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CONCENTRATION OF NITRATE AND NITRITE IN SOME SELECTED CEREALS SOURCED WITHIN KADUNA STATE, NIGERIA

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ABSTRACT: Concentrations of nitrate and nitrite ions were assessed in maize, millet, guinea corn and wheat obtained in Kaduna State, Nigeria. The results showed that nitrate ion concentration for white maize, red maize, agric millet, normal millet, white guinea corn, red guinea corn, and wheat were 4.5 mg/g, 4.2 mg/g, 15.5 mg/g, 21.3 mg/g, 6.3 mg/g, 4.0 mg/g, and 3.0 mg/g respectively. While the nitrite ion concentration for white maize, red maize, agric millet, normal millet, white guinea corn, red guinea corn, and wheat were 0.035mg/g, 0.030mg/g, 0.074 mg/g, 0.087 mg/g, 0.050 mg/g, 0.050 mg/g and 0.0154 mg/g respectively. The results obtained fall below the WHO's Acceptable Daily Intake (ADI) which is 40-100 mg/g for nitrates, and fall within range for nitrites which is 0.03 to 2.6 mg/g. This means that the nitrate and nitrite contents of maize, guinea corn, millet and wheat grown in Kaduna State may not presently pose a health hazard in the human population.

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INTRODUCTION

Nitrates are present naturally in soils, waters, all plant materials and in meats. They are also found in small concentrations (1-40/µg/m³) in air as a result of air pollution. Levels in water and cultivated soils, (which normally do not exceed 10mg/L), may be increased by the use of commercial nitrogenous fertilizers and by the return of waters derived from animal husbandry to the soil. Nitrate contents of crops are influenced by the plant species, and environmental factors. In certain crops the level may be very high (1000/mg/Kg or more) (Oztekin, 2002). Nitrate and nitrite are essential nutrients for plants protein synthesis and play a critical role in nitrogen cycle (Parkash et al., 1963). Nitrate is a naturally occurring form of nitrogen. Nitrate is formed from fertilizers, decaying plants, manure and other organic residues. It is also used as a food additive, mainly as a preservative and antimicrobial agent (Walker, 1990).

Due to the increased use of synthetic nitrogen fertilizers and livestock manure in intensive agriculture, vegetables and drinking water may contain higher concentrations of nitrate now than in the past (Santamaria, 2006) Nitrites are formed in nature by the action of nitrifying bacteria as an intermediate stage in the formation of nitrates, but concentrations in plant and water are usually very low. However, microbiological conversion of nitrate to nitrite may occur during the storage of fresh vegetables, particularly at room temperature, when nitrite concentrations may rise to exceptionally high levels (about 3600mg/Kg dry weight) (Oztekin, 2002). Both nitrates and nitrites are widely used in the production and preservation of cured meat products and of some fish. Such uses, which are controlled by law in many countries, are considered vital for the prevention of botulism caused by the growth of the toxin-producing strains of *Clostridium botulinum* that are sometimes present in raw meats and that may persist in cooked meats (Binstok, 1996).

Nitrates occur naturally in food crops and plants. The Nitrate levels commonly present in food is not toxic to humans but serve as the reservoir for conversion to nitrite by the intestinal flora. Nitrate per se has a relatively low toxicity. Nitrite is the compound that is of more health concern (Cassens, 1996).

The factors responsible for nitrate accumulation in plants are mainly nutritional, environmental and physiological. Nitrogen fertilization and light intensity have been identified as the major factors that influence the nitrate content in cereals (Cantliffe, 1973b). Diurnal changes in light intensity lead to a diurnal pattern of nitrate accumulation in plants. Similarly, nutrients such as chloride, calcium, potassium, sulphate and phosphorus are also involved in nitrate accumulation process in plants. Nitrate content varies in various parts of a plant (Santamaria et al., 1999, Anjana et al., 2006). Both nitrates and nitrites are widely used in the production and preservation of cured meat products and of some fish (Uwah et al., 2009). Human exposure to nitrate and nitrite result primarily from dietary ingestion particularly from food crops, vegetables, meat and water. The average adult intake from food

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has been estimated to be 40-100/mg for nitrate and 0.3-2.6mg for nitrite. Exposure estimates indicate that for more 99% of the adult populace, only 1-3% of nitrate and nitrite intake comes from drinking water, mainly in areas of notable contamination (Fann and Steinberg, 1996).

