STORAGE QUALITY OF LETTUCE LEAVES AS AFFECTED BY KINETIN AND ABSCISIC ACID

by

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Abstract

Some effects of post-harvest treatments of abscisic acid (ABA) and kinetin on the maintenance of quality and consumer appeal were studied on young lettuce plants. The treatments employed two concentrations of abscisic acid (1 and 5 ppm), one concentration of kinetin (20 ppm) and a combination of 5 ppm abscisic acid and 20 ppm kinetin. The plants were sprayed to the run-off point and placed in a storage chamber at $3 \pm 1^{o}C$ with relative humidity close to 100%. After 6 weeks of storage all lettuce including untreated controls were in good condition. The chemical treatments did not have any distinct effect on the quality of lettuce as evaluated by a panel of observers for visual quality rating. The 20 ppm kinetin retarded chlorophyll degradation when compared to the control or the ABA-only treatments. Considering chlorophylls A and B separately, the kinetin-treated plants showed a significantly higher chlorophyll A content than other treatments, including the control. The differences in chlorophyll B content followed the same trend but only approached the 5% level of significance. ABA in the 5 ppm + 20 ppm kinetin treatment had a mild antagonistic activity to kinetin, and hence reduced the effect of kinetin on both chlorophyll A and B. Measurement of chlorophyll contents and adjustment to the original fresh weight before the samples were put in storage, provided a common basis to make comparisons for the study of chlorophyll degradation as functions of storage time and chemical treatment. Means of chlorophyll contents reported on this basis showed a trend of degradation from the 5th week to the 7th week. Temperature at $3 \pm 1^{o}C$ and high relative humidity in the storage appear to be favourable for keeping lettuce. Hygenic preparation of the storage chamber also resulted in disease-free product even at the end of 7 weeks in storage.

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INTRODUCTION

Minimizing losses and conserving quality of vegetable crops in the post-harvest period is a challenge for growers, shippers and merchants who wish to get good quality produce to the consumer. Consequently, improved methods are constantly being sought for retarding the rates of transpiration, respiration, and chlorophyll degradation, thus lessening wilting and senescence, and extending the post-harvest salability of vegetable crops.

Present methods of fresh vegetable preservation include precooling, cold storage and special processing such as waxing and prepackaging; however, recent reports on the use of kinetin or abscisic acid suggested that these chemicals along with conventional cooling methods might be valuable to extend the post-harvest life of those vegetable crops even further.

Abscisic acid is known to induce stomatal closure and inhibit transpiration in some plants at the normal room temperature range (Little and Eidt, 1968; Mittelheuser and Van Steveninck, 1969; Horton, 1971; Cummin et al, 1971), and might be expected to inhibit transpiration in fresh vegetable crops and so extend their post-harvest life. If used in conjunction with conventional cold storage, the quality life of produce might then be significantly lengthened.

Kinetin was demonstrated by El-Mansy et al. (1967) to be an effective senescence-retardation agent under cold storage conditions, therefore, this chemical was also used in the present study. Furthermore, abscisic acid and kinetin have been known to interact in many physiological systems (Addicott and Lyon, 1969), therefore the effect of these two chemicals together on post-harvest quality was included.

In the present study, lettuce (*Lactuca sativa* L. var. capitata L.) was selected as the test vegetable. The rapid development and perishability of lettuce make it a convenient test plant for this type of research. Additionally, El-Mansy *et al.* (1967) used lettuce in his experiments with kinetin and these studies provide a valuable background for reference and comparison for the present study.

An experiment was planned to observe some effects of abscisic acid and kinetin, both separately and in combination, on the post-harvest quality of young lettuce plants.

LITERATURE REVIEW

A. Abscisic Acid

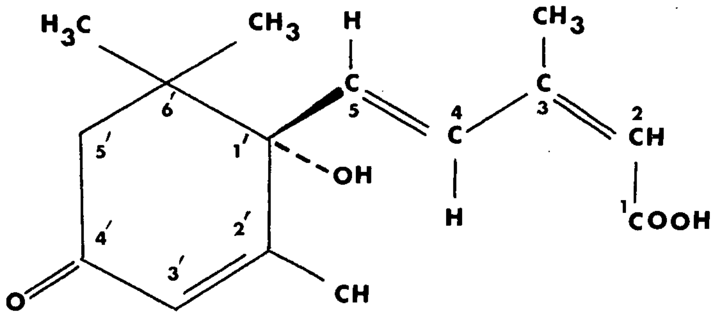


Figure 1. Structure of (S)-abscisic acid

The structural formula shown above is for a 3-methyl-5(1′-hydroxy-4′-oxo-2′-6′-6′-trimethyl-2′-cyclohexen-1′-yl)-cis, trans-2,4-pentadienoic acid - a hormone now known under the name "abscisic acid" and usually designated as ABA. This hormone is a relatively recent discovery; nevertheless, its physiological importance may rank with auxins, gibberellins or cytokinins (Addicott and Lyon, 1969). The substance was first isolated by Ohkuma *et al.* (1963) from young cotton fruit (*Gossypium hirsutum* L.). It was then named "abscisin II" because it promoted abscission activity. Almost at the same time, a group led by Wareing and Cornforth, being interested in dormancy-inducing substances, isolated an active substance from sycamore leaves (*Acer pseudoplatanus*). This substance was named "dormin" and later it was found to be the same substance as abscisin II (Cornforth *et al.* 1965a; Robinson and Wareing, 1964). This chemical was first synthesized by Cornforth *et al.* (1965b) and more contributions were added on its physical and biological activities (Cornforth *et al.* 1966). After the Sixth International Conference on Plant Growth Substances in 1967, the present term "abscisic acid", by mutual consent, is being used in place of the names "dormin" and "abscisin II" (Addicott et al. 1968). Figure 1 was derived from the latest revision on this chemical by Ryback (1972). ABA is widely distributed in plants (if not ubiquitous), and mostly found in very low concentrations, such as 40 μg/kg dry weight from Gossypium fruits (Ohkuma et al. 1963), and 9 μg/kg dry weight from Acer leaves (Cornforth et al. 1965a). The natural enantiomer of ABA has been found to be (S)-(+)-abscisic acid (Cornforth et al. 1966). The synthetic racemis substance is (RS)-(±)-abscisic acid, and this compound, on bioassay, showed approximately one-half the inhibitory activity of the natural hormone (Cornforth et al. 1965b).

ABA, like all other hormones, induces a wide spectrum of plant responses. Besides its activities in abscission and senescence, it is well recognized in various other significant phenomena including germination, dormancy, enzyme activities, and flowering. The general physiology of ABA, as well as its chemistry, historical discovery and development, is well reviewed by Addicott and Lyon (1969). Lately, it has been found that ABA affected stomatal diffusion resistance and transpiration (Little and Eidt, 1968; Mittelheuser and Van Steveninck, 1969; Mizrahi et al. 1970; Horton, 1971; Jones and Mansfield, 1970). This particular effect has given rise to the idea of using ABA as an antitranspirant which may be useful in prolonging post-harvest quality of some horticultural crops.

A.1. Effect of ABA on abscission and senescence

Leaf or fruit abscission is a common response to ABA treatment. This response is accepted as a part of the bioassay technique for ABA (Addicott and Lyon, 1969). Bornman et al. (1967) studied the nature of ABA-induced abscission in 14-day-old cotton explants. Comparisons were made among the effects caused by ABA, an abscission accelerant GA_3[1], and an abscission retardant IAA[2]. ABA was found to cause a breakdown of cells in a weakly defined separation layer and the separation could be commenced either ad- or abaxially, but it occurred abaxially in the control and in IAA-treated plants. The breakdown in a well defined separation layer of three or more rows of cells in width was observed in GA_3-treated plants. Cracker and Abeles (1969), working with explants of cotton and bean, suggested that the effect of ABA on abscission was two-fold. ABA appeared to cause an increase of ethylene production from explants which was found to account, at least in part, for the ability to accelerate abscission. There was also an increase in cellulase activity simultaneously, leading to an acceleration of abscission. Galston and Davies (1970, p.167) do not attribute the whole process of abscission to ABA only, but rather to the more complex system involving other hormones such as auxin and ethylene. Much evidence of hormone balance in connection with abscission has been reported (Salisbury and Ross, 1969, p.652).

Acceleration of senescence is another effect of ABA. Sankhla and Sankhla (1968a) demonstrated that ABA treatment proved a potent accelrator of senescence of Arabidopsis leaf disks. Within 24 hours, leaf disks floated on 5 ppm ABA lost 3 times more chlorophyll than the control. The mechanism whereby ABA promotes senescence is not yet clearly explained.

A.2. Effects of ABA on growth, dormancy and seed germination

Growth inhibition is the basis of several bioassays for ABA content. Such assays include growth inhibition of coleoptiles (Robinson and Wareing, 1964), hypocotyls (Aspinall et al. 1967; Eagles and Wareing, 1964),

[1] GA_3 = Gibberellic acid; [2] IAA = Indole acetic acid.

radicles (Aspinall _et al_. 1967), and leaf sections (Eagles and Wareing, 1964). ABA-induced dormancy in deciduous trees was reported by Eagles and Wareing (1964). ABA treatments by means of dipping, soil drench or spraying showed the same response by prolonging the bud break in several coniferous trees (Little and Eidt, 1968). Buds on potato tubers could be induced to go into their rest period by applying ABA (Shih and Rappaport, 1971).

Seed dormancy in many plants has been found to be associated with ABA. Aspinall _et al_. (1967) showed the inhibitory effect of ABA on the germination of lettuce seed. Germination of _Xanthium_ seed was inhibited by the same chemical (Khan, 1967a). It is of interest that this effect on seed germination is relatively transient; that is, germination can be promptly resumed after washing away the inhibitor (Sumner and Lyon, 1967, as cited by Addicott and Lyon, 1969).

A.3. Effects of ABA on RNA, DNA, enzyme and protein synthesis

ABA has been found to influence some of the fundamental biochemical mechanisms in plants. Crispeels and Varner (1967), working on isolated aleurone layer, found that the GA-promoted synthesis of the hydrolytic enzymes α-amylase and ribonuclease were inhibited by ABA within 2 to 3 hours after treatment. It was suggested that ABA might act by inhibiting the synthesis of enzyme-specific RNA molecules, or by preventing the incorporation of RNA into an active enzyme-synthesising unit. Working on intact barley seed, Khan and Downing (1968) reported inhibitions of growth response and α-amylase synthesis in treated seed. Van Overbeek _et al_. (1967) reported a blocking effect on specific DNA synthesis caused by ABA; this effect, as observed, seemed to precede the inhibition effect on RNA. Khan and Heit (1969) demonstrated that ABA inhibited the labelling of ^{32}P

into soluble RNA, DNA-RNA hybrid and light-ribosomal RNA fractions of germinating pear embryos. Khan and Anojulu (1970) found a greatly altered nucleotide composition in the rapidly labelled RNA species after ABA treatment in pear embryos. Khan et al. (1970) found the same response in the composition of rapidly labelled RNA species of excised lentil root. Also, Pilet (1970) showed that ABA caused a strong inhibition of total RNA accumulation and accelerated ribonuclear activity. ABA (10^{-6}M) was found to inhibit an increase of α- and β-amylase in excised bean cotyledons without affecting the ^{14}C-leucine incorporation activity or rate of respiration of cotyledons, and no inhibition occurred if the cotyledons were excised 3 days after germination (Yomo, 1971). Besides those inhibitors observed, promotions of some activities were reported, e.g. the increased development of invertase in slices of sugar beet, an increase of α-amylase activity (but not β-amylase) in a commercial enzyme preparation (Addicott and Lyon, 1969) and phenylalanine ammonia lyase in *Phaseolus* (Walton and Sondheimer, 1968). De Leo and Sacher (1970) reported that ABA accelerated the increase in activity of acid phosphate resulting in increase in free space of *Rhoeo* leaf sections. Srivastava (1968) also found the accelerated increase in chromatin-associated nuclease in senescing first leaves from 7-day-old barley seedlings which were floated on 10 ppm ABA in the dark.

A.4. Effects of ABA on transpiration and stomatal activity

ABA induced bud dormancy and simultaneously inhibited transpiration in red maple, white ash, balsam fir, and white spruce (Little and Eidt, 1968). Mittelheuser and Van Steveninck (1969) found the same inhibitory effect of ABA on transpiration in excised leaves of wheat, barley, oats and *Nasturtium*; and their studies of stomatal imprints from wheat and barley showed that ABA treatment induced stomatal closure. Jones and Mansfield (1970) demonstrated the same effect in *Xanthium* and tobacco leaves and found that the effect could not be reversed by flushing the leaves with CO_2-free air. They suggested that the effect was not due simply to an increase in the intercellular CO_2 concentration but a more direct effect on the stomatal apparatus itself. Horton (1971) sought to determine whether ABA changes stomatal aperture indirectly by altering water relations throughout the leaf or by acting directly on the mechanism of stomatal movement. He showed that ABA can inhibit stomatal opening in isolated epidermal strips of *Vicia faba*; thus, it was likely that ABA acted directly on the guard cells.

Activities of endogenous ABA have also been investigated. Wright and Hiron (1969) found that wilting induced a higher level of ABA in detached leaves of wheat, cotton, pea and dwarf bean; thus ABA may be acting as a part of a protective mechanism against drought. Mizrahi *et al.* (1970) found an increase of inhibitors (with similar chromatographic properties to ABA) while transpiration was inhibited through an osmotic stress applied to the roots. A wilty mutant of tomato "flacca" which tends to lack an ability to close its stomata was found to contain a much lower amount of the substance. Loveys and Kriedemann (1971) found that stomatal closure due to water stress was accompanied by an increased level of ABA. Closure caused by exogenous ABA was found to be initiated within minutes after treatment and completed within half an hour. This response appeared to be specific for ABA. They also found that exogenous applications of ABA caused stomatal closure in both attached and detached leaves, and the amount needed to trigger the response was dependent on species and was in the same order as the endogenous levels of those plants. Cummins *et al.* (1971) found that foliar application of ABA initiated stomatal closure within 5 minutes, and withdrawal of the hormone reversed the effect within 5 minutes, suggesting a rapid metabolism of ABA. They also suggested that ABA affected the stomatal apparatus directly.

A.5. Effects of ABA on other physiological behavior

ABA was found to inhibit flowering in long-day plants (Evans, 1966). Heide (1968) found that ABA stimulated the formation of adventitious buds in begonia leaves but reduced the number of roots, (the inhibitory effect on root formation occurred at high concentration only); root length was not significantly affected, but lamina expansion and petiole extension were reduced with increasing concentration of ABA. Sloger and Caldwell (1970) found that different cultivars of soybean had a different physiological response to applied ABA, and there was evidence that responsiveness was genetically controlled. Lichtenthaler and Becker (1970) found that ABA inhibited the synthesis of vitamin K_1, chlorophyll, and carotenoids in etiolated barley seedlings under illumination. They suggested that ABA interfered with thykaloid formation which then resulted in a reduced isoprenoid synthesis. Glinka (1971) found that ABA markedly raised the permeability to water of xylem disks from root of Daucus and stem tissue of Pelargonium. Gamborg and LaRue (1971) found that the ethylene production which actually occurred in rose and Ruta cell cultures was inhibited in the presence of ABA. Zeevaart (1971) found that when long-day spinaches were transferred from short-day to long-day condition, ABA content of the spinaches increased up to threefold during the first long day. It was found that ABA content was higher at the end of 8 hours high intensity light period than at the beginning in both short- and long-day conditions. Lieberman and Kunishi (1971) found that ABA, like ethylene, inhibited growth of isolated pea seedlings, but did not promote the "triple response" characteristic of ethylene. Application of both ABA and ethylene resulted in an increased inhibition of epicotyl growth. The results suggested that the inhibitory action of ABA and ethylene on growth of etiolated pea seedling was due to different mechanisms.

