with more glycerol concentrations than were used in the rest of the work. Although the authors have no explanation for this abnormal solubility effect, they have enough supporting data to be convinced that it is real and not merely a consequence of experimental error.

The tightness of the sleeve stoppers of the solution bottles was demonstrated by making Karl Fischer moisture determinations on four samples that had been immersed in the constant temperature bath for 46 days at 15° C. After deducting the dextrose, as determined with the saccharimeter, the water content of the solvent was found to be unchanged.

Sample	*a*	*b*	*c*	*d*
Original water concentration, %	75.1	49.9	37.4	25.1
Water by analysis after 46 days, %	75.0	51.1	37.65	25.0

Acknowledgment

The pure dextrose used in this work was generously supplied by George R. Dean, Corn Products Refining Co.

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Received for review April 13, 1953. Accepted June 22, 1953. Work sponsored by the Glycerine Division, Association of American Soap and Glycerine Producers, New York, N. Y.

ENZYME INACTIVATION

Relation of Rates of Inactivation of Peroxidase, Catecholase, and Ascorbase to Oxidation of Ascorbic Acid in Potatoes and Parsnips

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This study was undertaken to show whether the rate of enzyme inactivation could be correlated with ascorbic acid retention in cooked foods. Potatoes and parsnips were heated to various internal temperatures between 25° and 90° C. Each temperature was attained by four methods of cooking—pressure-cooking, steaming, boiling, and baking. At each temperature, potatoes were assayed for catecholase activity and parsnips for ascorbase and peroxidase activities; ascorbic acid was determined in both vegetables at each temperature. Statistical analysis of the data indicated that pressure-cooking caused a more rapid rate of enzyme inactivation than did baking; steaming and boiling caused intermediate rates which did not differ significantly from each other. The rate of enzyme inactivation, however, could not be related to the amount of ascorbic acid retained in either cooked potatoes or parsnips.

TWO TYPES OF OXIDATION OF ASCORBIC ACID in foods account for losses of this nutrient—autoxidation, catalyzed chiefly by copper but also by other metals and by metal complexes; and enzymatic oxidation, catalyzed by peroxidase, polyphenolases, ascorbase, and the cytochrome system. This study is concerned with the enzymatic oxidation of ascorbic acid in potatoes and parsnips during cooking.

The role of enzymes in relation to ascorbic acid retention has been investigated in cooked vegetables, in various fruits which show discoloration on injury, and in fruits and vegetables prepared for freezer storage (*1, 5, 8*). Green beans blanched for varying lengths of time at a low temperature retained less ascorbic acid than did similar beans blanched at higher temperatures (*2*). In experiments in which the enzyme activities and ascorbic acid content of various vegetables were determined before and after blanching for three different periods, and after freezer storage for varying lengths of time, no relationship was shown between enzyme activity and vitamin content of the frozen vegetables (*3*).

The present study is concerned with the difference in the rates of enzyme inactivation resulting from cooking potatoes and parsnips by various methods, and the relationship between rates of enzyme inactivation and retention of ascorbic acid. In this study the following have been determined: the catecholase activity of potatoes, the ascorbase and peroxidase activities of parsnips, and the ascorbic acid content of both vegetables cooked to various internal temperatures. As the cytochrome system is not an important terminal oxidase system in plants (*4*), it has not been considered in this study. The cooking methods for both vegetables were pressure-cooking, steaming, boiling in a covered saucepan, and baking.

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Experimental Methods

Selection and Storage Of Vegetables Potatoes were purchased in two lots. Bliss Triumph redskin potatoes were obtained on the day of harvest from a Gary, Ind., potato farm in October 1951 and tested during the following 3 months. The second lot consisted of South Dakota redskins that were purchased in February 1952 from a local Chicago market; these were assayed in the following 2 months. Parsnips harvested the previous autumn were obtained from a local wholesale market in Chicago in May 1952, and all determinations with parsnips were completed by July 1952. Upon delivery to the laboratory, the vegetables were sorted by size and weight, and were stored at 10° C. until the time of assay.

Table I. Average Cooking Times Required for Potatoes to Reach Several Internal Temperatures by Different Methods of Cooking

Cooking Method	Temp. of Raw Potato, ° C.	Temp. of Done Potato, ° C.	Wt. of Potato, G.	Time Required, Min., to Reach 25°	30°	35°	40°	45°	50°	55°	60°	70°	Doneness
Pressure-cooked	16	85	139	2.8	4.0	4.9	5.2	6.1	6.0	6.4	..	..	12.6
Steamed	18	90	114	5.1	6.6	7.1	8.1	9.7	10.1	11.3	12.6	15.5	27.5
Boiled	17	90	158	5.6	6.9	8.2	9.5	10.6	11.9	13.1	14.6	17.9	29.3
Baked	16	90	202	8.8	10.9	12.7	14.4	16.1	17.8	19.7	..	..	38.3

The potatoes kept very well under these conditions, but the parsnips underwent considerable dehydration.

