multiple pairwise comparison method. Significance level was determined at P<0.05. For all other experiments, main effects were tested for significance using a mixed effects model.

Ham - Product Procurement and Manufacture

Ready-to-eat ham was manufactured using fresh biceps femoris and semimembranosus with attached semitendinosus muscles obtained from a commercial slaughter/fabrication plant. The ham muscles were trimmed free of external fat and vacuum packaged prior to separation into five batches (11.34 kg each). Each batch consisted of approximately one-half biceps femoris muscles and one-half semimembranosus/semitendinosus randomly selected muscles. Treatments (TRT 1-4) and Control (C) were randomly assigned to the batches.

Three brines (Table 1) (Brine 1: TRT1, TRT2; Brine 2: TRT 3, TRT 4; Brine 3: C) were prepared according to treatment and control requirements. Salt and dextrose were held constant for all TRTs and C at ingoing injected levels of 2.75%
TABLE 1: Composition of brines used for the manufacture of no-nitrite-added (TRTs 1-4) and nitrite-added control (C) hams.

<table>
<thead>
<tr>
<th></th>
<th>Brine 1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Brine 2&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Brine 3&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>79.87</td>
<td>79.12</td>
<td>80.70</td>
</tr>
<tr>
<td>Salt</td>
<td>11.00</td>
<td>11.00</td>
<td>9.80</td>
</tr>
<tr>
<td>Dextrose</td>
<td>8.00</td>
<td>8.00</td>
<td>8.00</td>
</tr>
<tr>
<td>Vegetable Juice Powder</td>
<td>1.00</td>
<td>1.75</td>
<td>0.00</td>
</tr>
<tr>
<td>Starter Culture&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.1285</td>
<td>0.1285</td>
<td>0.00</td>
</tr>
<tr>
<td>Cure (6.25% Sodium Nitrite)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.00</td>
<td>0.00</td>
<td>1.28</td>
</tr>
<tr>
<td>Sodium Erythorbate&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.00</td>
<td>0.00</td>
<td>0.22</td>
</tr>
</tbody>
</table>

<sup>a</sup>Brine 1 used for TRTs 1 and 2 formulated for 0.20% vegetable juice powder of total batch.
<sup>b</sup>Brine 2 used for TRTs 3 and 4 formulated for 0.35% vegetable juice powder of total batch.
<sup>c</sup>Brine 3 used for nitrite added control (C). Salt was adjusted (1.20%) to compensate for salt included in cure mixture.
<sup>d</sup>Starter culture containing <i>Staphylococcus carnosus</i>.
<sup>e</sup>Cure mixture containing 6.25% sodium nitrite and 93.75% salt formulated for 200 mg/kg sodium nitrite.
<sup>f</sup>Sodium erythorbate added at 550 mg/kg.
and 2.00%, respectively. Concentrations of VJP (Vegetable Juice PWD NAT, Chr. Hansen Inc., Gainesville, Fla., U.S.A.) and starter culture (CS 299 Bactoferm™, Chr. Hansen Inc., Gainesville, Fla., U.S.A) for TRT brines were based on the total injected weight (meat block + added solution). Starter culture was added to TRTs at 35 g/kg of total injected weight. Concentrations of curing ingredients for the C, based on the total meat block, were 550 mg/kg sodium erythorbate and 200 mg/kg sodium nitrite. No phosphates were added to any TRTs or C because hams were intended to be similar to natural or organic products which restrict phosphate usage. TRTs and C fresh ham batches were injected using a multi-needle injector (Townsend injector, Model PI92-270, Townsend Engineering, Des Moines, IA., U.S.A) to 125% of green weight. After injection, additional brine was added or removed (by draining a portion of free brine) to achieve exactly 125% injection.

Ninety percent of the injected ham muscles were ground (Biro MFG Co. Marblehead, Ohio., U.S.A.) using a 2.54 cm plate and the remaining 10% were ground using a 4.76 mm plate. The ham mixtures (n=5) were then transferred to vacuum tumblers (DVTS Model 50, Daniels Food Equip. Inc., Parkers Prairie, MN., U.S.A) and were tumbled under vacuum continuously for 1 h to achieve adequate protein extraction and free brine pick-up. After tumbling was completed, the ham mixtures were transferred to a rotary vane vacuum-filling machine (Risco vacuum stuffer, Model RS 4003-165, Stoughton, Mass., U.S.A) and stuffed into 105.1 mm impermeable plastic casings (HC5 Clear, World Pac USA International Inc., Sturtevant, Wis., U.S.A.). The impermeable casings were used to control cross-contamination effects that any environmentally released nitric oxide gas could have
on the TRTs during thermal processing. The casings had an O₂ permeability rate of 12 cm³/m²/24 h at 1 atm and a water vapor permeability of 5 g/m²/24 h. TRTs and C were placed on separate smokehouse trucks based on incubation (MIN-HOLD) times (0 min or 120 min).

The stuffed hams were transferred to two single truck thermal processing ovens (Maurer, AG, Reichenau, Germany; Alkar, Model MT EVD RSE 4, Alkar Engineering Corp., Lodi, Wis., U.S.A). Incubation was conducted at 40.6 ºC dry bulb and 39.4 ºC wet bulb temperatures. MIN-HOLD times started when the internal temperature of the hams reached 37.8 ºC. Cooking was accomplished using a 60.0, 65.6, 71.1, 76.7 and 82.2 ºC ramped steam cook until an internal temperature of 70 ºC was achieved.

After thermal processing, hams were chilled for 10-12 h at 0-2 ºC. Intact hams were removed from casings, sliced (Bizerba Model SE12D Slicer, Bizerba GmbH & Co. KG., Balingen, Germany) to 2.5 and 20 mm slices and vacuum packaged using barrier bags (Cryovac B540, Cryovac Sealed Air Corp., Duncan, S.C., U.S.A.; Multivac Model A6800 vacuum packager, Multivac Inc., Kansas City, Mo., U.S.A.). The packaging film for the vacuum packaged slices had an O₂ transmission rate of 3-6 cc/m²/24 h at 1 atm, 4.4 ºC, and 0% RH, and a water vapor transmission rate of 0.5-0.6 g/645 cm²/24 h and 100% RH. The slicing and subsequent packaging of the samples were conducted in as little light as possible to minimize light-induced cured color fading.
Color Measurements

Color measurements were conducted using a Hunterlab Labscan spectrocolorimeter (Hunter Associated Laboratories Inc., Reston, Va., U.S.A.). The Hunterlab Labscan was standardized using the same packaging material as used on the samples, placed over the white standard tile. Values for the white standard tile were $X=81.72$, $Y=86.80$ and $Z=91.46$. Ham measurements were conducted while products were maintained in vacuum packaged conditions.

Illuminant A, $10^\circ$ standard observer with a 2.54 cm viewing area and 3.05 cm port size, was used to analyze internal surface color of ham samples. Commission International d’Eclairage (CIE) $L^*$ (lightness), $a^*$ (redness), and $b^*$ (yellowness) and cured meat color determined by reflectance ratio of wavelengths 650/570 nm (Hunt and others 1991; Erdman and Watts 1957) measurements were taken at 6 randomly selected areas on the samples ($n=2$) and the resulting average was used in data analysis (Hunt and others 1991).

Proximate Composition

Proximate composition was determined for the ham samples including crude fat (AOAC 1990), moisture (AOAC 1990a) and crude protein (AOAC 1993).

$\textit{pH}$ Determination

The pH of the ham samples was determined by blending the samples with distilled de-ionized water in a 1:9 ratio, then measuring the pH with a pH/ion meter (Accumet 925: Fisher Scientific, Fair Lawn, N.J., U.S.A.) equipped with an electrode (Accumet Flat Surface Epoxy Body Ag/AgCl combination Electrode Model 13-620-289, Fisher Scientific, Fair Lawn, N.J., U.S.A) calibrated with phosphate buffers 4.0
and 7.0, according to the method of Sebranek and others (2001). For each treatment, measurements were made in duplicate.

**TBARS Analysis**

Lipid oxidation was measured by the modified 2-thiobarbituric acid reactive substances (TBARS) test as described for cured meats (Zipser and Watts 1962). TBARS values were reported as mg of malonaldehyde equivalents/kg of meat sample. For each treatment, measurements were made in duplicate.

**Total and Cured Pigment Analysis**

Mononitrosylhemochrome (cured meat pigment) and total pigment concentrations were measured after extraction in 80% acetone and acidified acetone, respectively (Hornsey 1956). The experiment including sample preparation was done in subdued light, to reduce pigment fading. Samples were finely ground/chopped using a food processor (Sunbeam-Oskar Model 4817, Sunbeam Products Inc., Delray Beach, Fla., U.S.A.).

Cured pigment analysis was conducted using a modified method of Hornsey (1956). Duplicate 10 g samples were mixed with 40 ml of acetone and 3 ml of distilled de-ionized water with a Polytron mixer (PT 10/35, Kinematica GmbH, AG, Switzerland) for 1 min at speed setting 7. The sample was immediately filtered through a Whatman 42 filter paper, and the absorbance (540 nm) measured on the filtrate. Nitrosylhemochrome concentration was calculated as $A_{540} \times 290$ and was recorded in parts per million (ppm).
Total pigment analysis was conducted using a modified method of Hornsey (1956). The same finely ground/chopped samples used for cured pigment analysis were utilized for total pigment analysis. Duplicate 10 g samples were mixed with 40 ml of acetone, 2 ml of distilled de-ionized water and 1 ml of concentrated hydrochloric acid using a Polytron mixer (PT 10/35, Kinematica GmbH, AG, Switzerland) for 1 min at speed setting 7. The samples were allowed to stand for 1 h, then filtered through Whatman 42 filter paper and immediately analyzed. Absorbance was measured at 640 nm. Total pigment concentration was calculated as $A_{640} \times 680$ and was recorded in parts per million (ppm).

**Residual Nitrite Analysis**

Residual nitrite was determined by the AOAC method (AOAC 1990b). The same finely ground/chopped samples that were used for pigment analysis were also used for residual nitrite measurement. All residual nitrite assays were done in duplicate and all treatments within a block were analyzed at the same time to minimize variation in the analysis due to time.

**Residual Nitrate Analysis**

Sample preparation and nitrate determination methods were modifications of Ahn and Maurer (1987). Five grams of meat product samples were weighed in a 50-ml test tube and homogenized with 20 ml of distilled, de-ionized water (DDW) using a Polytron homogenizer (Type PT 10/35, Brinkmann Instruments Inc., Westbury, N.Y., U.S.A.) for 10 s at high speed. The homogenate was heated for 1 h in 80 °C water bath. After cooling in cold water for 10 min, 2.5 ml of the homogenate was transferred to a disposable test tube (16 x 100 mm). Carrez II (dissolve 10.6 g
potassium ferrocyanide in 100 ml DDW) and Carrez I (dissolve 23.8 g zinc acetate in 50 ml DDW, then add 3 ml glacial acetic acid and dilute to 100 ml with DDW) reagents were added (0.1 ml each) to precipitate proteins. The solution was diluted with 2.3 ml of DDW and mixed well. After precipitation, the supernatant was centrifuged at 10,000 x g for 20 min and the clear upper layer was used for nitrate measurement by high performance liquid chromatography (Agilent 1100 Series HPLC system, Agilent Technologies, Wilmington, Del., U.S.A.). The column used was Agilent Zorbax SAX (analytical 4.6 x 150mm, 5-micron) (Agilent, Wilmington, Del., U.S.A.) and the elution buffer was 15 mM phosphate buffer, pH 2.35, with isocratic elution. Flow rate was 1.0 ml/min and sample volume was 25 µL. The wavelength used was 210 nm. The area of nitrate peak was used to calculate nitrate concentration (ppm) using a nitrate standard curve.

*Trained Sensory Panel*

Hams were evaluated by a trained sensory panel for color, aroma, flavor and texture characteristics. Ten trained panelists, made up of Iowa State University students and staff, were used for each session. For training, three one-hour sessions were held using commercial and experimental products to develop descriptive terms for the desired attributes. Hams were evaluated for ham aroma, vegetable aroma, ham flavor, vegetable flavor, firmness and intensity of ham color.

Attributes were measured using a line scale (numerical value of 15 units) with graduations from 0 to 15 where 0 represented none (aroma, flavor and color intensity) and soft (firmness) and 15 represented intense (aroma, flavor, color), high (color) and hard (firmness).
Expectorant cups were provided to prevent taste fatigue and distilled de-ionized water and unsalted soda crackers were provided to clean the palate between samples. The presentation order was randomized for each session. A computer ballot was constructed and data was collected using a computerized sensory scoring system (COMPUSENSE five, Compusense, Inc. v.4.4, Guelph, Ontario, Canada, N1H3N4).

Refrigerated (3.3-5.6 ºC), sliced (2.5 mm) ham samples were evaluated by the panelists without reheating the samples, which would be characteristic for this product. Panelists were served one slice, 12 cm in diameter, of each treatment. The slice was cut into 2 quarter pieces and one half-piece, and presented to the panelists in a covered Styrofoam container. Panelists were asked to evaluate the intensity of ham flavor, vegetable flavor and firmness using the 2 quarter pieces and use the remaining half-piece to evaluate color intensity of the ham samples.

Results and Discussion

Ham Processing Attributes

Various product and processing parameters were measured during the manufacture of the hams (Appendix 7). The pH’s of ham brines used were as follows: brine 1 (TRTs 1 & 2) was 5.08, brine 2 (TRTs 3 & 4) was 4.88 and brine 3 (C) was 7.02. There were no differences (P>0.05) between brines 1 and 2, however, the pH of brine 3 was significantly (P<0.05) higher than brines 1 and 2. The average pH (n=3) of the water used for brine make-up was 9.07. Explanation of brine pH differences is unclear, but because all brine ingredients were held constant
except for the replacement of VJP and starter culture (TRT brines) with sodium nitrite and sodium erythorbate, it appears that VJP, having a pH 5.2, had an impact on brine pH’s. Phosphates were not included in the brines used for the TRTs and C. If included, phosphates would normally impact the brines by increasing the overall brine pH and subsequently injected product pH.

