SOME FACTORS INFLUENCING ETHYLENE INDUCTION OF BITTERNESS IN STORED CARROT ROOTS (DAUCUS CAROTA L.)

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THESIS

SOME FACTORS INFLUENCING ETHYLENE INDUCTION OF BITTER-NESS IN STORED CARROT ROOTS (DAUCUS CAROTA L.)

By

HAROLD WILLIAM SCHMALFELD

AN ABSTRACT

Submitted to the College of Agriculture, Michigan State University
of Agriculture and Applied Science in partial fulfillment of
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MASTER OF SCIENCE

Department of Horticulture

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HAROLD WILLIAM SCHMALFELD ABSTRACT

The effect of several factors on the formation of the bitter substance 3-methyl-6-methoxy-8-hydroxy-3, 4-dihydroisocoumarin in carrot roots in the presence of ethylene gas are examined. Varietal differences are found to significantly affect the quantity of the bitter principle formed.

In field trials carrot roots stored in cold storages with apples became bitter. Carrots placed in controlled storages, common storages, and an outdoor pit all in the absence of apples, remained free of bitterness.

Ethylene concentrations of 50, 100, and 200 parts per million of air were all effective in inducing the formation of the bitter principle. Exposure of the roots to 100 p.p.m. for as little as two days was effective in initiating the mechanism of bitter principle synthesis.

Carrots which had undergone four days of anaerobic conditions due to a pre-harvest flooding did not become bitter, but developed a musty, fermented taste and smell.

The physiological maturity of the carrot root at harvest time is proposed to be a factor in the disposition of the vegetable to bitterness formation.

The intensity of the fluorescence of the bitter compound in the fresh root under ultra-violet radiation is used as an indication of the quantity of the compound present. Fluorescence evaluations are correlated (r = .82) with spectrophotometric analysis of bitter-root extracts.

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INTRODUCTION AND REVIEW OF LITERATURE

The formation of a bitter compound in stored carrots, a problem of considerable economic importance in areas which produce carrots for the processing industry, has been under close scrutiny in Michigan and New York for the past several years.

The influence of preharvest conditions on the formation of the bitter substance was one of the first areas to be investigated. Bessey (4) and Atkins (2) were not successful in correlating any condition during the growing period with the bitterness that later developed in the roots. Bessey (4) proposed that the volatile emanations from apples might be the cause of the undesirable effect. This area was successfully investigated by Ells (12) who produced bitterness in carrots first by exposing them to gases emanating from apples, and later by exposing them to ethylene at an approximate concentration of 100 p. p. m. in the storage atmosphere.

The Effect of Ethylene on Plants and Plant Products

It has long been recognized that volatile hydrocarbons can adversely affect plants. Crocker and Knight (8) cite the observation by Girardin (17) in 1864 that illuminating gas is often injurious or fatal to plants. Other early investigators also cited by Crocker and Knight (28, 38, 40) described symptoms of plants injured by illuminating gas. Effects described are loss of turgor, yellowing of leaves, defoliation, and browning and death of the cambium. Crocker and Knight (8) discovered that traces of illuminating gas in the atmosphere would prevent the opening of carnation buds. They identified the most active component of the gas as ethylene.

Subsequently other aberrations in plant materials induced by ethylene have been observed. Wallace (42) reported the production of intumescences on Transparent apple cuttings when treated with ethylene in concentrations ranging from three parts of ethylene to one of oxygen to one part of ethylene in 100, 000, 000 of air. Exposure to 1. 5 percent of ethylene in air for seven and one-half minutes gave as definite a response as continuous exposure for two weeks. Zimmerman, Hitchcock and Crocker (45) demonstrated that epinasty, foliar abscission and interference of shoot elongation in roses developed in the presence of ethylene. Lumsden, Wright and Whiteman (31) describe injury by ethylene to cut flowers in cold storage rooms. Ferguson (14) attributed "sleepiness" in carnations to ethylene in Pintsch gas used in railroad cars. Extreme injury to unopened buds of Cattleya species in the presence of ethylene in concentrations as low as 0. 002 p. p. m. is reported by Davidson (10). Curtis and Rodney (9) found that ethylene in the atmosphere injured apple and pear nursery stocks. Lieberman and Spurr (29) relate the yellowing of harvested broccoli to endogenously produced ethylene, and Rood (33) showed brown spot of lettuce to be related to ethylene in the storage atmosphere.

The profound effects of ethylene are not limited to those considered to be entirely undesirable--some have been shown to be useful or potentially so. Mack (32) observed that ethylene hastened the blanching of celery although it also shortened its post-harvest life. Heinze and Craft (27) demonstrated the efficacy of ripening mature-green tomatoes with ethylene.

Working with rhubarb for early winter forcing, Bjornseth (5) broke dormancy by applying ethylene to crowns which had not been subjected to a cold period adequate to produce the effect. Vacha and Harvey (40) also report the breaking of dormancy in potato tubers, gladiolus corms and the seeds of several selected plants with ethylene.

Finch (15) reported that the application of ethylene to tented pecan trees advanced the harvest date by thirty days and was also effective in loosening the shucks from pecan fruits. Sorber and Kimball (37) report a similar result on the fruit of the Persian walnut.

Chace and Denny (6) in 1924 described the use of ethylene in promoting the development of color in citrus fruits. In 1927 Chace and Church (7) reported that coloring was the only consequence from an exposure to ethylene and that the composition of the fruit remained unchanged. Harvey (24) using a higher ethylene concentration described a true ripening effect on fruits and vegetables in addition to superficial color changes.

In more recent work Grierson and Newhall (22) found that large concentrations of ethylene were responsible for increased stem-end rot in citrus, and advocated the use of minimum atmospheric concentrations of the gas for the degreening process. In this connection Loucks and Hopkins (30) earlier advised the removal of metabolic by-products, such as ethylene, to reduce decay of citrus fruits.

The ripening and/or degreening of bananas and other tropical fruits with ethylene is described by Harvey (25). Degreening of otherwise mature pears and cranberries with ethylene is discussed by Hansen (23) and by Fudge (16), respectively.

Several workers have concerned themselves with the influence of ethylene on the composition of plant parts. Harvey (24) describes increases of sugars and a decrease of starch in bananas resulting from ethylene treatment. Working with celery, Babb (3) reported no effect on the vitamin B content of that vegetable, while Mack (32) reports no increase in the leaf catalase content of treated celery.

Thornton (39) found that the ascorbic acid content of ethylene-treated bananas was not changed from that of normally ripened fruits. Heid (26) found that less pectin could be extracted from ethylene-treated citrus than from that which was not so treated, and found it necessary to modify extraction methods to obtain the maximum amount of pectin from the treated fruit.

Working with bitter carrots, Sondheimer (36) successfully isolated and identified the bitter principle as 3-methyl-6-methoxy-8-hydroxy-3, 4-dihydroisocoumarin. This compound appears in carrot roots stored in the presence of ethylene gas or the emanations from ripening fruit as proposed by Bessey (4), and first demonstrated by Ells (12).

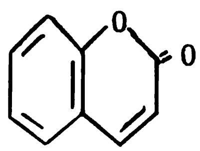
Fluorescence in Roots

Fluorescence is the emission of light by matter under the influence of an exciting agent. According to DeMent (11) the light emitted in practically every instance lies in the visible range of the spectrum and in nearly every case the radiation required to excite the fluorescence is shorter in wave-length than the fluorescent light.

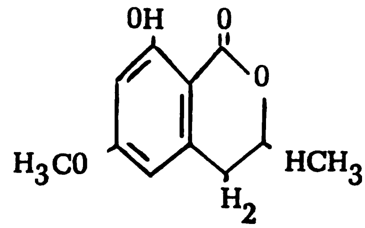
Goodwin and Kavanagh (18) found fluorescent substances in roots of 135 plant species representing 126 genera of 69 families. They identified the fluorescent substance in the roots of *Avena sativa* as scopoletin, a derivative of coumarin (6-methoxy-7-hydroxycoumarin). Later these investigators (19, 20) reported the fluorescence of many coumarin derivatives as a function of pH. The use of ultra-violet absorption spectra as a means of identifying several coumarin derivatives is discussed by Goodwin and Pollock (21). Umbelliferone (7-hydroxycoumarin), included in their investigation, had been isolated from the roots of several of the Umbelliferae. However, no mention of either isocoumarin or any of its derivatives was included in these reports.

