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Analytical Methods Section

A new, modified acid phosphatase assay for determining the extent of heat treatment in canned hams

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A modified acid phosphatase assay is presented in this paper for indication of heat treatment in canned hams. Adequate blending of the sample, increased substrate concentration and working at the pH optimum in the reaction mixture considerably improved the sensitivity of the method.

Difficulties arose with the use of concentrated meat slurries: high rate of adsorption of released phenol from phenyl phosphate and reduced activities for the high level of endogenous competitive inhibitors (phosphates). Taking the thermal processing curve of Lind as reference, a heat treatment equivalent F_c {70°C} = 80 min value is provisionally proposed (at $Z = 6{\cdot}94$°C) for the central area of the cans instead of the not unambiguous end-point temperature regulation (69°C/156°F). This F_c value assures the destruction of heat resistant *Streptococci* in the product. However, at present, such F_c and Z values are not available for the FMD virus. An adequate inspection system, based on mathematical statistical considerations is also briefly discussed here. In conclusion, results of this study indicate that the new, modified method has potential for the successful and rather simple measurement of the extent of heating in canned hams.

INTRODUCTION

Lind (1965, 1972) elaborated a method for the assessment of core temperature in canned hams by determining the residual acid phosphatase activity (E.C.3.1.3.2.). According to the USDA regulation (USDA-APHIS, 1982), a core temperature of at least 69°C (156°F) has to be reached during the pasteurisation of these products.

Lind's method, based partly on earlier results of Körmendy and Gantner (1960, 1967), was accepted by the USDA (Lind, 1987) in order to control the temperature attained in the central area (core) of canned hams during heat treatment. Results published by Cohen (1969), Finogerova *et al.* (1973) and Tyszkiewicz & Krysiak (1976) generally confirm the suitability of this method for the purpose mentioned above. However, two main problems have arisen concerning the acid phosphatase assay:

1. Is it possible to improve its sensitivity and reproducibility?
2. How to substitute the core temperature regulation of USDA with the correct heat treatment equivalent (F_c) concept which takes into account the *integral* heat treatment in the central area of the cans?

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MATERIALS AND METHODS

Selection of samples and heating procedures

Raw, cured ham samples were randomly taken from the production line of a Hungarian meat processing plant before packing the hams in cans. This type of product has a rather uniform proximate composition (water content 74–75%, protein content 21–22%, NaCl content 2·9–3·2%, added polyphosphate content 0·45–0·55%, fat content 2–3%). Proximate analysis was done according to Hungarian Standards (Lörincz & Lencsepeti, 1973). Approximately 20 g of samples were ground in the laboratory with a plate of 2 mm, placed in cellophane bags, pressed with a roller and vacuum-sealed. In this way, thin pouches with a diameter not exceeding 2 mm were obtained reaching the constant temperature of the water bath within 20 s (Gantner &

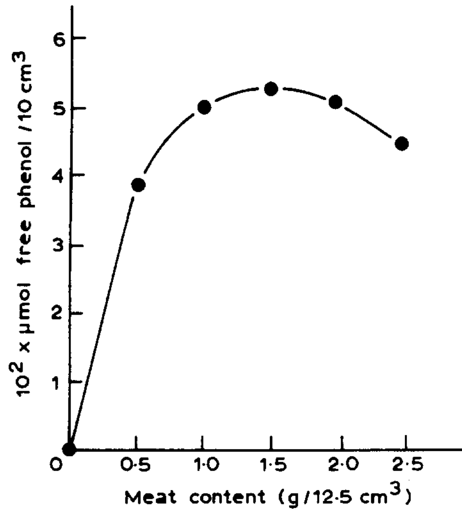

Fig. 1. Relationship between quantity of meat in the slurry and concentration of released phenol, measured with the method of Lind (1987) (S_0 = 0·01 mol/dm³; pH = 6·5; incubation: 60 min at 37°C; sample:cooked meat).

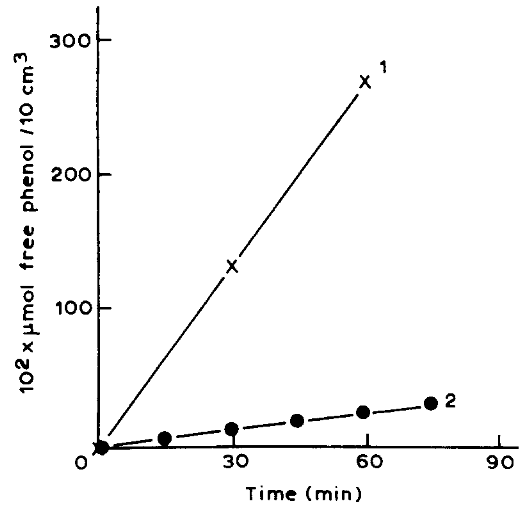

Fig. 3. Relationship between concentration of released phenol and time of incubation at 37°C, measured with the modified method (S_0 = 0·05 mol/dm³; pH = 5·4; samples: raw (1) and cooked (2) meat).

