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Evaluation of nitrate and nitrite contents in pickled fruit and vegetable products☆

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ABSTRACT

Our objective was to investigate nitrate and nitrite contents of acidified and fermented fruits and vegetables. L-ascorbic acid and total phenols were also examined based on the hypothesis that the presence of these antioxidant compounds may influence N-nitrosation reactions upon human consumption. The fermented and acidified vegetable products included 131 samples from multiple lots of 46 different commercially available products. Nitrite was detected in low concentrations (<1.5 mg/100 g) in four acidified (pickled green beans, red cabbage, pickled beets, and pickled mushrooms) and two fermented products (Greek olives and kimchi). Nitrate concentrations ranged from a mean value of 122 mg/100 g for kimchi to undetectable levels in acidified Brussels sprouts. Measures of antioxidant compounds showed that artichoke hearts had the highest total polyphenols (225 mg/100 g), and olive products had between 84 ± 5 mg/100 g (Spanish table olives) and 170 ± 8 mg/100 g (Greek olives). An acidified red pepper product had the highest L-ascorbic acid content of 32 ± 10 mg/100 g, with a low nitrate level of 0.1 ± 0.09 mg/100 g. These results provide new information for evaluating nitrate and nitrite contents in pickled fruit and vegetable products with regard to potential human dietary health consequences.

1. Introduction

Nitrate and nitrite are two anions in the nitrogen cycle that are widely distributed in nature. These compounds are found in the atmosphere, water, soil, microorganisms, plants, and animals. Dietary nitrite has been considered to be harmful to human health because of its association with fatal cases of methemoglobinemia upon consumption of foods or beverages with high concentrations of nitrite (>0.3 percent by weight) and possible links to some gastrointestinal cancers (Gorenjak & Cencic, 2013; Weitzberg & Lundberg, 2013). Nitrite may be involved in N-nitrosamine formation by N-nitrosation reactions with dietary-derived amines in the stomach, and dietary nitrite may be related to various forms of cancer, most notably esophageal and stomach cancers (Weitzberg & Lundberg, 2013). However, epidemiologic evidence for a link between nitrate or nitrite and cancer is lacking (Bryan, Alexander, Coughlin, Milkowske, & Boffetta, 2012; Eichholzer & Gutzwiller, 1998). Recently, attention has shifted toward the potential health benefits of dietary nitrate, due to *in vivo* formation of nitric oxide (NO) through a nitrite intermediate. *In vivo* nitrite has been found to be associated with beneficial health effects, including reduced risk of cardiovascular disease, myocardial infarction, stroke, gastric ulcer, renal failure, and metabolic syndrome (Archer, 2002; Gorenjak & Cencic, 2013; Habermeyer et al., 2015; Hord, Tang, & Bryan, 2009; Jonvik et al., 2016; Weitzberg & Lundberg, 2013). In the conversion of nitrate to nitrite, and subsequent formation of NO, reducing agents such as L-ascorbic acid (AA) and polyphenolic compounds facilitate the reduction of nitrite to NO and protect NO from being scavenged once it is produced. In addition, AA and polyphenols are potent inhibitors of nitrosation reactions (Habermeyer et al., 2015; Rocha, Nunes, Pereira, Barbosa, & Laranjinha, 2014). Because potentially harmful reactions with

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nitrite and nitrate may depend on the levels of antioxidants, evaluation of nitrite and nitrate concentrations in foods should also include AA and polyphenolic contents.

In the US, fermented and acidified vegetable products represent a market of over $2 billion, and fermented vegetables are growing in popularity. The nitrate and nitrite contents of fresh vegetables have been surveyed (Chung, Kim, et al., 2003; Chung, Tran, et al., 2011; Gonzalez, Martinez-Tome, & Isasa, 2010; Mor, Sahindokuyucu, & Erdogan, 2010; Nunez de Gonzalez et al., 2015), which enabled the development of a reference database for assessing the contributions of dietary nitrate from 178 vegetables and 22 herbs and spices (Blekkenhorst et al., 2017). Studies have also assessed the content of nitrate and nitrite in vegetables commonly marketed in frozen or canned forms (Bakr, El-Iraqui, & Huissen, 1986; Bednar, Kies, & Carlson, 1991; Leszczynska, Filipiak, Cieslik, Sikora, & Pisulewski, 2009; Siciliano, Krulick, Heisler, Schwartz, & White, 1975). The effect of specific cooking or preservation methods on nitrate content showed that substantial decreases in nitrate content were observed in boiled or canned vegetables, baking had little to no effect, and nitrate content was increased in fried products (Bakr et al., 1986; Prasad & Chetty, 2011; Schuster & Lee, 1987). However, there is very little information available on the concentrations of nitrate and nitrite in pickled vegetable products.

Processing fruits and vegetables by fermentation or acidification may influence nitrate and nitrite concentrations. During the fermentation of Chinese cabbage, nitrate decreased and then reached a stationary level, while the content of nitrite initially increased and then decreased when the pH was lower than 4.5 (Ji, Ji, Li, & Lu, 2009; Yang et al., 2014). Du, Wu, Sun, and Yue (2013) also found similar changes in nitrite in Chinese sauerkraut during fermentation, with an increase in the early stage, followed by decrease to a low level toward the end of fermentation. Lactic acid bacteria may metabolize nitrite during the fermentation of vegetables (C. K. Oh, Oh, & Kim, 2004; Yan et al., 2015; Yu & Zhang, 2013). The ability to metabolize nitrite was found in certain strains of *Lactobacillus brevis*, *Lactobacillus plantarum,* and *Leuconostoc mesenteroides* obtained from kimchi (Oh et al., 2004; Yu & Zhang, 2013). In contrast, coliform bacteria, which are displaced by the growth of lactic acid bacteria during the early stages of most vegetable fermentations (Breidt, McFeeters, Pérez-Díaz, & Lee, 2013), have been implicated in increasing nitrite levels in some vegetable fermentations (Hashimoto, 2001). Other ingredients in pickled fruits and vegetables, such as salt, sugar, ginger, and garlic, may affect the nitrate and nitrite contents directly or indirectly by affecting the fermentation process (Li et al., 2011). Fermented turnip, cucumber, mustard, and cabbage in northern China had nitrite levels of 0.40–0.60 mg/100 g (Hou, Jiang, & Long, 2013), while nitrite content of 0.017–0.198 mg/100 g was reported for the naturally fermented sour cabbage in Daqing, China (Zhang, Wu, Li, Yang, & Yue, 2013). In these fermented vegetables, nitrite levels were below 2.0 mg/100 g (calculated as sodium nitrite), a level that is considered safe under the Chinese National Food Standard regulation (GB2762-2017) and is below the World Health Organization's Acceptable Daily Intake for a healthy adult (0.06 mg/kg body weight per day ~3.6 mg per day for a 60- kg person). US regulations (21 CFR, part 172) do not specifically mention fruit and vegetable products, but specify that there can be no more than 20 mg/100 g nitrite or 50 mg/100 g nitrate in finished meat products where nitrite and nitrate are added as preservatives.

