

INTRODUCTION

It has been observed that greater losses of vegetables occur every year during the postharvest period which are not only in underdeveloped countries, but also in very well developed ones where modern technologies are available.

Vegetables are perishable products with active metabolism during the postharvest period. Proper postharvest handling plays an important role in increasing food availability, as documented by the increasing number of publications in the field of post harvest physiology, technology and handling during the past 10 years (Weichmann, 1987). Interestingly, most publications cover the topic of fruit postharvest physiology exclusively. Problems of vegetable postharvest physiology are studied much less often.

A comprehensive definition of vegetables has been given by Schuphan (1948), according to this definition vegetables are any plant products that are consumed fresh, raw or processed directly without substantial extraction. Vegetables cover a wide range of plant parts. Fresh vegetables are essential components of human diet. They contain a number of nutritionally important compounds especially the vitamins which cannot be synthesized, by the human body.

The immense importance of nutritional value in human diet, had led to preserve the quality of vegetables by using various ways and means. It is now axiomatic that storage can contribute to keep these characteristics as unchanged as possible but cannot improve them. Because of well recognised benefits in human nutrition, attributed to adequate consumption of fresh vegetables, there has been increased interest in improving commercial storage system for vegetables in the fresh state, so that large population inhabiting temperate climates may have such fresh vegetables regularly. However, in the endeavour to improve the storage system, the great diversity of plant materials used as food is a complication. Thus determining the storage potentials and then developing suitable techniques for exploiting these potentials seems to be the essence of storage improvement.

The knowledge of the changes that occur during harvest will therefore help to develop proper methods of handling the harvest produces in order to preserve their quality as intact as possible from the time they are harvested to the time they are stored. Knowledge of physiological changes that occur during harvest, is of vital importance.

'Turgidity' is certainly one of the master words in **postharvest** physiology, because it is the sine quanon condition for the fresh appearance of the produce and a guarantee of their health. Because of transpiration, the osmotic pressure, hence the suction pressure, increases. The source of water is cut off by the harvest, thus the deficit remains unbalanced and there is a loss of turgidity. This may then lead to biochemical damages. Another consequence is that the superficial cells become inert sacks of nutrients, which are then attacked by the pathogens. Therefore in the **postharvest** handling of plant organs, maintaining turgidity must be of the prime concern.

When an organ or a fruit is picked up during harvest there is a rupture of the cuticular barrier, through which inward flow of O_2 and outward flow of CO_2 of the same magnitude takes place. The consequence of this is that the cells show an abrupt increase in the respiration rate, a rapid depletion of respiratory metabolities - mainly reducing sugars and an increase in all the oxidative processes especially those affecting the phenolic compounds. Ethylene also casts its effect due to low CO_2 concentration. One striking instance of this is the destruction of chlorophylls in immature fruits, such as **peapods**

Green leafy vegetables pose a particular problem, which most harvesters are not aware of, the balance between respiration and photosynthesis. The data gathered by Phan and Tremblay (1982) showed a steady increase in the respiratory rate of head cabbage through the first seven days when placed in cold storage room at 4° C, where normally respiratory activity probably did decrease. As photosynthetic activity is generally much higher than respiratory activity, the reduction of the former will result in the apparent enhancement of the latter, even if this is also lowered by low temperature

As a general rule most mature organs like head lettuce, head cabbage, cauliflower, broccoli have stable green colour. Whereas, immature organs such as peapods or asparagus spears, undergo very drastic chlorophyll degradation. The enzyme chlorophyllase seems to become active as the amount of chlorophyllide increased noticeably 24 hours after harvest at room temperature (Phan, 1977). The other group sensitive to harvest is that of the phenolic compounds, phenol oxidation results in superficial browning, polymerization of tannins and latex blocking the vascular channels. In fact amino acids do not vary markedly but there might be a tremendous synthesis of proteins, mainly enzymes after harvest.

Taking into consideration the various aspects of physiological changes during postharvest period led to develop modern techniques for storage of essential commodities. Most of the techniques are concerned with storage of fruits. But very little attention has been paid to the vegetables. Especially in our country the postharvest physiology of leafy vegetables has been least dealt with, even though these vegetables form a common man's diet.

A number of workers have investigated storage methods for fruits and vegetables to overcome the physiological changes during the harvest period. One of the common and easily manageable method is the storage of necessary commodities at chilling temperature.

