

A STUDY OF THE INFLUENCE OF VINERY AND STORAGE CONDITIONS
UPON THE ACIDITY, VITAMIN C CONTENT, SUGAR CONTENT,
AND MICROBIAL NUMBERS OF PEAS AND LIMA BEANS.

By
WILLIAM A. NOLTE

Thesis submitted to the Faculty of the Graduate School
of the University of Maryland in partial
fulfillment of the requirements for the
degree of Doctor of Philosophy

1947

UMI Number: DP70508

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



UMI DP70508

Published by ProQuest LLC (2015). Copyright in the Dissertation held by the Author.

Microform Edition © ProQuest LLC.

All rights reserved. This work is protected against unauthorized copying under Title 17, United States Code



ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

TABLE OF CONTENTS

	Page
INTRODUCTION.....	1
HISTORICAL REVIEW.....	3
EXPERIMENTAL PROCEDURES	
A. Handling of Products.....	9
1. Peas.....	9
2. Lima Beans.....	10
B. Bacteriological Procedures.....	11
C. Total Sugar Determinations.....	11
D. Vitamin C Determinations.....	12
1. Effect of Handling and Frozen Storage Upon Vitamin C.....	12
2. Effect of Pure Cultures Isolated from Peas and Lima Beans Upon Pure Vitamin C.....	12
E. pH Determinations.....	15
F. Dry Weight Determinations.....	15
RESULTS.....	16
A. Effect of Handling on Microbial Numbers and Appearance	
1. Peas.....	16
2. Lima Beans.....	18
B. Effect of Handling and Frozen Storage on the Total Sugar Content of Peas.....	28
C. Effect of Handling and Frozen Storage on the Vitamin C Content...28	
1. Peas.....	28
2. Lima Beans.....	32
D. Effect of Pure Cultures of Microorganisms Upon Vitamin C	
1. Organisms Isolated from Peas.....	38
2. Organisms Isolated from Lima Beans.....	41
E. Effect of Handling and Storage on pH Changes	
1. Peas.....	46
2. Lima Beans.....	46
DISCUSSION	
A. Effect of Handling Upon Changes in Quality	
1. Peas.....	51
2. Lima Beans.....	55
B. Effect of Microorganisms Isolated from Peas and Lima Beans Upon Vitamin C.....	58

Table of Contents (Continued)

SUMMARY AND CONCLUSIONS.....	62
LITERATURE CITED.....	66
ACKNOWLEDGEMENTS.....	68
VITA.....	69

LIST OF TABLES

Number	Page
I Numbers of Bacteria on Vined Green Peas after Various Periods of Holding Under Conditions of the Vinery.....	17
IIa Characteristics of Pure Cultures of Organisms Isolated from Shelled Green Peas.....	19
IIb Characteristics of Pure Cultures of Organisms Isolated from Shelled Green Peas.....	20
III Number of Bacteria on Shelled Lima Beans after Various Periods of Holding Under Conditions of the Vinery.....	22
IV Number of Bacteria on Frozen Shelled Lima Beans Held in Locker Storage at -18°C . for One Year.....	23
Va Characteristics of Pure Cultures of Organisms Isolated from Unblanched and Blanched Shelled Lima Beans.....	25
Vb Characteristics of Pure Cultures of Organisms Isolated from Unblanched and Blanched Shelled Lima Beans.....	26
VI Characteristics of Pure Cultures of Organisms Isolated from Frozen Shelled Lima Beans Held in Locker Storage at -18°C . for One Year.....	27
VII Changes in Total Sugar Content of Green Peas after Various Periods of Holding Under Conditions of the Vinery and Frozen and Stored for Several Days at -18°C	29
VIII Changes in Vitamin C Content of Green Peas after Various Periods of Holding Under Conditions of the Vinery and then Frozen and Stored for Several Days at -18°C	30
IX Changes in the Vitamin C Content of Unshelled and Shelled Green Peas Held for Various Periods Under Conditions of the Vinery.....	32
X Early Baby Potato Variety of Freshly Shelled Lima Beans. Influence of a) holding in lug box at winery, b) freezing and c) frozen storage upon vitamin C.....	35
XI Henderson Associate Variety of Freshly Shelled Lima Beans. Influence of a) holding in lug box at winery, b) freezing and c) frozen storage upon vitamin C.....	36
XII Baby Fordhook Variety of Freshly Shelled Lima Beans. Influence of a) holding under running water at winery, b) freezing and c) frozen storage upon vitamin C.....	37

List of Tables (continued)

Number	Page
XIIIa The Effect of Pure Cultures of Microorganisms Isolated from Shelled Green Peas Upon Vitamin C in Vitro.....	39
XIIIb The Effect of Pure Cultures of Microorganisms Isolated from Shelled Green Peas Upon Vitamin C in Vitro.....	40
XIV The Effect of Mixtures of Bacteria Isolated from Shelled Green Peas Upon Vitamin C in Vitro.....	42
XV Descriptive Table of Members of the Coli-Aerogenes Group Isolated from Unblanched Shelled Green Peas.....	43
XVI The Effect of Variant Strains of the Coli-Aerogenes Group Isolated from Unblanched Green Peas Upon Vitamin C in Vitro.....	44
XVIIa The Effect of Pure Cultures of Microorganisms Isolated from Shelled Lima Beans Upon Vitamin C in Vitro.....	47
XVIIb The Effect of Pure Cultures of Microorganisms Isolated from Shelled Lima Beans Upon Vitamin C in Vitro.....	48
XVIII Influence of Holding Green Peas Under Conditions of the Vinery Upon the pH of the Peas.....	49
XIX Influence of a) holding at vinery, and b) frozen storage upon the pH of lima beans.....	50

INTRODUCTION

A number of reports have appeared in the literature since 1935 considering factors which influence the quality of fresh vegetables from the time they are harvested until they are canned or frozen for the market. The usual method of judging the quality of fresh vegetables is to examine them for good color, fresh odor, characteristic flavor, and firm texture. Such criteria are of value, but they are all primarily subjective tests and thus may indicate only general changes in quality which may be the result of handling. New tests for the determination of quality have also been introduced.

Among these tests, vitamin C content has been suggested as a good index to quality. It has been shown that the vitamin C content of some vegetables is greater in certain varieties than in others and that the concentration of this vitamin on the percentage basis decreases with increasing maturity.

Many food technologists have demonstrated a decrease in the concentration of vitamin C and other nutritional substances during the processing and storage of fresh vegetables. However, no single attempt has been made to test simultaneously for all of the changes that may indicate an alteration of quality during holding and processing operations prior to canning or freezing; nor to determine the relationship of microbial growth on vegetables to changes in nutritional values.

It is the object of this investigation to study the effect of holding under various conditions of blanching, and frozen storage on the quality of several varieties of peas and lima beans; and to determine the possible influence of microbial activity upon the destruction of vitamin C in these vegetables.

As indices of any variation in quality, and as possible explanations for any change that might occur, the pH, the microflora, and vitamin C and sugar content were studied at regular intervals. To demonstrate the possible relationship between microbial activity and the destruction of vitamin C in peas and lima beans, pure cultures of bacteria were isolated from these foods and tested for their ability to destroy vitamin C in vitro.

HISTORICAL REVIEW

The variety, the degree of freshness, and the stage of maturity have been shown to be important factors affecting the vitamin C content of vegetables.

Mack, Tressler and King (1936) demonstrated that early small-seeded varieties of peas are a better source of vitamin C than the late large-seeded varieties; likewise, that in a given variety the per cent of ascorbic acid was inversely proportional to the sieve size of the peas and that with increasing maturity the percentage of ascorbic acid decreased. These authors suggest that the actual amount of ascorbic acid does not decrease with growth, while the relative percentage decrease in vitamin C may be ascribed to a coincident large increase in the carbohydrate constituents of the pea. A similar relationship between variety, sieve size and degree of maturity to the vitamin C content was observed when Tressler, Mack, and Jenkins (1937) studied lima beans.

The effect on vitamin C of various methods of handling peas in preparation for and subsequent to freezing has been reported. Fenton and Tressler (1938) reported that washing, blanching, cooling and other commercial operations were responsible for an appreciable loss of ascorbic acid. King and Tressler (1940) found forty-five to sixty seconds of blanching time at 93°C. was required for the inactivation of the plant enzymes which oxidize the vitamin C. This blanch was found to cause a loss of approximately 16 per cent of the ascorbic acid content in Thomas Laxton and Alderman Telephone variety peas. When the blanching time was increased to one hundred and fifty-three seconds, a 35 per cent loss of ascorbic acid was noted. Jenkins, Tressler, and

Fritzgerald (1938) showed these same varieties of peas lose 30 per cent of their ascorbic acid content in preparation for freezing; of this, 10 per cent of the loss was the result of blanching. Moreover, they stated the minimum loss of vitamin C occurred when the blanching period used was just sufficient to inactivate the enzyme, ascorbic acid oxidase. A one minute blanch in boiling water was reported by Kertesz and co-workers (1936) to be sufficient to destroy the ascorbic acid oxidase in peas. Fenton and Tressler (1938) found the vitamin C content of the Thomas Laxton variety was reduced 38 per cent during the various processes up to the actual freezing. Studies by Tressler, Mack and Jenkins (1937) proved lima beans blanched in boiling water lost from 19 to 40 per cent of their ascorbic acid content, depending upon the length of the blanching period. A 19 per cent decrease occurred when the lima beans were blanched for 30 seconds, and when the blanch period was increased 150 seconds, a 40 per cent loss of ascorbic acid was detected.

The importance of refrigeration in preventing the loss of vitamin C from fresh vegetables has been demonstrated by Jenkins and co-workers (1938). They showed there was practically no loss in vitamin C from shelled peas when held at 4°C. for $9\frac{1}{2}$ hours, but when held at 27°C. a 14 per cent loss in this vitamin occurred in 3 hours.

Changes in sugar and starch concentrations in peas have been reported by Jones and Bisson (1932). Their studies indicated that during storage, sucrose decreased much more rapidly in shelled than in unshelled peas. At 0°C. the percentage of sucrose in shelled peas remained almost constant for the entire storage period of 168 days.

At 25°C. about 54 per cent of the sucrose was lost during the first 24 hours of storage and 86 per cent was lost during five days. Little change in the per cent of starch was noted by these authors in shelled peas stored at 0°C. At higher temperatures, they found that starch was more abundant. Jones and Blason interpreted these observations to indicate formation of the starch through the polymerization of the sugars, and to explain the loss of sugar. The greatest per cent increase in starch was noted in samples stored at 25°C.

The choice of satisfactory indices of quality in fresh and frozen vegetables is a problem. Tressler (1938) listed a low bacterial count, the absence of the enzymes catalase and peroxidase and a high vitamin C content as evidence of a high quality frozen product, and therefore, indicative that a good quality fresh product was used for freezing.

Types, as well as numbers of microorganisms, are of importance in the consideration of changes in quality since some bacteria can bring about more rapid decay of vegetables than others and thus cause greater loss in nutritional value. Brown (1933) showed the bacterial content of fresh vegetables such as peas, lima beans, and spinach included many non-pathogenic micrococci, some bacilli, and a few Achromobacter and Sarcinae. After the freezing of these same vegetables, Brown isolated many bacilli, some Flavobacteria, Achromobacter, diplococci, streptococci, and organisms of the colon group.

Studies on the microflora of fresh and frozen lima beans were conducted by Smart and Brunstetter (1938). They reported the isolation of species of Achromobacter, Aerobacter, Flavobacterium, Pseudomonas, Sarcinae, Phytomonas, Micrococcus, several species of bacilli, and many species of yeasts and molds from fresh and from blanched lima beans. After frozen

storage at 15°F. for from 3 to 15 months, portions of the same materials yielded a flora similar to that found on the fresh beans. Species from three of the generally, Achromobacter, Aerobacter, and Phytomonas, failed to survive the freezing. Moreover they showed that blanching, cooling and freezing operations reduced the microbial count of the lima beans more than 99 per cent.

In another report, Smart (1937) demonstrated the survival of species of yeasts, molds, and lactobacilli on fresh peas which had been scalded for one, two, and three minutes and then stored at -17.8°C. for five to seven months.

The possible influence of microbial activity upon the destruction of vitamin C in foods has not been overlooked. Lepkovsky, Hart, Hastings, and Frazer (1925) reported Streptococcus lactis had no effect on the vitamin C content of orange and tomato juices. Bifano and Seravazi (1935) infected lemons with Penicillium digitatum and found the vitamin C content was the same as non-infected lemons.

Many other investigators have demonstrated the action of pathogenic and non-pathogenic bacteria on the stability of ascorbic acid. Stepp and Schroder (1935) reported a strain of Escherichia coli capable of decomposing ascorbic acid, and Kendall and Chinn (1939) isolated members of the "Mucosus capsulatus" and Enterococcus group which fermented this vitamin. The latter workers expressed the belief that specific strains of bacteria rather than all strains of any one species were able to ferment ascorbic acid. For example, they found a strain of the Flexner bacillus, one of the "Mucosus capsulatus" group and one of Alealigenes that definitely inhibited the atmospheric oxidation of vitamin C while other strains attacked the vitamin.

Glucose was shown by them to exert a sparing action upon the fermentation of ascorbic acid by intestinal strains of enterococci and members of the "Mucosus capsulatus" group.

Esselen (1939) studied the effect of forty-five different strains of bacteria representing 39 species on ascorbic acid, and found the majority of these organisms retarded the oxidation of this vitamin to a noticeable degree, especially when they were growing in a medium containing a readily fermentable carbohydrate. He suggested that bacteria were not important as agents causing a loss of ascorbic acid in foods.