Körmendy, 1968). After heat treatment, they were immediately cooled in tap water. These experiments also served for the establishment of the heat inactivation curves (see Fig. 6 below). So, the constancy and uniformity of temperature inside the samples has been assured during heat treatment. Determination of activity was done after storage in a refrigerator for 48–72 h.

In Figs 1–5 and Tables 1 and 2 the term 'cooked meat' means that fresh cured ham samples were cooked in thin cellophane bags at 65°C or 70°C in a water bath. The cooking time was chosen according to the desired residual acid phosphatase activity range with the help of the data presented in Fig. 6 or in the paper of Gantner & Körmendy (1968), respectively.

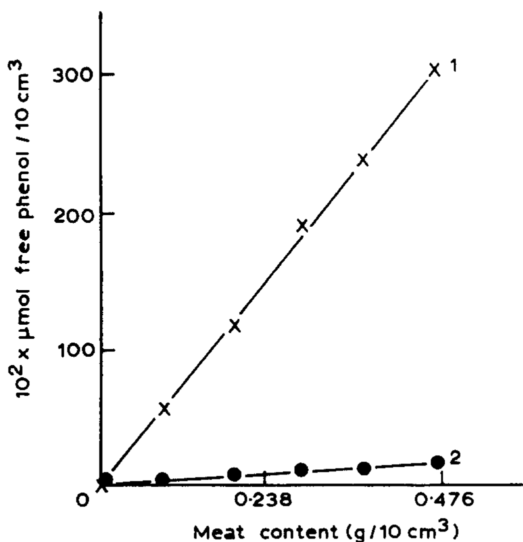

Fig. 2. Relationship between quantity of meat in the slurry and concentration of released phenol, measured with the modified method (S_0 = 0·05 mol/dm³; pH = 5·4; incubation: 60 min at 37°C; samples: raw (1) and cooked (2) meat).

Table 1. Comparison of Lind's (1987), method (EF) with the procedure of Gantner & Körmendy (1968) (A). (S_0 = 0·01 mol/dm³; pH = 6·5; sample; cooked meat; slurries: 0·2 g/cm³ and 0·0476 g/cm³, respectively). Number of replications: 5

Samples	Acid phosphatase activity			
	A		EF	
1	776	100%	304	39·2%
2	1317	100%	1083	82·2%

The thermal processing curve of Lind (1965), taken as a reference for the calculation of F_c values, had the following characteristics: oblong can of size, 166 × 103 × 320 mm (12 lb); constant temperature of water bath, 73°C; initial temperature of the canned ham, 9°C; max-

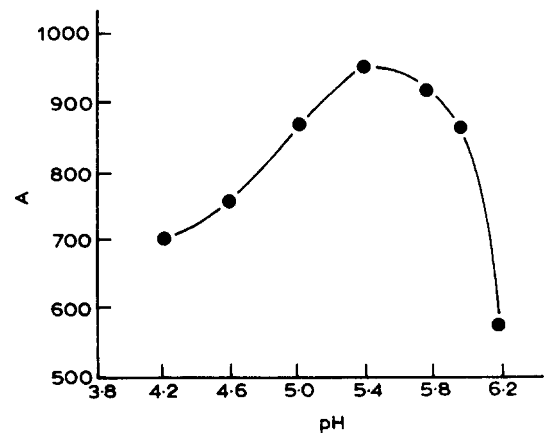

Fig. 4. Variation of acid phosphatase activity with pH (S_0 = 0·05 mol/dm³; 2·5 g sample + 50 cm³ buffer; incubation: 60 min at 37°C).