Preparation and Cooking Of Vegetables

Before being weighed and cooked, the potatoes and parsnips were rinsed with redistilled water and wiped dry. Stems and tips of parsnips were removed with stainless steel knives. Potatoes were cooked whole in their skins. Chicago tap water was used for cooking methods that required water.

Steaming was done in a 5-quart, aluminum steam cooker, the lid of which was equipped with a thermocouple wire extending 6 inches into the upper part of the cooker. The lower part of the steam cooker was also used for the boiling method, as the especially equipped lid fitted tightly on both parts of the utensil. The amount of water used for boiling varied with the size of the potatoes or parsnips, but the vegetables were always half-covered with water. Pressure-cooking was done at 15-pound pressure in a $2^1/_2$-quart cast aluminum pressure saucepan, the lid of which was equipped with a thermocouple wire extending 3 inches into the saucepan. A rack, $^1/_4$ inch high, was placed in the saucepan, and the smallest possible volume of water was used to prevent scorching before the end of the cooking period. Baking was done in a gas oven preheated to 425° F. and maintained at this temperature throughout the baking period.

Both potatoes and parsnips were cooked to a series of internal temperatures, which were measured by a Leeds and Northrup Precision potentiometer, using a thermocouple consisting of 30-gage iron and constantan wires.

For the determination of catecholase activity, steamed and boiled potatoes were heated to internal temperatures of 25°, 30°, 35°, 40°, 50°, 55°, 60°, and 70° C. The average cooking times required for potatoes to reach the different temperatures are recorded in Table I. With each temperature series, an unheated vegetable was assayed to give the enzymatic activity of the raw vegetable. For an experiment, 12 potatoes were matched for shape and for weight to within ±2.5 grams. One was withheld as the raw sample. The other 11 were weighed precisely and put into the top of the steam cooker, or into rapidly boiling water, the thermocouple having previously been inserted into one of the 11 and the converted e.m.f. recorded as the temperature of the raw sample. During the cooking process, all potatoes were assumed to be at the same temperature, and as the various desired temperatures were reached in the potato containing the thermocouple, a potato was taken out without disturbing the potato containing the thermocouple. The entire experiment was repeated nine times for steamed potatoes and nine times for boiled potatoes.

The temperature series for the baked and pressure-cooked potatoes consisted of 25°, 30°, 35°, 40°, 45°, 50°, and 55° C. The sampling procedure for the baked potatoes was the same as for steamed and boiled potatoes. However, in the pressure saucepan, for each temperature, two potatoes were put into the

Figure 1. Ascorbic acid content and enzyme activity of potatoes as affected by pressure-cooking to various internal temperatures

