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## A histochemical study of the green bean pod

John Marion Pearson

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Melvin R. Johnston, Major Professor

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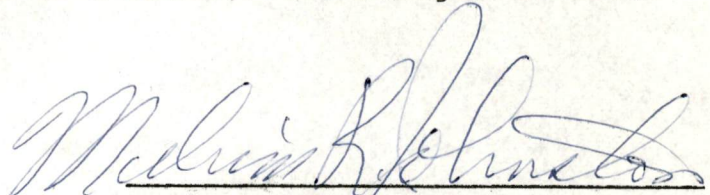
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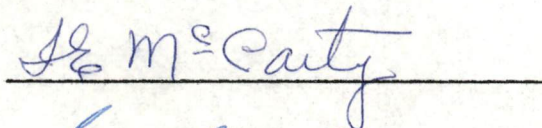
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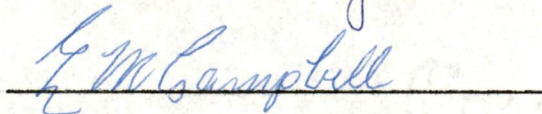
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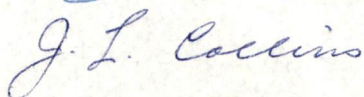
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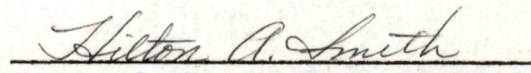
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Accepted for the Council:

  
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Dean of the Graduate School

A HISTOCHEMICAL STUDY OF THE GREEN BEAN POD

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A Thesis

Presented to

the Graduate Council of  
The University of Tennessee

---

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

John Marion Pearson

December 1965

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## CHAPTER I

### INTRODUCTION

As snap bean production continues to grow in economic importance in several areas of the United States, it is essential to determine, and if possible, correct the problems of sloughing and splitting of pods which results in down-grading of the processed product. The acreage of snap beans grown for processing has risen in Tennessee from a 1959-63 average of 9,040 to a 1965 acreage estimate of 13,700 which represents a 51.5 per cent increase. The estimated increase for 1965 in acreage for processing is 14 per cent over that of 1964 and marks the ninth consecutive year in Tennessee for an increase in acreage for processing (5). The problems of sloughing of the outer skin and splitting of pods in both canned and frozen snap beans have been recognized for some time. Though many research workers have explored the situation, the reason or reasons for sloughing and splitting of the pods have not yet been completely clarified.

The objectives of this study were: (1) to determine varietal differences in sloughing and splitting of pods,

(2) to determine if a relationship exists between amounts of water-soluble pectin in the bean and degree of sloughing and splitting of the pods, and (3) to determine the effects of certain post-harvest treatments on sloughing and splitting of pods.

## CHAPTER II

### REVIEW OF THE LITERATURE

#### I. AREAS OF SLOUGHING IN THE SNAP BEAN POD

The area of interest in studying the sloughing of the snap bean pod consists of four distinct layers of cells. There is a single epidermal (outside) layer consisting of small cells. Below this are two layers of hypodermal cells with thick walls. Next there are about ten rows of thin-walled parenchyma cells before reaching the vascular region (19).

The exact site of cell separation causing sloughing in the snap bean pod seems to vary, depending upon whether the bean is frozen or canned. Kaczmarzyk et al. (9) demonstrated two different types of cell separation in canned beans. Sloughing was evidenced both by a breaking away of the outer epidermal layer and a breaking which occurred in the cells between the epidermis and the large parenchyma cells. Sloughing of the outer epidermis was found to be the most prevalent type.

Strohmaier (19) showed that frozen and thawed snap beans slough primarily due to a break which occurs in the region of the large, thin-walled parenchyma cells just outside the vascular region. Apparently, freezing causes cell separation at a deeper cellular level than does canning.

## II. CAUSES OF SLOUGHING AND SPLIT PODS

Bean maturity (1), blanching time and temperature, time lapse between blanching and filling, and the presence of calcium salts have all been established as being influential on sloughing and splitting of pods (15, 20).

McConnell (11) has suggested that it is conceivable that breeding out of the string has weakened the entire skin structure, making more likely the occurrence of skin breakdown during processing.

Singleton (13), in his study of sloughing occurring in frozen snap beans found that varying the blanching time and temperature gave little success in preventing sloughing. He found a definite correlation between cavities around the seed and sloughing - the larger the cavity the more sloughing observed. Huffington (6) suggested that weather conditions at maturity, methods of harvesting and handling and

containers may have some effect on the sloughing incidence of canned snap beans. McConnell's (11) study indicated that cool, rainy weather at harvest time promotes sloughing.

Van Buren et al. (21) discussed the role of pectin methylesterase (PME) in sloughing of snap beans. PME catalyzes the deesterification of methyl galacturonide units of pectin to yield galacturonic acid and free methyl alcohol. The enzyme is relatively inactive in most intact plant tissue, but when activated by grinding or maceration the PME rapidly converts pectins to pectic acids. The results of PME action are a decrease in solubility of pectic substances, especially in the presence of calcium salts, and an increase in tissue firmness. This study showed that firmer beans exhibited a higher pectic acid or pectate content than did the softer beans. The authors suggest that a moderate blanching temperature might have the same effect on the enzyme as would maceration of the tissue. High blanching temperatures, however, inactivate PME and the authors further suggest that methods need to be developed to maintain or increase PME activity, since, in general, there is not enough pectate or pectic acid present in fresh beans to prevent a soft, sloughing character in the processed beans.

Post-harvest storage times and temperatures and their effect on sloughing have been studied previously. McConnell (11) found that the skin of the unprocessed snap bean became more resistant to sloughing at both room temperature and 40°F. Relative resistance to sloughing was found to increase with storage up to ten days.

Sistrunk (17) studied two varieties of snap beans which varied in sloughing due to storage. One variety (Earligreen) increased in sloughing the first day at all temperatures and then went through a period of decline in sloughing, followed by another increase in sloughing by the fifth day of storage. The other variety (Gallatin 50), however, increased in sloughing at all temperatures during storage. Generally, the higher the storage temperatures and the longer the storage period, the more the beans tended to slough. The results of this study are not in agreement with McConnell (11) who found the opposite to be the case.

### III. PECTIN COMPOUNDS RELATED TO SLOUGHING

The middle lamella area between the cells of the tissue is believed to be composed chiefly of pectin compounds. A stain, ruthenium red, has been used to show the

presence of pectic substances in snap beans (9). Jensen (7), however, states that a failure to stain with ruthenium red does not necessarily mean an absence of pectin. He states that if the pectic substances are present in high concentrations and if no interfering substances are present, staining with ruthenium red will result in staining the pectic substances red.

