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Title: THE INHERITANCE OF VOLATILE ALCOHOLS ASSOCIATED WITH FROZEN BUSH SNAP BEANS

Abstract approved: Dr. W. A. Frazier

Inheritance of the volatile components in bush snap beans, O. S. U. 58-110 X 'Romano' cross and reciprocal, was determined using gas-liquid chromatographic technique with gas-entrainment on-column trapping. A sample of 10 to 14 pods averaging 5 grams from a single plant was found to be adequate for F_1, F_2, and backcross studies.

The characteristic low concentration of 1-octen-3-ol in O. S. U. 58-110 was dominant over that of the higher concentration of 'Romano' in the F_1 generation. A chi square goodness of fit test for a 3:1 ratio in the F_2 and 1:1 ratio in the backcross of the F_1 to 'Romano' indicated a single dominant gene controlling the inheritance of 1-octen-3-ol. A better fit to a 9:7 ratio was shown for the F_2, but backcrosses to both parents did not indicate a two gene action. Backcross results have shown the importance of studying inheritance by

more than one method.

The difference in mean concentration and the amount of overlapping in the concentration of linalool found in the two varieties made it impossible to distinguish any genetic ratios.

Flower color inheritance for crosses involving 'Blue Lake' types (white flower) X 'Romano' (light purple) were shown to be controlled by two genes. Dominant gene $V_{\underline{lae}}$ produced the purple color while the recessive remained white. The second gene, Aeg, (with other dominant influencing genes) when dominant caused the banners to be a darker purple color than if the gene was recessive. No linkage was found between flower color intensifier and 1-octen-3-ol. It was not possible to determine linkage of the flower color, $V_{\underline{lae}}$, and 1-octen-3-ol.

An oval 'Blue Lake' mutant was found in the line O. S. U. 9025. Compared to the original round pod type, it was characterized by strings on both sutures, oval pod, glossy smooth light green outer skin, and light color inner flesh.

Thawing time, maturity, and processing affected the concentration of 1-octen-3-ol. Concentration of 1-octen-3-ol decreased with maturity (days from anthesis). The concentration in frozen beans was found to increase with longer thawing times. Processed samples, whether frozen or canned, were lower in concentration than fresh raw samples.

Processing influences on other volatiles were mostly quantitative, but several of the compounds were lost in the frozen samples. Increases in the "lower boiling" volatiles of the canned products were attributed to the heat induction and degradation of metabolites. A loss of volatiles in the frozen samples and "higher boiling" components in the canned product is believed to be through vaporization and leaching into the hot water bath during the blanching process. Reduction in quantity and losses of compounds with influence of thawing time made the freezing process less desirable for preserving samples for genetic studies. Fresh raw samples would be better for inheritance studies if the time required for analysis could be reduced.

Maturity and the nature of quantitative variation among varieties would limit the use of volatile flavor components for chemotaxonomy; yet analysis for phenolics, alkaloids, and flavonoids, combined with botanical means, should provide the most effective system for differentiating varieties.

The Inheritance of Volatile Alcohols Associated with Frozen Bush Snap Beans

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INTRODUCTION

In recent years there has been considerable emphasis on volatile flavor components of foods. More sensitive instruments and improved techniques have been mainly responsible (Teranishi, Lundin and Scherer, 1967; Weurman, 1969; Stevens, 1969). However, few of these studies have been concerned with the genetics of volatile flavor components (Stevens, 1970).

Knowledge of the inheritance of such flavor components can be useful to the plant breeder in affording him more control of flavor through selection of parent types and prediction of results in crossed progenies. The number of generations and amount of selection pressure required to obtain desirable flavors can be estimated. Studies of linkages of flavor compounds to more noticeable and simply inherited characters can also make breeding for flavor more predictable.

The objective of this study was to continue investigation of the genetics of flavor components involved in the development of bush 'Blue Lake' snap beans. In addition it was proposed to provide a method of using smaller quantities of sample (individual plants) that are closer to the raw product, and to improve the sampling technique. Hopefully the results will aid in studies of the inheritance of flavor

components, and may lead to the use of flavor chemotaxonomy of varieties.

REVIEW OF LITERATURE

Chemistry of Green Snap Bean Pods

Gross Composition

Flavor in foods has been defined as the complex sensation comprising taste, odor, and of lesser importance, temperature, texture, and psychological factors (Amerine, Pangborn and Roessler, 1965; Kulka, 1967). A survey of the gross composition of the green snap bean pod may be instructive in understanding the development of the overall flavor in the natural product and changes due to processing. Comparison of the composition in raw, frozen (unthawed), and canned green beans are as follows:

	100 grams edible portion		
	Raw	Canned (regular pack)	Frozen (unthawed)
(Watt and Merrill, 1963)			
Water	90.1%	93.5%	91.7%
Protein	1.9	1 0	1.7
Fat	0.2	0.1	0.1
Carbohydrate			
Total	7.1	4.2	6.0
Fiber	1.0	0.6	1.0

	Raw	Canned	Frozen
Ash	0.7	1.2	0.5
Calcium	0.056	0.034	0.042
Phosphorus	0.044	0.021	0.033
Iron	0.0008	0.0012	0.0008
Sodium	0.007	0.236[a]	0.0012
Potassium	0.243	0.095	0.167
Vitamin A	0.6	0 29	0.58
(Burger et al., 1956)			
Magnesium	-----	-----	0.021
Folic Acid	-----	-----	0.00002
Pantothenic Acid	-----	-----	0.00012
(Woodroof, Heaton and Ellis, 1962)			
Ascorbic Acid			
Uncut	0.00905	-----	-----
Cut	0.00771	-----	0.00485

[a]Salt was added for canning.

In general, protein and carbohydrates are low in raw or processed green beans, while other components tend to be typical of other vegetables. Clearly, processing has a considerable effect on the composition and undoubtedly accounts for some differences in flavor.

Inorganic compounds may be responsible for a number of factors affecting flavor directly such as structures of pigments (sight), salt formation (taste), binding and ionization in the cell walls (texture). Indirectly they can be responsible for the metabolism of volatile compounds.

Fiber content, protein, and total carbohydrate can also affect texture and taste. Sistrunk (1969) found canned sieve size 4 beans to have on a dry weight basis:

23.24% total sugars	11.423% cellulose
5.24 soluble starch	4.427 water soluble pectin
2.355 hemicellulose	2.754 cal-soluble pectin

Acids contribute a sour note or tartness, the variation depending on concentration, pH, and type of acid. Bakowski, Schanderl and Markakis (1964) reported on a fresh weight basis non-volatile acids present in a green bean line from a cross of 'Green Crop' X 'Romano'. Column and paper chromatography were used to identify:

malic	0.112%	pyrrolidone carboxylic	0.002%
phosphoric	0.042	malonic	trace
citric	0.034	amino acids	
succinic	0.004	aspartic	0.010
galacturonic	0.003	glutamic	0.004

Two more minor acids were also found but not identified.

Amino acids are usually sweet only in the alpha form. The

sweetness increases as the amino group and the carboxyl group become closer. Amino acids are considered precursors to carbonyl compounds in some instances as will be pointed out in the metabolism of volatiles. Rowlands and Corner (1963) have identified:

serine	proline
asparagine	tyrosine
alanine	glutamic acid
glutamine	aspartic acid

in the flowers of broad beans.

Volatile Components

Analysis of volatile compounds has been the approach many food chemists have taken in quest of understanding flavor (odor is believed to contribute most to flavor). Stevens (1967) reviewed studies on volatile compounds found in beans up to 1967. He also reported the identification of 40 compounds in canned snap beans by gas chromatography, infra-red, and mass spectroscopy. The following compounds were identified:

ethanol	hex-4-en-1-ol
cis-hex-3-en-1-ol	n-hexanol
2-methyl-2-hexanol	oct-1-en-3-ol
furfurol	benzyl alcohol
acetaldehyde	2-methyl propanal

3-methyl butanal	methylthioethanal
n-hexanal	hex-2-en-1-al
methional	furfural
5-methyl furfural	2-methoxy furfural
2-methyltetrahydrofuran	3-pentanone
diacetyl	2-heptanone
3-octanone	ethyl acetate
hex-3-en-1-yl acetate	ethyl phenyl ether
furfuryl methyl ether	methyl benzyl ether
veratrole	2-methoxy methyl benzyl ether
2-butoxy toluene	2-(2-methoxy ethyl) methoxy benzene
phenyl ether	
linalool	aryl-methoxy phenol
pulegone	α-terpineol
α-phellandrene	pyridine
biphenyl	

Stevens concluded that cis-hex-3-en-1-ol, oct-1-en-3-ol, linalool, α-terpineol, pyridine, and furfural are of primary importance in canned snap bean flavor and more particularly in the difference in flavor between varieties.

More recently MacLeod and MacLeod (1970) studied the volatiles of fresh and frozen runner beans after 10 minutes of cooking.

Compounds identified in fresh sample were:

methanethiol	acetaldehyde
dimethyl sulfide	propionaldehyde
acetone	n-butyraldehyde
ethyl methyl ketone	methyl alcohol
ethyl alcohol	diethyl ketone + diacetyl
but-2-en-1-al	dipropyl sulfide
dimethyl disulfide	allyl alcohol
dipropyl ketone	trans-pent-2-en-1-al
allyl cyanide	trans-but-2-en-1-ol
methyl propyl disulfide	cis-pent-3-en-1-ol
butyl methyl disulfide	cis-pent-2-en-1-ol
cis-hex-3-en-1-ol	butyl isothiocyanate
trans-hept-3-en-1-ol	

Trans-but-2-en-1-ol, cis-pent-2-en-1-ol, cis-hex-3-en-1-ol, and buty isothiocyanate were not identified in the frozen runner bean. However, acrolein, trans-hex-2-en-1-al, and dipropyl disulfide were identified in the frozen, but not in the fresh beans.

No single compound has the true characteristic of green bean flavor (Stevens, 1970). According to Hoffman (1961), 3-cis-hexenal, hexanal, and 3-trans-hexenal are reminiscent of green bean odor. Hewitt (1963) suggested 2-hexenal as being important.

Metabolism of Volatile Components

Enzymic processes producing the natural flavor substances and chemical reactions for the cooked flavors are the two essential mechanisms by which flavors may be produced according to Rohan (1970).

Konigsbacher and Hewitt (1964) believed the biosynthesis of odor to be part of the normal metabolic process of plants. This would entail many chemical routes by which odor substances are formed including synthesis by amino acids, isoprene groups, acetate moieties or breakdown products of more complex compounds such as glucosides, thioglycosides, or fatty acids.

The volatile terpene compounds are generally considered to be produced by the isoprenoid pathway (Bonner and Varner, 1965). No further discussion will be made of the terpene metabolism since the pathways are presented elsewhere in considerable detail (Pinder, 1960; Monick, 1968).

Yu, Olson and Salunkhe (1968) reported enzymes from red ripe tomato fruits reacted with alanine to give mainly carbonyl compounds (noted especially propanal), while leucine and valine gave mainly alcohols. They concluded that conversion of amino acids to volatile components appears to involve transamination.

More specifically, Meyers, Issenberg and Wick (1970) studied

radioactive L-leucin U^{14}-C in incubated ripe banana tissue discs. As much as 81% of the volatile radioactivity was found in isoamyl alcohol and 10% in isoamyl acetate, both compounds being major banana aroma constituents.

Most odor development studies have been limited to enzymatic enhancement of flavor from a processed product (Mackay and Hewitt, 1958; Schwimmer, 1963; Hewitt, 1963; Heatherbell and Wrolstad, 1971). It is assumed the flavor precursors survive processing while the enzyme of the food source and the natural flavor (through evaporation) are lost. By adding the appropriate enzyme preparation to the surving precursors the flavor of the processed product is reported to be similar to the raw commodity.

Cort et al. (1959) studied acid phosphatase, peroxidase, and dehydrogenase activity in the nonchloroplast preparations of fresh green bean juice. Only alcohol dehydrogenase with the cofactor triphosphopyridine nucleotide (perferable) or diphosphopyridine nucleotide (reaction much slower) added to the following substrates were found to be active:

ethanol	3-hexen-1-ol
1-hexanol	5-hexen-1-ol
1-propanol	2-hexen-1-ol
n-butyl alcohol	rhodinol
n-amyl alcohol	

Hewitt (1963) reported that the Thunberg tube assay on 2-hexen-1-ol resulted in 2-hexenal within eight minutes in the presence of the green bean enzyme and triphosphopyridine nucleotide. Ethanol was oxidized to 2-hexenal in 12 minutes by the green bean enzyme requiring only diphosphopyridine nucleotide as a cofactor.

Off-Odor Production

Chow and Watts (1969) concluded that lipid oxidation and fermentative changes contributed to the flavor deterioration of frozen beans. Malonaldehyde, an index to unsaturated fatty acid oxidation through action of lipoxidase, was related to the rancid odor. Acetaldehyde, a product of anaerobic fermentation, does not reproduce the full off-odor of anaerobically held samples. Secondary reactions of these aldehydes with other food constituents may be responsible. Anaerobic fermentation within plant tissues converts pyruvate principally to ethanol by way of acetaldehyde, but acetoin and diacetyl can also be produced.

