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Estimated dietary intake of nitrate and nitrite from meat consumed in Fiji

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ABSTRACT

To assess the risk posed by meat consumption to the Fiji and Pacific populace, the present study reports nitrate and nitrite in meat. Twelve commercially available meat products, with a total of 210 fresh and processed meat samples, were analysed for nitrate and nitrite by an optimised RP-HPLC technique with isocratic elution using n-octylamine in 20.0% methanol at pH 6.60. The nitrate content in the meat samples ranged from 0.00 to 124 mg kg^{-1} whereas the nitrite ranged from 0.00 to 164 mg kg^{-1}. The study shows that the nitrate and nitrite contents of meat samples in Fiji were below the maximum level proposed by European Union legislation but above the limit set by Food Standards Australia and New Zealand (FSANZ). The estimated dietary intake of nitrate and nitrite was calculated from a 24-h diet recall study as well as from Fiji's food balance sheets.

1. Introduction

The danger of food nitrate and nitrite still gives rise to debate in the scientific community. Many studies have suggested that high dietary nitrate and nitrite intake is an aetiological factor in the development of certain cancers. Nevertheless, some authors revised this risk downwards and claimed that nitrate and nitrite could have potential beneficial effects on human health by reducing hypertension and cardiovascular diseases (Bryan, Alexander, Coughlin, Milkowski, & Boffetta, 2012; Sindelar and Milkowsky, 2012).

The risk posed by the intake of nitrate and nitrite through diet has always been emphasised (Lee, 2018; Sindelar & Milkowski, 2012). The European Union has regulated the use of nitrate and nitrite in meat products (EC, 2006; EFSA, 2010). Other countries such as Australia have also established specific concentration limits for the accumulation of nitrate and nitrite salts in cured meat products (Adam, Mustafa, & Rietjens, 2017; FSANZ, 2013; FSAI, 2016; Hsu, Arcot, & Lee, 2009). The undesirable consequence of meat curing is the formation of nitrosamines. The first risk of nitrite is due to its reaction with secondary amines during food processing or gastrointestinal digestion to form nitrosamines, some of which are mutagenic. The second risk of nitrite is due to its reaction with the haem iron of myoglobin to form nitrosomyoglobin. The mutagenicity of nitrosylhaem has recently been reported. Nitrosylhaem could be involved in carcinogenesis *via* the production of mutagenic aldehydes and *via* the formation of various *N*-nitroso compounds (NOCs) which can react with DNA (Kuhnle & Bingham, 2007; Santarelli, Pierre, & Corpet, 2008). The endogenous formation of nitrosylhaem is strongly suspected in addition to the exogenous contribution. Indeed, it has been demonstrated that a red meat diet increased the level of nitrosylhaem at ideal level and in the stools of volunteers, compared with a vegetarian diet (Kuhnle & Bingham, 2007). The formation of nitrosamines was first detected in meat products in the USA in fried bacon (Andrée, Jira, Schwind, Wagner, & Schwägele, 2010). It is now well understood that endogenously acquired nitrate/nitrite can be converted to carcinogenic *N*-nitroso compounds upon digestion (Tamme, Reinik, & Roasto, 2010; Ward, Heineman, Markin, & Weisenburger, 2008).

It is well understood that maximum nitrate load comes from a diet rich in vegetables (Chetty & Prasad, 2009, 2011; Prasad & Chetty, 2008) whereas the maximum nitrite intake comes from consumption of processed meat, such as chicken and lamb sausages (Adam et al., 2017; Binkerd & Kolari, 1975; Mernard, Heraud, Volatier & Leblanc, 2008). It is worth mentioning that nitrate and nitrite are present in foods other than meat. For example, in fresh vegetables, studies reported low concentrations of nitrite but high concentrations of nitrate (generally less than 2000 mg kg^{-1}, but values around 10,000 mg kg^{-1} have occasionally been reported) (Thomson, Nokes, & Cressey, 2007). The human body is able to convert a large part of nitrate into nitrite. Saliva is the major site for the formation of this endogenous nitrite with conversion rates varying from one individual to another from 5% to 25% (van Maanen, van Geel, & Kleinjans, 1996). Due to their high nitrate concentrations and to the endogenous conversion of nitrate into

