DETERMINATION OF NITRATE LEVELS IN FRUITS AND VEGETABLES AND ASSESS THE POTENTIAL HEALTH RISKS IN CHATTOGRAM CITY

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Roll no : 0119/18

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TEACHERS

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G ……………………………………………………..…...Gram

PPM ………………………………………………...…Parts per million

UV …………………………………………….....…..Ultraviolet

Mg …………………………………....…………...Miligram

Kg ………………………………………………….....Kilogram

Kg/hm^2 ……………………………………...........Kilogram per square hectometer

ABSTRACT

A recognized dietary additive, nitrate is a significant and natural component of the human diet. It's a naturally occurring component of the nitrogen cycle found in fruits and vegetables. It is also in charge of defining the quality of vegetables. By evaluating the nitrate levels and the health hazards associated with excessive consumption of fruits and vegetables in Bangladesh, particularly in Chattogram city, this study seeks to establish a sensitive technique. Ion chromatography was used to test ten different fruit and vegetable species for nitrates. The cluster of fruits and vegetables evaluated for nitrate content was found using Ward's hierarchical cluster analysis. An assessment of the daily intake in terms of points was used to establish the health risks related to greater nitrate levels in fruits and vegetables. According to the findings, root and tuber vegetables accumulate much more nitrate than fruits and fruit vegetables ($P = 0.05$). In a cluster analysis, the nitrate buildup in fruits and vegetables can be divided into two distinct clusters that account for 89 and 11% of the sample's overall nitrate content, respectively. With the exception of radish, practically all of the trialed samples' risk assessments of Estimated Daily Intake (EDI) and Health Risk Index (HRI) were within the permitted range, showing that Bangladesh's nitrate intake is in conformity with safety standards. Nitrate may have been a concern factor for health, so the product should be carefully inspected throughout cultivation and storage.

Keywords: Cluster analysis, Ion Chromatography, Nitrate, Root and Tuber, Health Risk.

CHAPTER 1

INTRODUCTION

Contamination has become one of the most important global issues since the industrial revolution. Nitrate is one of the erratic chemical substances found in nature, and in recent years, its content in fruits and vegetables has risen above the permitted levels in all countries (Nifras et al., 2017). Since more than 50 years ago, dietary inorganic nitrates have gained a famously poor reputation (Lundbe et al., 2018). The ecology, the development of plants and animals, and human health have all been severely harmed by this filth (Nifras et al., 2017).

Fruits and vegetables make up a sizable portion of the diet for both adults and kids. Since they are an excellent source of vitamins, minerals, and biologically active substances, high quality agricultural goods are the cornerstone to human nutrition (Smyatskay, 2020). Fruits and vegetables are an essential component of a diet that is both quick and inexpensive for a person to consume.

The amount of nitrates in fruits and vegetables is one of the most important indications of their quality (Smyatskay, 2020). Compared to all other routes of entrance for nitrate consumption by humans daily, vegetables are the main way for it to enter and accumulate in the human body (Nifras et al., 2017). Nitrate is essential for plant nutrition and to maintain biological activity (Nifras et al., 2017). According to (Nifras et al., 2017), plants, particularly crops, may acquire undesirable behaviors when exposed to excess nitrate.

Therefore, the World Health Organization (WHO) and Food and Agriculture Organization (FAO) recommend ingesting at least 400 g of fruits and vegetables every day (WHO, 2004).Contrasted with,Bangladesh's per person consumption of fruits and vegetables is substantially lower than WHO recommendations, hence supporting local value chains is essential to provide the vast population of the nation with products that are both safe and affordable. (HIES, 2019).

At various locations within the same plant, the concentration of nitrate varies. According to studies, leafy vegetables and fruits in some societies account for roughly

85% of the diet's intake of potentially dangerous nitrates and have higher nitrate levels (Bahadoran et al., 2016; Bondonno et al., 2018; Adrian A Chetty et al., 2019).

The storage vegetable exhibits less concentration when observed because nitrate is continuously transported through the xylem to the other portions. The balance of nitrate in raw green leafy vegetables can be affected by agronomic methods such as the quantity, timing, and type of N fertilizer as well as environmental and genetic factors (Nifras and Riyas 2017).

In a study of 10 leafy vegetables grown at two different light intensities (200 and 400 mol m-2 s-1), the study found that the nitrate derivative was higher when the vegetables were grown at the lower light intensity of 200 mol m-2 s-1. On the other hand, frightening public health issues such as nitrate contamination from excessive pesticide residue (PR) in vegetables, chemical fertilizers, and contamination by microbes along with the value chain have evolved (Chung et al., 2011). The amount of nitrate in each serving of any vegetable is non-toxic, but the main health-related substances are its metabolites and byproducts, such as nitrite, nitric oxide, and N-nitrous compounds.

Nitrate exposure is a matter up for discussion. Given the large amount of contradictory information that appears in both the scientific research and the public press, it is unlikely that consumers' confusion and concern about nitrate is a surprise. Confusion is caused by problematic nutrition messages in the media, and this may make consumers less eager in learning about dangers and advantages. Increased usage of nitrogen fertilizers and nitrite-containing additives in processed food items, as well as increased exposure to nitrogen-containing chemicals like nitrate, are causing severe public health concerns, according to Chung et al. (2011).

1.1: RISKS ASSOCIATED WITH DIETARY NITRATE:

Concerns about nitrate consumption arise from the fact that they are linked to the development of various cancer types. Additionally, it causes methemoglobinemia, popularly known as "blue baby syndrome," a highly severe condition that affects newborns under three months of age. The iron in heme is oxidized to the ferric (Fe^{3+}) form in this situation, which is characterized by higher levels of hemoglobin (Suh et al., 2013).

When nitrite ion comes into contact with RBCs, ferrous iron in hemoglobin is oxidized to ferric state, rendering it incapable of carrying oxygen, leading to suffocation and death (Nifras and Riyas, 2017). Additionally, a high nitrate intake is connected to miscarriage (Afzali and Elahi, 2014).

N-nitroso compounds, such as nitrosamines, have been linked to mutagenicity, teratogenicity, and cancer are created in the stomach when nitrate combines with the secondary amines.This increases the chance of stomach and esophageal cancer (Cross et al., 2011; Loh et al., 2011; Ozdestan and Uren, 2010; Roohparvar et al., Shamspur, 2018).

Total nitrate from the diet enters the enterosalivary circulation and is subsequently changed into nitrites by oral bacteria and salivary enzymes. This conversion rate is between 5-7 percent in individuals who are in good health, but it is significantly greater in newborns and people who have gastroenteritis(Almasi et al., 2018).

Despite the fact that vegetables are a significant source of nitrate, it is typically advised to consume more of it due to its universally recognized positive health effects. Numerous studies have demonstrated a link between excessive nitrate intake and human stomach cancer (Nowrouz et al., 2012; Yang et al., 1998). According to this perspective, it is now crucial to check the amount of nitrate still present in foods like fruits and vegetables that people consume.

Dietary nitrate has gained popularity since it may provide advantages for cardiovascular health. Dietary nitrate is converted from nitrite to nitric oxide (NO) and other nitrogen oxide metabolites in saliva by bacteria on the tongue before being absorbed and circulated through the circulation (Keller, R.M., Beaver, L., Prater, M.C. and Hord, N.G., 2020).

1.2: ALTERNATIVE METABOLISM AND TURNOVER OF NITROGEN MONOXIDE, NITRITE AND NITRATE IN THE ORGANISM:

Due to the significance of nitric oxide in many physiological systems, which is formed from nitrate, several researchers are also emphasizing the positive effects of dietary intake of nitrate. Nitrite can be transformed into NO, a free radical gas that acts as a signaling particle required for endothelial function and metabolic control, in conditions of low oxygen availability such as high altitude living and exerciseinduced hypoxia in muscle. Dietary nitrate consumption increases plasma nitrite concentration, lowers resting blood pressure, and enhances vascular function via these pathways (Habermeyer, 2015).

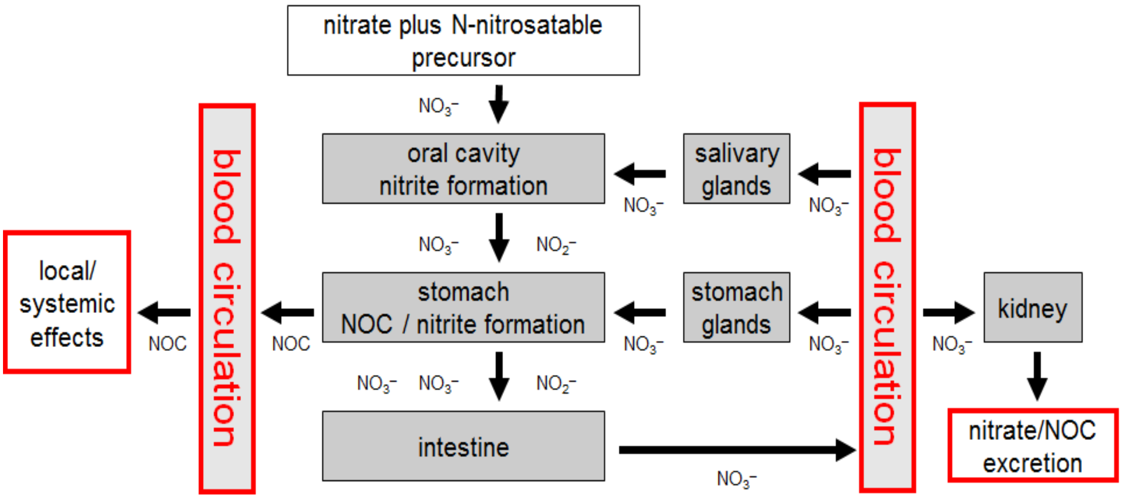


Fig.1: Nitrate ingestion, conversion to nitrite and gastric NOC formation

1.3: BENEFICIAL HEALTH EFFECTS OF DIETARY NITRATE FROM FOOD:

As a result of decreased NO synthesis and bioavailability in the vasculature, current research reveals that endothelial dysfunction is linked to a number of cardiovascular risk factors, including diabetes, obesity, hypertension, and atherosclerosis. NO endothelium homeostasis is restored by dietary sources of nitrate. Actually, nitrate concentrations in vegetables that increase NO bioavailability may be partially responsible for the blood pressure-lowering effects of the Dietary Approaches to Stop Hypertension (DASH) and other healthy dietary patterns. Human clinical experiments have shown that eating nitrate lowers blood pressure in people with high blood pressure as well as in those who are healthy (Habermeyer, 2015).

However, dietary nitrate may also improve performance and the assessed ability to withstand a heavy workload during maximal exercise, in part through lowering the oxygen cost of submaximal activity. A review of the potential health hazards and advantages connected with these foods is necessary in light of the demonstrated cardiovascular health benefits of dietary nitrate-containing veggies (Keller et al., 2020).