Fuleki and David (1963) found that at -21° C the formation of acetaldehyde and ethanol was almost completely inhibited, but only slowed off flavor development. Acetaldehyde and ethanol accumulation was highest early in storage then leveled off or decreased. Acetaldehyde and ethanol appeared not to provide an objective measure of off flavor development.

Genetics of Flavor Components

Vegetable Crops in General

Studies on the inheritance of flavor compounds are very limited. A review of these on vegetable crops are reported by Stevens (1970). An older review on related areas was presented by Stevens (1967). The attempt here will be to bring this review up to date on the inheritance of volatile compounds and add to the non-volatile components.

Stevens (1972) found that citrate and malate in tomatoes are controlled by a single gene for each compound. The dominant high concentration of citrate and the dominant low concentration of malate in plant introduction number (P. I.) 263713 are linked with a recombination of 18%. He concludes that there is no practical reason to breed for a specific citrate/malate ratio.

Inheritance of bitter substance elaterinide in watermelons was studied by Chambliss, Erickson and Jones (1968). A single recessive suppressor gene, $\underline{su}^{Bi}$, active in the presence of the plant bitterness gene, $\underline{Bi}$, results in nonbitter fruits. Quantitative differences in elaterinide content in bitter fruits is explained by a proposed modifer gene, $\underline{Mo}^{Bi}$.

In a study for insect resistance Da Costa and Jones (1971) found the bitter substance, cucurbitacin, to segregate in a 1:1 ratio

when the F_1 was backcrossed to the bitter parent. Feeding on the bitter plants (Bi) appeared to have a deleterious effect on the development and growth of the early larval stages.

Probably the most detailed study of gene control on individual compounds has been done on Mentha. Murray and Reitsema (1954) concluded that carvone, principal ketone of spearmint, was dominant over menthone, principal ketone of peppermint, in formation of their molecular structure. They suggested the mode of gene action determines the alternative methods of cyclization of a common antecedent ketone.

In a study of the conversion of methone to menthol, Murray (1960) suggested the action of the gene controls the reductase enzyme, which reduces the formation more in the dominant allelmorphs than in the recessive.

More recently Hefendehl and Murray (1972) have shown that the same dominant gene controlling the conversion of menthone to menthol is responsible for conversion of carvone to carveol and dihydrocarvone. They concluded that the multiple changes in oil composition are probably due to the difference in a single gene. They also confirmed the postulation that oxidation of pulegone to menthofuran is controlled by a single gene with incomplete dominance; the reduction of pulegone to menthone is also controlled by genes, but the number of genes involved was not given.

In snap beans Stevens (1967) reported the inheritance of oct-1-en-3-ol and linalool in canned varieties. The concentration of oct-1-en-3-ol is simply inherited with that of the 'Blue Lake FM-1L' variety dominant over both 'G-50' (Gallatin 50) and 'Romano'. Linalool appears to be simply inherited with an additive gene. Both compounds were influenced by stage of development in the pod.

Uses in Chemotaxonomy

Still in the infant stage, chemical taxonomical classification has made great strides. Although the number of compounds and varieties involved is not large, the accumulation of the reports would indicate the possibility for advancement. The review here will cover only the attempts of chemical classification in the genus Phaseolus.

Varietal differences in flavonoid compounds of leaves, flowers, and seeds of broad beans as well as in amino acids of the flower were shown by Rowlands and Corner (1963) using paper chromatography. Dwarf bean varieties also showed a difference in leaf flavonoids.

Qualitative paper chromatography was used by Singh and Thompson (1961) as a tool for classification of 13 varieties of beans based on their differences in flavonoid compound content. Four varieties were identified individually, while the others were split into a group of 5 and a group of 4. No attempt at identifying the flavonoids was made.

A suggestion has been made on possible uses of volatile flavor components in separation of varieties (Frazier, 1970). However, difficulties appear to limit their use on a quantitative basis as considerable variations of the parent lines were reported by Stevens (1967). Parent lines 'Romano', 'G-50', and 'Blue Lake FM-1L' showed 240±78.2, 15±7.7, and 160±12.1 p.p.b. mean concentration for oct-1-en-3-ol respectively and 40±10.0, 39±12.1, and 7±0.5 p.p.b. mean concentration for linalool.

The use of flavor volatiles would seem to need a standardizing method to reduce the variability in determination of the true concentration of compounds found in each variety.

Qualitatively (based on high and low concentration and not p.p b.) Stevens (1967) found by adding 0.2 p.p.m. linalool to the liquor of canned O.S.U. 9025 (bland flavored), the flavor of 'G-50' was closely approached. The rich beany flavor of 'Blue Lake FM-1L' was closely simulated by adding 0.4 p.p.m. 1-octen-3-ol and 1.6 p.p.m. cis-hex-3-en-1-ol to the liquor of O.S.U. 9025. These amounts of additional volatile compound needed to reach the respective variety flavor can be considered high since the natural concentrations are in p.p.b. These results indicate flavor compounds could be used in distinguishing varieties on a qualitative basis.

Specific Studies of 1-Octen-3-ol

Physical Properties

Emphasis is given here to 1-octen-3-ol because of its importance in the flavor of 'Blue Lake' snap beans.

Matsutake alcohol was first isolated by Murahashi (1938) from a Japanese mushroom (Armillaria matsutake) parasite growing on root hairs of Pinus densiflora. The more common name of this secondary aliphatic alcohol is 1-octen-3-ol (1-n-amylvinylcarbinol).

1-Octen-3-ol has a molecular weight of 128.21 and a structure of:

$$CH_3\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}\underset{\underset{OH}{|}}{CH}\text{-}CH{=}CH_2$$

Its boiling point of 742 mm Hg is 173.5°, refractive index N_D^{12} = 1.4391, and specific gravity of 13/4° C = 0.8395 (Monick, 1968).

Badenhop and Wilkens (1969) using soaked soybeans isolated a pure fraction of 1-octen-3-ol by gas chromatography. It was found to be levorotatory $\overset{17}{D}$ = -11.7. Based on this and other findings the mode of formation of 1-octen-3-ol in this case was presumed to be enzymatic.

Metabolism

1-Octen-3-ol formation appears to be through autoxidation of linoleic acid as found by Stark and Forss (1964) in oxidized dairy products, and by Smouse and Chang (1967) in reverted soybean oil. Both postulated the initial formation of an 18 carbon hydroperoxide and a concomitant allylic rearrangement followed by chain scission between C_{10} and C_{11}, but differ on resulting eight carbon fragments. Stark and Forss (1964) postulated a free radical undergoing allylic rearrangement followed by reaction with a hydroxyl free radical to produce 1-octen-3-ol. Smouse and Chang (1967) suggest the fragment, an olefin (2-octene), autoxidizes again with concomitant allylic rearrangement to 3-hydroperoxyl-1-octene. This reacts with another molecule of linoleic acid by cleavage of hydroxyl radical from the hydrogen peroxide. A subsequent acceptance of hydrogen ion from linoleic acid forms 1-octen-3-ol.

Hoffman (1962) believed also that the 1-octen-3-ol production was autoxidative from linoleic acid. However, he postulates the formation of a hemi acetal from unstable free radicals of linoleic acid via an unstable intermediate. An intermolecular cyclic rearrangement and cleavage of the hemi acetal would give a secondary unsaturated alcohol (1-octen-3-ol) and an unsaturated aldehydic acid.

Occurrence in Plants

Murray et al. (1968) felt hex-3-en-1-ol, a strong source of green leaf odor, and 1-octen-3-ol, a mushroom like odor, are important contributors to green pea flavor. These same two components were mentioned earlier as being responsible for the characteristic flavor of 'Blue Lake' snap beans.

Honkanen, Moisio and Karvonen (1969) also reported these same two compounds to be important in the volatile flavor substance in clover species. In review they reported the following to contain 1-octen-3-ol: Mentha species, Lavandula species, Chamaecyparis obtusa, Armillaria matsutake, Vaccinium vitis-idaea L., V. macrocarpon, and Ribes nigrum L.

Additionally, as reviewed by Stevens (1967), 1-octen-3-ol has been reported in the following plants: black current, bay, Salira sclarea, Perilla frutescens, and clover.

More recently 1-octen-3-ol has been found in: Psalliota campestris (Freytag and Ney, 1968), cocoa (Marion et al., 1967), French and Russian clary sage oil (Teisseira, 1961).

MATERIAL AND METHODS

Genetic Material and Crosses

Parent Materials

Three varieties of bush snap beans (Phaseolus vulgaris L.) were studied for the inheritance of linalool and 1-octen-3-ol production.

O. S. U. 58-110 was developed by Dr. W. A. Frazier from a single selection of a bulk planting in 1965 at the North Willamette Experiment Station near Aurora, Oregon Many complex crosses were involved in the bulk planting; consequently, the exact parentage is not known. The variety closely approximates the 'Blue Lake' pole bean in quality and possesses a moderately upright growth habit. Pods of the variety are characterized by being round, stringless, fleshy, dark green, large sieved, and closely resembling 'Blue Lake' pole bean in taste (taste panel evaluation). The plant is a bush, with white flowers, and appears less sensitive to day-length and to high temperatures than other O. S. U. lines (Frazier et al., 1968; Frazier, 1970).

Bush 'Romano FM-14' was selected from a cross between pole 'Romano' X 'Bachicha' in 1961 by John Moran of Ferry Morse Seed Company (McCabe, 1972). It resembels pole 'Romano' in the strong,

rich, bean flavor. It also is characterized by a flat pod and light purple flower color.

O. S. U. 9025 was selected by Dr. W. A. Frazier from O.S U. 6025, the original parents being pole 'Blue Lake' and 'White Seeded Tendercrop'. The 'Blue Lake' bush parent was in the seventh generation of backcrosses to pole 'Blue Lake'. Stevens (1967) found O. S. U. 9025 to be bland in volatile compounds, especially 1-octen-3-ol and linalool. It has a lighter colored bush plant, white flowers, with a more upright habit than O. S. U. 6025. The pods tend to be slightly oval, possibly more fibrous, and the smaller pods tend to have more color than the older ones.

Crosses

Crosses of O. S. U. 9025 × O. S. U. 58-110, O. S. U. 9025 × 'Romano' (bush), O. S. U. 58-110 × 'Romano' (bush), and their reciprocals were made in 1969. The F_1 progenies were planted on the Oregon State University Vegetable Research Farm in June, 1970. Individual F_1 plants were kept segregated to prevent mixing at harvest time.

All F_1 progenies were checked throughout the summer for possible selfing. 'Romano' crosses with either O. S. U. 9025 or O. S. U. 58-110 were checked by seed color and flower color intensity. Pod characteristics, growth habit, and plant color were used

to check O. S. U. 58-110 × O. S. U 9025 crosses.

Backcrosses of the F_1 to both parents were made towards the end of July and the first part of August. Additional F_1 crosses were also made. Crossing techniques used were described by Hikida (1961) with modification from the procedure of Wigton (1959).

Seeds from F_1 plants not used in the backcrosses were saved for the F_2 generations. Each plant of the F_2 was threshed and recorded separately.

Backcrosses, F_1 generation, F_2 generation, and parent lines were randomly planted in the field from May 10, 1971 to July 10, 1971. Parent lines were planted throughout this period for testing possible differences in concentration of each compound. Plantings were made in rows 36 inches apart with plant spacings 18-24 inches apart.

Processing Methods

Plant Preparation

All samples were based on individual plants. The first ten plants of each plot were selected to represent the individual plants of F_2 families. It was recognized that maturity would be difficult to control because of the varying pod shape (flat versus round). Sieve sizes and days from anthesis were used to show differences

in concentration and for indicating maturity of the pod. By comparing varieties using these two factors, maturity of the flat type beans could be judged in relation to other pods. Larger sieve sizes were selected for use because they were reported to have smaller changes in concentration of volatile material (Stevens, 1967). Sieve 4 and 5 pods were hand picked in the morning 8:00 A. M. to 10:00 A. M. Samples were then taken to the pilot plant of Food Science for processing.