The high demand for cereals by the increasing human population and their use for compounding livestock feed has necessitated the assessment of nitrate and nitrite in cereals. The levels of these compounds in food should be of much concern considering their toxicological health implications on humans and animals.

The aim of this study is to obtain information as to whether the level of nitrate and nitrite in the selected cereals is within the laid down standards by the World Health Organization (WHO).

MATERIAL AND METHODS

Sample Collection

A total of 8 samples, 2 samples per cereal, (Maize, Guinea corn, Millet and Wheat) were randomly collected from farm lands in Kaduna State. The cereals were crushed by a mortar and, pestle and cereals flours were put in nylon bags with shackles to prevent contamination with air. Therefore the effect of humidity on nitrate/nitrite content of the samples was reduced.

Extraction Procedure

For each sample, 1g of grounded cereal was used for the analysis; 4ml of hot water was added on the sample and blended for 5 min in a blender. The mixture was heated to 75ºC for the prevention of ascorbic acid interference and solution was transferred to a volumetric flask and 5ml hot water and 1.2 ml Sodium hydroxide (2% w/v in water) was added and blended again for another 10 mins and 1ml of Zinc hydroxide (7.2% w/v in water) was added and the mixture was agitated for 5min. Thereafter 0.5 ml Sodium hydroxide was then added and the mixture was blended for 5mins. Distilled water (8.3 ml) was added and mixed for 5min to obtain a final volume of 20 ml after which the mixture was then filtered using filter paper (Whatman No. 1) until the filtrate is completely clear (Pinar et al., 2009).

Determination of Nitrate (NO_3^-) and Nitrite (NO_2^-) concentrations in the cereals samples

The determination of Nitrate in each of the cereal sample solutions was performed using Spectrophotometer at a wavelength of 543nm. The result will be obtained as Nitrate-Nitrogen (NO_3^--N) and converted to ppm Nitrate (NO_3^-) by multiplying by 4.4 (conversion factor). The concentration levels of Nitrate (µg/g) was calculated from NO_3^- (µg/g) = C x V/M, where; C is the concentration of NO_3^- in the sample (ppm), V is the total volume of the sample solution (100ml), and M is the weight of the sample (1g). Nitrite levels in the sample solutions were similarly determined except that in this case, different reagents were used. The programmer number for Nitrite was 67 Nitrite-N and the reaction period was five minutes as against ten minutes in the case of Nitrate. Nitrite-Nitrogen (NO_2^--N) was converted to ppm Nitrite (NO_2^-) by multiplying by 3.3 (conversion factor). The concentration levels of Nitrite (µg/g) in the sample were calculated from:

NO_2^-(µg/g) = C x V/M

Where C is the concentration of NO_2^- in the sample (ppm), V is the total volume of the sample solution (100ml) and M is the weight of the sample (1g). Both nitrate and nitrite levels in all the samples were determined as described by (Uwah et al., 2009).