According to scientific evidence, having a lot of polyphenol and vitamin C in fruits and vegetables helps with the non-enzymatic conversion of harmful nitrate to good nitric oxide, which lowers the likelihood that nitrate will combine with secondary amines to generate nitrosamines (Erkekoglu and Baydar, 2010; Rocha et al., 2009). As a result, scientists are still grappling with the concepts of risk and benefit associated with dietary nitrate consumption.

Additional worrisome public health concerns include nitrate poisoning from excess pesticide residue, chemical fertilizers in fruits and vegetables, and contamination of microbes that affects the value chain (Chung et al., 2011). The main causes of the buildup of nitrates in fruits and vegetables is the compulsive usage of nitrogen fertilizers to facilitate the grow fruits and vegetables (Fewtrell, 2004). The biological characteristics of plants, soil characteristics, air humidity, lighting conditions, frequency of field planting, harvesting and processing intervals, geographic location, and fertilization are some examples of the factors that can greatly vary from region to region and contribute to the presence of nitrates in the produce (Correia et al., 2010; Parks et al., 2012; Reinik et al., 2008; Rose et al., 2014).

The hazard characterization of micronutrients must take this into account because adverse effects can occur from too low intakes (deficiency) as well as too excessive (toxicity) (Dybing et al., 2002).

1.4: FORMER RISKASSESSMENT OF NITRATE:

Chemicals present in our diet for establishing human safety standards like the TDI or the ADI, the NOAEL (No Observable Adverse Effect Level) value is used as the foundation. It is the highest dose (daily)in the most susceptible animal species or model system during chronic exposure, at which no adverse effects have been observed.

Nitrate consumption per day that reaches the body ranges from 11 to 41%. The average daily consumption from all additional nitrate sources must be considered in a true assessment. The Acceptable Daily Intake (ADI) for nitrate was established in 2002 by the Joint Expert Committee of Food and Agriculture (JECFA) and the

Scientific Committee on Food (SCF) of the European Commission. This value corresponds to the exogenous intake of 276 mg of nitrate or 5.25 mg of nitrite per day for an adult weighing 75 kg (bw) (Hmelak Gorenjak & Cenci, 2013). Then the World Health Organization determined the appropriate daily intake of nitrate at 3.7 mg/kg body weight/day, that is equal to 222 mg of nitrate for an adult of 60 kg/day. Studies have shown that the average adult daily intake (ADI) of various vegetables is around 400 g, which can be used to conclude that the average daily intake of nitrate is 157 mg/day (FAO/WHO, 2013)(Brkić et al., 2017).

The most popular chromatography methods to determine nitrate are high performance liquid chromatography, gas chromatography, and ion chromatography, despite the fact that their use was constrained by expensive column, expensive operator needs, and complex derivation methods. Ion chromatography (IC) with conductivity or UV detection has seen the most widespread use in recent years. Certain writers have described problems with process selectivity, such as the presence of interfering substances such sugar phosphates, which exhibit the same chromatographic properties as the nitrate ion (Saccani et al., 2006).

Despite, Bangladesh being primarily an agricultural-based nation, it grows a wide range of fruits and vegetables all year round. It's undeniably true that on the amounts of nitrate present in fruits and vegetables, it lacks data. However, the purpose of this study is to reveal and validate a quick and accurate method to measure the amount of nitrates in fruits and vegetables that are readily available in local markets. At the same time, based on the Acceptable Daily Intake (ADI) and Health Risk Index (HRI), as well as the established safety limits, we will also assess the health risk to people who consume nitrate-contaminated fruits and vegetables.

Numerous analytical techniques, such as ion exclusion chromatography, HPLC, spectrophotometric measurements, capillary electrophoresis, and ion chromatography, have been used to identify and quantify nitrate in fruits and vegetables (Croitoru, 2012; Gu et al., 2013; Kissner and Koppenol, 2005; Lopez-Moren et al., 2016). However, the majority of the procedures outlined in specialized literature are time-consuming, difficult, insensitive, expensive, and have downsides. Due to its speed, simplicity, and robustness, ion chromatography (IC) with conductivity or UV detection is currently the most popular technology (Amin et al., 2016).

The presence of interfering substances like sugar phosphate and high concentrations of chloride ions, which have the same chromatographic behavior as the nitrate ion, have been documented as problems with process selectivity by some authors (D'Amore et al., 2019; Saccani et al., 2006). Recent literature has reported using UPLC/MS because of its better sensitivity for nitrate analysis (Siddiqui et al., 2015). However, this type of more advanced gear cannot be used for all analytical methods in routine laboratories where a lot of analyses are typically needed. The fluorometric HPLC approach necessitates a challenging and time-consuming sample preparation procedure to eliminate matrix components (Singh et al., 2019).Additionally, because nitrate has an unstable nature, it may change to nitrous acid during the lengthy sample preparation process in this approach (Singh et al., 2019). The HPLC UV/Vis is the most flexible detector due to its ease of use and broad linear range (Hsu et al., 2009; Zuo et al., 2006). However, these detectors have a number of drawbacks. For example, when measuring nitrate, the absorbance at 210 nm is particularly vulnerable to interference from chloride, which is typically found in fruits and vegetables (Singh et al., 2019; Wang et al., 2017). Though, the electrochemistry detection is more sturdy than UV/Vis detection, it is subject to the interference of chloride and it’s not effective for routine analysis due to its poor sensitivity (Wang et al., 2017). In this study, we use ion chromatography to determine the nitrate content in fruits and vegetables. However, to minimizing analysis time and regents, avoiding the interference, maintaining minimal sample manipulation with nitrate in the detection spectra a highly sensitive, speedy, selective, and accurate method for the determination of nitrate is desirable.

The Study aims to

(i) examine the nitrate and nitrite levels of
vegetables on two known market in chattogram city,
(ii) explore the effects of preparation and
cooking methods on nitrate levels in vegetables, and
(iii) assess the associated health risk posed to the population through exposure to nitrate from vegetables. It covered 10 types of commonly consumed fresh fruits and

vegetables. The laboratory analysis was conducted by the Bangladesh Council of Scientific and Industrial Research (BCSIR) laboratory, ctg.

CHAPTER 2

REVIEW OF LITERATURE

Minerals, vitamins, and antioxidants are abundant in fruits and vegetables. They perform a variety of functions, including as acting as an antioxidant and providing protection in the body's acidic environment throughout the digestive process. It has been demonstrated that this substance has both anticancer and cardiovascular disease-reducing characteristics. Making sure that fruits and vegetables are grown in a very high-quality manner is crucial for a community's health. They have recently emerged as the most important source of nitrate in the human food chain. Nitrate is a common molecule found in our surroundings and is a component of the soil and plant building blocks (Salehzadeh, 2020).

2.1: FACTORS AFFECTING NITRATE CONCENTRATION:

There are nitrates in a huge variety of foods. Food products can include inorganic nitrates either naturally or as additions. The nitrate concentration in fruits and vegetables rises as a result of fertilizers, rot seeds, fertilizer, and other organic wastes. It makes up the majority of a nitrogen cycle that crops easily absorb and, as a result, is essential to plant development, function, and nutrition.

Due to agro-ecological conditions, the proportion of edible plants, and agricultural practices from the field to storage, the nitrate content in fruits and vegetables differed greatly from one location to another.The quantity and frequency of nitrogen-containing fertilizer applications for soil fertility, growth conditions, climate, season, temperature, light intensity, cultivation type (traditional versus greenhouse), harvesting time, moisture stress, plant species, plant age, soil pH, storage conditions, and post-harvest storage are some additional related factors.

A study found that compared to cereal crop soil, vegetable soil accumulated more nitrates in each layer from 0 cm to 200 cm. Within the 200 cm soil profile of typical vegetable fields, the total residual nitrate-nitrogen amount was 1358.8 kg/hm^2, while it was 1411.8 kg/hm^2 and 1520.9 kg/hm^2 in the plastic greenhouse fields after two and five years, respectively.However, there was only 245.4 kg/hm^2 in the fields of cereal crops. Vegetable growing regions face significant risks to underground water due to nitrate residue in the soil.

When nitrate intake rises beyond the Acceptable Daily Intake (ADI) limit and poses a risk of fatality, it becomes a problem. The European Commission's Scientific Committee on Food (SCF) and the Joint Expert Committee on Food and Agriculture (JECFA) have established regulations for (ADI) of nitrate, which is 3.70 mg/kg body weight/day (Santamaria, 2006; SCF, 1997). These results are equivalent to a daily exogenous intake of 276 mg of nitrate for an adult weighing 75 kg (Hmelak et al., 2013). According to studies, there is a wide range of nitrate content in fruits and vegetables, ranging from less than 0.1 mg per kilogram to more than 10,000 mg per kilogram (Hord et al., 2009; Reinik et al., 2008; Hsu et al. 2009; Kmecl et al., (2019).

The amount of nitrate in vegetables has been the subject of numerous research. As an illustration, Bahadoran et al. (2016) showed a significant difference in the amount of nitrate present in vegetables across a number of nations and areas, with a ceiling of 1000 mg/kg. Numerous investigations revealed that the nitrate concentration in various plant parts might be arranged as follows. leaf, root, stem, inflorescence, tuber, bulb, and petiole (Salehzadeh et al., 2020; Santamaria et al., 1999).

According to a survey done by Chung et al., (2004) to examine the nitrate content of Korean vegetables grown throughout various seasons, the mean nitrate content was higher for spinach (4259 mg/kg) and crown daisy (5150 mg/kg), whereas for soybean sprouts, onion, and sweet pepper the mean nitrate content was lower for other vegetables (56, 23 and 76 mg/kg respectively). The analysis also demonstrated that there had been no discernible variation in nitrate due to the change in cropping season.

According to research by Ayaz and colleagues, tomatoes have the lowest nitrate concentration, while parsley and spinach have the greatest levels. The highest nitrate concentration was reported in chicory (6250 mg/kg) and rocket (6120 mg/kg), which are consumed in significant numbers in specific regions of Italy. Nitrate content was observed in several edible vegetables in Italy between 1996 and 2002. Although lettuce and green salad had lower nitrate concentrations (the highest values were 4200 mg/kg and 3300 mg/kg, respectively), because they are often consumed, they accounted for nearly 60% of the overall nitrate intake. Only a small number of samples, with seasonal change, were above the permissible range. Green salad and rocket that was cultivated organically had a noticeably higher nitrate content than those that were grown traditionally. These data show the average daily intake of nitrate from leafy greens, less than the recommended level, which is 3.7 mg of nitrate ions per kilogram of body weight, but the total intake should be managed to safeguard vulnerable populations like children and vegetarians.

