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THE EFFECT OF VARIOUS HOLDING PERIODS ON THE CATALASE, PEROXIDASE, AND ASCORBIC ACID OXIDASE ACTIVITY OF SEVERAL VARIETIES OF BLANCHED CANNING PEAS

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INTRODUCTION

In recent years a great deal of attention has been given to the nutritive aspects of processed foods. The results of work done on dehydrated foods during World War II have stimulated a reexamination of the common canning practices and procedures.

In 1942, (4, 9) the National Canners Association and the Can Manufacturers Institute set up a jointly sponsored cooperative program with the objectives of establishing the nutritive values of commercially canned foods, determining the factors influencing their nutritive values, improving their nutritive properties, and investigating how well the commercially canned foods meet the human physiological needs. This program is still in operation.

One aspect of the nutritive value of canned foods now being investigated is the role that the various enzymes play in the retention of ascorbic acid during various handling and processing operations. In an effort to determine whether or not there are varietal differences in the enzyme activity of canning peas, a series of experiments were conducted and carried out during the 1948 canning season for peas.

REVIEW OF LITERATURE

"Enzymes in food products are like ants in the kitchen, the greatest problem is how to get rid of them".(3)

Enzymes are organic catalysts produced by living organisms and cells (8, 18). Enzymes are considered by most authorities to be either a protein or to be in colloidal combination or association with a protein (8, 18). Most enzymes are specific in nature, are thermolabile; and are sensitive to acids, alkalies, temperature changes, metal salts, pH, light, and radiation. Enzymes catalyze hydrolytic, oxidation-reduction, and fermentation reactions (8). Catalase (8) is one of the most widely distributed enzymes since it is found in nearly every living cell. Peroxidase (8) is an enzyme that is widely distributed in plants and also occurs in some animal products. Ascorbic acid oxidase is found in plants and particularly in the common vegetable plants (8). The three above mentioned enzymes catalyze oxidation-reduction reactions. Oxidation-reduction enzymes may be classified according to the following bases: 1. the chemical nature of the substrate, 2. the components of complete oxidation systems, and 3. the chemical nature of the prosthetic group (8). According to Green (8), peroxidase and catalase are oxidation enzymes with a Fe porphyrin prosthetic group; and ascorbic acid oxidase may be a copper protein. Both ascorbic acid oxidase and peroxidase are known to oxidize ascorbic acid.

Enzymatic action is involved in the determination of food (2, 3, 5). Many methods have been tried by man in his efforts to preserve food for future use. Modern methods are successful but not completely satisfactory (2). Much of the "Hay-like" and other undesirable odors and flavors of unblanched dehydrated and frozen vegetables

are attributed to enzymatic oxidation by Cruess and Joslyn (5) and Balls (2). Certain vitamin losses and discolorations in dehydrated and frozen vegetables may be caused by enzyme activity. The importance of enzymes in canned foods has not received the attention that it has in frozen and dehydrated foods. This lack of attention is mainly due to the fact that the heat process in canning inactivates the enzymes; therefore, enzymes aren't usually directly connected with the deterioration and spoilage of canned foods. In recent years, however, in investigating the losses in nutritive value of canned foods, attention has been directed toward the extent and causes of nutritive losses during handling and processing operations (6, 9, 10, 11, 13). Results of these studies by Jackson, et al (9), Lamb, et al (11), and others have indicated that considerable losses of ascorbic acid and carotene may take place during handling operations if measures aren't taken to inhibit or destroy enzyme activity early in the processing operation.

Blanching is the most common and practical means at present for inhibiting or inactivating enzyme action in vegetables. Many studies have been conducted on blanching methods and their effect on nutrient losses, and also on the proper times and temperatures to inactivate enzymes (5, 6, 9, 11). In studying the adequacy of the blanching process, tests have been made to determine at what temperature and time the most common enzymes were inactivated. Catalase, peroxidase, and ascorbic acid oxidase are the enzymes most often chosen to determine the adequacy of the blanch (5, 6, 14). Peroxidase has been most frequently selected as the test enzyme because it is generally the most difficult of the three enzymes to inactivate (5,

6, 14). There seems to be an indication that different blanching requirements may be necessary to inactivate this enzyme in the same vegetable under different environmental conditions (6). In general, all three enzymes were found to be inactivated by a blanch of 200°F. for 3 minutes; and in most products, 190-195°F. for 3 minutes was adequate (5, 6).

