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Incubation of curing brines for the production of ready-to-eat uncured ham

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Incubation of curing brines for the production of ready-to-eat uncured ham

by

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CHAPTER 1. GENERAL INTRODUCTION

Natural and organic processed meats have seen a dramatic increase in demand in recent years. Because these products are not allowed to contain chemical preservatives, traditional curing agents, nitrate and nitrite, are prohibited from natural and organic processed meat products. Nitrate and nitrite are unique ingredients used in the production of cured meats which produce typical properties such as desirable flavor and a distinctive color, as well as prevent the growth of spoilage bacteria and pathogens, most notably *Clostridium botulinum*.

Although it has many desired benefits, there are many negative perceptions of nitrite-cured meat products, most notably the formation of carcinogenic nitrosamines. This has allowed the natural and organic market to increase, as many consumers believe products labeled no nitrate/nitrite-added are safer and healthier. There are two types of products available to consumers that are labeled uncured. The first type has no intention of having any traditional cured meat characteristics, thus not having any direct or indirect form of nitrite. The second type is one which resembles a traditional cured product but where an indirect form of nitrite is added. Because nitrate and/or nitrite are being introduced to uncured products, the labeling is misleading and technically inaccurate.

Alternative processes have been identified to produce natural and organic meats labeled uncured, no-nitrate/nitrate-added, that resemble traditional cured products. It has been shown that uncured meat and poultry products can be manufactured with ingredients high in nitrate, and a nitrate reducing starter culture to produce cured meat products with similar sensory and quality characteristics as traditionally cured products. Several issues need

to be considered due to the fact that it is impossible to measure the amount of nitrite produced in this process. Low concentrations of nitrite bring up the concern for food safety, especially the survival and outgrowth of *C. botulinum* and excess amounts risk the formation of nitrosamines.

Manufacturing procedures must be standardized to prevent product variation, as well as maintain the quality and safety of products labeled no-nitrate/nitrite-added. One method to achieve this is to determine if pre-conversion of nitrate to nitrite in a brine will reduce product variation and assure product safety by allowing the amount of ingoing nitrite to be analytically measured and controlled.

Thesis Organization

This thesis is organized into four 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 the relevant topics pertaining to this research project. The third chapter is a manuscript to be submitted to Meat Science titled "Incubation of curing brines for the production of ready-to-eat uncured ham". The fourth chapter is a general summary of this research.

CHAPTER 2. LITERATURE REVIEW

History of Meat Curing

The curing of meat has been performed for centuries and can be traced back to the earliest recorded history. Historically, salt was the primary ingredient used for meat preservation because it was realized that cuts of meat would not spoil as fast when packed in dry salt. Salt prevents microbial growth due to its direct inhibition of bacteria and also because it decreases the water activity (Pegg & Shahidi, 2000). After centuries of salting meat, certain salts became more desirable because they created a distinct pink color and unique flavor. It is widely accepted that impurities of potassium nitrate were responsible for the characteristic cured meat pigment and special flavor (Binkerd & Kolari, 1975; Cassens, Ito, Lee & Buege, 1978). It is not known when ancient civilizations began curing meat intentionally, but history shows that the Romans were intentionally adding saltpeter (potassium nitrate) as a curing agent to obtain desirable cured meat characteristics during the 10th century B.C. (Pierson & Smoot, 1982).

Nitrate, either as a contaminant of salt or as saltpeter, was the primary ingredient used to cure meat for thousands of years before scientists closely examined the curing reaction. Interest began at the end of the 19th century after it was determined that pure salt (sodium chloride) did not produce a cured meat product. In 1891, Polenske (reported in Binkerd & Kolari, 1975; Townsend & Olson, 1987) discovered that the color change was caused by the sodium and potassium nitrates present as a contaminant in unpurified salt used in curing. Furthermore, the nitrate was converted to nitrite by naturally occurring bacteria. Soon after, Lehmann and Kisskalt (reported in Binkerd & Kolari, 1975; Townsend & Olson, 1987)

demonstrated that the characteristic cured color resulted from nitrite, and not nitrate as previously thought. Subsequent studies by Haldane in 1901 and Hoagland in 1908 (reported in Pegg & Shahidi, 2000) stated that it was essential for nitrite to be further reduced to nitric oxide and nitrous acid to produce a red color in cured cooked meats.

By 1917 curing mixtures containing nitrite were being marketed in Europe and a U.S. patent (Doran, 1917) for nitrite was issued. Several studies observed that products cured with nitrate alone produced extremely variable results, and often resulted in high nitrite levels in the product (Pegg & Shahidi, 2000). In 1923, the USDA authorized experiments using the direct addition of nitrite. One study demonstrated that the flavor and shelf life of meats cured solely with nitrite were equal to those cured with nitrate, and that curing with nitrites, rather than nitrates, shortened the curing period that was customary at that time. As a result of this study, the USDA authorized sodium nitrite as a curing agent in federally inspected establishments (Kerr, Marsh, Schroeder & Boyer, 1926).

Through the next few decades, meat processors gradually shifted from the use of sodium nitrate to the use of sodium nitrite with the main goal being a reduction in curing time to increase production capacity. As the curing process became better understood, the technology allowed processors to use less nitrite and have more control over the curing of meat (Sebranek, 1979). In the late 1960's and into the early 1970's, it became apparent that nitrite could result in the formation of carcinogenic n-nitrosamines in cured meat. This prompted a great deal of research and it was determined that a major factor of nitrosamine formation was the concentration of residual nitrite. As a result, nitrate was eliminated from most curing processes to decrease residual nitrite concentrations (Pegg & Shahidi, 2000).

Methods of Curing

There are several techniques used to achieve a cured product, however, all procedures fall into one of two basic methods of meat curing; dry curing and brine curing. Regardless of method used, the most important requirement is the even distribution of cure ingredients throughout the entire product. Sausage products can be cured with either method and the curing ingredients are incorporated during the mixing-comminution steps (Aberle et al., 2001).

Dry curing is the oldest method used to distribute the curing ingredients throughout the meat. In dry curing, the curing ingredients are rubbed on the surface of the meat. These ingredients dissolve in moisture drawn from the muscle tissue and diffuse through the specific cut of meat over a long period of time, diffusing at the rate of about 1 inch per week (Townsend & Olson, 1987; Aberle et al., 2001; Romans, Jones, Costello, Carlson & Ziegler, 2001). For large cuts the dry ingredients must be rubbed over the surface multiple times, making the process very labor intensive.

Brine curing entails the immersion of whole cuts of meat into solutions containing nitrite. Wet curing uses the same ingredients utilized in dry curing except that the ingredients are dissolved in water to form a pickle or brine. If sugar is included in the brine, it is referred to as a sweet pickle (Romans et al., 2001; Pegg & Shahidi, 2006). During brine curing, the initial outward flow of water and soluble proteins from the muscle tissue is reversed due to osmotic pressure. This is because the salt diffuses into the muscle forming a complex with the proteins of the meat, causing a higher osmotic pressure than the brine, resulting in an inward flow of water and curing ingredients to the muscle (Lawrie & Ledward, 2006). Although immersion curing is faster than dry curing, it is still relatively slow. When curing

large cuts of meat, it may take long periods of time for diffusion of the curing agents through the entire product. Due to the high water activity, microbial growth and spoilage can occur during brine curing even though the product is refrigerated and salt is present (Aberle et al., 2001; Pegg & Shahidi, 2006). Therefore, curing is often achieved by the injection of a brine directly into different meat cuts which achieve a more rapid and uniform distribution of pickle throughout the tissues (Townsend & Olson, 1987). These methods include artery pumping, which injects a pickle solution directly into the vascular system of a ham. With this technique, the curing agents travel throughout the tissue utilizing the arteries of the muscle for distribution of cure. Another common practice of pumping meat is stitch pumping where brine is injected through needles into various locations of the meat (Pegg & Shahidi, 2000). More recently, the process of multiple needle injection has become popular. In this process, a brine or pickle is prepared and then injected mechanically under pressure through needles. These machines inject brine at hundreds of locations throughout the meat ensuring rapid, continuous curing of meat with uniform distribution of the cure (Pegg & Shahidi, 2006). Mechanical methods can also be used to aid in the distribution of pickle in immersed or pumped products. Tumbling or massaging subjects products to agitation by massaging the pieces of meat against one another in rotating drums. This agitation helps disrupt tissue structure and increases the distribution of cure ingredients (Lawrie & Ledward, 2006). During the tumbling or massaging process the salt in the curing solution extracts salt-soluble myofibrillar proteins through the disruption of muscle tissue, increasing the water binding of proteins. As a result of the extracted proteins, a protein exudate forms which envelops pieces of meat and binds meat chunks together during thermal processing. (Aberle et al., 2001).

Current U.S. Regulations on Nitrite and Nitrate

Either sodium or potassium nitrite/nitrate may be used as a curing agent. However, they have the same limits based on weight (with the exception of bacon). Because potassium is a heavier element than sodium, this results in less nitrite in the product when potassium salts act as the carrier. Bacon also allows the use of either sodium or potassium nitrite, but there are different limits for the two salts. According to FSIS Directive 7620.3, the USDA requires a minimum of 120 parts per million for all cured meat products that are not shelf stable, unless the specific process is validated to be safe. There is no minimum ingoing nitrite level for shelf stable products, however 40 parts per million is recommended for preservation purposes. Current regulations on the maximum use of nitrite and nitrate vary depending on the method of curing used. All nitrite concentrations are based on the green weight of the meat block used in the formulation of the product. Table 2.1 displays the current regulations according to the USDA FSIS Processing Calculations Inspectors Handbook (1995). Due to the concern of nitrosamine formation, nitrite regulations are more stringent in bacon. Also, because of the same concern, nitrate is no longer permitted in any bacon (pumped and/or massaged, dry cured, or immersion cured) (USDA, 1995).

Bacon requires different regulations due to possible nitrosamine formation. The method of bacon processing determines the limits for nitrite. All limits are based on the green weight of the belly with the rind off. For immersion cured or massaged or pumped bellies (rind off), an amount of 120 ppm ingoing nitrite is required (148 ppm potassium nitrite). If the skin is not removed from the bellies, the ingoing nitrite level must be reduced because the skin is approximately 10% of the belly weight, and the skin retains virtually no curing

solution. Therefore nitrite usage must be decreased by 10%. The maximum ingoing nitrite

levels for sodium nitrite to cure bellies with the rind on are 108 ppm.

Curing Agent	Curing Method			
	Immersion Cured	Massaged or Pumped	Comminuted	Dry Cured
Sodium Nitrite	200	200	156	625
Potassium Nitrite	200	200	156	625
Sodium Nitrate	700	700	1718	2187
Potassium Nitrate	700	700	1718	2187

Table 1. Maximum ingoing nitrite and nitrate limits (in ppm) for meat and poultry products
(does not include bacon). This reprint is from USDA FSIS Processing Calculations
Inspector's Handbook (FSIS Directive 7620.3).

In some cases, a process may utilize both nitrite and nitrate in combination. In this

case, the finished product must not result in more than 200 ppm of nitrite, calculated as

sodium nitrite (USDA, 1995).

Cured Meat Color

Much like the characteristic bright red pigment in fresh meat, the characteristic pink

color of cured meat is important to the consumer because it is a major factor in the criteria

used to make purchasing decisions. Sebranek, Schroder, Rust and Topel (1977) reported that

a reduction in the amount of nitrite (156 mg/kg to 0 mg/kg) added to frankfurters resulted in

a decrease in consumer panel appeal. Myoglobin is the primary protein that is responsible for

the color of postmortem muscle tissue. Myoglobin is a relatively small protein with a complex structure. It is a globular heme protein consisting of approximately 153 amino acids, dependent on species. The heme group is comprised of four pyrrole groups bound to a central iron atom. The two additional bonds required by the iron atom are referred to as the 5th and 6th ligands. The 5th ligand is always attached to a histidine residue while the sixth ligand determines the meat pigment oxidation state (Dryden & Birdsall, 1980). The chemistry of the curing reaction is complex due to the extreme reactivity of the nitrite molecule, and is not entirely understood. Cured meat color is the result of the oxidation state of the iron atom (Fe^{2+} or Fe^{3+}) and occupant of the sixth ligand of the heme iron complex. When combined with nitrite, nitric oxide, and reductants, a number of reactions occur that lead to the cured meat pigment, nitrosohemochrome (Fox, 1966).

