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PRODUCTION OF BACTERIAL ALPHA-AMYLASE IN QUEENSLAND EGG PULP

By PATRICIA LOANE, B.Sc*

SUMMARY

Samples of raw and pasteurized egg pulp were examined for the presence of amylase-producing bacteria. From 194 colonies picked at random from plates of egg pulp, 50 proved to be starch hydrolysers. These were examined and tested for degree of dextrinizing activity. Ability to grow at the refrigeration temperature of 40°F and the effect of storage at 0°F over a period of 8 months were also examined. None of the starch hydrolysers produced sufficient amylase either to be evaluated by the method employed or to affect the negative results of the alpha-amylase test.

I. INTRODUCTION

In Queensland, egg is pulped, pasteurized at 148°F for 2½ min and tested (Loane 1964) for efficacy of heat treatment, using the absence or destruction of heat-labile alpha-amylase as the principle of the test (Shrimpton *et al.* 1962). Under these conditions of heating, both the enzyme and the most resistant strains of *Salmonella* are destroyed. The pulp is frozen for export, and on arrival at its destination it is retested for efficacy of pasteurization. The question of possible bacterial production of the enzyme during the interval between testings has arisen.

The production of amylase by different bacteria has been studied by a number of workers. Cultural conditions such as composition and pH of medium, temperature and time of incubation and other factors have been carefully worked out and controlled (Wallerstein 1939; Beckford, Kneen, and Lewis 1945; Beckford, Peltier, and Kneen 1946). The quantity and quality of amylase produced from bacterial isolates from a variety of sources have also been studied (Peltier and Beckford 1945; Kneen and Sandstedt 1946; Kneen and Beckford 1946).

* Dairy Research Laboratory, Queensland Department of Primary Industries.

The investigation of amylase-producing microflora in Queensland egg pulp and the dextrinizing activity of these organisms is described in this paper.

II. EXPERIMENTAL PROCEDURE

Sampling.—Thirty batches of pasteurized egg pulp were sampled in duplicate into sterile plastic bags at the factory. One sample was tested immediately and the other was frozen and stored at 0°F for 8 months. In addition, 8 samples of raw and 8 of pasteurized pulp (each pasteurized on the day of testing) were delivered to the laboratory for examination.

Alpha-amylase test.—All samples of pasteurized egg pulp were subjected to alpha-amylase test (Shrimpton *et al.* 1962). The stored frozen samples were tested before and after storage.

Isolation of cultures.—The method followed was that of Peltier and Beckford (1945). The pulps were plated on starch-heart infusion agar and incubated at 30°C for 3 days. Colonies were then picked off at random and repeatedly restreaked until pure. Representative colonies were subcultured into heart infusion broth, then back onto starch-heart infusion agar, and tested for hydrolyis of starch by addition of Lugol's iodine solution. All negative colonies were discarded.

Identification of cultures.—Determination of morphology, Gram reaction and motility were made from cultures grown at 30°C for 24 hr in both heart infusion broth and peptone water.

Starch-heart infusion agar was used for the growth of all cultures tested for growth at 40°F for 14 days; sensitivity to antibiotics (penicillin 2·5 i.u. and streptomycin 80 μg–"Sentests" Evans Medical Ltd.); oxidase (Gordon and McLeod 1928); and catalase (Topley and Wilson 1929).

Methyl red and Voges-Proskauer tests were performed on cultures grown in Difco MR.–V.P. medium incubated at 30°C for 4 days, using the O'Meara modification of the V.P. test (Skerman 1959).

The test for carbohydrate utilization was that of Hugh and Leifson (1953), using 1·5% agar to seal the "closed" tubes. The sugars used were glucose, lactose and sucrose.

Goré's modification (Goré 1921) was used for the determination of indole production. Ability to grow on Difco-desoxycholate agar was also determined.

The action of the cultures on litmus milk was examined after 7 days at 30°C.

Dextrinizing activity.—The activity of these starch hydrolysers was determined by the method of Kneen and Beckford (1946), using wheat bran agar slopes and broths. This activity is expressed in terms of "dextrinizing time", namely

the time in minutes required by 10 ml of wheat bran medium to convert 20 ml of 1% boiled starch (at 30°C and pH 6) to the point where the "red-brown" colour is given with iodine. Activity was gauged by the following times:—

1–10 min—active

10–20 min—fairly active

Over 60 min—inactive

III. RESULTS

(a) Incidence

The number of egg pulp samples examined and the number of colonies picked at random for subsequent testing for starch hydrolysis and dextrinizing activities are set out in Table 1.