A chilling temperature is any temperature below the critical threshold temperature (but above freezing) that causes injury. For most warm - season vegetable crops, these threshold temperatures are around 10° - 12° C. There is no generalised rule for chilling temperature. However, the optimum temperature for any plant process is not necessarily constant, but may vary with other conditions, even the species vary somewhat in tolerance with their region of origin.

Thus a chilling temperature can be defined as any temperature that is cool enough to produce injury but not cool enough to freeze the plant. Chilling or chilling treatment is the process of exposure of produce to a chilling temperature for a time period sufficient to cause permanent or irreversible damage. Many vegetables, primarily those of tropical or subtropical origin, are susceptible to a low temperature stress referred to as chilling injury.

Chilling injury in plants has been recognised since before the turn of the century (Molisch, 1897). It has become a topic of keen interest in recent years not just because of its importance as a postharvest concern, but because low temperatures limit the geographical areas and time of year for production of chilling - sensitive crops. A body of literature on the subject is referred to in several recent reviews [Ryall and Lipton, 1972 ; Lyons et al., 1979; Wade, 1979; Levitt, 1980; Bramlage, 1982; Graham and Patterson, 1982; Watada, 1982] where the horticultural aspects of chilling injury are well documented.

The sensitivity to low but non freezing temperatures possess serious postharvest problems in storage and handling, since refrigeration to slow metabolism is the most manageable way to extend storage

life of perishable commodities.

The symptoms shown by the plants in the field are quite different from those shown by them in storage. Three kinds of injuries occur. a) Direct injury, b) Indirect injury and c) Secondary injury (stress).

Direct injury is the direct effect of chilling temperature on plants. Seible (1939) divides plants that suffer chilling injury into two types according to the speed of the reaction. The first type (Episcia, Achimenes, Gloxinia) show injured spots after hours or at the latest after a day, due to death of the protoplasm and infiltration of the intercellular spaces. The second type (Tradescantia, Solanam, Coleus) are more resistant. They remain perfectly normal and turgid for a full day, but become soft and wilted only after chilling 5-6 days. Many of the fruits are included into this category.

Injury due to sudden chilling, which is very rapid is called a cold shock. Direct injury may occur as a result of several hours chilling, as well as when chilled abruptly for a few minutes. Increased leakage of K^+ may be the reason for pseudoplasmolysis in the cells at chilling temperatures. Membrane damage and apparent increase in cell permeability are the direct chilling injuries.

Indirect injury is the gradual and maintained stress. An increase in permeability has been indicated by increased leakage. Chilling - resistant cabbage showed no such increase in permeability. Light is essential for the injury to occur, the leaves becoming bleached and leaking amino acids. O_2 uptake is also decreased and therefore increase in leakage may be due to a decrease in active uptake. It is known that even in chilling resistant plants such as barley, active metabolism - dependent, ion uptake is sharply curtailed by chilling temperatures. Indirect injury is due to a metabolic upset. Several kinds of metabolic disturbances have been described.

In case of plants from temperate climates, a low temperature above freezing, produces only an elastic (reversible) strain and the plant is normally uninjured. At chilling temperatures in plants from tropical or subtropical climates, respiration rate may exceed the rate of photosynthesis and this may conceivably lead eventually to starvation. The reason for the sharp drop in photosynthesis is damage to the chloroplast thylakoids at the chilling temperature ($4^{\circ}C$). Thylakoid membranes of chilling sensitive species are less able to maintain a light induced high energy state at chilling temperatures. Photooxidation

at chilling temperatures may actually bring photosynthesis to a stop. Starvation of non photosynthesizing plant parts may result from the inhibition of translocation by chilling temperatures. eg. in sugarcane, translocation ceases completely at 5°C (Hartt, 1965). The inhibition of translocation may be due to an effect on the sink or on the translocation path. Exposure of maize to 2° C caused a sharp and continuing fall of soluble sugar level in the root tips and this was accompanied by reduced respiration and cessation of growth.