B. Kinetin

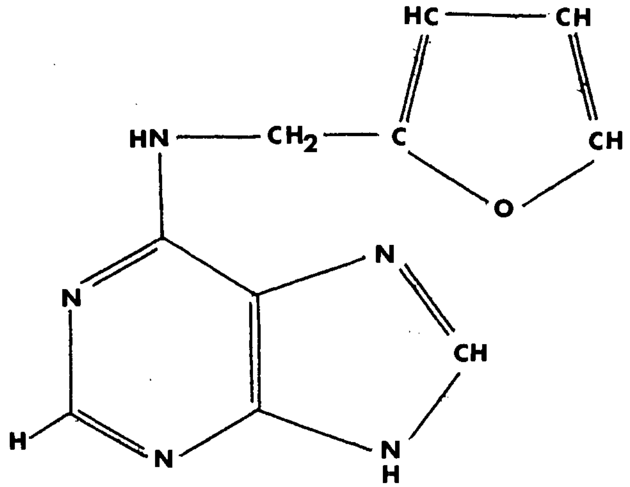


Figure 2. Structure of Kinetin

Miller found this chemical in 1954 and named it "kinetin" after its first-observed activity in association with cytokinesis. The substance was identified in 1955 as 6-furfurylaminopurine (Salisbury and Ross, 1969, p.461), the structure of which is shown in Figure 2. Kinetin itself has never been found in plants, although many other related purine derivatives do exist. Kinetin promotes cell division, and this activity in certain plants has been used in bioassay procedures. There is a large body of literature on physiological aspects of kinetin, and only selected works are reviewed here.

B.1. Effects of kinetin on senescence

The treatment of 20 ppm kinetin as a pre-harvest spray or post-harvest dip on mature head lettuce was shown to prolong the fresh appearance of lettuce heads under storage conditions of 40^{o}F and 85% R.H. and extend the shelf-life period (El Mansy et al. 1967). Better chlorophyll retention and higher moisture content were also observed. Abdel-Kader et al. (1966)

demonstrated a similar effect of kinetin in tomato fruit, where ripening of mature green fruits was delayed by 5 and 7 days with treatments of 10 ppm and 100 ppm kinetin respectively. Treatments on mature green tomatoes were more effective than on pink-ripe with both concentrations, and the higher concentration was more effective than the lower one. However, once the fully ripened stage was reached, those tomatoes with prior exposure to higher concentration deteriorated more rapidly than those with lower concentration. Von Abram and Pratt (1966) found that senescence was strongly retarded by kinetin and slightly influenced by NAA in broccoli leaves; the effect was markedly reduced by NAA when both kinetin and NAA were applied simultaneously. Boasson (1967) found chloroplast maturation in tobacco tissue culture to depend, in part, on kinetin activity. Kinetin was essential to, but kinetin alone would not support, chlorophyll synthesis unless sucrose was present, suggesting sucrose as a source of energy for the process. Shibaoka and Thimann (1970) experimented the mode of action of cytokinins and found evidence that the primary action of kinetin is to inhibit proteolysis rather than to promote protein synthesis. A correlation between senescence-postponing capability and the endogenous cytokinin was found in rose petals by Mayak and Halevy (1970). The endogenous cytokinin concentration in petals of a long-lived rose variety was higher than in a short-lived variety, and higher in young petals than in the old ones of the same variety. Application of N^6-benzyladenine lengthened the vase-life of a short-lived variety. This chemical had been tried and proved to yield similar effects to kinetin on post-harvest handling of many crops such as prolonging fresh appearance, reduced transpiration rate and weight loss in celery stalks (Zink, 1961; Wittwer et al. 1962), lettuce (Bessey, 1960; Zink, 1961; Lipton and Ceponis, 1962), cauliflower (Kaufman and Ringel, 1961), endive escarole, Brussels sprouts, sprouting broccoli, mustard greens, radish tops, parsley, green onions, and asparagus (Zink, 1961). Senescence was delayed and display-life of many cut flowers was prolonged by N^6-benzyladenine, e.g. carnations (MacLean and Dedolph, 1962; Waters, 1964; Heide and Øydvin, 1969), chrysanthemums (MacLean and Dedolph, 1962; Waters, 1964), asters and gerberas (Waters, 1964). Although the effectiveness of N^6-benzyladenine was widely demonstrated in the previous works, the work by El-Mansy et al. (1967) showed that kinetin was more effective than N^6-benzyladenine in prolonging storage life and subsequent shelf life of lettuce.

B.2. Effects of kinetin on RNA, DNA, enzyme and protein synthesis

Osborne (1962), working with detached *Xanthium* leaves and excised leaf disks, reported a kinetin-induced increase of ^{14}C-leucine incorporation into protein and of ^{14}C-orotic acid into RNA. Thus, kinetin can stimulate both RNA and protein synthesis. Osborne suggested that the retardation of senescence by kinetin is mediated through its action in sustaining nucleic and protein synthesis. Kuraishi (1968) obtained a similar effect of kinetin on *Brassica rapa*. He floated leaf disks on a medium containing ^{14}C-L-leucine in both the presence and absence of kinetin. The increase in radioactivity in the protein fraction of treated disks was almost linear with time, whereas the control, lacking kinetin, started to slow down. With leaf disks first incubated on ^{14}C-L-leucine then transferred to either solution or water, the radioactivity of treated disks decreased slower than in the case of the control. The slower decrease in radioactivity caused by kinetin was not due to an increased turnover rate, since the same phenomena were observed in the presence of cold leucine or casein hydrolysate solution. These results suggest that kinetin retards the decomposition rather than stimulates the synthesis of protein.

B.3. Effects of kinetin on respiration and stomatal activity

After kinetin treatments, a slight reduction in respiratory evolution of carbondioxide was observed by Dedolph et al. (1962); Katsumi (1963) as cited by Meidner (1967); and El-Mansy et al. (1967). Livné and Vaadia (1965) treated the excised, mature primary leaves of barley with kinetin (3×10^{-6}M) and observed an increased opening of stomatal apertures (the response in young leaves was not as noticeable as in mature leaves). They suggested that the subsequent increase in opening of stomatal aperture might be due to a lower carbondioxide concentration in the leaves. Meidner (1967) treated mature primary leaves of barley with kinetin and observed the increased rates of assimilation of carbondioxide. He suggested that the resulting reduction in the concentration of carbondioxide inside the leaves be considered as one factor causing the observed decrease in stomatal resistance, but, in addition, kinetin appeared to affect the stomatal mechanism directly. Tal et al. (1970) studied a kinetin-like activity in a wilty mutant of tomato using labelled leucine and a soybean callus bioassay. This specific mutant "flacca" wilts easily because its stomata resist closure. They found that kinetin-like activity in both leaf and root exudate was higher in the mutant than in the normal variety. It was also found that the actual decreased resistance to closure with age of this plant coincided with the decrease of kinetin-like activity in the leaf and root exudate at the time. Ben-Zioni et al. (1967) found evidence suggesting a lower level of endogenous cytokinin in osmotic stressed tobacco leaf disks.

B.4. Effects of kinetin on transpiration

An increase in stomatal aperture accompanied by a higher transpiration rate are reported by Livené and Vaadia (1965) in excised mature barley leaves treated with 10^{-5}M and 10^{-6}M kinetin. Luke and Freeman (1968), using cytokinins (including kinetin), observed the same phenomena in many gramineous, but not in dicotyledonous species. They also suggested that the increased transpiration of species of Gramineae should be considered as one of the biological activities specific to cytokinin. Besides, it was noticeable that the kinetin effect on stomata was relatively quick in comparison with the other known effects caused by kinetin (which always showed a time lag in the order of several hours). This increase in opening of the stomatal aperture discussed above (Section B.3.) might be one explanation for a higher rate of transpiration caused by kinetin and other cytokinins.

B.5. Effects of kinetin on other physiological behavior

Kinetin possessed the capability of retarding leaf abscission in *Phaseolus* (Chatterjee and Leopold, 1964). Wade and Brady (1971) found that, in transverse slices of green banana, kinetin hastened the peak of ethylene evolution and maximum rates were also 30% higher than the control. The respiration rate of kinetin-treated slices was found to exceed that of the control throughout the 48 hour period after slicing; peel degreening was also retarded. Street *et al*. (1967) found that the growth response of cultured sycamore cell suspensions to added kinetin depended on adequate carbohydrate (glucose) as the source of carbon energy.

C. Interaction of abscisic acid with kinetin, and with other hormones

Aspinall *et al*. (1967) exposed lettuce seed to far-red light in the presence of ABA and GA_3, or kinetin, and found that the effect of low concentration of ABA in suppressing GA_3-promoted germination was completely overcome by a high concentration of GA_3 and, in the case of kinetin, ABA was inhibitory only in the presence of a high concentration of this promoter. Khan (1967a) found that kinetin reversed the action of ABA inhibition of germination in lettuce and nondormant seed of Xanthium; also dormancy breaking action of kinetin on dormant seed of Xanthium was found to be affected by ABA. Khan (1968) found that an inhibitory effect of ABA on dark germination of Grand Rapids lettuce seed was reversed by kinetin but not by excess GA_3. Sankhla and Sankhla (1968b) showed that inhibition of seed germination caused by ABA was completely overcome by kinetin in both dark and light, whereas gibberellic acid and IAA showed no interaction with ABA.

Auxin-mediated growth of Avena coleoptile was found to be inhibited by ABA (Addicott et al. 1964, cited by Aspinall et al. 1967). Thomas et al. (1965) demonstrated that such an inhibition could be overcome by GA_3 but not by auxin, although the coleoptiles were responsive to auxin in the presence of ABA. They also found that ABA reduced the elongation of tall (but not dwarf) maize leaf sections, and GA_3 could overcome this effect. Aspinall et al. (1967) found that elongation of cucumber radicle, on the other hand, was promoted by ABA in the presence of a mixture of GA_4 and GA_7. Khan and Downing (1968) reported an inhibitory effect of ABA on the growth of barley coleoptile and the effect was reversed by kinetin. On the contrary, a synergistic inhibition of root growth was observed as affected by the combinations of kinetin and ABA. Khan (1969) also demonstrated that ABA inhibited coleoptile growth to a greater extent than the root growth, and although the increase in coleoptile growth by gibberellin plus ABA over ABA alone was observed, he did not think there was an interaction effect. Blumenfeld and Gazit (1970) reported that, in soybean callus culture, ABA (10 mg/l) acted as inhibitor when the kinetin level was low, but this inhibition was cancelled and changed to synergism when the kinetin level in the medium was raised. They stated that both the absolute quantities and ABA-kinetin ratio were important in the transition from inhibition to synergism. Pilet (1970) found that ABA inhibited the growth of lentil roots, but the effect was less noticeable than for IAA, and when both chemicals were applied simultaneously, ABA acted as a growth antagonist to IAA. IAA was found to have a synergistic effect on ABA-induced callus formation in the culture of citrus explants while ABA was much less effective. IAA and ABA alone or in combinations induced no callus formation in the absence of ABA (Altman and Goren, 1971). Blaydes found that ABA inhibited elongation of Avena coleoptile and the inhibition was lessened by kinetin.

Chrispeel and Varner (1967) found that GA enhanced the synthesis of α-amylase and ribonuclease in isolated aleurone layers of barley, and this process was inhibited by ABA. They suggested that ABA might exert its action by inhibiting the synthesis of α-amylase-specific RNA molecules or by preventing their incorporation into an active enzyme-synthesizing unit. Khan and Downing (1968) found that GA was far less effective than kinetin in reversing ABA inhibition of α-amylase synthesis in intact seed of barley, and a combination of GA and kinetin caused nearly complete reversal of ABA inhibition of α-amylase synthesis. They suggested that kinetin might act by removing the ABA inhibition of enzyme specific sites thereby allowing GA to function on α-amylase synthesis. Khan (1969), working on both intact and embryoless seeds, found that kinetin effectively reversed inhibition of α-amylase by ABA, but there was no reversal effect caused by excess GA or kinetin in the embryoless endosperm, thus cytokinin reversal of inhibition of enzyme synthesis probably depended on some factor(s) in the embryo. Srivastava (1968) found that ABA increased the chromatin-associated nucleases in excised barley leaves, and kinetin completely reversed the ABA effect with results comparable to the activity of these enzymes of barley leaves floated on solutions of kinetin alone. Pilet (1970) found that the IAA-induced RNA accumulation and inhibition of ribonuclease activities were reversed by ABA. Khan et al. (1970) showed that ABA induced changes in the nucleotide composition of rapidly labelled RNA species in excised lentil roots, and that the effect was reversed by kinetin. De Leo and Sacher (1970) found that ABA increased ribonuclease activity and inhibited the incorporation of uridine and leucine in leaf sections removed from plants grown under stress, and these effects were suppressed by NAA. A study of RNA synthesis in the *Avena* coleoptile by Blaydes (1971) showed that ABA decreased RNA synthesis (as measured by the incorporation of radio-active uracil and adding kinetin lessened the inhibition). Yomo (1971) found that ABA inhibited the increase of α-amylase and β-amylase activities, but not of ^{14}C-leucine incorporation or the respiration of excised bean and pea cotyledons during incubation. The inhibition was not reversed by kinetin, GA, or IAA.

Aspinall et al. (1967) found that high concentrations of kinetin overcame the capability of ABA to hasten senescence in radish leaf disks. Bhardwaj (1967) found the acceleration of abscission by ABA to be counter-acted almost completely by IAA and to a lesser extent by GA_3. Sankhla and Sankhla (1968a) also demonstrated that kinetin reversed the senescence accelerating effect of ABA on both leaf disks and whole leaves of *Arabidopsis*. Srivastava (1968) found the same kind of interaction between kinetin and ABA in excised barley leaves. Gamborg and La Rue (1971) found, in cell culture of rose and *Ruta*, that ABA inhibited growth and ethylene production in rose cells but only ethylene production in *Ruta* cells; and the addition of kinetin reversed the ABA inhibitory effect in rose cells but not in *Ruta* cells. Glinka (1971) found that ABA raised the permeability of tissue while kinetin decreased it and the effect of ABA dominated the system when both chemicals were applied simultaneously. Concerning ethylene production in plants, Lieberman and Kunishi (1971) found that ABA suppressed the IAA- and kinetin-induced stimulation of ethylene evolution in etiolated pea seedlings.

MATERIALS AND METHODS

A. Materials

Preceeding the present study, a small preliminary test had been carried out to investigate the effects of kinetin and ABA on the post-harvest quality of 5-week-old "Great Lakes" lettuce rosettes. The plants were cut, trimmed, and treated with 3 separate solutions; distilled water, 20 ppm kinetin, and 5 ppm ABA. The lettuce rosettes were dipped in the specified solution for one minute then allowed to drain and kept in a cold storage chamber with the temperature setting at $3 \pm 1^{o}C$ (no supplementary humidification). Twelve plants were used for each treatment and there was no replication. Post-harvest quality was observed once a week up to 5 weeks in storage, and the results revealed that the 5 ppm ABA-treated plants remained fresher and greener in comparison with the control and 20 ppm kinetin-treated plants. The results seemed encouraging for a further study of the potential use of these chemicals for retention of the fresh appearance of lettuce.

The interest of the present study was directed toward the uses of ABA (and/or kinetin) in preserving the post-harvest quality of lettuce in actual practice. Nevertheless, the present study could not be carried out to the fullest extent for 2 reasons. Firstly, the head lettuce industry uses the Great Lakes variety which in the Lower Mainland of British Columbia requires about 10 weeks from seeding to edible maturity in the growing season. That time period increases when lettuce is grown in the off-season in greenhouses. For example, even with supplementary lighting, it takes 7 weeks to reach the 10-leaf rosette stage, and the plants are still weeks away from head formation. Thus, it was expedient to use young plants in order to conserve time. Secondly, only a limited amount (25mg) of ABA was available at the time and this was not enough to establish an experiment using fully mature lettuce heads. Also, the chemical is very expensive.

