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Nitrite level of pickled vegetables in Northeast China

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ABSTRACT

Nitrite is commonly present in pickled vegetables. Nitrite has been proven to have adverse effects on health, including changing the normal form of hemoglobin and the formation of carcinogenic nitrosamines. The aim of our study was to determine the levels of nitrite in pickled vegetable samples consumed in Harbin, China. In this study, 218 samples of pickled turnip (PT) ($n = 42$, packaged (PK) = 19; unpackaged (UPK) = 23), pickled tuber mustard (PTM) ($n = 44$, PK = 23; UPK = 21), pickled cucumber (PCC) ($n = 48$, PK = 25; UPK = 23), and pickled cabbage (PCB) ($n = 84$, PK = 39; UPK = 45) were analyzed between October 2011 and December 2011, using colorimetric nitrite assay based on the Griess reaction. A nitrite level of <5 mg/kg was normally predominant in 147 (67%) samples of the investigated pickled vegetables, ranging from 0.01 mg/kg to 42.03 mg/kg, while a nitrite level of >20 mg/kg was detected in 9 (4%) samples. The overall nitrite content was 4.02 ± 0.62 mg/kg for PT, 4.52 ± 1.07 mg/kg for PTM, 3.91 ± 0.69 mg/kg for PCC, and 4.86 ± 0.80 mg/kg for PCB. The content of nitrite in unpackaged pickled vegetables (PT, 5.47 ± 0.89; PTM, 6.84 ± 2.02; PCC, 5.32 ± 1.27; PCB, 6.41 ± 1.28; and total, 6.08 ± 0.71 mg/kg) was significantly higher than those in packaged products (PT, 2.68 ± 0.72; PTM, 2.30 ± 0.55; PCC, 2.62 ± 0.54; PCB, 3.08 ± 0.81; and total, 2.73 ± 0.36 mg/kg) ($P < 0.05$). The variance in the concentration of nitrite in the pickled vegetables probably resulted from differences in quality, processing technique, and storage condition of the pickled vegetables. Our results indicated that, in China, low level nitrite is widely present in pickled vegetables. It is therefore important to assess the content of nitrite in pickled vegetables in China.

1. Introduction

Excessive quantities of nitrite and indirect nitrate consumption through food can be harmful to health (Chan, 2011; Leszczyńska, Filipiak-Florkiewicz, Cieślik, & Sikora, 2009). Nitrate may be endogenously transformed into nitrite which in turn can react with amines and amides to produce N-nitroso compounds (Santamaria, 2006; Yordanov, Novakova, & Lubenova, 2001). These have been related to an increased risk of gastric, esophageal, nasopharyngeal and bladder cancers (Choi, Chung, Lee, Shin, & Sung, 2007). Especially, the pickled vegetables have been included in the WHO list of possible carcinogens (WHO, 2012).

Methemoglobinemia is another health hazard attributed to nitrite, which is a condition where reduced iron (Fe^{2+}) in hemoglobin is oxidized by nitrite to become Fe^{3+}, thus reducing the total oxygen-carrying capacity of the blood (Greer, Shannon, American Academy of Pediatrics Committee on Nutrition, & American Academy of Pediatrics Committee on Environmental Health, 2005; Santamaria, 2006).

Nitrate and nitrite are natural constituents of plant materials (Correia et al., 2010; EFSA, 2008). Nitrate concentrations in vegetables depend on the biological properties of the plant culture, (day)light intensity, type of soil, temperature, humidity, frequency of plants in the field, plant maturity, vegetation period, harvesting time, size of the vegetable unit, storage time and source of nitrogen (Tamme et al., 2006). Even among different samples of the same vegetable varieties, the nitrate concentration may vary in a wide range (Prasad & Chetty, 2008; Tamme et al., 2006; Thomson, Nokes, & Cressey, 2007). In addition, cooking techniques may affect the final levels of nitrate and nitrite (Amal, 2000; Kmiecik, Lisiewska, & Słupski, 2004; Leszczyńska et al., 2009).