In order to form the color in cured meat, nitric oxide must first be produced from nitrite (NO_2^-). Because nitrite does not act as the direct nitrosylating agent, it must undergo several reactions where a variety of intermediates are formed. When nitrite is added to meat, it is found in two forms, the anion, NO_2^-, and the neutral nitrous acid, HNO_2 (Sebranek & Fox, 1985). Because the pH of meat, 5.5-5.6, is higher than the pK_a of nitrite, 3.36, the concentration of HNO_2 in cured meat is extremely low. In meat it is believed that the principal reactive species is the anhydride of HNO_2, dinitrogen trioxide, N_2O_3. Dinitrogen trioxide reacts with reductants found naturally in muscle tissue as well as added reducants, such as ascorbate, to form nitric oxide (Pegg & Shahidi, 2000). Other additives or conditions also influence the reduction of nitrite to nitric oxide. The addition of salt accelerates the reaction due to the formation of nitrosyl chloride (NOCl), which is a more effective

nitrosating agent than dinitrogen trioxide formed from nitrous acid (Møller & Skibsted, 2002).

The process of nitric oxide production is dependent upon several factors, including pH, temperature, and time. Acidity plays a big part in nitric oxide production due to the fact that a lower pH results in an increased reactivity of nitrous acid into nitric oxide. The reactants forming both nitrosating compounds, N_2O_3 and $NOCl$, must be in the protanated (acid) form, therefore the lower the pH the faster the reaction (Fox, 1987). This has been demonstrated by showing that the increase in pH, due to added phosphates, resulted in decreased nitric oxide production as shown by higher residual nitrite concentration in finished poultry products (Ahn & Maurer, 1989). It has also been reported that at minimum, a 2 hour period is necessary for 90% of nitrite to be converted to nitric oxide and to bind with myoglobin (Lee & Cassens, 1976).

Once nitric oxide (NO) is formed, the oxidation state of myoglobin changes due to the oxidation of the heme iron atom. The myoglobin molecule changes from the myoglobin state (Fe^{2+}) to the metmyoglobin state (Fe^{3+}). Upon formation of metmyoglobin, nitric oxide then binds to the sixth ligand position of the heme complex. Finally, the myoglobin protein is denatured during the cooking process resulting in the detachment from the heme complex. The resulting pigment is the characteristic cured meat pigment, nitrosohemochrome (Killday, Tempesta, Bailey & Metral, 1988).

Cured Meat Flavor

Nitrite is responsible for the production of the characteristic flavor of cured meat. Sensory data has shown a significant difference between uncured and cured pork flavor (Cho

& Bratzler, 1970). The role of nitrite in cured meat flavor is extremely complex and not entirely understood. The discussion of the volatile and non-volatile compounds that are responsible for the cured flavor is still on-going. The addition of salt during curing has a large impact on cured meat flavor as well. It has been demonstrated that increasing levels of salt results in an increased intensity of cured meat flavor when used in combination with nitrite (Froehlich, Gullet & Usborne, 1983). Part of the flavor difference appears to be related to the decrease in the rate of lipid oxidation that occurs after the cooking process when nitrite is added. However, other antioxidants do not create cured flavor (Gray, MacDonald, Pearson & Morton, 1981). Sensory research has suggested that cured flavor can not solely be attributed to decrease in oxidation, but is in combination with cured aroma and cured flavor (Shahidi, 1998). Because of this, there is no known substitute that can impart the flavor associated with sodium nitrite.

Antioxidant Role of Nitrite

Nitrite acts as a strong antioxidant in cured meats, therefore preventing lipid oxidation. Lipid oxidation is a common change in meat that results in a deterioration of its quality by causing discoloration, drip losses, off-odors, and off-flavors (Morrissey, Sheehy, Galvin, Kerry & Buckley, 1998). The rate and degree of lipid oxidation is related to the degree of unsaturated lipids present and degree of oxygen exposure (Vasavada & Cornforth, 2005). Salt adds to the problem as it is known as a pro-oxidant.

Lipid oxidation of an unsaturated fatty acid occurs in three phases. These include initiation, propagation, and termination. Initiation occurs when a hydrogen atom (H) is eliminated from an unsaturated fatty acid (RH) by bonding with oxygen (O_2) or other

catalysts. The propagation step results from the formation of a lipid free radical (R•) which reacts with oxygen, forming a peroxy radical (ROO•). It is the propagation step that sets off a chain reaction. Once a peroxy radical is formed, it eliminates a hydrogen atom from another unsaturated fatty acid molecule producing a hydroperoxide (ROOH) and a new lipid free radical. This fee radical may also react with oxygen, making this chain reaction self-sustaining until either oxygen or unoxidized lipids are depleted (Pegg & Shahidi, 2000).

The reactions that occur to develop the color in cured meat are likely to be important to the antioxidant function of nitrite. Nitrites function as an antioxidant by binding to heme iron released during the cooking process. Upon binding to the heme iron, the porphyrin ring is stabilized and hence prevents the release of Fe^{2+} (Pegg & Shahidi, 2000). This is important because free iron (Fe^{2+} and Fe^{3+}) can react with lipids to form free radicals that readily react with oxygen and act as a catalyst in reactions causing lipid oxidation (Morrissey et al., 1998). This was supported by Igene, Yamauchi, Pearson and Gray (1985) as they reported that uncured cooked beef samples had a significantly higher non-heme iron level than its raw counterpart while nitrite-cured beef samples non-heme iron levels did not change during the cooking process.

Antimicrobial Properties of Nitrite

Meat is an unstable product largely because microorganisms are able to thrive on its rich supply of nutrients and its high moisture content. The microorganisms that can grow in meat products often result in a decrease in shelf life due to spoilage. Nitrite is a bacteriostatic and bacteriocidal agent that inhibits the growth of bacteria that normally cause spoilage of fresh meats, as well as microorganisms that can cause food-borne illness (Tompkin, 1995).

Because of this, the addition of nitrite extends the shelf life of meat products as well as improves food safety. The microbiological safety that is assured from the addition of nitrite in cured meats is probably the most important function of the curing process (Romans et al., 2001).

Nitrite is a strong inhibitor of anaerobic bacteria and has been shown to contribute to the control of others such as *Listeria monocytogenes*. Nitrite affects different microorganisms in different ways and the mechanisms for inhibition differ between these microorganisms (Tompkin, 1995). Although nitrite is effective at controlling many microorganisms, the single bacterium that is of greatest concern relative to inhibition by nitrite is *Clostridium botulinum*. *C. botulinum* produces a heat stable spore that survives the typical cooking temperatures of thermally processed meats. Nitrite prevents germination and outgrowth of these spores and therefore prevents the production of a deadly toxin by vegetative cells. The toxins produced by *C. botulinum* are the most toxic substances known to man. If ingested, the toxin causes the disease known as botulism. Botulism is the most lethal of all food-borne diseases carrying a 20-50% mortality rate (Jay, 2000).

It should be mentioned that the antimicrobial activity of nitrite also depends on a number of other factors including pH and salt content. Salt is important because it works synergistically with nitrite to inhibit bacterial growth. Alone, salt inhibits microorganisms by decreasing the water available for their growth. It has been reported that 5% sodium chloride will effectively inhibit *C. botulinum* under optimal growth conditions (Lövenklev, Artin, Hagberg, Borch, Holst & Rådstöm, 2004). However this high of a level would result in a product that is unpalatable and therefore undesirable to consumers. Thus nitrite is needed in addition of salt to eliminate the growth of *C. botulinum*. The pH of the meat product is also

an important factor for controlling not only *C. botulinum*, but other pathogens and spoilage microorganisms as well. This appears to be associated with undissociated nitrous acid produced in the reaction to form nitric oxide, which becomes greater at reduced pH. As the pH is lowered one unit, the bacteriostatic effect is increased approximately ten fold. Therefore, in general, the lower the pH the stronger the antimicrobial effect of nitrite. However, as pH decreases, the stability of nitrite is decreased as well. Because of this instability, it is vital that salt be included in cured meats for its synergistic effect with nitrite (Urbain & Campbell, 1987).

Residual Nitrite

When nitrite is added to meat, it reacts with or binds to many constituents within the meat system, such as protein. Nitrite is extremely reactive, and the heat used in thermal processing of cured meat products increases the reactivity. Because of this, the amount of nitrite detectable in the finished product is much lower, often accounting for only about 10-20% of the amount added initially. The nitrite that is analytically detectable in cooked cured meat products is known as residual nitrite (Cassens, 1997b). The residual nitrite in cured meat decreases over time until levels are not detectable. Depletion initiates immediately upon addition to meat and is continuous (Pierson & Smoot, 1982).

The amount of residual nitrite present in cooked cured meat varies greatly between products and is dependent on product type and production procedures used. Since nitrite is toxic to humans at high levels and has the ability to produce carcinogenic compounds, it is very highly regulated by government agencies. Therefore, it is important to minimize the amount of nitrite used to cure meat while still providing enough nitrite to sufficiently insure a

safe product (Cassens, 1995). Some residual levels are beneficial for prolonged shelf life of the product because residual nitrite plays an important role in the stabilization of cooked cured meat products during distribution and storage. The residual nitrite serves as a reservoir of nitric oxide for stabilization of the cured color pigment until consumption (Dryden & Birdsall, 1980). Perhaps the most important role of residual nitrite is the botulinal control. A study performed by Christiansen, Tompkin and Shaparis (1978) observed that *C. botulinum* spores grew readily at both levels (50 and 156 μg/g) of nitrite upon depletion of residual nitrite. The authors concluded that the time of *C. botulinum* spore outgrowth was dependent on the level of residual nitrite.

There are several factors that affect the amount of residual nitrite and the rate of nitrite depletion. One variable that does affect this is the use of reducing agents such as ascorbate and erythorbate. These reducing agents accelerate the curing reaction which results in lower residual nitrite levels in the finished product (Lee & Shimakoa, 1984). A study by Brown, Hedrick and Bailey (1974) demonstrated that increasing levels of sodium ascorbate (0-568 ppm), resulted in decreased residual nitrite levels. Another important factor affecting the amount of nitrite detectable in cooked cured meat products is pH. Sebranek (1979), reported that as pH decreases, the reactivity of nitrite increases due to more favorable nitrite-myoglobin interactions resulting in lower residual nitrite concentrations. This is in agreement with Lee, Cassens and Fennema (1976) who demonstrated that a lower pH directly resulted in lower concentrations of residual nitrite.

Nitrosamines

Even though nitrite has many desirable effects, there is some concern about its safety. The issue arose in the late 1960's and into the 1970's when it became apparent that nitrite-cured meats, most notably bacon, had the potential to produce nitrosamines. Nitrite is an extremely reactive chemical and under appropriate conditions it can act as a nitrosating agent to produce nitroso compounds. Many of these nitroso compounds have been found to be carcinogenic (Cassens, 1995).Volatile nitrosamines can induce tumors throughout the body if present at appropriate concentrations (Ahn, Kim, Jo, Lee & Byun, 2002). Nitrosamines are formed from secondary amines in a slightly acidic environment. Nitrite itself is not the cause of the nitrosamines, rather it is one of nitrite's derivatives, nitrous acid (HNO_2). As stated earlier, nitrous acid's pK_a (3.36) is much lower than the pH of meat (5.5-5.6), making the concentration of nitrous acid in cured meat very low. Therefore, when nitrosamines are found in cooked cured meats, they are usually in very small quantities. Usually the levels, if detectable at all, are in the parts per billion range. However, because of their possible link to cancer in humans, a large amount of interest has been focused on the presence of nitrosamines in cured meats. There have been more than 300 nitroso compounds tested in laboratory animals, and greater than 90% have been shown to cause cancer. However, there has been no direct link to human cancer from the consumption of cured meats (Pegg & Shahidi, 2000).

