STUDIES ON THE OCCURRENCE, MEASUREMENT, AND CONTROL OF BITTERNESS IN CARROTS

By

Paul Mack Bessey

AN ABSTRACT

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ABSTRACT

Bitterness has recently become a serious problem for processors of stored carrots and has also been found in carrots on the fresh market. A series of studies was conducted to determine the factors involved in its occurrence and measures usable for its control.

Organoleptic, fluorescence and spectrophotometric ratings were used for bitterness evaluation. The first was based upon taste panel recognition of five intensities of bitterness ranging from 1- non-bitter to 5- exceedingly bitter. A yellowish-green fluorescence in tiny spots in phloem tissues of bitter roots exposed to short wave ultra violet light was found and was correlated significantly at .05 ($r = 0.645$) with bitterness by taste test. This phenomenon was adopted as a rapid bitterness evaluation technique using a 1-5, non-fluorescent to highly fluorescent, rating scale. Spectrophotometric evaluations were based upon energy absorbency of petroleum ether extracts of carrots at 240, 265 and 290 mu.

Early maturing varieties Nantes and Touchon harvested at the same chronological age as later maturing Danvers and Imperator became more bitter in cold storage. Most strains of the intermediate maturing Red Core Chantenay variety were similar in bitterness response to Nantes and Touchon. A progeny test of bitter and non-bitter selections from Red Core Chantenay resulted in non-significant differences in bitterness; however, the growing season may have reduced their susceptibility to bitterness. Short type Chantenay and Chanticler, seeded May 27 became more bitter in storage than when seeded July 6. Early Chanticler was

apparently more mature than early Chantenay in that more flower stalks were formed. It also became more bitter further substantiating the interpretation that maturity enhances bitterness. Late sown Chanticler appeared less mature in shape than Chantenay at harvest and became much less bitter during storage. In a comparison of long and short Chantenay strains from a single planting, bitterness differences did not correlate with types.

Carrots from mineral soils were higher in soluble solids than carrots from muck soils, yet evidenced no differences in bitterness that could be ascribed to soil type.

Applications of copper and manganese to field plots of carrots deficient in these two elements did not significantly affect bitterness. Deficiencies of copper and manganese did not induce bitterness. Mineral analyses of bitter carrots from an acid muck showed a higher content of iron but less manganese than non-bitter carrots from an alkaline muck.

Although not compared directly in the same study, early harvests resulted in more bitterness than late harvests.

The cool growing season of 1956 was more influential in reducing bitterness susceptibility than any varietal, field or storage treatment.

Injuries to roots during harvest and handling increased losses due to disease (Sclerotinia species), but did not increase the incidence of bitterness in storage. Conversely, injuries appeared to speed depletion of bitterness when present.

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ABSTRACT

Bitterness was three to five times as intense in carrots stored at 40° F as in carrots at 32° F. Carrots stored in the presence of apple emanations developed bitterness within six weeks while similar carrots stored in atmospheres free from fruit emanations remained non-bitter. Immature carrots and carrots stored for three months treated with apple emanations and ethylene were not induced to become bitter. Controlled atmosphere treatments with reduced oxygen (3 and 7 percent) and accumulated carbon dioxide (10 and 5 percent) retained their typical carrot flavor and developed no bitterness. Carrots stored under anaerobic conditions developed no bitterness, but a fermented flavor and aroma.

Bitterness appeared to increase to a peak during cold storage followed by a prolonged depreciation aided by ventilation and root injuries.

In summary, bitterness susceptibility appeared related to early maturity and a warm growing season. The presence of ethylene, or a similar substance in the storage atmosphere facilitated the development of bitterness in storage. Fluorescence of bitter roots under ultra violet light was found and related to bitterness as a rapid evaluation technique.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDIX TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Bitter compounds, flavor components of many foods, are recognized readily in coffee, chocolate, and grapefruit where they provide part or all of their characteristic flavor. Except for sugars, the principle flavoring component of the grapefruit is naringin, (Kesterson and Hendrickson, 1953) a bitter glycoside.

When bitterness appears abnormally in other products, consumers abruptly reject them because of poor quality.

The tubers of most varieties of potatoes, when exposed to light, are highly susceptible to greening. The chlorophyll that is developed in the exposed areas extends deeply into the tissues and is associated with the production of a glucosidal alkaloid, solanine (Henry, 1949), which in high concentration is bitter and poisonous.

Occasionally fruit of slicing cucumbers, particularly in greenhouse varieties, develops a bitterness which is more concentrated at stem end of the fruit. Most Dutch and Danish slicing cucumbers are sampled during the marketing process to check for bitterness. In this crop, bitterness often appears after a period of slow growth (Gram and Weber, 1953). The presence of elaterase, a relatively specific enzyme for the hydrolysis of bitter glycosides of the Cucurbitaceae, has been reported in

the cucumber and is thought to be related to bitterness. Genetic susceptibility to bitterness has been found in an African cucumber introduction (Barham, 1953).

A genetic line which produces bitter tomatoes was reported by Borchers and Nevin (1954) from a survey of plant introduction lines. The bitter principle was identified as an alkaloid and could be accurately measured by a chemical test.

Hot, dry weather and long days that often accompany slow vegetative growth and bolting in lettuce often result in a bitter flavor. However, the problem is not as serious as the other morphological changes that occur which make the plant unsalable. One explanation for the market disadvantage of eastern Pascal celery, as compared to the western grown crop, is the strong, bitterish flavor that is frequently present. Refrigerated storage of five to seven days, comparable to the shipping period of California celery, usually dissipates the strong flavor so that it becomes difficult to distinguish celery from the two places. Celery has often been stored or field blanched to remove color and objectionable off-flavors. An Ontario report, however, has mentioned the development of a strong or bitter flavor in celery stored for market (Truscott, 1954). According to Gram and Weber (1953) the bitter taste is associated with a high nitrogen content.

In carrots, several off-flavors have been found. Pesticide residues, particularly benzene hexachloride, have resulted in disagreeable, usually musty flavors. Herbicide spray oils with low volatility have

frequently left a disagreeable oily flavor. Green shoulders, resulting from chlorophyll formation with prolonged exposure to the sun during growth, give a strong, almost bitter flavor. An earthy flavor is occasionally found in carrots from muck soils and is probably related to fungal infection. Carrots which have developed a seed stalk usually are strongly flavored with a greater concentration in the core. A strong carrot flavor, as distinguished from bitterness, commonly appears in some roots in most plantings.

Bitterpit of apples, also known as stippin and Baldwin spot, is particularly troublesome with the varieties Baldwin, Northern Spy, Rhode Island Greening and York Imperial. Recent studies by Garman and Mathis (1956) have related an unbalanced supply of calcium to magnesium and potassium as the cause. Calcium salts applied as sprays or soil injections have given partially successful control. Bitter rot of apples, caused by the fungus Glomerella cingulata, is more of a problem than bitterpit of apples.

An additional off-flavor, recognized as bitterness, and for which there has been no apparent explanation, is a problem in carrots for processing and fresh market. This type of bitterness is the subject of this study which involves varieties, maturity, nutrition, harvest and post-harvest handling, storage temperatures and atmospheres.

CHAPTER II

REVIEW OF LITERATURE

Off-flavors in Carrots

When carrot bitterness studies were initiated by the author in the fall of 1954, the only published references pertaining directly to bitter flavors in the carrot were by Brown et al. (1944) and Hervey and Schroeder (1949). The former reported bitterness in apparently mature Chantenay carrots placed on the fresh market. Hervey and Schroeder indicated that yellows virus infection in carrots caused a bitter astringent flavor which persisted into the canned product. Yamaguchi et al. (1955) showed by tasting that yellows infected carrots could be readily distinguished from other bitter carrots. The off-flavor associated with green shoulders of carrots could be distinguished from the other two.

In an experiment comparing reconstituted dehydrated carrots for Michigan, Illinois and Wisconsin muck and upland sources, Newcombe and Alderman (1944) reported that muck-grown carrots developed greater oxidative rancidity than upland carrots and possessed a decided off-flavor not present in the upland carrots.

Barnell, Gooding and Wager (1955) indicate that dehydration or storage of dehydrated carrot products is responsible for oxidative breakdown of β-carotene to β-ionone with accompanying off-flavors and

odors. Weier (1944) showed complete carotene breakdown in 24 hours in carrot sections placed in moist air at 62° C. Carotene crystals dissolved in oil droplets in the carrot and gave a positive test for aldehydes when using Schiff's reagent. Reeve (1943) showed the disappearance of carotene into oil droplets under the microscope.

Benzene hexachloride and lindane, both insecticides, and DD, a soil fumigant and nematocide, have resulted in off-flavors in carrots according to Hinreiner and Simone (1956) and Lear et al. (1954).

Truscott (1954), Yamaguchi et al. (1955), Sondheimer et al. (1955) and Atkin (1956) all described the flavor under consideration as bitter with a persistent effect in the mouth.

Crocker (1945) in reviewing taste physiology, diagrammed the location of bitterness detection in the mouth as concentrated on the sides and back of the tongue with a few bitter sensitive tastebuds on the tip of the tongue and on the soft palate.

Effects of Environment on Carrots

Banga and DeBruyn (1954) and Banga, DeBruyn and Smeets (1955) showed temperature effects on carotene content and "degree of ripeness" of carrots. When grown at 8° C. roots had less carotene per gram dry weight than at 18° C. At the lower temperature total dry weight was lower, carrots were longer, more tapered and pointed at the tip than at the higher temperature. Degree of ripeness was indicated by the relationship between carotene/dry weight ratio and root shape.

Similar interpretations of growing temperature influence have been presented by Barnes (1936), Bremer (1931), Magruder et al. (1940), Miller et al. (1935), Hansen (1945), Lantz (1949) and Smith et al. (1944).

Drought and irrigation treatments by Yamaguchi et al. (1955) and Atkin (1956) resulted in no bitterness differences in the several varieties tried. With the latter, irrigation led to a definite off-flavor distinguishable from bitterness.

Yamaguchi et al. (1955) indicated that maturity could have an effect upon bitterness. Several varieties of carrots harvested at market maturity, minimum size for bunching, had developed no bitterness while those harvested later at processing maturity, after full size was reached, had a stronger taste and were sometimes slightly bitter.

Date of seeding was shown by Atkin (1956) to have little influence on subsequent bitterness. Harvest dates, October 2, November 2, and November 27 on the other hand, had a definite effect upon bitterness

development of carrots immediately placed in refrigerated storage and held until processing in December and January. The earlier the harvest, the more bitter the carrots became. His data indicate a slight increase in bitterness from December to January in carrots from the first two harvests. Atkin found that carrots from the late harvest had developed no bitterness by either December of January.

In comparing large and small roots for bitterness Atkin found slightly more bitterness in small roots and concluded that maturity was not a factor in controlling bitterness.

Seasonal differences in carrot composition have been noted by many workers including Miller et al. (1935), Smith et al. (1944), Hansen (1945), Lantz (1949), Janes (1949), Booth and Dark (1949) and Yamaguchi et al. (1952) who agree generally that for the same varieties, carotene content in the winter is one third to one half that of summer carrots. Aeration, reduced by a high water table was thought responsible for much of the poorer color in winter carrots according to Miller et al. Smith et al. (1944) found much higher carotene content in carrots from the south side of raised beds than from the north side in winter crop carrots.

Morris et al. (1946) in studying the seasonal variations in enzyme content of eleven varieties of carrots found ascorbic acid oxidase contents highest when growing conditions were most favorable. Peroxidase content in contrast was not markedly influenced by season. Thiamin and ascorbic acid contents were not particularly affected by season according to Smith et al. (1944). Lantz (1949) also found no seasonal difference for ascorbic acid.

Varieties and strains of carrots have been studied for bitterness response by both Yamaguchi et al. (1955) and Atkin (1956). Bitterness has been present in all tested and no marked differences were reported. Yamaguchi, however, in progeny testing selections from bitter and non-bitter parents found strongly flavored carrots appearing from the bitter selections and only mild flavored roots from the non-bitter. Atkin commented on the extreme differences that appeared from root to root within varieties and strains. Both suggested that breeding for bitterness immunity or resistance was a likely approach to eliminating the problem.

In a limited comparison Atkin (1956) found more bitterness in carrots from muck soils than from sands or loams. However, bitterness was found in carrots from all soil types.

Fertilization practices of carrot growers were surveyed by Atkin (1956). He found both bitter and non-bitter carrots produced on fields which were both very heavily fertilized and very lightly fertilized and concluded that the quantity of fertilizer had little or no bearing on bitterness development. In a preliminary minor element study Atkin and Sayre (1955) mentioned the application of a "shotgun" treatment of minor elements in frit at 0, 50 and 100 pounds per acre. The most bitterness appeared in the 0 treatment with progressively less at 50 and 100 pounds. The bitterness intensity at 100 pounds was still so high that the carrots would not have been used in processing.

Freeman and Harris (1951) reported progressive increases in carotene content of carrots following increasing increments of fertilizer nitrogen on a Monroe silt loam soil with a low test for nitrogen and phosphorus but high in potassium. Additions of phosphorus and potassium

were non-significant. The addition of chloride (KCl) depressed the carotene content while sulfate (K_2SO_4) did not have this effect.

Carotene content, color and sweetness of carrots were improved by addition of copper sulfate to copper deficient organic soil according to Harmer (1946).

Kelly, Somers and Ellis (1952) showed improvement in growth and carotene content of carrots grown on boron deficient soil following boron application. Warington (1940) found boron deficiency of carrot to result in tapering, poorly colored roots which appeared immature.

Bernstein and Ayers (1953) noted that carrots grown on saline soils were better flavored than those grown in low saline soils, and on a dry weight basis, contained 30 percent more sugar. They suggested that the influence of salinity on sugar content was probabably effected preponderantly through the osmotic properties of the soil solution than any specific effect of the added salts.

Storage Relationships

Atkin found in several studies that carrots placed in refrigerated storage tended to become bitter while common storage carrots showed no bitterness. The usual practices in operating storages for carrots in New York State according to Tyler (1944) are as follows. Cold storages are operated at about 31-34° F. with a relative humidity of 84-92%. Common storages are cooled by ventilation with cool outside air so that temperatures are slower to come down to holding levels of 36-45° F. Relative humidity ranges from 88-92%. Carrots kept better and longer in cold storage. He noted that disease organisms causing the most storage losses were the same in the two types of storages. Sclerotinia sclerotiorum and other Sclerotinia species were most destructive, followed by Erwinia carotovora (soft rot) and Botrytis species. There was less rot where crates, bins and storage rooms were well ventilated.

Rader (1952) found a similar disease situation in stored carrots.

Newhall (1953) found that the most important factor in reducing storage rots in carrots was rapid cooling to 31-32° F. immediately following harvest. Carrots from wet ends of fields spoiled more quickly than from drier areas. Mechanically harvested carrots developed more spoilage than hand topped ones. Roots with aster yellows usually developed a deep crown rot within a few months so were a bad storage risk. Washing was not recommended because of the danger of spreading several disease organisms. Carrots with excessive amounts of dirt on them as they came to storage were not predisposed to rot, nor did piling soil on several crates cause more rotting. There was no mention of bitterness.

Wright, Rose and Whiteman (1954) state that heat of respiration produced by topped carrots in BTU's per ton per 24 hours is 2130 at 32° F., 3470 at 40° F. and 8080 at 60° F. Their recommendations for best preservation of sugars and general quality of carrots is storage at 32° F. with relative humidity at 90-95 percent.

