ditions employed. The fastest migrating band had an electrophoretic mobility of 4.80 sq. cm. volt^{-1} sec.$^{-1}$ $\times$ 10^{-5}. Fractionation with ammonium sulfate (50 to 60% cut) removed bands 4, 6, 8, and 9. Further fractionation of the sample with zinc-ethanol (fraction A) removed all proteins except those migrating as bands 10 and 11. Protein in band 11 was extremely low in concentration compared to that in band 10, but was present in all fractions having cathepsin activity. Attempts to remove protein from the gel to determine which of the two bands contained the active enzyme were unsuccessful.

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SWEET POTATO AMYLASE

New Characteristic Alpha-Amylase in Sweet Potatoes

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A new amylolytic enzyme has been demonstrated in raw sweet potato juice. Its dextrinizing activity places it with the α-amylases. Among its unusual characteristics were high optimum activity temperature, heat stability, and low activity at ordinary temperatures. The optimum temperature was 70° to 75° C. The enzyme showed maximum resistance to heat inactivation at pH 6.0, while a much wider range of pH (3.6 to 8.1) was tolerated at 30° to 45° C. At these temperatures, however, the activity was low. Freshly harvested sweet potatoes contain relatively small amounts of this enzyme, which increases about sixfold after 9 months' storage. Unlike the sweet potato β-amylase, this enzyme is distributed almost uniformly throughout the inner tissues of the root and is more soluble in water than in sweet potato juice.

The occurrence of an active diastase in sweet potatoes was clearly shown in 1920 by Gore (*7*), who demonstrated that slow cooking of sweet potatoes through a range of 60° C. to the boiling point gave a very high conversion of starch into soluble carbohydrates. Giri (*6*) reported that sweet potato amylases were similar to malt β-amylase. Balls, Thompson, and Walden (*3*) crystallized sweet potato β-amylase and observed that the amylolytic activity was almost entirely due to β-amylase. Since that time many workers have concentrated or isolated β-amylase from sweet potato juice. Giri (*5*) and Ikemiya (*9*) have reported that sweet potatoes show some α-amylase activity.

This paper reports a series of experiments designed to show the nature of, and the cause for, observed changes in processing characteristics of sweet potatoes during storage. These experiments demonstrate that freshly pressed juice contains dextrinizing (α-amylase) activity which is readily apparent only at higher temperatures than are normally considered optimum for α-amylase activity. Some properties of the enzyme are presented: optimum pH and temperature, effect of the interaction of pH and temperature on the activity, and destruction of the enzyme. Preliminary data on the concentration of the enzyme, the yields from different extraction treatments, distribution in the roots, and increase in enzyme concentration in the juice during storage are also included.

Materials and Methods

Samples of several shipments of Goldrush variety sweet potatoes from the 1963

and 1964 crops grown in the vicinity of St. Francisville, La., were used in these experiments.

Sweet Potato Enzyme Preparations. The crude juices used as a source of enzyme in the experiments were prepared by water-washing the whole unpeeled sweet potatoes, shredding, and then pressing under 5000 p.s.i. in a hydraulic press. The pressed juice was filtered through a Whatman No. 1 paper. The batches of crude juice used in the various experiments were prepared at different times from sweet potatoes after various periods of storage.

An "enzyme concentrate" was prepared from a single large batch of fresh juice from sweet potatoes that had been cured for 5 days at 85% relative humidity and 32.2° C. and stored for 6 months at 15.6° C. This large batch of juice was filtered through Whatman No. 1 paper. In preparing the enzyme concentrate calcium chloride was added to give a 0.5% solution concentration. The resulting calcium chloride solution in sweet potato juice was then heated at 60° C. for 15 minutes. After cooling to room temperature the precipitate was removed by centrifugation at 10,000 r.p.m. in a Servall Model SS-3 centrifuge using an SS-34 head. Then the supernatant was treated with an equal volume of acetone, followed by centrifugation, and the precipitate formed was discarded. The supernatant water-acetone solution was then treated with another volume of acetone equal to that of the original water-acetone solution. This precipitate, which had a high α-amylase activity, formed when the acetone concentration was increased from 50 to 75%, could be separated by centrifugation. The acetone was eliminated by air drying. The dried residue was dissolved in sufficient distilled water to give an enzyme concentration of 200 sweet potato dextrinizing units (SDU) as estimated by the method described below. The solution was preserved by toluene and refrigerated at 4° C.