Six hundred samples of 15 vegetables—Chinese cabbage, radish, lettuce, spinach, soybean sprouts, onion, pumpkin, green onion, cucumber, potato, carrot, garlic, green pepper, and cabbage—cultivated at different times of year were examined for nitrate (NO3) level (Chung et al., 2003).

For a number of crops grown for both summer and winter harvests, there was no discernible variation in nitrate levels. When compared to other vegetables, the mean nitrate level was greater in spinach (4259 mg/kg) and tuberosum Roth (5150 mg/kg), lower in onions (23 mg/kg), soybean sprouts (56 mg/kg), and sweet pepper (76 mg/kg), and intermediate in radish (1878 mg/kg) and Chinese cabbage (1740 mg/kg).

The vegetables irrigated with treated wastewater performed better than those irrigated with rainwater, according to Muhaidat et al., (2019) Jammal's analysis of the impact of treated waste water on vegetables and soils along the reach of the Zarqa River in Jordan. The nitrate concentration in vegetables had a positive correlational statistics with that of plant nitrogen and water and soil nitrate content and had a negative correlation with nitrate reductase function, according to Tabande and Safarzadeh's (2018) evaluation of the nitrate accumulation and consequently the factors affecting in some leafy vegetables in Zanjan Province (Iran). Additionally, considerable variations in nitrate reductase enzyme activity were found across the examined vegetables. A substantial significance for soil nitrate concentration in nitrate buildup in vegetables was discovered among the investigated soil variables.

In order to quantify the quantities of nitrates in conventionally and organically farmed vegetables, Baran and Gruszecka-Kosowska (2017) conducted an analysis on 27 different types of vegetables. They discovered that nitrate levels in vegetables were higher in ancient farming than in organic farming. For leafy vegetables (lettuce, celery leaves, beet leaves, broccoli, chives, spinach), the concentration of nitrates was higher than for traditional organic cultivation.

According to studies by Bondonno et al., (2018), fruits and vegetables account for the majority of the nitrate in a person's diet. Beetroot, spinach, rocket, and lettuce are particular vegetables that are rich in nitrate, while peas, potatoes, and tomatoes are low in nitrate.

The nitrate level of vegetables was found to vary over the type of vegetable and to be comparable to that of vegetables cultivated in other nations. Four samples of industrial food and fifteen samples of home-prepared supplemental kid foods from Nigeria were found to have nitrate. The nitrate content of the industrial food samples ranged from 3.1 to 3.9 mg NO_3-N/100 g. With greatest values found in foods comprising vegetables and legumes, the nitrate content of the homemade supplemental infant feeds varied according to recipes. Vegetable pottage and yam had the greatest nitrate concentrations (25.1 mg NO_3-N/100 g). However, the supplemental foods' nitrate content was below the tolerance level, and they pose no health risks to newborns.

NRA was found to be higher in leaves, and the highest leaf NRA was observed in young leaves, according to a study on the comparison of the nitrate reductase activity (NRA) in different tissues of the poplar plant. Stem NRA remained constant whereas the NRA of the leaves and roots rose as the nitrate supply increased. At all external nitrate concentrations, NRA of leaf was at least ten times greater than NRA of root. With increasing nitrate availability, nitrate reductase abundance occurred in all tissues, and at all nitrate availabilities, nitrate reductase abundance was at least ten times more in leaves than in stems or roots. Increases in nitrate supply led to an increase in tissue nitrate concentration, which was higher in roots than stems and leaves.

NRA was regulated by photoperiod, and in nitrate-fertilized plants with short daily photoperiods, leaf NRA degenerated (8-h). It was discovered that the assimilation of nitrate by the 24 distinct tissues of the poplar varies greatly, with the roots assimilation of nitrate being the least and the leaves assimilation being the largest.

In 2002, Zhong, Hu, and Wang examined the nitrate levels in several vegetables purchased from a north China market during 1998 and 1999. Celery, Chinese cabbage, cabbage, wax gourds, and eggplant were found to contain the most nitrate. There was noticeable heterogeneity in the nitrate content across all of the examined entities. The estimated daily consumption of nitrate from these veggies is 422.8 mg.

In China, 48 different vegetable cultivars were tested for nitrate deposition during various seasons. According to the findings, 20 vegetables—or 41.7 percent of the total examined vegetables—had nitrate-nitrogen concentrations that were above Pollution Level 4 (NO_3-N > 325 mg/kg). These vegetables included all leafy vegetables as well as the majority of melons, roots, onions, and garlic. Five of the leafy vegetables (NO_3-N > 700 mg/kg) even exceeded level 4. Although leafy vegetables were typically prone to extensively collect nitrate, the majority of them had Level 3 (NO_3-N 325 mg/kg) or lower nitrate-nitrogen concentrations in their leaf blades.

Takruri and Humeid (1988) examined nitrate levels in 46 species of popular wild Jordanian plants and vegetables. Tetragonolbus palaestinus leaves had a nitrate content of 29 mg/kg while fenugreek seeds had a nitrate content of 6743 mg/kg (Trigonella stellata). The nitrate level of stems was often higher than nitrate level of leaves, which was found higher in turn of bulbs and roots. When compared to specimens taken from pasture, the forest, or non-irrigated farms, the same species from irrigated farms typically had higher nitrate contents.

In a different study, the nitrate content of various vegetable crops produced in the Jordan Valley was determined. The survey's findings showed that the nitrate levels in the analyzed fruits had generally increased. Compared to cucumber and squash, tomatoes showed lower nitrate concentrations. In comparison to vegetables cultivated in open fields or tunnels, vegetables growing in plastic homes exhibited greater amounts of nitrate. Additionally, the pulp of large cucumber and squash fruits had considerably (p0.01) lower nitrate levels than those of smaller fruits (88–98%), but the peels of the latter showed higher nitrate levels (52 - 79 percent).

Nitrate levels in squash varied from 63.9 to 248.7 mg/kg, in cucumbers from 20.0 to 160.5 mg/kg, and in tomatoes below the level of detection. The amounts of nitrate found in the vegetables examined for this study were found to be lower than those found in comparable products produced elsewhere in the world, particularly in European nations. Another study was conducted in Kenya by Gathungu et al., which examined the impacts of various nitrogen sources on the nitrogen content of potato plants (2000). It was discovered that nitrogen fertilizer applications made later led to increased accumulation in potato leaves and tubers.

On the nitrate concentration of numerous vegetables' edible sections, the effects of harvest season and cultivar were investigated. The nitrate level of the cabbage, spinach, and squash cultivated in open fields was found to be significantly influenced by the harvest date (P= 0.05). Late-harvested veggies have the lowest nitrate concentrations. The nitrate concentration of the greenhouse-grown vegetables was not significantly impacted by the harvest date. The nitrate content of the other vegetables, regardless of their cultivation method, was unaffected by cultivar, although the levels were found higher in greenhouse-grown vegetables than vegetables grown in open fields. Cultivar only had a significant impact (P= 0.05) on the nitrate content of squash and tomatoes. The squash cultivated in a greenhouse had the highest nitrate content (4.77 mg/100 g) whereas the cauliflower grown in an open field had the lowest nitrate content (0.13 mg/100 g).

In a review of the effects of environmental factors on nitrate accumulation in leafy vegetables that are grown in controlled environments, Bian et al. (2020) found that environmental regulation, controlled environmental conditions, and the best fertilizer and nutrient management all play a significant part in growing vegetables with low nitrate contents.

According to research by Abu-Dayeh (2007), vegetables cultivated in the winter tend to accumulate more nitrate than those grown in the summer due to environmental factors including temperature and drought.

In a 1995 study by Wu and Wang, the effects of shade, harvesting timing, culture method, and nitrate, phosphorus, and calcium concentrations on the formation of nitrate in Chinese cabbage leaves were examined. The leaf nitrate content of the plant that was harvested in the late afternoon was lower, the nitrate contents increased as the shade rate increased; this phenomena was more pronounced in autumn than in winter. Under a non-circulating system, the nitrate content of the nutritional solution rose, whereas under a circulating system, no appreciable variations were discovered. Increased calcium concentration significantly decreased nitrate levels in the leaves in the summer but not in the winter, whereas potassium concentration increased and nitrate level decreased in the leaves.

Another study measured the nitrate content of some common vegetables that were consumed uncooked. Numerous samples had nitrate concentrations that were higher than those found in spinach, which is known to be a major nitrate sources. Commercial lettuce likewise had very high nitrate levels, whereas lettuce grown without fertilizer had lower levels of nitrates.

A study was conducted in Poland to find out how much nitrate was present in samples of parsley root, carrot, beetroot, white cabbage, and potato. The mean nitrate concentration in traditional allotment goods range was calculated from 203 in potatoes to 843 in beetroot (mg/Kg wet wt), while the corresponding "ecological" products ranged from 145 to 350 (mg/Kg wet wt).

In the nitrate concentration of cucumber cultivated in a greenhouse, the impact of size of fruit and harvesting time was investigated. Fruit size had very little of an impact. The Sandra and Boloria varieties' respective nitrate contents decreased between the first and last harvesting by 51 percent and 71 percent, respectively, which indicates that harvesting
time had a substantial impact. The nitrate concentration of the Sandra var. was 62 percent higher than that of the Boloria var. Fruit and vegetable nitrate concentrations are also influenced by how they are cooked and stored. It was investigated, according to Adrian Avinesh et al., (2009), that the nitrate content of vegetables lowers when they are boiled. This is due to the cooking water's water-soluble nitrate leaching.
According to research by Prasad and colleagues, frying in soybean oil increases the nitrate concentration of green leafy leaves by 159–309 percent while boiling reduces it by 47–59%. Additionally, over the course of seven days, a little variation in the amount of nitrate in leafy vegetables was seen when utilizing the flash freezing

technique. Another study by Markowska et al. (1995) measured the amounts of nitrate in 33 samples and found that boiling reduced the nitrate content by about 50% while leaving the amount the same in both carrot juice and raw carrots.

Pickston et al., (1980) studied the impact of storage and pressure cooking on nitrate levels and discovered that cooked cabbage has 18% less nitrate than raw cabbage. Vahed et al., (2015) investigated the impact of various processing techniques on nitrate alterations in a variety of Iranian vegetables and found that nitrate levels varied between 34.67 to 198.09 mg kg-1.Traditional boiling reduces nitrate levels by 38.79 to 74.03 percent, according to studies on the effects of cooking techniques (boiling in the microwave, frying, and traditional boiling). The result of boiling veggies in the microwave was a less significant reduction in nitrate level when compared to traditional boiling.

The nitrate content is also regulated by storage conditions and time. Inappropriate storage conditions or lengthy storage of opened, thawed, cooked, or uncooked veggies can cause some of the nitrate to turn into nitrite, according to Daniel-Vedele, Filleur, and Caboche (1998) (Chung et al., 2004).

