Trends in the Levels of Residual Nitrite in Canadian Cured Meat Products over the Past 25 Years

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Nitrite is used widely as a preservative in the processing of cured meat products mainly for its antimicrobial properties against *Clostridium botulinum*. There is concern, however, over nitrite's role in the formation of various *N*-nitroso compounds in such products and for a suggested link between consumption of cured meats and incidence of childhood leukemia and brain tumors. The objective of this study was to obtain current data on residual nitrite levels in Canadian cured meat products that will be useful for health hazard assessment purposes and to determine trends in these levels. The average levels of nitrite (as $NaNO_2$) detected in Canadian cured meat products in various surveys over the past 25 years were as follows: 28 ppm (0−252 ppm; $n = 197$) in 1972; 44 ppm (0−275 ppm; $n = 659$) in 1983−1985; 31 ppm (1−145 ppm; $n = 76$) in 1993−1995; and 28 ppm (4−68 ppm; $n = 35$) in 1996. There appeared to be a noticeable decline in the incidence of samples containing high levels (>100 ppm) of nitrite, but the average residual levels might have decreased only slightly during this period. The latest data are in contrast to those reported in a recent U.S. study that indicated an average of only 10 ppm of residual nitrite ion (equivalent to 15 ppm of $NaNO_2$) in cured meats.

Keywords: *Nitrite; cured meats; residual nitrite levels*

INTRODUCTION

Nitrite, either alone or in combination with the nitrate salt, is widely used for curing meat products in North America, Europe, and many other countries in the world. Although the science and technology of meat curing have been developed relatively recently, the history of meat curing dates back to prehistoric times, an account of which has been reported by Kramlich et al. (1973). Nitrite is mainly used for its antimicrobial properties, especially in preventing the outgrowth of *Clostridium botulinum* spores that may be present in meats (Tompkin, 1983). It also serves a few other important functions such as imparting the typical cured meat color and flavor to the finished product and delaying rancidity development by acting as an antioxidant (Gray and Pearson, 1984). Nitrate, which was originally thought to be the principal antimicrobial agent, is no longer used, except in processing slow-cured products. In these cases, nitrate is gradually reduced to nitrite, the actual antimicrobial agent, by microbial flora in the meat. Thus, it serves as a reservoir of nitrite in these products during the long curing process.

Since nitrite is extremely toxic to man, causing methemoglobinemia and even death at relatively high doses, its usage for the processing of cured meats is strictly controlled by government regulations and monitored by both government and industry. In Canada, the maximum permitted levels of nitrite in most products is 200 ppm (as the Na^+ or K^+ salt) except for bacon, in which it is 150 ppm (Anonymous, 1984). In addition to 200 ppm of nitrite, the same level of nitrate is also permitted in processing of slow-cured products such as dry and semidry sausages. All of these levels relate to the amount added to the initial weight of the meat mixture before smoking, cooking, or fermentation. The nitrite ion at pH <7 is a very reactive species capable of interaction with various constituents of the meat (e.g., amines and amino acids, sulfhydryl and phenolic compounds, myoglobin, ascorbate, or erythorbate) (Fox and Nicholas, 1974; Walters and West, 1983; Toth, 1983). Therefore, residual levels of nitrite in the processed cured meats are much lower than those of the ingoing nitrite. The extent of nitrite loss, however, depends on various factors such as processing conditions, pH of the meat, and storage time and temperature. It has been reported that only 10−20% of the added nitrite can be detected analytically in cured meats immediately after processing, and the level gradually decreases with storage (Hill et al., 1973; Cassens, 1997). This poses a problem in detecting and prosecuting violators who inadvertently may have used >200 ppm levels of nitrite for such purposes. According to the present regulations in Canada, a relatively high residual nitrite level (say, 150 ppm) in a finished product would still be permissible, even though it would clearly indicate the addition of >200 ppm of nitrite in the product.