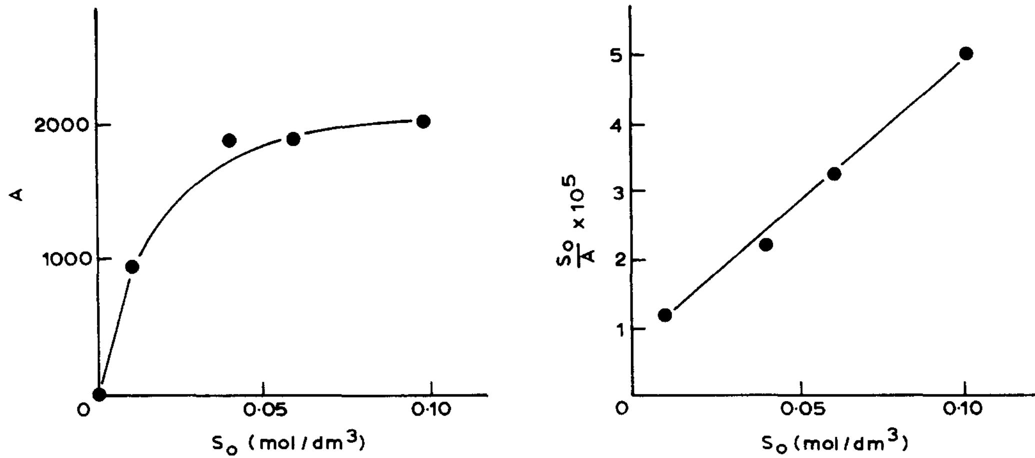

Fig. 5. Variation of acid phosphatase activity (A) with initial substrate concentration (S_o) at pH = 5·4 (incubation: 60 min at 37°C; 2·5 g sample + 50 cm³ buffer; sample: cooked meat).

imal core temperature attained during heat treatment, 70°C. The pasteurisation was stopped at a core temperature of 69°C after 277 min and the can was cooled in tap water. 60°C was attained after 360 min in the cooling phase.

The thermal processing curves used for the calculation of data presented in Fig. 7 were obtained with the same size of cans as above. However, the constant temperature of the water bath was 76°C and the initial temperatures of the cans was 5–9°C. After reaching the required core temperature, measured with inserted thermocouples, the cans were immediately cooled in tap water.

For determining the influence of meat quality on residual phosphatase activity, DFD (dark, firm, dry), normal or PSE (pale, soft, exudative) *M. semimembranosus* from *Post rigor* pork were used. Meat quality was evaluated with pH and reflectance measurements as well as by a sensory test according to Vada-Kovács (1974).

Table 2. Rate of phenol adsorption in meat slurry (pH = 6·5; sample: cooked meat)

Added phenol (µg/20 cm³)	Phenol recovery (%)	
	2·5 g + 50 cm³ buffer	2·5 g + 10 cm³ buffer
20·0	99·2	24·7
39·75	89·0	16·7
79·5	86·0	22·0

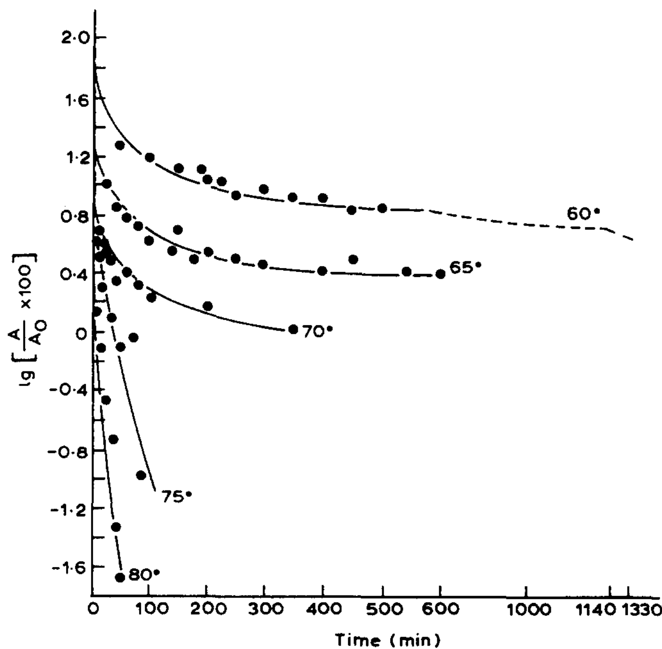

Fig. 6. Semilogarithmic plot of residual acid phosphatase activity as a function of heating time (t) and temperature, measured with the modified method (A_0 = activity at t = 0; A = activity at t min of heating).

Apparatus

This was Ultra-Turrax homogenizer (TP 18/2, 20 000 rev/min, Janke and Kunkel K. G., Staufen i. Br., GFR);

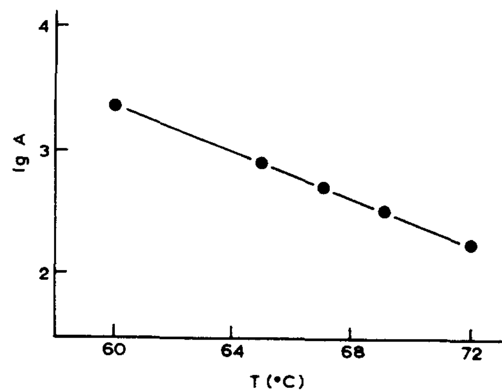

Fig. 7. Semilogarithmic plot of residual acid phosphatase activity (A) versus core temperature (T_c) in oblong cans with size: 166 × 103 × 320 mm (12 lb), measured with the modified method (temperature of water bath: 76°C; initial temperature of the cans 5–9°C).

magnetic stirrer; 100 cm³ Erlenmeyer flask and those, which are indicated in the USDA Guidebook (Lind, 1987).