Bacon has been a product that, in the past, was most frequently found to contain nitrosamines after cooking. The heat normally used to cook bacon is much higher than most cured meat products due to its desired crispness, and this increase in temperature may result in the formation of nitrosamines. The problem of nitrosamines in bacon was recognized by the USDA and after much research, a special regulation was implemented to decrease the

levels of residual nitrite in bacon, thus decreasing the levels of nitrosamines. The new regulation stated that ingoing nitrite levels could not exceed 120 ppm and that 550 ppm sodium ascorbate or erythorbate must be used in combination with the nitrite (McCutcheon, 1984).

A number of variables affect the amount of nitrosamines detected in cured meats such as ingoing and residual nitrite levels, method of cooking, smoking, temperature and time, and the presence of inhibitors. When the issue arose, the meat industry recognized the problem and dealt with it by eliminating the use of nitrate, except for specialty products, lowering the levels of nitrite used, and implementing tighter control of manufacturing processes. Also, ascorbate and erythorbate levels were used at maximum levels to inhibit formation of nitrosamines (Cassens, 1997a). The incorporation of reducing agents such as sodium ascorbate and sodium erythorbate reduces the formation of nitrosamines because they compete with the amines for nitrous acid. Ascorbate and erthythorbate reduce nitrous acid, therefore pushing the reaction away from the formation of nitrosamines (Townsend & Olson, 1987; Pegg & Shahidi, 2000).

The problem with nitrosamines died down after much research and discussion. The National Academy of Sciences issued two reports (National Academy of Sciences, 1981, 1982) that summarized all available scientific information at that time. These two reports noted the changes made in the previous decade solved the problem of the formation of nitrosamines in cured meat. These changes included the virtual elimination of nitrate, reduced use of nitrite, and change in the regulation of bacon along with mandatory use of ascorbate or erythorbate. However, issues during the last 30 years keep resurfacing regarding the safety of nitrite in cured meats. In the 1990's, several epidemiological studies reported

that the consumption of cured meats was linked to childhood leukemia and brain cancer (Peters, Preston-Martin, London, Bowman, Buckley & Thomas, 1994; Preston-Martin & Lijinsky, 1994; Sarasua & Savitz, 1994). Additionally, the state of California proposed nitrite as a developmental and reproductive toxicant under the Safe Drinking Water and Toxic Enforcement Act in 1998. However, subsequent research and thorough scientific review has largely resolved both issues of nitrite as a carcinogen and as a developmental and or reproductive toxicant (Sebranek & Bacus, 2007).

Toxicity

In addition to the nitrosamine issue, there are other safety concerns associated with the use of nitrites in foods. When not used properly, nitrite in high concentrations is toxic to humans (Cammack, Joannou, Xiao-Yuan, Martinez, Maraj & Hughes, 1999). Because of this, the USDA regulates the usage levels of nitrate and nitrite and both are considered restricted ingredients. Under the regulations enforced by the USDA, concentrations of nitrite used to cure meat products is not known to present a hazard to human health (Pierson & Smoot, 1982). The ingestion of high doses of nitrate and nitrite can lead to the condition of methemoglobinemia. Methemoglobinemia is the result of a high dose of an oxidant in the blood. In the case of nitrite, the iron atom of the hemoglobin molecule is oxidized from the ferrous Fe^{2+} to the ferric state Fe^{3+}. When the iron atom of hemoglobin is in the ferric state it is unable to bind oxygen, therefore the oxygen carrying capacity of the blood is reduced (Kennedy, Smith & McWhinney, 1997). The primary symptom of methemolobinemia is cyanosis. Cyanosis is the characteristic blue color of the skin and organs when the amount of unoxygenated hemoglobin in the blood is too high. In one case, it was reported that an

accidental overdose of nitrite from the consumption of cured meatballs resulted in the patient

having chocolate-brown colored blood (Khan, Adams, Simmons & Sutton, 2006). Nitrates

and nitrites can both cause methemoglobinemia but nitrites are more toxic due to their greater

oxidative potential. If not treated immediately or if ingested nitrite levels are too high, the

condition becomes fatal. The lethal oral doses of nitrate and nitrite for humans are

determined to be 80-800 mg nitrate/kg body weight and 22-250 mg nitrite/kg body weight

(Honikel, 2008). Newborn infants are particularly susceptible to methemoglobinemia

because the enzymes needed to counteract the effects of nitrite poisoning are not developed

at this time. This leads to "blue baby syndrome" in which the infants turn blue in color due to

lack of oxygen to internal organs and, if severe enough, can be fatal (Cammack et al., 1999).

For this reason, the USDA does not allow nitrates or nitrites in baby, junior, or toddler foods

(USDA, 1995).

Uncured Meat Products

Nitrite has been used for centuries to cure meat as it adds variety to the human diet and acts

as a preservative. Even though it has numerous benefits, its relationship with nitrosamines

and cancer over the past 40 years has given many consumers a negative perception of nitrite.

Heath concerns of consumers about the safety of nitrate and nitrite have encouraged the

production of uncured, no nitrate/nitrite-added meat and poultry products (Sindelar, Cordray,

Olson, Sebranek & Love, 2007a). Currently, there are two different classifications of

uncured, no nitrate/nitrite-added meat and poultry products in the marketplace. The first type

of product is one in which nitrate or nitrite is not included in the product formulation. These

products typically have undesirable quality characteristics as described earlier. The second

type of product is formulated with the intention of replacing nitrite to provide products that
are similar to typical cured meat products (Sindelar, Cordray, Sebranek, Love & Ahn,
2007b). In this type of product, a natural curing process is utilized in which naturally
occurring nitrates are reduced to nitrite in the meat products by specific microorganisms
(Bacus, 2006).

The term uncured is used by the USDA to label both types of products, ones that do
not contain nitrite and ones that are cured by naturally occurring nitrates and a starter culture.
USDA defines uncured in the Code of Federal Regulations Title 9, Parts 317.17 and 319.2.
The regulation states:

> "Any product which is required to be labeled by a common or usual name for
> which there is a standard and 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'' in the same size and style of lettering as
> the rest of such standard name: *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 and 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.'"

In the case of no nitrate/nitrite-added products that simulate the traditionally cured products
with naturally occurring nitrates, this labeling in technically inaccurate. The reason for the
designation of uncured is because products such as bacon, frankfurters, ham, bologna, and
other cured meats have a typical color and flavor widely recognized by consumers. These
product characteristics are standardized and are associated with being cured with the direct
addition of nitrite. Processing procedures are much different for "uncured" products and

therefore their label must clarify the differences between the two products. However, the term uncured used to describe products cured with indirect addition of nitrate is confusing to consumers. Nevertheless, processors are required to label the specific products as uncured due to existing USDA regulations. In April of 2006, the USDA verbally announced that new label submissions would require changes to the "No Nitrate or Nitrite Added" statement on uncured products. This announcement was not followed with documentation, though it was stated that new labeling changes will be reflected in the new Food Standards and Labeling Policy Book. The change in labeling was in response to the realization that many products labeled "uncured" actually contained naturally occurring nitrites that can be present in vegetable juices and sea salt. The changes that were made require new labels to contain the disclaimer "No Nitrates or Nitrites added except for naturally occurring nitrites found in" one of the ingredients present in the ingredient statement. These ingredients include celery juice powder, beet juice powder, carrot juice concentrate, sea salt, or any ingredient determined to contain nitrates or nitrites by USDA. Furthermore, the statement "No Preservatives" can not be used (Bacus, 2006).

Naturally occurring nitrates are very common in the environment and can be found in ingredients such as sea salt and vegetable sources. It is well known that vegetables are a source of relatively high nitrate concentrations. The National Academy of Sciences (1981) reported that vegetables contain nitrates in concentrations as high as 1500-2800 ppm in celery, lettuce, and beets. Vegetable juices and powders are commercially available and are being utilized to produce uncured, no nitrate/nitrite-added meat products. An analysis of commercially available vegetable juices reported that carrot, celery, beet, and spinach juice contained 171 ppm, 2114 ppm, 2273 ppm, and 3227 ppm of nitrate respectively (Sebranek &

Bacus, 2007). Vegetable powders are also available which can provide nitrate in concentrated form. Sindelar et al. (2007b) reported nitrate concentration of 27,462 ppm in commercially prepared celery juice powder. Celery powder is highly compatible with cured meats as it has relatively low vegetable pigment and flavor that does not cause discolorations or off flavors when used at lower levels.

Once naturally occurring nitrates are introduced into an indirect curing system, microorganisms positive for nitrate reductase are needed to enzymatically reduce the nitrate to nitrite. Starter cultures are commercially available for the reduction of nitrate in a meat curing system. Most of the applications use these cultures for dry sausages where a slow release of nitrite is desirable for the long curing process (Sebranek & Bacus, 2007). Coagulase negative, catalase-positive staphylococci are commonly applied as meat starter cultures (Casaburi, Blaiotta, Mauriello, Pepe & Villani, 2005; Gøtterup, Olsen, Knöchel, Tjener, Stahnke & Møller, 2008) and it is well established that most species of staphylococci possess enzymes involved in nitrate/nitrite metabolism (Neubauer & Götz, 1996). A study by Talon, Walter, Chartier, Barrièr and Montel (1999) measured the nitrate reductase of several different strains of staphylococci isolated from dry sausage. The authors found that strains of *Staphylococcus carnosus* had the highest nitrate reductase activity of all staphylococci tested. This is in agreement with Gøtterup et al. (2008) where the authors demonstrated that sausages inoculated with *S. carnosus* had a greater red intensity than compared to other strains of staphylococcus. It is also believed that staphylococcus strains are important for the generation of desirable flavor compounds in fermented sausages (Olesen, Meyer & Stahnke, 2004). Furthermore, it has been reported that certain strains of *S. carnosus* exhibit anti-

listerial activity against *Listeria monocytogenes* (Papamanoli, Kotzekidou, Tzanetakis & Litopoulou-Tzanetaki, 2002).

The nitrate reductase activity of *S. carnosus* is caused by a membrane-bound enzyme involved in respiratory energy conservation. The enzyme is only found in bacteria where nitrate is used as an alternative electron acceptor when oxygen is not available (Neubauer & Götz, 1996). After oxygen, the most widely used alternative electron acceptor is nitrate (Pantel, Lindgren, Neubauer & Götz, 1998). Neubauer and Götz (1996) showed that during the growth of *S. carnosus,* in the presence of nitrate, nitrite was accumulated in the growth medium, and the rate of nitrite accumulation was 8-10 fold higher under anaerobic conditions than under aerobic conditions. In addition, the authors demonstrated that the cells also have an enzyme that will further reduce the nitrite to ammonia. However, nitrite is only reduced by their inherent enzyme upon depletion of nitrate. It should also be noted that the authors reported that nitrite (up to 50 mM) did not inhibit the nitrate reductase activity. This is in agreement with Sanz, Vila, Toldrá, Nieto and Flores (1997) in which the authors reported that a starter culture of both *Lactobacillus sake* and *S. carnosus* was not greatly affected by the addition of nitrite as a curing salt.

