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Assessment of Consumers' Exposure to Vegetable Dietary Nitrate in Katima Mulilo, Namibia

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Abstract: *Leafy vegetables occupy a very important place in the human diet, but unfortunately constitute a group of foods which account for high dietary nitrate ingestion by human beings. Samples of spinach, rapes, kale chomolia, chomolia, 5-years, and cabbage highly traded in Katima Mulilo Open market were purchased directly from farmers and analysed for consumers' nitrate exposure. Samples were prepared for laboratory analysis using the in-house method LPFC-126 of the Health Protection Branch Laboratories, Bureau of Chemical Safety. Aqueous extracts of the vegetable sample were anaylsed for nitrate concentrations using MERCK reflectometer (RQflex) and each result reported in mg/kg. The concentrations of nitrate in the vegetables varied widely between 644.30 to 6,463.37 mg/kg wet weight basis. The vegetable consumption survey of 400 participants in the study area indicated that 58% of adult male and 60% of adult female (> 18 years) consumed 5-years as their preferred leafy vegetable while that of the children (12 – 17 years) showed 40% male and 57% female. Dietary nitrate exposures of the consumers via ingestion of these vegetables showed that 5-years recorded the highest mean level of 30.98 mg NO_3^-/day body weight while cabbage recorded the least (0.21 mg NO_3^-/day body weight). Generally, the levels of the vegetable dietary nitrate exposures obtained in this study are below the reported international estimates of intakes from food which varied between 31–185 mg NO_3^-/day in Europe and ≈ 40–100 mg NO_3^-/day in the United States. However, the bioavailability of dietary nitrate is 100% and this has implication for ingestion from a high nitrate diet source such as the 5-years vegetable. Therefore, producers' efforts should target a reduction in nitrate contents as this will add value to vegetable diets already very popular for their nutritional and therapeutic properties. This will involve among others, adopting appropriate strategies to determine the role of individual physiological factors and agronomic practices in the process of nitrate accumulations in vegetables in the study area.*

Keywords*: Dietary nitrate exposure, vegetables, consumption, Katima Mulilo*

I. Introduction

Nitrate is a naturally occurring compound and an important component of vegetables because of its potential to accumulate [1]. Nitrate plays a critical role in the nutrition and productivity of plants, especially vegetables. Due to the increased use of synthetic nitrogen fertilisers and livestock manure in intensive agriculture, edible vegetables and drinking water may contain higher concentrations of nitrate than in the past [2]. Inorganic nitrates and nitrites present in contaminated soil and water can be taken up by plants, especially green leafy vegetables and beet root [3]. Very high concentration (over 5000 mg/kg) of nitrate in vegetables; especially Leafy vegetables has been reported in the Mainland China and various countries in Europe [1, 4]. The Hong Kong Center for Food Safety (CFS), reported that nitrate concentrations of vegetables depend on factors such as species variation, season, light intensity, temperature, method of cultivation, and fertiliser usage [5]. Further, a vegetable nitrate content may also be affected by conditions of storage and processing prior to consumption.

The human exposure to nitrate is mainly exogenous through the consumption of vegetables (about 70-90%), and to a lesser extent water and other foods [1, 6, 7]. Dietary nitrate and nitrite have raised some concerns because of the reported implications for adverse health effects including methemoglobinaemia and possible increased risk of cancer. Reported populations that may become symptomatic at lower levels of methemoglobinaemia than predicted include patients with oxygen transport or delivery conditions like anemia, cardiovascular disease, lung disease, sepsis and presence of other structural hemoglobin variants [8]. There are reports that pregnant women and their fetuses might be more sensitive to toxicity from nitrites or nitrates at or near the 30th week of pregnancy [9, 10]. Conditions such as pregnancy with its high oxygen demand and increased levels of oxidative stress may overwhelm the body's ability to reconvert methemoglobin back to hemoglobin, resulting in increased methemoglobin levels [9]. It was also reported that health effects that were

significantly associated with nitrate or nitrite exposure during pregnancy include increased incidence of intrauterine growth retardation [11], cardiac defects [12], central nervous system defects [13-16], Sudden Infant Death Syndrome (SIDS) [17], and miscarriage [18].