Reagents

(a) Citrate buffer 0·05 mol/dm³, pH = 5·4: 8·036 g citric acid and 3·53 g NaOH were dissolved in distilled water and diluted to 1000 cm³ treated with 1 cm³ toluene and stored in a refrigerator.

(b) Citrate buffer 0·15 mol/dm³, pH = 5·0: 30·24 g citric acid and 11·76 g NaOH were dissolved in distilled water and diluted to 1000 cm³, treated with 1 cm³ toluene and stored in a refrigerator.

(c) Disodium phenyl phosphate (S_o = 0·05 mol/dm³): 1·2704 g disodium phenyl phosphate was dissolved in citrate buffer, pH = 5·0 and diluted with the same to 100 cm³. This was prepared immediately before use. Citrate buffer pH = 5·0 is to be used here in order to compensate alkaline disodium phenyl phosphate. So, a substrate stock solution (S_o) was obtained with pH = 5·4 which was then diluted threefold (5:15) in the reaction mixture.

(d) Working phenol solution: 5 cm³ of stock phenol solution (1 mg/cm³), prepared as indicated in USDA Guidebook (Lind, 1987), was transferred to a 500 cm³ volumetric flask. 50 cm³ of 50% TCA were added and was made up to volume with distilled water and shaken well.

Sodium carbonate, trichloroacetic acid (TCA) and 2,6-dibromoquinone chloroimide solutions and reagents for standardization of stock phenol solution were prepared as indicated in the USDA Guidebook (Lind, 1987).

Determination

(a) Samples taken from the central area of the product were ground through a 2 mm plate and thoroughly mixed.

(b) 2·5 g samples were weighed into a 100 cm³ Erlenmeyer flask and 50 cm³ cold citrate buffer pH = 5·4 were pipetted into it.

(c) The slurry was blended with an Ultra-Turrax at maximal speed (20 000 rev/min) 4 times for 10 s with breaks of about 20 s to avoid over-warming (Gantner & Körmendy, 1968; Körmendy *et al.*, 1968).

(d) 10 cm³ blended slurry were pipetted into each of three stoppered test tubes (A, B and C) assuring the uniformity of aliquots with a magnetic stirrer. Tubes A and B were used for duplicate determinations. Tube C was the control sample. (As demonstrated, the major part of acid phosphatase in cooked meat is attached to the undissolved, heat-coagulated proteins in the slurry (Körmendy & Gantner, 1967)).

(e) Test tube C (control sample) was placed in boiling water (100°C) for 30 min in order to fully inactivate the acid phosphatase enzyme. After this, it was cooled to room temperature with tap water.

(f) All tubes were placed in a water bath at 37·0°C for about 15 min (pre-incubation).

(g) 5 cm³ disodium phenyl phosphate solution were pipetted into each tube in turn at exactly 60-s intervals using a stop watch and shaken cautiously.

(h) All tubes were shaken at 150 min intervals during incubation.

(i) After exactly 60 min, 5 cm³ 20% TCA were added to each tube in turn at 60-s intervals (Tube A at 60 min, tube B at 61 min, etc).

(j) Each tube was removed from the water bath after addition of TCA, shaken well and, after standing for about 5 min, filtered. (Filtrates could be stored for some hours in a refrigerator if needed.)

(k) 5 cm³ of clear filtrates were pipetted into clean test tubes.

(l) 5 cm³ sodium carbonate (0·5 mol/dm³) were pipetted into each tube, and mixed.

(m) 0·1 cm³ 2,6-dibromoquinone chloroimide solution was pipetted into each tube, mixed well by swirling.

(n) Colour was developed at room temperature in the dark for at least 30 min (not overnight).

(o) Absorbances of each solution were read at 610 nm using 1 or 2 cm cells and water as reference for setting the spectrophotometer at 100% transmission.