EXPERIMENTAL MATERIALS AND METHODS

In an effort to duplicate commercial conditions and practices as much as possible, these experiments were carried out at the college canning factory during the regular pea canning operations.

Pride, Canner King, Shasta, Bonneville, and Perfection were the varieties of sweet (wrinkled-seeded) peas used in this experiment. All varieties were grown under the same conditions on the college farm. The peas were cut with a mower equipped with vine lifters and a windrow attachment, loaded by hand onto flat bed wagons, and hauled approximately one half mile from the field to be vined. Immediately after vining, the peas, in lug boxes, were hauled to the canning factory. The time from mowing until the vined peas reached the factory was approximately 2 to $2\frac{1}{2}$ hours.

On receipt at the canning factory, the peas were weighed and run through an air-blast cleaner to remove extraneous and foreign material. The cleaned peas were evenly distributed among six lug boxes, each of which held approximately 30 pounds of cleaned peas. The condition and maturity of the peas were noted. Then the temperatures of the peas in the lug boxes and of the air were noted, and the peas were divided into two lots of three lugs each. One lot was immediately blanched, and the other lot was set aside in the shade of the canning factory porch for approximately four hours. At the end of the holding period, the temperature of the peas in the lugs and the air temperature were again taken before the peas were blanched.

The peas were run over a riffle to remove any pebbles, then through a split pea pod remover and washer and were elevated to the blancher by a gooseneck elevator. The peas were blanched at 200°F. for two minutes, spray cooled in water for a few seconds, and run

over an inspection belt before the sample was removed.

The sample was collected in a stainless steel bucket from the hopper at the end of the inspection belt. Care was taken to get a representative sample of each lot of peas. The temperature of the sample peas in the bucket was noted, and a representative aliquot was taken for measuring the extent of enzyme inactivation. At the end of one-half hour, the temperature was recorded and another sample removed for the enzyme tests. A third sample was taken at the end of two hours, and the temperature was recorded. A sample of the unblanched peas was collected just before the peas were blanched for use as a measure of the fresh activity.

The remainder of the peas were run through the line, filled into number 10 cans along with a salt-sugar brine, exhausted for 8½ minutes to 171 to 176°F., closed, and then retorted for 55 minutes at 240°F. A random can was selected from this group for use as the control sample.

The activities of catalase, peroxidase, and ascorbic acid oxidase were assayed by the following methods. The percent inactivation of catalase was measured by a modification of the Thompson method (17), except that the method of preparation of the sample was modified so that a larger, more representative sample of the lot could be used. A 20 gram sample of the vegetable material was weighed accurately into a Waring blendor bowl containing 100 milliliters of distilled water and 12 grams of precipitated calcium carbonate. The sample was then blended until thoroughly macerated for approximately two minutes. The blended sample was then filtered through four thicknesses of cheesecloth, and the filtrate was refiltered through four

thicknesses of cheesecloth into a 400 milliliter beaker. From here on the procedure followed the method by Thompson.

Originally it was planned to use the Balls-Hale method (1) for determining the peroxidase activity, but the difficulty of getting consistent and reproducible results with it in another experiment led to the adoption of the Lucas-Bailey method (12). The preparation of the sample for the Lucas-Bailey method was modified to make it suitable for use on fresh and blanched vegetables. The sample was prepared in the same way as for the catalase assay; in fact, the same filtrate was used in both assays. The Lucas-Bailey method was further modified in that the filtrate from the sample of raw, unblanched peas was diluted 100 to 1 before assaying.