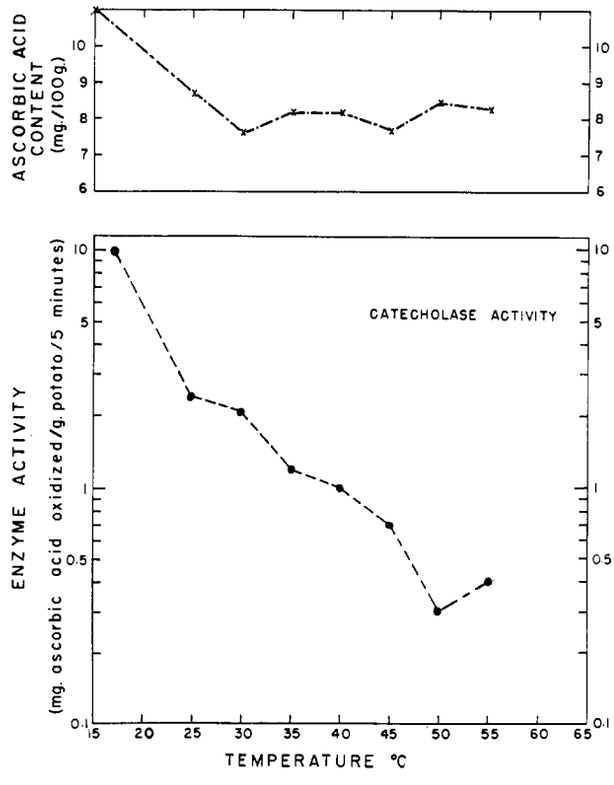
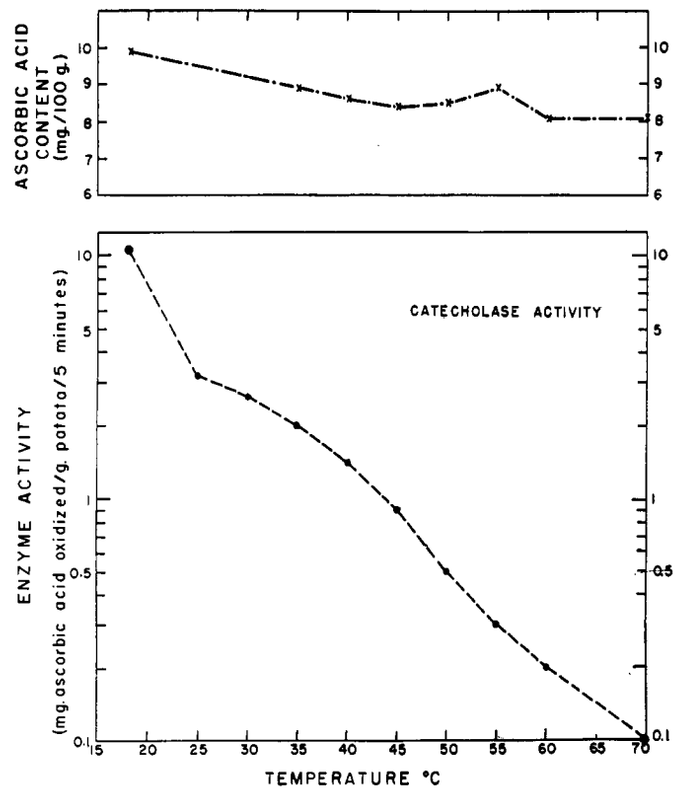


Figure 2. Ascorbic acid content and enzyme activity of potatoes as affected by steaming to various internal temperatures



saucepan, one to be used as the sample for that particular temperature and the other to contain the thermocouple. The entire experiment was repeated nine times for baked potatoes and eight times for pressure-cooked potatoes.

For parsnips, the temperature series for all four cooking methods consisted of 25°, 30°, 40°, 45°, 50°, 55°, 60°, 80°, and 90° C. Ascorbase activity was not determined at the last three temperatures. The thermocouple was always pushed vertically into the stem end of the parsnip to a depth of 1 to 1.5 inches. Matching the parsnips for shape was more difficult than in the case of potatoes, as there was considerable variation in the tapering of the roots; and to obtain reproducibility in successive runs of a temperature series, preliminary experiments indicated the necessity of inserting the thermocouple directly into each parsnip to be assayed. It was observed frequently that the potentiometer indicated falsely that the parsnip was at the temperature of boiling water or steam. This inaccuracy was due to the presence of a pith cavity through the longitudinal center of parsnips (stored) and to the many air spaces of the cortex. The thermocouple was reset to a different position in the parsnip whenever it was evident that this error existed, or, in the case of pressure-cooking, another parsnip was used.

Ascorbic acid determinations were made on potatoes and parsnips cooked by the same methods and to the same temperature as for the enzyme assays; ten replicate series were used for each cooking method.

Measurement of Enzyme Activities

Preparation of Crude Enzyme Filtrates. As suggested by Morris, Weast, and Lineweaver (*7*), 2% sodium chloride was the extractant for all enzyme determinations. The amount of extractant was adjusted to the size of the sample in the proportion of 100 grams of cold extractant and 1 gram of calcium carbonate for every 50 grams of potato, or for every 25 grams of parsnip. The raw weight of each vegetable was determined before cooking, and this weight was used in all calculations to avoid making moisture determinations, which would otherwise have been necessary because of the variation in moisture content resulting from the different cooking methods. The hot vegetable was weighed directly into a known amount of cold extractant in a blender cup and comminuted 3 minutes for potatoes and 5 minutes for parsnips. The blender was then put into an ice bath for 10 to 15 minutes and the slurry filtered through four layers of cheesecloth. The filtrate was stored at −20° C. for 30 to 45 minutes, at the end of which time catecholase activity was determined in potato filtrates and ascorbase and peroxidase activities were determined in parsnip filtrates. The hot potatoes cooled immediately as they always fell to the bottom of the blender, but because of the air spaces parsnips floated on the extractant and cooling was less rapid.