Measurement of Activity. The enzyme activities of different samples of sweet potato crude juice and the concentrate preparation were determined by a modification of the method outlined in AOAC "Official Methods of Analysis" (*1*), which is based on the method developed by Sandstedt, Kneen, and Blish (*11*). In the modification the temperature of the incubation is increased to 70° C. and all solutions are held at, or buffered to, a pH of 6.0. The dextrinizing activity measured in this manner is expressed as sweet potato dextrinizing units (SDU). All data on α-amylase activities, except where reaction temperature and/or pH were the variables under study, were determined in this manner. The specific activity is expressed in these α-amylase units per milligram of precipitated protein nitrogen. The protein was precipitated by trichloroacetic acid and the nitrogen was determined by the conventional Kjeldahl method.

Addition of Water in Extraction Procedure. The effect of the addition of distilled water to shredded material on quantity of extractable α-amylase was tested in two series of experiments. In one a representative sample of shredded sweet potatoes, equivalent to 220 grams of raw sweet potatoes, was pressed in the usual manner for preparing crude juice. The press cake was then washed with successive 50-ml. portions of distilled water, and repressed after each washing to give a series of additional extracts designated as first, second, third, and fourth water extracts. The α-amylase activities, protein nitrogens, and specific activities of the crude juice and water extracts were determined.

In a second series of experiments the pressed residues were not washed, but from 0 in the control to 100, 300, and 600 ml. of water, respectively, were added per 600 grams of shredded sweet potatoes. After standing covered at room temperature for 5 hours the four batches were pressed and the resulting liquid was filtered. α-Amylase, β-amylase, total solids, protein nitrogen, and specific activity were determined on each juice and juice-water extract. In the test for β-amylase (saccharifying activity) a mixture of 15 ml. of 2% soluble starch solution and 4 ml. of $0.1M$ acetate buffer, pH 5.0, was digested with 1 ml. of suitably diluted pressed juice at 35° C. for 10 minutes. The amount of reducing sugar produced by the enzyme action was determined by the Lane-Eynon method (*2*), and is expressed as milligrams of maltose produced per milliliter of original juice and juice-water extract per minute.

Distribution of α-Amylase Activity. Ten sweet potatoes, 7 to 10 cm. in maximum diameter, weighing 278 to 450 grams, were stored 11 months under the conditions described above. Each potato was cut into three portions: the outermost representing all of the cork layer and a minimum of inner tissue, about 0.2- to 0.3-cm. thickness from the surface; the middle representing the next immediate layer of 0.5- to 2-cm. thickness, depending on the diameter at the portion of the root; and the innermost representing the remaining part of the sweet potato root, the core. The three parts were shredded and pressed as described above and the crude juice obtained was analyzed for α- and β-amylase activity.

Sweet Potato and Commercial Enzyme Preparations. The α-amylase activities of three sweet potato and five commercial preparations were compared at a series of reaction temperatures. The sweet potato preparations included: crude juice from cured sweet potatoes stored approximately 3 months (1964 crop); crude juice from cured sweet potatoes stored approximately 14 months (1963 crop); and the enzyme concentrate. The commercial preparations included two fungal amylases, Mylase P and Rhozyme S, products of the Wallerstein Co., Staten Island, N. Y., and the Rohm & Haas Co., Philadelphia, Pa., respectively; two bacterial α-amylases, Amylase-Alpha and Rhozyme H-39, products of the Nutritional Biochemicals Corp., Cleveland, Ohio, and the Rohm & Haas Co., respectively; and malt diastase, a product of the Wallerstein Co. The commercial products were dissolved in water to make 1% solutions. The amylolytic activity of each was calculated on this basis. The 1% solutions and the sweet potato preparations were further diluted as required for the α-amylase determination. Acetate buffer solution was used at pH 6.0 for testing sweet potato and bacterial preparations, and at pH 5.0 for the fungal and malt preparations as recommended for these products. The incubation temperatures were 30°, 40°, 50°, 60°, 65°, 70°, 75°, and 78°, respectively.

