

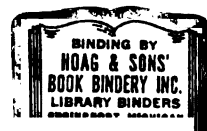
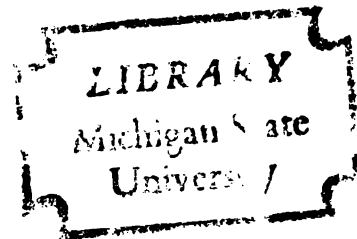


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EFFECT OF NITRITE ON THE FLAVOR
OF CURED PORK

Thesis for the Degree of M. S.
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IUE CHUNG CHO
1968

THESIS



ABSTRACT

EFFECT OF NITRITE ON THE FLAVOR OF CURED PORK

by Iue Chung Cho

The aim of the present study was concerned mainly with the effect of nitrite on the cured flavor of pork. It was not concerned with the constituents produced by the action of nitrite on pork.

The paired, left and right, Longissimus dorsi muscles of pork were used. Both samples were cured in a pickle, in which sodium nitrite was the only variable. Panelists were blindfolded and taste tests were conducted. Taste panels were of consumer type. The statistical treatment of the data obtained from the triangle taste tests was made using the table taken from the Guide Book for Sensory Testing by Ellis (1961). The statistical results showed that panelists were able to identify the different sample at a statistically significant level in the triangle taste tests. In the paired taste tests, a statistically significant number of panelists chose the sample containing sodium nitrite as having more cured flavor. Since sodium nitrite was the only variable in the curing pickle, these results indicate that sodium nitrite causes some change in the flavor of cured pork.

To determine the effect of nitrite on the flavor of cured pork without the influence of salt and sugar, the samples were cured in distilled deionized water, in which sodium nitrite was the sole curing agent. The results of the taste panel tests indicated that sodium nitrite alone produced a certain flavor. This action occurs during either curing or

cooking.

The effect of smoking on the flavor produced by sodium nitrite was also studied. Samples were cooked, sliced and smoked in that order. It appeared that smoking did not affect the ability of panelists to identify the different sample in the triangle taste test. Furthermore, panelists chose the samples containing sodium nitrite as having more cured flavor over those containing no sodium nitrite, even though both samples were smoked.

EFFECT OF NITRITE ON THE FLAVOR OF CURED PORK

By

Iue Chung Cho

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INTRODUCTION

The curing of meats has no definite beginning date. Salt curing and smoking of meats for preservation was practiced in the time of Homer (about 900 B.C.) (Jensen, 1945). He stated that the salting of meats was begun in the saline deserts of Hither Asia and along the sea coasts. In the old times, curing or smoking of meats was used merely as a method of preservation. Salt is the oldest known preservative and has been used in many countries as a meat preservative. Saltpeter also has been used for a long time to preserve meat from spoilage and discoloration (Hoagland, 1908). Today the public has awakened to an appreciation of flavor as one of the greatest attributes of an acceptable food product. As a result, more attention to flavor in food products has been paid by the manufacturers. The trend of curing has moved from preservation toward producing flavor to satisfy the consumer's taste.

Although nitrate has been used for a long time, little attention had been paid to the scientific aspects of the bright red color formation which occurs during the process of curing until 1890-1910. It was finally discovered then that it was the nitrite, resulting from bacterial reduction of nitrate, that was actually responsible for the formation of the thermally-stable cured meat pigment (American Meat Institute Foundation, 1960). It is now accepted that the development of the bright red color in cured meat is due to the interaction of nitrite with the myoglobin of the meat which yields a pigment, nitroso-myoglobin. Also, nitrite is known to act as a bacteriostatic agent. However, little is known about

its contribution to the flavor of cured pork (cured flavor). English researchers (Brooks et al., 1940) have reported that the characteristic cured flavor is due primarily to the action of nitrite on the meat. They claimed that a satisfactory bacon can be made by using only sodium chloride and sodium nitrite.

The investigation reported here was undertaken to verify their work and to obtain more detailed information on the effect of nitrite on cured flavor of meat. The objectives of the study were:

1. To determine what effect nitrite has on the flavor of cured pork.
2. To determine what effect wood smoke has on the flavor of cured pork cured with nitrite.

LITERATURE REVIEW

Curing Methods

Curing consists of the absorption of sufficient salt to preserve the meat, the fixation of color and the development of that distinctive flavor and texture which distinguish cured from fresh meat (Kerr et al., 1926). The ingredients commonly used in curing mixtures are sodium chloride, sugar, sodium and potassium nitrate, and sodium and potassium nitrite. Sometimes vinegar is used (Frazier, 1967).

Brandly et al. (1966) described three methods of curing meat. These include the sweet pickle cure, plain pickle cure, and dry cure. The sweet pickle cure consists of a 100° salometer strength solution of sodium chloride which is then diluted to the desired strength of pickle with water. To the diluted pickle solution the sugar and nitrate and/or nitrite are added. The meat cut may be submerged or covered in the pickle solution; or, the pickle may be injected or pumped into the body or through the arterial system of the meat cut. The internal temperature of the meat to be cured should not exceed 38°F. When using the plain pickle cure, no sugar is added to the pickle. Occasionally, however, nitrate or nitrite are added to the pickle as a variation of the plain pickle cure. The dry-curing method consists of rubbing all sides of the meat with the dry-cure mixture (salt, sugar, nitrate and/or nitrite).

Ziegler (1964) stated that there is not much difference in the shrinkage between dry and sweet pickle cured pork after 60 days of aging.

There will be a gain of an average of 5% in sweet pickle cured hams, whereas a loss of 5% to 7% occurs in dry cured hams. However, according to Skelly et al. (1960), shrinkage losses in dry cure during aging may be 30%. Furthermore, the quality of aged hams is not uniform. In the study of characteristics of hams during aging by Kemp et al. (1957) it was shown that shrinkage in dry cure increased at a decreasing rate each month and ranged from an average of 8.6% after smoking to 30.5% after 6 months aging.

Role of Curing Ingredients

Salt: Salt is the most important curing ingredient. Sufficient concentrations of salt inhibit microbial growth. Salt also contributes to the flavor of food (American Meat Institute Foundation, 1960).

Jensen (1945) reported that although salt was the most active preservative used in meat-curing solutions, it did not inhibit all bacteria. Its function is a selective action. Aerobes, facultative anaerobes, and micrococci were more resistant to salt than anaerobes. The rods were more easily suppressed by salt than cocci.

Bullman and Ayres (1952) showed that no spoilage was observed at concentrations exceeding 4.4% sodium chloride while varying degrees of bacteriostasis were shown by levels of 3.5% to 4.4% salt in pork trimmings. However, there are many conflicting reports regarding the effective preservation concentrations of curing salt against putrefactive anaerobes. Tanner and Evans (1933; 1934) explained some of these causes. Species and strains varied in susceptibility to the same salt concentration and similar organisms tolerate various levels of the same salt concentration in different substrates.

