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Analytical Methods

Nitrate and nitrite quantification from cured meat and vegetables and their estimated dietary intake in Australians

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ABSTRACT

High dietary nitrate and nitrite intake may increase the risk of gastro-intestinal cancers due to the *in vivo* formation of carcinogenic chemicals known as *N*-nitroso compounds. Water and leafy vegetables are natural sources of dietary nitrate, whereas cured meats are the major sources of dietary nitrite. This paper describes a simple and fast analytical method for determining nitrate and nitrite contents in vegetables and meat, using reversed-phase HPLC-UV. The linearity R^2 value was >0.998 for the anions. The limits of quantification for nitrite and nitrate were 5.0 and 2.5 mg/kg, respectively. This method is applicable for both leafy vegetable and meat samples. A range of vegetables was tested, which contained <23 mg/kg nitrite, but as much as 5000 mg/kg of nitrate. In cured and fresh meat samples, nitrate content ranged from 3.7 to 139.5 mg/kg, and nitrite content ranged from 3.7 to 86.7 mg/kg. These were below the regulatory limits set by food standards Australia and New Zealand (FSANZ). Based on the average consumption of these vegetables and cured meat in Australia, the estimated dietary intake for nitrate and nitrite for Australians were 267 and 5.3 mg/adult/day, respectively.

1. Introduction

It was estimated that 80% of human cancers were caused by environmental factors associated with food, water and air (Walters, 1980). In addition, malnutrition, dietary habits and lifestyle may be directly or indirectly related to 40% of the human cancers (Ologhobo, Adegede, & Maduagiwu, 1996). High dietary intakes of nitrate and nitrite have been implicated in the etiology of human gastric cancer based on epidemiology and clinical studies (Bartsch, Ohshima, Shuker, Pignatelli, & Calmels, 1990; Joossens et al., 1996).

Nitrate is naturally present in leafy vegetables and nitrite is usually added to meat as a preservative in the form of sodium or potassium salt (Cammack et al., 1999). In addition nitrate can be reduced to nitrite in the oral cavity and in the stomach (Duncan et al., 1997). Once in the stomach, nitrite can react with amines and amides, which are organics containing nitrogen such as amino acids, to form a group of carcinogens known as *N*-nitroso compounds (Archer, 1989). Stomach is most at risk from endogenous *N*-nitroso compound synthesis since stomach acid catalyses nitrosation reactions. High nitrate intake was associated with gastric cancer in England, Colombia, Chile, Japan, Denmark, Hungary and Italy (Forman & Shuker, 1997). Exposure to endogenously formed *N*-nitroso compounds had been associated with increased risks of cancer of the stomach, oesophagus and bladder (Bartsch et al., 1990).

Australia's food composition data were mostly based on overseas data especially those from the United Kingdom (UK) and the United States (US) till recently. However, in the revised Australian composition tables based on food analysis performed in Australia, the edible portion of fruit increased by 4% whereas in meat it decreased by 16% (Cashel & Greenfield, 1995). Thus dietary contribution of nitrate and nitrite may be over-estimated, whereas dietary intake of antioxidants such as vitamin C and vitamin E may have been underestimated.

The dietary intake of nitrates and nitrites in foods can vary greatly from region to region depending on factors such as farming practices, climate, soil quality, manufacturing processes and legislation. Nitrate and nitrite contents of foods are not available in Australia; hence values from overseas are commonly used. Due to the growing concern of *N*-nitroso compounds, accurate and robust methods are necessary for long-term monitoring of nitrate and nitrite concentrations in foods for susceptible populations.

It is therefore the aim of this study was to develop an accurate, simple and cost-effective method for quantifying the nitrate and nitrite contents in commonly consumed vegetables, cured meat and fresh meat produced in Australia.

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2. Materials and methods

2.1. Reagents

Analytical grade sodium nitrite and potassium nitrate from Univar (Ajax Finechem) were used as standards and for recovery studies. HPLC grade methanol from lab-scan was used and ion-pairing agent tetrabutylammonium phosphate was purchased from Waters.

2.2. Food samples

All vegetables were purchased at local supermarkets, produce shops or wholesale and kept at refrigeration temperature and analysed within 24 h. All cured and fresh meat products were also purchased at supermarkets and kept at refrigeration temperature and analysed within 48 h.