Influence of Storage of Sweet Potatoes. Since sweet potatoes change in processing characteristics during curing and storage, the following experiment was run to show whether there was a corresponding change in α-amylase activity. The sweet potatoes were cured 5 days at 85% relative humidity and 32.2° C., then stored at 15.6° C. Crude pressed juices were prepared from sweet potatoes stored for different intervals of time. Preparation procedures were held constant. The yield of juice was approximately 50% of the weight of the sweet potatoes. The α-amylase activity was determined at the intervals during the storage period shown in Table V.

Influence of pH at Different Reaction Temperatures. The enzyme concentrate preparation served as a source of sweet potato α-amylase in these experiments. The quantity used was held constant in each test and was the equivalent of 100 SDU at a reaction temperature of 70° C. and pH 6.0. There was a modification of the substrate, however. A 2% soluble starch solution was treated a day in advance with an excess of β-amylase for 7 hours at 40° C., and held overnight in the refrigerator. Two milliliters of a 0.2% calcium chloride solution were added to each of several 10-ml. portions of the β-amylase–treated substrate, followed by 2 ml. of an appropriate buffer (acetate and citrate phosphate) to give a selected pH from 3.6 to 8.1. The buffered substrates were brought to the desired temperature (30°, 45°, 55°, 70°, 76°, and 80° C.), then 1 ml. of enzyme solution was added and the reaction mixture was incubated for the determination of α-amylase activity.

Influence of Acidity on Stability of Sweet Potato α-Amylase Activity. A series of experiments was designed to show the effect of pH on α-amylase activity at optimum temperatures. The sweet potato enzyme concentrate was diluted to a concentration of 20 SDU per milliliter. One milliliter of the diluted enzyme solution was added to each of a series of tubes containing 1 ml. of 0.2% calcium chloride and 1 ml. of an appropriate buffer to give a selected pH, covering a pH range of 3.6 to 8.1 at 0.2-pH intervals. Each mixture was heated at 70° C. for 15 minutes, then cooled, and the remaining enzyme activity was estimated in the usual manner at pH 6.0 and 70° C.

Another series of experiments was designed to determine the extent of destruction caused by heating over a prolonged period at optimum pH. Enzyme–calcium chloride–buffer mixtures

were made in a manner similar to that described above, except that all were made to pH 6.0 by the addition of 1 ml. of acetate buffer. Two sweet potato enzyme preparations were tested: crude juice with an activity of 14 SDU per milliliter, prepared fresh from cured sweet potatoes which had been stored 6 months at 15.6° C., and the enzyme concentrate diluted to 20 SDU per milliliter. These enzyme–calcium chloride–buffer mixtures were heated in a 70° C. constant temperature water bath. Tubes were withdrawn for determination of remaining α-amylase activity at intervals of 30, 60, 90, 120, and 180 minutes.

Table I. α-Amylase Activities of Crude Juice and Successive Water Extracts

	Volume, Ml.	Activity per Ml.	Total Activity	Distribution, % in Extract	Protein Nitrogen, Mg./Ml.	Specific Activity[a]
Crude juice	76	7.50	570	70.2	3.62	2.07
Extract[b]						
1st	47	2.85	134	16.5	0.98	2.91
2nd	59	1.14	67	8.2	0.32	3.56
3rd	48	0.50	24	3.0	0.27	1.85
4th	52	0.33	17	2.1	0.16	2.06

[a] α-Amylase units per mg. precipitated protein nitrogen.
[b] Press cake washed with successive 50-ml. portions of distilled water and repressed after each washing.

