Conditions Under Which Bacterial Amylases Survive Ultrahigh Temperature Sterilization

J. E. ANDERSON, D. M. ADAMS, and W. M. WALTER JR.

ABSTRACT

The α-amylase of the thermophile *Bacillus stearothermophilus* 1518 was used as a model enzyme to examine the heat resistance of a bacterial amylase at ultrahigh temperature in a starch-based pudding. When heated in the laboratory pudding at 143°C for the equivalent of 22.2 sec, approximately 26% of the initial amylolytic activity was retained. The presence or absence of individual ingredient groups (starch, caseinate-nonfat dry milk, oil-emulsifiers, salts, or sucrose) of the pudding had no significant effect on amylase heat resistance. A combination of any two of the starch, caseinate-non-fat dry milk, or oil-emulsifier ingredients were found to be important for thermostability. The amylase of the mesophile *Xanthomonas campestris* 13957-2 exhibited similar thermal inactivation characteristics.

INTRODUCTION

THE USE OF ULTRAHIGH TEMPERATURE (UHT) sterilization methods, for a variety of food products, has increased in recent years. It has become evident, however, that the commercial sterility of UHT processed foods does not ensure extended shelf stability. The spoilage of various UHT processed dairy products has been attributed to heat resistant proteases and lipases that are produced by the psychrotrophic bacterial flora of raw milk (Law, 1979; Cogan, 1977; Bengtsson et al., 1973; Law et al., 1977, 1979; Ritcher et al., 1979; Malik and Swanson, 1974; Adams et al., 1975). When present in the raw product, these enzymes survive the UHT treatment and remain active in the sterile product, attacking the protein and fat components.

Recently, the thinning of UHT sterilized, starch-based pudding was reported to occur in 2 wk to 6 mo after processing (Barefoot and Adams, 1980). An amylase was extracted from spoiled, sterile pudding and characterized as an α-amylase (Barefoot and Adams, 1980). The amylase was postulated to be of bacterial origin because of its physical characteristics and the extreme thermostability necessary to have survived the UHT treatment. However, when the isolated amylase was added to sterile pudding or to a buffered starch solution and heated to 143°C, all activity was destroyed (Barefoot, 1979).

The heat resistance of bacterial α-amylases at UHT has not been investigated, although some are known to be quite heat stable at temperatures below the UHT range (Miller et al., 1953; Underkofler et al., 1965; Fullbrook, 1976; Wiseman, 1975; Medda and Chandra, 1980; Morgan and Priest, 1981; Madsen et al., 1973). The objectives of this study were to demonstrate the heat resistance of a bacterial α-amylase at UHT in a starch-based pudding formulation and to determine the factors important to the heat resistance of the amylase.

Author Anderson, formerly with North Carolina State Univ., is now affiliated with American Can Co., 433 N. Northwest Highway, Barrington, IL 60010. Author Adams, formerly with North Carolina State Univ., is now affiliated with the Campbell Institute for Research & Technology, Campbell Soup Co., Campbell Place, Camden, NJ 08701. Author Walter is affiliated with the USDA-ARS and North Carolina State Univ., Raleigh, NC 27650.

MATERIALS & METHODS

Amylase

Sterile culture filtrates of *Bacillus stearothermophilus* 1518 and of *Xanthomonas campestris* 13957-2 were used to evaluate the heat resistance of bacterial α-amylases at UHT.

Preparation of laboratory pudding

A laboratory formulation (Table 1) and procedure for pudding preparation (Fig. 1) were adapted from a commercial formulation (Table 1) and process for starch-based, UHT treated puddings on the market (Fig. 2). Flavors, colors, vitamins, and preservatives were omitted in the laboratory pudding because they were believed to play no significant role in the heat resistance of the amylase. Xanthan gum also was omitted from the formulation because of its implication as a possible source of heat resistant amylase. Carrageenan was omitted because the high viscosity of the pudding when it was used made the pudding difficult to work with. Nonfat dry milk was substituted for skim milk to allow enzyme to be added to the pudding without changing the final volume or concentration of ingredients.