Investigations by Rockwell and Ebertz (1924) showed that dehydration by salt is not the only preserving factor. They concluded that there are four other factors involved in the preserving action of sodium chloride: 1) direct effect of chloride ion, 2) removal of oxygen from medium, 3) sensitization of the test organism to CO_2 , and 4) interference with rapid action of proteolytic enzymes.

The biochemical mechanisms during curing were extensively studied by Callow (1947). Due to osmotic pressure, certain proteins diffuse into the salt solution. The flow of water is then reversed after the formation of a protein salt complex. After the diffusion of salt into muscular tissue, the micro-structure is altered. The texture becomes much more jelly-like, and the muscle juice is held more firmly. Grant and Gibbons (1948) also studied the biochemical aspects of curing. They stated that the chloride ions which were bound by the tissue were released by the bacterial action and the enzymatic breakdown of the muscle tissue. The chloride ion then re-united with the sodium ion in the outer tissue fluids to form sodium chloride. This action gives meat a salty taste.

The relationship between length of curing and the thickness of ham was studied by Miller and Ziegler (1939). They found that higher sodium chloride content was obtained by longer curing periods per unit of ham. Wistreich et al. (1959; 1960) showed that accumulation of sodium chloride increased with an increase in temperature and brine concentration, and that the accumulation of sodium chloride was related linearly to concentration, but logarithmically to time.

Investigations by Pearson et al. (1962) and Goembel (1962) showed that the optimal level of salt in cured ham was 2.5% combined with 1.1% sugar. Also, Marquardt et al. (1963) found that there were no significant differences in consumer preferences between 1.5% and 3.0% salt, whereas

there was a significant difference between 1% and 3% sugar.

Sugar: Sugar is used mainly to counteract the harshness of the salt cure and to improve the flavor and texture of the meat (Levie, 1967). It also provides energy for the growth of bacteria (Moulton and Lewis, 1940). According to Ashbrook (1955), sugar is broken down into organic acids by microorganisms. Lactic acid is one of these acids and gives a pleasant flavor to meat.

Urbain et al. (1940) studied the correlation between color changes of meat and the growth of microorganisms utilizing sugar. They observed that dextrose, mannose, and sucrose produced significant color changes in the blood pigments, oxymyoglobin and nitric oxide myoglobin. They stated that color change requires the reducing conditions which are provided by microbial growth.

Nitrite and Nitrate: The scientific study of color fixation of cured meat was started in Europe in 1890-1892 by Polenske, according to Lewis et al. (1925). They reported that Polenske found nitrite in cured meat and curing solutions, and that the nitrite was formed by bacterial reduction of nitrate. Lewis et al. (1925) further stated that Lehman and Kisskalt studied the function of nitrite. Lehman showed the color change by boiling fresh meat with nitrite in the presence of a little acid. Kisskalt proved that nitrate could fix the color if the meat was left in contact with the nitrate several days.

Hoagland (1908), in his study of the action of saltpeter (potassium nitrate) upon the color of meat, referred to the work by Weller and Riegel. These workers obtained a bright-red ether extract from sausage and other cured meat and found that the extract had an absorption spectrum similar to methemoglobin. This extract was also obtained from meat containing blood and treated with nitrate. Haldane (1901) extended Weller's and

Riegel's work. He identified the coloring matter of uncooked cured meat as nitroso-hemoglobin by its absorption spectrum. He showed that nitroso-hemoglobin and some methemoglobin were produced by the reaction of nitrites on blood. He concluded that the nitroso-hemoglobin was formed by the action of nitrite on hemoglobin in the absence of oxygen and in the presence of reducing agents. He further showed that the red color of cooked cured meat was due to the nitroso-hemochromogen produced by decomposition of nitroso-hemoglobin when heated. Ostertag (1904) stated that the red color of salt meat is not due to the saltpeter, but to nitrites, and perhaps nitric oxide which is formed from the saltpeter in the brine.

No attention was given to the changes which take place during the curing process in this country until Hoagland (1908) started his experiments on this subject. He verified and extended Haldane's (1901) work. Hoagland concluded that the nitric oxide which was formed by the reduction of the nitrites within the meat was an intermediate step between nitrates and the formation of nitroso-hemoglobin.

After the formal authorization of the first experiment involving direct use of nitrite, Kerr et al. (1926) undertook their experiments to prove that sodium or potassium nitrite can be substituted for sodium or potassium nitrate in the curing of meat. They reported that the nitrate must be reduced to nitrite before it becomes active in meat curing. They found that there was no difference in flavor between the nitrite cured meat and that cured with nitrate. In addition, meat cured with nitrite was not inferior in quality or wholesomeness to meat cured with nitrates. They observed a smaller amount of the residual nitrite in the nitrite cured meats than in the nitrate cured meat. As a result of their work, the use of sodium nitrite in meat curing was authorized by the Department

of Agriculture, through Amendment 4 to Bureau of Animal Industry Order 211 (revised) (USDA, 1926). The entire amendment is subject to meat-inspection regulation. The Bureau of Animal Industry issued Circular No. 1370 permitting the addition of 1/4 ounce of sodium nitrite per hundred pounds of meat. The Bureau further ruled that the finished product should contain no more than 200 parts per million of sodium nitrite (AMIF, 1960).

Anson and Mirsky (1925) investigated the reaction between nitric oxide and hemoglobin. They stated that hemoglobin in the presence of nitric oxide is oxidized to methemoglobin, which then forms nitric-oxide methemoglobin. Keilin and Hartree (1937) cite work by Haurowitz in which he claimed that methemoglobin was reduced by nitric oxide to hemoglobin which then combined with more nitric oxide to form nitric oxide hemoglobin. Keilin and Hartree (1937), however, contended that these interpretations were only partly correct. Their conclusion was that nitric oxide combines with both hemoglobin and methemoglobin. Nitric oxide forms a very stable compound with hemoglobin, whereas with methemoglobin it forms an easily reversible compound which is not stable and undergoes reduction to nitroso-hemoglobin on standing.

The chemical nature and reactivity of meat pigments were studied at the American Meat Institute Foundation by Schweigert (1956). His study concentrated on myoglobin which is referred to as muscle hemoglobin. He presented the schematic diagram showing the several chemical reactions involved in color changes of fresh or cured meats. (Fig. 1).