Preparation of standard curve

Duplicate aliquots of 0·0 cm³, 1·0 cm³, 2·0 cm³, 3·0 cm³ and 4·0 cm³ of working phenol solution were pipetted into test tubes (10 tubes total). 5·0 cm³, 4·0 cm³, 3·0 cm³, 2·0 cm³ and 1·0 cm³ respectively, of 5% TCA were pipetted making each tube equal in volume (5 cm³). Further steps are carried out according to steps (l), (m), (n) and (o). Absorbance is linearly related to phenol concentration in the range indicated above.

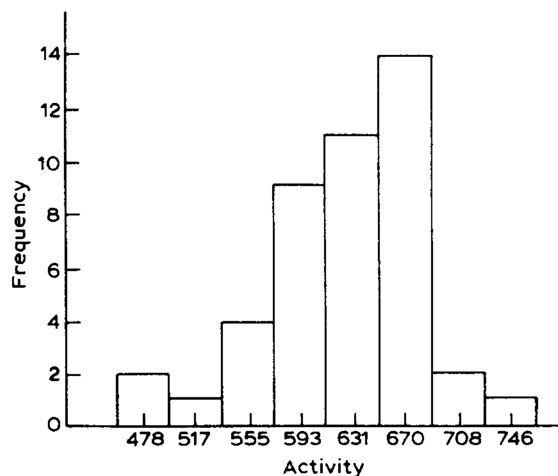

Fig. 8. Histogram of distribution of residual phosphatase activity (A) of samples cooked for 80 min at 70°C and measured with the modified method (n = 44).

(The millimolar absorbancy index of phenol is about 22 $cm^3/\mu mol$ at 610 nm.) (1 $\mu mol/cm^3$ = 1 $mmol/dm^3$). Determination of phenol concentration in stock phenol solution is carried out, if necessary, according to the USDA Guidebook (Lind, 1987). This modified acid phosphatase enzyme assay was used in experiments presented in Figs 2–8 as well as in Tables 4 and 5 (see below).

Calculations

Acid phosphatase activity is given here in arbitrary units. Under the conditions used, one unit of acid phosphatase was defined as the amount of enzyme which catalyses the production of 1 μmol phenol in 60 min from 1000 g sample:

$$[\mu\text{mol phenol/1000 g sample}] = \frac{a \times 4}{0{\cdot}476} \times \frac{1000}{f} \times 10 \quad (1)$$

where

$a = a_s - a_c$

a_s = absorbance of the sample at 610 nm, relative to a 1 cm cell

a_c = absorbance of the control at 610 nm, relative to a 1 cm cell,

f = millimolar absorbancy index of phenol obtained from standard curve.

Factor 4 in formula (1) indicates that the free phenol concentration is measured from a 5 cm^3 aliquot of 20 cm^3 final volume and 0·476 is the amount of sample (g) in 10 cm^3 slurry (2·5 g sample + 50 cm^3 citrate buffer; 2·5 g sample is taken for 2·5 cm^3 in the calculations). For the sake of comparability, activity measured with the method of Lind (1987) is given as follows:

$$[\mu\text{mol phenol/1000 g sample}] = \frac{a \times 4{\cdot}5}{2{\cdot}5} \times \frac{1000}{f} \times 10 \quad (2)$$

because a 5 cm^3 aliquot is taken from 22·5 cm^3 final volume (2·5 g $\approx$ 2·5 cm^3). Lind (1987) diluted a 2·5 g sample with 10 cm^3 citrate buffer (pH = 6·5) obtaining a 4·7 times more concentrated final solution than in this modified method or in our method published earlier (Körmendy & Gantner, 1960, 1967; Gantner & Körmendy, 1968; Körmendy *et al.*, 1968).

The sensitivity of the alternative methods was compared with the help of the sensitivity ratio (Mandel, 1964; ref. Körmendy *et al.*, 1989). If the sensitivity ratio $S = 1$, both methods are equally sensitive; if $S > 1$ or $S < 1$, either method is more sensitive than the other, respectively (Table 3). The standard deviations, necessary for the calculation, were measured with $k = 10$ replicates for each method to be compared. The first derivatives, contained also in the formula, were determined graphically in the non-linear cases.

Lind's method (1987) differs substantially from the procedure presented above in four steps:

— Slurry is much more concentrated: 0·2 g/cm^3 instead of 0·0476 g/cm^3 respectively.

— Blending of sample in citrate buffer with Ultra-Turrax is omitted. He only describes grinding through a 2 mm plate.

— Determination of acid phosphatase activity is carried out at pH = 6·5 and with a stock substrate solution S_o = 0·01 mol/dm^3 in accordance with our earlier method (Körmendy & Gantner, 1960, 1967; Gantner & Körmendy, 1968).

