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# α-Amylase in whole egg and its sensitivity to pasteurization temperatures

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## INTRODUCTION

Murdock, Crossley, Robb, Smith & Hobbs (1960) have concluded from the accumulated evidence of recent years not only that liquid egg in bulk contains organisms of the salmonella group, but also that these organisms can be destroyed by pasteurization at suitable temperatures. Nevertheless, as these authors have pointed out, many problems have to be solved before pasteurizing procedures can be satisfactorily applied to the production of liquid whole egg.

Internationally, the proportions of samples from liquid whole egg that are contaminated with salmonellae vary from country to country. Furthermore, many different serotypes are present and the minimum heat treatments necessary for the elimination of salmonellae may vary according to the origin of the egg. There is, also, little published information on the effect of heat treatments on the quality of the egg products; and no simple test is available for the effective control of a heating process. Clearly, the heat treatment of milk had analogous problems (cf. Kästli, 1957) and there, an effective control test has been developed based on the sensitivity to heat of the enzyme phosphomonoesterase (Kay & Graham, 1935; Aschaffenburg & Mullen, 1949).

A chemical test based on the destruction of the activity of an enzyme will be satisfactory for this purpose if it fulfils the following requirements: (1) the enzyme must be a natural constituent of the egg; (ii) there must always be an appreciable activity of the enzyme; (iii) destruction of the enzyme's activity by heat must be a first-order reaction; (iv) the influence of current procedures of processing on the test must be known.

The activities of diastase, protease, salicylase, lipase and oxidase were reported by Koga (1923). Lineweaver, Morris, Kline & Bean (1948) reported the presence of three esterases (tributyrinase, lipase and phosphatase), a peptidase and a proteinase, catalase, oxidase, cytochrome oxidase, peroxidase and an α-amylase similar to salivary amylase. The activity of all these enzymes was small; the phosphatase of yolk being only one twentieth as active, on a dry weight basis, as the phosphatase of dried skim milk. More recent work (Schormüller & Hothorn, 1956) has also shown the low activity of yolk phosphatase. It was not likely, therefore, that the phosphatase test of Kay & Graham (1935) for the control of pasteurized milk could readily be adapted for the control of pasteurized liquid whole egg. A comparable test using the inactivation of α-amylase by heat was

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proposed for egg by van Oijen (1940), but at that time the heat treatment considered necessary for adequate pasteurization was one of 20 min. at 65° C. in the presence of an anti-coagulant. At present in the United Kingdom the treatment proposed is $2\frac{1}{2}$ min. at 64·4° C. when no added anti-coagulant is necessary (Heller, Roberts, Amos, Smith & Hobbs, 1962).

For α-amylase it is clear that requirement (i) listed above is satisfactorily met from published information and van Oijen's results have indicated the practicability of the test for control procedures on the 'phosphate' pattern. In the experiments reported below the extent of the variation of the activity of the enzyme was studied in different samples of egg in the course of determining the rate of inactivation of the enzyme by heat. This procedure had two particular advantages, first it was necessary for this purpose to use a sensitive method for estimating activity and secondly, it involved a direct study of the relevant property of the enzyme. By this means the extent to which a test based on the heat-inactivation of α-amylase could fulfil requirements (ii), (iii) and (iv) was studied by applying it to many samples of whole egg collected over a long period.

## MATERIAL AND METHODS

### *Apparatus*

Because the temperatures (58°–67° C.) used were within the range of those which result in the coagulation of egg, it was essential for the time of heating to be closely controlled and for any period of warming up to be reduced to a minimum. These conditions were most satisfactorily achieved with a continuous flow apparatus in which the sequence was as follows:

(*a*) Pre-heater (at 45° C.) capacity 400 ml.

(*b*) Delivery tube and flowmeter (average rate of flow 13 ml./min.).

(*c*) Stainless steel heating tube (interior diameter 1·397 mm.).