Catecholase Activity. The method of Ponting and Joslyn (*8*) was used for the determination of catecholase activity. The reaction time was 5 minutes at 25° C., pH 5.0. Suitable quantities of the enzyme filtrate were added to the flasks, varying from 1 ml. for the unheated samples to 10 ml. for samples heated to 70° C. The ascorbic acid concentration at the end of the reaction time was measured spectrophotometrically after 9 ml. of 2,6-dichlorophenolindophenol had been added to 1 ml. of the reaction mixture in a cuvette.

Peroxidase Activity. Peroxidase activity was measured in a manner similar to that for catecholase activity (*8*). The reaction time was 5 minutes at 25° C., pH 5.0. The quantities of enzyme filtrate used varied from 1 ml. for filtrates of raw parsnips to 25 ml. for filtrates of parsnips cooked to 90° C. The peroxidase activities reported for parsnips are the experimental peroxidase activities minus the ascorbase activities at the respective temperatures, since substrates for both peroxidase and ascorbase were present in the peroxidase reaction mixture.

Ascorbase Activity. Ascorbase was determined in a similar manner (*8*). The reaction time for ascorbase was 5 minutes at 25° C., pH 6.0. The quantity of enzyme filtrate used varied from

Figure 3. Ascorbic acid content and enzyme activity of potatoes as affected by boiling to various internal temperatures

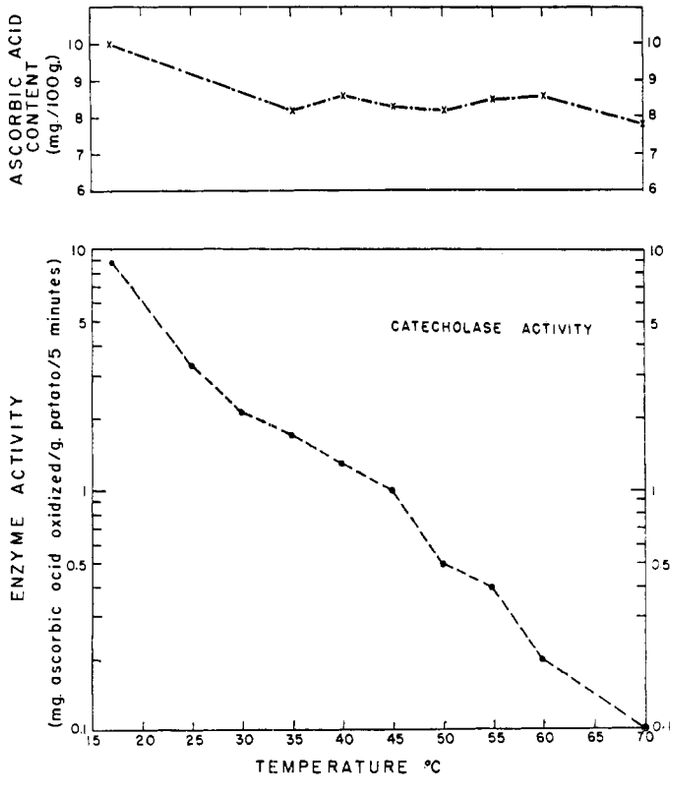
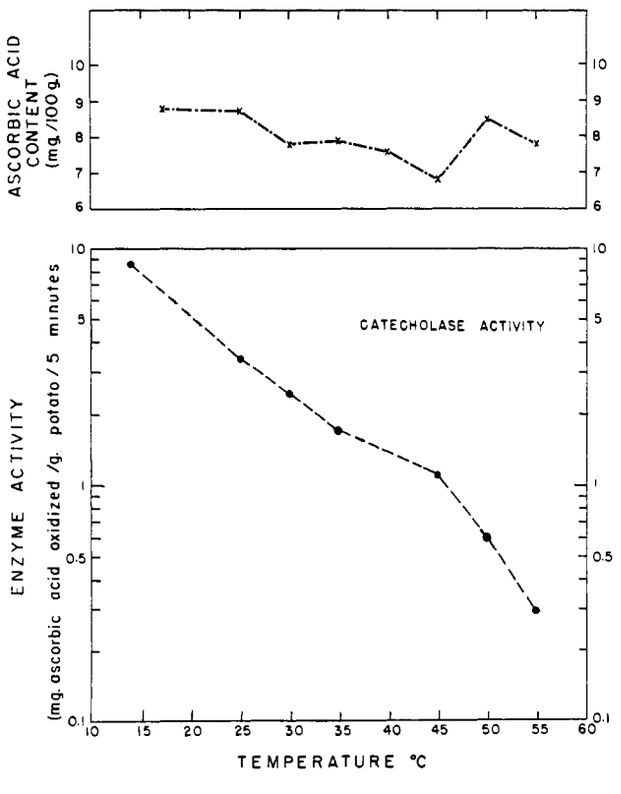