Raw ham muscles (inside and outside) were randomly selected for pH measurement. The pH of the raw ham muscles ranged between 5.66 and 5.92 and no significant (P>0.05) differences were found. Temperature and pH were measured after stuffing and before incubation (pre-incubate). Pre-incubate pH ranged from 5.50 to 5.60 while temperatures ranged between 7.3 and 7.8 ºC and no differences were found between any TRTs or C. The pH was also measured after the incubation step but prior to the cooking steps (post-incubate). These measurements were taken only for TRTs 2 and 4 because no incubation (0 min) was applied to TRTs 1, 3 or C. Post-incubate pH was 5.60 for TRT 2 and 5.53 for TRT 4 and no differences were found between the two treatments.

The time needed, at incubation temperatures, for the internal temperature of TRTs 2 and 4 to reach optimum conditions (37.8 ºC) ranged between 165 and 180 min. This difference in time was due to slight variation in stuffing temperatures between replications (n=3) as well as performance differences between the two thermal processing ovens. Total average thermal processing times (including come-up time to optimum incubation temperature) for TRTs 1, 3 and C was 215 min (no come-up time: 0 min incubation + cook) and for TRTs 2 and 4 was 493 min (come-
up time + 120 min incubation + cook). The diameter (105.1 mm) of the product was the primary factor in the relatively slow increase in temperature.

*Color Measurements*

The internal surface of ham slices (1.27 cm) was evaluated for CIE L*, a*, b* and cured meat color by reflectance ratio. No significant (P>0.05) interactions were found for any color measurements, but the main effect of time (day) was significantly (P<0.05) different for CIE L*, a*, b* and reflectance ratio (Table 2). For CIE L* values, ham slices were significantly (P<0.05) darker at day 0 than days 28, 56 or 90. The same ham slices were also redder (P<0.05) as confirmed by CIE a* values at day 0 and 14 compared to days 28, 56 and 90, while no differences were found between days 0 and 14. Significant differences (P<0.05) for CIE b* values were also observed but may not be meaningful because differences in values over time (day) were quite small. Cured meat color fading as indicated by measurement of reflectance ratios (Hunt and others 1991; Erdman and Watts 1957) revealed that the combined means of TRTs 1-4 and C had more (P<0.05) cured meat color at day 0 than at days 28, 56 or 90. However, cured meat color between days 0 and 14 was not found to be different. Over time cured meat color deceased as expected. It is worthy to mention that days 0 and 14 reflectance ratio values had a “excellent cured color” rating and days 28, 56 and 90 were above the “less intense but noticeable cured color” rating according to cured color intensity ratings outlined by Hunt and others (1991). These results indicate that some cured color was maintained over the 90-day period. Overall, color measurement values declined over time, as expected. If temperatures increase (Hustad and others 1973) or other packaging
TABLE 2: Least squares means\(^{a}\) for the main effects of time (Day 0, 14, 28, 56, 90) for objective color (L*, a*, b*)\(^{b}\), reflectance ratio (R/ratio)\(^{c}\) and total pigment\(^{d}\) of no-nitrite-added (TRT 1-4) and nitrite-added control (C) hams.

<table>
<thead>
<tr>
<th>DAY(^{e})</th>
<th>0</th>
<th>14</th>
<th>28</th>
<th>56</th>
<th>90</th>
<th>SEM(^{f})</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td>68.55(^{g})</td>
<td>69.64(^{gh})</td>
<td>70.02(^{h})</td>
<td>70.39(^{h})</td>
<td>70.74(^{h})</td>
<td>0.40</td>
</tr>
<tr>
<td>a*</td>
<td>18.37(^{g})</td>
<td>18.49(^{g})</td>
<td>17.59(^{h})</td>
<td>17.50(^{h})</td>
<td>17.26(^{h})</td>
<td>0.20</td>
</tr>
<tr>
<td>b*</td>
<td>13.92(^{ij})</td>
<td>14.15(^{gi})</td>
<td>13.69(^{hj})</td>
<td>13.54(^{hk})</td>
<td>13.29(^{k})</td>
<td>0.07</td>
</tr>
<tr>
<td>R/ratio</td>
<td>2.31(^{gi})</td>
<td>2.27(^{hi})</td>
<td>2.19(^{hk})</td>
<td>2.18(^{hl})</td>
<td>2.14(^{ijkl})</td>
<td>0.03</td>
</tr>
<tr>
<td>Total Pigment</td>
<td>70.1(^{g})</td>
<td>56.2(^{hk})</td>
<td>61.0(^{ijk})</td>
<td>63.6(^{gj})</td>
<td>64.2(^{gj})</td>
<td>1.85</td>
</tr>
</tbody>
</table>

\(^{a}\) Combined means of treatments 1-4 and control.  
\(^{b}\) Commission International D’Edairerage (CIE) L*a*b* where L* = lightness, a* = redness, and b* = yellowness on a 0-100 white scale.  
\(^{c}\) Cured meat color measurement by reflectance ratio of wavelengths 650/570 nm where 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.  
\(^{d}\) Total pigment analysis.  
\(^{e}\) DAY = 0, 14, 28, 56, 90 day vacuum packaged samples held at 0-2 °C.  
\(^{f}\) SEM = Standard error of the means for DAY for no-nitrite-added and nitrite-added hams.  
\(^{g}\) Means within same row with different superscripts are different (P<0.05).
methods are used (Møller and others 2003; Pexara and others 2002), these values would be expected to decrease more rapidly.

Proximate Composition

No significant (P>0.05) differences were observed between any treatment combinations for proximate composition (Appendix 8). Moisture ranged from 74.61 to 75.57% (standard error of the means = 0.44). Fat ranged from 2.13 to 3.47% (standard error of the means = 0.29) and protein ranged from 19.02 to 20.09% (standard error of the means = 0.30). These results show that treatment combinations were uniform in proximate composition.

pH Determination

A significant (P<0.05) difference for the interaction of treatment*day was observed for pH measurements (Table 3). TRT 1 pH was significantly (P<0.05) higher at day 0 than at day 14. This difference was unexpected as pH would not be anticipated to change post-thermal processing over time. Because different samples were utilized for pH determination on different days, error generated from sampling might explain some of the observed differences. The potential survival and slow growth of lactic acid-producing bacteria during and after product manufacture may also explain a decrease in pH over time. A significantly (P<0.05) lower pH was found at day 0 for TRT 2 compared to TRT 1, and at days 14, 56 and 90 for TRT 2 compared to C. Trends show that pH values were lower for TRTs with a longer incubation step (120 min) over time (day) regardless of VJP addition level and support the idea that the growth of lactic acid bacteria may be involved the pH differences reported. The pH results indicate that, regardless of VJP level,
### TABLE 3: Least squares means for the interaction of treatment combination (TRT 1-4, C) with storage time (Day 0, 14, 28, 56, 90) for pH\(^a\) of no-nitrite-added (TRT 1-4) and nitrite-added control (C) hams.

<table>
<thead>
<tr>
<th>TRT(^b)</th>
<th>DAY(^c)</th>
<th>0</th>
<th>14</th>
<th>28</th>
<th>56</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>6.41(^e)</td>
<td>6.20(^f)</td>
<td>6.30(^{ef})</td>
<td>6.28(^{ef})</td>
<td>6.27(^{ef})</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>6.16 (^n)</td>
<td>6.13</td>
<td>6.16 (^n)</td>
<td>6.12 (^n)</td>
<td>6.15 (^n)</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>6.33 (^{no})</td>
<td>6.26</td>
<td>6.28</td>
<td>6.27</td>
<td>6.21</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>6.20 (^{no})</td>
<td>6.22</td>
<td>6.24</td>
<td>6.27</td>
<td>6.28</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>6.31 (^{no})</td>
<td>6.36</td>
<td>6.32 (^o)</td>
<td>6.36 (^o)</td>
<td>6.40 (^o)</td>
</tr>
</tbody>
</table>

SEM\(^d\) = 0.04

\(^a\) pH of ham samples.
\(^b\) Treatment combination: TRT 1=low VJP + short MIN-HOLD; TRT 2=low VJP + long MIN-HOLD; TRT 3=high VJP + short MIN-HOLD; TRT 4=high VJP + long MIN-HOLD; C=200 ppm (mg/kg) sodium nitrite.
\(^c\) DAY = 0, 14, 28, 56, 90 day vacuum packaged samples held at 0-2 ºC.
\(^d\) SEM = Standard error of the means for no-nitrite-added and nitrite-added hams.
\(^e-f\) Means within same row with different superscripts are different (P<0.05).
\(^n-o\) Means within same column with different superscripts are different (P<0.05).
incubation affected the pH of cooked hams. Sebranek (1979) discussed the importance of pH on the residual nitrite level and stated that a pH decrease as little as 0.2 pH units during product manufacture can result in doubling the rate of color formation and other curing reactions. Several pH differences greater than 0.2 units were observed. However, pH's during processing (pre-incubate and post-incubate) were actually lower than the pH's of corresponding treatment combinations post-processing (Appendix 7 and Table 3). The pH differences at both pre-incubate and post-incubate between all treatment combinations were small (0.10 and 0.07, respectively). The pH of ham muscles decreased after injection with the treatment brines and larger pH differences (pre-inject vs. post-inject) were observed: TRT 1 = 0.24, TRT 2 = 0.11, TRT 3 = 0.23, TRT 4 = 0.17 and C = 0.32. Based on these differences, curing related reactions (color, pigment, nitrate, nitrite) of treatment combinations, especially TRTs 1, 3 and C, may have been accelerated by the pH change. However, treatment combinations with the same VJP level (TRT 1 & 2; TRT 3 &4) would be expected to have similar pH differences but this was not the case. The variation of the pH differences may be explained by a high buffering ability of ham muscles used or variation within and between the ham muscles.

**TBARS Analysis**

No significant differences were observed for any interaction but the main effects of treatment and day were significant (P<0.05) for lipid oxidation measured by TBARS (Table 4). Combined treatment combination means for the main effect of time show a decrease (P<0.05) in lipid oxidation between day 0 and day 56 as well
TABLE 4: Least squares means for the main effects of treatment combination (TRT 1-4, C) and storage time (Day 0, 14, 28, 56, 90) for lipid oxidation (TBARS)\(^a\) of no-nitrite-added (TRT 1-4) and nitrite-added control (C) hams.

<table>
<thead>
<tr>
<th>DAY(^c)</th>
<th>0</th>
<th>14</th>
<th>28</th>
<th>56</th>
<th>90</th>
<th>SEM(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (mg/kg)<strong>b</strong></td>
<td>0.2250(^{ij})</td>
<td>0.2275(^{hij})</td>
<td>0.2479(^{hij})</td>
<td>0.2107(^i)</td>
<td>0.2212(^{hij})</td>
<td>0.0134</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TRT(^e)</th>
<th>TBARS(^f) mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2441(^{no})</td>
</tr>
<tr>
<td>2</td>
<td>0.3044(^{n})</td>
</tr>
<tr>
<td>3</td>
<td>0.2380(^{no})</td>
</tr>
<tr>
<td>4</td>
<td>0.2463(^{n})</td>
</tr>
<tr>
<td>C</td>
<td>0.1296(^{o})</td>
</tr>
<tr>
<td>SEM(^g)</td>
<td>0.0238</td>
</tr>
</tbody>
</table>

\(^a\) 2-Thiobarbituric acid test reported as mg malonaldehyde/kg of sample.
\(^b\) TBARS means of combined TRTs (1-4 and C) for each DAY.
\(^c\) DAY = 0, 14, 28, 56, 90 day vacuum packaged samples held at 0-2 °C.
\(^d\) SEM = Standard error for Day for the combined means of TRT for no-nitrite-added and nitrite-added hams.
\(^e\) Treatment combination: TRT 1=low VJP + short MIN-HOLD; TRT 2=low VJP + long MIN-HOLD; TRT 3=high VJP + short MIN-HOLD; TRT 4=high VJP + long MIN-HOLD; C=200 ppm (mg/kg) sodium nitrite.
\(^f\) TBARS means of combined days of 0, 14, 28, 56 and 90 for each TRT and C.
\(^g\) SEM = Standard error for TRT for the combined means of DAY for no-nitrite-added and nitrite-added hams.
\(^h-j\) Means within same row with different superscripts are different (P<0.05).
\(^n-o\) Means within same column with different superscripts are different (P<0.05).
as from day 28 to day 56. A decrease in TBARS was unexpected, however, because the TBARS values were low, any error in the test or variation in the samples would be exaggerated. Between treatment combinations, the control had a significantly (P<0.05) lower TBARS value than TRT 2 or TRT 4 indicating that longer incubation times resulted in higher lipid oxidation. TRTs 2 and 4 both included 120 min of incubation in addition to the come-up time used to reach optimum incubation conditions. Favorable conditions for lipid oxidation were thus present for TRTs 2 and 4 and subsequently relative TBARS levels were observed. Although time (day) did not indicate that lipid oxidation progressively continued, it is worthy to mention as a possible hurdle to overcome for the success of this technology.

TBARS values were low for C, as expected, because sodium nitrite was added. Nitrite has been shown to be an effective antioxidant (Shahidi and others 1991). Higher values than observed and a greater number of differences between TRTs and days for TBARS values, especially over time, were expected for TRTs (1-4) because sodium nitrite was not directly added. These expectations were not realized, however, suggesting that TRTs containing VJP were comparable to the sodium nitrite control for limiting lipid oxidation over time.

Cured and Total Pigment Analysis

A significant (P<0.05) interaction of treatment*day was observed for cured pigment concentration as shown in Table 5. TRT 2 had a higher (P<0.05) cured pigment concentration at day 28 than day 14 and a lower (P<0.05) concentration at day 90 than day 28. No other differences were found between treatment combinations or time (day).
TABLE 5: Least squares means for the interaction of treatment combination (TRT 1-4, C) with storage time (Day 0, 14, 28, 56, 90) for cured pigment\(^a\) of no-nitrite-added (TRT 1-4) and nitrite-added control (C) hams.

