Impact Factors Influencing the Nitrate Accumulation of Leafy Vegetables in Plant Factory

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In recent years, cultivation of leafy vegetables such as lettuce and spinach using hydroponic systems in closed plant factories under light-emitting diodes (LEDs) lamps is becoming popular. Unfortunately, these vegetables may accumulate high level of nitrate which pose serious human health implications, upon being consumed by consumers. Finding solutions to lower the nitrate content through environmental control is important for vegetable quality control. Therefore, strategies are leaning towards to minimize the nitrate accumulation in leafy vegetables for agricultural product security. The aim of this review is to understand the factors leading to nitrate accumulation in a closed plant factory, to study the factors affecting nutrient ions management in hydroponic system, to review the cultural measures that may lead to minimize the nitrate content in leafy vegetables under controlled environment. Genetic, agronomic (e.g. supply, composition, timing, and form of nitrogen fertilizer), and environmental factors (e.g. temperature, light quality, intensity and photoperiod, carbon dioxide concentration) can significantly impact the nitrate level in leafy vegetables. To produce high quality vegetables especially in low nitrate content, regulatory methods during cultivation including light quality and other aspect of nutrient solution, can improve the value-added product.

Keywords: Environmental control, LED lighting, light intensity, nitrate reductase

INTRODUCTION

A plant factory with artificial lighting (PFAL) or indoor vertical farming system are a substitution for traditional greenhouses or open-field production and also the foundation of new markets and business opportunities. It’s a modern form of agriculture where the growth conditions of plants are carefully controlled. PFAL as a comprehensive agricultural production way is vital for profitable vegetable production to solve global concern. Contrary to the conventional vegetable cultivation, PFAL can extend the growing season to provide offseason vegetables (Kozai et al., 2015). This type of agricultural activity has been highly developed in many western and Asian countries since the 1970s, especially in Japan, China, Netherland, Israel, and the United States (Kozai et al., 2015) with advanced planting and pollution control technologies. The industry is interested in controlling the growth and quality of plants by fluctuating the biotic and abiotic factors such as light, temperature, carbon dioxide, and nutrient solution (Fang, 2015).

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Vegetable production in plant factory is about two to four times faster than by typical outdoor conventional system due to controlled of indoor conditions (Jones, 2004). In addition, vertical cultivation shelves (a multi-shelf system) are used in plant factories and in a small biological controlled facilitated cultivation space (Alejandro et al., 2016). Leafy vegetables are affluent in vegetarian diet and are commonly consumed as ready to eat salad foods in dietary intake of huge population. The sales of ready-to-use fresh vegetables have increased rapidly in recent decades as a result of changes in consumer preferences, especially in the consumption of prepared salads with fresh-cut vegetables. Loose-leaf lettuce which has become particularly popular over the last decade in Europe and is increasingly consumed as a side dish either without any other accompaniment or as part of ready-to-eat mixed salads. Lettuce contains high contents of vitamin C and antioxidants such as phenolic compounds and is also rich in dietary fiber. Red lettuce is becoming more popular than green lettuce in salad mixes because red lettuce is richer in anthocyanin (Blekkenhorst et al., 2017).

But unfortunately, it accumulates considerable amounts of nitrate which have both positive and negative effects on the human health (Danijel et al., 2017). Persistence solution to nitrate content in leafy vegetables is important because in the intellectual and academic community there are still conflicting evidence regarding potential long-term health risk of nitrate encountered in human diet (Santamaria, 2006; Sindelaar and Milkowski, 2012), such as “dietary nitrate – harmful or beneficial?” (Gilchrist et al., 2010) or “life-giving or death-dealing”? (Weightman and Hudson, 2013). Therefore, the European Union prescribed regulation (No. 1822/2005), became foundation stone to limit the nitrate content in leafy vegetables. Some investigations have recorded that a high nitrate accumulation is harmful to plant growth (Reddy and Menary, 1990) as well as to human health (Ishiwata et al., 2002).