Young and James (1942) emphasized the ease with which strains of Escherichia coli and Aerobacter aerogenes were able to decompose vitamin C in vitro. The rapidity with which the breakdown of the vitamin occurred was shown to be a variable with different strains. The presence of sugars, as glucose, lactose, and sucrose was demonstrated to retard the oxidation of ascorbic acid by these bacteria, apparently because their presence favored a more active metabolism of carbohydrates. Other cultures of bacteria studied, including several strains of Proteus and Alcaligenes, failed to attack the vitamin. These cultures demonstrated an ability to retard the oxidation of ascorbic acid by recognized non-bacterial means.

In another report, Young and Rettger (1943) made a more general survey of the ability of various species of bacteria to decompose vitamin C. He demonstrated that members of the genera Escherichia, Aerobacter, Salmonella, Eberthella, Streptococcus (both hemolytic species and enterococci), Encapsulatus, Vibrio, and the species Proteus morgani were able to oxidize ascorbic acid. Members of the genera Bacillus, Alcaligenes, Pseudomonas, Shigella, Staphylococcus, Brucella, Pasteurella, Serratia, Flavobacterium, Chromobacter, Achromobacter, Proteus, and the species Salmonella pullorum were unable to utilize ascorbic acid. Young and Rettger showed also that

the decomposition of ascorbic acid by these bacteria was carried beyond the reversible dehydro state, and that ascorbic acid was utilized readily as a carbon food when the medium contained a suitable source of organic nitrogen such as peptone.

EXPERIMENTAL PROCEDURE

A. Handling of Products.

1. Peas. The varieties of peas used in this study were the Dark Podded Thomas Laxton* and Gradus grown on the University of Maryland's experimental farm at Ridgely, Maryland. Each variety was separately vined, shelled, cleaned and graded for size in the commercial equipment at the experimental farm. Sizes five and six were used in these tests because they represented the largest percentage of the crop. After string, the peas were hand sorted to remove pieces of vine and crushed peas which passed the mechanical cleaner. Lug boxes 18" x 12" x 12" were filled about two-thirds full with peas of each variety.

Blanching was accomplished by placing the peas in a wire basket and immersing them in boiling water (100°C.) for one minute. During the immersion period, the peas were constantly agitated by moving the wire basket up and down in the boiling water. The basket was removed and the peas rinsed with untreated cool spring water at a mean temperature of 20°C. to stop the action of the heat and to rinse off the remainder of the blanch water.

After 0,2,5, and 8 hours holding at approximately 50°C., the average temperature of the vinery, portions of the samples were blanched and the following tests, bacterial counts, pH values, dry weight, sugar and vitamin C content were run on both the unblanched and blanched samples.

As there were no facilities at the farm for laboratory experiments, samples of unblanched and blanched peas collected after each holding period, were frozen in size No. 1 tin cans by packing the cans in a freezer chest

* In all subsequent discussion, the Dark Podded Thomas Laxton variety of peas will be referred to as the "Laxton variety."

containing dry ice. A temperature of -79°C . was reached. The samples were transported in the freezer chest to a locker storage warehouse, and stored at -18°C . until tested. The time required to freeze peas packed in one-pound flat tin cans has been shown by Joslyn and Marsh (1933) to be slightly less than one hour when dry ice was used.

To determine the effect of holding unshelled and shelled peas upon changes in vitamin C content, peas of the Laxton variety were also held in pods under the same conditions as the shelled peas of the same variety and analyzed for vitamin C content at the end of 24 hours holding.

2. Lima Beans. The varieties of lima beans used in this study were the Early Baby Potato, Henderson Associate, and Baby Fordhook which were grown on the experimental farm at Ridgely, Maryland.

As it was thought desirable to use samples of lima beans in the evaluation of the vitamin C content, all beans of which were at the same stage of maturity, a technique for tagging bean blossoms was employed. Two thousand pedicels of the blossoms of the three varieties of limas were marked with an enamel paint early in the blossoming season. Unfortunately, at harvest time approximately 99 per cent of the pedicels which were painted had dropped their blossoms without the development of beans. Therefore this approach was impracticable, and the experiment was carried out using vined and sifted samples which contained limas of different maturity. Two of the varieties of limas, Early Baby Potato (mixed size) and Henderson Associate (size No. 4) were held in lug boxes at a mean temperature of approximately 30°C . The third variety, Baby Fordhook (mixed size), was held in a porcelain tub into which cold water, at a mean temperature of approximately 20°C ., was continuously running. After 0, 5, 6, and 24 hours holding, samples of each variety were removed for analysis.

The method of blanching was the same as employed for peas with the exception that the time of blanching was two and one-half minutes. Vitamin C, pH, and dry weight analyses were made separately on samples of green and white beans individually, while bacterial analyses were made on composite samples of green and white limas. Portions of unblanched and blanched beans for each holding period were frozen in size No. 1 tin cans by packing the cans in dry ice. These frozen samples were transported in a frozen state to a locker storage warehouse, and stored at -18°C . for subsequent tests.

B. Bacteriological Procedures

Microbial numbers were determined on both unblanched and blanched fresh peas, and unblanched and blanched fresh and frozen lima beans for various holding periods. The analysis was made by weighing out 10 gram samples in duplicate, grinding them in sterile sand, and transferring them aseptically to dilution blanks. Plate counts were made with nutrient caseinate agar, and incubated at room temperature until well defined colonies developed. Colonies representing the most numerous types of bacteria that developed on these plates were isolated in pure culture and identified by the usual bacteriological procedures; namely, morphological, biochemical and cultural tests.

C. Total Sugar Determinations

The analyses for sugar on pea samples were carried out at the university laboratory on the frozen samples. Duplicate samples were used for each determination. As it has been shown by Jones and Bisson (1932) and Joslyn and Marsh (1933) that holding food products at temperatures below freezing does not affect the amount of total sugar present, this procedure was considered satisfactory.

Total sugar was determined by the Isbell, Pigman, and Frush (1940) modification of Seales cuprous-chloride-iodide method. The sugar was extracted from 20 gram samples of peas after they had been triturated in alcohol and digested over a water bath for one hour. Possible interfering protein substances were removed by precipitation with a saturated lead acetate solution. After removal of the lead with sodium oxalate, the extract was hydrolyzed with hydrochloric acid, and neutralized with anhydrous sodium carbonate. A 10 cubic centimeter aliquot was removed and employed in the actual analysis.

D. Vitamin C. Determinations

1. Effect of Handling and Frozen Storage Upon Vitamin C.

The ascorbic acid content of peas and lima bean samples was determined by Bessey and King's modification (1933) of Tillman's method in which sodium 2,6-dichlorophenolindophenol is used as the titrating reagent. The vitamin was extracted in an acid solution consisting of equal parts of 3 per cent meta-phosphoric acid and 2 normal sulphuric acid by grinding 20 gram samples with acid-washed sand (Thornton, 1938). Preliminary tests showed that a grinding period of one and one-half minutes was sufficient for thorough extraction. The extract was separated from the sand and other solid materials by filtration through cotton followed by centrifugation. A 1:10 dilution was prepared from the extract and 20 cubic centimeter aliquots were removed and titrated directly with sodium 2,6-dichlorophenolindophenol standardized to equal 0.12 mgs. of vitamin C per cubic centimeter of the dye. Duplicate samples were used for each test, and analysis were made on both fresh and frozen stored samples of peas and lima beans. Separate analysis for vitamin C were made on green and white beans.

2. Effect of Pure Cultures Isolated from Peas and Lima Beans Upon Pure

Vitamin C.

In order to demonstrate the relationship of bacteria on vegetables to the decrease of vitamin C in that vegetable, it was necessary first to isolate the organisms from the food product, and secondly to prove chemically their destructive action on this vitamin in vitro.

Nutrient broth containing ascorbic acid was employed in the study of bacterial activity upon vitamin C. An aqueous solution of ascorbic acid was sterilized by filtration and added to sterile standard nutrient broth in amounts that gave a final concentration of approximately 0.55-0.40 mgs. per cubic centimeter. After removing a portion for immediate analysis, the nutrient broth-ascorbic acid mixture was transferred aseptically in 100 cubic centimeter portions to 180 cubic centimeter bottles and inoculated with the microorganisms isolated from the vegetables.

An inoculum of 0.5 cubic centimeters of a 24 hour old nutrient broth culture of the test organism incubated at 37°C. was used in order to obtain a large microbial population in a short time. Incubation was at room temperature. Titrations were made at regular intervals and duplicate tests were made for each microorganism.

In making the determinations a one cubic centimeter portion of the broth to be tested was removed and placed in a large test tube containing 10 cubic centimeters of 2.4 per cent glacial acetic acid. The sample was titrated with freshly standardized sodium 2,6-dichlorophenolindophenol, one cubic centimeter of which was equivalent to 0.12 mgs. of ascorbic acid. The end point was reached when a faint pink coloration appeared and remained for approximately 20 seconds. The titration was completed within two minutes.

Two sets of controls were employed throughout this investigation. One set consisted of the nutrient broth plus ascorbic acid, which was uninoculated,

and was used to test for the rate of normal atmospheric oxidation of ascorbic acid under the conditions of the experiment. The second set of controls was inoculated, and contained nutrient broth plus the microorganism tested.

This set was employed to test the ability of the cultures to produce substances which would reduce the dye-indicator upon titration, which would result in an inaccurate determination. No such substance was detected.

Simultaneous with the vitamin C determinations, bacterial numbers were ascertained by the plate count method and the changes in pH were recorded using a Beckman pH meter.

Seven pure cultures and three mixed cultures isolated from peas were tested for their reaction towards vitamin C. The mixed cultures were prepared as follows: 1) a mixture prepared from 24 hour broth cultures of the seven pure cultures, Culture number M-P-3; 2) cultures obtained directly from the peas, Cultures M-U-1 and M-B-2. These latter cultures were prepared by placing about 10 gram of peas, unblanched and blanched respectively, into nutrient broth and incubating at room temperature for 48 hours. The mixed cultures thus obtained were used in these tests.

Eight pure cultures were isolated from lima beans. These cultures and a mixture of them prepared from 24 hour old broth cultures were tested for their action on vitamin C.

All results recorded on the course of microbial growth, changes in vitamin C content and in Hydrogen-ion concentration are based upon duplicate determinations. With the cultures isolated from peas, all tests were carried out at regular intervals for 31 hours; with the cultures from the lima beans, the tests were extended until the complete disappearance of vitamin C.

E. pH Determinations

Changes in the Hydrogen-ion concentration of peas and lima beans were determined by employing a quinhydrone-electrode calomel half-cell potentiometer. Hydrogen-ion determinations were made both on the skins and on the inside portion of the pea samples separately, and on the whole bean in the case of lima bean samples. The samples were triturated in a mortar with a few cubic centimeters of distilled water. About two-thirds of a cubic centimeter of the triturated sample was placed in a small glass cup to which a small amount of quinhydrone was added and mixed with the sample. The temperature was noted and the electrodes placed in the cup. The Eh value was read on the potentiometer and the pH obtained after temperature corrections were made by use of standard conversion tables.

F. Dry Weight Determinations

The dry weight of peas and lima beans was determined by weighing 20 gram samples of each lot in petri dishes. The material was crushed and to each dish a small amount of 70 per cent alcohol was added to facilitate rapid evaporation. The samples were placed in a vacuum oven which was held at a temperature of 75°C. and maintained at a vacuum of 25-29 inches. After a period of 24 hours, the samples were removed, cooled in a desiccator, reweighed, and then placed back in the oven. This was continued until constant weights were obtained. The samples were then considered free of moisture, and the per cent total solids calculated.

RESULTS

A. Effect of Handling on Microbial Numbers and Appearance

1. Peas. After holding shelled peas two hours, there was no great change in bacterial numbers on either the Laxton or the Gradus varieties. After 5 hours holding, the bacterial count increased 93.33 and 89.13 per cent on the Laxton and Gradus varieties respectively, and after 8 hours the population had increased over 99 per cent on both varieties of peas. The tremendous increase in bacterial population which was observed after the 8 hour holding period was accompanied by the appearance of a slimy and sticky surface coat on the peas of both varieties. These results are shown in Table I.

Blanching one minute in boiling water after each of the holding periods reduced the bacterial numbers more than 99 per cent.

After 24 hours holding at room temperature, the slimy coat on the unblanched peas, which was noticed previously, became more pronounced and appeared to be bacterial in origin. When samples of unblanched peas were added to flasks of nutrient broth and incubated for 24-48 hours at room temperature, a viscous and slimy growth developed and a vigorous proteolytic decomposition usually associated with bacterial activity occurred. Dilution plates prepared from these unblanched pea-nutrient broth cultures showed a predominant number of proteolytic bacteria as evidenced by a clear zone about the colonies growing on caseinate agar.

The surface of blanched peas after 24 hours holding at room temperature became covered with a heavy white mold-like growth that gave a yeasty odor. When samples of blanched peas were added to flasks of nutrient broth and held under the same conditions as the unblanched peas, the type of mold-like growth just described was noted. Dilution plates prepared from the blanched pea-nutrient broth culture showed a yeast-like mold which produced this

TABLE I

Numbers of Bacteria on Vined Green Peas after
Various Periods of Holding Under Conditions of the
Vinery

Variety	Method of holding	Holding period in hours	Effect of holding (unblanched peas)		Effect of blanching (blanched peas)	
			Column A Bacteria per gram	Column B % increase	Column C Bacteria per gram	Column D % decrease
Dark Podded Thomas Laxton	In lug box	0	5,400,000	-	900	99.98*
		2	6,000,000	10.00	1,890	99.96
		5	81,000,000	95.35	3,100	99.99
		8	580,000,000	99.07	120,000	99.98
Gradus		0	2,500,000	-	9,000	99.64
		2	4,000,000	37.50	11,000	99.72
		5	23,000,000	89.13	74,000	99.67
		8	705,000,000	99.64	3,100,000	99.56

* Column A compared to Column C.

characteristic yeasty odor on tomato-juice-peptonized milk agar. A single species of bacteria, which was later identified as Streptococcus fecalis, was also isolated from these cultures.

