CYTOKININ-LIKE AND GROWTH-PROMOTING ACTIVITY OF COCONUT WATER AS RELATED TO ITS EFFECT ON POLE SITAO POD DETERIORATION

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ABSTRACT

Cytokinin-like activity of coconut water was highest from 12-month old nuts and lowest from 8-month old nuts. Total growth-promoting activity showed a similar trend but was generally greater indicating the possible presence of other contributory compounds.

Coconut water from 10- and 12-month old nuts markedly retarded yellowing and chlorophyll loss in pole sitao pods. However, only the latter significantly improved the visual quality of the pods. All treatments did not affect weight loss and the textural properties of the pods. A slight stimulation of the respiratory activity was observed in all coconut water-treated pods.

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KEY WORDS: Pole sitao (*Vigna sesquipedalis* Fruw.). Pod deterioration. Coconut water. Stages of nut maturity. Cytokinin-like and growth-promoting activity.

INTRODUCTION

Pole sitao is a major legume vegetable in the Philippines. The pods, the economically useful part of the plant, are highly nutritious particularly the green ones (FNRC, 1964). However, their short shelf life and poor keeping quality greatly limit production and utilization. Pods quickly undergo senescent changes such as yellowing, shrivelling and loss of weight after harvest. These deteriorative changes can be delayed if the harvested crop is kept under low temperature conditions. This is the principle behind the use of the cold storage method in prolonging the consumer acceptability of commodities after harvest (Bautista and Data, 1976). The high cost of this method has been a deterrent

to its widespread adoption by majority of the farmers. Thus, the development of alternative low-cost storage techniques is important and necessary. It is for this reason that the potential use of coconut water in delaying rapid deterioration of pole sitao pods was explored.

Coconut water has been used to promote cell division in cultures of excised plant tissues. Its effect has been interchangeably related to that of kinetin in tissue cultures. It was also observed that coconut water treatment of detached leaves significantly inhibited senescent processes suggesting the presence of cytokinin-like substances (Shaw and Srivastava, 1964). Later, cytokinins such as zeatin, zeatin riboside and zeatin glucoside were identified in coconut water (van Staden and Drewes, 1975; van Staden, 1976). These naturally-occurring cytokinins were shown to be effective in extending the storage life of green vegetables (Fuller et al., 1977). However, the cytokinin content of coconut water as affected by nut maturity has not been ascertained. Likewise, the possible use of coconut water to prolong the shelf life of perishable crops like pole sitao pods has also not been tested to date. These are vital considerations in the assessment of the efficacy of coconut water in prolonging the marketability of harvested products.

This study investigated the proximate level of the cytokinin-like and total growth-promoting activity of coconut water from nuts at different stages of maturity and its possible relation to the effect of coconut water on the storage behavior of pole sitao pods.

MATERIALS AND METHODS

Determination of the Cytokinin-like Activity of Coconut Water

Water from 'Laguna Tall' coconut was obtained from the Coconut Breeding Project of the Department of Horticulture, University of the Philippines at Los Baños, College, Laguna. Protacio (Coconut Breeding Project, Department of Horticulture, UPLB, College, Laguna - Pers. comm.) claimed that a bearing, tall variety palm will initiate inflorescence approximately at monthly intervals. Therefore even without tagging, the ages of fruit branches below an inflorescence can be roughly determined from the date of their emergence. This was used as basis for determining the ages of nuts used in this study. Four stages of nut maturity were used: 6, 8, 10 and 12 months. Thirty nuts were utilized per stage per trial. Three trials with three replications each were done.

The procedure of del Rosario (1980) was essentially followed in the extraction, fractionation and partial purification of coconut water using an original volume of 3 liters. Cytokinin-like activity was determined using the cucumber cotyledon chlorophyll formation bioassay (Fletcher et al., 1982). Extracts equivalent to 500 ml coconut water were used. This was prepared by diluting 1.67 ml of the extract obtained from the original 3 liters of

coconut water to 10 ml with distilled water. A 5-ml extract solution was dispensed in a 5-cm diameter petri dish. The data presented represent the pooled activity of the ethyl acetate fraction and that of the eluate of coconut water.