A list of the varieties and lines prepared for raw, frozen, and canned products follows (all crosses included reciprocals):

Raw beans (refrigerated at 34° F [1. 11° C] until preparation for analysis, which was made the same day)

'Romano' (bush)
O. S. U. 949
O. S. U. 58-110
O. S. U. 190
'Gallatin 50'
'Blue Lake FM-1K'
O. S. U. 9025

Frozen beans

'Romano' (bush)
O. S. U. 58-110
'G-50'
O. S. U. 9025 and 'Blue Lake' oval mutant bush
'Romano' (bush) × O. S. U. 58-110 F_1 and F_2
O. S. U. 58-110 × O. S. U. 9025 F_1 and F_2

'Romano' (bush) × O. S. U. 9025 F_1 and F_2

Backcross ('Romano' × O. S. U. 58-110) F_1 × 'Romano'

Backcross (O. S. U. 58-110 × 'Romano') F_1 × 'Romano'

Backcross ('Romano' × O. S. U. 58-110) F_1 × O. S. U. 58-110

Backcross (O. S. U. 58-110 × 'Romano') F_1 × O. S. U. 58-110

Backcross ('Romano' × O. S. U. 9025) F_1 × 'Romano'

Backcross (O. S. U. 9025 × 'Romano') F_1 × 'Romano'

Backcross ('Romano' × O. S. U. 9025) F_1 × O. S. U. 9025

Backcross (O. S. U. 9025 × 'Romano') F_1 × O. S. U. 9025

Backcross (O. S. U. 58-110 × O. S. U. 9025) F_1 × O. S. U. 58-110

Backcross (O. S. U. 9025 × O. S. U. 58-110) F_1 × O. S. U. 58-110

Backcross (O. S. U. 58-110 × O. S. U. 9025) F_1 × O. S. U. 9025

Backcross (O. S. U. 9025 × O. S. U. 58-110) F_1 × O. S. U. 9025

Canned beans

O. S. U. 58-110

'Romano' (bush)

'G-50'

Freezing Procedure

Randomly selected 10 to 14 pods from each single plant were water blanched at 210° F (99° C) for 1 3/4 minutes. The blanched pods were cooled in cold water to room temperature. Samples were individually quick frozen (I. Q. F.) on trays in a -25° F (-31. 7° C) blast freezer. To minimize the amount of enzymatic activity, beans were frozen whole without trimming or cutting. After three to four hours the frozen beans were placed in #303 cans, sealed, and put into -10° F (-23. 3° C) room for storage. All samples were processed within six hours after being brought in from the field.

Enzyme inactivation of the blanched samples were tested with a modified method of Resende, Francis and Stumbo (1969) and Varseveld (1969). Samples were tested before freezing and after the storage period. Pod samples of 20 g and 60 ml of distilled water were blended and filtered through four layers of cheese cloth. Two ml of filtrate was placed into a test tube with 20 ml of distilled water. One ml of 0. 5% guaiacol and one ml 3% of hydrogen peroxide were added without mixing. Observation of brown coloring would indicate that the beans were not blanched long enough to inactivate the enzymes.

Canning Procedure

More than an individual parent plant was needed to supply enough pods to fill the #303 cans for processing. The beans were trimmed, cut, and sorted by hand. The procedure for canning is the same as that reported by Stevens (1967).

Headspace Analysis of Volatiles

Preparation of Sample

Frozen bean samples were allowed to thaw for three hours except in the studies of thawing time. Frozen and raw samples were prepared in the same manner for analysis. For canned products ten ml of the liquor was used.

Samples were weighed and placed in a Waring Blendor with an equal weight of distilled water (1:1 ratio). After one minute of blending the samples were filtered through four layers of cheese cloth. Ten ml of the filtrate was placed in a screw type vial (B-78105-3, Van Waters and Rogers). A few mg of tetradecanol to control foaming, 0.2 ml of internal standard, and a small magnetic stirrer were placed in the vial. A 25 p.p.m. concentration of 2,6-xylenol was used as the internal standard. Prior to gas entrainment on-column trapping, the original caps and liners were replaced with those specially prepared for this type of analysis

(Stevens, 1967).

On-Column Entrainment

The volatile compounds were compared by headspace gas-liquid chromatography (G. L. C.) using the gas-entrainment on-column trapping technique developed by Morgan and Day (1965) and modified by Stevens (1967). Slight modifications were necessary in this study. The vials were heated in a 90° C hot water bath for one minute before gas entrainment. This allows for a full ten minutes of entraining the sample in 90° C±3° C hot water bath. A small magnetic stirrer inside the vial, along with the purge carrier gas, kept the filtrate well agitated. The nitrogen carrier gas flow rate was 20 ml/min. The cold bath consisted of crushed Dry Ice in ethanol.

After each sample run the disconnected entrainment assembly was cleaned by drawing Labtone detergent (Van Waters and Rogers) and hot water through it with a water aspirator pump. After a final rinse of distilled water, the assembly was dried in an oven at 100° C until the next run. (Note: Tygon tubing was found to produce a large peak in the chromatograms between 1-octen-3-ol and linalool).

Gas-Liquid Chromatography

An F & M Model 810 gas-liquid chromatograph with a dual hydrogen flame ionization detector was used for these studies. It

was connected to a Barber-Colman Series 8000 chromocorder.

In preliminary experiments, packed columns of the following were tested for separation of compounds: Carbowax 20 M, O.V. 17, O.V. 1, Apiezon L, S.F. 9650 IGEPAL. A pair of columns, 19 feet × 1/8 inch outside diameter, packed with 2.5% Apiezon L on 80-100 mesh Chromosorb G AW DMCS (Chromosorb G acid washed dimethyldichlorosilane treated) gave the best results.

The following conditions were used:

Purge time and rate	10 min at 20 ml/min
Water bath temperature	90°C±3°C
Column temperature	
Initial	60°C for 2 min
Program	4° C/min
Final hold	130°C for 20 min
Flow rates	
Hydrogen	50 ml/min
Air	300 ml/min
Nitrogen	30 ml/min
Detector temperature	200°C
Injection port temperature	185°C
Range	10
Attenuation	X1

Quantitative Analysis of Data

Peak areas were calculated from chromatograms using height times width at half-height (McNair and Bonelli, 1968). Peak areas for sloping baselines were made in a similar manner (Hawkes and Russell, 1965). In some cases (influence of processing on concentrations and thawing time) peak areas were used directly for comparison of quantity. Where concentrations of 1-octen-3-ol were needed, known concentrations of this compound were chromatographed and the peak areas versus concentrations were charted (Appendix I). In all cases the peak areas of the internal standard, 2, 6-xylenol, were measured in order that all chromatograms could be compared on equal basis.

Mass Spectrometry and Retention Time Identification

Porapak Collection

For identification of compounds using the mass spectrometer (M. S.), it was necessary to decrease the amount of water eluting from the G. L. C. column and increase the amount of volatiles entrained. A Porapak Q collection method as suggested by Dravnieks and O' Donnell (1971) with modifications by Morgan (1972) met these requirements. The enlarged Porapak columns, 4 inches × 1/4 inch

outside diameter, were conditioned at 200°C for 20 minutes followed by 100°C for 12 hours with a nitrogen flow rate of 60 ml/minute. The ends of each Porapak column were labeled so that each column had a no. 1 end and a no. 2 end.

The Porapak column end No. 1 was connected to the headspace assembly for gas entrainment. A 250 ml reagent bottle filled with 50 ml of filtrate (prepared the same as previously mentioned in G. L. C. studies) was used in the entrainment of the volatiles. The following conditions were used:

Hot water bath temperature	
Frozen O. S. U. 58-110	90°C for 30 min
Fresh O. S. U. 58-110	65°C for 30 min
Nitrogen flow rate	30 ml/minute
Porapak trap temperature	55°C

Following the entrainment of the volatiles, the Porapak column was disconnected from the entrainment assembly and connected directly to the filtered carrier gas line. Using the same conditions as in the entrainment, except the time was increased to one hour, water was removed from the Porapak column.

In transferring the volatiles to the capillary trap, the Porapak column was reversed such that end No. 2 was connected to the carrier gas line. The following conditions were used:

Nitrogen flow rate	12 ml/min

Porapak column temperature	125° C
Time of collection	30 min
Cold bath	crushed Dry Ice

Mass Spectrometry

An Atlas CH-4 single-focusing mass spectrometer was used to separate and identify the high boiling compounds present in fresh and frozen pods. A 4:1 splitter in the G. L. C. unit allowed 80% of the volatiles to go to the membrane and 20% to the flame. A Llwellyn single-stage silicon rubber membrane separator (Varian V-5620, Varian Analytical Division, Palo Alto, Calif.) was used to enrich the gas chromatograph effluent in organics by excluding carrier gas. The following operating conditions were used:

Gas-Liquid Chromatography

Injection of sample	a special adaptaion was made on the G. L. C. for the capillary column (Scanlan, Arnold and Lindsay, 1968)
Instrument	F & M Model 810
Detector temperature	242° C
Injector temperature	200° C
Column	
Stationary phase	column coated with methylene

	chloride solution of Carbowax 20 M and Versamid 900 (Mon, 1971)
Tubing	500 ft. stainless steel 0.030 in diameter capillary column
Initial temperature	70° C for 5 min
Temperature program	1° C/min
Final hold	180° C
Flow rate	12 ml/min (Helium)
Range	10
Attenuation	X1
Mass Spectrometry	
Filament current	20 A
Electron voltage	70 eV
Accelerating voltage	3000 V
Analyzer pressure	9×10^{-7}
Multiplier voltage	1600 V
Scanning speed	2.5 sec from m/e 25 to m/e 250 and 5.0 sec from m/e 25 to m/e 250 on compounds with longer retention times on G. L. C. columns

Recorder	Honeywell Model 1508 Visicorder

Gas Chromatography Retention Time

Retention values were calculated by dividing the time for the internal standard, 2, 6-xylenol, to be eluted into the time of elution of the volatile compounds.

Factors Influencing Concentration of Volatiles

Effect of Maturity

Studies were made on the influence of maturity on concentrations of 1-octen-3-ol. 'Romano'(bush) and O. S. U. 58-110 were tagged on the day of anthesis. Harvests were made in 1971 at the 11th (or 12th), 16th, 21st, and 28th day from anthesis. Measurements of weights, lengths, diameters, and sieve sizes (O. S. U. 58-110) were made at the first three harvest dates and used to establish equivalence of maturity in the two varieties. Separation of the seed from the pod was done on fresh samples only.

Influence of Thawing Time

Frozen parent lines O. S. U. 58-110 and 'Romano'(bush) were prepared the same as those in the inheritance studies. G. L. C.

analyses were made of the filtrate at 0, 2, 4, and 21 hours thawing time. The filtrate was covered and left at room temperature between thawing times.

RESULTS AND DISCUSSION

Preliminary Investigation

Applying the technique of gas-entrainment on-column trapping proved effective for comparing volatile concentrations on a single plant basis. A sample of 10 to 14 pods averaging 5 grams was found to be adequate. In contrast, conventional procedures (distillation, extraction, etc.) would have entailed larger samples, consumed more time, and required elevated temperatures probably resulting in artifact formations.

As found in carrots by Heatherbell (1970), entrainment of bean volatiles at lower water bath temperatures, shorter purge time, and slower nitrogen purge rates resulted in insufficient quantities of higher boiling compounds. Higher temperatures, longer purge time, and higher purge rates caused excessive amounts of water to be entrained, stopping nitrogen flow rate at the Dry Ice bath. The best results occurred when samples were entrained at 90° ±3° C for 10 minutes with a purge rate of 20 ml/minute. Saturation of the samples with sodium sulfate was of no advantage under the conditions of this experiment.

The freezing procedure, I.Q.F., appeared to be superior to canning. In freezing, pods had more of the original green color,

less sloughing, and 10 to 14 whole bean pods could be used per can; whereas, for canned products the recommended method requires that each can contain 9 oz. of cut beans. This would require about 50 pods, which would be more than an individual plant could produce. Furthermore, chemical changes could occur from the time of cutting through blanching and to the final retorting process. Although the heat treatment was considerably less in freezing, 210° F (99° C) for 1 3/4 minutes blanching compared to 241° F (116.6° C) for 21 minutes retorting in canning, the enzymatic test showed that blanching was adequate to suppress any enzyme action before and after storage. The difference in volatile components influenced by the processing method will be covered later in the discussion.

Genetic Studies

Bush bean O. S. U. 58-110 was reported as closely resembling the pole 'Blue Lake' in flavor and other pod characteristics. Although the exact parentage is not known, it would appear that the variety contains a large number of genes governing 'Blue Lake' pod characteristics, including those responsible for 1-octen-3-ol formation. The chromatograms of fresh O. S. U. 58-110 closely resemble those of fresh pole 'Blue Lake FM-1K' and bush varieties O. S. U. 190 and O. S. U. 949, developed by Dr. Frazier from several backcrosses

to the 'Blue Lake' variety. 'Blue Lake' primary flavor compounds (Stevens, 1967), 1-octen-3-ol and cis-hex-3-en-1-ol, were positively identified from mass spectrometry and G. L. C. retention time (Appendix II).

Inheritance of 1-Octen-3-ol

The inheritance of 1-octen-3-ol was studied only in cross 'Romano' (bush) × O. S. U. 58-110 and reciprocal. O. S. U. 9025, being low in concentration, would have made an excellent parent for studying this compound. However, as discussed in a later section, a mutant was found mixed with this variety affecting many of the pod characteristics. On this basis the variety was dropped from further inheritance studies.

The concentrations of 1-octen-3-ol in the parent lines ('Romano' and O. S. U. 58-110) and the F_1 progeny are summarized in Table 1.

Table 1. 1-Octen-3-ol mean concentration and standard deviation found for sieve 4-5 frozen 'Romano', O. S. U. 58-110, and F_1 progeny.