The substantial nitrate content of leafy vegetable in plant factory depends on many factors, since there may be a significant difference in the level of nitrate between the same types of a vegetable which grew at same environmental conditions (Colonna et al., 2016). Factors responsible for higher nitrate accumulation in plant factories are connected with environmental, biological, nutritional, and physiological components. Among them light quality, lower light intensity and excessive nitrogen fertilization identified as major possible factors in affecting the process of nitrate accumulation in protected horticulture. Therefore, the nitrate content increases significantly when the rate of uptake exceeds the rate of its chemical reduction. This situation usually occurs in low light intensity and high level of nitrate in the medium (Cometti et al., 2011), which is specific to horticultural crops under covers, especially in autumn and winter time because light is the main factor stimulating the nitrate uptake and its reduction in plant tissues (Shao et al., 2007), supplemental illumination of horticultural plants during periods with shortage of sunlight improves the quantity and nutritional quality of yield. It is important to follow relevant strategies and determine the role of individual physiological factors in the process in order to limit accumulation of nitrate in vegetables, and important to optimize the use of nitrogen fertilizer (Santamaria et al., 1998). This review focuses on the input and contribution of leafy vegetables towards the dietary nitrate intake and its effects of nitrate on human health, and factors responsible for nitrate accumulation in plant factory. It also suggests and reviewed different approaches to decrease the nitrate content in leafy vegetables and its connected consumption by human beings.

HEALTH ASSESSMENT OF LEAFY VEGETABLE AS A SOURCE OF NITRATE DIETARY INTAKE

Leafy vegetables are ultimate and an important part of the human diet, approximately 80% of dietary nitrates are derived from vegetable consumption which means that human exposure to nitrate is usually associated with intake through vegetables (Temme et al., 2011). The vegetarian diets are rich in vital bioactive phytochemicals and as important source of consumption. These vegetables contain considerable amounts of nitrate, which can have both positive and negative effects on the human health. Leafy vegetable well-thought out to be a highest source of nitrate accumulation upon being consumed in dietary intake (Zhong et al., 2002), computing for 72%–94% of the total nitrate intake of humans (Walker, 1990; Dich et al., 1990; Umar and Iqbal, 2007). It has been postulated that the potential daily dietary intake of nitrate has significant effect on human health (Satnamand Andrew, 2013). This viewpoint is sustained by a large body of evidence (results and conclusions of a number of experimental and clinical studies) showing that dietary nitrate has a magnitude of beneficial cardiovascular effects (Tang et al., 2011) including reducing blood pressure (Larsen et al., 2005), improving platelet build-up (Bahra et al., 2012; Bonik et al., 2001), safeguard or repairing endothelial dysfunction (Webb et al., 2008), amplify the exercise performance in healthy and patients with secondary arterial disease (Sobko et al., 2010; Machha and Schechter, 2011). The valuable effects of nitrate also include reduction cardiovascular diseases and hypertension (McKnight et al., 1999). The book published in (2002) “Nitrate and man: Toxic, harmless or beneficial?” by L’hironde briefly described the various effects of nitrate on human health. A study reported by McKnight et al. (1999), dietary nitrate is an effective potential defensive shield defense against gastrointestinal pathogens. Nitrate have biological effects on human body (Duncan et al., 1997; McColl, 2005), it acts as mediator in digestion of food. Nitrate is relatively non-
toxic but its reaction products and metabolites such as nitrite, nitric oxide and N-nitroso compounds, have raised concern because of their implications for adverse health effects. Thus, documentation with reference to the effect of nitrate on human health is conflicting. It should be noted that nitrate to some extent by itself is nontoxic; however, it may be endogenously switch to nitrite, which rebound with amines and amides to produce N-nitroso compounds (Knobeloeh et al., 2000; Yordanovet et al., 2001). Their harmful effects are linked with the occurrence of methemoglobinemia, and some carcinogen effects have been held responsible to them as well (Keszei et al., 2013; Della et al., 2013). Nitrate is rapidly and effectively absorbed from the upper part of the small intestine in humans after digestion (Iijima et al., 2003).