The antitranspirational activity of this chemical has not been confirmed from any vegetable crops, thus, with the need to economize on time and quantity of ABA, it was logical at this initial stage to use a small laboratory model to gain more evidence before considering experiments in field production.

A.1. Test plants

The experiment was undertaken at The University of British Columbia from October 26, 1972 to February 3, 1973 at which time the field growing of lettuce was not feasible. The plants were instead grown under greenhouse conditions. "Great Lakes" head lettuce (Lactuca sativa L. var. capitata, L.) seeds were sown in steam-sterilized soil in 3" x 12" x 18" wooden flats on two different days (October 26 and 28, 1972) to provide 2 sets of seedlings for two replications. The plants were grown under a temperature setting of 20°C by day and 18°C by night and approximately 800 lux of supplementary light from fluorescent light banks 16 hours a day. Three weeks after sowing, seedlings were transplanted into standard flats using 2½" x 2½" spacing. Watering was done once a day and no fertilizer, pesticide or herbicide was used throughout the growing period. Because of the limiting factors of time and supply of chemicals as previously described, the plants were harvested when 7 weeks old, at which time they had already formed about 10 true leaves and weighed an average of 2.94 gm. No watering was done on the day of harvest to avoid possible inaccuracy in weight due to the extra water that might adhere to the leaf surface of the plants. Plants were harvested in the evening by cutting at root level just below the soil surface. Cotyledons, the first and the second outer leaves, and undesirable root portions were trimmed away. Foreign matter and soil were eliminated by means of a soft brush.

A.2. Chemical treatments

Concentrations of 1 ppm and 5 ppm ABA were chosen since they were within a range comparable to that used by previous researchers studying the effects of ABA on plant transpiration and stomatal activities. The selection of 20 ppm kinetin concentration parallels the study by El-Mansy (1967) on lettuce post-harvest quality. Finally, to study possible interaction of the two chemicals, a combination of 20 ppm kinetin plus 5 ppm ABA was used.

Aqueous solutions of ABA, kinetin or a combination of the two were made from the anhydrous forms of ABA and kinetin (bought from Sigma Chemical Company, St. Louis, Mo., U.S.A.). The chemicals were first made into stock solutions of 10 ppm ABA and 40 ppm kinetin, and then diluted to 500 ml each of the following with the corresponding designated abbreviations:

Treatment	Abbreviation
1. Control (distilled water)	0
2. 1 ppm ABA	A1
3. 5 ppm ABA	A2
4. 20 ppm kinetin	K
5. 5 ppm ABA + 20 ppm kinetin	A2K

The diluted solutions were prepared in the morning and kept in 500 ml flasks wrapped with aluminum foil and stored in a refrigerator (approximately $4^{o}C$) until the time of application in the evening of the same day. The solutions were sprayed on the plants which were spread on plastic sheets. The plants were sprayed thoroughly to the run-off point and let drain before they were shifted into cold storage. The spray application was chosen in preference to the dipping procedure used in the preliminary experiments, to conserve the chemical solutions, and thus permitting larger numbers of plants within treatments.

A.3. Cold storage facility and instruments

A Bell-Craft walk-in growth chamber was used as a cold storage facility. This chamber could be manipulated for conditions ranging from $-20^{o}C$ to $50^{o}C$ approximately. Temperature for the experiment was maintained between 3^{o} and $5^{o}C$. Humidity was kept as high as possible up to the saturation point using continuous humidification; a certain amount of water was fed into the humidifier via the pre-adjusted regulator valve. A thermohygrograph was placed in the chamber for continuous recording of temperature and humidity during the experiment. Ordinary dry-bulb and wet-bulb thermometers were used as a further check. The cold storage was tested and adjusted to meet the required conditions until there were 3 days of steady and reliable performance.

B. Methods

In addition to the quality observed by a panel, the study sought to investigate any correlation that might exist between this quality rating and other measurable phenomena which occurred during the post-harvest period, e.g. percent weight loss, percent moisture content, and chlorophyll content, since these might relate to the wilting and yellowing of the stored lettuce. Details of the experimental procedures and measurements follow.

B.1. Experimental design

A split plot design was employed. The experiment was composed of 3 main plots (40 plants each) which were randomly designated to be kept for 3 different lengths of time (5, 6 and 7 weeks) under storage. Each main plot was then subdivided into 5 groups of eight plants each and these groups were randomly assigned to the 5 different treatments. The experiment was replicated twice and each used plants from only one of the two seedings.

B.2. Treatment procedure

Randomization was exercised throughout the experiment wherever applicable. One of four flats of one seeding was randomly selected and set aside, and the entire population of 120 plants in the remaining 3 flats was used as one replication. In order to minimize physiological differences between plants within a treatment, plants were harvested in lots of 40. Each plant within the lot was weighed rapidly before being labelled and treated. Thus, time lapses between initial harvest and final weighing were reduced by using the lot of forty plants rather than harvesting the entire replication at one time. The longest lapse of time occurred in the weighing of individual plants. A pre-arranged randomization scheme was used to get plants distributed within a replication considering length of storage time, chemical treatment and plant number, and thence to determine where each plant was placed in the storage chamber. Plants were spread on 5 separate plastic sheets according to the groups to which the plants were assigned. Each group was then sprayed with the treatment solution (distilled water in the case of the control) up to the run-off point, then allowed to drain before being placed in the cold storage. The same procedure was repeated for the second and third sets (40 plants each), which represented the second and third main plots respectively.

The second replication was handled in the same manner using the succeeding sowing.

B.3. Visual quality rating

Rating of plants was done by a panel of 3 observers. The plants examined at the end of weeks 2, 3 and 4 in storage were used again at the end of 5, 6, and 7 weeks in storage in the following manner.

Five groups of eight plants(under 5 different treatments) with the same length of time in storage were inspected at a time. Numerical values were given to those groups for a pooled or group quality manifestation according to the following scheme.

Numerical Rating	Quality Description	
9	excellent:	field fresh, bright green appearance, free from all defects.
7	good:	green colour slightly decreased, still good retail sale appeal.
5	fair:	slightly wilted, some minor defects.
3	poor:	severely wilted, unsaleable.
1	very poor:	some decay, yellowing, would not be eaten.

In addition to the numerical record of quality, a representative plant from each treatment was photographed using a 35 mm single lens reflex camera. All settings (exposure time, aperture, distance) were fixed and all pictures were taken on the same roll of colour film. Plants subjected to photography were still kept continuously under the cold and humid experimental conditions and were disturbeded only by a slight touch during arrangement, since the photography was done in the same cold storage chamber. The chamber also served as a light-seal studio and helped eliminate all sources of light except the electronic flash equipment on the camera.

Domestic 110 volts A.C. electricity supplied power to the flash unit. Thus, it was assumed that there was a fairly constant illumination so that the photograph can be used as a valid record of visual comparison.

B.4. Measurement of weight loss

Each plant was weighed, as described already, at harvest. Then fresh weights were obtained after the storage periods. A modified styrofoam case was used to provide a low temperature and humid condition for the plant sample during transfer from the cold chamber to laboratory. A two-layer screen box was put in the middle of the styrofoam container and surrounded with at least 1½" layer of crushed ice in the bottom and all side-walls. The two-layer screen box provided good circulation of cold air and separated the samples from the melting ice. Plants were taken individually from the case and were rapidly weighed. Fresh weight after storage and original fresh weight were subsequently used to calculate the percent weight loss for each plant.

B.5. Moisture content measurement

Following the recording of fresh weight of a plant after storage, the fourth leaf (counting in spiral order from the outside in) of that plant was detached at a petiole base and kept in a plastic weighing dish with its identification tag attached. This leaf was set aside for chlorophyll extraction. The remaining portion of the plant was then weighed again for a fresh weight before drying. This portion was placed in a pre-labelled position in an aluminum foil tray. Five trays were used for all 40 plants to be dried simultaneously. Samples were placed in a vacuum dryer for 15 hours at $70^{o}C$.

Samples were removed from the dryer one tray at a time, and each dried plant was weighed as quickly as possible (the remaining samples were still in the dryer with the heater on but no more vacuum). Fresh weight before drying and the dry weight were used to calculate the percent moisture content.

B.6. Chlorophyll content measurement

The leaf samples from individual plants were used to measure the chlorophyll content in the following manner. A half gram sample was cut from the mid section of each leaf (eliminating leaf tip and base). Each sample was placed in an osterizer for 1½ minutes with approximately 30 ml of refrigerated-cold 80% acetone to yield a crude extract which was then filtered through 2 layers of Whatman No. 1 filter paper in a modified suction filtration apparatus as shown in Figure 3. Additional acetone was used to wash down the chlorophyll left on the filter papers and funnel to make up a final volume of 50 ml filtrate. The apparatus allowed the filtrate to flow directly into the 50 ml volumetric flask thus bypassing a few steps of the conventional method (that is no removal of stopper and funnel from the suction flask, no transferring and using acetone to wash down the filtrate from the suction flask into a volumetric flask and replacement of equipment to handle the next sample). This procedure made more efficient use of the acetone in that a larger volume was available for extraction and efficient washing of extract into the collective volumetric flask. The modified procedure allowed a large reduction in surface area of filtrate when the volumetric flask was used and thus lessened evaporation of the highly volatile acetone due to exposure to low pressures during filtration. The volumetric flask containing

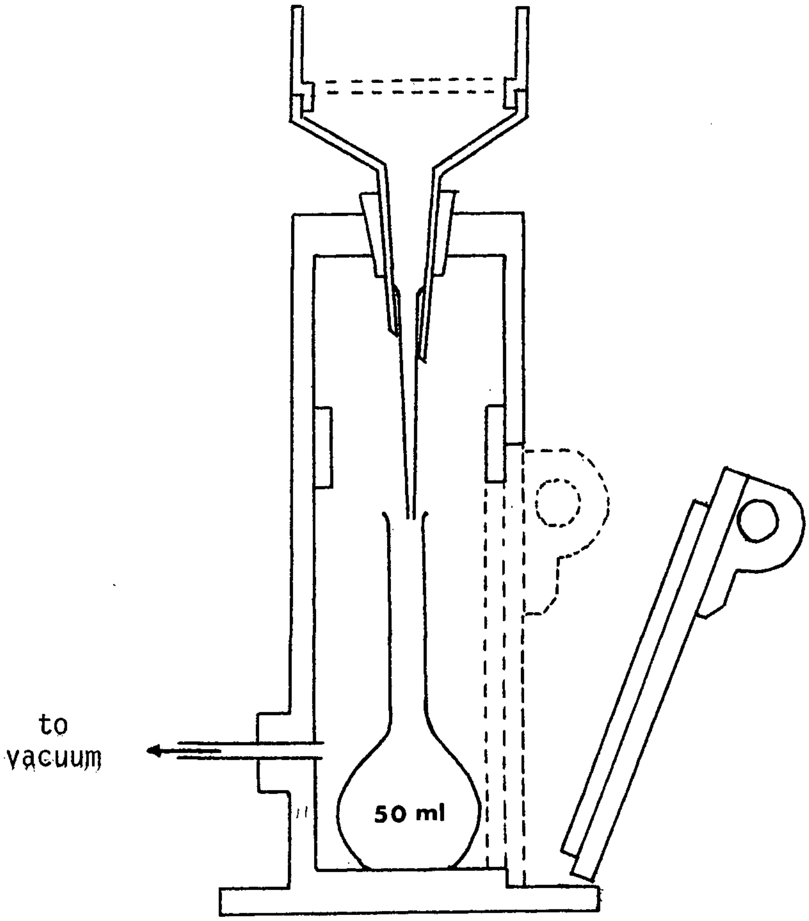


Figure 3 Modified vacuum filtration apparatus

chlorophyll extract was then plunged in crushed ice until the absorbancy measurement was made. Liquefaction and filtration were done in groups of 5 samples and each group was handled as quickly as possible.

A Perkin-Elmer double bean spectrophotometer (Model 124) was used to obtain the absorbancy measurements. Samples were read in a silica cell at 647, 664 and 700 mμ wavelengths with slit size of 0.5 mμ. Chlorophyll contents were calculated using the equations given by Ziegler and Egle (1965) as cited by Sestak (1971) in the following:

Chlorophyll A = $(11.78\ A_{664} - 2.29\ A_{647})$ mg/l

Chlorophyll B = $(20.05\ A_{647} - 4.77\ A_{664})$ mg/l

Chlorophyll A+B = $(7.01\ A_{664} + 17.76\ A_{647})$ mg/l

The results were subsequently calculated and are reported as mg of chlorophyll per gm fresh weight of leaf at the time of chlorophyll observation (designated as mg/gm FW), and also are reported as a mg of chlorophyll in mg per gm original fresh weight (designated as mg/gm OFW) - the fresh weight immediately before the same leaf sample had been treated and put in storage. The content based on gm FW is more or less parallel to the greenness of the leaf tissue, while the other based on gm OFW, is for the purpose of following the chlorophyll degradation with time. Using the OFW basis, the effect of weight loss is used in calculating chlorophyll contents, and these derived values make a common basis between original and subsequent determinations in spite of tissue shrinkage during the experiment. Hence the chlorophyll values on OFW basis measured at different periods of time, reveal the real picture of the possible degradation of chlorophyll from the original 1 gm samples regardless of the shrinkage due to weight loss.

RESULTS

1. Visual quality rating

The numerical values for lettuce quality were derived from the visual ratings made by the panel of observers, and only the values obtained after 5, 6 and 7 weeks in storage are presented. The panel made quality ratings at the end of 2, 3 and 4 weeks in storage, and the same plants were utilized in the same sequence for the 5, 6 and 7 week storage periods. The first cycle of observations for weeks 2, 3 and 4 showed no significant differences for treatments, and the inclusion of such data with the second cycle of observations presented a problem in statistical methods which would not permit sensible comparisons: therefore, data for weeks 2, 3 and 4 were omitted, and the values for weeks 5, 6 and 7 only were used to demonstrate the differences observed by the panel.

It is obvious in Table 1A that the quality of lettuce was decreasing as the storage period continued from 5 to 7 weeks. At the end of 7 weeks, all lettuce had reached an unsaleable condition with obvious yellowing of leaf tissue and severe wilting. The Duncan's multiple range test at the 5% level (Table 1B) shows no significant difference in quality between two replications, but time in storage and chemical treatment did have some effect on quality. After 5 weeks in storage, quality was significantly higher than that for 7 weeks but not for the 6 weeks of storage. The control and the 1ppm ABA-treated lettuce had a significantly higher quality than the 20 ppm kinetin and 5 ppm ABA + 20 ppm kinetin treatments. The 5 ppm ABA treatment did not differ significantly from any of the other treatments.