Despite being a major source of nitrate, increased consumption of vegetables is widely recommended because of their generally recognised beneficial effects for health; the WHO recommended vegetable and fruit intake of 400 g/person/day [1]. The Scientific Committee for Food reviewed the toxicological effects of nitrate and nitrite in foods and established an Acceptable Daily Intake (ADI) of 0-3.7 mg/kg body weight for nitrate in 1990 [19], and also retained this ADI in 1995 [20]. Further, the FAO/WHO Joint Expert Committee on Food Additives (JECFA) reconfirmed the nitrate ADI of 3.7 mg/kg body weight/day [21]. As a conservative base case, EFSA submitted that a person eating 400 g of mixed vegetables at typical median nitrate concentration levels would on average receive a dietary nitrate exposure of 157 mg/day [1].

Considering the high level of consumption of leafy vegetables by the residence of Katima Mulilo Namibia, it is quite pertinent to evaluate certain vegeable dietary components that have been identified as posting health risks to consumers. Thus, this study aimed to determine the consumers exposure to nitrate contents of six major leafy vegetables: cabbage, spinach, five-years, chomolia, kale chomolia and rapes highly consumed in Katma Mulilo, Namibia and compare the current dietary exposure level to regulatory guideline value to protect human health.

II. Materials And Method

Study area

Katima Mulilo is located on latitude 17°30′00″S and longitude 24°16′00″E based on the World Geodetic System (WGS) 84 coordinate reference system [22]. The area is blessed with arable land and sufficient rainfall (average annual rainfall varied between 550mm and 700mm [23], which greatly favour vegetables cultivation. The leafy vegetables: cabbage, spinach, five-years, chomolia, kale chomolia and rapes are among the food items highly consumed in Katima Mulilo, Namibia. However, the availability of these vegetables in the open market depend more on season; cabbage and five-years are the dominant leafy vegetables supply in the summer while spinach, chomolia, kale chomolia, and rapes provide the main leafy vegetables supply in winter. Cabbage, spinach and other non-indigenous vegetables are readily available in the shops such Pick N Pay and Shoprites which have facilities to preserve them all year round.

The vegetables focused on in this study are locally grown under diverse agronomic practices with growth enhancers such as chemical fertilizers and animal manure widely applied. Under improper usage, these inputs have the potential to accumulate excess nutrient elements such as nitrate and trace metals in the soil which are subsequently absorbed by crops and bioaccumulate into tissues. Thus, it is important to evaluate the vegetables dietary nitrate components which bother on food quality and safety for human health.

Sample Collection

Samples of the vegetables were purchased directly from farmers during the period of regular supply to the open market. Cabbage and five-years were purchased every fourth night between January and February, 2014 (summer) while spinach, chomolia, kale chomolia and rapes were purchased fourth-nightly from August to September, 2014 (winter).

Sample preparation and analyses

The non-edible parts were removed after which the vegetables were washed under tap water to remove adhered soil and dirty particles. The vegetables were then frozen prior to conveying to the laboratory for further processing and analysis. Samples preparation for laboratory analysis was based on the in-house method LPFC-126 of the Health Protection Branch Laboratories, Bureau of Chemical Safety, Ottawa, [24]. A measured 100 g sample of each vegetable was was cut into very small pieces and homogenised properly. Then, 10 g of the homogenate was blended with 70 ml deionised water and 12 ml 2 % NaOH solution in a blender until very smooth. The slurry was transferred into a 200 ml volumetric flask after which the blender was rinsed with 30 ml deionised water into the slurry and mixed properly. Then, 2 drops of the suspension was pipette and the pH checked to ensure it is within 8 – 10 [24]. Where the pH was more than 10, the suspension was heated in a water bath at 50^{o}C until its temperature reached 50^{o}C with occasional swirling to mix. Where the pH was less than 8, additional NaOH solution was added dropwise from a 25 ml pipette until the pH rose to 8; the suspension was heated as above and maintained at the 50^{o}C for extra 10 minutes. Thereafter, 10 ml of $ZnSO_4$ solution was added, swirl gently to mix and white precipitates of $Zn(OH)_2$ were formed.

$$Zn^{2+}_{(aq)} + 2OH^{-}_{(aq)} \longrightarrow Zn(OH)_{2(S)}$$

Then, the solution was cooled to room temperture in a water bath and the volume made up to 200 ml mark of the volumetric flask with deionised water. It was mixed thoroughly with a glass stirrer and filtered through

Whatman No. 1 filter paper, with the first 20 ml being collected in a glass stoppered flask for analysis of the nitrate concentrations.
The aqueous extracts were anaylsed for nitrate concentrations using MERCK reflectometer (RQflex) and the results obtained in mg/kg.