The corn oil and emulsifiers (mono- and digylcerides and sodium stearoyl-2-lactylate) were combined in a 30-ml beaker and heated

Table 1—Pudding ingredients[a]

Ingredients	Commercial pudding	Laboratory pudding
Fresh skim milk	75.96	NA[b]
Sucrose	9.80	9.80
Starch[c]	4.1	4.1
Nonfat dry milk	2.68	10.3
Sodium caseinate	1.98	NA
Vitamin-free casein	NA	1.98
Vegetable fat	1.70	1.70
Artificial flavors	0.311	NA
Sodium citrate	0.05–0.07	0.06
Magnesium carbonate	0.119	0.119
Xanthan gum	0.10	NA
Salt	0.10	0.10
Mono- and diglycerides	0.10	0.10
Magnesium sulfate	0.070	0.070
Sodium stearoyl-2-lactylate	0.05	0.07
Carrageenan	0.05	NA
Sodium ascorbate	0.0198	NA
Artificial color	0.0132	NA
Ferric orthophosphate	0.0073	NA
α-Tocopheryl acetate	0.004	NA
Sodium hexametaphosphate	0.003	NA
Niacinamide	0.0026	NA
Zinc oxide	0.0023	NA
Copper gluconate	0.0017	NA
Calcium pantothenate	0.0014	NA
Ascorbic acid	0.00066	NA
Vitamin A palmitate	0.00036	NA
Pyridoxine hydrochloride	0.00033	NA
Riboflavin phosphate	0.00032	NA
Thiamine hydrochloride	0.00025	NA
Folic acid	0.00007	NA
Biotin	0.00005	NA
Potassium iodide	0.00003	NA
Vitamin D_2	0.000001	NA
Vitamin B_{12}	0.00000095	NA

[a] Pudding ingredients expressed in percent (w/w).
[b] Not added to the formulation.
[c] Thin-n-Thik 99 (A. E. Staley Manuf. Co., Decatur, IL).

until the emulsifiers melted into the oil. The heated oil mixture was added to half of the required liquid for the formulation in a blender cup and blended on low speed for ca. 15 sec. Other ingredients were added gradually and blended at low speed for an additional 1 min.

Heat treatments

Amylase preparation was added to the concentrated pudding in the ratio of 0.38 ml enzyme per 0.62 ml of pudding. One milliliter of the enzyme-pudding mixture was added to each of two tubes of the starch assay substrate to measure amylolytic activity.

To swell or gelatinize the pudding starch, the enzyme pudding mixture was put in a glass beaker in a boiling water bath and heated with constant stirring to 80°C in 3 min. One milliliter of the swelled enzyme-pudding mixture was added to each of two tubes of the starch assay substrate and evaluated for amylolytic activity.

To assess the heat resistance of the amylase at UHT, 1 ml of swelled pudding was transferred to each of two, 8 mm o.d. Pyrex freeze-drying ampoules (Bellco Glass, Inc., Vineland, NJ). The ampoules were sealed with a gas flame, immersed in an oil bath (Haake, model NBS, Berlin, West Germany) at 143°C for 85 sec and cooled in ice water. The ampoules were aseptically opened under a bacteriological hood (Bioquest Biological Cabinet, Envirco, Albuquerque, NM), the contents of each removed and added to the starch assay substrate for evaluation of amylolytic activity.

A heat treatment of 143°C for 85 sec was estimated to be equivalent to a heat treatment of 22.2 sec at 143°C for the laboratory pudding, a solution of the protein and oil-emulsifier ingredients of the pudding, or water. This was similar to the commercial process of 143°C for 18-22 sec. The rate of heat penetration to the center of the ampoule was measured using a copper/constantin thermocouple sealed in a heating ampoule and heated at 143°C. The determination of the equivalent heat treatment was done by the lethal rate method or $L = 10^{(T - 143°C)/35°C}$. Based on the z-values (change in temperature yielding a 10-fold change in inactivation rate) of 32-39C° reported for heat resistant bacterial lipases and proteases (Adams and Brawley, 1981; Andersson et al., 1981; Adams et al., 1975), a z-value of 35C° was assumed.

To evaluate the effects of the major groups of pudding ingredients on amylase heat resistance, the protein group (nonfat dry milk and vitamin-free casein), oil and emulsifiers group (corn oil, mono- and diglycerides, and sodium stearoyl-2-lactylate), the sucrose, the starch, and the salts group (magnesium carbonate, magnesium sulfate, sodium chloride, and sodium citrate) were eliminated from the pudding, one at a time, with other factors maintained as standard. Amylolytic activity was determined using 1.0 ml of the enzyme-pudding mixture before swelling, after swelling and after the UHT treatment.