The ultra-violet absorption spectra of 3-methyl-6-methoxy-8-hydroxy-3, 4-dihydroisocoumarin was utilized as a measure of its relative concentration in carrot extracts by Sondheimer et al. (34) concurrent with investigations into the molecular structure of the substance. The fluorescence of the compound under ultra-violet radiation was used as a rapid indication of its concentration in fresh carrot roots by Bessey (4) and Ells (12), and correlated with bitterness by taste and spectrophotometric evaluations.

The structures of coumarin and 3-methyl-6-methoxy-8-hydroxy-3, 4-dihydroisocoumarin (36) are shown below.



Coumarin



3-methyl-6-methoxy-8-hydroxy-3, 4-dihydroisocoumarin

In the interest of brevity 3-methyl-6-methoxy-8-hydroxy-3, 4-dihydroisocoumarin will be designated "isocoumarin" when subsequently mentioned in this report.

STATEMENT OF THE PROBLEM

The prevention of the development of bitterness in stored carrot roots is a problem of some economic importance. It has been determined that ethylene in the storage atmosphere will result in the formation of the bitter principle. The relative quantity of the bitter principle (3-methyl-6-methoxy-8-hydroxy-3, 4-dihydroisocoumarin) has been measured spectrophotometrically and by fluorescence under ultra-violet radiation.

This investigation was undertaken to more clearly define the relation of duration of exposure to ethylene, ethylene concentration, variety of carrot, and kind of storage to the development of the bitter principle. The use of fluorescence techniques in the rapid determination of bitter principle concentration in fresh carrot roots as advanced by Ells (12) is further explored.

GENERAL METHODS

Storage Apparatus

Carrot samples consisting of six or eight roots in five-pound mesh onion bags were placed in cold storage in thirty-gallon air-tight drums with removable heads. Each drum was provided with an inlet and outlet tube. The inlet tube was connected to a source of compressed air and the flow regulated by means of a needle valve at approximately 79 ml. per minute, or enough to theoretically completely change the air every twenty-four hours. The outlet tube was attached to a rubber tube with its free end submerged in a water seal.

Definite volumes of ethylene were added to the test drums by filling a tygon tubing of known volume with ethylene and inserting the tubing between the source of air supply and the drum. Tubing capacities of 5.7, 11.4 and 22.8 ml. provided initial ethylene concentrations of approximately 50, 100 and 200 p.p.m. Since air movement through the drums was continuously reducing the ethylene concentration, ethylene was administered at 48 hour intervals.

Analytical Procedures

Sample bags of carrots were removed from each drum at random and the fluorescence of the roots observed in a dark room using an ultra-violet lamp with a 2537Å filter. Ratings of fluoresence were recorded using a zero to four scale. Zero indicated that no fluorescence was detected and four that the carrots were highly fluorescent.

The color of the fluorescence associated with bitter carrots is brilliant yellow-green. As reported by Bessey (4) it occurs within the carrot as innumerable miniscule specks. These are arranged in concentric circles when the root is examined in cross-section, Figure 1. When viewed in longitudinal section the specks appear in lines which parallel the root, as shown in Figure 2. All internal fluorescence occurs exclusively in the phloem.

Externally the fluorescence occurs localized as blotches and appears on any part of the surface as indicated in Figure 3. These areas tend to become larger and to coalesce as time in storage after exposure to ethylene increases, and in extreme instances the entire surface of the carrot may fluoresce. Two examples of highly fluorescent carrots are shown in Figure 4.

External fluorescence ratings were made on unwashed carrots. After determining the extent of the external fluorescence the carrots were washed, the tops were removed approximately one-half inch below the crown, and the remainder of the root cut in cross-section about midway of its length. Internal fluorescence ratings were then based on the amount of fluorescence exhibited by both ends of the central portion of the roots. Rating values of individual carrots were averaged for lots consisting of six or eight roots.

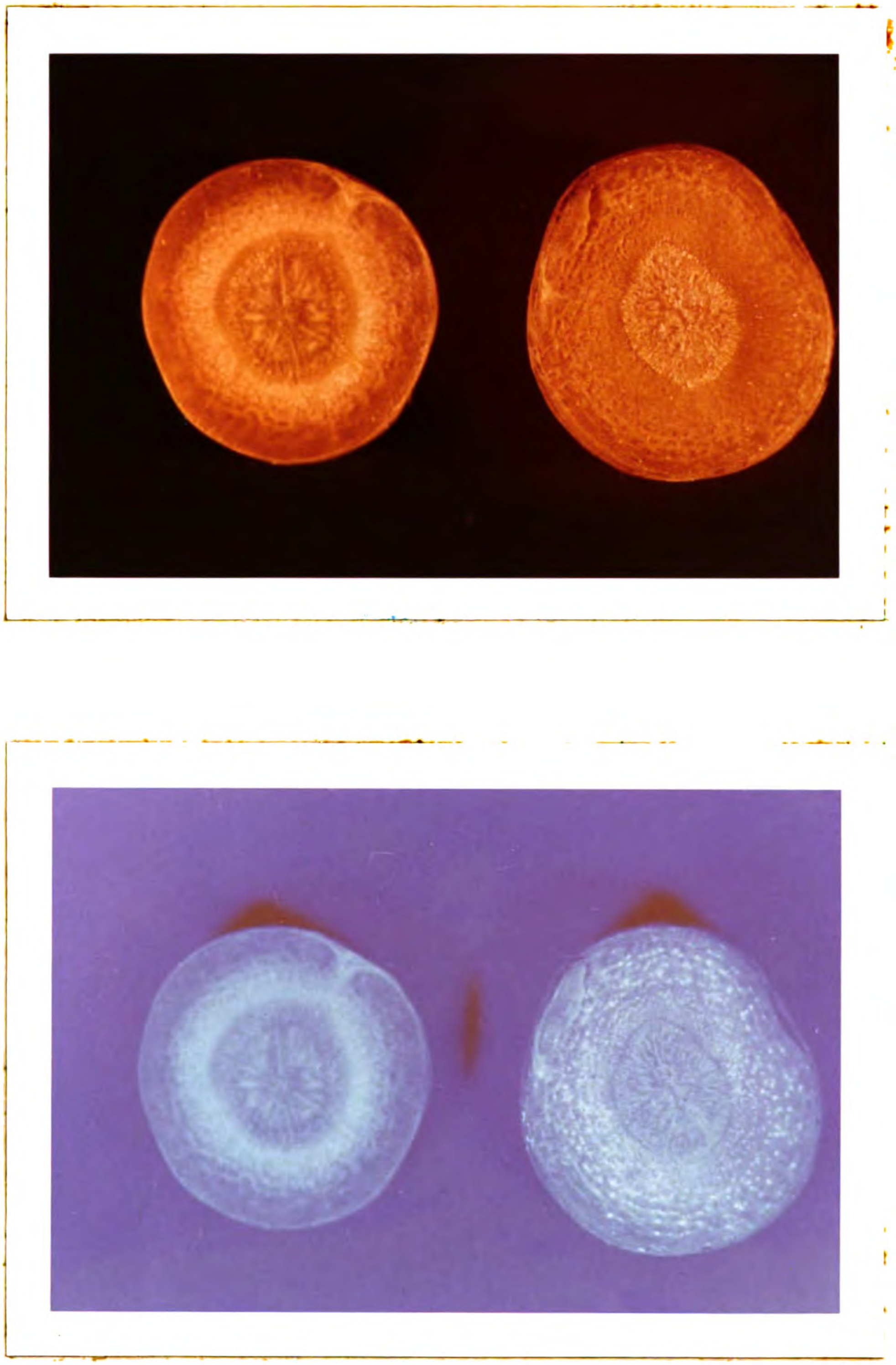


Figure 1. Above, cross-section of non-bitter and bitter carrot under white light. Below, same sections under 2537Å ultraviolet light.