Determination of the chlorophyll content of the cucumber cotyledons was done using the dimethyl sulfo-

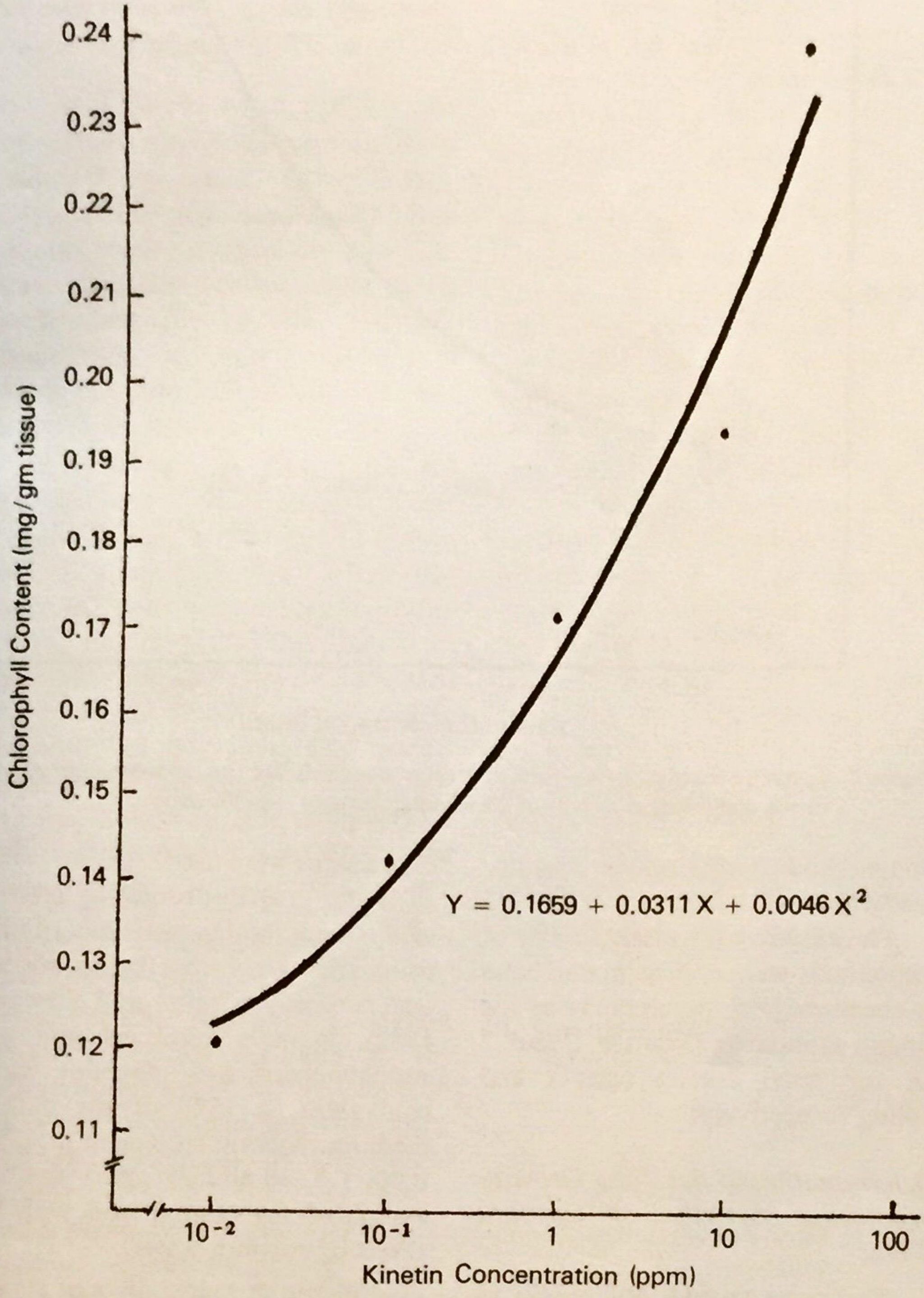

Figure 1. Cucumber cotyledon bioassay of kinetin standards for the determination of the kinetin equivalent of activity of the ethyl acetate extract of coconut water.