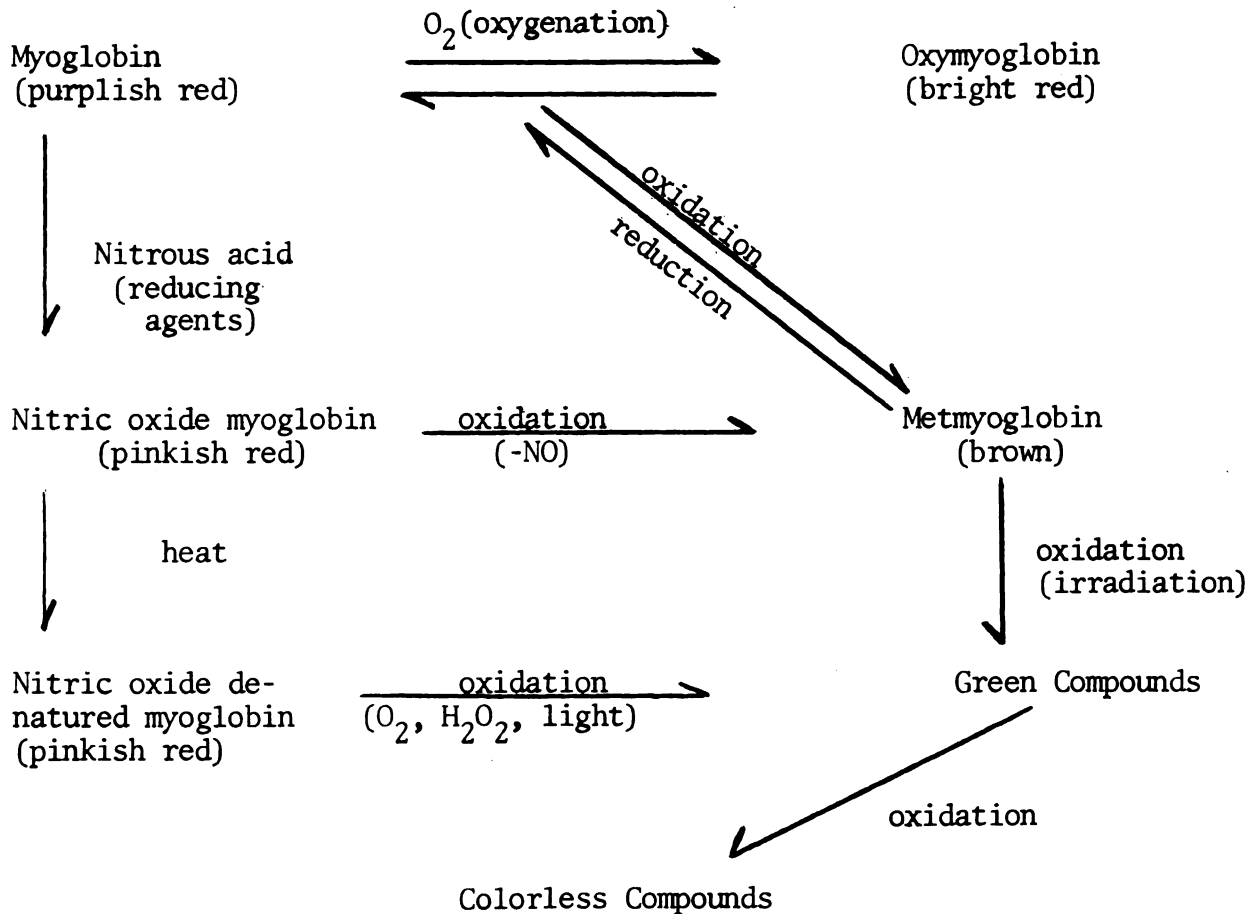
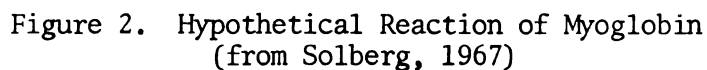


Figure 1. Chemical Reaction of Myoglobin
(from Schweigert, 1956)

Recently, extensive studies concerning the color development and color stability in meat processing have been performed. According to Fox and Thomson (1963), the myoglobin first had to be oxidized to metmyoglobin before it could combine with nitric acid. This report was supported by Taylor and Walter (1967). Their experiment showed that nitrite rapidly oxidized oxymyoglobin to metmyoglobin. They also suggested that reduction of nitric oxide metmyoglobin by mitochondria may be a possible mechanism for the formation of nitric oxide myoglobin during curing. However, a later report by Fox et al. (1967) indicated that nitroso-myoglobin may be formed by reducing agents such as ascorbate without being converted to nitroso-metmyoglobin. Solberg (1967) made an attempt to interpret a dual pathway in the formation of nitroso-myoglobin. Without added reductants



According to Frazier (1967), sodium nitrate is mildly bacteriostatic in acid solution, especially against anaerobes. Sodium nitrite also has some bacteriostatic effect in acid solution. Tarr (1941) found that low concentrations of nitrite can delay bacterial spoilage of fish in acid medium, but not above pH 7. Bullman and Ayres (1952) reported that nitrite inhibited the germination of the spores of putrefactive anaerobes (P.A. 3679) in inoculated emulsion. According to Castellani and Niven (1955), nitrites do not have any practical preservative action

on those organisms not killed by sodium chloride in cured meats.

Ingram (1939) attempted to determine the mechanism of inhibitory action of nitrite on bacteria. He found that nitrite very strongly inhibited the oxygen uptake of Bacillus cereus. Therefore, nitrite interferes with the cytochrome system. However, Tarr (1941) found that there was no relationship between inhibitory action of nitrite and its effect on respiration. Furthermore, the results obtained by Castellani and Niven (1955) did not support Ingram's explanation. Their results showed that nitrite was quite effective as a bacteriostatic agent in an anaerobic medium autoclaved with glucose. Their explanation was that undissociated nitrous acid is the active form in the inhibitory action.

Brooks et al. (1940) investigated the effect of nitrite on the cured flavor of bacon. In their experiments blindfolded tasters were asked to indicate their preferences. Three pieces of pork from the same pig, each weighing about 3,500 g., were cured in three different pickles:

	NaCl g./100g. water	NaNO ₃ g./100g. water	NaNO ₂ g./100 g. water
Pickle A	16	2.8	0.08
Pickle B	16	2.8	
Pickle C	16		

Their results showed that samples containing sodium nitrite were preferred, but there was no clear-cut preference for one particular concentration. In conclusion, they stated that the characteristic cured flavor of bacon is due primarily to the action of nitrite on the meat.

Smoking

The primary function of smoking originally was to enhance the preservative action of curing, but today smoking is used mainly to improve the flavor (Lawrie, 1966). In addition, smoking also improves the color

of the products (Tilgner, 1957). Lea (1933) reported that smoking acts as an antioxidant. His report was based on the peroxide value. The peroxide value of smoked bacon was lower than that of unsmoked bacon after 28 days of storage at -10°C .

