

AN ABSTRACT OF THE THESIS OF

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(Name) (Degree)  
in FOODS AND NUTRITION presented on March 15, 1974  
(Major) (Date)

Title: TEXTURAL CHANGES IN BROCCOLI AND CARROTS  
COOKED BY MICROWAVE ENERGY

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Carrots cooked by microwaves were judged less tender in both the core and external flesh area than those cooked by conventional boiling. Broccoli cooked by microwaves was less tender in the external layer but softer in the central flesh than that cooked conventionally. Cooking methods made essentially no difference in the concentration of water-insoluble pectic substances. The predominant factor responsible for the textural differences in vegetables cooked by microwaves seemed to be dehydration as evidenced by 1) a twofold water loss during cooking as compared to conventional method, 2) a significantly more shrunken contour of the vegetable piece, and 3) increased collapse of cells and extensive radial fissures in the parenchymatous tissues.

Textural Changes in Broccoli and Carrots  
Cooked by Microwave Energy

by

Doris Elaine Schrumpf

A THESIS

submitted to

Oregon State University

in partial fulfillment of  
the requirements for the  
degree of

Master of Science

June 1974

APPROVED:

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Date thesis is presented March 15, 1974

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## ACKNOWLEDGEMENTS

I wish to recognize and extend deep appreciation to Helen G. Charley, Professor of Foods and Nutrition, for her stimulation and counseling during my graduate course work and the research and writing of this thesis.

I also wish to thank Dr. Fred Rickson for making available equipment and facilities for the histological investigation and assistance in taking the photomicrographs, and Jiin Chiang Chen for statistical analysis and interpretation of the data.

Special appreciation is given to my husband, Barry, for his encouragement and understanding.

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# TEXTURAL CHANGES IN BROCCOLI AND CARROTS COOKED BY MICROWAVE ENERGY

## INTRODUCTION

The use of microwave energy for cooking was an outgrowth of work to improve radar for the military during World War II. Certain microwave frequencies were subsequently released for purposes other than military, and experimentation with their use for cooking accelerated (Proctor and Goldblith, 1948; Goldblith, 1966). By the 1950's microwave ovens were available for commercial and home use (Van Zante, 1973). In recent years commercial use in hospital, airline, and cafeteria food preparation processes has expanded rapidly. Rosen (1972) cited the number of microwave ovens sold for home use as more than doubling each year. Between 100,000 and 125,000 were sold in the U.S. in 1971 and by 1976 Van Zante (1973) predicts that microwave ovens will account for 25% of all cooking ovens in the U.S. One of the main factors favoring an increase in sales may be the decreasing price. Microwave ovens previously cost as much as \$1500 but are now available for less than \$500. Advertisement and promotion of microwave ovens have increased noticeably. With the onset of the "energy crisis," the reduced power requirement of the microwave oven may become as important a factor to some buyers as gas mileage has become in selecting a car. Other advantages claimed

for microwave cookery include speed, convenience, and retention of certain nutrients.

As might be expected, the difference in the way food is heated in a microwave oven as compared to conventional heating has resulted in differences in the color, taste, or textural characteristics of the cooked food. Many studies have been completed in the sensory evaluation of meats and baked products while much of the work involving vegetables was done on retention of nutrients. Although textural differences have been reported, no work to date has linked the changes in texture of vegetables cooked by microwaves with the factors responsible. The purpose of this study was to identify any textural differences occurring in broccoli and carrots cooked by microwaves as compared to conventional boiling and examine accompanying changes in pectic substances and cellular structure of both vegetables.

## REVIEW OF LITERATURE

### Microwave Energy

Principles of heating. The spectrum of electromagnetic energy covers a wide range of wavelengths. The wavelengths used for cooking have frequencies of 915 megahertz (MHz) or 2450 megahertz. Figure 1 shows the relationship of these frequencies to the remainder of the electromagnetic spectrum. The wavelengths used for cooking are a

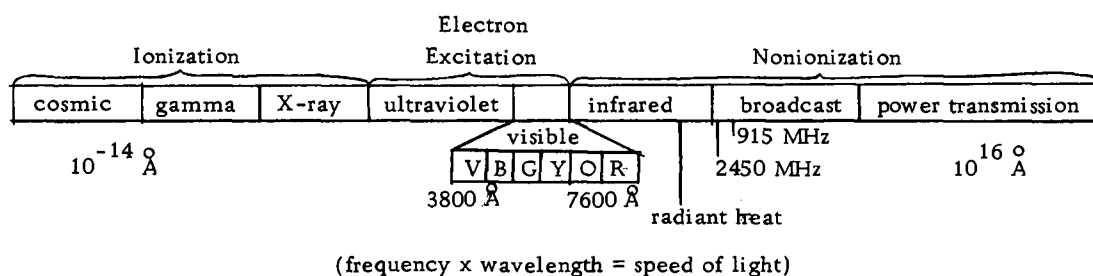


Figure 1. Electromagnetic Spectrum (adapted from Van Zante, 1973).

nonionizing form of electromagnetic energy. Only gamma rays, x-rays, ultraviolet light, and to some extent visible light have enough quantum energy to result in the breakage of chemical bonds to form ions or free radicals which react and form secondary products. The quantum energy of microwaves is only enough to break relatively weak hydrogen bonds. The "danger" of leakage of electromagnetic waves

from a microwave oven is often mistakenly assumed to be due to chemical changes such as those caused by ionizing radiation. Actually any damage to the human body by microwaves would be caused by the heat that they induce. The cornea of the eye and the testis are areas of the body particularly sensitive to excessive heat (Rosen, 1972).

The primary difference between microwave and conventional heating is the initial distribution of heat in the food. Conventionally, heat is brought to the surface of the food by conduction, convection, or radiant energy and transferred to the interior of the food mainly by conduction, although convection currents play a role in liquid portions. In microwave ovens, heat is created throughout the food. Only in foods which are thicker than the depth of penetration of the waves does conduction become the method of heat transfer.

As with all radiant energy, microwaves can be reflected, transmitted, or absorbed by the object with which they come in contact. Heat is created only in those media which absorb the waves. The absorption of microwave energy depends on the composition and polarity of the dielectric (nonconducting) substance. Any material containing free polar molecules will readily absorb microwaves. Foods are good absorbers of microwave radiation, with water the most strongly absorbing component (Macleod, 1972). The generation of heat within an absorbing medium is basically a result of friction caused when the charged asymmetric molecules of which the material is

composed are rotated in an attempt to align with the rapidly changing alternating current field (Goldblith, 1966). The amount of heat created is complicated by a gradual decrease of intensity as the energy is absorbed by successive layers of material and by the differing dielectric properties of the various materials. Further complications to even heating in foods are reflection and refraction of microwaves at interfaces between different food components and the influence of spatial arrangements of regions with high and low dielectric constants. Because foods are naturally nonhomogeneous, with numerous layers of materials varying in response to microwaves, hot spots may develop (Rosen, 1972).

Unlike conventional methods, temperature cannot be controlled when heating with microwaves. Timing is the chief means of determining extent of heating. This is affected by some of the same factors which influence doneness in conventional cooking such as initial temperature of the food, holding time, the specific and latent heats of food, and loss of moisture by evaporation. However, in addition there are some factors which are specific for the microwave method of cooking. These include the dielectric properties of the food, dipolar molecular action, depth of penetration of the microwaves, electromagnetic frequency of the oven used, the size and distribution of the load in the oven, shape of the food, and vapor pressure in the oven (Van Zante, 1973).

Effect on food constituents. Rosen (1972) points out that accumulating evidence supports the theory that thermal effects are sufficient to account for most if not all actions of microwaves on cells and their components. Any endangering of food constituents subjected to microwaves is mostly caused by overheating due to the high initial temperatures created by the microwaves. Proteins are very susceptible to denaturation by high temperatures. The gel-forming capacity of egg proteins is destroyed and the muscle fibers in meats toughen while connective tissues do not have the chance to solubilize. The carbonyl-amine browning reaction may occur within the meat wherever hot spots occurs. Carbohydrates are also affected by high temperature. A reduction in the thickening power of starch granules may occur because of overheating, and high temperature and rapid loss of water may cause rapid caramelization of sugar. The effect of microwave heating on fats varies because the capacity to absorb microwaves seems to vary according to the chemical structure of the fatty acids. Minerals in solution cause slower heating, and concentrations of minerals such as bones may reflect the waves (Van Zante, 1973).

Conservation of vitamins was among the early advantages claimed for microwave cookery. Proctor and Goldblith (1951) suggested that any improved retention of nutrients was due to a physical conservation of the nutrients or prevention of leaching rather than a difference between the effects of heat and microwave energy per se. Many

studies and conflicting results have followed. Ascorbic acid is one of the more labile vitamins because it is highly water soluble and also subject to oxidation. Sweeney et al. (1959) found that leaching of ascorbic acid into the cooking liquid rather than oxidation accelerated by heat was the chief factor responsible for loss of ascorbic acid regardless of the conventional cooking method used. Table 1 summarizes the results reported in several studies on the retention of ascorbic acid in cooked broccoli. In evaluating these results it is

Table 1. Retention of Ascorbic Acid in Broccoli Cooked by Microwave and Conventional Methods.

Investigators	Wt. of broccoli (g)	Microwave Method			Conventional Method		
		water (ml)	time (min)	retention (%)	water (ml)	time (min)	retention (%)
Campbell <u>et al.</u> (1958)	100	200	3	72	200	10	60
	100	300	6	64	200	20	55
Gordon and Noble (1959)	300	118	5	87	to cover <sup>a</sup>	6.5	45
Chapman <u>et al.</u> (1960)	454	236	6.5	89	300	13	72 <sup>b</sup>
Kylen <u>et al.</u> (1961)	300	150	8	79	150	11.7	83

<sup>a</sup>Followed recommendations of "Hows and Whys of Cooking," Halliday and Noble, 1946.

<sup>b</sup>Results for boiling cited from previous study, Sweeney et al., 1959.

necessary to keep in mind the length of cooking time and the proportion of water to vegetable. Campbell et al. (1958) cooked 100 g of broccoli in 200 ml water for 3 minutes by microwave energy and 10 minutes conventionally. They reported 72% retention of ascorbic acid

by the microwave method and 60% by the conventional method. When they doubled the cooking times and increased water to 300 ml, retention was less for both methods but that cooked by microwave lost more proportionately. Gordon and Noble (1959) cooked 300 g of broccoli in 118 ml of water by the microwave method and reported 87% retention of ascorbic acid compared to 45% retention for 300 g of broccoli cooked conventionally in enough water to cover. Chapman et al. (1960) reported 89% retention of ascorbic acid in 454 g of broccoli cooked by microwaves in 236 ml of water for 6.5 minutes. They compared this to 72% retention reported by Sweeney et al. (1959) for 454 g of broccoli cooked conventionally in 300 ml of water for 13 minutes. Kylen et al. (1961) are the only workers to report no significant difference between the two methods in retention of ascorbic acid. They cooked 300 g of broccoli in 150 ml of water for 8 minutes by microwaves and 11.7 minutes conventionally. They suggested that the results reported by earlier workers were largely because of the varying amounts of water and the length of heating times rather than any difference due specifically to the method of heating.

Thiamine is another vitamin that is lost during cooking. Several workers have investigated retention of thiamine in cooked meats and reported small but insignificant differences between meat cooked conventionally and that cooked by microwaves (Kylen et al., 1964; Apgar et al., 1959). Exposure of a solution of thiamine maintained



at 0°C to microwaves for extended periods of time caused no destruction of thiamine, but the loss did increase as length of exposure at 102.8°C increased (Goldblith et al., 1968). Thus it appears that the high initial temperatures reached in microwave cooking, not the microwaves per se, cause the destruction of thermally labile nutrients.