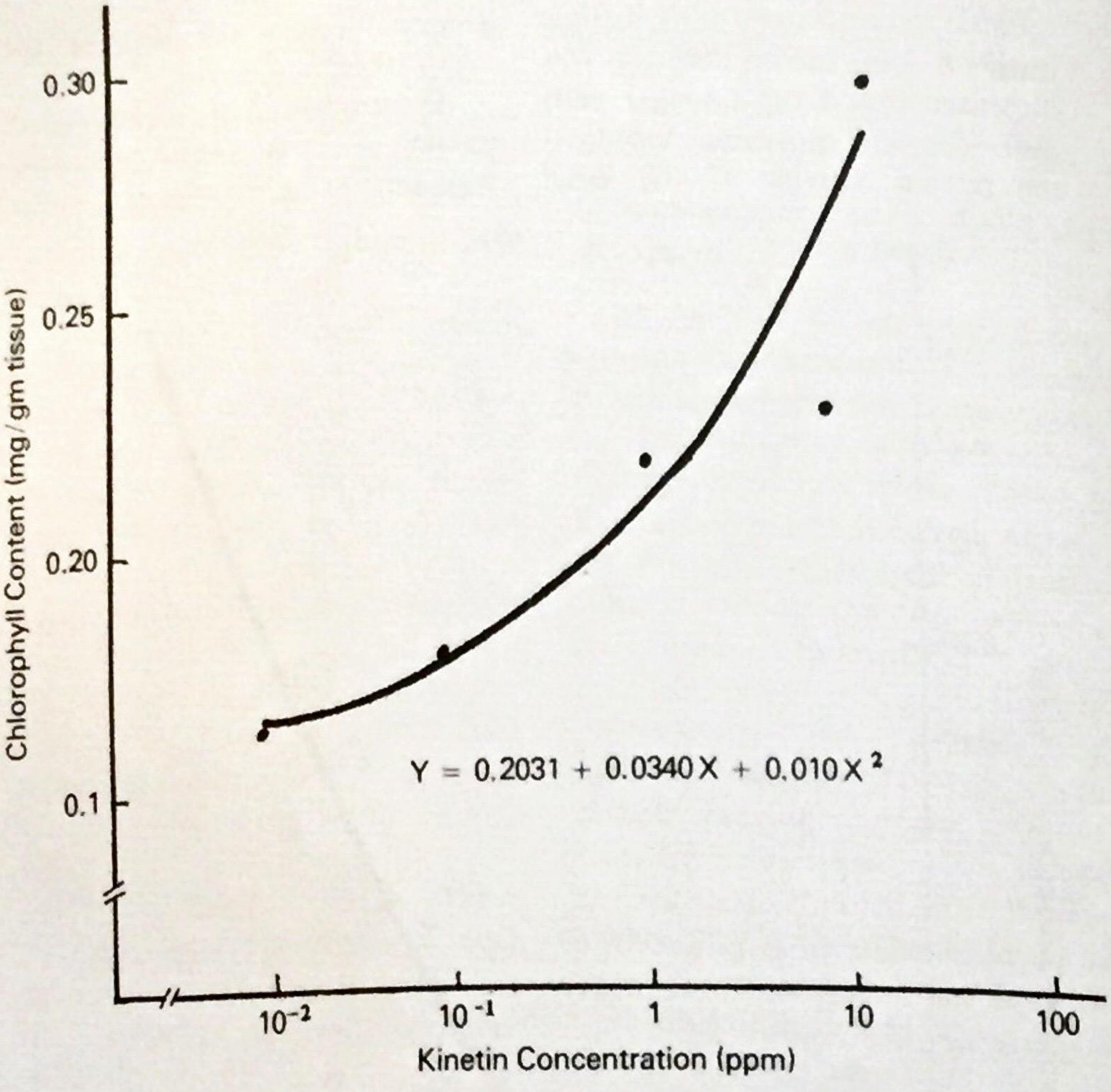

Figure 2. Cucumber cotyledon bioassay of kinetin standards for the determination of the kinetin equivalent of activity of the eluate fraction of coconut water.

xide method (Hiscox and Israelstam, 1980).

The kinetin equivalent activity of the extracts were approximated based on the average response curve of the kinetin standards (Figures 1 and 2 for the ethyl acetate extract and eluate, respectively).

Determination of the Total Growth-Promoting Activity of Coconut Water

The same variety and stages of nut maturity as that in the preceding experiment were used.

Total growth-promoting activity of coconut water was determined using the carrot root callus formation bioassay (Caplin and Steward, 1952). White's basal medium was supplemented with coconut water equivalent to 15% of the culture medium. Kinetin treatment (0, 0.01, 0.10, 1.0, 10 and 25 ppm) was also evaluated for comparison with the effect of coconut water.

Phloem or xylem discs of similar sizes were used with only one disc

inoculated per test tube. Ten replications were made. After one month, the fresh weight of each tissue was determined.

Storage Behavior of Pole Sitao Pods Treated with Coconut Water

Carefully harvested, healthy pole sitao (UPLBS$_1$) pods were obtained from Tuntungin, Los Baños, Laguna. The pods were sorted based on uniformity in maturity and size. Each treatment consisted of 500-g pods used for physical attribute evaluation, 30 pods for chemical analysis, and 10 pods for gas analysis.

The pods were dipped for 1.5 minutes in 4 liters of coconut water taken from 6-, 8-, 10- and 12-month old 'Laguna Tall' nuts. After dipping, the pods were placed in plastic trays, air dried, and then stored at ambient room conditions (temperature of 22-32.5°C and relative humidity of 64-95%). Tap water-treated and untreated control pods were also included for comparison. All treatments were replicated three times.