Cooking has a significant impact on reducing the nitrate level in vegetables, according to Bakr et al., (1986). The greatest nitrate loss during cooking was found in leafy vegetables. The study also demonstrated that while the nitrate level of potatoes was heat stable during cooking, losses occurred as a result of leaching into cooking water from potato tissues.

In a study published in Razgallah (2016) examined the level of nitrate and variables influencing nitrate accumulation in 22 vegetable species gathered from 40 farms in two regions of Tunisia. It was discovered that the amount of nitrate in vegetables varied based on the type of crop, the genetic component, the quantity of nitrogen fertilizer, and the amount of nitrogen in the soil.
Cintya et al., (2018) studied the effect of fertilization on nitrate, nitrite, and vitamin C contents in vegetables and showed that with increasing use of fertilizers the nitrate, nitrite and vitamin C contents have been increased.

Karnpanit et al. (2019) studied the effect of different cultivation practices, and evaluated the potential health risks of nitrate in Thailand. They observed that nitrate contents of most leafy vegetables grown under organic and GAP (Good Agriculture Practice) cultivation were lower than those grown with conventional production. They also explored that the vegetables did not exceed international standards thus assuring safe domestic consumption and allowing them to be competitive in international trade. In Nagpur and Bhandara districts of Maharashtra state, India, Taneja, Labhasetwar, and Nagarnaik (2019) assessed the health risk which is associated with the consumption of nitrate-containing vegetables. The largest amount of nitrate (1349.38 mg/kg) was produced by beetroot. With an assumption of 0.5 kg of vegetable consumption for an average weight of 60 kg, four varieties of vegetables exceeded the recommended daily intake (ADI) of the studied samples.

The impact of various farming practices on the nitrate level of several green leafy vegetables in Thailand was examined by Petpiamsiri et al., (2018). The vegetables grown using the hydroponic system outperformed the other three growing methods in terms of nitrate content Nascimento (2019) examined the nitrate levels of six commercial leafy vegetable samples grown under various conditions and discovered that the conventional method produced the highest levels of nitrate in all samples (239.26–7873.00 mg kg–1).

Vegetables were gathered and tested for nitrate residues in a Queensland study. Near the height of harvest, vegetables were harvested. Nitrate levels in potatoes, cabbages, and beets were greater in the survey's limited sample of hydroponically grown vegetables than they had been in previous studies.
It was discovered how much KNO_3 was present in samples of fresh and frozen veggies, apples, soft fruits, frozen fruits, and James. Some vegetables (maximum 3506 mg/kg in leek) contain more than 1000 mg KNO_3/kg apple has low quantities of nitrate while soft fruits have higher levels. The levels in the frozen goods were marginally lower than in the fresh goods.

The level of nitrate in commercially and home-processed beets and spinach was established by another investigation on the subject. Home-processed beets were shown to have higher nitrate contents than professionally processed beets, whereas home-frozen spinach indicated higher nitrate levels than commercially frozen spinach, however the difference was not statistically significant. The amount of nitrate in home-processed beets and spinach wasn't affected by how long they were stored.

2.2: EXPOSURE TO NITRATE AND IT'S HEALTH RISKS:

Nitrate exposure is a matter for discussion during negotiations. Given the large quantity of contradictory information found in both the scientific research and the public press, it is perhaps not unexpected that consumers are perplexed and afraid of nitrate. Consumers may become less interested in seeking out information regarding risks and benefits if they are presented with unclear nutrition messages in the public information environment. Increased usage of nitrogen fertilizers and nitrite-containing additives in processed foods, according to Chung et al. (2011), is creating a significant public health risk regarding increased exposure to nitrogen-containing chemicals like nitrate.

It was investigated whether eating foods high in nitrate caused the nitrosoamine chemicals, which are known to cause cancer. This study came to the conclusion that eating a high-risk nitrate diet increased the likelihood that carcinogenic nitrosoamines would form. Nitrate's potential endogenous conversion to nitrites and nitrosamines, as well as several acute and chronic toxicities such methemoglobinemia, thyroid conditions, and carcinogenesis, are the main causes for concern (Bahadoran et al., 2015; Gilchrist et al., 2010). High nitrate intake was also connected to Non-Hodgkin lymphoma, bolder cancer, pancreatic cancer, and stomach cancer, infection with diabetes, the occurrence of several birth malformations, and miscarriage, according to Coss et al., (2004) and Addiscott and Benjamin (2004); Van Maanen et al., (1998) investigated how nitrate interferes with the thyroid gland's ability to absorb iodine, which causes hypertrophy. Higher nitrate content foods have been linked in certain studies to an increased risk of thyroid cancer (Kilfoy et al., 2011; Ward et al., 2010).

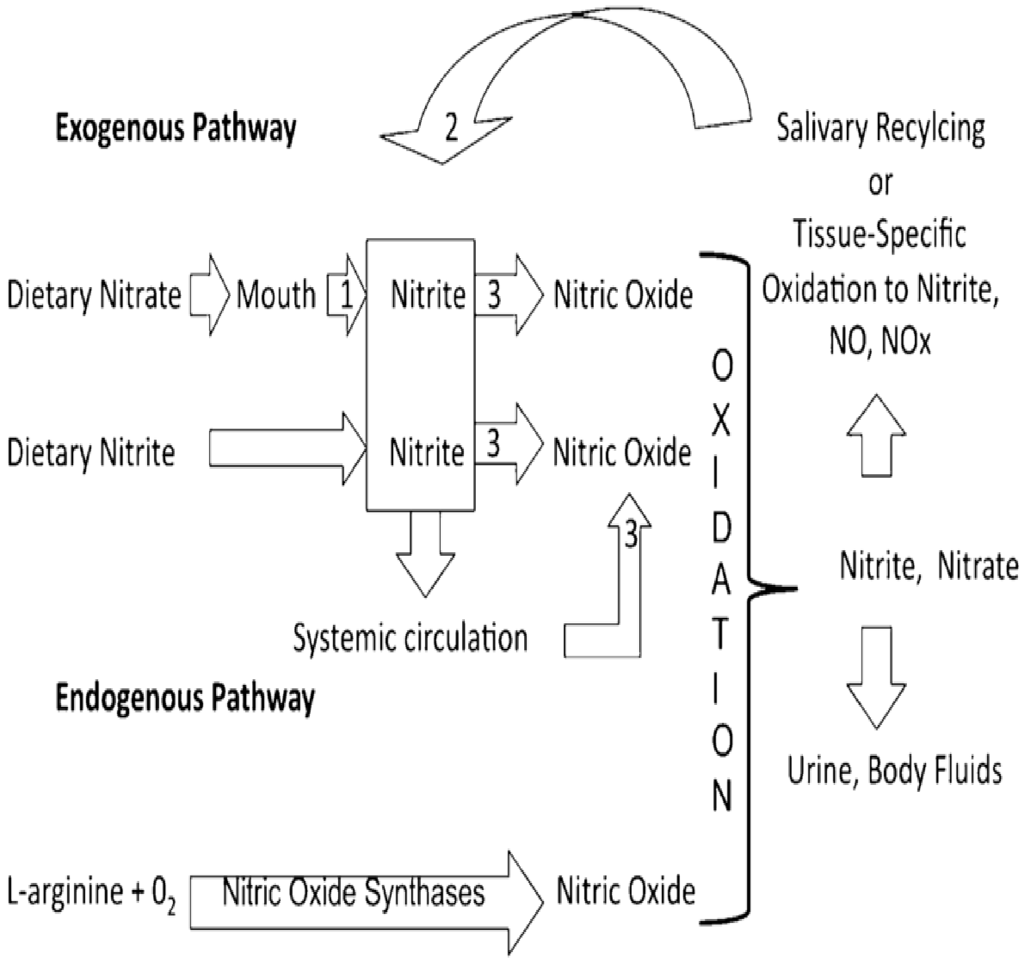


Fig. 2: A schematic representation of the exogenous (from food) and endogenous sources of nitrate, nitrite, and nitric oxide's metabolic destiny. The nitrate reductase activity of mammalian enzymes and bacterial nitrate reductases on the tongue are indicated by the number 1. Bacterial nitrate reductases are indicated by the number 2. Mammalian nitrite reductase-active enzymes are denoted by the number 3.

2.3: HEALTH BENEFITS OF NITRATE:

High nitrate/nitrite diets have been shown in studies to have a variety of possible health benefits, including the ability to prevent cardiovascular disease through having positive effects on blood pressure and the cardiovascular system (Hobbs et al., 2013).

Hord et al. (2009) explained the physiological background for potential health advantages and indicated that dietary nitrate, which is primarily found in vegetables, could improve the status of nitric oxide (NO), which is crucial for cardiovascular health. This substance contributes to the cardio protective action and is produced via many routes. According to Siervo et al., (2013) and Hord et al. (2009), a sufficient nitrate concentration improves endothelial dysfunction, lowers blood pressure, and improves exercise performance in patients with peripheral artery disease. Epidemiological data has lately shown reduction in various types of cancer and a decrease in the risk of heart disease (Joshipura et al., 2001; Terry & Wolk, 2001).

According to Bahraet al., (2012) and Tang et al., (2011), these benefits associated with nitrate cannot be fully explained by the antioxidant elements nonetheless, non-antioxidant components such as inorganic nitrate should be taken into account. Additionally, the capability of the data supporting the association between nitrate-containing plant meals and positive health benefits supports the classification of these advantageous chemicals as nutrients. Fruits and vegetables high in ascorbic acid, tocopherols, carotenoids, and flavonoids can prevent the synthesis of N-nitro compounds, according to Steinmetz and Potter (1991).

Researchers have developed a variety of techniques to measure and track the levels of nitrate in fruits and vegetables, including ion chromatography, HPLC, spectrophotometric assessments, ion exclusion chromatography, and capillary electrophoresis (Croitoru, 2012; Gu et al., 2013; Kissner & Koppenol, 2005; Lopez-M.). However, many of the techniques outlined in specialized literature are time-consuming, difficult, insensitive, expensive, and have downsides. Colorimetric techniques and spectrophotometry were the earliest and most basic identification techniques. Although straightforward and commonly used, spectrophotometry methods have limitations when trying to measure nitrate in non-uniform materials and encounter interference from various organic compounds (Ettre, 1993).

Due to its simplicity, speed, and toughness, ion chromatography (IC) with conductivity or UV detection has been the most popular technology in recent years (Amin et al., 2008; Lopez-Moreno et al., 2016).

The presence of interfering substances like sugar phosphate and high concentrations of chloride ions, which have the same chromatographic behavior as the nitrate ion, have been documented as problems with process selectivity by some authors (D'Amore et al., 2019; Saccani et al., 2006). Recent literature suggests using UPLC/MS for nitrate analysis because of their better sensitivity (Siddiqui, Wabaidur, ALOthman, & Rafiquee, 2015). However, in ordinary laboratories where a lot of tests are typically needed, this type of more advanced gear cannot be used for all analytical methods. To eliminate matrix components, the fluorometric HPLC approach necessitates a difficult and laborious sample preparation process (Singh, Singh, Beg, & Nishad, 2019). Additionally, because nitrate is unstable, it may convert to nitrous acid during the lengthy sample preparation process in this approach, introducing contamination from the environment or nitrite (Singh et al., 2019).