Besides the acute and chronic toxicity of nitrite itself as mentioned above, the practice of adding nitrite for curing meat products has come under severe criticism during the past 30 years for its role in the formation of various *N*-nitroso compounds, many of which are potent carcinogens (Preussmann and Stewart, 1984). Research over the past two decades has shown that traces to fairly high levels of approximately 18 *N*-nitroso compounds can form in meat products that have been preserved with nitrite (Sen, 1990). In addition, residual nitrite in the cured meat, after ingestion, can form traces of certain *N*-nitroso compounds in the acidic environment of the human stomach after reaction with secondary and tertiary amines that might also be present in the ingested food (O'Neil et al., 1984; Mirvish, 1995). Furthermore, several recent epidemiological studies have suggested that consumption of cured meats by

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pregnant mothers might be associated with a higher than normal incidence of childhood leukemia and pediatric brain tumors (Preston-Martin et al., 1982; Peters et al., 1994; Sarasua and Savitz, 1994). Although such a link has not been well established and the findings are inconsistent, the matter is of concern because *N*-nitrosoureas, which might be formed in the stomach due to in vivo nitrosation following consumption of cured meats, have been shown to induce specifically such cancers in experimental animals (Preussmann and Stewart, 1984). Therefore, it would be prudent to reduce the levels of both added and residual nitrite in cured meats to as low as technically feasible without reducing the protection nitrite offers against botulism and other harmful bacteria.

Although considerable amounts of Canadian and U.S. data on residual nitrite levels in cured meat products produced in the early 1970s have been reported in the literature (National Academy of Sciences, 1981), recent data are very limited. Cassens (1997), of the United States, reported a marked decline (~80%) in residual nitrite levels in U.S. cured meat products from those observed 20−25 years earlier. An average of only 10 ppm of residual nitrite (as NO_2^-) was detected in such samples in that study. The decline was attributed to various changes (e.g., increased use of ascorbate or erythorbate and addition of lower levels of nitrite) introduced over these years by the meat industry for processing such products in the United States. In the Canadian Health Protection Branch (HPB), we have also been monitoring nitrite levels in Canadian cured meat products over these years, but our results are somewhat different from those reported by Cassens (1997). In this paper, we present our recent and past (unpublished) findings and discuss trends in such data over the past 25 years.

MATERIALS AND METHODS

Meat Samples. In most of the surveys reported here, various cured meats were procured by the HPB inspectors directly from different meat-packing plants located throughout Canada. Following collection, the samples were stored at 4 °C and then transported by air cargo in dry ice packs to the various HPB regional laboratories for analysis. The only exception was in the most recent survey in which all of the samples (all domestic products) were purchased locally in the Ottawa area and analyzed in the authors' laboratory. Prior to analysis, each sample was cut into small pieces and mixed by hand, and a 200 g aliquot was homogenized thoroughly using a blender. The homogenized mixture was stored in a screw-cap glass container at 4 °C until analysis, which was carried out within 1−2 weeks. In the most recent 1996 survey, all samples were analyzed within 5 days of procurement.

Determination of Nitrite. All samples procured after 1977 were analyzed for nitrite using a Griess colorimetric method as described previously (Sen and Donaldson, 1978). It is based on diazotization of sulfanilic acid by nitrite, followed by coupling with *N*-(1-naphthyl)ethylenediamine to form an azo dye, which is measured spectrophotometrically at 550 nm. The method has been tested thoroughly in this laboratory and by others and found to give reliable and accurate results (Ohshima et al., 1987; Sen et al., 1979; Saul et al., 1981). In the earlier surveys, the method of Kamm et al. (1965) was used for the determination of nitrite. In addition, all of the samples in the 1996 survey were also analyzed by an HPLC−chemiluminescence detection method that is based on postcolumn chemical denitrosation of nitrite to nitric oxide, followed by detection of the liberated NO by thermal energy analyzer (TEA) (Sen et al., 1994).

Table 1. Summary of Residual Nitrite Levels in Canadian Cured Meat Products As Determined by the Canadian Health Protection Branch during the Period 1972−1996

		$NaNO_2$(ppm)	
survey period	*n*	mean	range
1972[a]	197	28	N[b]−252
1983−1985	659	43.6	N−275
1993−1995	76	30.8	1−145
1996	35	28	4−68

[a] Panalaks et al. (1973). [b] N, none detected (<1 ppm).