The following parameters were observed daily:

1. Percent cumulative weight loss - obtained by getting the difference in weight before and after each day of storage and expressing this as percentage of the initial weight;

2. Firmness - the equivalent pressure exerted in cutting the pod using a modified Effigi pressure tester and expressed in kilograms;

3. Yellowing - determined by counting the number of pods showing more than 50% yellow coloration and expressing this in percentage;

4. Percent chlorophyll loss - measured by getting the difference between the initial chlorophyll content and the chlorophyll content after each storage period using the acetone method of chlorophyll analysis (Yoshida et al., 1976). The subsequent chlorophyll readings and computation were done following the same procedure given in the dimethyl sulfoxide method of chlorophyll analysis;

5. Visual quality - the general appearance of the pods after each day of storage and assessed using the following visual quality index: 9 = excellent; 7 = good, defects slight; 5 = fair, defects moderate; 3 = limited marketability;

6. Respiration rate - measured by taking the CO_2 readings of the gas samples obtained one hour after 10 sample pods were allowed to respire inside a sealed cylinder and the rate in mg CO_2/kg/hr was calculated using the following formula:

$$\text{Respiration rate (mg } CO_2\text{/kg/hr)} = \frac{P \times A \text{ of sample}}{P \times A \text{ of standard}} \times C_1 - C_2 \times 10^{-2} \times \frac{MC}{T} \times \frac{V}{W}$$

where: P = peak height in mm
A = attenuation
C_1 = concentration of the CO_2 standard in %

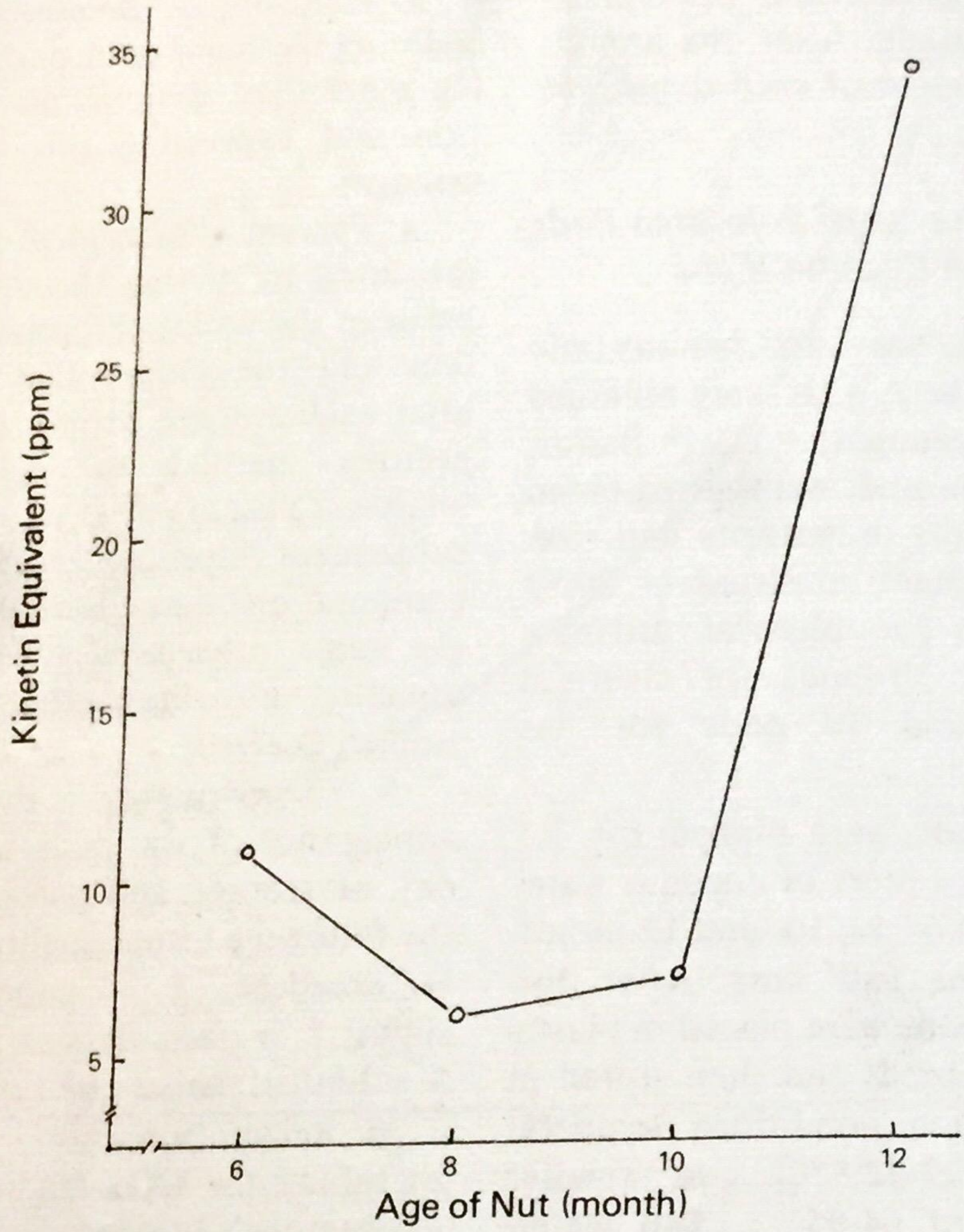

Figure 3. Total cytokinin-like activity (in kinetin equivalent) of 500 ml coconut water from nuts at different stages of maturity based on cucumber cotyledon chlorophyll formation bioassay.