<table>
<thead>
<tr>
<th>TRT(^b)</th>
<th>DAY(^c)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>14</td>
<td>28</td>
<td>56</td>
<td>90</td>
</tr>
<tr>
<td>1</td>
<td>35.9</td>
<td>33.7</td>
<td>35.5</td>
<td>34.6</td>
<td>31.5</td>
</tr>
<tr>
<td>2</td>
<td>41.3(^{efg})</td>
<td>34.7(^g)</td>
<td>48.0(^{0})</td>
<td>39.1(^{efg})</td>
<td>36.8(^{ig})</td>
</tr>
<tr>
<td>3</td>
<td>41.7</td>
<td>35.5</td>
<td>38.2</td>
<td>34.2</td>
<td>39.1</td>
</tr>
<tr>
<td>4</td>
<td>38.5</td>
<td>33.4</td>
<td>38.1</td>
<td>40.0</td>
<td>41.8</td>
</tr>
<tr>
<td>C</td>
<td>36.3</td>
<td>35.3</td>
<td>36.3</td>
<td>31.8</td>
<td>34.4</td>
</tr>
</tbody>
</table>

SEM\(^d\) = 2.33

\(^a\) Cured pigment (nitrosylhemochrome) analysis.
\(^b\) Treatment combination: TRT 1=low VJP + short MIN-HOLD; TRT 2=low VJP + long MIN-HOLD; TRT 3=high VJP + short MIN-HOLD; TRT 4=high VJP + long MIN-HOLD; C=200 ppm (mg/kg) sodium nitrite.
\(^c\) DAY = 0, 14, 28, 56, 90 day vacuum packaged samples held at 0-2 °C.
\(^d\) SEM = Standard error of the means for no-nitrite-added and nitrite-added hams.
\(^e-g\) Means within same row with different superscripts are different (P<0.05).
Over the length of the study, cured pigment concentrations for all treatment combinations increased and decreased with little or no explanation. The authors believe that this occurrence may be due to testing-induced error from non-uniformity of sample composition (varying proportion of inside and outside ham muscles in any given sample). Another explanation of the variance could be that residual nitrate present in TRTs was acting as a reservoir for nitrite-related reactions during storage, however, similar variation trends were also reported in the control.

No significant differences were observed for any interactions or main effects for total pigment measurements (Table 2) with the exception of day (P<0.05). Because the measurements were conducted on different days (day 0, 14, 28, 56 and 90) coupled with the previous mentioned variance possibility, error may have been introduced into the testing procedure because total pigment concentration would not be expected to change with time (day).

Residual Nitrite Analysis

Residual nitrite in hams was determined at pre-incubate, post-incubate and throughout a 90-day storage period (Table 6). At pre-incubate, C was the only treatment combination that contained nitrite. At post-incubate, TRT 4 had a significantly (P<0.05) higher amount of nitrite than TRT 2. Since TRTs 1, 3 and C did not have any incubation, post-incubate residual nitrite was not measured.

Significant (P<0.05) interactions of treatment*day (time) for treatment combinations were present for residual nitrite and are found in Table 7. As expected, residual nitrite levels diminished over time for all treatment combinations.
TABLE 6: Least squares means for the main effect of treatment combination (TRT 1-4, C) for residual nitrite and nitrate attributes during the manufacture of no-nitrite-added (TRT 1-4) and nitrite-added control (C) hams.

<table>
<thead>
<tr>
<th>TRT^a</th>
<th>PPM Residual Nitrite^b</th>
<th>PPM Residual Nitrate^c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Incubate^d</td>
<td>Post-Incubate^e</td>
</tr>
<tr>
<td>1</td>
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</tr>
<tr>
<td>2</td>
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</tr>
<tr>
<td>3</td>
<td>0.00^h</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>0.00^h</td>
<td>36.1^g</td>
</tr>
<tr>
<td>C</td>
<td>61.1^g</td>
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</tr>
<tr>
<td>SEM^f</td>
<td>1.16</td>
<td>0.97</td>
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^a Treatment combination: TRT 1=low VJP + short MIN-HOLD; TRT 2=low VJP + long MIN-HOLD; TRT 3=high VJP + short MIN-HOLD; TRT 4=high VJP + long MIN-HOLD; C=200 ppm (mg/kg) sodium nitrite.

^b Residual nitrite determination reported in ppm (mg/kg) of sample.

^c Residual nitrate determination reported in ppm (mg/kg) of sample.

^d Pre-Incubate = Samples collected randomly during stuffing and before incubation.

^e Post-Incubate = Samples collected randomly after incubation (MIN-HOLD) prior to cooking.

^f SEM = Standard error of the means for TRT for no-nitrite-added and nitrite-added hams.

^g-k Means within same column with different superscripts are different (P<0.05).
TABLE 7: Least squares means for the interaction of treatment combination (TRT 1-4, C) with storage time (Day 0, 14, 28, 56, 90) for residual nitrite (ppm)\(^a\) of no-nitrite-added (TRT 1-4) and nitrite-added control (C) hams.

<table>
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<tr>
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<th>56</th>
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<tr>
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<td></td>
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<td>(\text{n}23.6^{eh})</td>
<td>(\text{n}25.4^{eg})</td>
<td>(\text{n}19.4^{fg})</td>
<td>(\text{n}11.7^{l})</td>
</tr>
<tr>
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<td></td>
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<td>(\text{o}32.6^{eh})</td>
<td>(\text{o}32.8^{eg})</td>
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<td>(\text{o}21.3^{f})</td>
</tr>
<tr>
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<td></td>
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<td>(\text{p}61.1^{e})</td>
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<td>(\text{p}45.0^{l})</td>
<td>(\text{p}34.1^{g})</td>
</tr>
</tbody>
</table>

SEM\(^d\)= 1.72

\(^a\) Residual nitrite determination reported in ppm (mg/kg) of sample.

\(^b\) Treatment combination: TRT 1=low VJP + short MIN-HOLD; TRT 2=low VJP + long MIN-HOLD; TRT 3=high VJP + short MIN-HOLD; TRT 4=high VJP + long MIN-HOLD; C=200 ppm (mg/kg) sodium nitrite.

\(^c\) DAY = 0, 14, 28, 56, 90 day vacuum packaged samples held at 0-2 °C.

\(^d\) SEM = Standard error of the means for no-nitrite-added and nitrite-added hams.

\(^{e-h}\) Means within same row with different superscripts are different (P<0.05).

\(^{n-q}\) Means within same column with different superscripts are different (P<0.05).
This observation has been well documented by Jantawat and others (1993) who found a decreasing residual nitrite level with increased storage time relationship and by Hustad and others (1973) who reported that nitrite concentration was affected by both storage time and storage temperature. Since storage temperature was held constant in this study, storage time would be believed to be the principle factor in nitrite concentration decreases. Another explanation was suggested by Ahn and others (2002) who noted packaging effects in sausage samples stored in vacuum packages vs. aerobic packages. These authors reported that vacuum packaged sausages had lower residual nitrite than samples stored in aerobic conditions. The authors believed this phenomenon was caused by the product environment being in the reduced state thus allowing the conversion of nitrite to nitric oxide and resulting in the lower residual nitrite levels found.

No differences (P>0.05) in residual nitrite were observed between TRTs 1, 2 or 3 at any day throughout the 90-day storage period. Nor did nitrite differences (P>0.05) occur between TRTs 3 and 4 at any day except day 90. The control had significantly (P<0.05) higher residual nitrite values than all TRTs at all days measured. These results reveal that for TRTs 1-4, a higher concentration of VJP had a greater impact on residual nitrite concentration than did incubation time.

Residual Nitrate Analysis

Residual nitrate in hams was also determined pre-incubate, post-incubate and throughout a 90-day storage period. Least squares means for pre-incubate and post-incubate are reported in Table 6. TRTs 1 & 2 and TRTs 3 & 4 were formulated with VJP levels of 0.20 and 0.35%, respectively. Pre-incubate values for TRTs 1 & 2
or TRTs 3 & 4 for nitrate would then be expected to be very similar. Both TRTs 1 and 2 were significantly (P<0.05) lower in residual nitrate than TRTs 3 and 4, while C was different (P<0.05) than TRTs 1, 2 and 3. We expected TRT 1 to be different than C, however, variance in the samples tested (n=3) was large and affected statistical significance. Overall, residual nitrate differences were not surprising. Also, residual nitrate was detected in the control to which only nitrite was added. Cassens and others (1979) suggest that a portion of nitrite added to meat during the curing process is actually converted to nitrate.

No difference (P>0.05) was observed between TRTs 2 and 4 suggesting that VJP level had no effect on residual nitrite immediately after incubation but before cooking. Residual nitrate measurements of TRTs 1, 3 and C were not taken after incubation because those treatment combinations were not incubated (0 min). These values along with residual nitrite values during the pre- and post-incubation steps signify that conversion of nitrate to nitrite occurred. As residual nitrate for each TRT decreased from pre-incubate to post-incubate, residual nitrite increased. These results show that nitrate available in VJP was reduced to nitrite by the starter culture containing *S. carnosus*. Although this action can be accomplished by natural microorganisms found in the natural flora of the meat (Sanz and others 1997; Sebranek 1979; Pinotti and others 2001; MacDougall and others 1975), it can also be accomplished by intentional addition of microorganisms with nitrate-reducing properties (Sanz and others 1997). This additional reduction step in the curing reaction is necessary for a curing system with nitrate-containing VJP.
A significant (P<0.05) difference for the interaction of treatment*day was also observed for residual nitrate and the corresponding values are found in Table 8. As was found with residual nitrite, residual nitrate levels generally decreased over time for all treatment combinations. At day 0, no significant (P<0.05) differences were observed between TRTs 1, 2, 4 and C while TRT 4 had a higher (P<0.05) concentration of residual nitrate than all other treatment combinations. Furthermore, no differences (P>0.05) were identified between C and TRTs 1, 2 and 3 at days 14, 56 and 90 while C was not found different than TRTs 2 and 4 at day 28. TRT 3 resulted in the highest residual nitrate values because TRT 3 was formulated with a high VJP concentration and received no incubation time. As with pre-incubate measurements, the control showed residual nitrate values at each day sampled during storage.

Several researchers have reported the presence of nitrate in products of which only nitrite was added. Pérez-Rodríguez and others (1996), monitoring nitrite and nitrate in frankfurters, reported that in nitrite-cured, cooked and packaged frankfurters, approximately 50% of ingoing nitrite was present while about 10-15% of the added nitrite was found as nitrate. Interestingly, one theory is that a secondary oxidation involving nitrous acid could be involved in the conversion of nitrite to nitrate. This theory is also supported by Dethmers and Rock (1975) who proposed nitrate formation from the simultaneous oxidation-reduction of nitrous acid to yield nitric oxide and nitrate and from the oxidation of nitric acid by oxygen to yield nitrite which could subsequently react with water to yield nitrite and nitrate. Additionally, general decreases in residual nitrate over time for any TRTs could also be explained
TABLE 8: Least squares means for the interaction of treatment combination (TRT 1-4, C) with storage time (Day 0, 14, 28, 56, 90) for residual nitrate (ppm)\(^{a}\) of no-nitrite-added (TRT 1-4) and nitrite-added control (C) hams.

<table>
<thead>
<tr>
<th>TRT(^{b})</th>
<th>DAY(^{c})</th>
<th>0</th>
<th>14</th>
<th>28</th>
<th>56</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>(^{a})19.2(^{ef})</td>
<td>(^{p})20.7(^{ef})</td>
<td>(^{p})24.2(^{e})</td>
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<td>(^{o})14.6(^{f})</td>
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<tr>
<td>2</td>
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<td>(^{n})47.7(^{hi})</td>
<td>(^{n})50.5(^{eh})</td>
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<tr>
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<td></td>
<td>(^{o})12.4</td>
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<td>(^{op})10.5</td>
<td>(^{o})10.6</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>(^{o})16.6</td>
<td>(^{op})13.5</td>
<td>(^{o})14.7</td>
<td>(^{op})12.0</td>
<td>(^{o})12.4</td>
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</table>

SEM\(^{d}\) = 1.69

\(^{a}\)Residual nitrate determination reported in ppm of sample.
\(^{b}\)Treatment combination: TRT 1=low VJP + short MIN-HOLD; TRT 2=low VJP + long MIN-HOLD; TRT 3=high VJP + short MIN-HOLD; TRT 4=high VJP + long MIN-HOLD; C=200 ppm (mg/kg) sodium nitrite.
\(^{c}\)DAY = 0, 14, 28, 56, 90 day vacuum packaged samples held at 0-2 °C.
\(^{d}\)SEM = Standard error of the means for no-nitrite-added and nitrite-added hams.
\(^{e}-i\)Means within same row with different superscripts are different (P<0.05).
\(^{n}-p\)Means within same column with different superscripts are different (P<0.05).
by the previous comments. This is further supported by noting that residual nitrite levels generally decreased over time while residual nitrate levels generally remained constant. Research on nitrite to nitrate reactions performed by Hustad and others (1973) may explain the constant nitrate levels found in this study. Instead of both residual nitrite and nitrate levels diminishing over time, a portion of nitrite may have been continually converted to nitrate helping to maintain the levels of nitrate found.

The residual nitrate of VJP was measured and found to be 27,462 ppm (mg/kg) (n=3) or 2.75% of the VJP (w/w). Therefore, formulation nitrate (when injected into ham muscles) would be approximately 69 ppm for TRTs 1 & 2 and 120 ppm for TRTs 3 & 4. Hustad and others (1973) discussed loss of nitrite during processing and reported an average nitrite reduction of 16% after the meat was added to the bowl cutter. Similar trends were found in our results for both nitrate (Table 6 & 8) and nitrite (Table 6 & 7) indicating both nitrite and nitrite losses during the manufacturing process.

Trained Sensory Panel

Trained sensory analysis was performed on day 14 following product manufacture. Significant differences (P<0.05) for the main effects of treatment combinations for sensory attributes are reported as least squares means in Table 9. No differences in color intensity between any treatment combinations were shown. The control had a significantly (P<0.05) higher ham aroma score than TRTs 3 and 4 but was not different than TRTs 1 or 2. Because nitrite is directly related to cured color and aroma, we expected treatment combinations (TRTs 3 & 4) with higher ingoing VJP levels to be more similar to the control than the treatment combinations
TABLE 9: Least squares means of sensory attributes for intensity of ham color (color intensity), ham aroma, vegetable aroma (veg aroma), ham flavor, vegetable flavor (veg flavor) and firmness for no-nitrite-added (TRT 1-4) and nitrite-added control (C) hams.

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<th>Ham Aroma</th>
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<td>0.62</td>
<td>0.64</td>
<td>0.50</td>
<td>0.64</td>
<td>0.28</td>
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<sup>a</sup>Treatment combination: TRT 1=low VJP + short MIN-HOLD; TRT 2=low VJP + long MIN-HOLD; TRT 3=high VJP + short MIN-HOLD; TRT 4=high VJP + long MIN-HOLD; C=200 ppm sodium nitrite.

<sup>b</sup>SENSORY ATTRIBUTES = Trained panel scores using a line scale (numerical value of 15 units) with graduations from 0 to 15 where 0 represented none (aroma, flavor and color intensity) and soft (firmness) and 15 represented intense (aroma, flavor, color) and hard (firmness).