	Number of samples	Mean concentration (p.p.b.)	Standard deviation (s)
'Romano' (bush)	20	191.43	17.3767
O. S. U. 58-110	22	163.29	19.6614
'Romano' X O. S. U. 58-110 O. S. U. 58-110 X 'Romano'	13	146.85	23.3839

Although the difference in concentration between varieties is not as large as reported by Stevens (1967), the standard deviation is considerably less. The mean concentration between the two varieties is 177.36 p.p.b. Only two samples of O.S.U. 58-110 were found on the 'Romano' side, both at 178 p.p.b. Three samples of 'Romano' were below this value. Similar overlapping of 1-octen-3-ol concentrations were reported in parent lines used by Stevens (1967).

F_1 progeny mean concentration for 1-octen-3-ol was within the variation range of O.S.U. 58-110 and the range of F_1 progeny contained the O.S.U. 58-110 mean concentration. It would seem reasonable to conclude that the F_1 progeny mean concentration (146.85 p.p.b.) resembles O.S.U. 58-110 mean concentration (163.29 p.p.b.) more than the 'Romano' (191.43 p.p.b.). The discrepancy between the F_1 progeny and O.S.U. 58-110 may be due to inconsistency in maturity produced by the oval shaped pods of the F_1. A second possibility is the presence of minor genes influencing the production of the volatile. Stevens (1967) reported the possibility of an extrachromosomal influence on the synthesis of this compound leading to maternal influence. Distribution of sample concentration of 1-octen-3-ol in this study did not show the maternal influence. The data for 1-octen-3-ol does indicate the O.S.U. 58-110 level is dominant over that of 'Romano'.

A frequency distribution of 1-octen-3-ol concentrations of F_1

and F_2 progenies are shown in Figure 1. An arbitrary line was selected in which below 180 p.p.b. resembled O.S.U. 58-110 and above 180 p.p.b. resembled 'Romano'. This value is less than 3 p.p.b. from the mean between the two varieties and still allows easier division of concentrations into increments of 10 p.p.b. The differences in reciprocal crosses were not apparent; therefore, they were pooled.

Concentrations tend to deviate from the mean more in the F_1 than O.S.U. 58-110. In the F_2 progenies a further spread of the concentration from the expected resemblance of either parent occurred. Stevens (1967) reported a similar variation in the F_2 for the crosses 'Romano' (pole) × 'FM-1L' and 'G-50' × 'FM-1L'. He concluded a single gene pair was controlling the level of concentration in the parents, but the variation around each parent was influenced by maturity.

The F_2 data was chi square tested for a single gene pair with a 3:1 ratio and two pair of genes with 9:7 ratio. The results are reported in Table 2. The probability value showed a closer fit to the 9:7 ratio than the 3:1 ratio.

A backcross test was also made on the cross of 'Romano' (bush) × O.S.U. 58-110 and reciprocal. The frequency distribution for the backcross of F_1 to each of the parents is shown in Figure 2. A summary of backcross data is present in Table 3.

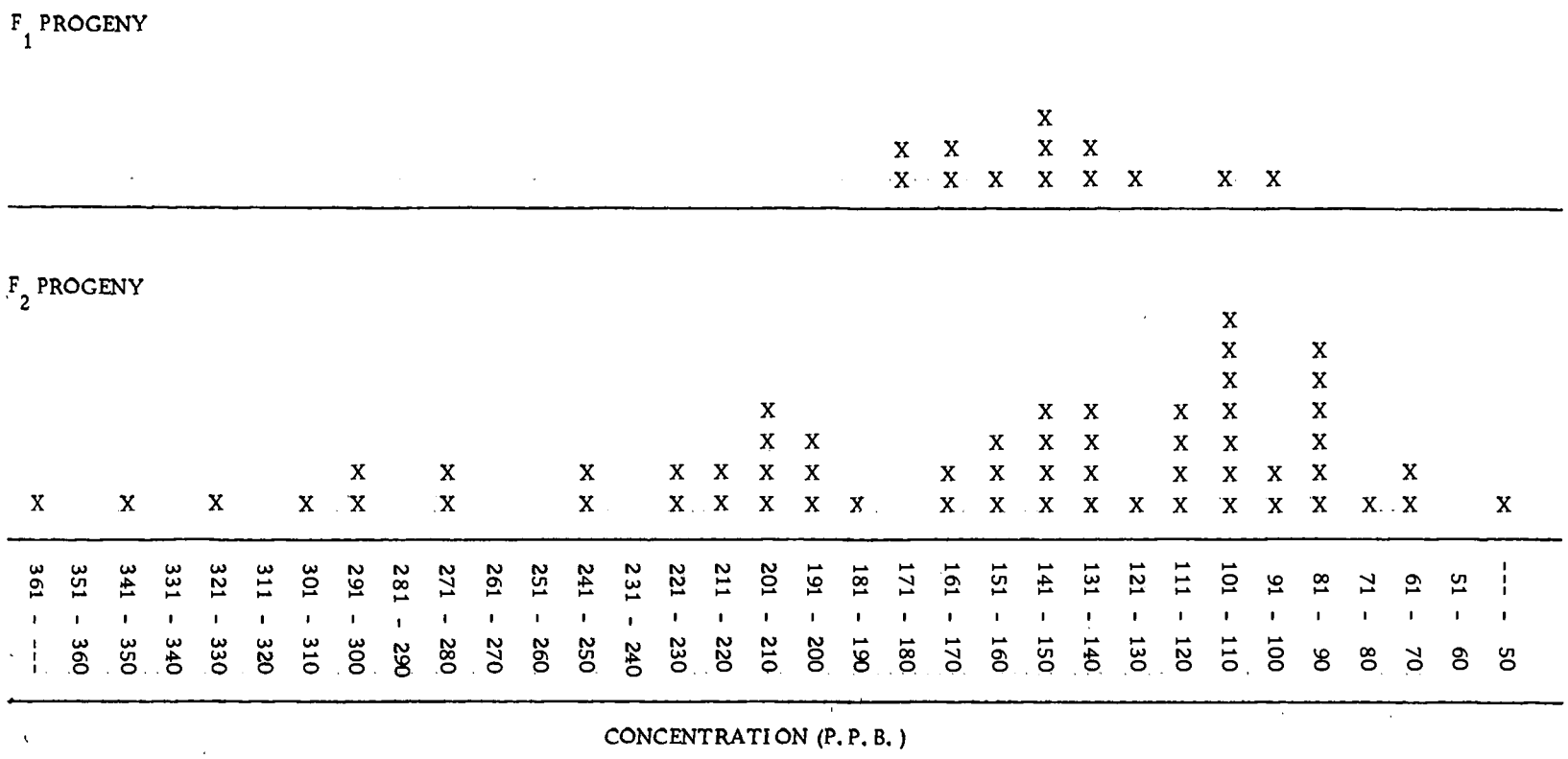

Figure 1. Frequency distribution of 1-octen-3-ol concentrations found in the F_1 and F_2 progenies of the cross 'Romano' (bush) X O. S. U. 58-110.

Table 2. Chi square test for goodness of fit of 1-octen-3-ol concentration from F_2 plants in crosses of 'Romano' X O. S. U. 58-110.

	No. of progeny resembling		Expected ratio	Chi square	P
	'Romano'	58-110			
'Romano' X 58-110 and reciprocal	22	38	3:1	4.3556	0.025-0.050
'Romano' X 58-110 and reciprocal	22	38	9:7	1.2231	0.250-0.500

Table 3. Chi square test for goodness of fit of 1-octen-3-ol concentration from backcrossed F_1 plants to the parents ('Romano' and O. S. U. 58-110).

	No. of progeny resembling		Expected ratio	Chi square	P
	'Romano	58-110			
F_1 backcrossed to O. S. U. 58-110	0	36			
F_1 backcrossed to 'Romano' (bush)	26	30	1:1	0.2856	0.500-0.750

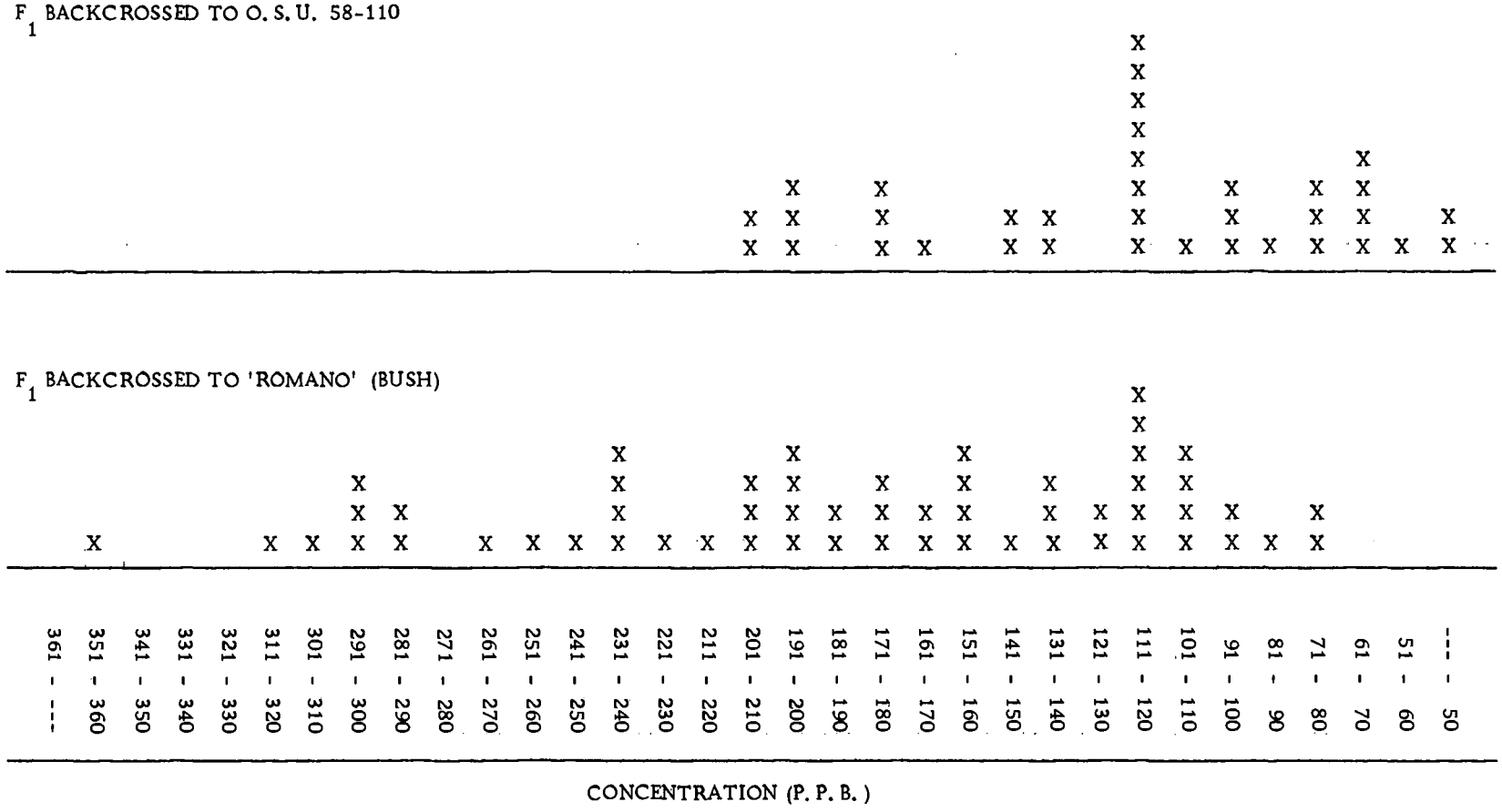

Figure 2. Frequency distribution of 1-octen-3-ol concentrations found in F_1 backcrossed to 'Romano' (bush) and O. S. U. 58-110.

In the backcross to O. S. U. 58-110 five samples were closer to the 'Romano' parent. The most divergent sample was 42 p. p. b. from the mean concentration for O. S. U. 58-110. Considering the variations found in parents, F_1, and F_2 progenies it would seem likely that these samples were influenced by maturity (thawing time may also be a slight factor as will be pointed out later).

If the five samples were considered 'Romano' types the results of the backcross to O. S. U. 58-110 would indicate that a complex inheritance would be involved. This would not agree with either the 3:1 ratio or 9:7 ratio found in the F_2 progeny.

In Table 3 all samples of the backcross to O. S. U. 58-110 were considered to resemble O. S. U. 58-110. In F_1 backcross to 'Romano' a good fit of the 1:1 ratio was obtained. From these results the inheritance of 1-octen-3-ol appears to be governed by the single gene pair. To confirm two gene pairs with the ratio of 9:7 in the F_2 generation, a 1:3 ratio should have been observed from the backcross to 'Romano'.

The results of F_1, F_2 and the backcrosses shows agreement with Stevens' (1967) studies in that a single gene controls the inheritance of 1-octen-3-ol. Although the sample of F_2 progenies was not large, a good fit to the 9:7 ratio was obtained. Importance of studying inheritance with a second method can be seen from the results of the backcross. The single gene pair, 3:1 ratio in the F_2,

being less favored but possible was confirmed in the backcross method.