Approximately estimated dietary exposure accounts in human daily diet ranged 11%-41% of daily intake, and Scientific Committee on Food (SCF) in 2002 estimated acceptable daily intake (ADI) for adult of weighing 60 kg ranged from 0 to 3.7 mg/kg body weight per day, which is equivalent to the intake of 222 mg nitrate per day. (Speijers, 1996; FAO/WHO, 2013). The European Commission adopted Regulation No. 1822/2005 has set the harmonized maximum levels of nitrate for fresh lettuce (Lactuca sativa L.) (protected and open-grown lettuce) in 2500-4500 mg/kg. Unfortunately, indoor cultivated leafy vegetables under different light conditions and systems accumulate more nitrate to potentially harmful contents (Vieira et al., 1998).

FACTORS AFFECTING THE PROCESS OF NITRATE ACCUMULATION IN PLANT FACTORIES

The world is facing global challenges in agriculture due to climatic changes, environmental and soil degradation, shortages of water, and quality concern in food products (Kozai, 2013). The stable supply food chain demand of products will be endangered due to these issues if concerns remained unsolved (Rosenzweig and Liverman, 1992). Quality of product, the safety and security of food in market is based on consumer requirement. Plant factory applications in controlled environment seem possible solution to meet the global food demand in future. Plant factories usually use hydroponic system including medium culture, in which nitrogen is considered as the essential nutritional demand, for proper metabolic control on the nitrate content and other nitrogen compounds. In this regard, nitrogen is an essential element required for successful plant growth (Chen et al., 2014). Nitrate accumulation in plants is affected greatly by environmental factors. Plant nitrate content is commonly viewed as an outcome of imbalance between its net absorption and assimilation rates (Cardenas et al., 1998). Light environment and nitrogen fertilization are distinguished major factors that influence nitrate accumulation in vegetables (Seginer et al., 1998).

Whereas on the other hand, nitrate uptake in aerial parts depends on availability of nutrition, and nitrate assimilation depends exclusively on photosynthetic photon flux density (PPFD), as it is partially a photosynthetic process in controlled environment (Ferrario et al., 1997). Alternatively, plant nitrate content in controlled environment might be either fixed through osmotic potential regulation (McIntyre, 1997) or regulated through negative feedback on its transport systems (Kacjan and Osvald, 2002). Therefore, in this regard, focusing on the environmental factors (light, temperature, photoperiod, composition of nutrient solution) and analyzing the effect of differences in nitrate ion can improve the quality of leafy vegetables. When these factors fluctuate plants have ability of adjusting morphological and physiological as well as their mineral composition and secondary metabolites (Niu et al., 2015).