The method used for assaying the ascorbic acid oxidase activity is essentially the method used by Robinson (16). It is a modification of the Robinson and Stotz indophenol-xylene extraction method for ascorbic acid (15). Due to inexperience and the lack of specific procedure for this method, very poor and unreliable results were obtained. After considerable trial and error, the writer believes that the method will henceforth give fairly reliable results.

RESULTS

The pea canning season at East Lansing began June 26 and lasted through July 9 of this year. The first variety canned was Glacier, but no tests were run. On June 29, the Thomas Laxton variety was canned, and a trial run of tests was made for another experiment. Since the data on Thomas Laxton was incomplete, it is not included in this paper. The regular series of tests were started July 1, with the Pride variety and continued to the end of the season as each succeeding variety was harvested. There were two plantings of Canner King variety harvested on separate dates. The Canner King variety was used in another experiment of which only two treatments were comparable to the treatments reported in this paper.

All varieties of peas were blanched at 200°F. for 2 minutes, then cooled to 105°F. $\pm$ 5° under water sprays. The normal blanching temperature for peas in this plant is 194°F. for 2 3/4 minutes.

As may be noted from Table I, there were no consistent differences in the percent inactivation of catalase or peroxidase, except that from 2 to 6 percent catalase activity was observed, irrespective of variety, in the samples blanched without delay with no holding period after blanching. The degree of catalase inactivation was considerably less for Shasta and Bonneville varieties when the peas were held for 4 hours prior to and two hours following blanching. These two varieties were considerably more mature than the other varieties tested. In general, there was more variability in the degree of catalase inactivation after blanching than in the degree of peroxidase inactivation.

No results are included for the ascorbic acid oxidase assays due to the writer's lack of experience with the method of assay and

mechanical difficulties in obtaining a calibration curve for the colorimeter used to measure the light transmission values. The necessary adjustments and modifications of the method have been made at this time, but the data taken on peas cannot be interpreted since there is no correlation between the results obtained under the original method and the present modifications.

TABLE I. Per Cent Inactivation of Catalase and Peroxidase

Variety Canned	PRIDE		CANNER KING		SHASTA		BONNEVILLE		CANNER KING		PERFECTION	
Date Canned	July 1		July 3		July 6		July 7		July 8		July 9	
Condition & Maturity	Young		Young		Medium Maturity		Medium Maturity		Young		Very Young	
TREATMENT	Catalase %	Peroxidase %	Catalase %	Peroxidase %	Catalase %	Peroxidase %	Catalase %	Peroxidase %	Catalase %	Peroxidase %	Catalase %	Peroxidase %
I. Blanched without delay Holding Period												
0 minutes	96.8	98.9	95.6	99.1	98.1	100	96.3	99.9	95.4	100	93.6	100
30 minutes	100	99.2	93.9	98.6	100	100	96.3	99.5	97.3	99.9	92.3	99.7
120 minutes	100	98.6			97.1	100	100	98.7			100	99.4
II. Blanched after 4 hours' delay Holding Period												
0 minutes	100	99.5			100	100	97.4	99.4			100	99.9
30 minutes	99.6	99.0			97.4	100	98.4	99.4			100	99.3
120 minutes	96.5	99.2			90.5	100	91.3	99.3			100	99.3

Note: Peas blanched at 200°F. for 2 minutes and then immediately water cooled to 105°F. ±5°.

DISCUSSION

In discussing the results obtained in this series of experiments, the first point to be considered is the adequacy of the blanching treatment used. The blanching temperature of 200°F. and time of 2 minutes was chosen to insure an adequate blanch so that any differences observed among the holding treatments could not be attributed to an inadequate blanch. For all practical purposes, the blanch can be considered adequate since the peroxidase assays indicated practically 100 percent inactivation for all treatments and varieties. This is in agreement with the findings of Cruess and Joslyn (5) and Davis, et al (6).

The observed catalase activity in the samples blanched without delay with no holding period after blanching may possibly be due in part to the error in the method and in part to the possibility that the catalase was not yet fully inactivated. Balls (2) states that a high initial temperature followed by lower temperature may serve to inactivate an enzyme. It may be that this happened in these experiments with catalase.