Figure 4. Ascorbic acid content and enzyme activity of potatoes as affected by baking to various internal temperatures



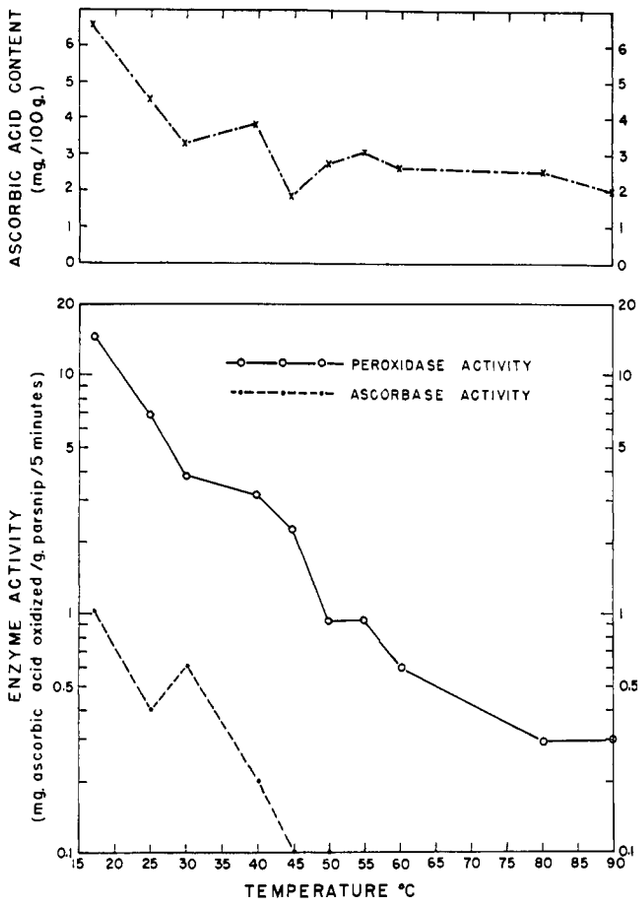


Figure 5. Ascorbic acid content and enzyme activity of parsnips as affected by pressure-cooking to various internal temperatures

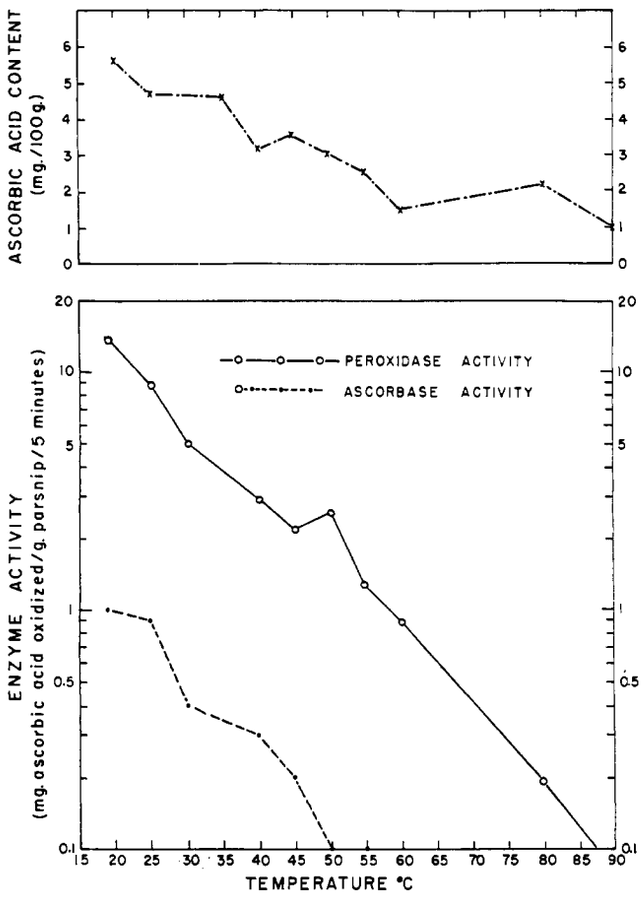


Figure 6. Ascorbic acid content and enzyme activity of parsnips as affected by steaming to various internal temperatures

10 ml. of raw parsnip filtrate to 25 ml. for the filtrate from vegetables cooked to 55° C.