EFFECT OF LIGHT QUALITY ON NITRATE CONTENT OF LEAFY VEGETABLE

The factors responsible for nitrate accumulation in closed plant factory system are mainly connected with environment, one of them is light (Gruda, 2005; Xiao and Kozai, 2004). Plants perceive changes in their lighting environment by sensing light quality using signal of photoreceptors (Smith, 2000). Light quality, mostly refers to the effects of red and blue lights on plant growth and development, recently it attracts more of the attentions due to the fact that these wavelengths are primarily absorbed by photosynthetic pigments and have the biggest influence on plant architecture and development (Goto, 2003; Abidi et al., 2013). Light quality impacts the formation of the key enzyme related to nitrate and its activity (Appenroth et al., 2000; Nemie et al., 2013). The phytochromes via photoreceptor involved in this process increases the activity of nitrate reductase as a result of dephosphorization of the enzyme. It has been demonstrated by Ohashi et al. (2006). When plants exposed to red and blue lights, the light quality stimulates the process of nitrogen assimilation in comparison to the exposure to only red or blue light. This means that the addition of blue light to red radiation results in increasing the activity of nitrate reductase. Nitrite reductase is regulated (NO\textsuperscript{−}\textsuperscript{−}, NO\textsuperscript{2}−), with regards to the amount of the enzyme and its activity (Aslam and Huffaker, 1989; Luo et al., 1993). The regulative effect of light quality on nitrate reductase activity, phytochrome potential aid in this phenomenon has been demonstrated too light quality regulates the nitrate uptake, translocation and incorporation into organic compounds (Lillo, 2004). Nitrate accumulation is directly or indirectly affected by light quality in indoor production facilities (Qi et al., 2007). It's reported that illumination cycle of 12 h of blue light increased nitrate reductase activity more than red light (Sasakawa and Yamamoto, 1979). Harmonizing/regulating/adjusting the red to blue light ratio
(R/B ratio) on photon flux density could significantly improve plant growth and development (Maevskaya and Bukhov, 2004). Previous results about quality control revealed that nitrate content in lettuce grown under the light with R/B ratio of 8 was lowest. The control of light quality usually is achieved by combining different kinds of lamps and regulating their relative intensities (Wen et al., 2009). Nitrate reductase activity is affected by light quality and blue light promotes stomatal opening which increases transpiration, and this phenomenon may promote nitrate uptake. Therefore, it may be strained to regulate nitrate content by only light quality (Hall et al., 2015).

EFFECT OF LIGHT INTENSITY ON NITRATE CONTENT IN PROTECTED LEAFY VEGETABLES

Light intensity seems to be among the environmental factors that most influences nitrate accumulation in plants, because nitrate accumulation in leafy vegetable is strongly affected by light intensity. Low light intensity is often reported in many studies to enhance nitrate accumulation. In contrast under high light intensity also increases the nitrate content when the temperature is high (Chadjaa et al., 2001). It has been reported in many studies that both light quality and intensity can influence the nitrate contents in vegetables. Nitrate content of leafy vegetables depends upon photosynthetic photon flux density (PPFD) because it is transported in the xylem through cell vacuoles (Fan et al., 2013). The xylem transports water and minerals from the roots zone to the leaves, whereas the phloem carries the products of photosynthesis (carbohydrates) from the leaves to the growth points of the plant. This mechanism affects the diffusion of nitrate between the leaves and cell vacuoles. The combination of nitrogen metabolism and photosynthetic electron transport in leaves signify that light intensity is the key factor in determining nitrate contents in leafy vegetables. Different PPFD in light intensity caused variations in nitrate contents of lettuce grown in plant factories (Yorio et al., 2001). Low light cycle has generally higher nitrate content than plants in the same environment with high PPFD. These differences can be explained by sampling lettuce at higher irradiance in tends to reduce nitrate content which coincide with periods of high irradiance and high temperatures (Di et al., 2008). The optimization of light quality and intensity influences also the nitrate level when vegetables are produced under glasshouse conditions (Pinto et al., 2014), this means e.g. shelter/shading of vegetables should be avoided.

EFFECT OF NITROGEN FERTILIZER ON NITRATE CONTENT

In plant factory, nitrogen fertilization is commonly used for maintaining proper green pigment retention and leaf growth. However, lettuce is characterized by its ability to accumulate high level of nitrate. The nitrate content in commercial lettuce vary considerably. Acceptable nitrate levels ranging from 1000 to 4500 mg/kg in fresh weight according to cultivation mode. Plants grown in hydroponic systems showed higher levels of nitrate compared to those grown in conventional systems (Gromaz et al., 2017). Nitrate in plant tissues occurs high when there is an imbalance between the absorption and assimilation of this ion or ammonium, and surplus quantities are stored in the vacuoles to be assimilated later (Andriolo, 1999). Nitrogen fertilization facilitates accumulation of nitrate in plant tissues as a result of an excess of nitrogen uptake over its reduction. When taken up in excess of immediate requirement, it is stored as free nitrate in the vacuole and can be remobilized subsequently when nitrogen supply is insufficient to meet the demand (Branimir et al., 2017). An adequate fertilization program may ensure sufficient plant growth without any risk of plant nitrate levels going too high (Vieira et al., 1998). Plants accumulate more nitrate as the nitrogen fertilization level increases (Nazaryuk et al., 2002; Leigh et al., 2016), whereas limiting the nitrogen availability reduces nitrate content significantly and nutritional quality of vegetables (Guo, 2016).