Table 1A

Means of quality ratings[1] by a panel of 3 observers, of lettuce subjected to 5 treatment[2], after 5, 6 and 7 weeks in storage

Week	Replication	Treatment					Mean
		0	A1	A2	K	A2K	
5	1	8.0	7.8	8.5	7.3	7.7	7.6
	2	7.8	8.7	7.7	6.2	6.2	
6	1	6.7	6.0	4.3	3.8	4.2	5.3
	2	6.2	6.2	5.7	4.0	5.3	
7	1	3.3	3.8	3.7	3.8	3.3	5.5
	2	3.6	4.0	3.0	5.0	4.0	
Mean		5.9	6.1	5.5	5.0	5.1	5.5

Mean of replication 1 = 5.4889 Mean of replication 2 = 5.5667

[1]Numerical ratings: 1 = very poor, 3 = poor, 5 = fair, 7 = good, 9 = excellent

[2]Treatments: 0 = control, A1 = 1 ppm ABA, A2 = 5 ppm ABA, K = 20 ppm kinetin, A2K = 5 ppm ABA + 20 ppm kinetin

Table 1B

Analysis of variance of numerical quality ratings of lettuce under 5 treatments after 5, 6 and 7 weeks in storage

Source	D.F.	S.S.	M.S.	F	Prob.
Replication	1	0.136	0.136	0.16	0.6936
Week (A)	2	231.810	115.900	40.39	0.0348
Error a	2	5.739	2.869	3.33	0.0414
Treatment (B)	4	16.361	4.090	4.71	0.0163
Week x Treatment (AB)	8	15.056	1.882	2.17	0.1095
Error b	12	10.417	0.868	1.01	0.4533
Error	60	51.667	0.861		
Total	89	331.180			

Duncan's test

Replication	1	2
Mean	5.48 a*	5.6 a

Time under storage (weeks)	5	6	7
Mean	7.6 b	5.3 b	3.7 a

Treatment	0	A1	A2	K	A2K
Mean	5.9 b	6.1 b	5.5 ab	5.0 a	5.1 a

* Mean separation in row by Duncan's multiple range test, 5% level

2. Total weight loss

Changes in weight of lettuce after 5, 6 and 7 weeks in storage were measured and calculated as percentage of total weight loss (% TWL). The record of these 240 observations is shown in Appendix 2. The data shown in Table 2A are averages of 8 Observations in the experimental unit. The comparisons among the 5 treatment means show only small and nonsignificant differences. The more obvious ones are % TWL of lettuce in the fifth week in comparison with the sixth or the seventh week of time under storage. The lettuce had a relatively lower percent weight loss in the fifth week than in the sixth or the seventh week. There was a very small difference between the latter two weeks. The analysis of variance shown in Table 2B reveals no significant differences in the % TWL at the 5% level between the replications, or among the treatments and different periods of time under storage

Table 2A

Percentages of total weight loss (means of 8 observations in each experimental lot) of lettuce under 5 treatments after 5, 6 and 7 weeks in storage

Week	Replica-tion	Treatment					Mean
		0	A1	A2	K	A2K	
5	1	-0.809	5.660	-5.716	-1.030	1.789	4.085
	2	7.844	4.061	8.407	9.540	11.106	
6	1	14.146	17.968	20.731	20.707	12.164	15.869
	2	15.690	15.121	15.800	11.625	14.738	
7	1	21.005	22.724	16.384	16.075	14.101	16.390
	2	17.183	12.694	10.522	14.506	18.704	
Mean		12.510	13.038	11.021	11.904	12.101	12.115

Mean of replication 1 = 11.727 Mean of replication 2 = 12.503

Treatments: 0 = control, A1 = 1 ppm ABA, A2 = 5 ppm ABA, K = 20 ppm kinetin, A2K = 5 ppm ABA + 20 ppm kinetin

Table 2B

Analysis of variance of total weight loss of lettuce under 5 treatments after 5, 6 and 7 weeks in storage

Source	D.F.	S.S.	M.S.	F	Prob.
Replication	1	36.14	36.14	1.66	0.1958
Week (A)	2	7747.60	3873.80	4.65	0.1795
Error a	2	1665.30	832.66	38.27	0.0000
Treatment (B)	4	107.94	29.98	0.23	0.9132
Week x Treatment (AB)	8	627.52	78.44	0.68	0.7043
Error b	12	1388.70	115.73	5.32	0.0000
Error	210	4569.40	21.76		
Total	239	16143.00			

3. Moisture content

Moisture content was fairly uniform for all the lettuce plants regardless of replications, treatments or different lengths of time under storage. The data for these observations are in appendix 2, and the means for eight-plant experimental units are in Table 3A. Uniformity can be observed throughout for every treatment and every week. The overall mean of 240 observations is 94.46% with a standard deviation of 0.6630%. The analysis of variance (Table 3B) showed no significant differences at the 5% level for replications, treatments, or times under cold storage.

4. Ch

Table 3A

Percentages of moisture content (means of 8 observations in each experimental lot) of lettuce under 5 treatments after 5, 6 and 7 weeks in storage

Week	Replica-tion	Treatment					Mean
		0	A1	A2	K	A2K	
5	1	95.33	94.76	95.44	95.36	94.99	94.94
	2	94.66	94.97	94.65	94.62	94.61	
6	1	94.35	93.88	93.22	93.78	94.50	94.17
	2	94.21	94.32	94.41	94.44	94.53	
7	1	94.02	93.72	94.26	94.56	94.48	94.26
	2	94.02	94.53	94.27	94.42	94.35	
Mean		94.43	94.37	94.38	94.53	94.58	94.46

Mean of replication 1 = 94.44 Mean of replication 2 = 94.47

Treatments: 0 = control, A1 = 1 ppm ABA, A2 = 5 ppm ABA, K = 20 ppm kinetin, A2K = 5 ppm ABA + 20 ppm kinetin

Table 3B

Analysis of variance of percent moisture content of lettuce under 5 treatments after 5, 6 and 7 weeks in storage

Source	D.F.	S.S.	M.S.	F	Prob.
Replication	1	0.035	0.035	0.15	0.7020
Week (A)	2	28.265	14.132	3.33	0.2320
Error a	2	8.483	4.242	17.38	0.0000
Treatment (B)	4	1.688	0.422	0.52	0.7232
Week x Treatment (AB)	8	5.655	0.707	0.87	0.5629
Error b	12	9.695	0.808	3.31	0.0002
Error	210	51.253	0.244		
Total	239	105.070			

4. Chlorophyll content

Measurements and calculations of the contents of chlorophyll A and B and the total were based on two different fresh weights:

1. one gm fresh weight of the sample leaf at the time of chlorophyll extraction after chemical and storage treatments.
2. one gm of the original fresh weight (before that same sample was treated and put into the storage).

The above two fresh weights are differentiated hereafter as gm FW and gm OFW respectively. Analyses of variance for chlorophyll A, B and A + B (appendices 4-9) were done on each of those two bases, and the results are summarized in Table 4B to 9B inclusive. (Tables 4A to 9A are means from 8 plant experimental lots, shown in appendices 4-9).

Table 4A

Chlorophyll A contents (means of 8 observations in each experimental lot) in mg/gm FW of lettuce under 5 treatments after 5, 6 and 7 weeks in storage

Week	Replica-tion	Treatment					mean
		0	A1	A2	K	A2K	
5	1	0.3409	0.5012	0.4229	0.4385	0.4734	0.4840
	2	0.5356	0.4730	0.4822	0.5769	0.5960	
6	1	0.4421	0.5229	0.5576	0.5661	0.5333	0.5246
	2	0.5328	0.4858	0.4836	0.5785	0.5435	
7	1	0.4729	0.4055	0.4131	0.4816	0.4676	0.4702
	2	0.4799	0.4103	0.4798	0.5710	0.5197	
Mean		0.4673	0.4664	0.4732	0.5354	0.5223	0.4929

Mean of replication 1 = 0.4693 Mean of replication 2 = 0.5166

Treatments: 0 = control, A1 = 1 ppm ABA, A2 = 5 ppm ABA, K = 20 ppm kinetin, A2K = 5 ppm ABA + 20 ppm kinetin

Table 4B

Analysis of variance of chlorophyll A content (mg/gm FW) of lettuce under 5 treatments after 5, 6 and 7 weeks in storage

Source	D.F.	S.S.	M.S.	F	Prob.
Replication	1	0.1341	0.1341	15.33	0.0002
Week (A)	2	0.1281	0.6403	1.36	0.4239
Error a	2	0.0943	0.0472	5.39	0.0054
Treatment (B)	4	0.2118	0.0530	3.18	0.0531
Week x Treatment (AB)	8	0.0870	0.0109	0.65	0.7219
Error b	12	0.1996	0.0166	1.90	0.0356
Error	210	1.8367	0.0087		
Total	239	2.6915			

Table 5A

Chlorophyll A contents (means of 8 observations in each experimental lot) in mg/gm OFW of lettuce under 5 treatments after 5, 6 and 7 weeks in storage

Week	Replication	Treatment					Mean
		0	A1	A2	K	A2K	
5	1	0.3442	0.4690	0.4498	0.4417	0.4663	0.4617
	2	0.4957	0.4542	0.4423	0.5235	0.5307	
6	1	0.3823	0.4297	0.4440	0.4493	0.4671	0.4411
	2	0.4417	0.4122	0.4093	0.5124	0.4628	
7	1	0.3740	0.3160	0.3475	0.4030	0.4036	0.3941
	2	0.3984	0.3599	0.4281	0.4884	0.4225	
Mean		0.4060	0.4068	0.4202	0.4697	0.4588	0.4323

Mean of replication 1 = 0.4125 Mean of replication 2 = 0.4521

Treatments: 0 = control, A1 = 1 ppm ABA, A2 = 5 ppm ABA, K = 20 ppm kinetin, A2K = 5 ppm ABA + 20 ppm kinetin

Table 5B

Analysis of variance of chlorophyll A content (mg/gm OFW) of lettuce under 5 treatments after 5, 6 and 7 weeks in storage

Source	D.F.	S.S.	M.S.	F	Prob
Replication	1	0.0942	0.0942	12.21	0.0007
Week (A)	2	0.1919	0.0960	9.08	0.1053
Error a	2	0.0211	0.0106	1.37	0.2553
Treatment (B)	4	0.1724	0.0431	4.18	0.0240
Week x Treatment (AB)	8	0.0464	0.0058	0.56	0.7897
Error b	12	0.1237	0.0103	1.34	0.1998
Error	210	1.6207	0.0077		
Total	239	2.2704			

Table 6A

Chlorophyll B contents (means of 8 observations in each experimental lot) in mg/gm FW of lettuce under 5 treatments after 5, 6 and 7 weeks in storage

Week	Replica-tion	Treatment					Mean
		0	A1	A2	K	A2K	
5	1	0.1735	0.2613	0.2110	0.2188	0.2405	0.2456
	2	0.2713	0.2550	0.2369	0.2963	0.2915	
6	1	0.2615	0.3170	0.3049	0.3234	0.2973	0.2898
	2	0.2884	0.2649	0.2540	0.3012	0.2853	
7	1	0.2641	0.2183	0.2133	0.2577	0.2450	0.2551
	2	0.2534	0.2275	0.2759	0.3118	0.2843	
Mean		0.2520	0.2573	0.2493	0.2849	0.2740	0.2635

Mean of replication 1 = 0.2538   Mean of replication 2 = 0.2732

Treatments: 0 = control, A1 = 1 ppm ABA, A2 = 5 ppm ABA, K = 20 ppm kinetin, A2K = 5 ppm ABA + 20 ppm kinetin

Table 6B

Analysis of variance of chlorophyll B content (mg/gm FW) of lettuce under 5 treatments after 5, 6 and 7 weeks in storage

Source	D.F.	S.S.	M.S.	F	Prob.
Replication	1	0.0224	0.0224	5.77	0.0164
Week (A)	2	0.0865	0.0432	1.57	0.3882
Error a	2	0.0549	0.0275	7.05	0.0012
Treatment (B)	4	0.0450	0.0112	2.27	0.1213
Week x Treatment (AB)	8	0.0301	0.0038	0.76	0.6436
Error b	12	0.0594	0.0050	1.27	0.2364
Error	210	0.8173	0.0039		
Total	239	1.1156			

Table 7A

Chlorophyll B contents (means of 8 observations in each experimental lot) in mg/gm OFW of lettuce under 5 treatments after 5, 6 and 7 weeks in storage

Week	Replication	Treatment					Mean
		0	A1	A2	K	A2K	
5	1	0.1750	0.2436	0.2249	0.2187	0.2366	0.2340
	2	0.2515	0.2450	0.2166	0.2687	0.2594	
6	1	0.2255	0.2595	0.2432	0.2566	0.2600	0.2432
	2	0.2386	0.2248	0.2150	0.2665	0.2428	
7	1	0.2090	0.1700	0.1795	0.2156	0.2112	0.2140
	2	0.2103	0.1994	0.2462	0.2668	0.2319	
Mean		0.2183	0.2237	0.2209	0.2488	0.2403	0.2304

Mean of replication 1 = 0.2219 Mean of replication 2 = 0.2389

Treatments: 0 = control, A1 = 1 ppm ABA, A2 = 5 ppm ABA, K = 20 ppm kinetin, A2K = 5 ppm ABA + 20 ppm kinetin

Table 7B

Analysis of variance of chlorophyll B content (mg/gm OFW) of lettuce under 5 treatments after 5, 6 and 7 weeks in storage

Source	D.F.	S.S.	M.S.	F	Prob.
Replication	1	0.0172	0.0172	5.28	0.0214
Week (A)	2	0.0358	0.0179	1.46	0.4060
Error a	2	0.0245	0.0122	3.75	0.0246
Treatment (B)	4	0.0345	0.0086	2.74	0.0782
Week x Treatment (AB)	8	0.0200	0.0025	0.80	0.6180
Error b	12	0.0377	0.0031	0.96	0.4861
Error	210	0.6856	0.0033		
Total	239	0.8553			

Table 8A

Chlorophyll (A+B) contents (means of 8 observations in each experimental lot) in mg/gm FW of lettuce under 5 treatments after 5, 6 and 7 weeks in storage

Week	Replication	Treatment					Mean
		0	A1	A2	K	A2K	
5	1	0.5143	0.7624	0.6339	0.6573	0.7138	0.7296
	2	0.8068	0.7280	0.7191	0.8733	0.8875	
6	1	0.7036	0.8399	0.8625	0.8895	0.8306	0.8144
	2	0.8211	0.7504	0.7376	0.8796	0.8288	
7	1	0.7370	0.6238	0.6264	0.7393	0.7126	0.7253
	2	0.7333	0.6379	0.7557	0.8829	0.8041	
Mean		0.7194	0.7237	0.7225	0.8203	0.7962	0.7564
Mean of replication 1 = 0.7231				Mean of replication 2 = 0.7897			

Treatments: 0 = control, A1 = 1 ppm ABA, A2 = 5 ppm ABA, K = 20 ppm kinetin, A2K = 5 ppm ABA + 20 ppm kinetin

Table 8B

Analysis of variance of chlorophyll (A+B) content (mg/gm FW) of lettuce under 5 treatments after 5, 6 and 7 weeks in storage

Source	D.F.	S.S.	M.S.	F	Prob.
Replication	1	0.2663	0.2663	11.86	0.0008
Week (A)	2	0.4035	0.2018	1.41	0.4140
Error a	2	0.2853	0.1426	6.35	0.0023
Treatment (B)	4	0.4444	0.1111	2.86	0.0702
Week x Treatment (AB)	8	0.2155	0.0269	0.69	0.6918
Error b	12	0.4655	0.0388	1.73	0.0625
Error	210	4.7147	0.0225		
Total	239	6.7951			

Table 9A

Chlorophyll (A+B) contents (means of 8 observations in each experimental lot) in mg/gm OFW of lettuce under 5 treatments after 5, 6 and 7 weeks in storage

Week	Replication	Treatment					Mean
		0	A1	A2	K	A2K	
5	1	0.5192	0.7126	0.6747	0.6604	0.7029	0.6957
	2	0.7471	0.6992	0.6590	0.7922	0.7900	
6	1	0.6077	0.6891	0.6872	0.7060	0.7271	0.6843
	2	0.6803	0.6369	0.6243	0.7789	0.7056	
7	1	0.5831	0.4861	0.5270	0.6181	0.6148	0.6081
	2	0.6087	0.5593	0.6743	0.7552	0.6543	
Mean		0.6244	0.6305	0.6411	0.7185	0.6991	0.6627
Mean of replication 1 = 0.6344				Mean of replication 2 = 0.6910			

Treatments: 0 = control, A1 = 1 ppm ABA, A2 = 5 ppm ABA, K = 20 ppm kinetin, A2K = 5 ppm ABA + 20 ppm kinetin

Table 9B

Analysis of variance of chlorophyll (A+B) content (mg/gm OFW) of lettuce under 5 treatments after 5, 6 and 7 weeks in storage