2.3. Apparatus

Waters HPLC controller model number 600 with photo array detector model number 996 and autosampler model number 717 plus were used. Phenomenex C_{18} 110A Gemini column (250 mm × 4.6 mm × 5 μm) was used for the separation. Injection volume was 10 μl with flow rate set at 1 mL/min and wavelength set at 214 nm. Mobile phase consisted of methanol: water (75:25) with 0.075 M of tetrabutylammonium phosphate (PIC-A).

2.4. Methods

2.4.1. Standards

Potassium nitrate (KNO_3) and sodium nitrite ($NaNO_2$) were mixed in MilliQ water in volumetric flasks to give a range between 5.0 and 100 mg/L for nitrite ions and 2.5–50 mg/L for nitrate ions.

2.4.2. Samples

Weighed 10–50 g of meat samples including salami, hot dogs, ham, bacon, Frankfurt and beef, which were purchased from the local supermarkets (at least two packets each) of two different brands, were blended with 300 mL distilled water for 1 min, then made up to 500 mL in volumetric flasks. The pH was measured and 1 mL was taken out for measuring nitrate and nitrite content before cooking using HPLC. Ten millilitre of mixture of each sample was transferred into 100 mL volumetric flasks and heated in a water bath at 75, 80, 90 and 100 °C for 5, 10 and 15 min. The mixture was made up to 100 mL with distilled water and was shaken. The mixture was allowed to settle and cool; then measured pH and nitrite and nitrate contents. The pH was adjusted with 0.1 M NaOH to neutral pH. Then the mixture was centrifuged at 10,000 rpm for 10 min; then supernatant was removed for ultra-filtration. The filtrate was used for further analysis including quality control such as recovery studies.

Fresh vegetables including English spinach, *buk choy*, *choy sum*, Chinese cabbage, *gai choy* and *iceberg* lettuce were purchased from the local supermarkets and produce stores. Three bunches each from two different locations with at least three replicates were used for the analysis including recoveries. To examine the effects of sample preparation and extraction conditions on nitrate and nitrite determination samples were chopped in thirds or blended or both and weighed between 25 and 100 g in 500 mL beakers. Spiking with standards was done before cooking in water bath between 60 and 100 °C for 5–30 min.

3. Results and discussion

Nitrate and nitrite can be unstable and different sampling methods and extraction procedures can influence their recoveries (Usher & Telling, 1975). Hence, the optimal extraction conditions were used for nitrate and nitrite determinations in fresh vegetables, cured meat and fresh meat. Mean recoveries were >92% for both nitrate and nitrite in all three food matrices tested (Tables 1 and 2). Factors affecting nitrate and nitrite recovery in foods include (1) temperature, since nitrate and nitrite are not stable at high temperatures, (2) cooking conditions, which can affect pH of the sample water and exposure to atmospheric oxygen, (3) pH of the sample water, since nitrite is readily converted to nitric acid or nitric oxide at acidic pH, and (4) sources of food samples can vary greatly and may contain interfering substances such as iron and magnesium (Usher & Telling, 1975).

The extraction and detection method would affect nitrite and nitrate quantification in meat and vegetables. This method was chosen because it was fast, sensitive and accurate. In both cases heat (hot water) was used to extract nitrite and nitrate, and in the case of vegetables blanching was a common cooking practice that was chosen in the present study. In addition, pH was monitored and maintained close to neutral pH to minimize conversion of nitrite to nitrous acid or nitrous oxide.

Nitrite levels in vegetables may increase during post-harvest storage by the action of indigenous bacteria and/or the presence of nitrate reductase (Hunt, 1994), especially when they are left at room temperature or higher. This may explain the small amount of nitrite (20 mg/kg) present in *Gai choy* during the preparation at room temperature (Table 1). Likewise, it was demonstrated that there was no detectable nitrite in 94% of edible fresh retail vegetables (Hunt & Turner, 1994).

Table 1
Mean nitrate and nitrite contents and their recoveries in cooked fresh vegetables.