It has been suggested that microwave energy can have an effect on bound water that is different from conventional heating. Although microwaves are a nonionizing form of energy, they do have enough quantum energy to break very low energy bonds such as the relatively weak hydrogen bonds (Rosen, 1972). Ruyack and Paul (1972) reported results supporting previous findings that cooking losses are greater for meat cooked by microwaves than that cooked conventionally and suggested that this may be partially due to the effect of microwave energy in breaking hydrogen bonds to release more bound water. Greater weight losses have also been reported for cakes baked by microwaves than for those baked conventionally in spite of the fact that internal temperatures were reported lower for the cakes baked in the microwave oven (Muck, 1960).

In early work Bollman et al. (1948) reported that vegetables tended to dehydrate unless protected by water or a natural covering such as corn husks. On the whole, fresh vegetables cooked by microwaves compared favorably with conventionally cooked vegetables if special precautions were taken. However, even when carrots and

broccoli were covered with water in a covered container, the loss of moisture was higher when the vegetables were cooked by microwaves than by other methods. One characteristic of vegetables cooked by microwaves was described as a "crispness of texture" which prolonged cooking did not decrease. An outer skin tended to form which became progressively thicker as the cooking time increased and was not completely prevented even when water sufficient to cover was used. When frozen peas left in the package with no added water were cooked in the microwave oven, there was a great difference in palatability between peas which were slightly undercooked and those slightly overcooked. The latter had a shriveled, dull appearance and a very hard texture (Stevens and Fenton, 1951). Kylen et al. (1961) cooked a variety of vegetables by both microwaves and conventional heating. Neither method yielded a consistently more palatable vegetable, but broccoli and cauliflower cooked by microwaves were judged significantly inferior in overall palatability. The data indicated that the major difference was due to texture. In recent work investigating microwave and conventional cooking at high altitudes, Bowman et al. (1971) reported that cauliflower, frozen broccoli, peas, and spinach cooked by microwaves rated medium in acceptability. Judges noted that vegetables cooked by microwaves frequently had tough stalks, tough skins, or seemed underdone. These authors also found that

increasing the amount of cooking water did not always prevent dehydration or "certain other deleterious effects on texture."

### Texture in Vegetables

Plant structure. Texture is very intimately associated with the structural makeup of raw or processed fruits and vegetables. The living plant is an intricate association of both living and dead cells in complex patterns. The contribution of these cells to the texture of a food varies with individual construction, location, and relative abundance (Sterling, 1963). Figure 2 shows cross sections of the carrot root and the broccoli stalk. As is the case with broccoli and

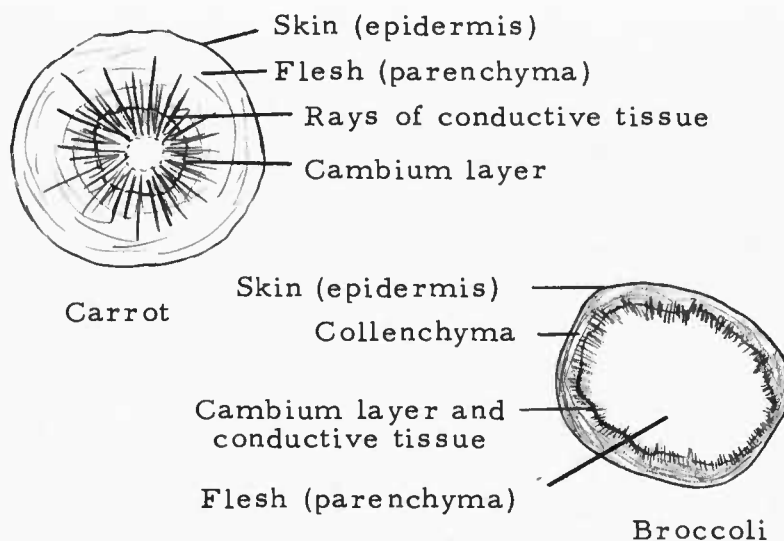


Figure 2. Gross Morphology of Carrot and Broccoli.

carrots, most edible portions of vegetables are composed largely of the fleshy parenchymatous ground tissue with conducting tissues forming a network throughout (Weier and Stocking, 1949).

Parenchymatous cells generally have very thin walls and multi-angular surfaces which cause intercellular spaces to occur between adjacent cells (Figure 3). Some vegetables, such as celery, have collenchyma cells which form a supportive tissue that may be of importance in some young vegetables. These cells are similar to parenchyma cells except they have walls which are irregularly thickened with pectic substances mainly at the inner angles of their wall facets. These cells also contain especially large amounts of water (Feinberg et al., 1964; Ledbetter and Porter, 1970).

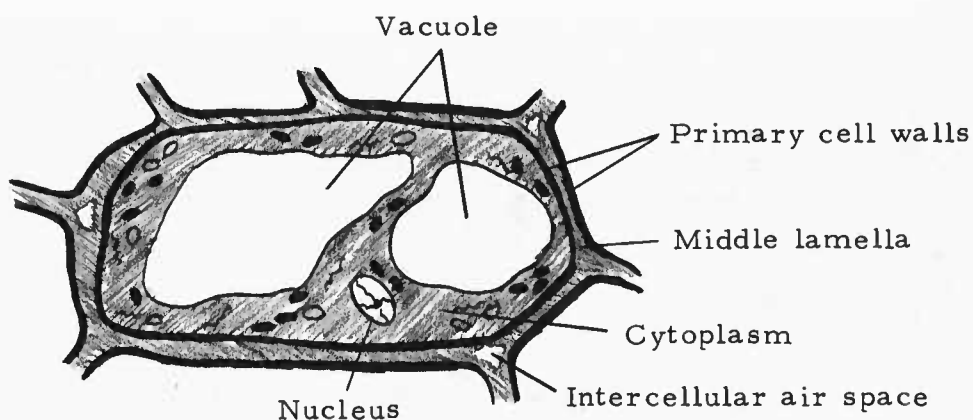


Figure 3. Generalized Plant Cell.

Vascular tissues are of two types: xylem (water conducting) and phloem (food conducting). The principal water conducting elements, vessels, are long tubes made of a series of dead cells. The cell contents have been replaced by an aqueous salt solution and the terminal walls of each cell have dissolved to form a continuous column. The remaining walls of these tubes have been irregularly strengthened with lignin in the form of rings, spirals or pitted sheets. The food conducting elements, sieve tubes, are generally not lignified. However, fiber cells may occur in phloem tissue, especially in more mature plants, and these cells have thick lignified walls (Weier and Stocking, 1949).

All vegetables are protected by an epidermal layer of some sort. This is generally a layer of specialized parenchyma cells covered by a thick cuticle substance. The cuticle allows transpiration to take place through the outer surface but resists wetting from the outside (Weier and Stocking, 1949; Ledbetter and Porter, 1970).

In young plants and even mature herbaceous plants, the mechanical tissues alone are not sufficient to support the leaves and stems. Rigidity is the result of internally developed hydrostatic pressure (turgor) which is a feature of the "biochemical activity of the living parenchyma cell" (Sterling, 1963). The vacuole of the cell can hold as much as 90% of the water in the plant due to osmotic pressure developed across the semipermeable vacuolar membrane (Feinberg

et al., 1964). This pressure distends the walls of individual cells, causing them to press against each other and hold many of the weak plant organs erect. Turgor pressure can amount to as much as nine atmospheres or more and is responsible for the desired crispness, firmness and succulence of raw fruits and vegetables. The turgid state depends on living cells. When the cell is killed, turgor pressure and the related textural characteristics are irretrievably lost (Sterling, 1963).

Among the remaining factors which can affect the texture of vegetables is the cell wall. The cell wall is a non-living, semi-elastic structure enclosing the protoplasm or living portion of the cell. When fully developed, it is composed of three layers. The middle lamella is the cementing layer common to adjacent cells. It is the first separatory layer formed between two daughter cells at the time of cell division. The most common theory is that this layer consists principally of a firm gel of pectic substances. Evidence also indicates the presence of protein in the middle lamella which may chelate metals such as calcium in a cross-linked gel structure. In older tissue, lignin often appears in the middle lamella before it is deposited in the remainder of the cell wall (Sterling, 1963).

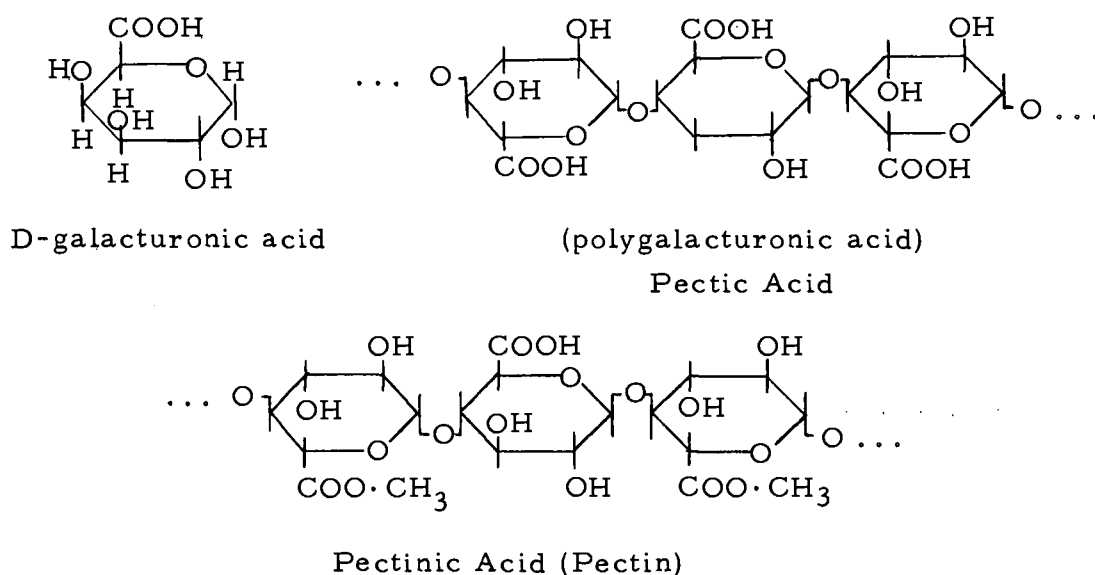
The primary cell wall is a layer adjacent to the middle lamella, but it has a much higher degree of organization. It is generally rather thin and consists mainly of firm gels of pectic materials, cellulose,

and hemicelluloses. The pectic materials and hemicelluloses are the most prevalent. These form an amorphous matrix in which microfibrils of cellulose are arranged perpendicular to the long axis of the cell. These microfibrils are composed of both crystalline micelles and amorphous cellulose (Sterling, 1963).

Most vegetables are consumed at a point in their development before the formation of a secondary wall. This structure is a highly stratified and often rather thick layer formed on the inside of the primary wall. In the secondary cell wall, microfibrils of cellulose are embedded in an amorphous matrix of lignin and/or hemicelluloses (Sterling, 1963).

Of all the constituents that make up the layers of the cell wall, pectic substances and their physical properties are probably most closely related to the textural qualities of raw and cooked vegetables. The precise structure of the pectic substances is still to be elucidated. The main component has been established as polymers of 1-4 linked  $\alpha$ -D galacturonic acid in various degrees of esterification or neutralization. However, polysaccharides composed solely of this sugar seem rare. Numerous papers have reported small proportions of neutral sugars, especially L-arabinose, D-galactose, and L-rhamnose, as components of exhaustively purified pectic substances, although their structural significance is still not known (Aspinall, 1963; McCready, 1970).

Actually, the term pectic substances is a group designation for a range of complex colloidal carbohydrate derivatives. The group is conventionally subdivided on the basis of physical characteristics, and standardized nomenclature has been adopted for these sub-groups. Pectic acids are the simplest of the pectic substances, having just enough anhydrogalacturonic acid units to attain colloidal properties. They are essentially free from methyl ester groups. Salts of pectic acid are normal or acid pectates. Pectinic acids are colloidal polygalacturonic acids with more than a negligible proportion of methyl ester groups. They can form gels, and their salts are normal or acid pectinates. "Pectin" refers to those water soluble pectinic acids which are capable of forming gels with sugar and acid. Protopectin is the water insoluble parent pectic substance which yields pectinic acids upon restricted hydrolysis (Kertesz, 1951).