Studies of changes in the carotene content of carrots during storage show differing trends. Langley, Richardson and Andes (1933) found no appreciable change in vitamin A values. Werner (1941) and Hansen (1945) found carotene content to remain stable followed by a decrease as they sprouted in the spring. Barnes (1936), and Lee and Tapley (1947) found carotene content to decrease from the beginning of storage. Lantz (1949) and Lipton (1953) found that data expressed on fresh weight at analysis and on dry weight indicated an increase in carotene content, but when corrected to original fresh weight, there was a decrease in carotene content during storage. Increases in carotene following harvest were reported by Anon. (1944), Lachman (1944), Brown (1947), McKillican (1948), Wharton and Ohlson (1949), Rygg (1949), Kelley et al. (1950), and Booth (1951). The latter found carotene content, regardless of variety, age or initial pigmentation, to increase by 11 percent in about sixty days and to decrease thereafter. According to Lipton, there is strong evidence that there is an increase in carotene concentration in carrots following harvest. Both Lipton and Booth suggested that the apparent increase in carotene was due to the presence at harvest of unconverted precursors which are changed in the roots during storage to measurable carotenes.

Observations indicated that carrots which are stored with apples frequently become bitter. Therefore, it was considered advisable to review briefly the literature relative to apple emanations. Difficulties resulting from the storage of carrots with apples have not been reported. However, apples have caused certain problems in other commodities stored with them.

Curtis and Rodney (1953) found that dormant nursery stock in cold storage could be damaged in the presence of ethylene gas in the storage atmosphere at concentrations as low as 1 ppm. Cambial proliferation followed by death required about two months at 35° F., and occurred in about ten days at 55° F.

The source of ethylene in one case seemed to be from an apple storage on the other side of a well insulated wall.

Apples and many other fruits as they ripen produce ethylene and other volatiles. Gane (1934) indicated from biological tests that one apple would produce a volume of about 1 ml. of ethylene, and that the amount would vary with variety and size of the fruit. This was confirmed chemically by Hansen and Christensen in 1939.

Smock (1943) demonstrated that stored apples influenced the rate of ripening of other apples stored with them. He indicated that although ethylene did have an effect, other unknown volatile materials from apples were probably responsible for storage scald and other apple troubles.

Several sources of ethylene other than ripening fruits have been established. Young, Pratt and Biale (1951) identified ethylene as a volatile product of the fungus Penicillium digitatum. The same organism has been found to be a minor storage disease of carrots according to Rader (1952).

Certain diseased tissues have been shown by Williamson (1951) and Ross and Williamson (1951) to produce physiologically active emanations in greater amounts than those produced by comparable healthy tissues. Emanations were biologically evaluated for physiological activity by a pea seedling test and were presumed to be ethylene. The increased evolution of volatiles was apparently a response to injury and occurred only as long as the infected tissue was alive. In Physalis floridana infected with potato virus Y, they found greater ethylene production at 70° F. than 80° F. which corresponded with an increased incidence of necrosis. Necrotic lesions were induced in leaves of P. floridana and Nicotiana Tobacum by treatment with phytotoxic chemicals, eg. copper sulfate; which also increased ethylene production.

Denny (1935) showed that immature and maturing fruit, seeds in green pods, parts of flowers, leaves, stems and roots produced volatiles which caused epinasty of young potato plants similar to that obtained with low concentrations of ethylene. Pratt (1954) gave direct chemical proof of ethylene production by detached leaves. Fifty-three pounds of thistle leaves produced 16.8 ml. of ethylene in two experimental periods of four days each. The presence of unsaturated compounds with physiological action similar to ethylene in self-blanching celery was found by Nelson and Harvey (1935). Non-self-blanching varieties did not have these compounds.

In summarizing the effects of ethylene on fruits, Thornton (1940) listed the destruction of chlorophyll thereby allowing the characteristic ripe color to predominate as in citrus and tomatoes. Respiration rate is speeded as well as certain metabolic changes, for instance the conversion of starch to sugar in the ripening of the banana.

Severe internal brown spotting of lettuce has resulted from storage with apples at 38 and 44° F. according to Rood (1956). The ethylene fraction of the atmosphere was determined as the agent responsible for the damage. Tests with pure ethylene at 20 ppm. produced severe symptoms at 38 and 44° F. in a few days but not at 32° F.

Biochemical Composition Studies

According to Atkin (1956) processors have observed that bitter carrots are lighter in color than non-bitter carrots. He divided ten lots of carrots from variety trials each into three sampels. One sample was canned at harvest and the other two following refrigerated and common storage respectively. Bitterness was found only in the sample stored under refrigeration, which was also less red in color than the other two as measured by a Hunter Color Difference Meter.

Truscott (1954) reported two off-flavors in processing carrots. One he described as hot and peppery, the other as bitter. Both were decidedly objectionable in the raw product. The hot and peppery flavor did not persist after cooking so was not considered a problem for processing carrots. The bitter flavor, however, did remain after cooking. By placing carrot slices in beakers of boiling water and evaluating a peculiar "flat" aroma arising, he felt that he was able to arrange the samples in order of bitterness intensity. The use of this test has not been reported in subsequent literature. Following overnight storage of the raw carrots in the laboratory at room temperature little bitterness could be found. From this experience, he suggested a practice of high temperature, ventilated conditioning of bitter carrots for several days before they were to be processed.

Yamaguchi et al. (1955), Sondheimer et al. (1955) and Atkin (1956) all reported the bitter flavor to be found in phloem tissues, not the xylem.

A syrupy bitter glycoside has been isolated from leaves of wild carrots by v. Gizycki and Hermanns (1951), but was not identified. From seeds and stems of red carrots, Reeb (1923) reported a bitter glucoside which he called daucusin. Neither of the substances was crystalline so their identity remains questionable.

Dodson et al. (1956) reporting on isolation and identification studies on a bitter principle isolated from the roots of bitter tasting carrots found it to have a molecular weight of 268. Percentage composition of carbon and hydrogen were 63.94 and 5.68 respectively. An emperical formula of $C_{15}H_{15}O_5$ was given to the compound. The ultra violet absorption spectra was found to have a peak at 268 mμ and minima at 242 and 287 mμ. This is slightly in variance with the 265, 240 and 290 mμ values given by Sondheimer et al. (1955) for their tentative spectrophotometric procedure. Paper chromatographic Rf values in five solvents were the same for the bitter crystalline principle and for acetone extracts of bitter carrots. Spots were detected on filter paper by their fluorescence in ultra violet light.

Blue fluorescence in roots of carrot seedlings was reported by Goodwin and Kavanagh (1948) when tissues were exposed to 3650Å ultra violet light.

Zechmeister and Sandoval (1945) separated chromatographically a pale orange oily substance, phytofluene, from carrot root extracts. This substance was found to have a greenish fluorescence under ultra violet light. Another polyene which was colorless was reported by Porter and Zscheile (1946) and was obtained in the same manner from carrots.

Goodwin and Kavanagh (1950, 1952) noted an increase in fluorescence intensity in solutions at pH levels of 8 to 10 and above with a number of fluorescent compounds, especially coumarin derivitives.

Sondheimer, Phillips and Atkin (1955) reported a high spectrophotometric absorption peak of bitter carrot extracts in the ultra violet range at 265 mμ and a lower, but always present peak at 290 mμ. Extracts from non-bitter roots show little or no absorption from about 220 mμ up to about 400 mμ. They also found a bitter, orange residue upon evaporation of the solvent from petroleum either extracts of bitter carrots.

Chemical analyses by Yamaguchi et al. (1955) showed less α-carotene, 4.8 mg per 100 grams, in bitter carrots than non-bitter, 7.3 mg per 100 grams. β-carotene, total sugars, starch and protein showed negligible differences as did analyses for calcium, iron, phosphorus and vitamins C, B_1, B_2 and niacin. They concluded that the large α-carotene difference between bitter and non-bitter carrots, plus petroleum ether solubility and magnesium oxide column adsorption of the bitter principle, suggested that bitterness may be caused by metabolic products of the carotenoids.

CHAPTER III

STATEMENT OF PROBLEM

This investigation involved studies on the occurrence, measurement, and possible methods of control of bitterness in fresh market and processing carrots.

The role of variety, genetic selection, soil type, mineral nutrition, physiological age, harvest and post-harvest handling, and storage treatment in relation to bitterness are aspects of the problem that will be evaluated.

The primary objectives were the identification of conditions resulting in carrot bitterness and the development of usable controls or preventative measures.

CHAPTER IV

METHODS OF BITTERNESS EVALUATION

For the evaluation of bitterness in these experiments, organoleptic, spectrophotometric, and fluorescence tests were used.

Organoleptic Test

Tasting was used in practically all of the studies conducted, either by a panel or by the writer. Carrots were selected at random from each treatment, washed, peeled, quartered and coarsely ground and mixed for taste testing. Spectrophotometric analyses were made on the same samples. Except for one preliminary experiment where samples were dried, all lots were handled in the fresh state at room temperature. Treatments were replicated and rated in comparison with reference samples by taste panels which ranged from one to thirty persons depending upon the experiment.

A rating scale of 1 to 5 was devised with the following designations: 1- not bitter; 2- just detectably bitter; 3- moderately bitter; 4- strongly bitter; and 5- exceedingly bitter. A rating form, Figure 1, was used and a summary sheet compiled. Each bitterness intensity level was given a weighted value. Ratings from all tasters were totaled and multiplied by their scale values. For each treatment these products were summed and divided by the number of persons participating, as shown by the example in Figure 1. Sample A would be given an over-all rating of 2.0, or just detectably bitter. Sample B, with an over-all rating

TASTE TEST RATING FORM

Name_____ Date _____ Experiment_____

Sample	Not Bitter				Most Bitter	Comments
	1	2	3	4	5	
X						
A						
B						
C						
D						

Instructions: Rate samples as to their bitterness in comparison with Sample X which has already been checked. Thank you.

SUMMARY TABULATION OF ORGANOLEPTIC RATINGS

Sample	Bitterness Intensity Rating and Value					Total	Average
	1	2	3	4	5		
A	4	18	6	4		32/16	2.0
B		4	15	28	10	57/16	3.6
C	16					16/16	1.0

Figure 1. Rating Form for Organoleptic Bitterness Evaluation and Example of Summary Tabulation.

of 3.6 would be quite bitter, ranging between moderate and strong. Sample C would be recognized by all tasters as non-bitter.

This rating system is considerably different from that used by Sondheimer et al. who rated bitterness on a 1 to 10 basis with 1 indicating the highest level of bitterness. A subsequent report by Atkin, referring to the same experimental data rated bitterness by taste ranging from 0 to 9 with the 0 on the non-bitter end.

Spectrophotometric Tests

The spectrophotometric procedure developed by Phillips (1954) was used to supplement taste test evaluations in the early studies, but because of its elaborate extraction procedure, the laboratory evaluation capacity was limited to about eight samples a day.

The basic steps were:

> (1) extraction with acetone in a blender, (2) centrifugation to remove all solids, (3) dehydration by combination with anhydrous Na_2SO_4, (4) transfer of bitter principle to Skellysolve B by separation with a separatory funnel, (5) concentration by evaporation, (6) chromatographic separation on a 2:1 by weight MgO:Celite column under vacuum followed by washing with Skellysolve B, (7) removal of the upper portion of the column on which the bitter principle is adsorbed, (8) elution with spectrograde methyl alcohol, (9) determination of transmittance values at 248, 268, and 290 mμ, and (10) the plotting of points on plain graph paper (10 x 10 to the inch). Measurement of the depression in cm. of the 268 mμ value below a line drawn between the 248 and 290 mμ values was fitted to a formula to give "degrees of bitterness" (B°).

A B° score below 5 to 7 was considered non-bitter, above 10 definitely bitter.

After May 1956 treatments were evaluated by using a modification of this method by Sondheimer, Phillips and Atkin (1955) which allowed laboratory capacity to be increased to approximately 50 samples a day.

Modified spectrophotometric method:

> (1) Samples were pureed in a blender, (2) 5 gram aliquots were placed in a 50 ml ground glass stoppered Erlenmeyer, then (3) shaken with 40 ml of spectro grade Skellysolve B, and (4) measured directly in a Beckman DU spectrophotometer and later a Beckman DK2 Recording spectrophotometer. (5) Light absorption (optical density) at 240, 265, and 290 mμ was measured. Values were fitted to a formula which gives a bitterness reading in terms of "height of 265 mμ peak" as follows:
>
> $$\frac{\text{o.d. at 265 m}\mu - \frac{\text{o.d. at 290 + o.d. at 240 m}\mu}{2}}{\text{grams of sample}} \times \text{ml solvent}$$
>
> A height of 265 mμ peak of about 0.75 would be close to the taste threshold of bitterness.

A typical bitterness absorption curve is shown in Figure 2.

Fluorescence Test

In January 1956 it was observed that bitter carrots fluoresced in short wave ultra violet light (2537Å) and that this phenomenon was correlated ($r = 0.64$) significantly with their bitter taste. Since fluorescence correlated with taste testing about as well as the spectrophotometric methods did, fluorescence estimations were used as a third means of evaluation. Fluorescence appeared yellowish-green and was patterned in tiny spots present only in the phloem region which was also the site of bitterness by organoleptic and spectrophotometric observations.

The system of rating devised paralleled the taste test rating on a 1 to 5 basis with 1 being non-bitter and non-fluorescent, and 5 being exceedingly bitter and highly fluorescent. Vertical sectioning of the roots gave the best representation of fluorescence concentrations and distribution.

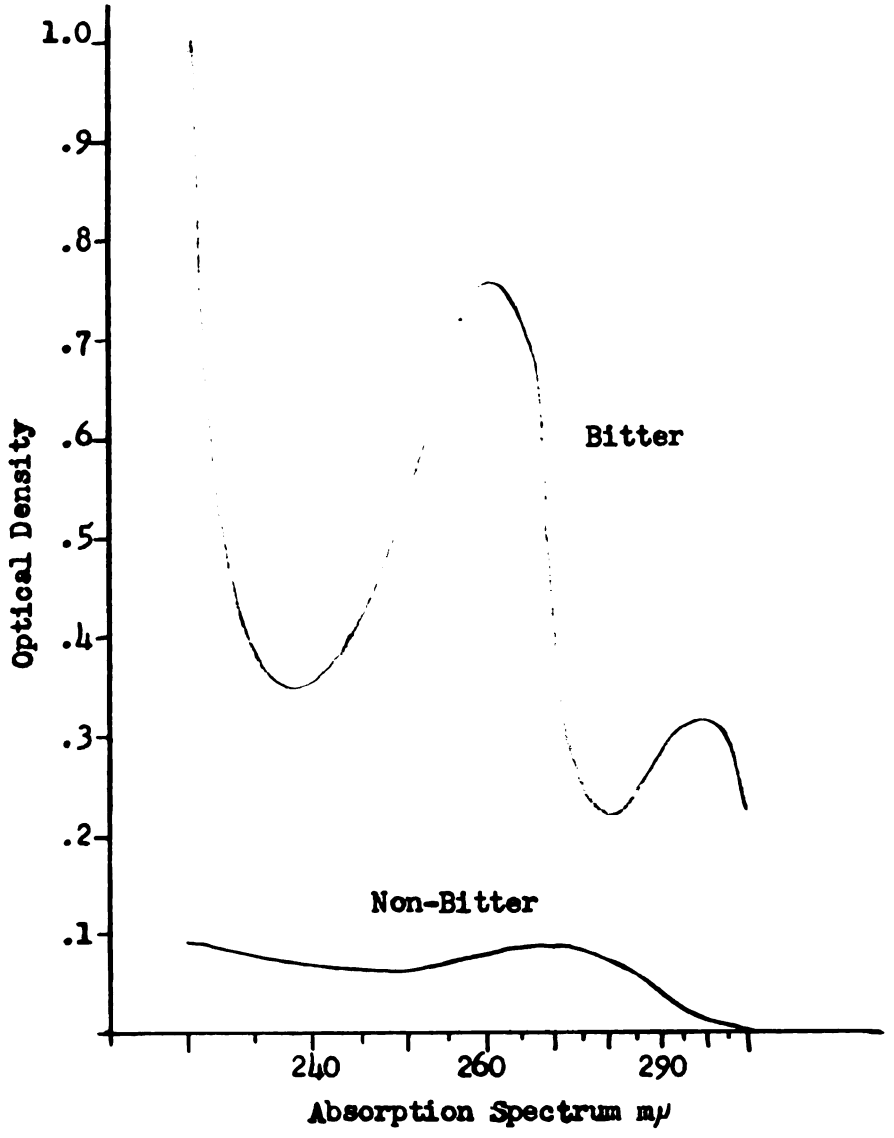

Figure 2. Typical Spectrophotometric Absorption Curves for Hexane Extracts from Bitter and Non-Bitter Carrots.