Measurement of Reduced Ascorbic Acid The extractant for ascorbic acid determinations was 3% oxalic acid. Approximately 100 grams of cold extractant per 50 grams of potato, or per 40 grams of parsnip, was weighed into a tared blender. The vegetables were cooked to the various temperatures and comminuted for 5 minutes and the blends were filtered through Whatman No. 12 filter paper. Because these filtrations were slow, they were carried out in the refrigerator at 2° C. Ascorbic acid content was determined by the method of Loeffler and Ponting (*6*), using 1 ml. of the vegetable filtrate and 9 ml. of dye.

Results and Discussion

Effect of Cooking Method On Rate of Catecholase Inactivation in Potatoes. The rates of catecholase inactivation in potatoes heated to various internal temperatures by four methods of cooking are represented in Figures 1 to 4. An analysis of variance in catecholase activities over the range 25° to 50° C. (Table II) showed a significant difference at the 5% level in the rates of catecholase inactivation by the four cooking methods. Comparison of the mean differences between enzyme activities at the various temperatures indicated that inactivation of catecholase was significantly more rapid for pressure-cooked potatoes than for steamed, boiled, or baked potatoes. Among the latter three, there were no significant differences in the rates of enzyme inactivation. The expected decrease in enzyme activities with increasing internal temperatures was shown by the large F value to be significant.

On Rate of Ascorbic Acid Oxidation in Potatoes. The variation in ascorbic acid content of potatoes heated by different methods over the temperature range 25° to 70° C. is represented in the upper portions of Figures 1 to 4. Since the difference in ascorbic acid content of raw potatoes and potatoes heated to the lowest temperature was of greater

Table II. Analysis of Variance

Data on enzyme activity and ascorbic acid content as affected by cooking potatoes by four different methods to various internal temperatures (nine replicates)

Constituent	Temp., ° C.	Source of Variation	Degrees of Freedom	F	Significance
Catecholase	25–50	Cooking method, C	3	4.26	At 0.05 level
		Replication within C	32		
		Temperature, T	5	128.83	At 0.01 level
		$T \times C$	15	1.65	Not significant
		Residual	160		
		Total	215		
Ascorbic acid	17, 35	C	3	1.56	Not significant
		Replication within C	32		
		T	1	17.47	At 0.01 level
		$T \times C$	3	0.89	Not significant
		Residual	32		
		Total	71		
Ascorbic acid	35–55	C	3	1.61	Not significant
		Replication within C	32		
		T	4	1.38	Not significant
		$T \times C$	12	0.56	Not significant
		Residual	128		
		Total	179		

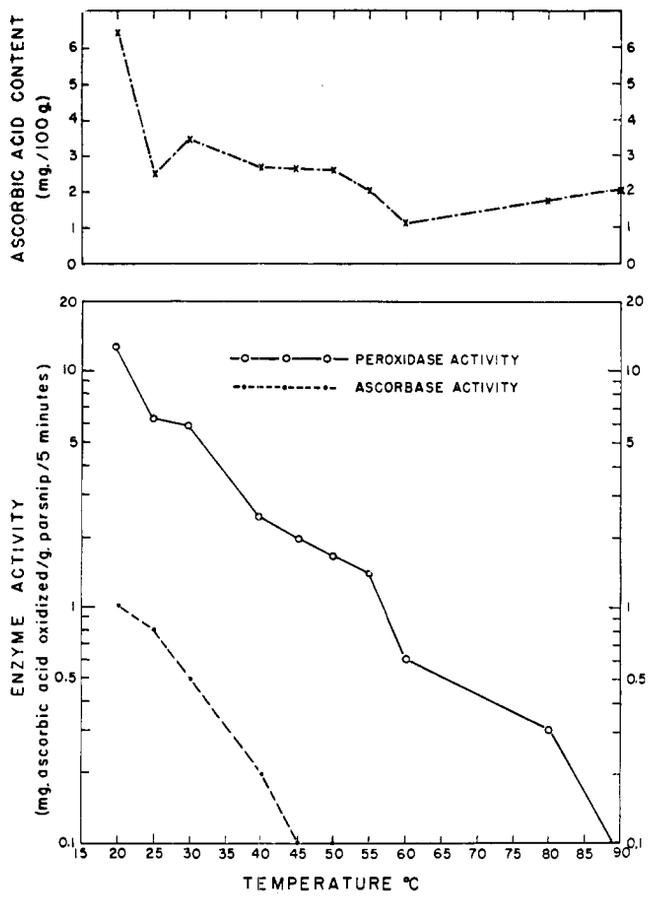


Figure 7. Ascorbic acid content and enzyme activity of parsnips as affected by boiling to various internal temperatures

