Physiological and Biochemical Studies on Parsley during Postharvest Storage

by

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THESIS

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P R E S E N T A T I O N

TO THE GREAT MASTER OF ALL HUMANITY

OUR PROPHET MOHAMED

A C K N O W L E D G M E N T S

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I - INTRODUCTION

Since a long time ago parsley has been one of the important leafy vegetable crops known in Egypt. It is favoured in the diets of Middle Eastern people, particularly in the United Arab Republic. Parsley is also known by the pharmaceuticologists and physiologists for its valuable importance in the relief of several bacterial and fungal diseases. The prolongation of market acceptability period for leafy vegetables is one of the most important problems. This is because of their rapid senescence and deterioration which begins at harvest time.

The new trend to increase the area cultivated with vegetables for exportation introduced several problems for investigation; one of which is how to store vegetables to extend their shelf-life with a minimum loss of quality and nutritive value. Harvesting at the ideal stage of development, fast precooling after harvest, modern packaging methods, and maintaining a low temperature during transit and storage, are widely used practices to extend the marketing life of fresh vegetables through the reduction of transpiration and/or respiratory rate.

The main postharvest deterioration factors for leafy vegetables are water loss (transpiration) and green colour loss (chlorophyll degradation). Water loss can be minimized by low temperature and high relative humidity during transit and storage.

Several additional postharvest treatments have been investigated. Kinetin and N^6 benzyladenine, a synthetic kinin-like chemical applied as a pre- or post-harvest spray or dip, have been found to delay yellowing and other manifestations of senescence in harvested leafy vegetables.

The purpose of this study was to investigate the effect of postharvest application of N^6 benzyladenine at various concentrations on quality, water loss, chlorophyll degradation, amino acids content, and total amino nitrogen content of harvested parsley plants.

II - REVIEW OF LITERATURE

A - Effect of Kinetin and Benzyladenine on Leaf Senescence

Spot application of radioactive kinetin to leaf tissue have been shown by Mothes *et al*. (1959) to delay leaf senescence, but the effect was localised in the treated areas. They reported that nitrogen compounds were actually mobilized to the point of application from elsewhere in the leaf. Mothes (1960) concluded that the aging(yellowing) process of *Nicotiana rustica* leaves can be reserved by the application of Kinetin which stimulated protein synthesis.

Osborne and Hallaway (1961) reported similar results using auxins to those previously mentioned by Mothes *et al*. (1959). Spot application of 2,4-D and 2,4,5-T delayed leaf senescence. They suggested that the auxin treated spots acted as "metabolic sink" draining nitrogenous and carbon compounds from surrounding untreated area of the leaf.

Parthier (1961) found that when leaves of *Nicotiana tabacum* were treated with 10^{-6} - 10^{-5} M for 24 hours, they showed an increased ^{14}C incorporation into proteins from labelled glycine. This effect was more marked in darkness than in light. Higher concentrations of kinetin were inhibiting.

Sugiura *et al.* (1962) working on leaf discs of tobacco floating in water in darkness, reported that leaf discs of *Nicotiana rustica* did not respond to kinetin unless brought into the light or supplied with sugar. Kinetin induced a considerable accumulation of nucleic acids in the microsomal and supernatant fractions.

Banaerji and Laloraya (1963) studied the effect of 5 ppm kinetin solution on protein and soluble nitrogen in leaf discs of *Nicotiana rustica*. Kinetin maintained the protein level and green colour of leaf samples taken from flowering plants. On the other hand, the leaf protein content decreased with increasing node number from the apex. Thus, they concluded that the physiological age of the leaf may be a controlling factor in the kinetin regulated protein level.

Osborne (1962) reported that kinetin addition to detached Xanthium leaves and to exised discs temporarily arrested the senescent changes and maintained a relatively high ratio of RNA (or protein) to DNA. The increased incorporation of ^{14}C-leucine into protein and ^{14}C-orotic acid into RNA was indicative of a stimulation in both RNA and protein synthesis. Thus, Osborne (1962) suggested that the effect of kinetin in retarding senescence in Xanthium leaf cells is mediated through its action in sustaining nucleic acid and protein synthesis.

Kulaeva *et al.* (1963) mentioned that yellowed mahorka leaves treated with kinetin turned green again provided the nuclei of the cells have not been destroyed. During the process of regreening the size of the nuclei more than doubled and there was a considerable increase in the protein and RNA contents.

Leopold and Kawase (1964) working on the effect of benzyladenine on bean leaf growth and senescence, found a marked stimulation of leaflet growth and green colour in treated plants, whereas untreated leaves were quickly forced into a yellowing condition.

Halevy and Wittwer (1965) reported that N^6 benzyladenine, CCC and B-Nine were active in preservation of chlorophyll in detached leaves of bean and some other plants.

Yordanov and Papov (1966) reported that mature phaseolus bean leaves when stored for 8 days in dark, showed little chlorophyll degeneration, either in control or kinetin treated plants. Meanwhile, when the leaves were kept in the dark for 16 days, kinetin markedly preserved the chlorophyll content as compared with the untreated plants.

Von Abrams and Pratt (1966) found that senescence (measured as loss of chlorophyll) was strongly retarded by kinetin to leaf disks, localised areas of laminae, and petioles of detached leaves of broccoli and Xanthium. The response to kinetin was as great in young leaves as in old leaves of broccoli.