CHAPTER V

THE OCCURRENCE OF BITTERNESS IN CARROT VARIETIES AND STRAINS AS AFFECTED BY SOIL, LOCATION AND STORAGE

Introduction

In this study the range of bitterness susceptibility within the varieties and strains of carrots was investigated. It had been noted that reports of bitterness primarily involved carrots stored for processing, of which Red Core Chantenay and its strains are the most important. This suggested that these strains should be surveyed as well as market types which could be used for either purpose.

Most of the processing carrots and a large part of the fresh market carrots in Michigan are grown on muck soils. Lipton (1953) and Newcombe and Alderman (1944) indicated that quality of carrots grown on mineral soils was generally superior to those grown on muck, therefore, bitterness responses of carrots from both soils were evaluated.

It had also been observed that bitterness more often appeared in some storages than others which directed attention to the influence of different storage conditions.

1954 Survey

Methods and Materials

Carrot varieties were available in the fall of 1954 from one mineral and two muck soils. Eight varieties and strains were selected from each

source. Five of the University muck and mineral trial lines were identical. Bulk lots were obtained at harvest and were held in a 32° F. storage. Eight 5-pound samples of each of the 24 lots were packed in 8-pound polyethylene bags, half of which were sealed and the remainder punched for ventilation. Duplicate samples were placed in two storage rooms at 32 and 40° F. In addition, samples from the muck trial near Grant, Michigan which had been held from harvest in two commercial storages were transferred in December to a 32° F. storage at East Lansing.

All carrots remained in storage until April 7 to 16, 1955 when they were removed and evaluated with a hand refractometer for soluble solids content, and subsequently processed in No. 1 carrot enamel cans. Canning was used to eliminate differences that might be due to variations in length of storage resulting from use of the complicated original spectrophotometric evaluation procedure and in running statistically comparable taste tests.

Bitterness evaluations were actually delayed until September 1956 when the rapid spectrophotometric test modified by Sondheimer et al. could be run on the canned samples.

Results

Carrots in ventilated polyethylene bags stored at 40° F. gave the highest spectrophotometric bitterness ratings, as shown in Table 1. In the University mineral and muck trials, the early maturing varieties, Nantes and Touchon became much more bitter at 40° F. in punched bags than the late varieties Gold Spike and Imperator. The second-early Chantenay strains rated close to Nantes and Touchon in bitterness response.

TABLE 1

BITTERNESS RATINGS AS INFLUENCED BY VARIOUS FACTORS

(Height of 265 mμ peak[1])

Source, Varieties & Strains	Storage Temperatures 32° F. Ventilated	32° F. Sealed	40° F. Ventilated	40° F. Sealed	Averages
A. Mineral Soil-M.S.U.					
Nantes (a)[2]	.32	.20	4.42	.18	1.23
Touchon (b)	.55	.24	2.94	.42	1.04
Chantenay (c)	.64	.30	1.90	.36	.80
Nantes	.18	.21	2.21	.21	.70
Danvers	.50	.20	1.48	.39	.64
Danvers (d)	.60	.22	.70	.22	.44
Long Chant.	.52	.18	.64	.26	.40
Gold Spike (e)	.22	.12	.56	.08	.24
Averages	.44	.21	1.86	.26	.69
B. Muck Soil-M.S.U.					
Nantes (a)	.27	.27	1.21	.22	.50
Touchon (b)	.25	.10	2.21	.18	.68
Chantenay (c)	.28	.33	.97	.18	.44
Danvers (d)	.20	.15	.53	.10	.24
Gold Spike (e)	.31	.20	.55	.15	.30
Royal Chant.	.11	.19	1.39	.23	.48
Red Core Chant.	.17	.22	1.20	.27	.46
Imperator	.25	.28	.61	.28	.36
Averages	.23	.23	1.09	.20	.43
C. Muck Soil-Grant					
Chanticler	1.86	.55	1.73	.61	1.19
Red Core Chant.	.53	.48	2.18	.37	.89
Royal Chant.	.55	.67	1.42	.32	.74
Short Type Chant.	.60	.60	1.01	.73	.74
Red Core Chant.	.41	.43	1.25	.78	.72
Long Type Chant.	.42	.78	1.10	.50	.70
Long Type Chant.	.58	.66	1.09	.37	.68
Short Type Chant.	.44	.29	.64	.39	.44
Averages	.55	.56	1.36	.51	.76

1 Spectrophotometric values are expressed as height of 265 mμ absorption peak and for this experiment were determined on canned samples. Carrots with values below 0.75 are probably not bitter to taste.

2 Varieties with the same letter in the University mineral and muck trials came from identical seed sources.

Comparing the three sources, the highest over-all average was found in the carrots from the muck trial at Grant. The highest individual bitterness ratings, however, were in the early maturing varieties in the University mineral soil trial which also included the widest differences in bitterness response among varieties. The lowest bitterness average was in carrots from the University muck trial.

Carrots from the Grant muck stored by the Gerber company in the two commercial storages produced a marked difference in bitterness. The average bitterness rating for the eight Chantenay strains in storage A was 1.29 while in B the value was 0.72. Carrots and apples had been stored together in A. In B, carrots were stored alone. Bitterness response could not be separated on the basis of long and short types of Chantenay.

Data in Table 2 on soluble solids show a general decrease where carrots were stored at 40° F. as compared to 32° F. At 32° F. the percent of soluble solids was lower in carrots from sealed than from ventilated bags while at 40° F. there was little consistent difference.

For the three sources, percent soluble solids was highest - 6.06 for carrots from the mineral soil, followed by 5.66 and 4.32 for the University and Grant mucks respectively. The highest soluble solids values for carrots from the Grant muck were below the lowest from the University muck and mineral trials.

Varietal differences in soluble solids from the mineral soil were not consistent; but from the University muck soil, the late varieties, Gold Spike and Imperator averaged much higher in soluble solids than the earlier maturing varieties.

TABLE 2

VARIATION IN THE SOLUBLE SOLIDS CONTENT OF CARROTS AS INFLUENCED BY SOURCE, STORAGE TEMPERATURE AND PACKAGE VENTILATION. (Percent)

Source, Varieties & Strains	Storage Temperatures 32° F. Ventilated	32° F. Sealed	40° F. Ventilated	40° F. Sealed	Averages
A. Mineral Soil-M.S.U.					
Nantes (a)[1]	6.75	6.20	5.80	5.75	6.12
Touchon (b)	6.30	6.05	4.40	5.65	5.60
Chantenay (c)	7.40	6.10	5.80	5.60	6.22
Nantes	7.25	6.20	6.50	5.55	6.38
Danvers	6.90	6.80	6.10	5.65	6.36
Danvers (d)	6.25	6.85	5.00	5.35	5.86
Long Chant.	6.90	6.05	6.15	5.05	6.04
Gold Spike (e)	6.60	5.90	5.50	5.60	5.90
Averages	6.79	6.27	5.66	5.52	6.06
B. Muck Soil-M.S.U.					
Nantes (a)	5.90	5.62	4.88	5.05	5.36
Touchon (b)	6.10	6.12	5.80	5.65	5.92
Chantenay (c)	5.70	5.62	5.38	5.60	5.58
Danvers (d)	6.10	6.12	4.75	5.50	5.62
Gold Spike (e)	7.70	6.00	5.65	6.35	6.42
Royal Chant.	5.05	5.00	4.80	4.60	4.86
Red Core Chant.	5.25	5.25	5.75	5.15	5.35
Imperator	7.60	6.62	5.25	5.85	6.33
Averages	6.18	5.79	5.28	5.47	5.66
C. Muck Soil-Grant					
Chanticler	4.85	4.00	4.35	3.70	4.22
Red Core Chant.	4.50	4.75	4.35	4.50	4.52
Royal Chant.	4.50	4.15	3.85	3.70	4.05
Short Type Chant.	5.15	4.40	3.80	4.00	4.34
Red Core Chant.	4.35	4.00	3.70	3.70	3.94
Long Type Chant.	5.05	5.00	-	-	-
Long Type Chant.	4.50	4.95	4.35	4.65	4.61
Short Type Chant.	4.90	4.90	4.20	4.35	4.59
Averages	4.72	4.52	4.08	4.08	4.32

[1] Varieties with the same letter in the University mineral and muck trials came from identical seed sources.

From the muck trial at Grant, soluble solids differences between long and short Chantenay types were less than within the respective types. The long-short type characteristic is apparently not a valid indicator for soluble solids contents. Data for bitterness and soluble solids appear highly variable and not related.

1955 Trials

Methods and Materials

The 1955 trials involved chiefly processing types and included seed of several of the strains and varieties evaluated in 1954.

Seventeen seed lots were planted at the University muck farm June 2, three at the Horticulture farm on Hillsdale sandy loam June 16 both in replicated, randomized, complete block designs. Six more were planted in early May by the Gerber Products Company on muck soil near Grant, Michigan.

Carrots from the University muck trial were dug by hand and mechanically topped October 18. Diseased and damaged roots were discarded and samples of sound carrots placed in 10 pound onion bags. Carrots from the mineral soil were dug and topped by hand November 3. Samples from both trials were stored under refrigeration at 32-35° F. until bitterness was evaluated during July 1956. There was some loss due to mold growth, but in general, the carrots were in acceptable condition.

The carrots planted at Grant were mechanically dug and topped September 28, 1955. About one ton of each strain was stored at 32° F. in each of the two commercial storages compared in 1954. Samples were removed to the carrot storage in East Lansing in January 1956. At that time, bitterness was detected in some lots.

Results

No bitterness was detected in stored carrots from the mineral soil trial (Table 3). Their color and flavor were superior to that of any of the muck grown varieties. A low to moderate level of bitterness developed in the carrots from the University muck farm. The highest level of bitterness developed in the carrots from the muck trial at Grant.

Variety and strain differences were apparent in both muck trials. Red Core Chantenay (Coreless) from Northrup, King was by far the most bitter in the group of six strains grown in both places. No strains were consistently low in bitterness from both muck trials. In the University muck trial, the two market varieties, Imperator and Gold Spike, were rated as essentially non-bitter. Three Chantenay strains were rated as low.

By comparing bitterness development by taste test in long and short Chantenay strains, it was found that the short type tended to be more bitter than the long in both muck trials (Table 4). Short strains grown on University muck averaged 2.62 by taste test against 2.15 for the long strains. The Grant muck showed 3.50 for the shorts and 2.62 for the longs. The range for bitterness went from slight to moderate in each group, however.

Muck trial carrots placed in the two commercial storages again revealed a significant difference between storages (Table 5). Carrots in storage A became markedly more bitter than in storage B with taste test values of 3.17 and 2.67 and spectrophotometric values of 2.95 and 2.52 respectively. As in 1954, carrots and apples had been stored together in storage A. Apples were not in the carrot room in storage B but were stored in adjoining rooms.

TABLE 3

BITTERNESS RATINGS OF CARROT VARIETIES AND STRAINS, 1955-56

Variety or Strain	Seed Source	Bitterness Ratings		
		University Muck Taste Test 1-5 Rating(1)	Grant Muck Taste Test 1-5 Rating(1)	Grant Muck Spectrophotometric Ht. 265 mμ peak(2)
Red Core Chantenay	Northrup,King	3.3	4.5	3.83
Long Type Chantenay	Woodruff	2.7	2.5	1.59
Short Type Chantenay	Woodruff	2.3	2.5	1.40
Royal Chantenay	Northrup,King	2.2	2.0	3.31
Royal Chantenay	Corneli	2.0	2.5	3.39
Long Type Chantenay	Corneli	1.7	3.5	2.89
Red Core Chantenay	Harris	3.5		
Red Core Chantenay	Asgrow	3.0		
Chanticler	Asgrow	2.7(3)		
Red Core Chantenay	Corneli	2.5		
Long Type Chantenay	Northrup,King	2.5		
Long Type Chantenay	Ferry-Morse	2.2		
Short Type Chantenay	Ferry-Morse	2.0(3)		
Chantenay	Northrup,King	1.7		
Imperator Long Type	Ferry-Morse	1.7		
Long Chant. Special	Harris	1.3		
Gold Spike	Ferry-Morse	1.3(3)		
Averages		2.41	2.92	2.73

(1) Taste test values of 1 - non-bitter, 5 - exceedingly bitter. Panel of 6.

(2) Spectrophotometric values expressed as height of 265 mμ absorption peak. Carrots with values below 0.75 are probably not bitter to taste.

(3) These three varieties were also tested on Hillsdale sandy loam. No bitterness could be detected after storage by taste or spectrophotometric tests.

TABLE 4

BITTERNESS RATINGS OF FIFTEEN RED CORE CHANTENAY CARROT STRAINS COMPARED BY LONG AND SHORT ROOT TYPES, 1955-56

Root Type	Strain	Source	Bitterness by Taste (1-5)	Averages (1-5)
Long				
	Long Type Chantenay	Ferry-Morse	2.2	
	Long Type Chantenay	Corneli	1.7	
	Long Type Chantenay	Northrup,King	2.5	
	Long Type Chantenay	Woodruff	2.7	
	Long Chantenay Special	Harris	1.3	
	Chanticler	Associated	2.7	
	Royal Chantenay	Corneli	2.0	
	Royal Chantenay	Northrup,King	1.2	2.1
Short				
	Short Type Chantenay	Ferry-Morse	2.0	
	Short Type Chantenay	Woodruff	2.3	
	Red Core Chantenay	Associated	3.0	
	Red Core Chantenay	Harris	3.5	
	Red Core Chantenay #2	Corneli	2.5	
	Red Core Chantenay, Coreless	Northrup,King	3.3	
	Chantenay	Northrup,King	1.7	2.6

TABLE 5

BITTERNESS RATINGS OF SIX STRAINS OF RED CORE CHANTENAY CARROTS STORED IN TWO LOCATIONS IN 1955-56

No.	Strain of Chantenay(3)	Bitterness Ratings: Taste Test(1) Storages A	B	Averages*	Height of 265 mμ peak(2) Storages A	B	Averages
1	Royal	2	2	2.0	3.28	3.34	3.31
2	Long Type	4	3	3.5	3.15	2.62	2.89
3	Royal	3	2	2.5	4.95	1.83	3.39
4	Red Core	5	4	4.5	3.16	4.50	3.83
5	Short Type	2	3	2.5	1.44	1.36	1.40
6	Long Type	3	2	2.5	1.69	1.50	1.59
	Averages	3.17	2.67	-	2.95	2.52*	-

(1) Taste test ratings on a 1-5 basis with 1 - non-bitter and 5 - exceedingly bitter. Panel of 6.