(*d*) Holding tube (at selected pasteurization temperature). Chosen from a set of tubes with capacities from 13·2 to 94·5 ml. thus giving a range of average holding times. A tube with a capacity of 34·5 ml. was used for an average holding time of $2\frac{1}{2}$ min.

(*e*) Chilling tube through ice chips.

(*f*) Three-way delivery tap to collecting vessel.

### *Analysis*

The analytical procedure was in two parts. First the rates of hydrolysis of different concentrations of starch by the α-amylase in variously pasteurized samples of liquid whole egg were determined experimentally. Secondly, these values were used to calculate the corresponding activities of the α-amylase and the rate of its inactivation by heat.

#### *Hydrolysis of starch*

B.D.H. soluble starch was used at concentrations of 2–6 % selected according to the anticipated activity of the α-amylase. 120 g. of whole egg pulp were

incubated with 20 ml. of the starch solution at 37° C. Samples of 60 ml. were withdrawn after 30 min. and 1 hr. A serum was prepared from these mixtures by shaking each for about 1 min. with 8 ml. of basic lead acetate (prepared from neutral lead acetate and litharge according to the A.O.A.C.—Association of Official Agricultural Chemists—method, 1955). 20 ml. of chloroform was added and, after shaking for about 15 sec., centrifuged.

When the pulp was deproteinized some starch was precipitated with the protein. The extent of this 'binding' was different for soluble starches of different origins.

*Determination of residual starch*

The amount of unhydrolysed starch remaining was determined by treating 5 ml. of the serum with 0·5 ml. of 0·5N nitric acid and 5 ml. of 0·003N iodine and measuring the absorption at 585 mμ in 0·2 cm. cells.

Because the colour of the iodine-starch complex faded, the absorption was determined at intervals of 5, 10, 15 and 20 min. after the addition of the iodine and the initial value was determined by extrapolation.

The initial amount of starch in the incubation mixture was determined by extrapolation from the amount remaining after 1 hr. incubation through the amount found after 30 min. to the start of incubation.

*Activity of α-amylase*

The velocity constant of the destruction of the activity of α-amylase for any given condition of pasteurizing was calculated by comparing the rates at which soluble starch were hydrolysed by pasteurized and raw egg.

First, the velocity constants $k_1$ and $k$ were calculated for the hydrolysis of starch by pasteurized and raw egg respectively, using the equation for a reaction of the first order, i.e. $K = 2{\cdot}303 \log 10(a/a{-}x)$, where $a$ is the initial concentration of the starch and $x$ is the amount reacting in the time $t$. Secondly, the concentration of the α-amylase after pasteurizing was calculated in relation to an initial concentration arbitrarily taken to be 100 from the expression $[(k-k_1)/k] \times 100$. This can also be regarded as the percentage destruction of the enzyme's activity by the pasteurizing treatment. Thirdly, the velocity constant of the destruction of α-amylase during pasteurization, $K_p$, was calculated assuming that within this range of temperatures the reaction was one of the first order. Finally, the Arrhenius relation between rate and temperature of reaction was tested by plotting the log 10 of the different values obtained for $K_p$ for different times and temperatures of pasteurizing sixty samples of whole egg obtained over 11 months against the reciprocals of the pasteurizing temperatures in degrees absolute: conformity with this relation gave confidence in the basis of calculation adopted for $K_p$.

*Flow characteristics of pasteurizing apparatus*

The sodium nitrite injection method of Botham (1952), which was modified for use in commercial egg plants by Murdock *et al.* (1960), was used. The nitrite concentrations were determined in deproteinized samples of egg by the sensitive method of Eddy (1958) using the α-naphthylamine–acetic acid–sulphanilic acid reagent.

*Determination of* pH

A glass electrode and a pH meter was used.

*Origin of the eggs*

Fresh eggs were bought from shops in Cambridge over a period of 11 months and a pulp was prepared from each purchase (usually 12) using an 'Ato-Mix'. On some occasions commercially prepared pulp was also used.