RESULTS AND DISCUSSION

Table 1 shows the concentration of nitrate and nitrite in some selected cereals obtained in Kaduna state, Nigeria. The results from this study show that all the cereals analyzed contain detectable amount of nitrate/nitrite ion. The result indicated higher levels of nitrate in the range of 3.0 mg/g in wheat to 21.3 mg/g normal millet. The trend of nitrate variation was in the order: Normal millet >Agric millet > White guinea corn > White maize > Red maize > Red guinea corn > Wheat. The Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2003) and Scientific Committee on Food (SCF) have proposed an acceptable daily intake (ADI) for nitrite of 0.3-2.6/mg/g in humans. Ezeagu (2006), conducted a similar work on rice, maize, and cowpea with maize varieties having a higher value (1000/mg/kg) which is above 500/mg/kg nitrate limit recommended by WHO/FAO (WHO, 1973). Similarly, Gilbert et al. (1946) reported high concentrations of nitrates in air-dried samples, ranging from 12200.0/mg/kg in millet to 30000.0/mg/kg in oats. However, they speculated that these high figures are artifact and probably reflect contamination during drying rather than actual levels.

The concentrations of nitrite in the cereals were generally low compared to the nitrate contents. The concentrations of nitrite in the cereals analyzed were in the range of 0.030 mg/g in red maize, to 0.154 mg/g in wheat. The trend of nitrite variation in the cereals was also in the order: wheat > normal millet > agric millet > red and white guinea corn > white maize > red maize. These levels are low and within the limits of the recommended normal acceptable daily intake (ADI) level (0.1/mg/kg body weight). Dietary exposure to nitrite is normally very low. Exceptionally, higher levels may result from microbial reduction of nitrate in hygienically poor quality well water or in foods rich in nitrate stored under inappropriate conditions (Heisler et al., 1974; Ezeagu, 1996).

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Table 1 - The concentration (mg/g) of NO_3^- and NO_2^- ions from cereal varieties

Cereals	NO_3^-	NO_2^-
White maize	4.5	0.035
Red maize	4.2	0.030
Agric Millet	15.5	0.074
Normal Millet	21.3	0.087
Guinea Corn White	6.3	0.050
Guinea Corn Red	4.0	0.050
Wheat	3.0	0.154

Generally, low levels of nitrate/nitrite are reported for grains and seeds (McNamara et al., 1971). Differences in species, strain and agro-technical operations as well as environmental pollution could account for the wide differences of nitrate and nitrite concentrations in this study vis-à-vis other related studies. It could also be as a result of soil type of the farmland and agricultural management practices.

Nitrates are soluble in water; they are easily washed off fields into rivers where they cause water pollution problems. The rate of absorption of dissolved nitrates from underground and surface water differs from plant to plant, and specie to specie, and this could be responsible for the difference in concentration of nitrate/nitrite as obtained from the current study. Also, the application of artificial fertilizers and animal waste is also seen as a major source of nitrates to plant crops. Furthermore, in farmlands where these practices are adopted, there is a possibility of having high levels of nitrate and nitrite ion contents in the plant crops.

The low concentration of nitrate and nitrite reported in this study compared to other related studies could be as a result of differences in geographical location of the cereals, geographical location of farmlands also determines the levels of nitrate in plant. Farmlands situated in industrialized areas are prone to pollution by the release of chemicals into the farmlands leading to contamination of plant crops and the wash off of nitrate contents in the sub-soils, whereas those farmlands situated in non-industrialized areas are free from contamination and may have higher nitrate contents. In natural system, nitrates in the soil are lost by denitrification, erosion, leaching and replaced by fixation and the application of either inorganic or organic manure. Human intrusion in the nitrogen cycle can result in fewer nitrates being cycled, or in an overload of the system. For example, the cultivation of croplands, harvesting of crops and cutting of forests are known cause of steady decline in nitrate content of the soil. The consumption of supplement sources of nitrate in diet (e.g. legumes, vegetables, and some water sources) and other classes of food along with cereals could allow the Acceptable Daily Intake (ADI) to increase above the set values recommended by world health organisation (WHO).

CONCLUSION

Nitrate levels present in the cereals analysed falls below the acceptable standards of 40-100/mg/g, while nitrite levels fall within the acceptable standard of 0.03-2.6/mg/g, which is below the toxicity level. This means that the nitrate and nitrite content of maize, millet, guinea corn and wheat grown within Kaduna State may not presently, pose a health hazard in the humans' population.

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