Currently, there is little information available on the levels of nitrite or nitrate together with levels of antioxidant compounds in fermented and acidified (pickled) vegetables. The objective of this study was to investigate the levels of nitrite, nitrate, and antioxidants, including AA, and polyphenols in pickled vegetable products to provide information to consumers, health professionals, and food producers. The results may be useful in product design and in estimating intake levels for research on the effects of dietary nitrite and nitrate on human health.

2. Materials and methods

2.1. Samples and reagents

Fermented and acidified products were purchased from a variety of commercial sources including retail groceries in Raleigh, N.C., U.S.A., and online suppliers. These products included fruit, seed, leaf, flower, stem, bulb and root vegetables, and mushrooms. Certain products were analyzed from more than one producer because they are popular commercial items in the U.S., such as pickled cucumber, pickled pepper, and sauerkraut. The names of the producers have been coded as shown in Tables 1 and 2. For most products, samples from three independent lots (one jar per lot) were obtained. A 200-g portion of pickled vegetable or fruit product was cut into small (<0.5 g) pieces and homogenized as described below. A 5.0 g representative sample of the homogenate was used for the analysis of nitrite, nitrate, AA, and total phenols.

Analytical grade reagents were used for all assays. Nitrate standard (1000 mg/100 g nitrate in water), nitrite standard (1000 mg/100 g nitrite in water), gallic acid, L-ascorbic acid, and zinc (powder) were purchased from Sigma-Aldrich (St Louis, Mo., U.S.A.). Other chemicals were also purchased from Sigma-Aldrich, including disodium tetraborate decahydrate, potassium hexacyanoferrate (II) trihydrate, zinc sulfate heptahydrate, cadmium acetate, ammonium hydroxide solution, sulfanilic acid, N-(1-naphthyl) ethylenediamine dihydrochloride, glacial acetic acid, *meta*-phosphoric acid, sulfuric acid, acetone, methanol, sodium carbonate, and Folin-Ciocalteu reagent.

2.2. Determination of nitrite and nitrate

Contents of nitrite and nitrate were determined following the method of Ozdestan and Uren (2010) with some modifications. Briefly, 5.0 g of pickled product was homogenized with 30 mL distilled water using a Tissuemizer (TekMar; Watts Water Technologies Inc., North Andover, Ma., U.S.A.), with addition of 1.2 mL of 2.6% (weight/volume) $Na_2B_4O_7$ solution, followed by heating at 70 °C for 15 min. After cooling on ice for 15 min, the mixture was clarified by adding 0.5 mL of 30% w/v $ZnSO_4\ 7H_2O$ solution, shaken by hand for 15 s, then 0.5 mL of 15% (w/v) $K_4Fe\ (CN)_6\ 3H_2O$ solution was added with shaking for an additional 15 s. After holding at room temperature for 30 min to allow separation, the mixture was centrifuged at 5000 × g (SS-34 rotor) for 30 min at 4 °C (Sorvall RC-5B centrifuge, Thermo Electron Corp, Asheville, N.C., U.S.A.). The supernatant was filtered through Whatman filter paper (No. 2). Then the clear supernatant (32 mL) was analyzed for nitrite and nitrate.

For the nitrite analysis and calibration curve, a 6.0 mL aliquot of the sample extract or diluted extract in water was mixed with equal volumes (0.76 mL) glacial acetic acid and Griess reagent (Ozdestan & Uren, 2010). After standing at room temperature for 30 min, absorbance of the mixture was read at 538 nm with a spectrophotometer (Pharmacia LKB Novaspec II, Pharmacia, LKB Biochrom, UK). A nitrite calibration curve was prepared by treating 6.0 mL of standard nitrite solutions prepared in water with concentrations ranging from 0.5 to 4.0 mg/L, with 0.76 mL glacial acetic acid and 0.76 mL Griess reagent. Griess reagent (100 mL) was prepared by combining sulfanilic acid and N-(1-naphthyl) ethylenediamine solutions prepared as described: sulfonic acid was prepared by dissolving 0.42 g sulfanilic acid in 50 mL of a 15% (by volume) acetic acid solution with heating on a hotplate; N-(1-naphthyl)

Table 1
Products containing acetic acid (acidified).