The predominating bacteria growing on peas were isolated from these dilution plates by the procedure already described. Arranged in order of descending occurrence on dilution plates, the organisms isolated were found to be closely related to the following species described by Bergey (1939): Achromobacter candidans, Escherichia coli, Flavobacterium aurantiacum, Flavobacterium fulvum, Kurthia zenkeri, and Streptococcus fecalis, and to the genus Rhodotorula described by Harrison. These microorganisms were designated as cultures Nos. 1, 2, 3, 4, 5, 6, and 7 respectively.

The tests used and the results obtained in the identification of these predominant types are presented in Tables IIa and IIb.

It seemed strange that none of the predominant bacterial types isolated from the fresh unblanched peas were spore-formers. This fact may partially explain the decided reduction in bacterial numbers by the blanching process.

2. Lima Beans. A constant increase in microbial numbers during storage of 24 hours was demonstrated for the two varieties of lima beans held in lug boxes, the Early Baby Potato and the Henderson Associate. The third variety, Baby Fordhook, which was held under running water, showed a decrease in bacterial numbers at the 6 hour period and then a tremendous increase in bacterial population at the 24 hour period. Although this last variety showed a great increase in bacterial numbers, the per cent increase at the end of the 24 hour period was not as great as with the other varieties. This increase was 93.33 per cent as compared with 98.99 per cent

TABLE IIa

Characteristics of Pure Cultures of Organisms Isolated from Shelled Green Peas

Cultures	No. 1	No. 2	No. 3	No. 4
MORPHOLOGY				
Shape and grouping	rods single pairs	short rods single pairs	short rods single pairs	short rods single pairs
Gram Reaction	negative	negative	negative	negative
Capsule	absent	absent	absent	absent
Spore	absent	absent	absent	absent
Motility	negative	positive	positive	negative
Potato Slant	opaque grayish viscid growth	opaque growth	bright yellow-orange butyrous growth	yellow butyrous growth
PHYSIOLOGY				
Dextrose	no change	AG**	A*	no change
Mannite	no change	AG	A	no change
Maltose	no change	AG	A	no change
Sucrose	no change	AG	A	no change
Lactose	no change	AG	A	no change
Indol	negative	positive	positive	negative
Nitrite	negative	positive	negative	negative
Litmus Milk	alkaline	acid slow curd	acid	slight acid
Nutrient Broth	3+ *** sedimented growth	4+ turbid growth	2+ turbid growth	3+ turbid growth
Gelatin Liquefaction	negative	negative	negative	negative
Starch Hydrolysis	negative	negative	negative	negative
Hydrogen Sulfide	negative	negative	negative	negative
Microorganism most closely related to	<u>Achromobacter</u> <u>candicans</u>	<u>Escherichia</u> <u>coli</u>	<u>Flavobacterium</u> <u>aurantiacum</u>	<u>Flavobac-</u> <u>terium</u> <u>fulvum</u>

* A - acid

** AG - acid & gas

*** + - degree of growth

TABLE IIb

Characteristics of Pure Cultures of Organisms
Isolated from Shelled Green Peas

Cultures	No. 5	No. 6	No. 7
MORPHOLOGY			
Shape and grouping	rods single pairs chains	cocci pairs chains	large oval cells
Gram Reaction	positive	positive	positive
Capsule	absent	absent	absent
Spore	absent	absent	absent
Motility	positive	negative	negative
Potato Slant	spreading gray-yellow butyrous growth	faint white growth	pink butyrous growth
PHYSIOLOGY			
Dextrose	slight A*	slight A	slight A
Mannite	no change	slight A	no change
Maltose	slight A	slight A	slight A
Sucrose	no change	slight A	slight A
Lactose	no change	slight A	no change
Indol	negative	negative	negative
Nitrite	negative	negative	negative
Litmus Milk	no change	acid slight curd reduction	acid reduction
Nutrient Broth	4+ *** turbid growth	1+ turbid growth	2+ turbid growth
Gelatin Liquefaction	negative	negative	negative
Starch Hydrolysis	negative	negative	negative
Hydrogen Sulfide	negative	negative	negative
Microorganism most closely related to	<u>Kurthia</u> <u>senkeri</u>	<u>Streptococcus</u> <u>fecalis</u>	<u>Rhodotorula</u> (Harrison)

* A - acid
*** + - degree of growth

and 99.98 per cent for the first two varieties respectively. Table III presents the above figures.

Blanching the beans for two and one-half minutes in boiling water (100°C.) and then cooling them immediately with running spring water at approximately 20°C. resulted in a reduction in bacterial numbers of greater than 99 per cent for each holding period. There was only one exception to the above statement. The Baby Fordhook beans which were held in running water for 6 hours showed a decrease in bacterial population of only 72 per cent as a result of blanching. The decrease in count between the beans examined at the beginning of the test and those examined after 6 hours of holding in running water may be explained by the dilution effect of the running water. The organisms remaining must have adhered firmly to the surface of the beans and this adherence may explain the decreased efficiency of the blanch in lowering bacterial numbers.

A comparison of the numbers of bacteria found upon the frozen beans after prolonged storage with those present on the beans before freezing indicated an increase in the bacterial numbers (Table IV). Although this increase was noted in 21 out of 22 determinations, the percentage increase was a variable and thus no general statement may be made at this time. Among the possible explanations for these observations, the following may be given: a) the breaking up of clumps of bacteria during freezing, b) the accidental defrosting of the samples during storage with accompanying microbial growth, or c) to the variations of sampling itself. As mentioned previously, blanching the fresh product resulted in a great reduction in bacterial numbers. The influence of blanching was still apparent in the frozen stored samples.

The predominating types of microorganisms present on unblanched and blanched beans were isolated by the serial dilution method. Tables Va and

TABLE III

Number of Bacteria on Shelled Lima Beans after Various Periods of Holding
Under Conditions of the Vinery

Variety	Method of holding	Holding period in hours	Effect of holding (unblanched lima beans)		Effect of blanching after holding (unblanched lima beans)	
			Column A bact./gm.	Column B % increase	Column C bact./gm.	Column D % decrease
Early Baby Potato	In lug box	0	201,000	-	1,200	99.41*
		3	800,000	74.88	800	99.92
		6	2,000,000	89.95	1,300	99.93
		24	20,000,000	98.99	10,000	99.95
Henderson Associate	In lug box	0	29,300	-	900	96.29
		3	80,000	63.75	1,100	98.62
		6	500,000	94.14	3,000	99.40
		24	200,000,000	99.98	35,000	99.98
Baby Fordhook	Under running water	0	120,000	-	800	99.50
		6	10,000	-	2,800	72.00
		24	1,800,000	93.33	7,000	99.61

*Based on bact./gm. in Column A and Column C.

TABLE IV

Number of Bacteria on Frozen Shelled Lima Beans Held in Locker
Storage at -18°C . for One Year

Variety	Treatment Prior to Freezing		Effect of storage			
	Method of holding	Holding period in hours	Unblanched		Blanched	
			before storage bact./gm.	after storage bact./gm.	before storage bact./gm.	after storage bact./gm.
Early Baby Potato	In lug box	0	201,000	250,000,000	1,200	60,000
		3	800,000	1,720,000	600	5,000
		6	2,000,000	2,760,000	1,300	5,400
		24	20,000,000	2,760,000,000	10,000	60,000
Henderson Associate	In lug box	0	29,300	690,000	900	2,500
		3	80,000	580,000	1,100	40,000
		6	500,000	720,000	3,000	10,000
		24	200,000,000	1,785,000,000	35,000	100,000
Baby Fordhook	Under running water	0	120,000	500,000	600	7,500
		6	10,000	17,000	2,800	6,700
		24	1,800,000	39,000,000	7,000	2,000

Vb give the identity of these types based upon their morphological, cultural and biochemical characteristics.

Strains were isolated from the unblanched beans which were closely related to the following species described by Bergey (1939): Bacillus megatherium, Bacillus subtilis, and Staphylococcus albus, cultures Nos. 8, 9, and 10 respectively.

The blanched samples showed a greater variety of bacterial species compared to the unblanched vegetable. The different types of bacteria isolated from blanched limas had characteristics similar to the following species described by Bergey (1939): Achromobacter gaminum, Achromobacter nitrificans, Aerobacter aerogenes, Alcaligenes metalcaligenes, and Bacillus parvus, cultures Nos. 11, 12, 13, 14, and 15 respectively.

The finding of a greater variety of bacteria on the blanched limas is worthy of note. As untreated water was used in rinsing the beans after the blanch, it is possible that the water might have recontaminated the limas with these bacterial types. Several of the types isolated are commonly found in untreated spring water. No examinations were made on the purity of the rinse water.

Isolations of the predominating types of bacteria also were made from the beans held in frozen storage at -13°C . for one year. The cultures of bacteria were purified and identified by the usual methods. Table VI gives the morphological and biochemical characteristics of the four representative cultures. Cultures Nos. 16, 17, 18, and 19, were identified as species related to Bacillus subtilis, Aerobacter aerogenes, Aerobacter slovacae, and Flavobacterium butyri respectively. The first two species were also isolated and identified as predominating types on the unfrozen product.

TABLE Va

Characteristics of Pure Cultures of Organisms
Isolated from Unblanched and Blanched Shelled Lima Beans.

Cultures	No. 8	No. 9	No. 11	No. 12
MORPHOLOGY				
Shape and grouping	rods single pairs chains	rods single pairs chains	short coccoid rods pairs	short coccoid single pairs
Gram Reaction	positive	positive	negative	negative
Capsule	absent	absent	absent	absent
Spore	oval, central	oval, central	absent	absent
Motility	negative	positive	positive	positive
Potato Slant	growth white creamy glistening	growth white creamy glistening	growth grayish creamy glistening	growth grayish creamy glistening
PHYSIOLOGY				
Dextrose	A*	A	A	A
Mannite	A	alkaline	A	A
Maltose	A	A	A	A
Sucrose	A	A	A	A
Lactose	no change	alkaline	no change	alkaline
Indol	negative	negative	positive	negative
Nitrite	negative	positive	positive	positive
Koser Citrate	2+ growth****	1+ growth	2+ growth	3+ growth
Litmus Milk	pept.***only	pept. only	alkaline	no change
Nutrient Broth	4+ flaky growth	4+ flaky growth	4+ turbid growth	4+ turbid growth
Gelatin Liquefaction	weak positive	positive	negative	positive
Starch Hydrolysis	weak positive	positive	negative	negative
Hydrogen Sulfide	negative	negative	negative	negative
Microorganism most closely related to	<u>Bacillus megatherium</u>	<u>Bacillus subtilis</u>	<u>Achromobacter gementum</u>	<u>Achromobacter nitrificans</u>

* A - acid

*** pept.-peptonization

**** + - degree of growth

TABLE Vb

Characteristics of Pure Cultures of Organisms Isolated
from Unblanched and Blanched Shelled Lima Beans.

Cultures	No. 13	No. 14	No. 10	No. 15
MORPHOLOGY				
Shape and grouping	short coccoid rods single pairs	short rods single pairs	cocci in clusters single pairs	rods single pairs chains
Gram Reaction	negative	negative	positive	positive
Capsule	absent	absent	absent	absent
Spore	absent	absent	absent	oval, central
Motility	positive	negative	negative	positive
Potato Slant	growth grayish creamy glistening	growth white creamy glistening	growth white creamy glistening	growth yellow rough dull
PHYSIOLOGY				
Dextrose	AG**	no change	A*	A
Mannite	AG	no change	A	A
Maltose	AG	no change	A	A
Sucrose	AG	no change	A	A
Lactose	AG	no change	A	A
Indol	negative	negative	negative	negative
Nitrite	positive	positive	positive	positive
Koser Citrate	4+ **** growth	no growth	2+ growth	4+ growth
Litmus Milk	acid, curd, gas	alkaline	acid curd	pept ***
Nutrient Broth	4+ granular growth	2+ sediment growth	2+ granular growth	4+ flaky growth
Gelatin Liquefaction	negative	negative	negative	negative
Starch Hydrolysis	negative	negative	negative	negative
Hydrogen Sulfide	negative	negative	negative	negative
Microorganism most closely related to	<u>Aerobacter aerogenes</u>	<u>Alcaligenes metalcaligenes</u>	<u>Staphylococcus albus</u>	<u>Bacillus parvus</u>

* A - acid
** AG - acid and gas

*** pept.-peptonization
**** + degree of growth

TABLE VI

Characteristics of Pure Cultures of Organisms Isolated from Frozen Shelled Lima Beans Held in Locker Storage at -18°C . for One Year.