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nitrite, the contribution of plant foods to the global ingestion of nitrites is generally higher than 90%. Thus, quantification of nitrate and nitrite and related consumer consumption pattern studies have become quite popular in recent years (Honikel, 2008; Hsu, Arcot, & Alice Lee, 2009; Larsson, Darnerud, Iiback, & Merino, 2011; Leth, Fagt, Nielsen, & Andersen, 2008; Rezaie, Shariatifar, Jahed & Javadzadeh, 2013; Reinik et al., 2005; Soumi et al., 2015; Temmea et al., 2011; Thomson et al., 2007; Zhong, Hu, & Wang, 2002). However, processed meat has commonplace in the diet of Pacific Islanders. Fiji and Pacific consumers are increasingly becoming aware of the health risks associated with a diet of processed meats. In processed meat products, nitrate and nitrite are used for the purpose of preservation, antimicrobial action and for colour fixation (Andrée et al., 2010). The addition of nitrite and nitrate also contributes to the characteristic flavour of cured meats.

Certain foods such as salami, ham, bacon, saveloy, cheese and cheese products usually contain nitrate and nitrite as food additives. Nitrate and nitrites are added as salts of sodium or potassium. Generally, nitrites are used in curing of meats although nitrates are sometimes also used, as they act as an *in-situ* reservoir of nitrites (Honikel, 2008). Various researchers have reviewed the history of meat curing and the use of nitrate and/or nitrite and thus good reviews are available (Benedict, 1980; Binkerd & Kolari, 1975; Gray, Macdonald, Pearson, & Morton, 1981; Roberts, Gibson, & Robinson, 1981).

Antimicrobial activity through nitrites is seen in foods at 100–200 mg L^{-1}, where its activity increases with decreasing pH (Binkerd & Kolari, 1975). Over the years, various types of cured meat products (salami, ham, bacon, turkey sausage, pork sausage, chicken sausage, egg sausage, etc.) have been introduced to the consumers. The record of bacteriological safety due to nitrate and nitrite in these meats is believed to be excellent (Hammes, 2012; Sindelar & Milkowski, 2012). Product stability has further been enhanced with the increasing use of refrigeration and direct application of nitrite. Thus, nitrite has been extremely effective in preventing the growth of *Clostridium botulinum*, the causative agent of food poisoning known as botulism (Armenteros, Aristoy, & Toldrá, 2012; de Klein & Hoven, 1984). Various outbreaks of botulism have been recorded in many parts of the world where meat products are cured without nitrate and/or nitrite (Gray & Randall, 1979). Cured meats are kept in a strict anaerobic environment which results in the reduction of nitrites to nitric oxides (NO). The NO in turn react with haem pigments in the muscle tissue to form the pink-coloured pigment nitrosohaem, and keep the processed meats pink rather than turning brown, which heightens the commercial acceptability of the product (Lee, 2018).

Fiji's food composition table does not contain data on nitrate/nitrite of food products available to consumers in Fiji or in the Pacific. As such, there is a lack of incidence data in Fiji as well as in the Pacific, which gives rise to an imminent need to investigate the nitrate and nitrite levels in processed as well as fresh meat products available in the supermarkets. Thus, this paper reports the optimisation of a reversed-phase high-performance liquid chromatographic (RP-HPLC) method to determine the nitrate and nitrite contents in meat products and their dietary intake arising from meat consumption to assess their potential risk in comparison to international standards.

2. Materials and methods

2.1. Reagents and standards

Only analytical grade reagents were utilised in this study. Ultra-pure water was used in preparation of all solutions and sample extraction. The solvents for HPLC were filtered through 0.220 μm (NL17) polyamide-based filters and degassed under vacuum for an appropriate time. Sodium nitrate ($NaNO_3$), sodium nitrite ($NaNO_2$), *n*-octylamine ($C_8H_{19}N$) and HPLC-grade methanol (CH_3OH) were purchased from Sigma-Aldrich Chemicals Co. (St Louis, MO), whereas activated charcoal was from Merck (Darmstadt, Germany).

n-Octylamine (1.29 g) was dissolved in 20.0% MeOH and made to 1.00 L in a volumetric flask with the same solvent. The pH was adjusted to 6.60 using 10.0% (*v/v*) phosphoric (H_3PO_4) acid solutions. Carrez reagent I was prepared by dissolving 11.0 g of zinc acetate dihydrate and 1.50 mL of glacial acetic acid in a 50 mL volumetric flask. Carrez reagent II comprised an aqueous solution of 5.30 g of potassium hexacyanoferrate(II) trihydrate ($K_4[Fe(CN)_6].3H_2O$) in a final volume of 50.0 mL. The stock solutions of nitrate and nitrite (1000 μg mL^{-1}) were separately prepared using $NaNO_3$ (0.137 g) and $NaNO_2$ (0.150 g) respectively in 100 mL volumetric flasks. Subsequent dilutions were performed to acquire standards to construct calibration curves from 0.250 to 6.00 μg mL^{-1} for nitrate and nitrite separately.