The effects of single ingredient groups of the pudding on enzyme heat resistance were examined using aqueous solutions of each component group at the same concentration as it was used in the pudding formulation. Solutions were prepared using the same procedure as described for the pudding. Activity of the amylase was measured using 1 ml of the enzyme-solution mixture before swelling, after swelling and after UHT treatment.

The effects of two or three groups of pudding components in combination on heat resistance were examined to determine if an interaction between the components was significant to the heat resistance of the amylase. The protein, oil and emulsifiers, and starch elements were selected for evaluation because they were reported to enhance amylase heat stability at sub-UHT (Heinen and Lauwers, 1975; Manning and Campbell, 1961; Medda and Chandra, 1980; Pfueller and Elliot, 1969). The solutions were prepared using the procedure described previously for pudding. Amylolytic activity was measured using 1 ml of the enzyme-solution mixture before swelling, after swelling and after the UHT treatment.

Assay for amylolytic activity

Amylolytic activity was measured as dextrinizing power (i.e., the loss in the ability of starch to bind iodine) by the method of Fuwa (1954) as modified by Manning and Campbell (1961). The substrate used was 8% Lintner modified starch (Sigma Chemical Co., St. Louis, MO) in 0.08M acetate buffer with 20 mM calcium chloride and pH 6.8-7.0. To each of two screw cap tubes containing 3.0 ml sterile starch substrate were added 2.0 ml sterile distilled water, 1.0 ml of the enzyme or enzyme-pudding mixture and
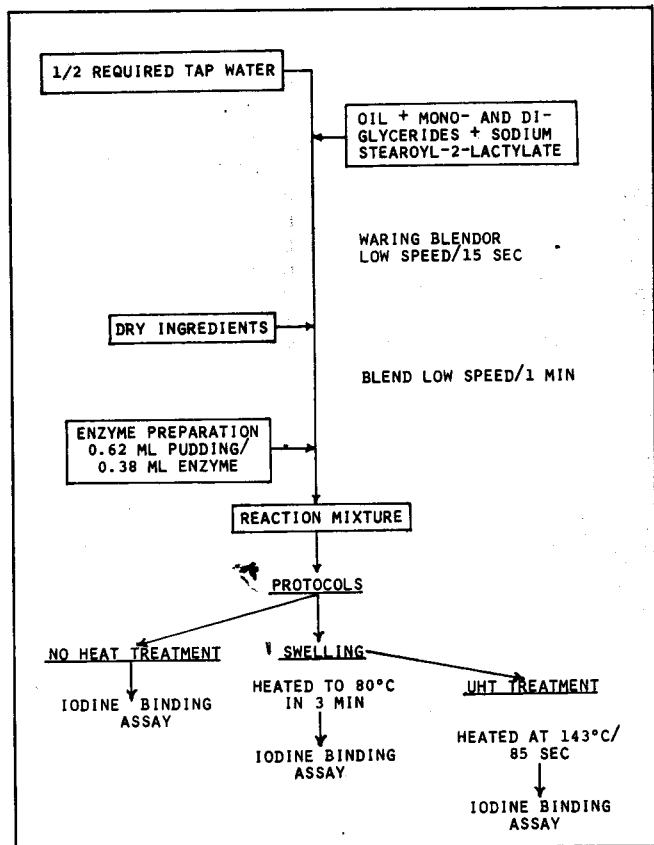