Cultures	No. 16	No. 17	No. 18	No. 19
MORPHOLOGY				
Shape and grouping	rods single pairs	small rods single pairs	small rods single pairs	small rods single pairs, chains
Gram Reaction	positive	negative	negative	negative
Capsule	absent	absent	absent	absent
Spore	absent	absent	absent	absent
Motility	positive	positive	positive	positive
Agar slant	grayish, creamy glistening	opaque glistening	opaque glistening	yellowish creamy, dull
PHYSIOLOGY				
Dextrose	A*	AG**	AG	no change
Mannite	no change	AG	AG	no change
Maltose	A	AG	AG	no change
Sucrose	A	AG	AG	no change
Lactose	no change	AG	AG	no change
Indol	negative	negative	negative	negative
Nitrite	positive	positive	positive	negative
Methyl Red test	negative	negative	negative	negative
Koser Citrate	no growth	3+****growth	3+ growth	no growth
Voges-Proskaur test	negative	positive	positive	negative
Litmus Milk	pept.***	acid, curd	acid, curd	no change
Gelatin Liquefaction	positive	negative	positive	negative
Starch Hydrolysis	positive	negative	negative	negative
Microorganism most closely related to	<u>Bacillus subtilis</u>	<u>Aerobacter aerogenes</u>	<u>Aerobacter cloacae</u>	<u>Flavobacterium butyri</u>

- * A - acid
 ** AG - acid and gas
 *** pept. - peptonization
 **** + - degree of growth

B. Effect of Handling and Frozen Storage on the Total Sugar Content of Peas.

The course of total sugar change as influenced by holding peas 2, 5, and 8 hours is presented in Table VII. The per cent loss calculated on both fresh and dry weight basis is indicated, and the results recorded are the average of duplicate samples.

The initial total sugar content of fresh green peas was found to be 308 and 285 mgs. per gram dry weight basis for the Laxton and Gradus varieties respectively. The amount of sugar lost increased with the length of the holding period. Fresh peas of the Laxton variety showed a drop in total sugar from 308 mgs. per gram to 267 mgs. per gram, a loss of 13.32 per cent during 5 hours of holding. The Gradus variety, on the other hand, showed a loss of 6.72 per cent for the same period. A greater difference between varieties is observed after the 8 hour period when the per cent loss of sugar was 24.67 per cent for the Laxton variety compared to a 13.78 per cent sugar loss for the Gradus variety.

The blanching process did not materially influence the sugar loss; as no definite change in the trend is apparent from the results obtained (Table VII). However, a slight deviation is noted in the total sugar content of some of the blanched pea samples. This may be due to slight variation inherent within the samples of the same lot of peas, since the content of individual peas may vary slightly according to degree of maturity, growth conditions and other factors, Tressler, Mack, and Jenkins (1957).

C. Effect of Handling and Frozen Storage on the Vitamin C Content.

1. Peas. The initial vitamin C content of the Laxton variety of peas was slightly higher than that of the Gradus variety (Table VIII, Column C). Vitamin C loss from the Thomas Laxton peas is observed to increase with the

TABLE VII

Changes in Total Sugar Content of Green Peas Held for Various Periods Under Conditions of the Vinery and Frozen and Stored for Several Days at -13°C .

Variety	Holding period in hours	Total sugar content after freezing and holding several days at -13°C .									
		Fresh basis				Dry basis					
		Unblanched		Blanched		Unblanched			Blanched		
		mg. /gm.	% loss	mg. /gm.	% loss	Total Solids %	mg. /gm.	% loss	Total Solids %	mg. /gm.	% loss
Dark Podded Thomas Laxton	0	63.6	-	60.4	5.46**	20.6	308	-	18.8	321	none**
	2	63.2	.62*	58.8	6.96	21.0	301	2.27*	19.7	299	0.66
	5	56.4	11.32	56.3	0.14	21.1	267	13.32	19.1	295	none
	8	49.8	21.70	49.8	none	21.6	233	24.67	24.2	212	9.01
Gradus	0	58.8	-	54.4	7.49	20.8	233	-	19.9	274	3.18
	2	55.6	5.44	56.4	none	21.4	260	8.12	18.7	301	none
	5	55.2	6.12	55.6	none	20.9	264	6.72	19.8	281	none
	8	52.4	10.88	46.8	10.89	21.5	244	13.78	19.0	246	none

* % loss due to holding

** % loss due to blanching after holding.

TABLE VIII

Changes in Vitamin C Content of Green Peas after various Periods of Holding Under Conditions of the Winery and then Frozen and Stored for Several Days at -18°C .

Variety	Holding period in hours	Total solids %		Ascorbic acid content															
		Unblanched	Blanched	After holding period								After freezing and holding several days in frozen state							
				Fresh basis				Dry basis				Fresh basis				Dry basis			
				Unblanched Col. A		Blanched Col. B		Unblanched Col. C		Blanched Col. D		Unblanched Col. E		Blanched Col. F		Unblanched Col. G		Blanched Col. H	
				mgs./ gm.	% loss*	mgs./ gm.	% loss**	mgs./ gm.	% loss*	mgs./ gm.	% loss**	mgs./ gm.	% loss*	mgs./ gm.	% loss**	mgs./ gm.	% loss*	mgs./ gm.	% loss**
Dark Podded Thomas Laxton	0	20.6	18.8	.25	-	.17	32.00	1.21	-	.91	24.79	.07	-	.13	none	.34	-	.69	none
	2	21.0	19.7	.24	4.00	.17	29.20	1.14	5.79	.36	24.56	.06	14.29	.13	none	.29	14.71	.66	none
	5	21.1	19.1	.23	8.00	.16	30.10	1.09	9.91	.84	22.94	.07	none	.12	none	.33	none	.63	none
	8	21.6	24.2	.20	20.00	.15	25.00	0.94	22.31	.62	34.25	.07	none	.10	none	.33	none	.41	none
Gradus	0	20.8	19.9	.22	-	.14	36.41	1.03	-	.70	35.18	.12	-	.13	none	.58	-	.66	none
	2	21.4	18.7	-	-	-	-	-	-	-	-	.12	none	.13	none	.56	3.45	.68	none
	5	20.9	19.8	-	-	-	-	-	-	-	-	.06	50.00	.11	none	.27	53.45	.56	none
	8	21.5	19.0	-	-	-	-	-	-	-	-	.05	58.33	.10	none	.23	60.35	.47	none

* % loss due to holding

** % loss due to blanching after holding.

length of the holding period (Columns A & C). For the 2 hour holding period, this variety showed a loss of 5.79 per cent vitamin C dry weight basis, for the 5 hour period a 9.91 per cent, and for the 8 hour period a 22.31 per cent loss.

Because of inadequate laboratory facilities at the experimental farm, it was impossible to determine the vitamin C content on fresh samples of the Gradus variety for the different holding periods. Therefore, only the initial determinations were made on unblanched and blanched samples of this variety.

The blanching and washing process reduced the vitamin content of the Laxton variety from 1.21 mgs. to 0.91 mgs. per gram dry weight basis, a loss of 24.79 per cent (Column D). For the Gradus variety the vitamin content was reduced from 1.08 mgs. to 0.70 mgs. per gram, a loss of 35.18 per cent (Column D). Similar losses in ascorbic acid content of the Thomas Laxton peas due to blanching is observed after 2, 5, and 8 hours holding.

Examination of Table VIII shows that after samples of peas were frozen and held at freezing temperatures, considerable loss in vitamin C occurred in both unblanched and blanched samples when compared with comparable unfrozen samples (Column C compared with Column G, and Column D compared with Column H). For both varieties, the blanched frozen samples retained a higher percentage of the ascorbic acid than the unblanched frozen samples. An excessive loss of the vitamin is noted for the unblanched peas of the Laxton variety.

A comparison of the vitamin C content of unshelled versus shelled peas is striking (Table IX). Peas of the Laxton variety held in pods for 24 hours showed a reduction from an initial content of 0.25 mgs. to 0.22 mgs. of ascorbic acid per gram, a loss of 12 per cent. On the other hand when the same variety of pea was shelled, the vitamin was reduced from an initial content of 0.25 mgs. to 0.11 mgs., a 56 per cent loss. No significant loss in

ascorbic acid was noted after holding for 2 and 5 hours in either the unshelled or shelled samples; but after 8 hours, a 20 per cent loss occurred in shelled peas.

TABLE IX

Changes in the Vitamin C Content of Unshelled and Shelled Green Peas Held for Various Periods Under Conditions of the Vinery.

Variety	Holding period in hours	Unshelled Peas		Shelled Peas	
		Ascorbic acid content mgs./gm.	% loss	Ascorbic acid content mgs./gm.	% loss
Dark Podded Thomas Laxton	0	.25	-	.25	-
	2	.24	4	.24	4
	5	.23	8	.23	8
	8	.23	8	.20	20
	24	.22	12	.11	56

2. Lima Beans. A comparison of the ascorbic acid content of fresh green limas with white limas was desirable. In every test the green beans contained considerably more ascorbic acid than the white (Tables X, XI, and XII). On dry weight basis, the initial ascorbic acid content per gram of sample of green limas compared with white limas was 0.63 mgs. to 0.22 mgs. for the Early Baby Potato variety, 0.91 mgs. to 0.43 mgs. for the Henderson Associate variety, and 0.90 mgs. to 0.32 mgs. for the Baby Fordhook variety.

Fresh beans of the three varieties showed considerable loss of vitamin C as the result of the blanch. The average initial ascorbic acid content of duplicate composite samples of green and white limas of the Early Baby Potato variety was 0.42 mgs. per gram. After blanching for 2½ minutes at a temperature

of 100°C., the ascorbic acid content was lowered to 0.32 mgs. per gram which represented a loss of 23.8 per cent. Composite samples of beans of the Henderson Associate contained initially 0.67 mgs. per gram of vitamin C. After the blanch, the ascorbic acid content was lowered to 0.43 mgs. per gram, a loss of 35.8 per cent. The Baby Fordhook variety contained initially 0.61 mgs. of ascorbic acid per gram for a composite sample of lima's. Comparable material after the blanch showed 0.42 mgs. per gram, a loss of 31.1 per cent.

The loss in ascorbic acid shown by the initial sample of unblanched frozen beans held for several weeks in locker storage (-16°C.) is notable (Tables X, XI, and XII). The average initial ascorbic acid content of duplicate composite unblanched samples of the Early Baby Potato variety decreased from 0.42 mgs. per gram for unfrozen beans to 0.30 mgs. per gram, dry weight basis, for the frozen, a 28 per cent loss (Columns B & D).

Similar losses are also observed for both the Henderson Associate and Baby Fordhook varieties. No such loss in ascorbic acid is observed when comparing the blanched frozen beans with the blanched unfrozen beans. The loss of ascorbic acid in the fresh frozen samples may be explained by the oxidation of ascorbic acid by oxidase during storage (Jenkins, Tressler, and Fitzgerald, 1938). In general, the blanched frozen samples of each variety contained less ascorbic acid after the various holding periods than the unblanched frozen samples (Tables X, XI, and XII, Cols. B & D).

Since all samples of frozen beans were subjected to the same conditions of frozen storage, the effect of holding upon vitamin C content should indicate a trend in the frozen samples similar to the trend that existed in the fresh beans for the various holding periods. The following results indicate the possible effect of holding upon loss in vitamin C.

Unblanched frozen green and white lima's of the Early Baby Potato variety

(for the 24 hour holding period) showed considerable loss in ascorbic acid. The initial ascorbic acid content of a composite frozen sample of this variety contained 0.30 mgs. per gram (Column D). Comparable materials (held 24 hours prior to freezing) contained but 0.09 mgs. per gram. This represented a 70 per cent loss. In the case of the Henderson Associate, the initial ascorbic acid content of a composite frozen sample was 0.50 mgs. per gram (Column D). Comparable materials for the 24 hour period contained 0.30 mgs. per gram, a loss of 40 per cent due to holding. For the Baby Fordhook variety, the loss represented the difference between the initial vitamin C content, which was 0.50 mgs. per gram and the vitamin C content of frozen limas held for 24 hours prior to freezing (Column D). A composite sample of limas for this period contained 0.34 mgs. per gram, and the loss represented a 32 per cent.

During locker storage at -18°C . for one year, there was a considerable loss in vitamin C for both unblanched and blanched beans of all varieties. Considering the Early Baby Potato variety, the unblanched green and white limas soon after freezing (Table X, Column D) contained 0.30 mgs. of vitamin C per gram, dry weight basis, whereas comparable material after frozen storage (Column F) contained only 0.20 mgs. per gram. This represented a 33.3 per cent loss during storage. Comparable samples for the 3, 6, and 24 hour periods also show losses (Columns D & F). Similar changes in vitamin C content is observed for frozen stored samples of both the Henderson Associate and the Baby Fordhook varieties (Tables XI and XII, Columns D & F).

Blanched frozen samples of the three varieties after locker storage for one year showed less loss of ascorbic acid than comparable unblanched frozen samples.

TABLE X

Early Baby Potato Variety of Freshly Shelled Lima Beans

Influence of a) holding in lug box at winery, b) freezing and c) frozen storage upon vitamin C.

Condition of the Lima beans	Holding period in hours	Ascorbic acid content in mg. per gram														
		Total solids %	After holding in fresh state				Total solids %	After freezing & short holding time		Total solids %	After freezing & holding 1 yr. at -18°C.					
			fresh basis		dry basis			fresh basis	dry basis		fresh basis	dry basis				
Col. A	Col. B	Col. C	Col. D	Col. E	Col. F											
Unblanched																
Green	0	54.5	.34	.24*	.63	.42*	54.5	.27	.16*	.50	.30*	43.9	.14	.10*	.32	.20*
White		62.5	.14		.22		62.5	.06		.10		53.4	.04		.07	
Green	3						38.2	.27	.18	.70	.41	41.9	.15	.10	.36	.22
White							47.8	.09		.22		58.0	.04		.07	
Green	6						40.3	.15	.11	.37	.25	53.0	.12	.08	.23	.14
White							46.3	.06		.13		58.5	.03		.05	
Green	24						44.8	.05	.04	.11	.09	45.8	.04	.02	.09	.05
White							51.6	.03		.06		57.4	.002		.004	
Blanched																
Green	0	37.0	.22	.16	.39	.32	37.0	.22	.16	.39	.32	35.3	.19	.12	.53	.31
White		40.5	.10		.25		40.5	.10		.25		62.4	.05		.08	
Green	3						32.8	.24	.16	.48	.34	52.2	.19	.12	.35	.21
White							36.2	.07		.19		55.0	.04		.07	
Green	6						24.5	.20	.12	.58	.34	40.3	.17	.11	.43	.25
White							37.5	.03		.09		46.6	.04		.06	
Green	24						39.6	.14	.08	.08	.05	40.4	.13	.07	.31	.17
White							46.2	.01		.02		47.6	.01		.02	

* Ascorbic acid content of composite sample of green and white beans.