Acidified product name	Company[a]	Organic acids and sugars (g/100 mL)[b]			pH[c]	NaCl (%)[c]
		Acetic	Glucose	Fructose		
Artichoke Hearts	C	0.13	1.12	1.02	4.0 ± 0.1	1.5 ± 0
Pickled Asparagus	H	1.30	2.30	2.49	3.7 ± 0.1	2.8 ± 0.2
Pickled Asparagus	N	1.17	3.36	3.68	3.7 ± 0.1	1.5 ± 0.2
Pickled Baby Corn	N	1.11	2.16	2.36	3.5 ± 0.2	1.8 ± 0.1
Pickled Beets	A	0.88	8.44	7.06	3.6 ± 0.1	0.9 ± 0.1
Pickled Beets	H	1.30	8.18	5.81	3.8 ± 0	1.2 ± 0.1
Pickled Brussels Sprouts	H	1.33	0.99	0.80	3.5 ± 0.1	2.2 ± 0.2
Pickled Brussels Sprouts	O	1.17	1.17	1.03	4.1 ± 0.1	1.3 ± 0.1
Red Cabbage	A	0.66	11.0	9.12	3.6 ± 0	1.2 ± 0.2
Pickled Capers	D	0.83	0.07	bdl	3.2 ± 0.1	2.4 ± 0.1
Cayenne Carrots	O	1.30	1.34	1.11	3.7 ± 0.1	1.9 ± 0.3
Hot Cauliflower	I	0.69	0.18	0.12	3.1 ± 0	3.7 ± 0.2
Sweet Cauliflower	H	0.98	14.8	6.56	3.8 ± 0.1	1.0 ± 0.1
Petite Dill Pickles	L	0.72	0.19	0.20	3.7 ± 0.2	2.5 ± 0.1
Dill Pickled Spears	L	0.82	0.87	0.95	3.6 ± 0.1	2.2 ± 0.3
Dill Pickled Spears	U	0.83	0.84	0.90	3.8 ± 0	1.9 ± 0.1
Pickled Ginger	V	1.19	bdl	bdl	2.7 ± 0	2.6 ± 0.1
Pickled Green Beans	H	1.19	2.77	2.86	3.9 ± 0.2	1.7 ± 0.1
Marinated Mushrooms	B	1.20	bdl	0.17	3.9 ± 0.2	3.1 ± 0.3
Marinated Mushrooms	H	0.66	bdl	bdl	3.6 ± 0.1	3.6 ± 0.6
Cocktail Onions	K	2.42	1.05	2.34	3.0 ± 0	4.2 ± 0.6
Hot Pepper Rings	E	2.86	0.67	0.59	3.1 ± 0.1	2.2 ± 0.4
Hot Cherry Peppers	C	2.19	0.43	0.38	3.6 ± 0.2	2.6 ± 0.1
Jalapeno Peppers	H	1.94	0.77	0.83	3.0 ± 0.1	3.5 ± 0.1
Pepper Rings	L	0.89	1.68	1.32	2.9 ± 0	3.1 ± 0.1
Sweet Cherry Peppers	K	3.01	0.50	0.42	3.0 ± 0.1	2.9 ± 0.1
Pickled Plums	V	1.89	1.23	1.18	3.4 ± 0.1	4.7 ± 0.2
Pickled Green Plums	P	0.47	0.66	0.16	1.9 ± 0	8.2 ± 0.4
Pickled Green Tomatoes	H	0.21	0.39	0.27	3.2 ± 0	4.2 ± 0
Malt Vinegar Walnuts	M	1.42	21.8	18.9	2.8 ± 0	3.5 ± 0.1
Pickled Watermelon Rinds	H	1.77	9.50	9.23	3.4 ± 0.2	1.2 ± 0.1

[a] Company code.
[b] Organic acid and sugar concentrations, in g/100 mL for brine, bdl = below the detection limit.
[c] Mean and standard deviation of two or more lots.

Table 2
Products containing lactic acid (fermented).

Acidified product name	Company[a]	Organic acids and sugars (g/100 mL)[b]				pH[c]	NaCl (%)[c]
		Lactic	Acetic	Glucose	Fructose		
Kimchi	S	1.2	0.4	bdl	bdl	3.7 ± 0.2	1.9 ± 0.1
Kalamata Olives	I	0.31	0.24	bdl	bdl	3.8 ± 0.1	4.7 ± 0.2
Kalamata Olives	K	0.13	0.97	bdl	bdl	3.4 ± 0.1	5.1 ± 0.4
Spanish Olives	E	0.87	0.2	bdl	bdl	3.2 ± 0.1	7.2 ± 0.3
Spanish Olives	F	0.78	0.2	bdl	bdl	3.4 ± 0.1	4.5 ± 0.2
Sauerkraut	R	1.31	0.33	0.17	bdl	3.2 ± 0.1	1.6 ± 0.2
Sauerkraut	J	1.26	0.47	0.16	0.15	3.3 ± 0	2.3 ± 0.3
Sauerkraut	H	1.69	1.33	0.14	bdl	3.2 ± 0	2.1 ± 0.5
Pickled Garlic[d]	N	0.5	0.56	0.16	0.46	2.8 ± 0.1	3.4 ± 0.2
Pickled Okra[d]	T	0.1	1.28	0.86	0.87	3.6 ± 0.1	3.6 ± 0.6
Pickled Turnips[d]	W	0.07	0.24	1.28	0.8	3.8 ± 0.1	4.1 ± 0.6

[a] Company code.
[b] Organic acid and sugar concentrations in g/100 mL, bdl = below the detection limit.
[c] Mean and standard deviation of two or more lots.
[d] These products had lactic acid but may not have been fermented.

ethylenediamine solution was prepared by dissolving 0.15 g of the reagent in 6.0 mL hot distilled water in 50 mL of a 15% (v/v) acetic acid solution. Griess reagent was prepared weekly and stored protected from light at 4 °C. Linearity was obtained between 0.5 and 4.0 mg/L of nitrite and absorbance values of 0.1–0.8 (slope = 0.1809, $R^2 = 0.999$). The limit of detection for nitrite was 0.5 mg/L.

For the nitrate analysis and calibration curve, the principle of the method included reduction of nitrate to nitrite with cadmium acetate solution and zinc powder, followed by colorimetric determination of nitrite with Griess reagent, as described above (Ozdestan & Uren, 2010). A 10-mL aliquot of the sample extract or diluted extract was transferred into a 50-mL tube and mixed with 0.4 mL of a 25% ammonium hydroxide solution, then 100 mg zinc powder was added. The suspension was mixed by vortexing and 0.2 mL of 5% (w/v) cadmium acetate solution was added to the sample. After vigorous shaking, the sample was allowed to stand at room temperature for 5 min, was filtered through a syringe filter (0.45 μm). The filtrate was used for nitrite analysis. Reported nitrate concentrations were based on measurements with standard nitrate solutions (0.5–8.0 mg/L). Linearity was obtained between 0.5 and 8.0 mg/L of nitrate and absorbance values of 0.07–0.90 (slope = 0.102, $R^2 = 0.999$). The lower limit of quantitation for nitrate was 1.0 mg/L, and the recovery of nitrate was approximately 100% for concentrations between 1 and 100 mg/L.

