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Determination of Nitrate and Nitrite Residues in Smoked Caspian Kutum, *Rutilus frisii kutum* and Mullet, *Liza auratus* in the North of Iran

[1]L. Karimzadeh, [1]F. Koohdani, [2]M. Mahmodi, [3]F. Safari and [3]Z. Babaee

[1]Department of Nutrition and Biochemistry, [2]Department of Static,
School of Public Health and Institute of Public Health Research,
Tehran University of Medical Science, Tehran, Iran
[3]Mazandaran Food and Drug Organization,
Mazandaran University of Medical Science, Sari, Iran

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. In this study nitrate and nitrite residues in smoked Caspian kutum, *Rutilus frisii kutum* and mullet, *liza auratus* in the local markets (considered as batch no. 1, 2 and 3) of north of Iran were determined using Spectrophotometer-UV. In smoked Caspian kutum, nitrate content ranged from 12.3 to 19.5 mg/kg and nitrite was between 0 and 12.8 mg/kg. In this species batch No. 2 had the lowest levels of nitrite (2.2±0.3 mg/kg) compared to batch 1 and 3 ($P<0.05$) while nitrate values were approximately the same for three batches. The highest amount of nitrite was observed in batch number one of smoked mullets while in batch number 2 and 3 nitrite levels were lower than 1.9 mg/kg. Nitrate contents were between 9.8 and 25.2 mg/kg with batch No. 2 showed lower values ($P<0.05$) than the other samples. In conclusion, the present result has shown that smoked fish available in local markets present a wide range of nitrite and nitrate contents which is some samples was above the threshold tolerance that can be of risk concern for consumption.

Key words: Fish % Dry-salted % Mazandaran province

INTRODUCTION

Around 80 percent of human cancers are originated from environmental factors associated with food, water and air [1]. High nitrate intake from food has been reported to associate with gastric cancer in some countries [2] due to the formation of N-nitroso compounds in the stomach [3]. Gastric cancer is the third leading cause of cancer death in men after lung and prostate cancers and is the fourth leading cause of cancer death in women worldwide [4]. 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 [5]. Information on the nitrate and nitrite contents in fishery products in Iran is scare in the literature. Tolerance threshold for the sum of nitrite and nitrate is reported to be 20 mg/kg [6]. However due to increasing concern on N-nitroso compounds, monitoring of nitrate and nitrite contents in fishery products is necessary.

Smoking is one of the oldest methods of food preservation and is still widely used in fish processing. In Europe approximately 15% of the total fish production is offered on the market as smoked products [7]. Caspian kutum (*Rutilus frisii kutum*) and Mullet (*Liza auratus*) are two commercially important fish species exist in the southern waters of the Caspian Sea with high market acceptance. They are caught from the coastal waters and rivers mouth using beach seine, gill or cast nets during autumn to mid in spring [8] and consumed as fresh or smoked.

The aim of this study was to assess the concentrations of nitrate and nitrite residues in smoked products of Caspian kutum and mullet from the local markets in the north of Iran.

Corresponding Author: Farib Koohdani, Department of Nutrition and Biochemistry, School of Public Health and Institute of Public Health Research, Tehran University of Medical Science, Tehran, Iran

MATERIALS AND METHODS

Fish Samples: Smoked Caspian kutum (weight: 500-800 g) and mullets (weight: <400 g) were purchased at local markets of different cities (Mazandaran province, north of Iran) three times (from April to July, 2008). At each sampling time 7 smoked kutum and 7 mullets were purchased (total 42 samples). Smoked fish that sampled at first, second and the third sampling time was regarded as batch number 1, 2 and 3. Samples were kept at refrigeration temperature and analyzed within 24 hours.

Nitrate and Nitrite Analysis: Nitrite and nitrate contents were determined according to the method described by Zanardi *et al.* [9] using enzymatic method. In this method, nitrate is reduced to nitrite by NADPH in the presence of the enzyme nitrate reductase. 10g of minced and homogenized sample was placed into wide mouth Erlenmeyer-flask containing 50 ml redist water and homogenized for 30-60 second with Ultra-Turrax. The shaft of homogenizer was rinsed with 50 ml hot redisttalled water. Then 0.2 ml bromothymol blue solution was added and titrated with NaOH (1 M) until the color changed (but pH<8.5) and dincubated for 15 min in a bath with boiling water. The flask was cooled to 20-25°C and transferred the contents quantitatively into a 200 ml volumetric flask. Then 2 ml concentrated Carrez-I solution (15.0 g $K_4[Fe(CN)_6]\times 3H_2O$/100 ml) and 2 ml concentrated Carrez-II solution (30.0 g $ZnSO_4\times 7H_2O$/100 ml) were added one after the other and mixed. Then flask was filled up to the mark with redist. Water, mixed and filtrated. The first few ml of filtrate were discarded and clear filtrate was used for assay.

To determine the amount of nitrite, 2 ml of sample solution, 1 ml redistilled water and 3 ml color reagent were mixed in a tube. Color reagent was prepared daily by mixing 1.5 ml of sulfanilamide and 1.5 ml of N-(1-naphthyl)-ethylene-diammonium dichloride. It was then incubated at room temperature for 30 min. After that absorbance of the solution was read at 540 nm. Nirate was determined as follow: 2 ml of sample solution, 1 tablet of NADPH, 1ml buffer/enzyme solution were mixed and incubated at room temperature for 60 min. After that, 3ml of color reagent added and incubated at room temperature for 30 min and absorbance of the solution was read at 540 nm.

Statistical Analysis: Significant difference in nitrite and nitrate contents of different batches were analyzed using one-way analysis of variance (ANOVA) followed by Duncan multiple range test (P<0.05). All data are expressed as mean±S.E.M (standard error of the mean).