<sup>c</sup>SEM = Standard error of the means for sensory attributes for no-nitrite-added and nitrite-added ham.

<sup>d-f</sup>Means within same column with different superscripts are different (P<0.05).
(TRTs 1 & 2) with lower nitrate-containing VJP levels. This, however, was not shown in the results and may be explained by an overpowering effect of VJP on aroma as indicated by vegetable aroma results. Because ham normally has less spice and comprises a more delicate flavor profile compared to other processed meats such as frankfurters, vegetable aroma and flavor may have a greater impact. These sensory attributes were included in the sensory evaluation for that reason. Vegetable aroma scores showed that C was not different (P>0.05) than TRTs 1 or 2 but both TRT 1 and C had less (P<0.05) vegetable aroma than TRTs 3 and 4. These results indicate that vegetable aroma, as a result of the addition of VJP, was detectable at the higher concentration (0.35%) used in this study regardless of a short or long incubation treatment. Whether this is objectionable is not clear and would have to be determined by consumer sensory evaluations.

For ham flavor, the control was scored the highest and was significantly (P<0.05) higher than TRTs 3 or 4, yet not different than TRTs 1 or 2. Based on these results, hams manufactured with a low level of VJP may have a more desirable ham flavor than those manufactured with a high level of VJP. As was shown for ham aroma, this may be due to an overpowering effect of the VJP on ham flavor. Vegetable flavor scores displayed no differences between TRTs 1, 2 and C, however TRT 1 and C had a lower (P<0.05) score for vegetable flavor than TRTs 3 and 4. Interestingly, trends from the sensory results showed that as VJP level increased, regardless of incubation time, characteristic ham aroma and flavor decreased while vegetable aroma and flavor increased. TRT 1 was firmer (P<0.05) than TRT 4 but no other differences for firmness were observed.
differences would not be expected unless muscle variation (inside and outside ham muscles) was present between samples presented to panelists affecting firmness assessment. However, nitrite-curing reactions have been suggested to increase firmness (Pegg and Shahidi 2000).

Conclusions

Treatment combinations containing vegetable juice powder (VJP) and starter culture containing S. carnosus were shown to be comparable to a sodium nitrite-added control for color, lipid oxidation, cured pigment and trained sensory measurements. All TBARS values reported were below the detectable threshold of lipid oxidation indicating that all treatment combinations were effective in controlling lipid oxidation, regardless of VJP level or incubation time. Differences in color, cured pigment, residual nitrate and residual nitrite measurements for the TRTs validated that curing reactions occurred, though the relative extent of many nitrite-related curing reactions in the VJP products is still unclear. Neither incubation nor VJP level was found to affect cured color and limited differences were observed for cured pigment concentrations.

Although a large number of differences existed in residual nitrate and nitrite analyses, those differences appeared to have little impact on lipid oxidation, color and pigment measurements. While TRT 4 (containing a high level of VJP) showed a greater change from pre-incubate nitrate to post-incubate nitrite than TRT 2 (containing a low level of VJP), the anticipated extra nitrite available for curing reactions did not necessarily improve cured color or cured pigment concentrations.
Sensory data demonstrated the concern for adding a high level (0.35%) of VJP to hams with the highest scores for undesirable vegetable aroma and flavor corresponding to those treatment combinations with the high level of VJP. Treatment combinations with a low VJP level (0.20%) were comparable to a sodium nitrite-added control for all sensory attributes.

The VJP used in this study was an effective replacement for sodium nitrite at the tested levels for the manufacture of uncured, no-nitrate/nitrite-added hams. At the VJP concentrations (0.20% and 0.35%) tested, it appears that concentrations closer to 0.20% of the total formulation provided adequate quality attributes and acceptable sensory attributes compared to a sodium nitrite-added control regardless of incubation time. The slow temperature increase during thermal processing of a large diameter product such as ham may provide ample time for nitrate to nitrite reduction reactions to occur and therefore short vs. long incubation time differences may have been partially negated. Optimum starter culture temperatures may occur for sufficient time during the slow temperature increases of the cooking process and that time/temperature period could provide enough nitrate conversion to allow for results comparable to those found for the long incubation (120 min) time.

The ingoing nitrate levels of 69 ppm (TRTs 1 & 2) and 120 ppm (TRTs 3 & 4) determined in this study would result in considerably less nitrite than the USDA FSIS maximum allowable limit of 200 ppm nitrite, even if the nitrate was 100% converted. Since USDA FSIS requires a minimum of 120 ppm nitrite in cured meat products labeled “Keep Refrigerated” (USDA 1995), the low ingoing levels found in this study
could result in microbiological concerns, specifically relative to *C. botulinum* survival and outgrowth.

Further research regarding the effects of VJP on the consumer acceptability of these treatment combinations and the effect of product diameter on nitrate-to-nitrite conversion rates and efficiency is needed to generate a better understanding of this technology. Further research regarding the effects of this technology on microbiological control and shelf life of these products is also needed.

**Acknowledgement**

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References


CHAPTER 6. GENERAL CONCLUSIONS

The intent of the consumer evaluation of uncured no nitrate/nitrite added hams, frankfurters and bacons was to provide insight on consumer reactions to those products as well as possible analytical reasoning for why consumer differences did or did not exist. As hypothesized, a great deal of variation existed not only between product types but also within each investigated product type. Although replication by brand interactions existed for many attributes investigated making data interpretation difficult, it was clearly demonstrated that variation in uncured no nitrate/nitrite added processed meat products does in fact exist. From this study, we may pose questions regarding product uniformity and what product attributes are deemed important by consumers in regards to purchasing decisions.

Investigating an uncured no nitrate/nitrite added manufacturing system for the manufacture of emulsified frankfurter style cooked (EFSC) sausages and hams utilizing vegetable juice powder (VJP) and a starter culture containing Staphylococcus carnosus has shown that the resulting products are in fact comparable to a sodium nitrite added control for color, lipid oxidation, cured pigment and trained sensory measurements. Furthermore, residual nitrate and nitrite content during product manufacture as well as over the 90-day storage period showed similar levels to those of nitrite added controls. Although post-incubation levels of nitrite varied significantly between treatments, post thermal processing levels did not show the same proportional differences.
An important discovery, regardless of VJP level, was that incubation time is a critical step during the thermal processing by allowing time for adequate conversion of nitrate to nitrite and for subsequent curing reactions. For the EFSC sausages study, the longer incubation time (120 minutes) was found to be more critical and resulted in products comparable to a sodium nitrite added control regardless of VJP level (0.20% or 0.40%). For the ham study, levels of VJP closer to 0.20% of the total formulation provided adequate quality attributes and acceptable sensory attributes compared to a sodium nitrite added control regardless of incubation time. This is believed to be due to the diameter of the product. The slow temperature come-up time of the hams may have provided adequate time/temperature conditions for starter culture function and thus ample time for nitrate to nitrite reduction reactions to occur and therefore short vs. long incubation differences may have been partially negated.

A challenge with the use of VJP is to find a balance between high enough formulated ingoing levels to result in adequate nitrate and subsequent nitrite content without affecting finished product sensory characteristics. This research has shown that VJP aromas and flavors may be detected at high formulated levels in hams while none were detected for EFSC sausages. This suggests that product non-meat ingredient composition and level may play an important role in negating or masking the VJP aroma and flavor attributes in finished products. It can be concluded that VJP is an effective replacement for sodium nitrite at the tested levels for the manufacture of uncured, no nitrate or nitrite added EFSC sausages and hams.
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Appendix 1: Product brand designations and product ingredient statements for commercial uncured, no-nitrate/nitrite-added (Brand A-D) and nitrate-added (Brand E) hams used in study.

Appendix 2: Product brand designations and product ingredient statements for commercial uncured, no-nitrate/nitrite-added (Brand A-D) and nitrite-added (Brand E) frankfurters used in study.

Appendix 3: Product brand designations and product ingredient statements for commercial uncured, no-nitrate/nitrite-added (Brand A-D) and nitrite-added (Brand E) bacon used in study.

Appendix 4: Means for processing attributes (PROC ATTRIBUTES) between replications (REP) for no-nitrite-added (TRT 1-4) and nitrite-added control (C) EFSC sausage.

Appendix 5: Least squares means for processing attributes of pre-incubation (pre-incubate) pH and temperature (temp) and post-incubation (post-incubate) pH for no-nitrite-added (TRT 1-4) and nitrite-added control (C) EFSC sausage.

Appendix 6: Least squares means for proximate composition of cooked no-nitrite-added (TRT 1-4) and nitrite-added control (C) EFSC sausage.

Appendix 7: Least squares means for processing attributes of brine pH and temperature (temp), raw ham muscle pH and temperature (temp), before incubation (pre-incubate) pH and temperature (temp) and after incubation (post-incubate) pH for no-nitrite-added (TRT 1-4) and nitrite-added control (C) hams.

Appendix 8: Least squares means for cooked proximate composition of no-nitrite-added (TRT 1-4) and nitrite-added control (C) hams.

Appendix 9: Objective Color Analysis (CIE L* a* b*)

Appendix 10: Objective Color Analysis (Reflectance Ratio)

Appendix 11: Pigment Measurement by Absorbance (Cured Meat Pigment)

Appendix 12: Pigment Measurement by Absorbance (Total Meat Pigment)

Appendix 13: Measurement of pH
Appendix 14: TBARS Analysis

Appendix 15: Nitrites in Cured Meat – Colorimetric Method

Appendix 16: Nitrate Determination – HPLC Method

Appendix 17: Proximate Analysis

Appendix 18: Consumer Test of Ham (Ballot)

Appendix 19: Consumer Test of Bacon (Ballot)

Appendix 20: Consumer Test of Frankfurters (Ballot)

Appendix 21: Uncured No Nitrate/Nitrite Added EFSC Sausage Experiment Formulations

Appendix 22: Uncured No Nitrate/Nitrite Added Ham Experiment Formulations

Appendix 23: CHR Hansen CS 299 Bactoferm™ Product Information

Appendix 24: CHR Hansen Vegetable Juice PWD NAT Product Information

Appendix 25: World Pac International EFSC Sausage Casing – WP-E

Appendix 26: World Pac International Ham Casing – HC5

Appendix 27: Sensory Evaluation of Frankfurters (Ballot)

Appendix 28: Sensory Evaluation of Ham Products (Ballot)
APPENDIX 1: Product brand designations and product ingredient statements for commercial uncured, no-nitrate/nitrite-added (Brand A-D) and nitrate-added (Brand E) hams used in study.

<table>
<thead>
<tr>
<th>Product Brand Designation</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
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<td>Brand A</td>
<td>Pork, water, seasonings (sea salt, raw sugar, honey, spices), maple sugar, sodium lactate (from fermented corn)</td>
</tr>
<tr>
<td>Brand B</td>
<td>Pork, water, sodium lactate (from beets), vinegar, sea salt, evaporated cane juice, celery juice concentrate, dextrose, lactic acid starter culture, spice extractives</td>
</tr>
<tr>
<td>Brand C</td>
<td>Pork, water, sea salt, turbinado sugar, natural spices, lactic acid starter culture</td>
</tr>
<tr>
<td>Brand D</td>
<td>Pork, water, salt, dextrose, sodium phosphates, natural flavor, lactic acid starter culture</td>
</tr>
<tr>
<td>Brand E</td>
<td>Pork, water, salt, sugar, dextrose, sodium erythorbate, sodium nitrite</td>
</tr>
</tbody>
</table>
### APPENDIX 2: Product brand designations and product ingredient statements for commercial uncured, no-nitrate/nitrite-added (Brand A-D) and nitrite-added (Brand E) frankfurters used in study.

<table>
<thead>
<tr>
<th>Product Brand Designation</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand A</td>
<td>Organic beef, water, sodium lactate (from beets), sea salt, organic honey, organic evaporated cane juice, organic spices, celery juice, organic paprika, lactic acid starter culture (not from dairy)</td>
</tr>
<tr>
<td>Brand B</td>
<td>Beef, water, salt, mustard powder, raw sugar, white pepper, paprika, nutmeg, garlic powder, coriander, onion powder, ground allspice</td>
</tr>
<tr>
<td>Brand C</td>
<td>Beef, water, salt, honey and natural spices</td>
</tr>
<tr>
<td>Brand D</td>
<td>Beef, water, sea salt, dried honey, spices, sodium lactate (from fermented corn), natural flavorings</td>
</tr>
<tr>
<td>Brand E</td>
<td>Beef, water, contains less than 2% of salt, corn syrup, dextrose, flavor, sodium lactate, hydrolyzed beef stock, sodium phosphates, autolyzed yeast, sodium diacetate, sodium erythorbate (made from sugar), sodium nitrite, extractives of paprika</td>
</tr>
</tbody>
</table>
APPENDIX 3: Product brand designations and product ingredient statements for commercial uncured, no-nitrate/nitrite-added (Brand A-D) and nitrite-added (Brand E) bacon used in study.

<table>
<thead>
<tr>
<th>Product Brand Designation</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand A</td>
<td>Pork, water, sea salt, turbinado sugar, natural spices, lactic acid starter culture</td>
</tr>
<tr>
<td>Brand B</td>
<td>Organic pork, water, sea salt, organic honey, organic beet powder for color, organic spices</td>
</tr>
<tr>
<td>Brand C</td>
<td>Organic pork, water, sea salt, organic evaporated cane juice, celery juice, lactic acid starter culture (not from dairy)</td>
</tr>
<tr>
<td>Brand D</td>
<td>Pork, sea salt, raw sugar and spices</td>
</tr>
<tr>
<td>Brand E</td>
<td>Pork, water, salt, sugar, dextrose, sodium erythorbate, sodium nitrite</td>
</tr>
</tbody>
</table>
Appendix 4: Means for processing attributes (PROC ATTRIBUTES) between replications (REP) for no-nitrite-added (TRT 1-4) and nitrite-added control (C) EFSC sausage.