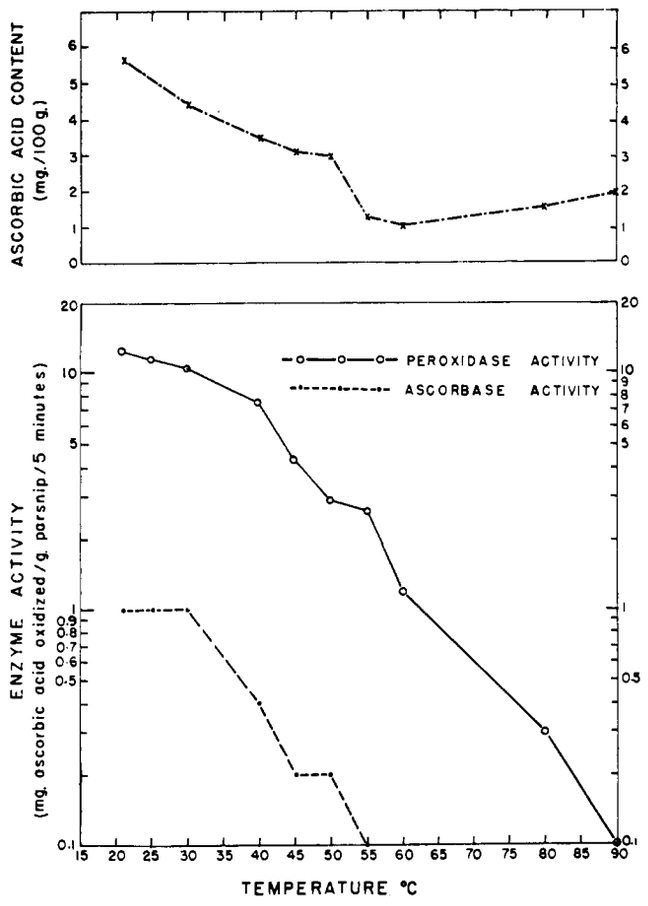


Figure 8. Ascorbic acid content and enzyme activity of parsnips as affected by baking to various internal temperatures

magnitude than that between any other pair of consecutive temperatures, one analysis of variance was made on the data for these first two temperatures and a second analysis on the data over the temperature range 35° to 55° C. (Table II). Neither analysis showed significant differences in the rates of oxidation of ascorbic acid due to different cooking methods. There was a significantly smaller amount of ascorbic acid in potatoes heated to 35° C. than in raw potatoes, but no difference was shown among potatoes heated to temperatures between 35° and 55° C.

On Rate of Enzyme Inactivation in Parsnips. The rates of inactivation of ascorbase and peroxidase are represented in Figures 5 to 8. Ascorbase activity was approximately one tenth the magnitude of peroxidase and catecholase activities. Since at lower temperatures the variation between replicates was considerably greater than at higher temperatures, the ascorbase data were analyzed in two groups, 25° to 40° and 45° to 55° C. (Table III). The rates of peroxidase inactivation were measured and analyzed over the range 25° to 60° C. The analyses showed that the activity of both enzymes decreased significantly with increasing temperature, and that the speed of inactivation of both enzymes differed significantly with the method of cooking, except for ascorbase in the temperature range 45° to 55° C. The difference between cooking methods was due to the slower inactivation of both enzymes in baking than in the other three methods, which did not differ significantly among themselves.

On Rate of Ascorbic Acid Oxidation in Parsnips. The ascorbic acid content of parsnips heated by various methods over the temperature range 25° to 90° C. is shown in the upper portions of Figures 5 to 8. Comparison of the raw parsnips with those heated to 25° C. indicated no difference in the rate of ascorbic acid oxidation resulting from method of cooking (Table III), but there was a significantly smaller amount of ascorbic acid in parsnips heated to 25° C. than in raw parsnips. Over the temperature range 25° to 90° C., the rate of oxidation of ascorbic acid was significantly higher (5% level) in the steamed parsnips than in parsnips cooked by the other methods, but this difference was not certain, because the interaction between cooking methods and temperature was significant at the 1% level. The amount of ascorbic acid in parsnips cooked to various temperatures between 25° to 90° C. showed a significant decrease at the 1% level with increasing temperature.

Comparison of Enzyme Inactivation With Ascorbic Acid Oxidation

The speed of inactivation of catecholase in potatoes was significantly more rapid in pressure-cooking than in steaming, boiling, or baking; whereas, in parsnips, both ascorbase and peroxidase were inactivated significantly more rapidly in pressure-cooking, steaming, and boiling than in baking. The rates of ascorbic acid oxidation within the temperature range studied did not differ among the four cooking methods in either potatoes or parsnips, and hence, no relationship was shown between speed of enzyme inactivation and rate of ascorbic acid oxidation.