Chilling temperatures strongly inhibit the aerobic phase of respiration without any inhibition of the anaerobic phase of the overall process of respiration. This is true in chilling sensitive plants. However, chilling resistant plants show a different kind of respiratory change at chilling temperatures. They develop a greater involvement of the pentose phosphate pathway. In case of fruits the respiratory rise is associated with chilling injury, which might be due to increased production of ethylene. Twenty to twenty-five times increase in ethylene production was recorded in citrus fruits when chilled by a temperature regime of 20° Day / 5° night. Effects of chilling on cytoplasmic streaming may possibly be related to these respiratory changes. In all the chilling sensitive plants tested (tomato, watermelon, tobacco, sweet potato) streaming

was just perceptible in the trichomes or ceased completely after 1-2 min at 10° C. In the chilling resistant plants (radish, carrot), on the other hand streaming continued even at 0 - 2.5° C. However, some attempts have indicated that both oxidative activity and phosphorylation are inhibited by the chilling stress.

Accumulation of α - keto acids, browning (polyphenol) and accumulation of peroxides has been reported in the peel of the fruits, around vascular tissues and in the leaf tissue respectively.

Protein break down at low temperature without an equally rapid resynthesis has been suggested as a cause of injury due to a deficiency of proteins or a toxicity by the products of hydrolysis (amino acids, NH_3). Decreased photorespiratory activity with decreased supply of ATP may be responsible for decreased rate of protein synthesis.

Deficiency of ATP, inhibition of ATP synthesis, inhibition of the reaction - lutein \rightarrow violoxanthin, loss of ascorbic acid, nitrogen deficiency, increase in the level of chlorogenic acid, increase in the activity of hydroxycinnamyl transferase (an enzyme of chlorogenic acid metabolism) are some of

the suggested events as the biochemical lesions of chilling injury. The photooxidation may be thought of as producing a toxic product or as preventing the synthesis of an essential component chlorophyll, and thus producing a biochemical lesion. It may also damage membrane system.

Development of secondary water stress at low temperatures was first indicated by Sachs (1864). Kramer (1942) found that low temperature decreased the water absorption more in chilling-sensitive than in chilling resistant crops. He concluded that this was due to a chilling induced decrease in permeability. Some evidence indicates that a secondary O₂ deficiency stress may also develop at chilling temperatures.

Significant changes may occur at chilling temperatures in the relative rates of glycolysis and mitochondrial respiration in chilling sensitive vegetables, which may result in higher respiration rates at the chilling temperatures than at the non-chilling temperatures for certain commodities, such as okra and cucumber. Another important symptom of chilling injury is the abnormally high respiration rate upon transfer to a non chilling temperature. Sustained increase in respiration rate usually indicates occurrence of irreversible damage. This enhanced

respiration may be due to the tissue's efforts to repair damage to membranes and subcellular structures and / or to eliminate toxic metabolic intermediates that may have accumulated during exposure to chilling temperatures (Wang, 1982).

Wang and Adams (1980,1982) have shown that the detrimental effects of chilling observed in fresh cucumbers held at 2.5° C is due to increased synthesis of ACC (1-aminocyclopropane - 1 - carboxylic acid) synthase, resulting in increased Acc and ethylene levels which occurs only after the fruits are transferred to a higher temperature of 25° C. However, if the chilling period is prolonged for more than four days, large amounts of ACC are synthesized but little ethylene is produced, suggesting that excessive chilling irreversibly damaged the conversion of ACC to ethylene. Under most commercial conditions chilling is not likely to exceed four days, and the ethylene generated, upon warming, probably is responsible for the loss of colour and pitting noted in cold - damaged cucumbers.

Van Staden and Dimalla (1977) investigated the endogenous cytokinins in potato tubers stored in the dark for nine months at 5° C, during which they developed a storage disorder called "little potato". Wurr et al., (1980) demonstrated higher levels

not only of cytokinins but also of gibberellins in extracts from little potato tubers than from normal tubers.

Another metabolic transformation, sensitive to temperature, is the interconversion of carbohydrates in case of irish potatoes. They become 'sugary' when kept at low temperature. Pressey (1966) showed that this phenomenon has its cause in the increase of the activity of the enzyme invertase at low temperature. This characteristic of invertase activity has been found in other tissues such as carrots (Ricardo and ApRees, 1970) and is now believed to be universal.

Vitamins, especially ascorbic acid, are decreased on storage at temperatures below 12.5° C, the loss increases with decreasing temperature due to chilling injury. Sweet potatoes are chilled at 10° C and symptoms of injury become clearly apparent when they are stored for 10 weeks at 7.5° C. This loss of ascorbic acid accompanies senescence and deterioration Lieberman et al., (1959).