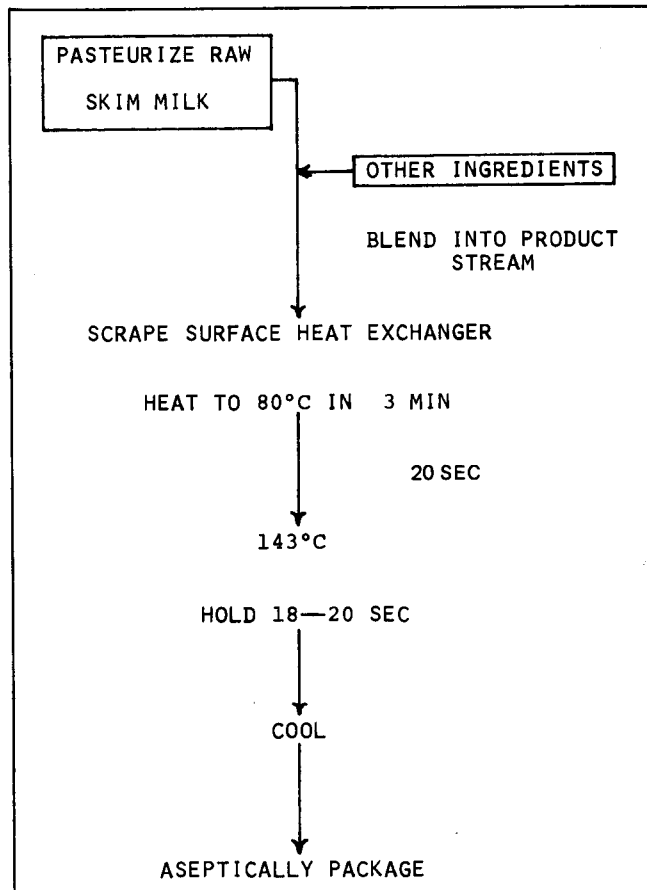Fig. 1—Flowchart showing laboratory procedure for UHT treated pudding.

Fig. 2—Flowchart showing the commercial procedure for UHT processed puddings.

sodium azide to 0.04% to inhibit microbial growth. The tubes were incubated at 37°C at approximately a 30° angle, in a Psychrotherm gyrotory shaker-incubator (New Brunswick Scientific Co., Inc., New Brunswick, NJ) at 100 rpm. Activity was measured over a 48-hr period and was expressed as the change in optical density (O.D.) at 620 nm per hour per milliliter of enzyme added. Values were calculated from the slope of the linear regression of $O.D._{620}$ on time.

RESULTS & DISCUSSION

Amylase heat resistance in pudding and in starch assay substrate

The activity of the *B. stearothermophilus* amylase, when added to the starch assay mixture, was unchanged from the amylolytic activity of the preparation added to distilled water, but when mixed with the concentrated laboratory pudding, the activity was 32.5% lower. The initial amylolytic activity of the *X. campestris* amylase was 28.3% lower when it was mixed with the laboratory pudding rather than distilled water. The reductions in activity suggested that interactions between pudding ingredients and the amylases may occur.

There was no appreciable change in the activity of the *B. stearothermophilus* amylase after swelling when the enzyme was present in the starch assay substrate or the laboratory pudding (Table 2). After UHT treatment at 143°C for 85 sec, there was no detectable amylolytic activity remaining in the starch assay substrate; activity remaining in the laboratory pudding averaged 25.9% of the original. For the *X. campestris* amylase, 77.8% of the initial activity was lost during swelling. Of the activity remaining, 80.6% survived the UHT treatment. After both heat treatments, 17.9% of the initial activity was retained.

The inability of the *B. stearothermophilus* amylase to survive UHT treatment when heated in the starch-acetate buffer-$CaCl_2$ enzyme assay medium confirmed the findings of Barefoot and Adams (1980). It also indicated that Ca^{++} and starch alone could not stabilize the amylase and sug-

gested that the pudding was unique in its ability to confer heat resistance. Preliminary studies, however, showed that the amylase was not heat resistant when heated at UHT in sterile, commercially made pudding (data not presented), indicating that the presence of the amylase in the raw product during swelling and UHT treatment was critical to enzyme heat resistance. This suggested that the amylase interacted with pudding ingredients prior to UHT treatment to form a heat stable complex. Supporting this is the observation that amylase activity was reduced when the amylases were mixed with pudding ingredients.

The effects of pudding ingredients on the heat resistance of the *B. stearothermophilus* amylase were examined by eliminating each ingredient, as a group, one at a time while the others were maintained at normal concentrations. Elimination of single ingredient groups had no statistically significant effect on the amount of activity surviving swelling or UHT treatments (P = 0.01) (Table 3). The increase in amylolytic activity noted after heat treatments in the absence of the protein group may be due to the ability of the amylase to better interact with pudding ingredients without the competitive influences of another protein component present.

These results suggested that more than one ingredient provided heat resistance for the enzyme. When both the protein and starch components of the pudding were eliminated, a statistically significant (P = 0.01) reduction in the amount of enzyme activity surviving the UHT treatment was observed (Table 3). This suggested that the protein and starch were important to heat resistance.