Table 1. Cytokinin-like activity of the ethyl acetate extract and eluate fraction of coconut water.[1]

Age of Nut (month)	Cytokinin Activity (ppm kinetin)	
	Ethyl Acetate Extract[2]	Eluate Fraction[2]
6	0.90	10.00
8	0.07	6.00
10	0.14	7.00
12	9.00	25.00

[1] Activity obtained from extracts was equivalent to 500 ml coconut water.

[2] Determination of the cytokinin-like activity of the ethyl acetate extract and eluate fraction was done separately.

C_2 = concentration of atmospheric CO_2 in %
MC = molecular weight of CO_2
V = volume of free space in ml
W = weight of pods in kg

Data analysis and comparison of treatment means were done using the completely randomized design (CRD) and the Duncan's Multiple Range Test (DMRT), respectively.

RESULTS AND DISCUSSION

Cytokinin-like Activity of Coconut Water

Total cytokinin-like activity of coconut water varied with nut maturity (Fig. 3). Highest cytokinin-like activity (34 ppm kinetin) was obtained in coconut water from 12-month old nuts while the lowest (6.07 ppm kinetin equivalent) was obtained in coconut water from 8-month old nuts. Coconut water from 6- and 10-month old nuts had cytokinin-like activity equivalent to 10.9 and 7.14 ppm kinetin, respectively. These values were the sum total of the cytokinin-like activity in kinetin equivalent of the ethyl acetate extract and eluate of coconut water. Both the extract and the eluate showed a similar activity trend at the different nut age treatments although the latter exhibited a greater degree of activity (Table 1).

The results suggest that the high cytokinin-like activity of coconut water from 12-month old nuts may be a consequence of the increased level of cytokinin compounds which are among the growth substances which accumulate in the liquid endosperm prior to embryo growth. It has been established that the liquid endosperm provides the nutritive fluid for the immature embryo as well as that for the embryo's subsequent rapid development which commences at the twelfth month of nut development (Steward and Caplin, 1952). On the other hand, the higher activity in coconut water from 6-month old nuts coincided with the onset of active cell division and the cellularization of the liquid endosperm which heralds solid endosperm formation (Cedo, 1983). It has been demonstrated that cytokinin activity is high in developing fruits and seeds where active cell division predominates (Gazit and Blumenfield, 1970; Oritani and Yoshida, 1971; Sandstedt, 1971). It is therefore probable that the exhaustive utilization of the growth substances such as the cytokinins as a result of solid endosperm formation may have decreased the cytokinin-like activity of the coconut water from 8- and 10-month old nuts.

Total Growth-Promoting Activity of Coconut Water

Growth of carrot root tissues was markedly enhanced by coconut water. Callus weight was significantly higher in all treatments with coconut water than in the control

Table 2. Average fresh weight of carrot root tissues cultured in White's basal medium supplemented with 15% coconut water from nuts at different stages of maturity or with different concentrations of kinetin.

Treatment	Average Fresh Weight[1] (mg)
Control (White's basal medium alone)	159ab
Coconut water from:	
6-month old nuts	544e
8-month old nuts	440d
10-month old nuts	533e
12-month old nuts	634f
Kinetin concentration:	
0.01 ppm	262bc
0.10 ppm	329cd
1.00 ppm	386cd
10.00 ppm	444d
25.00 ppm	126a

[1] Means of three trials with 10 replicates per trial. Means within the column followed by a common letter are not significantly different at 5% level, DMRT.

(Table 2). This result further demonstrates the promotive effect of coconut water on callus proliferation and thus confirms the early findings of Caplin and Steward (1948), Nickell (1950), Morel and Westmore (1951) and del Rosario (1980).

The total growth-promoting activity of coconut water varied with the stage of nut development (Table 2). Coconut water from 6-month old nuts induced callus growth comparable to that with coconut water from 10-month old nuts. A significantly lesser callus growth was obtained when coconut water from 8-month old nuts was used. Growth-promoting activity was significantly highest with coconut water from 12-month old nuts.