Table II. Effect of Added Water on Extraction of α-Amylase Fraction from Sweet Potatoes

	Water Added, Ml.[a]			
	0	100	300	600
Amount of pressed juice, ml.	270	375	585	875
α-Amylase, SDU/ml.	20.0	14.5	12.0	8.0
Total SDU	5400	5437	7020	7000
% gained	...	+0.7	+30.0	+29.6
Saccharifying activity,[b] mg. maltose/ml./min.	1425	1000	675	450
Total activity × 10^{-3}	384.7	375.0	394.8	393.7
% gained	...	−2.5	+2.6	+2.3
Protein nitrogen, mg./ml.	1.41	1.42	1.02	0.68
Total protein nitrogen, mg.	380.7	532.5	596.7	595.0
% gained	...	+39.3	+56.7	+56.3
Specific activity, SDU/mg. prot. N	14.18	10.21	11.76	11.76
Solids, g./ml.	0.144	0.125	0.088	0.062
Total solids extracted, g.	38.88	46.88	51.48	54.25
% gained	...	+20.6	+32.4	+39.5

[a] Water added to 600-g. portions of shredded sweet potatoes.
[b] Saccharifying activity, mg. of maltose produced by 1 ml. of pressed juice per min. at 35° C. from 15 ml. of 2% soluble starch solution.

Table III. Distribution of Amylase in Sweet Potato

	Portion of Sweet Potato		
	Cork layer	Middle	Innermost
Weight of portion, g.	700	1535	1285
Pressed juice, ml.	288	695	578
α-Amylase			
SDU per ml.	8.22	14.70	17.00
SDU per g. original material	3.38	6.66	7.65
β-Amylase[a]			
Activity per ml.	1120	1280	2080
Activity per g. original material	460	580	940

[a] Saccharifying activity, mg. of maltose produced by 1 ml. of pressed juice per min. at 35° C. from 15 ml. of 2% soluble starch solution.

Results and Discussion

Addition of Water in Enzyme Extraction Procedure. Data presented in Table I on the extractability of the α-amylase from shredded Goldrush sweet potatoes show that most of the enzyme was extracted in the crude juice and the first water extract. The relatively high specific activities in the first and second water extracts may indicate that the α-amylase–active fraction is somewhat less soluble in crude juice than some of the other albuminoid compounds. Balls *et al.* (3) reported that the residue from the hydraulic press contained practically no β-amylase. Thus there is an indication of a difference in the relative amounts of the α- and β-amylases extracted in pressed sweet potato juice—i.e., apparently the α-amylase is less soluble in the juice. The specific activity of the α-amylase is highest in the second water extract. If the enzyme is less soluble in juice, it might be postulated that at this step most of the remaining enzyme is available to solubilization in water, while in later steps the relative amounts of other soluble proteins result in lower specific activity values. About 85% of the total activity was observed to be in the crude juice and first extract.

In the second experiment (Table II), where different amounts of water were added to the shredded roots prior to pressing, the indication of solubility differences between the two types of enzymes was confirmed. The added increments of water did not materially increase the total saccharifying power of the extract, while the addition of 300 ml. of water to 600 grams of shredded sweet potato increased the α-amylase extracted by almost one third. Protein nitrogen extraction was increased even more than α-amylase by the water addition.

Distribution of α-Amylase Activity. The difference in the distribution of α- and β-amylase activity as measured in the pressed juice from the outer corky layer, the intermediate layer, and the inner core, is striking. Table III shows that while the α-amylase activity is distributed uniformly throughout the inner tissues of the roots with an indication of minor concentration in the inner core portion, the β-amylase is definitely concentrated in this layer. The juice from the outer cork layer and skin was low in both amylolytic enzymes, and the true outer layer probably contained even less amylolytic activity than is indicated in the table, since in separating the sweet potato parts for pressing, a special effort was made to include all of the lighter colored outer layer, which necessarily caused a considerable portion of the inner orange colored tissue to be included. Giri (*6*) also demonstrated that the innermost core of the sweet potato had more than three times the saccharifying power of the outer layer.