(2) Spectrophotometric rating of bitterness in terms of height of 265 mμ absorption peak with a bitterness taste threshold at about 0.75. Since these tests were obtained from dried carrot tissues, values are a little lower than would be expected from this procedure if run on fresh tissues.

(3) Source of Chantenay strains: 1- Northrup,King; 2- Corneli 1951; 3- Corneli 3355; 4- Northrup,King, Coreless; 5- Woodruff, BG 1-5520; 6- Woodruff, BG 1-6510.

* Statistical differences significant at the 5 percent level for storages under the spectrophotometric test and closely approaching the 5 percent level for varieties under the taste test.

Discussion of the 1954 and 1955 Trials

Variety and strain differences in bitterness response were clearly present with the earliest maturing lines developing the most bitterness. Nantes and Touchon, which reach market maturity about 10 days earlier than most strains of Red Core Chantenay, definitely were more susceptible to becoming bitter. Imperator and Gold Spike, which require about a week longer to reach market maturity than Red Core Chantenay strains, consistently developed the least bitterness. Although the bitterness response of the Red Core Chantenay strains varied from almost non-bitter to exceedingly bitter, most tended to be close to the response of the early varieties, in other words, quite bitter. One strain, Red Core Chantenay-Coreless from Northrup, King, was the most bitter of the Red Core Chantenay strains in all three of the tests in which it was included. Chanticler, an Associated strain rated highly bitter in two trials. Three strains (Long Type Chantenay - Corneli, Chantenay - Northrup, King, and Long Chantenay-Special - Harris) rated essentially non-bitter in a single comparison (University muck trial - 1955). Although 1954 trials showed no differences between averages of long and short type strains, in 1955 more bitterness was found in the short types.

Atkin in 1956 cautiously indicated that muck soils produced more bitterness in carrots than mineral soils. This view was supported in general by observations of processors; however, data from this study

does not support this contention. Of all the 1954 comparisons, the most intense bitterness developed in carrots from mineral soil. In 1955, mineral grown carrots were not bitter while some muck carrots were exceedingly bitter. In both the 1954 and 1955 trials the highest levels of bitterness were found in the earliest harvested carrots regardless of soil type effects, with the conclusion that muck carrots are not necessarily more bitter than mineral grown carrots. This suggests that date of harvest is a more important factor.

This study indicated that mineral soil grown carrots contain more soluble solids than muck grown carrots, agreeing with the work of Lipton. There was a lack of correlation between sugar content and bitterness.

The 1954 temperature-packaging study revealed that only the carrots stored at 40° F. became bitter. Almost all of the bitterness developed in carrots in polyethylene bags which had been punched for ventilation. Although carrots in the 32° F. room were essentially non-bitter, spectrophotometric data showed generally higher values in punched bags, as found at 40° F.

Two possibilities are apparent to explain the interaction. First, the reaction to form the bitter principle operates as a function of temperature, with carrots in sealed bags inhibited from developing bitterness because of high CO_2 and/or low O_2 content. Second, the incidence of bitterness could be a function of presence of physiologically active volatiles in the storage atmosphere affecting carrots in vented bags but not those in sealed bags.

Varietal differences were also present in respect to the temperature-ventilation interaction in that the earliest maturing varieties became most bitter while the late varieties were almost free from bitterness.

Commercial storage A resulted in more bitterness in processing carrots in both years of the study than storage B. The observed difference in management between them was the inclusion of apples and carrots in the storage A. Apples were kept in the same building but not in the same room in B. It is suggested that the higher incidence of bitterness in A was due to the presence of physiologically active volatiles emanating from the stored fruit.

CHAPTER VI

STUDY OF INHERITANCE OF CARROT BITTERNESS

It has been reported by Atkin (1956) and observed by the writer that bitterness frequently varies markedly from root to root within the same variety and strain. This suggests the possibility of genetic differences which might be eliminated during a breeding program. To determine the presence of a genetic factor, both bitter and non-bitter roots would have to be selfed or grown in isolation and their progeny evaluated.

Methods and Materials

In the winter of 1955, bitter, long-type Chantenay carrots were screened for five intensities of bitterness. The rating of 1- non-bitter to 5- exceedingly bitter was based on a taste evaluation of chips removed from the phloem region of the roots.

Each intensity level included 20 roots which were planted in pots in the greenhouse. Those which survived were moved to a cold frame and later to the field. At flowering, bags were placed over each plant enclosing at least two umbels. Fly pupae were introduced as often as necessary for about two weeks to promote pollination. Seven plants from all five levels of bitterness produced viable seed.

For evaluation of this first inbred generation, seed was sown on muck soil in May 1956 and the carrots harvested in mid-September. After storage in a refrigerated root cellar at about 35° F. for four and a half months, bitterness evaluations were made.

Results and Discussion

The results, Table 6, indicate that bitterness was not present to any appreciable extent. Despite the negative results, this experiment does not allow the conclusion that bitterness either is or is not genetically controlled. As observed in other studies, weather during root development was apparently more influential in affecting bitterness than any treatment applied. Also, very little bitterness was detected in the entire commercial processing crop in 1956, and in addition, the carroty flavor was generally low and in some cases undetectable.

Work reported by Yamaguchi et al. (1955) on comparison of bitter and non-bitter progenies indicated that the bitter selections produced strong and slightly bitter roots while the non-bitter selections produced mild flavored roots. Thus it would appear that selection of roots on the basis of carroty flavor, strong versus mild, might produce differences in bitterness response. Strong flavored roots are objectionable, yet much of this flavor is dissipated in cooking by volatilization; on the other hand, carrots which are too mild, lack characteristic carrot flavor. It is possible that a low content of these flavoring materials may not only result in the development of bitterness, but also affect keeping quality in storage, particularly favoring the development of Sclerotinia as evidenced by the poor flavor and high incidence of disease in the 1956 crop.

TABLE 6

BITTERNESS RATINGS OF SELECTED INBRED CARROTS, 1956 CROP
(Rated February 14, 1957 by the Author)

Original Bitterness Rating by Taste (1-5 basis)	Inbred Plot Number 1956	Bitterness Ratings		
		Taste Test (1-5 basis)	Fluorescence (1-5 basis)	Spectro-photometric (Ht 265 mμ peak)
1	476	1.0	1.0	0.24
1	477	2.0	1.0	0.10
2	478	1.0	1.0	0.20
2	480	2.0	1.0	0.19
3	481	1.0	1.0	0.24
4	482	2.0	1.2	0.14
5	483	1.0	1.0	0.12
Wild*	484	1.0	1.0	0.31

* This was not an inbred, but was grown from locally harvested wild carrot seed planted and handled with the inbreds.

CHAPTER VII

BITTERNESS STUDY ON SEEDING DATE AND DEVELOPMENT TRENDS THROUGHOUT THE STORAGE SEASON

The stage of physiological maturity at harvest has been suggested by Drewes (Reath, 1953) as a possible factor contributing to the subsequent development of carrot bitterness.

Red Core Chantenay strains are usually used for processing while Imperator types are grown for the fresh market; however, both may be used for either purpose. Carrots for processing in the northern states are seeded as early as feasible and to obtain maximum yields, are left in the field until freezing weather. As Red Core Chantenay strains tend to mature earlier than Imperator types, they are frequently overmature at harvest. Market carrots, however, are seeded by schedule to provide a continuous supply of tender maturing roots during the season. Bitterness has been more frequently observed in canning than fresh market carrots, which may possibly be due to differences in their physiological ages.

Methods and Materials

Two strains of Red Core Chantenay carrots, Short Type Chantenay (Ferry-Morse) and Chanticler (Associated), and Gold Spike were seeded on muck May 27 and July 6, 1955 in a complete randomized block design. Some bolting appeared in the May planted Chantenay and Chanticler varieties. All plants with flower stalks were removed prior to harvest on October 19.

The carrots were dug and topped by machine and were sorted to eliminate diseased and badly damaged roots. Two bushel lots were stored at 32° F. from November 1 until July 10, 1956. Samples were removed at monthly intervals and were evaluated for bitterness by a panel and by spectrophotometric analysis to obtain a pattern for bitterness development during the storage season. Fresh weight, dry weight and ash content were obtained at the first evaluation period. Total sugars and nitrogen content for each period were determined by the Agricultural Chemistry Department.

Results and Discussion

At harvest, all samples were comparatively non-bitter by taste test; however, Chanticler from the early planting had a tendency to develop more bitterness during the storage season than Short Type Chantenay (Table 7). By taste test the average bitterness value of Short Type Chantenay was 2.73 for the early and 2.15 for the late seeding, while Chanticler was 3.12 and 1.87 respectively. Spectrophotometric values in "degree of bitterness" from the analytical procedure by Phillips (1954) were 4.57 and 5.24 for Short Type Chantenay and 6.24 and 1.97 for Chanticler for early and late plantings.

Monthly bitterness ratings by the taste panel gave relatively constant values through the storage season as shown in Table 7 and Figure 3. Carrots from early seedings of both strains were more bitter than from late seedings. Roots from late seedings of both strains were just detectably bitter with Chanticler tending to be slightly less bitter than Chantenay; however, in roots from the early seedings, Chanticler was

TABLE 7

STORAGE INFLUENCE ON BITTERNESS RATINGS OF TWO STRAINS OF CARROTS SEEDED ON TWO DATES, 1955 CROP

Chantenay Strain	Seeding Date	Bitterness Ratings - Taste Test (1-5 Scale)(1)						
		Dec.	Jan.	Feb.	Mar.	April	May	Averages
Short Type	Early	3.1	2.2	2.8	2.4	2.6	3.3	2.73
Short Type	Late	2.5	1.8	2.2	2.0	2.0	2.4	2.15
Chanticler	Early	2.0	3.0	3.6	3.1	3.6	3.4	3.12
Chanticler	Late	1.8	1.8	2.0	2.0	1.7	1.9	1.87

(1) Bitterness ratings by taste test: 1 = non-bitter, 5 = exceedingly bitter. Number of members in panel: Dec. 15, Jan. 14, Feb. 24, Mar. 24, April 15, and May 20.

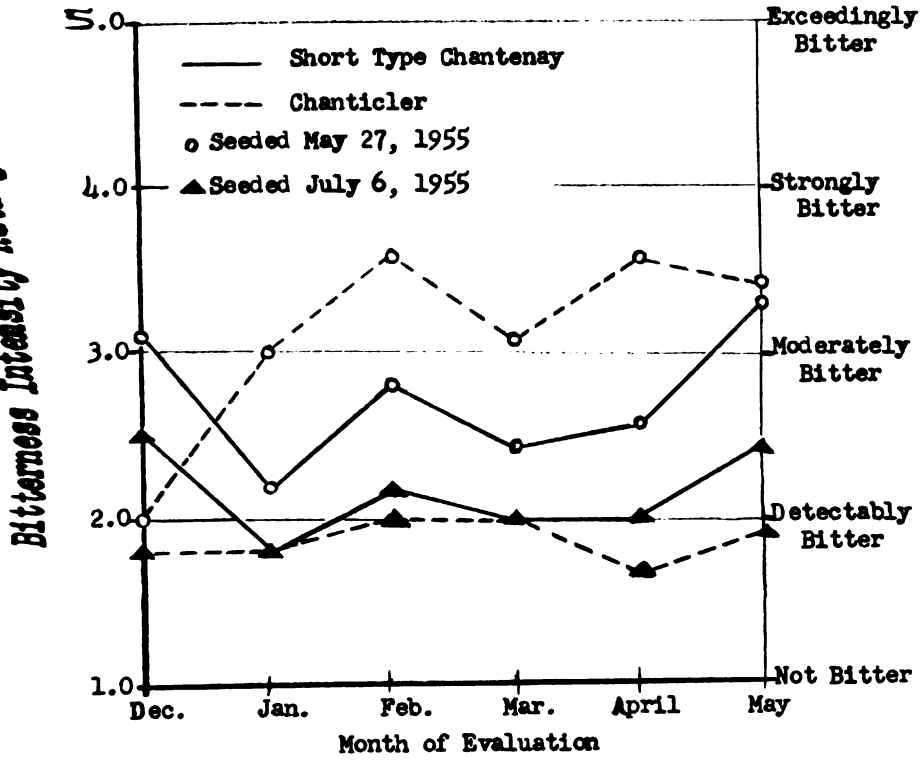

Figure 3. Storage Influence on Bitterness by Taste Ratings of Two Strains of Carrots Seeded on Two Dates.

considerably more bitter except at the first taste evaluation period. Gold Spike was tested for bitterness by the panel from January through May but was not checked for other characteristics. Roots from the early planting developed a low level of bitterness, but those from the late planting were practically free of bitterness.

Early seeded carrots contained considerably less sugars on a dry weight basis than the same strains seeded later, Table 8. Late seeded Chanticler had a higher sugar content in November than Short Type Chantenay; however, by May it had lost much more sugar than Short Type Chantenay. This difference suggests that late Chanticler was of a younger physiological age than late Short Type Chantenay and that it had a higher rate of respiration.

The first seeding developed a small number of carrots with flower stalks and carrots infected with aster yellows. Chanticler had more seeders, 2.71 to 0.64 percent, while Short Type Chantenay had more yellows, 1.33 to 0.81 percent.

TABLE 8

DRY MATTER, ASH, TOTAL SUGARS AND NITROGEN COMPOSITION OF TWO STRAINS OF CARROTS SEEDED ON TWO DATES, MAY 27 AND JULY 6, 1955

	Analysis Date	Short Type Chantenay Early	Short Type Chantenay Late	Chanticler Early	Chanticler Late
Dry Matter - %	Nov.	10.15	10.45	9.55	11.30
Ash — % D. Wt.	Nov.	9.45	8.12	8.37	7.46
Ash — % F. Wt.	Nov.	0.96	0.85	0.75	0.83
Total Sugars - % D. Wt.	Nov.	31.9	36.5	32.8	41.2
	May	29.2	37.1	33.1	36.7
Nitrogen - % D. Wt.	Nov.	2.64	2.16	2.58	2.01
	May	2.88	2.24	2.70	2.33

CHAPTER VIII

HARVEST AND POST-HARVEST HANDLING STUDY

In the date of seeding study, it was noted that large, overmature carrots were more severely damaged by mechanical harvesting than smaller, less mature roots. Bitterness appeared related to size, maturity and injury at harvest. This handling experiment was designed to explore the relationship between bitterness, injury, and the incidence of storage diseases.

Methods and Materials

Long type Chantenay carrots were seeded on muck June 16, 1956, grown conventionally and harvested in different ways on October 11. Two lots were dug and topped with a Chickering mechanical harvester, so operated as to cause heavy and extensive injury to the roots of one lot, and as little damage as possible with another lot. A third lot was carefully dug and topped by hand.

The carrots were sorted to remove obviously diseased roots, then held in a 35° F. refrigerated root crop storage from October 18 to 25, when the carrots were washed and subsequently stored at 32° F. These carrots were packed in bushel apple crates lined with polyethylene (Hardenburg, 1956) to retard moisture loss and escape of volatiles. On October 30, additional lots from the same source were treated with Beta rays with a total radiation of 150,000 rep. (Roentgen equivalent physical). Carrots were spread on trays, irradiated, turned over, and irradiated on the other side, then packed in polyethylene crate liners and returned to the storage.

Results and Discussion

Bitterness evaluations were made October 26, January 14 and March 21 using all three measurement techniques.

