Histological Findings in String Beans during Cooking in Relation to its Change in Color Pigment

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Summary — String beans raw, cooked for 5, 15 and 30 min. were studied microscopically. It was found that as the time for cooking was increased, the green pigment decreased and substances within each cell tended to loosen and coagulate as compared to the raw bean where the chloroplasts were situated around the periphery of the palisade cells. When cooking was up to 30 min. breaks in the epidermal and parenchyma cells and extreme shrinkage within the endodermis were observed.

Introduction

Many studies on vegetables during cooking have been made. Simpson and Halliday (*1*) have made a chemical analysis on carrots and parsnips in the raw state, steamed 20 min. and 45 min. and have found a decrease in the total pectic substances as steaming progressed. They confirmed and extended these studies by histological findings which showed a less thick and less continuous cell walls in the cooked samples than those in the raw. Texture changes in canned beans also appeared to be related to changes in pectic substances during blanching and canning (*2*).

The solution in which the vegetable is held during cooking must also be taken into account, since Van Buren, et al (*3*) have found that by soaking in a solution containing calcium ion the degree of sloughing of beans was decreased whereas it was increased by soaking them in oxalate and Calgon solution prior to blanching. Vegetables boiled in water required less time to obtain desirable tenderness than those boiled in water to which sodium chloride was added (*4*).

Changes which occur in plant pigments while cooking are among the most important of changes from the standpoint of appearance of cooked vegetables. Sweeney and Martin (*5*) have determined the percentages of chlorophyll in raw broccoli, cooked for 5, 10, 15 and 20 min. and have found a decrease in the chlorophyll content as the

cooking time was increased. Although many studies on green beans have been made (*2*), (*6*), (*7*), (*8*), (*9*), (*10*), in the present paper the total chlorophyll has been determined in raw and cooked string beans and each sample has been histologically observed to find whether there are any outstanding differences in cell structure among the samples during the process of cooking in relation to the undesirable color changes.

Materials and Methods

Determination of Chlorophyll: Fresh string beans of uniform size were selected and divided into four groups—raw, cooked for 5, 15 and 30 min. For samples to be cooked, 100 ml. of distilled water was brought to boil before the samples, each weighing 1.5 g., were placed into the water. For extraction of chlorophyll, 0.1 g. of $CaCO_3$ was added before the tissue was macerated and 10 ml. of 85 % acetone was used to extract the chlorophyll according to the method described in A. O. A. C. (*11*). Colorimetric measurements were made by using Shimadzu photo-electric spectrophotometer, type QR-50.

Histological Procedure: Samples—raw, cooked for 5, 15 and 30 min. were fixed and embedded in paraffin as previously reported (*12*). Paraffin ribbons were cut 12 microns thick with a Yamato sliding microtome, stained with Heidenhain's iron-alum haematoxylin according to Chamberlain (*13*) and mounted with balsam. For photomicrographs equal exposure times were used. Development of the photographs was by conventional means, and absolutely uniform procedures were used throughout.

Results and Discussion

Effect of heating on the color pigment: — The figures for the maximal absorption in the four samples were similar to the trend of the standard chlorophyll a and b (*14*). A typical absorption curve is given in Fig. 1. From the figure, the solution extracted can be considered as a mixture of chlorophyll a and b.

Table I shows the variation of absorption with cooking time at 642.5 mμ. and 660 mμ., corresponding to the content of chlorophyll a and b, respectively.

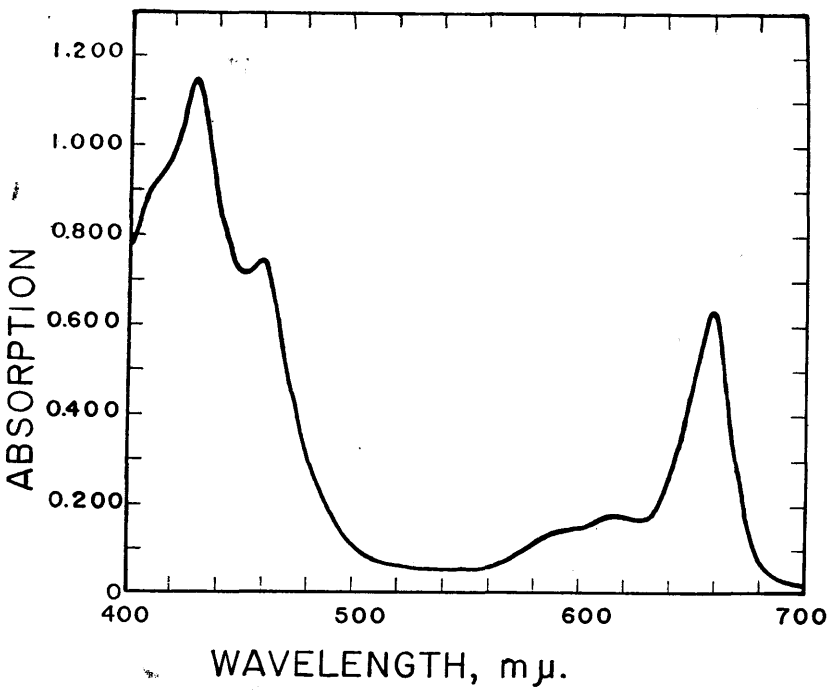

Fig. 1. —Absorption spectrum of acetone extracts from raw string beans.