2.2. Analytical procedure and equipment

A Crison pH meter (BASIC 20+), Stuart Scientific shaker SF1, Retsch GM 200 Grindomix and Fungilab ultrasonic cleaner were used for eluent and sample preparation. Nitrate and nitrite analysis was carried out using an analytical HPLC unit equipped with a Gilson 305 pump (Gilson, France). The HPLC system also included a 7125i Rheodyne injector with a 20.0 μL loop, a Gilson 118 variable long-wave ultra-violet detector (λ = 220 nm) and Gilson 712 HPLC system controller software. The nitrate/nitrite separation was accomplished using an ACE C18 (5.00 μm diameter) chromatographic column.

2.3. Sample extraction

The study design involved two categories of meat: fresh and processed. Samples were sourced from three well-recognised food chain outlets (supermarkets) in Suva, the capital city of Fiji. Eleven dissimilar meat-based foods were scrutinised; 210 samples were bought in total. Composite samples were prepared from 210 collected samples and analysed, giving a total of 70 samples. Each type of meat sample (25.0 g) was taken to make an individual composite sample, which was stored in acid-prewashed (snap-lock) plastic bags. All frozen meat samples were freeze-dried within 24 h. Freeze-drying times depended on water content of meat samples. After freeze-drying all samples were vacuum-sealed and labelled.

Approximately 50.0–100.0 mg of the freeze dried meat samples were weighed in 90.0 mL glass vials on an analytical balance. Equal amount of activated charcoal was added to each vial containing sample. Subsequently 10 mL water (≈100 °C) were added, followed by additions of 0.500 mL of Carrez reagents I and II. The mixture was shaken on a Stuart Scientific Shaker SF1 for 30 min. Part of the mixture was filtered through HPLC syringe filters (0.220 μm) into polypropylene vials ready for analysis.

2.4. 24 h diet recall and food balance sheets

Food balance sheets (FBS) were collated from the Food and Agriculture Organization (FAO) of the United Nations website (http://www.fao.org/faostat/en/#data/FBS). FBS data for five years starting from 2009 to 2013 were collected and analysed. The dietary survey (24-Hr DR) data were collected from 150 individuals through questionnaires and personal interviews. The age-group of sampled individuals was 18–65 years. Fifteen participants were chosen from a pool of twenty tertiary (chemistry) students. These participants were trained as interviewers to administer the structured 24-Hr DR questionnaires. Each interviewer was asked to find ten respondents from ten different households within the Suva–Nausori corridor. The Suva–Nausori corridor houses Fiji's largest urban population. The respondents were assured of their privacy and names of participants were not recorded. Data on the type and source of food, portion size, beverage intake and time of day were recorded. Consumption patterns, with regards to the acute intake of fresh and processed meats only, were analysed for this study. The estimated dietary intake (EDI) was calculated based on body

weight and nitrate/nitrite content of analysed meat samples.

2.5. Statistical analysis

The statistical analysis was accomplished by means of Microsoft Excel 2010. Study data were exposed to one-way analysis of variance (ANOVA) and Pearson Product-Moment Correlation. The mean values and corresponding standard deviations were considered for each sample.

3. Results and discussion

Acceptable daily intake (ADI) for nitrate is 3.70 mg kg^{-1} body weight/day and for nitrite is 0.0700 mg kg^{-1} body weight day^{-1} as regulated by the Joint Expert Committee of Food and Agriculture (JECFA) and the European Commission's Scientific Committee on Food (SCF) (Hambridge, 2003; Santamaria, 2006; SCF, 1997). These values are analogous to the exogenous consumption of 276 mg nitrate or 5.25 mg nitrite per day for a 75.0 kg body weight (bw) adult (Hmeljak & Cencič, 2013).