TABLE XI

Henderson Associate Variety of Freshly Shelled Lima Beans

Influence of a) holding in lug box at vinery, b) freezing and c) frozen storage upon vitamin C.

Condition of the Lima Beans	Holding period in hours	Ascorbic acid content in mg. per gram														
		Total solids %	After holding in fresh state		Total solids %	After freezing & short holding time		Total solids %	After freezing & holding 1 yr. at -18°C.							
			fresh basis Col. A	dry basis Col. B		fresh basis Col. C	dry basis Col. D		fresh basis Col. E	dry basis Col. F						
Unblanched																
Green	0	40.7	.37	.39*	.91	.67*	40.7	.28	.22*	.69	.50*	39.1	.19	.12*	.48	.29*
White		49.2	.21		.43		49.2	.15		.31		45.8	.05		.10	
Green	3						37.5	.28	.20	.75	.50	39.5	.18	.11	.46	.27
White							46.5	.11		.24		49.8	.04		.08	
Green	6						42.0	.28	.19	.67	.44	40.4	.12	.08	.30	.19
White							47.6	.10		.21		45.8	.03		.07	
Green	24						50.1	.23	.16	.46	.30	48.8	.11	.06	.23	.13
White							56.8	.08	.14	.14		50.4	.01		.02	
Blanched																
Green	0	36.8	.23	.16	.63	.43	36.8	.23	.16	.63	.42	36.2	.19	.12	.53	.33
White		40.0	.09		.23		40.0	.08		.20		39.8	.05		.12	
Green	3						36.3	.22	.15	.61	.40	39.7	.18	.11	.45	.27
White							39.6	.07		.18		43.8	.04		.09	
Green	6						34.8	.21	.14	.61	.40	37.1	.17	.11	.45	.28
White							37.6	.07		.18		40.8	.04		.10	
Green	24						42.2	.19	.12	.45	.28	39.4	.16	.10	.40	.24
White							47.8	.05		.11		43.5	.03		.07	

* Ascorbic acid content of composite sample of green and white beans.

TABLE XII

Baby Fordhook Variety of Freshly Shelled Lima Beans

Influence of a) holding under running water at vinery, b) freezing and c) frozen storage upon vitamin C.

Condition of the Lima Beans	Holding period in hours	Ascorbic acid content in mg. per gram														
		Total solids %	After holding in fresh state				Total solids %	After freezing & short holding time				Total solids %	After freezing & holding 1 yr. at -18°C.			
			fresh basis		dry basis			fresh basis		dry basis			fresh basis	dry basis		
Col. A	Col. B	Col. C	Col. D	Col. E	Col. F											
Unblanched																
Green	0	38.9	.35	.25*	.90	.61*	38.9	.30	.22*	.77	.50*	39.2	.20	.13*	.51	.32*
White		47.2	.15		.32		47.2	.11		.23		49.8	.06		.12	
Green	6						31.1	.27	.18	.37	.55	31.5	.19	.11	.60	.35
White							37.0	.08		.22		43.4	.03		.10	
Green	24						42.9	.24	.15	.56	.34	31.9	.16	.09	.50	.27
White							50.2	.06		.12		40.8	.01		.03	
Blanched																
Green	0	35.2	.23	.15	.65	.42	35.2	.23	.15	.65	.42	34.3	.19	.12	.56	.33
White		39.2	.07		.18		39.2	.07		.18		41.1	.04		.10	
Green	6						36.2	.18	.10	.50	.28	32.3	.16	.09	.50	.27
White							40.1	.02		.05		33.2	.01		.03	
Green	24						30.1	.17	.10	.56	.32	32.4	.12	.07	.37	.20
White							36.3	.03		.08		38.3	.01		.02	

* Ascorbic acid content of composite sample of green and white beans.

D. Effect of Pure Cultures of Microorganisms Upon Vitamin C.

1. Organisms Isolated from Peas. An inspection of the data in Tables XIIIa and XIIIb shows that of those cultures isolated from unblanched and blanched peas, only one pure culture, Escherichia coli, was able to utilize vitamin C. This species destroyed the vitamin completely within ten hours whereas the uninoculated control retained approximately 45 per cent of the vitamin for the same period. Three of the cultures protected the vitamin from atmospheric oxidation. The test medium inoculated with the culture resembling Kurtzia zenkeri retained 75 per cent of the original content of ascorbic acid after 31 hours of incubation, while the cultures resembling Flavobacterium aurantiacum and Flavobacterium fulvum retained 50 and 45 per cent respectively for the same period. The three cultures related to Achromobacter caudatus, Streptococcus fecalis and the yeast, Rhodotorula sp., did not affect the stability of the vitamin significantly. The ascorbic acid concentrations in these tests followed the approximate course of non-bacterial oxidation as shown in the uninoculated ascorbic acid control.

The relation between bacterial counts and oxidation or protection of vitamin C shows that a large number of organisms must be present before the vitamin is attacked or before a definite protective action is exerted.

The influence of a mixed microflora from unblanched and blanched peas upon ascorbic acid is presented in Table XIV. A comparison of the mixed cultures, M-U-1, M-B-2, and M-P-3 to the uninoculated control indicates that the vitamin was rapidly destroyed in each of the mixtures in 10 to 15 hours. The mixed culture prepared from 24 hour broth cultures of the seven pure cultures, M-P-3, shows that when strains of bacteria which protect the vitamin grow with a single strain that destroys the vitamin, the vitamin is rapidly destroyed. The bacterial counts of the mixed cultures show that the

TABLE XIIIa

The Effect of Pure Cultures of Microorganisms Isolated from Shelled Green Peas Upon Vitamin C in Vitro

Cultures	<u>Flavobacterium aurantiacum</u>				<u>Kurthia zenkeri</u>				<u>Escherichia coli</u>				<u>Escherichia coli var. Y-15*</u>				Uninoculated control		
	Ascorbic acid content		pH values	Bacterial count nos./cc. in T**	Ascorbic acid content		pH values	Bacterial count nos./cc. in T	Ascorbic acid content		pH values	Bacterial count nos./cc. in T	Ascorbic acid content		pH values	Ascorbic acid content		pH values	
	mg./cc.	percent present			mg./cc.	percent present			mg./cc.	percent present			mg./cc.	percent present		mg./cc.	percent present		mg./cc.
0	.36	100.0	6.35	12,700	.36	100.0	6.35	6,800	.36	100.0	6.35	4,250	.36	100.0	6.35	19,700	.36	100.0	6.35
5	.27	75.0	-	37,000	.31	86.6	-	12,000	.28	77.8	-	255,000	.30	83.3	-	251,000	.26	72.2	-
10	.23	63.9	6.50	135,000	.29	80.6	6.68	88,000	.00	00.0	6.20	505,000	.16	44.5	6.25	550,000	.16	44.5	6.35
15	.22	61.1	-	209,000	.29	80.6	-	149,000	.00	00.0	-	490,000	.00	00.0	-	620,000	.15	41.7	-
24	.18	50.0	6.68	500,000	.29	80.6	6.96	202,000	.00	00.0	6.48	460,000	.00	00.0	6.46	720,000	.04	11.1	6.00
31	.18	50.0	6.74	990,000	.27	75.0	6.98	243,000	.00	00.0	6.50	450,000	.00	00.0	6.50	700,000	.00	00.0	6.00

* A stock culture of Escherichia coli was used for comparison of vitamin C oxidizing ability.

** T denotes thousands.

TABLE IIIb

The Effect of Pure Cultures of Microorganisms Isolated from Shelled Green Peas Upon Vitamin C in Vitro

Cultures	<u>Achromobacter</u> <u>candicans</u>				<u>Flavobacterium</u> <u>fulvum</u>				<u>Rhodotorula</u> <u>(Harrison)</u>				<u>Streptococcus</u> <u>fecalis</u>				Uninoculated control		
	Ascorbic acid content		pH values	Bacterial count nos./cc. in T*	Ascorbic acid content		pH values	Bacterial count nos./cc. in T	Ascorbic acid content		pH values	Bacterial count nos./cc. in T	Ascorbic acid content		pH values	Bacterial count nos./cc. in T	Ascorbic acid content		pH values
	mg./cc.	percent present			mg./cc.	percent present			mg./cc.	percent present			mg./cc.	percent present			mg./cc.	percent present	
0	.36	100.0	6.35	480	.36	100.0	6.35	880	.36	100.0	6.35	3,220	.36	100.0	6.35	25	.36	100.0	6.35
5	.25	72.2	-	1	.26	72.2	-	1.5	.24	66.7	-	209,000	.24	66.7	-	57	.26	72.2	-
10	.20	55.6	6.40	15	.21	58.3	6.42	97,000	.18	50.0	6.58	294,000	.15	41.7	6.32	71	.16	44.5	6.35
15	.12	33.3	-	330	.20	55.6	-	100,000	.11	30.6	-	320,000	.08	22.2	-	235	.15	41.5	-
24	.08	22.2	6.35	4,900	.18	50.0	6.38	73,000	.04	11.1	6.32	283,000	.03	08.4	6.02	1,600	.04	11.1	-
31	.01	02.8	6.06	10,000	.16	44.5	6.34	42,000	.02	05.6	6.32	319,000	.00	00.0	6.06	1,000	.00	00.0	6.00

* T denotes thousands.

vitamin was completely oxidized when the bacterial population had increased from a few million to a billion. When the mixed cultures designated as M-U-1 and M-B-2 were streaked onto plates of Endo agar, varying types of coliform colonies developed on the plates streaked with culture M-U-1. Colonies which appeared different (based on size, shape, and degree of reddening of the medium) were fished and restreaked on Endo agar. To insure purity, multiple restreakings of the different variants were made. Five distinct strains of the coliform group were isolated based upon the data presented in Table XV.

A study was made to test these five cultures for their reaction towards the oxidation of vitamin C. The tests were conducted according to the procedure previously described. All five strains were found to destroy vitamin C, but the rapidity of destruction varied with the different cultures (Table XVI). A repetition of the test gave the same results. It is interesting to note that each culture offered varying degrees of protection from atmospheric oxidation during the first 5 to 10 hours incubation. After this period, rapid destruction of the vitamin occurred.

There appears to be no correlation between vitamin C destruction and microbial growth to pH changes during the 31 hours of incubation used. The control test showed that with loss of vitamin C there was slight increase in acidity.

2. Organisms Isolated from Lima Beans. A study of the data in Tables XVIIa and XVIIb show that the pure cultures related to Aerobacter aerogenes and Achromobacter nitrofileans were able to utilize vitamin C. The species, Aerobacter aerogenes destroyed the vitamin completely within 24 hours, while the species Achromobacter nitrofileans required 56 hours to bring about destruction. Two cultures, one similar to Bacillus megatherium, and the other similar to Staphylococcus albus did not seem to affect the stability of ascorbic acid.

TABLE XIV

The Effect of Mixtures of Bacteria Isolated from Shelled Green Peas Upon Vitamin C in Vitro

Cultures*	M-U-1				M-B-2				M-P-3				Uninoculated control		
	Ascorbic acid content		pH values	Bacterial count nos./cc. in T**	Ascorbic acid content		pH values	Bacterial count nos./cc. in T	Ascorbic acid content		pH values	Bacterial count nos./cc. in T	Ascorbic acid content		pH values
	μgs./cc.	percent present			μgs./cc.	percent present			μgs./cc.	percent present			μgs./cc.	percent present	
0	.36	100.0	6.35	24,000	.36	100.0	6.35	23,200	.36	100.0	6.35	35,000	.36	100.0	6.35
5	.31	86.1	-	182,000	.34	94.5	-	310,000	.29	80.6	-	320,000	.26	72.2	-
10	.10	27.8	6.51	680,000	.12	33.3	6.72	1,000,000	.00	00.0	6.67	1,040,000	.16	44.5	6.35
15	.00	00.0	-	1,000,000	.00	00.0	-	1,500,000	.00	00.0	-	1,310,000	.15	41.7	-
24	.00	00.0	6.94	2,450,000	.00	00.0	7.00	2,700,000	.00	00.0	7.02	2,450,000	.04	11.1	6.00

* M-U-1: Mixed microflora isolated from unblanched peas.

M-B-2: Mixed microflora isolated from blanched peas.

M-P-3: Mixed culture prepared from the seven pure cultures of bacteria isolated from peas.