Source	D.F.	S.S.	M.S.	F	Prob.
Replication	1	0.1920	0.1920	9.89	0.0021
Week (A)	2	0.3629	0.1814	4.03	0.2008
Error a	2	0.0901	0.0451	2.32	0.0986
Treatment (B)	4	0.3561	0.0890	3.69	0.0351
Week x Treatment (AB)	8	0.1251	0.0156	0.65	0.7270
Error b	12	0.2898	0.0241	1.24	0.2547
Error	210	4.0785	0.0194		
Total	239	5.4945			

Table 10

Tabulation of the means (mg) of chlorophyll A, B and A+B contents, based on 1 gm fresh weight (gm FW) and 1 gm original fresh weight (gm OFW), for 2 replications, under 5 treatments, and after 5, 6 and 7 weeks in storage

Basis of measurement		FW			OFW		
Chlorophyll		A	B	A+B	A	B	A+B
Replication	1	0.4693A[1]	0.2538a	0.7231A	0.4125A	0.2219a	0.6344A
	2	0.5166B	0.2732b	0.7897B	0.4521B	0.2389b	0.6910B
Time in storage (weeks)	5	0.4840a[2]	0.2456a	0.7296a	0.4617a	0.2340a	0.6957a
	6	0.5246a	0.2898a	0.8144a	0.4411a	0.2432a	0.6843a
	7	0.4702a	0.2551a	0.7253a	0.3941a	0.2140a	0.6081a
Treatment	0	0.4673α[3]	0.2520a	0.7194α	0.4060a	0.2183α	0.6244a
	A1	0.4664α	0.2573a	0.7237α	0.4068a	0.2237α	0.6505ab
	A2	0.4732α	0.2493a	0.7225α	0.4202ab	0.2209α	0.6411ab
	K	0.5354α	0.2849a	0.8203α	0.4697c	0.2488α	0.7185c
	A2K	0.5223αβ	0.2740a	0.7962α	0.4588bc	0.2403α	0.6991bc

Mean separation in column by Duncan's multiple range test:

[1] separation by upper case letter- significant at 1% level

[2] separation by lower case letter- significant at 5% level

[3] separation by Greek symbol- approaching the 5% level of significance

Treatments: 0 = control
A1 = 1 ppm ABA
A2 = 5 ppm ABA
K = 20 ppm kinetin
A2K = 5 ppm ABA + 20 ppm kinetin

Chlorophyll A, B and A + B contents of replication 2 were significantly higher than that of replication 1 in all respects regardless of pigment or basis of measurement, but the differences in chlorophyll A and total chlorophyll (A + B) contents were highly significant at the 1% level, whereas differences in chlorophyll B contents were significant at the 5% level.

There were small differences among the contents of chlorophylls in lettuce kept under storage for 5, 6 and 7 weeks in both gm FW and gm OFW bases, but the differences were not significant. Nevertheless, the two bases showed different characteristics as illustrated in Figure 5 where the content based on gm OFW showed a gradual decrease of contents from week 5 to week 7, whereas the other base (gm FW) had high contents at week 6 and lower contents at weeks 5 and 7.

Treatment effects were revealed only in cases of chlorophyll A and A + B contents as measured on gm OFW basis, and some of the differences were significant at the 5% level. In the case of chlorophyll B, some of the differences approached the 5% level of significance.

Considering chlorophyll A content per gm OFW, there was a significantly higher content in the 20 ppm kinetin treatment than in the 1ppm ABA, 5 ppm ABA, and the control, but not significantly higher than the 5ppm ABA + 20 ppm kinetin treatment. The 5 ppm ABA + 20 ppm kinetin treatment had a higher chlorophyll A content than 1 ppm ABA and the control, but the difference between the 5ppm ABA + 20 ppm kinetin and 5 ppm ABA alone was not significant.

The total chlorophyll (A + B) contents per gm OFW showed the same response to the treatments as did chlorophyll A alone with the exception that the combination treatment of 5 ppm ABA + 20 ppm kinetin was not significantly different from either of the ABA treatments.

Chlorophyll A contents were roughly twice those of chlorophyll B. Figure 4 shows the relative comparison and also shows the trend of chlorophyll contents under the 5 different treatments which varied from 5 to 7 weeks in storage.

Figure 4

Chlorophyll A and B in mg/gm OFW as affected by treatment and storage time

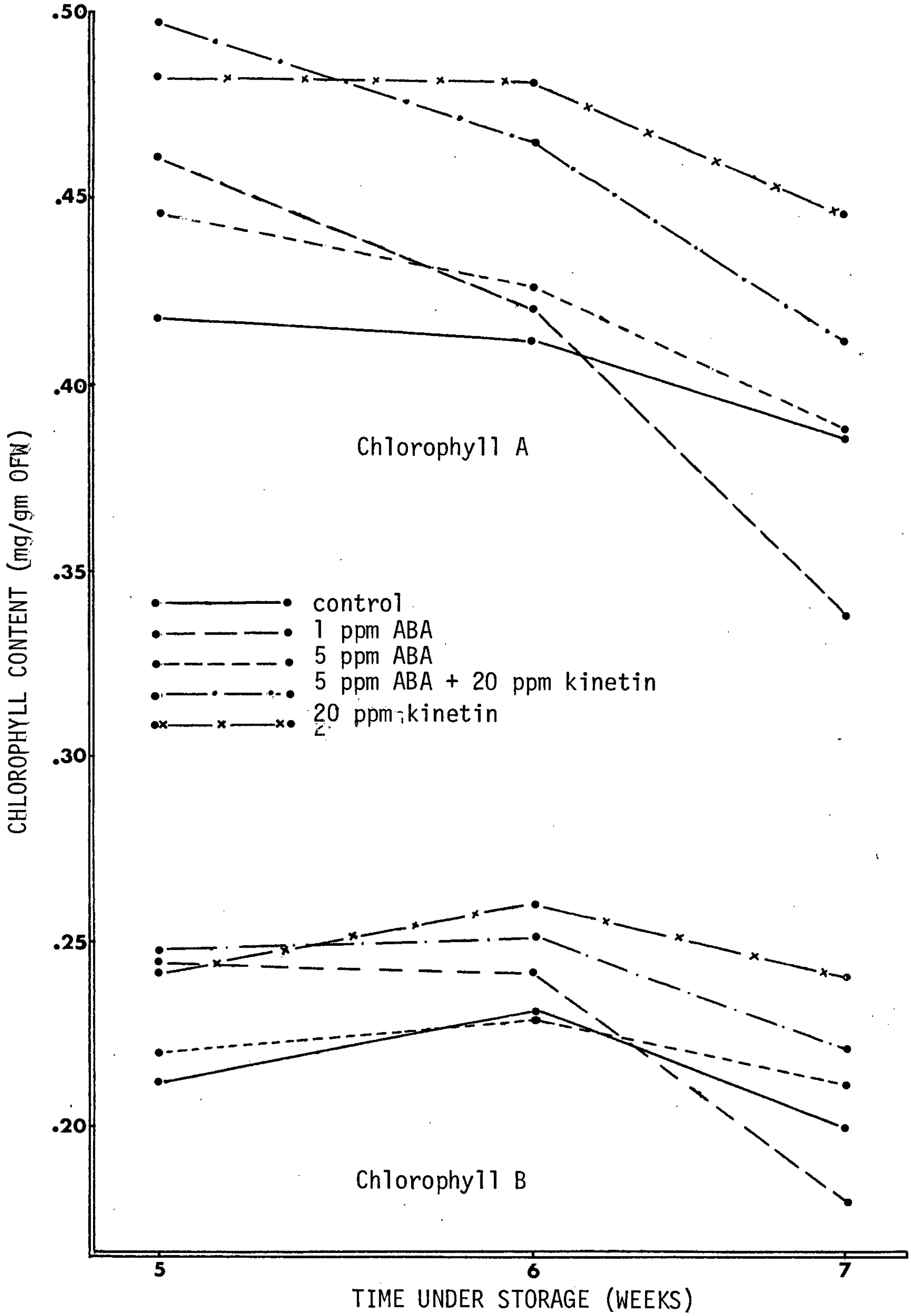
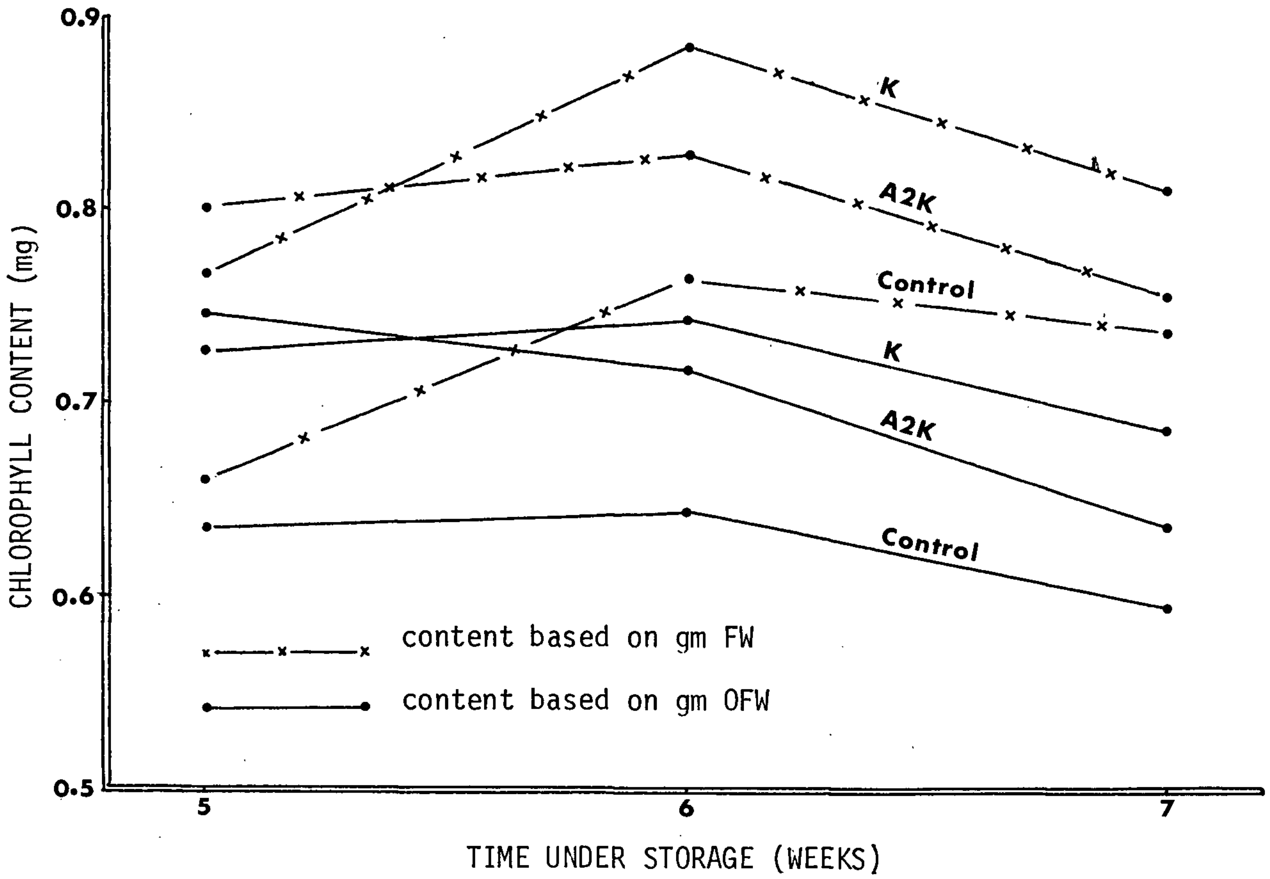


Figure 5

Chlorophyll (A+B) in mg/gm FW and mg/gm OFW of lettuce under control, 20 ppm kinetin and 5ppm ABA + 20 ppm kinetin treatments at the end of 5, 6 and 7 weeks in storage



5. Correlation and simple linear regression

The data were further studied using correlation and linear regressions. Only results which were deemed useful are presented. In each case, there are three correlation and regression values. One for the total experiment using 240 pairs of observations and the other two are for the individual replications each employing 120 paired observations.

5.1. Chlorophyll A and chlorophyll B contents

Correlations of chlorophyll A with chlorophyll B within the same leaf sample showed very high correlation coefficients, as can be seen in the r^2 (coefficient of determination) values in Table 11. Arbitrarily designating chlorophyll A as the dependent variable X, and chlorophyll B as the independent Y, the simple linear regression equations were obtained as shown in Table 11.

Table 11

Linear regression equations for chlorophyll B content* (Y) on chlorophyll A content* (X)

Source	Regression equation	F-Prob		Standard error			
		(b)	(a)	(b)	(Y)	r^2	N
2 Reps.	Y = 0.5551X - 0.01014	0.0	0.0107	0.0211	0.0347	0.7435	240
Rep. 1	Y = 0.6480X - 0.05026	0.0	0.0152	0.0315	0.0356	0.7820	120
Rep. 2	Y = 0.4702X - 0.03028	0.0	0.0143	0.0272	0.0301	0.7167	120

* content in mg/gm FW

The regression equations show a very high degree of association between these 2 chlorophylls within the same sample. Nevertheless, the three linear regression equations are not identical. This means that in spite of the strong correlation, a different quantity of chlorophyll B in association with a changed quantity of chlorophyll A is different when the affecting conditions are different, as in this case of the two replications producing different effects.

5.2. Percent weight loss and storage time

The simple regression equations (Table 12) show a high coefficient of regression (F probability = 0) which means that such a linear relation existed between percent weight loss and the storage time in weeks. The coefficient of determination was not very high in these cases but all the equations imply that the percent weight loss of a plant tended to increase with time in storage.

Table 12

Linear regression equations of percent weight loss (Y) on storage time in weeks (X)

Source	Regression equation	F-Prob. (b)	Standard error (a)	(b)	(Y)	r^2	N
2 Reps.	Y = 6.152X - 24.80	0.0	3.116	0.5147	6.510	0.3752	240
Rep. 1	Y = 9.040X - 42.51	0.0	4.804	0.7933	7.096	0.5239	120
Rep. 2	Y = 3.265X - 7.09	0.0	3.283	0.5422	4.850	0.2351	120

5.3. Percent moisture content and storage time

The equations in Table 13 imply a progressive loss in moisture content of lettuce during storage, but provide no information on possible differences which might exist among the treatments.

Table 13

Linear regression equations of percent moisture content (Y) on storage time in weeks (X)

Source	Regression equation	F-Prob. (b)	standard error (a)	(b)	(Y)	r^2	N
2 Reps.	Y = 96.48 - 0.3372X	0.000	0.2892	0.0477	0.6042	0.1732	240
Rep. 1	Y = 97.34 - 0.4825X	0.000	0.4556	0.0752	0.6729	0.2584	120
Rep. 2	Y = 95.62 - 0.1920X	0.001	0.3416	0.0564	0.5045	0.0894	120

DISCUSSION AND CONCLUSION

Lettuce held at $3 \pm 1^{o}C$ and relative humidity close to 100% in the experiment maintained the marketing quality of lettuce satisfactorily up to 5 or 6 weeks in the storage, regardless of the chemical treatment used. At the end of 6 weeks in storage, the numerical quality rating of all lettuce in the experiment averaged 5.3, and in the scale employed, this valued indicated "fair condition". A severe wilting and yellowing occurred only in the seventh week. No disease was observed on any lettuce plant throughout the seven week period of storage. This freedom from disease might be due to the growing conditions in the greenhouse, the hygenic handling of the specimens and clean cold storage facilities. The above mentioned conditions which are generally recommended for storage of mature lettuce appeared to be favourable for the juvenile, 7-week-old lettuce used in this experiment. Kinetin, which has been shown effective in prolonging the storage and shelf life of various vegetables (as previously described in the literature review) did not result in any significant improvement in lettuce quality as observed by the rating panel. The other treatments, 1 ppm ABA, 5 ppm ABA and 5ppm ABA + 20 ppm kinetin showed no effects which the panel could observe.