Growth promotion by coconut water from nuts at different stages of maturity follows a trend similar to their cytokinin activity (Table 1, Fig. 3). However, the degree of growth promotion by coconut water cannot be solely attributed to its cytokinin content. Other substances such as phenylalanine (van Staden and Drewes, 1974), hexitols (Pollard et al., 1961), sugars (Nathanael, 1952; del Rosario, 1978), auxins (Paris and Duhamet, 1954 as cited by Tulecke et al., 1965), and gibberellins (Radley and Dear, 1958) have been isolated from coconut water and all enhanced cell division. This may account for the greater growth promotion in carrot root tissues treated with coconut water irrespective of the stage of nut

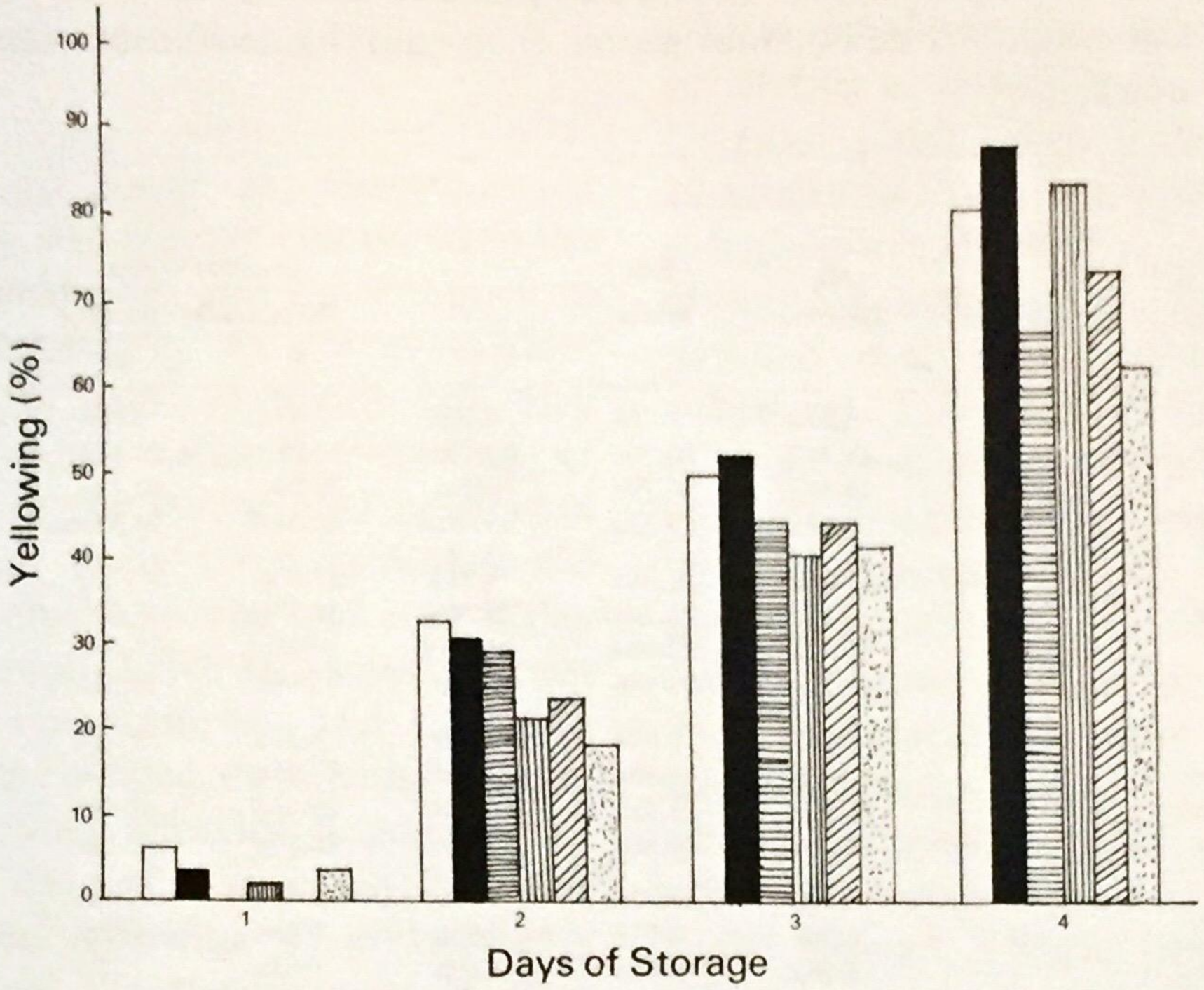

Figure 4. Percent yellowing of pole sitao pods given no treatment (□); pods dipped in tap water (■); and pods dipped in coconut water from 6-month old (▤); 8-month old (▥); 10-month old (▨) and 12-month old (▩) nuts.