The insolubility of protopectin was previously thought to be due to the extended length of the polygalacturonic acid chain (Bonner, 1950). Later research suggests more complicated factors may be involved. Covalent bonding between pectic substances and other cell wall constituents, especially the hemicelluloses, and association with other cell wall components by secondary bonds is thought to occur. The presence of cations, especially  $\text{Ca}^{++}$ , would lead to the insolubility of low ester pectic substances and to reduction of swelling of the pectic substances with a higher degree of esterification. And finally, the filamentous macromolecules of pectic substances may be mechanically enmeshed with each other and other polymers of the cell wall (Sterling, 1963). Figure 4 shows a suggested model for protopectin.

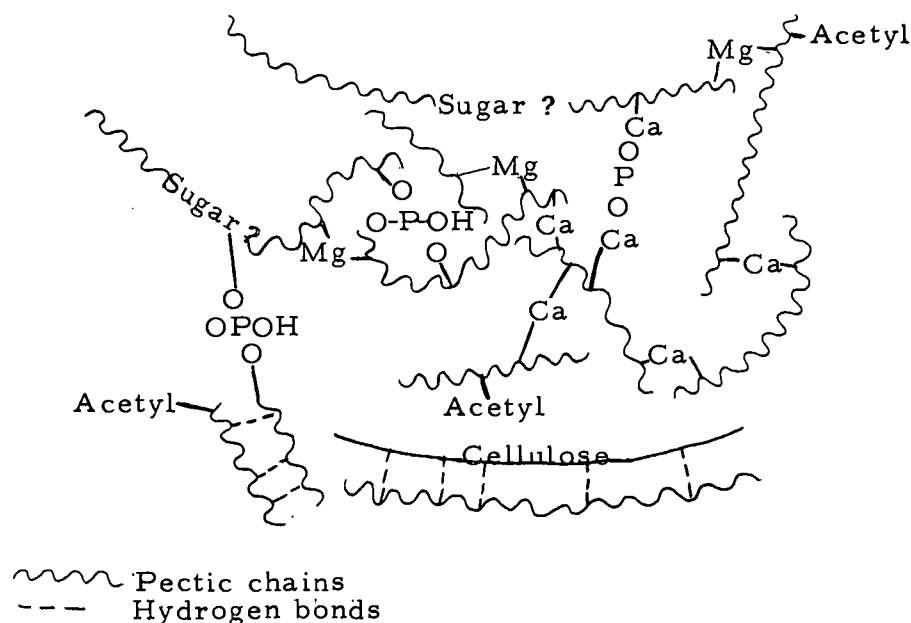


Figure 4. Suggested Model for Native Protopectin (Duckworth, 1966).

On the basis of solubility and staining, pectic substances in the cell wall appear to differ from those in the middle lamella. The primary cell wall is thought to be mainly protopectin, while the middle lamella contains calcium and magnesium pectates (Bonner, 1950; Joslyn, 1962). It is often possible to cause separation of cells by treating tissues with calcium sequestering agents. However, some workers report that when calcium is extracted from the tissues, separation is not complete. This suggests that calcium bridges may not be as important in the formation of calcium pectinate gel as hydrogen bonds and other secondary valence forces (Sterling, 1963).

Factors affecting textural characteristics. Changes in the texture of a vegetable are related in varying degrees to alteration of all of the constituents of the cell which contribute to structure of the plant. Various enzymes in the tissues are capable of altering texture. Softening of fruits and vegetables as they mature is due to the solubilization, catalyzed by pectic enzymes, of the pectic substances of the middle lamella and cell walls. The enzyme pectin methylesterase can also produce firmness in tissues by catalyzing the de-esterification of pectin to produce a larger amount of low methoxyl pectin or pectic acids, which react with calcium ions to form insoluble calcium pectate and so increase the adhesion between cells (Isherwood, 1955). This enzyme can also be activated by the proper blanch temperature to control firmness of green beans and cauliflower during canning

(Van Buren et al., 1960, 1962; Hoogzand and Doesburg, 1961). The enzyme phytase in garden peas may inhibit tenderizing during cooking if the peas are handled in such a way as to permit the enzyme to catalyze the hydrolysis of phytin so that it no longer can bind the calcium from insoluble calcium pectate in the middle lamella (Isherwood, 1955).

Physical and chemical treatments affect the various components of the layers of the cell wall in different ways. On heating in water, cellulose undergoes only limited rupture of hydrogen bonds and swelling and a slight decrease in crystallinity. Hemicelluloses, which include a variety of substances including xylans, galactans, galactomannans, mannans, glucomannans, and others, are also resistant to heat but more or less soluble in strongly alkaline solutions and occasionally in acidic solutions (Sterling, 1963). Lignin is unaffected by heating in water or by hydrolysis during digestion (Weier and Stocking, 1949). Pectic substances have weaker covalent bonds than does cellulose. The glycosidic bond in pectic substances is readily broken at temperatures below the boiling point. This hydrolysis is catalyzed actively by the presence of acids, bases, and pectic glycosidases. The ester bond can be hydrolyzed readily by various reagents, especially bases, even in the cold, but heat alone can also cause demethylation (Sterling, 1963). Depolymerizing and breaking of hydrogen bonds by heat lead to a greater amount of soluble pectic

substances and a decrease in total pectic substances during cooking (Simpson and Halliday, 1941).

The rapid softening of parenchymatous tissues during cooking is due to the loss of integrity of the middle lamella. Cell walls remain intact due to the resistance of cellulose and hemicelluloses to heat (Sterling, 1955). As the protoplasmic proteins are coagulated by heat, the cell membranes lose their semipermeability. When turgor pressure is released, the cell wall shrinks inward and liquid is forced out into the intercellular spaces or even out of the tissue (Gane and Wager, 1958).

Any treatment which alters the disposition of water, including heating, salting, or freezing, can cause tissue dehydration. In all cases the polysaccharide gels (cellulose, hemicellulose, and pectic substances) will become more crystalline. As crystallinity increases, the gels become tougher, less reactive, and swell less (Sterling, 1963). During dehydration of a tissue, a moisture gradient occurs so that each succeeding layer from the center is a little drier than the layer below it (Van Arsdell, 1963). When drying is very rapid the outside surface of the tissue becomes hard, shrunken, and rather impervious to the passage of gases and water vapor. Slower drying preserves a greater amount of amorphous material. In any case, the total amount of moisture that is present within a gel definitely determines its texture (Sterling, 1963).

Although pectic substances seem to be the most readily affected constituent, textural changes in vegetables are a series of interrelationships between the cell and cell wall components and their reactions to various physical, chemical, and biochemical treatments. To date, none of these constituents have been directly related to the specific textural changes reported for vegetables cooked by microwaves. This investigation examined cell structure and the concentration and solubility of various pectic substances to determine the importance of the role of each of these factors in the textural characteristics of vegetables cooked by microwaves.

## EXPERIMENTAL METHODS

### Preparation of the Vegetables

Sampling. Broccoli and carrots of good quality were purchased locally each week and refrigerated until used. For each replication, five carrots or five stalks of broccoli were used. Six replications were completed for each vegetable. The stalks of broccoli were washed, trimmed of woody tissue and leaves, and separated from the flower heads. Carrots were washed, scraped, and trimmed to a four inch central section of the root. From this point, treatment of both vegetables was the same. Each piece of vegetable was cut in half lengthwise. One half was assigned to the lot to be cooked by microwave and the other half to the lot cooked conventionally. Four such halves in each lot were weighed prior to cooking. These four paired halves were used for sensory evaluation. The two halves of the fifth stalk or root provided both raw and cooked subsamples for histological observations and for analysis of pectic content. After subsamples were taken from the raw tissue, the remainder of each half was weighed prior to cooking. Figure 5 shows the positions of the various subsamples taken both before and after cooking.

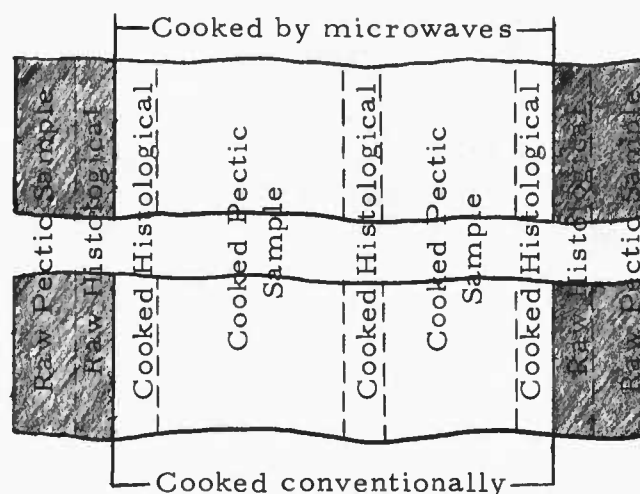


Figure 5. Sampling of Vegetable for Objective Tests.

Cooking. The halves used for objective evaluations were marked with a loose loop of string before being added to their respective lots for cooking. The other individual stalks or roots were sufficiently unique in shape that marking was unnecessary to pair matching halves again after they were cooked. The lot cooked by microwaves<sup>1</sup> (1600 watts; 2450 MHz) was added to 500 ml boiling water in a covered two quart pyrex baking dish and timing was started. The casserole was sufficiently large to allow the pieces to be arranged in a single layer. The lot cooked conventionally was added to 500 ml boiling water in an aluminum saucepan and boiled rapidly until tender. The lid was placed on the saucepan immediately after the carrots were added and,

<sup>1</sup> Amana Radarange, Amana Refrigeration Inc., Amana, Iowa.

in the case of broccoli, 5 minutes after the vegetable was added.

Preliminary trials were used to determine equivalent cooking times for the two methods and the volume of cooking water necessary to assure that water remained around the pieces of vegetable at the end of the conventional cooking period. This amount of water was also adequate to cover the lot cooked by microwaves during the entire cooking period. Table 2 gives the average weights of the cooking lots and the average cooking times.

Table 2. Raw Weights of Vegetables and Cooking Times.<sup>1</sup>

	Total Weight (g)	Cooking Water (ml)	Cooking Time (min)
Carrots			
Microwave	81.1	500	8
Conventional	80.8	500	20
Broccoli			
Microwave	67.5	500	5
Conventional	67.5	500	12

<sup>1</sup> Average of six replications.

Vegetables were removed from the cooking water as soon as they were done, placed cut side down on absorbent towels, and covered with plastic wrap to avoid excessive evaporation during a 30 minute cooling period. Preliminary trials showed that cooling was necessary to eliminate the rapid change in weight as the subsamples were weighed and that it did not interfere with sensory evaluation of



texture. The four halves for sensory evaluation in each lot were weighed, as was each of the paired halves used for objective tests. Then, from the latter, subsamples for objective tests were removed and weighed.

### Weight Loss

Weight loss during cooking was calculated as a percentage of fresh weight for the entire lot cooked by each method and also for those halves used for objective evaluation. The latter was used to calculate the retention of pectic substances in the cooked stalk or root.

### Sensory Evaluation

A trained panel of four members was used to detect any differences in texture between each vegetable cooked by each of the two methods. To elicit an unbiased decision, many factors including equality of sample size, temperature, and form as well as size and neutrality of the container were considered (Amerine et al., 1965). Each panelist was served randomly coded, paired halves to eliminate differences due to variations between individual stalks or roots and to minimize differences due to sample size, shape, and form. The panelists were asked to evaluate the change in the outline of the halves by matching the shape at the end and center of the piece with sketches on the score card provided. The sketches were drawn for each

vegetable from trial observations. In addition to shape, carrots were ranked for tenderness of outer flesh and inner core and broccoli was ranked for tenderness of inner flesh and outer layer. Descriptive terms for tenderness of flesh ranged from mushy to spongy for carrots and mushy to firm for broccoli. The core of carrots was ranked from tender to very fibrous and the outer layer of broccoli was ranked from tender to very tough. Judges were asked to indicate any samples which were unacceptable. In addition, the cooked broccoli with the thicker outer layer was also indicated.