The amount of time needed for the reduction of nitrate is dependent on many variables such as concentration of the reactants, temperature, pH, and other environmental conditions. The temperatures used for these cultures vary with the strain. Casaburi et al. (2005) demonstrated that staphylococcus strains were able to reduce nitrate to nitrite at temperatures as low as 15 °C but the activity increased as temperature increased, with maximum activity at temperatures over 30 °C. It is recommended that commercially available strains used for nitrate reduction should be used at temperatures of 38-42 °C.

Recent research has shown that time is a critical factor for the conversion of nitrate to nitrite. Sindelar, Cordray, Sebranek, Love and Ahn (2007c) concluded that incubation time (at 38 °C) was more critical than the amount of vegetable juice powder to produce no nitrate/nitrite-added emulsified cooked sausages that had characteristics similar to a nitrite cured control. However, a parallel study of hams showed that the amount of vegetable juice powder added was more critical than incubation time for production of this product (Sindelar et al., 2007b). It was also reported that a high concentration of vegetable juice powder (0.35%) resulted in a more intense undesirable vegetable flavor and aroma than the lower concentration of vegetable juice powder (0.20%) in no nitrate/nitrite-added hams. Furthermore, the lower concentration of vegetable juice powder resulted in sensory characteristics similar to the nitrite cured control (Sindelar et al., 2007b). The authors concluded that the slow temperature increase in the large diameter hams provided sufficient time for nitrite production regardless of concentration of vegetable juice powder. In the emulsified cooked sausages, the small diameter resulted in a quick temperature increase, therefore making incubation time a more critical factor.

Although the use of vegetable juice powder and a starter culture have been found to produce uncured meat products that are acceptable to consumers (Sindelar et al., 2007a, 2007b, 2007c), there are several concerns associated with the process. The most important concern is the safety of these products. It is well documented that nitrite prevents the outgrowth of *C. botulinum* spores that produce the deadly toxin which causes botulism (Pierson & Smoot, 1982; Tompkin, 1995). The amount of ingoing and residual nitrite is important for the control of this microorganism. It has been reported that often in meat products cured with natural sources of nitrate, the amounts of both ingoing and residual

nitrite are lower than nitrite cured-products (Sindelar et al., 2007b, 2007c). This raises the concern for food safety, especially regarding *Clostridium botulinum* growth, as well as other microorganisms whose growth is inhibited by nitrite. However, data have also shown that it is possible to produce uncured meat products with higher than normal residual nitrite levels (Sindelar et al., 2007c). High residual nitrite concentrations have been directly related to the formation of nitrosamines. Also reducing agents such as ascorbate and erythorbate are not used in many uncured meat products, which increases the chance of nitrosamine formation. These reductants have been found to reduce the amount of nitrosamines formed, and are required in nitrite cured bacon (Pegg & Shahidi, 2000). The conversion of nitrate to nitrite is extremely variable when converted in a meat system, therefore, more research is needed to find a more controlled environment for nitrate to nitrite conversion.

Recently, pre-converted vegetable juice powders have become commercially available for the production of uncured meat and poultry products. In these pre-converted powders the nitrate is reduced with microorganisms before adding it to the meat block. The pre-converted powders can be added directly to comminuted products, or can be mixed in a brine and injected or tumbled into different meat cuts. These powders can be purchased already containing nitrite at concentrations as high as 10,000 – 15,000 parts per million. As a result, no starter culture or incubation step is required for the production of no-nitrate-or-nitrite-added meat products. There is concern about the use of pre-formed nitrite in vegetable juice powders because nitrite alone is a restricted ingredient. Conversely, the use of vegetable juice powders containing nitrite is not regulated, as the USDA has no restrictions on the use of pre-converted vegetable juice powders. This also adds to the confusion about the labeling of these products. It could be argued that the statement of "no nitrates or nitrites added"

should not be used when pre-converted vegetable juice powders are used due to the fact that nitrite is being added directly. Nevertheless, because the nitrite is derived from naturally occurring nitrates, the product is still required to be labeled as uncured with the statement of no nitrates or nitrites added. Another issue to consider with pre-converted vegetable juice powders is that at the recommended usage rate, they still only result in an ingoing nitrite concentration of approximately 20-60 parts per million. This is much lower than the concentrations used in nitrite-cured meat products which raises concerns in regards to food safety and shelf life. Therefore, it is necessary to establish a standard processing procedure that provides more consistency and better control over nitrite levels. One possible method of achieving this would be to convert the nitrate found naturally in vegetable juice powders into nitrite with the use of a nitrate reducing starter culture in the brine prior to injection. This approach could offer better control over the amounts of nitrite injected into the product by development of brine incubation processes that would produce a consistent, desired nitrite concentration. For example, nitrite accumulation in the brine is dependent on many variables, such as temperature, salt content, time, and pH. If these variables could be held constant, the amount of nitrite produced would be consistent between batches. Pre-converting a brine before injecting is hypothesized to allow more consistent nitrite concentrations, faster product cure, and better control over nitrite levels in the product.

Summary

Recent consumer interest for healthier perceived foods has prompted consumer demands for uncured, no-nitrate-or-nitrite-added meat and poultry products. Curing processes that produce uncured meat products utilize natural ingredients high in nitrate such

as vegetable products, and a nitrate-reducing starter culture to produce nitrite from nitrate. The labeling of these products is technically inaccurate and confusing to consumers because many processed meats labeled uncured have the same characteristics of nitrite-cured meats. Nitrite concentration produced by natural processes is impossible to determine because of high reactivity of the nitrite molecule. This raises concern for the safety of these products because nitrite is a highly effective antimicrobial, particularly against the growth of *Clostridium botulinum*, and excess nitrite provides a risk for the formation of nitrosamines. Consequently, manufacturing procedures that maintain the quality and safety of products labeled no-nitrate/nitrite added must be utilized. Further research is needed to find alternative methods to produce meat products cured with naturally occurring nitrates to assure product safety and to control the amount of nitrite produced from these processes. Therefore, the first objective of this research was to investigate the optimum conditions needed during the incubation of curing brines containing vegetable juice powder and a starter culture to produce a sufficient amount of nitrite for the production of uncured meat products. The second objective was to investigate the effects of these incubated brines on quality characteristics and residual nitrite concentrations on uncured, no-nitrate-or-nitrite-added ham.

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CHAPTER 3. INCUBATION OF CURING BRINES FOR THE PRODUCTION OF READY-TO-EAT UNCURED HAM

A paper to be submitted to Meat Science

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Abstract

The effect of salt level, vegetable juice powder (VJP) and temperature were investigated in a preliminary study to determine optimum conditions needed during the incubation of curing brines including VJP and a starter culture containing *Staphylococcus carnosus* for the production of "uncured" no nitrate/nitrite-added meat products. Subsequently, incubated curing brines were utilized to produce no-nitrate/nitrite-added ham in which quality characteristics and residual nitrite concentrations were measured. Two ham treatments (SC: VJP and starter culture containing *Staphylococcus carnosus*; PC: pre-converted VJP) and a nitrite-added control (C) were used for the study. No differences ($P>0.05$) were found between treatments and control for CIE L* or TBARS values. Residual nitrite concentration was greater ($P<0.05$) in the control hams during the first week of storage. Although nitrite in the control remained at relatively greater concentration throughout storage, the difference was not statistically significant after the first week. At day 42 of storage, the control (C) treatment retained significantly ($P<0.05$) greater a* (redness) values than either the SC or PC treatments.

Introduction

The curing of meat has been performed for centuries and can be traced back to the earliest recorded history. Historically, salt was the primary ingredient used for meat

preservation because it was realized that cuts of meat would not spoil as fast when packed in dry salt (Pegg & Shahidi, 2000). After centuries of salting meat, certain salts became more desirable because they created a distinct pink color and unique flavor. It is widely accepted that impurities of potassium and sodium nitrate were responsible for the characteristic cured meat pigment and special flavor (Binkerd & Kolari, 1975; Cassens, Ito, Lee & Buege, 1978). Furthermore, it was determined that the sodium and potassium nitrate in these salts were converted to nitrite by naturally occurring bacteria (Townsend & Olson, 1987).

Today, nitrite is added directly to processed meats to offer consumers flavor and appearance typical of cured meat. Nitrite is responsible for development of cured color and flavor, for imparting antioxidant activity and extending shelf life, and for suppressing the outgrowth of *Clostridium botulinum* (Pegg & Shahidi, 2006).

Even though nitrite has many desirable effects, there is some concern about its safety. The issue arose in the late 1960's and into the 1970's when it became apparent that nitrite-cured meats had the potential to produce nitrosamines. Nitrite is an extremely reactive chemical and under appropriate conditions it can act as a nitrosating agent to produce nitroso compounds. Many of these nitroso compounds have been found to be carcinogenic (Cassens, 1995). Because of nitrite's relationship with nitrosamines and cancer over the past 40 years, many consumers have a negative perception of nitrite.

Although it has numerous benefits, health concerns of consumers about the safety of nitrate and nitrite have encouraged the production of no nitrate/nitrite-added meat and poultry products that simulate typical cured meats (Sindelar, Cordray, Olson, Sebranek & Love, 2007a). Currently, there are two different classifications of no nitrate/nitrite-added meat and poultry products in the marketplace. The first type of product is one in which nitrate or nitrite

is not included in the product formulation and is truly uncured. The second type of product is formulated with the intention of replacing nitrite with natural sources of nitrate to provide products that are similar to typical cured meat products (Sindelar, Cordray, Sebranek, Love & Ahn, 2007b). The term uncured is used by the United States Department of Agriculture (USDA) to label both types of products. USDA defines uncured in the Code of Federal Regulations Title 9, Parts 317.17 and 319.2. The regulation states:

> "Any product which is required to be labeled by a common or usual name for which there is a standard and 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'' in the same size and style of lettering as the rest of such standard name: *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 and 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.'"

Nitrite is a unique ingredient used to cure meat for which there is no substitute. However, because nitrite is a preservative, direct addition of nitrite to natural or organic processed meats is not permitted. As a result, different processes are needed to produce natural or organic processed meats that have similar characteristics of nitrite-cured meat (Sebranek & Bacus, 2007). Therefore, a natural curing process is typically utilized in which naturally occurring nitrates are reduced to nitrite in the meat products by specific microorganisms (Bacus, 2006). It is well known that vegetables contain considerable amounts of nitrate (Siciliano, Krulick, Heisler, Schwartz & White, 1975), and when vegetable sources are added to processed meats in addition to a nitrate reducing starter culture, a sufficient amount of

nitrite may be produced to achieve curing reactions. However, nitrite concentration produced by natural processes is impossible to determine because of high reactivity of the nitrite molecule. This raises concern for the safety of these products because nitrite is a highly effective antimicrobial, and a minimum amount of nitrite is considered to be important to antimicrobial effectiveness. On the other hand, excess nitrite might provide a risk for the formation of nitrosamines.

Recently, pre-converted vegetable juice powders have become commercially available for the production of uncured meat and poultry products. These powders can be purchased already containing nitrite at concentrations as high as 10,000 – 15,000 parts per million. This raises questions about the use of pre-formed nitrite in vegetable juice powders because nitrite alone is a restricted ingredient. Conversely, the use of vegetable juice powders containing nitrite is not regulated, and the USDA has no restrictions on the use of pre-converted vegetable juice powders. Therefore, it is necessary to establish a standard processing procedure that provides more consistency and better control over nitrite levels in the natural and organic products. Cured meats such as hams and bacon are typically injected with brine containing curing agents. Because the brine is formulated prior to injection, this may offer a processing step for improved control of nitrite concentration in these products. One method of achieving this in the natural and organic products would be to convert the nitrate found naturally in vegetable juice powders into nitrite with the use of a nitrate-reducing starter culture in the brine prior to injection. This approach could offer better control over the amounts of nitrite subsequently injected into the product by development of brine incubation processes that would produce a consistent, desired nitrite concentration appropriate for a specific product application.