Fiji being a developing country, a number of new processed meat products enters the market frequently. Since Fiji does not have an established limit for nitrate and nitrite in food products, the nitrate and nitrite content of meat products could very well exceed the limits set by international regulatory bodies, such as Food Standards Australia and New Zealand (FSANZ, 2013), Food and Agricultural Organization (FAO), World Health Organization (WHO) and European Food Safety Authority (EFSA, 2010). Therefore, estimating dietary intake and comparing with the regulatory data helps to keep control of nitrate/nitrite levels and disease patterns.

3.1. Analytical and quality assurance

The pH of the mobile phase (0.01 M *n*-octylamine in 20.0% methanol) was stabilised using monobasic and dibasic sodium phosphate based buffers (NaH_2PO_4 and Na_2HPO_4) and adjusted to 6.60 using 10% (*v*/*v*) phosphoric (H_3PO_4) acid solutions. The isocratic elution was achieved using 0.01 M *n*-octylamine in 20.0% methanol as mobile phase. A stable baseline was obtained after allowing the ion-pairing agent to flow through the HPLC system at a flow rate of 0.5 mL min^{-1}. At the end of the analysis the HPLC column was regenerated by passing 75.0% CH_3OH at a flow rate of 0.1 mL min^{-1} overnight. RP-HPLC peak profiles for the nitrate and nitrite calibration standards were obtained within a 12.0 min run. The calibration graphs were acquired for each run by injecting five different concentrations of nitrate and nitrite in duplicate in the range of 0.250–6.00 μg mL^{-1}. The linear calibration curves were obtained by plotting five data points each for nitrate and nitrite.

For quality assurance measures, the relative standard deviation (RSD) for peak areas was calculated to be below 1.00% for both the nitrate and nitrite calibration curves. The samples that exceeded the determined calibration range were diluted and re-analysed. The calibration standards were analysed each day before each batch of sample runs. Blank samples were run before and after six consecutive sample analyses.

The figures of merit for the quantification of nitrate and nitrite in the meat samples are presented in Table 1. The limits in the current assay are defined by the limit of detection (LoD) and the limit of quantification (LoQ) (Erkekoglu, Sipahi, & Baydar, 2009). Thus, the LoD and LoQ for nitrate were calculated to be 0.162 and 0.747 mg kg^{-1}, whereas for nitrite LoD and LoQ were 0.105 and 0.484 mg kg^{-1}, respectively (Table 1).

The accuracy of the methodology was assessed through the results of the spiked recovery of nitrate and nitrite from three real samples: corned mutton, chicken luncheon meat and frankfurter lamb sausages. Only the samples with processed meat matrix were used for the spiked recovery analysis, where 5.00, 10.0 and 20.0 μg mL^{-1} of nitrate and nitrite solutions were added as internal standards. The internal standards were added to the freeze dried meat samples (50.0–100.0 mg) and run through the whole extraction process as described in Section 2.3. The results of the recovery analysis are presented in Table 2. The typical recoveries of spiked nitrate residues ranged from 97.3 to 104% giving an average recovery of 100 ± 2.15% whereas the nitrite recovery ranged from 94.4 to 110% with an average recovery of 98.8 ± 4.83%, showing excellent recoveries.

3.2. Sample analysis

The results of the investigation of the nitrate and nitrite contents (mean values) of the selected commercially available meat products in Fiji are shown in Table 3. Table 3 also reports the minimum and the maximum contents of nitrate and nitrite in studied samples with standard deviation. The nitrate contents of the eleven meat samples assayed ranged from 0.870 to 124 mg kg^{-1}, while the nitrite contents in the same set of samples ranged from 10.7 to 164 mg kg^{-1}. Corned beef samples had the highest nitrate content (105 mg kg^{-1}) within the studied meat samples followed by corned mutton, chicken frankfurters, lamb frankfurters, chicken luncheon meat, lamb luncheon meat, chicken sausage, lamb, chicken, pork and beef, in decreasing order (Table 3). Nitrite was not detected (nd) in any of the non-processed meat samples, i.e., lamb, chicken, pork, and beef. The chicken sausage had the highest nitrite content within the studied samples, followed by lamb sausage, lamb luncheon meat, chicken luncheon meat, chicken frankfurters, lamb frankfurters, corned beef and corned mutton in decreasing order. Standard deviations ranged from 5.39 to 22.9 μg mL^{-1}.