TABLE 1

INCIDENCE OF STARCH HYDROLYSERS AND DEXTRINIZING ACTIVITY

Description of Samples	No. of Samples	No. of Colonies Picked at Random	No. of Starch Hydrolysers	No. with Dextrinizing Times of Less Than 60 min
Pasteurized stored	30	55	8	0
Pasteurized ..	8	45	24	0
Raw	8	94	18	0
TOTALS ..	46	194	50	0

(b) Alpha-amylase Test

All pasteurized samples gave readings indicating effective pasteurization treatment at the factory. The results of the stored pulps had not altered from those of their duplicates, tested immediately after pasteurization prior to storage.

(c) Classification of Isolates

Representative colonies were picked from plates streaked with positive starch-hydrolysing cultures, purified and classified provisionally by the use of the tests detailed above. A summary of reactions is set out in Table 2. The cultures appeared to fall into five groups, as follows:

TABLE 2

SUMMARY OF BIOCHEMICAL REACTIONS

Name of Group	Biochemical Reaction														
	Glucose		Lactose		Sucrose		Reaction to Penicillin	Reaction to Streptomycin	Catalase	Oxidase	Indole	Methyl Red	V.P.	Litmus	Growth at 40°F
	O	C	O	C	O	C									
(i) *Bacillus* ..	A	A	—	—	A	A	R	S	++	+	—	+	—	Red	—
(ii) *Aeromonas* ..	A	A/G	—	—	A	A/G	R	S	++	+	+	—	+	Red/Dig	+
(iii) *Achromobacter*	—	—	—	—	—	—	R/S	S	+	+	—	—	—	—	+
(iv) *Xanthomonas*	—	—	—	—	—	—	R	S	+	+	+/—	—	—	+/—	—

A = Acid production.
G = Gas production.
R = Resistant.
S = Sensitive.

Red = Reduction.
Dig = Digestion.
+/— = Variable reaction.

(i) *Bacillus group.*—There were 10 cultures in this group, 7 isolated from freshly pasteurized pulp and 3 from raw pulp. All organisms were large, motile, Gram-positive, spore-forming bacilli. Agar colonies were large, flat, rough and irregular. They all fermented glucose and sucrose with the production of acid only. Lactose was not attacked. They were resistant to penicillin but sensitive to streptomycin, catalase and oxidase positive, indole negative, methyl red positive and V.P. negative. They were unable to grow at 40°F. Litmus milk was reduced.

The dextrinizing activity test showed that the cultures were inactive in 60 min.

(ii) *Aeromonas group.*—Twelve cultures (9 from raw and 3 from freshly pasteurized pulp) appear to belong to the genus *Aeromonas.* All organisms were short, motile, Gram-negative and rod-shaped. Carbohydrates were fermented, i.e. acid and gas produced anaerobically from glucose and sucrose. Lactose was not utilized. They were resistant to penicillin and sensitive to streptomycin; catalase, oxidase, indole and V.P. tests were all positive. They were able to grow on desoxycholate agar at 30°C and on starch-heart infusion agar at 40°F. Litmus milk was reduced and the curd digested.

The dextrinizing activity time of these organisms was greater than 60 min, so they can be termed inactive.

(iii) *Achromobacter group.*—Ten cultures (3 from raw and 7 from freshly pasteurized pulp) would appear to fall into the *Achromobacter* group. These were non-pigmented, short, stout, coccoid, motile rods. There was very little or no utilization of carbohydrates. They gave variable reactions to penicillin and were sensitive to streptomycin. They were catalase and oxidase positive; indole, methyl red and V.P. negative. There was growth at 40°F and very little reaction in litmus milk.

The dextrinizing activity time was greater than 60 min for these organisms.

(iv) *Xanthomonas group.*—Seven cultures (3 from raw and 4 from freshly pasteurized pulp) can provisionally be assigned to the genus *Xanthomonas.* These were Gram-negative, thin, long, non-motile rods, pigmented yellow. There was no fermentation and very little oxidation of carbohydrates. They were resistant to penicillin and sensitive to streptomycin; catalase and oxidase tests were positive; indole production was variable; methyl red and V.P. tests were negative. No growth occurred at 40°F or on desoxycholate agar at 30°C, and the effect on litmus milk was variable.