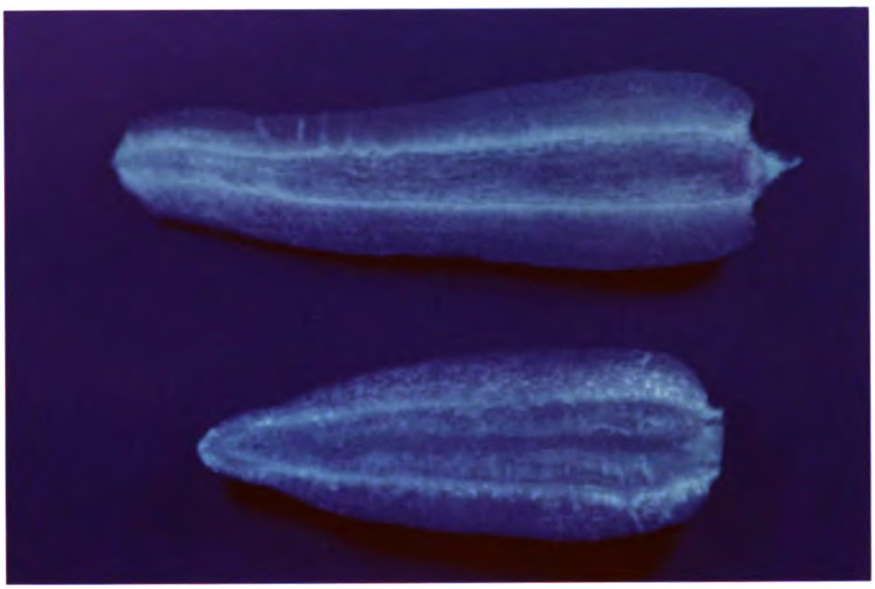


Figure 2. Above, longitudinal section of non-bitter and bitter carrot in white light. Below, same sections under 2537Å wavelength ultra-violet light.

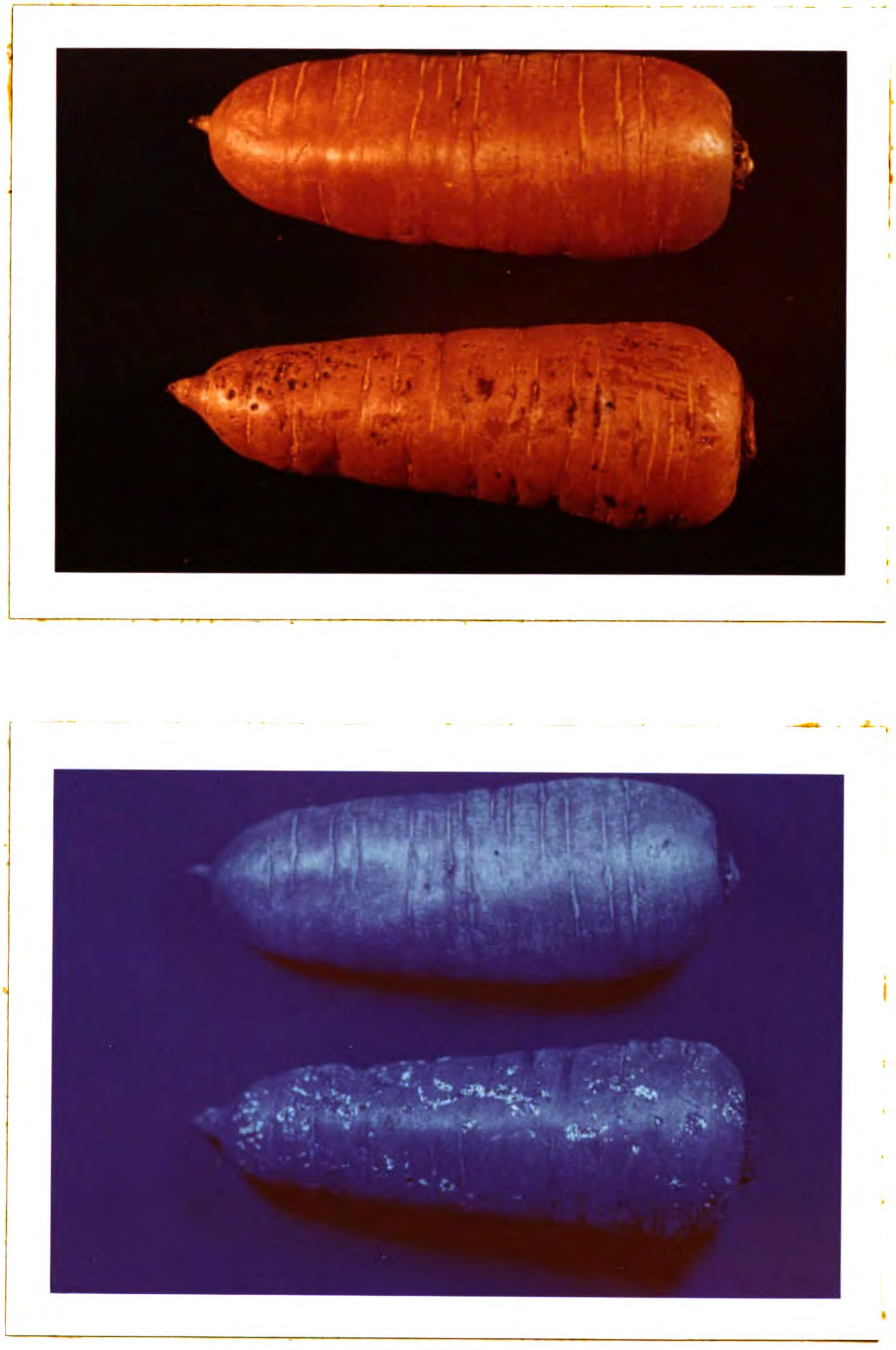


Figure 3. Above, non-bitter and bitter carrot in white light. Below, same carrots under 2537Å wavelength ultra-violet light.

Figure 4. Two extremely fluorescent carrots under 2537Å wavelength ultra-violet light.

Each carrot was then tasted and rated on a zero to four scale, ranging from zero (non-bitter) to four (extremely bitter). Ratings were again averaged for the lots.

Spectrophotometric analyses were made using longitudinally cut quarters of each carrot in the sample. The sample quarters were homogenized in a Waring blendor with a quantity of water equal to their weight. Five-gram aliquots of the homogenate were weighed into 50 ml., ground-glass stoppered Erlenmeyer flasks. Forty ml. of "Skelly-solve B"[1/] were added to the flasks to extract the bitter principle. The extract was decanted into a spectrophotometer silica absorption cell with a 1 cm. light path, and the absorption of ultra-violet light measured at 240, 265 and 290 mμ using a Beckman DK-2 ratio recording spectrophotometer. The relative amount of isocoumarin in the sample lots was then calculated by Sondheimer's formula (35).

Spectrophotometric ratings ranged from approximately zero to 13.5. The bitter taste threshold for these ratings had been established as 0.75 by Ells (12).

[1/] Skelly-solve, a product marketed by the Skelly Oil Company.

THE EFFECT OF ETHYLENE CONCENTRATION AND TIME OF EXPOSURE ON DEVELOPMENT OF BITTERNESS

Several low concentrations of ethylene gas in air were administered to carrot roots in storage for varying lengths of time to determine the length of time and the concentration required to induce the formation of the bitter principle.

Materials and Methods

Large-sized California-grown carrots of the Imperator type obtained locally on July 2, 1958 were divided into lots of six carrots and stored at 36° F in atmospheres containing 0, 50, 100 and 200 p. p. m. of ethylene in air for two, four, seven and fourteen days. At the end of the storage periods lots were rated for bitterness by taste test and fluorescence. Longitudinal sections were frozen and later analyzed spectrophotometrically.