#### Extraction of the Pectic Substances

The heterogeneity of the pectic substances makes essential their fractionation into groups differing in solubility (colloidal) before attempting to relate any measurement of pectic substances to texture in foods. Many methods for extraction are available, but care must be exercised not to depolymerize, degrade, or chemically modify the material present in the plant tissue. To isolate fractions of pectic substances comparable to those in situ, a cold extraction procedure is best (McCready, 1970).

Such a procedure was used in this study to obtain fractions of pectic substances with solubility characteristics as nearly the same as they existed in the plant tissue (Dietz and Rouse, 1953; Ruiz, 1958; Mackey et al., 1973). First the tissue was heated to 85°C in alcohol

to inactivate pectic enzymes, remove interfering carbohydrates, and precipitate pectic substances. Then progressive extractions and centrifugations were used to fractionate the pectic substances according to their solubility in water, sodium hexametaphosphate, or sodium hydroxide. Those pectic molecules having 8 to 11% methyl ester content were removed with water. Low methoxyl calcium and magnesium pectinates were released by sodium hexametaphosphate due to the sequestering effect of polyphosphates on the calcium and magnesium. Extraction with sodium hydroxide removed protopectin and the calcium and magnesium pectates not removed by sodium hexametaphosphate. Because of the overlapping of solubility characteristics, these three fractions are not discrete entities.

Reagents. The following reagents were used in the extraction of the three pectic fractions:

0.4% sodium hexametaphosphate ( $\text{NaPO}_3$ )<sub>6</sub> (4 g/liter).

1N sodium hydroxide (40 g/liter).

0.05N sodium hydroxide (2 g/liter).

Ethanol (95%, 80%, and 70%).

Diatomaceous silica filter aid (Celite).

Procedure. Two subsamples were taken from each raw and cooked tissue as previously indicated in Figure 5. Subsamples of cooked broccoli weighed 2 g each and those of raw broccoli weighed 5 g each. A larger weight of raw tissue was necessary to obtain a

yield of water-soluble pectic fraction sufficient to measure. Sub-samples of 2 g of cooked carrot and 3 g of raw carrot were used. Each subsample was diced into 20 ml 70% ethanol and blended<sup>2</sup> for 10 minutes. Several washings with 70% ethanol were used to transfer the slurry to a 50 ml graduated pyrex centrifuge tube and bring the volume to 40 ml. Purification and extraction was carried out according to the following directions as outlined by Dietz and Rouse (1953), Ruiz (1958), and Mackey et al. (1973):

Precipitation of the pectic substances and removal of the carbohydrate impurities:

1. Heat the tubes in an 85°C water bath for 10 minutes, stirring occasionally. The mixture in the tubes should simmer the last five minutes.
2. Centrifuge at 2400 rpm for 10 minutes.
3. Decant and discard the supernatant.
4. Dilute the residue in each tube to 40 ml with 80% ethanol; stir until evenly dispersed.
5. Heat the tubes in an 85°C water bath for 10 minutes, stirring occasionally; simmer last 5 minutes.
6. Centrifuge at 2400 rpm for 10 minutes.

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<sup>2</sup>Virtis "45" Hi-Speed Homogenizer, The Virtis Company Inc., Gardiner, New York 12525.

7. Decant and discard the supernatant.
8. Repeat steps 4 through 7. Last supernatant should be free of plant pigments.

#### H<sub>2</sub>O extraction:

1. Dilute residue in the tubes to 40 ml with distilled water; add approximately 1/8 tsp filter aid (Celite) and stir until evenly dispersed.
2. Allow to stand 10 minutes; stir again.
3. Centrifuge at 2400 rpm for 10 minutes.
4. Decant each tube into a labeled 100 ml volumetric flask.
5. Dilute residue in tube to 40 ml with distilled water, stir until evenly dispersed.
6. Repeat steps 2 and 3.
7. Decant into the same volumetric flasks.
8. To each volumetric flask add 5 ml of 1N NaOH and dilute to volume with distilled water.

#### (NaPO<sub>3</sub>)<sub>6</sub> extraction:

1. Dilute residue in the tubes to 40 ml with 4% (NaPO<sub>3</sub>)<sub>6</sub> and stir until evenly dispersed.
2. Let stand 10 minutes; stir again.
3. Centrifuge 10 minutes (2400 rpm).
4. Decant each tube into a labeled 100 ml volumetric flask.

5. Repeat steps 1 through 3.
6. Decant into the same volumetric flasks.
7. Add 5 ml 1N NaOH to each volumetric flask and dilute to volume with 4%  $(\text{NaPO}_3)_6$ .

#### NaOH extraction:

1. Dilute residue in each tube to 40 ml with 0.05N NaOH. Stir until evenly dispersed.
2. Allow to stand 15 minutes; stir again.
3. Centrifuge 10 minutes (2400 rpm).
4. Decant each tube into a labeled 100 ml volumetric flask.
5. Repeat steps 1 through 3.
6. Decant into the same volumetric flasks.
7. Dilute to volume with 0.05N NaOH.

#### Colorimetric Determination of Pectic Substances

A modified uronic acid-carbazole method developed by Bitter and Muir (1962) was used to estimate the concentration of pectic substances in each of the fractions. The method is based on the reaction of concentrated sulfuric acid and D-galacturonic acid to form 5-carboxy-2-furfural which reacts with carbazole to form a purplish red color (Stutz and Deuel, 1956). Through modifications of the reagents and procedure, several advantages have been gained over

procedures developed since the use of the reaction was first reported by Dische (1947). With this modification, there is an approximate doubling of sensitivity, and optical density is a linear function of concentration between 4 and 40 micrograms/ml. There is greater reproducibility and reduction of interference by chloride ions and oxidants.

Reagents. The following reagents were used for the colorimetric determination of pectic substances:

0.025M sodium tetraborate  $\cdot$  10 H<sub>2</sub>O in concentrated sulfuric acid

(9.55 g Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>  $\cdot$  10 H<sub>2</sub>O/liter concentrated H<sub>2</sub>SO<sub>4</sub>).

0.125% carbazole in purified ethanol (0.125 g (C<sub>6</sub>H<sub>4</sub>)<sub>2</sub>NH/100 ml).

Stable for 12 weeks at 4°C in the dark.

Purified ethanol. (Refluxed 1 liter of reagent grade 95% ethanol with 4 g zinc dust and 2 ml conc H<sub>2</sub>SO<sub>4</sub> for 24 hours. Distilled using all glass equipment. Added 4 g zinc dust and 4 g potassium hydroxide to 1 liter of the distillate and redistilled.)

Galacturonic acid monohydrate standard (120.5 mg galacturonic acid monohydrate + 10 ml 0.05N NaOH diluted to 1 liter with distilled water). This contains 100 micrograms anhydrogalacturonic acid/ml.

Carbazole reaction. Two aliquots from each pectic fraction, one of which served as a blank, were used to measure the concentration of pectic substances in the following manner:

1. For each pectic fraction analyzed, two pyrex test tubes (25 ml capacity) were cooled to below  $0^{\circ}\text{C}$  in an ice bath. Rock salt was added to the ice to hold the temperature of the brine at  $-15^{\circ}\text{C}$ . Care was taken that the contents of the tube were not contaminated by the brine which would result in a green color when carbazole was added.
2. Using a syringe pipette, 5 ml concentrated sulfuric acid was added to each tube and allowed to cool.
3. Each one ml aliquot of pectic fraction was carefully layered onto the acid in a cooled tube.
4. The tubes were stoppered and the contents shaken gently, then vigorously, while cooling continued.
5. The tubes were warmed to room temperature, rinsed with distilled water to remove the salt brine, processed in a vigorously boiling distilled water bath for 10 minutes, and then cooled to room temperature.
6. Using an automatic pipette, 0.2 ml carbazole reagent was added to one of the tubes and 0.2 ml purified alcohol only was added to the other to serve as the blank.
7. The contents of each tube was shaken again, heated in the boiling distilled water bath for 15 minutes, and then cooled to room temperature.



8. The optical density<sup>3</sup> of the red solution was read at 530 nm.

The OD of the blank against concentrated sulfuric acid should be below 0.025.

Calculation. Optical density readings for the aliquots were converted to micrograms of anhydrogalacturonic acid/ml of extract from a standard curve. For the latter, two standard solutions were prepared from vacuum dried (30°C for 5 hours) galacturonic acid monohydrate. The solutions were allowed to stand overnight before two sets of working standards covering the range of 10 to 60 micrograms/ml were prepared from each standard solution. The carbazole reaction was carried out using two test aliquots and one blank for each working standard. The average of all eight optical density readings for each concentration was plotted to obtain the standard curve (Figure 6).

Concentration of anhydrogalacturonic acid (AGA) in each pectic fraction was calculated as % of fresh weight of vegetable according to the following equation:

$$\frac{\% \text{ AGA of fresh weight}}{= \frac{\mu\text{g AGA} \times \text{dilution volume (ml)} \times 100}{1,000,000 \mu\text{g/g} \times \text{fresh sample weight (g)}}$$

The weight of the cooked subsample was converted to a fresh weight basis as follows:

$$\text{fresh sample weight} = \frac{\text{cooked sample weight}}{100 - \% \text{ loss of weight}} \times 100$$

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<sup>3</sup> Spectronic-20 Spectrophotometer, Bausch and Lomb Inc., Analytical Systems Division, Rochester, New York 14625.

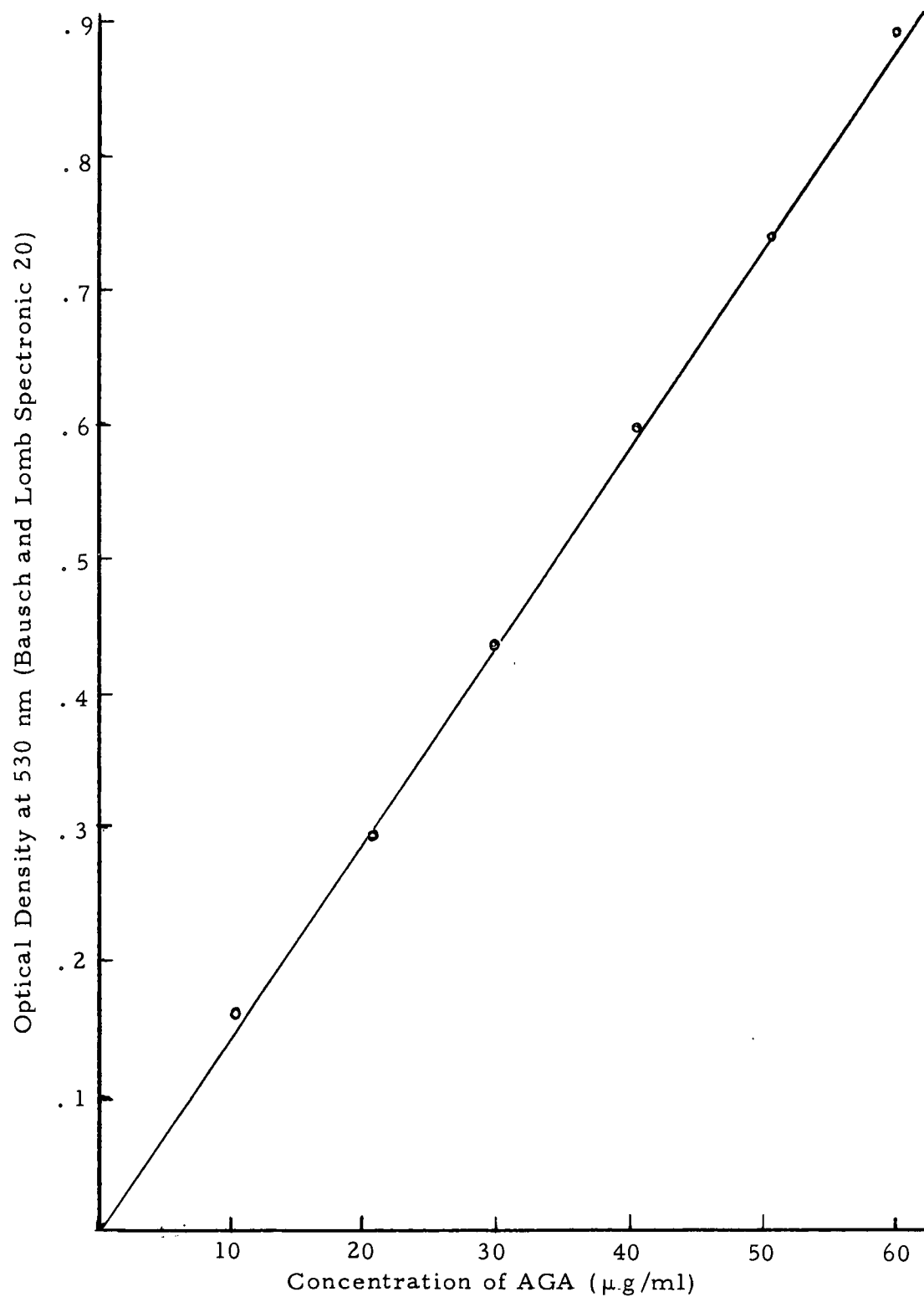


Figure 6. Standard Curve for Anhydrogalacturonic Acid.