Many researchers now believe that pectic substances in the middle lamella consist primarily of calcium pectates as described by Kertesz (10). The calcium bond between polygalacturonic acid molecules is believed to be responsible for increasing or maintaining the "cementing" power between adjacent cells (2). Protopectin, specifically, is believed responsible for holding adjacent cells together since it is the water-insoluble, parent pectin. During maturation and processing the water-insoluble pectin is hydrolyzed to water-soluble pectin in the snap bean pod (15). Sistrunk (17) found that low water-soluble pectin values in the bean were in most cases, indicative of less sloughing.



## CHAPTER III

### MATERIALS AND METHODS

#### I. TREATMENTS

The five snap bean varieties (Wadex, Slenderwhite, Tender Crop, Early Harvest, and Provider) used in this study were grown on the University of Tennessee Cumberland Plateau Experiment Station Farm near Crossville. They were harvested at comparable stages of maturity during the period of August 3-7, 1964. One-half of the beans were mechanically harvested and the other half were harvested by hand.

Both the mechanically harvested and hand harvested beans were divided into two equal lots. One lot was hydrocooled in the field to remove the field heat. This procedure consisted of submerging the beans in a large container of ice water for ten minutes, which resulted in lowering the temperature of the beans from approximately 85°F. to about 50°F. The remaining beans were not hydrocooled. All beans were taken to Knoxville where they were processed.

Lots of both the hydrocooled and non-hydrocooled beans were processed (frozen and canned) immediately upon

arrival in Knoxville. The remaining hydrocooled and non-hydrocooled beans were each divided into two equal lots. One lot was stored at 45°F. and the other lot was stored at 95° ± 5°F. At intervals of 24, 48, and 72 hours a sample from each storage temperature was taken and divided into two lots for processing. One lot was frozen and the other lot was canned (See Table I).

All samples were snipped and cut using commercial equipment and were sized manually. The 3-5 sieve size beans for canning were blanched in boiling water for one and one-half minutes, filled into plain 303 size cans and covered with boiling water. A teaspoon of salt was added to each can before closing. With closing temperature of about 190°F. the beans were processed for twenty-one minutes at 250°F. and cooled to 100°F. in running water. The canned beans were stored at room temperature for approximately twelve months before opening.

The beans for freezing were snipped, cut, sized, and blanched in boiling water for one and one-half minutes, as were the beans to be canned. They were put into plastic freezer bags and freezer boxes, frozen in an air blast freezer at -20°F. and stored at 5°F. for about twelve months.

TABLE I

HARVEST, TREATMENT, AND PROCESSING TABLE FOR SNAP BEANS HARVESTED  
AUGUST 3-7, 1964, CUMBERLAND PLATEAU

Harvest Method	Field Treatment	How Processed (Fresh Beans)	Temp. of Storage (°F.)	Length of Storage (Hrs.)	How Processed (Stored Beans)
Hand Harvested & Mechanically Harvested	Hydrocooled	Frozen	45	24	Frozen
				48	Canned
				72	Frozen
		Canned	24	Frozen	
			48	Canned	
			72	Frozen	
	Non-Hydrocooled	Frozen	45	24	Frozen
				48	Canned
				72	Frozen
		Canned	24	Frozen	
			48	Canned	
			72	Frozen	

## II. METHODS OF ANALYSIS

The data reported herein represent five varieties of mechanically harvested canned snap beans. Evaluations included both hydrocooled and non-hydrocooled beans stored at 45°F. and 95° ± 5°F. for periods of 0, 24, and 48 hours. The beans were evaluated as to split pods, sloughed material, and gammas of anhydrogalacturonic acid (AGA) per milliliter of bean liquor. Each determination was performed in duplicate.

To check the amount of sloughed tissue and number of split pods, twenty pieces of canned beans and 100 mls. of water were placed in a 250 ml. Erlenmeyer flask. The flask was shaken on a Burrell Wrist-Action Shaker at setting No. 5 (350 shakes per minute with an amplitude of 1.5 cm.) for five minutes. The water, with its suspended material, was poured into a 100 ml. graduate cylinder. After one hour, the level of the sediment in the cylinder was checked and the results expressed as milliliters of sediment. The beans were then removed from the flask, the split pods counted, and the results expressed as per cent split pods (20).

The AGA or water-soluble pectin determinations were performed on the liquor from the canned beans. Sistrunk and Cain (15) showed the amount of water-soluble pectin in the liquor to be a better indicator of sloughing ( $r = +0.878$ ) than the amount of water soluble pectin in the bean pod. A 3 ml. sample of liquor was diluted to a 20 ml. volume with distilled water. This mixture was precipitated once in 70 per cent ethanol and twice more in 60 per cent ethanol at 60°C., centrifuging and decanting the ethanol after each heating period (16). This served to remove interfering carbohydrates and at the same time concentrate the pectic material (3). After heating with ethanol, two 40 ml. water extractions were made. Five mls. of 1N NaOH were added to the water extracts and the volume was made to 100 mls. This solution was analyzed using the colorimetric procedure of Dische (4) as modified by Sistrunk and Cain (15). The results were expressed as gammas AGA/ml. of canned bean liquor (water extraction from 70 per cent ethanol precipitate).

### III. HISTOLOGICAL PROCEDURE

All samples to be evaluated histologically were cut into one-quarter inch lengths, deaerated and fixed in FAA

(formalin-glacial acetic acid-alcohol) (8). They were then stored in 70 per cent ethanol until ready for dehydration. Dehydration was carried out using a tertiary-butyl alcohol series and the specimens were paraffin-infiltrated. After embedding and trimming, cross sections of the pod 15 microns in thickness were made using a Spencer AO rotary microtome. The slides were prepared for staining according to Sass (12) and were submerged in a 0.05 per cent aqueous solution of ruthenium red for sixty minutes to stain the pectic substances (9).

## CHAPTER IV

### RESULTS AND DISCUSSION

#### I. SLOUGHING

Analysis of variance of the sloughing evaluations on the canned snap beans showed significant differences among the five varieties and between the 24 and 48 hour storage treatments. Slight differences were found in the other treatments, but these were not significant. Table II indicates that a significantly greater amount of sloughing was found after the 48 hour storage period. The Duncan's Multiple Range Test results found in Table III reveal no significant sloughing differences between Wadex and Slenderwhite or between Slenderwhite and Tender Crop varieties. There were, however, large differences among varieties in general, ranging from a mean sloughing value of 3.31 for Wadex to 18.50 for Provider.