The temperatures used for staphylococcus starter cultures vary with the strain. Casaburi et al. (2005) demonstrated that staphylococcus strains were able to reduce nitrate to nitrite at temperatures as low as 15 °C but the activity increased as temperature increased, with maximum activity at temperatures over 30 °C. It is recommended that commercially available strains used for nitrate reduction should be used at temperatures of 38-42 °C. Casaburi et al. (2005) also demonstrated that staphylococcus strains survived in the presence of up to 20% salt and at a pH as low as 5.0.

Therefore, the objectives of this research were to investigate the optimum conditions needed during the incubation of curing brines containing vegetable juice powder and a starter culture to produce a sufficient amount of nitrite for the production of uncured (using the USDA labeling terminology for these products) meat products. Furthermore, these incubated brines were compared with a pre-converted vegetable juice powder and a nitrite-cured control to assess quality characteristics and residual nitrite concentrations on uncured, no-nitrate-or-nitrite-added ham.

Materials and Methods

Preliminary Study

Experimental Design

Vegetable juice powder (VJP) concentration, salt concentration, and temperature were used to first investigate effects of conditions in curing brines used for no-nitrate-or-nitrite-added meat products. Brines containing two levels of VJP and four concentrations of salt were held at three temperatures resulting in a 2 x 4 x 3 factorial design with 24 treatment combinations. Due to limited starter culture activity in the presence of salt at the low

temperature, only treatments of 0% salt were measured only at the low temperature, resulting in a total of 18 brine treatments. Brines were measured for concentrations of nitrate, nitrite, pH and number of live cells of starter culture over time until observed nitrite concentrations started to decrease.

Brine Manufacture

Brines were prepared according to the experimental design (Table 1) to represent typical injection brines as much as possible within the treatment variables. Dextrose was held constant at 8% for all treatments. The two levels of vegetable juice powder (Vegetable Juice Powder NA 20, Chr. Hansen Inc., Gainsville, Fla., USA) used were calculated to be equivalent to 0.2% and 0.4% of total product weight (meat block + brine solution) when used at a 25% injection level. Four treatments of salt were added at concentrations of 0%, 3%, 6%, and 9% in the brine solution. Starter culture consisting of *S. carnosus* (CS 299 Bactoferm™, Chr. Hansen Inc., Gainseville, Fla., USA) was added at 0.1285% of the brine for all treatments. Brines were mixed by hand, adding the ingredients to the water while mixing. VJP was added first followed by dextrose, salt (if included), and finally starter culture. After mixing, brines were immediately placed in holding areas at one of three temperatures. Treatments included a high (38 °C), medium (24 °C), and low temperature (6 °C) incubation of the brine. High temperatures (38 °C) were achieved by placing brine in a water bath (Model WPC95, Sheldon Manufacturing Inc., Cornelius, OR., USA). Medium temperatures (24 °C) were achieved by holding the brine at room temperature and low temperatures (6 °C) were achieved by placing the brine in a refrigerated processing area maintained at 6 °C at the Iowa State University Meat Laboratory.

pH Determination

The pH was recorded during incubation for each brine treatment until observed nitrite concentrations started to decrease. To measure pH, approximately 50 ml of brine was transferred into a 100 ml beaker. The pH was then measured with a pH/ion meter (Acumet 950: Fisher Scientific, Fair Lawn, N.J., USA) equipped with an electrode (Accumet Flat Surface Epoxy Body Ag/AgCl combination Electrode Model 13-620-289, Fisher Scientific, Fair Lawn N.J., USA) calibrated with phosphate buffers at pH 4.0 and pH 7.0. At each time interval for each treatment, measurements were made in duplicate.

Nitrite Analysis

Nitrite was determined with modifications to the AOAC method ([AOAC] Association of Official Analytical Chemists, 1990). Five ml of brine solution was transferred to a 500 ml volumetric flask using a pipette. The flask was then filled to volume with distilled, de-ionized water (DDW). Approximately 30 ml of sample was then transferred to a 50 ml volumetric flask. Under a fume hood, 2.5 ml sulfanilamide reagent (0.5g sulfanilamide in 150 ml 15% acetic acid) was added. After 5 minutes, 2.5 ml NED reagent (0.2 g N-(1-naphthyl) ethylenediamine .2HCl in 150 ml 15% acetic acid) was added and filled to volume with sample. Color was allowed to develop for 15 minutes. Solution was transferred to a spectrophotometer cuvette and absorbance was measured at 540 nm against a blank of 45 ml DDW, 2.5 ml sulfanilamide reagent, and 2.5 ml NED reagent. Nitrite was determined by comparing sample reading with a standard curve as described in the official method. All nitrite assays were done in duplicate.

Nitrate Analysis

Brine samples used for nitrate analysis were transferred to sterile test tubes, frozen, and stored at -5 °C. Nitrate determination methods were modifications of Ahn and Maurer (1987). After thawing, samples were diluted between 4 and 11 times with DDW depending on expected nitrate concentrations. The sample was then heated for 1 hour in an 80 °C water bath. After cooling in cold water for 10 min, 2.5 ml of the solution was transferred to a disposable test tube. Carrez II (10.6 g potassium ferrocyanide in 100 ml DDW) and Carrez I (23.8 g zinc acetate in 50 ml DDW, with 3 ml glacial acetic acid added, then diluted 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 (Afilent, Wilmington Del., USA). The column used was Agilent Zorbax SAX (analytical 4.6 x 150 mm, 5-micron) (Agilent, Wilmington Del., USA) 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.

Microbiological Analysis

Brine samples used for bacterial counts were transferred to sterile test tubes, frozen, and stored at -20 °C until measurements were taken. After thawing, 1 ml of the sample was used to make appropriate serial dilutions. These dilutions were prepared in sterile peptone water. The population of viable cells was determined by spread plating onto Tryptic Soy Agar (TSA) (Becton, Dickinson, and Company, Franklin Lanes, NJ, USA) and staphylococci population was measured using Baird Parker (Becton, Dickinson, and Company, Franklin

Lanes, NJ, USA) media with EY Tellurite Enrichment. TSA plates were incubated at 35 °C

for 24 hours and the Baird Parker media was incubated at 35 °C for 48 hours.

Ham Study

Experimental Design and Data Analysis

Two forms of vegetable juice powder used for the manufacture of ham were

investigated and a conventional nitrite-cured control was included. The three ham treatments

were as follows:

SC: vegetable juice powder as a nitrate source and starter culture containing *S.*

carnosus;

PC: pre-converted vegetable juice powder containing 10,000-15,000 ppm nitrite;

Control (C): conventional cure with sodium nitrite added.

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 main plot that consisted of 2 blocks (replication) and 3

ham treatments resulting in 6 observations for processing attributes. 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 8 sampling periods (day 0, 1, 3, 7, 14, 21, 28, and 42) and combined with

the main plot resulted in a total of 48 observations for color, nitrite, 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 was determined at $P<0.05$.

Brine Preparation

Hams with the three brine treatments (Table 2) were manufactured according to the experimental design. The first treatment (SC) consisted of water, vegetable juice powder (Vegetable Juice Powder NA 20, Chr. Hansen Inc., Gainsville, Fla., USA), dextrose, salt, and starter culture containing *Staphylococcus carnosus* (CS 299 Bactoferm™, Chr. Hansen Inc., Gainseville, Fla., USA). The second treatment (PC) contained water, pre-converted vegetable juice powder (Veg Stable™ 504, Florida Food Products, Inc., Eustis, Fl), salt and dextrose. The third treatment (C) was a nitrite-cured control containing water, salt, dextrose, sodium erythorbate and sodium nitrite. Brines were mixed by hand by adding the dry ingredients to the water while mixing. The SC treatment was mixed without salt and incubated in a thermal processing unit (Alkar, Lodi, WI., USA) at 38 °C for 12 hours. Salt was then added after incubation. These conditions and procedures were selected based on the results of the preliminary experiment.

Product Manufacture

Fresh ham muscles were purchased from a local supplier and kept refrigerated until used. Ham muscles were ground (Biro MFG Co., Marblehead, Ohio, USA) using a 6.35 mm plate. The ground ham was then separated into three 20 lb batches. Treatments (SC, PC, and C) were randomly assigned to each batch. Each treatment was then added to a vacuum mixer (Fatosa vacuum mixer, Model AV80, Barcelona, Spain), brine was added at 25% of the meat

weight according to treatment, and was mixed for 5 minutes under vacuum. After mixing, ham was transferred to a rotary vane vacuum stuffer (Risco SPA, Model 1040C, Thiene, Italy) and stuffed into 3.47" diameter fibrous casings. All three treatments were then hung on a smokehouse truck and cooked in a single truck thermal processing oven (Maurer AG, Reichenau, Germany). The final internal temperature of the product was brought to 70 °C using the cooking schedule found in Table 3. After thermal processing, the cooked ham treatments were chilled for 18 hours at 0-2 °C. The hams were then sliced (Model SE12D Slicer, Bizerba GmbH & Co. KG., Balingen, Germany) to a thickness of 6 mm. The slices were placed in barrier bags with four slices per bag (Cryovac B2470, Cryovac Sealed Air Corp., Duncan, S.C., USA) and vacuum packaged. The vacuum packaged ham slices were then stored on shelves under 7.0 lux of Deluxe Cool White florescent lighting (constant lighting for 24 hours per day) at 2-4 °C. Lights were suspended 61 cm above the packages during the storage period.

Color Measurements

Color measurements were conducted using a Hunterlab Labscan spectrocolorimeter (Hunter Associated Laboratories Inc., Reston, Va., USA). The Hunterlab Labscan was standardized using the same packaging material used on the samples, placed over the white standard tile. Illuminate D65, 10° standard observer light source with a 1.27 cm viewing area were used to analyze the ham samples. Commission International d'Eclairage (CIE) L* (lightness), a* (redness), and b* (yellowness) measurements were taken at a random location of each of the 4 slices in a randomly selected package and the resulting average was used in data analysis. Color was measured immediately after slicing (day 1) and subsequently at day 3, 7, 14, 21, 28, and 42.

pH Determination

The pH was measured with a pH meter (HI 99161, Hanna Instruments, Woonsocket, RI) equipped with a pH probe (FC202D, Hanna Instruments, Woonsocket, RI) calibrated with standard buffers at pH 4.0 and pH 7.0. Measurements of pH were performed on the raw meat sample, each brine treatment, each treatment after the brine was added, and on the cooked ham after chilling. The pH was determined by inserting the probe directly into either the brine solution or in the meat itself. 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. Measurements were conducted at day 1 after packaging and subsequently at day 3, 7, 14, 21, 28, and 42. For each treatment, measurements were made in duplicate.

Residual Nitrite Analysis

Residual nitrite was determined by the AOAC method (AOAC, 1990). Residual nitrite was measured before thermal processing (Day 0) and subsequently at Day 1, 3, 7, 14, 21, 28, and 42. All residual nitrite measurements were done in duplicate.