<table>
<thead>
<tr>
<th>PROC ATTRIBUTES(^a)</th>
<th>REP 1</th>
<th>REP 2</th>
<th>REP 3</th>
<th>SEM(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef Trim Fat (%)</td>
<td>16.7</td>
<td>NA</td>
<td>16.8</td>
<td>0.55</td>
</tr>
<tr>
<td>Beef Trim pH</td>
<td>5.57</td>
<td>5.51</td>
<td>5.51</td>
<td>0.02</td>
</tr>
<tr>
<td>Beef Trim Temp (ºC)</td>
<td>-0.5</td>
<td>-0.8</td>
<td>-0.7</td>
<td>0.01</td>
</tr>
<tr>
<td>Pork Trim Fat (%)</td>
<td>56.7</td>
<td>NA</td>
<td>54.8</td>
<td>0.58</td>
</tr>
<tr>
<td>Pork Trim pH</td>
<td>6.20</td>
<td>6.22</td>
<td>6.30</td>
<td>0.03</td>
</tr>
<tr>
<td>Pork Trim Temp (ºC)</td>
<td>3.2</td>
<td>3.1</td>
<td>3.8</td>
<td>0.01</td>
</tr>
<tr>
<td>Water pH</td>
<td>8.92</td>
<td>8.85</td>
<td>8.88</td>
<td>NA</td>
</tr>
<tr>
<td>Water Temp (ºC)</td>
<td>5.1</td>
<td>3.3</td>
<td>6.6</td>
<td>NA</td>
</tr>
</tbody>
</table>

\(^a\) PROC ATTRIBUTES = Processing attributes measured during the manufacturing process of EFSC sausage TRTs.

\(^b\) SEM = Standard error of the means for REP for no-nitrite-added and nitrite-added EFSC sausage processing attributes.
Appendix 5: Least squares means for processing attributes of pre-incubation (pre-incubate) pH and temperature (temp) and post-incubation (post-incubate) pH for no-nitrite-added (TRT 1-4) and nitrite-added control (C) EFSC sausage.

<table>
<thead>
<tr>
<th>TRT&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Pre-Incubate&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Post-Incubate&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Temp&lt;sup&gt;(ºC)&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>5.46</td>
<td>12.2</td>
</tr>
<tr>
<td>2</td>
<td>5.45</td>
<td>12.9</td>
</tr>
<tr>
<td>3</td>
<td>5.46</td>
<td>13.6</td>
</tr>
<tr>
<td>4</td>
<td>5.46</td>
<td>13.0</td>
</tr>
<tr>
<td>C</td>
<td>5.44</td>
<td>13.3</td>
</tr>
<tr>
<td>SEM&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<sup>a</sup>Treatment combinations where: TRT 1=low VJP + short min-hold; TRT 2=low VJP + long min-hold; TRT 3=high VJP + short min-hold; TRT 4=high VJP + long min-hold; C=156 (mg/kg) ppm sodium nitrite.

<sup>b</sup>PROCESSING ATTRIBUTES<sup> </sup>taken during the manufacturing process of EFSC sausage TRTs.

<sup>c</sup>Pre-incubate = Measurements taken randomly during stuffing and before incubation.

<sup>d</sup>Post-incubate = Measurements taken randomly after incubation (MIN-HOLD) prior to cooking.

<sup>e</sup>pH of ham samples (Sebranek and others 2001).

<sup>f</sup>SEM = Standard error of the means for TRT for no-nitrite-added and nitrite-added EFSC sausage processing attributes.
Appendix 6: Least squares means for proximate composition of cooked no-nitrite-added (TRT 1-4) and nitrite-added control (C) EFSC sausage.

<table>
<thead>
<tr>
<th>TRT&lt;sup&gt;a&lt;/sup&gt;</th>
<th>MOISTURE&lt;sup&gt;%&lt;/sup&gt;</th>
<th>FAT&lt;sup&gt;%&lt;/sup&gt;</th>
<th>PROTEIN&lt;sup&gt;%&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61.76</td>
<td>21.11</td>
<td>13.26</td>
</tr>
<tr>
<td>2</td>
<td>61.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.65</td>
<td>13.54</td>
</tr>
<tr>
<td>3</td>
<td>61.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.42</td>
<td>13.24</td>
</tr>
<tr>
<td>4</td>
<td>61.59</td>
<td>21.09</td>
<td>13.47</td>
</tr>
<tr>
<td>C</td>
<td>61.98&lt;sup&gt;d&lt;/sup&gt;</td>
<td>21.11</td>
<td>13.36</td>
</tr>
<tr>
<td>SEM&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.11</td>
<td>0.16</td>
<td>0.23</td>
</tr>
</tbody>
</table>

<sup>a</sup>Treatment combinations where: TRT 1=low VJP + short min-hold; TRT 2=low VJP + long min-hold; TRT 3=high VJP + short min-hold; TRT 4=high VJP + long min-hold; C=156 (mg/kg) ppm sodium nitrite.

<sup>b</sup>SEM = Standard error of the means for TRT for no-nitrite-added and nitrite-added EFSC sausages.

<sup>c-d</sup>Means within same column with different superscripts are different (P<0.05).
Appendix 7: Least squares means for processing attributes of brine pH\(^a\) and temperature (temp), raw ham muscle pH and temperature (temp), before incubation (pre-incubate) pH and temperature (temp) and after incubation (post-incubate) pH for no-nitrite-added (TRT 1-4) and nitrite-added control (C) hams.

<table>
<thead>
<tr>
<th>TRT(^b)</th>
<th>Brine(^d) pH</th>
<th>Brine(^d) Temp((^°)C)</th>
<th>Raw Ham Muscle(^e) pH</th>
<th>Raw Ham Muscle(^e) Temp((^°)C)</th>
<th>Pre-Incubate(^f) pH</th>
<th>Pre-Incubate(^f) Temp((^°)C)</th>
<th>Post-Incubate(^g) pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.08(^k)</td>
<td>4.8</td>
<td>5.74</td>
<td>-0.3</td>
<td>5.50</td>
<td>7.6</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>5.08(^k)</td>
<td>4.8</td>
<td>5.66</td>
<td>-0.1</td>
<td>5.55</td>
<td>7.6</td>
<td>5.60</td>
</tr>
<tr>
<td>3</td>
<td>4.88(^k)</td>
<td>4.6</td>
<td>5.80</td>
<td>-0.2</td>
<td>5.57</td>
<td>7.3</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>4.88(^k)</td>
<td>4.6</td>
<td>5.74</td>
<td>-0.1</td>
<td>5.57</td>
<td>7.6</td>
<td>5.53</td>
</tr>
<tr>
<td>C</td>
<td>7.02(^l)</td>
<td>4.2</td>
<td>5.92</td>
<td>-0.3</td>
<td>5.60</td>
<td>7.8</td>
<td>NA</td>
</tr>
<tr>
<td>SEM(^h)</td>
<td>0.10</td>
<td>0.01</td>
<td>0.09</td>
<td>0.01</td>
<td>0.05</td>
<td>0.01</td>
<td>0.03</td>
</tr>
</tbody>
</table>

\(^a\) pH of ham samples (Sebranek and others 2001).

\(^b\) Treatment combinations where: TRT 1=low VJP + short min-hold; TRT 2=low VJP + long min-hold; TRT 3=high VJP + short min-hold; TRT 4=high VJP + long min-hold; C=200 ppm (mg/kg) sodium nitrite.

\(^c\) PROCESSING ATTRIBUTES taken during the manufacturing process of Ham TRTs.

\(^d\) Brine = used for 25% injection of ham muscles. Same brine was used for TRT 1 & 2 and TRT 3 & 4.

\(^e\) Raw pork ham muscles were randomly selected for pH and temperature measurements.

\(^f\) Pre-Incubate = Measurements taken randomly during stuffing and before incubation.

\(^g\) Post-Incubate = Measurements taken randomly after incubation (MIN-HOLD) prior to cooking.

\(^h\) SEM = Standard error of the means for TRT for no-nitrite-added and nitrite-added ham.

\(^k-l\) Means within same column with different superscripts are different (P<0.05).
Appendix 8: Least squares means for cooked proximate composition of no-nitrite-added (TRT 1-4) and nitrite-added control (C) hams.

<table>
<thead>
<tr>
<th>TRT&lt;sup&gt;a&lt;/sup&gt;</th>
<th>MOISTURE (%)</th>
<th>FAT (%)</th>
<th>PROTEIN (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75.11</td>
<td>2.13</td>
<td>20.05</td>
</tr>
<tr>
<td>2</td>
<td>74.61</td>
<td>3.47</td>
<td>19.02</td>
</tr>
<tr>
<td>3</td>
<td>74.83</td>
<td>2.23</td>
<td>20.09</td>
</tr>
<tr>
<td>4</td>
<td>75.25</td>
<td>2.60</td>
<td>19.49</td>
</tr>
<tr>
<td>C</td>
<td>75.57</td>
<td>2.17</td>
<td>19.94</td>
</tr>
<tr>
<td>SEM&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.44</td>
<td>0.29</td>
<td>0.30</td>
</tr>
</tbody>
</table>

<sup>a</sup> Treatment combination where: TRT 1=low VJP + short min-hold; TRT 2=low VJP + long min-hold; TRT 3=high VJP + short min-hold; TRT 4=high VJP + long min-hold; C=200 (mg/kg) ppm sodium nitrite.

<sup>b</sup> SEM = Standard error of the means for TRT for no-nitrite-added and nitrite-added hams.
APPENDIX 9: OBJECTIVE COLOR ANALYSIS (CIE L*a*b*)

Internal color analysis was performed using a Hunter Labscan spectrocolorimeter instrument (Model LS, 1500)

Ham:
1. Use the following settings:
   CIE L*a*b*
   Illuminant A light source
   10° standard observer
   4.45 cm viewing area
   5.08 cm port size

2. Calibrate the Hunterlab Labscan by covering the calibration plates with same packaging material used for samples. A white tile was used for standardizing colorimeter.

3. Two measurements across the cut surface from 2 vacuum packaged sliced samples were taken for a total of 4 measurements.

Frankfurters:
1. Use the following settings:
   CIE L*a*b*
   Illuminant A light source
   10° standard observer
   1.27 cm viewing area
   1.78 cm port size

2. Calibrate the Hunterlab Labscan by covering the calibration plates with Saran film. A white tile was used for standardizing colorimeter.

3. Samples were sliced in half longitudinally and immediately covered with Saran film and readings were taken.

4. Two measurements across the cut surface from 2 vacuum packaged sliced samples were taken for a total of 4 measurements.
**Bacon:**

Bacon measurements were taken on only the lean portion and also on the entire slice.

**Bacon – only lean portion**

1. Use the following settings:
   - CIE L*a*b*
   - Illuminant A light source
   - 10º standard observer
   - 0.64 cm viewing area
   - 1.02 cm port size

2. Calibrate the Hunterlab Labscan by covering the calibration plates with same packaging material used for samples. A white tile was used for standardizing colorimeter.

3. Four to six measurements across the cut surface from 2 vacuum packaged sliced samples were taken for a total of 9-12 measurements.

**Bacon – entire slice**

1. Use the following settings:
   - CIE L*a*b*
   - Illuminant A light source
   - 10º standard observer
   - 2.54 cm viewing area
   - 3.05 cm port size

2. Calibrate the Hunterlab Labscan by covering the calibration plates with same packaging material used for samples. A white tile was used for standardizing colorimeter.

3. Two-three measurements across the cut surface from 2 vacuum packaged sliced samples were taken for a total of 5-6 measurements.
APPENDIX 10: OBJECTIVE COLOR ANALYSIS (Reflectance Ratio)


Internal color analysis was performed using a Hunter Labscan instrument (Model LS, 1500)

Reflectance Ratio:
Measure sample at wavelengths of both 650 nm and 570 nm.
Divide 650 and 570 to get a reflectance ratio

Reference values 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
- excellent cured color 2.2 to 2.6

Hunter colorimeter settings

The same calibration and measurement procedures outlined for the CIE L*a*b* objective color analysis were used for reflectance ratio measurements.
APPENDIX 11: PIGMENT MEASUREMENT BY ABSORBANCE
(Cured Meat Pigment)


Materials needed:
- Acetone
- Food Processor
- Polytron
- Filter Paper – Whatman No. 42
- Spectrophotometer

Procedure:
*** the entire experiment should be done in as reduced light as possible to prevent light induced pigment changes
*** care should be taken to control acetone evaporation throughout entire experiment

1. Work in cooler with as little light as possible to prevent changes in the pigment. Grind sample with food processor. Use 10 grams of cured meat sample. Place 10 grams of sample into 125 ml flask. Add 40 ml of acetone and 3 ml of distilled, de-ionized water.

2. Mix with Polytron (setting 7) for 1 minute.

3. Immediately filter the solution using Whatman No. 42 filter paper. Be certain filtrate is clear.

4. Measure the absorbance of the solution at 540 μm. The blank should be an 80% acetone and 20% water solution.

5. Multiply the absorbance at 540 μm (1 cm cell) x 290 to convert ppm nitrosylhemochrome concentration.
APPENDIX 12: PIGMENT MEASUREMENT BY ABSORBANCE
(Total Meat Pigment)


Materials needed:
- Acetone, Food Processor, Polytron, Filter Paper – Whatman No. 42, HC1 – concentrated, Spectrophotometer

Procedure:
***care should be taken to control acetone evaporation throughout entire experiment

1. Grind sample with food processor in cooler with as little light as possible. Use 10 grams of lean cured meat sample [for bacon, separate lean from fat and use lean portion]. Place 10 grams of sample into 125 ml flask. Add 40 ml of acetone, 2 ml of distilled, de-ionized water and 1 ml of concentrated HC1.

2. Mix with a polytron (setting 7) for 1 minute. Place stopper in flask and label (including time).

3. Allow the mixture to stand for 1 hour before proceeding. This converts virtually all of the pigments to one form.

4. Filter the solution using Whatman No. 42 filter paper. Be certain the filtrate is clear.

5. Measure the absorbance of the solution at 640 m\( \mu \). The blank should be a mixture of 80% acetone, 18% water and 2% HC1.

6. Multiply the absorbance at 640 m\( \mu \) (1 cm cell) x 680 to convert the reading to ppm of total pigment.

7. As a final check, read absorbance at 512 m\( \mu \) also. The ratio of absorbance at 512 m\( \mu \) over absorbance at 640 m\( \mu \) should not be more than 1.9 to ensure that all pigment has been converted to one form.
APPENDIX 13: MEASUREMENT OF pH


Equipment: Scale
           pH meter
           150 ml beakers
           Polytron
           Whatman No. 1 filter paper

Procedure:

1. Weigh 10 grams (± 0.02) of sample into 150 ml beaker. Do this in duplicate.

2. Add 90 ml of distilled, de-ionized water to beaker.

3. Mix (homogenize) with polytron on speed setting 7 for 45 seconds.

4. Insert a folded piece of Whatman No. 1 filter paper (11.0 or 12.5 cm) into the beaker, pushing it down into the slurry.