Sweet Potato and Commercial Enzyme Preparations. The relative activities of α-amylases from three sweet potato preparations and five commercial sources are shown in Table IV for several reaction temperatures. Optimum temperature of enzyme reaction is dependent upon the conditions of the reaction; however, most amylase enzymes have their optimum temperatures below 50° C. Under the conditions of these experiments the optimum reaction temperatures of the fungal and bacterial α-amylases were at or near 50° C. The most heat-stable bacterial amylases showed reduced activity at 70° C. The malt diastase had its optimum tempera-

Table IV. α-Amylase Activities of Sweet Potato and Commercial Preparations at Different Reaction Temperatures

	Reaction Temperature, ° C.								Activity Ratio[a]
	30	40	50	60	65	70	75	78	
Sweet potato									
Crude juice[b]	0.4	0.7	2.5	3.8	7.5	8.8	11.0	10.0	27.5
Crude juice[c]	1.6	3.0	12.0	16.0	27.0	28.0	28.0	27.0	17.5
Enzyme concn.[d]	4.2	8.8	28.4	36.5	68.5	70.6	70.6	60.5	16.6
Commercial preparations									
Mylase-P[e]	60.0	109.0	133.0	30.5	0				2.2
Rhozyme-S[e]	17.1	28.8	38.2	40.0	18.4	0			2.3
α-Amylase[f]	41.0	77.0	99.0	68.0	62.0	10.2	0		2.4
Rhozyme H-30[f]	60.3	121.5	170.0	120.0	120.0	82.0	0		2.8
Malt diastase	5.2	10.0	17.1	18.5	15.0	3.3	0		3.6

[a] Ratio of activity at optimum temperature to that at 30° C.
[b] 1964 crop of sweet potatoes, cured and stored 3 months.
[c] 1963 crop of sweet potatoes, cured and stored 14 months.
[d] Diluted enzyme concentrate, see text.
[e] Fungal α-amylase.
[f] Bacterial α-amylase.

ture at 60° C. and exhibited a considerably reduced activity at 70° C.

In sharp contrast to the amylases in the commercial preparations, those from sweet potatoes exhibited their optimum activity in the 70° to 75° C. range; activity was very low at 30° C. The ratio of the optimum temperature activity to that at 30° C. is much higher for the sweet potato amylases than for the others, again emphasizing the unusually low activity of this enzyme at ordinary incubation temperatures.

The fact that the optimum reaction activities of the two sweet potato crude juice preparations corresponded to that for the sweet potato enzyme concentrate is evidence that inhibitors are not responsible for the low activities of the sweet potato α-amylase in the 30° to 45° C. range, since removal of the bulk of extraneous material did not change its pattern of activity.

Influence of Storage of Sweet Potatoes. α-Amylase activity increases in sweet potatoes with length of storage (Table V). Further studies are needed to establish whether the increase occurs in a stepwise fashion when storage conditions are held constant, or progressively to reach a maximum. The protein nitrogen decreases during storage, which checks with the observation made by Heinze and Appleman (*8*).

Earlier studies (*4, 9*) have shown that saccharifying activity also increases in sweet potatoes during storage. Culpepper and Magoon (*4*) also reported that on cooking sweet potatoes the proportion of starch which is converted to maltose and dextrin by the action of the amylase in the root is generally higher in cured and stored sweet potatoes than in freshly harvested roots. Others have reported conversion of the starch to sugar. Previously this conversion of starch into sugars and dextrins was believed to be due to the activity of the intrinsic β-amylase of sweet potatoes.

The role of the α-amylase in the conversion of starch in sweet potatoes will now have to be reconsidered; and perhaps, a reassessment of the mechanism of the conversion of starch during storage and processing will be necessary. One may speculate that the firmness of sweet potatoes canned within a few days of digging may be attributed to their low α-amylase activity. In contrast, the excellent processing characteristics of cured and stored sweet potatoes for flake production may be attributed to the increase in the intrinsic α-amylase in the stored sweet potato.