Inheritance of Linalool

An attempt was made to study the inheritance of linalool. However, the difference in mean concentration and the amount of overlapping in concentration of the two varieties made it impossible to distinguish any genetic difference and the inheritance study of linalool was terminated. It appears that the compound is influenced by processing methods and will be discussed in more depth in that section (peak No. 10).

Flower Inheritance and Possible Linkage with 1-Octen-3-ol

Flower color was used as a genetic marker to check for selfs in crosses involving purple flowered 'Romano' (bush) × white flowered 'Blue Lake' bush types. A darker purple than that of 'Romano' was noticed in the F_1 generation and several F_2 segregates (Figure 3). The use of the backcross technique for the inheritance of 1-octen-3-ol provided an excellent opportunity to study this in detail.

The inheritance of flower color in crosses involving 'Romano' are shown in Table 4. The probability values for chi square were 0.50 to 0.75 for both a 3:1 ratio of the F_2 generation and 1:1 ratio in the backcross to the white flower parent. A single dominant gene

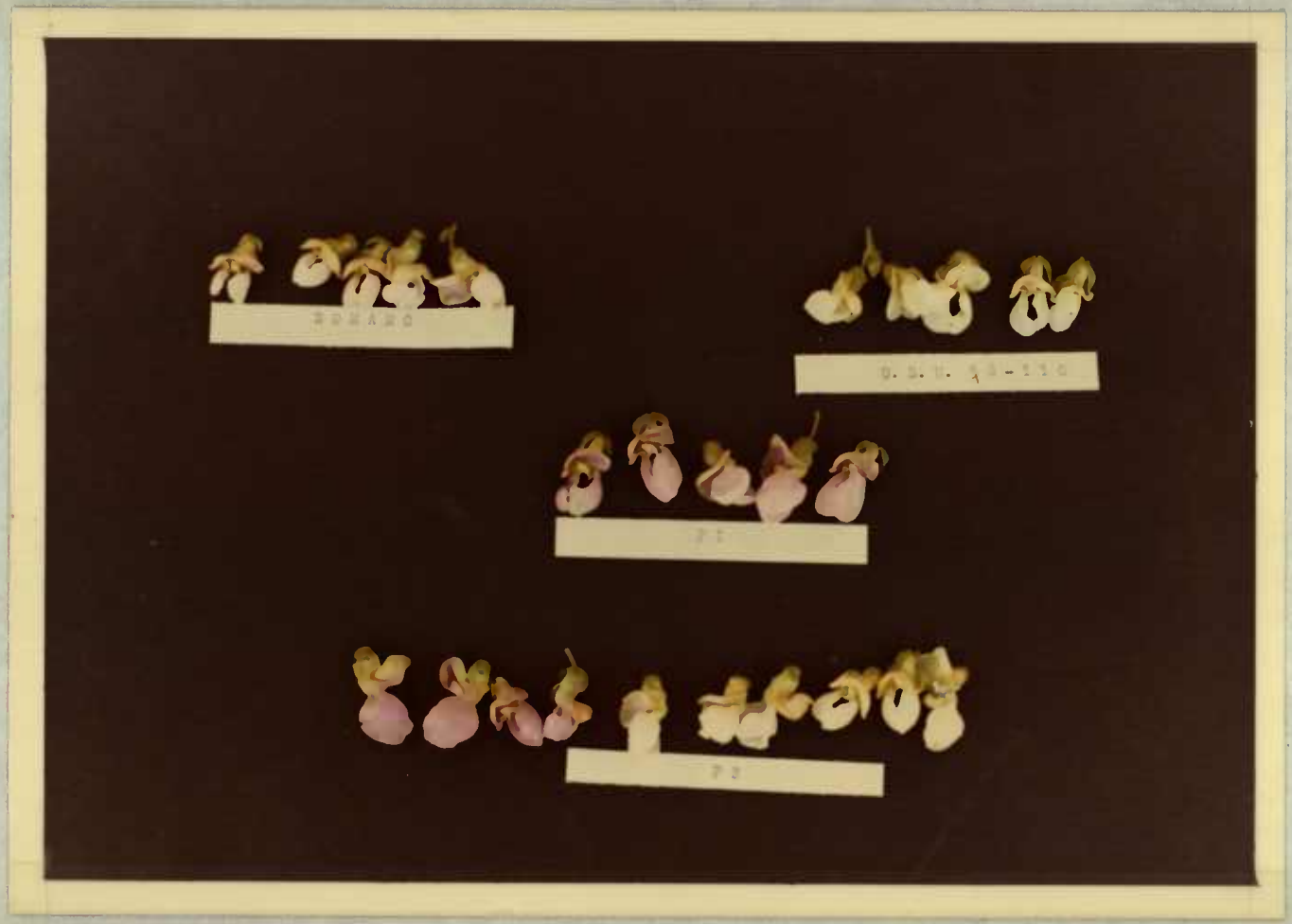

Figure 3. Inheritance of flower color in the F_1 and F_2 generations of the cross 'Romano' X O. S. U. 58-110.

Table 4. Inheritance of flower color in F_2 and backcross generations of white flowered 'Blue Lake' bush X 'Romano'.

	No. of progenies consisting of:			Expected	Chi		
	Dark purple	Light purple	White	ratio	square	P	
Parents							
'Romano' (bush)	0	165	0				
O. S. U. 58-110	0	0	144				
O. S. U. 9025	0	0	85				
F_1 generation							
'Romano' X white flowered 'Blue Lake' bush parents	55	0	0				
F_2 generation (1971 and 1972)	326	106	154	3:1	0.1280	0.50-0.75	1 d. f.
				9:3:4	0.5603	0.75-0.90	2 d. f.
Backcrosses							
To white flower parent	38	0	43	1:1	0.3086	0.50-0.75	1 d. f.
To 'Romano'	30	46	0	1:1	3.3684	0.05-0.10	1 d. f.

controls the inheritance of colored flowers in 'Romano' over the white flowers of the 'Blue Lake' bush types. This agrees with Stevens (1967) and Hong (1968).

A second gene is involved in the intensity of the purple flower color in F_1 and F_2 generations. A chi square test shows that the F_2 data fit a 9:3:4 ratio (dark purple, light purple, and white respectively). Backcrosses of the dark purple F_1 flower to the white flowered parents produced only dark purple and white flowered progenies. Backcrosses to 'Romano' produced no white flowered plants, but had progenies of light and dark purple at a ratio of 1:1.

Therefore, it appears two genes are involved, one modifying the action of the second. A single dominant gene, $V_{\underline{lae}}$ from 'Romano' is responsible for the presence of the purple pigment. A second dominant gene, Aeq, from the white flowered parent acts as a promoter enhancing the color of the first gene. Similar results were reported by Miyake, Imai and Tabuchi (1930). The inheritance pattern and assignment of gene symbols for flower colors are presented in Figure 4.

For a study on possible linkage between flower color and concentration of 1-octen-3-ol, the gene for colored versus non-colored was eliminated from consideration. Since the dominant gene for flower color came from 'Romano' and dominance for 1-octen-3-ol came from O. S. U. 58-110, a simultaneous test cross for both

Parents

'Romano' FM-14 (bush)		X	O. S. U. 58-110
P T V_{1ae} aeq	light purple		P T v Aeq white

F_1

	P T V_{1ae} Aeq	dark purple

F_2

9/16	P T V_{1ae} Aeq	dark purple
3/16	P T V_{1ae} aeq	light purple
3/16	P T v Aeq	white
1/16	P T v aeq	white

Backcrosses

	To 'Romano'		To O. S. U. 58-110	
1/2	P T V_{1ae} Aeq	dark purple	1/2 P T V_{1ae} Aeq	dark purple
1/2	P T V_{1ae} aeq	light purple	1/2 P T v Aeq	white

List of Genes

P - basic color gene with T. P without T and color genes is colorless as is p.

T -Lamprecht's equivalence to Gri, second basic color factor.

V_{1ae} -gives laelia flower color when dominant. v gives white flowers.

Aeq -darkens the banner petal. For the expression as an intensifier the gene must be accompanied by the dominant genes E Unc Uc and Rst or Rma (Lamprecht, 1948; Yarnell, 1965).

Figure 4. Assignment of gene symbols for the inheritance of flower color in the cross involving 'Romano' and O. S. U. 58-110.

characters was not possible. Thus, a backcross test would not show whether the genes were linked or independent. Since F_2 data for this relationship was incomplete, it was not possible to determine linkage.

Linkage studies therefore involved only the genes modifying flower color. Both this factor and the flavor component were dominant in O. S. U. 58-110 and F_1 generation. Linkage relationships studied in the backcrosses are presented in Table 5.

If genes controlling flower intensity and 1-octen-3-ol concentration are linked, then backcrosses to the recessive parent should produce only dark purple flowers resembling O. S. U. 58-110 concentration and light purple flowers resembling 'Romano' concentration. The results of the backcross showed these two types plus dark purple flowers with 'Romano' concentration and light purple flowers with O. S. U. 58-110 concentration. The chi square test showed a good fit to the independent gene ratio 1:1:1:1. It was concluded that no linkage between 1-octen-3-ol concentration and flower color intensity exist in this particular cross.

Pod Mutant in O. S. U. 9025

As mentioned earlier, O. S. U. 9025 was characterized by two distinctly different pod types, oval and round (Figure 5). The original O. S. U. 9025 selected by Dr. W. A. Frazier was described earlier. The round podded strain is probably the original line. It

Table 5. Summary of data for possible linkage between 1-octen-3-ol concentration and flower color in backcross to 'Romano' and O. S. U. 58-110.

	No. of progenies consisting of:						
	Dark purple		Light purple		White		
	58-110[a]	'Romano'[b]	58-110	'Romano'	58-110	Romano	Total
Backcrosses							
To O. S. U. 58-110	16	0	0	0	20	0	36
To 'Romano'	17	9	13	17	0	0	56
Expected no.[c]	14	14	14	14	0	0	56
		Chi square value		3. 142			
		Probability value		0. 25-0. 50 (3 d. f.)			

[a]Represents the level of 1-octen-3-ol resembling O. S. U. 58-110.

[b]Represents the level of 1-octen-3-ol resembling 'Romano'.

[c]Expected no. of progenies from segregating independent genes for backcross to 'Romano' (1:1:1:1 ratio).

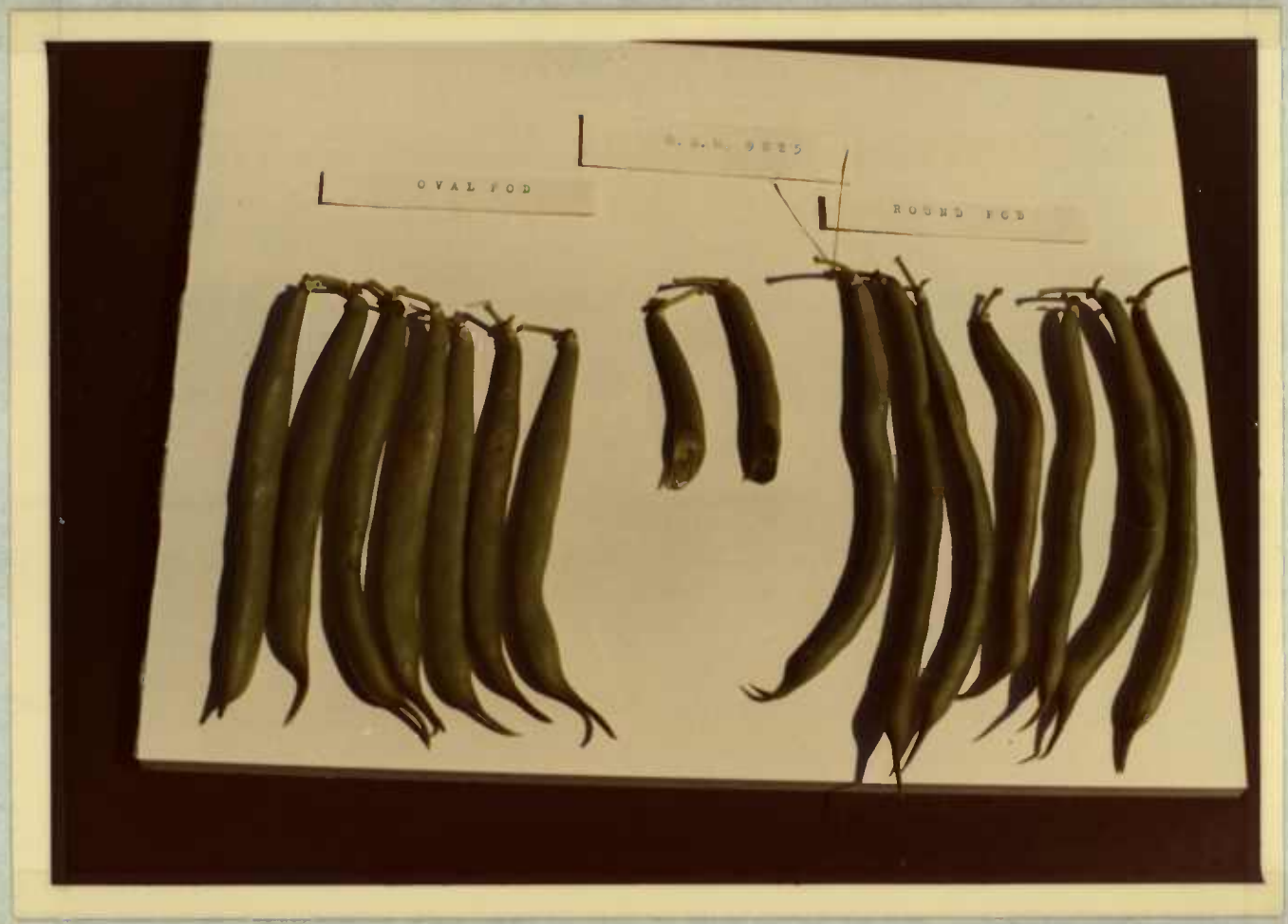

Figure 5. Pod shape and flesh color characteristic found in the normal and mutant O. S. U. 9025 bush bean.

is characterized by having a dark colored outer skin, with a flesh color typical of 'Blue Lake' varieties, lacking strings and possessing a bland taste. The oval pod has strings on both sutures, the pod has a glossy smooth light green skin, with a light colored flesh. Stringlessness and pod shape, used as main distinguishing factors, are summarized in Table 6.