EFFECT OF TEMPERATURE ON NITRATE CONTENT

Temperature is one of the critical factors determining the growth rate of plants, and the nutrient solution and air temperatures impact a variety of physiological processes. Literatures concerning the effect of temperature on nitrate content of vegetables are conflicting, this might due to inability to distinguish between uptake of nitrate and its reduction at particular temperatures of solution and atmosphere. Photosynthetic functions and most enzymes are also influenced by temperature which then in response affect nitrate uptake. It has been reported in many studies that temperature of nutrient solution affects uptakes of water and nutrient ions. Generally, more uptake of NO₃⁻ is reported with cold solution due to less water uptake, whereas high temperature with high light intensity also increases the NO₃⁻ uptake rate. Temperature also affects solubility of fertilizer and uptake capacity of oxygen, by roots which ultimately affect the nitrate uptake. Many studies demonstrated significant interaction between temperature and nitrate supply, which means nitrate uptake is temperature dependent (Liu et al., 2016).

EFFECT OF PHOTOPERIOD ON NITRATE CONTENT

Nitrate content in leafy vegetables is not constant throughout the day, it increases during the dark period and decreases during the photoperiod. Photoperiod plays an important role in nitrate assimilation. It was observed in many studies that nitrate content found in higher when plants treated with low cycle of photoperiod. It means that lengthening the photoperiod decreases the nitrate content. Nitrate uptake in leafy vegetables is sensitive to light and dark cycles, plants tissues accumulate more nitrates especially when grown in high NO₃⁻-N availability and long
dark cycle. It was observed that with low nitrogen supply and long continuous light for 72 h reduce the nitrate content significantly. Growing cycle and nitrate concentration of nutrient solution has been demonstrated in a number of studies that carried out in field and growth chamber studies on changes in nitrate contents over a 24 h period for spinach, sweet basil, and scallions (Wen et al., 2009). The authors demonstrated that the nitrate content fluctuated over the 24 h period, and these variations were strongly correlated to the changes in light intensity over the same period and were also species dependent. The authors concluded that the reduction of nitrate content could be possible, by harvesting the vegetables at the right time of the day. Nevertheless, little information is available concerning the effect of light intensity at the time of harvest on desirable and undesirable compounds in baby leaf vegetables, especially under greenhouse conditions (Zhou et al., 2013).

**NUTRIENT IONS MANAGEMENT IN SOILLESS SYSTEM**

High quality products with low nitrate content in soilless system are only possible if nutrition is optimized. This prevail upon accurate management of all factors involved in nutrition, which include nutrient solution composition, electrical conductivity (EC) and pH, dissolved oxygen, and temperature of nutrient solution, water supply (Bongekile et al., 2017). Plants may suffer inconsistency in nutrient uptake, if these factors are under non-optimal conditions. In order to diagnosis distortion in yield and quality due to incorrect management in controlled environment, these above-mentioned factors need to be focused.