According to Hess (1928), the smoking of fish fillets inhibited the growth of microorganisms. Also, White et al. (1942) reported that the smoking of bacon reduced the number of surface bacteria "by 10,000 times" of those of unsmoked bacon. In a study of lethal effect of smoke on cheddar cheese, Hedrick et al. (1960) found that hardwood sawdust smoke had bactericidal action on the surface bacteria of freshly cut cheddar cheese. Jensen (1949) stated that smoking greatly increased the keeping qualities of meat. This statement was supported by White et al. (1944). They found that smoking of bacon extended the storage period to 2 months from 1 month at the same temperature.

Phenolic compounds play a major role in smoke flavor (Husani and Cooper, 1957). Consequently, total phenolic content in smoked foods has been used as the index of the degree of smoking (Hanley et al., 1966). Shewan (1949) found more phenols at the surface than under or at the center of fish fillet and sausage. Tucker (1942) also found phenolic compounds concentrated at the surface. All of these results indicate that most of the flavoring components of smoke are deposited at or near the surface of the smoked products.

Meat Flavor

Lawrie (1966) defines flavor as a complex sensation comprising odor, taste, texture, temperature and pH. Odor and taste are most difficult to define objectively. Four basic tastes, sweet, salty, bitter, and sour, are generally recognized today (Hornstein and Teranish, 1967).

Due to the greater range of olfactory responses, there are many types of odor classifications. Amerine et al. (1965) gave a classification of odors which included 7 types; spicy, flowers, fragrant, resinous, putrid, fruit, and ethereal.

Crocker (1948) stated that the flavor of cooked meat arises from the chemical changes occurring in the fiber. Kramlich and Pearson (1958) reported that the water soluble fraction contained large portions of cooked and raw beef flavor. Later (1959) they separated the volatile components from cooked meat and identified carbon dioxide, methyl mercaptan, acetone, acetaldehyde, methyl sulfide and water. Similar results were obtained by Bender and Ballance (1961), Hornstein et al. (1960), and Yueh and Strong (1960). Ockerman et al. (1964) isolated volatile compounds from dry-cured hams by gas chromatography retention times and further verified the compounds by infrared spectroscopy. These compounds were formaldehyde, acetaldehyde, propionaldehyde, isobutylaldehyde, n-valeraldehyde, isovaleraldehyde, acetone, diacetyl, methyl ethyl ketone, formic acid, acetic acid, propionic acid, butyric acid, and isocaproic acid. They also identified hydrogen sulfide and trace amounts of disulfides and/or monosulfides by selective trapping.

More recently, Cross and Ziegler (1965) compared some of the volatile components of cooked hams, both cured and uncured. The examination of the volatile compounds of uncured ham by gas chromatography showed that appreciable quantities of hexanal and valeraldehyde were present in the uncured product. These compounds were barely detectable in the volatile compounds of the cured meat.

Taste Panel Methodology

According to Caul (1957), the trained taster was first employed in the wine, tea and coffee industries. Primarily he rates the quality

of his products. Boggs and Hanson (1949) suggested that the trained taster should be used for detecting differences in flavor. Thurstone (1950) agreed with the previous work. He stated that a large group of untrained tasters is best for preference tests and a small group of trained tasters is best for difference tests.

Caul (1957) stated that the use of difference tests presupposes a thorough knowledge, on the part of the person conducting the test, of flavor in general, and of the flavor of the products under test. Having such knowledge, he can determine if the products are merely different and/or in what respect they differ. Amerine et al. (1965) illustrated 5 methods of difference tests. They are single-stimulus, paired-stimuli, duo-trio, triangle, and multi-sample tests.

The triangle and paired tests were compared by Dawson and Dochterman (1951). They found that there were no differences in obtaining correct answers between these two tests. Therefore, they concluded that the two tests were equal in their precision as a basis for selecting reliable panel members. However, the triangle test can eliminate judges who cannot identify duplicate samples. Byer and Abrams (1953) also compared the two methods. Differing concentrations of quinine or dextrose were used in their experiment. Their statistical results showed that the paired test gave more clear-cut evidence of a taste difference than the triangular test. In quality judgments the paired test also achieved higher significance than the triangular test.

EXPERIMENTAL PROCEDURES

Samples

Paired pork samples from the right and left Longissimus dorsi muscle were used in this study. The samples were either from the 10th to the last rib portion or from the last rib to the last lumbar vertebra section. The samples were frozen and stored at 0°F. until needed. One day before the curing process, the samples were thawed at 38° F. After thawing, the samples were weighed and placed in Cry-O-Vac bags in which the meats were cured.

Curing and Curing Yields

Stock pickles of the desired concentrations of sodium chloride and sugar were equally divided into two parts. Sodium nitrite was added to one of the parts. One of the paired samples was cured in the pickle containing sodium nitrite. The other sample was cured in the pickle containing no sodium nitrite. Left and right samples were cured alternately in the pickle containing nitrite. The curing pickle to sample ratio was always 2:1 (w/w). The meat was allowed to cure 3 days at 36-40°F. The position of Cry-O-Vac bags was changed every day.

The concentration of sugar in the pickle was always 1.2% where it was used. The concentration of nitrite in the pickle containing nitrite was always 300 ppm. Two levels of sodium chloride used in the pickles were 4.70% and 2.35%. The composition of pickles used throughout this experiment is summarized in Appendix IV.

After curing, the samples were again weighed to determine per cent change during curing. Weight change during curing was calculated by dividing the weight gained during curing by the weight before curing.

Cooking

The cured meats were cooked in a 350°F. electric oven to an internal temperature of 185°F. The external fat was removed (after cooking in all samples except samples 1 and 2). The samples were sliced with a Hobart meat slicer to a thickness of 5 mm.

Smoking

After cooking and slicing the samples, the samples were placed on a 1/2 inch wire mesh screen on a rack in the smoking chamber. This arrangement was used to provide uniform distribution of smoke on all sides of each piece. The samples were smoked for 10 minutes. During the smoking process the temperature was 128°F. and relative humidity was 34%.

Panel Presentation

Each slice of meat was cut into 3 pieces. The samples were randomized and coded for the triangle panel test. The triangle tests were carried out with samples No. 1 through No. 5 under red light to mask color differences. In each test, panel members were given three samples, two of which were the same and one different. Figure 3 shows the questionnaire used in this test.

Name _____ Plate No. _____ Date _____

Two of these samples are identical and the other different. Please check the different sample. Disregard tenderness. Consider flavor only.

Sample	Different Sample (indicate by /)
_____	_____
_____	_____
_____	_____

Figure 3. Example of questionnaire used in triangle test.