Analysis of variance (Table IV) shows field treatment and length of storage interaction to be significant at the .05 level. Field treatment and variety, storage temperature and variety, and length of storage and variety interactions

TABLE II  
 EFFECTS OF CERTAIN POST-HARVEST TREATMENTS  
 ON SLOUGHING OF CANNED SNAP BEANS

Treatment	Sloughing (mls. sed.) Means of All Varieties	Statistical Significance at .05 Level
Hydrocooling	8.85	
Non-hydrocooling	8.52	
Storage at 95°F.	8.87	
Storage at 45°F.	8.50	
Storage time 24 hours	8.35	
Storage time 48 hours	9.02	*



TABLE III  
 DIFFERENCES IN SLOUGHING AMONG FIVE VARIETIES  
 OF CANNED SNAP BEANS

Variety	Wadex	Slender- white	Tender Crop	Early Harvest	Provider
Mean Sloughing Values (mls. sed.)	3.31	5.94	6.37	9.31	18.50
Statistical Significance at .05 Level	_____	_____	_____	_____	_____

TABLE IV  
ANALYSIS OF VARIANCE OF SLOUGHING EVALUATION  
OF CANNED SNAP BEANS

Source	D.F.	M.S.	F. Ratio
1 <sup>a</sup>	1	2.11	1.11
2 <sup>b</sup>	1	2.81	1.49
3 <sup>c</sup>	1	9.11	4.83*
4 <sup>d</sup>	4	553.90	193.46**
12	1	2.11	1.12
13	1	9.11	4.84*
14	4	31.26	16.62**
23	1	0.61	0.32
24	4	11.90	6.32**
34	4	123.39	65.63**
123	1	0.01	0.00
124	4	3.51	1.86
134	4	3.20	1.70
234	4	4.76	2.53
1234	4	12.98	6.90**
Within Replicates	40	1.88	
Total	79		

<sup>a</sup>Field treatment.

<sup>b</sup>Storage temperature.

<sup>c</sup>Length of storage.

<sup>d</sup>Variety.

were highly significant at the 0.01 level. All highly significant interactions have variety included as a factor.

## II. SPLITTING OF PODS

Analysis of variance of split pod values on canned snap beans showed significant differences among varieties and between hydrocooling and non-hydrocooling values. The slight differences among the other treatments were not significant. The incidence of splitting was significantly less when the beans received the hydrocooling treatment (Table V). The results of the Duncan's Multiple Range Test (Table VI) show there were no significant differences among Wadex, Slenderwhite, Tender Crop, and Early Harvest varieties in per cent split pod values. There was, however, a wide difference between Provider and the other four varieties. Again the wide range of values is shown here as in the case of sloughing values. The per cent of split pods ranges from 0.0 per cent (Wadex) to 16.87 per cent (Provider). The order of varietal ranking from lowest to highest split pod values was identical to the ranking of the varieties according to sloughing values. Storage temperature and length of storage, and length of storage and variety were significant interactions (Table VII).

TABLE V  
EFFECTS OF CERTAIN POST-HARVEST TREATMENTS ON  
SPLITTING OF PODS IN CANNED SNAP BEANS

Treatment	<u>% Split Pods</u> Average of All Varieties	Statistical Significance at .05 Level
Hydrocooling	3.12	
Non-hydrocooling	4.62	*
Storage at 95°F.	4.00	
Storage at 45°F.	3.75	
Storage time 24 hours	4.50	
Storage time 48 hours	3.25	

TABLE VI  
DIFFERENCES IN PER CENT SPLIT PODS AMONG FIVE  
VARIETIES OF CANNED SNAP BEANS

Variety	Wadex	Slender- white	Tender Crop	Early Harvest	Provider
Mean Split Pod Values (%)	0.00	0.31	0.67	1.56	16.87
Statistical Significance at .05 Level					

TABLE VII  
ANALYSIS OF VARIANCE OF SPLITTING OF PODS  
OF CANNED SNAP BEANS

Source	D.F.	M.S.	F. Ratio
1 <sup>a</sup>	1	45.00	4.5*
2 <sup>b</sup>	1	1.25	0.1
3 <sup>c</sup>	1	31.25	3.1
4 <sup>d</sup>	4	850.46	85.0**
12	1	20.00	2.0
13	1	5.00	0.5
14	4	8.28	0.8
23	1	61.25	6.1*
24	4	6.71	0.6
34	4	36.71	3.6*
123	1	20.00	2.0
124	4	8.28	0.8
134	4	2.65	0.2
234	4	63.59	6.3**
1234	4	39.53	3.9*
Within Replicates	40	10.00	
Total	79		

<sup>a</sup>Field treatment.

<sup>b</sup>Storage temperature.

<sup>c</sup>Length of storage.

<sup>d</sup>Variety.

### III. ANHYDROGALACTURONIC ACID

Significant differences in amounts AGA leached from the canned beans into the liquor were found among treatments (Table VIII) and among varieties. Table VIII shows that significantly less AGA was leached from the beans when they were hydrocooled, stored at a lower temperature, and when stored for a shorter period of time.

Table IX shows wide varietal differences in gammas AGA leached into the liquor. There were no significant differences between Early Harvest and Slenderwhite or among Slenderwhite, Tender Crop, and Wadex. Provider liquor contained a significantly higher amount of AGA than the other varieties. If pectic substances are important in holding the cells of the pod together, varieties with less AGA leached into the liquor should have more remaining in the pod and should show less sloughing and splitting. Sistrunk and Cain (15) found a correlation coefficient of + 0.878 between per cent AGA in the liquor and sloughing. In this study, however, such was not the case. A correlation coefficient of + 0.575 was found between gammas AGA found in the liquor and sloughing of the tissue. However, since

TABLE VIII  
EFFECTS OF CERTAIN POST-HARVEST TREATMENTS ON AMOUNT  
OF ANHYDROGALACTURONIC ACID LEACHED INTO LIQUOR  
FROM CANNED SNAP BEANS

Treatment	<u>γ AGA/Ml. Bean Liquor</u> Average of All Varieties	Statistical Significance at .05 Level
Hydrocooling	429.93	*
Non-hydrocooling	488.57	*
Storage at 95°F.	468.13	*
Storage at 45°F.	450.37	*
Storage time 24 hours	435.77	*
Storage time 48 hours	458.94	*



TABLE IX

DIFFERENCES IN AMOUNT ANHYDROGALACTURONIC ACID  
LEACHED INTO LIQUOR AMONG FIVE VARIETIES  
OF CANNED SNAP BEANS

Variety	Early Harvest	Slender- white	Tender Crop	Wadex	Provider
Mean AGA Values ( $\gamma$ AGA/ml. liquor)	405.77	434.17	440.88	458.94	556.52
Statistical Significance at .05 Level	_____		_____		

a total pectin determination was not made; it is still possible that a greater per cent AGA leached from the varieties showing the most sloughing. Note the highly significant interactions in Table X. Highly significant two-way interactions were found between field treatments, lengths of storage, and varieties. The interaction which was not highly significant was field treatment and storage temperature.