Due to its large linear range and simplicity, the HPLC UV/Vis detector is the most flexible (Hsu et al., 2009; Zuo et al., 2006). However, these detectors have a number of issues. For instance, when nitrate is being detected, absorbance at 210 nm is being recorded, and chloride, which is typically present in fruits and vegetables, might cause interference (Singh et al., 2019; Wang et al., 2017). Although electrochemistry detection is more resistant to interference from chloride than UV/Vis detection, it is also less useful for regular analysis due to its low sensitivity (Wang et al., 2017). Because of its precision, simplicity, and ease of use, ion chromatography by conductivity is growing in popularity as a method for determining nitrate levels. However, a highly sensitive, selective, quick, and accurate approach for determining nitrate is preferred to preserve minimal sample manipulation, save analysis time and regents, and eliminate interference with nitrate in the detection spectrum.

There have been numerous studies on this crucial topic in the region, but none have been done in our area (Chattogram city, in Bangladesh). The summary of the relevant work is provided below.

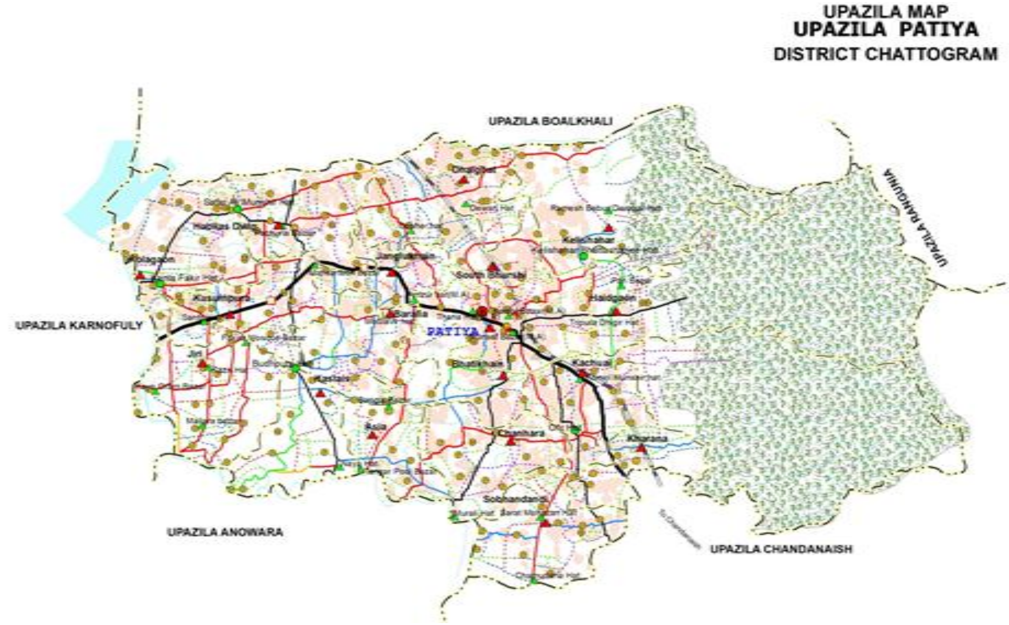
CHAPTER 3

METHODS AND MATERIALS

3.1: STUDY AREA AND SAMPLE COLLECTION:

The initial stages of this study were carried out in Bangladesh's Chittagong division's two most agriculturally productive areas, Shantir Hat, Patiya Upazila, and Kachimar Battali Bazar (Fig. 3). The chosen longitudes for Kachimar Battali Bazar were 22.30° N and 91.50° E, and 22.20° N and 91.55° E for Shantir Hat.

Depending on the crops raised and the fertilizers used, as well as suggestions from locals and officers related to agriculture, the sites were chosen. Two presumptions guided the selection of the vegetables and fruits: (i) that fruits and vegetables cultivated in Bangladesh have a high nitrate content; and (ii) that Bangladeshis consume a lot of fruits and vegetables.All samples were chosen at random during the growth season when they reached biological maturity (October 2021 to January 2022). The samples were divided into three categories: fruits, fruit vegetables, and root and tuber vegetables. After being labeled, the samples were put in a plastic bag and brought to the lab. Until the analysis the following day, the samples were stored over night at a temperature below 4 °C.



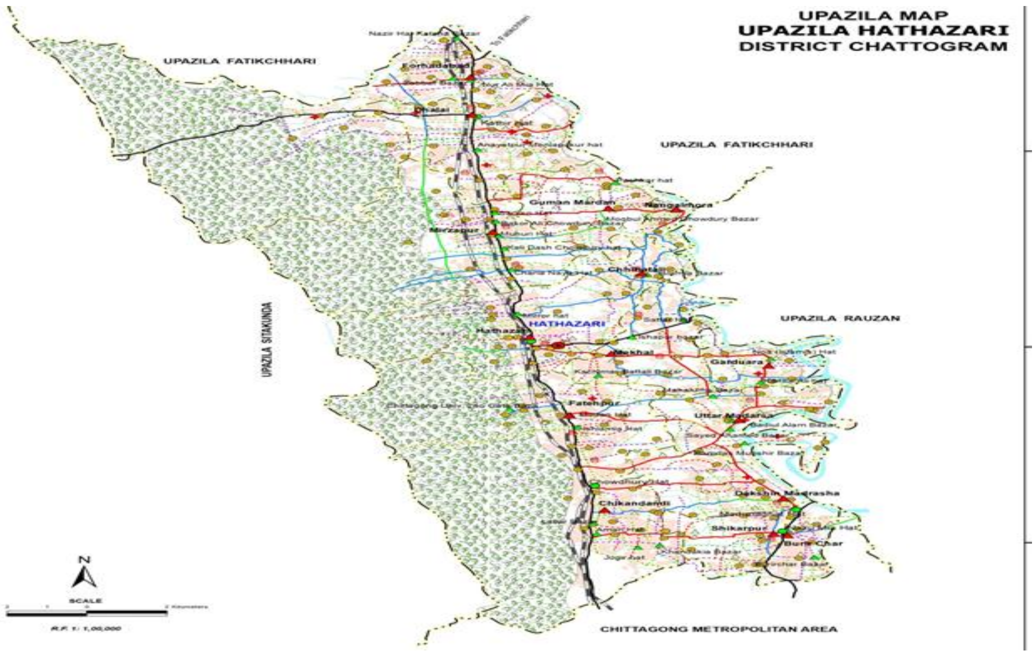


Fig.3: The study areas: Shantir Hat in Patiya Upazila and Kachimar Battali Bazar in Hathazari Upazila

Source: (http://www.lged.gov.bd/ViewMap.aspx).

3.2: INSTRUMENTATION AND CHROMATOGRAPHIC CONDITION:

Ion Chromatography (Sykam Chromatography Anion Separation Column ION-A06 S/N 1990075) with Conductivity detector was used in this study. A column (Cat No. S007270, Type: Anion Exchange, Particle Size: 9 m, Column Size: 4.0x250 mm) was used for the separation. Suppressed CD was the detector (conductivity detector). Standard and sample injection volumes were 40 L each, and the flow rate was 1 mL/min. The column oven's operating parameters were 60 0C, 50 bar of pressure, and 10 minutes. Isocratically conducted analysis was used.

Multielement Ion Chromatography Anion Standard Solution (Product name: 89886, Lot number: BCCC9257, Description of CRM: High purity water (1.8 M.cm, 0.22 m filtered, expiration date: FEB 2024, storage: 20°C or less) and high purity beginning materials was employed as the buffering agent. Standard KNO3 (162.70 mg) was weighed in a 100 mL volumetric flask and then dissolved in deionized water to produce a 1000 ppm nitrate solution. To create the standards needed to create the calibration curve for nitrate, additional serial dilutions were created. By injecting standard nitrate solutions in five distinct concentrations of 2, 4, 6, 8, and 10 ppm and graphing peak area opposed to concentration, the calibration curve was created (ppm). The slope of the curve, which emerges at 13690.2 use a typical correlation coefficient of R^2 = 0.9998, indicated a good level of linearity. For peak locations, the Relative Standard Deviation (RSD) was less than 1%. As a result, we calculated the nitrate concentration in a few samples of fruits and vegetables using the calibration curve, and we finally published the results as mgkg-1. The radish nitrate was first calculated for method development using a standard calibration curve, and the resulted nitrate was 2501mg/kg.

Until an equilibrium standard signal was reached, the mobile phase solution was allowed to pass through the column. With 1 mL/min flow rate and length of the wave was 225 nm, volume of the injection was 40 L. The column oven ran for 10 minutes at a temperature of 60 °C. Once the standard solution injections produced repeatable peak regions and retention times and for analysis, each sample of the solution was injected. By comparing the sample's peaks to the respectable standards' peaks, the sample's peaks were recognized.

3.3: SAMPLE PREPARATION AND EXTRACTION:

The samples were cut into pieces and then homogenized using a cutter and a homogenizer, respectively, to eliminate the inedible components. Then, before analysis, the homogenized samples were immediately kept at -20 °C. 2 g of the homogenized material were placed in a volumetric flask of 100 mL along with 50 mL of deionized water. The flask was then transferred to a boiling water bath for 20 minutes at 80 °C, agitated, and placed on a table to cool. It was then further diluted with deionized water to a final volume of 100 mL. Finally, a 0.45 filter was used to pass 10 ml of the sample extract through. Three milliliters of the initial filtrate were discarded, and the remaining filtrate was preserved for nitrate analysis. Within an hour of preparation and extraction, every sample was examined.