The lettuce lost weight with time, but the percentage of total weight loss varied greatly from plant to plant within the same treatment. All lettuce under 5 different treatments lost an average of 16.4% of its original fresh weight at the end of week 7 in storage. In contrast with the great variability in percentage of total weight loss, all plants tended to have a percent moisture content around 94.45% (standard deviation of 240 observations = 0.66%) regardless of storage times (5, 6 and 7 weeks) treatments, or the subsequent variability in terms of percent total weight loss. These particular results, if not just a coincidence, imply that a certain relationship and some harmony between the transpiration and other biological processes, particularly respiration, existed so that the plant could maintain its level of percent moisture content at about 94.5% throughout the period of 5, 6 and 7 weeks in storage.

Apparently, the expected antitranspirant characteristic of ABA showed no beneficial effect under the conditions of this experiment. Other experiments using the same chemical treatments at room temperatures or the conditions normally existing on the shelf of a retail store may be useful because the value of ABA as an antitranspirant was observed by Mittelheuser and Van Steveninck (1969), Jones and Mansfield (1970) in experiments carried out under normal room temperatures. Hofstra and Hesketh (1969) found that the change of air temperature affected stomatal opening and transpiration in various plant species. Stomata closed and transpiration was reduced at a low temperature (the experiment was carried out in the 15^{o} to 36^{o}C range). Under the conditions of the present experiment, it was likely that the low temperature of the cold storage affected stomatal activity to favour moisture conservation. This low temperature plus the high relative humidity in the cold storage provided such good storage conditions for the lettuce that the applications of ABA were ineffective and unnecessary.

Furthermore, ABA has been reported as highly subject to rapid biological breakdown - an inactivation process (Walton and Sondheimer, 1971; Milborrow, 1970). Also, most of the previous work on the antitranspirant effect of ABA was studied under short periods of time such as a few days up to one week, thus it was possible that the ABA effect did not last as long as 6 or 7 weeks. No attempt was made to investigate the breakdown of

ABA in the present study. The only conclusion is that ABA at the concentrations used in this experiment was ineffective as a quality-preservation agent.

It is possible that additional ABA was not needed to conserve quality in the present experiment. Wright and Hiron (1969) reported an increase in ABA content in detached wheat leaves induced by wilting; (they also found similar increases in excised leaves of cotton, pea and dwarf bean). This phenomenon may reduce the severity of wilting in nature, and similarly ABA-treated lettuce may thus appear to be little different from the untreated.

High humidity is definitely recommended for storage of young lettuce. In this experiment, extra moisture which sometimes condensed on lettuce leaves did no harm. However, this condition might be questionable if the subsequent shelf life quality was studied. Keeping relative humdity within a range of 93-95% with no fluctuation to the saturation point, could eliminate excess moisture within a few days. Certainly the storage of lettuce is dependent largely on the time lapse between cutting at harvest and being put in a cold storage, and obviously the shortest time lapse is best. The lack of large differences between treatments and storage time was undoubtedly due to the rapid placement of freshly harvested lettuce in high humidity storage.

The chlorophyll analyses showed roughly a 2 to 1 ratio of chlorophyll A to chlorophyll B. Regression equations of chlorophyll B on A (Table 11) show a high association between these two substances. Nevertheless, the relationship was subject to alteration to some degree by exogenous factors and surroundings.

The chemical treatment, particularly kinetin, retarded the degradation of chlorophyll A and possibly chlorophyll B. The treatment effect on chlorophyll A content was apparent at the 5% level but, for chlorophyll B, the difference was just approaching the 5% level of significance.

ABA in the 5 ppm ABA + 20 ppm kinetin treatment appeared to have mild antagonistic activity against kinetin so that the chlorophyll content in the combination treatment was lower than that of the kinetin only treatment, but the difference was not significant. The differences of chlorophyll contents between the two replications were also less obvious in the case of chlorophyll B than A.

Comparisons of chlorophyll contents (A, B or A + B) of lettuce with 5, 6 and 7 weeks under storage showed no significant differences among the three different periods of storage, regardless of the basis (mg/gm FW or mg/gm OFW) used. The mg/gm OFW basis was more useful in following the degradation trend of chlorophyll content with storage time.

The quantitative measurements of chlorophyll were more objective than the subjective visual ratings of green colour as a quality component. Nevertheless, the small differences in the chlorophyll measurements could not be detected visually; therefore such differences cannot be of any importance to influence consumer acceptance. Slight differences in green colour do exist in lettuce on the market, but of greater concern is the freshness of appearance and crispness of the commodity on sale.

The present investigation indicates that the use of abscisic acid and kinetin were of little practical value to maintain quality in leaf lettuce beyond what is commonly achieved in conventional cold storage, and that good storage conditions including good hygiene would prolong the post harvest life of lettuce for periods up to 6 weeks.

It is also significant that the present experiment is far from simulating the actual lettuce production conditions which involve different environmental conditions and cultivation practices, (e.g. fertilization, herbicide and pesticide applications) - factors that might complicate the effect of the intended post-harvest quality prolonging agent.

Up to this stage, ABA is unlikely to work as an antitranspirant under the low temperature and high humidity of cold storage but it is still possible that it might be beneficial in retarding transpiration rate and help prolong the quality of the commodity under normal room temperature ranges in places and under certain situations where cold storage facilities are not available.

Further studies should be considered and carried out before concluding that ABA, as well as kinetin, has any value in the post-harvest handling of lettuce. The response of the chemicals may be affected by (1) age and maturity of plant tissue, (2) concentrations of chemicals, (3) mode of application (spraying, dipping, single- or multi-application), (4) temperature, and (5) relative humidity. All these factors should be studied, and particularly in the variable environments encountered in the handling, storing and retailing of lettuce.

LITERATURE CITED

Abdel-Kader, A.S., L.L. Morris, and E.C. Maxie. 1966. Effect of growth-regulating substances on the ripening and shelf-life of tomatoes. HortScience 1: 90-91.

Addicott, F.T. and J.L. Lyon. 1969. Physiology of abscisic acid and related substances. Ann. Rev. Plant Physiol. 20: 139-164.

_______________________, K. Ohkuma, W.E. Thiessen, H.R. Carns, O.E. Smith, J.W. Cornforth, B. V. Milborrow, G. Ryback, and P.F. Wareing. 1968. Abscisic acid: a new name for abscisin II (dormin). Science 159: 1493.

Altman, A. and R. Goren. 1971. Promotion of callus formation by abscisic acid in citrus bud cultures. Plant Physiol. 47: supplement no. 138.

Aspinall, D., L.G. Paleg and F.T. Addicott. 1967. Abscisin II and some hormone-regulated plant responses. Austral. J. Biol. Sci. 20: 869-882.

Ben-Zioni , A., C. Itai and Y. Vaadia. 1967. Water and salt stresses, kinetin and protein synthesis in tobacco leaves. Plant Physiol. 42: 361-365.

Bessey, P.M. 1960. Effects of a new senescence inhibitor on lettuce storage. Univ. Ariz. Exp. Sta. Rep. 189: 5-8.

Bhardwaj, S.N. and Y.P. Abrol. 1967. Interaction of abscisin II with some growth regulating substances. Indian J. Exp. Biol. 5: 264-265.

Blaydes, D.F. 1971. The effect of abscisic acid and growth substances on _Avena_ coleoptile growth. Plant Physiol. 47: supplement No. 137.

Blumenfield, A. and S. Gazit. 1970. Interaction of kinetin and abscisic acid in the growth of soybean callus. Plant Physiol. 45: 535-536.

Boasson, R. and W.M. Laetsch. 1967. Chlorophyll synthesis in tobacco callus: interaction of sugar and kinetin. Experientia 23: 968.

Bornman, C.H., A.R. Spurr and F.T. Addicott. 1967. Abscisin, auxin and gibberellin effects on the developmental aspects of abscission in cotton (_Gossypium hirstutum_). Amer. J. Bot. 54: 125-135.

Chatterjee, S.K. and A.C. Leopold. 1964. Kinetin and gibberellin actions on abscission processes. Plant Physiol. 39: 334-337.

Chrispeels, M.J. and J.E. Varner. 1967. Hormonal control of enzyme synthesis: on the mode of action of gibberellic acid and abscisin in aleurone layers of barley. Plant Physiol. 42: 1008-1016.

Cornforth, J.W., B.V. Milborrow, and G. Ryback. 1965a. Chemistry and physiology of "dormin" in sycamore. Nature 205: 1269-1270.

____. 1965b. Synthesis of (±)-abscisin II. Nature 206:715.

____. 1966. Identification and estimation of (+)-abscisin II (dormin) in plant extracts by spectropolarimetry. Nature 210: 627-628.

Cracker, L.E. and F.B. Abeles. 1969. Abscission: role of abscisic acid. Plant Physiol. 44: 1144-1149.

Cummins, W.R., K. Raschke, and H. Kende. 1971. Abscisic acid: specific, rapid and reversible effects on stomata. Plant Physiol. 47: supplement No. 133.

Dedölph, R.R., S.H. Wittwer, V. Tuli, and D. Gilbart. 1962. Effect of N^6-benzylaminopurine on respiration and storage behavior of broccoli (Brassica oleracea var. italica). Plant Physiol. 37:509-512.

De Leo, P. and J.A. Sacher. 1970. Control of ribonuclease and acid phosphatase by auxin and abscisic acid during senescence of rhoeo leaf sections. Plant Physiol. 46: 806-811.

Eagles, C.F., P.F. Wareing. 1964. The role of growth substances in the regulation of bud dormancy. Physiol. Plant. 17: 697-709.

El-Mansy, H.I., D.K. Salunkhe, R.L. Hurst, and D.R. Walker. 1967. Effects of pre- and post-harvest applications of 6-furfurylaminopurine and of N^6-benzyladenine on physiological and chemical changes in lettuce (Lactuca sativa L.). Hort. Res. 7: 81-89.

Evans, L.T. 1966. Abscisin II: inhibitory effect on flower induction in a long-day plant. Science 151: 107-108.

Galston, A.W. and P.J. Davies. 1970. Control mechanisms in plant development. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

Gamborg, O.L. and T.A.G. La Rue. 1971. Ethylene production by plant cell cultures: the effect of auxins, abscisic acid, and kinetin on ethylene production in suspension cultures of rose and Ruta cells. Plant Physiol. 48: 399-401.

Glinka, Z. 1971. Abscisic acid raises the permeability of plant cells to water. Plant Physiol. 48: 103-105.

Heide, O.M. 1968. Stimulation of adventitious bud formation in begonia leaves by abscisic acid. Nature 219: 960-961.

Heide, O.M. and J. Oydvin. 1969. Effects of 6-benzylaminopurine on the keeping quality and respiration of greenhouse carnations. Hort. Res. 9: 26-36.

Hofstra, G. and J.D. Hesketh. 1969. The effect of temperature on stomatal aperture in different species. Can. J. Bot. 47: 1307-1310.

Horton, R.F. 1971. Stomatal opening: the role of abscisic acid. Can. J. Bot. 49: 583-585.

Jones, R.J. and T.A. Hansfield. 1970. Suppression of stomatal opening in leaves treated with abscisic acid. J. Exp. Bot. 21: 714-719.

Kaufmann, J. and S.H. Ringel. 1961. Tests of growth regulators to retard yellowing and abscission of cauliflower. Proc. Amer. Soc. Hort. Sci. 78: 349-352.

Khan, A.A. 1967. Antagonism between dormin and kinetin in seed germination and dormancy. Amer. J. Bot. 54: 639.

__________. 1968. Inhibition of gibberellic acid-induced germination by abscisic acid and reversal by cytokinins. Plant Physiol. 43: 1463-1465.

__________. 1969. Cytokinin-inhibitor antagonism in the hormonal control of α-amylase synthesis and growth in barley seed. Physiol. Plant. 22: 94-103.

__________. 1970. Abscisic acid-induced changes in nucleotide composition of rapidly labeled ribonucleic acid species of lentil root reversal by kinetin. Plant Physiol. 46: 494-495.

__________. 1970. Abscisic acid induced changes in nucleotide composition of rapidly labelled RNA species of pear embryos. Biochem. Biophys. Res. Commun. 38: 1069-1075.

__________. and9R8D. Downing. 1968. Cytokinin reversal of abscisic acid inhibition of growth and α-amylase synthesis in barley seed. Physiol. Plant. 21: 1301-1307.

__________ and C.E. Heit. 1969. Selective effect of hormones on nucleic acid metabolism during germination of pear embryos. Biochem. J. 113: 707-712.

Kuraishi, S. 1968. The effect of kinetin on protein level of Brassica leaf disks. Physiol. Plant. 21: 78-83.

Liehtenthaler, H.K. and K. Becker. 1970. Inhibition of the light-induced vitamin K_1 and pigment synthesis by abscisic acid. Phytochemistry 9: 2109-2113.

Lieberman, M. and A.T. Kunishi. 1971. Abscisic acid and ethylene production. Plant Physiol. 47: supplement No. 129.

Lipton, W.J. and H.J. Ceponis. 1962. Retardation of senescence and stimulation of oxygen consumption in head lettuce treated with N^6-benzyladenine. Proc.Amer.Soc.Hort.Sci. 81: 379-384.

Little, C.H.A. and D.C. Eidt. 1968. Effect of abscisic acid on budbreak and transpiration in woody species. Nature 220: 498-499.

Livné, A. and Y. Vaadia. 1965. Stimulation of transpiration rate in barley leaves by kinetin and gibberellic acid. Physiol. Plant. 18: 658-664.

Loveys, B.R., P.E. Kriedmann, G.L. Fuller and A.C. Leopold. 1971. Abscisic acid-induced stomatal closure. Plant Physiol. 47: supplement No. 134.

Luke, H.H. and T.E. Freeman. 1968. Stimulation of transpiration by cytokinins. Nature 217: 873-874.

MacLean, D.C. and R.R. Dedolph. 1962. Effects of N^6-benzylaminopurine on post-harvest respiration of Chrysanthemum morifolium and Dianthus carophyllus. Bot. Gaz. 124: 20-21.

Mayak, S. and H. Halevy. 1970. Cytokinin activity in rose petals and its relation to senescence. Plant Physiol. 46: 497-499.

Meidner, H. 1967. The effect of kinetin on stomatal opening and the rate of intake of carbon dioxide in mature primary leaves of barley. J. Exp. Bot. 18: 556-561.

Milborrow, B.V. 1970. The metabolism of abscisic acid. J. Exp. Bot. 21 (66): 17-29.

Mittelheuser, C.J. and R.F.H. Van Steveninck. 1969. Abscisic acid induces stomatal closure and inhibits transpiration. Nature 221: 281-282.

Mizrahi, YY,AA. Blumenfeld, and A.E. Richmond. 1970. Abscisic acid and transpiration in leaves in relation to osmotic root stress. Plant Physiol. 46: 169-171.

Ohkuma, K., J.L. Lyon, F.T. Addicott and O.E. Smith. 1963. Abscisin II, an abscission-accelerating substance from young cotton fruit. Science 142: 1592-1593.

Osborne, D.J. 1962. Effect of kinetin on protein and nucleic acid metabolism in Xanthium leaves during senescence. Plant Physiol. 37: 595-602.

Pilet, P.E. 1970. The effect of auxin and abscisic acid on the catabolism of RNA. J. Exp. Bot. 21(67): 446-451.

Robinson, P.M. and P.F. Wareing. 1964. Chemical nature and biological properties of the inhibitor varying with photoperiod in sycamore (Acer pseudoplatanus). Physiol. Plant. 7: 314-323.

Ryback, G. 1972. Revision of the absolute chemistry of (+)-abscisic acid. Chem. Comm. p.1190-1191.