At the end of each storage interval a lot from the 100 p. p. m. treatment was placed in an ethylene-free atmosphere for an additional period of two weeks, after which it was analyzed.

Results and Discussion

Roots from the 100 and 200 p. p. m. ethylene treatments showed small amounts of internal fluorescence after four days, but carrots from the 50 p. p. m. treatment exhibited no fluorescence until the roots had been stored for fourteen days.

Regardless of the ethylene concentration in the storage atmosphere and the observation of fluorescence, carrots did not taste bitter until they had been stored for two weeks. At that time only a few roots exhibited bitterness-- several from the 50 p. p. m. and the 200 p. p. m. treatments.

Carrots which had received 100 p. p. m. of ethylene during a one-week initial storage period and were subsequently stored in an ethylene-free atmosphere for two additional weeks fluoresced internally, but did not taste bitter. Carrots receiving a two-week initial ethylene treatment fluoresced both internally and externally at the end of the supplemental two-week period in the non-ethylenic atmosphere, and were bitter to taste.

Spectrophotometric ratings for all lots are found in Table I. Each concentration of ethylene was effective in inducing isocoumarin formation. The analyses through the first storage week indicate that sufficient time had not elapsed for isocoumarin to accumulate although its formation had evidently been induced as indicated by fluorescence observations and the spectrophotometric values observed after two additional weeks in air storage. The data indicate that approximately equal amounts of isocoumarin had formed at the end of two week's storage in roots from all ethylene treatments.

This confirms the interesting phenomenon noted by Ells (12) wherein carrots removed from an atmosphere containing ethylene continued to accumulate isocoumarin. It might be postulated that the ethylene either initiates

TABLE I

Spectrophotometric Ratings for Carrot Roots as Influenced by Ethylene Concentration and Length of Storage Period*

Ethylene Concentration (parts per million)	Length of Storage Period (Days) 2	4	7	14
0	0.00	0.00	0.00	0.15
50	0.00	0.00	0.04	0.94
100	0.03	0.04	0.00	0.94
200	0.00	0.00	0.05	1.15
100 p.p.m. followed by 0 p.p.m.**	0.32	0.49	0.89	2.21

*Determined from frozen samples.

**Samples removed from 100 p.p.m. treatment after length of time indicated at head of columns and stored in air for two weeks before analysis.

self-sustaining aberrant metabolism, or is retained within the tissue and continues to serve as a substrate or co-substrate, or acts as a catalyst in the synthesis of isocoumarin. Since the absolute amount of ethylene present is minute compared to the amount of isocoumarin formed, the postulation that it is a substrate or co-substrate is the least tenable.

THE INFLUENCE OF HARVEST DATE AND HANDLING ON BITTERNESS DEVELOPMENT IN ROYAL AND RED-CORED CHANTENAY CARROTS

Carrot samples were taken from a field at two different dates to ascertain the influence of physiological age on the formation of isocoumarin. The carrots were harvested carefully by hand, and also by machine in an attempt to discover the effect of careful or rough handling on subsequent development of the undesirable bitter compound.

Materials and Methods

Muck-grown carrots of Red-cored and Royal Chantenay varieties planted April 4, 1958 were sampled on July 25. Two bushels of each variety were harvested, one of which was hand pulled and topped. The other lot of the Royal Chantenay was taken from a 40-bushel crate of freshly, mechanically harvested carrots. Since the Red-cored Chantenay variety was not yet being harvested by the grower, a second bushel was hand pulled and passed through the mechanical harvester to approximate a mechanically harvested condition.

These carrots were treated with either zero or 100 p.p.m. of ethylene in air and stored at 36°F. Analyses were made following harvest, and after one, two, four, seven, fourteen and twenty-one days of storage. A second sample from each lot was retained, as in the preceding experiment, in an ethylene-free atmosphere for an additional period of two weeks and then analyzed.

A second harvest of hand-pulled and topped carrots was made on August 22 from the same field. These roots were stored in 100 p.p.m. of ethylene in air and in air alone for one, two, four, seven, and fourteen days at 36° F. Samples were analyzed at the end of the treatment period and again after storage in air for an additional two-week period.

Results and Discussion

Carrots which were stored in an ethylene-free atmosphere throughout their storage period did not develop bitterness. Tables II and III indicate the relative amounts of isocoumarin developed in each ethylene-stored sample as revealed by spectrophotometric analysis. The variable results obtained with the two varieties of carrots between harvest methods indicates that rough handling at harvest time is apparently not a determining factor in pre-disposition to isocoumarin formation.

Carrots of the Royal differed from the Red-cored Chantenay that were stored for one week in an atmosphere containing ethylene and subsequently stored in a non-ethylenic atmosphere for two weeks in that they accumulated appreciable amounts of isocoumarin and were detectably bitter (Table II). This is probably related to the fact that Royal Chantenay carrots were more mature than the Red-cored Chantenay at the time of sampling. Both varieties were bitter after two weeks in ethylene followed by two weeks in normal air storage.

TABLE II

Spectrophotometric Ratings for Hand and Machine Harvested Chantenay Carrots Harvested July 25, 1958 and Stored in 100 p.p.m. of Ethylene in Air for Various Periods*

Strain and Method of Harvest	Days of Storage in 100 p.p.m. Ethylene in Air					
	1	2	4	7	14	21
Rc C Mch	0.00	0.00	0.00	0.00	0.17	1.97
Rc C Hand	0.00	0.00	0.00	0.00	0.00	0.71
RC Mch	0.00	0.00	0.00	0.03	0.39	1.10
RC Hand	0.00	0.00	0.00	0.06	0.47	1.54
	Following Air Storage for Two Additional Weeks					
Rc C Mch	0.00	0.00	0.00	0.29	1.33	2.05
Rc C Hand	0.00	0.00	0.00	0.15	1.13	2.61
RC Mch	0.00	0.01	0.12	0.89	2.35	2.36
RC Hand	0.00	0.01	0.22	0.91	1.25	4.21

Rc C = Red-cored Chantenay　　Mch = Machine harvested
RC = Royal Chantenay　　Hand = Hand harvested

*Determined from frozen samples.

TABLE III

Spectrophotometric Ratings for Chantenay Carrots Harvested August 22, 1958 and Stored in 100 p. p. m. of Ethylene in Air for Various Periods*

Strain	Days of Storage in 100 p. p. m. Ethylene				
	1	2	4	7	14
Rc C	0.00	0.00	0.00	0.00	0.13
RC	0.00	0.02	0.00	0.04	0.82
	Following Air Storage for Two Additional Weeks				
Rc C	0.00	0.11	0.00	0.05	--
RC	0.00	0.12	0.29	1.38	--

Rc C = Red-cored Chantenay

RC = Royal Chantenay

*Determined from frozen samples.

Both varieties developed bitterness at approximately equal rates and to approximately equal degrees as evaluated by taste tests. Fluorescence was first observed after seven days storage at which time taste tests also revealed bitterness. The early taste evaluation averages were approximately one-half those of the fluorescence rating averages.

The levels of isocoumarin accumulation in Red-cored Chantenay roots in the second harvest were all low, and they developed a musty, fermented taste and smell. These carrots had been flooded by a five-inch rainfall several days before harvest which probably disturbed the roots' metabolism due to the exclusion of oxygen from the soil. It is assumed that they were less physiologically active than the Royal Chantenay and were unable to synthesize isocoumarin.

The Royal Chantenay carrots from the later harvest developed isocoumarin earlier in their storage than those of the same variety from the earlier harvest (Table III). The increased rate of isocoumarin development may be the result of the increased age of the roots and a more advanced state of physiological maturity.

THE INFLUENCE OF VARIOUS STORAGE PERIODS IN AN ETHYLENE-CONTAINING ATMOSPHERE ON SEVEN VARIETIES OF CARROTS

Seven varieties of carrots were evaluated to ascertain the influence of inheritance on the rapidity of isocoumarin formation. The varieties were analyzed at regular intervals to determine also whether genetic variations might exert their influence at different times during the storage period.