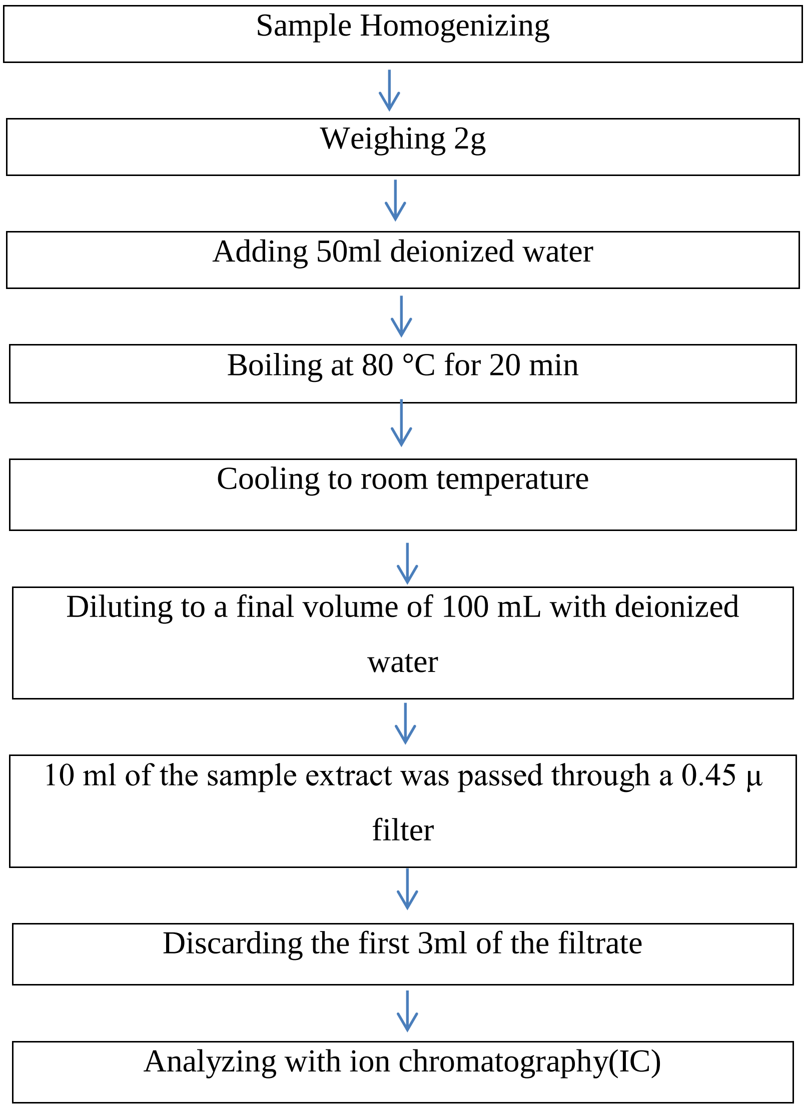


Fig.4: Procedure for detection of the nitrate of Fruits and Vegetables

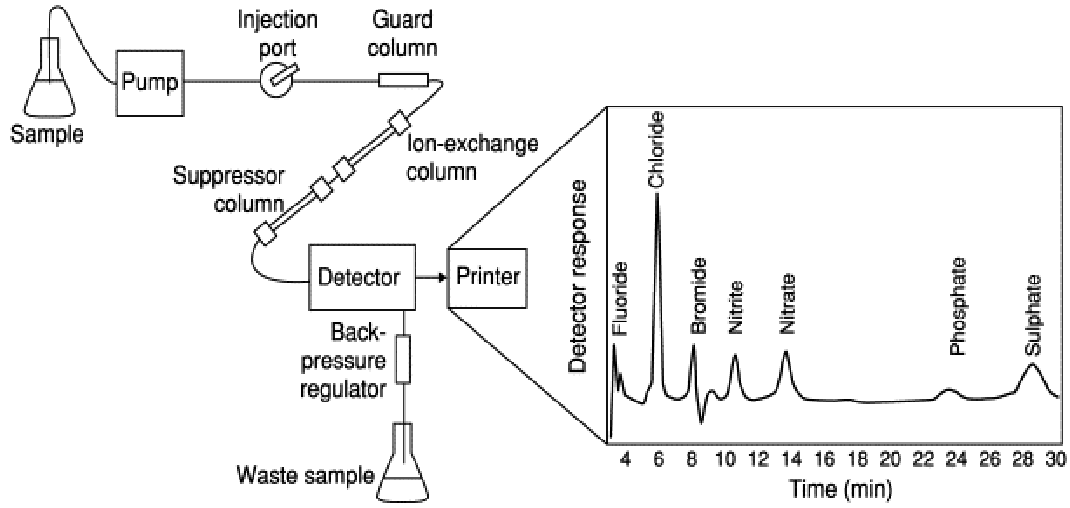


Fig.5 : Schematic diagram of an ion chromatography

3.4: HEALTH RISK ESTIMATION:

If nitrate intake from fruits and vegetables surpasses the Acceptable Daily Intake (ADI) limit or toxicity level, it becomes a substance of concern that could be fatal (Du et al., 2007). The Joint Expert Committee of Food and Agriculture (JECFA) along with the Scientific Committee on Food (SCF) of the European Commission have established 3.7 mg/kg body weight as the acceptable daily intake (ADI) for dietary nitrates (Hambridge, 2003; Santamaria, 2006; SCF, 1997).The amount of nitrate consumed each day was calculated in order to determine the relative bioavailability of nitrate as well as the average daily nitrate build up in a person of a given body weight. The Estimated Daily Intake (EDI) simply takes into consideration the potential ingestion rate and ignores any potential nitrate metabolic excretion. The EDI was calculated based on the following equation

$$\text{EDI} = \frac{\text{Average daily consumption of fruits and vegetables} \times \text{concentration of nitrate in fruits and vegetables (mg/kg)}}{\text{Body weight} \times 1000} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

According to the most current Expenditure Survey and Household Income, the average (per capita) consumption of fruits and vegetables was taken into account in equation 1 as 232 and 35.5 grams, respectively (HIES, 2019). In accordance with the recommendations of the WHO and FAO (FAO/WHO, 2004), we also estimated the EDI and HRI for someone who consumed 400 grams of fruits and vegetables. In both instances, the body weight of adults (60 kg) and children (27 kg) was used (Shaheen et al., 2016). The computed EDI and ADI were then compared to determine the potential health risks of consuming nitrate-containing fruits and vegetables.

3.5: HEALTH RISK INDEX (HRI):

If HRI >1, it indicates that there may be a health risk to the general public for any amount of nitrate. The EDI value of the food goods' and the oral Reference Dose (RfD) determine the Health Risk Index (HRI) value. The oral Reference Dose (RfD) is a quantitative assessment of the daily oral nitrate intake for humans which is not anticipated to have adverse consequences over the course of a person's lifetime, including vulnerable populations like children (Barnes et al., 1988). The HRI for nitrate exposure from ingestion was determined using the equation, as reported by earlier investigations (2).(Abtahi et al., 2018; Rahmani et al., 2018)

$$HRI = \frac{EDI}{Rfd} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots... (2)$$

Where EDI stands for estimated daily intake (mg/kg body weight/day) and Rfd is the reference dosage (mg/kg body weight/day). The oral Rfd for nitrate nitrogen, according to the United States Environmental Protection Agency (USEPA), is 1.6 mg/kg body weight/day, which is equivalent to 7.09 mg/kg body weight/day of nitrate (USEPA, 2013).

3.6: STATISTICAL ANALYSIS:

Three replicates of the nitrate determination process were used. R program for Windows version was used to conduct the analysis (version 4.0.2). Statistical significance was deemed to be present when the p values were less than 0.05. The studied fruits and vegetables were divided into groups based on their nitrate levels using Ward's hierarchical cluster analysis.

CHAPTER 4

RESULT

It is well known that a substantial amount of the nitrates in the diet come from fruits and vegetables. The samples that were evaluated for this investigation were divided into the three categories described earlier and given with their corresponding nitrate contents.

All of the fruits and vegetables samples that were tested for nitrates contained it, and the findings indicate that the nitrate content varies considerably among root and tuber vegetables, fruit vegetables, and fruits (p= 0.05). It was discovered that the nitrate level of fruit vegetables (7.33mg/kg) was much lower than that of root and tuber vegetables (1722.23mg/kg) (Fig. 6). In addition, it was observed thatnitrate content is lower in the fruit samples (5.25mg/kg) when compared to the vegetables.

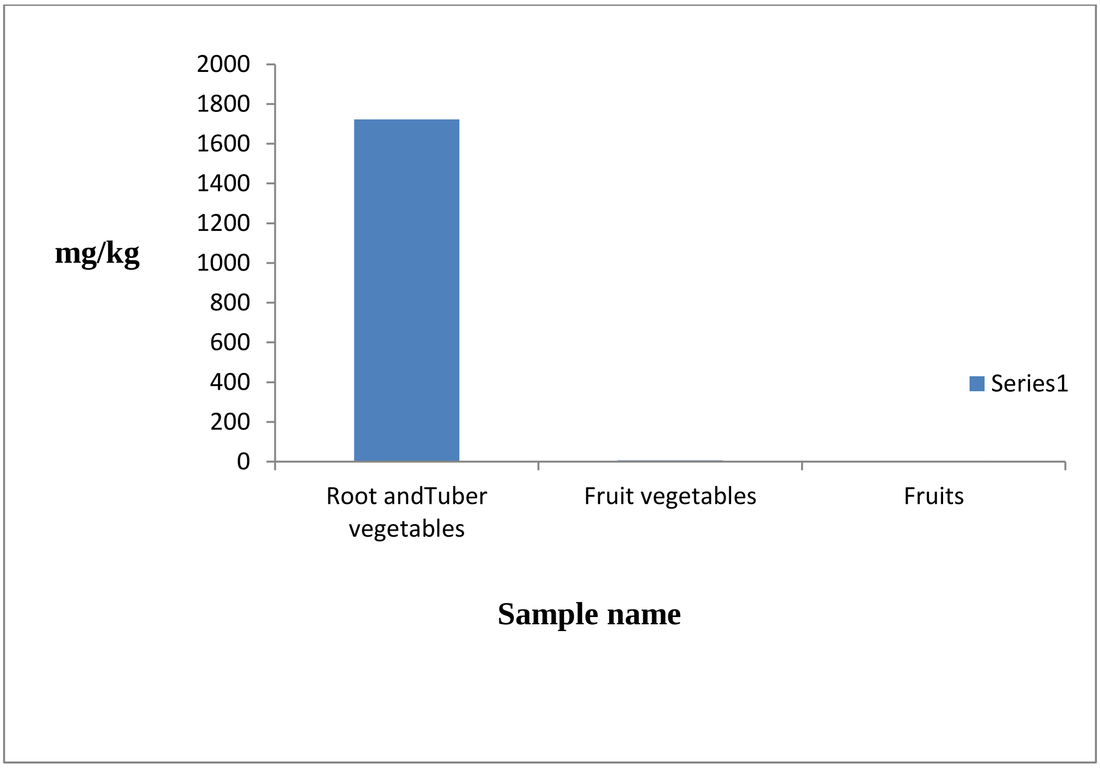


Fig. 6: Nitrate concentrations in various root and tuber vegetables, fruit vegetables, and fruit samples that were analyzed. Standard deviation(SD) is shown via error bars. At p-value = 0.05, identical letter columns are not statistically distinct from other columns.