Results and Discussion

Preliminary Brine Study

Varying levels of salt, vegetable juice powder, and incubation temperature were used in the preliminary investigation to determine favorable conditions for the production of nitrite

by a nitrate reducing starter culture. To determine the optimum conditions for nitrite production in curing brines, nitrite and nitrate concentrations, pH, and starter culture populations were measured over time. However, because the preliminary work was not replicated, statistical analysis of this portion of the study was not conducted.

pH Determination

The pH in all of the brines appeared to change over time (Tables 4 and 5). Initial pH of the curing brines ranged from 5.16 for the treatment of 38 °C, 9% salt, and 0.4% VJP to 6.01 for the treatment of 38 °C, 0% salt, and 0.2% VJP. Initial pH was lower, in general, in treatments with the higher level of VJP. The average initial pH for the treatments containing 0.2% VJP was 5.60 while the average initial pH for the treatments containing 0.4% was 5.38. This could be due to the VJP which had a pH of 5.2. Another factor affecting initial pH was salt. As salt concentration increased in the brine, the measured pH of brines appeared to decrease. Over time pH decreased in all treatments, most notably for the treatments at the high and medium temperatures suggesting that the culture produced enough acid to affect brine pH. The pH of the curing brines at the low temperature decreased but only slightly. This suggests that the starter culture was not very active at the low temperature, as indicated by the limited amount of nitrite produced, and therefore generated very little acid. Excluding the low temperature treatments, the ending pH of the brines ranged from 4.33 to 4.86 with a mean of 4.51. A major factor in determining the reactivity of nitrite in a curing system is the pH. Sebranek (1979) stated that a pH decrease as little as 0.2 pH units could result in doubling the rate of curing reactions. Therefore, as the curing brines become more acidic, the nitrite that is produced is likely to become more reactive.

Nitrite Analysis

Nitrite concentrations changed over time for all treatments and between treatments (Figures 1 and 2). The variation was not only in peak nitrite concentration, but also in the time it took to achieve the peak concentration. Nitrite generated from low temperature (6 °C) treatments was virtually none regardless of VJP level (data not shown) and never exceeded 4 ppm at most. Generally, the medium temperature (24 °C) treatments seemed to result in a higher nitrite concentration than the high temperature (38 °C) treatments. However, the high temperature treatments reached their peak nitrite level on an average of approximately 40 hours sooner than the medium temperature, most likely because of the increased activity of the starter culture at the higher temperature. The higher concentration of nitrite at the medium temperature may be attributed to the increased reactivity of nitrite at the highest temperature. As temperature increases, nitrite becomes more reactive which results in the nitrite molecule being further reduced to nitric oxide. Therefore, the medium temperature treatments may have had less nitrite depletion than the high temperature, resulting in a higher ultimate nitrite concentration accumulated in the brine. Nitrite concentrations also appear to be affected by salt levels in the brine. As the concentration of salt increased from 0% to 9%, the peak nitrite concentration decreased. This might be due to the effect of chloride on nitrite reactivity. Sebranek and Fox (1985) demonstrated increased nitrite reactivity as chloride concentrations increased. This could also be attributed to the antimicrobial properties of salt. Salt increases the osmotic pressure in the brine (Lawrie and Ledward, 2006) resulting in a lower starter culture activity. This prevents the starter culture from converting nitrate to nitrite. The level of VJP also had an effect on nitrite concentrations. At 0% salt, the treatments with 0.4% VJP resulted in considerably more nitrite than the treatments with 0.2% VJP. This was expected because the higher concentrations of VJP would increase the amount

of nitrate available and therefore would allow more nitrite generation in the brine. However, in the treatments containing 3 %, 6 %, and 9% salt, the higher level of VJP resulted in lower nitrite concentrations than the lower level of VJP. This was unexpected and suggests that further research is needed to determine the affect of the interaction between salt and VJP on nitrite production during brine incubation.

Nitrate Analysis

Nitrate concentrations were measured initially (0 hrs), at peak nitrite concentration, and again after nitrite levels started to decrease (Tables 4 and 5). Nitrate concentrations decreased as nitrite concentrations increased for all treatments indicating that the starter culture converted nitrate into nitrite. As would be expected, the treatments resulting in the highest nitrite concentrations had the lowest detectable nitrate concentrations. VJP also affected the amount of nitrate present in the brine as expected. Treatments containing 0.2% VJP had initial nitrate concentrations from 168.0 to 186.8 ppm with an average of 179.2 ppm. Treatments containing 0.4% VJP had initial nitrate concentrations from 234.2 to 374.4 ppm with an average of 339.6 ppm. This explains the increased amount of nitrite generated with the higher VJP concentrations as the higher quantity of nitrate would allow for additional nitrite production.

Microbiological Analysis

Plate counts of viable cells and staphylococci were conducted immediately after brines were mixed (0 hrs), at peak nitrite concentration, and again after observed nitrite levels started to decrease (Tables 4 and 5). Starter culture populations did not increase regardless of treatment, and often declined to undetectable levels. Generally, the population decreased as pH decreased. It appears that the acid produced by the starter culture is directly correlated to

the decrease in starter culture population. Therefore, the pH should be closely regulated during the incubation of curing brines for uncured, no-nitrate-or-nitrite-added meat products. A buffer added to the brine may help the starter culture survive longer during the incubation step and consequently increase the amount of nitrite generated by the nitrate reductase enzyme of the starter culture. Another possible way to increase the amount of nitrite produced would be to exclude dextrose during the incubation step. The starter culture does not grow during brine incubation, therefore a food source may not be necessary. The omission of dextrose would decrease the osmotic pressure and may allow for a higher enzyme activity. Because the cells of *S. carnosus* do not grow, the nitrate reductase enzyme is all that is of any concern, therefore the ingoing level of starter culture is extremely important to the amount of nitrite produced.

As a result of the preliminary experiment, the conditions of 0.4 % VJP, 38 °C, 0 % salt and an incubation time of 12 hours were chosen to investigate brine incubation prior to injection into hams to assess curing effectiveness. This appeared to be the best combination of incubation time and temperature for practical applications.

Ham Study

Various processing attributes were measured during the production of hams. These include the pH of the brine, raw ham muscles, ham after mixing, and of the finished product as well as the temperature of the brine, raw ham muscles and ham after mixing. Product yield was also recorded (Table 6). The pH of ham brines was measured immediately before addition to the ham muscles. SC, PC, and C brines were all significantly different (P<0.05) with pH values of 4.24, 9.22, and 8.02, respectively. The acidic pH of the SC brine is more than likely attributed to the lactic acid probably produced by the starter culture used for

nitrate reduction (Gøtterup, Olsen, Knöchel, Tjener, Stahnke & Møller, 2008). Temperature of the brines was also measured immediately before addition to the raw ham. The temperatures were 36.6, 3.7, and 3.8 °C for the SC, PC, and C, respectively. The SC brine was significantly (P<0.05) higher than the PC and C brines due to the incubation step at 38 °C, whereas the PC and C brines were not different. Raw ground ham muscles were measured for pH and temperature prior to the addition of brine. The pH of the raw ham ranged from 5.98 to 6.07 while temperatures ranged from 1.5 to 1.7 °C and no significant (P>0.05) differences were found. Temperature and pH of meat was also measured after mixing of the ham, just prior to stuffing. The pH of the SC, PC, and C treatments was 5.83, 5.95, and 5.90 respectively with no differences between the treatments. The temperatures after mixing for the SC, PC, and C treatments were 8.9, 8.2, and 6.8 °C, respectively. There were no differences between the SC and PC treatments, but the C treatment was lower (P<0.05) than both the SC and PC treatments. Final pH and product yield of the hams were measured 24 hours after thermal processing. There were no significant differences (P>0.05) in pH or in yield for the three ham treatments. These results indicate that the differences in brine pH and temperature had no effect on the final pH or product yield of the ham treatments.

Residual Nitrite

The residual nitrite measurements are presented in Table 7. The results showed a significant (P<0.05) difference in the amount of measured nitrite at day 0. Calculated concentrations of ingoing nitrite were 39, 60, and 200 ppm for the SC, PC, and C treatments respectively. As expected, residual nitrite concentrations decreased throughout the 42 day storage period for all treatments. Before thermal processing (Day 0), residual nitrite concentration was 21.2,

40.8, and 112.4 ppm for the SC, PC, and C treatments respectively. The control (C) treatment had significantly more residual nitrite than the SC or PC treatments whereas the SC and PC treatments were not different. On Days 1, 3 and 7, the control was significantly greater than the SC treatment. After day 14, no significant differences were found between treatments. Although not always significant, it is interesting to note that the residual nitrite concentrations were numerically greater for the nitrite-added control across the entire 42 day storage period. Because nitrite forms a variety of reaction products in cured meat, this could have implications for the growth of both pathogens and spoilage bacteria

Color Measurements

The Hunterlab was used to objectively measure the CIE L* (lightness), a* (redness), and b* (yellowness) characteristics for each ham treatment. There were no significant (P>0.05) interactions or significant differences for the main effects of treatment or day for L* values (Table 8). There was a significant (P<0.05) interaction for treatment and day for a* values (Table 9). As expected, a* values generally decreased over time regardless of treatment which suggests that the redness of ham slices declined for all treatments. This is in agreement with Sindelar et al. (2007) who found uncured, no-nitrate-or-nitrite-added ham slices to be redder at 7 and 14 days than at 28 and 56 days of storage. At day 42, the control (C) treatment resulted in significantly (P<0.05) higher a* values than either the SC or PC treatments. This indicates that the control (C) treatment had a more intense cured color at the end of the 42 day storage period than other treatments. The higher a* values is more than likely due to the higher concentration of ingoing nitrite in the control treatment, which is responsible for the stability of the cured color. There was also a significant interaction for treatment and day for b* values (Table 10). At day 3, 7, 14, and 21, there were no significant

(P>0.05) differences in b* values across treatments. However, on day 1, 28, and 42 the control (C) treatment had lower b* values than the SC treatment indicating that the SC treatment was more yellow than the control. There were no differences between the PC and SC treatments on any day.

TBARS

TBARS values ranged from 0.1039 to 0.1410 and no significant interactions or significant (P>0.05) differences for the main effects of treatment or day for were found (Table 11). This was not unexpected because sodium nitrite has been shown to be an effective antioxidant with as little as 40-50 ppm ingoing nitrite (Pegg and Shahidi, 2000). Therefore the calculated levels of ingoing sodium nitrite of 39, 60, and 200 ppm for the SC, PC, and C treatments respectively would be enough to prevent or reduce lipid oxidation. Another factor that could limit lipid oxidation in the SC treatment is the presence of catalase. Catalase degrades hydrogen peroxide and is an important antioxidant enzyme. A study by Talon, Walter, Chartier, Barrièr and Montel (1999) measured several strains of staphylococci for the production of catalase to establish its role in lipid oxidation during sausage manufacturing. In the study, sausages inoculated with *S. carnosus* had the lowest amount of volatiles from lipid oxidation than all other strains of staphylococci, which was attributed to the high amount of catalase present in the cells. Interestingly, strains of *S. carnosus* also generated significantly more catalase in the presence of nitrate. Therefore, catalase could have a major impact on TBARS values in the SC treatment.

Conclusions

The results of this study show that incubating curing brines containing vegetable juice powder (VJP) and starter culture (*S. carnosus*) prior to injection resulted in hams that were

comparable to a sodium nitrite added control for cured color and suppression of lipid oxidation. The differences observed in residual nitrite appeared to have little impact on lipid oxidation. Color values and residual nitrite for the SC and PC treatments throughout the 42 day storage period confirmed that curing reactions occurred in all treatments. The calculated maximum levels of ingoing sodium nitrite of 39 and 60 ppm for the SC and PC treatments respectively would result in considerably less nitrite than the USDA FSIS maximum allowable limit of 200 ppm. The USDA FSIS requires a minimum of 120 ppm nitrite in cured meat products labeled "Keep Refrigerated" (USDA, 1995), therefore the low ingoing levels of nitrite in the "no-nitrate-or-nitrite added" products in this study could be a cause for concern regarding food safety, especially the growth of *C. botulinum.*

Further research is needed to determine if the use of a buffer and the omission of dextrose to decrease the pH change during the incubation of a curing brine for uncured meat and poultry products is effective for producing higher levels of nitrite in the brine. Also, further research is needed regarding the effects of this technology on microbiological shelf life of these products.