Vegetable consumption data

The leafy vegetables consumption data were collected over one week using a 24-hour dietary recall by the participants. The consumption survey was carried out for cabbage, spinach, five-years, chomolia, kale chomolia and rapes respectively using two sets of human population in the study area: children (12 to 17 years) and adult above 18 years of age. The participants consisted of 100 males and 100 females making 200 in each age bracket and a total of 400 persons surveyed. They were selected based on anonymous household address sampling procedure [5].

Estimation of consumers' exposure to the vegetable nitrate contents

Consumers' exposure to the vegetable's nitrate (NO_3^-) was calculated to determine if the levels recorded in this study present an unacceptable risk to public health and safety. A modified health risk index assessment was used to calculate the consumers' exposure [25]. Each vegetable's nitrate concentration was multiplied by its average daily intake based on 24-hour recalls and the result divided by the mean body weight. Put in a formula;

$$\text{Exposure to vegetable } NO_3^- = \frac{\text{Sample } NO_3^- \text{ concentration x Average daily intake}}{\text{Mean body weight}}$$

In this formula, each sample (vegetable) nitrate concentration was measured in mg/kg, the average daily intake of vegetable was given in kg and mean body weight also expressed in kg.

III. Results

The results presented in Figure 1 shows the mean nitrate concentrations of the leafy vegetables. It can be seen that 5-years recorded the highest mean nitrate concentration of 6,463.37 mg/kg while cabbage recorded the lowest level of 644.30 mg/kg. Spinach, rapes, kale chomolia, and chomolia recorded nitrate contents of 3,964.40 mg/kg, 841.70 mg/kg, 2,635.60 mg/kg and 2,824.80 mg/kg respectively.

Table 1shows the vegetable consumption data of the study population. The results indicated that greater proportion of the participants consumed 5-years as their favourite vegetable followed by cabbage. Kale chomolia was the least consumed vegetable with the results showing only 2 % dietary recall by the male adults and 0 % by female adults, male and female children respectively. The survey data showed a consistent mean quantity of 290 g consumed per serving while the frequency of consumption showed twice per week across the study population.

Table 2 presents the daily mean nitrate exposure levels of the consumers based on the ingestion of spinach, rapes, kale chomolia, chomolia, 5-years and cabbage investigated. Samples of 5-years recorded the highest (22.86 to 38.48) dietary nitrate exposure followed by spinach while cabbage recorded the least (0.16 to 0.26).

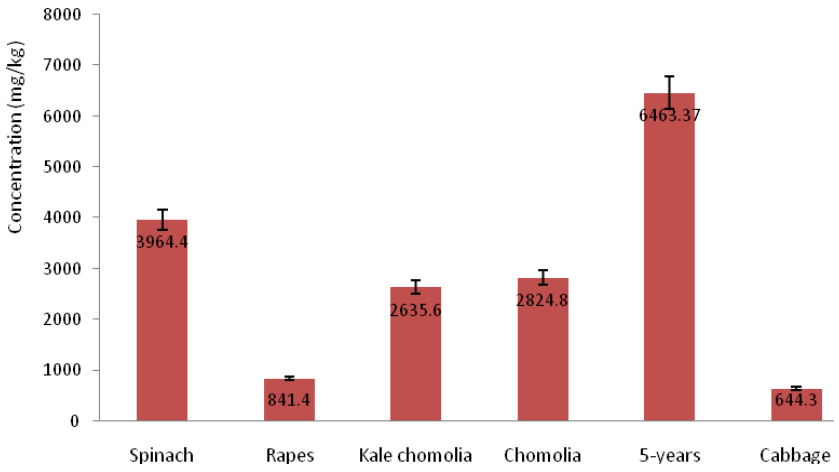

Figure 1. Mean concentrations (mg/kg) of nitrate in the leafy vegetables

Table 1. Vegetable consumption data of two sets of human population in Katima Mulilo