Salisbury, F.B. and C. Ross. 1969. Plant Physiology. Wadsworth Publishing Company, Inc., Belmont, Calif.

Sankhla, N. and D. Sankhla. 1968a. Abscisin II - kinetin interaction in leaf senescence. Experientia 24: 294-295.

____________________. 1968b. Reversal of (±)-abscisin II induced inhibition of lettuce seed germination and seedling growth by kinetin. Physiol. Plant. 21: 190-195.

Sestak, Z. 1971. Determination of chlorophyll a and b. In Plant photosynthetic production. pp. 672-698. Ed. Z. Šestak, J. Catsky, and P.G. Jarvis. Dr. W. Junk N.V. Publishers, The Hague.

Shibaoka, H. and K.V. Thimann. 1970. Antagonisms between kinetin and amino acids; experiments on the mode of action of cytokinins. Plant Physiol. 46: 212-220.

Shih, C.Y. and L. Rappaport. 1971. Regulation of bud rust in tubers of potato, Solanum tuberosum L. VIII. Early effects of gibberellin A_3 and abscisic acid on ultrastructure. Plant Physiol. 43: 31-35.

Sloger, C. and B.E. Caldwell. 1970. Response of cultivars of soybean to synthetic abscisic acid. Plant Physiol. 45: 634-635.

Srivastava, B.I.S. 1968. Acceleration of senescence and of the increase of chromatin-associated nucleases in excised barley leaves by abscisin II and its reversal by kinetin. Biochim. Biophys. Acta 169: 534-536.

Street, H.E., H.A. Collin, K.C. Short, and I. Simpkins. 1967. Hormonal control of cell division and expansion in suspension culture of Acer pseudoplatanus L. The action of kinetin. In Biochemistry and physiology of plant growth substances, pp. 489-504. Ed. F. Wightman and G. Setterfield. Runge Press Ltd., Ottawa.

Tal, M., D. Imber, and C. Itai. 1970. Abnormal stomatal behaviour and hormonal imbalance in flacca, a wilty mutant of tomato. I. Root effect and kinetin-like activity. Plant Physiol. 46: 367-372.

Thomas, T.H., P.F. Wareing, P.M. Robinson. 1965. Action of the sycamore "Dormin" as a gibberellin antagonist. Nature 205: 1270-1272.

Van Overbeek, J., J.E. Loeffler and M.I.R. Mason. 1967. Dormin (abscisin II), inhibitor of plant DNA synthesis? Science 156: 1497-1499.

Von Abrams, G.J., and H.K. Pratt. 1966. Interaction of napthaleneacetic acid and kinetin in the senescence of detached leaves. Plant Physiol. 41: 1525-1530.

Wade, N.L., and C.J. Brady. 1971. Effect of kinetin on respiration, ethylene production, and ripening of banana fruit slices. Austral. J. Biol. Sci. 24: 165-167.

Walton, D.C. and Sondheimer. 1968. Effects of abscisin II on phenylalanine ammonia-lyase activity in excised bean axes. Plant Physiol. 43: 467-469.

________________________. 1971. Metabolism of 2-C^{14}-(RS)-abscisic acid in bean axes. Plant Physiol. 47: supplement No. 132.

Waters, W.E. 1964. Influence of chemical preservatives on keeping quality of asters, carnations, chrysanthemums, and gerbera daisies. Proc. Fla. St. hort. Soc. 77: 466-470.

Wittwer, S.H., R.R. Dedolph, V. Tuli and D. Gilbart. 1962. Respiration and storage deterioration in celery (Apium graveolens L.) as affected by post-harvest treatments with N^6-benzylaminopurine. Proc. Amer. Soc. Hort. Sci. 80: 408-416.

Wright, S.T.C. and R.W.P. Hiron. 1969. (±)-abscisic acid, the growth inhibitor induced to detached wheat leaves by a period of wilting. Nature 224: 719-720.

× Yomo, H. 1971. Inhibition of amylase formation by abscisic acid in excised pea and bean cotyledons. Plant Physiol. 47: supplement No. 135.

Zeevaart, J.A.D. 1971. (+)-Abscisic acid content of spinach in relation to photoperiod and water stress. Plant Physiol. 48: 86-90.

Zink, F.W. 1961. N^6-benzyladenine, a senescence inhibitor for green vegetables. J. Agr. Food Chem. 9: 304-307.

APPENDIX 1

Visual rating of lettuce quality under 5 different treatments after 2,3,4,5,6, and 7 weeks in storage.

Week	Rep.	Treatment 0	A1	A2	K	A2K
2	1	9.0	8.0	9.0	9.0	9.0
		9.0	8.5	9.0	9.0	8.0
		9.0	8.0	9.0	9.0	8.0
	2	8.0	9.0	9.0	9.0	8.0
		8.0	9.0	9.0	9.0	8.5
		8.0	9.0	9.0	9.0	9.0
3	1	7.0	7.0	9.0	8.0	9.0
		7.0	7.0	8.0	8.0	8.0
		7.0	7.0	8.0	8.0	8.0
	2	6.0	7.0	7.0	5.0	5.0
		6.0	7.0	7.0	6.0	6.0
		6.0	7.0	7.0	6.0	6.0
4	1	6.5	7.0	5.5	5.5	6.0
		4.5	7.0	4.0	4.0	5.0
		5.0	7.0	5.0	5.0	5.0
	2	7.0	5.0	5.5	5.5	7.0
		6.0	7.5	4.0	6.0	5.0
		6.0	7.0	5.0	5.0	5.0
5	1	9.0	8.0	9.0	4.5	7.0
		7.0	7.5	7.5	3.0	8.0
		8.0	8.0	9.0	4.0	8.0
	2	8.5	9.0	9.0	7.0	7.5
		7.0	8.0	7.0	4.0	5.0
		8.0	9.0	7.0	4.0	6.0
6	1	7.0	7.0	5.0	5.0	4.5
		7.0	6.0	3.0	3.5	4.0
		6.0	5.0	5.0	3.0	4.0
	2	7.5	7.5	7.0	4.0	6.5
		6.0	6.0	5.0	4.0	4.5
		5.0	5.0	5.0	4.0	5.0
7	1	3.0	4.5	4.0	5.0	4.0
		4.0	3.0	3.0	3.5	3.0
		3.0	4.0	4.0	3.0	3.0
	2	4.0	5.0	3.0	4.0	5.0
		4.0	4.0	3.0	4.0	4.0
		3.0	3.0	3.0	4.0	3.0

APPENDIX 2

Percent total weight loss of lettuce under 5 different treatments after 5,6, and 7 weeks in storage

Week	Rep.	Treatment 0	A1	A2	K	A2K
5	1	0.255	6.522	-0.784	5.463	7.837
		9.459	2.239	-9.145	12.987	4.051
		-1.027	-3.341	-7.527	-13.123	4.955
		1.916	1.881	-6.000	0.000	-0.357
		-5.740	15.790	-4.947	-2.222	-1.522
		-1.190	8.585	-16.466	-4.018	0.431
		-9.914	9.366	-1.435	0.443	5.856
		-0.235	4.235	0.580	-7.7?2	-6.936
	2	3.182	7.237	9.350	11.470	14.220
		8.571	2.198	7.347	11.245	10.933
		[illegible]	[illegible]	8.537	10.622	11.524
		11.480	0.000	10.860	9.705	14.057
		7.692	2.491	10.432	7.960	9.662
		7.179	5.351	9.091	6.731	12.500
		12.605	3.965	5.098	7.023	10.476
		3.947	4.530	6.539	11.562	5.479
6	1	15.288	12.245	24.561	26.720	6.468
		17.355	16.432	18.692	24.101	17.624
		22.187	15.471	24.242	18.214	7.018
		20.231	30.153	24.092	24.939	9.554
		12.315	12.800	11.314	19.098	13.008
		6.590	16.320	18.944	18.983	7.750
		8.140	19.333	17.472	19.626	19.355
		11.060	20.988	26.531	13.975	16.538
	2	77.029	17.284	13.406	10.876	14.783
		13.514	12.462	14.222	12.544	13.726
		15.849	10.638	15.790	11.312	15.306
		15.522	15.816	13.208	10.945	14.286
		15.938	18.142	11.917	9.730	10.891
		9.259	15.970	21.519	9.524	12.081
		11.507	16.964	16.337	16.235	19.324
		36.905	13.693	20.000	11.832	17.508
7	1	26.525	24.201	19.198	151686	11.027
		21.622	25.347	10.749	15.517	10.928
		17.608	18.779	17.266	14.563	21.116
		18.400	27.386	21.401	12.625	14.727
		23.724	23.922	18.919	12.523	14.011
		19.802	21.429	20.488	14.173	17.003
		20.120	16.794	6.941	22.247	11.350
		20.238	23.936	16.110	21.265	12.648
	2	3.774	25.630	9.091	17.365	15.723
		13.873	13.566	12.158	12.202	25.714
		21.073	8.929	13.636	9.247	20.588
		21.000	13.014	10.163	13.043	27.132
		20.648	10.791	10.853	15.041	14.395
		19.167	12.602	7.738	17.699	15.152
		14.545	12.459	10.109	16.908	12.217
		23.383	4.563	10.425	14.545	18.750

APPENDIX 3

Percent moisture contents of lettuce under 5 different treatments after 5,6, and 7 weeks in storage.

Week	Rep.	Treatment				
		0	A1	A2	K	A2k
5	1	95.635	94.793	95.128	95.181	94.945
		94.628	94.898	95.332	94.779	94.947
		95.227	95.233	95.914	95.897	94.985
		95.655	95.454	95.423	95.061	95.274
		95.825	94.231	95.642	95.556	94.814
		94.996	94.326	95.650	95.517	94.947
		95.618	94.274	95.338	95.055	94.806
		95.053	94.894	95.053	95.810	95.146
	2	94.040	95.891	95.128	94.750	94.840
		95.267	93.345	95.782	95.079	94.826
		94.831	94.647	95.474	95.469	95.119
		94.776	95.832	95.007	94.557	95.089
		94.352	95.189	94.503	94.242	93.841
		94.837	94.966	92.225	94.182	94.861
		94.596	94.712	93.995	94.071	94.969
		94.582	95.185	95.095	94.600	93.314
6	1	94.251	94.243	93.125	93.303	95.016
		94.412	94.092	93.718	94.494	94.532
		93.911	93.884	93.815	94.057	95.226
		94.016	93.016	92.467	93.127	95.242
		94.632	94.436	93.869	93.751	93.585
		94.355	94.107	92.973	94.122	94.473
		94.988	93.835	93.007	93.235	93.821
		94.260	93.439	92.813	94.150	94.110
	2	94.676	94.744	94.177	94.911	94.484
		94.415	94.856	94.115	93.960	95.238
		94.219	93.916	94.471	94.488	94.638
		94.368	93.980	94.472	94.317	94.404
		94.075	94.318	94.340	94.701	94.789
		93.964	94.315	94.785	94.294	94.391
		94.530	94.246	94.160	94.445	94.604
		93.464	94.215	94.783	94.443	93.683
7	1	93.781	93.388	93.940	94.240	94.570
		93.596	93.721	93.966	94.618	94.772
		94.168	93.770	94.134	94.254	94.020
		94.190	93.870	94.074	96.851	94.923
		94.509	93.935	94.155	94.251	94.547
		94.000	93.972	93.756	94.381	93.852
		93.886	93.583	95.553	94.103	94.107
		94.057	93.537	94.512	93.809	95.020
	2	93.264	94.409	94.618	94.518	94.226
		93.879	94.902	93.985	94.913	94.287
		94.179	94.678	94.079	94.318	93.911
		94.258	94.250	94.472	93.952	94.379
		93.711	94.108	94.065	94.019	94.570
		93.921	94.993	94.951	94.184	94.478
		94.469	94.377	94.268	94.737	94.398
		94.455	94.511	93.711	94.690	94.560

APPENDIX 4

Chlorophyll A contents (mg/gmFW) of lettuce under 5 different treatments after 5,6, and 7 weeks in storage.

Week	Rep.	Treatment				
		0	A1	A2	K	A2K
5	1	0.3512	0.4509	0.3055	0.3462	0.2859
		0.3269	0.4589	0.4647	0.4858	0.6374
		0.2944	0.4383	0.4841	0.3974	0.4111
		0.3704	0.4067	0.4192	0.4083	0.5671
		0.3315	0.6125	0.3653	0.4127	0.4060
		0.4664	0.5067	0.5330	0.4807	0.4383
		0.3764	0.7032	0.3745	0.5913	0.5165
		0.2096	0.4240	0.4372	0.3854	0.5245
	2	0.7138	0.3799	0.4787	0.5130	0.6145
		0.4220	0.8271	0.4518	0.4348	0.4379
		0.5320	0.4526	0.3426	0.5315	0.4628
		0.4175	0.3598	0.4496	0.5637	0.5926
		0.7149	0.3689	0.4763	0.6710	0.8713
		0.4974	0.5203	0.5236	0.6215	0.5979
		0.4585	0.4379	0.6008	0.7625	0.5317
		0.5284	0.4372	0.5340	0.5174	0.6593
6	1	0.3995	0.6007	0.4713	0.5206	0.5450
		0.3519	0.4345	0.5570	0.5355	0.5142
		0.3836	0.4642	0.6301	0.6502	0.4046
		0.4726	0.4660	0.6025	0.6207	0.4730
		0.3621	0.5436	0.6946	0.5253	0.6407
		0.5969	0.5050	0.4797	0.5502	0.5481
		0.5083	0.5351	0.5591	0.5418	0.5487
		0.4622	0.6339	0.4662	0.5845	0.5921
	2	0.4859	0.4718	0.4462	0.5249	0.4681
		0.5420	0.4264	0.6000	0.6789	0.5336
		0.4883	0.5304	0.4831	0.6060	0.5978
		0.5342	0.4532	0.5899	0.6561	0.5304
		0.4226	0.5672	0.5417	0.5884	0.5007
		0.5295	0.4742	0.3370	0.6418	0.5312
		0.4747	0.4756	0.4463	0.3999	0.5211
		0.7848	0.4872	0.4245	0.5319	0.6651
7	1	0.3697	0.4970	0.4008	0.4601	0.7249
		0.6393	0.2647	0.3722	0.4363	0.3745
		0.4960	0.5587	0.3723	0.5566	0.3196
		0.3563	0.2548	0.4575	0.4048	0.3182
		0.4420	0.4698	0.2616	0.4666	0.5094
		0.5179	0.4555	0.3834	0.4559	0.5514
		0.6501	0.4751	0.5706	0.5713	0.5709
		0.3118	0.2686	0.4865	0.5013	0.3722
	2	0.5091	0.3468	0.3380	0.4775	0.4383
		0.5266	0.4751	0.5385	0.4539	0.4328
		0.5120	0.4400	0.5901	0.6588	0.5106
		0.4857	0.3600	0.4835	0.5202	0.5964
		0.5731	0.4453	0.5077	0.6436	0.5103
		0.4424	0.4574	0.3580	0.5851	0.5603
		0.4298	0.3016	0.4615	0.6480	0.5371
		0.3602	0.4566	0.5614	0.5815	0.5720

APPENDIX 5

Chlorophyll A contents (mg/gmOFW) of lettuce under 5 different treatments after 5,6, and 7 weeks in storage.