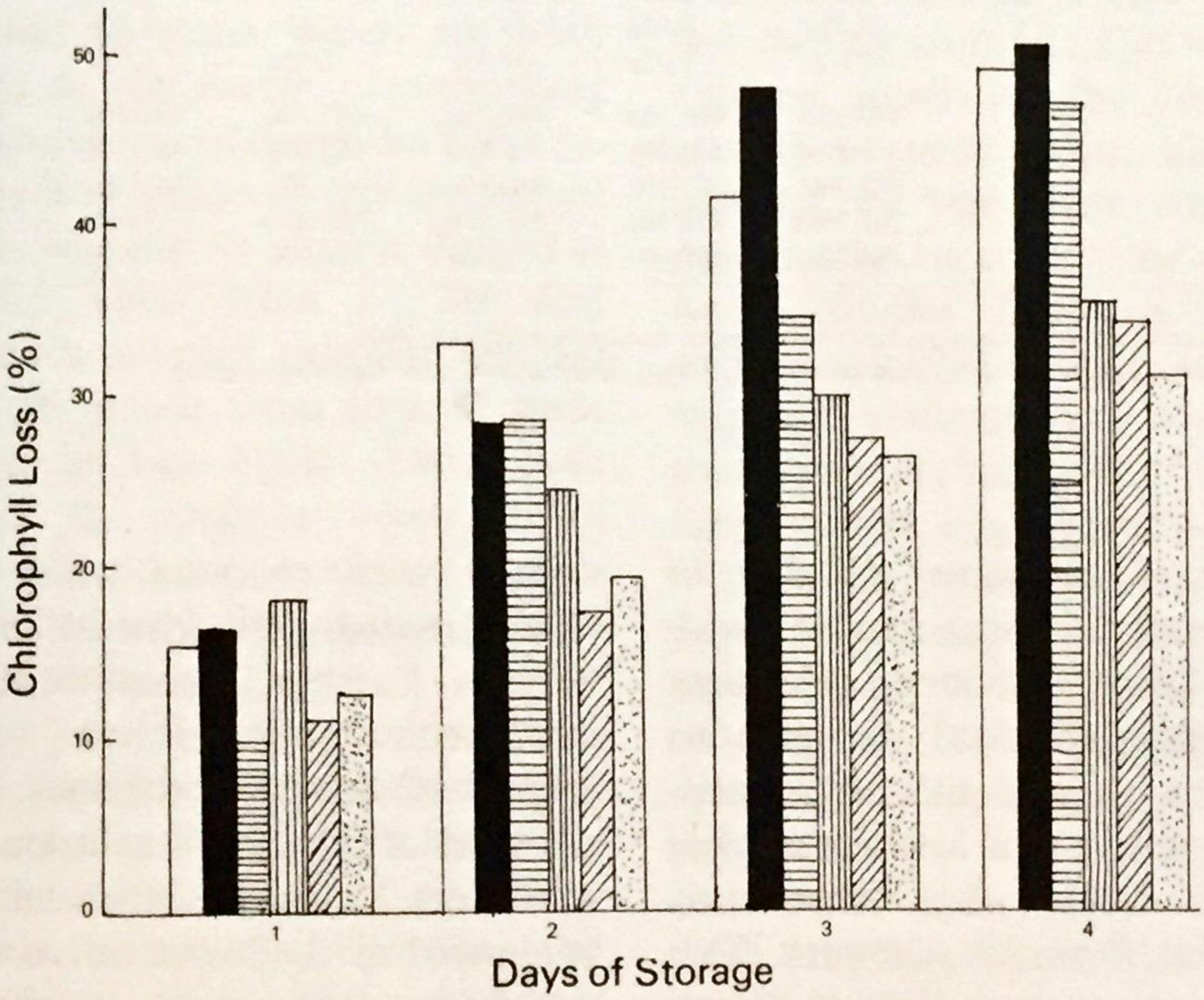

Figure 5. Percent chlorophyll loss in pole sitao pods given no treatment (□); in pods dipped in tap water (■); and in pods dipped in coconut water from 6-month old (▤); 8-month old (▥); 10-month old (▨) and 12-month old (▩) nuts.

Table 3. Postharvest changes in undipped pole sitao pods, in pods dipped in tap water, or in coconut water from nuts at different stages of maturity.[1]