#### IV. CANNED FRESH SNAP BEANS AS COMPARED WITH BEANS STORED FOR TWENTY-FOUR HOURS BEFORE CANNING

Tables XI through XIII show the effects of hydrocooling and 24-hour storage time on canned snap beans. It is interesting to note that canned fresh beans sloughed more and had a higher per cent split pods than either the hydrocooled or non-hydrocooled beans which were stored for 24-hours before canning. These differences, however, were not statistically significant at the 0.05 level. Table XIII shows that the lowest amount AGA leached into the liquor occurred in beans which had been hydrocooled and stored for 24-hours before canning. These results and those shown in

TABLE X

ANALYSIS OF VARIANCE OF GAMMAS OF ANHYDROGALACTURONIC  
ACID LEACHED INTO LIQUOR FROM CANNED SNAP BEANS

Source	D.F.	M.S.	F. Ratio
1 <sup>a</sup>	1	68792.34	290.21**
2 <sup>b</sup>	1	6308.88	26.61**
3 <sup>c</sup>	1	45111.22	190.31**
4 <sup>d</sup>	4	53150.58	224.21**
12	1	768.11	3.24
13	1	18181.35	76.70**
14	4	2348.72	9.90**
23	1	6019.23	25.39**
24	4	8578.37	36.18**
34	4	66543.90	280.72**
123	1	872.84	3.68
124	4	3157.86	13.32**
134	4	4671.90	19.70**
234	4	13077.23	55.16**
1234	4	11907.28	50.23**
Within Replicates	40	237.04	
Total	79		

<sup>a</sup>Field treatment.

<sup>b</sup>Storage temperature.

<sup>c</sup>Length of storage.

<sup>d</sup>Variety.

TABLE XI  
EFFECTS OF 24 HOUR STORAGE AND HYDROCOOLING  
ON SLOUGHING IN CANNED SNAP BEANS

Treatment	<u>Non-Hydrocooled</u> 24 Hr. Storage	<u>Hydrocooled</u> 24 Hr. Storage	Fresh
Mean sloughing values all varieties	7.90	8.60	9.10
Statistical Significance at .05 Level	_____		

TABLE XII

EFFECTS OF 24 HOUR STORAGE AND HYDROCOOLING  
ON SPLITTING IN CANNED SNAP BEANS

Treatment	<u>Hydrocooled</u> 24 Hr. Storage	<u>Non-Hydrocooled</u> 24 Hr. Storage	Fresh
Mean split pod values (%) all varieties	3.00	4.00	6.50
Statistical Significance at .05 Level	_____		

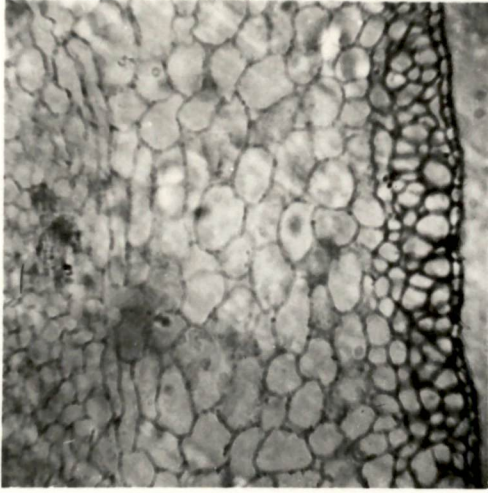
TABLE XIII  
 EFFECTS OF 24 HOUR STORAGE AND HYDROCOOLING ON  
 LEACHING OF ANHYDROGALACTURONIC ACID INTO  
 LIQUOR OF CANNED SNAP BEANS

Treatment	<u>Hydrocooled</u> 24 Hr. Storage	Fresh	<u>Non-Hydrocooled</u> 24 Hr. Storage
Mean AGA Values ( $\gamma$ AGA/ml. liquor)	414.65	450.99	455.95
Statistical Significance at .05 Level	_____		

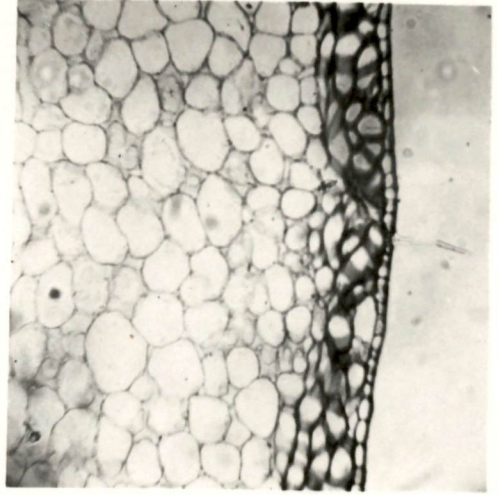
Table II, page 15, generally agree with Sistrunk's findings (17). Although there was a decline in sloughing the first day of storage, it was not statistically significant and by the end of the second day of storage there was a significant increase in sloughing values.

#### V. HISTOLOGICAL RESULTS

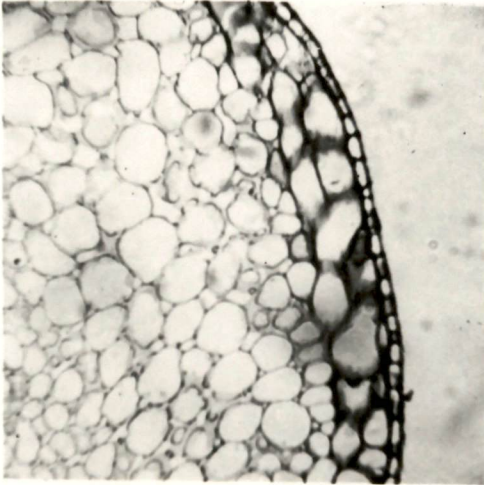
There was very little damage evident from the prepared slides in either the fresh beans or those which were blanched. Normal cell structure with little or no cell separation was apparent although there were isolated cases where sloughing was observed. The normal, unsloughed pods are shown in Plate I(a), (b), and (c). Pectic substances stained more readily in the fresh beans which received no blanch treatment. Some of the slides showed no pectin stain, but Jensen (7) has stated that this does not mean an absence of pectic substances. It is possible that the pectic substances were not present in high enough concentrations or that certain interfering substances were present preventing staining. These possibilities stated by Jensen could explain the difficulty encountered in staining certain slides.