Preparation of Tissue for  
Histological Observation

Two or three sections approximately 1/8 inch thick were removed with a new double edge razor blade from the areas previously indicated in Figure 5.

Reagents. The following reagents were used to clear and dehydrate the tissue:

Craf II fixative. Mix equal volumes of solutions A and B immediately before using.

A - 20 parts 1% chromic acid + 10 parts 10% acetic acid +  
20 parts distilled water

B - 5 parts formalin (37% to 40% aqueous formaldehyde) +  
45 parts distilled water

Ethanol (10% and 30%).

Tertiary butyl alcohol solutions (TBA):

1 - 1000 parts  $H_2O$  + 800 parts 95% ethanol + 2 parts TBA

2 - 600 parts  $H_2O$  + 1000 parts 95% ethanol + 400 parts TBA

3 - 300 parts  $H_2O$  + 1000 parts 95% ethanol + 700 parts TBA

4 - 900 parts 95% ethanol + 1100 parts TBA

5 - 1500 parts TBA + 500 parts 100% ethanol

6 - 100% TBA

7 - 50 parts TBA + 50 parts Paraoil

Paraoil (liquid paraffin)

Paraplast

Procedure. Tissue was covered with Craf II fixative. Because intercellular air had already been removed from the cooked tissue, it was not subjected to vacuum infiltration. This eliminated a possible cause of breakage of cell walls in the softened cooked tissue. Slices of raw tissue were vacuum infiltrated for 24 hours. Tissue remained in the fixative until the latter turned from amber to green (approximately seven days). Tissue was then cleared and dehydrated according to the following steps:

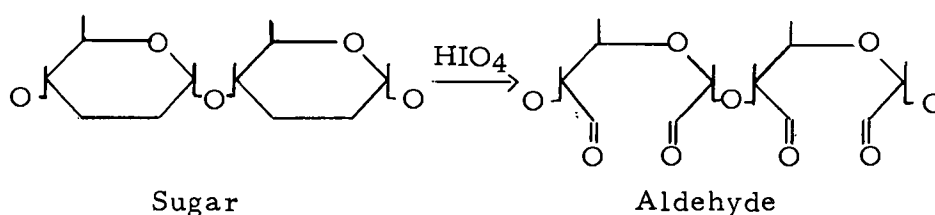
1. Rinsed in three changes of tap water over a 24 hour period.
2. Held for a minimum of 2 hours in 10% ethanol and then 30% ethanol. (Twelve hours or longer were used in all cases.)
3. Moved through TBA solutions 1 through 5; held for a minimum of 2 hours in each one.
4. Held in TBA 6 in a warm place. Changed the solution three times over a 12 hour period with the last change for overnight.
5. Added the tissue to enough TBA 7 to cover; held for a minimum of 2 hours.
6. Added an equal volume of paraoil to the TBA 7 covering the tissue.
7. Added the solution containing the tissue to solidified paraplast in a vial and held at 60°C until the paraplast melted.

Replaced the paraplast solution with melted paraplast three times over a 48 hour period.

The fixed and cleared tissue was then embedded in paraplast and sectioned at 12 microns. Sections were affixed to slides with Haupt's reagent with the aid of 4% formalin.

### Localization of the Cell Wall Polysaccharides

Periodic acid-Schiff's reaction. Despite the prominence and importance of the carbohydrates in cell structure, relatively few histochemical methods are available to estimate them quantitatively. Only one method is available which will localize all the insoluble polysaccharides in the cell (Jensen, 1962). The basis of the reaction is the production of aldehydes by the action of an oxidative acid. For this reaction to occur, the hydroxyl groups of the sugar must be free; if they are substituted or involved in a linkage, they will not react (Hotchkiss, 1948; Jensen, 1962). The most commonly used oxidative acid is periodic acid which splits the glycol grouping of carbohydrates (McManus, 1948):



Color is developed by the reaction between aldehydes and the dye fuchsin. Fuchsin can be transformed to a colorless leucofuchsin in acid solution, commonly called Schiff's reagent. Leucofuchsin reacts with the aldehydes to form stable, highly colored complexes. The polysaccharides become an intense purplish red while the cytoplasm remains colorless (Jensen, 1962).

The periodic acid-Schiff's reaction (PAS) is highly suitable for histochemical localization because it is specific and the reaction is understood. The glycosidic linkage is not broken and there is little or no possibility of interference or false localization (Jensen, 1962).

Reagents. The following reagents were used for PAS staining of the cell wall polysaccharides:

Xylene.

Ethanol (100%, 95%, 70%, and 50%).

0.5% periodic acid in distilled water.

Schiff's reagent. (Basic fuchsin (0.5 g) and 0.5 g potassium or sodium metabisulfite were dissolved in 100 ml of 0.15N HCl by shaking at intervals of 2 to 3 hours or until the dye was converted to fuchsin-sulfurous acid. Then 300 mg fresh decolorizing charcoal was added, and the reagent shaken for at least 5 minutes before it was filtered through hard filter paper. The solution should be clear and colorless. Stored in the refrigerator, stability varies from 6 weeks to a year.)

Procedure. Chemically fixed tissue sectioned at 12 microns was used and the slides carried through the following steps:

1. Removed paraffin in xylene and passed through a graded alcohol series (100%, 95%, 70%, 50%) to distilled water.
2. Placed in the periodic acid solution at room temperature for 5 to 30 minutes.
3. Washed in multiple changes of distilled water for 10 minutes.
4. Stained with Schiff's reagent for 10 to 15 minutes.
5. Rinsed in tap water for 5 minutes.

## RESULTS AND DISCUSSION

### Sensory Evaluation

Appearance. In evaluating the contour of the halves of cooked root or stalk, the panelists matched the cross-sectional shape of the pieces of vegetable with one of seven sketches on the score card. The shape most nearly resembling the raw piece of vegetable was assigned a score of one and the most shrunken, deformed shape, a score of seven. Figure 7 shows on the rating scale the average contour of carrots cooked by both methods. The shapes of the ends of carrot

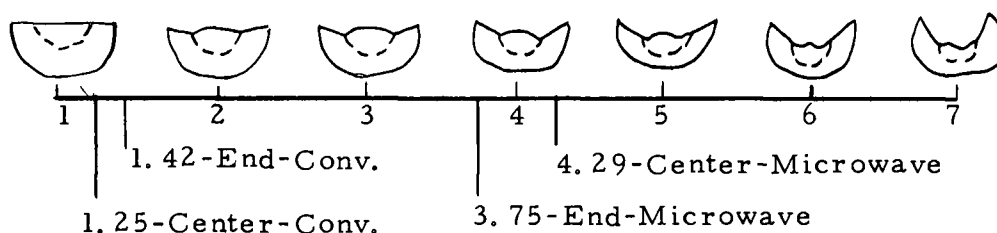


Figure 7. Contour of Cooked Carrots.

halves cooked by microwave averaged 3.75 and of the center averaged 4.29. Halves of carrots cooked conventionally averaged 1.42 for appearance of the end and 1.25 for appearance of the center. The difference in contour of the two halves due to cooking method was highly significant (see Table 3). Difference in shape between end and center of the root was not significant for either method of cooking (Table 3).



Table 3. Analysis of Variance for Sensory Evaluation.

Variable	Degrees of Freedom	Random Error	F test*	
			Carrots	Broccoli
Appearance				
micro/conv	1	85	202.79	177.68
end/center	1	85	2.03	2.99
judges	3	85	4.84	8.39
replications	5	85	1.14	0.90
Tenderness of flesh				
micro/conv	1	38	109.26	44.64
judges	3	38	0.33	7.50
replications	5	38	1.02	1.67
Tenderness of core or outer layer				
micro/conv	1	38	84.45	73.08
judges	3	38	3.39	2.44
replications	5	38	1.75	2.19

\* Minimum F values for 95% or 99% significance levels are as follows:

	F (1. 85)	F (3. 85)	F (5. 85)	F (1. 38)	F (3. 38)	F (5. 38)
95%	3. 96	2. 71	2. 33	4. 08	2. 84	2. 65
99%	6. 97	4. 04	3. 26	7. 31	4. 31	3. 51

Figure 8 shows on the rating scale the average shapes of halves of broccoli stalks cooked by each method. For those halves cooked by

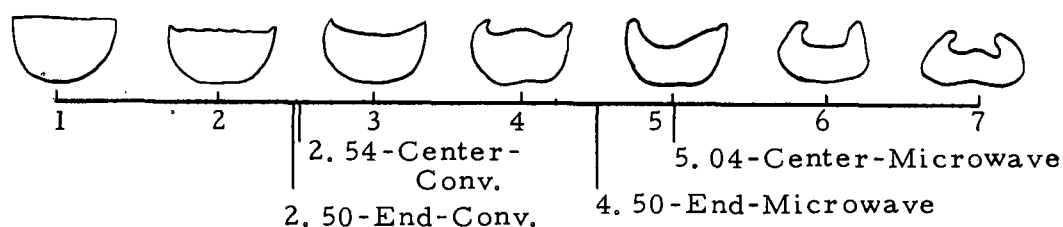


Figure 8. Contour of Cooked Broccoli.

microwaves, the contour of the end averaged 4.50 and of the center 5.04. The stalks cooked conventionally averaged 2.50 for the end and 2.54 for the center. Differences in shape due to cooking method were highly significant, but differences between end and center of the piece were not significant (Table 3).