Vegetables	Nitrite (mg/kg)	Nitrate (mg/kg)
English spinach	0	4849.6 ± 573.6
Recovery (%)	89	74
Buk choy	0	1841.1 ± 84.0
Recovery (%)	97	97
Choy sum	0	1376.9 ± 56.0
Recovery (%)	111	102
Chinese cabbage	0	236.2 ± 27.4
Recovery (%)	91	97
Gai choy	19.6 ± 10.8	1642.3 ± 126.0
Recovery (%)	102	100
Iceberg lettuce	0	48.0 ± 30.2
Recovery (%)	92	110

Values are means of at least four replicate determinations from two sources and up to 15 determinations.

Table 2
Mean raw nitrate and nitrite contents and their recoveries in cured and fresh meat from Sydney supermarkets after pH adjustment.

Meat	Nitrite (mg/kg)	Nitrate (mg/kg)
Hot dog	78.6 ± 16.4	69.9 ± 11.3
Recovery (%)	109	103
Ham	34.2 ± 5.5	19.0 ± 8.1
Recovery (%)	97	87
Salami	0	142.5 ± 36.3
Recovery (%)	91	102
Bacon	15.7 ± 14.5	23.3 ± 8.2
Recovery (%)	91	82
Frankfurt	83.9 ± 10.1	54.9 ± 8.7
Recovery (%)	96	94
Minced beef	0	18.7 ± 6.2
Recovery (%)	80	104
Beef medallion	0	38.5 ± 14.9
Recovery (%)	80	75

Values are means of at least four replicates from two to four brands with up to five determinations.

Cultivar and harvest date can affect the nitrate and nitrite levels of selected vegetables (Amr & Hadidi, 2001). This may explain the high variability between findings presented in this study also contributing to high standard deviation particularly in English spinach as observed in Table 1. However, meat samples with low levels of nitrates had smaller standard deviation (Table 2), most likely because the low levels of nitrates in general meant less chance of reacting to the conditions they were exposed to.

English spinach had the highest nitrate content (4850 mg/kg) compared to other vegetables (Table 1). This finding correlated well with the literature (Öztekin, Nutku, & Erim, 2002). However, according to Gaiser, Rathjen, and Spiess (1996), spinach blanched for 3 min can contain in the range of 50–5600 mg/kg nitrates with a mean nitrate concentration of approximately 2000 mg/kg and a large standard deviation of 1411.4 mg/kg. This demonstrated the high variability of nitrate content in spinach and other green leafy vegetables. Excluding spinach, other vegetables tested had nitrate ranging 48–1841 mg/kg, which was less than half that of spinach (Table 1). Thus it can be concluded that spinach contributed to the highest dietary nitrate intake from leafy green vegetables. Lettuce contained lowest amount of nitrate in this study (48.0 mg/kg, Table 1), which was significantly lower to earlier studies that demonstrated high nitrate content in lettuce at 2500 mg/kg (Marshall & Trenerry, 1996). This dissimilarity may be due to horticultural practices such as the use of nitrate-based fertilizers.

Different countries have set their maximum limits for the addition of nitrate and/or nitrite salts in cured meat. Under the Australian Food Standard Code 1.3.1 schedule 1, 125 mg/kg of nitrite in a form of potassium or sodium salt is permitted in cured, dried, and slow dried cured meat; whereas in commercially sterile and canned cured meat, the maximum nitrite (potassium or sodium salts) permitted is 50 mg/kg. For slow dried cured meat, the maximum allowed nitrate (potassium or sodium salts) is 500 mg/kg (FSANZ, 2007–2008). Given the established antimicrobial effect of nitrite salts, particularly in reference to *Clostridium botulinum* in cured meat, its level should remain sufficient enough to prevent the occurrence of foodborne illnesses, but also kept to the minimum to minimize dietary nitrite intake in light of its potential adverse health effects based on epidemiological and clinical studies.

Seven types of meat tested in this study had at least four replicates each from at least two brands. Nitrate and nitrite contents in various cured meat products were below the maximum allowable limit set by Food Standards Australia and New Zealand (FSANZ) at 125 mg/kg (Table 2). However, there was no limit set for fresh meat. Continuous monitoring of nitrite used in cured meat products is important to ensure that the dietary intake of nitrite is kept to below the limit set by FSANZ.