Taste tests, spectrophotometric and fluorescence analyses at the beginning of the study indicated no sign of bitterness. A low level of typical bitterness as measured by fluorescence did appear in a few carrots during storage but no further evidence of bitterness could be detected (Table 9).

As proposed in the experiment, different harvest and post-harvest treatments did influence the incidence of disease (Table 9 and Figure 4). After five months of storage, visible infection rates ranged from 5.8 to 78.1 percent. The primary organism was identified as a Sclerotinia species described by Rader (1952) as one of the more serious storage diseases of carrots.

Rough machine harvesting was responsible for more infection than gentle machine harvesting. Digging and topping by hand resulted in the least infection. Irradiation of unwashed roots reduced disease incidence by an average of 10.6 percent while washing reduced it by 19.6 percent.

The fluorescence observed was for the most part related to injury apparently received from Beta irradiation used to destroy surface microorganisms. Damage to the affected carrots appeared as dried blackened tissue on one side of the roots. Only a few roots showed damage and it is suspected that they were not turned between irradiation treatments so that they received a double exposure. An examination of cross sections through the blackened area of the roots indicated that fluorescence was present in apparently sound tissue, centripetally located to the injury appearing as typical bitterness fluorescence spots.

TABLE 9

BITTERNESS RATINGS AND FUNGAL DISEASE INFECTION OF RED CORE CHANTENAY CARROTS AS AFFECTED BY HARVEST AND POST-HARVEST TREATMENTS, 1956-57

Harvest and Post-Harvest Treatments	Bitterness Ratings(1)				Visible Disease Infection		
	Spectrophotometric Height 265 mμ peak(2)		Fluorescence 1-5 Rating(3)		Roots with Rot	Roots with Surface Mold	Total Infected
	Jan.	Mar.	Jan.	Mar.	%	%	%
Unwashed							
Rough Harvested	.10	.22	1.0	1.0	32.6	45.5	78.1
Gentle Harvested	.13	.16	1.0	1.0	26.2	33.8	60.0
Hand Harvested	.08	.17	1.0	1.0	13.3	10.7	24.0
Irradiated							
Rough Harvested	.13	.16	1.5	1.6(4)	34.3	30.6	64.9
Gentle Harvested	.14	.13	1.0	1.6(4)	35.2	18.0	53.2
Hand Harvested	.28	.12	1.2	1.6	9.2	2.9	12.1
Washed							
Rough Harvested	.19	.13	1.2	1.0	15.0	43.7	58.7
Gentle Harvested	.18	.15	1.0	1.0	26.1	13.7	39.8
Hand Harvested	.12	.14	1.0	1.0	1.2	4.6	5.8

(1) Bitterness evaluations at harvest by taste test, spectrophotometric and fluorescence analyses all indicated a lack of bitterness.

(2) Height of 265 mμ peak values of carrots below 0.75 indicate an absence of bitterness to taste.

(3) 1= non-bitter, non-fluorescent; 5= exceedingly bitter and fluorescent.

(4) Several roots had black sides suggesting irradiation damage. These were the only roots with fluorescence in their respective lots and the fluorescence was concentrated on the black sides.

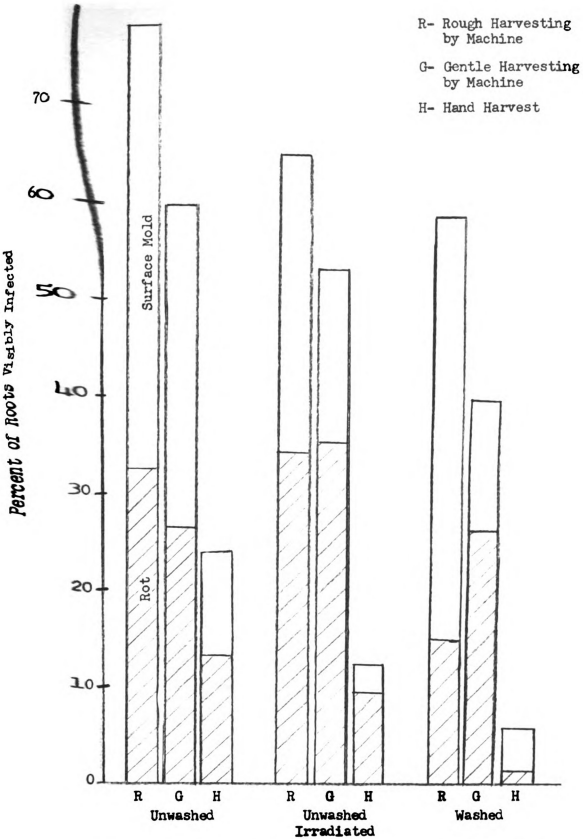

Figure 4. Percent of Carrot Roots Infected by Rot and Surface Mold as Influenced by Harvest and Post-Harvest Treatments, after Five Months of Cold Storage, 1956-57.

CHAPTER IX

CONTROLLED AND MODIFIED STORAGE ATMOSPHERE STUDIES

In the fall of 1955 a 40 ton lot of bitter carrots which had temporarily been stored with Bartlett pears was examined. Ripening Bartlett pears have a high rate of respiration and are also a prolific source of volatile emanations, particularly ethylene (Hansen and Christensen, 1939). This would tend to cause a build up of CO_2 and ethylene and a reduction of O_2 within a self contained storage atmosphere. The carrots had the appearance and darkness of color of roots which might have been stored for six months. Three weeks previously, however, these carrots were of non-bitter, acceptable processing quality.

This observation indicated that bitterness might have a connection with products given off by fruits and led into the following series of experiments.

Storage Atmosphere Study 1955-56

Methods and Materials

To study these factors in relation to bitterness, six treatments were devised to evaluate the effects of controlled atmospheres with high CO_2 and low O_2 contents, and the influence of apple emanations.

Six 5-kilogram samples of short type Chantenay carrots were selected from a planting seeded July 6, 1955 and harvested on October 20. They were placed in large-mouth 3-gallon jars designed for respiration studies

and then held in a 32° F. apple storage. The treatments were as follows:

A - Sealed

B - Controlled atmosphere - 3% CO_2:10% O_2

C - Controlled atmosphere - 7% CO_2:5% O_2

D - Apple emanations in a 220 ml/minute air stream

E - Air - 220 ml/minute stream

F - Open to storage room atmosphere

Treatment A was sealed so that ready exchange of CO_2 and O_2 would not occur. The controlled atmospheres in treatments B and C were maintained by flushing with N_2 gas to lower CO_2 concentration and adding compressed air to raise O_2 to the desired levels. In treatment D, pictured in Figure 5, the air flow was regulated by adjusting the pressure on an air flow manometer board. Jonathan apples were placed in a gallon jar which was sealed, except for air tubes, and kept in a laboratory at about 75° F. to speed respiration and emanation rates. An air tube was passed from this jar into the cold room to discharge in a humidifying jug which also served to cool the air as it passed into the carrot chamber. Apples were replaced at the end of three weeks. The air treatment E, or control, was similarly set up except for the elimination of the apple chamber. The final treatment F was left open to the atmosphere of the storage room which contained uncovered apples.

The carrots were placed under the various treatments on October 29 and all treatments were checked daily for CO_2 and O_2 content with a Haynes-Orsat gas analyzer. December 9, after a period of 43 days, the carrots were evaluated for bitterness.

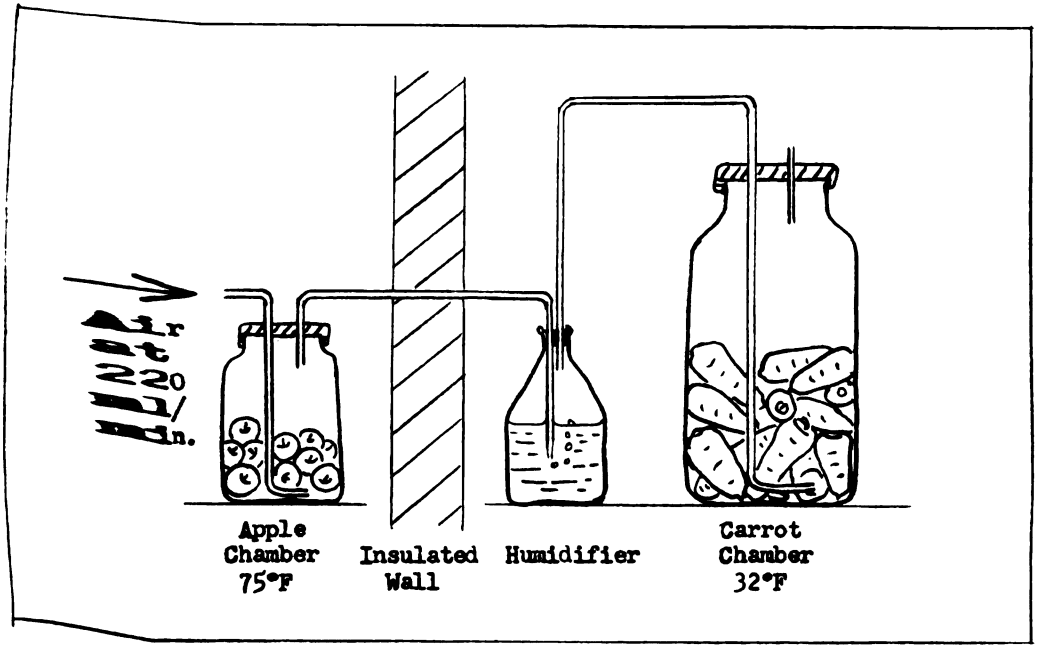

Figure 5. Experimental Setup for Apple Emanation Treatment for Inducing Bitterness in Carrots.

Random samples were washed and peeled and portions of five individual roots were checked for soluble solids with a hand refractometer. The remaining material from the samples was then ground and part evaluated by a taste panel, the remainder dried and analyzed spectrophotometrically. Other evaluations included rating of amount of mold, darkening of the peel and loss of weight during storage.

The remaining unsampled carrots were left in open jars which were transferred to a carrot storage room at about 35° F. A final bitterness evaluation was made July 6 on stored carrots from the apple emanation and air treatments.

To test the practicability of a quick test for bitterness susceptibility by combined storage of apples and carrots, and experiment was set up and run in December 1955. Six 1500-gram lots of carrots from the same source as above were sealed with two crisp, ripe Jonathan apples in 8-pound polyethylene bags at 32, 36 and 75° F. Evaluations were made at the end of one and two weeks.

Ethylene gas was used in an experiment started in February 1956, in another attempt to induce bitterness development. Two 5-pound lots of medium to small roots, from the July 6 plantings used above, were placed in wide-mouth 3-gallon respiration jars, and a third lot placed in a paper bag and stored at 32° F. as a check. To one jar, ethylene was introduced under pressure through a rubber tube to the bottom of the jar with air vented out through a glass tube at the top. The daily procedure was to first flush the atmosphere of both jars for five minutes with compressed air, then to apply ethylene to one jar for 30 seconds at five pounds pressure. Ethylene and ventilation treatments were discontinued February 26.

Results and Discussion

Where apple volatiles were added to a constant air stream passing through a container of carrots, bitterness, which was rated by a taste panel as intense, developed within a six week period (Table 10). A lower level of bitterness occurred in the container open to the apple storage atmosphere but none of the other treatments in the controlled and modified atmosphere study caused the development of a detectable bitter flavor. Spectrophotometric analysis on the dried tissues in January substantiated these evaluations. A further evaluation in July on treatments D and E gave the same results by both the taste test and spectrophotometric analysis.

No bitterness appeared in carrots from any treatment which developed over one percent of CO_2, suggesting the possibility that added CO_2 may inhibit the development of bitterness. However, where apples or pears and carrots have been stored together, the CO_2 content would be expected to be above normal, yet bitterness has appeared under such circumstances. Thus, the inhibition of bitterness by CO_2 accumulation is unlikely.

In color of peel, a rough measure of freshness and quality, the carrots exposed to apple emanations became darker than those under a constant air flow alone or controlled atmospheres. This condition was similar to that observed the previous September where carrots became bitter and dark in color in a pear storage. The brightest color of peel and the sweetest carrots were found in the two controlled atmosphere treatments where CO_2 content was above normal while darkest color and lowest sweetness and soluble solids values occurred in the carrots stored under anaerobic conditions.

TABLE 10

QUALITY FACTORS IN SHORT TYPE CHANTENAY CARROTS AND PERCENTAGE CARBON DIOXIDE AND OXYGEN ATMOSPHERE COMPOSITION AS AFFECTED BY STORAGE ATMOSPHERE TREATMENTS

Quality Factors and Atmosphere Composition(1)	Storage Atmosphere Treatments					
	A Sealed	B 3%CO_2 10%O_2	C 7%CO_2 5%O_2	D Apple Emanations	E Air	F Open
Bitterness: 1-5 taste rating	1.0	1.0	1.0	5.0	1.0	3.0
Sweetness: 1-6 rating	6(2)	1	1	masked(3)	3	4
Soluble Solids: %	6.08	7.36	7.66	7.84	7.60	6.78
Color of Peel: 1-6 ranking	6	1	2	5	3	4
Weight Loss: %	0.50	0.15	0.25	0.25	0.25	1.00
Mold: 1-5 rating	1	2	2	5	3	3
CO_2 Content: %	80	3	7	0.5	1-4	0.3
O_2 Content: %	0	10	5	19-20	19-20	20-21

(1) The lowest value in the rating and ranking scales indicates the most favorable characteristic.

(2) Flavor and aroma of this sample were that of fermented, dead tissue.

(3) Sweetness was present but could not be rated by the panel of six.

A low incidence of an unidentified mold developed on the surface of carrots in all except the sealed treatment (A). The highest mold rating was in treatment D which also developed the highest intensity of bitterness. Since mold ratings for treatments E and F were both 3, and bitterness ratings for these treatments were 1 and 3, respectively, it is thought that the appearance of mold was not related to bitterness development. The concentrated apple emanations in treatment D may have stimulated mold growth giving it the highest mold rating.

Results of the test of combined storage of Jonathan apples and carrots in polyethylene bags at 32, 36 and 75° F. (Table 11) in December 1955, show that bitterness was not induced by any of the treatments tried - The only marked flavor changes occurred in carrots from the 75° F. treatment where nearly a quarter of the roots developed a fermented flavor in one week and all were slightly soured in two weeks.

Several possibilities are suggested for the lack of bitterness development although the carrots and apples used were from the same sources as in the previous tests. 1. By December, the precursor of the bitter principle in the carrot had been dissipated or converted to a more stable compound so that bitterness induction was impossible. 2. Apples effective in causing bitterness in November could have passed their peak production of ethylene and therefore were ineffective. 3. The reaction to develop bitterness may take more than the two weeks allowed in this test. 4. Within the polyethylene bags the atmospheres tended in the direction of high CO_2 content and might be responsible for an inhibition of bitterness.

TABLE 11

INFLUENCE ON CARROTS OF COMBINED STORAGE WITH APPLES AT 32, 36 and 75° F.(1)

	Storage Temperature		
	32° F.	36° F.	75° F.
	One Week		
Flavor:	% of Roots	% of Roots	% of Roots
Normal or Sweet	41.7	63.7	46.2
Strong	50.0	27.3	23.1
Other	8.3 (earthy)	9.0 (earthy)	23.1 (fermented)
Bitter	0.0	0.0	7.7(2) (slightly)
Sprouting	Slight	Slight	Moderate(3)
Mold Rating(4)	1	1	2
Aroma	None apparent	Turpentine-like	Apple-NH_3-like
Condensation	Slight	Slight	Heavy(5)
Apple Condition	Hard, crisp	Fairly firm Skin soft	Fairly firm Skin soft
	Two Weeks		
Flavor:			
Normal or Sweet	54.5	50.0	0.0
Strong	36.4	40.0	0.0
Other	9.1 (flat)	10.0 (musty)	100.0 (slightly sour)
Bitter	0.0	0.0	0.0
Sprouting	Slight	Slight	No record
Mold Rating(4)	1	2	5
Aroma	Slight, musty, carroty	Sharp-apple	Sharp-NH_3-like
Condensation	Slight	Very slight	Moderate
Apple Condition	Firm	Firm	Soft

(1) Evaluations made by Dr. James A. Cook.