The dextrinizing activity of these organisms showed that they also were inactive in 60 min.

(v) *Remainder.*—The remaining 11 organisms remain unidentified. Their reactions to the above tests were varied. They were not sporing rods and unable to either dextrinize 20 ml starch solution in 60 min or to grow at 40°F.

IV. DISCUSSION

The Gram-positive bacilli have been assigned to the species *Bacillus megaterium*. The characteristics of these cultures follow closely those proposed by Smith, Gordon, and Clark (1952). They stipulate that the Voges-Proskauer reaction is an important and a reliable test for distinguishing some species, if certain modifications of the usual method are followed. These organisms gave a negative V.P. reaction.

The bacteria belonging to group ii appear to follow closely the description of the genus *Aeromonas* given by Eddy (1960). Using his key to the species, these organisms would appear to be *Aeromonas liquefaciens*.

Shewan, Hobbs, and Hodgkiss (1960) set out a determinative scheme for the identification of certain genera of Gram-negative, asporogenous, rod-shaped bacteria. Their work and discussions proved helpful in classifying and differentiating group iii as *Achromobacter*.

The presence of *Xanthomonas* and the unidentified bacteria is not important in this investigation, as they can neither survive pasteurization nor are they psychrophilic. The latter point is important because refrigeration rather than freezing temperatures may, at some stage, be used in the storage of egg pulp.

Group iv has been classified as *Xanthomonas* because it agrees with *Phytomonas* of Bois and Savary (1945), which name was later changed to *Xanthomonas* (see "Bergey's Manual of Determinative Bacteriology," 4th and 7th editions).

The two types of bacterial amylase are alpha (dextrinizing) and beta (saccharifying). The test for effectiveness of pasteurization of egg pulp is based on the destruction of alpha-amylase.

Although 25% of the colonies isolated from pulp hydrolysed starch, they were shown not to be dextrinizing. In addition, they did not produce sufficient alpha-amylase to affect the alpha-amylase test either in fresh or in stored egg pulp.

None of the active bacilli—*B. subtilus, B. macerans, B. polymyxa*—was found in the the pulp.

It is therefore concluded that the alpha-amylase test provides an effective assessment of pasteurization of liquid whole egg pulp at 148°F for $2\frac{1}{2}$ min.

REFERENCES

BECKFORD, L. D., KNEEN, E., and LEWIS, L. H. (1945).—*Industr. Engng Chem.* 37:692.

BECKFORD, L. D., PELTIER, G. L., and KNEEN, E. (1946).—*Industr. Engng Chem.* 38:232.

BOIS, E., and SAVARY, J. (1945).—*Canad. J. Res.* 23:208.

EDDY, B. P. (1960).—*J. Appl. Bact.* 23:216.

GORDON, J., and MCLEOD, J. W. (1928).—*J. Path. Bact.* 31:185.

GORÉ, S. N. (1921).—*Indian J. Med. Res.* 8:505.

HUGH, R., and LEIFSON, E. (1953).—*J. Bact.* 66:24.

KNEEN, E., and BECKFORD, L. D. (1946).—*Arch. Biochem.* 10:41.

KNEEN, E., and SANDSTEDT, R. M. (1946).—*Arch. Biochem.* 9:235.

LOANE, PATRICIA (1964).—*Qd J. Agric. Sci.* 21:153.

PELTIER, G. L. and BECKFORD, L. D. (1945).—*J. Bact.* 50:711.

SHEWAN, J. M., HOBBS, G., and HODGKISS, W. (1960).—*J. Appl. Bact.* 23:463.

SHRIMPTON, D. H., MONSEY, J. B., HOBBS, BETTY, and SMITH, MURIEL (1962).—*J. Hyg., Camb.*, 60:153.

SKERMAN, V. B. D. (1959).—"A Guide to the Identification of the Genera of Bacteria." (Williams and Wilkins: Baltimore).

SMITH, N. R., GORDON, RUTH, and CLARK, F. E. (1952).—Monograph U.S. Dep. Agric. No. 16.

TOPLEY, W. W. C., and WILSON, G. S. (1929).—"Principles of Bacteriology and Immunity." (Edward Arnold: London).

WALLERSTEIN, I. (1939).—*Industr. Engng Chem.* 31:1218.

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