Interferences naturally present or added additives in cured meat products may account for differences in nitrate and nitrite recovery. For example, Butt, Riaz, and Iqbal (2001) demonstrated that the presence of 50-fold sulphate and chloride did not affect the resolution and percent recovery of nitrite, but did reduce the resolution and recovery of nitrate. In addition, the presence of magnesium, iron and calcium significantly reduced the percentage recovery of both anions, which should be removed to ensure accurate determination of nitrate and nitrite. Furthermore, Butt et al. (2001) also demonstrated that under optimized HPLC conditions, both nitrate and nitrite peaks began to merge when the concentration of nitrite was above six-fold of nitrate concentration, hence nitrite used in calibration curve and for recovery were half the concentration of nitrate to minimize the merging of nitrite and nitrate peaks.

Using similar detection method as Reinik et al. (2005), they found the mean sodium nitrite and nitrate concentrations in ham were 20.8 and 68 mg/kg, respectively. However in this study, the nitrite concentration in ham averaged at 34.2 ± 5.6 mg/kg and nitrate concentration was lower at 19.0 mg/kg (Table 2). Some manufacturers add less nitrite but more nitrate as a nitrite reserve. This may also explain the differences in the findings by Öztekin et al. (2002), where the nitrite and nitrate contents in ham were 4.0 and 35.6 mg/kg, respectively.

Dionex Corporation (1998) found the nitrite and nitrate contents in ham to be 11.6 and 5.4 mg/kg, respectively, whereas salami contained 108.0 mg/kg nitrite and 98.5 mg/kg nitrate. Using capillary electrophoresis, the nitrite and nitrate content in salami detected were 24.3 and 43.6 mg/kg, respectively (Öztekin et al., 2002). Compared to their findings, the current study showed that the salami contained no nitrite but much more nitrate at 142.5 mg/kg (Table 2). Although the extraction methods were similar the temperature used in our study was higher, apart from the differences that may be attributed to the manufacturing practices. Stalikas, Konidari, and Nanos (2003) used similar extraction temperature and reported that nitrate and nitrite contents in salami were 54 and 84 mg/kg, respectively. Thus differences are more likely to be due to the manufacturing processes.

It was reported by Dennis, Key, Papworth, Pointer, and Massey (1990) that the mean nitrite content in bacon was 24.0 mg/kg and for nitrate was 43.0 mg/kg, whereas nitrite and nitrate in ham were 56.0 and 22.0 mg/kg, respectively. They used similar extraction and detection methods but with an anion exchange column. Both bacon and ham products in this study contained less nitrate and nitrite (Table 2) in comparison. Siu and Henshall (1998) who found that nitrite and nitrate contents in salami were 108.0 and 98.5 mg/kg, respectively, and 11.6 and 5.4 mg/kg for ham, respectively. Sample extraction procedures used in the current study were similar to Marshall and Trenerry (1996), but they omitted the heating step. This may explain the low nitrite content of less than 10 mg/kg in salami, leg ham and bacon. However the nitrate contents were higher at 141.5, 132.5 and 48.0 mg/kg, respectively. Different cured meat products may require different ratio of nitrite and nitrate as preservatives. Since fresh meat does not naturally contain nitrite (Table 2), its nitrite and nitrate contents have not been extensively tested. However, based on this study, the nitrate content in minced beef and medallion beef were within the range found in cured meat products (Table 2).

It was demonstrated that recovery increases as the meat solids decreases (Usher & Telling, 1975). Hence using smaller meat samples should reduce the effects of interfering substances, which was demonstrated in this study (Table 2). Furthermore, most interference can be eliminated by UV detection. However, chloride ions maybe detected by UV as positive or negative peaks in the wavelength used for nitrate and nitrite and are eluted before nitrite (Di Matteo & Esposito, 1997). Chloride peaks were not present at 214 nm in this study, which suggests that chloride ions did not interfere with nitrite quantification since nitrite recovery was above 92% for both meat and vegetable samples (Tables 1 and 2).