It is believed that the oval type is a mutant 'Blue Lake' rather than a product of seed mixture. Chromatograms of the mutant showed a similarity in 'Blue Lake' pods except the concentration of 1-octen-3-ol was low.

Table 6. Pod shape and string characteristic found in O. S. U. 9025.

	Round Pod		Oval Pod		% Oval
	String	Stringless	String	Stringless	Mutant
O. S. U. 9025 used in this study	0	107	23	0	17.7
Dr. Frazier's Stock No. 1 O. S. U. 9025	0	95	0	0	0.0
Dr. Frazier's Stock No. 2 O. S. U. 9025	0	19	3	0	13.6

Flat and oval mutants have been reported in 'Blue Lake' beans (Frazier et al., 1968). 'Blue Lake' mutants are also characterized by strings and light color flesh (Frazier, 1970).

Atkins (1960) reported a flat podded rogue arising from a round pod parent. The flat pod appeared to be due to a single gene difference with intermediate dominance. He adds that once the mutation

occurs the rogues are favored by a higher seed yield.

The mutant was not noticed until 1971. A test in 1972 showed its presence in one of two original stock seeds. The mutant is believed to be in at least the third generation, accounting for its high percentage in 1971.

Factors Influencing Concentrations of 1-Octen-3-ol

Effect of Maturity

Maturity has been shown to influence greatly the concentration of 1-octen-3-ol (Stevens, 1967). This becomes a more complicating factor because of the difficulty in harvesting flat and round pods at the same maturity.

The effect of maturity on 1-octen-3-ol concentration based on the number of days from anthesis is shown in Figure 6. The rate of decrease in concentration tends to be slower at the larger number of days from anthesis. The difference between mean concentrations of 'Romano' and O. S. U. 58-110 at the last two harvest dates were nearly the same. This may suggest that both varieties are reaching the same state of maturity at the same time.

The decrease in concentration of 1-octen-3-ol was discussed in detail by Stevens (1967). He explained that part of the decrease was due to a decrease in percentage of snap bean pod tissue. It was

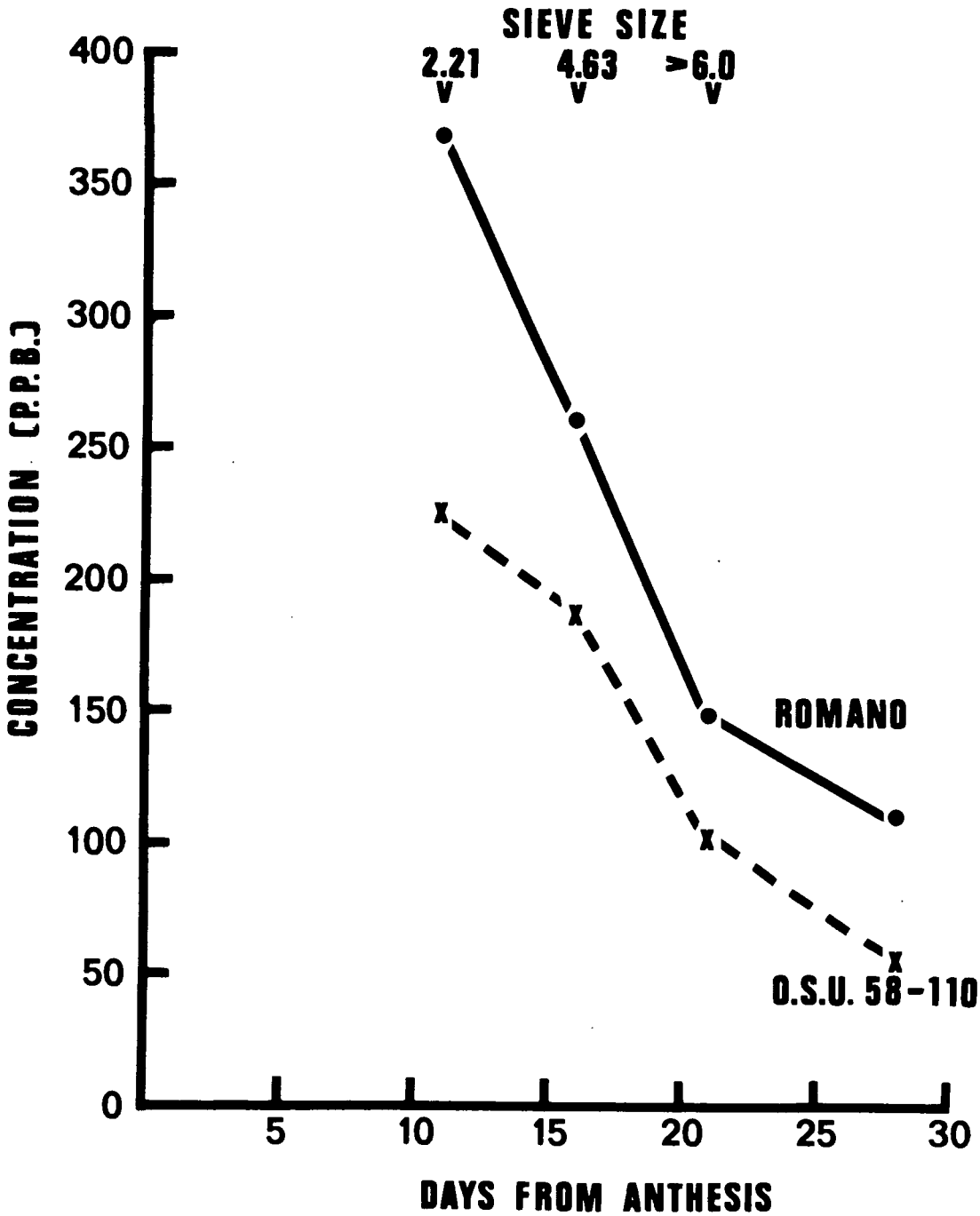

Figure 6. Effect of maturity (based on days from anthesis) on the concentration of 1-octen-3-ol in 'Romano' and O. S. U. 58-110.

believed that the pod contained the compound, while the embryo and cotyledons did not.

A separation of seeds from the pod tissues were made for both 'Romano' and O. S. U. 58-110. G. L. C. chromatograms of the seed showed small amounts of 1-octen-3-ol were present. Seeds were found to contribute 1.36% and 0.95% of the concentration found in O. S. U. 58-110 and 'Romano', respectively.

If 1-octen-3-ol is produced from the precursor linoleic acid, as hypothesized by many (cited earlier), it would not be surprising to find traces in seeds. Fats of developing seeds are formed at the expense of sugars which are translocated to this tissue from leaves (Salisbury and Ross, 1969). The production of the volatile compounds may be limited due to the activity of the enzyme responsible for the conversion or from other controlling factors. Or, the decrease in 1-octen-3-ol concentration may be due to a dilution caused by growth of cells.

The concentration of 1-octen-3-ol was shown to decrease rapidly early in the period of studies, then the rate slowed considerably beyond 21 days. At the same time pod growth increased rapidly until around sieve size 6 (21 days from anthesis) then tapered off. This apparent inverse relationship was not tested statistically.

There appears to be no strong relationship between the

decrease in 1-octen-3-ol reported here and the respiration pattern reported by Watada and Morris (1967). As 1-octen-3-ol was decreasing rapidly they found a decrease in the respiration rate of the young fruits (6-8 days from anthesis). The respiration rate began to increase when the fruit was near maximum length and continued to increase until the seeds consisted of 22% of the fruit weight (21 days from anthesis). Respiration rates did not decrease until after the seed weight reached 30% (over 30 days).

It was speculated that these volatile compounds may serve as attractants for pollinating insects and natural seed disseminators and in some cases as retardants of pests. In any case, 1-octen-3-ol appears to be produced in high concentration early in the development of the pod and is possibly the highest at the time of flowering.

Influence of Thawing Time

Earlier studies indicated that duration of thawing time influenced the final concentration of 1-octen-3-ol. All studies thereafter were made with a three hour thawing period varying not more than twenty minutes.

A brief study (not statistically tested) was made on the influence of thawing time. Since it appeared that most compounds were not affected, only the last three peaks of the chromatograms are shown in Figure 7. Changes in peak no. 8 were typical for most

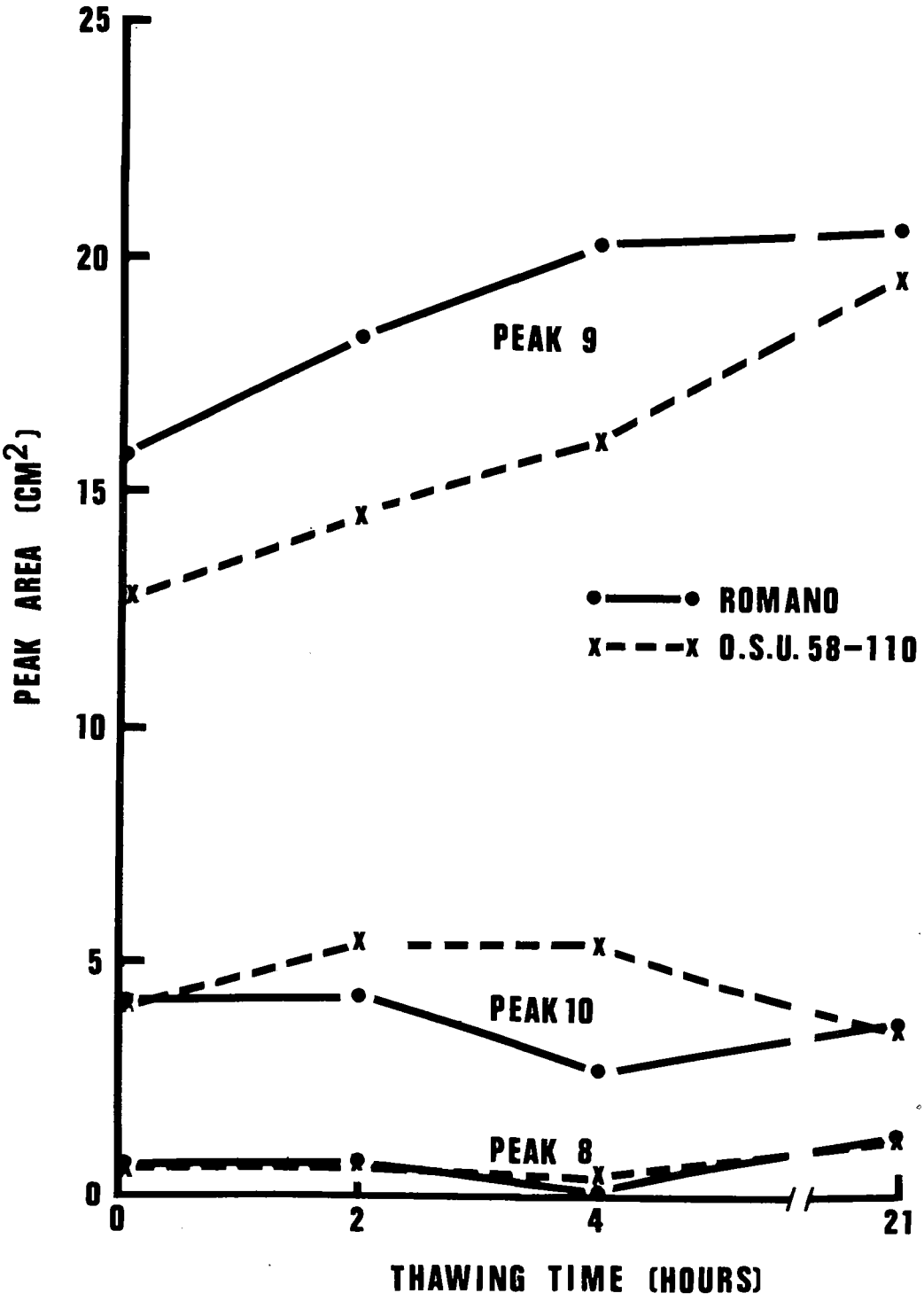

Figure 7. The effect of thawing time on the peak areas for chromatogram peaks 8, 9, and 10 in the varieties 'Romano' and O. S. U. 58-110.

compounds. Almost all the peaks increased slightly up to two hours thawing time. Small changes in compound concentration in either direction occurred up to four hours. In some of the compounds a more drastic change occurred up to 21 hours. Without positive identification of these compounds it would be difficult to postulate the mechanisms responsible for their changes.