Storage conditions may affect the amino acid contents of Vegetables. The increase in nonprotein nitrogen was enhanced by chilling injury during storage of tomatoes (Abou Aziz - et al., 1975).

Chilling resistance is due to an ability of cells to maintain the membrane lipids in the liquid crystalline state at chilling temperatures. Early workers observed that plants of warm climates contained more saturated fatty acids than plants of cooler climates. A degree of unsaturation of fatty acids has been correlated to chilling resistance. Maintenance of unsaturation or increase in the amount of unsaturated fatty acids in the membrane system and thus maintaining or increasing the fluidity of lipids in the membrane system caused to increase the chilling resistance in plants. Increased level of cholesterol and sterol contents may resist the chilling damage. Increased protein sulfhydryl content may be taken as one of the mechanisms of chilling resistance.

Activity of soluble enzymes, not associated with membranes may be involved in chilling resistance mechanism. According to the ability of the plants to withstand chilling temperature they are classified as follows.

a) Chilling - insensitive species which have their origins in temperate zones, continue to grow and develop slowly and even complete their life cycle at temperatures down to or near 0° C.

b) Chilling sensitive plants :- when exposed to temperatures below the chilling threshold, inevitably result into their death. Among chilling - sensitive plants, there is variability in the rate of expression of injury, and a resistance or a tolerance range can be exhibited. Species can in some instances, express only slight symptoms of injury in response to prolonged or repeated period of chilling temperature because, they have the ability to either better endure the insult of the low temperature impact upon the primary temperature sensor or under intermittent exposure, repair the trauma before irreversible damage occurs.

From this foregoing account of the literature on plants and the chilling temperature effects on them, it is evident that most of the work is on the fruits stored at chilling temperatures. Very few vegetables like tomato, potato, cabbage, onion and lettuce are so far studied. There is almost no work on the physiology of leafy vegetables stored at chilling temperatures. Hence, in the present investigation an attempt has been made to study the physiological changes imposed by chilling temperature in two leafy vegetables namely Amaranthus blitum L. Var. Oleracea, Hooker (Tambada Pokla) and Trigonella foenum - graecum L. (Fenugreek - Methi). Both these plants are very popular

leafy vegetables, which form a common man's diet. They are rich in nutritive and medicinal values.

The present investigation deals with the influence of chilling temperature during storage of these vegetables for 48 and 96 hours, on moisture content, chlorophylls, carbohydrates, polyphenols, soluble proteins, proline, nitrate and ascorbic acid contents. Changes in the level of peroxidase, catalase and nitrate reductase as influenced by storage were also investigated. Absorption spectrum of photosynthetic pigments was also determined

The plants were subjected to chilling temperature in the refrigerator with the temperature recorded $5^{\circ} \pm 2^{\circ} \text{C}$. The plants were kept in two sets, one covered with polyethylene bag, while the other uncovered. Simultaneously the plants from the same harvest were kept covered with polyethylene bag, in the laboratory at normal (room) temperature. In the same manner, plants sprayed with NaCl were also subjected to chilling and room temperatures. For analysis the most recent methodology and techniques have been followed.

The thesis has been divided into the following parts :

The first part of the thesis (chapter I) reveals the brief idea regarding the terms, chilling

temperature, chilling injury, and review of chilling injury studies of fruits and vegetables.. Later part deals with the types of chilling injury, physiological effects, horticultural manifestation, mechanism of chilling injury, mechanism of chilling resistance in plants and finally with the various methods of alleviation of chilling injury. The chapter ends with a brief resume of morphology, nutritional and medicinal importance of the vegetables undertaken.

Second part of thesis (chapter II) denotes the source of material obtained and the methodology adopted to study the chilling effect on the two vegetables undertaken for study.

The significant findings of the present investigation have been statistically analysed and discussed critically in the light of recent Literature which was obtained in the form of reprints, research articles, research papers, monographs and books. All this has been included in third part of the thesis (chapter III).

The problem, perspectives and significant findings of the investigation have been summarised briefly in the fourth part of the thesis (chapter IV).

The various research papers, articles, monographs and books used extensively for the discussions have been listed in Bibliography which formed the last part of the thesis.