** T denotes thousands.

TABLE XV

Descriptive Table of Members of the Coli-Aerogenes Group
Isolated from Unblanched Shelled Green Peas

Cultures	No. 20	No. 21	No. 22	No. 23	No. 24
Colonial characteristics on Endo agar	Small size faint pink color	Medium size faint pink color	Medium size dark pink color	Medium size center dark pink color	Giant size faint pink color
MORPHOLOGY					
Shape and grouping	short rods single pairs	short rods single pairs	short rods single pairs	short rods single pairs	short rods single pairs
Gram Reaction	negative	negative	negative	negative	negative
Capsule	absent	absent	absent	absent	absent
Motility	negative	negative	negative	negative	negative
PHYSIOLOGY					
Dextrose	AG*	AG	AG	AG	AG
Mannite	AG	AG	AG	AG	AG
Maltose	AG	A**	AG	AG	AG
Sucrose	no change	AG	AG	AG	AG
Lactose	AG	AG slow	AG	AG	AG
Dextrin	AG slow	AG	AG	AG	AG
Glycerol	AG	AG	AG	AG	AG
Indol	positive	positive	positive	positive	positive
Nitrite	positive	positive	positive	positive	positive
Citrate broth	no growth	growth 3+****	no growth	growth 4+	growth 4+
Uric acid broth	no growth	growth 3+	no growth	growth 4+	growth 4+
Brilliant Green Lactose Bile Broth	***	G	G	G	G
Methyl Red Test	positive	negative	positive	positive	negative
Voges-Proskauer test	negative	negative	negative	positive	negative
Hydrogen Sulfide	weak positive	weak positive	negative	negative	negative
Gelatin Liquefaction	negative	negative	negative	negative	negative
Litmus milk	▲ only	▲ only	▲ only	▲ only	▲ only
Name of bacterium	<u>Es. coli</u> (atypical)	<u>Es. coli</u> (atypical)	<u>Es. coli</u> (typical)	<u>Es. coli</u> (atypical)	<u>A. aerogenes</u> (atypical)

* AG - acid & gas

** A - acid only

*** G - gas formed

**** + - degree of growth

TABLE XVI

The Effect of Variant Strains of the Coli-Aerogenes Group Isolated from Unblanched Green Peas Upon Vitamin C in Vitro.

Cultures	No. 8 <u>Esch. coli</u> atypical			No. 9 <u>Esch. coli</u> atypical			No. 10 <u>Esch. coli</u> typical			No. 11 <u>Esch. coli</u> atypical			No. 12 <u>Aero. aerogenes</u> atypical			<u>Esch. coli</u> * typical			Uninoculated control		
	Ascorbic acid content		pH values	Ascorbic acid content		pH values	Ascorbic acid content		pH values	Ascorbic acid content		pH values	Ascorbic acid content		pH values	Ascorbic acid content		pH values	Ascorbic acid content		pH values
	mg./cc.	percent present		mg./cc.	percent present		mg./cc.	percent present		mg./cc.	percent present		mg./cc.	percent present		mg./cc.	percent present		mg./cc.	percent present	
0	36	100.0	6.68	36	100.0	6.68	36	100.0	6.68	36	100.0	6.68	36	100.0	6.68	36	100.0	6.68	36	100.0	6.68
5	35	97.0	-	33	91.7	-	35	97.0	-	30	83.3	-	31	86.6	-	34	94.5	-	26	72.2	-
10	21	58.3	-	30	83.3	-	33	91.7	-	19	52.8	-	21	58.3	-	32	88.9	-	20	55.6	-
15	00	00.0	-	11	30.6	-	23	63.9	-	00	00.0	-	00	00.0	-	17	47.2	-	16	44.5	-
24	00	00.0	6.42	00	00.0	6.72	00	00.0	6.42	00	00.0	6.60	00	00.0	6.55	00	00.0	6.42	07	19.4	6.22

* Stock culture of Escherichia coli used for comparison of vitamin C oxidizing ability.

The loss of the vitamin in these latter tests followed the approximate rate of non-bacterial oxidation that occurred in the uninoculated controls. Four species were found to exert a definite protective action against the oxidation of ascorbic acid. The cultures resembling Alcaligenes metalcaligenes, Bacillus subtilis, Bacillus parvus, and Achromobacter ginseng exhibited varying degrees of protection against ascorbic acid oxidation. For example, Bacillus subtilis and Bacillus parvus both retarded the oxidation of vitamin C up to 373 and 350 hours respectively; while the culture Achromobacter ginseng protected the vitamin up to 252 hours and the culture Alcaligenes metalcaligenes showed protection up to 195 hours. Destruction of ascorbic acid by the mixed culture isolated from lima beans was shown to be at a rate similar to that demonstrated for the species Aerobacter aerogenes. Vitamin C destruction by this mixed culture paralleled the destruction of the vitamin by the mixture isolated from peas. This would seem to indicate that the ability of certain organisms to destroy the vitamin is more pronounced than the ability of the other organisms to protect the vitamin when growing together.

The correlation between the decrease in ascorbic acid and the pH was interesting. With the uninoculated control, there is a drop in the pH during the initial 5 hours incubation, followed by a relatively constant pH level. The ascorbic acid content decreased at a fairly constant rate until it disappears.

With the inoculated tubes the pH shows an initial drop during the first 5 hours of incubation with a subsequent increase in pH level, dependent upon the particular culture. The rate of disappearance of the vitamin C varied with the cultures used. Thus there can be no relationship between the loss of vitamin C and change in pH.

It appears that the destruction of vitamin C and also the protection of vitamin C from non-bacterial oxidation is operative only when the bacterial populations have increased to millions per cubic centimeter. The initial growth of two species of bacteria, one similar to Bacillus parvus and the other similar to Bacillus megatherium appear to be somewhat retarded by vitamin C, as indicated by a lowering of the viable count during the early adjustment phase of growth.

E. Effect of Handling and Storage on pH Changes

1. Peas. The following general statements may be made concerning the changes in pH observed in the unblanched peas: 1) as the period of holding increased, the pH readings of both the inside and outside portions decreased; 2) after holding for 8 hours, the decrease in pH readings did not exceed 0.3 of one pH with the inside portion of the peas, while the skins showed a marked change under the same conditions. Blanching did not lower the pH of the skins or cause any other detectable change. The greater increase in acidity observed with portions of the skin compared to that of the inside portion of peas may have been caused by the activity of the bacteria on the skins (Table XVIII).

2. Lima Beans. No significant change was observed in pH values in either the unblanched or blanched frozen samples of lima beans of the three varieties (Tables XIX).

Since the pH was determined on the whole bean, it is possible that any change in acidity or alkalinity which might have taken place on the seed coat due to bacterial growth, was masked by the buffering effect of the protein content of the bean when the sample was triturated.

TABLE XVII a

The Effect of Pure Cultures of Microorganisms Isolated from Shelled Lima Beans Upon Vitamin C in Vitro

Cultures	<u>Bacillus megatherius</u>				<u>Staphylococcus albus</u>				<u>Achromobacter nitrificans</u>				<u>Aerobacter aerogenes</u>				Mixed cultures*				Inoculated control		
	Ascorbic acid content		pH values	Bacterial count nos./cc. in T**	Ascorbic acid content		pH values	Bacterial count nos./cc. in T	Ascorbic acid content		pH values	Bacterial count nos./cc. in T	Ascorbic acid content		pH values	Bacterial count nos./cc. in T	Ascorbic acid content		pH values	Ascorbic acid content		pH values	
mg./cc.	percent present	mg./cc.			percent present	mg./cc.			percent present	mg./cc.			percent present	mg./cc.			percent present	mg./cc.		percent present	mg./cc.		percent present
0	39	100.0	6.35	6.5	39	100.0	6.35	440	39	100.0	6.35	3,000	39	100.0	6.35	2,550	39	100.0	6.35	1,400	39	100.0	6.35
5	29	74.4	6.00	2.8	31	79.5	5.78	1,620	31	79.5	5.52	113,000	31	79.5	5.65	98,000	29	74.4	5.50	54,000	28	71.8	6.00
12	26	66.7	6.10	.1	27	69.2	5.96	11,000	28	71.8	5.84	116,000	17	43.6	6.08	373,000	06	15.4	6.15	340,000	24	61.5	6.04
24	16	41.0	6.12	6.8	25	64.1	5.84	34,000	21	53.8	5.80	292,000	00	00.0	6.26	392,000	00	00.0	6.24	-	19	48.7	6.00
36	12	30.8	6.12	090	20	51.3	6.50	24,000	01	02.8	5.98	710,000
56	08	20.5	6.08	343,000	08	20.5	6.56	-***	00	00.0	6.55	1,360,000
73	02	05.1	6.83	207,000	00	00.0	7.42	-
98	00	00.0	6.94	374,000

* Mixed cultures prepared from the eight pure cultures of bacteria isolated from lima beans.

** T denotes thousands.

*** No reliable count due to flaky or sedimented growth.

TABLE XVII b

The Effect of Pure Cultures of Microorganisms Isolated from Shelled Lima Beans Upon Vitamin C in Vitro

Cultures	<u>Bacillus subtilis</u>				<u>Bacillus parvus</u>				<u>Achromobacter genium</u>				<u>Alcaligenes metalcaligenes</u>				Uninoculated control		
	Ascorbic acid content		pH values	Bacterial count nos./cc. in T*	Ascorbic acid content		pH values	Bacterial count nos./cc. in T	Ascorbic acid content		pH values	Bacterial count nos./cc. in T	Ascorbic acid content		pH values	Bacterial count nos./cc. in T	Ascorbic acid content		pH values
	mg./cc.	percent present			mg./cc.	percent present			mg./cc.	percent present			mg./cc.	percent present			mg./cc.	percent present	
0	.39	100.0	6.35	750	.39	100.0	6.35	4.2	.39	100.0	6.35	700	.39	100.0	6.35	600	.39	100.0	6.35
5	.32	82.1	6.30	25,000	.29	74.4	5.82	.3	.30	76.9	5.90	9,000	.28	71.8	5.86	710	.28	71.8	6.00
12	.30	76.9	6.36	24,000	.24	61.5	6.08	80.	.29	74.4	6.02	77,000	.28	71.8	6.00	750	.24	61.5	6.04
24	.30	76.9	6.50	**	.21	53.8	5.96	3,900	.28	71.8	5.95	65,000	.26	66.7	6.28	140,000	.19	48.7	6.00
36	.29	74.4	6.64	-	.20	51.3	6.48	12,000	.26	66.7	6.22	57,000	.23	59.0	6.72	44,000	.13	33.3	6.04
56	.27	69.2	6.82	-	.20	51.3	6.64	**	.25	64.1	6.52	1,680,000	.23	59.0	6.96	-	.04	10.0	6.00
73	.25	64.1	7.42	-	.20	51.3	7.18	-	.24	61.5	7.02	67,000	.20	51.3	7.64	-	.00	00.0	6.02
96	.24	61.5	7.10	-	.19	48.7	7.18	-	.21	53.8	6.90	101,000	.19	48.7	7.30	-
122	.23	59.0	7.42	-	.17	43.6	7.55	-	.20	51.3	7.25	**	.18	46.2	7.52	-
151	.21	53.8	7.70	-	.16	41.0	7.50	-	.16	41.0	7.40	-	.15	38.5	7.70	-
181	.19	48.7	7.70	-	.15	38.5	7.70	-	.13	33.3	7.60	-	.02	05.1	7.70	-
195	.18	46.2	7.70	-	.14	35.9	7.70	-	.07	17.9	7.60	-	.00	00.0	8.10	-
224	.16	41.0	7.95	-	.09	23.1	8.00	-	.02	05.1	7.55	-
252	.13	33.3	8.05	-	.06	15.4	8.10	-	.00	00.0	7.55	-
273	.09	23.1	8.10	-	.04	10.3	8.10	-
300	.07	17.9	8.10	-	.03	07.6	7.90	-
326	.04	10.3	7.90	-	.02	05.1	7.70	-
350	.02	05.1	7.30	-	.00	00.0	8.20	-
373	.00	00.0	8.05	-

* T denotes thousands.

** No reliable count due to flaky or sedimented growth.

TABLE XVIII

Influence of Holding Green Peas Under Conditions of
the Vinery Upon the pH of the Peas.

Variety	Condition of peas	Holding period in hours	pH Values	
			skin	inside
Dark Podded Thomas Laxton	Unblanched	0	7.00	6.47
		2	6.40	6.80
		5	6.15	6.87
		8	5.49	6.47
	Blanched one minute at 100°C.	0	7.00	6.85
		2	6.80	7.00
		5	6.87	6.87
		8	6.52	6.70
Gradus	Unblanched	0	6.67	6.80
		2	6.62	6.81
		5	6.40	6.70
		8	6.00	6.70
	Blanched one minute at 100°C.	0	6.89	6.90
		2	6.73	6.89
		5	6.75	6.90
		8	6.40	6.70

TABLE XIX

Influence of a) holding at vinery, and b) frozen storage upon the pH of lima beans.

Variety	Holding period in hours	Condition of lima beans	pH values			
			After freezing and holding several weeks		After freezing and holding 1 yr. at -18°C.	
			Unblanched	Blanched	Unblanched	Blanched
Early Baby* Potato	0	Green	6.98	7.05	6.72	6.96
		White	6.96	6.98	6.73	6.83
	3	Green	6.98	6.98	6.79	6.84
		White	6.98	6.98	6.74	6.87
	6	Green	6.79	6.98	6.85	6.83
		White	6.98	6.98	6.87	6.82
24	Green	6.92	6.98	6.69	6.78	
	White	6.98	6.96	6.90	6.83	
Henderson Associate*	0	Green	6.76	6.24	6.74	6.82
		White	6.77	6.58	6.80	6.80
	3	Green	6.80	6.77	6.80	6.82
		White	6.80	6.77	6.82	6.85
	6	Green	6.79	6.77	6.74	6.74
		White	6.87	6.79	6.72	6.79
24	Green	6.65	6.63	6.75	6.77	
	White	6.84	6.77	6.80	6.80	
Baby Fordhook**	0	Green	6.82	6.98	6.83	6.81
		White	6.91	6.98	6.83	6.90
	6	Green	6.53	6.65	6.34	6.48
		White	6.78	6.88	6.72	6.68
	24	Green	6.48	6.54	4.98	6.15
		White	6.74	6.88	5.84	6.52

* Shelled lima beans held in lug box at vinery.