Pearson product-moment correlation (PPMC) test was used to appraise the presence of a direct relationship between the nitrate and nitrite values in the processed meat samples. The PPMC coefficient, $r > 0$ advocates that a confident linear correlation exists between the nitrate and the nitrite content of the similar processed meat samples of the different brands. The processed meat samples from similar brands were subjected to a one-way ANOVA (analysis of variance) test. As seen from the results ($p > 0.05$), there are no statistically significant differences between the nitrate and nitrite contents of the meat samples.

3.3. Cluster analysis

Fig. 1 depicts the pair-wise dissimilarity between the studied meat samples through a dendrogram based on hierarchical clustering. Ward linkage and Euclidean distance arising out of the 2 variables (nitrate and nitrite) of selected meat samples clearly show that the clusters generated form two groups. The cluster tree is made up of nodes which contain a group of similar data. The first group is made up of fresh meat samples (beef, pork, chicken and lamb), whereas the second group is made up of the processed meat samples (chicken sausage, lamb sausage, lamb luncheon, corned beef, corned mutton, lamb frankfurters, chicken frankfurters, and chicken luncheon meat). The studied samples of beef, pork, chicken and lamb have low nitrate/nitrite (3.02–7.51/0.00 mg kg^{-1}) content and thus form one clade. On the other hand chicken sausages, lamb sausages and lamb luncheon meat, with nitrate and nitrite values ranging from 0.00 to 18.0 mg kg^{-1} and nitrites values of 119 to 142 mg kg^{-1}, make up the second clade. A third clade is made up of corned mutton and corned beef (nitrate/nitrite 87.4–105/24.0–30.2 mg kg^{-1}). The final clade comprises lamb frankfurters, chicken frankfurters and chicken luncheon meat because their nitrate and nitrite contents ranged from 40.7 to 65.5 mg kg^{-1} and 56.7 to 103 mg kg^{-1}, respectively. It can be clearly seen that the 2nd to 4th clusters which are made up of processed meat samples show a clear degree of similarity as the clusters at one level join with clusters at the next level.

Table 1
Quantification parameters of RP–HPLC (Gilson, France) and figures of merit for the analysis of nitrate and nitrite.

Facet	Parameter for nitrate	Parameter for nitrite
Separation column	ACE C18 (5 μm diameter) chromatographic column	ACE C18 (5 μm diameter) chromatographic column
Pump; injector	Gilson 305; 7125i Rheodyne	Gilson 305; 7125i Rheodyne
Detector; wavelength	UV–Vis; 220 nm	UV–Vis; 220 nm
Sample volume; flow rate	20.0 μL; 0.500 mL min^{-1}	20.0 μL; 0.500 mL min^{-1}
Limit of detection	0.0032 μg mL^{-1}; 0.162 mg kg^{-1}	0.0021 μg mL^{-1}; 0.105 mg kg^{-1}
Limit of quantification	0.0149 μg mL^{-1}; 0.747 mg kg^{-1}	0.00970 μg mL^{-1}; 0.484 mg kg^{-1}
Dynamic range of detection	0.250–6.00 μg mL^{-1}	0.250–6.00 μg mL^{-1}
Coefficient of determination	0.996	0.999
Run time	12.0 min	12.0 min

Table 2
Determination of nitrate and nitrite extracted from the original meat samples and recovery study from the corresponding spiked meat samples with three different nitrate/nitrite concentrations.

Types of meat samples	Nitrate (μg mL^{-1})		Recovery (%)	Nitrite (μg mL^{-1})		Recovery (%)
	Spiked	Content ± SD		Spiked	Content ± SD	
Corned mutton	–	103 ± 6.16	–	–	13.8 ± 2.14	–
	5.00	108 ± 5.68	100	5.00	18.5 ± 2.34	94.4
	10.0	113 ± 5.53	97.7	10.0	23.3 ± 2.56	94.7
	20.0	123 ± 5.24	98.0	20.0	33.4 ± 2.35	98.0
Chicken luncheon meat	–	42.7 ± 5.96	–	–	97.0 ± 4.86	–
	5.00	47.9 ± 4.18	104	5.00	102 ± 3.74	96.3
	10.0	52.6 ± 5.03	99.5	10.0	107. ± 4.76	102
	20.0	62.4 ± 4.54	98.5	20.0	119 ± 5.37	110
Frankfurter lamb sausages	–	66.1 ± 21.1	–	–	61.9 ± 6.26	–
	5.00	70.9 ± 19.6	97.3	5.00	66.8 ± 5.23	99.6
	10.0	76.4 ± 18.5	103	10.0	71.3 ± 5.77	94.8
	20.0	86.1 ± 15.7	100	20.0	82.0 ± 6.54	101
Average recovery			100 ± 2.15			98.8 ± 4.83

SD: Standard deviation.