Also occurring at the 21 hour time was the presence of ethanol and acetaldehyde (retention time and mass spectrometry), which were not detected at any other time. Both of these are believed to be fermentation products (Chow and Watts, 1969).

Peak no. 9 shown on the graph appears to be increasing continuously for both parents. The compound is positively identified, by retention time and mass spectrometry, as 1-octen-3-ol. Associated with anaerobic fermentation in the deterioration of processed beans is fatty acid oxidation. Oxidative cleavage of linoleic acid (and oils containing this acid) was found to produce 1-octen-3-ol (Hoffman, 1962). He described the brown bean mushroom odor in oxidized soybean as having the components 2-trans-heptenal + 1-octen-3-ol, and an unknown substance.

Peak no. 10, linalool (positively identified), was found to remain relatively constant. This compound has been found to be produced by melvalonic acid in higher plants (Suga, Shisibori and Bukeo, 1971). The number of possible enzymes in this pathway,

that may have been inactivated by blanching, would suggest no further production of linalool. Only a decrease would be expected because of conversions to limonene and α-terpineol (Attaway and Buslig, 1969).

Comparison of Processing Methods

A comparison of volatiles in canned, frozen, and fresh raw snap beans were made on the varieties, 'G-50', O. S. U. 58-110, and 'Romano' (latter two are shown in Figures 8 and 9 respectively). Differences between canned and raw samples were almost entirely quantitative. Frozen compared to raw samples appeared to have lost compounds qualitatively as well as quantitatively. The volatiles are reported quantitatively in Table 7 as peak areas and peak heights. Mass spectrometry and retention time identification on several of these peaks are presented in Appendix II.

The early peaks 1b, 1d, 1c, and 2 (considered "lower boiling" volatiles) were considerably higher in canned products compared to the raw samples. Heatherbell, Wrolstad and Libbey (1971) reported 7 out of 8 "lower boiling" carrot volatiles increased in canned products. Strecker degradation, lipid auto-oxidation, non-enzymatic browning, and thermal degradation were discussed as possiblities for the increase. Self, Casey and Swain (1963) reported cooking time of 30 minutes (21 minutes at 241° F [116.6° C] was used here)

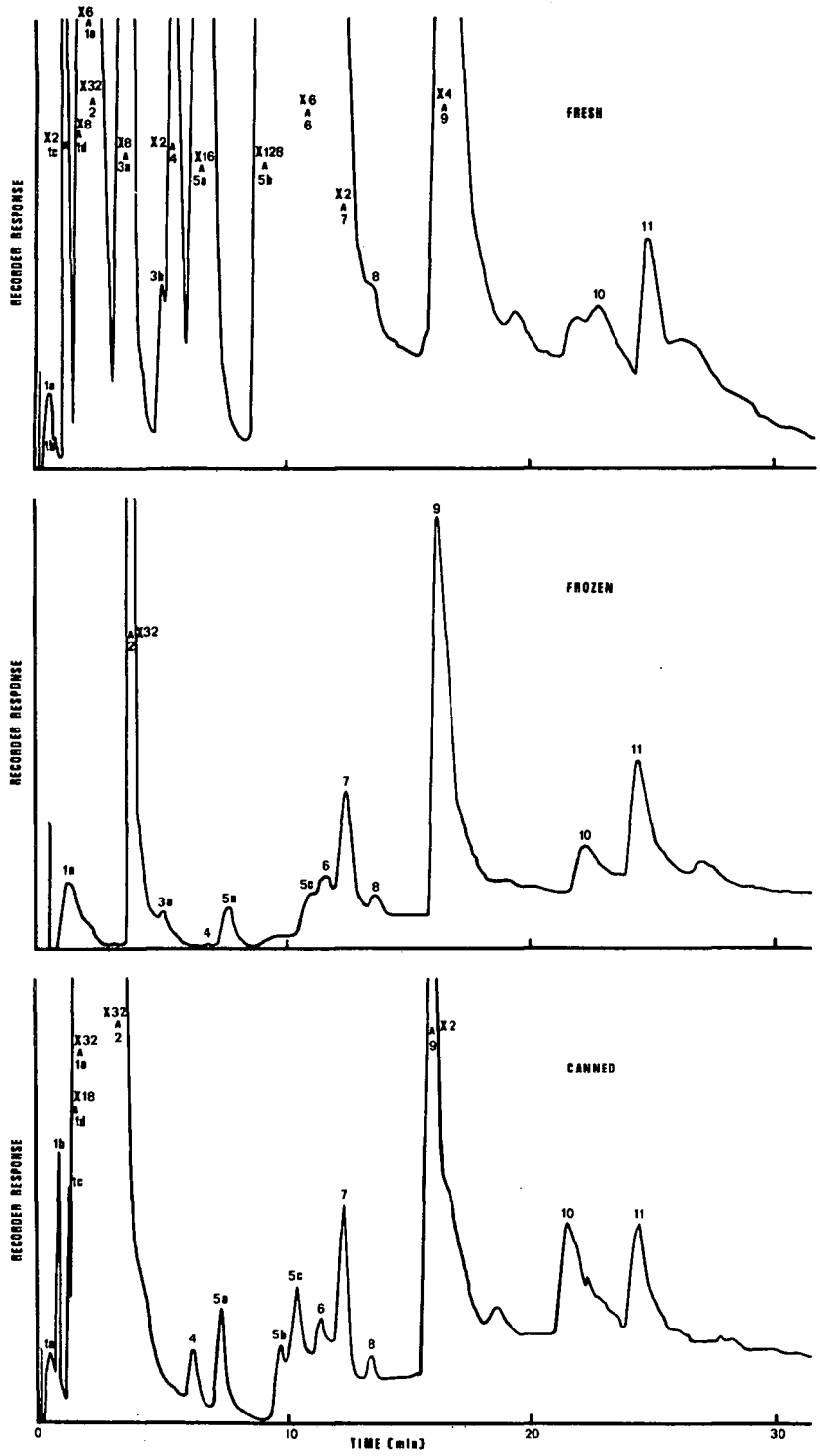

Figure 8. Chromatograms showing differences between canned, frozen, and fresh O. S. U. 58-110 bean samples.

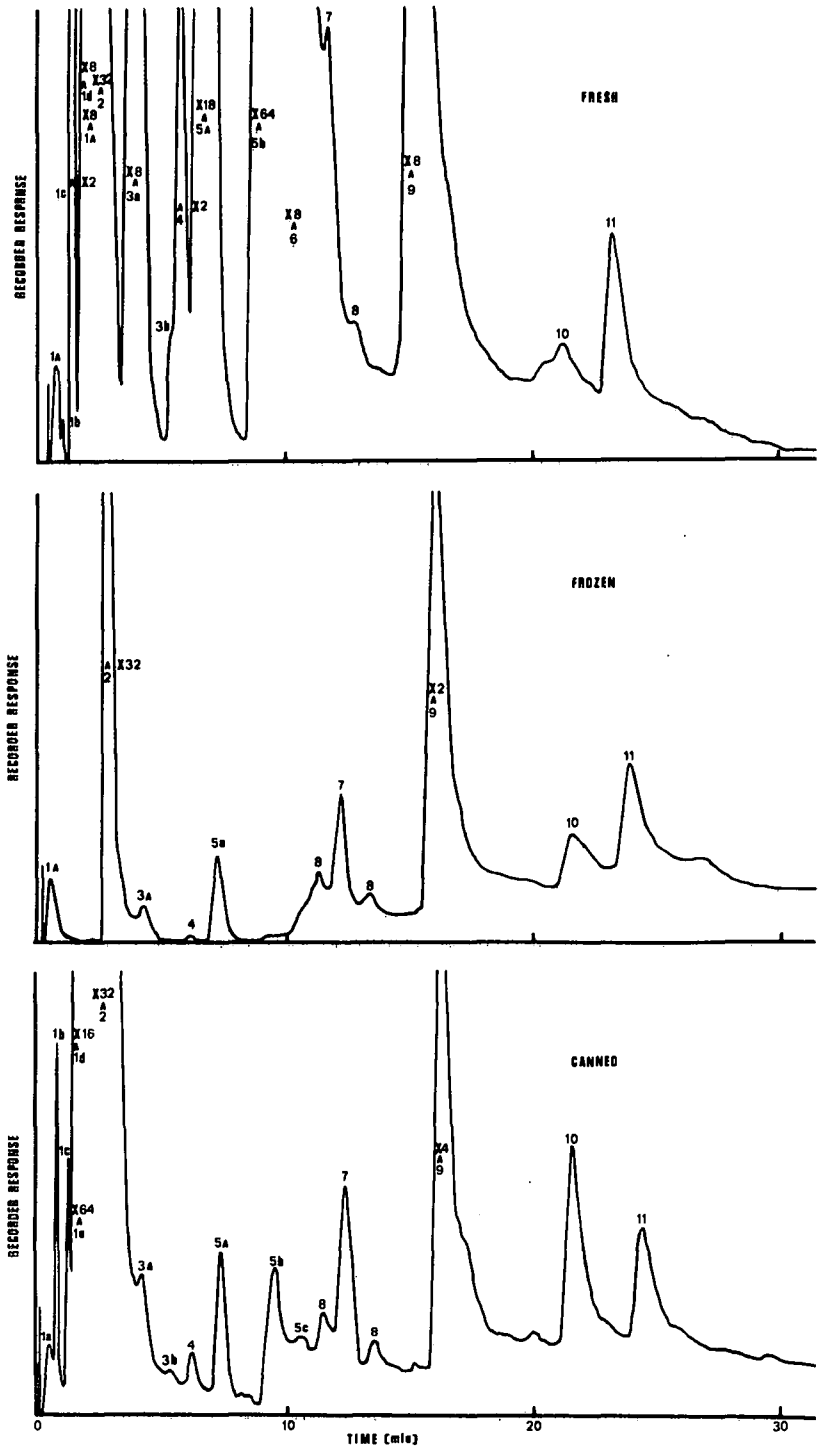

Figure 9. Chromatograms showing differences between canned, frozen, and fresh 'Romano' bean samples.

Table 7. Effect of processing on bean volatiles.

Peak[a]	'Romano'			O. S. U. 58-110			'Gallatin 50'		
	Canned[b]	Frozen	Fresh	Canned	Frozen	Fresh	Canned	Frozen	Fresh
1a	3.55	2.66	2.20	3.02	2.52	2.15	2.63	1.76	2.10
1b	1.56	----	tr[c]	1.14	----	tr	tr	----	tr
1c	2.93	----	7.25	1.36	----	5.04	2.39	tr	3.26
1d*	285.87	----	161.60	246.76	----	137.60	288.71	----	119.20
1e*	603.02	----	143.20	583.11	----	184.80	608.71	----	164.00
2*	632.89	547.20	635.20	622.93	555.20	614.40	672.71	601.60	518.40
3a	tr	tr	85.68	----	tr	93.00	----	11.25	67.08
3b	tr	----	tr	----	----	tr	----	----	2.25
4	tr	tr	8.50	1.20	1.32	13.95	tr	----	5.68
5a	3.69	2.21	215.04	2.49	3.22	278.40	3.51	3.03	598.40
5b*	5.51	----	1075.20	1.60	----	1843.20	tr	tr	1638.40
5c*	tr	tr	----	3.47	tr	----	tr	----	----
6*	1.42	2.10	91.60	1.60	1.60	131.20	1.22	tr	60.18
7	4.20	4.80	3.30	3.69	4.36	4.01	3.29	3.58	3.90
8	tr	tr	tr	tr	tr	tr	tr	tr	tr
9	35.05	15.88	124.32	32.71	14.58	58.96	2.84	2.40	4.55
10	8.01	4.20	2.70	6.61	5.35	5.08	12.60	6.30	4.34

a * values reported are peak heights. Other peaks are based on peak area.

b Canned products were at a ratio of 9 oz. of beans to 7 oz. of water. The value given is adjusted to be equal to the 1:1 ratio used in frozen and fresh samples.

c Traces (tr) are peak areas that are detected but have a value less than 1.

produced six additional low boiling volatiles in potatoes. They believed a similar pattern on the low boiling volatiles would occur in most cooked foods. These volatiles were thought to be produced by degradation of metabolites.

Peak 4, 5a, 5b, 6, and 9 showed large losses of volatiles due to canning. Heatherbell, Wrolstad and Libbey (1971) noted 50% reduction in total volatile content of the "higher boiling" compounds found in canned carrots. They accounted for part of the loss of volatiles due to the step prior to the retorting process. Linalool, peak 10, was one of the few high boiling compounds that increased upon processing Simonsen (1931) reported the partial conversion of geraniol to linalool when heated to 200° C. Williams (1959) considered linalool in citronella oil as an artifact formed by the isomerization of geraniol in the extraction more than a primary constituent of the oil. The greatest difference of linalool among the bean varieties appeared in the canned products. Without knowledge of the geraniol content in snap beans it would be difficult to speculate that linalool is a product of isomerization of geraniol.