Plant factories practice hydroponics as growing plants substrate with the addition of essential nutrients and considered as a successful used method in nutrient-delivery systems. Excessive amount of nitrogenous fertilizers is applied against yield loss, when inputs of nitrogen increases the demand, plants are no longer to absorb it, this causes imbalance in nutrient uptake in nutrient solution. That’s why among factors affecting nitrate accumulation in plant factories, the nutrient solution is considered to be one of the most important determining factors in terms of quality control. The fundamental component in hydroponic system is represented by the nutrient solution. The concentration control of nutrient solution, referred as EC or osmotic pressure, allows the culture of a great diversity of species. Moreover, the accurate control of nutrient supply to the plant represents the main advantage of soilless culture. Additionally, the regulation of pH, root temperature among others factors, leads to increased yield and quality. Nitrate accumulation in leafy vegetables often depends on, nitrogenous fertilizers, mainly of nitrate variety (Wang, 2004). An adequate program of applying nitrogen at once at the beginning based on potassium, ammonia, or mixture of potassium, ammonium nitrate is effective in controlling nitrate accumulation. In addition, it is essential to have a good knowledge of plant mineral requirement in order to formulate optimum nutrient solution. Several studies have documented that when nitrite content is too high or low quality and yield may even be decreased. Therefore, it is crucial to formulate nutrient solution with balanced relationship among the different ions. Some ions in excess can cause nutrient deficiencies by interfering with the uptake of other ions, which is called antagonism. Studies of antagonism highlights the importance of nutrient ions management instead of monitoring EC level in nutrient management. In order to formulate the optimum nutrient solution for particular leafy vegetable, it’s necessary to understand the factors that regulates nutrient ions absorption by that particular vegetable, and first step is measuring that vegetable nutrient absorption under different conditions. In soilless system, many studies reported that nutrient uptake is proportional to nutrient supply and plants regulates its uptake according to its needs.

In commercial production of plant factories, symptoms of specific deficiencies by limited nutrient ions supply may appear in plant tissues. Therefore, visual symptoms are not the correct method to diagnose nutrient deficiencies, because limiting level of specific nutrient ions affect metabolic process in which they involved. Therefore, many nutrient ions are having been identified in literatures such as calcium, chlorides, potassium, phosphorus, sulfate, are involved in nitrate accumulation.

**CULTURAL MEASURES TO REDUCE NITRATE**

The availability of current technologies makes it possible to expand an optimal control of nitrate contents for leafy vegetables grown in plant factories and some of approaches can help to maintain an acceptable limit. The impact of nitrogen dressings on the nitrate contents in leafy vegetables has been studied in several countries in indoor experiments conducted both in greenhouses and growth chambers. Nitrate content in plants is commonly reported as result of imbalance between endogenous and exogenous factors in both uptake and assimilation and that seems genetically determined. Therefore, in plant factory, the factors affecting of nitrate uptake and its assimilation are totally dependent on nutritional, environmental and physiological factors. Therefore, for indoor cultivation to limit the nitrate accumulation in vegetables, it’s important to adopt appropriate strategies and determine the role of individual physiological factors in the process. Regulatory methods to produce low nitrate vegetables depend on light quality, light intensity, and composition aspects of nutrient solution. Light intensity is the key factor of nitrate accumulation during the cultivation process. Light quality also plays a significant role to drive biochemical photosynthetic activity (Du et al., 2007; Liu et al., 2016).
Arrangements for good agriculture practice (GAP) with regard to nitrate were established to aid grower respond to the European nitrate rules and the need to curtail nitrate contents in vegetables. These policies were shaped by the agronomist or commercial growers. Each GAP includes an assembly of presently existing knowledge, including reference based on experiments, and is projected to address agricultural, economic, and health issues in on-farm production and the processing of produce beyond the farm gate.

Previous studies highlighted following culture measures to avoid nitrate accumulation in lettuce by dosed nitrogen supply of nutrient solution (Kowalska, 1997; Santamaria et al., 1997), inhibiting the nitrogen supply of solution with growth stages (Andersen and Nilsen, 1992; McCall and Willumsen, 1999; Seginer, 2003), withdrawal of nitrate in fertilization prior to harvest (Stagnani et al., 2015), replacing and balancing nitrate ions with chloride before crop maturity (Gunes et al., 1994), optimization of environmental factors with respect to nitrate accumulation (Gaudreau et al., 1995; Nazaryuk et al., 2002). Predicting and manipulating nitrate deficiency in relation to other fertilization chemicals (Ahmed et al., 2000; Chen et al., 2014). It is reported in several studies that stopping the nitrogen supply in growth medium few days before crop maturity, resulted in yield losses and poor leaf quality.