5. After allowing the fat free solution to come through the filter paper, insert the tip of the pH meter electrode into the solution and record pH reading.

6. Follow the manufactures directions regarding the pH meter and electrode.
APPENDIX 14: TBARS ANALYSIS


1. TBA Reagent

Prepare the amount of TBA Reagent needed for your samples according to the table below:

<table>
<thead>
<tr>
<th>Thiobarbituric Acid</th>
<th>Total Vol. DI Water and TBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5768 g</td>
<td>200 ml</td>
</tr>
<tr>
<td>0.4326 g</td>
<td>150 ml</td>
</tr>
<tr>
<td>0.2884 g</td>
<td>100 ml</td>
</tr>
<tr>
<td>0.1442 g</td>
<td>50 ml</td>
</tr>
</tbody>
</table>

Dissolve the Thiobarbituric Acid in the distilled, de-ionized water. Use portion of total water to rinse TBA weighing boat. Use stirring bar and stirrer to mix.

2. HCl Solution:

Make volume as needed; 1:2, HCl : D-H₂O (v/v).

3. Sulfanilamide Solution:

Dissolve 1 gram sulfanilamide in 40 ml of concentrated HCL and 160 ml of distilled water.

4. Antifoam:

Use Dow antifoam C emulsion. Mix 1:1 ratio with D-H₂O.

Procedure (for cured meats):

1. Add 10 g (± 0.02) of thawed and diced sample to round bottom flask.
2. Add 1 ml sulfanilamide solution and swirl to mix.
3. Add 97.0 ml of distilled, de-ionized water.
4. Add 2.0 ml of HCL solution.
5. Add 5 drops of antifoam and some boiling beads (7).
6. Swirl to mix.

7. Turn on condenser water.

8. Attach flasks to distillation apparatus and place 50 ml beaker under apparatus outlet.

9. Start burners on 6, then turn to high after they have begun to boil.

10. Distill until 50 ml of distillate has been collected. Replace beaker with 250 ml beaker of D-H$_2$O and allow water to backwash (this will clean condenser temporarily, but the condensers should be cleaned with hot soapy water periodically).

11. While samples are distilling the TBA reagent can be prepared and allowed to mix. The TBA reagent should be used within 2 hours of mixing because it will deteriorate with time.

12. Place 5 ml of distillate and 5 ml TBA reagent in test tubes. The blanks are 5 ml D-H$_2$O and 5 ml TBA reagent.

13. Cap firmly and mix each sample with Vortex mixer for 10 seconds.

14. Turn water bath on 100$^\circ$C.

15. Place tubes in test tube rack and immerse into boiling water bath for 35 minutes.

16. Turn Spectrophotometer to VISIBLE ON (must warm up 30 min.)

17. When the tubes are done heating in the water bath cool them in cold water for at least 10 minutes.

18. Transfer sample to disposable 4.5 ml cuvette (done in duplicates).

19. Read the absorbance of the sample at 532 nm.

20. Convert % T to optical density and multiply by the constant 7.8 (7.6 for poultry) to convert to mg malonaldehyde/1000 g of sample, i.e. TBARS Number.
APPENDIX 15: NITRITES IN CURED MEAT – COLORIMETRIC METHOD


Directions for Nitrite Determination

Preliminary steps (Reagents and Apparatus):

1. Make up 1000 ml or 500 ml of the 15% CH₃COOH (acetic acid) solution
2. Make up NED reagents and Sulfanilimide reagents as described in AOAC 973.31.
   a. Store them in brown glass bottles in dark cabinet.
   a. Stock solution – dissolve 1.000 g of sodium nitrite in a 1000 ml. volumetric and shake it up thoroughly diluting with distilled, de-ionized water.
   b. Intermediate solution – take 100 ml. of the stock solution add it to another 1000 ml. volumetric and dilute with distilled, de-ionized water.
   c. Working solution – take 10 ml. of the Intermediate solution and put in a 3rd 1000 ml. volumetric and dilute to volume with distilled, de-ionized water.
4. Get some filter paper, test for nitrite according to method.

Procedure:

Heat distilled, de-ionized water in big beakers or Erlenmeyer flasks and put them onto a warming plate approximately 30-50 minutes before beginning experiment. The water will need to be 80 degrees Centigrade before use.

1. Chop up sample out of the presence of light as much as possible using food processor.
2. Weigh 5 g of sample into a 50 ml beaker.
3. If you want to do duplicates you will need to weigh up two samples per treatment.
4. After the samples are weighed add roughly 40 ml of the hot water to the sample and stir vigorously with a glass stir rod. Be careful not to slop the sample out of the beaker.
5. After the sample has been stirred adequately, transfer the beaker contents to a 500 ml volumetric flask. You will need to add some more hot water to the beaker to clean out all the sample and what sample is left on the funnel you will use. Be very liberal with the hot water. Don’t worry if it takes you 100-200 ml to get all of the sample cleaned off of the beaker and into the flask.

6. After all of the samples in the beakers are transferred to their own 500 ml flask make sure that each flask has about 350 ml of solution in it.

7. Cap the flasks with rubber corks.

8. Put the flasks under the steam hood and crank the steam full blast.

9. Swirl the flasks every 30 minutes to aid in extraction.
   a. Be careful handling the hot flasks and be sure to let the pressure out of the flask before shaking.

10. After 2 hours take the flasks out of the steam and set them on the bench top to cool (2 hours). It is important that you let them cool before filling the flask to volume with room temperature water. If you add the water before the flask has cooled, the volume may change once temperature equilibration has been done.

11. Again, once the flask has cooled fill to volume.

12. Once you have all the flasks filled, shake them up good so you get a representative sample.

13. Filter about 50 ml of the flask contents into 2 separate 50 ml beakers. You could also use 1, but I preferred splitting the sample in two at this point so that any discrepancies on the spec would show up right away.

14. Transfer about 30 ml of the sample to a 50 ml volumetric flask.

15. Under a fume hood, add 2.5 ml of sulfanilamide solution and wait 5 min. Use a pipette set for 2.5 ml.

16. Pipette 2.5 ml of NED reagent and then fill the flask to volume with some more sample that you had in the beaker.

17. Let the sample set for 15 min. to allow the color to develop.

18. After color development, run samples on spectrophotometer.

19. Set the spec at 540 nm as described in the method.

20. Prepare a blank right along with your other samples, 2.5 ml sulfanilamide, 45 ml water, and 2.5 ml NED then standardize the spec with the blank.
Standard curve preparation.

1. Follow directions as described in the official method.

2. Add 10, 20, 30, and 40 ml of working solution with 2.5 ml sulfanilamide and NED just like you did with the regular samples and top off with water to make 50 ml (again just like a blank).

3. By reading them on the spec you should get a relatively straight line.

4. You can then determine the slope of the line so that you can make an equation \( y = mx + b \). You will need the slope to extrapolate the line when you get down to smaller residual nitrite values.
APPENDIX 16: NITRATE DETERMINATION – HPLC METHOD


Sample preparation and nitrate determination methods were modifications of Ahn and Maurer (1987).

1. Five grams of meat product samples were weighted in a 50-ml test tube and homogenized with 20 ml of distilled de-ionized water (DDW) using a Polytron homogenizer (Type PT 10/35, Brinkmann Instruments Inc., Westbury, NY, USA) for 10 s at high speed.

2. The homogenate was heated for 1 h in 80°C water bath.

3. After cooling in cold water for 10 min, 2.5 ml of the homogenate was transferred to a disposable test tube (16 x 100 mm). Carrez II (dissolve 10.6 g potassium ferrocyanide in 100 ml DDW) and Carrez I (dissolve 23.8 g zinc acetate in 50 ml DDW, then add 3 ml glacial acetic acid and dilute to 100 ml with DDW) reagents were added (0.1 ml each) to precipitate proteins.

4. The solution was diluted with 2.3 ml of DDW and mixed well.

5. After precipitation, the supernatant was centrifuged at 10000 x g for 20 min and the clear upper layer was used for nitrate measurement by high performance liquid chromatography (Agilent 1100 Series HPLC system, Agilent Technologies, Wilmington, DE, USA). The column used was Agilent Zorbax SAX (analytical 4.6 x 150mm, 5-micron) (Agilent, Wilmington, DE, USA) and the elution buffer was 15 mM phosphate buffer, pH 2.35, with isocratic elution. Flow rate was 1.0 ml/min and sample volume was 25 µL. The wavelength used was 210 nm. The area of nitrate peak was used to calculate nitrate concentration (ppm) using nitrate standard curve.
APPENDIX 17: Proximate Analysis


Sample Preparation (Modified)

1. Section meat samples into very small (<1 cm squares) pieces.
2. Grind (chop) the sample into mixed substance using a food processor.
3. Grind 2 to 3 minutes until the sample has been ground into a mixed substance, transfer the sample to a labeled plastic bag and secure by tying a knot.
4. Store the sample in refrigerated conditions 0 °C (32 °F) until analysis.

Moisture Analysis

1. Label the thimbles with a pencil (not pen) before drying. Thimbles should be handled with tongs or while wearing gloves. Work rapidly so thimbles won’t collect excess moisture. For fatty (approximately > 20% fat) samples, place a ball of cotton in the bottom of the thimble before drying.
2. Check that the analytical balance is clean and level.
3. After zeroing, record the number of the thimble, weigh and record the weight of the thimble. Zero the scale again.
4. Weigh approximately 5.0 grams of sample into the thimble (place it in small pieces, not one large piece) with a spatula. Record the weight of the sample. For fatty samples, use approximately 4.0 grams.
5. Place the samples into the gravity oven for 18 hours at 100-102 °C.
6. Transfer the sample directly to a desiccator and allow to cool for 30 minutes.
7. Weigh and record the dried weight of the samples. Determine the percent moisture content as follows:

\[
\text{Moisture (\%)} = \left( \frac{\text{wet sample wt.} - \text{dried sample wt.}}{\text{wet sample wt.}} \right) \times 100
\]

8. For each treatment measurements were made in duplicate.

**Fat Analysis Using Ether Extraction**

1. Take samples from moisture analysis and place into the Soxhlet fat extraction tubes. Make sure that all the samples are below the level where the ether drains off (curved glass on outside of tube).

2. Add 200ml (if using small 500 ml flask) of petroleum diethyl ether to clean boiling flasks until about ¾ full. Add 2 to 3 glass beads as a boiling aid.

3. Connect the extraction flask to the boiling flask and Soxhlet apparatus. Place lubriseal on the joint. Mount both to the condensing units on top of extraction flasks using lubriseal around joint.

4. Turn on condensing water so it runs at a steady stream.

5. Set Rheostats on high and run for 6 hours.

6. Place ether soaked samples onto a rack in a fume hood for at least 10-15 minutes to allow any remaining ether to dissipate.

7. Place samples in drying oven for 4 hours to remove any possible moisture then place in dessicator for 1/2 hour to cool.

8. Weigh and record the weight of the samples. Calculate fat on wet basis with the following equation:

\[
\text{Fat (\%)} = \left( \frac{\text{dried sample wt.} - \text{extracted sample wt.}}{\text{wet sample wt.}} \right) \times 100
\]

9. For each treatment measurements were made in duplicate.

**Protein Analysis**

1. Protein was analyzed by using a nitrogen analyzer (LECO TruSpec Nitrogen/Protein determinator, LECO Corp., St. Joseph, MI).

2. A combustion method was used to determine the nitrogen released at high temperature and measured by thermal conductivity.

3. A nitrogen-to-protein conversion factor of 6.25 was used.
4. Weigh out approximately 250-350 mg of meat sample into the tared piece of foil. The sample ID is entered into the machine and the weight is recorded.

5. Protein analysis is conducted using a Leco Protein Analyzer. The Leco Protein Analyzer is run according to the manufacturer’s directions.

6. For each treatment, measurements were made in duplicate.
APPENDIX 18: CONSUMER TEST OF HAM (Ballot)

Please read the informed consent form and, if you agree to participate, sign and date it and pass it through the door at the front of the booth. An attendant will give you a registration code and your first sample. Please answer all questions. Your name is not on the questionnaire and you will not be identified with your answers.

Registration Code ________

1. What is your age?
   18-24 ____
   25-34 ____
   35-44 ____
   45-54 ____
   55-64 ____
   >64 ____

2. How often do you typically consume ham?
   At least once per week_______
   Two times per month_______
   Once per month___________
   Less than once a month_____

3. What is your most important consideration when purchasing ham (choose only one)?
   Price________
   Brand name____
   Nutritional value____
   Ingredients________

Now we want you to indicate how much you like some sensory attributes of five ham products. You will also be asked to tell us anything you liked or disliked about each sample. After you have evaluated each sample you will be asked whether you would purchase that product.

1. Enter the Code Number of the First Sample ___

Smell the sample and check the box that indicates how much you like or dislike the AROMA of the ham.

- dislike extremely
- neither like or dislike
- like extremely
Look at the sample and check the box that indicates how much you like or dislike the SURFACE COLOR of the ham.

- □ dislike extremely
- □ neither like or dislike
- □ like extremely

Now we would like you to taste the ham. Please rinse your mouth with water before starting and in between samples. Feel free to re-taste the product as often as needed and indicate how you feel about the ham.

**Overall Acceptance**

- □ dislike extremely
- □ neither like or dislike
- □ like extremely

**Texture**

- □ dislike extremely
- □ neither like or dislike
- □ like extremely

**Flavor**

- □ dislike extremely
- □ neither like or dislike
- □ like extremely

If you wish, please indicate anything you liked or disliked about the ham.

Comments:  ________________________________________________________________

Would you purchase this ham product?

Yes_______
No_______
APPENDIX 19: CONSUMER TEST OF BACON (Ballot)

Please read the informed consent form and, if you agree to participate, sign and date it and pass it through the door at the front of the booth. An attendant will give you a registration code and your first sample. Please answer all questions. Your name is not on the questionnaire and will not be identified with your answers.

Registration Code ________

1. What is your age?
   18-24 ____
   25-34 ____
   35-44 ____
   45-54 ____
   55-64 ____
   >64  ____

2. How often do you typically consume bacon?
   At least once per week________
   Two times per month_________
   Once per month______________
   Less than once a month_______

3. What is your most important consideration when purchasing bacon (choose only one)?
   Price________
   Brand name____
   Lean to fat ratio___
   Ingredients/Nutritional Label________

Now we want you to indicate how much you like some sensory attributes of five both uncooked and cooked bacon products. You will also be asked to tell us anything you liked or disliked about each sample. After you have evaluated each sample you will be asked whether you would purchase that product.