## RESULTS AND DISCUSSION

### *The activity of α-amylase in raw whole egg*

*Variation in eggs from different sources*

Values for the velocity constant, $k$, for the hydrolysis of starch by whole egg from different sources were calculated and the results are shown in Table 1. It was concluded that the variation was sufficiently small to be neglected for the purpose in hand.

Table 1. *The rate of hydrolysis of starch by whole egg from different sources*

| Origin of whole egg | No. of eggs | $K$* |
|---|---|---|
| Retailer A, sample 1 | 12 | 5·14 |
| Retailer A, sample 2 | 16 | 4·92 |
| Retailer A, sample 3 | 7 | 5·30 |
| Retailer A, sample 4 | 12 | |
| Retailer B | 12 pooled | 5·88 |
| Retailer C | 12 | |
| Wholesaler A (U.K.) | Bulk sample 1 | 4·76 |
| Wholesaler A (U.K.) | Bulk sample 2 | 4·78 |
| Wholesaler B (U.K.) | Bulk sample | 4·96 |
| Wholesaler C (imported) | Bulk sample | 5·80 |

* The velocity constant for the hydrolysis of starch (10 % solution).

*Influence of* pH

The pH of liquid whole egg is usually near 7·5 (Brooks & Taylor, 1955). The activity of the enzyme measured by the velocity constant ($k$) for the hydrolysis of starch was determined over a range of pH from 6 to 9 and the results are shown in Fig. 1. The optimum pH, between 7 and 8, is near to that of whole egg which is a convenience for the purpose of an 'amylase test'.

*Influence of packing station procedures*

No effect on the activity was observed due to commercial freezing, thawing, homogenization (by an orifice procedure at a pressure of 200 lb./sq. in.) or spray drying and the activity was not influenced by freeze drying under laboratory conditions. The addition of citrate at 1 % or sucrose at 13 % apparently significantly increased the activity of the enzyme.

*The influence of heat on the activity of α-amylase*

*Flow characteristics of the laboratory pasteurizer*

It was not practicable to pass egg through the pasteurizer at a greater average velocity than 13 ml./min. Taking into account the dimensions of the apparatus, it was clear that no operating conditions could be envisaged in the laboratory in which the value of the Reynolds Number ($N$) could exceed 2000, the maximum value for stable laminar flow in pipes. The possibility of establishing stable turbulent flow ($N > 3000$) was discounted completely.

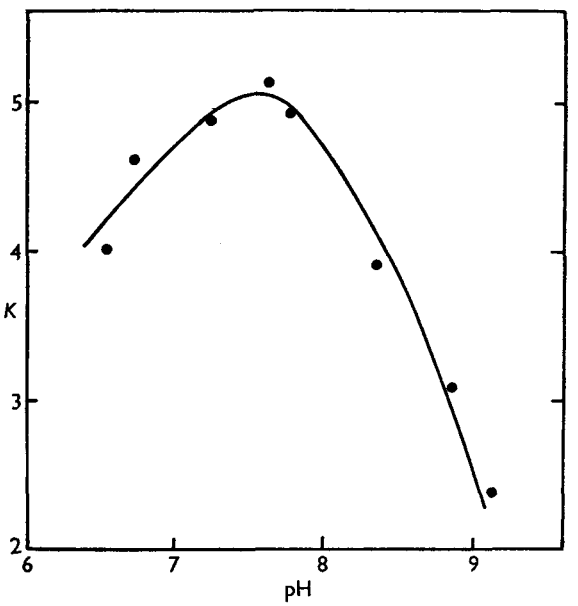


Fig. 1. Influence of pH on the activity of α-amylase of whole egg.

Since flow through the laboratory pasteurizer was laminar rather than turbulent, all holding times were calculated as average holding times and not in the customary way for commercial pasteurizers, as minimum holding times. The time/temperature relations are therefore not comparable with those obtaining for commercial pasteurizers.