RESULTS AND DISCUSSION

Effect of enzyme concentration on results

As previously mentioned, Lind (1987) used about 4·2 times more concentrated slurries than used in our experiments. However, as Table 1 shows, the acid phosphatase activities obtained by his procedure are surprisingly lower than those obtained with the method of Gantner & Körmendy (1968). (Differences are highly significant.) If enzyme concentration and amount of released phenol were proportionally related, about four times higher free phenol concentrations should be obtained with Lind's procedure after incubation for 60 min at 37°C. Accordingly, activities (related to 1000 g sample) should be approximately the same.

This phenomenon can be partly explained by the high extent of adsorption of released phenol from phenyl phosphate in more concentrated slurries during the incubation (Table 2). As Table 2 shows, the rate of phenol adsorption by the meat particles is much higher in the more concentrated slurry used by Lind (1987).

Table 3. Average residual acid phosphatase activity ($\bar{A}$) standard deviation (s), coefficient of variation (CV) and sensitivity ratio (S) of alternative methods. (Number of replications for each method: k=10)

Heat treatment of samples	Methods											
	'x'			'z'			'y'					
	$\bar{A}$	s	CV	$\bar{A}$	s	CV	$\bar{A}$	s	CV	$S\left\{\frac{z}{x}\right\}$	$S\left\{\frac{y}{z}\right\}$	$S\left\{\frac{y}{x}\right\}$
60 min at 70°C	256	27·5	10·7	485	27·5	5·7	848	37·6	4·4	1·80	1·29	2·06
30 min at 70°C	329	27·5	8·3	604	36·3	6·0	1073	42·0	3·9	1·57	1·51	1·81
50 min at 65°C	592	64·9	11·0	867	50·8	5·8	1536	54·6	3·6	1·01	1·63	1·25

It was also shown that phosphates, present originally in meat and added also during the curing procedure, behaved as competitive inhibitors in the acid phosphatase assay (Schonheyder, 1952; Körmendy & Gantner, 1960; 1967). For this reason, a more concentrated meat slurry means evidently a more concentrated phosphate solution in the reaction mixture. Therefore, in addition to phenol adsorption, this phenomenon also produces lower acid phosphatase activities in the more concentrated slurries and also a non-linear relationship between enzyme concentration (which is proportional to the quantity of meat in the slurry) and amount of released phenol during the enzyme assay (Fig. 1).

On the other hand, in diluted slurries (up to 0·0476 g/cm³) the same relationship is linear (Fig. 2) and the amount of released phenol is also proportionally related to the time of incubation at 37°C within 60 min (Fig. 3).

This is the consequence of the fact that the rate of substrate hydrolysis is low; it is less than 5% even in the case of raw meat with high acid phosphatase activity. Such a level indicates the initial, approximately linear phase of the enzymic reaction.

Increase of sensitivity of the acid phosphatase assay

The former acid phosphatase assay published by Gantner & Körmendy (1968) used pH = 6·5 and a substrate (phenyl phosphate) stock solution S_o = 0·01 mol/dm³. However, the pH optimum of the acid phosphatase is at pH = 5·4 if S_o = 0·05 mol/dm³ (Fig. 4).

Moreover, by increasing S_o, reaction rate also increases and, as Fig. 5 shows, the near stationary region of the saturation curve (activity versus initial substrate concentration) is not far from S_o = 0·05 mol/dm³.

The original procedure ('x') published by Gantner & Körmendy (1968) was compared with two alternative methods:

— method 'x': pH = 6·5; S_o = 0·01 mol/dm³;
— method 'z': pH = 5·4; S_o = 0·01 mol/dm³;
— method 'y' presented here in Materials and Methods: pH = 5·4; S_o = 0·05 mol/dm³.

Blending was carried out with an Ultra-Turrax: 2·5 g sample + 50 cm³ citrate buffer in each case. (For further experimental conditions see Materials and Methods except that pH and S_o were adjusted accordingly.) Lind's procedure was not included in this study because of the reaction kinetic considerations discussed previously.

Results are presented in Table 3. As Table 3 shows, the sensitivity is considerably improved by working at the pH optimum 5·4 and S_o = 0·01 mol/dm³. The sensitivity ratio of method 'y' compared to method 'x' is about 2 with sample heated at 70°C for 60 min, that is, the former method is twice as sensitive as the latter one.

Further improvement of the acid phosphatase assay was not successful. Increase of S_o above the level 0·05 mol/dm³ causes, among other problems, too high blank values at the spectrophotometric determination of phenol. An increase of the temperature of incubation from 37 to 45°C gives rise to spontaneous hydrolysis of phenyl phosphate and, in the case of raw meat samples, to a slight denaturation of proteins. Elimination of endogenous inhibitors (phosphates) from the slurry in order to increase activity, complicates the procedure excessively. Mg^{2+} activates only the alkaline phosphatase (Bergmeyer, 1962).