For the remainder of the panel tests, the tasters were blindfolded. Both paired and triangle tests were carried out, but only the paired test was used with samples 9, 10, and 11. In the paired test, the tasters were asked to indicate the sample which had more cured flavor. The samples were presented to panelists according to the plan shown in Figure 4.

Panelist	Order of Serving Triangle			Order of Serving Paired	
1	w ^(a)	w	w/o ^(b)	w	w/o
2	w	w/o	w	w/o	w
3	w/o	w	w	w	w/o
4	w/o	w/o	w	w/o	w
5	w/o	w	w/o	w	w/o
6	w	w/o	w/o	w/o	w
7	w	w/o	w/o	w/o	w

(a) = with nitrite

(b) = without nitrite

Figure 4. Order of sample presentation

Statistical Analyses

The data obtained from the triangle and the paired tests were evaluated using the table taken from Bengtsson, R. (1953) in Guide Book For Sensory Testing (1961) (See Appendix I).

Chemical Analyses

Samples: Deionized distilled water was used throughout the study unless otherwise indicated. The samples for chemical analyses were always obtained from the middle section of the Longissimus dorsi after cooking and trimming the fat. Each sample was ground twice through a meat grinder (2mm plate).

Moisture: Moisture was determined according to the method described

in A.O.A.C. (1965). Five g. of sample were placed in an aluminum foil dish and dried to a constant weight for 24 hours at 100°C. The moisture content was calculated using the following formula:

$$\%H_2O = \frac{\text{Original sample wt.} - \text{sample wt. after drying}}{\text{Original sample wt.}} \times 100$$

Ether Extract: The dried sample used in the moisture analysis was placed in an extraction thimble. The fat was extracted with anhydrous ether for 3 1/2 hours in a Goldfish Fat Extractor. The ether extract was calculated using the following formula:

$$\% \text{ Ether Extract} = \frac{\text{Wt. of fat extracted}}{\text{Original sample wt.}} \times 100$$

Sugar: Determination of glucose was first carried out by the method described by Folin and Wu (1920). One g. of meat was placed in a 25 ml. centrifuge tube. Ten and one-half ml. of water and 6.4 ml. of 0.2 N. sulfuric acid were then added. After 2 minutes of standing at room temperature, 2 ml. of 10% sodium tungstate was added slowly with shaking. After setting for 5 minutes, the tube was centrifuged at a speed of 2,800 r.p.m. for 5 minutes.

Folin-Wu tubes were used for color development. Two ml. of Folin's copper reagent were added to 2 ml. of the aliquot and placed in boiling water for 8 minutes. The tube was then cooled in running tap water and 2 ml. of phosphomolybdic acid were added. Again the tube was placed in boiling water for 2 minutes and then diluted to 25 ml. with water. The optical density was read against a reagent blank at 420 mμ, with a Bausch and Lomb Spectronic 20 spectrophotometer. Using a standard glucose solution which most closely matched the unknown, the concentration of glucose was calculated.

The sucrose in the sample was inverted by the method described by Harrow et al. (1955). One g. of the sample was placed in a test tube

containing 10 ml. of water. Two drops of concentrated sulfuric acid were added. After 3 minutes the solution was neutralized with saturated sodium carbonate solution, using litmus as the indicator. Then the amount of glucose was determined using the method previously described.

The amount of sugar in the meat was determined as the difference obtained by subtracting the amount of glucose before inversion from the total amount of glucose in the sample after inversion.

Salt: The sodium chloride content was determined by the Volhard method as outlined in A.O.A.C. (1965). Three g. of meat were placed in a 300 ml. Erlenmeyer flask. Fifty ml. of 0.1 N. silver nitrate were added to precipitate all chloride and to leave an excess of silver nitrate. After adding 15 ml. of concentrated nitric acid, the solution was mixed and boiled until all the meat disintegrated. During boiling 5% potassium permanganate was added, a few drops at a time, until a few ml. had been added. After boiling, the solution was diluted to approximately 150 ml. with water. Then 25 ml. of diethyl ether were added to coat the silver chloride. Five ml. of ferric indicator $[\text{Fe NH}_4 (\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}]$ and 5 ml. of a 1:1 solution of nitric acid and water were added. Excess silver was then titrated with potassium thiocyanate until a permanent light brown color appeared. From the volume (ml.) of silver nitrate used, the quantity of chloride was calculated using the following formula:

$$\% \text{NaCl} = \frac{\text{ml. of 0.1 N. AgNO}_3 \times 0.58}{\text{Sample wt.}}$$

Nitrite: The nitrite content was determined by the procedures outlined in A.O.A.C. (1965). Five g. of the meat sample were placed in a 500 ml. volumetric flask. Approximately 300 ml. of warm water (80°C.) were added. The flask was placed in a steam bath for 2 hours with occasional shaking. Five ml. of a saturated mercuric chloride solution were added

immediately after removal from the steam bath and the resulting solution mixed thoroughly. The solution was cooled to room temperature, brought to volume with water, and filtered through S&S #560 filterpaper. Ten ml. of the filtrate were added to 2 ml. of Griess reagent in a 50 ml. volumetric flask. The optical density was obtained at 520 mu. using a Beckmann D.U. spectrophotometer. The nitrite content was determined by comparison with a standard nitrite curve.

Protein: The Kjeldahl method outlined in A.O.A.C. (1965) was used to determine protein content. One-half g. of the meat sample was weighed on N free parchment paper squares. The paper was placed in a digestion flask. One-half g. of sodium sulfate, 1 ml. of 10% copper sulfate, 7 ml. of concentrated sulfuric acid and one glass bead were added to the flask. The flask was boiled with occasional swirling until the solution cleared. The flask was cooled and water was added to about the middle of the lower bulb of the flask. The flask was then connected to distilling equipment. After adding approximately 5 ml. of cold 40% NaOH, the distillate was collected in a 125 ml. Erlenmeyer flask containing 10 ml. of 2% boric acid and several drops of bromo-cresol-green indicator. The distillation period was 7 minutes while the tip of the condenser was beneath the surface of the boric acid and 4 minutes while the tip of the condenser was above the surface. The excess boric acid was back titrated with 0.1 N. sulfuric acid. The protein content was calculated using the following formula:

$$\% \text{ protein} = \frac{\text{N. of acid} \times 14 \times 6.25 \times \text{ml. of acid}}{\text{wt. of sample}}$$

Phenol: The estimation of total phenols was made by the colorimetric method of Tucker (1942). Twelve and one-half g. of the meat were blended for 5 minutes with 50 ml. of 1:1 alcohol water solution. The

solution was filtered through S&S #560 filterpaper. After 24 hours storage at 38°F. the filtrate was refiltered through Whatman No. 2 filterpaper at the same temperature. Twelve and one-half ml. of the filtrate were diluted to 25 ml. with water. Five ml. aliquot of the sample was pipetted into a 15 x 180 mm. test tube. Five ml. of 0.5% sodium borate buffer and 1 ml. of N, 2, 6-Trichloro-p-benzoquinoneimine were added to the tube. The tube was shaken and then allowed to stand for 1 hour at room temperature to allow for color development. The colored complex was extracted from the aqueous solution with 15 ml. of n-butanol in a small separatory funnel. The butanol layer was transferred into a 25 ml. graduated test tube and the volume increased to 21 ml. with n-butanol. Two ml. of n-butanol saturated with ammonia were then added, and after mixing by shaking, its optical density was read against a reagent blank at 635 mμ. on a Bausch and Lomb Spectronic 20 spectrophotometer. The estimation of total phenols (mg./100g.) in the collected solution was obtained with the phenol standard curve.