According to Table 1, radish had the highest mean content of nitrate (5166 mg/kg) among root and tuber vegetables, followed by potatoes (0.3 mg/kg) and carrots (0.4 mg/kg), all of which are statistically significant (p=0.05). Tomato had the greatest nitrate content among fruit vegetables, followed by bean, cauliflower, and eggplant (0–20 mg/kg). Fruits were examined, and it was found that bananas had a mean nitrate content of 10.05 As can be observed from Fig. 7, which shows the findings of the cluster analysis for nitrate buildup in fruits and vegetables, the examined fruits and vegetables may be divided into two distinct clusters. Radish makes up the first cluster. Other samples like carrot, potato, tomato, cucumber, cauliflower, bean, banana, and papaya are included in the second cluster. The two groups are in charge of 89% and 11%, respectively, of the overall nitrate content.

Table.1: Mean concentrations of nitrate in the tested fruits and vegetables

Fruits and vegetables	**Nitrate ±S.D. (mg/kg)**
Root and tuber vegetables	
Carrot	0.4±0.0
Potato	0.3±0.0
Radish	5166±0.1
Fruit vegetables	
Tomato	15.60 ±0.58
Cucumber	0.34 ±0.0
Cauliflower	6.54 ±0.25
Egg plant	6.33±0.5
Bean	7.86±0.27
Fruits	
Banana	10.05 ±0.38
Papaya	0.45±0.0

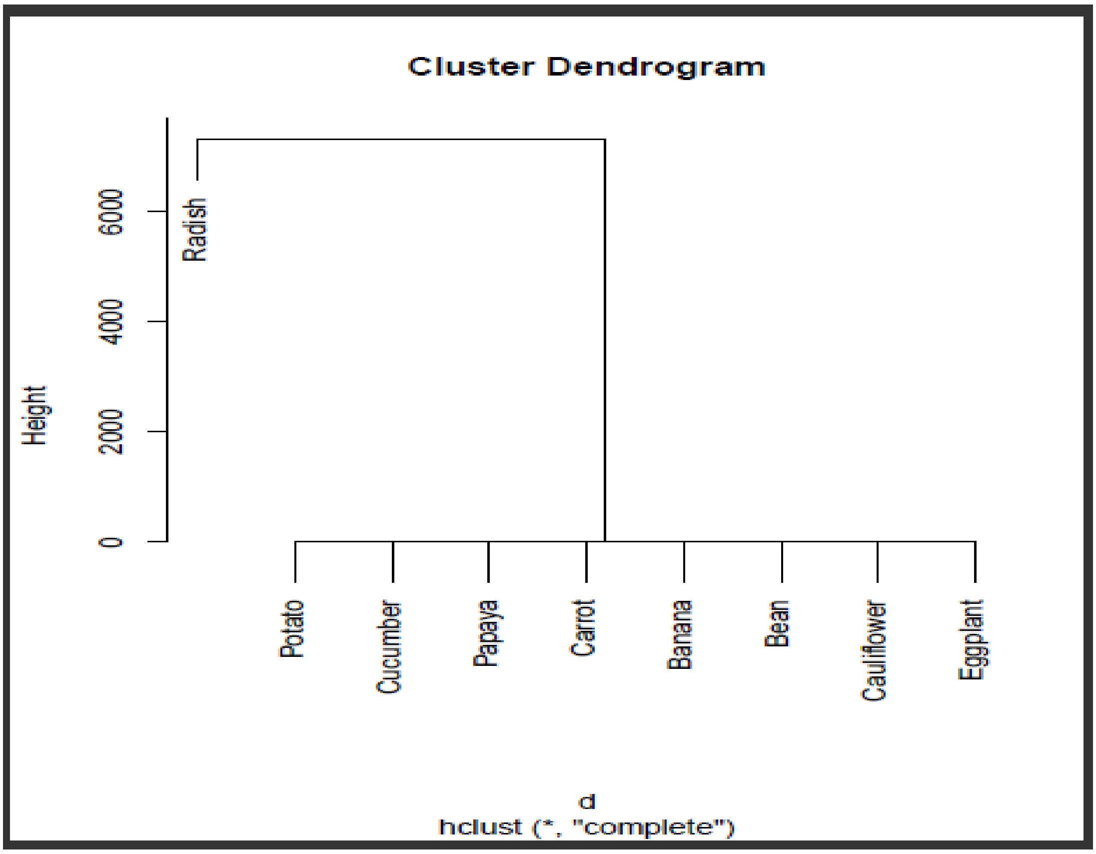


Fig.7: Dendrogram showing hierarchical clustering for nitrate content

4.1: HEALTH RISK ASSESSMENT:

There is a relationship between nitrate intake daily and its level of toxicity. In accordance with equations (2) and (1), the health risk exposure to nitrate was determined as the Health Risk Index (HRI) and Estimated Daily Intake (EDI) in this study. The results are shown in Table 2.

The greatest adult EDI of nitrate among the tested root and tuber vegetables was found in radish, while values for carrot and potato were below 0.5 mg/kg, or what is currently consumed daily and what the WHO recommends. Only in the instance of radish does the Health Risk Index exceed the standard range of 1 for both adults and children. According to estimates, adults consume about 1 mg of nitrate per kilogram of body weight each day, whereas children consume between 0.05 and 0.13 mg. If consumption complies with WHO recommendations, the value for youngsters varies below 1mg NO_3/Kg bodyweights. For both adults and children, the EDI for bananas is 0.01 mg NO_3/Kg body weight. The HRI is under the limit of less than 1 for each of the tested samples of fruits and vegetables.

Table.2: Health Risk Index (HRI) and Estimated Daily Intake (EDI) for the Fruits and vegetables according to the current daily consumption and WHO/FAO recommendation for children

Fruit and vegetable samples	According to current daily consumption						According to WHO recommendation of consumption of 400 g/day					
	EDI (mg NO_3/Kg Body weight/day)		%ADI		HRI		EDI (mg NO_3/Kg Body weight/day)		%ADI		HRI	
	Adult	**Children**	**Adult**	**Children**	**Adult**	**Children**	Adult	**Children**	Adult	Children	Adult	Children
Root and tuber vegetables												
Carrot	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Potato	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Radish	**19.98**	**44.39**	-	-	**2.82**	**6.26**	**34.44**	**76.53**	-	-	**4.86**	**10.79**
Fruit vegetables												
Tomato	0.06	0.13	1.63	3.62	0.01	0.02	0.10	0.23	6.25	6.21	0.01	0.03
Cucumber	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cauliflower	0.03	0.06	0.68	1.52	0.00	0.01	0.04	0.09	1.18	2.61	0.01	0.01
Egg plant	0.02	0.05	0.66	1.47	0.00	0.01	0.04	0.09	1.08	2.43	0.01	0.01
Bean	0.03	0.06	0.84	1.82	0.00	0.01	0.05	0.12	1.42	3.15	0.01	0.01
Fruits												
Banana	0.01	0.01	0.16	0.36	0.00	0.00	0.05	0.15	1.81	4.02	0.01	0.02
Papaya	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

CHAPTER 5
DISCUSSION

According to the findings of this study, fruit vegetables and fruits had the highest concentration of nitrate, followed by root and tuber vegetables. Fruits in Bangladesh may have lower nitrate amounts since less fertilizer is used during fruit growing. These outcomes are consistent with those of the studies conducted by Suin et al., (2006) in Slovenia and Bahadoran et al. (2016) in Iran.

This study clearly shows that the mean nitrate concentration values (mg/kg) found in Bangladeshi fruits and vegetables were decreased compared to those noted in other research. Roila et al., 2018; Stavroulakis et al., 2018; Temme et al., 2011; Bahadoran et al., 2016; Colla et al., 2018; De Martin & Restani, 2003; Feng et al., 2006). In comparison to Taghipour et al. (2019) and Taneja et al., (2019), the nitrate concentration of the cauliflower, beans, eggplant, and cucumber was reduced (2017). Bangladesh experiences tropical monsoons., which is distinguished by significant seasonal fluctuations in high temperatures, precipitation, and lengthened durations of sunshine. Escobar-Gutierrez et al., (2002) found a correlation between higher temperatures and more sunshine exposure and reduced nitrate levels in fruits and vegetables. It could provide an explanation for the overall decreased nitrate levels found in the current study. Contrarily, a high level of variability, a significant levels of nitrates across the results, and in some vegetable and fruit samples, a large standard deviation may be caused by the type of soil, the use of fertilizers, the timing of harvest, groundwater nitrate contamination, and agricultural methods (Abdulrazak et al., 2014; Amr & Hadidi, 2001; Mahvi et al., 2005). However, more research is necessary in this aspect.

It appears that the EDI values for tomato and cauliflower were greater in our research. The results were lower for potatoes, carrots, and cucumbers (0.84, 0.03, and 0.16 mg NO_3/Kg body weight, respectively), in comparison to the results (0.04, 0.03 mg NO_3/Kg body weight, respectively).of Polish researchers Gruszecka-Kosowska and Baran (2017). In a 2019 study from Iran, Mehri, Heshmati, Moradi, and Khaneghah revealed high EDI values for tomato (0.17 mg NO_3/Kg body weight) and carrot (0.19 mg NO_3/Kg body weight), which are somewhat similar to our findings. The results from our investigation were lower than the EDI values published for the potato samples (0.13 mg NO_3/Kg body weight), nevertheless (Mehri et al., 2019). Radish was found to contain amounts that are higher than permissible limit for both adults and children, signaling risks from nitrates, utilizing 3.7 mg/kg body weight/day as the reference value for the ADI threshold (as per the guidelines of the WHO) (Hambridge, 2003); and HRI$<$1 (as the benchmark of the HRI threshold).Previous studies, like those by the Korean researchers Suh et al., the Poles Gruszecka-Kosowska and Baran, and the Egyptian researchers Sebaeia and Refai, have revealed that no examined entity outperforms the standard value of ADI (Gruszecka-Kosowska & Baran, 2017; Sebaei & Refai, 2019; Suh et al., 2013). The use of nitrate-contaminated water, intense fertilization, or high nitrate buildup rates may all be contributing factors to radish in our study exceeding the ADI threshold value.

Based on a daily consumption rate of 400 g, the projected daily consumption and each fruit and vegetable's health risk score were determined. In relation to this, the maximum permitted level for both adults and children, the WHO's recommended ADI of 3.7 mg/kg body weight/day was found to be exceeded by radish; whereas for HRI, the only food found to surpass the limit was radish. The negative effects of nitrate pollution in Bangladesh on fruits and vegetables are therefore within an acceptable range, according to this study. However, obtaining relatively lower EDI and HRI values may be caused by reduced nitrate contamination of fruits and vegetables as well as a lower intake of fruits and vegetables than what is advised (400 g/person/day) (Organization, 2008).

Although fruits and vegetables are the main sources of nitrate, it is important to remember that there are other ways to absorb nitrate in our bodies, therefore a Health Risk Index(HRI) of less than 1 cannot be used to determine an appropriate quantity of nitrate intake. To ascertain the health risks of nitrate, it is necessary to investigate other potential nitrate sources, such as drinking water and other foods.