Heat inactivation of acid phosphatase

Figure 6 shows the semilogarithmic plot of residual acid phosphatase activity as a function of duration of heat treatment. These experiments were performed at temperatures of 60°C, 65°C, 70°C, 75°C and 80°C with ground cured hams in thin pouches (see Materials and Methods).

As can be seen from Fig. 6, relationships are not linear; consequently, the heat inactivation process does not exhibit a first order reaction kinetics. The general form of the rate equation (Hill & Grieger-Block, 1980):

$$v = kE^n \quad (3)$$

where v = reaction rate,
k = rate constant,
E = enzyme concentration,
n = order of reaction,
is not valid because the log-log plot of the data does not give parallel lines, i.e. n is temperature-dependent.

Beckman (ref. Boyer, 1971) separated heat-labile and heat-stable acid phosphatase isoenzymes in meat by electrophoresis. Such type of isoenzymes can produce a biphasic heat inactivation process. Biphasic heat inactivation curves were obtained with horseradish peroxidase (Ling & Lund, 1978; Chang *et al.*, 1988), kohlrabi peroxidase (Schmidt & Vámos-Vigyázó, 1985) and papaya acid phosphatase (Carreno & Chan, 1982). However, as demonstrated by Körmendy & Gantner (1967), the originally homogeneous distribution of acid phosphatase in the sarcoplasmic fraction of meat is transformed to a heterogeneous one during heat treatment, i.e. the major part of the enzyme will be attached to the undissolved, coagulated meat proteins. So, an intermediary, active form of the enzyme appears as the first step in a consecutive reaction followed by a completely inactive form.

The first step is more rapid than the second. The superposition of these two phenomena (presence of isoenzymes + consecutive reactions) seems to produce the heat-inactivation curves as shown in Fig. 6 (Reiner, 1969).

With the help of these data, calculations were performed in order to determine the Z value, which is the increase of temperature required to reduce the enzyme

activity by one order of magnitude. Körmendy (1966) has demonstrated that, under certain conditions, the Z value will be constant even if the heat-inactivation process does not follow first order kinetics, i.e. the decimal destruction time of the enzyme is not constant at a given temperature. (Theory and calculation are not given here.) This assumption is fulfilled in this case and an average Z value of 6·94°C was obtained for the acid phosphatase in cured ham.

The USDA regulation for the heat processing of canned hams (core temperature should attain at least 69°C) intends to kill the foot-and-mouth disease (FMD) virus in the product. Unfortunately, there are neither heat destruction curves nor Z and D values published for FMD virus so far (Heidelbaugh & Graves, 1968; Blackwell *et al.*, 1982, 1988). Melo & Vigario (1987) found $Z = 4{\cdot}5°C$ for African swine fever (ASF) virus in Portuguese sausages. In previous experiments, Körmendy *et al.* (1987) obtained a Z value of 5·85°C for canned hams; however, they used the method of Lind (1987) at pH = 6·5 and $S_0 = 0{\cdot}01$ mol/dm^3.

Adequate heat treatment and USDA regulation

Lind (1965) found a close correlation between residual phosphatase activity and core temperature in 12-lb oblong cans. The same results were obtained by Hvass (1983*a,b*) for canned luncheon meat and canned pork shoulder. However, the residual phosphatase activity does not depend on the core temperature itself, but on the integral heat treatment of the core. It is evident that, for example, 1-lb cans reach the required end-point core temperature of 156°F/69°C more rapidly than 12-lb ones, so that the residual phosphatase activity can be considerably higher in the former case.

The determination of heat treatment equivalent (F_c) performed with Bigelow's method, therefore gives a clear indication of the extent of heating (ref. Ball & Olson, 1957; Reichert *et al.*, 1979; Körmendy *et al.*, 1987; Müller, 1989). The heat treatment equivalent (F_c) is generally referred to 70°C at pasteurized meat products (Reichert, 1980; Reichert *et al.*, 1979, 1988).