Tenderness of flesh. Tenderness of the flesh of the root or stalk was assessed subjectively by the resistance of the piece of vegetable to cell separation when chewed. In carrots, the flesh area was considered as the general parenchyma tissue surrounding the central core. In broccoli, flesh referred to the central parenchyma tissue inside the ring of cambium cells near the skin (Figure 2). The cooked carrots were ranked for tenderness of flesh from spongy, assigned a numerical value of one, to mushy, assigned a value of five. The average rankings of carrots cooked by each method are given in Figure 9. The numerical average of the rankings for carrots cooked

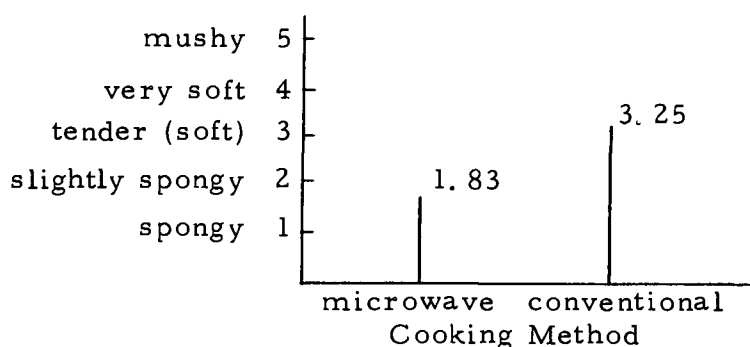


Figure 9. Average Tenderness Rankings for Flesh of Cooked Carrots.

by microwaves was 1.83 and for carrots cooked conventionally was 3.25. The descriptive terms associated with these values were slightly spongy, inclined to spongy, for carrots cooked by microwaves and tender, inclined to very soft, for carrots cooked conventionally.

Flesh of broccoli was ranked from firm to mushy. A value of one was assigned to firm and a value of five was assigned to mushy. Figure 10 gives the average rankings for tenderness of flesh of broccoli cooked by both methods. Broccoli cooked by microwaves had

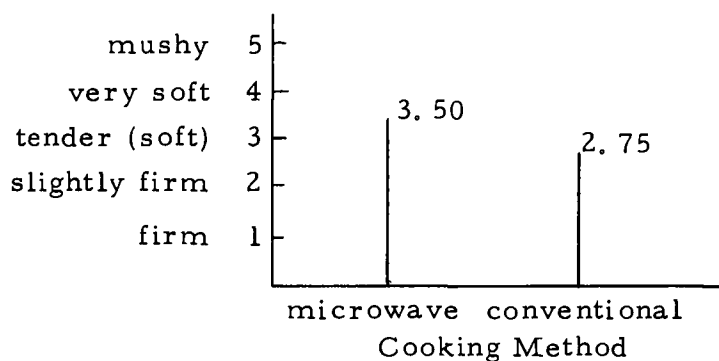


Figure 10. Average Tenderness Rankings for Flesh of Cooked Broccoli.

an average ranking of 3.50 while that cooked conventionally had an

average ranking of 2.75. Although stalks cooked by both methods were ranked near tender, the flesh of broccoli cooked by microwaves was ranked slightly softer than tender while that cooked conventionally was slightly firmer than tender. As was true for carrots, differences in tenderness due to cooking method were highly significant but in the case of carrots the flesh was less tender when cooked by microwaves than conventionally while in broccoli the reverse was true (Table 3).

Tenderness of core or outer layer. Tenderness of the central core of carrots and the outer ring of tissue in broccoli was assessed by the amount of pressure needed to cut or chew it. These were areas of the vegetables where supportive and vascular tissue predominated. The core of carrots was ranked on a scale from very fibrous (1) to tender (5). The numerical average of rankings for each cooking method is shown in Figure 11. The core of carrots cooked by micro-

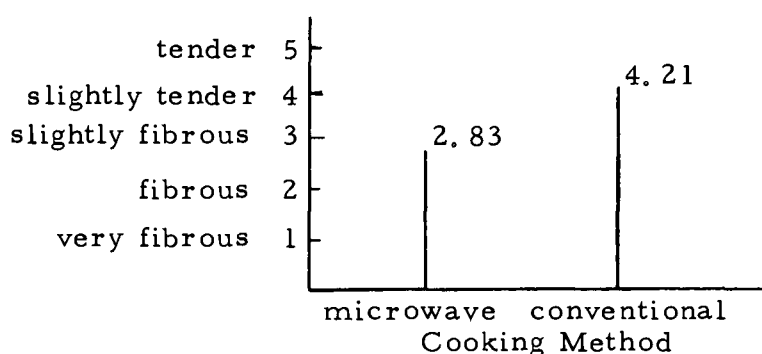


Figure 11. Average Tenderness Rankings for Core of Cooked Carrots.

waves had an average value of 2.83 and those cooked conventionally had an average value of 4.21. These values correspond to the

descriptive terms slightly fibrous for carrots cooked by microwaves and slightly tender for carrots cooked conventionally. Differences between the cooking methods were highly significant (Table 3).

The exterior tissue of the stalks of broccoli was ranked on a scale from very tough (1) to tender (5). The average rankings are given in Figure 12. Broccoli cooked by microwaves had an average

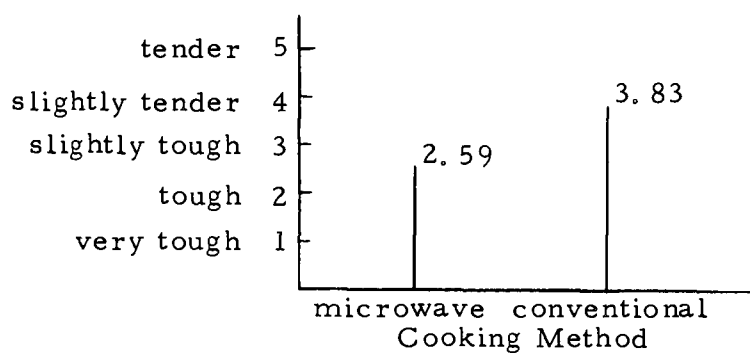


Figure 12. Average Tenderness Rankings for Exterior Tissue of Cooked Broccoli.

value of 2.59 and that cooked conventionally had an average value of 3.83. The exterior area of broccoli cooked by microwaves was judged significantly tougher than that of broccoli cooked conventionally (Table 3). Broccoli cooked by microwaves was described as slightly tough to tough while that cooked conventionally was borderline between slightly tender and slightly tough. Thus only in the case of the flesh of broccoli did cooking by microwaves produce a more tender vegetable.

Acceptability. Each panelist was asked to indicate whether the root or stalk cooked by either method was unacceptable. Carrots cooked by microwaves were judged unacceptable 58% of the time (24

total judgements) while those cooked conventionally were rejected only once or 4% of the time. Comments of panelists indicated that texture and/or appearance were the main reasons for rejecting the carrots cooked by microwaves but these carrots were sweeter, had more carrot-like flavor and better color.

The broccoli cooked by microwaves was judged unacceptable 67% of the time while none of the stalks cooked conventionally were unacceptable. Broccoli cooked by microwaves was judged as having the thicker tough outer layer 92% of the time. This suggests that greater toughness of the exterior layer of broccoli cooked by microwaves involves more than the skin.

The composite picture presented by the results of the sensory evaluation has several implications. First of all, there was an obvious difference in the appearance of both vegetables cooked by each method. Those samples cooked by microwaves had a rather collapsed and shrunken appearance while the samples cooked conventionally more nearly resembled the shape of the raw stalk or root. In regard to texture, both parts of both vegetables cooked conventionally more nearly approached the just tender stage. Halves of carrots cooked by microwaves had a more spongy flesh and a more fibrous core than did the matching halves cooked conventionally. Halves of stalks of broccoli cooked by microwave energy had a softer center flesh area but a tougher outer area than the matching halves cooked conventionally.

These results support the earlier reports of Bollman et al. (1948) and Bowman et al. (1971) that even though the vegetables cooked by microwaves were surrounded by water, they were not protected from "deleterious effects on texture."

### Weight Loss

Average percent weight loss for carrots and broccoli cooked by each method is given in Tables 4 and 5, respectively. Because these losses were based on weights after the cooling period, they are higher than those reported in other literature. But the cooked vegetables were cooled under identical conditions so any differences in weight loss should be due to the cooking method. In both cases, the weight loss in the vegetables cooked by microwaves was more than double the weight loss of the matching halves cooked conventionally.

Considering that water is the component of food which most strongly absorbs the microwave energy, it seems reasonable that more of the water content of a vegetable cooked by this method would be converted to steam and lost from the tissue. Any effect of microwaves on bound water is probably of secondary importance in explaining the large differences between cooking methods. Although there was more than enough water to cover the vegetable, the halves cooked by microwaves floated during the entire cooking period instead of becoming submerged as did the halves cooked conventionally. This suggests

Table 4. Weight Loss of Cooked Carrots.<sup>1</sup>  
(% of fresh weight)

Replication	Cooking Method	
	Microwave	Conventional
1	31.1	15.7
2	33.8	13.6
3	37.5	16.2
4	36.8	15.9
5	34.7	18.5
6	<u>31.3</u>	<u>14.3</u>
Average	34.2	15.7

<sup>1</sup> Average of combined loss of five pieces for each of six replications.

Table 5. Weight Loss of Cooked Broccoli.<sup>1</sup>  
(% of fresh weight)

Replication	Cooking Method	
	Microwave	Conventional
1	29.3	12.4
2	28.7	12.5
3	28.7	9.5
4	25.0	10.5
5	27.5	13.4
6	<u>21.6</u>	<u>7.6</u>
Average	26.8	11.0

<sup>1</sup> Average of combined loss of five pieces for each of six replications.



that the intercellular air in the tissue was being replaced by another gas, possibly steam, which maintained the buoyancy of the half instead of by water which would permit it to sink.

### Content of Pectic Substances

Carrots. Table 6 gives the average content of anhydrogalacturonic acid in three fractions of pectic substances extracted from carrots. All values were expressed as percentage of fresh weight.

Table 6. Content of Anhydrogalacturonic Acid in Three Fractions of Pectic Substances from Carrots.<sup>1</sup>  
(% fresh weight)

Sample	H <sub>2</sub> O extraction	(NaPO <sub>3</sub> ) <sub>6</sub> extraction	NaOH extraction	Total
Raw	0.10	0.27	0.34	0.71
Cooked				
Microwave	0.21	0.24	0.12	0.57
Conventional	0.18	0.28	0.08	0.53

<sup>1</sup> Average of two subsamples for each of six replications.

Raw carrots had a total content of pectic substances of 0.71% of the fresh weight. Of this amount, 0.10% was in the water-soluble fraction, 0.27% was in the sodium hexametaphosphate-soluble fraction, and 0.34% was in the sodium hydroxide-soluble fraction. Carrot halves cooked by microwaves had a total of 0.57% pectic substances. This was the sum of the water-soluble fraction (0.21%), the sodium hexametaphosphate-soluble fraction (0.24%) and the sodium

hydroxide-soluble fraction (0.12%). Carrots cooked conventionally contained 0.18% pectic substances in the water-soluble fraction, 0.28% in the sodium hexametaphosphate-soluble fraction, and 0.08% in the sodium hydroxide-soluble fraction, adding to a total of 0.53% pectic substances. These results are depicted graphically in Figure 13.

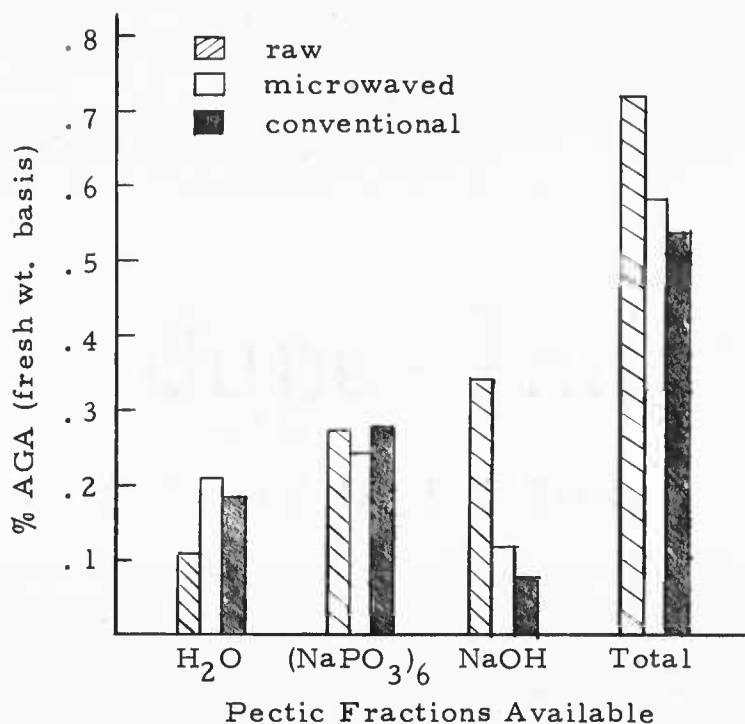


Figure 13. Content of Anhydrogalacturonic Acid in Three Fractions of Pectic Substances from Carrots.