Table I. Variation of Chlorophyll in String Beans with Cooking Time.

Cooking Time	Absorption	
	642.5 mμ.	660 mμ.
0 min.	0.345	0.625
5	0.325	0.570
15	0.290	0.490
30	0.265	0.410

It can be clearly observed from the above table that there is a distinct decrease in chlorophyll content as the cooking time is increased. This decrease has also been found in the case of broccoli (*5*).

Effect of heating on the cell structure: — Various dyes have been recommended for specific studies (*15*). Ruthenium red has been used by several workers (*1*), (*9*) to observe the pectin in vegetables. Among these workers, Kaczmarzk, et al (*9*) have

used 0.05 % aqueous solution of ruthenium red for 60 min. and washed in water in order to locate the pectic substances, determine the area of breakdown during sloughing and to examine any possible changes from blanching. They have presumed that the small dark specks, appearing at random throughout the photographs are crystals rising from the unstable staining solution. In preliminary work for the present study, when the author used Delafield's haemotoxylin, results similar to the above were obtained. But when Heindenhain's iron-alum haematoxylin, which is highly recommended for cytological investigation (*13*), was used in place of the former, the site of the chloroplasts was easily located, indicating that the specks may have been due, in part at least, to the chloroplasts.

When green vegetables are dropped into boiling water, an instantaneous change to a brilliant green is obtained due to the translucency of the tissue (*17*), but no change in the degree of absorbance of the stain by the epidermal cells could be noted among the samples in this experiment.

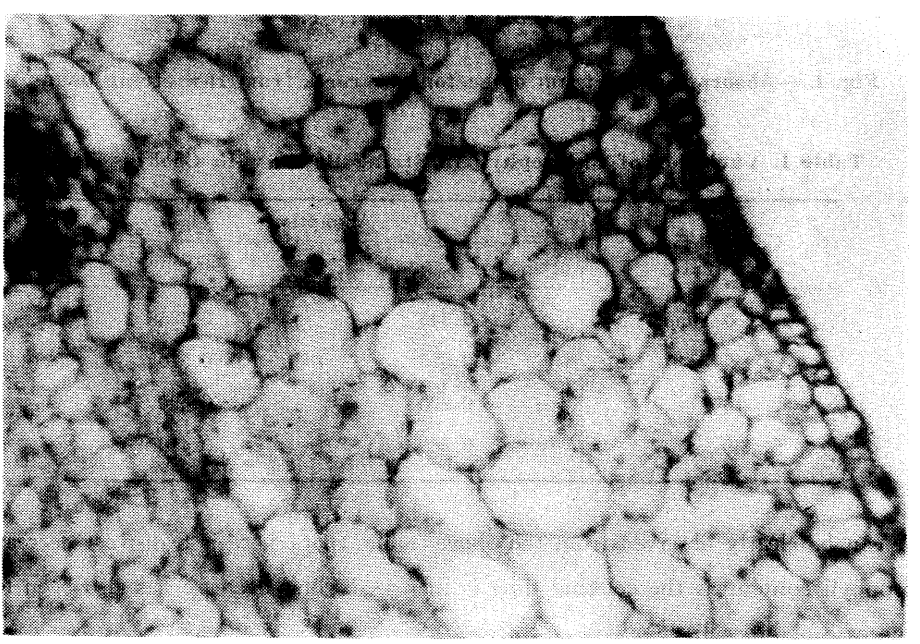

Fig. 2 Transverse section of a raw string bean embedded in paraffin; cut 12 microns thick; stained with Heidenhain's haematoxylin. Magnification ×150.