The effects of individual ingredient groups alone on heat resistance of the *B. stearothermophilus* amylase were evaluated using aqueous solutions of each component group as the heating medium. After the swelling treatment, there was no measurable amylolytic activity remaining in the solution of pudding salts (Table 4). This loss of activity may be due to a pH effect, the effect of ionic strength on the enzyme or a binding of salt components to the enzyme. With the exception of the salts, enzyme activities in the

Table 2—Heat resistance of the B. stearothermophilus and X. campestris amylases at UHT

| Amylase | Heating medium | Enzyme activity[a] | | | % Amylase activity surviving | |
		Before swelling	After swelling	After UHT	Swelling & UHT treatments	UHT treatment[b]
B. stearothermophilus	Starch assay substrate	−0.016	−0.017	<−0.0001	<1.00	<1.00
B. stearothermophilus	Laboratory pudding	−0.0113	−0.0108	−0.003	25.9	26.7
X. campestris	Laboratory pudding	−0.016	−0.004	−0.003	17.9	80.6

[a] $\Delta O.D._{620}$/hr/ml enzyme.
[b] Based on the activity remaining after swelling.

Table 3—Surviving activity of the B. stearothermophilus amylase in pudding with different ingredients eliminated

| Ingredient eliminated | % Amylase activity surviving | |
	Swelling & UHT treatments	UHT treatment[a]
None	25.9	26.7
Protein	38.3	49.7
Starch	13.3	11.7
Salts	15.7	15.7
Oil-emulsifiers	13.3	14.9
Sucrose	12.4	15.3
Protein/starch	5.1[b]	5.1[b]

[a] Based on the activity remaining after swelling.
[b] Significant reduction in amylolytic activity (P = 0.01).

Table 4—Activity of B. stearothermophilus amylase in solutions of single ingredient groups

| Solution | Enzyme activity[a] | | | % Amylase survival after swelling and UHT |
	Before swelling	After swelling	After UHT	
Distilled water	−0.013	−0.013	<−0.0001[b]	<1.0[c]
Oil-emulsifiers	−0.015	−0.020	−0.0004[b]	2.7[c]
Protein	−0.013	−0.014	−0.0001[b]	1.1[c]
Salts	−0.004	<−0.0001[b]	<−0.0001[b]	<1.0[c]
Starch	−0.012	−0.010	<−0.0001[b]	<1.0[c]
Sucrose	−0.020	−0.012	<−0.0001[b]	<1.0[c]

[a] $\Delta O.D._{620}$/hr/l ml enzyme.
[b] Significant reduction in amylolytic activity (P = 0.01).
[c] Significant reduction in amylase survival (P = 0.01).

Table 5-effect of ingredient interactions on B. stearothermophilus amylase heat resistance

| | % Amylase activity surviving | |
Solution	Swelling & UHT treatments	UHT treatment[a]
Protein/oil-emulsifiers	21.1	18.9
Protein/starch	13.9	14.2
Starch/oil-emulsifiers	12.8	15.0
Starch/oil-emulsifiers/protein	18.6	23.7

[a] Based on the activity remaining after swelling.

other solutions were not appreciably affected by the swelling treatment. After the UHT treatment, little or no amylolytic activity was detected in the solutions, confirming that no single ingredient group confers heat resistance to the enzyme, but that the thermostability at UHT depended on the interactions of two or more ingredient groups.

To evaluate this hypothesis, the B. stearothermophilus amylase was heated in a solution of protein, starch and oil-emulsifiers in mixtures of two or all three. An average of 7.0% of the activity was lost during the swelling treatment in each solution. After the UHT treatment, more than 12% of the amylolytic activity was retained in all of the solutions examined (Table 5). The surviving activities were not statistically different from the activity surviving in pudding (P = 0.01). The highest levels of surviving activity were observed in the mixture of protein and oil-emulsifiers and in the mixture of all three components, retaining 21.2% and 18.6% of the original activity after both heat treatments, respectively. It appeared that any two of these three components of the pudding were essential for the heat resistance of the amylase at UHT. Likewise, the amylase of X. campestris survived swelling and UHT treatment as well in the mixture of starch, oil-emulsifiers, and protein as in the complete pudding (Table 2); with 18.9% of the initial amylase activity surviving the swelling and UHT treatments; 72.9% of the activity remaining after swelling survived the UHT treatment.