Balls (2) further states that enzymes may be "destroyed" by heat and yet reappear on cooling. The increased activity of catalase in the samples of Shasta and Bonneville varieties that were held four hours before blanching and two hours after blanching may be an example of the regeneration of catalase. This apparent regeneration occurred only in the two varieties that were most mature at harvest and consequently contained larger peas than the other varieties. The larger size peas might possibly have had some residual catalase enzyme that was only temporarily inactivated by blanching.

Though no results were obtained from the ascorbic acid oxidase

assays, it may be assumed that the blanching treatment was adequate to insure the inactivation of the ascorbic acid oxidase since Davis, et al (6) found that ascorbic acid oxidase was inactivated for most products in about a third of the time that it took to inactivate peroxidase.

Greater differences in treatments and more regeneration might have been obtained in these experiments if a lower blanching temperature had been selected.

SUMMARY AND CONCLUSIONS

The problem of the losses of vitamins and other nutrients during the processing of canned foods is the object of investigation at this and other institutions. The role that enzymes play in these losses is one aspect of the problem now being investigated. This experiment was initiated in an effort to learn whether there are any significant differences in enzyme activity between varieties of canning peas. Pride, Canner King, Shasta, Bonneville, and Perfection canning varieties of peas, grown on the college farm, were used in this experiment.

Customary methods were employed in harvesting, vining, and cleaning the peas. Approximately six lugs of well mixed peas were divided into two lots, one of which was set in the shade for four hours before processing;and the other lot was immediately blanched at 200°F. for 2 minutes and then water-cooled. Samples were taken of the unblanched, blanched, and canned peas. Catalase and peroxidase assays were run immediately on all samples, and at the end of one-half and two hour holding periods for the blanched samples. These assays were repeated for the lot of peas that was held four hours in the shade.

The results obtained indicated that there is no significant differences between treatments or varieties under the conditions of this experiment. It is concluded that for the varieties of peas used in this experiment, there are no apparent differences in enzymatic activity of the adequately blanched peas and very little apparent regeneration of enzymes even under conditions of delay seldom encountered in commercial canning.

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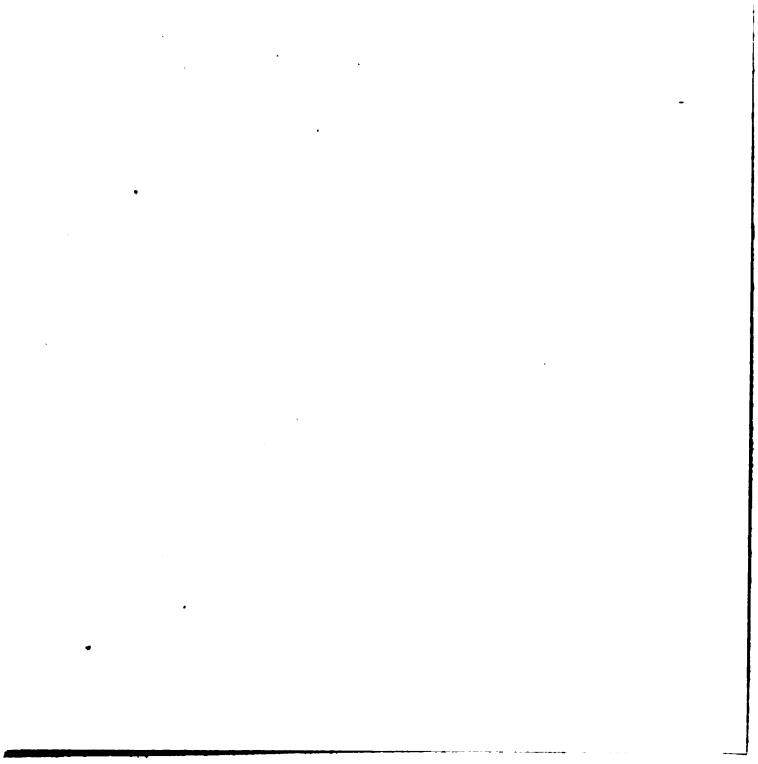


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