Many authors have suggested that additions of moderate amount of chloride to the growing medium replacing nitrogen supply during the last week prior to harvest decreases nitrate content (Maynard et al., 1976; Cerezo et al., 1990; Urrestarazu et al., 1998). Increase rate of potassium and phosphorus application facilitates and promotes metabolism accompanied by nitrate assimilation (Buwalda and Warmenhoven, 1999; Ahmed et al., 2000).

Furthermore, the increased light intensity supplied more energy to fix carbon dioxide to accelerate nitrate assimilation in plant leaves (Demsar et al., 2004). In hydroponic culture broken nitrogen treatment before harvest can decline the nitrate accumulation in leafy vegetables (Liu and Yang, 2012; Zhou et al., 2013). The morphological, physiological adaptation of plants depends on light quality, the spectral composition of light sources must be adjusted to physiological requirements of plants to drive photosynthesis efficiently for growth and development (Lee et al., 2007). The nitrate content of leafy vegetable is negatively correlated with photosynthetic end product (carbohydrates). The amount of said product can be uplifted by higher light intensity whereas nitrate stored in vacuoles (Abidi et al., 2013). In term of light quality that refers to effect of red and blue lights on plant architecture and development attracts more attention due to specific wavelength and color (Johkan et al., 2010), which ultimately affects plants photosynthetic capacity and CO₂ assimilation to reduce nitrate content (Yorio et al., 2001; Dougher and Bugbee, 2004; Hernandez and Kubota, 2016). Nitrate content in indoor facilities is greatly affected by environmental factors, interaction between light quality, light intensity, temperature, and nitrogen supply. Unfortunately, different experimental conditions and designs restrain an efficient comparison of the listed methods.

**CONCLUSIONS**

Leafy vegetable production in plant factory using hydroponics represents substitute to traditional farming with benefits for producers, consumers and environment. Leafy vegetables are the main source of nutrition and contributes to 80% of dietary intake of human diet. Leafy vegetables especially grown in indoor need further studies to understand the specific role of nitrate and its derivatives with respect to human health because ingestion of high nitrate level though vegetables may lead to carcinogenic effects. Leafy vegetables such as lettuce grown under distinctive production systems may accumulate different nitrate content which may reach to the levels potentially toxic to humans. Thus, uncertainty to human health due to nitrate intake may be minimized according to European commission regulation No. 1822/2005 by determining the exact mechanism for nitrate accumulation in leafy vegetables. It is more important to educate the farmer and consumer to understand the human health implications of nitrate in dietary intake. They must be motivated to adopt relevant agricultural practices that help in minimizing nitrate content. Nitrate and nitrite levels in raw agricultural commodities can be influenced by a number of factors such as storage time and conditions (i.e. ambient, refrigerated, frozen), and food processing (i.e. washing, peeling, blanching, boiling). Therefore, various approaches are suggested and may be adopted to decrease the nitrate level.

- Leafy vegetables grown in hydroponic system needs balanced fertilization scheme according to supply and release of nutrient ions. Nutrient management strategies should be implemented with simulation models for proper uptake and its reduction, because the time variation of nitrate content in whole lettuce plants during photoperiod and dark period throughout cultivation is different. Controlled nutrition results in sufficient reduction in leaf nitrate of leafy vegetables.
- Nitrate uptake in aerial parts of leafy vegetables may be reduced by partial adjustment of nitrogen solution with chloride before harvest, under controlled environmental conditions increasing the light intensity over diluted nitrogen solution to standard effects the nitrate content.
- A computerized system is proposed which supplies different light condition (slight, medium, strong) in accordance with growth stages and changeable nitrate content. Effect of light quality on micronutrients, cultural conditions, physiological mechanism need to be investigated by molecular tools to understand the over expression of nitrate.
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