I. **Project Title and NPPC ID No.** A Survey of Nitrate and Nitrite in Selected Food in the USA (NPPC Project #98-179)

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II. Abstract:

Twenty six commonly consumed food items were randomly selected from four grocery stores of major chains in three U.S. metropolitan areas (Tampa, Fl; Minneapolis, MN; and Los Angeles, CA) during the spring season of 2,000. Sampled products were shipped with frozen ice packs by overnight express delivery service to Covance Laboratories in Madison, WI, for analysis for nitrate and nitrite by ion exchange chormatography. These samplings and analyses were conducted to provide current information on the nitrate and nitrite content of commonly consumed non-meat products in the American diet, in order to provide perspective on how the nitrate and nitrite content of cured pork and other cured meat/poultry products contribute to overall nitrate and nitrite ingestion by Americans. The results of this study confirm that a wide range of foods contain substantial amounts of nitrate, and low levels of nitrite. Nitrate was detected in all 26 products sampled, with 15 products containing average nitrate concentrations greater that 100 ppm, and four of those product having levels greater than 1000 ppm. There was frequently wide variation in nitrate concentration among different samples of a particular food. Nitrite was found to be present in 19 of the products examined, but all had average concentrations of 12 ppm or lower (8 of the 19 averaged less than 1 ppm). In comparison, a 1997 report by Cassens and co-workers at the University of Wisconsin described the sampling of cured meats (bacon, sliced ham, wieners and bologna) produced by leading U.S. manufacturers for residual nitrate and nitrite content. Those studies found residual nitrite to average between 5 and 10 ppm (range 0 to 48 ppm) across three trials, with no residual nitrate detected in any of the products tested in the one trial which included nitrate analysis (10 ppm nitrate was detection limit of procedure used).

III. Introduction:

Over 50% of pork production is marketed as "cured" products containing the functional food additive, sodium nitrite (contributes to product safety, color and flavor). Although potassium nitrate (saltpeter) was the original historical curing ingredient, today very few cured items are manufactured with added nitrate.

Having complete and current data about dietary sources of nitrite and nitrate in the USA is important because cured meats have come under attack in the past, and were currently challenged in the late 1990s, as dangerous to human health because of their residual nitrite content. The most recent publication about dietary sources of nitrite and nitrate is

by White (1975). Cassens (1997) conducted a recent survey on residual nitrite in processed meats and found an approximate 80% reduction from the values reported in 1975. The contribution of other foodstuffs to human intake of nitrite and nitrate in the USA remains based on the values published nearly a quarter century ago. Other countries, notably Great Britain (1994) and The Netherlands (1990) maintain up-to-date databases, but extending their data to the USA is not acceptable.

This project was designed to determine the current nitrite and nitrate content in foods other than processed meats. Commonly consumed foods were retrieved from four stores in each of three cities (Los Angeles, Minneapolis and Tampa) during Spring, 2000. The foods were shipped to Covance Laboratories in Madison, WI for analysis of nitrite and nitrate using an ion chromatography method. The selected foods to be analyzed were:

Vegetables
1. Beans (green snap)
2. Beets
3. Broccoli
4. Cabbage (green)
5. Carrots
6. Cauliflower
7. Celery
8. Cucumber
9. Iceberg head lettuce
10. Packaged mushrooms
11. Onion (yellow)
12. Pepper (green)
13. Potato (white)
14. Radish (red)
15. Spinach
16. Tomato (red)
17. Prepared salad greens (bagged)

Fruits
18. Apples (red delicious and MacIntosh)
19. Bananas
20. Grapes (red and green)
21. Oranges
22. Strawberries

Dairy
23. Milk (2%, white)

Miscellaneous
24. Beer
25. Dried beans
26. Bread

This project is being jointly sponsored by the National Pork Producers' Council and the American Meat Institute Foundation.

IV. Objectives:

The overall objective of this project is to assess the contribution of processed meats to other selected foods as sources of nitrite and nitrate in the diet in the USA. Information is in hand regarding the current levels of nitrite and nitrate in processed meats (Cassens, 1997). Therefore, the specific objective of this work is to determine current nitrite and nitrate content of other foods commonly consumed in the USA. Foods surveyed represent products which are a significant part of American diet (quantity) and/or are known historically to contain significant amounts of nitrate and nitrite.

V. Procedures:

1. Cities: Samples were collected in three major metropolitan areas: Los Angeles, Minneapolis and Tampa. These areas are geographically dispersed, to reflect different distribution channels, and potentially different brands. Los Angeles and Tampa are near regions of major fruit and vegetable production. They are also situated to be potentially more accessible to imported products. Minneapolis represents a northern climate, in which locally grown products will represent a smaller share of what is offered for sale in stores.