2.3. Determination of total phenols

Total phenolic content was estimated using the Folin-Ciocalteu reagent and an Oasis HLB cartridge (Waters, Milford, Mass., U.S.A.) to eliminate the interference of water-soluble reducing compounds (George, Brat, Alter, & Amiot, 2005). Five-gram samples were homogenized in 30 mL of 70% acetone in water (v/v). The slurry was centrifuged at 5000 × g for 30 min at 4 °C (Thermo Scientific, Sorvall legend XTR, Germany), then 1 mL of supernatant was added to 9 mL of distilled water to reduce the proportion of acetone to 7%, the diluted supernatant was filtered (Whatman filter paper, No. 2) under vacuum. The filtrate constituted the raw extract. Separation of interferences was then performed by loading an aliquot (2 mL) of raw extract onto an Oasis HLB cartridge installed on an extraction manifold (Vac Elut and washing cartridge, Agilent, Santa Clara, Calif., U.S.A.) and washed with 2 × 2 mL deionized water. The used cartridge was regenerated by washing three times with 2 mL methanol and two times with 2 mL de-ionized water, and reused five times.

A 0.5-mL sample of untreated or prepared extract or was added to 2.5 mL Folin-Ciocalteu reagent (0.2 N, water-diluted reagent from 2 N Folin-Ciocalteu reagent, used on the day of preparation). After incubation at room temperature for 2 min, 2.0 mL of sodium carbonate solution (7.5%) was added. The solution was incubated at 50 °C for 15 min in a water bath (ISOTEMP, Fisher Scientific) and then cooled on ice. The absorbance of the reaction solution was measured at 760 nm using a spectrophotometer (Pharmacia LKB Novaspec II, Pharmacia, England). Total phenolic content was estimated by subtracting gallic acid equivalents (untreated minus prepared extract values). Results were expressed as mg gallic acid/100 g fresh pickled vegetable or fruit, based on a calibration curve with 2.0–32.0 mg/L of gallic acid and absorbance values of 0.019–0.294 (slope = 0.0092, $R^2 = 0.999$). The recovery of gallic acid was approximately 100% for concentrations between 2 and 16 mg/L.

2.4. Determination of L-ascorbic acid

The extraction and determination of AA in pickled products were performed as described by Klimczak and Gliszczynska-Swiglo (2015) and Grace et al. (2014), respectively, with some modifications. Fermented or acidified fruits and vegetables (5.0 g) were homogenized in 30 mL of 10% *meta*-phosphoric acid (on ice), and the slurry was centrifuged at 5000 × g for 30 min at 4 °C (Thermo Scientific, Sorvall legend XTR centrifuge, Germany). The supernatant was filtered using a syringe filter (0.45 μm), and the eluent was analyzed by high-performance liquid chromatography (HPLC, Shimadzu LC-20 series) using a Synergi Polar reverse-phase column (Phenomenex, 250 × 4.6 mm, 4 micron). The isocratic mobile phase consisted of 2.5 mM H_2SO_4 with a flow rate of 0.3 mL/min, and an injection volume of 10 μL. AA was determined by measuring absorbance at 242 nm using a photodiode array detector (SPD-20MA, Shimadzu). Quantification of AA was done using an external calibration curve with standards ranging from 1.0 to 32.0 mg/L in deionized water (slope = 8556.4, $R^2 = 0.999$). Validation of the method was performed by addition of AA to the brine prior to analysis. The recovery was approximately 100% with added AA (8 and 32 mg/L), while it was 85.1% at a concentration of 2 mg/L. Using this method, an L-ascorbic acid content of less than 8 mg/L would be underestimated.

2.5. Quantification of organic acids and sugars

Organic acids and sugars were quantified for one jar from one lot of each product. A representative sample was prepared by homogenization in water, centrifugation and filtration (0.45 μm). Quantification of glucose, fructose, D/L-lactic acid, and acetic acid was conducted as described by McFeeters and Barish (2003) with minor modifications. Briefly, the high performance liquid chromatography (HPLC) system (Shimadzu Scientific Instruments, Columbia, Md., U.S.A.) consisted of a model LC-20AD pump, with a SIL-20AC HT autosampler and a Shimadzu column oven, a Bio-Rad HPX-87H column (300 mm × 7.8 mm; Bio-Rad, Hercules, Calif., U.S.A.) with a refractive index detector (RID-10A, Shimadzu) and a UV detector (SPD-20A, Shimadzu) in series for detection of the sugars and acids, respectively. Operating conditions of the system included a sample tray at 4 °C, a column temperature of 65 °C, and a mobile phase of 0.01 N sulfuric acid at a flow rate of 0.9 mL/min. Shimadzu LabSolutions software (V 5.71 SP1) was used to monitor the system and analyze the data, using peak heights for quantification, based on an 8- point calibration curve with authentic standards between 0.1 and 100 mM concentrations. Concentrations were reported as g/100 mL based on brine samples from pickled fruit and vegetable products.

2.6. Measurement of pH and sodium chloride (NaCl)

Measurement of brine pH was performed at ambient temperature using an Accumet AR25 pH meter (Fisher Scientific), which was calibrated with standards of pH 2.00, 4.00, and 7.00 (Fisher Scientific). NaCl concentration in brine was determined by chloride ion titration using 0.171 N silver nitrate titrant and 0.5% fluorescein dye indicator as previously described for pickle products (Fleming, McFeeters, & Breidt, 2001).