EXPERIMENTAL RESULTS AND DISCUSSION

Effect of Nitrite on Cured Flavor of Pork

The major portion of this study was undertaken to determine if nitrite has an effect on the cured flavor of pork. A study was initiated using 300 ppm of sodium nitrite in the pickle as the only variable. Table 1 shows the composition of the pickle used in paired samples 1 through 5.

Table 1. Composition of the pickle used in the paired samples 1 - 5.

Paired Sample	Sodium Chloride	Sugar	Sodium Nitrite*
Right	4.70%	1.2%	300 ppm
Left	4.70%	1.2%	-----

*Sodium nitrite was added to left and right samples alternately.

After cooking, the color of the samples was compared to insure uniform distribution of sodium nitrite. A pink color was uniformly prevalent throughout the meat cured with nitrite. No pink color was observed in the meat cured without sodium nitrite. No nitrite burn was observed.

Triangle taste tests were carried out under a red light to mask the color difference between the meat containing sodium nitrite and the one containing no sodium nitrite. Eighteen untrained panelists participated in the taste panel tests. The data collected from this taste panel were analyzed using the table in Appendix I. The results of the taste panel and the statistical analysis of the data are summarized in Table 2.

Table 2. Results of statistical analyses of the data obtained from the triangle taste test.

Trials	No. of Panelists	No. of panelists who identified different sample	Significance
1	18	9	n.s. [a]
2	18	7	n.s.
3	18	12	**
4	18	10	*
5	18	12	**

* $P < 0.05$

[a] not significant

** $P < 0.01$

In trials 1 and 2 external fat was not removed prior to the taste panel. The results of the triangle tests showed that fewer than a significant number of panelists were able to select the different sample. In the trials 3, 4, and 5 external fat was removed before the taste panel tests were carried out. This time, the results of the triangle tests were significant. Panelists were able to identify the different sample in the triangle taste tests at a statistically significant level.

To determine whether the panelists distinguished the difference between samples because of the saltiness in the triangle test, paired tests were carried out using the same samples used in the triangle test. The panelists were asked to indicate the saltier sample. No significant difference in saltiness between the two samples was noted.

Further studies were initiated to confirm that sodium nitrite affected cured flavor. Panelists were blindfolded to insure that the judgment in the tests was not influenced by the color of the meat. The concentration of sodium chloride in the pickle was decreased from 4.70% to 2.35%. The results of the triangle tests and the paired tests are summarized in Table 3. The results of the triangle tests show that panelists were able to identify the different sample at a statistically significant level in the triangle taste tests.

Table 3. Results of statistical analysis of the data obtained from the triangle taste tests and paired taste tests.

Trial	No. of panelists	Triangle# taste test	Significance	Paired## taste test	Significance
6	18	11	*	15	**
7	18	10	*	14	*
8	36	21	**	32	***

#No. of panelists who identified the different sample * P<0.05
 ##No. of panelists who chose the sample containing ** P<0.01
 NaNO₂ as having more cured flavor ***P<0.001

As Table 3 shows, a statistically significant number of panelists chose the sample containing sodium nitrite as having more cured flavor.

To determine whether the judgment in the paired tests was based on saltiness, the following experiment was carried out. The concentration of sodium chloride was doubled in the pickle containing no sodium nitrite. The composition of the pickle used was as follows:

Table 4. Composition of the pickle used in paired samples 9, 10, and 11.

Paired sample	Sodium chloride	Sugar	Sodium Nitrite*
Right	2.35%	1.2%	300 ppm
Left	4.70%	1.2%	-----

*Sodium nitrite was added to left and right alternatively.

The panelists were blindfolded and asked to indicate the sample having more cured flavor. The results of the taste panel tests are summarized in Table 5.

Even though no statistical significance was found in these paired tests, a larger number of panelists selected the sample containing sodium nitrite as having more cured flavor despite the fact that the sample was less salty. These results indicated that saltiness was not the only factor

responsible for the production of the cured flavor of pork. Obviously, sodium nitrite, as well as salt, contributes to cured flavor.

Table 5. Results of statistical analyses of the data obtained from the paired taste tests (9, 10, & 11).

Trials	No. of panelists	No. of panelists#	Significance
9	36	21	n.s. [a]
10	36	23	n.s.
11	36	20	n.s.
#No. of panelists who chose the sample containing sodium nitrite as having more cured flavor.			[a] not significant

An attempt was made to determine whether sodium nitrite brings about some change in the flavor of pork without the influence of salt and sugar. This trial was not designed to isolate or characterize the factors responsible for the production of the cured flavor by sodium nitrite. Neither sugar nor salt was used in the curing process. Sodium nitrite was the only variable in the curing pickle. One of the paired pork samples was cured in deionized distilled water containing 300 ppm of sodium nitrite as the sole curing agent and the other was cured in deionized distilled water. Table 6 gives the results of the triangle and paired taste tests.

Table 6. Results of statistical analyses of the data obtained from the triangle and paired taste tests.

Trials	No. of panelists	Triangle# taste test	Significance	Paired## taste test	Significance
12	23	14	**	19	**
13	19	11	*	16	*
14	18	11	*	14	*

No. of panelists who identified the different sample.

* $P < 0.05$

** $P < 0.01$

##No. of panelists who chose the sample with NaNO_2 as having more cured flavor.

The results of the triangle taste tests showed that the panelists identified the different sample at a statistically significant level. In paired taste tests a significant number of panelists chose the sample containing sodium nitrite. These results indicated that the sodium nitrite causes some change in flavor of pork without the influence of salt and sugar.

It was interesting to note the performance of the panelists in the taste panel tests. As pointed out by Mrak et al. (1959) and Baker et al. (1961), some panelists were very sensitive in distinguishing the different sample in the triangle tests and others were very insensitive. Some panelists were actually unable to distinguish the difference between the samples containing sodium nitrite and those containing no sodium nitrite. The author's opinion is that results can be obtained at a higher significant level if panelists are previously trained for the purpose of this experiment. This statement is supported by Boggs and Hanson (1949). They suggested that the trained taster should be used for detecting differences in flavor.