Taking the thermal processing curve of Lind (1965), described schematically in Materials and Methods, as a reference, the following F_c values were obtained in the core with Bigelow's method (ref. Körmendy *et al.*, 1987):

$$F_c\{70°C, Z = 6{\cdot}94°C\} = 80 \text{ min and}$$
$$F_c\{70°C, Z = 10°C\} = 94 \text{ min,}$$

$Z = 6{\cdot}94°C$ is the actual value obtained with the modified phosphatase assay presented in this paper and $Z = 10°C$ is the value generally accepted for pasteurized canned meat products (Reichert, 1980; Reichert *et al.*, 1979, 1988). Reichert *et al.* (1979, 1988) found F_c values from 20 to 80 min (referred to 70°C) at $Z = 10°C$ with *S. faecium* strains for cured meat products. (F_c is the heating time necessary for complete destruction of *S. faecium*.) According to Magnus *et al.* (1988), *S. faecium* P-1A is the most heat resistant vegetative microorganism occurring in meat with $Z = 7{\cdot}47°C$ and $F_c = 95$ min. Houben (1980, 1982) found $Z = 10°C$ and $F_c = 89$ min with the same microorganism for canned hams. As can be seen, the $F_c\{70°C, Z = 6{\cdot}94°C\} = 80$ min value, calculated from the thermal processing curve of Lind (1965) for canned ham and taken unintentionally as a reference curve by the USDA, is sufficient to kill the heat resistant Streptococci and, hopefully, the FMD virus, too. So, the provisional acceptance of this F_c value should be reasonable by the competent authority (USDA) until reliable data for the heat destruction parameters of FMD virus will be available.

For the sake of comparison, following the experiments of Lind (1965) and Hvass (1983*a,b*), a relationship between core temperature (T_c) and logarithm of residual phosphatase activity (log A) was determined with oblong cans under experimental conditions as described in Materials and Methods. As shown in Fig. 7, the relationship was rigorously linear with a standard error of 0·27°C around the regression line. These results are in agreement with those obtained by Lind (1965) and by Hvass (1983*a,b*).

Inspection of the adequate heat treatment equivalent

Forty-four raw hams were randomly taken from the production line and thin pouches were prepared with them as described in Materials and Methods. After this, they were cooked in a water bath at 70°C for 80 min. This histogram of distribution of residual phosphatase activity measured with the new, modified method can be seen in Fig. 8. Table 4 shows the statistical parameters of the samples and the upper control limits (one sided test: $\alpha = 0{\cdot}05$) for sample sizes 1 and 2. Naturally, the precision of the inspection system can be further improved with more than two items (cans) taken randomly from the lot (Hald, 1962). Taking the second type error for $\beta = 0{\cdot}1$, a heat treatment equivalent $F_c = 30$ min can already be detected with 90% probability if $n = 2$.

Needless to say, as well as the effect of the heat treatment, the inactivation of the acid phosphatase is also influenced by the chemical composition of the meat

Table 4. Statistical parameters of 44 cured ham sample units taken randomly from the production line and cooked at 70°C for 80 min in thin pouches (equivalent to F_c = 80 min). (n = sample size; $\bar{A}$ = mean activity; s = standard deviation; CV = coefficient of variation; one sided test: α = 0·05)

n	$\bar{A}$	s	CV	Upper limits	
				$\bar{A} + 1{\cdot}65\,s$	$\bar{A} + 1{\cdot}65\,\frac{s}{\sqrt{2}}$
44	627·6	59·55	9·49%	725·9	697·1

Table 5. Influence of the type of post rigor *M. semimembranosus* of pork (DFD, normal, PSE) on acid phosphatase activity ($\bar{A}$ = mean activity; s = standard deviation; CV = coefficient of variation; n = sample size)

Type of meat	n	$\bar{A}$	s	CV
DFD	12	24 416	1 706	6·99
normal	15	24 412	1 329	5·44
PSE	9	22 954	1 549	6·75

product in question: NaCl, polyphosphate and protein content, pH value, etc., also effect the heat-inactivation of this enzyme (Körmendy & Gantner, 1960, 1967). So, the stability of the manufacture of these products is of primary importance in controlling the extent of heating with this method. Any systematic change in the technological process influencing the composition of the product may alter the statistical parameters of the samples cooked for $F_c = 80$ min (see Table 4 and Fig. 8).

Fortunately, this condition for stability is fulfilled in practice, in the production of canned hams. Table 5 shows that the type of pork meat (*M. semimembranus*: DFD, normal and PSE, excised 2–3 days after slaughter and stored at refrigeration temperature) has no significant effect on acid phosphatase activity, measured with the modified method. The coefficients of variation are not excessively high. This fact is very important since the residual activity in the final product after heating is strongly dependent on the unknown initial one.

There is no evidence that acid phosphatase is reactivated in cooked meat and meat products after storage at refrigeration temperatures (Körmendy & Gantner, 1960; Lind, 1965; Körmendy *et al.*, 1968).

The results of this study indicate that the new, modified phosphatase assay could be used successfully in the control of heating of canned hams or other meat products.

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