Because most vegetables are used at an immature stage in their growth, enzyme-catalyzed reactions to solubilize the pectic substances of the middle lamella have not yet occurred. As would be expected, the raw tissue contained a high proportion of water-insoluble pectic

substances and a small proportion that were water-soluble. During cooking, the water-soluble fraction increased and total content of pectic substances decreased, as was previously reported by Simpson and Halliday (1941). Both methods resulted in more water-soluble fraction, i. e., pectins, microwaves more than conventional, and both methods decreased the sodium hydroxide-soluble fraction, presumed to be mainly protopectin, conventional more than microwaves. These differences were statistically significant (Table 7). It appears that microwave cooking resulted in less hydrolysis of pectic substances as evidenced by greater total pectic substances and significantly more pectic substances in both the pectin and protopectin fractions. Although there are some hydrogen bonds holding pectic molecules together, they are not the predominant bonding force so the capability of microwaves to break hydrogen bonds is probably of small importance in causing hydrolysis. Assuming hydrolysis is dependent mainly upon length of exposure to heat, then it is reasonable that the longer cooking period for the conventional method would result in greater hydrolysis of the protopectin and also greater hydrolysis of water soluble pectins to non-colloidal molecules which would result in a lower total pectic content. This theory might be tested by exposing a vegetable slurry to microwaves at controlled temperatures similar to the work done with thiamine.

Table 7. Analysis of Variance for Pectic Analysis.

Variable	Degrees of Freedom	Random Error	F test*	
			Carrots	Broccoli
H <sub>2</sub> O Fraction				
raw/cooked	2	27	67.31	148.58
top/bottom	1	27	0.13	1.06
replications	5	27	14.62	3.51
raw: top/bottom	1	50	14.22	8.68
raw: replications	5	50	9.63	1.75
micro/conv	1	16	43.66	4.03
method: top/bottom	1	16	3.66	0.81
method: replications	5	16	63.27	7.46
(NaPO <sub>3</sub> ) <sub>6</sub> Fraction				
raw/cooked	2	27	1.85	15.69
top/bottom	1	27	3.90	20.14
replications	5	27	4.45	0.61
raw: top/bottom	1	50	10.34	79.47
raw: replications	5	50	13.31	0.69
micro/conv	1	16	3.67	0.26
method: top/bottom	1	16	1.86	4.92
method: replications	5	16	3.75	0.90
NaOH Fraction				
raw/cooked	2	27	250.68	17.26
top/bottom	1	27	0.62	0.61
replications	5	27	13.03	0.42
raw: top/bottom	1	50	8.48	1.28
raw: replications	5	50	39.70	1.34
micro/conv	1	16	12.93	5.93
method: top/bottom	1	16	0.00	0.06
method: replications	5	16	6.86	1.02

\* Minimum F values for significance at the 95% or 99% level are as follows:

	F(1.27)	F(2.27)	F(5.27)	F(1.50)	F(5.50)	F(1.16)	F(5.16)
95%	4.21	3.35	2.57	6.61	5.05	4.49	2.85
99%	7.68	5.69	3.78	16.26	10.97	8.53	4.44

Broccoli. The average content of anhydrogalacturonic acid in three fractions of pectic substances from broccoli is given in Table 8.

Table 8. Content of Anhydrogalacturonic Acid in Three <sup>1</sup>  
Fractions of Pectic Substances from Broccoli.  
(% of fresh weight)

Sample	H <sub>2</sub> O extraction	(NaPO <sub>3</sub> ) <sub>6</sub> extraction	NaOH extraction	Total
Raw	0.02	0.16	0.24	0.42
Cooked				
Microwave	0.11	0.26	0.18	0.55
Conventional	0.12	0.25	0.17	0.54

<sup>1</sup> Average of two subsamples for each of six replications.

The total value for raw broccoli was 0.42% of fresh weight. Of this amount, 0.02% was water-soluble, 0.16% was soluble in sodium hexametaphosphate, and 0.24% was soluble in sodium hydroxide. Broccoli cooked by microwaves contained 0.11% anhydrogalacturonic acid in the fraction soluble in water, 0.26% in the fraction soluble in sodium hexametaphosphate, and 0.18% in the fraction soluble in sodium hydroxide. This added to a total of 0.55%. Broccoli cooked conventionally contained 0.12% in the fraction soluble in water, 0.25% in the fraction soluble in sodium hexametaphosphate, and 0.17% in the fraction soluble in sodium hydroxide, adding to a total of 0.54%. These results are depicted graphically in Figure 14.

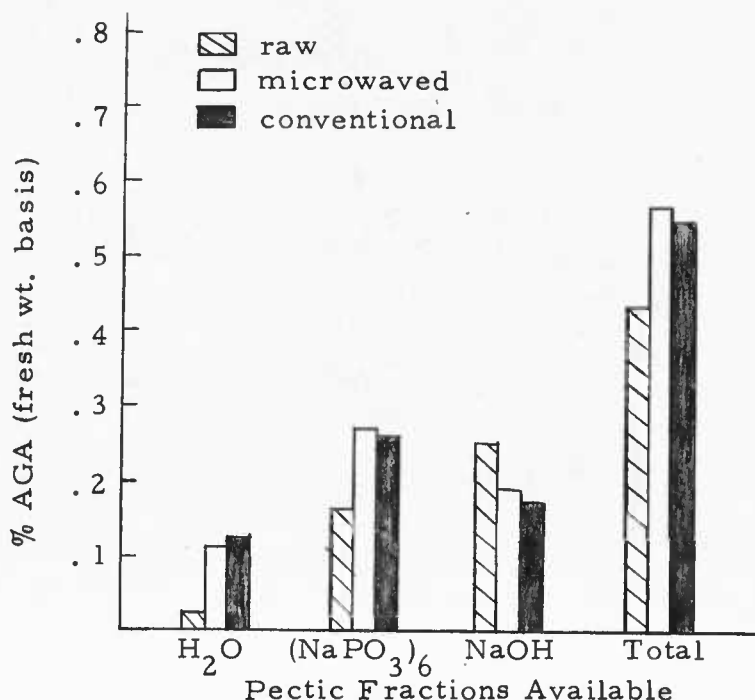


Figure 14. Content of Anhydrogalacturonic Acid in Three Fractions of Pectic Substances from Broccoli.

As in carrots, raw broccoli had a higher content of pectic substances which were insoluble in water than ones which were soluble. The water-soluble pectic substances in the cooked tissue increased at the expense of the sodium hydroxide-soluble pectic substances. Only in the sodium hydroxide-soluble fraction was there a significant difference due to method of cooking (Table 7). The results also indicated more total pectic substances in the cooked broccoli than in the raw. No mechanism is known for degrading or otherwise converting other carbohydrate constituents of the cells to pectic substances by heating. The results suggest incomplete extraction of the pectic substances from the raw tissue. Subsamples of raw and cooked broccoli taken

exactly opposite each other from the same stalk were analyzed. The results of this trial indicated that although the total pectic substances extracted from cooked tissue was greater than that from the raw, an average of 85% or more of the amount removed by three extractions was present in the first two extractions. This was true for each of the three solvents. These results indicated that the problem lies in the extractability of the pectic substances in the raw tissue rather than saturation of the extractant.

In reviewing the nature of protopectin, Joslyn (1962) pointed out that any of the explanations for the insolubility of protopectin are only theoretical. However, in different plants or different parts of the same plant, the forces which chemically bond or physically anchor the pectic substances may differ. Because of this, the pectic substances of some plants such as sugar beets are not as readily extracted as those of others, such as apple or citrus. It is most likely the protopectin which was not completely removed from the broccoli, because this is the most tightly bound pectic substance. Heat has a limited effect on cellulose and hemicelluloses of the cell wall but it could break the bonds joining them to pectic substances. Heat also has a depolymerizing effect on pectic molecules, and breaking glycosidic bonds of the protopectin could account for the increase in measurable pectic substances after cooking.

Although this method of extraction is recommended for estimation of pectic content of foods, it obviously has limitations. However, an exhaustive method of extraction with heat would cause extensive depolymerization and other chemical modification. No other method can fractionate pectic substances into groups according to solubility similar to that existing in the tissues. The cold extraction method does give an indication of the degree of insolubility of the pectic substances in broccoli and perhaps the extent to which they are tightly incorporated into the plant structure. For a more complete analysis, another method of extraction to measure total pectic content of the raw tissue would be necessary.

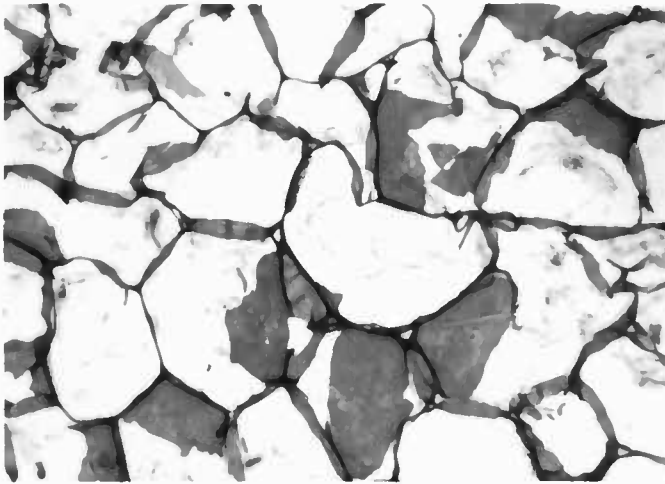
Although there were some significant differences in the content of pectic substances in vegetables cooked by each method, they do not seem great enough to account for the differences in textural changes observed by the sensory evaluation panel. There seems to be no way to distinguish subjectively between firmness of texture due to those pectic substances in the middle lamella which form insoluble salts with calcium or magnesium and that due to those pectic substances in the primary wall classified as protopectin. For carrots the total of the two fractions of insoluble pectic substances was the same for both methods. The higher total amount of pectic substances remaining in carrots cooked by microwaves was primarily water-soluble pectic substances so this would not contribute to greatly increased adhesion.



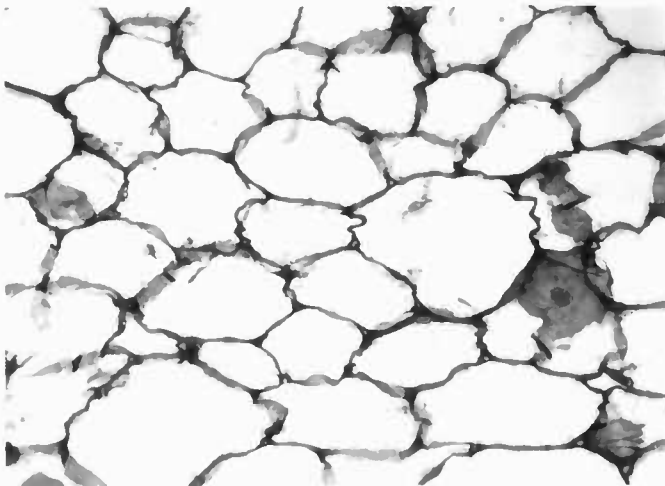
In broccoli cooked by microwaves there was slightly more insoluble pectic substances. It is unlikely that this alone is responsible for the textural differences observed. However, the concentration of the pectic substances in the walls of collenchyma cells near the skin in broccoli may have an effect on adhesion of the outer layer. In any case, the evidence is not substantive enough to say that in any of the differences, the method of heating rather than conditions inherent in the method such as length of cooking time or difference in temperature is the factor.