Effect of Smoking on the Cured Flavor of Pork Due to Nitrite

The samples were cured in the pickle in which sodium nitrite was the only variable. The samples were cooked and smoked. The results of the statistical analyses of the data obtained from the taste panel tests are shown in Table 7. As the results in Table 7 indicate, the panelists were able to identify the different sample in the triangle test at a highly significant level, even though both samples were smoked. These results indicated that smoking did not affect the ability of the panelists to identify the different sample in the triangle taste tests.

Table 7. Results of statistical analyses of the data obtained from the triangle and paired taste tests.

Trials	No. of panelists	Triangle#		Paired##	
		taste tests	Significance	taste test	Significance
15	26	17	***	22	***
16	22	14	**	18	**
17	18	13	***	17	***
# No. of panelists who identified the different sample.				**P<0.01	
##No. of panelists who chose the sample containing NaNO ₂ as having more cured flavor.				***P<0.001	

In the paired tests, panelists chose the sample containing sodium nitrite as having more cured flavor at a highly significant level. Thus, smoking may not affect the flavor constituent produced by the action of sodium nitrite on pork.

Weight Change During Curing

Changes in weight during curing were observed. In all cases, there was a weight increase during the curing (Appendix II). The weight gained during the curing varied from 5.00% to 18.80%.

There are many factors that could affect weight changes during curing. One of the most important factors is the physical state of the meat. Lewis (1966) pointed out that meat, in which the structure was watery, had a poorer absorption of salt compared with normal meat. The weight of brine absorbed was about 7% in the former whereas it was 40% in the latter. The temperature of the curing room may also effect the weight change during curing. The rate of diffusion of the curing ingredients can be greatly facilitated by increasing the temperature. However, if the temperature is too high, bacterial spoilage may result (Amer. Meat Inst. Found., 1960). Moulton and Lewis (1940) claimed that bacterial

spoilage increased if the temperature of the curing room was raised above 40°F.

Chemical Analysis

The purpose of the chemical analyses was to compare the concentrations of the curing ingredients within the paired samples. The effect of the difference in the concentration on the judgment of the panelists was determined.

Representative paired samples taken from the center section of the cooked sample were analyzed. Appendix III gives the results of the chemical analyses.

A paired taste test was used to determine whether the panelists could detect a variation of 0.18% salt concentration at 2%, and 0.08% salt concentration at 1% salt levels in the cooked meat. The results obtained indicated that the panelists could not significantly differentiate either of the two levels (Tables 8, 9). Therefore, their flavor evaluation was not based on saltiness.

Table 8. Results of statistical analyses of the data obtained from the paired taste tests (meat containing 2% sodium chloride).

Paired samples	% Sodium Chloride	No. of# panelists	No. of## panelists	No. of### panelists	Significance
1R ^[1]	2.11				
1L ^[2]	2.20	4	7	7	n.s. ^[3]
2R	2.08				
2L	2.26	6	5	7	n.s.
3R	2.09				
3L	2.02	5	9	5	n.s.
4R	1.72				
4L	1.86	4	8	6	n.s.
5R	1.73				
5L	1.89	7	2	9	n.s.

Note: See bottom of Table 9 for code.

Table 9. Results of statistical analyses of the data obtained from the paired taste tests (meat containing 1% sodium chloride).

Paired samples	% Sodium Chloride	No. of# panelists	No. of## panelists	No. of### panelists	Significance
6R ^[1] 6L ^[2]	1.01 1.08	7	2	9	n.s. ^[3]
7R 7L	1.15 1.14	7	4	7	n.s.
8R 8L	0.95 1.08	11	15	10	n.s.
[1] right [2] left [3] not significant # Indicated there was no difference in saltiness ## Chose R sample as being saltier ### Chose L sample as being saltier					

Sugar analyses showed that the highest concentration of sugar in the cured meat was 0.35% (Appendix III). Mills et al. (1960) stated that the threshold level of sugar for detection of sweetness in hams appeared to be 0.5%-0.75%. Since 0.35% of sugar is less than the reported threshold level, apparently the concentration of sugar did not affect the panelist's judgment in the taste tests.

Difference in phenol content between the paired samples ranged from 0.19ppm to 0.26ppm. It is doubtful if the panelists were able to detect such a small difference in phenol content. Results showed that panelists chose the samples containing sodium nitrite and less phenol content as having a more cured flavor than the samples containing no sodium nitrite and a higher phenol content (Table 10). These results seem to suggest that the phenol content probably did not influence the judgment of the taste panel.

Table 10. Results of statistical analyses of the data obtained from the paired taste tests (smoked sample).

Paired samples	Phenols (ppm)	Sodium nitrite (ppm)	No. of panelists	No. of# panelists	Significance
15R ^[1]	3.12	32	26	22	***
15L ^[2]	3.31				
16R	9.11	19	22	18	**
16L	9.34				
17R	6.38	26	18	17	***
17L	6.02				
[1] right		**p<0.01			
[2] left		***p<0.001			
#	Chose the sample containing sodium nitrite as having more cured flavor.				

The cooked samples were also analyzed for moisture, fat and protein to observe the diversity of the biological system (Appendix III). The results obtained from an analysis of variance showed no significant difference in moisture, fat and protein between the right and left samples. These results indicated that the moisture, fat and protein content of the samples did not influence the judgment of the panelists in the triangle and paired taste tests. However, there was a significant difference in moisture, fat and protein between sample pairs.

SUMMARY AND CONCLUSIONS

The experimental work described strongly indicates that sodium nitrite contributes to the cured flavor of pork. Sodium nitrite alone, without the influence of salt and sugar, reacts with constituents of the tissue, either during curing or during cooking to produce a certain flavor. Characterization of the flavor and identification of the flavor constituents produced by the action of sodium nitrite on pork remain to be determined.

Even though both samples, the sample containing sodium nitrite and the sample containing no sodium nitrite, were smoked, panelists were still able to identify the different sample in the triangle taste tests. In the paired taste tests using the samples as above, a statistically significant number of panelists chose the sample containing sodium nitrite as having more cured flavor. Therefore, smoking may not have any effect on the flavor produced by sodium nitrite in pork.

It was observed that some panelists were very accurate and consistent in identifying the different sample in the triangle taste test, whereas some were actually unable to identify the different sample.