1. Enter the Code Number of the First Sample ___

Look at the packaged sample and check the box that indicates how much you like or dislike the COLOR of the LEAN IN THE UNCOOKED BACON.

- dislike extremely
- neither like or dislike
- like extremely
Smell the sample on the plate and check the box that indicates how much you like or dislike the AROMA of the COOKED BACON.

- dislike
- extremely
- neither like or dislike
- like
- extremely

Now we want you to taste the cooked bacon. Please rinse your mouth with water before starting and in between samples. Feel free to re-taste the product as often as needed and indicate how you feel about the bacon.

**Overall Acceptance**

- dislike
- extremely
- neither like or dislike
- like
- extremely

**Texture**

- dislike
- extremely
- neither like or dislike
- like
- extremely

**Flavor**

- dislike
- extremely
- neither like or dislike
- like
- extremely

If you wish, please indicate anything you liked or disliked about the uncooked or cooked bacon.

Comments: ________________________________________________________________

Would you purchase this bacon?

Yes_______  No_________
APPENDIX 20: CONSUMER TEST OF FRANKFURTERS (Ballot)

Please read the informed consent form and, if you agree to participate, sign and date it and pass it through the door at the front of the booth. An attendant will give you a registration code and your first sample. Please answer all questions. Your name is not on the questionnaire and will not be identified with your answers.

Registration Code ________

1. What is your age?
   - 18-24 ____
   - 25-34 ____
   - 35-44 ____
   - 45-54 ____
   - 55-64 ____
   - >64 ____

2. How often do you typically consume frankfurters?
   - At least once per week________
   - Two times per month________
   - Once per month________
   - Less than once a month_______

3. What is your most important consideration when purchasing frankfurters (choose only one)?
   - Price_______
   - Brand name_______
   - Nutritional value_______
   - Ingredients__________

Now we want you to indicate how much you like some sensory attributes of five frankfurter products. You will also be asked to tell us anything you liked or disliked about each sample. After you have evaluated each sample you will be asked whether you would purchase that product.

1. Enter the Code Number of the First Sample ___

Smell the sample and check the box that indicates how much you like or dislike the AROMA of the frankfurter.

- dislike extremely
- neither like or dislike
- like extremely
Look at the sample and check the box that indicates how much you like or dislike the INTERNAL COLOR of the frankfurter.

[ ] [ ] [ ] [ ] [ ] [ ] [ ] [ ] [ ]
  dislike extremely
  neither like or dislike
  like extremely

Now we would like you to taste the frankfurter. Please rinse your mouth with water before starting and in between samples. Feel free to re-taste the product as often as needed and indicate how you feel about the frankfurter.

Overall Acceptance

[ ] [ ] [ ] [ ] [ ] [ ] [ ] [ ] [ ]
  dislike extremely
  neither like or dislike
  like extremely

Texture

[ ] [ ] [ ] [ ] [ ] [ ] [ ] [ ] [ ]
  dislike extremely
  neither like or dislike
  like extremely

Flavor

[ ] [ ] [ ] [ ] [ ] [ ] [ ] [ ] [ ]
  dislike extremely
  neither like or dislike
  like extremely

If you wish, please indicate anything you liked or disliked about the frankfurter.

Comments:  ____________________________________________________________

Would you purchase this frankfurter?
  Yes_______
  No_______
APPENDIX 21: Uncured No Nitrate/Nitrite Added EFSC Sausage Experiment Formulations

TRT 1: Low VJP (0.20%) & Short Incubation (30 minutes)  
TRT 2: Low VJP (0.20%) & Long Incubation (120 minutes)

Meat Block:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>TRT 1 Quantity</th>
<th>TRT 2 Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef Trim 80/20</td>
<td>37.5 lb</td>
<td></td>
</tr>
<tr>
<td>Pork Trim 50/50 (42%)</td>
<td>12.5 lb</td>
<td></td>
</tr>
</tbody>
</table>

Ingredients:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>TRT 1 Quantity</th>
<th>TRT 2 Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water/Ice</td>
<td>10.0 lb</td>
<td></td>
</tr>
<tr>
<td>A.C. Legg Spice Pack</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blend TG-05-405-000</td>
<td>14.30 oz</td>
<td></td>
</tr>
<tr>
<td>Salt (2.25%)</td>
<td>1.12 lb</td>
<td>508.40 gm</td>
</tr>
<tr>
<td>Dextrose (2.0%)</td>
<td>1.00 lb</td>
<td>454.00 gm</td>
</tr>
<tr>
<td>Vegetable Juice Powder (CHR Hansen)</td>
<td>0.126 lb</td>
<td>57.22 gm</td>
</tr>
<tr>
<td>- (0.20% of total formulation)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starter culture (CS 299 Bactoferm)</td>
<td>7.35 gm</td>
<td></td>
</tr>
<tr>
<td>(35 gm / 300 lb meat)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TRT 3: High VJP (0.40%) & Short Incubation (30 minutes)  
TRT 4: High VJP (0.40%) & Long Incubation (120 minutes)

Meat Block:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>TRT 3 Quantity</th>
<th>TRT 4 Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef Trim 80/20</td>
<td>37.5 lb</td>
<td></td>
</tr>
<tr>
<td>Pork Trim 50/50 (42%)</td>
<td>12.5 lb</td>
<td></td>
</tr>
</tbody>
</table>

Ingredients:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>TRT 3 Quantity</th>
<th>TRT 4 Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water/Ice</td>
<td>10.0 lb</td>
<td></td>
</tr>
<tr>
<td>A.C. Legg Spice Pack</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blend TG-05-405-000</td>
<td>14.30 oz</td>
<td></td>
</tr>
<tr>
<td>Salt (2.25%)</td>
<td>1.12 lb</td>
<td>508.40 gm</td>
</tr>
<tr>
<td>Dextrose (2.0%)</td>
<td>1.00 lb</td>
<td>454.00 gm</td>
</tr>
<tr>
<td>Vegetable Juice Powder (CHR Hansen)</td>
<td>0.252 lb</td>
<td>114.43 gm</td>
</tr>
<tr>
<td>- (0.40% of total formulation)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starter culture (CS 299 Bactoferm)</td>
<td>7.35 gm</td>
<td></td>
</tr>
<tr>
<td>(35 gm / 300 lb meat)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Control: Nitrite added

Meat Block:
- Beef Trim 80/20: 37.5 lb
- Pork Trim 50/50 (42%): 12.5 lb

Ingredients:
- Water/Ice: 10.00 lb
- A.C. Legg Spice Pack Blend TG-05-405-000: 14.3 oz
- Salt (2.25%): 1.00 lb, 454.00 gm
- Dextrose (2.0%): 1.00 lb, 454.00 gm
- Sodium Nitrite (6.25% cure): 0.125 lb, 56.60 gm
- Sodium Erythorbate: 0.0275 lb, 12.49 gm

Processing Schedule:
1. Grind lean (beef) and fat trimmings (pork) through ½" plate.
2. Place lean trimmings, salt, ½ of water (½ water / ½ ice) and vegetable juice powder into bowl chopper.
3. Add rest of dry ingredients into chopper and chop to 36 °F.
4. Add 50/50 pork trim and remaining water and chop to 55-59 °F.
5. Stuff into plastic casing.
6. Split TRTs into two equal batches and incubate. Incubation time begins when internal temperature of product reaches 100 °F.
7. Thermal process to internal temperature of 160 °F.
8. Place in cooler and chill to below 40 °F.

Short Incubation (TRT 1 & 3) Smoke House Schedule:

<table>
<thead>
<tr>
<th>Step</th>
<th>Time</th>
<th>Dry Bulb (°F)</th>
<th>Wet Bulb (°F)</th>
<th>Relative Humidity</th>
<th>Dampers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (cook)</td>
<td>Int = 100</td>
<td>105</td>
<td>103</td>
<td>93%</td>
<td>Auto</td>
</tr>
<tr>
<td>2 (cook)</td>
<td>30 min.</td>
<td>105</td>
<td>103</td>
<td>93%</td>
<td>Auto</td>
</tr>
<tr>
<td>3 (cook)</td>
<td>20 min.</td>
<td>140</td>
<td>110</td>
<td>38%</td>
<td>Auto</td>
</tr>
<tr>
<td>4 (cook)</td>
<td>20 min.</td>
<td>160</td>
<td>145</td>
<td>67%</td>
<td>Auto</td>
</tr>
<tr>
<td>5 (cook)</td>
<td>Int = 160</td>
<td>185</td>
<td>178</td>
<td>85%</td>
<td>Auto</td>
</tr>
<tr>
<td>6 (shower)</td>
<td>15 min.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Long Incubation (TRT 2 & 4) Smoke House Schedule:

<table>
<thead>
<tr>
<th>Step</th>
<th>Time</th>
<th>Dry Bulb ($^\circ$F)</th>
<th>Wet Bulb ($^\circ$F)</th>
<th>Relative Humidity</th>
<th>Dampers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (cook)</td>
<td>Int = 100</td>
<td>105</td>
<td>103</td>
<td>93%</td>
<td>Auto</td>
</tr>
<tr>
<td>2 (cook)</td>
<td>120 min.</td>
<td>105</td>
<td>103</td>
<td>93%</td>
<td>Auto</td>
</tr>
<tr>
<td>3 (cook)</td>
<td>20 min.</td>
<td>140</td>
<td>110</td>
<td>38%</td>
<td>Auto</td>
</tr>
<tr>
<td>4 (cook)</td>
<td>20 min.</td>
<td>160</td>
<td>145</td>
<td>67%</td>
<td>Auto</td>
</tr>
<tr>
<td>5 (cook)</td>
<td>Int = 160</td>
<td>185</td>
<td>178</td>
<td>85%</td>
<td>Auto</td>
</tr>
<tr>
<td>6 (shower)</td>
<td>15 min.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX 22: Uncured No Nitrate/Nitrite Added Ham Experiment Formulations

Control (C)

Meat Block:

25 lb  Lean pork boneless inside and outside ham muscles

Injection:

25% Injection

Brine formulation for Control ham:

<table>
<thead>
<tr>
<th>Ingredient (25% Injection)</th>
<th>Percent</th>
<th>Weight (lb)</th>
<th>PPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>80.70 %</td>
<td>100.88 lb.</td>
<td></td>
</tr>
<tr>
<td>Salt</td>
<td>9.80 %</td>
<td>12.25 lb.</td>
<td>24,500 ppm</td>
</tr>
<tr>
<td>Dextrose</td>
<td>8.00 %</td>
<td>10.00 lb.</td>
<td>20,000 ppm</td>
</tr>
<tr>
<td>Sodium Erythorbate</td>
<td>0.22 %</td>
<td>0.275 lb.</td>
<td>550 ppm</td>
</tr>
<tr>
<td>Cure mix (6.25% nitrite)</td>
<td>1.28 %</td>
<td>1.60 lb.</td>
<td>200 ppm</td>
</tr>
<tr>
<td>Total</td>
<td>100.00 %</td>
<td>125.0 lb.</td>
<td></td>
</tr>
</tbody>
</table>

Processing procedures:

1. Prepare meat ingredients: use skinless, boneless ham muscles. Trim ham muscles practically free of fat.
2. Prepare brine by adding ingredients to water while mixing. Add the ingredients in the following order: salt, sodium nitrite, dextrose and sodium erythorbate.
3. Inject hams to 25% over their initial weight.
4. Grind 90% of ham muscles through kidney plate.
5. Grind remaining 10% of the meat through 3/16” plate.
6. Weigh meat and add any additional brine to equal 31.25 lbs (25% gain).
7. Place all the meat in a tumbler.
8. Tumble under vacuum on medium speed continuously for one hour.
9. Stuff hams into plastic impermeable casings.
10. Thermal process hams using attached smokehouse schedule.
11. Chill in cooler (30-32 °F) and store under refrigeration.
TRT 1: Low VJP (0.20%) & Short Incubation (0 minutes)
TRT 2: Low VJP (0.20%) & Long Incubation (120 minutes)

Meat Block:
50 lb  Lean pork boneless inside and outside ham muscles

Injection:
25% Injection

Brine formulation for Low VJP ham:

<table>
<thead>
<tr>
<th>Ingredient (25% Injection)</th>
<th>Percent</th>
<th>Weight (lb)</th>
<th>PPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>79.87%</td>
<td>99.84 lb.</td>
<td></td>
</tr>
<tr>
<td>Salt</td>
<td>11.00%</td>
<td>13.75 lb.</td>
<td>27,500 ppm</td>
</tr>
<tr>
<td>Dextrose</td>
<td>8.00%</td>
<td>10.00 lb.</td>
<td>20,000 ppm</td>
</tr>
<tr>
<td>Vegetable Juice Powder (0.20%)</td>
<td>1.00%</td>
<td>1.25 lb.</td>
<td>2500 ppm (meat only) 2000 ppm total (meat + water)</td>
</tr>
<tr>
<td>Starter Culture (35g/300 lb) (7.29 g per 100 lb of brine)</td>
<td>0.1285%</td>
<td>0.1606 lb.</td>
<td>321 ppm</td>
</tr>
<tr>
<td>Total</td>
<td>100.00%</td>
<td>125.0 lb.</td>
<td></td>
</tr>
</tbody>
</table>

Processing procedures:
2. Prepare brine by adding ingredients to water while mixing. Add the ingredients in the following order: salt, sugar, vegetable juice powder and starter culture.
3. Inject hams to 25% over their initial weight.
4. Grind 90% of ham muscles through kidney plate.
5. Grind remaining 10% of the meat through 3/16" plate.
6. Weigh meat and add any additional brine to equal 31.25 lbs (25% gain)
7. Place all the meat in a tumbler.
8. Tumble under vacuum on medium speed continuously for one hour.
9. Stuff hams into plastic impermeable casings.
10. Thermal process hams using smokehouse schedule.
11. Chill in cooler (30-32 ºF) and store under refrigeration.
TRT 3: High VJP (0.35%) & Short Incubation (0 minutes)
TRT 4: High VJP (0.35%) & Long Incubation (120 minutes)

Meat Block:
50 lb  Lean pork boneless inside and outside ham muscles

Injection:
25% Injection

Brine formulation for High VJP ham:

<table>
<thead>
<tr>
<th>Ingredient (25% Injection)</th>
<th>Percent</th>
<th>Weight (lb)</th>
<th>PPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>79.12 %</td>
<td>98.90 lb.</td>
<td></td>
</tr>
<tr>
<td>Salt</td>
<td>11.00 %</td>
<td>13.75 lb.</td>
<td>27,500 ppm</td>
</tr>
<tr>
<td>Dextrose</td>
<td>8.00 %</td>
<td>10.00 lb.</td>
<td>20,000 ppm</td>
</tr>
<tr>
<td>Vegetable Juice Powder (0.35%)</td>
<td>1.75 %</td>
<td>2.1875 lb.</td>
<td>4375 ppm (meat only) 3500 ppm total (meat + water)</td>
</tr>
<tr>
<td>Starter Culture (35g/300 lb) (7.29 g per 100 lb of brine)</td>
<td>0.1285 %</td>
<td>0.1606 lb.</td>
<td>321 ppm</td>
</tr>
<tr>
<td>Total</td>
<td>100.00 %</td>
<td>125.0 lb.</td>
<td></td>
</tr>
</tbody>
</table>

Processing procedures:
2. Prepare brine by adding ingredients to water while mixing. Add the ingredients in the following order: salt, sugar, vegetable juice powder and starter culture.
3. Inject hams to 25% over their initial weight.
4. Grind 90% of ham muscles through kidney plate.
5. Grind remaining 10% of the meat through 3/16” plate.
6. Weigh meat and add any additional brine to equal 31.25 lbs (25% gain)
7. Place all the meat in a tumbler.
8. Tumble under vacuum on medium speed continuously for one hour.
9. Stuff hams into plastic impermeable casings.
10. Thermal process hams using smokehouse schedule.
11. Chill in cooler (30-32 °F) and store under refrigeration.
### Smoke House Schedules

#### Short Incubation (TRT 1, TRT 3) & Control (C) Smoke House Schedule:

<table>
<thead>
<tr>
<th>Time</th>
<th>Dry Bulb (°F)</th>
<th>Wet Bulb (°F)</th>
<th>Relative Humidity</th>
<th>Humidity</th>
<th>Dampers</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>105</td>
<td>103</td>
<td>93%</td>
<td>Steam</td>
<td>Auto</td>
</tr>
<tr>
<td>30 min</td>
<td>140</td>
<td>140</td>
<td>100%</td>
<td>Steam</td>
<td>Closed</td>
</tr>
<tr>
<td>60 min</td>
<td>150</td>
<td>150</td>
<td>100%</td>
<td>Steam</td>
<td>Closed</td>
</tr>
<tr>
<td>45 min</td>
<td>160</td>
<td>160</td>
<td>100%</td>
<td>Steam</td>
<td>Closed</td>
</tr>
<tr>
<td>45 min</td>
<td>170</td>
<td>170</td>
<td>100%</td>
<td>Steam</td>
<td>Closed</td>
</tr>
<tr>
<td>Int = 158°</td>
<td>180</td>
<td>180</td>
<td>100%</td>
<td>Steam</td>
<td>Closed</td>
</tr>
<tr>
<td>20 min C. shower</td>
<td>50</td>
<td>50</td>
<td>-----</td>
<td>Off</td>
<td>Auto</td>
</tr>
</tbody>
</table>

#### Long Incubation (TRT 2, TRT 4) Smoke House Schedule:

<table>
<thead>
<tr>
<th>Time</th>
<th>Dry Bulb (°F)</th>
<th>Wet Bulb (°F)</th>
<th>Relative Humidity</th>
<th>Humidity</th>
<th>Dampers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Int = 100°F</td>
<td>105</td>
<td>103</td>
<td>93%</td>
<td>Steam</td>
<td>Auto</td>
</tr>
<tr>
<td>120 min</td>
<td>105</td>
<td>103</td>
<td>93%</td>
<td>Steam</td>
<td>Auto</td>
</tr>
<tr>
<td>30 min</td>
<td>140</td>
<td>140</td>
<td>100%</td>
<td>Steam</td>
<td>Closed</td>
</tr>
<tr>
<td>60 min</td>
<td>150</td>
<td>150</td>
<td>100%</td>
<td>Steam</td>
<td>Closed</td>
</tr>
<tr>
<td>45 min</td>
<td>160</td>
<td>160</td>
<td>100%</td>
<td>Steam</td>
<td>Close</td>
</tr>
<tr>
<td>45 min</td>
<td>170</td>
<td>170</td>
<td>100%</td>
<td>Steam</td>
<td>Closed</td>
</tr>
<tr>
<td>Int = 158°F</td>
<td>180</td>
<td>180</td>
<td>100%</td>
<td>Steam</td>
<td>Closed</td>
</tr>
<tr>
<td>20 min C. shower</td>
<td>50</td>
<td>50</td>
<td>-----</td>
<td>Off</td>
<td>Auto</td>
</tr>
</tbody>
</table>
# CS 299 Bactoferm™

**Product Information**

## Description

Meat culture for enhancing color and flavor formation in cured, spreadable and fermented meat products acidified by natural or chemical means. This culture is a meat starter cultures producing lactic acid and other flavor compounds.

CS 299 is a highly concentrated single strain culture containing *Staphylococcus carnosus* in a convenient freeze-dried form. *S. carnosus* enhances the formation of a pleasant mild flavor profile and a good stable color.

## Application

CS 299 is recommended in the production of all fermented cured sausages, sliceable as well as spreadable, in which the acidification is obtained by means of lactic acid bacteria or chemical acidulants (GDL, citric acid or likewise).

## Packing

<table>
<thead>
<tr>
<th>Packing size</th>
<th>Material number</th>
</tr>
</thead>
<tbody>
<tr>
<td>25g for 225kg</td>
<td>673911</td>
</tr>
</tbody>
</table>

## Storage and shelf life

Freeze-dried cultures should be stored at below -17°C (0°F) to have a shelf life for at least 18 months. At -5°C (41°F) the shelf life is at least 6 weeks.

## Instructions for use

Addition to sausage mince: The contents of the pouch should be added directly to the bowl chopper early in the process together with the dry ingredients. According to USDA regulations/Flavoring Agents this product is labeled as "Lactic Acid Starter Culture".

## Dosage

Follow the information on the pouch.

## Production temperature

Recommended production temperature is 20°C (68°F) as a minimum in order to ensure activity. The fermentation and drying procedures should be adapted to the specific recipes.
## Technical data

<table>
<thead>
<tr>
<th>Culture</th>
<th>CS 299</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria strain composition</td>
<td><em>Staphylococcus carnosus</em></td>
</tr>
<tr>
<td>Temperature</td>
<td>30°C/45°C/10°C (86°F/113°F/50°F)</td>
</tr>
<tr>
<td>Salt limit</td>
<td>16% salt-in-water</td>
</tr>
</tbody>
</table>
| Characteristics | Facultative anaerobic  
                 | Catalase positive  
                 | Nitrate reductase positive  
                 | Lipolytic  
                 | Proteolytic  
                 | DL(+/−) lactic acid producing |
| Fermentoriable sugars | Glucose (dextrose)  
                        | Fructose  
                        | Maltose  
                        | Lactose  
                        | Saccharose (sucrose)  
                        | Starch  |
| Carrier       | Glucose (dextrose) |
| Appearance    | White powder with brownish particles |
| Packing       | Freeze-dried culture packed in water and air proof aluminum foil pouch |

## Technical service

Chr. Hansen's worldwide facilities and the personnel of our Application and Technology Centers are at your disposal with assistance, instructions and guidance for your choice of culture and needs for spice and seasoning blends.

## References

References and analytical methods are available upon request.

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The information contained herein is to our knowledge true and correct and presented in good faith. However, no warranty, guarantee, or freedom from patent infringement is implied or inferred. This information is offered solely for your consideration and verification.
APPENDIX 24: CHR Hansen Vegetable Juice PWD NAT Product Information

August 2005: CWR

Product Description: Vegetable Juice Powder Natural is a dried powder derived from the flesh of celery. It retains the typical sweet flavor of raw celery.

Ingredients: Natural Flavor (Celery Powder).

Storage: To assure best quality, storage and shipment in tightly closed containers at 50° to 80° F (10° to 27° C) is recommended. Store in a cool, clean, and dry area with relative humidity at 70% maximum.

Stability: If stored in sealed containers under proper conditions, this product should remain stable for twelve (12) months.

Packaging: Code # Package Size
672303 44.09 Lb (20 kg) Foil Pouch

Applications: Vegetable Juice Powder Natural can be used in meats, dry soups, beverages, and seasoning blends.

Usage Level: 0.2% or higher.

Approval: Vegetable Juice Powder Natural complies USDA regulations 9 CFR 317.2 (f) (1) (l).

Labeling: Natural Flavor, or Celery Powder.
APPENDIX 25: World Pac International EFSC Sausage Casing – WP-E

WP-E

TENSILE STRENGTH AT BREAK [MPa]  40 - 50

ELONGATION AT BREAK [%]  300

OXYGEN PERMEABILITY (O₂) [cm³/m²·d·bar]  6 - 7

WATER VAPOUR PERMEABILITY [g/m²·d]  130

WATER ABSORPTION AT EQUILIBRIUM 23°C  9 - 10

STORAGE TIME:
SHIRRED IN NET  max. 3 months
SHIRRED, READY TO STUFF  max. 6 months

The storage time is applied principally for transparent products; colored product have to be tested for a longer period to guarantee the storage time.

STORAGE CONDITIONS:
Normal conditions (15 - 25°C; 50 - 70% rel. humidity), don’t expose it directly to irradiation (sun).

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Date: 9/29/03
7900 Durand Ave. Bldg. #4 Northwest / P.O. Box 742 / Sturtevant, WI 53177-0742
Phone: (262) 598-0062  Fax: (262) 598-0064
APPENDIX 26: World Pac International Ham Casing – HC5

HC5

THICKNESS  
45 micron, tolerance +/- 5 micron

LAYFLAT  
According to detailed specification between 35 and 280mm  
Tolerance +/- 1mm  
Layflat of shrinkable casing measured directly after production  
During converting, transportation, storage or soaking, the layflat may shrink by a  
maximum of 3%. The product will reach its designated filling diameter despite the  
temporary shrink.

SHRINKAGE  
10 - 14% in both directions

BURSTING PRESSURE  
> 50 kPa

WATER VAPOUR  
PERMEABILITY  
5 g/m²d (DIN 53122), tolerance +1 point

OXYGEN  
PERMEABILITY  
12 cm³/m²d*bar (DIN 53380), tolerance +1 point

TEMPERATURE  
STABILITY  
121° C

**This information is confidential and proprietary to World Pac International USA, Inc. or its affiliates. It is intended only for the recipient and for the express purpose(s) described therein. Any other use is prohibited**

Date: 9/29/03
7900 Durand Ave. Bldg. #4 Northwest / P.O. Box 742 / Sturtevant, WI 53177-0742  
Phone: (262) 598-0062  
Fax: (262) 598-0064
APPENDIX 27: Sensory Evaluation of Frankfurters (Ballot)

Registration Code____
Date_______
Sample______

Please indicate the intensity of the following attributes. Rinse your mouth with water before tasting the sample.

Cured Frankfurter Aroma

none  intense

Internal Cured Frankfurter Color

low  high

Uniformity of Internal Frankfurter Color

not uniform  uniform

Cured Frankfurter Flavor

none  intense

Firmness

soft  hard

Comments:
<table>
<thead>
<tr>
<th>Attribute</th>
<th>None</th>
<th>Intense</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ham Aroma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetable Aroma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ham Flavor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetable Flavor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Firmness</td>
<td>Soft</td>
<td>Hard</td>
</tr>
<tr>
<td>Intensity of Ham Color</td>
<td>None</td>
<td>Intense</td>
</tr>
</tbody>
</table>

Comments:
ACKNOWLEDGEMENTS

It has been an enjoyable, busy, at times frustrating and predominantly enjoyable experience here at ISU for the past 4+ years. Although the burden of achieving my goals was ultimately placed on me, there are many great people that I wish to acknowledge for their contributions towards helping me attain my goals.

I would like to begin by thanking Dr. Joe Cordray, my academic advisor. From the moment our conversation took place, while I pulled off the interstate in MI to receive the call, when he offered me an opportunity to work under him, my excitement and gratitude has never dwindled. Although nearly impossible to do appropriately here, I would like to say thanks for simply...everything. Your guidance, advice, life lessons, examples and continual support have been sincerely appreciated and have instilled qualities in me that will no doubt greatly contribute to my future success. I also appreciate all the opportunities that you have given me to travel across the country and world teaching and learning and Meat Science.

I would like to thank all the members of my graduate committee. To Dr. Jane Love, for all your contributions and support you have given me about food sensory. To Dr. Aubrey Mendonca, who I consider one of the finest instructors I've every had, for all your thought provoking questions and support throughout my academic time. To Dr. Dennis Olson, for all your advice and recommendations for my projects. To Dr. Dong Ahn, for all your advice, recommendations and support, I truly appreciate. I have learned a great deal about scientific approach and methodology from you.
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I would like to especially thank Professor Bob Rust for all your help, teaching and mentoring. Thank you for serving on my committee as well as all the other things you’ve done for me. I will truly miss manufacturing processed meat products with you…especially the ones made for no reason except for the fun of it.

I would like to recognize the ISU Meat Laboratory staff and student employees for all the assistance provided to me during my project as well as the countless other times I’ve done things in the Meat Lab. I’ve enjoyed the support and friendship given by Randy Petersohn, Steve Bryant, Jeff Mitchell, Vail Olson and Mike Holtzbauer who I consider all top-notch individuals. A special thanks to Deb Michel for your invaluable never-ending support. I can’t begin to tell you how much I’ve appreciated all you’ve done for me.

Thank you to Macia King-Brink and Elaine Larson for all you’ve done to make my research possible and as successful (my opinion) as it was. Without your expertise, I may still be doing lab work.

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stated the importance of surrounding oneself with good people and these individuals easily fit that bill.

To my parents and family who have been supportive throughout my life and my academic career. Although what I do is not always easy to understand, I appreciate all the positive support you’ve given me doing my years in college.

Finally and most importantly, I’d like to thank my wife, Connie, for all her support. You have been my rock throughout my college endeavors and I sincerely appreciate that. Your words of encouragement, scientific advice and support of my Meat Science interests are impossible to acknowledge in a couple statements.

In conclusion, if I were asked to do it all over again, I’d be ready in a heart-beat.