5.1: INTERNATIONAL EFFORTS TO EVALUATE THE SAFETY OF FRUITS AND VEGETABLE NITRATES:

Numerous studies have found that plant-based antioxidants such ascorbate, atocopherol, b-carotene, phenolic compounds, and indole have strong suppressive effects on the generation of nitrosamines, which reduces the risk of cancer. Additionally, the IFT (Institute of Food Technologists Expert Panel on Food Safety and Nutrition) panel has rejected any scientific data that would support the need for the establishment of a regulatory framework for foods containing nitrates. Additionally, the following is the final judgment reached by the EERO (European Environmental Research Organization) of the EU, which is made up of toxicology experts from the Netherlands, England, Belgium, Germany, and MIT, USA: Vegetables are a major source of nitrates, but they also include critical nutrients like vitamin C and E, carotenoids, and other antioxidants that stop the body from turning nitrites into the dangerous form known as nitrosamine and cyanosis. Furthermore, a substantial body of data indicates that veggies may prevent cancer, and there isn't any strong proof that vegetable nitrates are dangerous. But neither the USA nor the EU have found any solid evidence suggesting that the present nitrate levels in vegetables are harmful to people's health. While the EU has established norms, the USA has no legislation governing the amount of nitrates in vegetables. It should be noted that the EU member states were obligated to establish the rules in order to prevent trade conflicts.

CHAPTER 6
CONCLUSION

This study's analysis of nitrate levels makes it abundantly evident that almost all samples had an average nitrate concentration that was much lower than the WHO recommendation cutoff. This is due to the fluctuating concentration of nitrates in combination of various fruits and vegetables that are required for subsequent survival and growth. The level of nitrate variation is influenced by environmental factors as well as genetic ones, such as the varieties or strains of vegetables and the nitrogen concentration of soil, sunlight, temperature, and growing and storage conditions. Whatever the case, it's important to remember that lengthy and inadequate storage, using chemical fertilizers too frequently, and water tainted with nitrate all lead to high nitrate levels in fruits and vegetables. The HRI values for nitrate in the samples other than radish were 1, indicating that there were no appreciable health concerns from nitrate exposure.

As a result, consumers' nitrate intake from fruits and vegetables might be regarded as safe. In addition, it is thought that the antioxidant compounds that are present in all vegetables act favorably as an inhibitor of the nitrosamine synthesis from nitrites.So, it is believed that nitrates consumed from fruits and vegetables are safe for human health. This study is being done to measure the nitrate levels and evaluate the health hazards related to them in Bangladesh, which will give industry and health specialists vital information for future research.

CHAPTER 7

RECOMMENDATION AND FUTURE PROSPECTUS

We examined the nitrate levels in a few common fruit and vegetable samples that were on hand during the period of our study. The content of nitrate of fruits and vegetables available in Bangladesh is not fully represented by this small-scale analysis. It is necessary to do more extensive research with a larger sample size in order to have a broad grasp of the nitrate levels found in fruits and vegetables available in Bangladesh.

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Appendix-1

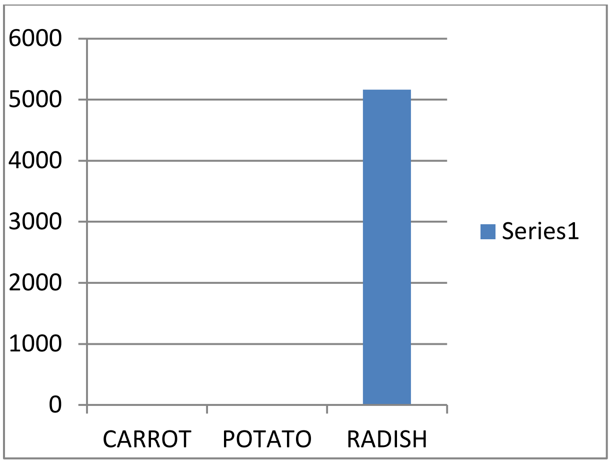

Fig.8: Nitrate levels in root and tuber vegetables

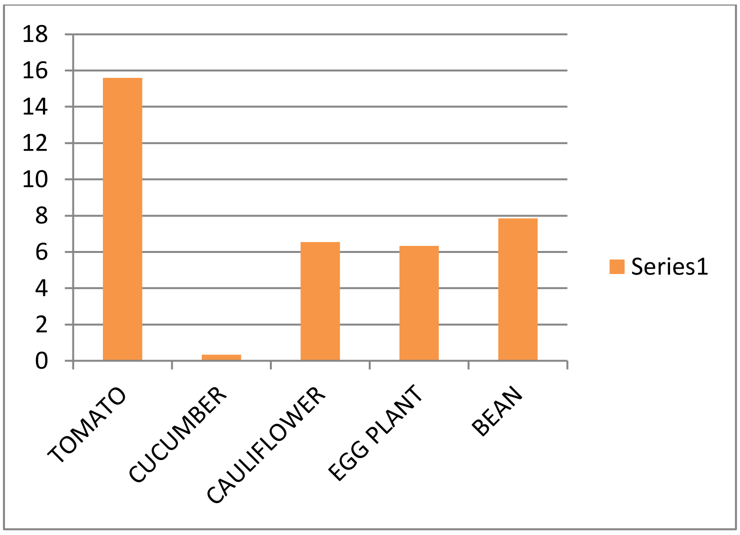

Fig.9: Nitrate levels in fruit vegetables

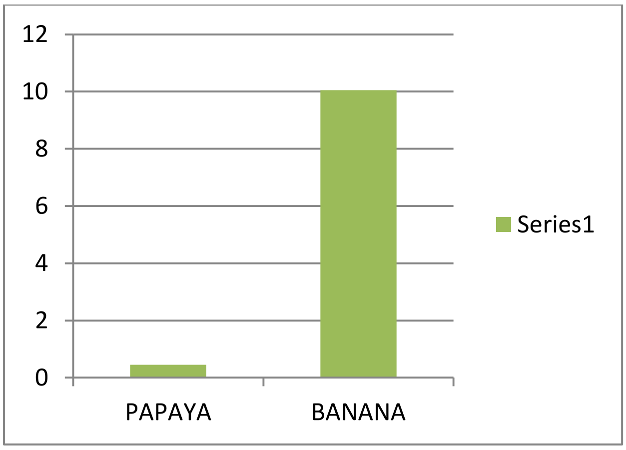

Fig.10: Nitrate levels in Fruit

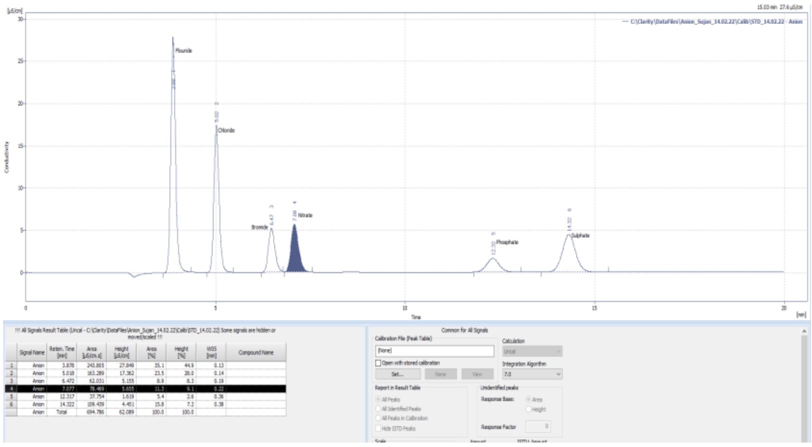

Fig.11: Chromatogram_10mgkg each anion

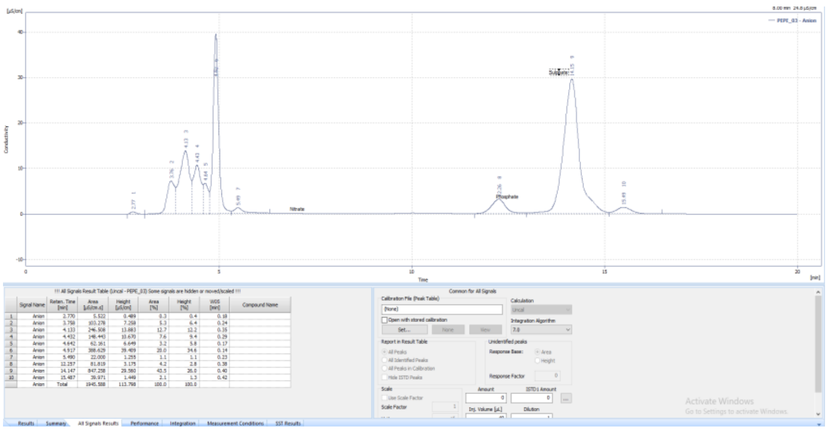

Fig.12: Chromatogram_Papaya

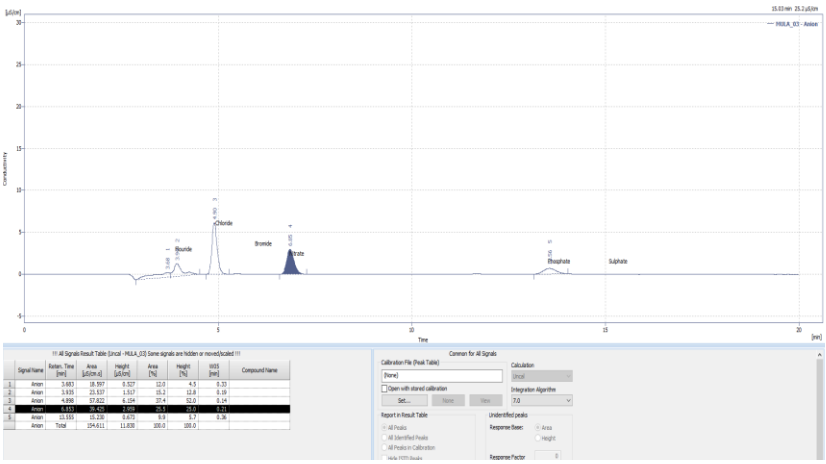

Fig.13: Chromatogram_Radish

Appendix - 2





Fig.14: Some laboratory activities

Brief Biography

Umme Salma Arju passed the Secondary School Certificate Examination in 2010 and then Higher Secondary Certificate Examination in 2012. She obtained her B.Sc.(Hon’s) in Food Science and Technology from the Faculty of Food Science and Technology of Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. Now, she is a candidate for the degree of Master of Science in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition , Faculty of Food Science and Technologhy, Chattogram Veterinary and Animal Sciences University (CVASU). She has immense interest to work in improving health status of poor through proper guidance and suggestions and create awareness among people about Food Science and Nutrition.