(2) Since there was a low incidence of bitterness in the carrot population used for this experiment, the appearance of this specimen is discounted.

(3) These carrots also seemed more woody than any of the others.

(4) The mold intensity rating is based on a 1 to 5 scale with 1- no mold and 5- severe rotting.

(5) The bag was also puffed indicating anaerobic respiration.

Ethylene treated carrots tasted by a 17 member panel in February were rated at 1.8, or questionably bitter, while carrots with daily ventilation were rated 2.3, or slightly bitter. The control lot held in storage was rated at 1.0, or non-bitter. When the three lots were tasted again by the writer at the end of March, no bitterness could be detected in any of the three lots. Extensive surface pitting and discoloration, indicating injury, occurred to the ethylene treated carrots.

Storage Atmosphere Study 1956-57

The previous studies suggest that carrots from the 1955 crop might no longer be induced to become bitter after six to eight weeks of storage. Therefore, the determination of the effect of ethylene on bitterness development was delayed until a new crop of carrots was available.

The objectives of this study were: 1- to determine whether ethylene might be a causal factor in bitterness development; 2- to repeat the observed effects of apple emanations in inducing bitterness; 3- to determine whether ventilation could be effective in inhibiting or preventing the development of bitterness; 4- to determine whether harvest injuries influence bitterness susceptibility; 5- to compare varieties for bitterness response; and 6- to check the tendency for bitterness development in western packaged carrots.

Methods and Materials

Long type Chantenay and Gold Pak carrots were seeded June 11, 1956 on muck and dug by hand and machine on October 11. Those dug by hand were sorted carefully to eliminate any injured, diseased, or off type roots and were handled carefully to avoid further injury. The machine dug roots were bruised intentionally in the harvesting process and were sorted to remove uninjured, diseased, or off type roots. The Gold Pak variety was also machine harvested but in the usual manner so that the amount of injury was considerably less than with the machine harvested Chantenay variety.

The harvested roots were held for seven days in a common storage, then stored in a refrigerated root crop storage at 35° F. until placed under experimental conditions December 17.

Mineral soil grown Colorado Imperator carrots were obtained from a local store in mid-November. Sprouts and dry leaf tissues attached to the crown indicated that these carrots had been stored previously.

The basic design of the experiment included four primary treatments: apple emanations, ethylene gas, air or ventilation, and an open control, each consisting of: uninjured Chantenay, injured Chantenay, machine harvested Gold Pak, and the western sample from the market. The experimental set up was similar to that in the 1955 study with treatments replicated twice with a total of eight jars.

The purpose of the experiment was to store carrots in an atmosphere containing about 100 ppm of ethylene which was based on a calculated estimate of the ethylene concentration in the pear storage where bitterness had appeared in 1955. Following two weeks of unsuccessful attempts to produce a continuous flow of an atmosphere of this concentration which could be readily maintained and adjusted, it was decided to use an intermittant treatment. Ethylene was introduced to the constant air flow at the rate of 3½ ml per minute for five minutes each day.

Delicious apples were used to test the effect of emanations which were passed through the system by a controlled air flow of about 220 ml per minute.

Results and Discussion

Bitterness was evaluated by fluorescence, taste, and spectrophotometric tests. The bulk lots of Chantenay and Gold Pak were evaluated one week previous to starting the treatments and were rated as non-bitter. The western carrots were not sampled prior to the test.

The carrots were placed under treatment from December 17 to January 9 when five roots from each lot were removed and evaluated for bitterness; the remainder were left under treatment until February 5 when all jars were opened and stored in place. A second evaluation for bitterness was made February 9 on the first replication and March 18 on the second.

Results in Table 12 show a slightly lower bitterness value for carrots in the open control. This was due almost entirely to lower values for the Colorado Imperator. Circulation of air in the storage room and in the open containers was probably greater than circulation through the constant air flow treatments, thus effecting a more rapid dissipation of the bitter principle. Ratings by all three evaluation techniques showed a tendency that had been previously noted for a decrease in bitterness from the second to the third evaluation.

A summary of the influence of variety and handling sub-treatments, Table 13, shows the locally grown Chantenay and Gold Pak carrots to be essentially free from bitterness while Imperator, grown and stored in Colorado were moderately bitter. Some individual roots from this lot were strongly bitter. Spectrophotometric values for the harvest injured Chantenay carrots were slightly lower than for those carefully harvested by hand. This suggests that mechanical injury may reduce the amount of

TABLE 12

INFLUENCE OF STORAGE ATMOSPHERES ON BITTERNESS DEVELOPMENT IN FOUR LOTS OF CARROTS, 1956-57

Storage Atmosphere Treatment	Variety and Handling Treatment	Bitterness Ratings(1) Taste 1-5 Rating		Fluorescence 1-5 Rating		Spectrophotometric Ht 265 mμ peak	
		2nd	3rd	2nd	3rd	2nd	3rd
Apple Emanations	S.T.C.-Hand	1.0	1.0	1.0	1.4	.21	.26
	S.T.C.-Rough	1.5	1.0	1.4	1.0	.28	.11
	Gold Pak	1.5	1.0	1.0	1.1	.39	.28
	Western Imper.	3.2	2.0	2.9	2.7	2.68	1.81
	Averages	1.80	1.25	1.58	1.55	.89	.62
Ethylene	S.T.C.-Hand	1.0	1.0	1.0	1.0	.38	.23
	S.T.C.-Rough	1.2	1.5	1.0	1.0	.34	.20
	Gold Pak	1.5	1.5	1.0	1.0	.40	.27
	Western Imper.	3.5	2.0	2.6	2.4	2.91	1.46
	Averages	1.80	1.50	1.40	1.35	1.01	.54
Air	S.T.C.-Hand	1.2	1.0	1.0	1.0	.43	.28
	S.T.C.-Rough	1.0	1.0	1.0	1.0	.28	.13
	Gold Pak	1.0	1.0	1.0	1.0	.43	.27
	Western Imper.	2.5	1.8	2.3	3.0	2.56	1.84
	Averages	1.42	1.20	1.32	1.50	.92	.63
Control (open)	S.T.C.-Hand	1.0	1.0	1.0	1.0	.23	.22
	S.T.C.-Rough	1.0	1.0	1.0	1.0	.22	.18
	Gold Pak	1.2	1.0	1.0	1.0	.26	.30
	Western Imper.	2.0	1.5	3.0	2.5	1.11	1.28
	Averages	1.30	1.12	1.50	1.38	.46	.50

(1) Taste, fluorescence and spectrophotometric evaluations on Short Type Chantenay and Gold Pak on November 2 indicated no bitterness to be present. The western sample was not tested at that time. The second and third organoleptic tests were made by panels of 11 and 14 individuals respectively.

TABLE 13

VARIETY AND HANDLING TREATMENT INFLUENCE ON BITTERNESS RATINGS OF STORED CARROTS, 1956-57

Variety and Handling Treatment	Bitterness Ratings(1)					
	Taste 1-5 Rating		Fluorescence 1-5 Rating		Spectrophotometric Ht 265 mμ peak	
	2nd	3rd	2nd	3rd	2nd	3rd
S.T.C. Hand	1.1	1.0	1.0	1.1	.31	.25
S.T.C. Rough	1.2	1.1	1.1	1.0	.28	.16
Gold Pak	1.3	1.1	1.0	1.0	.37	.28
Western Imperator	2.8	1.8	2.7	2.6	2.32	1.60

(1) Taste, fluorescence and spectrophotometric evaluations on Short Type Chantenay and Gold Pak on November 2 indicated no bitterness to be present. The western sample was not tested at that time. The second and third organoleptic tests were made by panels of 11 and 14 individuals respectively.

potential bitterness by allowing more ready escape, removal, or conversion of the precursor of the bitter principle, assuming it to be present in the carrot at the time of harvest.

A possibility for the absence of induced bitterness may be due to the fact that the study was initiated so late in the storage life of the roots that the precursor of the bitter principle had been lost by volatilization. However, short type Chantenay roots placed in an apple storage on November 10, shortly after harvest, developed a low intensity of bitterness by taste, fluorescence, and spectrophotometric analysis in three weeks.

The effect of season was also a factor, in that there was no bitterness reported in the commercial crop in Michigan for 1956. Two factors in the weather could have affected potential bitterness. First, the growing season was exceptionally cool through August with a possible deficiency in production of the precursor. Second, the harvest and early storage season was unseasonably warm and comparatively dry, possibly resulting in field volatilization or reorganization of the precursor of bitterness.

CHAPTER X

MINOR ELEMENT NUTRITION AS RELATED TO BITTERNESS IN CARROTS

Harmer (1946) found that carrots were moderately responsive to copper fertilization on copper deficient muck soil, and below a minimum level of copper, carotene and sugar contents of the roots were low.

Manganese deficiency in carrots is a recognized problem in muck soils with symptoms appearing as interveinal chlorosis and yellowing of the younger leaves. Foliar sprays with $MnSO_4$ at 2 and 4 pounds per acre quickly and effectively alleviate the symptoms.

Manganese toxicity symptoms on many plants appear as iron deficiency symptoms and can be helped by foliar applications of iron salts or iron chelates.

Methods and Materials

Three studies to determine whether or not minor element deficiencies were related to bitterness were carried out in 1955 and 1956.

Copper deficient muck soil plots treated with 0, 6.25, 12.5, 25 and 50 pounds of copper per acre were planted with Red Core Chantenay carrots. At harvest, samples were obtained and stored in 50 pound onion bags at 32° F. until January 12, 1956 when a taste panel evaluation was made.

At about this same time two samples of carrots, one bitter, the other non-bitter, were obtained from a commercial carrot storage where they had been held for the same length of time. The bitter sample had been grown on an acid muck and the non-bitter one had come from an alkaline muck in which marl was present. These samples were analyzed for minerals to reveal differences which might be associated with the development of bitterness.

In a subsequent study, Long Type Chantenay carrots were seeded on June 16, 1956 in a muck field which was heavily limed to pH 6.2 in 1954. Manganese deficiency symptoms had regularly appeared on several crops previous to 1956. The seeding was band fertilized with 800 pounds per acre of 5-10-20.

Manganese deficiency appeared in the carrot seedlings and was corrected in certain plots by $MnSO_4$ spray treatments at both 3 and 6 pounds of elemental manganese to the acre. A foliar application of $FeSO_4$ at 4.2 pounds of iron per acre resulted in foliage damage wherein "burning" appeared. Growth of new leaves masked the damage within two weeks. The crop was harvested October 11, and placed in refrigerated storage at 32° F. on October 18. Bitterness was evaluated at harvest by tasting, and again November 1, December 12 and January 27 by tasting, fluorescence and spectrophotometric analysis.

Results and Discussion

Carrots from all plots of the copper experiments were tasted at harvest and found free of bitterness. After three months of cold storage, a taste panel again rated all lots as non-bitter. Spectrophotometric evaluations made on the same samples the following July showed no essential differences.

Mineral analyses of bitter and non-bitter carrots from a commercial cold storage room showed marked differences in content of manganese, iron and sodium (Table 14). The sample from the alkaline muck soil which was not bitter was higher in manganese but lower in iron and sodium than the bitter sample from the acid muck soil. Although these differences could not be considered as evidence that deficiencies of any of the elements were directly related to bitterness, the manganese-iron inversion suggested as promising further investigation on the influence of manganese nutrition.

In the subsequent evaluation of foliar treatments with manganese and iron salts, no consistent differences were found among spectrophotometric bitterness ratings of the stored carrots.

Under the conditions of these experiments, deficiencies of neither copper nor manganese caused bitterness to develop in carrots. As already pointed out, this does not necessarily mean that a shortage of these elements in carrot nutrition will prevent bitterness.

Some carrots from the same farm, but not in the experiment, although stored in the same refrigerated room as those in the copper study, became bitter indicating that storage conditions developed which promoted

TABLE 14

MINERAL ANALYSIS OF CARROTS FROM TWO SOURCES, 1955 CROP
(% dry weight)

Elements	Non-bitter(1)	Bitter(2)
Ca	0.25	0.25
Cu	0.0010	0.0014
Fe	0.0083	0.0112
K	4.41	4.54
Mn	0.0151	0.0036
Na	0.10	0.25
P	0.46	0.39
Co	0.10	0.09

(1) From an alkaline muck soil.

(2) From an acid muck soil.

bitterness. As no bitterness appeared in the carrots from the copper study, copper had no apparent effect on bitterness.

The manganese and iron study was conducted in 1956, a year in which no bitterness was reported in commercial carrots in Michigan. This indicated that the trend of the weather for the growing season was probably of greater importance than nutritional factors, and that regardless of the nutritional situation, the other conditions required for bitterness development were not present. The possibility that a manganese deficiency may allow bitterness development under other seasonal conditions cannot be ruled out.

CHAPTER XI

FLUORESCENCE STUDIES

The possibility that the metabolic changes associated with the formation of the bitter principle in carrots might be related to the development of fluorescent substances in the roots (Zechmeister and Polgar, 1944) led to an examination of tissues from bitter and non-bitter carrots under ultra violet light. Yellowish green fluorescent spots were concentrated in the phloem tissue of bitter roots, Figure 6, but were not discernable in normal carrots. The area of fluorescence in the phloem was the site of the most intense bitterness by organoleptic and spectrophotometric tests.

As fluorescence appeared to have considerable promise as a fairly accurate rapid means of evaluating bitterness and perhaps aid in the interpretation of some aspects of the physiological response associated with the development of a bitter metabolite, its relation to bitterness was investigated.

Methods and Materials

After discovering that bitter carrots fluoresced under ultra violet excitation from a 2537Å Mineralight source, a correlation test was made with 64 muck grown short type Chantenay carrots which ranged from non-bitter to exceedingly bitter. The size, shape, height of crown, kind and extent of injury, amount of mold present, relative proportion of

Figure 6. Location of Fluorescence in a Bitter Red Core Chantenay Carrot under Excitation of 2537Å Ultra Violet Light.

xylem and phloem, shoulder color and surface fluorescence were measured and tested statistically for correlation with internal fluorescence and bitterness intensity evaluated by taste test. A center slice removed from each carrot was rated in a dark room under a 2537Å ultra violet light for intensity of fluorescence according to a 1 to 5, non-fluorescent to highly fluorescent scale. An additional slice was removed from each carrot, peeled and organoleptically rated by the writer for intensity of bitterness using again a 1 to 5, non-bitter to exceedingly bitter scale.

A second correlation study was conducted on 10 roots from the same source evaluated for the above characteristics, but based upon longitudinal sections with fluorescence ratings upon five arbitrarily selected concentric levels of the phloem and xylem. Bitterness ratings were made by tasting parings from each of the fluorescence levels. Correlation values were determined upon summations of fluorescence and bitterness ratings for individual roots.

Imprints of bitter carrots upon Whatman #1 filter paper showed fluorescence under ultra violet excitation and could be used to quickly evaluate fluorescence without interference from reflections or the orange pigments of the carrots and provide a semi-permanent record of its intensity and distribution. Photographs were taken of patterns of fluorescence produced by ultra violet sources of 2537 and 3660Å.