Postharvest Changes	Days of Storage	Treatments: No Dipping	Tap Water	Coconut Water, Nut Maturity (month) 6	8	10	12
Yellowing (%)	1	6.17a	3.70a	0.00a	2.47a	0.00a	3.70a
	2	31.74a	30.56a	29.17a	20.78b	23.61b	18.95b
	3	48.41a	50.79a	44.44a	39.80a	44.44a	40.87a
	4	78.15ab	86.82a	65.69bc	80.84ab	70.37abc	61.56c
Chlorophyll Loss (%)	1	15.20a	16.38a	9.94a	17.93a	11.11a	12.48a
	2	33.12a	28.26ab	28.46ab	24.37abc	17.54c	19.49bc
	3	41.71ab	47.95a	34.50bc	29.63bc	27.68c	26.12c
	4	48.93a	50.58a	47.17a	35.09b	34.41b	31.19b
Cumulative Weight Loss (%)	1	9.44a	8.87a	9.75a	9.27a	9.10a	8.89a
	2	16.31a	16.46a	16.80a	17.20a	17.30a	16.65a
	3	25.30a	25.21a	25.52a	25.46a	25.59a	24.79a
	4	36.05a	39.86a	36.66a	36.85a	37.24a	36.20a
Firmness (kg)	0	0.92a	0.92a	0.92a	0.92a	0.92a	0.92a
	1	1.00a	0.97a	0.98a	0.95a	0.99a	0.98a
	2	1.08a	1.02a	1.00a	1.03a	1.00a	1.02a
	3	1.02a	1.03a	1.09a	1.03a	1.12a	1.02a
	4	1.09a	1.08a	1.11a	1.06a	1.09a	1.06a
Visual Quality[2]	0	8.70a	8.70a	8.70a	8.70a	8.70a	8.70a
	1	6.50a	6.93ab	7.17b	7.30b	7.40b	7.20b
	2	5.27a	5.80b	5.67ab	6.00b	6.00b	6.07b
	3	4.73a	4.93a	4.17a	4.50a	4.60a	5.00a
	4	2.00a	1.97a	1.93a	2.00a	1.93a	2.43b
Respiration Rate (mg CO_2/kg/hr)	0	437.30a	416.10a	460.20a	469.50a	424.50a	434.60a
	1	349.80a	346.80a	350.60a	362.20a	326.30a	366.50a
	2	301.30a	322.20a	318.80a	308.20a	341.10a	322.90a
	3	291.30ab	255.50a	343.50b	293.00ab	336.00b	322.80b
	4	168.50a	197.90a	213.60a	194.70a	294.50b	226.80ab

[1] Means within a row followed by a common letter are not significantly different at 5% level, DMRT.
[2] Visual quality index: 9 = excellent; 7 = good, defects slight; 5 = fair, defects moderate; 3 = limited marketability.

maturity as compared to that in carrot root tissues treated with kinetin (Table 2). Kinetin treatment at 10 ppm produced the greatest callus growth and this was statistically similar to the lowest response obtained with coconut water treatment using 8-month old nuts. This finding concurs with that of Kuraishi and Okumura (1961) that growth of leaf discs was greater when treated with coconut water than when treated with kinetin or gibberellin. Further increase in kinetin concentration inhibited callus growth of carrot root tissues. Kinetin level of 25 ppm was apparently inhibitory to callus formation as evidenced by the lower callus weight which was comparable to the control.

Storage Behavior of Pole Sitao Pods Treated with Coconut Water

Coconut water-treated pods exhibited lower percent yellowing throughout storage compared to the undipped pods and those dipped in tap water (Fig. 4). Coconut water from 8- and 10-month old nuts significantly reduced yellowing of pods on the second day of storage whereas water from 12-month old nuts, on the second and fourth day of storage (Table 3). However, only the water from 10- and 12-month old nuts elicited significantly higher chlorophyll retention in pods after 3 and 4 days of storage (Fig.5, Table 3). The yellowing of pods mainly accounts for the changes in their visual quality. This assumption is based on the insignificant effect of coconut water treatment on weight loss and firmness which are both related to the extent of shrivelling and tenderness of the pods (Table 3). After 1 and 2 days of storage, visual quality was higher in pods dipped in coconut water from 8-, 10- and 12-month old nuts although this did not vary much from that of pods dipped in tap water. Only pods dipped in coconut water from 12-month old nuts exhibited significantly better visual quality after 4 days of storage.

The results presented in Figures 4 and 5 indicate that coconut water from 12-month old nuts markedly retarded deterioration of pole sitao pods in terms of yellowing and chlorophyll loss, and ultimately improved the visual quality of the pods. This might manifest its greater cytokinin-like and growth-promoting activity (Fig. 3, Table 2). Coconut water taken from 6- and 10-month old nuts had comparable cytokinin-like and growth-promoting activity but the latter retarded pod deterioration more effectively. It is most probable that the high level of growth substances in coconut water from 6-month old nuts is essential mainly for cell division which is predominant at this stage of nut development.

The characteristic effect of cytokinins on the depression of respiration (Goldthwaite, 1974) was not observed in pods dipped in coconut water. Instead, a slight stimulatory effect was noted especially when coconut water from 10- and 12-month old nuts was used (Table 3). This was noted at 2 days until the last day of storage. This effect of coconut water on the respiratory activity of pods might have been induced by the sugar component specifically sucrose in coconut water . Studies have shown that sucrose at 0.02M strongly stimulates respiratory metabolism and raises the respiratory quotient of detached leaves which consequently resulted in inhibition of protein and chlorophyll breakdown (Goldthwaite, 1974). Analysis of sucrose content of 'Laguna Tall' coconut water revealed that sucrose can be isolated only when the nut possesses solid endosperm and that it increased from 0.13% in 7-month old nuts to 0.96% in 12-month old nuts (Vista, 1915; Nathanael, 1952).

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