Table 3
Nitrate and nitrite content of fresh and processed meat samples.

Meat sample	Nitrate (mg kg^{-1})				Nitrite (mg kg^{-1})			
	Min	Max	Av	SD	Min	Max	Av	SD
Lamb[A,B,C]	4.07	12.6	7.51	3.44	nd	nd	nd	nd
Chicken[A,B,C]	2.80	8.08	5.05	2.58	nd	nd	nd	nd
Pork[A,D]	0.870	6.24	3.99	2.24	nd	nd	nd	nd
Beef[D,E]	0.990	7.34	3.02	2.41	nd	nd	nd	nd
Corned mutton[A,B,C,F*]	67.9	110	87.4	20.6	10.7	47.5	24.0	17.1
Corned beef[A,B,C*]	71.3	124	105.2	24.5	16.3	47.0	30.2	13.5
Chicken luncheon meat[A,B,C]	17.9	48.8	40.7	11.8	87.8	111	103	8.78
Lamb luncheon meat[A,B,C]	7.36	23.2	18.0	7.80	103	140	119	18.4
Chicken sausage[A,B,C*]	nd	17.4	7.92	7.79	110	164	142	22.9
Lamb sausage[A,B,C*]	nd	nd	nd	nd	127	140	135	5.39
Lamb frankfurters[A,B,C*]	29.8	81.4	49.1	25.7	48.0	74.6	56.7	13.4
Chicken frankfurters[A,B,C*]	56.9	71.2	65.5	6.12	59.5	76.2	68.5	6.53

Min: minimum; Max: maximum; nd: not detected; Av: average (n = 6); A, B, C, D, E, F: different shops/brands.
*Different sample brands.

3.4. Estimated dietary intake of nitrate and nitrite

Table 4 presents the EDI calculated from Fiji's FBS data, obtained from the Food and Agriculture Organization (FAO) of the United Nations website (http://www.fao.org/faostat/en/#data/FBS). Currently, information on Fiji was available only till 2013. The total meat consumption increased from 2009 to 2012 but reduced in 2013. On the whole, poultry meat, which included processed poultry meat, such as chicken sausages, chicken frankfurters and chicken luncheon meat, is the most commonly consumed commodity. The EDI for the five years was 0.170 ± 0.020 mg NO_3^- kg^{-1} bw day^{-1} whereas the EDI for nitrite was 0.350 ± 0.060 mg NO_2^- kg^{-1} bw day^{-1}. The bodyweight used to calculate the EDI was taken from the 24-Hr DR study (n = 150) which was 72.6 kg.

Table 5 presents a comparison of the compiled dietary data from two distinct sources i.e. FBS and 24-Hr DR study. The dietary exposure to nitrate and nitrite was calculated as accurately as possible. It is clearly evident that the total meat consumption (per capita) for the FBS is more in comparison to the current specifically designed individual nutritional survey undertaken through 24Hr DR study. The FAO-amassed FBS estimates data on a number of factors, such as total imports, exports, annual food production, losses on storage and transportation on a population level (Naska et al., 2009; Rodrigues, Lopes, Naska, Trichopoulou, & de Almeida, 2007). The data may be subject to the accuracy of the individual countries' data-collecting agencies. The disadvantage for estimating dietary intake from FBS's for a specific analyte, thus lies in the fact that data on the primary form of the food commodity are given and not on the consumption of a specific food item, as is evident from Table 4. Thus, a dietary survey, such as a 24-Hr DR study, affords better accuracy, as consumption of individual food items is highlighted. The disadvantage is that only a small section of the community was sampled, due to the ensuring research costs. Also the amount of food/meat consumed is solely reliant on the information provided by the subject.