Effects of potassium hydroxide, hydrochloric acid, sulfuric acid, when applied to bitter fluorescent carrots were observed.

Photomicrographs were taken of hand sections of fresh bitter carrots with white transmitted light and under 2537Å ultra violet incident light showing the location and extent of fluorescent spots.

Results and Discussion

Results of the correlation study of sixty-four roots (Appendix Table 1) showed the correlation coefficient between fluorescence of transverse sections and bitterness by taste test both rated on a 1 to 5 basis to be a non-significant 0.28. By sectioning a number of other carrots longitudinally and transversely, it was noted that fluorescence was not always uniformly distributed at a given transverse level. By sectioning roots longitudinally, more representative sections were obtained for fluorescence evaluation.

Other observations (Appendix Table 2) from this study included a positive correlation between both injury and mold, and external fluorescence but no correlation between mold or injury and bitterness. Roots with large cores and/or thin cortices averaged 3.9 on the bitterness scale by taste while roots with small cores and/or thick cortices averaged 2.3 in bitterness. The large cored roots also tended to have much taller growing points which suggested an earlier initiation of bolting. Color of xylem or phloem and color of shoulder had no apparent effect upon bitterness; however, carrots with green shoulders tended to have a slightly higher than average external and a lower internal fluorescence rating. Carrots with purple shoulders showed no correlation with either internal or external fluorescence. There was no relationship between carrot diameter or length and bitterness. A small number of roots were bitter to taste but exhibited little or no fluorescence indicating the presence of more than one bitter principle.

Data from the second fluorescence-bitterness correlation study, (Appendix Table 3), showed an improvement in correlation coefficient from 0.28, when using transverse sections, to 0.64 when using longitudinal sections.

Bitterness and fluorescence were shown to be located in the same morphological tissue, the phloem, and were both lacking in the core or xylem. A tendency towards increasing bitterness and fluorescence from the outside to the cambial layer was recorded.

No correlation was present between root size, and bitterness or fluorescence in either this or the previous study (Appendix Table 4). With molds, a correlation of 0.45 was found with surface injuries, 0.39 with surface fluorescence, but only 0.05 with bitterness. A correlation coefficient of -0.46 was found between surface injury and bitterness, indicating the greater the injury, the less the bitterness.

By pressing cut carrots onto sheets of soft filter paper, Whatman #1, semi-permanent records (some of which have lasted over a year) of fluorescence intensity and distribution were obtained. A photograph of carrot imprints representing the five fluorescence intensity levels used in rating bitterness is shown in Figure 7. The root section at the upper left shows no fluorescence indicating freedom from bitterness, while the highly fluorescent root section at the lower right indicates an exceedingly bitter flavor.

Differences in fluorescence pattern produced by ultra violet sources of 2537 and 3660Å are shown in Figure 8.

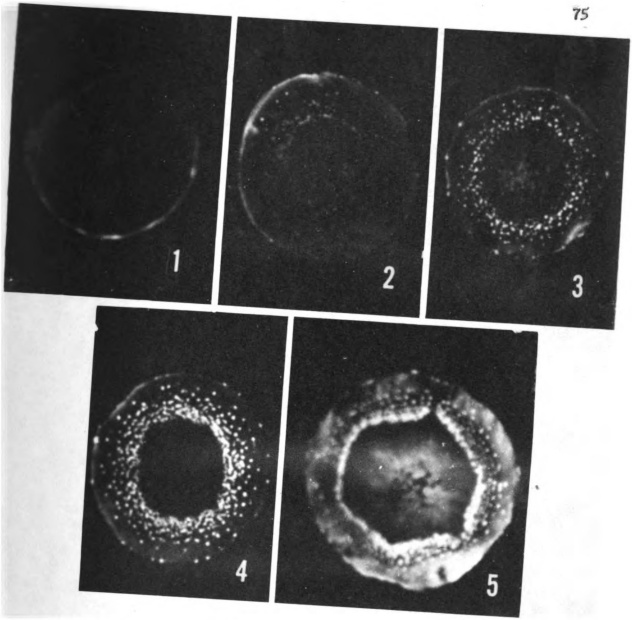

Figure 7. Typical Fluorescence Scale of 1-Non-Bitter, 2- Just Detectably Bitter, 3- Moderately Bitter, 4- Strongly Bitter, and 5- Exceedingly Bitter Carrots under Ultra Violet Light Excitation at 2537Å.

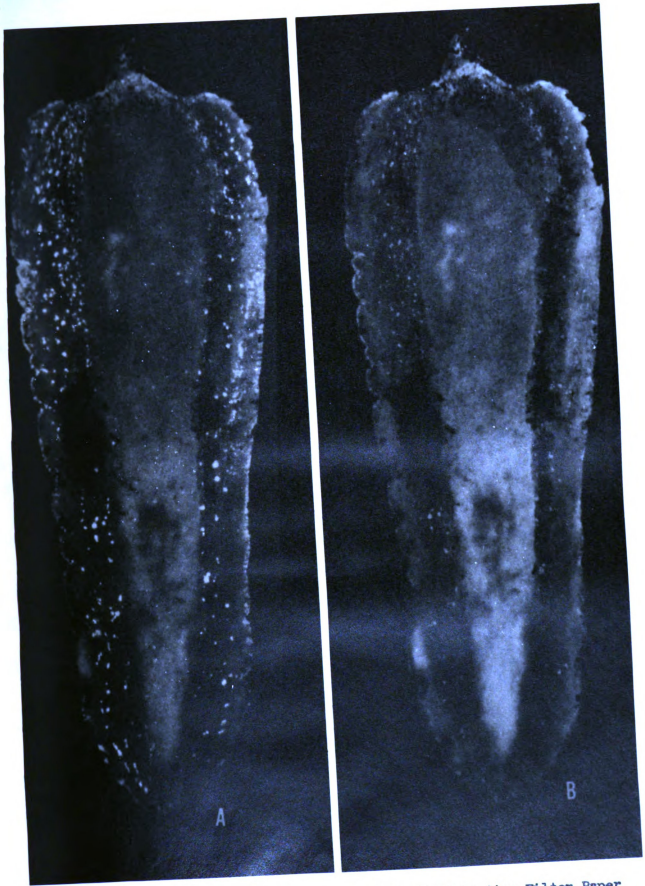

Figure 8. Fluorescence Variations of a Longitudinal Section Filter Paper Impression of a Bitter Red Core Chantenay Carrot under (A) 2537Å and (B) 3660Å Ultra Violet Excitation.

Some of the yellow-green fluorescent spots in the phloem of bitter carrots under excitation of 2537Å ultra violet light are missing under excitation at 3660Å which suggests the presence of at least two fluorescent compounds. A general over-all fluorescence is more common at 3660Å and appears more intense in senescent carrots stored for a long time.

Application of five percent potassium hydroxide by drops or as a fine mist over cut bitter carrots enhanced fluorescence under 2537Å ultra violet presumably much in the same manner as Goodwin and Kavanagh (1950, 1952) found in their pH studies of coumarin compounds. Application of dilute hydrochloric acid and sulfuric acid reduced fluorescence materially. Potassium hydroxide, hexane, and water applied to fluorescent spots caused them to spread.

Photomicrographs, Figures 9A and 9B show fluorescent areas of a fresh, hand sectioned bitter carrot under incident ultra violet and transmitted white light. Fluorescence appeared to be concentrated in groups of cells thought to be oil glands and in areas beginning to break down.

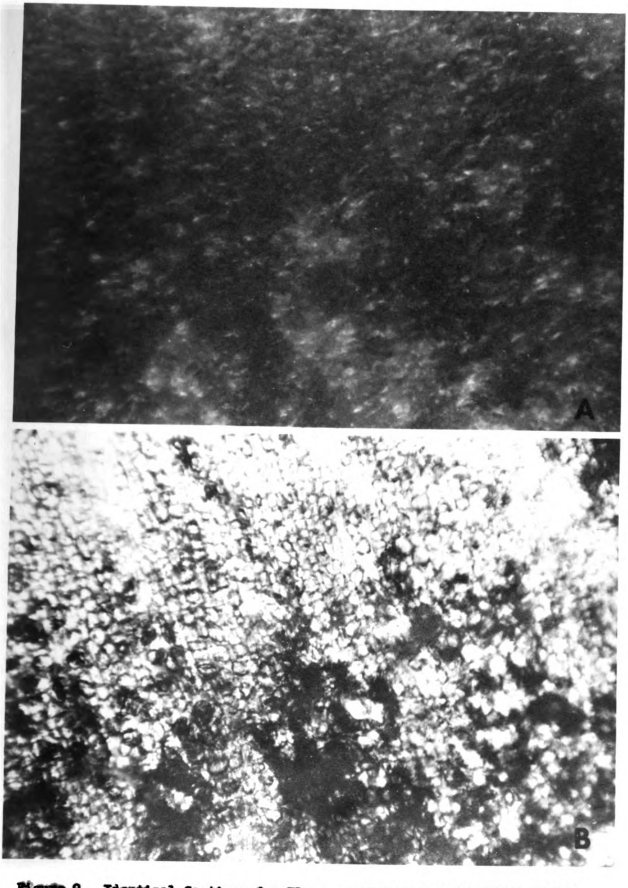

Figure 9. Identical Section of a Fluorescent Carrot under Ultra Violet Light (A) and Transmitted White Light (B).

CHAPTER XII

GENERAL DISCUSSION

Bitterness in carrot roots depreciates the edible quality by causing an unexpected biting flavor which persists through cooking and processing.

The bitter principle has been morphologically located in the phloem and its intensity evaluated by small spots of yellowish-green fluorescence under excitation from short wave 2537Å ultra violet light.

In the discussion that follows, an attempt will be made to interrelate the role of heredity and pertinent environmental factors during growth and storage to the development and possible control of bitterness.

Genetic Relationships

Differences among commercial varieties and strains of carrots in their susceptibility to bitterness was suggested by Atkin, 1956, but was not clearly supported by his experiments. In this study, using carrots of the same chronological age, the early maturing fresh market varieties Nantes and Touchon developed the most bitterness, while later maturing Danvers, Imperator, Gold Spike and Gold Pak showed the least bitterness. The intermediate Red Core Chantenay strains varied in bitterness, but tended to fall closer in response to that of Nantes than Imperator. A similar trend in maturity was noted by Dickson (1957) in studies of temperature induced bolting response.

Earliness of maturity was thought by Banga et al. (1955) to be due to a rapid attainment of the maximum contents of carotenoids and dry matter and the typical mature shape for the variety.

The precursor of the bitter principle may be a usual metabolic intermediary compound, accumulating near the end of the first season of growth. Briefly, the earlier the maturity of the carrot, the longer the synthesis of the precursor could continue if the plants remain in the field.

In progeny tests of bitter and non-bitter selections from Red Core Chantenay, Yamaguchi et al. (1955) found that bitter parents produced strongly flavored progeny and that mild flavored progeny were produced from non-bitter parents. In this work, in similar tests conducted from January 1955 through January 1957, no differences in progeny evaluations for bitterness were observed. However, this is not in contradiction of Yamaguchi's observation since the over-all level of bitterness in the 1956 Michigan crop was negligible, probably due to unfavorable weather for synthesis of the bitterness precursor. The development of non-bitter, strongly flavored progeny from bitter parents suggests that the precursor may be one of the oily flavoring components of carrots.

Environmental Factors

Soil Type

Carrots for processing and fresh market are grown on many soil types, but most of the carrots in the northeastern states are produced on muck soils. Atkin (1956) indicated from limited tests that carrots grown on muck soils were generally more bitter than those from upland loams.

In his study, carrots from sandy soils showed a greater variation in bitterness than those from either mucks or loams but were lower in bitterness than those from mucks. Analyses by Lipton (1955) showed greater total sugars and carotene content in upland than in muck grown carrots. Newcombe and Alderman (1944) pointed to a greater oxidative rancidity of dehydrated carrots from muck than from upland soils. Results in this investigation supplement Lipton's observation that upland carrots tended to be of higher quality; yet in bitterness response, soil types appeared to be of little consequence. In one series of harvests, the most bitter carrots came from an upland trial and in another, from muck soils.

Nutrition

In a nutritional study related to bitterness in carrots, Atkin and Sayre (1955) reported that the level of bitterness in muck grown Red Core Chantenay carrots was reduced by an application of 50 or 100 pounds per acre of fritted minor elements. In this study, copper and manganese deficient carrots developed no difference in bitterness as compared with carrots amply supplied with these elements, indicating that deficiencies of neither are the direct cause of bitterness. However, the possible role of minor elements in the enzymatic activities resulting in the development of bitterness warrant further investigation.

Physiological Age

Carrots seeded at varying times were shown by Atkin (1956) to differ but little in bitterness response, and he found that early harvest, regardless of seeding date, was most influential in effecting bitterness occurrence. In this study, May 27 seeded carrots developed considerably more bitterness in storage than carrots seeded July 6 indicating the

possibility that at a certain stage of physiological maturity, the metabolic processes in the root may be altered in some manner by harvesting and storage which results in the development of bitter products. Early and late seeded short type Chantenay were similar in bitterness response, while early seeded Chanticler was considerably more bitter. Late seeded Chanticler was decidedly less bitter than short type Chantenay sown at the same time. This suggested that short type Chantenay tended to mature earlier than Chanticler, thus allowing the late Chantenay seeding to catch up with the earlier one. Late seeded Chanticler in addition to being least bitter, accumulated the most total sugars by the time of harvest and lost the most total sugars during storage, suggesting that it had the highest metabolic rate of any carrots in the test.

Date of harvest was not directly involved in this study; however, it was noted both in 1954 and 1955 that the highest intensities of bitterness occurred in the earliest harvested carrots. As found both by Atkin (1956), and in this work, harvests of processing carrots as late as November, resulted in little or no bitterness. From a commercial point of view, September harvested carrots placed in cold storage have provided the largest total source of bitterness. Thus, a delay in harvest of carrots to be stored for a prolonged period appears justified to reduce bitterness occurrence, despite a potential increase in susceptibility due to carrot maturity. To interpret this apparent divergence, over-maturity versus early harvest as a cause of bitterness, the evaluation of storage factors should be considered.

Seasonal Influence

As noted by Yamaguchi et al. (1955) marked differences in bitterness of commerical carrots from year to year are probably affected by weather. Koch (1957) of the Gerber Products Company stated that there has been no bitterness in their carrots in California since 1954. In 1956 the commercial carrot crop in Michigan exhibited an almost complete lack of bitterness which appeared to be related to a deficiency in the typical oily carrot flavor. It may not be a coincidence that in 1956 the peach crop in Central Michigan was also deficient in typical peach flavor and that the central Wisconsin McIntosh apples showed the same defect (Roberts, 1957). A study by Biggs and Leopold (1954) on the influence of temperature on synthesis of menthol and mentholated esters in peppermint -- essential oils similar to some of those in the carrot -- revealed that high temperatures (80° F. day, 70° F. night) resulted in greater yields than lower temperatures.

Although rainfall was near the long term average in East Lansing in 1956, temperatures during the growing season were considerably below normal through August. During September, October and early November, temperatures were above average and precipitation was light. Even though conclusive evidence is lacking, it is suggested that the precursor of the bitter principle may be an essential oil or of hydrocarbon nature and is favored in its formation by comparatively high growing temperatures.