Table 2. Residual Nitrite Levels in Various Cured Meat Products As Observed in the 1983−1985 Cross-Canada Survey

		$NaNO_2$ (ppm)	
product analyzed	*n*	mean	range
bacon (1983−1985)	52	33.7	N[a]−178
hot dog/frankfurter	109	60.9	1−178
sausages	102	33.8	1−132
hams	88	48.9	4−146
corned beef	59	31.5	1−192
meat loaf	12	18.5	2−66
bologna	39	65.5	N−137
picnic hams (shoulders)	18	39.2	4−122
sliced cooked meats	42	31.4	6−120
uncooked cured meats (pastrami, smoked beef, spiced beef)	5	72.9	11−275
miscellaneous	20	38.8	N−168
salami and European-type sausages	90	37.6	3−174
pepperoni	23	62.5	10−206
total	659	43.6	N−275

[a] N, none detected (<1 ppm).

RESULTS AND DISCUSSION

A summary of the data on residual nitrite levels in cured meat products analyzed by the Canadian Health Protection Branch since 1972 is been presented in Table 1. Of these, the data reported by Panalaks et al. (1973) have already been published and are reported here for comparison. The data generated since 1983 have not been published previously, and, therefore, are discussed below in detail.

In the 1983−1985 survey (Table 2), a total of 659 samples of various cured meats were analyzed. The overall mean of residual nitrite (expressed as the Na salt) was found to be 43.6 ppm (range = <1−275 ppm). Of these, hot dogs/frankfurters, bolognas, pepperonis, and uncooked cured meats contained higher than average mean residual nitrite levels. Over 10% of the samples (70 of 659) in various categories contained >100 ppm of residual nitrite, suggesting that >200 ppm of nitrite—the maximum permissible level permitted under the Canadian Food and Drug Regulation—was added to these products. Hill et al. (1973) have shown that in frankfurters and sausages only 10−25% of the added nitrite can be detected in the final product after processing and storage for a week at 2−5 °C.

In the 1993−1995 survey (Table 3), only 76 samples were analyzed. The mean residual nitrite level and the range were 30.8 and 1−145 ppm, respectively. Of the different meat products, hot dogs/frankfurters contained the highest average levels of residual nitrite (mean = 65.5 ppm). Since the number of samples analyzed in this category was only four, no conclusion can be drawn and additional confirmatory studies are needed. Only 2 of 76 samples in the 1993−1995 survey exceeded 100 ppm of residual nitrite as opposed to 70 of 659 samples in the 1983−1985 survey. This suggested that the

Table 3. Levels of Nitrite in Various Cured Meats As Detected in the 1993−1995 Cross-Canada Survey

meat product	*n*	$NaNO_2$ (ppm) mean	$NaNO_2$ (ppm) range
hot dog/frankfurter	4	65.5	23−112
ham	7	28.6	1−61
sausages and salami	31	30.2	4−145
bacon	11	33.8	7−81
pepperoni	8	35	10−58
miscellaneous (corned beef, pastrami, bologna, marinated cubes, head cheese, smoked turkey)	15	18.6	8−36
total	76	30.8	1−145

Table 4. Comparison of Residual Nitrite Levels in Cured Meat Products As Determined by Two Different Methods in the 1996 Survey Carried out in the Ottawa Area

		$NaNO_2$ (ppm) colorimetric		$NaNO_2$ (ppm) chemi-luminescence	
product analyzed	*n*	mean	range	mean	range
hot dog/frankfurter	8	24	10−63	26	10−67
bacon	6	34	4−68	36	5−68
bologna	2	33	31−35	35	28−42
pepperoni	3	31	10−53	31	9−57
smoked ham	4	24	9−40	24	10−44
miscellaneous	4	31	19−43	33	18−50
sausages and salami	8	26	9−37	29	13−43
total	35	28	4−68	30	5−68

industry was exercising a better control of nitrite addition to such products.