Due to its reactive nature, nitrite analysis from food does not give a true representation of the total nitrite added. Furthermore, nitrite added to meat is usually present as nitric oxide bound with other food components such as myoglobin (5–15%), sulphydryl groups (5–15%), lipids (1–5%), proteins (20–30%), as nitrate (<10%), and as free nitrite (10–15%) (Zanardi, Dazzi, Madarena, & Chizzolini, 2002). Therefore recovery range may be quite large as a result of nitrite's reactive nature and its attachment to other food components. However, because only free nitrite can participate in nitrosation, other methods of food extraction estimate the total nitrite present by releasing food-bound nitrite. This may over estimate the significance of dietary nitrite and the etiology of gastric cancer. Hot water extraction to quantify free nitrite available to participate in nitrosation was used in this study.

Regarding relevance to incidence of gastric cancer, based on age-standardized statistics, diagnosed gastric cancer rate per 100,

000 in males and females worldwide is 22% and 10.3%, respectively, with mortality rate of 14.3% and 8.3%, respectively. Gastric cancer is the third leading cause of death in men after lung and prostate cancer, and is the fourth leading cause of death in women worldwide (Forman & Burley, 2006). Overall gastric cancer rate is declining, especial in more developed countries, with the exception of Miyagi prefecture of Japan still having the highest gastric cancer rate. Korea, East Asia, South America and Eastern Europe also sustained a high gastric cancer rate. However, Bombay in India always maintained a low gastric cancer rate between 1953 and 1997 (Forman & Burley, 2006). This may be due to higher consumption of antioxidant rich fruits and vegetables and herbs and spices, which have been shown to reduce the risk of gastric cancer. Joossens et al. (1996) studied dietary salt, nitrate and gastric cancer mortality in 24 countries and demonstrated that nitrate intake became an increased risk factor for gastric cancer when salt intake was also high.

It was predicted that with increasing population numbers and increasing longevity, it would cause a net increase in gastric cancer rate worldwide. Since diagnosis often occurs between the ages of 60 and 80, with up to 30% mortality rate after five years diagnosis (Forman & Burley, 2006), it is vital to make dietary and lifestyle changes to decrease gastric cancer rate and to increase survival rate with better diagnostic facility and education. Risk factors to be avoided include *Helicobacter pylori* infection, smoking, high consumption of cured meat and salt, and low consumption of fruits and vegetables.

Once the nitrate and nitrite contents in food were established, one can estimate the intake of these anions based on national dietary surveys. Gangolli et al. (1994) estimated that the mean daily intake of nitrate and nitrite in the US were 106 and 1.5 mg/kg, respectively, and in the UK were 104 and 1.5 mg/kg, respectively. In 1994, van Vliet, Vaessen, van de Burg, and Schothorst (1997) estimated the mean intake of nitrate in the Dutch population to be 80 mg/day per person, and the median intake of nitrite to be 0.1 mg/day per person. In comparison, Italy had a mean daily intake of nitrate of 245 mg/day, whereas Poland and Switzerland recorded mean daily nitrate intakes of 178 and 125 mg/day, respectively. France's mean daily intake of nitrate and nitrite were 150.7 and >3 mg/day, respectively, followed by Netherlands, Germany and Norway where mean daily nitrate intakes were 71, 68 and 43 mg/day, respectively. The mean daily nitrite consumption in those countries was 0.6, 2.6 and 1.8 mg/day, respectively (Gangolli et al., 1994). According to Cornée, Lairon, Velema, Guyader, and Berthezene (1992), the average daily nitrate intake per person per day was 121 mg (85% from vegetables, 5% from preserved and cured meat, and 5% from cereal products). For the average daily nitrite intake per person per day, it was found to be 1.88 mg (43% from vegetables, 28% from cured meat, and 16% from cereals). The remaining 13% of nitrite must come from non-dietary sources of nitrite such as atmospheric contamination.

In summary, Pennington (1998) estimated that the daily nitrate intake ranges between 53 and 350 mg/day depending on the type and quantity of the vegetable consumed and the level of nitrate in drinking water. Whereas daily nitrite consumption was between 0 and 20 mg/day depending on the levels of nitrite present in cured meat and much of it was consumed. The acceptable daily intake (ADI) for nitrate was set at 3.7 mg/kg body weight by the European Union Scientific Committee for Food (1995) and since nitrite has higher acute toxicity than nitrate its ADI was set at 0.06 mg/kg body weight (Reinik et al., 2005).