2.7. Data analysis

For most products, samples with 3 independent lot codes were obtained, and data were expressed as the mean value with standard deviation. Molar ratios of AA or total phenols to *in vivo* nitrite (N_i) were calculated based on the estimated *in vivo* nitrite concentrations of 6.5% of the mean nitrate for all lots of a given product (Habermeyer et al., 2015). This method was based on the estimated *in vivo* reduction of nitrate to nitrite following consumption of products containing nitrate. One-way analysis of variance (ANOVA) was performed for nitrate, nitrite, AA, and total phenols measurements modeled with vegetable commodity, followed by a Tukey's HSD test for separation of means (JMP version 12.0.1, SAS Institute, Cary, N.C., U.S.A.).

3. Results

3.1. Composition of products

Acidified and fermented products subjected to chemical analysis are shown in Tables 1 and 2 (respectively). The pH of pickled fruit and vegetable products ranged from 1.9 to 4.1 (Tables 1 and 2), with a mean pH of approximately 3.4. Most products (35) had pH values above pH 3.0. NaCl concentrations ranged widely from 0.9% (162 mM, pickled beets) to 8.2% (1.4 M, pickled green plums). The mean NaCl concentration was approximately 2.9%, with most products (33) containing 1.5%–4.5% NaCl. The four different olive products, and two pickled plum products had the highest NaCl concentrations, being the only products with NaCl concentrations of 4.5% or above.

A survey of organic acid and sugar contents of selected products used in this study showed that most products were acidified, containing acetic acid concentrations greater than 0.13 g/100 mL with no detectable lactic acid (Table 1). Several products, including cocktail onions and pickled peppers, had acetic acid concentrations over 2 g/100 mL. Alternatively, artichoke hearts and Spanish olives (Table 2) had the lowest acetic acid concentrations (between 0.1 and 0.2 g/100 mL), but they may have contained additional inorganic or organic acid acidulents, such as phosphoric or citric acids, which were not analyzed. Sweetened pickle products with glucose or fructose concentrations exceeding 8 g/100 mL included pickled beets, red cabbage, sweet cauliflower, malt vinegar walnuts, and watermelon rinds. Several products had little or no detectable glucose or fructose, including pickled ginger, mushrooms, and capers. Traditionally fermented products, including kimchi, sauerkraut, and several types of fermented olives, had lactic acid concentrations between 0.13 g/100 mL for Kalamata olives and 1.69 g/100 mL for sauerkraut (Table 2). Pickled okra and turnips contained low levels of lactic acid (<0.11 g/100 mL), and may have been acidified products that had undergone some degree of fermentation during processing or storage (Table 2).

3.2. Nitrate and nitrite

Nitrite was only detected in six of the 46 pickled fruit and vegetable products analyzed (producer codes in parenthesis), including green beans (H), red cabbage (A), kimchi (S), pickled beets (H), marinated mushrooms (B), and Greek olives (I), with concentrations of 0.1, 0.2, 1.4, 0.6, 0.2, and 0.3 mg/100 g, respectively (data not shown). The majority of pickled vegetables (39) had nitrate concentrations below 25 mg/100 g (Tables 3 and 4). Products having nitrate in excess of 25 mg/100 g included fermented cabbage (sauerkraut and kimchi), pickled beets and turnips, with kimchi having approximately 122 mg/100 g. In contrast, other fermented products, namely the fermented olives, Kalamata olives (companies I and K) and Spanish olives (companies E and F), had relatively low levels of nitrate (0.9, 0.9, 0.6, and 1.9 mg/100 g), respectively. Several acidified vegetable products had nitrate levels between 10 and 21 mg/100 g, including dill pickle spears, pickled red cabbage, sweet cherry peppers, dill pickle halves, pickled green beans, and pickled watermelon rind. The differences in nitrate content among pickled vegetables (Table 5) were largely attributable to the raw vegetable commodities used for the product, based on published data (Blekkenhorst et al., 2017). An ANOVA of nitrate content modeled with vegetable type showed that the differences in products' nitrate contents were significantly different across commodities ($p < 0.001$), and 87% of the variability in nitrate levels across several products from multiple producers could be explained by a linear model of the raw commodity used as the main ingredient in each pickled vegetable. In general, the nitrate contents of pickled vegetables were lower than those of the median literature values for the corresponding raw commodities.

3.3. L-ascorbic acid and total phenols

AA was detected in all products except pickled beets (H), and ranged from an estimated 0.1 mg/100 g for pickled ginger, to over 20 mg/100 g for some pickled pepper products, including banana peppers, sweet pepper rings, and over 30 mg/100 g for hot cherry peppers (L), malt vinegar walnuts and pickled plums (Tables 3 and 4). It is interesting to note that other pepper products, including hot and sweet cherry peppers and Jalapeno peppers (producers C, K, and H, respectively) had much lower AA concentrations (3.9 mg/100 g to 4.4 mg/100 g) than the pepper products mentioned above. Products with the highest concentrations were malt vinegar walnuts (M) and pickled green plums (P), which both had approximately 32 mg/100 g. Total phenols (TP) were determined on all products, and varied widely from less than 2 mg/100 g for dill pickle spears and cocktail onions to greater than 100 mg/100 g for malt vinegar walnuts and Kalamata olives (Tables 3 and 4). Pickled artichoke hearts had the highest total phenol measurements with approximately 225 mg/100 g. However, most products (39 of 46) had total phenols below 50 mg/100 g, with a mean value of 30.3 mg/100 g.

3.4. Antioxidant/in vivo nitrite ratios

The concentration of predicted *in vivo* nitrite (N_i), was estimated as described by Habermeyer et al. (2015), and was used to calculate the ratio of AA and TP to N_i (Tables 3 and 4). Products having high AA or TP to N_i ratios included pickled artichoke hearts, which had a ratio of 18.1 for AA/N_i, and 3142.0 for TP/N_i. Hot cherry peppers (L) had ratios of 1241.0 and 234.1 for AA/N_i and TP/N_i, respectively. Kalamata olives, Spanish olives, pickled plums, pickled asparagus, baby corn, and malt vinegar walnuts all had AA or TP to N_i ratios over 100, principally because of low (<2 mg/100 g) nitrate levels. Products having low AA or TP to N_i ratios (<2) included kimchi, pickled beets, cucumber pickle halves and spears, pickled watermelon rinds, and pickled turnips. The high nitrate content in kimchi, pickled beets, and pickled turnips contributed to the low AA and TP to N_i ratios of these products, whereas the low AA and TP contents of large-sized pickled cucumbers (halves, G) and pickled watermelon rind were responsible for their low AA and TP to N_i ratios.