The pickled vegetable is one of the traditional fermented vegetables in China, especially in northern China. Pickled vegetables are popular food items in China (Li et al., 2011; Yan, Xue, Tan, Zhang, & Chang, 2008). The process is that the fresh vegetable is

Abbreviations: PT, Pickled turnip; PTM, pickled tuber mustard; PCC, pickled cucumber; PCB, pickled cabbage; PK, Packaged; UPK, Unpackaged.

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washed with tap water and drained, then immersed in 6–8% NaCl (W/v) in earthernware pots in layers and airproofed with water. However, the accumulation of nitrite is a common problem during vegetable fermentations (Toshirou, 2001; Yan et al., 2008). During fermentation, nitrate present in the plant tissue is converted to nitrite. The concentration of nitrite rises over time to reach a peak value, and then decreases (Park & Cheigh, 1992; Spoelstra, 1985). Seel et al. (1994) reported that a potential link for the high gastric cancer rate in southwest Korea was associated with the high regular consumption of salted pickled cabbage and salted seafood sauce. In addition, Li et al. (2011) also reported that the risk of neural tube defects in baby was positively associated with the frequency of the consumption of pickled vegetables by the mother. The World Health Organization has listed pickled vegetable as a possible carcinogen. For this reason, the measurement of nitrite content in pickled vegetables has received increasing attention. In the present study, we analyzed the content of nitrite in pickled vegetables readily available in China.

2. Materials and methods

2.1. Samples

Samples of pickled turnip (PT), pickled tuber mustard (PTM), pickled cucumber (PCC), and pickled cabbage (PCB) were purchased from 6 local supermarkets (packaged, $n = 78$; unpackaged, $n = 45$) and 8 wholesale stores (packaged, $n = 28$; unpackaged, $n = 67$) in Harbin, China. Of the total 218 pickled vegetable samples (packaged, $n = 106$; unpackaged, $n = 112$), 42 were PT (Packaged, $n = 19$; Unpackaged, $n = 23$), 44 were PTM (Packaged, $n = 23$; Unpackaged, $n = 21$), 48 were PCC (Packaged, $n = 25$; Unpackaged, $n = 23$), and 84 were PCB (Packaged, $n = 39$; Unpackaged, $n = 45$). The packaged pickled vegetables were hermetically sealed within the plastic film and mainly kept under refrigerated storage, while the unpackaged products were exposed to air without any packaging materials. All pickled vegetables were stored at a refrigeration temperature and analyzed within 24 h. The samples were stored in dark bottles without headspace at room temperature for analysis.

2.2. Nitrite content of pickled vegetables

The nitrite content of the pickled vegetables was determined using colorimetric nitrite assay based on the Griess reaction (Ito, Yodoshi, Tanaka, & Iwaida, 1979) using a Shimadzu UV2401 spectrophotometer (Shimadzu Inc., Tokyo, Japan). In order to reduce the variation caused by the effect of the water content on the nitrite content of the samples, the same effect was made to the pickled products before the chemical analysis. Approximately 5 g pickled vegetable sample was crushed, deproteinated and defatted by precipitation with 10 mL $ZnSO_4$ (0.42 mol/L) followed by filtration. 1 mL each of the three color development reagents, 0.2% sulfanilamide, 0.1% *N*-1-naphtyethylene diamine dihydrochloride), and 44.5% HCl, was added sequentially to the filtrates. The mixtures were then kept at room temperature for 5 min. The OD value of the colored mixtures was read at 538 nm against the reagent blank. A standard curve of $NaNO_2$ solutions (0–2000 mg/L) was constructed, which was subjected to the similar color development and OD measurement. The nitrite analysis was repeated three times for each prepared vegetable sample. All reagents were analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

2.3. Statistical analysis

All values are shown as the means and standard deviations for three replicates. The statistical analyses were performed using the ANOVA function of SPSS 13.0 for Windows software. Statistical significance was determined as $P < 0.05$ using Student's *T*- test.