Based on the Australian Bureau of Statistics (Australian Bureau of Statistics, 1998–1999), Australians consumed 8.7 kg of bacon and ham combined per capita per year in 1998–1999. Assuming half of each product was consumed at 4.35 kg per capita per year (or 12 g per capita per day) based on the finding in this study, nitrite from bacon per capita per day was 0.19 mg, and for nitrate was 0.41 mg. For ham (12 g per capita per day) nitrite consumed per capita per day was 0.28 mg and for nitrate was 0.23 mg. Thus combined nitrite and nitrate intake from bacon and ham per capita per day were 0.47 and 0.64 mg, respectively. At the upper extreme, assuming 100 g of bacon or ham was consumed every day, the nitrite and nitrate intake from bacon would be 1.57 mg and 3.42 mg per capita per day, respectively. Similarly for ham the nitrite and nitrate intake would be 2.33 and 1.90 mg nitrite and nitrate per capita per day, respectively, giving a total of 3.9 mg of nitrite and 5.32 mg nitrate per capita per day. This is significantly lower than the ADI set by the European Union Scientific Committee for Food in 1995.

However, taking endogenous formation of nitrate into account, this means additional 70 mg of nitrate for an average 70 kg adult (Gangolli et al., 1994). Furthermore, it was estimated approximately 25% of dietary nitrate is converted to nitrite by bacteria and nitrate reductase in the oral cavity (Gangolli et al., 1994). Thus assuming two servings (150 g) of vegetables comes from green leafy vegetables, again taking the analytical data from this study (Table 1), this means approximately 727.5 mg of nitrate from English spinach (Fig. 1) is ingested, of which 181.9 mg of nitrite can participate in nitrosation in the stomach. Based on the above assumptions, the total nitrite and nitrate burden for an Australian adult of 70 kg body weight, the intakes per day is approximately 184.4 and 617.7 mg, respectively, sourced from cured meat, spinach and endogenous nitrate formation. This exceeds the ADI of 4.2 mg for nitrite by 44 times per 70 kg adult per day, and by 2.4 times of ADI of 259 mg nitrate per 70 kg adult per day. However, it must be noted that the above prediction assumed that nitrite only came from one serving (50 g) each of bacon and ham (Fig. 2), and that nitrate intake only came from two servings of English spinach. Since English spinach had the highest nitrate content, this predicts the upper extreme of dietary nitrate intake. Furthermore, the ADI do not include the 25% conversion of dietary nitrate to nitrite in the oral cavity, which underestimate the total ingested dietary nitrite.

Japan has seven times the rate of gastric cancer than the United States and is also significantly higher compared to the United Kingdom and Germany. There is little evidence that genetic differences contributed to the different gastric cancer rates (Davies & Sano, 2001). Thus this epidemiological study suggests that diet and lifestyle may play an important role in gastric cancer etiology besides effective screening and management. Countries such as South Korea, Japan and China had the highest stomach cancer mortality for men, whereas countries with the highest stomach cancer mortality for women were South Korea, China and Columbia. Canada and Denmark had the lowest stomach cancer mortality for men and women, respectively (Joossens et al., 1996).

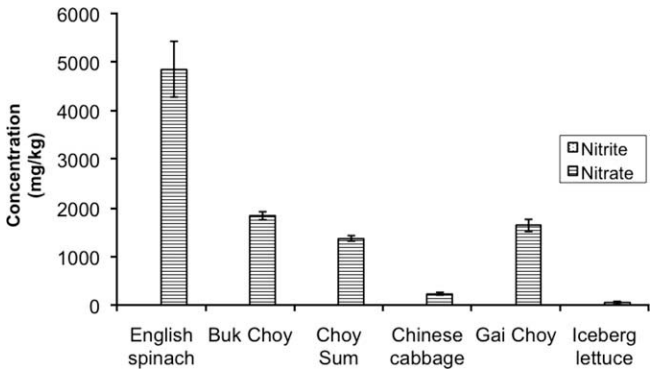

Fig. 1. Mean nitrate and nitrite contents and recoveries in fresh vegetables after 5 min boiling. Values are means of at least four replicate determinations.