Materials and Methods

Seven varieties of carrots, Imperator, Gold Pak, Danvers 126, Long Nantes, Scarlet Nantes and two strains of Red-cored Chantenay were planted on the university muck farm June 16, 1958 and harvested 90 days later. At the time of harvest roots of all varieties except the Gold Pak and Chantenay were of acceptable marketable size.

The roots were stored at 36°F in air and with 50 p.p.m. of ethylene. Samples were taken for analysis at harvest and from the stored lots at weekly intervals for six weeks.

Non-fluorescent roots in this, as in previous experiments, were non-bitter and extremely low in spectrophotometric value. Non-fluorescent roots were therefore not evaluated spectrophotometrically after the second week of this experiment; and after four weeks, except for the random tests, were no longer taste-evaluated.

Results and Discussion

The trends in the increase in bitterness, averaged for all varieties for the three methods of evaluation, are graphically illustrated in Figure 5. The trend lines show a sharp rise in the rate of isocoumarin formation from the third to fourth week as determined by spectrophotometric and fluorescence ratings. The data indicate that the rate slowed again after the fourth week. The sharp rise might be explained on the basis of a climacteric peak in respiratory activity with more of the bitter substance formed at an increased rate of physiological activity. No climacteric peak has been reported for carrots, nor was respiratory activity studied as such in this investigation. Appleman and Smith (1) report that the carbon dioxide production of stored carrots decreases steadily with storage time, reducing the plausibility of this theory.

An explanation of the observed phenomena might rest in the concept of a threshold wherein the carrots, after exposure to a specific quantity of ethylene or time of exposure, produce isocoumarin at an increased rate.

There was a high degree of correlation ($r = .82$) between internal fluorescence evaluations and spectrophotometric ratings through the duration of this trial. Correlations between taste and spectrophotometric analysis ($r = .52$) and between taste and internal fluorescence evaluations ($r = .67$) were also found. The predicted lines of regression for these correlations are shown in Figures 6, 7 and 8.

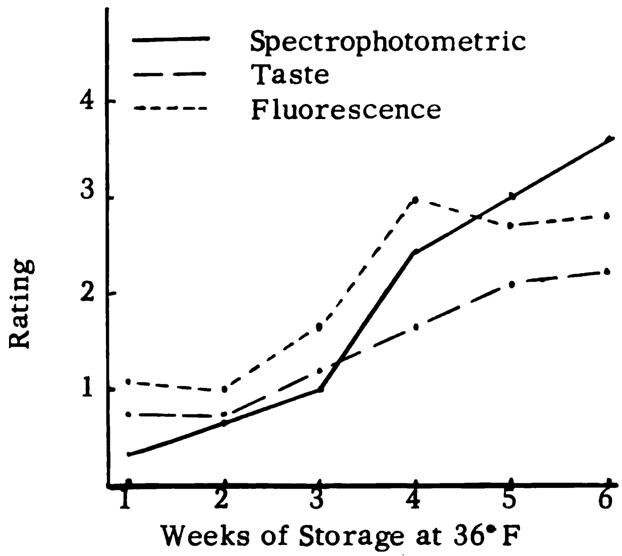


Figure 5. Averages of spectrophotometric, internal fluorescence and taste evaluations for seven varieties of carrots.

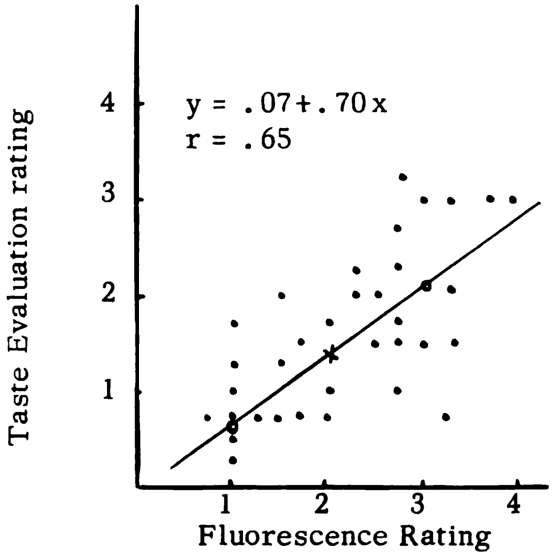


Figure 6. Predicted line of regression for taste and internal fluorescence ratings.

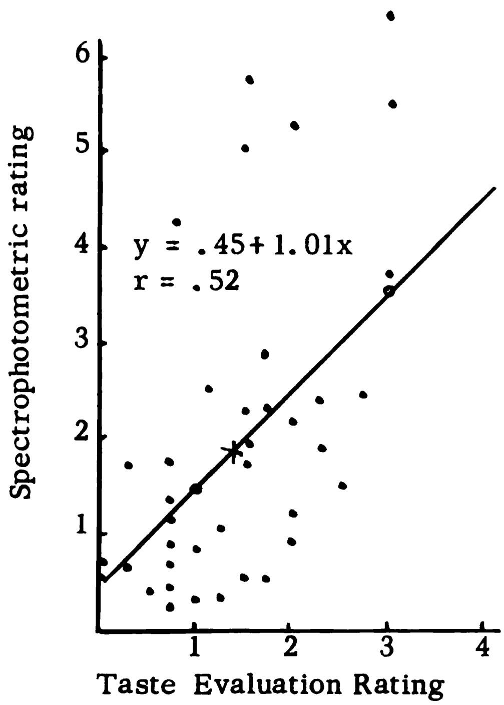


Figure 7. Predicted line of regression for taste and spectrophotometric ratings.

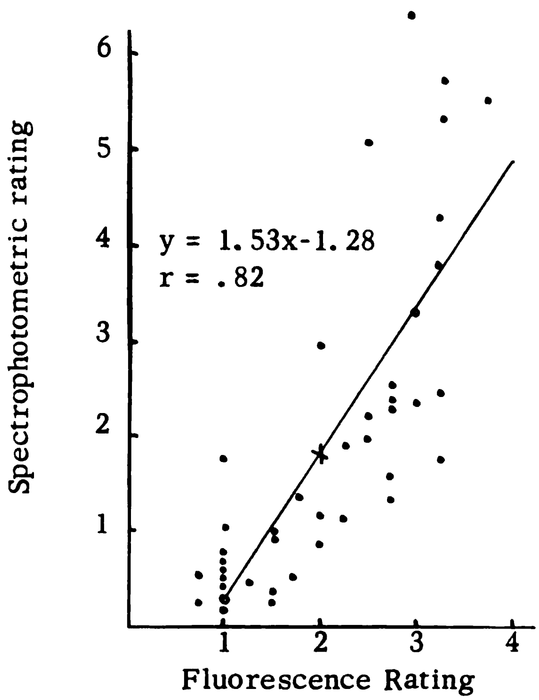


Figure 8. Predicted line of regression for fluorescence and spectrophotometric ratings.

Although the subjective evaluations correlate with the objective spectrophotometric analysis it must be remembered that the taste and fluorescence ratings are on a zero to four scale. At high spectrophotometric ratings these evaluations would run beyond the rating scale which, being based on sensually perceivable gradients, cannot be readily expanded. Nevertheless, taste and fluorescence evaluations are valuable in determining the relative quantity of isocoumarin content at low levels.

The values in Table IV show that the relative amount of isocoumarin generally increased with storage time to the end of the experiment. The concentration of isocoumarin indicated by these analyses at the end of one, two, or three weeks storage was not necessarily related to the quantity present at the end of the six-week period. After six weeks of storage the values for the Gold Pak and Nantes were considerably above those of the other varieties.

The varietal differences in isocoumarin accumulation might suggest genetic susceptibility to the disorder. Ells (12) however, has postulated that the physiological maturity of the harvested carrot is a factor in the amount of isocoumarin formed. Although the carrots in this study were sowed and harvested at the same times, those varieties with a longer season to maturity were presumably at different stages of physiological maturity. If such be the case, the plants' genetic make-up would only be indirectly responsible for the differences shown between varieties.