The results indicate that the amylases of both a thermophile and a mesophile will survive UHT processing when heated in mixtures of protein (casein and nonfat dry milk) and starch, protein and oil-emulsifiers, starch and oil-emulsifiers, or protein and oil-emulsifiers, but not in any single one of these ingredients. The mechanism of heat resistance appears to be more complex than the single requirement for CA^{++} reported for heat stable proteases (Adams, 1980) and at least as complex as that indicated for a heat stable lipase (Adams and Brawley, 1981). Calcium, starch and bovine serum albumen (BSA) stabilize amylases, but at temperatures much lower than 143°C (Heinen and Lauwers, 1975; Manning and Campbell, 1961; Medda and Chandra, 1980; Pfueller and Elliot, 1969; Ogasahara et al., 1970a). Ogasahara et al. (1970a) found that the B. stearothermophilus amylase was completely inactivated in the presence of 0.005M EDTA, a chelating agent, at 60°C for 15 min. With the addition of 0.0025M $CaCl_2$, the enzyme maintained the full or slightly higher activity than the original. Pfueller and Elliot (1969) found that the removal of Ca^{++} from B. stearothermophilus amylases had no effect on the enzyme below 50°C. Above 50°C, rapid loss of enzyme activity occurred.

Miller et al. (1953) reported the increased thermostability of a bacterial α-amylase at temperatures up to 95°C when added to a flour substrate used in bread making. The amylases produced by B. licheniformis CUMC 305 and B. coagulans CUMC 512 were reported to tolerate temperatures of 110°C and 90°C, respectively, for up to 1 hr in the presence of a starch substrate (Medda and Chandra, 1980).

Bovine serum albumen was found to protect the α-amylase produced by B. stearothermophilus 15034 (Pfueller and Elliot, 1969) and the B. subtilis amylase (Ogasahara et al., 1970b) from heat denaturation above 50°C and at 60°C, respectively. The effects of other proteins on amylase thermostability have not been investigated.

The mechanism of amylase thermostability at UHT in the pudding formulation must involve interactions between the amylase and the protein, starch and oil-emulsifiers. Protein-starch interactions have been implicated as enhancers of thermostability, by the increased viscosity of the pudding mixture obtained after the swelling step in the presence, rather than the absence of casein (Merritt, personal communication), and emulsifiers interact with starch and protein to regard the starch gel retrogradation. The enzyme may participate in a starch-protein and starch-protein-oil-emulsifier interaction no differently than the casein and whey proteins but in the process become stabilized against inactivation at UHT.

The source of heat stable amylase in UHT treated puddings is unknown. The heat resistance of the X. campestris amylase in the laboratory pudding and in the protein-starch-oil-emulsifier mixture suggests that xanthan gum could be a source of amylase. Some processors have overcome the problem of thinning by eliminating xanthan gum from the pudding formulation. However, other manufacturers have experienced thinning when not using xanthan gum, but when microbial counts of the nonfat dry milk were very high. Barefoot (1979) reported that 0.4–15% of the microflora of raw milk produce amylases and we have confirmed these findings (data not presented). Identifying the source of these amylases leading to their full characterization should be possible now that a procedure has been developed to identify their most unique characteristic — their extreme heat resistance.

REFERENCES

Adams, D.M. 1980. The role of heat resistant bacterial enzymes in UHT processing. In "Proceedings of International Conference on UHT Processing and Aseptic Packaging of Milk and Milk Products." North Carolina State Univ., Raleigh, NC, 1979.

Adams, D.M., Barach, J.T., and Speck, M.L. 1975. Heat resistant proteases produced in milk by psychrotropic bacteria of dairy origin. J. Dairy Sci. 53: 828.

Adams, D.M. and Brawley, T.G. 1981. Factors influencing the heat resistance of a heat-resistant lipase of Pseudomonas. J. Food Sci. 46: 673.

Andersson, R.E., Danielsson, G., Hedlund, C.B., and Svensson, S.G. 1981. Effect of heat-resistant microbial lipase on flavor of ultrahigh-temperature sterilized milk. J. Dairy Sci. 64: 375.