Week	Rep.	Treatment				
		0	A1	A2	K	A2K
5	1	0.3503	0.4290	0.3079	0.3273	0.2635
		0.2960	0.4486	0.5072	0.4227	0.6116
		0.2974	0.4529	0.5205	0.4495	0.3907
		0.3633	0.3991	0.4443	0.4083	0.5691
		0.3505	0.5158	0.3833	0.4219	0.4121
		0.4719	0.4632	0.6208	0.5000	0.4364
		0.4137	0.6373	0.3799	0.5887	0.4863
		0.2101	0.4060	0.4346	0.4153	0.5609
	2	0.6910	0.3524	0.4339	0.4542	0.5271
		0.3858	0.8089	0.4186	0.3859	0.3900
		0.4889	0.4222	0.3134	0.4751	0.4095
		0.3696	0.3598	0.4008	0.5090	0.5093
		0.6599	0.3597	0.4266	0.6176	0.7872
		0.4617	0.4924	0.4760	0.5796	0.5232
		0.4007	0.4205	0.5702	0.7090	0.4760
		0.5076	0.4174	0.4991	0.4576	0.6232
6	1	0.3385	0.5271	0.3556	0.3815	0.5097
		0.2909	0.3631	0.4529	0.4064	0.4236
		0.2985	0.3924	0.4773	0.5318	0.3762
		0.3770	0.3255	0.4574	0.4659	0.4278
		0.3175	0.4740	0.6160	0.4250	0.5573
		0.5576	0.4226	0.3888	0.4458	0.5056
		0.4669	0.4317	0.4614	0.4354	0.4425
		0.4111	0.5009	0.3425	0.5028	0.4942
	2	0.4518	0.3903	0.3864	0.4678	0.3989
		0.4687	0.3732	0.5147	0.5937	0.4604
		0.4109	0.4740	0.4068	0.5374	0.5064
		0.4513	0.3815	0.5120	0.5843	0.4546
		0.3552	0.4643	0.4771	0.5311	0.4462
		0.4804	0.3985	0.2645	0.5807	0.4671
		0.4201	0.3949	0.3734	0.3350	0.4204
		0.4952	0.4205	0.3396	0.4689	0.5486
7	1	0.2717	0.3767	0.3239	0.3879	0.6450
		0.5011	0.1976	0.3322	0.3686	0.3336
		0.4087	0.4538	0.3080	0.4756	0.2521
		0.2907	0.1850	0.3596	0.3537	0.2713
		0.3371	0.3574	0.2121	0.4082	0.4380
		0.4154	0.3579	0.3049	0.3913	0.4577
		0.5193	0.3953	0.5310	0.4442	0.5061
		0.2487	0.2043	0.4081	0.3947	0.3251
	2	0.4899	0.2579	0.3073	0.3946	0.3694
		0.4536	0.4107	0.4730	0.3985	0.3215
		0.4041	0.4007	0.5096	0.5979	0.4055
		0.3837	0.3131	0.4344	0.4523	0.4346
		0.4548	0.3973	0.4526	0.5468	0.4370
		0.3576	0.3998	0.3303	0.4816	0.4754
		0.3673	0.2640	0.4149	0.5384	0.4715
		0.2760	0.4358	0.5028	0.4969	0.4648

APPENDIX 6

Chlorophyll B contents (mg/gmFW) of lettuce under 5 different treatments after 5,6, and 7 weeks in storage

Week	Rep.	Treatment 0	A1	A2	K	A2K
5	1	0.1714	0.2310	0.1287	0.1658	0.1328
		0.1181	0.2119	0.2497	0.2718	0.4494
		0.1275	0.1859	0.1884	0.1603	0.1586
		0.2000	0.2751	0.2548	0.1636	0.2484
		0.1373	0.3620	0.2154	0.2230	0.1836
		0.2548	0.1773	0.2814	0.1687	0.1859
		0.1574	0.4438	0.1639	0.4299	0.2880
		0.2211	0.2031	0.2054	0.1671	0.2772
	2	0.3612	0.2152	0.2651	0.3086	0.2560
		0.2498	0.4510	0.2741	0.2064	0.1994
		0.2607	0.2297	0.1481	0.2648	0.2562
		0.1713	0.1947	0.2539	0.2881	0.3299
		0.3607	0.2331	0.2565	0.3421	0.4274
		0.2193	0.2846	0.2853	0.2838	0.2933
		0.2255	0.2090	0.1698	0.4064	0.2627
		0.3215	0.2245	0.2427	0.2705	0.3067
6	1	0.2398	0.3477	0.2604	0.2520	0.2650
		0.1845	0.2486	0.3367	0.3186	0.2985
		0.2711	0.2538	0.3759	0.4060	0.1957
		0.2886	0.2875	0.2628	0.3988	0.2655
		0.1938	0.2771	0.4110	0.3189	0.3716
		0.3511	0.3004	0.2074	0.2878	0.3460
		0.2914	0.3513	0.3186	0.2931	0.3114
		0.2718	0.4700	0.2664	0.3121	0.3244
	2	0.2507	0.2564	0.2247	0.3229	0.2694
		0.3504	0.2213	0.3537	0.3715	0.2658
		0.1905	0.2748	0.2557	0.3015	0.2742
		0.3000	0.2028	0.2851	0.3348	0.2748
		0.2152	0.2675	0.2740	0.2589	0.2983
		0.2828	0.2746	0.1771	0.3023	0.2477
		0.2706	0.2625	0.2438	0.2167	0.3168
		0.4471	0.3573	0.2182	0.3009	0.3350
7	1	0.2155	0.3017	0.1991	0.2707	0.3719
		0.3339	0.1223	0.1839	0.2325	0.1734
		0.3002	0.2902	0.1935	0.2909	0.1823
		0.2057	0.1397	0.2240	0.2032	0.1752
		0.2322	0.2247	0.1197	0.2528	0.2718
		0.2856	0.2516	0.2138	0.2380	0.2873
		0.3774	0.2570	0.3063	0.3194	0.3138
		0.1625	0.1571	0.2658	0.2541	0.1839
	2	0.2547	0.2000	0.1882	0.2751	0.2642
		0.2878	0.2665	0.3021	0.2560	0.2435
		0.2689	0.2789	0.3424	0.3796	0.2809
		0.2623	0.1832	0.2919	0.2655	0.2767
		0.2842	0.2423	0.2763	0.3456	0.2733
		0.2091	0.2546	0.2203	0.3463	0.3181
		0.2600	0.1628	0.2874	0.3075	0.3141
		0.2002	0.2320	0.2986	0.3191	0.3038

APPENDIX 7

Chlorophyll B contents (mg/gmOFW) of lettuce under 5 different treatments after 5,6, and 7 weeks in storage

Week	Rep.	Treatment				
		0	A1	A2	K	A2K
5	1	0.1710	0.2160	0.1297	0.1567	0.1224
		0.1069	0.2072	0.2726	0.2365	0.4312
		0.1288	0.1921	0.2025	0.1814	0.1508
		0.1961	0.2699	0.2701	0.1636	0.2493
		o.1452	0.3048	0.2261	0.2279	0.1864
		0.2578	0.1621	0.3277	0.1755	0.1851
		0.1730	0.4023	0.1662	0.4280	0.2712
		0.2216	0.1945	0.2042	0.1801	0.2964
	2	0.3497	0.1997	0.2403	0.2732	0.2196
		0.2284	0.4411	0.2539	0.1832	0.1776
		0.2396	0.2126	0.1355	0.2366	0.2367
		0.1517	0.1947	0.2264	0.2601	0.2835
		0.3329	0.2273	0.2298	0.3149	0.3861
		0.2036	0.2694	0.2593	0.2647	0.2567
		0.1971	0.2007	0.1611	0.3779	0.2352
		0.3088	0.2144	0.2268	0.2392	0.2899
6	1	0.2031	0.3051	0.1965	0.1846	0.2479
		0.1525	0.2077	0.2737	0.2418	0.2459
		0.2110	0.2145	0.2848	0.3321	0.1819
		0.2302	0.2oo8	0.1995	0.2994	0.2401
		0.1699	0.2416	0.3645	0.2580	0.3233
		0.3280	0.2513	0.1681	0.2331	0.3192
		0.2677	0.2834	0.2629	0.2356	0.2511
		0.2417	0.3713	0.1957	0.2685	0.2707
	2	0.2331	0.2121	0.1946	0.2878	0.2296
		0.3031	0.1937	0.3034	0.3249	0.2293
		0.1603	0.2455	0.2153	0.2674	0.2323
		0.2534	0.1707	0.2475	0.2981	0.2355
		0.1809	0.2190	0.2414	0.2338	0.2658
		0.2566	0.2307	0.1390	0.2735	0.2177
		0.2394	0.2180	0.2040	0.1815	0.2556
		0.2821	0.3083	0.1746	0.2653	0.2763
7	1	0.1584	0.2287	0.1609	0.2283	0.3309
		0.2617	0.0913	0.1642	0.1965	0.1545
		0.2473	0.2371	0.1642	0.1965	0.1545
		0.1678	0.1014	0.1760	0.1776	0.1494
		0.1771	0.1710	0.0971	0.2211	0.2337
		0.2290	0.1976	0.1700	0.2043	0.2384
		0.3014	0.2138	0.2850	0.2483	0.2782
		0.1296	0.1195	0.2230	0.2000	0.1607
	2	0.2451	0 1487	0.1711	0.2274	0.2227
		0.2478	0.2304	0.2654	0.2248	0.1809
		0.2122	0.2540	0.2957	0.3445	0.2231
		0.2072	0.1593	0.2622	0.2309	0.2016
		0.2256	0.2161	0.2463	0.2936	0.2341
		0.1690	0.2225	0.2033	0.2850	0.2699
		0.2222	0.1425	0.2583	0.2555	0.2757
		0.1534	0.2214	0.2674	0.2727	0.2469

APPENDIX 8

Chlorophyll (A+B) contents (mg/gmFW) of lettuce under 5 different treatments after 5,6, and 7 weeks in storage

Week	Rep.	Treatment 0	A1	A2	K	A2K
5	1	0.5226	0.6900	0.4342	0.5120	0.4187
		0.4451	0.6708	0.7144	0.7576	1.0868
		0.4219	0.6241	0.6724	0.5577	0.5697
		0.5704	0.6819	0.6740	0.5719	0.8155
		0.4688	0.9745	0.5807	0.6357	0.5896
		0.7212	0.6840	0.8144	0.6494	0.6241
		0.5338	1.1470	0.5384	1.0212	0.8045
		0.4307	0.6271	0.6426	0.5525	0.8017
	2	1.0749	0.5951	0.7438	0.8216	0.8705
		0.6718	1.2781	0.7259	0.6412	0.6373
		0.7927	0.6805	0.4907	0.7963	0.7191
		0.5888	0.5545	0.7036	0.8518	0.9225
		1.0756	0.6020	0.7328	1.0131	1.2987
		0.7167	0.8049	0.8088	0.9053	0.8912
		0.6840	0.6469	0.7706	1.1689	0.7945
		0.8499	0.6618	0.7767	0.7879	0.9660
6	1	0.6393	0.9484	0.7318	0.7726	0.8100
		0.5364	0.6831	0.8937	0.8541	0.8127
		0.6547	0.7180	1.0060	1.0562	0.6003
		0.7612	0.7535	0.8653	1.o195	0.7385
		0.5559	0.8207	1.1056	0.8442	1.0123
		0.9480	0.8053	0.6870	0.8380	0.8941
		0.7996	0.8864	0.8777	0.8349	0.8600
		0.7340	1.1039	0.7325	0.8967	0.9165
	2	0.7366	0.7282	0.6709	0.8478	0.7375
		0.8924	0.6477	0.9537	1.0503	0.7995
		0.6788	0.8052	0.7388	0.9075	0.8721
		0.8342	0.6560	0.8750	0.9909	0.8052
		0.6377	0.8347	0.8157	0.8473	0.7990
		0.8123	0.7488	0.5142	0.9441	0.7789
		0.7452	0.7381	0.6901	0.6166	0.8379
		1.2319	0.8445	0.6427	0.8328	1.0000
7	1	0.5853	0.7987	0.5999	0.7308	1.0968
		0.9733	0.3870	0.5561	0.6689	0.5480
		0.7962	0.8507	0.5657	0.8475	0.5019
		0.5619	0.3945	0.6815	0.6081	0.4934
		0.6742	016945	0.3813	0.7194	0.7812
		0.8035	0.7071	0.5972	0.6939	0.8387
		1.0275	0.7321	0.8769	0.8907	0.9947
		0.4743	0.4252	0.7523	0.7553	0.5561
	2	0.7638	0.5468	0.5262	0.7527	0.7026
		0.8144	0.7417	0.8406	0.7099	0.6764
		017808	0.7189	0.9325	1.0384	0.7915
		0.7480	0.5431	0.7753	0.7857	0.8732
		0.8574	0.6876	0.7804	0.9892	0.7837
		0.6514	0.7120	0.5783	0.9314	0.8784
		0.6899	0.4644	0.7489	0.9555	0.8512
		0.5605	0.6886	0.8599	0.9066	0.8758

APPENDIX 9

Chlorophyll (A+B) contents (mg/gmOFW) of lettuce under 5 different treatments after 5,6, and 7 weeks in storage

Week	Rep.	Treatment				
		0	A1	A2	K	A2K
5	1	0.5213	0.6450	0.4367	0.4840	0.3859
		0.4030	0.6558	0.7798	0.6592	1.0428
		0.4262	0.6450	0.7230	0.6309	0.5415
		0.5594	0.6690	0.7144	0.5719	0.8184
		0.4957	0.8207	0.6094	0.6498	0.5986
		0.7289	0.6253	0.9485	0.6755	0.6215
		0.5867	1.0396	0.5461	1.0166	0.7574
		0.4317	0.6005	0.6389	0.5954	0.8573
	2	1.0407	0.5512	0.6743	0.7274	0.7467
		0.6142	1.2500	0.6726	0.5691	0.5676
		0.7286	0.6348	0.4488	0.7117	0.6362
		0.5212	0.5545	0.6271	0.7691	0.7928
		0.9929	0.5870	0.6564	0.9325	1.1732
		0.6652	0.7618	0.7352	0.8443	0.7798
		0.5978	0.6212	0.7313	1.0868	0.7113
		0.8163	0.6318	0.7259	0.6968	0.9131
6	1	0.5416	0.8322	0.5520	0.5662	0.7576
		0.4433	0.5708	0.7266	0.6482	0.6695
		0.5095	0.6069	0.7621	0.8638	0.5581
		0.6072	0.5263	0.6568	0.7653	0.6680
		0.4874	0.7156	0.9805	0.6830	0.8806
		0.8855	0.6739	0.5569	0.6789	0.8248
		0.7346	0.7150	0.7243	0.6710	0.6936
		0.6528	0.8722	0.5382	0.7714	0.7649
	2	0.6849	0.6024	0.5810	0.7556	0.6285
		0.7718	0.5670	0.8180	0.9186	0.6897
		0.5712	0.7195	0.6221	0.8048	0.7386
		0.7047	0.5522	0.7594	0.8824	0.6901
		0.5361	0.6833	0.7185	0.7649	0.7120
		0.7370	0.6292	0.4035	0.8542	0.6848
		0.6595	0.6129	0.5774	0.5165	0.6706
		0.7773	0.7298	0.5142	0.7343	0.8249
7	1	0.4300	0.6054	0.4848	0.6162	0.9759
		0.7628	0.2289	0.4964	0.5651	0.4881
		0.6560	0.6910	0.4681	0.7241	0.3959
		0.4585	0.2865	0.5356	0.5313	0.4207
		0.5142	0.5284	0.3092	0.6293	0.6717
		0.6444	0.5555	0.4749	0.5956	0.6961
		0.8207	0.6092	0.8160	0.6926	0.7843
		0.3783	0.3237	0.6311	0.5947	0.4858
	2	0.7350	0.4066	0.4784	0.6220	0.5921
		0.7014	0.6411	0.7384	0.6233	0.5024
		0.6163	0.6547	0.8053	0.9424	0.6285
		0.5909	0.4724	0.6965	0.6832	0.6363
		0.6803	0.6134	0.6989	0.8404	0.6711
		0.5266	0.6223	0.5335	0.7666	0.7453
		0.5895	0.4065	0.6732	0.7939	0.7472
		0.4294	0.6572	0.7703	0.7696	0.7116