Table 5 clearly shows that the total meat (per capita) consumed under the 24-Hr DR study is lower in comparison to the FBS and the EDI (mg kg^{-1} bw day^{-1}) of nitrate and nitrite. The range of the EDI (FBS and 24-Hr DR) of nitrate in terms of the lower bound (LB) and upper bound (UB) percentage with respect to the SCF-determined ADI (SCF, 1997; Santamaria, 2006) is 3.24–5.14%, whereas the EDI of nitrite

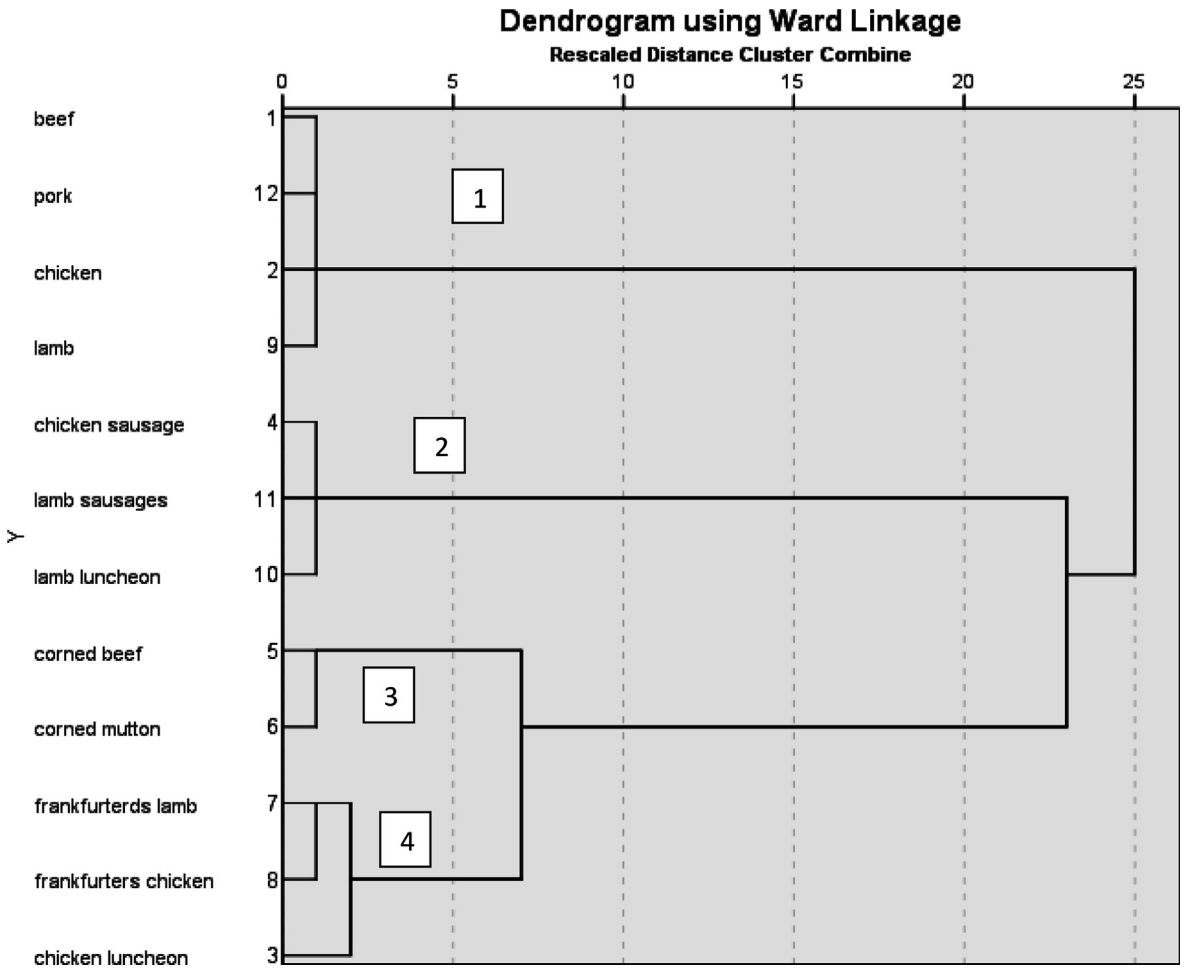

Fig. 1. Dendrogram showing hierarchical clustering for nitrate and nitrite contents in the selected meat samples (mg kg^{-1}).