2. Seasons. To determine if season of the year affects the nitrate and nitrite content of foods, the sampling of products were planned to be repeated over four seasons. Information collected to date represents only one season (Spring, 2000).

3. Supermarket Chains. In each of the three metropolitan areas, four stores were sampled. This sampling was done from the leading supermarket chains in the area. The chain or chains selected cumulatively provided for 60% or more of the grocery sales in that area. The number of stores from each chain to sample within an area reflected the market shares of the various chains in that area.

4. Store Selection. The stores sampled from within each qualifying chain were assigned in a random manner. The stores were geographically dispersed within the metropolitan area. Selection of stores was made by consulting chain listings of their stores, or regional phone books, and then using a suitable random procedure for selecting the geographically-dispersed stores to visit.

5. Product Retrieval. The co-investigators picked up samples in the Minneapolis, Los Angeles and Tampa areas.

6. Identification of Selected Products. Each product selected and purchased was placed in a pre-labeled ziplock-type bag (Glad-Lock “Zipper Storage Bags” -- 1 gallon size). At sampling, a log was filled out listing: product name, city, store, date, name of company producing or distributing the product, domestic or import designation (if available), and any other pertinent information which should be recorded for particular products.

7. Product Shipment. Samples were shipped from Los Angeles and Tampa by an overnight express carrier (AirBorne Express). Products from stores were placed in hard-sided picnic coolers, containing three to six pre-frozen "blue-ice" coolants. Shipment was made directly from sampled cities to Covance Laboratories in Madison.

Product picked up by project personnel in Minneapolis was packed in exactly the same manner as described above. However, the coolers were brought back directly by car and delivered to Covance Laboratories.

8. General Store Sampling Procedures:

A. Product was selected from the non-organic sections of the store. If a needed products was not available in the non-organic form, an organic product was selected in its place.

B. When several "types" of the same product were available, the various types or displays were numbered, and a random process was used to select the type to pick up.

C. Most product displayed had so many possible sampling units that it was overly tedious to try to precisely randomly determine which unit to select. The system used was to face the display, and then make a random selection from a prescribed area of the display, according to a defined repeating sequence.

D. All products selected were fresh and wholesome. A product with obvious quality defects would not likely be purchased by a consumer.

E. The product was shipped as purchased, in the zip-lock type resealable plastic bags. Products which were purchased unpackaged from bulk displays were placed directly into such bags. Products which were purchased in packaging (such as cauliflower, celery, some carrots, etc.) retain their original packaging when placed into the zip-lock type bags.

F. Upon arrival at Covance Laboratories, products were placed under refrigeration, and the zip-lock type storage bags were opened to allow free air circulation with the atmosphere. Final trimming and preparation to a final consumable form was done by personnel at Covance Laboratories, according to a defined protocol. Laboratory personnel processed products in an order reflecting their expected perishability, with more perishable products processed first, and more stable processed last. Laboratory personnel ground products under liquid nitrogen and stored them frozen until analyses were performed.

9. Preliminary Test: In advance of the start of actual the project in May of 2000, a preliminary sampling/product analysis was conducted, utilizing a store in the Madison area.

V. Results:

Initially it must be stated that while this sampling and analysis of foods for nitrite and nitrate was to be conducted over four different seasons, only the Spring 2000 sampling has been conducted to date. The reason for this delay is the discovery by Covance Laboratories that results obtained for some products by the prescribed ion exchange chromatography procedure were, in some cases, different than results obtained by previously established AOAC procedures utilizing colormetric techniques. The newer ion chromatography procedure was selected for this study because of its greater speed and lower cost (and presumed greater accuracy) than the older AOAC methods. Initial comparisons by Covance of the two methods on several products gave similar results, suggesting that the two methods were equivalent. However, some check samples run during the course of analyses on the Spring 2000 samples found discrepancies between the two methods on several food products. Since that time Covance Laboratories has been doing internal testing to determine the relationship of these two methods across all foods sampled in this test. When there are two analytical methods which do not agree in certain circumstances, it can be difficult to determine which method is correct. Until this question is resolved, it is of little use to continue to conduct samplings and run analyses, since the results will remain in question until the validity of the results is confirmed.