Barefoot, S.F. 1979. Amylolytic activity in spoiled ultrahigh temperature sterilized pudding. M.S. thesis, North Carolina State Univ., Raleigh, NC.

Barefoot, S.F. and Adams, D.M. 1980. Amylase activity in sterile spoiled pudding. J. Food Sci. 45: 1658.

Bengtsson, K., Gardhage, L., and Isaksson, B. 1973. Gelation in UHT treated milk, whey and casein solution. The effect of heat resistant proteases. Milchwissenschaft 28: 495.

Cogan, T.M. 1977. A review of heat resistant lipases and proteinases and the quality of dairy products. Ir. J. Food Sci. Technol. 1: 95.

Fullbrook, P.D. 1976. Recent advances in the use of enzymes in starch processing. Proc. Inst. Food Sci. Technol. (UK) 9: 105.

Fuwa, H. 1954. A new method for microdetermination of amylase activity by the use of amylose as the substrate. J. Biochem. 41: 583.

Heinen, W. and Lauwers, A.M. 1975. Amylase activity and stability at high and low temperatures depending on calcium and other divalent cations. In "Enzymes and Proteins from Thermophilic Microorganisms," (Ed.) H. Zuber. Birkhauser Verlag, Basel and Stuttgart.

Law, B.A. 1979. Reviews of the progress of dairy science: enzymes of psychrotrophic bacteria and their effects on milk and milk products. J. Dairy Sci. 46: 573.

Law, B.A., Andrews, A.T., Cliffe, A.J., Sharpe, M.E., and Chapman, H.R. 1979. Effect of proteolytic raw milk psychrotrophs on cheddar cheese-making with stored milk. J. Dairy Res. 46: 497.

Law, B.A., Andrews, A.T., and Sharpe, M.E. 1977. Gelation of ultrahigh temperature-sterilized milk by proteases from a strain of Pseudomonas fluorescens isolated from raw milk. J. Dairy Res. 44: 145.

Madsen, B.G., Norman, B.E., and Slott, S. 1973. A new heat-stable bacterial amylase and its use in high temperature liquefaction. Die Starke 25: 304.

Malik, A.C. and Swanson, A.M. 1974. Heat-stable proteases from psychrotrophic bacteria in milk. J. Dairy Sci. 57: 591.

Manning, G.B. and Campbell, L.L. 1961. Thermostable α-amylase of Bacillus stearothermophilus. 1. Crystallization and some general properties. J. Biol. Chem. 236: 2952.

Medda, S. and Chandra, A.K. 1980. New strains of Bacillus licheniformis and Bacillus coagulans producing thermostable α-amylase active at alkaline pH. J. Appl. Bacteriol. 48: 47.

Miller, B.S., Johnson, J.A. and Palmer, D.L. 1953. A comparison of cereal, fungal, and bacterial alpha-amylases as supplements for breadmaking. Food Technol. 7: 38.

Morgan, F.J. and Priest, F.G. 1981. Characterization of a thermostable α-amylase from Bacillus licheniformis NC1B 6346. J. Appl. Bacteriol. 50: 107.

Ogasahara, K., Imahishi, A., and Isemura, T. 1970a. Studies on thermostable α-amylase from Bacillus stearothermophilus. 2. Thermal stability of thermophilic α-amylase. J. Biochem. 67: 77.

Ogasahara, K., Katsuhide, Y., Imanishi, A., and Isemura, T. 1970b. Studies on thermophilic α-amylase from Bacillus stearothermophilus. 3. Effect of temperature on the renaturation of denatured thermophilic α-amylase. J. Biochem. 67: 83.

Pfueller, S.L. and Elliot, W.H. 1969. The extracellular α-amylase of Bacillus stearothermophilus. J. Biol. Chem. 244: 48.

Ritcher, R.L., Schmidt, R.H., Smith, K.L., Mull, L.E., and Henry, S.L. 1979. Proteolytic activity in ultra-pasteurized aseptically packaged whipping cream. J. Food Prot. 42: 43.

Underkofler, L.A., Denault, L.J. and Hou, E.F. 1965. Enzymes in the starch industry. Die Starke 17: 179.

Wiseman, A. 1975. Enzyme utilization in industrial processes. In "Handbook of Enzyme Biotechnology." Halsted Press, New York, NY.