Table 1: Mean nitrate and nitrite contents (mg/kg) in smoked Caspian kutum, *Rutilus frisii kutum* (n=7 for each batch)

	Nitrite(mg/kg)	*Range*	*Nitrate(mg/kg)*	Range
Batch 1	9±1.7^a	7.6-12.8	16.6±2^a	15-18.3
Batch 2	2.23±0.3^b	0-4.5	15.5±0.4^a	12.3-15.8
Batch 3	6.3±1.2^a	2.8-9.8	17.5±2.4^a	15.6-19.5

*- Different letters in line indicate difference among groups (P<0.05)

Table 2: Mean nitrate and nitrite (mg/kg) contents in smoked mullet, *liza auratus*. (n=7 for each batch)

	Nitrite(mg/kg)	*Range*	*Nitrate(mg/kg)*	Range
Batch 1	8±1.7^a	2.5-13.5	19.3±3.3^a	13.8-25.2
Batch 2	1.1±0.5^b	0-1.9	14±2.4^b	9.8-17.6
Batch 3	0.9±0.18^b	0-1.1	19.3±2^a	18-21

*- Different letters in line indicate difference among groups (P<0.05)

Table 3: Total amount of nitrate and nitrite (mg/kg) contents in smoked Caspian kutum and Mullets. Data expressed as mean±S.E.M (standard error of the mean)

	Smoked Caspian kutum	Smoked mullet
Batch 1	25.6±3.8	27.3±3.1
Batch 2	17.7±2.1	15.1±1.8
Batch 3	23.8±2.7	20.2±1.3

RESULTS

Mean nitrite and nitrate contents of smoked Caspian kutum are shown in Table 1. Significant difference (P<0.05) in nitrite contents was observed among samples of different batches with batch number 2 contained the lowest amount of nitrite. Mean nitrate values did not reveal any significant difference among samples. Samples of batch number 2 and 3 of smoked mullets had low levels of nitrite contents compared to that of batch number 1 samples (Table 2). On the other hand, batch number 2 had the lowest nitrate value (14±2.4 mg/kg) compared to other samples (P<0.05; Table 2). Sum of nitrite and nitrate values in the both smoked products are shown in Table 3.

DISCUSSION

It has been established that there is an “cancer belt ”, originating in the Far East (Japan, Korea, China) and extending through Middle Asian countries (Uzbekistan, Turkmenistan etc.) as far as the Near East (Iran, Caucasian and Eastern Anatolia regions of Turkey) [10]. In these endemic cancer regions, the people are of low socioeconomic status and have poor nutritional habits

(salty foods, hot tea consumption, lack of fruit and vegetables in diet etc.) along with several environmental and geographic risk factors (barren land, radioactivity, heavy metals etc.) [11]. The highest gastric cancer incidence may be due to the consumption of specified food that is high in nitrites. In China, certain salted fermented fish products including fish sauce were associated with the highest gastric cancer mortality [12]. In Southwest Korea; potential link for the high gastric cancer was associated with regular high consumption of salted sea food [13]. Information on the nitrate and nitrite contents of sea foods (as fresh or smoked product) is scare in Iran. Literature review showed also few pertinent nitrite and nitrate levels in smoked fish [14]. In dry-salted smoked salmon, nitrite contents was between 1.3 and 2.3 mg/kg and after storage up to 45 days, low levels of nitrite was found in the samples. Nitrite values in raw and dry-salted (with no smoke) salmon were <1 and 1.1 mg/kg, respectively [14]. Nitrite and nitrate contents in other foods like ham were reported to be 4 and 35.6 mg/kg, respectively [15]. Hsu *et al.* [16] determined nitrite and nitrate contents in several vegetables which contained <23 mg/kg nitrite, but as much as 5000 mg/kg of nitrate. Our data on nitrite values is comparable to the finding of Pedersen and Meyland [17] which reported the nitrite levels in pickled herring about 10 mg/kg. Values of nitrite in batch number 2 (smoked caspian kutum, Table 1) and batch number 2 and 3 of smoked mullet (Table 2) are approximately the same as that reported by Ofstad *et al.* [14] on smoked salmon. Low values of nitrite in some samples in this study (batche number 2 of smoked Caspian kutum and batch 2 and 3 of smoked mullet) may be due to the age of the samples as nitrite contents will decrease with increasing product storage time [18] which in turn depends on storage temperature. However the age of smoked fish used in this study is not known. Tolerance threshold for the sum of nitrite and nitrate is reported to be 20 mg/kg [6] of which nitrite values may not be more than 7 mg/kg and they rejected two samples out of fourthly surveyed samples according to the threshold [6]. In this study nitrite contents in batch number one of both products are higher than 7 mg/kg (9±1.7 and 8±1.7 mg/kg, respectively). Total nitrate and nitrite (mg/kg) contents in smoked Caspian kutum (batch 1 and 3, Table 1) and smoked mullets (batch1, Table 2) were higher than 20 mg/kg which can be of risk concern for consumption. Our previous study indicated that nitrite and nitrate levels in red meat (cow and sheep meat) were lower than that observed in smoked fish of this study (6.5 and 4.9 mg/kg for nitrate and 0.44 and 0.46 mg/kg for nitrite in cow and sheep meats respectively [19]. This high amount of nitrite and nitrate contents in smoked fish may increase the risk of high these ions intake when consuming smoked products (to provide recommended dietary protein).

We suggest assessing the effect of different processing methods on the levels of nitrate and nitrite in the final smoked products, to determine their levels in raw fillets of commercial fish species and also regularly monitoring of nitrate and nitrite concentrations in various fishery products to estimate the dietary intake.

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