The variety Scarlet Nantes was not as bitter to taste after three or four weeks in storage (Table V) as would be expected from the increased spectrophotometric and fluorescence ratings (Tables IV and VI). This discrepancy may lie in the inherent subjectivity of taste evaluations.

TABLE IV

Spectrophotometric Ratings for Seven Varieties of Carrots Stored in 50 p. p. m. of Ethylene in Air for Various Periods*

Variety	Number of Weeks in 50 p. p. m. of Ethylene in Air					
	1	2	3	4	5	6
Imperator	0. 22	0. 58	1. 17	1. 36	1. 97	2. 20
Gold Pak	0. 56	0. 76	0. 89	2. 34	2. 87	5. 73
Danvers 126	0. 44	0. 27	0. 54	1. 76	1. 12	1. 55
Nantes, Long	0. 25	1. 05	0. 91	3. 77	5. 50	6. 41
Nantes, Scarlet	0. 38	0. 64	1. 74	4. 23	5. 06	5. 32
Chantenay, 25673	0. 17	0. 57	0. 84	1. 30	2. 43	1. 86
Chantenay, 17010	0. 31	0. 64	1. 36	2. 29	2. 50	2. 33
Average	0. 33	0. 64	1. 06	2. 44	3. 06	3. 63

*Averages of two fresh samples.

TABLE V

Taste Evaluation Ratings for Seven Varieties of Carrots Stored in 50 p.p.m. of Ethylene in Air for Various Periods

Variety	Number of Weeks in 50 p.p.m. of Ethylene in Air					
	1	2	3	4	5	6
Imperator	0.75	0.00	0.75	1.00	1.50	2.00
Gold Pak	0.75	0.00	0.75	1.50	1.75	1.50
Danvers 126	0.75	1.00	1.50	1.50	2.00	2.25
Nantes, Long	0.75	1.25	2.00	3.00	3.00	3.00
Nantes, Scarlet	0.50	0.25	0.25	0.75	1.50	2.00
Chantenay, 25673	0.75	1.75	1.00	3.25	2.25	2.25
Chantenay, 17010	1.25	0.75	0.75	1.50	2.75	1.75
Average	0.79	0.71	1.18	1.64	2.11	2.25

TABLE VI

Fluorescence Evaluation Ratings for Seven Varieties of Carrots Stored in 50 p. p. m. of Ethylene in Air for Various Periods*

Variety	Number of Weeks in 50 p.p.m. of Ethylene in Air					
	1	2	3	4	5	6
Imperator	0.75	1.00	2.00	2.75	2.50	2.50
Gold Pak	0.75	1.00	1.50	3.00	2.00	3.25
Danvers 126	1.25	1.00	1.75	3.25	2.25	2.75
Nantes, Long	1.50	1.00	1.50	3.25	3.75	3.00
Nantes, Scarlet	1.00	1.00	1.00	3.25	2.50	3.25
Chantenay, 25673	1.00	1.00	2.00	2.75	3.25	2.25
Chantenay, 17010	1.50	1.00	1.75	2.75	2.75	2.75
Average	1.11	1.00	1.64	3.00	2.71	2.82

*All carrots stored without ethylene did not fluoresce.

A COMPARISON OF RELATIVE AMOUNTS OF ISOCOUMARIN FORMED IN CARROTS STORED IN THREE COMMERCIAL STORAGES

Previous experiments having been conducted under controlled conditions this experiment represents an extension to field conditions. Varietal differences were studied, the number of strains being increased to ten.

Materials and Methods

Ten varieties of carrots were harvested from October 10 to October 24, 1958 from plots seeded June 15 and 16 on the university horticulture and muck farms. They were temporarily held in common storage at East Lansing to October 27, and then placed in commercial storages. One of the storages, at St. Johns, Michigan, was a quonset-type building operated as a common storage, the storages at Sparta and Martin, Michigan, were refrigerated storages. The storage at St. Johns contained only carrots, while at the other two locations, apples were held in rooms adjoining those in which the carrots were stored. The samples were removed on January 20, 1959 and analyzed on January 21.

Results and Discussion

Carrots from the common storage exhibited external fluorescence ratings ranging from 0. 17 to 0. 83 with the exception of Long Nantes which had an average external rating of 1. 83. Internal fluorescence ratings averaged lower, but ranged from zero to 1. 17. Fluorescence values may be too high in this case since in most instances only a few specks of fluorescence were observed in each root--these were given the minimum rating of one. Carrots showing internal fluorescence were generally non-bitter to the taste. The variety Gold Pak, grown on muck soil, however, had an average taste evaluation of 0.83 for six roots.

External fluorescence ratings were high for roots from the cold storages, with a large number of individual roots receiving the maximum rating of four. The range was from one to four, averaging 3.03. No taste evaluations were made on complete lots in these two storages. Random taste evaluations indicated bitterness in all lots.

The spectrophotometric ratings for the ten varieties, two soils and three storages are listed in Table VII. There was a marked difference in the development of bitterness in carrots from the common storage as compared with that found in roots from the two cold storages. From preceding experiments it is evident that this difference in bitterness was not due to the cold storage *per se,* but to ethylene introduced into the storages by apple emanations, gasoline exhaust fumes (13) and perhaps other sources.

Ethylene was probably present in the common storage since gasoline engine powered machinery was used within the building and some roots in the storage had been attacked by fungi. According to Williamson and Dimock (42) plant tissues attacked by various pathogens evolve ethylene. Young *et al.* (43) have

TABLE VII

Spectrophotometric Ratings for Ten Varieties of Carrots Stored in Three Commercial Storages*

Storage	Supreme Half-long	Morse Bunch-ing	Gold Pak	Chante-cler	Spec. Buncher	Imper-ator	Chantenay 25673	Chantenay 17010	Nantes Scarlet	Nantes Long	Average
Sandy Loam Soil											
St. Johns**	0.00	0.04	0.07	0.07	0.04	0.18	0.21	0.37	0.12	0.81	0.19
Sparta***	1.97	2.92	1.97	4.43	1.31	2.77	1.79	1.60	3.23	4.56	2.55
Martin***	4.62	1.97	3.11	4.57	5.83	3.08	3.12	3.46	1.55	4.31	3.56
Muck Soil											
St. Johns	0.90	0.06	0.10	0.11	0.00	0.03	0.04	0.19	0.06	0.16	0.18
Sparta	1.30	1.33	1.57	2.40	0.91	0.60	1.81	1.17	3.61	2.63	1.73
Martin	3.19	2.53	3.57	2.59	1.00	1.53	2.47	2.74	2.98	6.95	2.96

*Averages of two fresh samples.
**Common storage.
***Refrigerated storage.

shown that the fungus _Penicillium digitatum_ produces ethylene. The amount of ventilation necessary for the operation of the common storage, however, may have been a factor in reducing the amount of bitterness observed.

FORMATION OF ISOCOUMARIN IN CARROTS PLACED IN APPLE, PIT, COMMON AND CONTROLLED STORAGES

The trials under field conditions were extended in this experiment to include a number of apple storages, an outdoor pit, and a common storage. A controlled ethylene storage was also used for comparison with the various storages. Due to the extensive scope of this experiment and the number of lots involved, it was necessary to sample at varying periods to make the analytical work possible.

Materials and Methods

The carrots for this examination were harvested between October 28 and November 10, 1958 from the same sandy loam and muck areas from which the roots reported in the previous studies were taken. During the harvest period temporary storage was in an outdoor pit covered with loose straw. Lots of ten varieties from each soil were distributed on November 15 to four commercial apple storages. Groups of samples were also placed in an outdoor pit storage, in the common storage on the horticulture farm in East Lansing, and in 50 p. p. m. of ethylene in air at 36° F.

Analyses were made after varying storage intervals. The controlled ethylene storage lots and those from two apple storages (A and B) were examined February 15, 1959. On February 25 analysis was accomplished on carrots stored in the pit and common storages. A second analysis was completed March 15 on the carrots from storage A at which time those in storages C and D were also analyzed. The second analysis of roots from storage B was made on April 1.