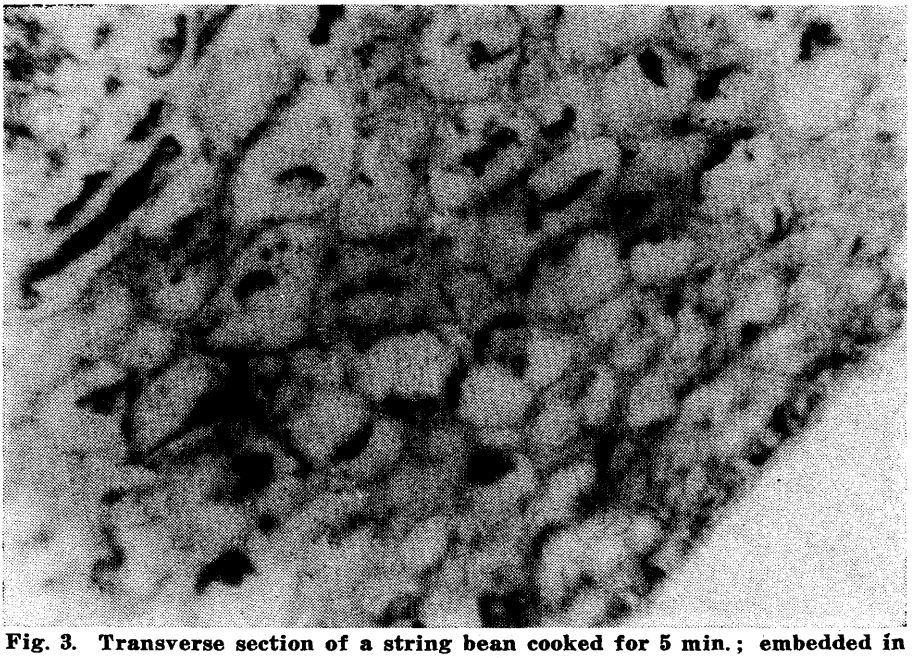

Fig. 3. Transverse section of a string bean cooked for 5 min.; embedded in paraffin; cut 12 microns thick; stained with Heidenhain's haematoxylin. Magnification ×150.

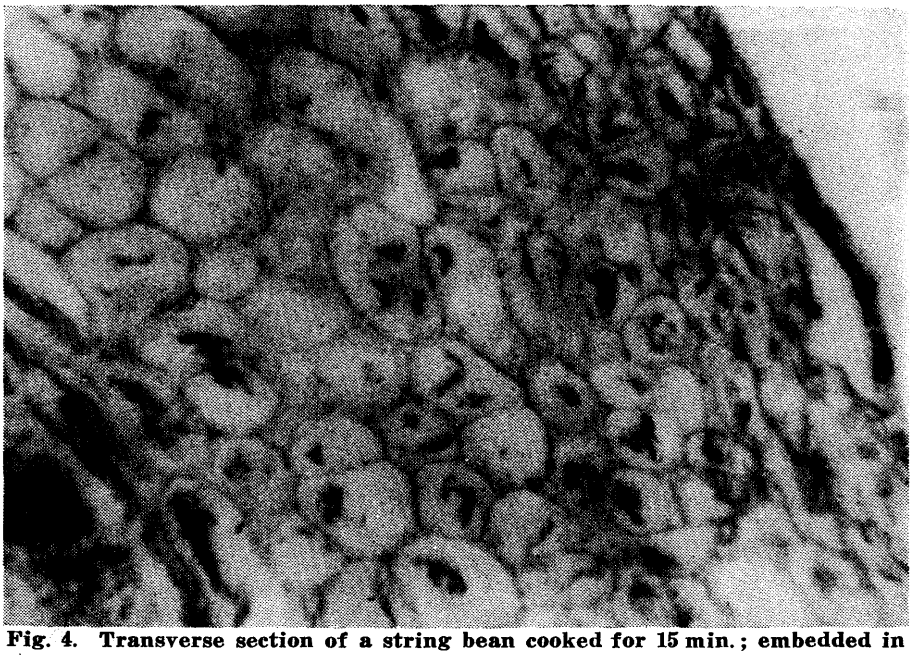

Fig. 4. Transverse section of a string bean cooked for 15 min.; embedded in paraffin; cut 12 microns thick; stained with Heidenhain's haematoxylin. Magnification ×150.