### Cell Structure

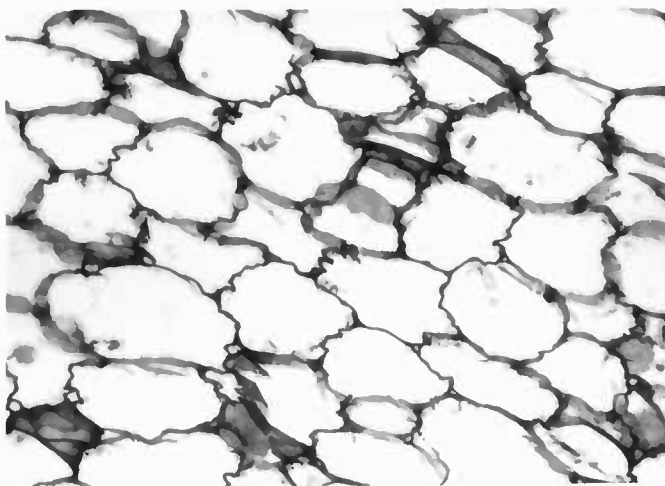
Raw broccoli. Photomicrographs showing the cell structure and contour of broccoli and carrots in both the raw and cooked state are shown in Figures 15 and 16, respectively. In raw broccoli several cell types were evident. The skin was one layer of small rectangular shaped cells. Next to the epidermal cells were collenchyma cells which formed a wide tissue area towards the bottom of the stalk and thin towards the top. These cells appeared quite round with very heavily thickened walls. Inter cellular spaces were most evident here. Cambium cells and bundles of conductive tissue formed a ring just inside the collenchyma tissue. The cells in this area were very small except for the large, round, lignified vessel elements. The tissue from the cambium and conductive ring towards the center of the stalk



Raw Broccoli



Broccoli Cooked Conventionally



Broccoli Cooked by Microwaves



Figure 15. Cell Structure (41X) and Cross Section (2X) of Broccoli.

was made up of thin walled parenchyma cells which increased in size towards the center. These were by far the largest cells in the broccoli tissue. They were irregularly shaped and intercellular spaces were less evident. The parenchymatous tissue seemed to undergo some rupture of cell walls during fixing and dehydration. Possibly vacuum infiltration was the cause, because it was evident to some extent in all the stalks observed and there was no indication of tearing during sectioning.

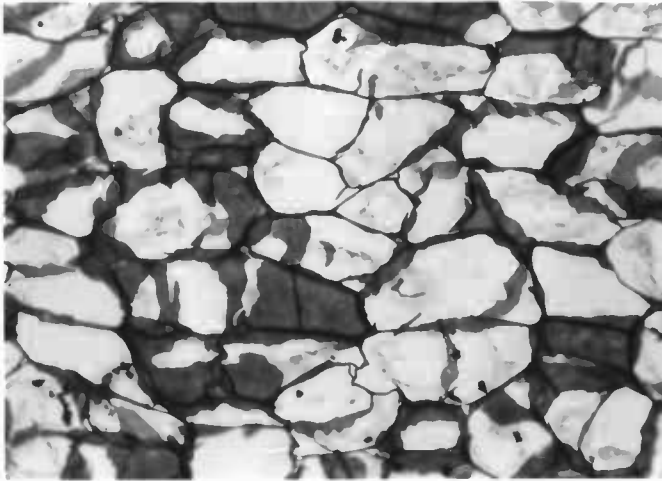
Cooked broccoli. In the conventionally cooked broccoli tissue (which did not need vacuum infiltration) there was little evidence of rupture of cell walls. Occasionally a radial split developed in the parenchymatous flesh, but this was between cells. The cell walls of both collenchymatous and parenchymatous tissues appeared thinner and cell size was somewhat diminished as was indicated by wrinkling of the walls of the parenchyma cells. The walls of collenchymatous and conductive tissue remained relatively smooth and unwrinkled.

Cooking by microwaves also resulted in thinner cell walls than in the raw broccoli tissue. The parenchyma cells were more collapsed than in conventionally cooked tissue and had predominant folding of the cell wall. The collapse of cells was especially extensive along the radial fissures which occurred abundantly between cells in broccoli cooked by microwaves. The collenchyma cells assumed more of an oval shape but the walls were still intact and smooth rather than folded.

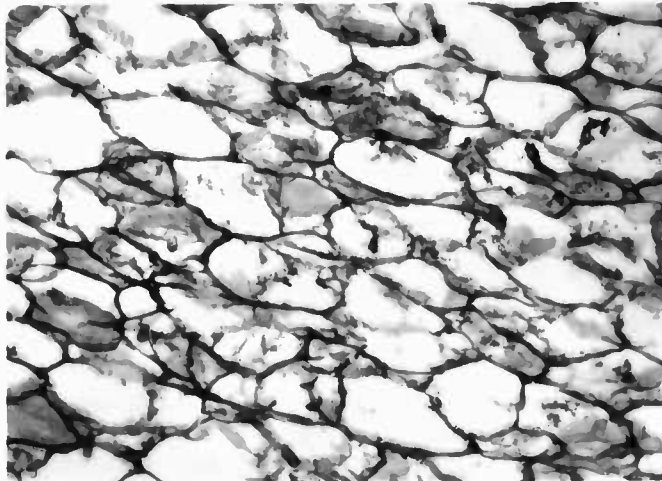
Occasional separations between cells' did occur in the collenchymatous tissue, although these were not nearly as long or wide as those fissures which occurred in the parenchymatous tissue. The conductive tissues were also relatively unaffected by cooking, as would be expected due to their lignin content. However, the small cells surrounding the vessel elements did appear to have folds in the cell wall when microwave were used.

Raw carrots. The raw tissue of carrots was predominantly parenchyma cells in both the outer layers and the very inner circle of the core. The parenchyma cells were very large, with those of the central core the largest. Because the carrots had been scraped, no skin layer was evident. Rays containing vessel elements penetrated both inward and outward from the cambium layer which differentiated the core from the flesh. No collenchymatous tissue was present and intercellular spaces were neither large nor abundant.

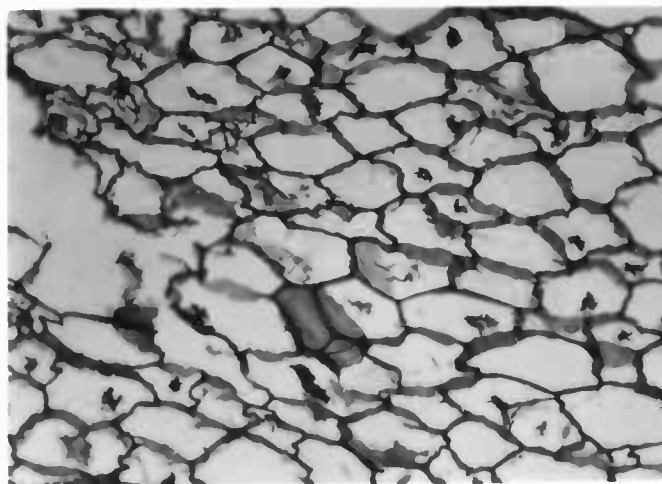
Cooked carrots. The parenchyma cells of carrots cooked conventionally appeared much the same as did those of the broccoli flesh. The cells in the outer layer showed thinner walls and slight reduction in size with corresponding wrinkling of the walls. The parenchyma cells of the core seemed less affected by cooking although this may be due to the presence of lignified conductive tissues which would tend to hold the other cells in place.



Raw Carrots



Carrots Cooked Conventionally



Carrots Cooked by Microwaves



Figure 16. Cell Structure (41X) and Cross Section (2X) of Carrots.

Cooking by microwaves caused much more extensive collapse of cells and radial fissures between cells. Occasionally the fissures passed through the cambium and into the core parenchyma. Cells along these splits were almost entirely collapsed. In all parenchyma cells the walls were more folded and cell area smaller when cooked by microwaves than when cooked conventionally.

The results of several of the tests indicated that a more advanced state of dehydration occurred in vegetable tissue cooked by microwaves. The measurable water loss was more than double that which was lost during a longer cooking period by conventional boiling. As a result, the contour of the vegetables was quite shrunken and the walls of cells of parenchymatous tissues folded as the cell collapsed. Both the contour of the piece and the cell structure correspond to physical changes occurring in dehydrated vegetables. Reeve (1942, 1943) reported that as more of the water that holds the walls apart was removed during dehydration, the highly vacuolated parenchyma cells of carrots collapsed until they were indistinguishable. Photomicrographs of carrots (Reeve, 1942) at different stages of dehydration show cell structure comparable to that occurring in the carrots cooked by microwaves. Van Arsdel (1963) reported that surface cells are the first to diminish in volume and collapse. As drying continues, corners become dry and firm while the center is still losing moisture. This results in concave curvatures on the cut surfaces. The more rapidly dehydration

occurs, the more shrunken the final form of the piece. This was apparent in the contour of both broccoli and carrots cooked by microwaves. In carrots, the lignified xylem cells in the rays help maintain less severe shrinkage there than in the outer cylinder (Reeve, 1943). Huxsoll and Morgan (1968) reported that the use of microwaves for dehydration of potato and apple pieces caused fissures to develop due to internal vapor pressure. As drying proceeded, further shrinkage occurred, resulting in an open porous structure.

The connection between dehydration and the less tender texture observed in the vegetables is most likely due to changes in the crystallinity of the carbohydrate gels. The extent of increase in crystallinity which occurs during dehydration is dependent upon both the rate and extent of removal of water and the physical state of the polysaccharide gel prior to dehydration. Not only can the proportion of crystalline micelles of cellulose increase but formerly amorphous hemicelluloses and pectic substances can become crystalline (Sterling, 1963). Increased crystallinity imparts increased mechanical strength or toughness to tissues. Although increased adhesion due to the greater retention of pectic substances in broccoli and carrots cooked by microwaves was discounted as the major cause of toughness, increased crystallinity of these pectic substances could accentuate their effect on texture. X-ray diffraction techniques would be necessary to assess the degree of crystallinity in each of the carbohydrate constituents

and therefore the responsibility for increased toughness in carrots and broccoli cooked by microwaves.



## SUMMARY

Differences in textural characteristics of broccoli and carrots cooked by microwaves and conventional boiling were identified by a sensory evaluation panel. Cell structure, water loss during cooking, and concentration of three fractions of pectic substances were examined as factors responsible for the textural differences. The results were as follows:

1. Halves of carrots cooked by microwaves appeared much more shrunken than did corresponding halves cooked conventionally. The same was true for halves of stalks of broccoli.
2. Carrots cooked by microwaves were less tender than those cooked conventionally in both the core and exterior flesh areas. The core was more fibrous and the outer flesh more spongy. Broccoli cooked by microwaves was less tender in the exterior layer but softer in the interior flesh than that cooked conventionally.
3. Carrots were judged unacceptable 58% of the time when cooked by microwaves and 4% of the time when cooked conventionally. Only the broccoli cooked by microwaves was judged unacceptable (67%).
4. Weight loss for both vegetables cooked by microwaves was more than double that which occurred during conventional cooking.
5. Both cooked vegetables contained more water-soluble pectic substances and less sodium hydroxide-soluble pectic substances than

did the raw tissue. In comparison to conventional cooking, cooking by microwaves resulted in more sodium hydroxide-soluble pectic substances for both vegetables and also more water-soluble pectic substances in carrots. Cooking method made essentially no difference in the concentration of combined non-water-soluble pectic substances.

6. Cooking by microwaves resulted in greater collapse of the parenchyma cells and extensive radial fissures between almost completely collapsed cells in both broccoli and carrots.

These results suggest that the toughening effect of microwave cooking is due to dehydration as evidenced by a twofold loss of water in the vegetables so cooked, extensive radial fissures, and marked collapse of cells.

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