A total of 35 samples were analyzed in the 1996 survey (Table 4). The mean and range of residual nitrite levels detected in these samples were 28 and 5−68 ppm, respectively. The average levels of residual nitrite in the different categories seemed to be comparable (24−34 ppm), and none contained >70 ppm. In contrast, Cassens (1997), in the United States, analyzed a total of 164 samples of various cured meat products. The overall mean residual nitrite levels (as nitrite ion) detected was about 10 ppm (equivalent to 15 ppm of $NaNO_2$), which is approximately half of that found in the latest Canadian survey (Table 4). If one combines the number of samples in the last two Canadian surveys (Tables 3 and 4), the total number (111) is closer to that analyzed by Cassens (1997). Even then, the picture does not change much because the mean residual nitrite levels remains about the same.

As mentioned earlier, the cured meat samples in the 1996 survey were analyzed according to two different methods. The main purpose was to verify the results obtained by the Griess colorimetric method, which is and has been widely used by many labratories. The chemiluminescence (TEA) method is based on a completely different principle from the other method and is extremely sensitive (to 5 μg/kg) and highly specific. An analysis of variance was performed on the two sets of data (Table 4). An ANOVA model, with a main effects term for type of meat product, was used to analyze the differences between the chemiluminescence (TEA) and colorimetric results for each sample (individual results not shown in Table 4). There was no effect of meat product on the differences ($p = 0.93$), but there was evidence that the differences were different from zero ($p = 0.036$), i.e., that the results for one method are biased with respect to the results from the other method. A plot of the TEA results versus the colorimetric results for each sample is shown in Figure 1. The line that

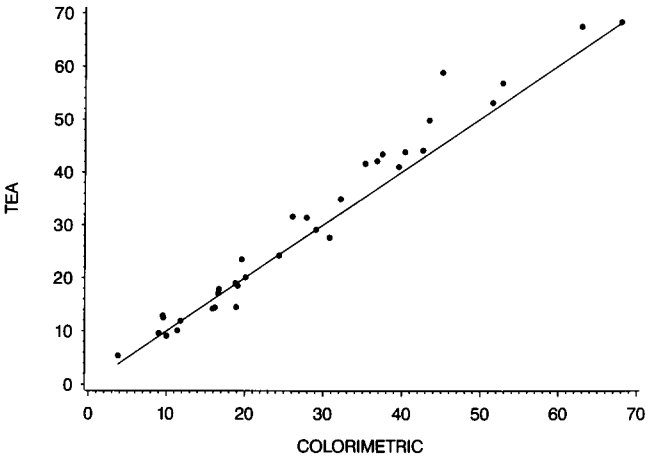

Figure 1. Plot of TEA vs colorimetric results; values represent levels (parts per million) of $NaNO_2$ detected.

Table 5. Trends in the Residual Nitrite Levels in Raw Bacon in Canada during the Period 1972−1996

year	*n*	$NaNO_2$ levels mean	$NaNO_2$ levels range
1972[a]	20	38	4−252
1973[b]	12	41	12−94
1975[c]	14	52	10−101
1976[d]	12	62	2−131
1983−1985	52	34	N[e]−178
1993−1995	11	34	7−81
1996	6	34	4−68

[a] Panalaks et al. (1973). [b] Sen et al. (1974). [c] Sen et al. (1976). [d] Sen et al. (1977). The rest of the data are from this paper. [e] N, none detected (<1 ppm).

represents equality is also on the graph. The TEA results are consistently greater than the colorimetric results when values are >35 ppm, which explains why a statistically significant outcome was obtained.

Below 35 ppm, however, the two sets of results agree very well with each other. The exact reason for this discrepancy at higher concentrations is not clear, but it is possible that the high dilutions needed to bring the TEA readings on scale may have affected somewhat the chemistry of the system. Since the TEA method is ~200-fold more sensitive than the colorimetric one, the meat extracts had to be diluted 100−1000 times more, especially for the high-nitrite-containing samples, prior to HPLC−TEA determination. Further research might be advisable to determine if this or other factors are responsible for the observed differences. Nevertheless, these results at least indicate that the colorimetric method commonly used in our laboratories does not yield false-high results.