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Table 1. Brine Formulations

Ingredient	0.2% Vegetable Juice Powder				0.4% Vegetable Juice Powder			
	Salt Level				Salt Level			
	0	3	6	9	0	3	6	9
Water	90.87%	87.87%	84.87%	81.87%	89.87%	86.87%	83.87%	80.87%
Salt	0%	3%	6%	9%	0%	3%	6%	9%
Dextrose	8%	8%	8%	8%	8%	8%	8%	8%
Vegetable Juice Powder	1%	1%	1%	1%	2%	2%	2%	2%
Starter Culture	0.1285%	0.1285%	0.1285%	0.1285%	0.1285%	0.1285%	0.1285%	0.1285%
Total	100%	100%	100%	100%	100%	100%	100%	100%

Table 2. Brine formulations for the production of ham

	Treatment[a]		
Ingredient	SC	PC	C
Water	78.87 %	79.00 %	80.70 %
Salt	11.00 %	11.00 %	9.80%
Dextrose	8.00 %	8.00 %	8.00 %
VJP	2.00 %[b]	2.00 %[c]	--------
Starter Culture[d]	0.1285%	--------	--------
Sodium Erythrobate	--------	--------	0.22 %
Curing Salt[e]	--------	--------	1.28%
Total	100.00 %	100%	100.00 %

[a] Treatments:

        SC = Vegetable juice powder and a starter culture (*Staphylococcus carnosus*)

        PC = Pre-converted vegetable juice powder

        C = nitrite-cured control

[b] Vegetable Juice Powder NA 20, Chr. Hansen Inc., Gainsville, Fla., USA

[c] Veg Stable™ 504, Florida Food Products, Inc., Eustis, Fl

[d] CS 299 Bactoferm™, Chr. Hansen Inc., Gainseville, Fla., USA

[e] 6.25% Sodium Nitrite

Table 3. Cooking Schedule for Ham

Step Type	Step Time	Dry Bulb (°C)	Wet Bulb (°C)	RH (%)	IT* (°C)
Cook	00:40	74	0	0	-------
Cook	00:30	77	0	0	-------
Smoke Cook	00:45	79	0	0	-------
Smoke Cook	01:00	79	72	71	-------
Cook	-------	82	71	62	60
Steam Cook	-------	85	85	100	70
Cold Shower	00:10	10	0	0	-------

*IT = Internal Temperature

Table 4. Effects of salt and temperature on nitrate concentration, nitrite concentration, cell counts, and pH over time on curing brines containing 0.2% vegetable juice powder and a starter culture containing *Staphylococcus carnosus*.

| | High Temperature (38 °C) | | | | | | Medium Temperature (24 °C) | | | | | | Low Temp (6 °C) | | | | | |
| | | | | Log CFU/mL | | | | | | Log CFU/mL | | | | | | Log CFU/mL | | |
Salt	Hours	ppm Nitrate	ppm Nitrite	TSA[a]	Baird Parker[b]	pH	Hours	ppm Nitrate	ppm Nitrite	TSA[a]	Baird Parker[b]	pH	Hours	ppm Nitrate	ppm Nitrite	TSA[a]	Baird Parker[b]	pH
0%	0	168.0	2.7	8.05	8.11	5.73	0	183.6	3.1	7.95	8.09	5.85	0	181.4	1.2	7.79	7.82	6.01
0%	6	15.5	147.8	5.70	6.00	4.51	26	16.6	143.1	2.86	2.94	4.86	120	175.1	2.6	7.07	7.53	5.81
0%	48	19.9	128.7	NG	NG	4.41	100	17.4	126.1	1.88	1.65	4.75						
3%	0	176.1	3.1	7.84	8.02	5.57	0	181.7	0.1	7.81	7.95	5.63						
3%	24	16.0	136.5	7.00	7.55	4.35	48	56.1	126.8	5.98	4.93	4.64						
3%	77	17.4	100.2	NG	NG	4.37	101	48.9	117.8	7.20	5.15	4.63						
6%	0	174.0	3.4	8.00	7.93	5.36	0	184.4	0.6	7.98	7.83	5.46						
6%	23	67.9	100.1	7.34	7.63	4.35	96	76.6	106.0	7.27	7.22	4.48						
6%	100	60.7	79.4	10^1	NG	4.33	192	69.3	87.7	3.23	2.88	4.42						
9%	0	177.2	5.3	7.98	7.95	5.30	0	186.8	1.1	7.58	7.87	5.47						
9%	47	146.5	36.1	7.34	7.54	4.53	94	24.0	94.0	5.66	6.03	4.54						
9%	96	148.3	30.6	6.28	6.91	4.52	212	24.2	127.7	1.30	1.30	4.48						

[a] Plate counts on Tryptic Soy Agar incubated at 35 °C for 24 hours
[b] Plate counts on Baird Parker media with EY Tellurite Enrichment incubated at 35 °C for 48 hours

Table 5. Effects of salt and temperature on nitrate concentration, nitrite concentration, cell counts, and pH over time on curing brines containing 0.4% vegetable juice powder and a starter culture containing *Staphylococcus carnosus*.

| | High Temperature (38 °C) | | | | | | Medium Temperature (24 °C) | | | | | | Low Temp (6 °C) | | | | | |
| | | | | Log CFU/mL | | | | | | Log CFU/mL | | | | | | Log CFU/mL | | |
Salt	Hours	ppm Nitrate	ppm Nitrite	TSA[a]	Baird Parker[b]	pH	Hours	ppm Nitrate	ppm Nitrite	TSA[a]	Baird Parker[b]	pH	Hours	ppm Nitrate	ppm Nitrite	TSA[a]	Baird Parker[b]	pH
0%	0	314.7	3.2	7.93	7.77	5.49	0	358.3	3.5	8.14	8.02	5.56	0	363.7	1.5	7.60	7.72	5.72
0%	12	88.5	208.5	1.70	2.27	4.58	26	69.2	229.6	1.65	1.40	4.89	120	350.8	3.4	7.26	7.69	5.50
0%	48	87.6	187.2	NG	NG	4.52	100	33.4	217.1	1.00	NG	4.86						
3%	0	354.1	2.9	7.63	7.84	5.34	0	329.1	0.4	8.29	8.19	5.43						
3%	24	195.4	122.2	7.43	8.01	4.51	48	200.6	123.4	6.87	6.46	4.79						
3%	77	199.3	75.7	NG	NG	4.48	101	206.0	118.3	7.56	7.31	4.70						
6%	0	374.4	4.3	7.69	7.89	5.17	0	234.2	1.1	7.94	8.07	5.28						
6%	23	301.2	61.7	7.51	7.65	4.62	96	251.0	98.1	6.80	7.19	4.72						
6%	100	283.5	50.0	1.40	1.00	4.54	192	232.6	78.9	2.74	NG	4.66						
9%	0	369.2	0.3	7.51	7.97	5.16	0	358.7	1.2	7.15	7.68	5.23						
9%	47	358.4	14.5	7.05	7.37	4.78	94	223.9	122.3	6.99	7.23	4.75						
9%	96	367.4	8.0	5.32	6.30	4.78	212	220.1	111.3	1.30	NG	4.69						

[a] Plate counts on Tryptic Soy Agar incubated at 35 °C for 24 hours
[b] Plate counts on Baird Parker media with EY Tellurite Enrichment incubated at 35 °C for 48 hours
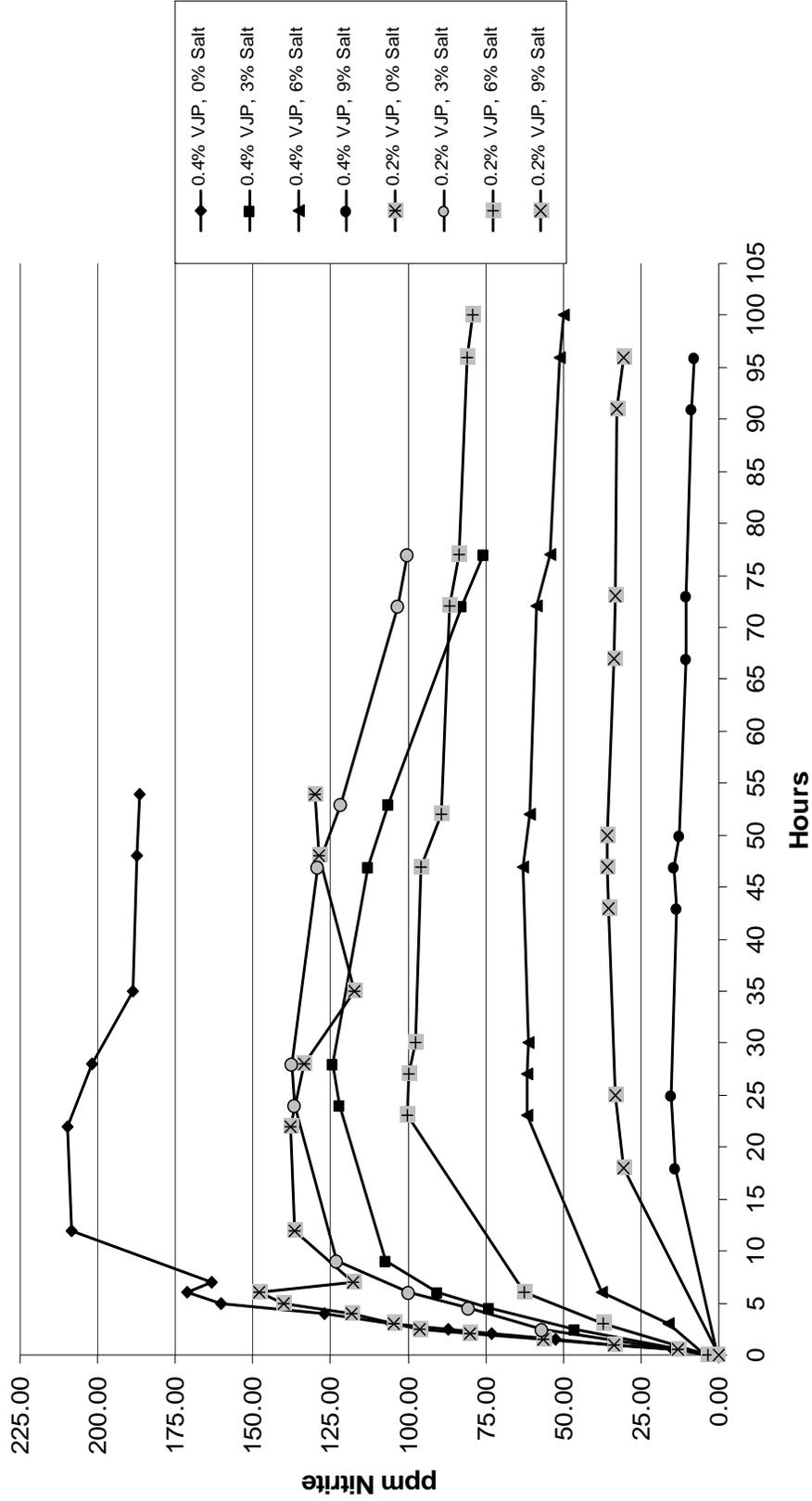
Figure 1. Nitrite concentrations of curing brines with varying levels of vegetable juice powder and salt over time incubated at 38°C.
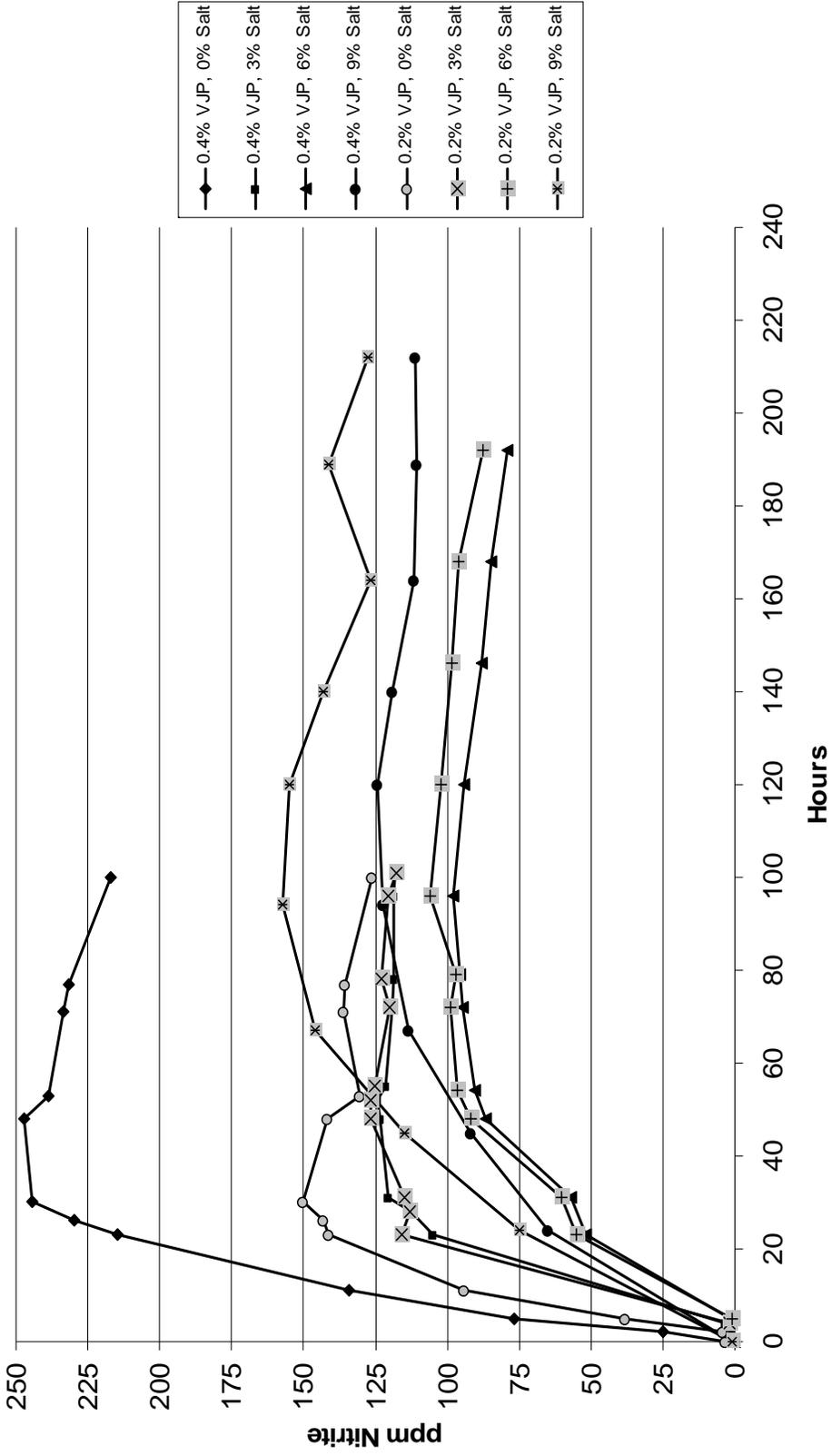
Figure 2. Nitrite concentrations of curing brines with varying levels of vegetable juice powder and salt over time incubated at 24°C