Influence of pH at Different Reaction Temperatures. Figures 1 and 2 show the effect of pH on the activity of sweet potato α-amylase. The pH data shown in these curves were taken after the enzyme-substrate reaction. It is

Table V. Influence of Storage of Sweet Potatoes on α-Amylase Activity of Their Crude Juice Extracts

Days after Harvest[a]	SDU/Ml.[b]	Protein Nitrogen, Mg./Ml.	Specific Activity[c]
4	3.40	...	...
16	3.60	...	...
42	3.85	3.82	1.0
95	7.50	3.62	2.1
161	10.40	3.33	3.1
197	14.10	3.05	4.6
218	14.00	2.90	4.8
245	18.40	2.43	7.6
301	20.00	1.41	14.2

[a] Harvested August 31, 1964.
[b] Pressed juice equivalent to about 50% of pulp weight.
[c] SDU per mg. precipitated protein N.

apparent from the curves in Figure 1 that the sweet potato α-amylase is stable at low temperatures through a wide range of pH. At 30° and 45° C. the sweet potato α-amylase appears to be stable at a pH as low as 4.0, which is considerably lower than for most plant α-amylases. The relatively small differences in the activities of the juice at 30° or 45° C. and in the pH 4 to 8 range, are changed abruptly when the temperature is increased (Figure 2). The optimum pH range becomes narrower

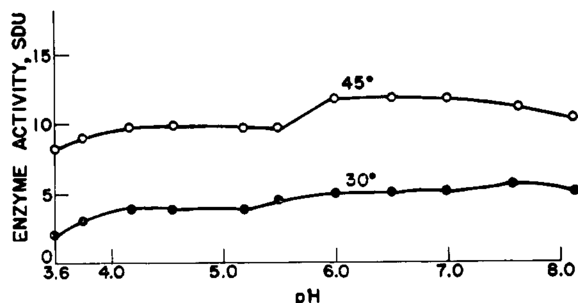

Figure 1. Effect of pH on enzyme activity at 30° and 45° C.

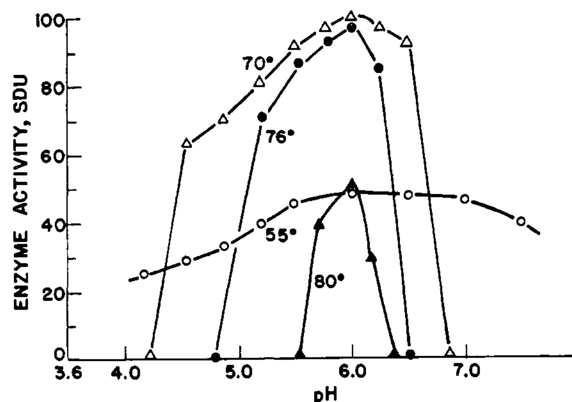

Figure 2. Effect of pH on enzyme activity at 55°, 70°, 76°, and 80° C.

the higher the temperature. At 80° C. there is a maximum activity only at pH 6.0 with very little stability on either the acid or base side of this pH. The optimum pH will depend on the duration and temperature of the reaction. For higher temperatures the pH must be carefully controlled to as near 6.0 as possible, whereas at lower temperatures a much wider range of pH without any specific optimum may be used. The higher the reaction temperatures, the easier it is to determine the optimum pH of maximum stability closely.