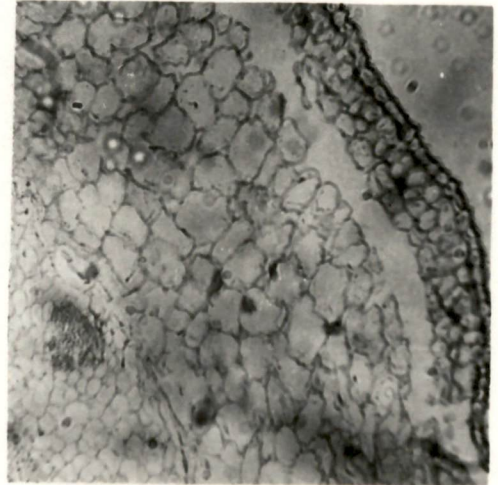
a. Fresh bean, Wadex variety.



b. Fresh, blanched bean, Wadex variety.



c. Fresh bean, Slender-white variety.



d. Canned bean after 24-hours storage at 95°F., Early Harvest variety.

PLATE I

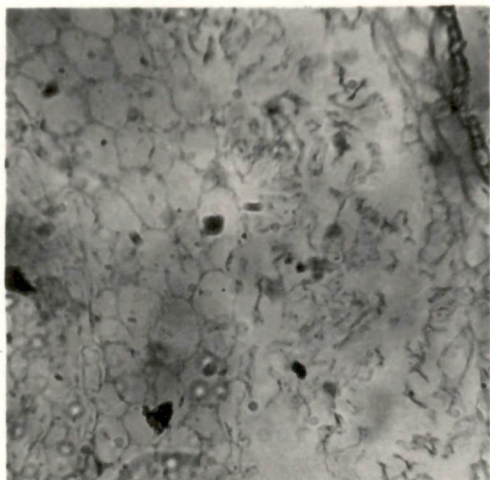
PHOTOMICROGRAPHS OF MACHINE-HARVESTED, NON-HYDROCOOLED  
SNAP BEAN PODS. 100X



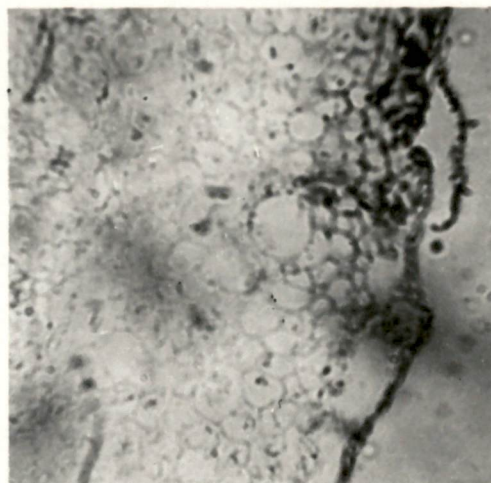
The slides of the fresh and blanched beans show the pectic substances stained most readily in an area about 60 degrees either side of the suture and staining was most apparent in the epidermal and hypodermal cell layers.

The prepared slides of the canned beans demonstrated the location of cell separation which causes sloughing. Plate I(d) shows a break occurring between the hypodermal and parenchymal cell layers. This was a fairly common separation site. More breaks were evident in this area than in the parenchymal area, but the most common breakage was between the epidermal and hypodermal cell layers, as shown in Plate II(c). This concurs with the study of Kaczmarzyk et al. (9) who also observed that breaking away of the epidermis was the most prevalent type of separation in canned beans.

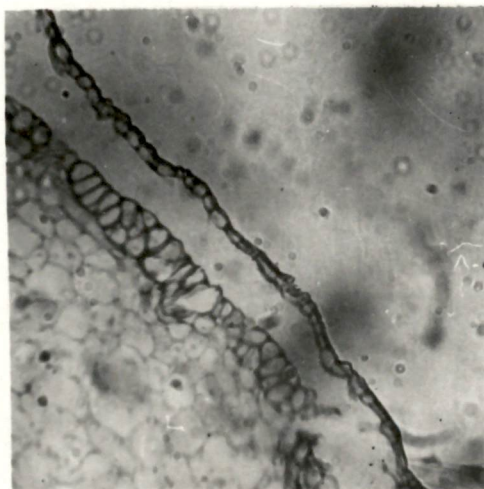
Breaks in the parenchymal area were observed in some instances, but would not contribute to the amount of sloughing since in many cases the epidermal layer remained intact. Plate II(a) shows cellular separation at all three outer cellular levels. This variety, Provider, showed more extensive cell disruption than did the other varieties. It also gave more difficulty in staining, strengthening the belief that pectic substances are involved in holding the cells together.



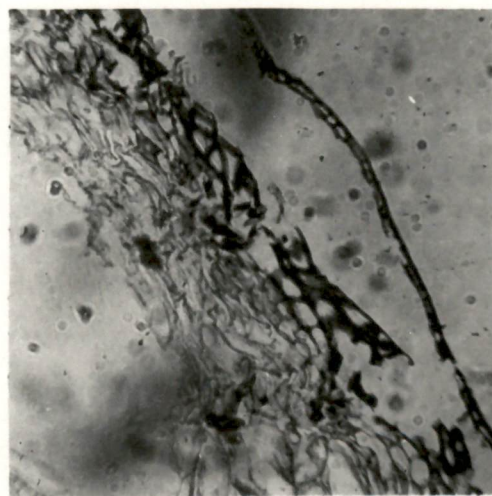
a. Machine-harvested, non-hydrocooled bean canned after 24-hours storage at 95°F., Provider variety.



b. Hand-harvested, hydrocooled bean canned after 72-hours storage at 95°F., Wadex variety.



c. Machine-harvested, non-hydrocooled bean canned after 48-hours storage at 95°F., Tender Crop variety.



d. Machine-harvested, non-hydrocooled, fresh frozen bean, Wadex variety.