Of various cured meat products, bacon probably has been most thoroughly investigated for both nitrite and nitrosamine contents (Fazio et al., 1973; Hotchkiss 1987, Pensabene et al., 1974). This has been done because of concern due to the formation of consistently high levels of nitrosopyrrolidine during frying of bacon and due to nitrite's role in such formation (Gray, 1977; Sen et al., 1974). As a result, more complete data on residual nitrite levels are available for bacon, and some of them have been summarized in Table 5. In this context, it should be mentioned that in April 1975, Health and Welfare Canada reduced the permissible level of nitrite in bacon from 200 to 150 ppm (Anonymous, 1975). Although this change occurred between the 1975 and 1976 studies (Table 5), and the samples in the 1976 study were obtained 6 months after promulgation of the

above law, no statistically significant difference in residual nitrite levels was observed between the two sets of data (Sen et al., 1977). Perhaps the industry took a longer time to comply with the regulation. There appeared to be a slight drop in the mean nitrite levels in bacon analyzed in 1983−1985, and this has remained unchanged since then (Table 5). In contrast, Cassens (1997) reported the presence of only an average of 4.7 ppm of nitrite ion (equivalent to 7 ppm of $NaNO_2$) in raw bacon ($n = 20$), which is much lower than levels observed in our latest two surveys.

It might be mentioned in this context that Canada also allows the addition of ascorbate or erythorbate in cured meat products, but there are no limits on the maximum level of their use (Anonymous, 1982). They are allowed to be used on the basis of "good manufacturing practice". Nor is it mandatory to add them to any specific type of product (e.g., for bacon). Very little information on the input or residual levels of ascorbate in Canadian cured meats is, however, available in the literature. In any event, this would not have had any effect on our nitrite results because the analytical method used in our studies to measure residual nitrite levels is not affected by ascorbate (up to a level of 2000 ppm) (Sen and McPherson, 1978). The method contains a built-in feature that oxidizes residual ascorbate before it can destroy any residual nitrite during the analytical workup.

At present, we do not have a definite explanation for the lower residual nitrite levels in U.S. cured meats as reported by Cassens (1997). There might be a variety of reasons such as increased use of ascorbate in the United States compared with Canada, longer storage prior to analysis, differences in analytical methodology, and differences in processing conditions. Cassens (1997) reported that some of the samples analyzed were found to contain high levels (up to 483 ppm) of ascorbate. Since ascorbate is known to react with nitrite at a wide pH range (Mirvish et al., 1972), it is possible that it could have destroyed the bulk of the nitrite during processing as well as storage. It has also been demonstrated that high residual ascorbate levels result in false-low measurements of nitrite in cured meats when analyzed according to the official AOAC method (Sen and McPherson, 1978; Sen et al., 1979). Additional information is needed before any definite conclusion as to the reason for the differences in results observed between the U.S. and Canadian studies can be reached.

The incidence of high (say >100 ppm) nitrite-containing samples in the 1983−1985 survey, which was 70 of 659, was somewhat disturbing because in spite of the concern about nitrite's role in the formation of nitrosamines in such products during the 1970s and the lowering of the permissible level of nitrite in bacon in 1975, the industry's control over nitrite input during the processing of cured meats was less than ideal. Although such incidences of high-nitrite samples in the 1993−1996 survey's were much lower, it is difficult to say whether this is apparent or real because only a total of 111 samples were analyzed during this period. It will be highly desirable to carry out a more extensive survey, and if such high levels of nitrite are found in some products, then additional investigations will be warranted to determine the reason behind it.

In summary, it appears that the overall mean residual nitrite levels in Canadian cured meat products have decreased only slightly over the past 20−25 years. The incidence of samples containing high levels of nitrite (say >100 ppm), however, has decreased sharply during this time. Additional surveys are recommended to confirm if this is true. Also, if it is found that lower residual nitrite levels in U.S. cured meat products have resulted from the increased use of ascorbates/erythorbates, mandatory inputs of 500 ppm levels of these antioxidants to cured meat products might be considered in the future.

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