4. Discussion

The content of nitrate was lower than 10 mg/100 g in 32 of the 46 products tested, and was higher than 10 mg/100 g in 14 products. This includes four products (two beet products, turnip, and kimchi) that contained nitrate over 50 mg/100 g. Nitrate concentrations within product types had large variances, which were likely due to variations in products produced from different batches of raw materials. The nitrate content in pickled products was generally lower than that reported for fresh fruits and vegetables (Zhou, Wang, M. J., & Wang, 2000; Zhong, Hu, & Wang, 2002; Gorenjak & Cencic, 2013; Blekkenhorst et al., 2017). Ji et al. (2009) showed that both nitrate and nitrite contents decreased as pH decreased in cabbage during fermentation. Our data showed no correlation between nitrate levels and pH of finished products (data not shown). Besides cabbage, most other pickled fruits and vegetables are brined during preservation or in finished product processing, which has the potential to dilute the nitrate content of the finished products by 30–70% compared to raw vegetable content. This is consistent with observations of nitrate levels in canned vegetables that were not acidified (Siciliano et al., 1975). Losses in nitrate content during boiling of various vegetables ranged from 13 to 79% (Bakr et al., 1986), and a previous study of canned carrots showed that about 64% of nitrate content was lost due to both heat treatment and subsequent equilibration of the remaining nitrate with the cover liquid (Schuster & Lee, 1987). Acidified, shelf-stable, pickle products are brined and then pasteurized, so a reduction in vegetable nitrate content would be expected in these products.

Nitrate can be reduced to nitrite by oral commensal bacteria. The recycling of endogenous nitrate and conversion to nitrite by oral bacteria plays an important role in blood plasma nitrite levels and, thereby, on physiological control of vascular NO homeostasis and blood pressure. With blood circulation, nitrate reaches the salivary glands and is actively transported into saliva.

Table 3
The contents of nitrate, L-ascorbic acid, and total phenols in acidified products.

Product Name	Co.[a]	N[b]	Concentration (mg/100 g)[c]			Ratios[d]	
			Nitrate	L-ascorbic acid (AA)	Total phenols (TP)	AA/N_i	TP/N_i
Artichoke Hearts	C	3	0.4 ± 0.2	1.4 ± 2.5	224.9 ± 31.5	18.1	3142.0
Pickled Asparagus	H	3	0.3 ± 0.03	4.2 ± 2.6	27.6 ± 2.7	75.0	518.0
Pickled Asparagus	N	3	0.4 ± 0.3	1.1 ± 1.0	30.6 ± 4.7	25.5	613.0
Pickled Baby Corn	N	3	0.3 ± 0.2	0.9 ± 0.8	18.0 ± 4.5	14.5	424.0
Pickled Beets	H	3	67.3 ± 26.0	ND	18.8 ± 0.7	<0.1	1.7
Pickled Beets	A	3	76.9 ± 37.2	0.3 ± 0.5	19.0 ± 2.1	<0.1	1.6
Pickled Brussels Sprouts	H	3	ND	12.9 ± 0.6	19.9 ± 1.4	NA	NA
Pickled Brussels Sprouts	O	3	2.3 ± 2.4	2.8 ± 1.2	46.9 ± 8.1	16.4	215.0
Pickled Red cabbage	A	3	11.4 ± 9.0	0.3 ± 0.4	57.6 ± 11.9	0.1	82.7
Pickled Capers	D	3	2.5 ± 0.8	0.5 ± 0.2	47.7 ± 15.4	1.1	112.0
Pickled Carrots	O	3	7.1 ± 6.6	0.8 ± 0.2	21.7 ± 4.5	0.9	32.9
Hot Cauliflower	I	3	0.7 ± 0.5	0.4 ± 0.1	3.6 ± 0.4	5.9	54.5
Sweet Cauliflower	H	2	6.3 ± 4.9	3.2 ± 3.0	9.7 ± 1.9	5.5	13.5
Petite Dill Pickles	U	3	1.4 ± 0.3	0.8 ± 0.9	2.7 ± 0.8	2.9	11.1
Petite Dill Pickles	L	3	4.6 ± 0.9	0.6 ± 0.3	4.3 ± 1.5	0.7	5.5
Dill Pickled Halves	G	3	15.3 ± 2.2	0.8 ± 0.1	3.1 ± 0.8	0.3	1.1
Dill Pickled Spears	U	3	10.2 ± 1.3	0.3 ± 0.5	1.4 ± 0.2	0.2	0.8
Dill Pickled Spears	L	3	20.9 ± 2.9	0.4 ± 0.2	1.8 ± 0.7	0.1	0.5
Pickled Ginger	V	3	4.9 ± 1.6	0.1 ± 0.1	20.8 ± 1.6	0.1	26.4
Pickled Green Beans	H	3	17.9 ± 13.4	0.5 ± 0.9	9.9 ± 0.5	0.1	7.2
Marinated Mushrooms	B	3	3.8 ± 2.3	2.5 ± 1.4	5.9 ± 0.6	5.0	11.2
Marinated Mushrooms	H	3	2.9 ± 0.3	1.8 ± 1.3	4.1 ± 0.9	3.2	8.0
Cocktail Onions	K	3	2.1 ± 3.4	0.7 ± 0.1	1.5 ± 0.7	19.5	33.4
Hot Cherry Peppers (Green)	C	3	1.5 ± 0.7	3.9 ± 5.0	12.5 ± 2.2	15.7	52.8
Hot Cherry Peppers (Red)	L	3	0.1 ± 0.1	31.8 ± 10.5	8.4 ± 3.5	1241.0	2341.0
Jalapeno Peppers	H	3	6.5 ± 2.7	4.3 ± 3.7	6.3 ± 1.0	4.1	6.1
Banana Peppers	L	3	1.0 ± 0.8	21.7 ± 4.0	13.9 ± 2.0	161.0	123.0
Hot Pepper Rings	E	3	4.6 ± 0.9	16.1 ± 4.1	10.1 ± 3.1	19.8	12.7
Sweet Pepper Rings	L	3	2.2 ± 1.5	28.4 ± 3.1	12.5 ± 1.8	101.0	43.5
Sweet Cherry Peppers	K	3	14.8 ± 4.1	4.4 ± 3.7	18.5 ± 4.4	2.0	7.8
Pickled Green Plums	P	2	1.5 ± 0.02	32.4 ± 11.6	18.4 ± 1.3	117.0	68.9
Pickled Plums	V	2	0.2 ± 0.05	0.4 ± 0.1	26.5 ± 3.4	10.4	668.0
Pickled Green Tomatoes	H	2	1.1 ± 0.1	1.3 ± 0.6	8.2 ± 0.7	6.2	42.5
Malt Vinegar Walnuts	M	2	1.9 ± 1.0	32.1 ± 37.8	103.8 ± 15.8	74.1	350.0
Pickled Watermelon Rinds	H	2	19.9 ± 1.5	0.2 ± 0.3	3.0 ± 0.04	0.1	0.9