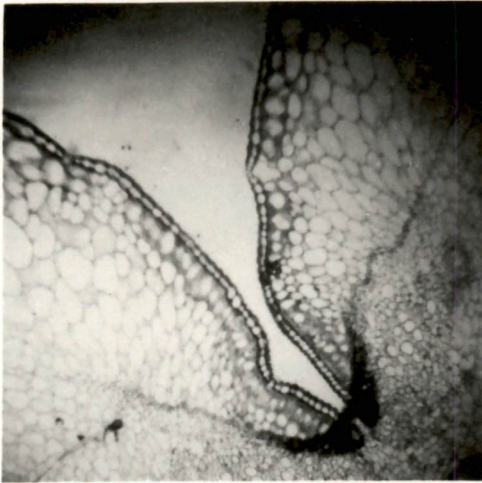
PLATE II

PHOTOMICROGRAPHS OF SNAP BEAN PODS. 100X

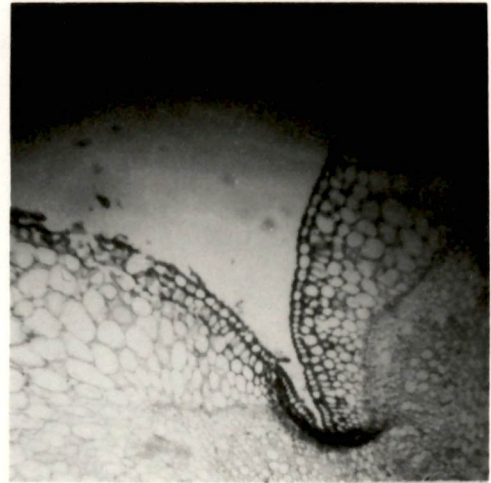
Plate II(b) shows extensive cell disruption, demonstrating the effect of a long storage period (72 hours) at an elevated temperature (95°F.).

All slides of the frozen beans showed extensive cell separation at all cellular levels ; see Plate II(d). The epidermal layer was broken away in all instances. In most cases, however, the hypodermal layer was found to be intact and stained most readily. Strohmaier (19) found that frozen and thawed snap beans sloughed primarily due to cell separation in the parenchymal area. This study, however, showed that breaking away of the epidermis was as frequent as was cellular separation in the parenchymal region.

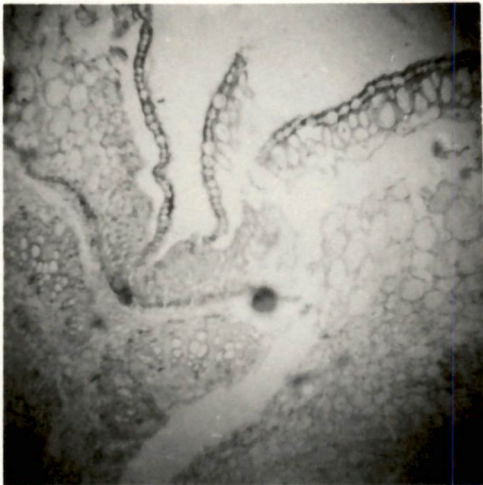
Plate III shows the suture area where cell separation occurs, causing splitting of pods. Plate III(a) and III(b) show fresh and blanched beans, respectively, demonstrating no splitting. The cell separation causing splitting in the canned bean, Plate III(c), does not occur directly on the suture line, but to one side where the separation extends through all cellular levels. The suture area of the split frozen pod, Plate III(d), generally was disrupted and cell separation was extensive allowing the pod to split apart.



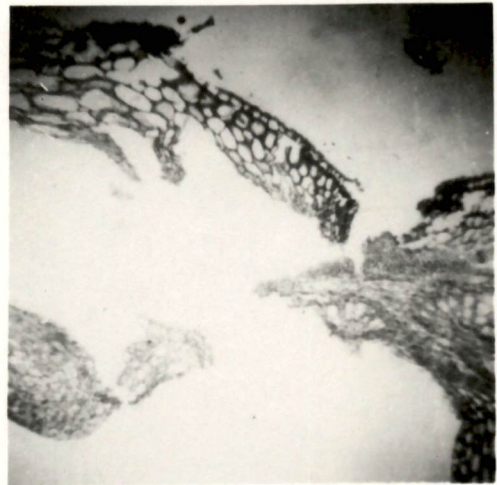
a. Suture area of normal, fresh pod. Tender Crop variety.



b. Suture area of blanched, fresh pod. Provider variety.



c. Suture area of pod canned after 24-hours storage at 95°F. Provider variety.



d. Suture area of pod frozen after 24-hours storage at 95°F. Wadex variety.

PLATE III

PHOTOMICROGRAPHS OF SUTURE AREAS OF SNAP  
BEAN PODS. 100X

From all slides examined, Slenderwhite and Wadex stained more satisfactorily than the other varieties. Slenderwhite showed the best staining of pectic substances and Provider the poorest staining. It may be that Slenderwhite and Wadex varieties retained a larger percentage of pectic substances in the pod or that there was an absence of interfering substances which were present in the other varieties, preventing their proper staining with ruthenium red. Less sloughing was evident in Wadex and Slenderwhite than in other varieties which indicates that these two varieties retained more pectic substances in their pods.

## CHAPTER V

### SUMMARY

A study was made of five varieties of snap beans and several post-harvest treatments which might affect sloughing and splitting of pods in canned snap beans. Sloughing and split pod evaluations were performed on the canned beans and anhydrogalacturonic acid was determined on the liquor of the canned beans using a colorimetric procedure. Photomicrographs were prepared of cross sections of the bean pods for histological examination.

From the data and observations of the slides, it was found under the conditions of this study that:

1. Hydrocooling treatment and canning within 24 hours of harvest produced a minimum amount of sloughing and splitting of pods.

2. There was a low correlation between gammas of AGA in the liquor and sloughing or splitting of pods; however, study of the slides showed Slenderwhite and Wadex varieties to stain more readily than the other varieties which would indicate less tissue breakdown.

3. Histology was found to be a useful method in studying varietal differences in cell separation caused by the treatments in this study.

4. There were wide varietal differences in sloughing and splitting of pods in canned snap beans and wide varietal differences in amounts of water-soluble AGA in the canned bean liquor.