Influence of Acidity on Heat Stability of Sweet Potato α-Amylase. Preheating solutions of sweet potato amylase which had been adjusted to different pH's (range pH 3.6 to 8.1 at 0.2 pH intervals) for 15 minutes at 70° C. demonstrated that minimum destruction (maximum heat stability) of the enzyme occurred at pH 6.0 (Figure 3). The pH data shown are those determined after heating. The chart showing the influence of pH at 70° C. (Figure 2) is reproduced in Figure 3 for comparison. These data confirm greater stability of the sweet potato α-amylase at pH 6.0 than at the other pH's investigated. Destruction of the enzyme is progressively greater on either side of the optimum but more so on the basic side. No activity was detected in the preheated mixtures in which the pH was adjusted to pH 6.6 and higher, or in those adjusted to pH 4.5 and lower. As the curves in Figure 3 show, preheating the enzyme solution for 15 minutes at 70° C. does damage the enzyme, although the extent of damage at pH 6.0 may not be detectable by the methods employed. Adjustment of the pH's to the optimum prior to performing the activity test indicated that the inactivation effect of pH on the stability of the enzyme at the preheat and test temperatures was irreversible. Nakayama and Kono (*10*) reported similar findings for sweet potato β-amylase. Employing a similar technique, they observed that β-amylase was most stable against heat inactivation at pH 5.4.

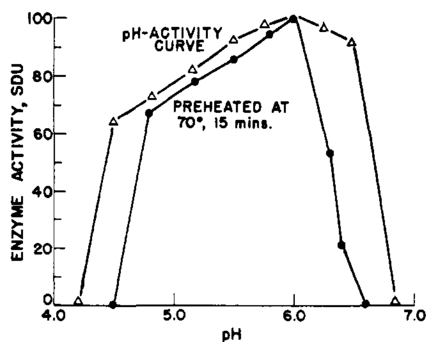

Figure 3. Effect of pH on heat stability and pH-activity curve

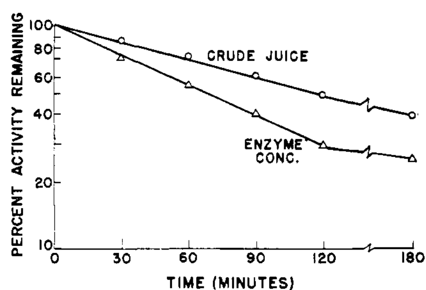

Figure 4. Effect of heating time on inactivation of α-amylase

Heating the sweet potato α-amylase preparations for long periods at 70° C. causes a progressive destruction of the enzyme (Figure 4). The residual activity plotted on a logarithm scale against time of heating resulted in a straight line for 120 minutes for either the crude or the purified sweet potato α-amylase, indicating that the heat inactivation of the α-amylase follows first-order kinetics with respect to time. This has already been reported for β-amylase in the sweet potato (*10*). These results suggest that heat inactivation in both of these enzymes may be an intramolecular denaturation phenomenon. Further work will be needed to indicate the nature of the breakdown of these protein molecules.

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CHEESE FLAVORS

Quantitation, Evaluation, and Effect of Certain Microorganisms on Flavor Components of Blue Cheese

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Efforts to characterize the unique flavor properties of Blue-veined cheese and to define the microbiology of ripening have been the subject of numerous investigations. The list of compounds comprising Blue cheese volatiles has become extensive (*2*) and limited quantitative data have been reported on a few classes of compounds—i.e., methyl ketones (*11*) and fatty acids (*1*).

This paper reports on the quantitative analysis of two major classes of compounds in Blue cheese volatiles. The quantitative data were evaluated by preparing mixtures of selected compounds and determining their similarity to Blue cheese flavor. Since the ratios of the major classes of compounds might be altered by the microflora of the cheese, the effect of bacteria, yeasts, and molds, common to the cheese during ripening, upon ketone-alcohol interconversion was determined.

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Experimental Procedure

Quantitation of Methyl Ketones. Hexane, benzene, and chloroform solvents were treated to remove carbonyls and redistilled. Nitromethane was distilled over boric acid, and ethylene chloride was distilled and stored over anhydrous potassium carbonate.

The quantitation procedure was similar to that described for fat and oils (*10*) and cheese (*11*). Ten grams of cheese and 15 grams of Celite 545 were ground with a mortar and pestle, and the mixture was placed in a chromatographic column plugged with glass