Results and Discussion

The internal fluorescence values for these carrots are illustrated in Tables VIII and VIIIa. The Gold Pak variety from the 50 p. p. m. of ethylene in air treatment received an internal fluorescence rating of zero, and was also non-bitter by taste evaluation, while ratings for other carrots in the same storage were fairly high. Since spectrophotometric ratings were generally low for this variety, it is possible that the physiological maturity was such that the formation of the bitter principle was delayed, was stopped at a low concentration, or was developed more slowly than in other carrots.

Fluorescence was found in four samples of carrots from the pit and common storages, the amount of fluorescence was minimal, and the taste evaluations and spectrophotometric analyses were negative.

Several spectrophotometric ratings were considerably higher than the average. Probably dessication of these samples in storage had the effect of concentrating the bitter principle. Tables IX and IXa indicate that the quantity of isocoumarin formed in most commercial apple storages is approximately twice that formed in carrots stored in 50 p. p. m. of ethylene in air.

TABLE VIII

Internal Fluorescence Ratings for Carrots Grown in Sandy Loam Soil and Stored in Selected Storages for Prolonged Periods

Storage	50 p.p.m	A-1	B-1	Pit	Common	A-2	C	D	B-2	Avg. (7 stgs)
No. days in storage	90	90	90	100	100	120	120	120	135	
Variety										
Spec. Buncher	3.00	2.00	2.75	0.00	0.00	2.25	1.25	1.75	1.66	2.09
Gold Pak	0.00	2.00	1.50	0.00	0.00	2.00	1.25	0.75	1.75	1.32
Imperator	1.75	3.25	3.25	0.00	0.00	2.75	2.25	0.75	3.00	2.43
Chantenay 25673	2.50	3.50	2.75	0.00	0.00	2.75	2.50	2.00	3.33	2.76
Chantenay 17010	3.50	3.50	3.25	0.00	0.00	3.25	2.75	2.75	2.50	3.07
Danvers 126	2.75	2.25	2.75	0.00	0.50	2.50	2.75	1.25	--*	2.38
Chantecler	2.50	3.25	3.25	0.00	0.00	2.00	3.00	1.25	3.00	2.61
Nantes, Strong Top	2.50	3.00	2.50	0.00	0.00	2.75	3.50	3.25	3.25	2.96
Nantes, Scarlet	4.00	3.25	2.75	1.00	0.75	2.50	3.25	3.25	3.25	3.25
Nantes, Long	3.75	2.75	3.50	0.00	0.50	3.00	2.75	2.25	--	3.00
Average	2.32	2.88	2.73	0.10	0.18	2.58	2.52	2.02	2.72	2.61

*Sample destroyed by fungi.

TABLE VIIIa

Internal Fluorescence Ratings for Carrots Grown in Muck Soil and Stored in Selected Storages for Prolonged Periods

Storage	50 p.p.m.	A-1	B-1	Pit	Common	A-2	C	D	B-2	Avg. (7 stgs)
No. days in storage	90	90	90	100	100	120	120	120	135	
Variety										
Spec. Buncher	3.25	2.75	3.75	--*	0.00	3.25	1.75	2.75	3.50	3.00
Gold Pak	1.75	2.75	1.75	0.00	0.00	1.75	--	1.75	2.66	2.07
Imperator	1.25	2.25	3.25	--	--	3.50	--	3.00	1.50	2.46
Chantenay 25673	3.00	3.75	4.00	--	0.00	3.75	3.50	3.00	--	3.50
Chantenay 17010	2.75	3.75	2.25	--	0.00	3.75	3.75	3.00	--	3.21
Danvers 126	3.50	3.50	3.50	0.00	0.00	3.75	3.75	3.25	3.25	3.50
Chantecler	3.25	3.25	4.00	--	0.00	3.25	3.25	1.50	3.75	3.18
Nantes, Strong Top	2.25	4.00	4.00	--	0.00	2.75	3.75	3.75	3.75	3.46
Nantes, Scarlet	3.25	3.75	3.50	--	0.00	2.50	2.00	2.75	3.50	3.04
Nantes, Long	3.50	2.25	3.50	--	--	3.00	--	2.50	3.00	2.96
Average	2.75	3.20	3.25			3.12	3.05	2.72	3.11	2.83

* -- Indicates sample destroyed by fungi.

TABLE IX

Spectrophotometric Ratings for Carrots Grown in Sandy Loam Soil and Stored in Selected Storages for Prolonged Periods*

Storage	50 p.p.m.	A-1	B-1	Pit	Common	A-2	C	D	B-2	Avg. (7 stgs)
No. days in storage	90	90	90	100	100	120	120	120	135	
Variety										
Spec. Buncher	0.68	1.85	3.77	0.00	0.00	1.70	1.23	0.99	1.84	1.63
Gold Pak	0.14	1.70	1.95	0.00	0.00	2.06	2.15	1.53	1.33	1.55
Imperator	0.74	2.51	5.39	0.00	0.00	2.57	3.96	0.84	2.32	2.62
Chantenay 25673	1.16	2.72	5.40	0.00	0.00	2.37	3.26	2.26	2.85	2.86
Chantenay 17010	1.12	4.91	4.64	0.00	0.00	2.60	3.80	1.50	1.31	2.84
Danvers 126	0.46	4.54	3.38	0.00	0.00	3.18	2.34	0.79	--**	2.45
Chantecler	2.17	2.48	2.84	0.00	0.00	2.20	5.20	0.91	1.75	2.51
Nantes, Strong Top	1.78	3.55	6.04	0.00	0.00	3.92	12.75	3.69	9.37	5.87
Nantes, Scarlet	5.02	5.78	9.38	0.00	0.00	6.16	10.46	7.92	13.55	8.18
Nantes, Long	2.32	5.00	11.98	0.00	0.00	5.96	6.96	3.53	--	5.96
Average	1.56	3.50	5.38			3.27	4.50	2.40	4.29	3.66

*Average of two fresh samples.
** -- Indicates sample destroyed by fungi.

TABLE IXa

Spectrophotometric Ratings for Carrots Grown in Muck Soil and Stored in Selected Storages for Prolonged Periods*

Storage	50 p.p.m.	A-1	B-1	Pit	Common	A-2	C	D	B-2	Avg. (7 stgs)
No. days in storage	90	90	90	100	100	120	120	120	135	
Variety										
Spec. Buncher	0.39	1.78	2.62	--**	0.00	1.61	2.13	2.48	2.66	1.95
Gold Pak	1.26	1.94	2.23	0.00	0.00	2.22	--	1.26	5.40	2.39
Imperator	1.08	2.14	5.52	--	--	3.86	--	3.64	1.86	3.02
Chantenay 25673	2.12	5.91	5.46	--	0.00	3.78	7.01	3.67	--	4.66
Chantenay 17010	1.87	5.26	4.43	--	0.00	4.50	4.86	2.51	--	3.91
Danvers 126	0.56	3.02	3.85	0.00	0.00	3.30	2.98	2.67	4.92	3.04
Chantecler	1.68	4.70	3.65	--	0.00	3.86	2.45	1.92	4.88	3.31
Nantes, Strong Top	1.42	5.53	5.96	--	0.00	5.75	4.38	4.33	9.74	5.30
Nantes, Scarlet	1.61	7.14	5.64	--	0.00	6.56	5.09	2.94	6.76	5.11
Nantes, Long	1.51	3.31	3.92	--	--	3.98	--	5.61	3.25	3.60
Average	1.35	4.07	4.33			3.94	4.13	3.10	4.93	3.97

*Averages of two fresh samples.

** -- Indicates sample destroyed by fungi.

An analysis of variance was performed to determine, in so far as possible, the effect of the various controlled factors on the carrots and to note possible interactions. Storages used in the analysis of variance were selected by elimination of those which had missing values because of molds, or had obviously elevated values as a result of dessication. The common and pit storages were not included since they were represented by zero values and could not readily enter the analysis. The two storages E and F represent the storages at Martin and Sparta which were used in the preceding experiment for a 90-day period of storage as were several storages in this trial. Varieties that were common to both experiments were used.