Post-Harvest and Storage Factors

Injuries

Reference to injuries sustained by carrots in mechanical harvesting in relation to bitterness is missing from the literature. Observations in carrot harvesting by machine in 1954, 1955 and 1956 showed that early planted, over-mature, and large roots tended to be bruised, scuffed, cut and cracked. Studies in 1956 on controlled injuries and correlation of factors in the fluorescence experiments indicated that injury tended to reduce bitterness. This may possibly be due to an acceleration of respiration or other reactions which affected the precursor, or to the effect of increased volatilization of precursor or bitter principle from the injured root.

Sclerotinia rot infection was intensified by injury and affected storability, but not bitterness.

Storage

Atkin (1956) showed that carrots kept in refrigerated storage became bitter, while carrots held in air cooled common storage did not. Among storages there are differences in temperatures, humidities, and atmospheric composition. Carrots in common storage reach a desirable holding temperature more slowly than in cold storages held at or near 32° F., and consequently higher respiratory and disease losses occur. Common storages show no marked deviation from normal air, while cold storages involve a more or less self-contained atmosphere which permits depletion of oxygen and accumulation of carbon dioxide and volatile materials.

Controlled atmosphere tests in this study with depleted oxygen and accumulated carbon dioxide did not induce bitterness. At the end of six weeks, edible root quality was actually superior under controlled atmospheres. Anaerobic conditions resulted in a fermented flavor and odor, but no bitterness.

Mature carrots stored in atmospheres containing pear or apple emanations became bitter in a few weeks. In atmospheres supplemented with apple emanations or ethylene, long stored and immature carrots did not become bitter, but darkened and developed surface pitting similar to that of fresh, mature carrots stored with fruit.

Bitterness development in carrots appeared in a few weeks, followed by gradual depletion during storage.

On the basis of this study, it is suggested that carrots for storage be seeded late to provide immature roots at harvest and harvested as late as possible to escape storage conditions condusive to bitterness development. Less bitterness should occur in late than in early maturing varieties when harvested at the same time. Carrots should not be stored with fruit or where fruit volatiles are present. Since it is possible that injured or diseased carrots may produce volatiles which have a bitterness inducing effect, it is suggested that all diseased or badly injured roots be discarded and that the remainder be handled carefully.

CHAPTER XIII

SUMMARY

Bitterness has been found in carrots held in cold storage for later processing or packaging following full attainment of maturity in the field.

Early maturing varieties, Nantes and Touchon became more bitter in storage than the late maturing Danvers, Imperator, Gold Spike and Gold Pak, when harvested at the same chronological age. Strains of Red Core Chantenay were intermediate in bitterness response, yet were highly variable and showed a marked interaction with seeding date. A progeny test of bitter and non-bitter parents showed no differences in a season in which full maturity was probably not reached.

Minor element fertilizer tests with copper and manganese indicated that deficiencies were not a direct cause for bitterness and that ample copper nutrition did not affect bitterness. The effect of manganese was masked due to immaturity at harvest.

Injuried received by roots in digging and topping did not increase subsequent bitterness but tended to decrease it probably through stimulated metabolism.

Refrigerated storage of carrots with Bartlett pears and apples resulted in bitterness development within three weeks. Apple emanations passed over refrigerated roots caused bitterness while carrots in air free from volatiles retained acceptable quality. The induction of

bitterness in immature and long stored carrots with ethylene was unsucessful; however, discoloration and surface pitting were similar to such damage received in commercial storages. Controlled atmospheres of 3% CO_2: 10% O_2 and 7% CO_2:5% O_2 resulted in retention of sweetness but no bitterness. Anaerobically stored carrots became fermented, but not bitter. Bitterness development in the absence of stored fruit may possibly be brought about by naturally formed volatiles from carrots, from disease organisms or from seepage of fruit emanations through storage room walls.

A phenomenon of yellowish-green fluorescence under short wave 2537Å ultra violet light was significantly correlated with bitterness in the phloem tissues of the carrot. Microscopic study indicated that the fluorescence was located in oil glands and in senescent areas. A quick bitterness rating test employing fluorescence was developed.

CHAPTER XIV

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CHAPTER XV

APPENDIX

APPENDIX TABLE 1

RATINGS OF FLUORESCENCE OF TRANSVERSE SECTIONS AND BITTERNESS BY TASTE OF A PORTION OF THE CORTEX OF SIXTY-FOUR INDIVIDUAL SHORT TYPE CHANTENAY CARROTS, MARCH 1, 1956

Root No.	Fluorescence(1) Ext.	Phloem	Bitterness	Root No.	Fluorescence(1) Ext.	Phloem	Bitterness
1	5	2	2	33	2	4	2
2	2	3	3	34	1	1	1
3	3	3	3	35	3	2	2
4	4	2	3	36	1	1	1
5	5	2	5	37	2	2	5
6	5	2	4	38	1	1	2
7	4	2	5	39	5	3	4
8	2	1	1	40	2	4	3
9	3	2	4	41	5	2	2
10	2	2	3	42	5	3	3
11	2	3	4	43	5	3	3
12	5	4	2	44	2	1	5
13	3	3	5	45	5	3	3
14	4	2	4	46	4	3	3
15	2	3	5	47	3	2	3
16	4	3	5	48	4	3	2
17	5	3	5	49	1	2	1
18	4	5	5	50	5	4	4
19	3	4	5	51	5	2	5
20	2	2	3	52	5	2	2
21	5	3	4	53	3	2	3
22	5	2	3	54	4	3	2
23	4	4	5	55	4	3	2
24	2	2	5	56	5	3	3
25	1	2	2	57	3	2	2
26	3	3	1	58	2	2	4
27	5	3	4	59	4	2	5
28	2	2	4	60	5	3	3
29	5	2	3	61	3	2	3
30	3	3	4	62	2	2	3
31	3	1	3	63	4	2	1
32	4	4	5	64	3	3	3

Correlation coefficient, r = .28 with statistical significance at .05 on phloem fluorescence and bitterness.

(1) 1 to 5 Scales: 1 = non-fluorescent and non-bitter, 5 = highly fluorescent and highly bitter.

APPENDIX TABLE 2

CHARACTERISTICS OF SIXTY-FOUR SHORT TYPE CHANTENAY CARROTS, MARCH 1, 1956

Root No.	Length (cm)	Di-ameter (cm)	Shape		Crown Height (1-5) (3)	Rel. Size Core: Cortex (4)	Injury(5)		Mold (1-5) (6)	Color		
			Taper (1)	Tip (2)			Scuffs Bruises (1-5)	Cuts Gouges Cracks (1-5)		Core (1-5) (7)	Cortex (1-5) (7)	Shoulder (8)
1	12	3.2	-	++	1	-XP	3	1	2	3	2	O
2	10	4.7	+	+	2	XP	3	3	2	4	2	O
3	15	6.0	-	++	3	+XP	3	2	2	4	2	GO
4	11.5	4.5	-	-	2	+XP	2	3	3	4	2	GO
5	16	6.3	+	-	2	+XP	3	4	3	3	2	GO
6	11	4.5	-	+	2	XP	4	2	4	4	2	GO
7	13	5.0	++	-	4	XP	2	3	3*	2	2	O
8	11	4.5	+	+	2	XP	2	3	2*	3	2	O
9	12.5	4.5	+	+	2	XP	3	1	3	3	2	PO
10	14	6.0	++	-	1+	XP	2	3	2	3	2	O
11	9.5	4.0	+	+	2	XP	3	2	1	5	2	O
12	10.5	5.2	-	+	3	XP	4	4	3	2	2	GO
13	11	4.5	-	+	5	+XP	2	2	2	2	2	GO
14	11	4.3	-	+	2-	XP	3	3	2	3	2	O
15	13	4.7	++	-	2	XP	4	3	3	1	1	G
16	14	3.7	+	-	2	X-P	4	3	4*	4	2	O
17	10.5	3.5	-	-	1	XP	4	5	3	2	2	O
18	11	4.2	-	+	5+	+X-P	4	2	2+	3	1	O
19	13	4.8	-	++	2	XP	3	2	2	3	2	P
20	12	4.7	-	++	2	XP	3	3	3	5	3	GO
21	12	6.0	++	-	4	+XP	4	2	5	4	2	O
22	10	3.2	-	+	2	XP	4	2	2	4	2	O
23	13	3.5	-	++	3	XP	4	2	2	2	2	P
24	11.5	5.4	+	+	1	+XP	3	4	3	4	2	GP
25	12	4.5	-	+	3	XP	3	2	2	4	2	PO
26	11	3.5	-	-	1	XP	2	1	1+	3	2	O

APPENDIX TABLE 2 - Continued

Root No.	Length (cm)	Di-ameter (cm)	Shape		Crown Height (1-5) (3)	Rel. Size Core: Cortex (4)	Injury(5)		Mold (1-5) (6)	Color		
			Taper (1)	Tip (2)			Scuffs Bruises (1-5)	Cuts Gouges Cracks (1-5)		Core (1-5) (7)	Cortex (1-5) (7)	Shoulder (8)
27	12	4.2	-	+	1	XP	4	4	3*	5	2	GO
28	11	5.5	+	-	2	+XP	4	3	3	4	2	O
29	9	5.5	++	+	2	+XP	5	2	4	4	2	O
30	10.5	4.5	+	-	2	XP	3	2	3-	5	2	GO
31	10	4.8	+	-	2	XP	3	3	3*	3	2	GO
32	11.5	4.0	+	-	4	XP	4	4	2+	3	2	PO
33	15	4.0	-	+	2	XP	3	4	3	3	2	P
34	13.5	5.0	-	+	2	XP	4	3	3	3	3	P
35	12	5.4	-	++	3	XP	3	3	2*	3	2	P
36	11	5.5	++	-	1	XP	3	1	2	3	2	O
37	9.5	4.0	+	+	3+	XP	3	3	2*	5	2	O
38	13	5.9	+	+	3	XP	3	3	2	3	2	G
39	14	4.6	-	+	4+	XP	4	2	2*	2	2	GP
40	10	4.0	+	+	2	XP	3	3	1*	3	2	PO
41	9	3.5	++	+	2	XP	4	2	3	3+	2	O
42	12	5.0	++	-	2	XP	3	4	3*	2	2	PO
43	13.5	6.7	+	+	5	+XP	3	3	2*	3	1½	G
44	9	3.5	+	+	2	X-P	3	2	2*	5	3	PO
45	15	5.3	-	+	3	X+P	3	4	3	4	2	PGO
46	11	4.0	-	+	2	-X+P	4	3	3	4	2	O
47	12	3.8	-	+	5	XP	4	2	2	5	2	G
48	7.5	3.8	+	+	2	XP	3	2	2	3	2	O
49	13	6.0	+	+	4	+XP	3	3	2	2	2	PO
50	13	3.5	+	-	1+	XP	3	3	2	3	2	O
51	8	4.3	+	+	2	+X-P	4	2	3	4	2	O
52	13	5.0	+	+	2	X+P	4	2	3	3	2	GO
53	13	6.0	-	+	5	+XP	3	5	4	3	2	PG
54	9	3.8	+	+	1	-XP	4	3	2	3	2	PO
55	8	3.7	+	+	2	-XP	4	3	4	3	2	O
56	14	5.7	-	+	2	XP	4	4	4*	4	2	PO

APPENDIX TABLE 2 - Continued

Root No.	Length (cm)	Diameter (cm)	Shape: Taper (1)	Shape: Tip (2)	Crown Height (1-5) (3)	Rel. Size Core: Cortex (4)	Injury(5): Scuffs Bruises (1-5)	Injury(5): Cuts Gouges Cracks (1-5)	Mold (1-5) (6)	Color: Core (1-5) (7)	Color: Cortex (1-5) (7)	Color: Shoulder (8)
57	11	5.3	+	-	3	-X+P	3	3	3*	3	2	G
58	8.5	3.2	-	+	2	XP	3	2	3*	2+	2	O
59	10.5	5.3	++	-	2	+XP	3	4	2	3	2	O
60	10.5	4.0	-	+	3	+X-P	4	3	3*	3	2	O
61	8	3.8	+	+	2	XP	4	3	3*	3	2	PO
62	12	4.1	+	-	3	+XP	3	3	2	4	2	G
63	12	5.9	+	+	3	XP	3	3	3	3	2	O
64	11.5	3.4	+	+	2	XP	4	5	3*	2	1½	O

(1) Taper: ++ = yes, much; + = yes, a little; - = no.

(2) Tip: how blunt, same values as 1.

(3) Crown Height: height of growing point above shoulder, 1 = level with shoulder, 5 = presence of flower stalk.

(4) Relative Size of Core and Cortex: XP = average, -P = thin phloem, +P = thick phloem, -X = small diameter xylem or core, +X = large diameter core.

(5) Injury: 1 = no injury, 5 = heavy or extensive injury.

(6) Mold: 1 = no mold, 5 = beginnings of rot; * = depression in side of root, probably Violet Root Rot, a Rhizoctonia species.

(7) Color: 1 = light yellow, 5 = dark orange.

(8) Color Shoulder: O = orange, G = green, P = purple.

APPENDIX TABLE 3

FLUORESCENCE, BITTERNESS AND AROMA RATINGS OF TEN INDIVIDUAL SHORT TYPE CHANTENAY CARROTS, MARCH 28, 1956 (1 to 5 ratings[1])

Root No.	Fluorescence of Whole Root by Layers[2]					Bitterness by Taste of Parings of Layers[2]					Bitterness by Taste on Whole Root	Aroma
	1	2	3	4	5	1	2	3	4	5		
1	1	2	2	2	1	2	1	3	3	1	3	1
2	2	2	3	4	1	3	2	3	4	2	5	3
3	2	2	2	2	1	1	3	5	5	1	5	5
4	1	2	2	2	1	2	2	3	4	1	3	1
5	1	2	2	2	1	2	1	2	2	1	3	1
6	1	2	3	3	1	1	2	3	5	1	4	4
7	1	2	2	2	1	1	1	1	2	1	2	3
8	2	4	4	4	1	2	3	4	5	1	5	5
9	1	2	2	2	1	1	3	4	5	1	5	4
10	2	2	2	2	1	2	2	3	4	1	4	3

(1) Ratings of 1 are most favorable, 5 least favorable.

(2) Layer 1 is outside peeling; layer 4 is tissue about the cambium; layer 5 is xylem tissue or core.

Correlation coefficients significant to .05 by t test: 1-Summation of layers of phloem (1 to 4) for fluorescence and bitterness r = 0.645; 2-Summation of layers of phloem and bitterness of whole root r = 0.588; Bitterness of whole root and aroma r = 0.712; Bitterness on summation of layers and on whole root r = 0.914.

APPENDIX TABLE 4

CHARACTERISTICS OF TEN SHORT TYPE CHANTENAY CARROTS, MARCH 28, 1956(1)

Root No.	Root Wt. Grams	Root Size		Crown Height (1-5)	Rel. Size Core: Cortex	Injury		Mold (1-5)	Color	
		Length (cm)	Diameter (cm)			Scuffs (1-5)	Cuts (1-5)		Xylem (1-5)	Phloem (1-5)
1	126	14	4.2	2	XP	4	4	3*	5	3
2	158	12	4.8	4+	XP	2	2	1*	3	2
3	150	12	4.5	2	XP	3	2	5	4	2
4	164	11.5	5.3	4+	XP	3	2	1	4	3
5	222	11	6.4	3	+XP	4	3	5*	2	3
6	154	11	5.2	4	XP	2	1	2	4	3
7	76	11	3.5	1	XP	2	2	2*	3	3
8	82	9.5	3.9	2	XP	2	1	3	3	2
9	58	9	3.4	4	XP	3	1	2	3	2
10	48	8.4	3.6	2	XP	3	2	3	3	2

(1) See footnotes, page 99.