As shown in Table IV, most of the loss of ascorbic acid from both potatoes and parsnips occurred by the time the temperature reached 55° C. During this rise in temperature, the enzyme activities were reduced to about one tenth of their original activity. As shown by the average retentions of ascorbic acid in potatoes and parsnips cooked to 55° C., two and one-half times as much of the original ascorbic acid was retained in potatoes as in parsnips. The parsnips were not well preserved during these experiments; this may have been a factor contributing to the low retentions of ascorbic acid in cooked parsnips. The retention at 55° C.

Table III. Analysis of Variance

Data on enzyme activity and ascorbic acid content as affected by cooking parsnips by four different methods to various internal temperatures (ten replicates)

Constituent	Temp., ° C.	Source of Variation	Degrees of Freedom	F	Significance
Ascorbase	25–40	Cooking method, *C*	3	4.91	At 0.01 level
		Replication within *C*	36		
		Temperature, *T*	2	31.04	At 0.01 level
		T × *C*	6	2.91	At 0.05 level
		Residual	72		
		Total	119		
Ascorbase	45–55	*C*	3	1.93	Not significant
		Replication within *C*	36		
		T	2	7.81	At 0.01 level
		T × *C*	6	1.62	Not significant
		Residual	72		
		Total	119		
Peroxidase	25–60	*C*	3	23.54	At 0.01 level
		Replication within *C*	36		
		T	6	20.46	At 0.01 level
		T × *C*	18	1.77	Not significant
		Residual	216		
		Total	279		
Ascorbic acid	20, 25	*C*	3	2.73	Not significant
		Replication within *C*	36		
		T	1	27.29	At 0.01 level
		T × *C*	3	1.88	Not significant
		Residual	36		
		Total	79		
Ascorbic acid	25–90	*C*	3	3.08	At 0.05 level
		Replication within *C*	36		
		T	8	17.47	At 0.01 level
		T × *C*	24	2.08	At 0.01 level
		Residual	288		
		Total	359		

Table IV. Retention of Ascorbic Acid in Potatoes and Parsnips at 55°, 85°, and 90° C.

Cooking Method	Potatoes, %			Parsnips, %	
	55° C.	85° C.	90° C.	55° C.	90° C.
Pressure-cooked	75	73	..	47	30
Steamed	90	..	..	35	22
Boiled	85	..	..	31	31
Baked	89	..	108	25	35
Av.	85	..	..	35	30

for parsnips ranged from 25% for baked parsnips to 47% for pressure-cooked parsnips, whereas the range was from 75% for pressure-cooked potatoes to 90% for steamed potatoes. Preparation of the vegetables for cooking differed in that the tip and stem ends of the parsnips were removed, whereas in all cases the potatoes were cooked in their skins. However, the difference in average vitamin retention for the two vegetables could not be explained on the basis of leaching, as baked parsnips retained less ascorbic acid than did boiled parsnips cooked to 55° C.

While ascorbic acid losses in potatoes and parsnips cooked to an internal temperature of 55° C. were considerable, losses of the vitamin over the range 55° to 90° C. were slight. Retentions of ascorbic acid in parsnips cooked to 90° C. are reported in Table IV. Comparable retentions in potatoes are available only for pressure-cooked and for baked potatoes. Comparison of retentions for parsnips at 55° and at 90° C. shows that a small decrease in ascorbic acid content resulted from pressure-cooking and steaming between 55° and 90° C., boiling caused no change, and baking caused an apparent gain. These data are further indication that leaching was not the reason for low ascorbic acid retentions in parsnips; and they also indicate that both potatoes and parsnips, when baked, show between 55° and 90° C., an apparent gain in ascorbic acid content of 19 and 10%, respectively.

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Received for review April 1, 1953. Accepted June 19, 1953. Data presented by Johnnie H. Watts to the Faculty of the Division of the Biological Sciences of the University of Chicago in partial fulfillment of the requirements for the degree of doctor of philosophy.

FRUIT COLOR LOSS

Effects of Carbohydrates and Other Factors On Strawberry Products

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STRAWBERRY PRESERVES represent about 26% of all fruit preserve flavors, or about 12% of the total production of all fruit spreads, and consequently the largest volume of a single variety of fruit spread in the country.

When strawberry preserves are made by modern vacuum pan methods, the finished product is a bright red or bright maroon-red, depending on the variety of strawberry used. As the preserve is stored at room temperature in a ware-