[a] Company code.
[b] Number of product lots examined.
[c] Concentration of analyte in mg/100 g of nitrate, L-ascorbic acid (AA), total phenols (TP); ND, not detected.
[d] Ratios of antioxidant concentration (AA and TP) to estimates of *in vivo* nitrite (N_i), where N_i represents the calculated *in vivo* nitrite estimated as 6.5% of nitrate content on a molar basis. NA is not applicable.

Table 4
The contents of nitrate, L-ascorbic acid, and total phenols in fermented products.

Product Name	Co.[a]	N[b]	Concentration (mg/100 g)[c]			Ratios[d]	
			Nitrate	L-ascorbic acid (AA)	Total phenols (TP)	AA/N_i	TP/N_i
Kimchi	S	3	121.9 ± 36.8	2.3 ± 0.1	20.9 ± 1.6	0.1	1.0
Kalamata Olives	I	3	0.9 ± 0.3	0.8 ± 0.1	169.8 ± 7.9	5.1	1063.0
Kalamata Olives	K	3	0.9 ± 0.5	0.7 ± 0.1	133.7 ± 25.1	4.6	1002.0
Spanish Olives	E	3	0.6 ± 0.1	1.2 ± 0.2	83.9 ± 5.2	11.4	820.0
Spanish Olives	F	3	1.9 ± 0.5	1.6 ± 0.4	83.1 ± 9.5	4.5	249.0
Sauerkraut	H	3	26.9 ± 14.2	7.2 ± 4.1	5.9 ± 0.7	1.5	1.5
Sauerkraut	R	3	30.1 ± 3.7	9.8 ± 6.9	5.8 ± 0.5	1.7	1.1
Sauerkraut	J	3	47.2 ± 16.3	11.2 ± 4.9	5.4 ± 0.9	1.6	0.7
Pickled Garlic	N	3	1.6 ± 0.3	0.7 ± 0.1	2.9 ± 0.3	2.5	10.5
Pickled Okra	T	3	6.6 ± 2.8	1.6 ± 0.6	34.1 ± 2.9	1.5	32.0
Turnips	W	2	64.5 ± 24.9	3.1 ± 0.6	7.1 ± 1.8	0.3	0.7

[a] Company code.
[b] Number of product lots examined.
[c] Concentration of analyte in mg/100 g of nitrate, L-ascorbic acid (AA), and total phenols (TP).
[d] Ratios of antioxidant concentration (AA and TP) to estimates of *in vivo* nitrite (N_i), where N_i represents the calculated *in vivo* nitrite estimated as 6.5% of nitrate content on a molar basis.

Approximately 25% of the nitrate originally ingested is secreted through the salivary glands, and 6–7% of the total nitrate is converted to nitrite (Habermeyer et al., 2015). Therefore, 6.5% of the total food nitrate may be used for calculation of the predicted *in vivo* nitrite produced by reduction of dietary nitrate, and the ratios of AA to reduced nitrite and total phenols to reduced nitrite can be used to assess the effects of nitrate in pickled products on human health.

Table 5
Nitrate contents of pickled vegetable products by commodity.

Vegetable	# Samples	Product Type	Nitrate[a] (mg/100 g)	Raw commodity nitrate content[b]		
				Median (mg/100 g)	IQR (mg/100 g)	N_{obs}/N_{median}[c]
Napa Cabbage	3	Fermented	121.9 ± 36.8 a	104	86–143	1.2
Beet	6	Acidified	72.1 ± 29.2 b	143	91–192	0.5
Turnip	2	Fermented[d]	64.5 ± 24.9 bc	66	38–199	1.0
Cabbage	9	Fermented	28.9 ± 16.7 cd	43	22–80	0.7
Watermelon Rind	2	Acidified	19.9 ± 1.5 de	NA	NA	NA
Bean, Garden	3	Acidified	18.0 ± 13.4 de	34	16–49	0.5
Red Cabbage	3	Acidified	11.4 ± 9.0 de	NA	NA	NA
Cucumber	15	Acidified	10.5 ± 7.4 e	19	7.2–34	0.6
Carrot	3	Acidified	7.1 ± 6.6 e	15	7.0–27	0.5
Okra	3	Acidified	6.6 ± 2.8 e	11	4.0–25	0.6
Ginger	3	Acidified	4.9 ± 1.6 e	130	51–203	0.0
Pepper	20	Acidified	4.1 ± 5.1 e	9.5	4.3–20	0.4
Mushroom	6	Acidified	3.4 ± 1.5 e	2.2	0.8–4.9	1.5
Cauliflower	5	Acidified	2.9 ± 3.9 e	17	8.7–32	0.2
Capers	3	Acidified	2.5 ± 0.8 e	NA	NA	NA
Onion	3	Acidified	2.1 ± 3.4 e	7.7	3.1–11	0.3
Walnut	2	Acidified	1.9 ± 1.0 e	NA	NA	NA
Garlic	3	Fermented[d]	1.6 ± 0.3 e	12	6.9–23	0.1
Green Plum	2	Acidified	1.5 ± 0.02 e	NA	NA	NA
Brussel Sprouts	6	Acidified	1.1 ± 2.0 e	3.2	2.3–6.5	0.3
Olive	12	Fermented	1.1 ± 0.6 e	NA	NA	NA
Tomato	2	Acidified	1.1 ± 0.2 e	6.0	2.5–19.3	0.2
Plum	2	Acidified	0.2 ± 0.05 e	NA	NA	NA
Artichoke	3	Acidified	0.4 ± 0.2 e	NA	NA	NA
Asparagus	6	Acidified	0.3 ± 0.2 e	8.3	1.8–14	0.0
Corn	3	Acidified	0.3 ± 0.2 e	1.2	0.6–82	0.3