Likewise, it should be mentioned that much of the recent controvery over the safety of nitrite and nitrate in cured meats has been diminished for the time being. The controversial research reports of the mid-1990s which associated cured meat consumption with childhood cancer gave rise to extensive testing by the National Toxicological Program. Three levels of nitrite were fed to male and female rats and mice over a prolonged period of time. The results of those tests, announced in May, 2000, found no evidence that nitrite consumption increased cancer in test animals. The aforementioned 1990's epidimiological reports also had led the state of California to propose a warning label for packages of nitrite-cured processed meats, under their Proposition 65. However, the results of the National Toxicological Program tests persuaded California in June 2000 to drop their proposal for labeling of cured meats in June, 2000. Therefore, much of the current strong interest in defining dietary sources of nitrite and nitrate has been temporarily diminished. However, just as concerns of nitrite consumption have surfaced in the past, they are likely to again arise in the future. This project has the potential to provide a valuable update of the nitrate and nitrite content of many consumed foods in the U.S., and would be a valuable scientific contribution for any near-future safety considerations. The results of this study could likewise be included in the USDA's Database for Standard Reference, the authoritative reference for the composition of foods consumed by Americans. At this time there is no nitrate/nitrite information in that database, although such information exists in the nutrient databases of some other countries.

Below are mean nitrate and nitrite concentrations found in the 26 food items sampled from four stores in each of the three metopolitan areas, including results of a preliminary test in Madison, during Spring, 2000. Products are listed in order of decreasing nitrate concentration. Bear in mind that the accuracy of these values is currently still being verified by Covance Laboratories.

	Nitrate	**Nitrite**

Food	Mean (ppm)	S.D./(range)* (ppm)	Mean (ppm)	S.D./(range) (ppm)
Beets (11)**	2756	713/(1390-3590)	10	9.3/(2.1-30)
Spinach (13)	2,333	1145/(535-3660)	7.0	4.5/(0-12.9)
Radishes (13)	1680	525/(764-2500)	0.1	0.3/(0-1)
Celery (14)	1543	804/(316-3320)	1.6	1.5/(0-5.2)
Iceberg lettuce (13)	786	194/(347-1080)	0.2	0.6/(0-1.7)
Bag salad (13)	746	309/(289-1210)	2.3	2.8/(0-8.3)
Green beans (13)	386	149/(165-611)	0.5	0.9/(0-2.5)
Broccoli (13)	319	272/(20-595)	2.3	3.9/(0-12)
Potato -- w/peel (7)	205	74/(100-296)	1.1	1.0/(0-2.2)
Cauliflower (13)	182	53/(117-313)	2.7	3.5/(0-13)
Strawberries (13)	173	51/(105-293)	2.0	2.6/(0-7.1)
Bananas (13)	136	38/(91-214)	2.1	2.6/(0-9.5)
Cucumbers (13)	127	85/(27-230)	0.0	----------
Cabbage (132)	122	107/(4.5-222)	0.0	----------
Potato -- w/o peel (7)	118	51/(70-226)	0.3	0.8/(0-2.2)
Mushrooms (13)	59	25/(24-101)	8.0	9.7/(0-38)
Green peppers (13)	33	19/(8-73)	0.4	1.1/(0-2.6)
Dry beans (13)	32	36/(2-93)	0.1	0.3/(0-1.2)
Beer (13)	28	63/(0-41)	0.0	----------
Tomatoes (13)	22	13/(5.7-47)	0.4	0.8/(0-1.6)
On ions (13)	13	8.7/(3.2-29)	0.0	----------
Bread (13)	7.8	7.0/(1.3-21)	12.3	9.4/(5.6-40)
	Nitrate		**Nitrite**	

Food	Mean (ppm)	S.D./(range) (ppm)	Mean (ppm)	S.D./(range) (ppm)
Grapes (13)	5.8	7.8/(0-9.8)	0.0	----------
Oranges (13)	2.6	1.5/(0-4.8)	0.0	----------
Apples -- w/o peel (7)	2.6	6.1/(0-16.4)	0.5	1.4/(0-3.7)
Apples -- w/peel (7)	0.7	1.4/(0-3.6)	0.0	----------

* S.D./(range) = standard deviation/(range of values for individual samples)
** number of samples analyzed

The results of this study to date confirm that a wide range of foods contain substantial amounts of nitrate, and low levels of nitrite. Nitrate was detected in all 26 products sampled, with 15 products containing average nitrate concentrations greater that 100 ppm, and four of those product having levels greater than 1000 ppm. There was frequently wide variation in nitrate concentration among different samples of a particular food. Nitrite was found to be present in 19 of the products examined, but all with average concentrations of 12 ppm or lower (8 of the 19 averaged less than 1 ppm). These results provide a valuable current perspective of food sources contributing nitrate and nitrite to the American diet.

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