	Percent (%) of consumers by sex and age				
	Adult (> 18 years)		Children (12 – 17 years)		Mean (%)
Vegetable	Male	Female	Male	Female	
Spinach	6	3	0	7	4.00
Rapes	2	0	0	7	2.25
Kale chomolia	2	0	0	0	0.50
Chomolia	12	17	0	14	10.75
5-years	58	60	40	57	53.75
Cabbage	20	20	60	15	23.75
Average Weekly consumption	2 x	2 x	2 x	2 x	2 x
Average quantity per serving (g)	290	290	290	290	290
Average body weight (kg)	82.00	70.10	52.30	48.70	63.28

Key; 2 x = twice

Table 2. Levels (mg NO_3^-/day) of the vegetables dietary nitrate exposure of consumers

	Adult (> 18 years)		Children (12 – 17 years)		Mean
Vegetable	Male	Female	Male	Female	exposure
Spinach	23.61	21.98	14.02	16.40	19.00
Rapes	5.01	4.67	2.98	3.48	4.04
Kale chomolia	15.69	14.61	9.32	10.90	12.63
Chomolia	16.82	15.66	9.99	11.69	13.54
5-years	38.49	35.84	22.86	26.74	30.98
Cabbage	0.26	0.25	0.16	0.18	0.21

Key: b.w = body weight

IV. Discussion

Levels of nitrate in the leafy vegetables

The results of the concentrations of nitrate in the spinach, rapes, kale chomolia, chomolia, 5-years, and cabbage investigated varied beween 644.30 to 6,463.37 mg/kg. Traditional spinach (5-years) recorded the highest mean nitrate concentration of 6,463.37 mg/kg followed by spinach with 3,964.40 mg/kg NO_3^- content. Cabbage recorded the lowest nitrate level of 644.30 mg/kg. Apart from the nitrate level of 5-years, the other results are within the range of nitrate concentrations in vegetables (1 mg/kg in peas to 4,800 mg/kg in rucola) reported in Europe in a comprehensive survey of 41,969 analytical results from 20 member states and Norway [1]. The wide variation observed in nitrate concentrations of the vegetables may be attributed to factors such as different physiological properties as well as the agronomic conditions of the crops cultivation. In the study area, vegetables cultivation involve the applications of nitrogen fertilizers and livestock manures under different soil conditions and this may cause varying nitrate absorption and bio-accumulation by vegetables. Under excessive application of nitrogen fertilizer, these vegetables can accumulate high levels of nitrate and, upon being consumed by living beings, pose serious health hazards [26]. It was also stated that nitrogen fertilization facilitates accumulation of nitrate in plant tissues as a result of an excess nitrogen uptakes over its reduction. Plant's nitrate content is the outcome of imbalance between its net absorption and assimilation rates [27]. Both the uptake and assimilation systems are genetically determined [28, 29], explaining the variability in plant's nitrate content among species and cultivars [26]. The role of agricultural chemicals in regulating the nitrate accumulation in plants has also been shown to depend on three major factors: application of mineral fertilizers, treatment with physiologically active substances and sorbents, as well as the natural and anthropogenic changes in the soil environment [30]. Although, there are research reports which indicate that nitrate on its own is relatively nontoxic; however, its metabolites: nitrite, nitric oxide, and N-nitroso compounds make nitrate of regulatory importance because of their potentially adverse health implications [1]. The International Agency for Research on Cancer (IARC) classifies nitrates and nitrites as "probably carcinogenic to humans" (Group 2A) under certain conditions (i.e. ingested nitrate or nitrite under conditions that result in endogenous nitrosation) which could lead to the formation of known carcinogens such as N-nitroso compounds [31]. Thus, a reduction in nitrate content can add value to vegetable products already very popular for their nutritional and therapeutic properties [2].