The freezing process resulted in the greatest loss of volatiles. Peaks 1b, 1d, 1e, and 3b were not found in frozen samples of any of the three varieties. No peak was found to increase substantially during the freezing process. Woodroof, Heaton and Ellis (1962) reported an appreciable loss of aroma and flavor of precooked beans

during the freezing, thawing, and rewarming procedures.

The blanching process for both frozen and canned samples appeared to influence peaks 3a, 5a, 5b, and 6. These peaks were at least six times higher in the unblanched raw samples as compared to either of the processed samples. Mathews (1960) showed by G. L. C. analysis a definite reduction in the higher boiling components of oil from the blanched bean pods. Woodroof, Heaton and Ellis (1962) reported the flavor of beans, either partially or fully cooked was retained slightly better when they were covered with the cooking liquor. Throughout these studies the hot water from the blanching procedure for each sample of beans was discarded. This hot water more than likely contained some of the flavor compounds that may have been leached from the pods. Adding to the loss are the volatiles that were vaporized during the heating period.

Johnson et al. (1968) found that without exception, processing tomato juice reduced the amount of iso-amyl alcohol, n-pentanol, and cis-3-hexenol-1. Variety appeared to have an influence on the percent of each compound; 'ES 24' had the highest loss for all three compounds, while 'KC 146' was the lowest for all three. A similar varietal influence might be expected in green bean samples. Quality changes and composition influenced by processing have been shown to vary with variety (Sistrunk, 1969; Woodroof, Heaton and Ellis, 1962). These changes would undoubtedly influence the metabolism

and ultimately the flavor of the processed beans.

Evaluation of Frozen Samples for Genetic Studies

The most ideal procedure for analyzing bean samples for genetic studies would be use of the raw product. The quantities of "higher boiling" volatiles were in moderate amounts compared to the processed samples. The use of raw bean samples would entail less influence of processing variability on volatile concentrations. The ten minute cooking period at 90° C for entrainment simulates the actual preparation for consumer consumption.

However, under the procedure described for obtaining an adequate sample for study, it would hardly be possible to analyze 150-200 samples with equal maturity during the harvest season. The use of canning for preserving the samples was done by Stevens (1967). Heat induced formation of furfural was reported. Other volatile compounds as reported earlier are also believed to be produced during the canning process.

A freezing method was used as a means of preserving the bean pod samples in this study. Results of the influence of thawing time and processing methods indicated that this method could account for the considerable variability in concentrations in the F_2 and backcrosses. In the fresh bean pod samples 1-octen-3-ol (peak 9) showed a greater difference in quantity among varieties than in the frozen

samples. The influence of thawing time was apparent with only some of the volatiles, one of these being 1-octen-3-ol.

The parameters in the frozen sample analysis were kept as constant as possible. But the deviation in the genetic studies of the expected progenies are believed to be still influenced by some of the parameters of the procedure. For these reasons freezing probably would not be the best method for preserving plant material for genetic studies.

Uses in Chemotaxonomy

For chemotaxonomy purposes it would seem most reasonable to study the volatile components with the least amount of procedural influence. Fresh raw samples with 14 measurable peaks contained the highest amount of "higher boiling" volatiles and moderate amount of "low boiling" compounds. Differences in concentration between varieties appeared the largest in the fresh bean pod analysis compared to the processed samples. Since the number of samples required for chemotaxonomy would not be as much as required for genetic inheritance studies, it would be possible to use the fresh raw samples for this type of study.

Maturity presents probably the only major problem. If equivalence in maturity could be achieved between varieties, the use of volatile flavor components for chemotaxonomy would be a valuable

asset.

Due to mostly quantitative differences, volatile flavor components alone do not appear to be adequate for differentiation of varieties. Even in combination with phenolics, alkaloids. flavonoids, and other possible substances, the use of chemotaxonomy would be more meaningful if it were integrated with botanical means.

SUMMARY AND CONCLUSIONS

Preliminary investigations indicated that a sample of 10 to 14 pods averaging 5 grams was sufficient for analysis. Freezing as a method of preserving the samples showed less visual deterioration of quality factors.

The inheritance studies on volatile components in the bush bean cross O. S. U. 58-110 × 'Romano' and reciprocal, gave these results:

1. A low concentration of 1-octen-3-ol resembling O. S. U. 58-110 was dominant in the F_1 generation over the higher concentration of 'Romano'.
2. The F_2 generation showed a close fit to a two gene pair ratio of 9:7. Although considerably lower in probability value, a 3:1 ratio was also acceptable.
3. A backcross test of F_1 to 'Romano' and reciprocal showed a good fit of the chi square value for a 1:1 ratio. Backcrosses to the O. S. U. 58-110 parent showed only the low concentration typical of this parent. These results confirmed the 3:1 ratio in the F_2 and the results obtained by Stevens (1967), indicating that the concentration of 1-octen-3-ol is controlled by a single gene. The results of the backcross indicates the importance of studying inheritance by more than one method.
4. The difference in mean concentration and the amount of

overlapping in the concentration of linalool found in the two varieties made it impossible to distinguish any genetic difference.

Flower color inheritance was shown to be controlled by two genes. The first gene, $V_{\underline{lae}}$, produced the purple color when dominant and white when recessive. The second gene, Aeq (along with other dominant influencing genes), intensifies the purple color when it is dominant and has no effect when recessive. No linkage was found between flower color intensifier and 1-octen-3-ol. It was not possible to determine linkage of the flower color, $V_{\underline{lae}}$, and 1-octen-e-ol.

A 'Blue Lake' mutant was found in the variety O. S. U. 9025. It was typified by strings on both sutures, oval pod, glossy smooth light green skin, and a light colored inner flesh compared to the typical 'Blue Lake' type of bean.

Thawing time, maturity, and processing were all shown to influence the concentration of 1-octen-3-ol. Concentration of 1-octen-3-ol decreased with maturity (reported on the days from anthesis). Frozen bean pods were found to increase in concentration with longer thawing times. The processed samples, whether frozen or canned, were both lower in concentration than the fresh raw samples.

Processing influences on other volatiles were mostly quantitative with losses occurring in a few of the volatiles in frozen samples.

In canned samples lower boiling volatiles were found to increase in concentration. Heat induced degradation of metabolites is believed to result in the higher amounts of "lower boiling" volatiles. The "higher boiling" compounds were found to decrease in concentration due to processing. These losses in concentration appear to occur prior to retorting. During the blanching period for both frozen and canned beans, volatiles are lost through vaporization and leached out into the hot water bath.

Reduction in quantity and loss of compounds along with the influence of thawing time made the freezing process a less desirable method for preserving sample for genetic studies. It appears that a better method would be to use fresh raw samples and reduce the time needed to analyze them. The main advantage would be the minimum number of variables introduced. Concentrations of "higher boiling" volatiles were found to be highest in the raw product, while the "lower boiling" volatiles were found in moderate amounts. The ten minute cooking period at 90° C for gas-entrainment simulates the actual preparation for consumer consumption.

Maturity problems and quantitative differences would limit the use of volatile flavor components alone for chemotaxonomy. However, this method in combination with analysis of phenolics, alkaloids, flavonoids, and with botanical methods would probably constitute a useful tool for differentiating varieties.

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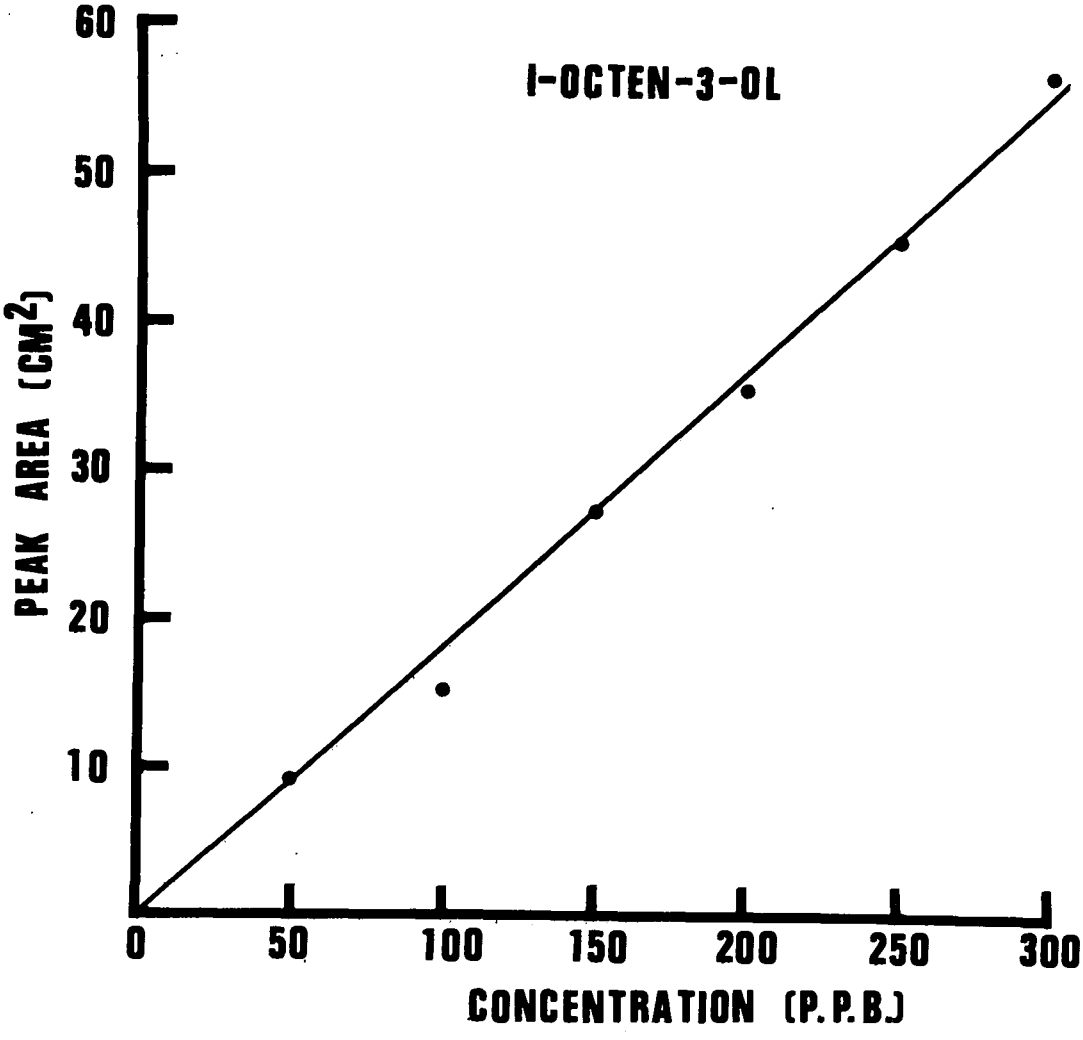
APPENDICES

APPENDIX I

Peak Areas Obtained with Various Concentrations of 1-octen-3-ol



APPENDIX II

Positive identification of chromatogramed peaks by mass spectrometry and relative retention time.

Peak Number	Compound	Mass spectr al Identification	Relative Retention Time[a]	
			Snap beans	Authentic
1a			0.15	
1b	Acetaldehyde	+	0.27	0.33
1c	Ethanol	+	0.46	0.49
1d			0.60	
1e			0.74	
2			1.05	
3a	3-Pentanone	+	1.60	1.50
3b			2.21	
4			2.45	
5a	n-Hexanol	+	2.89	3.05
5b			3.89	
5c			4.29	
6	cis-Hex-3-en-1-ol	+	4.57	4.70
7			5.00	
8	trans-2-hexen-1-ol	+	5.49	5.41
9	1-Octen-3-ol	+	6.69	6.60
10	Linalool	+	8.99	9.15
11	2, 6-Xylenol	Internal Standard	10.000	10.00

[a] t_R/t_R of 2, 6-Xylenol = 10.00.

Appendix III

List of Abbreviations

This appendix gives the abbreviations used in this thesis which are not in standard use in chemical and biochemical literature.

'FM-1L'	'Ferry Morse 1L Blue Lake' pole bean
'FM-1K'	'Ferry Morse 1K Blue Lake' pole bean
'FM-14'	'Ferry Morse 14 Blue Lake' bush bean
'G-50'	'Gallatin 50'
G. L. C.	Gas liquid chromatography
I. Q. F.	Individual quick frozen
M. S.	Mass spectrometry
O. S. U. 190	Oregon State University 190
O. S. U. 949	Oregon State University 949
O. S. U. 9025	Oregon State University 9025
O. S. U. 58-110	Oregon State University 58-110
OV-1	Methyl silicone
OV-17	Methyl phenyl silicone
P	Probability values (R. G. D. Steel and J. H. Torrie. 1960. Principles and procedures of statistics. New York, McGraw-Hill. p. 435)