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APPENDIX I

TABLE OF SIGNIFICANCE LEVELS OF TASTE-TEST RESULTS

No. of Tasters	TWO SAMPLE TEST			TRIANGLE TEST			No. of Tasters
	No. of Concurring Choices Necessary to Establish Significance			No. of Concurring Choices Necessary to Establish Significance			
	*	**	***	*	**	***	
1	--	--	--	--	--	--	1
2	--	--	--	--	--	--	2
3	--	--	--	3	--	--	3
4	--	--	--	4	--	--	4
5	--	--	--	5	5	--	5
6	6	--	--	5	6	--	6
7	7	--	--	5	6	7	7
8	8	8	--	6	7	8	8
9	8	9	--	6	7	8	9
10	9	10	--	7	8	9	10
11	10	11	11	7	8	10	11
12	10	11	12	8	9	10	12
13	11	12	13	8	9	11	13
14	12	13	14	9	10	11	14
15	12	13	14	9	10	12	15
16	13	14	15	9	11	12	16
17	13	15	16	10	11	13	17
18	14	15	17	10	12	13	18
19	15	16	17	11	13	14	19
20	15	17	18	11	13	14	20
21	16	17	19	12	13	15	21
22	17	18	19	12	14	15	22
23	17	19	20	12	14	11	23
24	18	19	21	13	15	16	24
25	18	20	21	13	15	17	25
26	19	20	22	14	15	17	26
27	20	21	23	14	16	18	27
28	20	22	23	15	16	18	28
29	21	22	24	15	17	19	29
30	21	23	25	15	17	19	30
31	22	24	25	16	18	20	31
32	23	24	27	16	18	20	32
33	23	25	27	17	18	21	33
34	24	25	27	17	19	21	34
35	24	26	28	17	19	22	35
36	25	27	29	18	20	22	36

*Significant (above the 5% level of probability).

**Highly significant (above the 1% level of probability).

***Very highly significant (above the 01.% level of probability).

APPENDIX II

WEIGHT CHANGE DURING CURING

Paired sample	Wt. before curing (lbs.)	Wt. after curing (lbs.)	Yields(%)
1R	1.60	1.80	12.50
1L	1.40	1.60	14.30
2R	1.70	1.90	17.60
2L	1.70	1.90	17.60
3R	1.60	1.80	12.50
3L	2.00	2.20	10.00
4R	1.80	2.00	11.10
4L	1.40	1.60	14.30
5R	0.80	0.95	18.80
5L	1.00	1.18	11.80
6R	1.80	2.00	11.11
6L	2.40	2.60	8.33
7R	1.10	1.25	13.65
7L	1.20	1.40	16.66
8R	1.80	2.00	11.11
8L	1.80	2.00	11.11
9R	1.60	1.70	6.25
9L	1.50	1.60	6.67
10R	1.80	1.90	5.55
10L	1.80	1.90	5.55
11R	1.80	1.90	5.55
11L	1.80	1.90	5.55
12R	1.40	1.50	7.14
12L	1.00	1.05	5.00
13R	1.60	1.80	12.50
13L	1.60	1.80	12.50
14R	1.60	1.80	12.50
14L	1.60	1.80	12.50
15R	2.00	2.20	10.00
15L	1.80	2.00	11.11
16R	1.80	2.00	11.11
16L	1.60	1.80	12.50
17R	1.80	2.00	11.11
17L	2.00	2.20	10.00

APPENDIX III

CONCENTRATIONS OF CURING INGREDIENTS IN COOKED SAMPLES

Paired Sample	% Sodium Chloride	% Sugar	Nitrite (ppm)	Phenol (ppm)	% Moisture	% Protein	% Fat
1R	2.11	0.20			37.75	26.01	7.22
1L	2.20	0.22	37		36.44	25.78	7.65
2R	2.08	0.24	32		37.16	25.78	6.88
2L	2.26	0.19			36.18	25.24	6.73
3R	2.09	0.25			40.75	27.99	6.41
3L	2.02	0.25	41		39.96	28.93	6.87
4R	1.72	0.09	30		50.02	33.80	9.09
4L	1.86	0.09			50.39	34.93	8.80
5R	1.73	0.11			53.12	36.08	8.00
5L	1.89	0.10	30		50.66	38.15	7.71
6R	1.01	0.22			43.21	27.08	7.27
6L	1.08	0.25	39		40.98	31.90	6.96
7R	1.15	0.30	42		51.01	37.95	6.87
7L	1.14	0.28			53.47	39.27	6.97
8R	0.95	0.31	19		53.66	33.02	6.78
8L	1.07	0.26			53.28	33.67	7.11
9R	1.11	0.16	39		49.70	28.54	6.81
9L	2.20	0.19			55.55	29.40	6.34
10R	2.11	0.14			55.95	26.95	6.63
10L	1.00	0.16	35		54.09	28.27	6.47
11R	1.21	0.15	38		52.71	29.01	7.35
11L	2.14	0.14			54.76	30.66	7.17
12R			23		48.57	28.60	7.74
12L					51.00	27.99	7.45
13R					55.01	33.48	7.10
13L			22		52.62	33.80	8.94
14R			22		58.00	29.46	9.17
14L					59.33	30.85	8.40
15R	0.95	0.35	32	3.12	55.54	28.67	8.63
15L	1.00	0.31		3.31	59.30	29.23	7.97
16R	1.13	0.31		9.11	55.09	31.88	9.31
16L	1.18	0.34	19	9.34	56.42	28.65	7.98
17R	1.04	0.21		6.38	54.25	36.38	5.82
17L	1.01	0.29	26	6.02	51.63	36.03	5.86

APPENDIX IV
COMPOSITION OF PICKLES

Paired Samples	% Sodium Chloride	% Sugar	Nitrite (ppm)	Smoked
1R	4.70	1.2	0	
1L	4.70	1.2	300	
2R	4.70	1.2	300	
2L	4.70	1.2	0	
3R	4.70	1.2	0	
3L	4.70	1.2	300	
4R	4.70	1.2	300	
4L	4.70	1.2	0	
5R	4.70	1.2	0	
5L	4.70	1.2	300	
6R	2.35	1.2	0	
6L	2.35	1.2	300	
7R	2.35	1.2	300	
7L	2.35	1.2	0	
8R	2.35	1.2	300	
8L	2.35	1.2	0	
9R	2.35	1.2	300	
9L	4.70	1.2	0	
10R	4.70	1.2	0	
10L	2.35	1.2	300	
11R	2.35	1.2	300	
11L	4.70	1.2	0	
12R			300	
12L			0	
13R			0	
13L			300	
14R			300	
14L			0	
15R	2.35	1.2	300	yes
15L	2.35	1.2	0	yes
16R	2.35	1.2	0	yes
16L	2.35	1.2	300	yes
17R	2.35	1.2	0	yes
17L	2.35	1.2	300	yes

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