Table 6. Least squares means for processing attributes of no nitrite-added (SC and PC) and nitrite-added control (C) ham.

Treatment[a]	pH			Temperature (°C)			Final pH[e]	Yield[f]
	Brine[b]	Raw Ham[c]	Post Mixing[d]	Brine[b]	Raw Ham[c]	Post Mixing[d]		
SC	4.24[h]	6.05[h]	5.83[h]	36.6[h]	1.7[h]	8.9[h]	5.97[h]	91.5%[h]
PC	9.22[i]	5.98[h]	5.95[h]	3.7[i]	1.6[h]	8.2[h]	6.01[h]	91.6%[h]
C	8.02[j]	6.07[h]	5.90[h]	3.8[i]	1.5[h]	6.8[i]	6.12[h]	91.3%[h]
SEM[g]	.0394	.0501	.0558	.2191	.0924	.0214	.0562	.0901

[a] Treatments:
      SC = Vegetable juice powder and a starter culture (*Staphylococcus carnosus*)
      PC = Pre-converted vegetable juice powder
      C = nitrite-cured control

[b] Brine = used for 25% gain of ham muscles
[c] Ground raw pork ham muscles were randomly selected for pH and temperature measurements
[d] Post Mixing = measurements for pH and temperature were taken immediately after ham came out of mixer
[e] Final pH = pH taken after chilling for 18 hours at 0-2 °C
[f] Yield = weight of cooked product/weight of raw product after chilling for 18 hours at 0-2 °C
[g] Standard error of means.
[h-j] Means within same column with different superscripts are different (P<0.05)

Table 7: Least square means of residual nitrite concentrations (ppm) of no-nitrite-added (SC and PC) and nitrite-added control (C) hams.*

					Day			
TRT[a]	0	1	3	7	14	21	28	42
SC	21.2[b]	15.4[b]	15.5[b]	14.5[b]	12.0[b]	9.0[b]	6.0[b]	2.0[b]
PC	40.8[b]	28.3b[c]	30.2[bc]	30.0[bc]	25.2[b]	20.6[b]	15.7[b]	7.3[b]
C	112.4[c]	52.4[c]	54.1[c]	54.2[c]	46.8[b]	38.0[b]	33.7[b]	26.1[b]

* Pooled Standard Error of Means = 7.0514

[a] Treatments:

    SC = Vegetable juice powder and a starter culture (*Staphylococcus carnosus*)
    PC = Pre-converted vegetable juice powder
    C = nitrite-cured control

[b-c] Means within same column with different superscripts are different (P<0.05)

Table 8: Least square means for L* values of no-nitrite-added (SC and PC) and nitrite-added control (C) ham.*

TRT[a]	Day						
	1	3	7	14	21	28	42
SC	61.85	60.49	61.05	61.06	60.45	61.27	61.26
PC	60.01	59.84	60.13	60.76	58.17	59.10	60.43
C	60.47	60.56	61.07	60.41	59.72	59.61	59.44

* Pooled Standard Error of Means = 1.4046

[a] Treatments:
  SC = Vegetable juice powder and a starter culture (*Staphylococcus carnosus*)
  PC = Pre-converted vegetable juice powder
  C = nitrite-cured control

Table 9: Least square means for the a* values of no-nitrite-added (SC and PC) and nitrite-added control (C) hams.*

TRT[a]	Day						
	1	3	7	14	21	28	42
SC	6.77^{b}	8.61^{b}	8.41^{b}	8.09^{b}	8.10^{b}	7.49^{b}	6.64^{b}
PC	7.90^{bc}	8.35^{b}	8.21^{b}	8.08^{b}	8.26^{b}	8.12^{b}	7.56^{b}
C	8.62^{c}	8.81^{b}	8.83^{b}	8.93^{b}	9.10^{b}	8.41^{b}	9.23^{c}

* Pooled Standard Error of Means = 0.1823

[a] Treatments:

SC = Vegetable juice powder and a starter culture (*Staphylococcus carnosus*)
PC = Pre-converted vegetable juice powder
C = nitrite-cured control

[b-c] Means within same column with different superscripts are different (P<0.05)

Table 10: Least square means for the b* values of no-nitrite-added (SC and PC) and nitrite-added control (C) hams.*

				Day			
TRT[a]	1	3	7	14	21	28	42
SC	7.71^c	6.37^b	6.03^b	6.55^b	6.72^b	6.95^c	7.57^c
PC	6.57^{bc}	6.15^b	6.17^b	6.14^b	6.14^b	6.11^{bc}	6.24^{bc}
C	6.28^b	5.91^b	5.97^b	5.49^b	5.66^b	5.46^b	5.82^b

* Pooled Standard Error of Means = 0.1501

[a] Treatments:
    SC = Vegetable juice powder and a starter culture (*Staphylococcus carnosus*)
    PC = Pre-converted vegetable juice powder
    C = nitrite-cured control
[b-c] Means within same column with different superscripts are different (P<0.05)

Table 11: Least square means for the TBARS values of no-nitrite-added (SC and PC) and nitrite-added control (C) hams.*

	Day						
TRT[a]	1	3	7	14	21	28	42
SC	0.1158	0.1102	0.111	0.108	0.1238	0.1289	0.1219
PC	0.1285	0.1371	0.1143	0.1125	0.108	0.1131	0.109
C	0.1176	0.1197	0.1094	0.1115	0.1039	0.119	0.141

* Pooled Standard Error of Means = 0.008

[a] Treatments:
    SC = Vegetable juice powder and a starter culture (*Staphylococcus carnosus*)
    PC = Pre-converted vegetable juice powder
    C = nitrite-cured control

CHAPTER 4. GENERAL CONLUSIONS

The biggest concern with incubating brines to convert nitrate to nitrite prior to injection is the low levels of nitrite that were produced. For this study, the maximum amount of nitrite generated in an incubated brine was 247.2 ppm, occurring after 48 hours of incubation. At this concentration, the ingoing amount of nitrite at a 25% gain would be 61.8 ppm nitrite. This is significantly lower than the USDA FSIS maximum allowable limit of 200 ppm. Since USDA FSIS requires a minimum of 120 ppm nitrite in cured meat products labeled "Keep Refrigerated", the low ingoing levels from this process found in this study could result in microbiological concerns, especially regarding *C. botulinum*. It has been shown that the amount of vegetable juice powder affects the amount of nitrite produced. The higher the amount of vegetable juice powder, the more nitrite is able to be generated. However, high levels of vegetable juice powder can cause off odors and off flavors in the finished product.

One way to possibly increase the amount of nitrite in an incubated brine would be the addition of a buffer. Brine's pH had a large impact on nitrite generation by affecting the population of the starter culture. If pH was held constant, it may allow for more of the nitrate in the system to be converted to nitrite. Also, if pH did not decrease to the point where the cells of *S. carnosus* became depleted, it may allow for additional conversion during the cooking process when the internal temperature of the product increases to provide adequate time/temperature conditions for nitrite production.

Another possible way to increase the amount of nitrite produced would be to exclude dextrose during the incubation step. It appears that the starter culture does not grow during

brine incubation, therefore a food source may not be necessary. The omission of dextrose

would decrease the osmotic pressure and may allow for a higher enzyme activity. Because

the cells of *S. carnosus* do not grow, the nitrate reductase enzyme is all that is of any

concern, therefore the ingoing level of starter culture may be extremely important to the

amount of nitrite produced.

The process of incubating curing brines with vegetable juice powder and a nitrate

reducing starter culture containing *Staphylococcus carnosus* for the production of uncured

ready-to-eat ham resulted in a product that was inferior in a* values and ingoing nitrite levels

to a nitrite-cured control. However, it was similar to a product produced with a pre-converted

vegetable juice powder. It is also important to note that after 7 days of storage, residual nitrite

between the three treatments was not found to be statistically different, although the control

had higher levels throughout the 42 day storage period. Also, all three treatments had similar

TBARS values indicating that all methods of producing a cured product were effective in

controlling lipid oxidation. Catalase production may provide an additional antioxidant effect

to the treatment that used *S. carnosus* as nitrate reducing starter culture. This could be

important due to the low concentrations of residual nitrite throughout the storage period.

Further research is needed to determine if the use of a buffer and the omission of

dextrose during the incubation of a curing brine for uncured meat and poultry products is

effective at producing higher levels of nitrite. Also, further research is needed regarding the

effects of this technology on microbiological control and shelf life of these products.

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