The analysis of variance shown as Table X indicates that the soils were identical in their effect on the development of bitterness. No significant interactions were observed, but the effect of variety and of storage was significant.

Group mean comparisons of varieties, identified in Table XI, indicate that most of the variance due to variety was between the three groupings as shown in Table X.

The correlation between the intensity of internal fluorescence and spectrophotometric analysis was low, r = .46. This is probably due to the fact that the isocoumarin content was generally high and the fluorescence ratings were generally close to the maximum where variations in degrees of intensity could not be differentiated visually.

TABLE X

Analysis of Variance for the Isocoumarin Content of Eight Varieties of Carrots on Two Soils from Five Storages

Source	DF	Sum of Squares	M. Sq.	F
Total	79	199.04		
Variety	7	62.13	8.87	3.11*
$\bar{X}_{a, b, c}$ vs. $\bar{X}_{d, e, f}$ vs. $\bar{X}_{g, h}$	2	60.68	30.34	10.67**
$\bar{X}_{a, b, c}$ vs. $\bar{X}_{d, e, f}$	1	5.42	5.42	1.90NS
$\bar{X}_{a, b}$ vs. $\bar{X}_{c}$	1	.28		NS
Storage	4	39.87	9.97	3.50*
Soil	1	.17		NS
Variety x storage	28	2.57		NS
Variety x soil	7	6.33		NS
Storage x soil	4	8.09		NS
Storage x variety x soil	28	79.88	2.85	

TABLE XI

Varieties and Mean Spectrophotometric Ratings Used in the Analysis of Variance in Table X

	Variety	Mean Spectrophotometric Rating
a	Special Buncher	1.69
b	Gold Pak	1.87
c	Imperator	2.07
d	Chantenay 17010	2.31
e	Chantenay 25673	2.46
f	Danvers 126	2.67
g	Scarlet Nantes	4.06
h	Long Nantes	4.14
	GROUP	
	a, b	1.78
	a, b, c	1.87
	c	2.22
	d, e, f	2.48
	g, h	4.10

THE RELATION OF ROOT COLOR TO ISOCOUMARIN FORMATION

In an attempt to discover whether carotene had any influence on the induction of isocoumarin formation in carrots, a number of variously pigmented roots obtained from the University of Wisconsin were evaluated.

Materials and Methods

Six intensities of pigmentation were presented, two varieties of which were identified. In order of increasing color intensity the carrots were: white, cream, yellow, intermediate (between orange and yellow), orange (Danvers 126), and dark orange (German Red). These carrots were analyzed upon arrival and after three months storage at 36° F in zero and 100 p. p. m. of ethylene in air.

Results and Discussion

Taste tests proved to be inconclusive due to the strong natural flavors inherent in some of the varieties. The results of fluorescence and spectrophotometric analysis are shown in Table XII.

Intensity of pigmentation, and presumably intensity of carotene content, was apparently not linearly related to the amount of accumulated isocoumarin as indicated by spectrophotometric analysis. The white carrots containing no carotene developed more isocoumarin than the cream, and as much as the orange. The greatest amount of isocoumarin was developed by the yellow colored carrot.

TABLE XII

The Relation of Intensity of Pigmentation to Isocoumarin Development as Influenced by Ethylene and Indicated by Fluorescence and Spectrophotometric Analysis*

Color	0 p. p. m.		100 p. p. m.	
	Spec.	Int. Flu.	Spec.	Int. Flu.
White	0. 08	0. 00	2. 36	2. 00
Cream	0. 00	0. 00	0. 91	3. 00
Yellow	0. 00	0. 00	5. 90	4. 00
Intermediate	0. 00	0. 00	3. 27	4. 00
Orange (Danvers 126)	0. 05	0. 00	2. 36	4. 00
Dark Orange (German Red)	0. 09	0. 00	4. 43	4. 00

*Averages of two fresh samples.

GENERAL DISCUSSION

An exposure of California Imperator carrots to 100 p. p. m. of ethylene in air for two days was effective in initiating bitterness formation as measured spectrophotometrically after two additional weeks of storage. However, using locally grown Chantenay carrots, the effect was not noticeable until after the initial exposure to the same ethylene concentration reached four days. Other Chantenay carrots harvested from the same field one month later showed a more rapid increase in isocoumarin formation than the earlier harvest. Data derived from analyses show that the variety Gold Pak formed more total isocoumarin when harvested early than late. The explanation for these differences is based primarily upon the concept of physiological maturity as advanced by Ells (12) wherein it is postulated that carrots at the peak of maturity are more susceptible to isocoumarin formation than those that are under- or over-mature.

Imperator carrots throughout this examination were relatively low in accumulated isocoumarin, while Nantes strains were consistently high. This, with other varietal differences noted, might suggest an hereditary difference in susceptibility to isocoumarin formation. It is possible that varieties influence isocoumarin bitterness as a result of varying rates of metabolic activity as related to stage of maturity, or as a result of the presence of suitable quantities of substrate materials.

Although many varied effects of ethylene on plant materials are noted in the review of literature, ethylene may affect some basic metabolic process which manifests itself differently in different organisms. The minute quantities of ethylene required might also suggest that some basic process is affected in all plants. Wallace's (42) demonstration that a seven and one-half minute exposure of Transparent apple cuttings to ethylene was as effective in formation of intumescences as two weeks continual exposure, and Ells' (12) description of the effect of an initial ethylene exposure in inducing bitterness, which was borne out in this investigation, would lend credence to the theory that some basic process is affected, and that this effect is irreversible.

A marked increase in the rate of isocoumarin formation in carrots stored in 100 p.p.m. of ethylene in air was noted between the third and fourth storage week. This might be due to the removal of some essential metabolite being used as the substrate for isocoumarin synthesis, with more of the metabolite being produced as a consequence, and also formed into isocoumarin.

Very little isocoumarin was produced in the roots which had undergone anaerobic conditions prior to harvest. It is assumed that these anaerobic conditions, or the water which produced them so altered the roots' normal physiology that they were unable to produce the bitter principle.

In analyses employing fluorescence under ultra-violet light no isocoumarin was discovered in the absence of fluorescence. The value of internal fluorescence as a rapid, practical method of bitterness identification is indicated by the fact that when fluorescence ratings are low, taste evaluations are lower. Since fluorescence occurs at very low levels of isocoumarin content it will be useful in discovering bitterness at a time when the roots are still edible.

The correlations found between internal fluorescence and spectrophotometric analysis indicate, too, that internal fluorescence, within limits, may be used as an indication of relative isocoumarin concentration.

This investigation has indicated that the physiological condition of the carrots is an important factor on the formation of the bitter principle. Further detailed physiological investigations will be necessary to discover the nature of the processes involved and the compounds formed.

SUMMARY AND CONCLUSIONS

Carrots subjected to ethylene in low concentrations in air develop bitterness which is detectable by fluorescence under ultra-violet light. The fluorescence intensity has been correlated with the relative quantity of iso-coumarin present as revealed by spectrophotometric analysis. Internal fluorescence appears just prior to, or concurrent with, the presence of taste-detectable bitterness lending itself to use in discovery of bitterness in early stages of its development.

Carrots placed in apple storages invariably became bitter indicating that such storages probably contain ethylene in the atmosphere from apple emanations and should not be employed for carrot storage. Carrots placed in pit or common storage remained free of the bitter principle.

Rough handling at harvest time does not appear to result in increased production of the bitter principle. It does appear that reduced physiogical activity is effective in decreasing the quantity of isocoumarin formed as evidenced by the trial in which the physiology of some carrots had been altered by flooding prior to harvest.

An increased rate of bitterness production occurred between the third and fourth weeks of continual exposure to 100 p. p. m. of ethylene in air indicating that there is perhaps a peak in physiological activity under the influence of ethylene.

It is postulated that ethylene may affect some basic metabolic process in carrot roots. The identity of metabolites entering into the formation of isocoumarin is unknown. Neither intensity of pigmentation nor presumably, quantity of carotene appear to be necessary for bitterness production, since white and light-colored roots developed as much isocoumarin as those of orange-fleshed varieties.

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