Table 4
Estimated Dietary Intake (EDI) of nitrate and nitrite (mg kg^{-1} bw day^{-1}) calculated from Fiji's food balance sheets (2009–2013).

	Food balance sheets					
Sample (kg $year^{-1}$)	2013	2012	2011	2010	2009	average
Total meat	42.4	55.8	47.8	42.5	41.3	45.9
Bovine	8.96	10.6	9.71	9.70	9.03	9.60
Mutton and goat	5.90	5.90	5.64	5.90	9.43	6.60
Pigmeat	4.31	4.50	4.60	4.50	4.52	4.50
Poultry	20.3	31.4	24.6	18.7	15.0	22.0
Meat other	0.150	0.130	0.100	0.130	0.040	0.110
Offal edible	2.77	3.22	3.15	3.61	3.27	3.20
mg NO_3^- kg^{-1} bw day^{-1}	0.150	0.200	0.170	0.150	0.150	0.170
mg NO_2^- kg^{-1} bw day^{-1}	0.320	0.460	0.370	0.310	0.310	0.350

ranges from 357 to 614%. In terms of nitrate consumption, the data are underestimated as the nitrate intake from vegetables and fruits are not taken into account. With respect to nitrite intake the current data are fairly accurate as processed meats are the major source of nitrites. The EDI calculated from the FBS and the 24-Hr DR study clearly indicates that due to the high consumption of meat, the Fiji adult population has exceeded the nitrite ADI. Despite this, it must be considered that food consumption studies can themselves be sources of uncertainties, for example, the food left on an individual's plate was not considered. Also a seven-day study in comparison to a 24Hr study would give more accurate results, as individuals are likely to consume more meat over the weekends than the working days.

4. Conclusion

The RP-HPLC methodology illustrated herein allows a rapid, accurate and simultaneous measurement of nitrate and nitrite in meat samples. The excellent recoveries of nitrate and nitrite reflected the accuracy and the robustness of the optimised RP-HPLC methodology. Elution of both analytes occurs within 12 min. The present study presents primary data on the nitrate and nitrite content of commercially available fresh and processed meat products marketed in Fiji. The evaluated samples were identified as the most common meat types in the diet of the Fiji populace. FBS and a 24-Hr DR was undertaken to estimate the nitrate/nitrite dietary intake. It is clearly evident that the EDI (0.130 mg kg^{-1} bw day^{-1}) of nitrate from meat consumed in Fiji is lower than the internationally established (3.70 mg kg^{-1} bw day^{-1}) ADI (Hambridge, 2003; Santamaria, 2006; SCF, 1997). On the other hand, the EDI of nitrite from meat consumption from both the studies undertaken (FBS and 24-Hr DR) exceeded the established (0.070 mg kg^{-1} bw day^{-1}) ADI (Hambridge, 2003; Santamaria, 2006; SCF, 1997). There is an urgent need for Fiji to set maximum limits for the addition of nitrates and nitrites in processed meats, to reduce the probability of the negative impact associated with exceeding the nitrate/nitrite ADI.

Conflict of interest

None.

Table 5
Comparison of nitrate and nitrite data collected from food balance sheets (45.9 kg per capita) and 24 h diet recall study (30.0 kg per capita) of meat products.

Stats	Nitrate		Nitrite	
	FBS	24-Hr DR	FBS	24-Hr DR
Meat per capita (kg)	45.9 ± 6.05	30.0 ± 17.3	45.9 ± 6.05	30.0 ± 17.3
Meat (kg day^{-1})	0.130 ± 0.020	0.080 ± 0.050	0.130 ± 0.020	0.080 ± 0.050
Nitrate/nitrite (mg kg^{-1} day^{-1})	12.0 ± 1.61	9.14 ± 4.00	25.7 ± 4.65	19.9 ± 10.0
EDI (mg kg^{-1} bw day^{-1})	0.170 ± 0.020	0.130 ± 0.060	0.350 ± 0.060	0.270 ± 0.140
CL (95%)	0.030	0.010	0.080	0.0200
LB	0.140	0.120	0.270	0.250
UB	0.190	0.130	0.430	0.300
SCF ADI (mg kg^{-1} bw day^{-1})	3.70	3.70	0.070	0.070
LB/ADI (%)	3.78	3.24	386	357
UB/ADI (%)	5.14	3.51	614	429

CL (95.0%): confidence level (95.0%); LB: lower bound; UB: upper bound.

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