Pozsar et al. (1967) reported that protein synthesis increased in both kinetin and benzyladenine treated half leaves of beans relative to untreated samples, as measured by the incorporation of ^{14}C-glycine into the protein fraction. The incorporation of ^{14}C-adenine into the nucleic acid fraction of bean leaves was increased by benzyladenine treatment.

Kesslar et al. (1967) observed that protein breakdown in leaf disks from Phaseolus vulgaris plants was retarded by CCC and kinetin solutions. Retardation was enhanced by the presence of actinomycin D. Chlorophyll levels were similarly maintained by these growth substances.

Lagerstedt and Langston (1967) found that radioactivity from kinetin-8-^{14}C freely moved in the vascular system of several types of the leaves, including those of beans. This movement was usually distal to the point of application and seemed to occur with the transpiration stream. The transloc- ated material was extracted from vein tissue and was shown to be radioactive and to be able to retard senescence.

B - Kinetin and Benzyladenine as Senescence Inhibitors For Harvested Green Vegetables

Kinetin and N^6 benzyladenine, an active chemical analog of kinetin, have been used as senescence inhibitors for green vegetables by many workers since 1960. However, these growth regulators have not yet been approved for commercial application because of their questionable safety in human consumption.

Bessey (1960) reported that dips and sprays of benzyladenine (5-25 ppm) on lettuce retarded green colour and extended marketing life as compared to untreated lettuce heads.

Zink (1961) obtained similar results by using 5 ppm benzyladenine as a postharvest spray on Great Lakes lettuce variety. Benzyladenine treatment also increased the storage life of leaf and butterhead lettuce varieties. Comparing different methods, rates, and timing of benzyladenine application, the same author concluded that best results were obtained with applications as near harvest as possible or with postharvest applications. He also found that the response of endive, escarole, spinach, and mustard greens to benzyladenine (5 ppm) was striking in delaying senescence symptoms such as chlorophyll loss, quality deterioration, and infection by fungi and bacterial soft rots. Preliminary studies by Zink (1961) showed that benzyladenine was also effective as a senescence inhibitor for Brussels sprouts, sprouting broccoli, radish and carrot tops, celery, parsley, green onions and asparagus. In general, concentrations in the range of 2.5 to 10 ppm showed satisfactory results whereas higher doses (20 ppm) may cause phytotoxicity, as evidenced by marginal necrosis and wilting.

Guzman (1961) sprayed benzyladenine (2.5 and 10 ppm) in water at 150 galons/acre on celery, endive, lettuce and brassica crops before harvesting. All treated plants retained their colour and remained fresh longer than untreated plants. A concentration of 5 ppm was recommended for practical purposes.

Kaufman and Ringel (1961) treating freshly harvested cauliflower heads with 10 ppm benzyladenine or a combination of 10 ppm benzyladenine and 50 ppm 2,4-D found that benzyladenine treated cauliflower heads had a longer marketable life than untreated heads. On the other hand, Benzyladenine and 2,4-D treatment prevented abscission and retarded yellowing for 28 days in storage at 38°F.

Salunkhe and Dhaliwal (1962) used benzyladenine (5,10, and 20 ppm) at weekly intervals as 1-minute dip for cauliflower, endive, parsley, beans, lettuce, radishes, onions and cabbages during postharvest storage at 5°C. The storage life of these vegetables was extended by a period that ranged from 2 days for cauliflowers to 8 days for cabbages.

Van Overbeek and Loeffler (1962) found that kinin or benzyladenine treated leaves fed with $^{14}CO_2$ had a composition of resulting metabolites which resembled a growing leaf rather than a leaf in storage, which show that Kinins may act as maintenance hormones, Postharvest Kinin treatment prevented or substantially reduced the breakdown of cellular constituents usually associated with stored produce and reduced respiration. Thus, by maintaining cellular integrity, kinin treated plants retain their colour,flavour, resistance to invading micro-organisms, and even against source of kinin production in the intact plant.

Atlee (1962) indicated that benzyladenine (2.5, 5 or 25 ppm) treatments caused Brussels' sprouts to retain their chlorophyll longer and lose their quality more slowly than untreated sprouts, Respiration rates for treated sprouts were about 30% lower than rates for untreated ones except for the 25 ppm benzyladenine treatment, which gave an initial higher rate of respiration. This may be due to some toxicity at the higher concentration.

Hansen (1963) reported similar results with Brussels sprouts. In addition, he reported that benzyladenine treatment preserved quality of cauliflower and radishes for a longer time than untreated plants during cold storage.

Wittwer and Dedolph (1962) reported that benzyladenine treatment reduced respiratory rate during storage and extended the shelf-life of asparagus, braccoli, and celery. Asparagus spears which were dipped in 10 ppm benzyladenine after harvesting were satisfactorily stored at 40°F for 3 weeks. Yellowing of broccoli and celery heads was delayed by a postharvest dip in a 10 ppm benzyladenine solution.

Wittwer et al. (1962) found that postharvest dipping of freshly harvested stalks of two varieties of celery in 10 ppm benzyladenine extended the duration of visual freshness, green coloration and market acceptability of both varieties. Weight losses were reduced during storage at 50 and 60°F, but not at 40 or 70°F. The effectiveness of benzyladenine in preserving freshness in celery appeared to be a consequence of respiration inhibition.

Sigall et al. (1964) reported that benzyladenine increased the shelf-life of escarole (Chicorium endivum) by two or more days.

Dedolph et al. (1962) studied the effect of benzyladenine (10 ppm) on respiration and storage behaviour of broccoli (Brassica oleracea var. italica). They found that