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APPENDIX

TABLE XIV

SLOUGHING DATA FOR CANNED SNAP BEANS  
(SLOUGHING IN MILLILITERS  
OF SEDIMENT)

Variety	Rep.	TREATMENT						
		Hydrocooling	Non- Hydrocooling	95°F. Storage	45°F. Storage	24-Hr. Storage	48-Hr. Storage	
Early Harvest	1	10.0	11.0	14.0	11.0	11.0	11.0	8.0
	2	11.0	11.0	20.0	11.0	11.0	11.0	9.0
	1	10.0	14.0	10.0	10.0	14.0	14.0	7.0
	2	11.0	20.0	11.0	11.0	20.0	20.0	7.0
	1	4.0	8.0	7.0	8.0	10.0	10.0	4.0
	2	4.0	9.0	7.0	9.0	11.0	11.0	4.0
	1	6.0	7.0	6.0	4.0	10.0	10.0	6.0
	2	6.0	7.0	6.0	4.0	11.0	11.0	6.0
Wadex	1	5.0	4.0	4.0	4.0	4.0	4.0	2.0
	2	4.0	4.0	4.0	4.0	4.0	4.0	2.0
	1	4.0	4.0	4.0	5.0	4.0	4.0	4.0
	2	5.0	4.0	5.0	4.0	4.0	4.0	4.0
	1	1.0	2.0	4.0	2.0	5.0	1.0	1.0
	2	1.0	2.0	4.0	2.0	4.0	4.0	1.0
	1	4.0	4.0	4.0	1.0	4.0	4.0	4.0
	2	1.0	4.0	1.0	1.0	5.0	5.0	1.0
Slender- white	1	7.0	5.0	0.0	5.0	5.0	5.0	9.0
	2	8.0	6.0	0.0	6.0	6.0	6.0	8.0
	1	6.0	0.0	6.0	7.0	0.0	0.0	9.0
	2	5.0	0.0	5.0	8.0	0.0	0.0	7.0

TABLE XIV (continued)

Variety	Rep.	TREATMENT							
		Hydrocooling	Non- Hydrocooling	95°F. Storage	45°F. Storage	24-Hr. Storage	24-Hr. Storage		
Slender- white	1	10.0	9.0	9.0	9.0	7.0	10.0	7.0	10.0
	2	7.0	8.0	7.0	8.0	8.0	7.0	8.0	7.0
	1	8.0	9.0	8.0	10.0	6.0	8.0	6.0	8.0
	2	7.0	7.0	7.0	7.0	5.0	7.0	5.0	7.0
Tender Crop	1	2.0	1.0	3.0	1.0	1.0	1.0	1.0	12.0
	2	2.0	2.0	2.0	2.0	2.0	2.0	2.0	11.0
	1	1.0	3.0	1.0	2.0	3.0	11.0	3.0	11.0
	2	0.0	2.0	0.0	2.0	2.0	9.0	2.0	9.0
	1	9.0	12.0	11.0	12.0	2.0	9.0	2.0	9.0
	2	10.0	11.0	9.0	11.0	2.0	10.0	2.0	10.0
	1	10.0	11.0	10.0	9.0	1.0	10.0	1.0	10.0
	2	10.0	9.0	10.0	10.0	0.0	10.0	0.0	10.0
Provider	1	17.0	18.0	15.0	18.0	18.0	16.0	18.0	16.0
	2	20.0	17.0	16.0	17.0	17.0	14.0	17.0	14.0
	1	27.0	15.0	27.0	17.0	15.0	16.0	15.0	16.0
	2	22.0	16.0	22.0	20.0	16.0	19.0	16.0	19.0
	1	17.0	16.0	16.0	16.0	17.0	17.0	17.0	17.0
	2	21.0	14.0	19.0	14.0	20.0	21.0	20.0	21.0
	1	22.0	16.0	22.0	17.0	27.0	22.0	27.0	22.0
	2	19.0	19.0	19.0	21.0	22.0	19.0	22.0	19.0



TABLE XV (continued)

Variety	Rep.	TREATMENT							
		Hydrocooling	Non-Hydrocooling	95°F. Storage	45°F Storage	24-Hr. Storage	48-Hr. Storage		
Slender-	1	0.0	5.0	0.0	5.0	0.0	0.0	0.0	
	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Tender Crop	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	1	0.0	0.0	0.0	0.0	0.0	0.0	10.0	
	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	1	0.0	0.0	10.0	0.0	0.0	0.0	0.0	
	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	1	0.0	10.0	0.0	0.0	0.0	0.0	0.0	
	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Provider	1	10.0	30.0	25.0	30.0	30.0	30.0	20.0	
	2	15.0	10.0	20.0	10.0	10.0	10.0	15.0	
	1	25.0	25.0	25.0	10.0	25.0	25.0	15.0	
	2	25.0	20.0	25.0	15.0	20.0	20.0	15.0	
	1	25.0	20.0	15.0	20.0	10.0	10.0	25.0	
	2	15.0	15.0	15.0	15.0	15.0	15.0	15.0	
	1	5.0	15.0	5.0	25.0	25.0	25.0	5.0	
	2	0.0	15.0	0.0	15.0	25.0	25.0	0.0	

TABLE XVI

 AGA DATA FOR CANNED SNAP BEANS  
 (γAGAmL. CANNED BEAN LIQUOR)

Variety	Rep.	TREATMENT					
		Hydrocooling	Non-Hydrocooling	95°F. Storage	45°F. Storage	24-Hr. Storage	48-Hr. Storage
Early Harvest	1	413.00	429.52	446.04	429.52	429.52	429.52
	2	413.00	388.22	446.04	388.22	388.22	421.26
	1	454.30	446.04	454.30	413.00	446.04	495.60
	2	429.52	446.04	429.52	413.00	446.04	495.60
	1	289.10	429.52	495.60	429.52	413.00	289.10
	2	289.10	421.26	495.60	421.26	413.00	289.10
	1	330.40	495.60	330.40	289.10	454.30	330.40
	2	322.14	495.60	322.14	289.10	429.52	322.14
Wadex	1	446.04	528.64	495.60	528.64	528.64	462.56
	2	479.08	545.17	561.68	545.17	545.17	446.04
	1	479.08	495.60	479.08	446.04	495.60	462.56
	2	446.04	561.68	446.04	479.08	561.68	479.08
	1	355.10	462.56	462.56	462.56	446.04	355.10
	2	330.40	446.06	479.08	446.04	479.08	330.40
	1	413.00	462.56	413.00	355.10	479.08	413.00
	2	413.00	479.08	413.00	330.40	446.04	413.00
Slender-white	1	305.62	528.64	181.72	528.64	528.64	528.64
	2	305.62	479.08	181.72	479.08	479.08	512.12
	1	289.10	181.72	289.10	305.62	181.72	619.50
	2	280.84	181.72	280.84	305.62	181.72	619.50