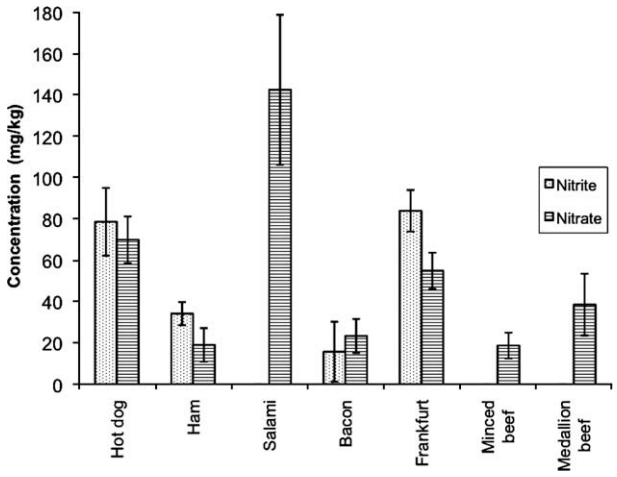

Fig. 2. Mean nitrate and nitrite contents in cured and fresh meat from Sydney supermarkets. Values are means of at least four replicate determinations.

The high gastric cancer incidence in the Far East may be due to the consumption of specific foods that are high in nitrates such as Korean Kimchi or high in salt as in many traditional Japanese dishes, or particular food preparation methods such as broiling of meats (Duncan et al., 1997). Regions of high risk to gastric cancer often coincide with a low intake of foods containing vitamin C. Other risk factors for human gastric cancer include residence in areas with high nitrate-containing soil due to many factors such as the addition of fertilizers and foods pickled with salt (Weisburger, 1981). Certain salted fermented fish products including fish sauce were associated with the high gastric cancer mortality in Fujian province of China (Chen et al., 1992). Similarly, a potential link for the high gastric cancer rate in Southwest Korea was associated with regular high consumption of salted pickled cabbage and salted seafood sauce (Seel et al., 1994). The former, also contained high levels of total *N*-nitroso compound precursors, and cabbages, which are known to contain high levels of nitrate than any other vegetables.

In addition of forming carcinogens in the stomach, nitrite is also genotoxic and can readily induce methaemoglobinaemia especially in babies (Gangolli et al., 1994). Furthermore, the lethal dose for nitrite in adults was estimated to be between 2 and 9 g $NaNO_2$ per day, or 33–250 mg/kg body weight (Gangolli et al., 1994), whereas the lethal dose for nitrate ions was estimated at 20 g per day, or 330 mg nitrate ions/kg body weight (Gangolli et al., 1994). Although it is unlikely to reach these toxic levels from dietary intake alone, the long-term effects may be detrimental based on epidemiological and clinical studies.

Homogenization of food is difficult but often necessary prior to chromatographic analysis. Factors such as variable texture, structure and the presence of immiscible phases may hinder the homogenization process (Lichon, 1996). Because these properties are inherent properties of the food product, it cannot be changed during the manufacturing process. The sampling of food and their preparation must therefore be considered carefully prior to analysis to ensure representative and accurate results. This means using traditional cooking practices so that factors that affect nitrate and nitrite determination and their recovery are consistent and applicable to dietary exposure of these anions.

4. Conclusion

Different authors attributed different percentage of dietary nitrate and nitrite to the major food groups, but the consensus is that vegetables contributed to the majority of dietary nitrate and that cured meat products contributed to the majority of dietary nitrite. This study agreed with the literature that vegetables are the major dietary nitrate contributor and that meat and especially cured meat provided the majority of dietary nitrite. The extraction and detection method used in this study was demonstrated to be simple, fast, sensitive and applicable to both meat and vegetable samples.

Nitrate and nitrite content of foodstuff should be monitored in the long-term to estimate the dietary intake and to provide insights into the effects of new horticultural and manufacturing technologies on the levels of nitrates and nitrite in foods and in the etiology of gastro-intestinal cancers. It was demonstrated that nitrate and nitrite content tested were below the guideline set by FSANZ and their intake were within the range reported in the Western countries and were below the ADI. This study will be the first to quantify the levels of nitrate and nitrite in foods in the Sydney supermarket and will provide useful data to industry and health professionals.

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