*Activity of α-amylase after various heat treatments in the laboratory pasteurizer*

The temperatures used ranged from 59° to 66° C. for periods from 1·7 to 6·7 min. The value of $K_p$ (the velocity constant of the destruction of α-amylase during pasteurizing) was calculated for each of these conditions in many samples of eggs collected over a period of 11 months and the results are summarized in Fig. 2, where $\log_{10} K_p$ is plotted against $1/T$ (the reciprocal of the pasteurizing temperature in °A.). A regression line has been plotted and the broken lines indicate the 1 and 5% limits of confidence. It was concluded that with all samples of eggs studied a linear relationship existed under these conditions between $\log_{10} K_p$ and

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$1/T$. The results also show that the assumption made in the calculation of $K_p$, namely that the heat inactivation of the $\alpha$-amylase is a first order reaction, is supported by the experimental evidence.

The regression line has been calculated from observations on sixty samples, each consisting of the blended contents of twelve eggs purchased randomly over nearly a year. Thus, it is unlikely that samples of eggs will be encountered in which the $\alpha$-amylase responds differently, so that heat inactivation of the enzyme is a promising test for controlling a pasteurizing process.

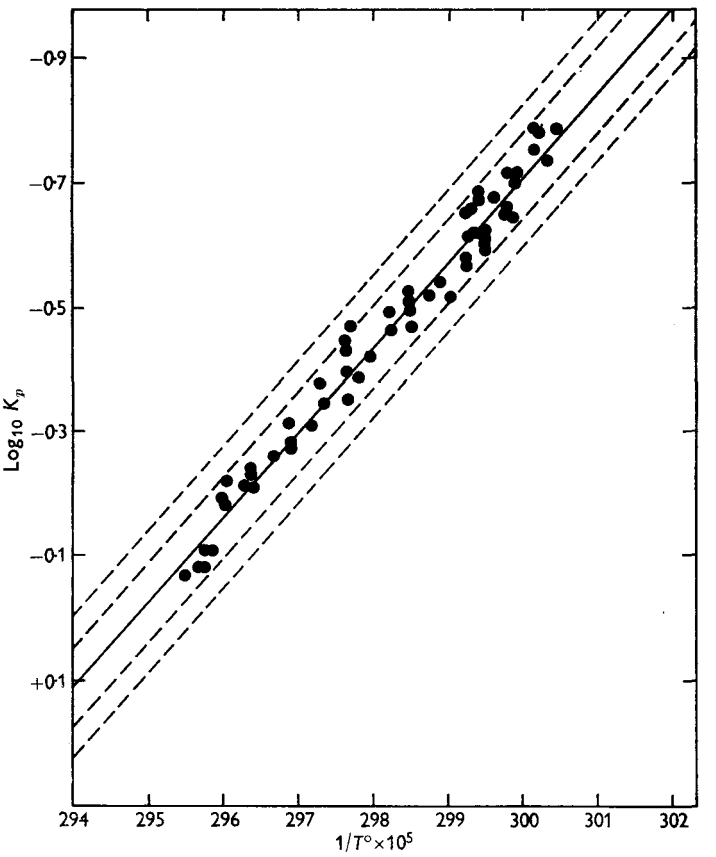


Fig. 2. The effect of different conditions of pasteurizing on the activity of the $\alpha$-amylase of whole egg (sixty samples, each of twelve eggs, obtained over a period of 11 months).

SUMMARY

The activity of the enzyme $\alpha$-amylase of whole egg is known to be sensitive to pasteurizing temperatures and the possibility that this property could be used as a control test for the adequacy of pasteurization has been examined.

The occurrence of the enzyme, the physico-chemical characteristics of its heat inactivity, the consistency of its behaviour in large numbers of eggs and the influence on it of packing station procedures has been studied.

By sampling eggs from many sources for 11 months it was concluded that the

activity of the enzyme was consistent, the inactivation by heat was a first-order reaction, and freezing, thawing, homogenization, spray-drying and in the laboratory freeze-drying did not affect its inactivation. But there was some interference from added citrate and sucrose.

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