[a] Nitrate content (average ± standard deviation) of pickled fruit and vegetable products by commodity. Different lowercase letters represent statistically different means using an ANOVA by commodity followed by a *t*-test for comparison of means.
[b] Median and interquartile range (IQR) values for nitrate content of raw vegetables (Blekkenhorst et al., 2017).
[c] Ratio of nitrate content of pickled vegetables (N_{obs}) to the median nitrate content of the corresponding raw vegetable commodity (N_{median}).
[d] These products contained lactic acid, however it is unclear if they were fermented.

When nitrite is consumed, it may survive passage through the stomach and enter the circulatory system. Under acidic gastric conditions or in blood and tissue, a variety of reactive nitrogen oxide species, most of which are highly bioactive, are formed from nitrite by 3 redox-dependent pathways: nitrosation reactions, nitration reactions, and NO formation. Nitrosamines, which are suspected to be carcinogenic, can be formed by nitrosation reactions if there are secondary amines present in the stomach. However, in the presence of dietary AA the generation of NO from nitrite is greatly enhanced, and the formation of nitrosamines is significantly inhibited (Weitzberg & Lundberg, 2013). In model systems, the process of nitrosamine formation was completely blocked when the molar ratio of AA to nitrite was greater than 2:1. This ratio was used to assess whether nitrite or nitrate content in vegetables may be considered harmful to human health (Chung, Chou, & Hwang, 2004; Fan & Tannenba, 1973; Gray & Dugan, 1975).

In most of the fermented and acidified products in this study, the content of AA was low: only five products had concentrations greater than 20 mg/100 g. L-ascorbic acid has been shown to decrease during cabbage fermentation (Martinez-Villaluenga et al., 2009), and it is generally known to be unstable during processing and storage in acid or acidified foods. Nonetheless, 27 of 46 pickled fruit and vegetable products had molar ratios of AA to predicted *in vivo* nitrite (AA to N_i) greater than 2. Dietary polyphenols may enhance reduction of nitrite, leading to the production of NO, and were proposed to modulate redox signaling pathways (Rocha et al., 2014). The protection *in vivo* against genotoxic damage induced by endogenous nitrosation was evidently improved by combinations of AA plus dietary polyphenols (Abraham & Khandelwal, 2013). It is possible that hypoxia resulting from the reaction between nitrite and hemoglobin may be reduced by the improved production of NO from nitrite. Although further research is needed, these results indicate that the ratios of AA to nitrate and total phenols to nitrate in pickled vegetables and fruits may influence the beneficial effects of nitrite and nitrate on human health.

In this study, 34 of the 46 pickled vegetables had AA to N_i or TP to N_i ratios greater than 2, suggesting that this product category, may have a relatively low risk of nitrosamine formation. If both AA and TP are considered together, the three sauerkraut products also fall into this category. Interestingly, the kimchi in this study had a higher nitrate content and lower AA to N_i ratio, but similar TP to N_i ratios than sauerkraut. Both products are made from fermented cabbage, but vary in type of cabbage, ingredients, and processing methods. Since the quantities of nitrate and antioxidant compounds can vary widely among cabbages, research to determine whether different fermentation processes influence the composition of nitrate and antioxidants may help to improve AA or TP to N_i ratios.

5. Conclusions

Pickled fruits and vegetables had wide ranges of nitrite and nitrate contents. Among the 46 pickled products, nitrite was only detected in low levels (<1.5 mg/100 g) in six products, while nitrate was present in most products at levels that were consistent with the primary vegetable in the pickled product. Of the products studied, ratios of antioxidants (AA or TP) to N_i were greater than one for 42 of 46 (91%) of products. Approximately 80% of products had AA or TP to N_i ratios (>2) known to be sufficient to prevent nitrosamine formation. Products with very high (>50) AA or TP to predicted N_i ratios, included pickled artichokes, pickled red cherry pepper, banana pepper, Brussels sprouts, Spanish olives, plums, green cherry peppers, asparagus, and Kalamata olives. Although the specific health effects of dietary nitrate remain unclear, these data will be useful for estimating intakes of nitrate and antioxidants from processed vegetables to support future studies on nitrate and human health.

Author contributions

Ding and Breidt formulated the initial plan for the study, and the experimental design was developed by Ding, Truong, Johanningsmeier, and Breidt. Ding conducted the analyses of nitrate, nitrite, NaCl, pH, and antioxidants, with laboratory assistance from Price, Reynolds, and Conley Payton. Price did the HPLC analysis, with assistance from Reynolds, Johanningsmeier, and Conley Payton. Breidt, Johanningsmeier, Ding, and Truong contributed to writing the manuscript, with all authors assisting in interpreting the results and reviewing the manuscript.

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