** Shelled lima beans held under running water at vinery.

DISCUSSION

A. Effect of Handling Upon Changes in Quality

1. Peas. The increase in bacterial counts, lowering of the pH reading, loss of vitamin C, and loss of total sugar provide a fairly accurate picture of the changes occurring in shelled green peas held for varying periods under conditions of the vinery. A two hour holding resulted only in slight changes in the peas analyzed. After 5 hours holding, a slight depreciation in quality was noted. These changes in quality were more marked with peas of the Lerton variety. The tests on samples of peas held under conditions of the vinery for 8 hours show that they undergo definite microbial spoilage within that time.

The most obvious change in quality during 8 hours holding was the alteration in appearance of the peas. The surface became covered with a grayish viscous growth of a bacterial nature and the normal pea green color developed a bleached appearance. Berry (1937) observed a similar correlation between the appearance of bacterial growth and of a bleached appearance on peas. He believed this to be due to the instability of chlorophyll in the presence of acid produced by acid-forming bacteria which were present. It has been shown in our tests that the skins of the peas became much more acid than the centers after holding 8 hours, which may be interpreted as the result of microbial activity on the surface of the peas. It was observed also that as the microbial count increased, the pH reading decreased, and because of the high carbohydrate content of the peas, the drop in pH may have been caused by bacterial activity. The results suggest that microbial activity is the main cause of spoilage or deterioration of peas after holding 8 hours or longer.

It is probable that the vining machine is responsible for the large initial contamination of shelled peas. Morris and Baker (1936) compared

bacterial counts of peas shelled aseptically with forceps with peas shelled by hand, and with peas shelled by commercial shelling machines. Their results indicated that the intact pea in a sound pod was practically sterile, and that the large numbers of bacteria on the outside of the pod leads to the heavy infestation noted on commercial shelled peas. In a preliminary experiment in this investigation, a hand-shelled sample of peas was found to contain only 9,000 bacteria per gram, while the initial bacterial content of vined peas used in this study varied between 2.5 and 5 million microorganisms per gram.

The predominating species of bacteria resembling Achromobacter candicans, Flavobacterium aurantiacum, Flavobacterium fulvum, and Kurthia zenkeri, isolated from unblanched peas probably contributed to the slimy condition of the peas after several hours holding, for the colonies formed by them upon artificial medium were butyrous and even slightly viscid; although no capsules were demonstrated by the staining procedure.

The pink yeast isolated from the unblanched peas resembled those of the genus Rhodotorula. Stained slides showed no spores to be present when cultured on caseinate agar; however, some budding was noted.

A one-minute blanch followed by immediate cooling, brought about 99 per cent reduction in the microbial content of the peas. From a sanitary point of view, the blanching process is indispensable for it destroyed Escherichia coli, one of the predominating species isolated from fresh peas. Presumably the blanching process would cause similar destruction of related intestinal pathogens that might be present concurrently with Escherichia coli. A species of yeast-like fungus and a species of bacteria, Streptococcus fecalis, were found to predominate on blanched peas. The presence of these two microbial forms on the blanched peas may be explained by contamination of the product by dust in the air or by direct human contact subsequent to blanching or both.

In brief, the spoilage of vined unblanched peas followed the course of common proteolytic decomposition effected by most soil bacteria, whereas spoilage of blanched peas followed the course of typical carbohydrate decomposition effected by mold and certain bacteria. A typical putrefactive, foul odor accompanied the former type of decomposition and a yeasty odor the latter.

Preliminary experiments demonstrated that both the Laxton and Gradus varieties contained little reducing sugar, quantities so small as to be undetectable by the method employed. Thus determinations of reducing sugars as an index of quality would give little information and is of no value in this regard. Therefore, the change in total sugar was considered.

A gradual decrease in total sugar after holding for 8 hours was noted in both the Laxton and Gradus varieties. Whether the loss of total sugar was due to microbial action, to enzymatic activity of the plant tissue, or both, is not known. Kertesz (1930) noted a striking decrease in the sucrose content of peas and an increase in the percentage of alcohol-insoluble residue immediately after shelling. He concluded that this was due to the enzyme sucrase and diastase found in the peas. Jones and Blisson (1932) observed that during storage, the starch content of peas increased with increase in storage temperature. They suggested that this increase in starch was possibly due to the condensation of sugar. Kertesz and Green (1932) showed that blanching prior to chilling prevented loss of sugar that normally occurred when the peas were not blanched. They concluded this change to be the result of enzymic activity. Thus while decrease in the total sugar may be a valuable index of quality in a food product, it is not necessarily a direct measure of bacterial activity.

The loss in vitamin C from unshelled Laxton peas held at the vinery for

one day was found to be negligible in comparison to the loss in this vitamin from shelled peas of this same variety held under the same conditions. Fenner and co-workers (1937) observed from their studies on seasonal variation in vitamin C content, that peas showed less fluctuation in ascorbic acid than did the other vegetables used. These workers suggested that the pod of the pea is responsible for the greater retention of this vitamin. Jenkins, et al (1938) reported a 12 per cent loss in vitamin C from Laxton peas (in pods) held for 18 hours at 32°C. This percent loss is exactly the same as was observed in our studies.

The protective action of the pod may be two fold; 1) the exclusion of air, and 2) the prevention of injury, either of which would expose the vitamin in the cells to enzymatic-oxidative activity. Moreover the pod keeps out microorganisms that may be able to attack the vitamin in the skin tissues.

The chief loss of vitamin C was found to occur during blanching and subsequent cooling. Between 24 and 35 per cent of the vitamin C content in both varieties of peas was lost in these processes. Similar losses in vitamin C from peas during commercial preparation for freezing have been reported by Jenkins and co-workers (1938). In spite of the loss of vitamin C by blanching, the amount retained in blanched samples remained at a fairly constant level after the blanching when held at low temperatures, probably because of the destruction of ascorbic acid oxidase by the blanching temperature. Samples of unblanched peas that were frozen after various holding periods and held several days at freezing temperatures showed an excessive loss in ascorbic acid; whereas samples of blanched frozen peas kept under the same conditions showed only a slight loss. This demonstrated again the value of blanching in preventing oxidative decrease in vitamin content. Jenkins, Tressler, and King (1938) reported a similar observation in which they compared the vitamin C content of unblanched and blanched peas held at -18°C. for varying periods.

When the ice crystals around 10 gram samples of frozen blanched peas were washed off and titrated with indophenol dye, vitamin C varying from 0.03 to 0.07 mgs. per gram was detected from all of the samples. No vitamin C was ever detected in the frozen water about unblanched frozen peas. The presence of the vitamin in the crystals about the blanched peas further supports the fact that the rinse water used in this blanching process removes some of the vitamin from the peas without actually destroying it.

The heat of the blanch water undoubtedly renders the pea tissues more permeable so that the vitamin is more easily removed from the peas by subsequent rinsing. Fenton and Tressler (1938) found that after cooking 283 grams of frosted peas in 292 cubic centimeters of boiling water for 8 minutes, the cooking water contained from 0.07 to 0.08 mgs. of vitamin C per cubic centimeter. The same authors found that 36 per cent of vitamin C from a sample of frozen Thomas Laxton peas passed into the cooking water, 59 per cent remained in the peas, and 5 per cent was actually destroyed. Thus it appears that while the heat of the blanching process destroys ascorbic acid oxidase and other undesirable enzymes, yet the water itself (during blanching and cooling), removes considerable vitamin C by simple diffusion from the plant tissues. The optimum blanching period would be the minimum length of time required to destroy all of the enzymes and destroy the minimum amount of vitamin C. Kertesz, Dearborn, and Mack (1938) found that heating peas one minute in boiling water most nearly meets the optimum conditions.

2. Lima Beans. The increase in microbial numbers and loss of vitamin C are good indices of changes in quality of freshly shelled lima beans during holding. The changes in pH were so variable that it was impossible to associate pH changes with loss in quality.

It is common practice to hold lima beans under running water at the vinery or cannery when considerable time will lapse before the beans will be processed. Such practice has been employed in an attempt to keep the beans at a low temperature thus retarding enzymatic and microbial activity, and also to keep the beans clean and of good fresh color.

The general appearance of the Baby Fordhook variety of beans held under running water was good at the end of the 24 hour period. These beans had a fresh green color and showed no indication of bacterial decomposition. The two varieties of beans, Early Baby Potato and the Henderson Associate, held in lug boxes began to show loss of color at the six hour period, and at the 24 hour period, a large percentage were brown in color and were covered with a sticky coat which was high in bacterial numbers.

From the unblanched beans species of bacteria resembling Bacillus megatherium, Bacillus subtilis, and Staphylococcus albus were isolated. Bacteria similar to Achromobacter kininum, Achromobacter nigrificans, Aerobacter aerogenes, Alcaligenes metalcaligenes, and Bacillus parvus, were isolated from blanched beans. From samples of beans which were frozen and held in locker storage at -18°C . for one year, organisms related to Bacillus subtilis, Aerobacter aerogenes, Aerobacter cloacae, and Flavobacterium butyri were isolated. The species Bacillus subtilis and Aerobacter aerogenes were isolated and identified as predominating types present on both fresh and frozen lima beans. Many species of bacteria similar to these were isolated by Smart and Brunstetter (1938) from fresh and frozen lima beans.

Blanching was found to be effective in reducing the bacterial numbers 99 per cent. This percentage reduction was also noted in the tests with peas. Thus it would seem that blanching is important not only in the processing of foods as a means of inactivating plant enzymes, but also as a means of reducing

the bacterial numbers on vegetables and preventing loss in quality due to microbial activity.

The effect of freezing as a means of reducing bacterial numbers on vegetables is not indicated in this experiment. Considerable irregularity was noted in the microbial counts on the frozen beans. Possible explanations for these irregularities in counts are: 1) accidental defrosting of the samples during storage with accompanying microbial growth, 2) the breaking up of dumps of bacteria during freezing, and 3) the variations of sampling itself. These results are not in agreement with the findings of Wallace and Tanner (1933), who demonstrated a reduction in the numbers of microorganisms as the result of freezing and holding at -18°C . for varying periods. These workers noted that there was a marked drop in numbers during the first week. Following this drop, there was a slow steady decrease in numbers, but never did the bacterial numbers reach zero.

Since samples of beans for the various holding periods were frozen and held for several weeks in the frozen state before analysis of vitamin C, it was difficult to determine directly the effect of holding upon changes in the vitamin C content of the beans. Because of inadequate laboratory facilities at the experimental farm, it was impossible to determine the vitamin C content of the fresh beans at the end of each holding period. Analyses were run upon the beans frozen at each holding period. It is assumed that the changes in vitamin C content detected in the frozen samples would indicate the changes that occurred in the fresh specimens.

In every test, the green beans contained considerably more ascorbic acid than the white. Since green beans are younger than white beans, these results confirm previous findings of Tressler, Mack, and King (1936), Tressler, Mack, and Jenkins (1937), and others, namely that the vitamin C content of most vegetables decreases with increasing maturity.

Little or no loss in vitamin C is observed for the three varieties of beans during 6 hours of holding, but after 24 hours holding, losses in vitamin C range from 32 to 70 per cent in fresh beans. Blanching the fresh beans for $2\frac{1}{2}$ minutes at 100°C . reduced the vitamin C content approximately one-fourth to one-third. Tressler and co-workers (1937) reported similar losses in lima beans to those observed in this study due to blanching.

Unblanched and blanched frozen limas stored at -18°C . for one year showed a decrease in vitamin C, a loss which was less marked in the blanched samples. This would be expected since blanching inactivates the ascorbic acid oxidase and thus retards the rate and degree of vitamin C reduction. It is probable that the reduction in ascorbic acid in the blanched samples during frozen storage was due to autooxidation of ascorbic acid, whereas the loss in ascorbic acid in the unblanched samples during frozen storage was due to enzymatic activity, Jenkins and co-workers (1938).

B. Effect of Microorganisms Isolated from Peas and Lima Beans Upon Vitamin C.

There is very little information concerning the action of bacteria commonly associated with foods on the oxidation of ascorbic acid. Esselen (1939) reported that there was no evidence which would indicate that bacteria are destructive to ascorbic acid, and therefore it would appear that bacteria are not of importance in causing a loss of ascorbic acid in foods.

The results of the in vitro tests in which pure cultures of microorganisms isolated from peas and lima beans were grown in a medium containing vitamin C demonstrated that certain microorganisms could destroy vitamin C. The organisms which showed this destructive action towards vitamin C were mainly members of the coliform group. In our in vitro tests, it was observed that during the first 5-10 hours incubation, the numerous strains of coliform

bacteria exhibited a protective action towards the oxidation of vitamin C. After this period rapid destruction of the vitamin occurred. Our findings are substantiated by Young and James (1942), and Young and Rettger (1943) who reported the ease with which strains of the coliform group were able to destroy vitamin C in vitro.

The discrepancies between our results and those of Esselen may be explained by the fact that Esselen incubated his tests for only 6 hours. Since at that hour his controls showed a greater loss in ascorbic acid than his tests, he concluded that bacteria did not destroy vitamin C. Our tests indicated that bacteria which were destructive to vitamin C first demonstrated a protection of this vitamin from atmospheric oxidation during the first 5-10 hours incubation. After this period rapid destruction of the vitamin occurred. It is quite probable that if Esselen carried out his tests for a period of 24 hours or longer, he would have discovered that many of his cultures would destroy vitamin C.

From the results of our tests, it can be stated that the bacteria isolated from peas and lima beans may be classified into three groups as regarding their effect on the stability of ascorbic acid; namely, 1) those organisms which destroy ascorbic acid; 2) those which protect ascorbic acid from non-bacterial oxidation; and 3) those which have no effect on ascorbic acid. These tests also demonstrated that before any protective activity of vitamin C by bacteria occurs, the bacterial population must increase to numbers in the millions per cubic centimeter. Moreover when microorganisms which protect and microorganisms which destroy vitamin C grew together, the destructive mechanism appears to be the stronger.