Vegetables consumption in the study area

The vegetables consumption survey data based on two categories of human population in the study area (Table 1) above showed that a greater proportion of the participants consume 5-years as their favourite vegetable. A total of 58% of male and 60% of female adult (> 18 years) consumed 5-years as their preferred leafy vegetable while that of the children (12 – 17 years) showed 40% male and 57% female. Generally, the trend of the vegetable consumption data based on the consumers' mean preference showed that 5-years > cabbage > chomolia > spinach > rapes > kale chomolia. In a study on dietary exposure models for nitrates and nitrites, the author noted that dietary nitrate intake is determined by the type of vegetable consumed, levels of

nitrate in the vegetables (including nitrate content of the fertilizer applied), the amount of vegetables consumed, and the level of nitrate in the water supply [32]. Generally, vegetables which are eaten more frequently may lead to significant dietary nitrate intake [26]. In a related study, the ESFA also submitted that the critical driver for a high dietary exposure to nitrate is not the absolute amount of vegetables consumed but the type of vegetable (e.g. leafy vegetables) and the concentration of nitrate related to the conditions of production. Thus, there is higher potential for consumers' exposure to vegetable nitrate via ingestion of 5-years which is the most preferred leafy vegetable in the study area.

Vegetable nitrate daily exposures of consumers

The levels of vegetable nitrate daily exposures of the consumers via ingestion of spinach, rapes, kale chomolia, chomolia, 5-years, and cabbage investigated showed that 5-years recorded the highest mean dietary nitrate exposure of 30.98 mg NO_3^-/day while cabbage recorded the least (0.21 mg NO_3^-/day). The trend of the dietary nitrate exposure via ingestion of the vegetables showed that 5-years > spinach > chomolia > kale chomolia > rapes > cabbage. Generally, the levels of the vegetable nitrate exposures obtained in this study are below the reported international estimates of intakes from food which varied between 31–185 mg NO_3^-/day in Europe and ≈ 40–100 mg NO_3^-/day in the United States [33, 34]. However, the bioavailability of dietary nitrate is 100% [14]. This has implication for ingestion from a high nitrate diet source such as the 5-years vegetable. The Joint Food and Agricultural Organization/World Health Organization (FAO/WHO) has set the Acceptable Daily Intake (ADI) for the nitrate ion at 3.7 mg/kg body weight [1]. Similarly, the Environmental Protection Agency (EPA) set a Reference Dose for nitrate of 1.6 mg nitrate nitrogen kg body $wt^{-1} \cdot d^{-1}$ (equivalent to ≈7.0 mg nitrate ion/kg body weight per day). The range of dietary nitrate exposures of 5-years, spinach, chomolia and kale chomolia exceed both the FAO/WHO and EPA regulatory limits; rapes' level exceeds the FAO/WHO's limit but below that of EPA while cabbage' level was less than both recommendations. However, experts have variously questioned the veracity of the evidence supporting the hypothesis that nitrates and nitrites are toxic to healthy adolescent and adult populations [35, 36, 1]. Thus, there is need for an intensive context specific re-evaluation of the set nitrate exposure regulation because not all the food sources of nitrate and nitrite may be treated equally when considering their potential health benefits or risks.

V. Conclusion

The concentrations of nitrate in the spinach, rapes, kale chomolia, chomolia, 5-years, and cabbage investigated varied widely beween 644.30 to 6,463.37 mg/kg wet weight basis. This may be attributed to the dinstinct physiologic properties of the vegetables as well as the different agronomic practices variously employed by the farmers. Apart from nitrate level of 5-years, the other results are within the range of nitrate concentrations in vegetables (1 mg/kg to 4,800 mg/kg) reported in Europe in a comprehensive survey of 41,969 analytical results from 20 member states and Norway. Even though, some research reports indicated that nitrate on its own is relatively nontoxic, the International Agency for Research on Cancer (IARC) classified nitrates and nitrites as "probably carcinogenic to humans" (Group 2A) under conditions that ingested nitrate and nitrite result in endogenous nitrosation which could lead to the formation of known carcinogens such as N-nitroso compounds. The trend of the vegetable consumption data based on a 24-hour dietary recall over one week showed that a greater percentage of the sampled population preferred 5-years. Generally, the levels of the vegetable dietary nitrate exposures obtained in this study are below the reported international estimates of intakes from food in Europe and the United States. However, the bioavailability of dietary nitrate is 100% and this has implication for ingestion from a high nitrate diet source such as the 5-years vegetable. Therefore, producers' efforts should target a reduction in nitrate contents as this will add value to vegetable diets already very popular for their nutritional and therapeutic properties.

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