3. Results and discussion

Among 218 samples analyzed, a nitrite concentration of <5 mg/kg was detected in 147 samples (67%), which is normally predominant in the investigated pickled vegetables. In China, for trade in pickled vegetables, the limit of a maximum nitrite level of 20 mg/kg has been established (GB 2762-2005). The WHO has set the acceptable daily intake (ADI) limit for the nitrite ion at 0.06 mg/kg body weight (WHO, 1995, p. 29). In present study, the number of >20 mg/kg nitrite positive samples were 1 (2%) for PT, 2 (4%) for PTM, 1 (2%) for PCC, and 5 (6%) for PCB (Table 1). Similar results were reported by Correia et al. (2010) that the nitrite level was lower than 5 mg/kg in 70% of the analyzed samples and lower than 20 mg/kg in 90% of the total. In the present study, the nitrite concentrations observed in total PT, PTM, PCC and PCB were 4.02 ± 0.62, 4.52 ± 1.07, 3.91 ± 0.69, 4.86 ± 0.80 mg/kg, ranging between 0.25 and 20.18, 0.15–38.15, 0.14–25.36, and 0.01–42.03 mg/kg, respectively (Table 2). However, these values were higher than those reported for fresh vegetable samples (Menard, Heraud, Volatier, & Leblanc, 2008; Zhong, Hu, & Wang, 2002). Hence, fresh vegetables should be consumed as fresh produce anyway.

It is commonly assumed that the nitrite levels in fresh leafy vegetables are usually less than 2 mg/kg (Santamaria, 2006). It has been shown that nitrite concentrations in fresh, uninjured, well-stored vegetables are extremely low, possibly because the nitrite reductase activity is in equilibrium with one of the nitrate reductases under proper storage conditions (Chung, Chou, & Hwang, 2004). In addition, the accumulation of nitrite is a common problem for vegetable fermentation (Park & Cheigh, 1992; Spoelstra, 1985). During fermentation, nitrate present in the plant tissue is converted to nitrite. The concentration of nitrite rises over time to reach a peak value (after fermentation 3–5 days) and then decreases (Chung et al., 2004; Prasad & Chetty, 2008). During fermentation, the nitrite concentrations may increase dramatically via microbiological degradation of nitrate in vegetables while the nitrate content decreases during storage at ambient temperature. Under refrigerated storage, however, nitrite accumulation tends to be inhibited but may still occur (Chung et al., 2004; Prasad & Chetty, 2008). Therefore, a poor storage could result in bacterial growth which in turn can contribute to the increasing accumulation of high nitrite levels (Chung et al., 2004).

Table 1
Detection of nitrite in pickled vegetable samples.

Salted vegetable sample		Number of samples	Content of nitrite (mg/kg)			
			<5	5–10	10–20	>20
Pickled turnip	Packaged	19	16 (84%)	2 (11%)	1 (5%)	–
	Unpackaged	23	11 (48%)	10 (43%)	1 (4%)	1 (4%)
	Total	42	27 (64%)	12 (29%)	2 (5%)	1 (2%)
Pickled tuber mustard	Packaged	23	19 (83%)	4 (17%)	–	–
	Unpackaged	21	13 (62%)	4 (19%)	2 (10%)	2 (10%)
	Total	44	32 (72%)	8 (18%)	2 (5%)	2 (5%)
Pickled cucumber	Packaged	25	20 (80%)	5 (20%)	–	–
	Unpackaged	23	13 (57%)	8 (35%)	1 (4%)	1 (4%)
	Total	48	33 (69%)	13 (27%)	1 (2%)	1 (2%)
Pickled cabbage	Packaged	39	33 (85%)	3 (8%)	1 (3%)	2 (5%)
	Unpackaged	45	23 (51%)	16 (36%)	4 (9%)	3 (7%)
	Total	84	55 (65%)	19 (23%)	5 (6%)	5 (6%)
Total	Packaged	106	88 (83%)	14 (13%)	2 (2%)	2 (2%)
	Unpackaged	112	59 (53%)	38 (34%)	8 (7%)	7 (6%)
	Total	218	147 (67%)	52 (24%)	10 (5%)	9 (4%)