It is noted that the vitamin C content of the controls used in the tests with pea cultures decreased more rapidly than the vitamin C content in the

controls used in the tests with lima bean cultures. The same basic ingredients were used in the nutrient broth, but the two batches of broth were prepared at different times and it is probable that different lots of peptone and beef extract were used. Thus, this reaction was probably due to slight differences in composition of the ingredients used in preparation of this broth medium and indicates that it would be highly desirable to use a medium of known chemical composition in testing for the destructive action of vitamin C by bacterial activity.

Early observation of changes in the vitamin C content of peas and lima beans during holding at the vinery indicated that little loss of this vitamin occurred during 6 and 8 hours, but after 24 hours holding considerable loss was noted. This loss in vitamin C was caused partially through oxidation by plant enzymes as suggested by Mack and co-workers (1936). However, there is reason to believe that bacterial activity might be responsible for some loss of this vitamin. Shelled peas and lima beans held for 24 hours in lug boxes both became brown in color, and were covered with a sticky coat. Bacterial analysis of these peas and beans at this 24 hour period showed that the numbers had increased to millions per gram of sample. It was at this period that significant loss in vitamin C in these vegetables was observed, and also it was observed in the in vitro tests that rapid destruction of vitamin C took place in mixed cultures when the bacterial population had increased to millions per cubic centimeter. It is highly probable that the destruction of ascorbic acid in peas and lima beans held for 24 hours or longer was the result of both enzymatic oxidase activity by the plant tissue and bacterial activity.

During the first 5-6 hours of holding peas and lima beans in lug boxes microbial activity was of little significance in bringing about decrease in

vitamin C or gross changes in physical appearance. By the end of 24 hours bacteria may have hastened the reduction of vitamin C and will have changed the physical appearance of the products. Beans held under running water showed a similar reduction in vitamin C, but the loss was not as great and they were of superior appearance.

Regardless of bacterial numbers and vitamin C content, all peas and lima beans held in lug boxes for 24 hours would be rejected on the basis of appearance and unpalatability. Therefore, any correlation between bacterial activity and loss of vitamin C in vegetables during food processing operations prior to canning or freezing detected by laboratory means is of questionable practical significance.

SUMMARY AND CONCLUSIONS

Studies were made to determine the influence of vinery and storage conditions upon the quality of freshly shelled peas and lima beans in an attempt to detect any correlation between bacterial activity and loss of vitamin C from these vegetables during holding at the vinery. The following general statements may be made from the results obtained.

A. Studies of freshly shelled peas.

1. The increase in bacterial numbers, lowering of pH readings, and the loss of total sugar and of vitamin C provided fairly accurate indices of the quality of peas held for varying periods under the conditions of the vinery.
2. By the use of the above indices, it has been shown that shelled green peas should not be held more than five hours under the conditions which prevailed at the vinery in this test, namely warm and humid weather. A holding period of 2 hours was shown to have no adverse effect detectable by the tests used, but a holding period of 8 hours rendered the peas inferior in quality, due namely to changes brought about by increased bacterial numbers.
3. The most obvious change in quality after 8 hours was alteration in appearance of the peas. The surface became covered with a sticky coat, and bleaching of the normal pea-green color was evident. The blanching period for one minute at 100°C. was effective in destroying enzymes that oxidize ascorbic acid and in killing the majority of microbial species. The normal flora of the unblanched peas caused proteolytic decomposition, while the flora from the blanched peas brought about carbohydrate decomposition.

- A gram negative short rod, closely related to Achromobacter candicans, was most frequently encountered in the flora of the unblanched peas. Two species related to Flavobacterium aurantiacum and Flavobacterium fulvum, and a species closely related to Kurthia zenkeri were present in large numbers and may have contributed to the sliminess of the peas. A pink yeast, Rhodotorula sp. was found growing in mixed cultures isolated from unblanched peas. This organism failed to survive the blanching process. Escherichia coli also was isolated from the unblanched samples of peas. A yeast-like mold and Streptococcus fecalis were isolated from the blanched vegetables.
4. Blanching of the peas caused a decrease in vitamin C content of 24 to 35 per cent.
 5. When held for 8 hours, unblanched shelled peas lost 20 per cent of the vitamin but when held for 24 hours, they lost 56 per cent. Unshelled peas held under similar conditions for 24 hours, lost 12 per cent of the ascorbic acid.

B. Studies of lima beans.

6. Increase in bacterial numbers and a decrease in vitamin C were good indices of changes in quality of shelled lima beans held for varying periods under vinery conditions.
7. After holding two varieties, the Early Baby Potato and the Henderson Associate, in lug boxes for 24 hours, the beans showed definite browning, were covered with a sticky coat and yielded high bacterial counts. The Baby Fordhook variety, held under running water for 24 hours, gave no obvious indication of deterioration in quality.
8. Blanching for $2\frac{1}{2}$ minutes at 100°C . was effective in reducing the bacterial count 99 per cent and brought about 23.8 to 35.6 per cent reduction in ascorbic acid of the beans.

9. The predominating microorganisms isolated from unblanched lima beans were similar to Bacillus megatherium, Bacillus subtilis, and Staphylococcus albus. From the blanched beans, organisms were isolated which were like Achromobacter geminum, Achromobacter nitrificans, Aerobacter aerogenes, Alcaligenes metalocaligenes and Bacillus parvus, and from beans held in frozen storage organisms were isolated which were related to Bacillus subtilis, Aerobacter aerogenes, Aerobacter cloacae, and Flavobacterium butyri.
10. Blanched frozen lima beans held in storage at -18°C . for several weeks contained the same ascorbic acid content as the blanched fresh beans. Unblanched frozen beans showed a decided loss in ascorbic acid when compared with unblanched fresh beans. The loss of ascorbic acid in the latter was possibly due to the oxidation of ascorbic acid by the oxidase, which in the former was inactivated by the blanch.
11. During locker storage at -18°C . for one year, there was a considerable loss in ascorbic acid from both unblanched and blanched beans of all varieties. This loss in ascorbic acid was probably due in part to autooxidation as suggested by Mack and Tressler (1937).
- C. Bacterial utilization of vitamin C in vitro.
- These studies were made to determine the ability of various bacterial strains isolated from peas and lima beans to protect or destroy vitamin C in vitro in an effort to correlate any possible significance between bacterial activity and loss of this vitamin from these vegetables during holding at the vinery.
12. On the basis of these studies pure cultures of bacteria may be grouped as follows on their reaction with the vitamin.
- a) All strains of the coliform group isolated from peas and lima beans

and the strain Achromobacter nitrificans isolated from lima beans destroyed vitamin C rapidly in vitro, but the rate varied with the different strains.

b) Kurthia zenkeri, Flavobacterium aurantiacum and Flavobacterium fulvum isolated from peas; and Alcaligenes metalcaligenes, Bacillus subtilis, markedly protected vitamin C from non-bacterial oxidation.

c) Achromobacter caudicans, Streptococcus fecalis, and Rhodotorula (Harrison) isolated from peas and Bacillus megatherium and Staphylococcus albus isolated from lima beans were indifferent towards vitamin C stability.

13. When mixtures of the above cultures contained a "vitamin C utilizer", the vitamin was destroyed rapidly.

D. General conclusions.

14. From the above observations, it must be concluded that:

- a) During a shorter holding period in which the peas and beans are satisfactory on the basis of organoleptic tests, the bacterial numbers have not increased to a point where they would materially affect the vitamin C content of the vegetables.
- b) In the judging of the quality of shelled peas and lima beans during holding at the vinery, laboratory determinations of bacterial numbers and of vitamin C changes brought about by bacterial activity are of questionable practical significance.

LITERATURE CITED

1. Bergey, D. H., et al, 1939. Bergey's manual of determinative bacteriology. 5th ed. Williams and Wilkins Co., Baltimore.
2. Berry, J. A., 1937. Microbiology of frozen-pack vegetables. West. Can. and Packer, 29: 4, 14-16.
3. Bessey, O. A. and King, C. G., 1933. The destruction of vitamin C in plant and animal tissues, and its determination. J. Biol. Chem. 103: 687-698.
4. Biffano, M., and Servazzi, O. A., 1935. Effect of various biological agents on vitamin C. Arch. ist. biochim, ital. 7, 151-156. Cited from Chem. Abst., 29: 7399, 1935.
5. Brown, E. B., 1933. Bacterial studies of defrosted peas, spinach, and lima beans. J. Home Econ., 25: 887-892.
6. Esselen, W. B., Jr., 1939. Influence of bacteria on oxidation of ascorbic acid. Food Res., 4: 329-334.
7. Feener, S. L., Palmer, V. W., and Fitzgerald, G. A., 1937. Seasonal variations in vitamin C content of fresh vegetables. Paper presented at the 24th spring meeting of The American Society of Refrigerating Engineers, French Lick, Ind., June 8th.
8. Fenton, F., Tressler, D. K., and King, G. G., 1936. Losses of vitamin C during the cooking of peas. J. Nut., 12: 285-295.
9. Fenton, F., Tressler, D. K., 1938. Losses of vitamin C during commercial freezing, defrosting, and cooking of frosted peas. Food Res., 3: 408-416.
10. Harrison, F. C., 1927. Cheese Torulae. From the transaction of the Royal Society of Canada, Third Series, Vol. XXI, Section V: 341-379.
11. Isbell, H. S., Pigman, W. W., and Frush, H. L., 1940. Reducing powers of various sugars with alkaline copper-citrate reagent. J. Res. of Nat. Bu. Stds., 24: 241-246.
12. Jenkins, R. R., Tressler, D. K., and Fitzgerald, G. A., 1938. Vitamin C content of vegetables. VIII Frozen peas. Food Res., 5: 133-140.
13. Jones, H. A., and Bisson, C. S., 1932. Changes in the composition of the garden pea after harvest. Plant Physiol., 7: 273-283.
14. Joslyn, M. A., and Marsh, G. L., 1933. Changes occurring during freezing storage and thawing of fruits or vegetables. Calif. Agr. Exp't Sta., Berkeley, Calif., Bulletin 551, 1-40.
15. Kendall, A. I., and Chinn, H., 1938. The decomposition of ascorbic acid by certain bacteria. Studies in bacterial metabolism. J. Infect. Dis., 62: 330-336.

16. Kertesz, Z. I., 1930. The chemical changes in peas after picking. *Plant Physiol.*, 5: 399-412.
17. Kertesz, Z. I., and Green, E. L., 1932. Deterioration of shelled green peas held a few days in storage prior to canning. *J. Agr. Res.*, 45: 361-370.
18. Kertesz, Z., I., Dearborn, R. B., and Mack, G. L., 1936. Vitamin C content of vegetables. *J. Biol. Chem.*, 116: 717-725.
19. King, C. G., Tressler, D. K., 1940. Effect of processing on the vitamin C content of foods. *N. Y. State Agr. Exp't Sta. J. Paper No. 389*.
20. Lepkovsky, S., Hart, E. B., Hastings, E. G., and Brazier, W. C., 1925. The effect of fermentation with specific microorganisms on the vitamin C content of orange and tomato juices. *J. Biol. Chem.*, 66: 49-56.
21. Mack, G. L., Tressler, D. K., and King, C. G., 1936. Vitamin C content of vegetables. II Peas. *Food Res.*, I: 231-235.
22. Morris, T. W., and Baker, J., 1936. Frozen Vegetables. *Gt. Brit. Dept. Sci. & Indus. Res. Food Invest. Bd. Ann. Rpt. for 1935, Section 5*, 149-151.
23. Smart, H. F., Brunstetter, B. C., 1936. Lima Beans in frozen pack. *The Canner*, 83: 5, 14-16.
24. Smart, H. F., 1937. Types and survival of some microorganisms in frozen peas, beans, and sweet corn grown in the east. *Food Res.*, 2: 515-528.
25. Stepp, W., and Schroder, H., 1935. Das schicksal des vitamin C in verdauungskanal. L. Uber die einwirkung von darmbakterine auf vitamin C. *Klin. Wochschr.*, 14: 147-148.
26. Thornton, N. C., 1938. Extraction and determination of vitamin C in plant tissue. *Bull. Boyce Thompson Inst. Plant Res.*, 9: 273-281.
27. Tressler, D. K., Mack, G. L., and King, C. G., 1936. Factors influencing the vitamin C content of vegetables. *Am. J. Pub. Health*, 26: 905-909.
28. Tressler, D. K., Mack, G. L., and Jenkins, R. R., 1937. Vitamin C in vegetables. VII. Lima Beans. *Food Res.*, 2: 175-181.
29. Tressler, D. K., 1938. Bacteria, enzymes, and vitamins--indices of quality in frozen vegetables. *Refrig. Eng.*, 36: 5, 319-321.
30. Wallace, G. I., and Tamer, F. W., 1935. Microbiology of frozen foods. III. Longevity of pure cultures of microorganisms frozen in various menstra. *Fruit Prod. J.*, 14: 235-237.
31. Young, R. M., and James, L. H., 1942. Action of intestinal microorganisms on ascorbic acid. *J. Bact.*, 44: 75-84.
32. Young, R. M., and Rettger, L. F., 1943. Decomposition of vitamin C by bacteria. *J. Bact.*, 46: 351-363.

ACKNOWLEDGEMENT

The author wishes to express his appreciation to Dr. L. H. James* for his advice in directing this investigation, to Dr. C. H. Mahoney** who supplied the raw materials used, and to Dr. Norman C. Laffer for his advice and assistance in the writing of this dissertation.

* Dr. L. H. James was formerly Head of the Department of Bacteriology, University of Maryland.

** Dr. C. H. Mahoney was formerly Head of the Department of Horticulture, University of Maryland.