TABLE XVI (continued)

Variety	Rep.	TREATMENT											
		Hydrocooling		Non-Hydrocooling		95 °F.		45 °F.		24-Hr.		48-Hr.	
		Hydrocooling	Storage	Hydrocooling	Storage	Storage	Storage	Storage	Storage	Storage	Storage	Storage	Storage
Slender-white	1	545.16	528.64	528.64	619.50	528.64	619.50	528.64	305.62	545.16	305.62	545.16	
	2	512.12	512.12	512.12	619.50	512.12	619.50	512.12	305.62	512.12	305.62	512.12	
	1	512.12	619.50	619.50	512.12	545.16	512.12	545.16	289.10	512.12	289.10	512.12	
	2	545.16	619.50	619.50	545.16	512.12	545.16	512.12	280.84	545.16	280.84	545.16	
Tender	1	363.44	338.66	338.66	454.30	338.66	454.30	338.66	338.66	578.20	338.66	578.20	
	2	363.44	313.88	313.88	429.52	313.88	429.52	313.88	313.88	578.20	313.88	578.20	
	1	371.70	454.30	454.30	371.70	363.44	371.70	363.44	454.30	561.68	454.30	561.68	
	2	371.70	429.52	429.52	371.70	363.44	371.70	363.44	429.52	561.68	429.52	561.68	
	1	446.04	578.20	578.20	561.68	578.20	561.68	578.20	363.44	446.04	363.44	446.04	
	2	429.52	578.20	578.20	561.68	578.20	561.68	578.20	363.44	429.52	363.44	429.52	
	1	462.56	561.68	561.68	462.56	446.04	462.56	446.04	371.70	462.56	371.70	462.56	
	2	429.52	561.68	561.68	429.52	429.52	429.52	429.52	371.70	429.52	371.70	429.52	
Provider	1	528.64	512.12	512.12	619.50	512.12	619.50	512.12	512.12	569.94	512.12	569.94	
	2	528.64	495.60	495.60	619.50	495.60	619.50	495.60	495.60	569.94	495.60	569.94	
	1	578.20	619.50	619.50	578.20	528.64	578.20	528.64	619.50	578.20	619.50	578.20	
	2	578.20	619.50	619.50	578.20	528.64	578.20	528.64	619.50	578.20	619.50	578.20	
	1	520.38	569.94	569.94	578.20	569.94	578.20	569.94	528.64	520.38	528.64	520.38	
	2	495.60	569.94	569.94	578.20	569.94	578.20	569.94	528.64	495.60	528.64	495.60	
	1	569.94	578.20	578.20	569.94	520.38	569.94	520.38	578.20	569.94	578.20	569.94	
	2	561.68	578.20	578.20	561.68	495.60	561.68	495.60	578.20	561.68	578.20	561.68	

TABLE XVII

EFFECTS OF POST HARVEST TREATMENTS ON CANNED  
SNAP BEAN QUALITY, CUMBERLAND PLATEAU

Field Treatment	TREATMENTS			VARIETIES				
	Temp. of Storage	Length of Storage	Evaluation	Early Harvest	Wadex	Slender-White	Tender-Crop	Pro-vider
Oven 90-100 °F.	24-Hrs.	Sloughing	10.5*	4.5	5.5	1.0	24.5	
		Split pods	0.0	0.0	0.0	0.0	25.0	
	48-Hrs.	YAGA/ml.	441.91	462.56	284.97	371.70	578.20	
		Sloughing	6.0	2.5	7.5	10.0	20.5	
	Hydrocooled	48-Hrs.	Split Pods	0.0	0.0	0.0	0.0	2.5
			YAGA/ml.	326.27	413.00	528.64	446.04	565.81
Cooler	24-Hrs.	Sloughing	10.5	4.5	7.5	2.0	18.5	
		Split pods	2.5	0.0	0.0	0.0	12.5	
	48-Hrs.	YAGA/ml.	413.00	462.56	305.62	363.44	528.64	
		Sloughing	4.0	1.0	8.5	9.5	19.0	
	Non-Hydrocooled	48-Hrs.	Split pods	0.0	0.0	0.0	0.0	20.0
			YAGA/ml.	289.10	342.70	528.64	437.78	507.99
Oven 90-100 °F.	24-Hrs.	Sloughing	17.0	4.0	0.0	2.5	15.5	
		Split pods	7.5	0.0	0.0	0.0	22.5	
	48-Hrs.	YAGA/ml.	446.04	528.64	181.72	441.91	619.50	
		Sloughing	7.0	4.0	8.0	10.0	17.5	
	Non-Hydrocooled	48-Hrs.	Split pods	2.5	0.0	0.0	2.5	15.0
			YAGA/ml.	495.60	470.82	619.50	561.68	578.20

TABLE XVII (continued)

Field Treatment	TREATMENTS			VARIETIES				
	Temp. of Storage	Length of Storage	Evaluation	Early Harvest	Wadex	Slender-White	Tender-Crop	Pro-vider
Non-Hydrocooled (continued)	Cooler	24-Hrs.	Sloughing	11.0	4.0	5.5	1.5	17.5
			Split pods YAGA/ml.	0.0	0.0	0.0	0.0	20.0
		48-Hrs.	Sloughing	8.5	2.0	8.5	11.5	15.0
			Split pods YAGA/ml.	0.0	0.0	2.5	0.0	17.5
Fresh Beans, Hydrocooled and Canned Immediately After Harvest			Sloughing	425.39	454.30	520.38	578.20	569.94
			Sloughing	14.0	2.0	4.0	8.5	17.0
			Split pods YAGA/ml.	15.0	0.0	5.0	0.0	20.0
		After Harvest	446.04	404.74	660.80	379.96	363.44	

\*All numbers are average of two replications.

## VITA

The author was born May 4, 1941, in Bells, Tennessee. He was graduated from Bells High School, Bells, Tennessee, in 1959. He attended the University of Tennessee, Martin Branch, from 1959 to 1962 and received a B.S. degree in Liberal Arts from the University of Tennessee, Knoxville, in June, 1963. In September of the same year, he was appointed Assistant in Food Technology at the University of Tennessee, Knoxville. Since that time he has been working to complete the requirements for the degree, Master of Science.