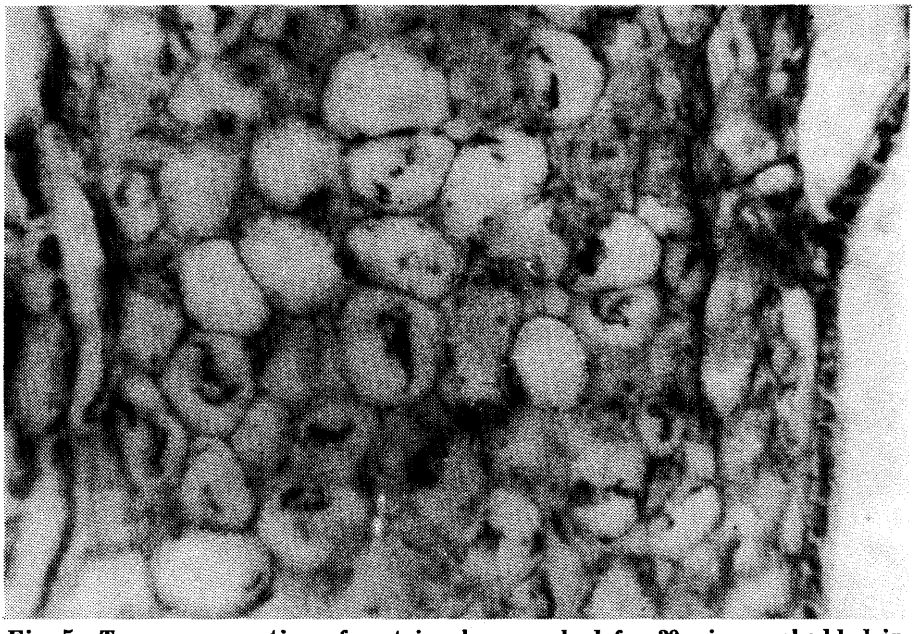

Fig. 5. Transverse section of a string bean cooked for 30 min.; embedded in paraffin; cut 12 microns thick; stained with Heidenhain's haematoxylin. Magnificatin ×150.

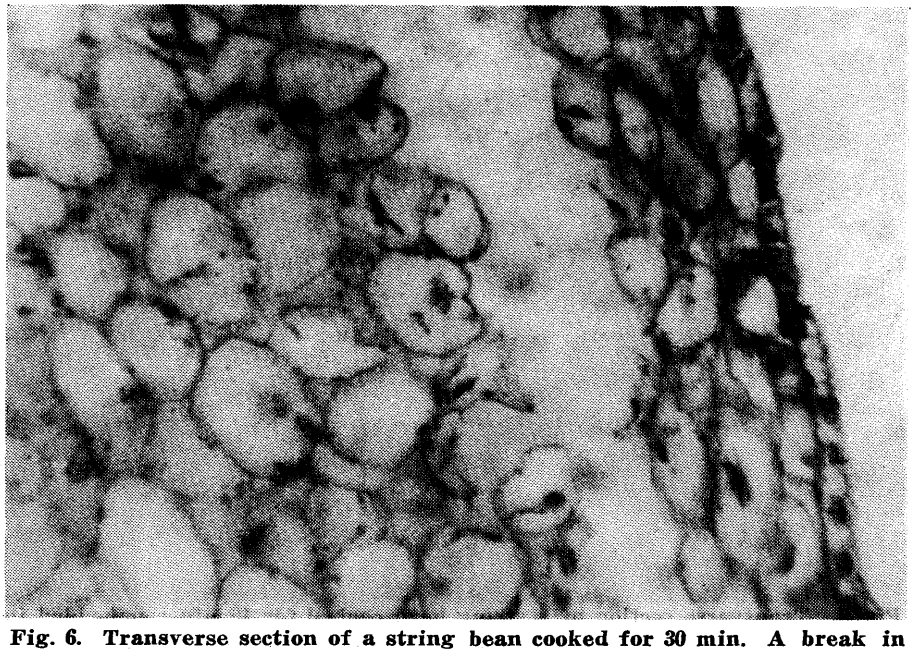

Fig. 6. Transverse section of a string bean cooked for 30 min. A break in the parenchyma cells is noted. Magnification ×150.

Figs. 2—5 are photomicrographs of string beans, raw, cooked for 5, 15 and 30 min., respectively. In the freshly harvested raw state of string beans the "chloroplasts" or "chloroplastids" appear to be distributed around the periphery of the palisade cells. The nucleus is also found near the cell wall (Fig. 2). This arrangement of the cells resembles that of the diagram illustrated by Robbins and Rickett (*16*). After 5 min. of cooking, the chloroplasts are seen scattered at random, some near the nucleus. This may be due to the heat softening the cell walls and in turn causing the chloroplasts to loosen. At 15 min. the epidermal cells showed a separation, and a coagulation of the chloroplasts together with the nucleus has occurred, although a distinction among the cells can be made (Fig. 4). Further cooking for 30 min. showed a complete coagulation and a marked separation of the epidermal cells from the rest of the cells. It was observed that as time for boiling was increased a gradual shrinkage within the endodermis appeared. At some sections a break in the region of the parenchyma cells can be seen (Fig. 6). This is similar to the breakage found in frozen and thawed snap beans (*7*).

Thus, from the above findings it can be said that as the content of chlorophyll decreased during the process of cooking a marked change within the cell membrane could be seen.

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